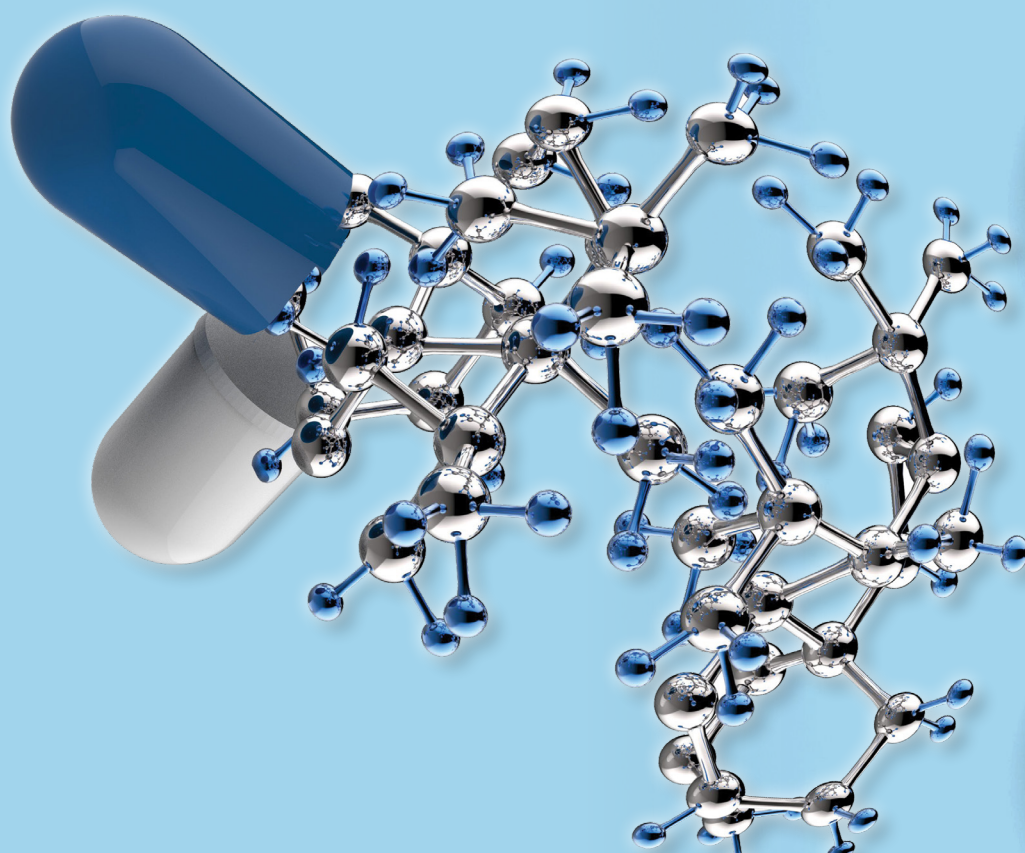


ISSN 1105-4999



ΦΑΡΜΑΚΕΥΤΙΚΗ PHARMAKEFTIKI

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
A QUARTERLY EDITION ON PHARMACEUTICAL SCIENCES' TOPICS



Proceedings of the 2nd International Babylon
Conference on Clinical and Experimental
Pharmacological Research

19-20 APRIL 2025, HILLAH, IRAQ

TOMOS
VOLUME

37

TEYXOS
ISSUE

2s • 2025

ΦΑΡΜΑΚΕΥΤΙΚΗ

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ
ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
ΤΟΜΟΣ 37, ΤΕΥΧΟΣ 2S, 2025

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PHARMAKEFTIKI

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ON PHARMACEUTICAL SCIENCES' TOPICS
VOLUME 37, ISSUE 2S, 2025

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PHARMAKEFTIKI

A QUARTERLY JOINT EDITION OF
THE HELLENIC SOCIETY OF
MEDICINAL CHEMISTRY &
THE HELLENIC PHARMACEUTICAL SOCIETY



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PROCEEDINGS OF THE 2ND INTERNATIONAL BABYLON CONFERENCE ON CLINICAL AND EXPERIMENTAL PHARMACOLOGICAL RESEARCH

edited by

APOSTOLOS ZARROS
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2nd International Babylon Conference on Clinical and Experimental Pharmacological Research

(College of Pharmacy, University of Babylon, Hillah, Iraq; April 19–20, 2025)

organized under the auspices of



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The 2nd International Babylon Conference on Clinical and Experimental Pharmacological Research: focusing on drug development and repurposing, drug safety, and public health

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KEY WORDS:

Iraq; pharmacological research; drug development; drug safety; public health

ARTICLE INFO:

Received: March 31, 2025

Revised: April 02, 2025

Accepted: April 03, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

The proceedings of the 2nd International Babylon Conference on Clinical and Experimental Pharmacological Research (taking place at the College of Pharmacy of the University of Babylon, in Hillah, Iraq; April 19-20, 2025) are hereby hosted by *Pharmakeftiki*; a journal founded by the Hellenic Society of Medicinal Chemistry (HSMC) in 1988 and currently published independently with the support of both the HSMC and the Hellenic Pharmaceutical Society. This year's conference proceedings are more extensive in terms of both the number of papers included in them and the thematic representation of the field. In that respect, the conference's scientific programme exhibits an encouraging enrichment that also signifies the importance of this event for the Iraqi pharmacological community. The proceedings cover the main messages of the keynote speakers' talks, present the findings of a large number of clinical, epidemiological, and experimental research studies, and also host interesting (mini-)reviews on important pharmacological topics that, in most cases, are penned by post-graduate students and their supervisors, as part of their degree prerequisites. The conference's committees have successfully established an international forum for the discussion of critical developments in pharmacological research, with this year's conference focusing on drug development and repurposing, drug safety, and public health.

The International Babylon Conference on Clinical and Experimental Pharmacological Research is not only an annual international forum for the communication of pharmacological research and the discussion of the latest developments in the field, but also a major event for the Iraqi medical, biomedical, pharmaceutical, and wider scientific communities. This year's (second) conference builds on the success of the first conference (that took place at the College of Pharmacy of the University of Babylon on the 4th and the 5th of May 2024) with a significantly larger scientific programme and an impressive thematic enrichment. Under the guidance and leadership of Prof. Hussam W. Al-Humadi (the Dean of the College of Pharmacy of the University of Babylon), the members of the steering, the organizing, and the scientific committees of this year's conference have put together what is expected to be a structurally, thematically, and scientifically excellent international meeting, thereby facilitating the development of this recurring event into an institution of national and international importance for the communication of research findings in the field of pharmacology. In my view, this year's remarkable interest for the conference brings us all also a step closer to the establishment of a national pharmacological society for Iraq; a development that we should consider immediately after the conclusion of the conference works.

This year, the 2nd International Babylon Conference on Clinical and Experimental Pharmacological Research takes place at the College of Pharmacy of the University of Babylon, in Hillah, Iraq, on the 19th and the 20th of April 2025, under the auspices of the Ministry of Higher Education and Scientific Research of the Republic of Iraq, the University of Babylon and its College of Pharmacy, as well as the University of Hilla. The conference proceedings are hereby gracefully hosted by *Pharmakeftiki*; a journal founded by the Hellenic Society of Medicinal Chemistry (HSMC) in 1988 and currently published independently with the support of both the HSMC and the Hellenic Pharmaceutical Society. In a similar manner to last year's conference proceedings, the current proceedings cover

the main messages of the keynote speakers' talks, present the findings of a large number of clinical, epidemiological, and experimental research studies, and also host interesting (mini-)reviews on important pharmacological topics that, in most cases, are penned by postgraduate students and their supervisors, as part of their degree prerequisites.

As far as the objectives of this year's conference are concerned, these remain unaltered and can be summarized as follows: (i) to facilitate the mapping of the Iraqi pharmacological research activity and priorities, and (ii) to facilitate global collaborations. However, readers will notice that, in contrast to last year's thematic dominance of papers communicating the findings of ethnopharmacological research, alarming data regarding antibiotic misuse, or promising applications of nanotechnology in drug formulation and delivery¹, this year's conference proceedings are significantly enriched with papers focusing on drug development and repurposing, exploring critical drug safety issues, or attempting to address important public health challenges. This thematic enrichment is – in my opinion – an encouraging indicator of a swift of focus towards the long-awaited embracing of pharmaceutical innovation for the benefit of the Iraqi pharmaceutical sector^{2,3}, the strengthening of pharmacovigilance for the improvement of drug safety standards⁴⁻⁶, as well as the study of critical public health issues related to social pharmacology and healthcare system preparedness⁷⁻⁹.

My work as editor has focused again on ensuring that all proceedings papers are edited for accuracy, clarity, and consistency. This task has been greatly facilitated by the contributors' patience, enthusiasm, and collaboration, as well as by the unwavering support of the conference's organizing committee, the journal's editorial board, and the publisher's staff. I am certain that the proceedings in hand convey the intended messages in the best possible manner, and that the readers will enjoy and appreciate the remarkable thematic diversity, the interesting research findings, and the academic potential recorded in them.

Acknowledgments

The proceedings editor is grateful to Prof. Anna Tsantili-Kakoulidou, Prof. George Albert Karikas, Mr Gerasimos Kouloumpis, Mrs Alexandra Platania, Prof. Hussam W. Al-Humadi, and Dr Mohammed Obies for their enthusiastic help and continuous support. The proceedings editor acknowledges acting as the British Pharmacological Society Ambassador to the University of Babylon.

Conflicts of interest

As of March 2025, the proceedings editor (and author of the current editorial) is an honorary professor at the University of Babylon.

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HOW TO CITE:

Zarros A. The 2nd International Babylon Conference on Clinical and Experimental Pharmacological Research: focusing on drug development and repurposing, drug safety, and public health. *Pharmakeftiki* 37(2s), 1–3, 2025. <https://doi.org/10.60988/p.v37i2S.129>

Targeting the amyloid precursor protein interactome in Wnt signalling provides novel therapeutic strategies for Alzheimer's disease

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KEY WORDS:

Alzheimer's disease;
drug discovery;
Wnt signalling;
drug repositioning;
transcriptomics

ARTICLE INFO:

Received: April 14, 2025

Revised: n/a

Accepted: April 16, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

Alzheimer's disease (AD) is characterised by progressive synaptic dysfunction, cognitive decline, and pathological hallmarks including amyloid-beta ($A\beta$) accumulation and tau hyperphosphorylation. $A\beta$ disrupts Wnt signalling by inducing Dickkopf-1 (Dkk1); a secreted antagonist that promotes a pathological shift from canonical Wnt/ β -catenin to non-canonical planar cell polarity signalling. Crucially, the amyloid precursor protein (APP) interacts with key components of the Wnt pathway, including the co-receptor LRP6 (low-density lipoprotein receptor-related protein 6). This places APP at the centre of a regulatory network – the APP interactome – that modulates synapse stability, $A\beta$ production, and transcriptional homeostasis. In our recent studies, we explored how therapeutic modulation of this network restores synaptic health and cognitive function in AD models. We show that the ROCK (Rho-associated coiled-coil kinase) inhibitor fasudil reverses AD-associated transcriptional signatures *in vivo* and blocks $A\beta$ -induced synaptotoxicity. Furthermore, we report a novel Dkk1-LRP6 disruptor peptide that restores canonical Wnt signalling, reduces $A\beta$ generation, and prevents cognitive deficits *in vivo*. These data position the APP-Wnt interactome as a rich source of therapeutic targets, and establish new strategies for the restoration of synaptic resilience in AD.

1. Introduction

Alzheimer's disease (AD) remains an unmet clinical challenge, with currently-available treatments offering only modest symptomatic relief. Synapse loss, rather than plaque or tangle burden, is the best pathological correlate of cognitive decline¹. Understanding how amyloid-beta (A β) contributes to synaptic dysfunction is critical to the development of disease-modifying therapies. The amyloid precursor protein (APP) is cleaved by β - and γ -secretases to generate A β , but emerging data show that APP itself is not a passive substrate². Instead, it functions as a modulator of cellular signalling through its interactions with Wnt pathway components, especially LRP6 (low-density lipoprotein receptor-related protein 6). This APP-Wnt interactome governs synaptic stability and A β production³. Dysregulation of this network – particularly *via* a Dkk1 (Dickkopf-1)-mediated antagonism of the canonical Wnt signalling – leads to synapse loss, increased A β generation, and cognitive impairment³⁻⁵. In this context, we investigated how targeting the APP interactome in Wnt signalling could yield a therapeutic benefit. Our studies have identified two promising approaches: (i) the pharmacological inhibition of downstream Wnt-PCP (planar cell polarity) effectors using fasudil^{3,5} and (ii) the disruption of the Dkk1-LRP6 interaction using a novel peptide, thereby restoring canonical Wnt signalling and APP homeostasis⁶.

2. Mechanistic basis: fasudil blocks A β -driven synaptotoxicity *via* Wnt-PCP inhibition

To explore fasudil's mechanistic action, we examined the synaptic effects of A β in rodent primary neuronal cultures. A β was found to rapidly increase Dkk1 expression³⁻⁵, which in turn activated the non-canonical Wnt-PCP pathway *via* RhoA and ROCK (Rho-associated coiled-coil kinase), leading to cytoskeletal destabilisation and dendritic spine loss. We demonstrated that both A β and Dkk1 induce spine retraction through this pathway, and that fasudil robustly blocks this effect, restoring synaptic density^{3,5}. *In vivo*, fasudil administration rescued performance in

the novel object recognition task in rats exposed to intracerebroventricular A β , indicating cognitive benefit⁵. These results position fasudil as a potent modulator of Wnt-PCP-dependent synaptotoxicity. Its effects converge on the RhoA/ROCK axis downstream of APP and Dkk1, thereby disrupting the pathological APP-Wnt-cytoskeleton cascade^{3,5}.

3. Fasudil reverses neurodegenerative disease-associated pathways in the AD brain

Fasudil is a clinically-approved Rho-associated protein kinase (ROCK) inhibitor with established use in cerebral vasospasm. Given ROCK's downstream role in Wnt-PCP signalling – a pathway activated by the A β -Dkk1 signalling – we hypothesised that fasudil could correct synaptic dysfunction in AD. To test this, we administered fasudil systemically for two weeks to AD transgenic mice (3xTg), which model both amyloid and tau pathology. Global transcriptomic analysis of brain tissue revealed fasudil-induced gene expression changes that strongly anti-correlated with signatures from postmortem AD, Parkinson's, and Huntington's disease brains⁷. Pathway analysis revealed an upregulation of oxidative phosphorylation, mitochondrial function, and canonical Wnt signalling; pathways typically suppressed in AD⁷. Conversely, pathways upregulated in AD, including neuroinflammation and Wnt-PCP signalling, were suppressed by fasudil. These transcriptional effects highlight fasudil's disease-reversing activity at the level of molecular networks. Furthermore, fasudil restored the expression of many of the top 25 most-downregulated genes in AD, particularly those related to synaptic function⁷. This global transcriptomic reversal suggests that fasudil can re-establish a homeostatic set point in the diseased brain.

4. Disrupting Dkk1-LRP6 interaction: a novel strategy to maintain canonical Wnt signalling

While fasudil targets the downstream effectors of Wnt-PCP, we next explored whether direct interference with the upstream A β -induced shift from canonical to non-canonical signalling could offer an

even more targeted therapeutic strategy. We identified the Dkk1-LRP6 interaction as a critical switch point in this process⁶. Using peptide array mapping, alanine scanning, and docking simulations, we developed an 11-amino-acid disruptor peptide (DLDP) that binds the first β -propeller domain of LRP6^{6,8}, thereby blocking Dkk1 binding with nanomolar affinity.

Functionally, the DLDP restored canonical Wnt/ β -catenin signalling in luciferase reporter assays, even in the presence of Dkk1. It also rescued the APP-LRP6-dependent suppression of A β generation, suggesting a restoration of non-amyloidogenic APP processing. In primary rat cortical neurons, the DLDP prevented the dendritic spine loss caused by Dkk1 and reduced endogenous A β production, while *in vivo*, the intracerebroventricular delivery of DLDP prevented the A β -driven cognitive impairment in the novel object recognition task⁶.

5. Targeting the APP-Wnt signalling hub: a therapeutic framework

Together, our findings define the APP-Wnt signalling axis as a key mediator of synaptic pathology in AD and a promising site for therapeutic intervention. Our findings can be summarised as follows: (i) APP's interaction with LRP6 is crucial for maintaining canonical Wnt signalling and promoting non-amyloidogenic processing, (ii) A β induces Dkk1, which disrupts this interaction, shifts signalling to the Wnt-PCP axis, and promotes both synapse loss and A β production *via* a feed-forward loop, (iii) fasudil acts downstream by inhibiting ROCK, thereby reversing AD-related transcriptional changes and protecting synapses and cognition, and (iv) DLDP (a peptide disruptor of the Dkk1-LRP6 interface) acts upstream, thereby preserving the APP-LRP6-mediated canonical signalling and reducing A β toxicity. These strategies converge on

the preservation of synaptic integrity by restoring Wnt pathway homeostasis. By targeting the APP interactome – either at the level of upstream ligand-receptor interactions or downstream cytoskeletal effectors – we have demonstrated a systems-level therapeutic approach to modify the course of AD.

6. Conclusion

Our data identify the APP-Wnt interactome as a central hub linking A β toxicity to synapse degeneration and cognitive decline. Fasudil and DLDP represent two mechanistically distinct, yet complementary, approaches to restoring network homeostasis. Fasudil, already approved for human use, offers immediate translational potential for AD. DLDP exemplifies a novel peptide-based approach targeting a specific protein-protein interaction upstream of synaptic degeneration. These therapeutic strategies offer a new framework for tackling AD by focusing on synaptic resilience and network repair, rather than amyloid clearance alone. Future studies should evaluate their efficacy in chronic models of AD and consider combination therapies that modulate the APP interactome at multiple levels.

Acknowledgements

The author acknowledges all collaborators who have made this work possible, with special mention of Dr Richard Killick of King's College London.

Conflicts of interest

None exist.

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HOW TO CITE:

Elliott C. Targeting the amyloid precursor protein interactome in Wnt signalling provides novel therapeutic strategies for Alzheimer's disease. *Pharmakeftiki* 37(2s), 4-7, 2025. <https://doi.org/10.60988/p.v37i2S.130>



Recent advances in the acute management and long-term secondary prevention in transient ischaemic attack and ischaemic stroke

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KEY WORDS:

ischaemic stroke; transient ischaemic attack; reperfusion treatment; antiplatelet treatment; anticoagulant treatment

ARTICLE INFO:

Received: April 27, 2025

Revised: n/a

Accepted: April 28, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

Ischaemic stroke is by far the most frequent condition that neurologists routinely face in the hospital setting, accompanied by significant morbidity and long-term loss of function for the survivors. The approach of the potential stroke patient should start at the pre-hospital level, since time is of essence. Any focal neurological deficit of sudden onset should raise the suspicion of stroke or transient ischaemic attack (TIA), especially if no signs of seizures are present. After prompt brain imaging, in the setting of the emergency department, treatment mainly aims at reperfusing the tissue originally supplied by the occluded artery. This is achieved by either intravenous thrombolysis or mechanical thrombectomy. Certain criteria must be fulfilled in order to be able to apply these treatments. Moving away from the emergency approach, an important concern of the subacute (in-hospital) and long-term (post-hospitalisation) treatment of stroke patients surrounds the challenges regarding the proper use of antiplatelet and anticoagulant agents. The latter have a place in the long-term secondary prevention of cardioembolic stroke or TIA, while the former (either as a monotherapy or as a dual antiplatelet treatment) are mainly used in the management of the hospitalised patients and the secondary prevention of non-cardioembolic stroke and TIA.

1. Introduction

Ischaemic stroke is by far the most frequent condition that neurologists routinely face in the hospital setting, accompanied by significant morbidity and long-term loss of function (i.e., significant drop in quality of life) for the survivors. Diagnosis and management of the condition nowadays follows internationally-acknowledged guidelines aiming at maximising the benefit for the patients, which depends on both the prompt and proper intervention of the paramedical and medical teams in the acute and subacute stage, as well as the best-fitting, long-term management of the patients. Prompt and proper intervention entails detection of patients with suspected stroke (ideally even before them reaching the emergency department, by the pre-hospital health-care team) and guiding them timely through the proper diagnostic channels so as to confirm the diagnosis and the best therapeutic intervention (based on the type of ischaemic stroke and the temporal window). The best-fitting, long-term management of the patient relates to two main concerns: (i) identifying the underlying aetiology of the ischaemic stroke, that would assist in (ii) deciding on the most fitting treatment scheme for secondary prevention, taking also into consideration other parameters from the medical history of the patient (such as other co-morbid conditions and related drugs they may have to take). In this short paper, I will summarise the treatment options used at the acute, subacute, and chronic level of management of ischaemic stroke (Figure 1).

2. Approaching ischaemic stroke and transient ischaemic attack in the acute setting

Any focal neurological deficit of sudden onset should raise the suspicion of stroke or transient ischaemic attack (TIA), especially if no signs of seizures are present. The pattern of neurological semiology should guide clinical thinking on whether a potential lesion may originate from the brain (and not from the spinal cord or the peripheral nervous system), and subsequently, whether the potential stroke / TIA originates from the anterior circulation or the posterior circulation¹. Additionally, patients should be promptly scheduled

for brain imaging, with brain computed tomography (CT), CT angiography, and perfusion CT in order to (i) exclude the possibility of intracranial haemorrhage, (ii) capture evidence of lesion(s) compatible with an ischaemic stroke (although such evidence is usually absent at the acute stage), (iii) capture evidence of a large vessel occlusion (LVO), and (iv) estimate the extent of the potentially salvageable brain region (penumbra)². In cases of TIA, patients should be classified as low- or high-risk (for future development of ischaemic stroke) based on the ABCD² scale. If the possibility of an ischaemic stroke cannot be excluded by the diagnostic approach in the emergency setting, the patient should be treated as such. The three main therapeutic concerns are to achieve timely recanalization of the occluded artery (i.e., reperfusion of the ischaemic tissue), optimise collateral flow, and avoid secondary brain injury.

Guidelines regarding the application of reperfusion treatments are available for adult patients (unfortunately there are limited data regarding paediatric stroke) and concern ischaemic stroke cases that either cause disabling symptoms (e.g. aphasia, hemianopia, or hemiparesis) or have a National Institutes of Health Stroke Scale (NIHSS) score above 4. Such patients are scheduled for mechanical thrombectomy, if there is evidence of LVO, and the treatment can be administered within 6 h of symptom onset. Patients with no evidence of LVO, are scheduled for intravenous thrombolysis if treatment can be administered within 4.5 h of symptom onset and no contraindications exist. Various trials are on-going in order to establish whether certain subgroups of patients may benefit from these reperfusion treatments, even if they do not fall within the aforementioned timeframes³.

3. Basic principles of antiplatelet and anticoagulant use in the post-emergency stage of the ischaemic stroke

Moving away from the emergency approach of the stroke patient, an important concern of the subacute (in-hospital) and long-term (post-hospitalisation) treatment surrounds the challenges regarding the proper use of antiplatelet and anticoagulant agents. Generally, all ischaemic stroke patients are treated

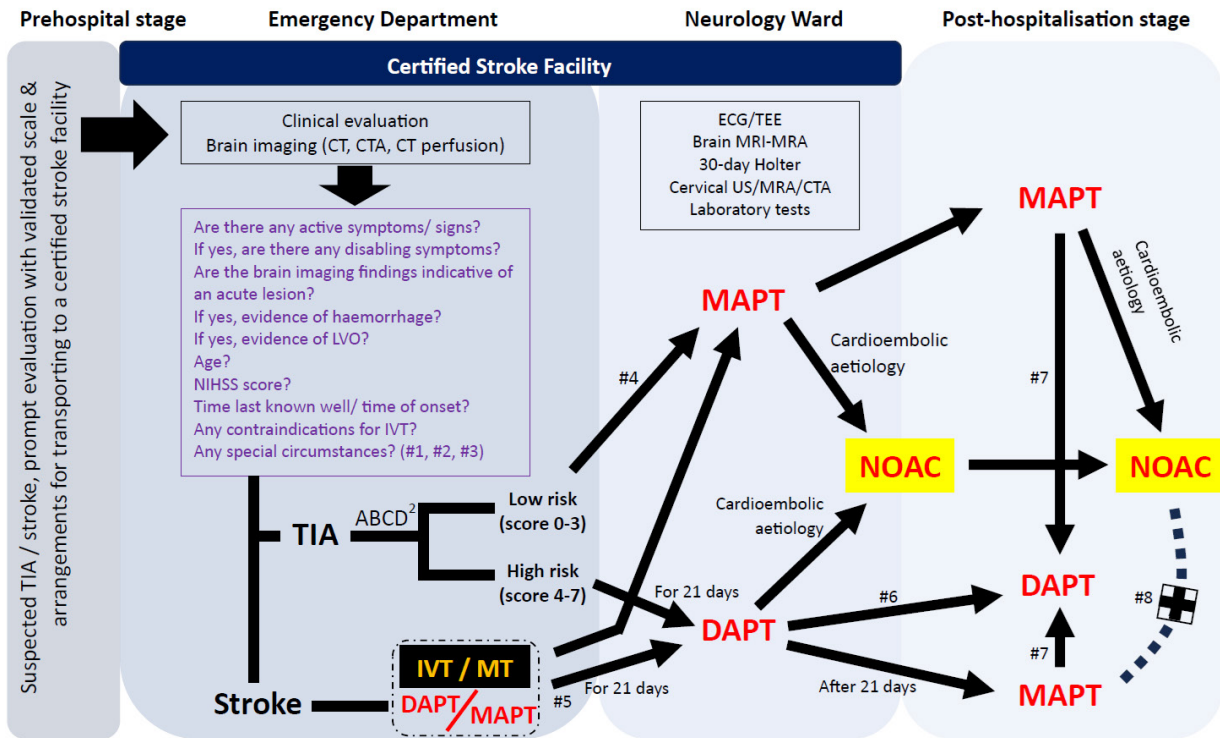


Figure 1. Integrative management of TIA and ischaemic stroke. Approach of the patient with potential TIA / stroke should start at the prehospital level by the paramedical team responsible for promptly transporting the patient to a certified stroke facility. In the setting of the emergency department, stroke experts should evaluate the patient clinically and with brain imaging (CT, CTA, and perfusion CT). Based on this initial investigation, the patient will be categorised as suffering from a haemorrhagic stroke (outside the scope of this paper) or potentially TIA or ischaemic stroke. In suspected stroke, the age of the patient, the presence of disabling symptoms, the NIHSS score, any evidence of LVO, the presence of any contraindications of reperfusion treatment, and the time of the symptoms' onset will determine whether the patient is eligible for a reperfusion treatment (MT, IVT) or a conservative approach with immediate start of DAPT or MAPT in higher initial doses. The temporal window for IVT is 4.5 h after symptom onset. A subset of patients though might benefit from IVT even if conducted outside the 4.5 h window. These include wake-up strokes or strokes of unknown onset time with high diffusion-perfusion mismatch in MRI (up to 9 h) or, in cases of basilar artery occlusion, ineligible for MT, who do not have a large established pontine or cerebellar infarction, especially if they have fluctuating deficits (#1). The temporal window for MT is 6 h after the symptom onset. A subset of patients with LVO though might benefit from MT even if conducted outside the 6-h window. These include wake-up strokes or strokes of unknown onset time with high diffusion-perfusion mismatch in MRI (up to 24 h) (#2) or, in cases of basilar artery occlusion who do not have a large established pontine or cerebellar infarction, especially if they have fluctuating deficits (even >24 h) (#3). If no clinical or imaging evidence of ischaemic lesion is present, the symptoms of the patient have been completely resolved and the value of the ABCD² scale of the patient is below 4, then the patient can be considered as having experienced a low-risk TIA and can be discharged with instructions for undergoing further diagnostic evaluation (brain MRI, cervical US, ECG, 30-day Holter) and starting MAPT (and statin) (#4). If the ABCD² scale of the patient is above 3, then the patient is considered as having experienced a high-risk TIA, and the suggestion would be to be hospitalised, undergo the same diagnostic evaluation (in the hospital

setting), initiate DAPT (and statin) for 21 days, and switch to MAPT (and statin) after that period of time. DAPT is usually also administered for 21 days, instead of reperfusion treatment, in stroke cases with an NIHSS score <5 and non-disabling symptoms (#5). DAPT may exceed 21 days (it can go up to 90 days or even longer) in cases of extra- / intracranial major artery stenosis (#6). If the diagnostic approach reveals a cardioembolic origin of the TIA / stroke, antiplatelet treatment is converted to anticoagulant treatment (preferably NOAC). In cases of recurrent stroke of non-cardioembolic origin under MAPT, change in the long-term prevention strategy (from MAPT to DAPT) may be considered (#7). The addition of clopidogrel to the anticoagulant regime could potentially be useful for secondary prevention in cases of post-stroke patients who also display unstable coronary heart disease (angina) in the presence of atrial fibrillation (#8). Abbreviations used: ABCD², clinical scale to evaluate whether TIAs should be considered as low- or high-risk; CT, computed tomography; CTA, computed tomography angiography; DAPT, dual antiplatelet therapy; ECG, echocardiogram; IVT, intravenous thrombolysis; LVO, large vessel occlusion; MAPT, antiplatelet monotherapy; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; NOAC, non-vitamin K antagonist oral anticoagulant; TEE, trans-oesophageal echocardiogram; TIA, transient ischaemic attack; US, ultrasound.

in-hospital with an antiplatelet scheme independent of whether a reperfusion treatment was conducted at the acute stage, and are switched to an anticoagulant scheme if the diagnostic approach reveals a potential cardioembolic cause of the stroke. Another important question about the use of antiplatelet agents is whether a monotherapy (MAPT) or a dual antiplatelet therapy (DAPT) should be applied, and what its duration should be.

4. Antiplatelet agents and their use in the context of MAPT or DAPT

Various meta-analyses from multiple available randomised controlled trials have established that DAPT given within 24 h of a high-risk TIA or a non-cardioembolic mild stroke, for 21 days, can effectively reduce the risk of recurrent stroke and major adverse cardiovascular events compared to MAPT⁴. DAPT can be implemented in various occasions in the management of the ischaemic stroke patients: in the acute setting, DAPT can be used (instead of intravenous thrombolysis) in those patients that show no disabling symptoms, no evidence of LVO or capsular warning syndrome and have a NIHSS score below 6, while in the sub-acute setting, DAPT is indicated for 3 weeks in patients with an NIHSS score below 6 and for longer duration (90 days or

even longer) in patients with symptomatic stenosis of carotid arteries. DAPT is not recommended as a long-term secondary prevention strategy, but may be considered for only very selected cases; these include: (i) recurrence of ischaemic stroke in patients under MAPT, when other preventive strategies have been unsuccessfully implemented (e.g., adherence to the MAPT has been ensured, no drug interactions exist that may render MAPT ineffective, investigation consistently reveals no cardioembolic origin of the stroke, and variables such as arterial hypertension, diabetes mellitus, hypolipidemic treatment, and lifestyle have been tightly controlled for) and (ii) patients with non-valvular atrial fibrillation unsuitable for anticoagulant treatment⁵.

MAPT is implemented in the remaining (and large majority) of ischaemic stroke and TIA cases; these include low-risk, non-cardioembolic TIAs, non-cardioembolic ischaemic strokes of greater severity (NIHSS score above 5) or disabling symptoms, following reperfusion treatment, or in the long-term, after the end of the DAPT period (usually 21 days) for secondary prevention.

Among the different types of antiplatelet agents that have been tested in randomised controlled clinical trials, aspirin (an irreversible cyclooxygenase inhibitor, which reduces platelet aggregation by inhibiting the synthesis of the procoagulant thromboxane A₂) and

clopidogrel (a prodrug metabolised by the hepatic cytochrome P450 system to its active form, an irreversible inhibitor of the P2Y₁₂ class of adenosine diphosphate / ADP receptors on the surface of platelets, preventing ADP-mediated activation of the downstream glycoprotein IIb/IIIa complex, thereby resulting in reduced platelet aggregation) are those whose use has been widely established, either as MAPT or combination therapy (DAPT). Ticagrelor, with similar mode of action to clopidogrel, can replace the latter in the context of DAPT in cases of poor response to clopidogrel (usually due to reduced bioavailability in a subset of patients who possess loss of function polymorphisms in CYP2C19). Dipyridamole (with multiple modes of action, among which a cyclic adenosine monophosphate / cAMP-phosphodiesterase inhibitor, which also inhibits the reuptake and breakdown of adenosine by platelets, thereby enhancing prostaglandin I₂ biosynthesis) can also be used in the context of DAPT (with aspirin) for the secondary prevention of stroke in patients with recurrent stroke(s) under MAPT (either only aspirin or clopidogrel). Finally, cilostazol (a selective inhibitor of phosphodiesterase 3, which increases the activation of intracellular cAMP and, thereby, inhibits platelet aggregation and causes vasodilation) may have a role in the context of DAPT (with either aspirin or clopidogrel) in the long-term secondary prevention of stroke in patients with (extra- / intracranial) major artery stenosis either ≥50% or less significant but with 2+ additional risk factors (i.e., smoking, age ≥65 years, hypertension, diabetes mellitus, chronic kidney disease, recurrent stroke, ischaemic heart disease, peripheral vascular disease)⁶.

5. Anticoagulant agents and their role in secondary prevention of stroke

Anticoagulant treatment (through the use of non-vitamin K antagonist oral anticoagulants or, rarely, vitamin K antagonists) has its place in the long-term secondary prevention after TIA or ischaemic stroke of cardioembolic origin. Its use can start as soon as 1 day after TIA, 4 days after a minor stroke (NIHSS score: <8), 7 days after a moderate stroke (NIHSS score: 8–15), and 14 days after a severe one. In cases of haemorrhagic trans-

formation or after mechanical thrombectomy, anticoagulant treatment can start after 4–14 or 5–14 days, respectively, following an individualised assessment of risks / benefits⁷. The aforementioned guidelines are not affected by the application of intravenous thrombolysis. There is only one case, where a combined antiplatelet (clopidogrel)-anticoagulant therapy may have benefits that outweigh the risks: this is the case of the long-term secondary prevention of recurrent stroke in patients who also suffer from unstable coronary heart disease (angina) in the presence of atrial fibrillation. However, more research is required on the topic.

6. Conclusion

Stroke constitutes the second cause of death worldwide, with a quarter of the cases being recurrent incidences. There have been significant advancements in the therapeutic approach and overall management of the condition over the last 2.5 decades. The approach of the potential stroke patient should start at the pre-hospital level, since time is of essence. In the acute setting, reperfusion treatment needs to be decided about. In the subacute, in-hospital setting antiplatelet treatment plays an important role, while in the chronic setting, stroke aetiology will dictate secondary prevention treatment (i.e., continuation of antiplatelet agents or switching to anticoagulant regimes).

Acknowledgements

The author would like to express his gratitude towards his colleagues in the Neurology Department of the General Hospital of Athens “Pammakaristos” for their valuable support in the proper management of stroke patients.

Conflicts of interest

None exist.

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HOW TO CITE:

Kalafatakis K. Recent advances in the acute management and long-term secondary prevention in transient ischaemic attack and ischaemic stroke. *Pharmakeftiki* 37(2s), 8-13, 2025.
<https://doi.org/10.60988/p.v37i2S.131>

Managing diabetes mellitus in a complex world: navigating the challenges of polypharmacy

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KEY WORDS:

diabetes mellitus; comorbidities; polypharmacy; side effects; challenges

ARTICLE INFO:

Received: March 27, 2025

Revised: n/a

Accepted: March 28, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

Diabetes mellitus (DM) presents complex challenges for both patients and healthcare providers. DM can lead to a range of complications and often coexists with other comorbidities. The need to effectively manage these complications, coupled with the presence of comorbidities, contributes to polypharmacy in DM patients. Polypharmacy can increase the risk of adverse drug events and drug interactions. As most serious adverse effects of polypharmacy in DM patients arise from drug interactions, the importance of careful medication management is paramount. Physicians must consider individual patient factors and conduct thorough pharmacological assessments in order to prevent unwanted effects. Furthermore, medication errors are a significant concern, particularly in elderly diabetic patients who are often prescribed multiple drugs due to comorbidities. Adhering to the Beers Criteria as well as regular monitoring for drug-drug and drug-food interactions, side effects, and medication appropriateness is essential for the optimization of the treatment and the reduction of the risk of adverse events in DM patients.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that presents complex challenges for both patients and healthcare providers. It is one of the leading global health concerns, ranking as the eighth leading cause of death and disability world-

wide. Currently, 537 million adults are living with diabetes; a number that is expected to rise to 783 million by 2045¹. DM can lead to a range of complications, including both macrovascular and microvascular issues, and often coexists with other comorbidities². Around 50% of individuals with DM also experience comorbidities, such as heart

disease, hypertension, and dyslipidaemia. These conditions, along with the risk of cardiovascular, renal, gastrointestinal, neurological, endocrine, and ophthalmological complications, result in a significantly reduced quality of life compared to those without DM³.

The need to manage these complications, coupled with the presence of comorbidities, contributes to polypharmacy in DM patients. Although polypharmacy has been an important factor in reducing the incidence of type 2 DM-related complications and all-cause mortality, it can increase the risk of adverse drug events and drug interactions. Furthermore, managing complications with additional medications further complicates adherence to treatment plans, thereby increasing the likelihood of non-compliance. It is also important to note that the risk of adverse events rises by approximately 50% when five drugs are used, and by over 95% when eight or more drugs are prescribed.

2. Pharmacological treatment of type 2 DM

A variety of drug classes are used to treat type 2 DM, including insulin, biguanides, sulfonylureas, meglitinides, sodium-glucose transport protein-2 (SGLT-2) inhibitors, thiazolidinediones, alpha-glucosidase inhibitors, amylin analogues, dipeptidyl peptidase-4 inhibitors, and incretin mimetics. Each class works through different mechanisms, but all come with potential adverse reactions. Severe side effects, as outlined in the summary of product characteristics, include haematological disorders (e.g., leukopenia, agranulocytosis, thrombocytopenia), anaphylactic reactions, severe skin reactions (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis), and the potential for pancreatitis, among other serious conditions.

Certain adverse reactions require special attention: (i) euglycemic diabetic ketoacidosis (a serious and potentially life-threatening reaction associated with SGLT-2 inhibitors)⁴, (ii) Fournier's gangrene (although extremely rare, it is particularly dangerous), (iii) medullary thyroid cancer (MTC; the incidence of MTC is slightly elevated in patients using

semaglutide; the latter should be avoided in individuals with a family history of MTC and it should be used cautiously in those with nodular goiter), (iv) bladder cancer (the risk of bladder cancer associated with pioglitazone is time- and dose-dependent), (v) bone fractures (an increased risk of fractures, particularly in postmenopausal women, is linked to pioglitazone), and (vi) adverse reactions related to tirzepatide (still under evaluation).

3. Lipid management in DM patients

For DM patients with established coronary artery disease, maintaining low-density lipoprotein (LDL) levels is crucial. LDL levels should be reduced to less than 55 mg/dL or to less than 35 mg/dL if heart attacks persist despite treatment. Common medications used to achieve these targets include statins, ezetimibe, bempedoic acid, and combinations of these drugs. Additional therapies, such as PCSK9 (proprotein convertase subtilisin / kexin type 9) inhibitors, colestevam, or fish oil derivatives, may also be used⁵.

However, lipid-lowering drugs, particularly statins, are not without risks. Serious, albeit rare, side effects include skin reactions like the Stevens-Johnson syndrome and the DRESS (drug reaction with eosinophilia and systemic symptoms) syndrome. Patients should also be monitored for muscle-related issues, including myopathy, rhabdomyolysis, and severe necrotizing myopathy⁶. Additionally, statins may exacerbate conditions like myasthenia gravis and have been linked to an increased risk of developing DM. Other rare side effects of lipid-lowering medications, particularly of rosuvastatin, include tubular proteinuria.

4. Hypertension and diabetic comorbidity

Hypertension is a common comorbidity in individuals with type 2 DM. Treatment often begins with a combination of two or three medications, depending on the severity of hypertension. First-line therapies include diuretics (e.g., hydrochlorothiazide or furosemide), angiotensin II receptor antagonists or

angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers (e.g., amlodipine), and beta-blockers (e.g., bisoprolol).

It is essential to monitor for potential adverse effects associated with antihypertensive and diuretic medications. Key concerns include electrolyte disturbances and cardiovascular symptoms, such as angina pectoris, atrioventricular block (1st, 2nd, or 3rd degree), sinus bradycardia, sinus pause, malleolar oedema, palpitations, and tachycardia. Additionally, rare but serious adverse reactions like the DRESS syndrome have been reported with furosemide. ACE inhibitors may also cause mood changes, including depression, while the relationship between calcium channel blockers and depression remains unclear.

5. Drug interactions and considerations

Most serious adverse effects result from drug interactions, and can be classified as either pharmacokinetic or pharmacodynamic⁷. Some notable examples of drug interactions include the co-administration with: (i) hypoglycaemic drugs (this can lead to significant hypoglycaemia), (ii) drugs metabolized by cytochrome P450 enzymes (particular attention is needed for drugs metabolized by these enzymes, such as sulfonylureas and pioglitazone; significant pharmacokinetic interactions can occur with inducers or inhibitors of cytochrome P450; cytochrome P450 inhibitors include ketoconazole, itraconazole, ritonavir, clarithromycin, erythromycin, amiodarone, diltiazem, verapamil, fluconazole, and grapefruit juice; cytochrome P450 inducers include rifampicin, carbamazepine, phenytoin, phenobarbital, and St John's wort)^{8,9}, (iii) contraceptives (requires caution due to the potential for reduced contraceptive effectiveness; for example, tirzepatide significantly reduces the plasma concentration of oral contraceptives), (iv) verapamil or quinidine (this combination can be harmful for patients with hypertrophic obstruc-

tive cardiomyopathy, potentially leading to pulmonary oedema), (v) dicoumarin anticoagulants (close monitoring of the international normalized ratio / INR levels is essential for safe anticoagulation), (vi) digitalis or lithium (close monitoring of drug levels is essential due to the risk of toxicity), and (vii) antihypertensives and nonsteroidal anti-inflammatory drugs (caution is advised, particularly in patients with compromised renal function, as this combination can further impair kidney function).

6. Conclusion

Most serious adverse effects arise from drug interactions, thereby highlighting the importance of careful medication management. Physicians must consider individual patient factors and conduct thorough pharmacological assessments in order to prevent unwanted effects. Furthermore, medication errors are a significant concern, particularly in elderly DM patients who are often prescribed multiple drugs due to comorbidities. Adhering to the Beers Criteria for prescribing can help minimize adverse drug reactions and improve patient outcomes¹⁰. Regular monitoring for drug-drug and drug-food interactions, side effects, and medication appropriateness is essential for the optimization of the treatment and the reduction of the risk of adverse events.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Liapi C. Managing diabetes mellitus in a complex world: navigating the challenges of polypharmacy. *Pharmakeftiki* 37(2s), 14-17, 2025. <https://doi.org/10.60988/p.v37i2S.132>

Enhancing pharmacovigilance through patient engagement: perspectives and strategies

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KEY WORDS:

patient engagement; pharmacovigilance; patient safety; real-world evidence; digital health technologies

ARTICLE INFO:

Received: January 29, 2025

Revised: March 04, 2025

Accepted: March 06, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

Pharmacovigilance, as defined by the World Health Organization, is the science and activities related to the detection, assessment, understanding, and prevention of adverse events or any other possible drug-related problem. The Council for International Organizations of Medical Sciences Working Group XI has provided a comprehensive definition of patient engagement, highlighting mechanisms related to patient engagement such as direct patient reporting of adverse events, patient advisory roles, and established feedback channels. In recent years, patient roles in different aspects of healthcare, including medicine development, regulations, and the safe use of medicines are witnessing increased attention and expansion. This presentation sheds light on the importance of engaging patients in different pharmacovigilance activities, discusses patient engagement mechanisms contributing to drug safety, and addresses the strategies and the challenges associated with enhancing patient engagement.

Patient engagement is currently a cornerstone for monitoring and evaluating the safety of medicinal products, particularly since its emergence as a key element of pharmacovigilance¹. Patient participation in different activities brings valuable perspectives and unique insights, thereby enhancing pharmacovigilance efforts and supporting the real-world experiences². However, addressing accessibility, awareness, and trust barriers dictates a need for

meaningful patient participation. The objectives of my presentation are to highlight the importance of patient engagement in pharmacovigilance, to discuss the patient engagement mechanisms contributing to drug safety, and to outline the strategies and challenges in supporting patient engagement.

My presentation highlights the definition of patient engagement based on the work of the Council for International Organizations of Medical Sciences Working

Group XI and its relationship with the World Health Organization's definition of pharmacovigilance. Examples describing the evolving practices in the global pharmacovigilance systems that demonstrate the value of patient participation are presented, and so are the importance and the methods of this engagement. I also discuss the challenges facing patient engagement in pharmacovigilance and the strategies allowing us to address these challenges.

Patient engagement in pharmacovigilance through the incorporation of real-world evidence and patient perspectives has been shown to enhance the quality and the relevance of safety data. A more robust pharmacovigilance system could be established through direct patient reporting, participation in advisory roles, and the application of feedback mechanisms. However, widespread engagement is still a challenge due to issues related to accessibility, trust, and patient awareness. Strategies that could be implemented in an effort to address these challenges include improving patient education, leveraging digital tools, and fostering collaborative environments in which patients feel valued as partners.

Patient engagement is a very crucial step forward

in a new era in the field of pharmacovigilance. The direct and active patient involvement in different aspects related to drug safety monitoring and assessment paves the way to improved and comprehensive pharmacovigilance data quality and, consequently, to improved patient health outcomes. Finally, the evolving innovative approaches employing digital health technologies will support and strengthen the role of patients in pharmacovigilance. Ensuring patients' voices are reached out loud and their experiences are shared is a crucial step in shaping the regulatory decision-making for the new future of safety monitoring.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Younus M.M. Enhancing pharmacovigilance through patient engagement: perspectives and strategies. *Pharmakeftiki* 37(2s), 18-19, 2025. <https://doi.org/10.60988/p.v37i2S.133>

Mitigative effects of a topically-applied combination of cimifugin and vinpocetine on a murine psoriasis-like model

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KEY WORDS:
cimifugin; vinpocetine;
TNF- α ; IL-17; IL-23

ARTICLE INFO:
Received: January 15, 2025
Revised: February 20, 2025
Accepted: February 20, 2025
Available online: October 10, 2025

*** CORRESPONDING
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ABSTRACT

The chromones found in the dried roots of *Saposhnikovia divaricata* include cimifugin; in fact, *Saposhnikovia divaricata* is a main source of cimifugin. Vinpocetine is a synthetic version of vincamine derived from periwinkle; it is characterized by potent anti-inflammatory properties that allow it to reduce immune cell infiltration and suppress the release of pro-inflammatory cytokines. The objective of this study was to investigate the potential influence of a topically-applied combination of cimifugin and vinpocetine gels on a model of a psoriasis-like inflammatory skin reaction. To this end, we divided 48 albino BALB/c mice into six groups. All groups except for the negative control group received imiquimod topically (daily, for 7 days) for the induction of psoriasis-like skin lesions. A group received imiquimod (5%) only (positive control), while four other groups were also treated with a clobetasol (0.05%) cream, a cimifugin (3%) gel, a vinpocetine (3%) gel, or a combination of cimifugin (3%) and vinpocetine (3%) gels, once daily, for 7 days; the aforementioned treatments were first applied 7 days after the pharmacological induction of the lesions. Our findings revealed that the topically-applied combination of cimifugin- and vinpocetine-containing gels had an important anti-psoriatic effect, as seen by the diminishing of the skin levels of tumour necrosis factor-alpha, interleukin-17, and interleukin-23, thereby improving the imiquimod-induced histological changes in

mice. We conclude that a topically-applied combination of cimifugin and vinpocetine can exert substantial anti-psoriatic activity.

1. Introduction

Psoriasis is a common, chronic skin disorder passed on through genetics. It is an illness that is proliferative and inflammatory. There is no known cause of psoriasis. Clinically speaking, there are five main types of psoriasis: guttate, plaque, inverse, pustular, and erythrodermic. In addition, interleukin (IL)-17 (IL-17), tumour necrosis factor-alpha (TNF- α), and interferon are known to promote keratinocyte development. Moreover, the TNF- α /Th17/IL-23 pathway is a key component of the inflammatory signalling pathway that promotes psoriasis¹.

Saposhnikovia divaricata's roots contain chromones, including cimifugin, which are essential for treating inflammatory diseases. Cimifugin has anti-inflammatory, antiproliferative, and immunoregulatory properties². Vinpocetine is a synthetic version of vincamine, an alkaloid derived from the leaves of the periwinkle plant *Vinca minor*, which belongs to the Apocynaceae family; it has vasodilating and anti-inflammatory effects. Vinpocetine also has immunomodulatory and antioxidant properties, and it is marketed as a nutritional supplement for the improvement of cognition and memory. Finally, imiquimod rapidly prompts psoriasis-like inflammatory skin reactions in rodents that are similar to human psoriasis, by strongly relying on the IL-23/IL-17 axis. This quick and easy model can be used in order to assess novel therapeutic strategies and drugs³. This study sought to ascertain the beneficial effects of cimifugin and vinpocetine gels when used alone and in combination on the imiquimod-induced model of psoriasis in mice, through their effects on the skin levels of TNF- α , IL-17, and IL-23.

2. Methodology

2.1. Drugs and reagents

Meda AB (Sweden) is the producer of imiquimod.

Cimifugin and vinpocetine were provided by HyperChem (China), while the clobetasol (0.05%) cream was obtained from GSK plc (UK).

2.2. Cimifugin and vinpocetine gel preparation

Carbopol 940 (1%) and an appropriate volume of distilled water were combined in order to create the gel, which was then thoroughly stirred. Cimifugin (or vinpocetine) was dissolved in ethanol (96%) and was sonicated so as to produce a solution. As this solution was gradually added, the Carbopol 940 dispersion was continuously agitated. While being continually mixed, triethanolamine was gradually incorporated into the mix (up to 1%). The gel's final volume was up to 50 g after adding the distilled water and agitating in order to make a homogeneous gel.

2.3. Experimental design

The work was done at the Al-Nahrain University between February 2024 and June 2024. The institution's reviewing board at the Al-Mustaqbal University College of Pharmacy has surveyed the comprehensive research plan and has licensed the hereby presented experiments. The study was conducted following the ethical principles outlined in the Declaration of Helsinki, while a local ethical board has reviewed and approved the research protocol (approval protocol number: 1025; date: 4/11/2024).

In total, 48 albino BALB/c mice with an average body weight of 28–40 g and an age of 8–12 weeks were used. The shaving of the backs of the mice took place before applying 62.5 mg of imiquimod daily to their skin. The mice were then randomly assigned into the following six groups (n=8 per group): (i) group I (negative control group; healthy mice without any treatment), (ii) group II (imiquimod 5%; mice that received 62.5 mg of the imiquimod 5% cream on the shaved dorsal skin, daily, for 7 days; induction of psoriasis-like lesions),

(iii) group III (imiquimod 5% > clobetasol 0.05%; mice that, after the induction of the psoriasis-like lesions, have been treated with a clobetasol 0.05% cream, daily, for 7 days), (iv) group IV (imiquimod 5% > cimifugin 3%; mice that, after the induction of the psoriasis-like lesions, have been treated with a cimifugin 3% gel, daily, for 7 days), (v) group V (imiquimod 5% > vinpocetine 3%; mice that, after the induction of the psoriasis-like lesions, have been treated with a vinpocetine 3% gel, daily, for 7 days), and (vi) group VI (imiquimod 5% > cimifugin 3% + vinpocetine 3%; mice that, after the induction of the psoriasis-like lesions, have been treated with a combination of cimifugin 3% and vinpocetine 3% gels, daily, for 7 days).

2.4. Animal sacrifice and sampling

All mice were sacrificed on day 14 of the experiment by diethyl ether inhalation. Skin tissue samples were obtained and homogenized; the supernatants were stored in Eppendorf tubes (at -80°C) for the undertaking of ELISA assessments. Other skin tissue samples were also placed under a 10% formalin preservation process for the undertaking of histological assessments⁴.

2.5. Measurement of IL-17, TNF- α , and IL-23 levels in skin tissue homogenates

An ELISA kit was used in order to estimate the skin levels of TNF- α , IL-17, and IL-23 according to the producer's instructions.

2.6. Statistical analysis

Data enrolled in this study were analysed using the SPSS v. 16 software. Comparison of mean levels among the study groups was done by using one-way ANOVA. The level of significance was set at $p < 0.05$.

3. Results and Discussion

The application of imiquimod on the shaved murine skin (group II) produced substantial histopathological alterations, characterized by Munro abscesses,

hyperkeratosis, parakeratosis, acanthosis, abnormal thickness of the epidermis, the presence of rete ridges, and marked lymphocyte infiltration and papillary congestion (results not shown). On the other hand, the histological features of the skin samples obtained from the mice treated with the combination of cimifugin and vinpocetine gels (group VI) are characterized by the absence of Munro abscesses, the absence of hyperkeratosis, the absence of parakeratosis, acanthosis, the absence of rete ridges, mild lymphocyte infiltration, and the absence of papillary congestion; similarly to the negative control group (group I; results not shown).

Table 1 provides an overview of the skin levels of TNF- α , IL-17, and IL-23 among the experimental groups of the study. In the present study, the induction of psoriasis-like skin lesions resulted in raising the skin levels of TNF- α ; these levels were reduced following treatment. The best results were associated with the use of a combination of cimifugin and vinpocetine gels (group VI), while the best monotherapy results were associated with the use of the cimifugin gel (Table 1). Our results are in agreement with those of Liu *et al.*⁵, since we have demonstrated the ability of cimifugin in reducing the levels of TNF- α . However, the point of originality in our study is found in the fact that we have shown that the application of a combination of cimifugin and vinpocetine gels can be associated with an even more intense reduction of the skin TNF- α levels.

In this study, the administration of imiquimod has resulted in a rise in the skin levels of IL-17 and IL-23, while the treatments have resulted in the reduction of these biomarkers' levels (Table 1). The combined treatment has again resulted in a better reduction in the skin IL-17 and IL-23 levels than the single agents. According to Liu *et al.*⁵, cimifugin can efficiently invert the imiquimod-induced up-regulation of proinflammatory cytokines (including TNF- α , IL-6, IL-17A, IL-1 β , and IL-22); therefore, our study results are in line with regard to the effect of cimifugin on IL-17 levels. It is also noteworthy mentioning that vinpocetine has been suggested to be able to prevent the occurrence of COVID-19, as it potentially exerts anti-inflammatory and antioxidant properties through the amelioration of the MAPK/NF- κ B transcription factor pathway⁶. On the other hand, the topical administration of imiquimod to

Table 1. Comparison of the skin tumour necrosis factor-alpha (TNF- α), interleukin-17 (IL-17), and interleukin-23 (IL-23) levels (in pg/g) among the experimental groups of the study (n=8 mice per group). Different letters (as superscripts) are used in order to express statistically-significant differences among the biomarker levels of the studied groups within each column.

Treatments / study groups (n=8 mice per group)	TNF- α levels (pg/g)	IL-17 levels (pg/g)	IL-23 levels (pg/g)
negative control / I	146.27 $\pm$ 9.85 ^c	198.70 $\pm$ 13.90 ^b	190.88 $\pm$ 38.65 ^b
imiquimod 5% (induction of psoriasis) / II	372.41 $\pm$ 52.72 ^a	457.79 $\pm$ 84.78 ^a	496.61 $\pm$ 37.71 ^a
imiquimod 5% > clobetasol 0.05% / III	218.72 $\pm$ 33.97 ^b	246.02 $\pm$ 12.62 ^b	161.61 $\pm$ 37.71 ^b
imiquimod 5% > cimifugin 3% / IV	158.96 $\pm$ 13.04 ^b	268.98 $\pm$ 35.60 ^b	248.64 $\pm$ 34.80 ^b
imiquimod 5% > vinpocetine 3% / V	168.86 $\pm$ 14.09 ^b	258.78 $\pm$ 33.50 ^b	263.52 $\pm$ 32.30 ^b
imiquimod 5% > cimifugin 3% + vinpocetine 3% / VI	116.35 $\pm$ 56.66 ^c	222.58 $\pm$ 24.11 ^b	154.64 $\pm$ 18.24 ^b
p-value (one-way ANOVA)	<0.001	<0.001	<0.001

laboratory animals is known to not only produce cutaneous inflammatory reactions, but to also produce an excess of inflammatory mediators⁷.

In our experiments, imiquimod was found to up-regulate the release of inflammatory mediators, but vinpocetine suppressed their levels. Nevertheless, as compared to the imiquimod group (group II), the administration of the vinpocetine 3% gel significantly reduced the skin levels of inflammatory cytokines such as IL-17 and IL-23 (Table 1). Al-Kuraishy *et al.*⁸ have suggested that vinpocetine can reduce inflammation by suppressing the NF- κ B signalling pathway and the release of pro-inflammatory cytokines (such as IL-1 β , IL-6, IL-8, IL-12, and TNF- α). Since vinpocetine can inhibit the NF- κ B activation in order to produce its anti-inflammatory action, it could be a promising adjunctive and a valuable drug in the treatment of inflammatory and immune-mediated diseases⁹. Finally, our experimental data regarding the skin levels of IL-17 and IL-23 suggest that vinpocetine could act as an anti-psoriatic agent. The pathogenesis of psoriasis includes IL-23; a cytokine believed to have a role in the production of IL-17 and IL-22 by the Th17 cells¹⁰.

4. Conclusion

A topically-applied combination of cimifugin (3%) and vinpocetine (3%) gels exerts a restorative effect on the histopathological (psoriasis-like) changes induced by

the application of imiquimod (5%) on the murine skin. This anti-psoriatic activity could be mediated by the anti-inflammatory and immunomodulating properties of these compounds, as evidenced in our study by the effects that they had on the skin levels of TNF- α , IL-17, and IL-23.

Acknowledgements

The authors would like to express their gratitude towards the College of Pharmacy of Al-Mustaqbal University for providing all the facilities they needed in order to undertake this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Abbas A.H., Hassan Z.M., Albarki M.A., Zigam Q.A., Ridha-Salman H., Abbas H.A.M., AbdulAemah M.A., Abbas W.J., Raheem A.K., Al-Athari A.J.H., Abbas Z.H., Hajwal S.K., Al-Tae M.H. Mitigative effects of a topically-applied combination of cimifugin and vinpocetine on a murine psoriasis-like model. *Pharmakeftiki* 37(2s), 20-24, 2025. <https://doi.org/10.60988/p.v37i2S.134>

Hepatocyte nuclear factor-4 α and sex hormone-binding globulin levels in overweight polycystic ovary syndrome patients from the Babil Province

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KEY WORDS:

PCOS; SHBG; HNF- α ;
ELISA; BMI

ARTICLE INFO:

Received: January 02, 2025

Revised: February 13, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

Polycystic ovary syndrome (PCOS) is an endocrine condition that comprises two major elements: metabolic irregularities and elevated male hormone levels. Sex hormone binding-globulin (SHBG) acts as an essential androgen regulator; however, its expression and metabolic functions are controlled by the hepatocyte nuclear factor-4 α (HNF-4 α). This study has investigated the relationship between the serum levels of SHBG and those of HNF-4 α in overweight women with PCOS in the Babil Province of Iraq. To this end, we enrolled 45 women with PCOS (patient group) and a control group consisting of 45 participants. This study was conducted at the Babylon Teaching Hospital for Maternity and Children in Hilla City and at private clinics from May to December 2024. A microplate reader was used in order to determine the serum levels of glycated haemoglobin (HbA1c), SHBG, total testosterone, insulin, and HNF-4 α . The PCOS group demonstrated significant elevations of serum HbA1c levels ($p < 0.05$) together with higher serum testosterone ($p < 0.05$), insulin ($p < 0.05$) and HNF-4 α ($p < 0.05$) levels, as compared to those of the control group. The SHBG levels displayed a significant relationship with the HNF-4 α levels in women with PCOS, thereby indicating that these markers may serve as metabolic regulators. Further studies are required in order to shed more light on both the biological processes and the potential treatment applications of our findings.

1. Introduction

One of the common reproduc-

tive disorders that women face is the polycystic ovary syndrome (PCOS). It is a syndrome that oc-

curs through the convergence of several homogeneous conditions, such as metabolic disorders and reproductive issues, as well as their combination. Most women with PCOS present with higher androgen levels, which is the case behind irregular menstrual cycles, multiple small cysts in the ovaries, and erratic ovulatory functions. It should be emphasized that polarity refers to the presence of cysts; however, for diagnosis purposes, these are not essential¹.

A major factor in the pathophysiology of PCOS is the sex hormone-binding globulin (SHBG); a glycoprotein that is mostly produced in the liver. The SHBG's effect on testosterone and oestradiol levels is critical, because it modulates their levels in circulation. Most of the time, there is a decrease in the levels of SHBG among PCOS patients, which is usually related to coexisting insulin resistance. Insulin resistance worsens metabolic derangements, which are associated with type 2 diabetes and cardiovascular disease². The hepatocyte nuclear factor-4 α (HNF-4 α) is a key transcription factor that regulates the SHBG expression. Its interaction with distinct promoter regions also modulates the production of hepatic SHBG, thereby impacting glucose and lipid metabolism in the liver. As far as the polycystic ovaries are concerned, the altered regulation of HNF-4 α may provide a potential explanation for the metabolic disorders in this condition³.

Recently, several proteins and peptides have gained increasing attention in energy balance research, classifying them as possible biomarkers for obesity, diabetes, and PCOS. The role of SHBG relative to HNF-4 α , particularly in metabolic dysfunction, remains under investigation⁴. This relationship is important for elucidating the mechanisms underlying the pathophysiology of PCOS, and may open up new forms of treatment for the additional metabolic problems associated with PCOS.

Our study has aimed at exploring the relationship between the serum SHBG and HNF-4 α levels in patients with PCOS and obesity living in the Babil Province of Iraq.

2. Methodology

The study involved 90 women aged between 19 and 42 years that enrolled in the study; these women were subdivided into two groups: 45 with a diagnosis of PCOS and a control group consisting of 45 healthy women. In the sample of women with PCOS, additional structured interviews were conducted in order to obtain self-reported information on smoking, medical history, family history, and the body mass index (BMI) of each respondent. This study was conducted at the Babylon Teaching Hospital for Maternity and Children in Hilla City and at private clinics from May to December 2024. Verbal consent was obtained from all subjects before sample collection. The specific procedures for the study as well as the information and the informed consent of the participants, were approved by the University of Babylon Medical College's ethics committee (document number: A0012; date: 5/8/2024).

ELISA was used in order to quantify the serum levels of glycated haemoglobin (HbA1c), SHBG, testosterone, insulin, and HNF- α , while we employed a spectrophotometric method for measuring fasting blood glucose. The data were analysed through the employment of Student's *t*-test and linear regression through the use of the SPSS v. 20 analysis software.

3. Results and Discussion

Women with PCOS exhibited significantly higher serum levels of HbA1c, total testosterone, insulin, and HNF-4 α compared to controls (Table 1). SHBG levels were found to be significantly lower in patients with PCOS (Table 1). These findings suggest a strong reverse correlation between SHBG and HNF-4 α levels in PCOS, potentially contributing to metabolic dysfunction.

PCOS is considered a complicated hormonal imbalance that, along with metabolic disarray and sexual dysfunction, revolves around the reproductive system. A clinical appraisal of women diagnosed with PCOS reveals the presence of insulin resistance, which is key to the pathophysiology of the disease

Table 1. Hormonal and metabolic profiles of the herein studied patients with polycystic ovary syndrome (PCOS) and of the control group participants. Abbreviations used: BMI, body mass index; FAI, free androgen index; HbA1c, glycated haemoglobin; HNF- α , hepatocyte nuclear factor-alpha; HOMA-IR, homeostatic model assessment of insulin resistance; NS, non-significant; SHBG, sex hormone-binding globulin.

Parameters (mean $\pm$ SD)	PCOS patients (n=45)	Control group (n=45)	P-value
BMI (kg/m ² ; range)	28.2 $\pm$ 1.3 (26.0–29.2)	27.4 $\pm$ 3.5 (25.6–28.2)	>0.05 (NS)
HbA1c (%)	6.4 $\pm$ 1.1	5.0 $\pm$ 0.4	<0.05
SHBG (nmol/L)	45.1 $\pm$ 1.2	52.7 $\pm$ 12.6	<0.05
Total testosterone (nmol/L)	0.6 $\pm$ 0.1	0.3 $\pm$ 0.1	<0.05
FAI	4.5 $\pm$ 0.9	1.6 $\pm$ 2.2	<0.05
Fasting insulin (mIU/L)	14.7 $\pm$ 3.2	4.2 $\pm$ 0.6	<0.05
Fasting glucose (mg/dL)	123.1 $\pm$ 29.3	80.7 $\pm$ 16.9	<0.05
HOMA-IR	6.2 $\pm$ 2.0	1.4 $\pm$ 0.2	<0.05
HNF- α (pg/mL)	650.7 $\pm$ 60.3	77.7 $\pm$ 10.3	<0.05

and contributes to hyperandrogenism and metabolic derangement⁵. Supporting this view, we noted significant relationships between SHBG and HNF-4 α levels in overweight PCOS women. SHBG is a glycoprotein that is synthesized in the liver and plays a key role in the availability of sex hormones such as androgens and oestrogens⁶.

Women with PCOS generally tend to exhibit low SHBG levels, which can lead to elevated free androgen levels that may worsen the features of the syndrome. Our research reflects the work of earlier authors, who have also pointed out that low SHBG levels are markers of metabolic distress or insulin resistance, and are common in this group of women. Moreover, the relationship between insulin and reproductive hormones is further complicated by the presence of the insulin-like growth factor system, which is known to play a role in regulating reproduction. Such a dysfunction can contribute to the derangements in hormonal levels that are seen in PCOS cases, thereby resulting in clinical issues.

Based on the observations made in this study, the menstrual cycle controlled by the hypothalamus provides insight into the neuroendocrine aspects of PCOS^{7,8}. Therefore, dietary issues (such as fructose consumption) have been shown to have a negative effect on glucose utilization in insulin-resistant indi-

viduals. Hence, it can be inferred that some lifestyle changes that focus on diet could have a positive effect on the management of insulin resistance and, therefore, the metabolic components of PCOS.

Our results on HNF-4 α are particularly interesting because this transcription factor is essential for the synthesis of SHBG and participates in the regulation of a variety of metabolic processes. However, the heightened levels of HNF-4 α in our PCOS patients may indicate metabolic compensation. In this regard, it seems that clarifying the significance of HNF-4 α for SHBG regulation could be helpful in developing therapeutic approaches related to PCOS from both hormonal and metabolic perspectives.

It has also been emphasized in the literature that insulin-sensitizing therapies are important for the treatment of women with PCOS, and some systematic reviews have suggested that an enhanced control of insulin resistance may improve reproductive performance. This combination may increase the effectiveness of treatment and the quality of life in women with PCOS⁹.

4. Conclusion

This study highlights the complex and interrelated nature of SHBG, HNF-4 α , and insulin resistance in

women with PCOS. These findings indicate the importance of understanding the bidirectional coupling of the dysregulation of hormonal metabolism that characterizes this syndrome. In future studies, special emphasis must be placed on explaining the mechanisms of action for these relationships, so that interventions of interest toward the improved clinical management of PCOS in women can be initiated.

Acknowledgements

The authors express their gratitude to the College of Pharmacy of the University of Babylon for its sup-

port.

Conflicts of interest

None exist.

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HOW TO CITE:

Abbas S.H., Ali Z.A., Najm Z.M., Alshimary F. Hepatocyte nuclear factor-4 α and sex hormone-binding globulin levels in overweight polycystic ovary syndrome patients from the Babil Province. *Pharmakeftiki* 37(2s), 25-28, 2025. <https://doi.org/10.60988/p.v37i2S.135>

Interplay between BMI, gender, and gallstone formation: insights from an Iraqi cohort

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KEY WORDS:

**gallstone; gender;
body mass index; BMI;
cholelithiasis**

ARTICLE INFO:

Received: January 13, 2025

Revised: February 15, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Gallstone disease (GD; also known as cholelithiasis) is a prevalent illness with many aggravating factors; it is firmly connected with metabolic abnormalities associated with insulin resistance, being overweight, hypertriglyceridaemia, and dietary propensities. Patients at risk of developing cholelithiasis are usually women, of increasing age, and suffering from diabetes mellitus. This study was performed at the Al-Yarmouk Teaching Medical Hospital in Baghdad, Iraq, between January 2020 and December 2021. It involves 250 participants: 103 obese, 100 overweight, and 47 normal-weight patients. The patients' gender, weight, height, and body mass index (BMI) were recorded and studied. Statistical analysis revealed a strong association ($p < 0.05$) between BMI and gallstone formation. Gender also plays a significant role, where women exhibited a higher incidence ($p = 0.002$) than men. Age also significantly correlated with GD development risk ($p = 0.02$). Although demographics and hormonal factors were significant, clinical evaluations revealed a high correlation between BMI and the incidence of GD. The increase in BMI leads to an increase in the incidence of cholelithiasis. Further study into the genetic and environmental factors associated with Middle Eastern populations are essential in order to enhance our understanding regarding this pathological condition and to inform our interventions.

1. Introduction

Cholelithiasis is considered among the most pervasive and exorbitant gastrointestinal diseases, with a significant load forced on medical service frameworks. Cholelithiasis can occur due to various ailments. The debilitation of the digestion of cholesterol and bile acids is a major cause of gallstone disease (GD)¹. One of the most common causes of GD is obesity, which is always accompanied by an excess production and elimination of cholesterol into the bile. Simultaneously, being overweight is straightforwardly relative to the quantity of cholesterol being synthesized in one's body^{1,2}.

Gender differences play a pivotal role in GD epidemiology; the reason behind the high incidence of GD in women might be the existence of high levels of oestrogen, which causes the liberation of a high quantity of cholesterol into the bile. Furthermore, the body mass index (BMI; an indicator used for the quantification of obesity) is a crucial factor in this context. The risk of GD development is enhanced by a higher BMI, which in turn is correlated with a higher bile cholesterol saturation³.

The multifactorial nature of GD emphasizes the importance of having a thorough understanding of its risk factors. In addition to BMI and gender, eating habits, advanced age, and rapid weight loss play a crucial role in GD formation. Normal-weight people are not immune to the increased bile cholesterol saturation caused by obesity, thereby suggesting the participation of other, less well-studied mechanisms^{4,5}.

Our study aimed at illustrating the role of BMI and gender on the cholelithiasis incidence among Iraqi patients. This study also provides insights on the burden of GD in an Iraqi cohort.

2. Methodology

2.1. Study design and participants

A cross sectional study was conducted in Baghdad (Iraq), at the Al-Yarmouk Teaching Medical Hospital, from January 2020 to the end of December 2021. Since the hospital provides services to the metropolitan ar-

ea's population, its patients are considered a reliable representation of the wider Iraqi population. This study included 250 patients suffering from GD. The study considered patients aged from 20 to 75 years with symptomatic GD (as verified by ultrasonography). The exclusion criteria were the following: patients with chronic diseases unrelated to GD, asymptomatic GD, or patients with prior cholecystectomy.

2.2. Data collection

Demographic information regarding all patients, including age and gender were collected. Anthropometric measurements were taken for all patients in light cloth and without shoes. A digital scale was used in order to measure their weight, and a stadiometer was used in order to measure their height. The BMI was calculated as follows: $BMI = \text{weight (kg)} / (\text{height (m)})^2$.

2.3. Diagnostic assessment

All participants are assessed by ultrasonography and liver function tests. If the stone in the common bile duct was not detected by ultrasonography, then a magnetic resonance cholangiopancreatography was employed. If the diagnosis was not made by magnetic resonance cholangiopancreatography, then an endoscopic ultrasound was used to confirm the diagnosis instead.

2.4. Ethical considerations

The Al-Yarmouk Teaching Medical Hospital's Institutional Review Board has examined and approved the study's protocol (approval number: YTMH5122019; date: December 2019). All participants provided written informed consent before being included in this study, thereby guaranteeing compliance with ethical guidelines for studies involving human participants.

2.5. Statistical analysis

Data were analysed using the SPSS v. 22 software.

Table 1. Gallstone disease patient characteristics and statistical correlations. The table presents the demographic distribution of the study's participants based on their body mass index (BMI; categories: healthy, overweight, and obese), their respective age groups, and their gender. Note: * denotes statistical significance.

Age						
	N	Mean	Standard deviation	Minimum value	Maximum value	
Age (years)	250	46.3	11.14	20	75	
Gender and age						
	N	Mean	Standard deviation	Minimum value	Maximum value	p-value
Female	169	46.5	10.9	20.5	39.4	0.002*
Male	81	45.8	11.4	23.3	35.9	0.06
Age and BMI categories						
Age (years)	Healthy	Overweight	Obese	Total	p-value	
<30	4	9	6	19	0.08	
30–50	26	55	66	147	0.02*	
>50	25	28	31	84	0.04*	
BMI categories and gender						
		Gender		Total	p-value	
		Female	Male			
BMI categories	Healthy	33	14	47	0.09	
	Overweight	76	24	100	0.04*	
	Obese	60	43	103	0.04*	

Descriptive statistics were used in order to summarize the participants' characteristics. Logistic regression models were employed in order to assess the relationship between the GD presence, gender, and BMI, adjusting for potential confounders. Statistical significance was set at $p < 0.05$.

3. Results

Our study involved 250 GD patients of both sexes. The male patients were fewer ($N=81$; 32.4%) than the female ones ($N=169$; 67.6%). The age of the patients included in our study ranged from 20 to 75 years and the mean of their age was 46.3 years (Table 1). Most patients in this study were between 30–50-years-old ($N=147$; 58.8%). According to the BMI classification, most of the GD patients were obese ($N=103$; 41.2%), followed by patients who were overweight ($N=100$; 40%), and patients of "healthy" weight ($N=47$; 18.8%). Statistical analysis has confirmed a significant association between BMI and gallstone forma-

tion (Table 1). Women exhibited a higher incidence of GD compared to men ($p=0.002$). Moreover, age also significantly correlated with a GD development risk (Table 1).

4. Discussion

Gallstone prevalence increases with age, with most cases being asymptomatic for extended periods. This study reaffirms the strong association between BMI, gender, and GD, in a manner consistent with global research. Our study has shown that women exhibit a 2:1 ratio of GD prevalence compared to men ($p=0.002$), thereby aligning with the belief that oestrogen-related mechanisms increase biliary cholesterol. This finding is also consistent with previous studies^{6,7} that have reported a higher incidence of GD in women (compared to men), suggesting that the female gender is a significant risk factor for GD development.

BMI has emerged as a critical determinant of gall-

stone pathogenesis. An elevated BMI correlates with increased risk of GD formation. Our study has confirmed a significant association between BMI and gallstone formation ($p<0.05$), consistently with the studies by Kim *et al.*⁸, Zhang *et al.*⁹, and Xie *et al.*¹⁰, that have identified similar patterns of BMI-related risk of GD development across diverse populations.

Age remains an independent risk factor, as evidenced by the predominance of patients above 35 years in this study ($p<0.05$). Lifestyle factors, including diet and physical activity, further influence GD development risk. This underscores the importance of targeted prevention strategies, particularly in high-risk populations. Identifying and managing these factors early could substantially reduce the disease burden.

5. Conclusion

This study focuses on an Iraqi cohort and concludes that obesity significantly increases the GD development risk. Gender and age also substantially

seem to influence gallstone formation, with women and older individuals being at a higher risk. Public health initiatives should prioritize lifestyle interventions in an attempt to reduce the GD burden in Iraq.

Acknowledgements

We would like to express our deepest gratitude to Al-Bayan University, the Al-Farabi College University, and Tikrit University for the support that made this study possible.

Conflicts of interest

None exist.

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HOW TO CITE:

Abbood M.K., Khalaf H.A., Ali H.M., Hasan A.K. Interplay between BMI, gender, and gallstone formation: insights from an Iraqi cohort. *Pharmakeftiki* 37(2s), 29-33, 2025. <https://doi.org/10.60988/p.v37i2S.136>

Solubility enhancement of ebastine through natural solid dispersion: formulation and pharmacokinetics

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KEY WORDS:

ebastine; sodium alginate; natural solid dispersion; solvent evaporation; xyloglucan

ARTICLE INFO:

Received: December 07, 2024

Revised: January 14, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

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ABSTRACT

Natural solid dispersion (NSD) is a clever pharmaceutical technology technique that is used in order to improve the physico-chemical characteristics of pharmaceuticals, such as their solubility and dissolution. Ebastine (EBS) is a medication used for the treatment of pruritic skin problems and the symptoms of allergic illnesses, such as rhinitis. The aim of this work was to improve the solubility and rate of dissolution of EBS by using an NSD system. Utilizing solvent evaporation as a preparation technique, eight EBS formulations were created as solid dispersions through the use of two natural polymers, namely sodium alginate (SA) and xyloglucan, at various drug to polymer ratios (1:0.5, 1:1, 1:2, and 1:3). The produced formulations were assessed for their *in vivo* pharmacokinetics, their solubility, and their dissolution. According to our results, the employed polymers improved the drug solubility. In comparison to pure EBS, the optimal formula (EBS:SA at a 1:1 ratio) exhibited a significant increase in solubility. Additional analysis of the ideal formula revealed that EBS is amorphous. *In vivo* experiments in rats revealed a superior pharmacokinetic performance in terms of bioavailability of the ideal formula compared to pure EBS. It may be inferred that the NSD can be used in order to address the issue of EBS solubility under the chosen conditions, which may lead to improved EBS bioavailability.

1. Introduction

The adoption of natural solid dispersion (NSD) is a smart way of improving a drug's absorption rate and solubility¹. Solid dispersion (eutectic mixture) is the term used when a drug (hydrophobic component) is disseminated in an inert carrier or matrix (hydrophilic part) in a solid state. Ebastine (EBS) helps with pruritic skin problems and the symptoms of allergic conditions including rhinitis; it is a prodrug that undergoes significant first-pass metabolism allowing it to produce carebastine (an active metabolite of carboxylic acids). Carebastine is responsible for almost all of the H₁-antihistaminic activity, and is more potent than the parent medication. The administration is performed orally. The disadvantage of this molecule is that it is mostly hydrophobic, which results in reduced water solubility and lower medication bioavailability²: in fact, it is sparingly soluble in methanol, practically insoluble in water, and highly soluble in methylene chloride. With a pKa of 8.8 and a logP of 7.64, it is a basic compound with a tertiary amine group. According to the Biopharmaceutics Classification System, it falls within class II³. The aim of this work was to create an NSD of EBS by using natural polymers in varying ratios, so as to improve its solubility and bioavailability.

2. Methodology

2.1. Production of EBS solid dispersion formulas

We employed solvent evaporation in order to create NSDs of EBS. To create a transparent solution, 500 mg of the medication were dissolved in 25 mL of methanol. The natural polymers xyloglucan (XG) and sodium alginate (SA) were then suspended in the medication solution. The drug to carrier ratios of 1:0.5, 1:1, 1:2, and 1:3 were used in order to mix the drug and the carriers. NSD1–NSD4 represent EBS:SA formulas, while NSD5–NSD8 represent EBS:XG formulas. At room temperature, the suspension was continuously agitated with a magnetic stirrer until all of the solvent had evaporated. For additional assessments, the produced NSDs were placed in a des-

iccator with calcium chloride for at least 24 h after passing through a #20 sieve⁴.

2.2. Assessment of EBS solid dispersion formulas

2.2.1. Study of saturation solubility and *in vitro* dissolution and solubility

Excess NSD formulations were added to distilled water in simple tubes in order to measure the saturation solubility of prepared NSDs in water through the use of a water bath shaker for 48 h, at 25°C. The USP type II dissolution device was filled with precisely weighed samples equal to 5 mg of EBS. Pure drugs and NSDs with the highest solubility values were the subjects of an *in vitro* dissolution assessment. At 37°C (±0.5°C), 1,000 cc of 0.1 N HCl (pH 1.2) and a paddle speed of 100 rpm were used for this assessment. After filtering, a 5-mL sample was obtained at 10, 15, 20, 30, 40, 50, and 60 min. The samples were then evaluated spectrophotometrically at the designated λ_{max} of EBS and were replaced with an equivalent volume of fresh dissolution media. Subsequently, the plotting of the drug dissolution profiles was undertaken^{5,6}.

2.2.2. Study of *in vivo* pharmacokinetics

We assessed the pharmacokinetic properties of the pure EBT suspension and of the NSD suspension in male Wistar rats. Rats were randomly assigned into two groups (A and B), each consisting of three rats (n=3). All animals received the samples orally. In each session, the EBT dosage was 10 mg/kg of body weight. Every rat received ether anaesthesia. A nasogastric tube was used in order to administer the optimized formula diluted with water and the pure EBT suspension. The tail vein was used in order to obtain blood samples at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 h after the administration. Centrifugation (Centurion Scientific, Chichester, UK) was performed on the blood samples (that were collected in heparinized plastic tubes) for 5 min at 1,500 rpm. For additional assessments, the plasma was carefully pipetted out and kept at -20°C in Eppendorf plastic tubes. We centrifuged equal amounts

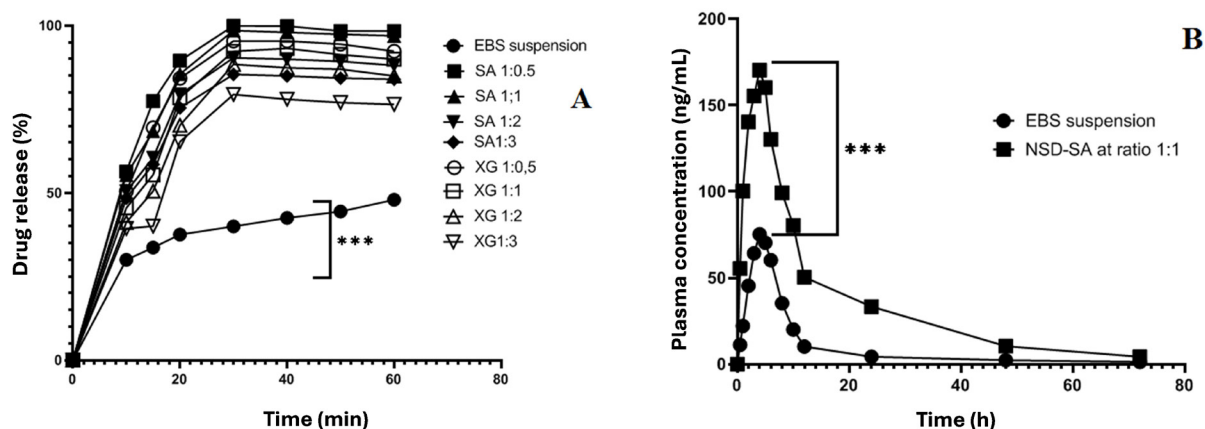


Figure 1. (A): Dissolution profiles of the herein employed EBS suspension and of various NSDs. (B): Mean plasma concentration-time profile for carebastine following the oral administration of an EBS suspension and of the optimized NSD formula (EBS:SA at a 1:1 ratio). After 4 h, the C_{max} of the EBS suspension was 75.33 ng/mL, while the C_{max} of the EBS:SA NSD (at a ratio of 1:1) indicated a peak plasma concentration of 170.32 ng/mL. Compared to the oral suspension that exhibited an area-under-the-curve (AUC) value of 701.9 ng/mL/h, the optimized formula had a considerably greater AUC of 2,541.33 ng/mL/h. Abbreviations used: EBS, ebastine; NSD, natural solid dispersion; SA, sodium alginate; XG, xyloglucan.

of absolute methanol and plasma for 5 min at 10,000 rpm. Drying the supernatant involved applying a nitrogen stream at -40°C . To acquire the supernatant for subsequent analysis using Knauer HPLC (Germany), the obtained residue was reconstituted with methanol and centrifuged at 10,000 rpm. Acetonitrile, methanol, and ammonium acetate buffer (20:30:50) made up the mobile phase. The HPLC with a C-18 column (4.6 x 250 mm) used a flow rate of 1 mL/min and a wavelength of 257 nm in order to evaluate the levels of carebastine, while samples that included 1.0 mg/mL of cetirizine hydrochloride acted as internal standard. The column's temperature remained constant at 40°C . The final sample and standard concentrations (40 $\mu\text{g/mL}$) were prepared. The samples were injected for elution after the baseline was established. The maximum plasma concentration (C_{max}), the time to attain the C_{max} (T_{max}), and the area-under-the-curve (AUC_{0-t}) were all determined by using the Microsoft Excel PKSolver (2016) software.

3. Results and Discussion

EBS is sparingly soluble in methanol, nearly insol-

uble in water, and insoluble in 0.1-N HCl. Our findings concurred with those of earlier studies^{7,8}. All assessed formulations exhibited superior dissolving rates in 0.1-N HCl, as shown in Figure 1A. Compared to XG, SA dissolves better in water. This implies that it can more readily envelop drug particles in a hydrated layer, potentially aiding in their dispersion and disintegration in the aqueous medium. Based on the earlier findings, the NSD of EBS:SA at ratio of 1:1 improved the EBS dissolution and solubility. As a result, this NSD was examined further *in vivo*.

Following the administration of a single oral dose, the concentration of EBT in the rat plasma was measured (Figure 1B). EBT underwent substantial first-pass metabolism to become carebastine; its active metabolite. The AUC's findings, which are based on variations between comparable items, were statistically significant. Carebastine was measured, since the concentration of EBS in the plasma was not found. The *in vivo* pharmacokinetic assessment of carebastine confirmed that the EBS:SA NSD (at a ratio of 1:1) significantly increases the bioavailability of EBT. The significant

absorption across transmucosal membranes may have contributed to the high C_{max} value observed (Figure 1B). The developed optimized formula resulted in a considerable increase in the AUC value. When compared to pure EBT, the optimized formula's oral bioavailability rose about 2.95 times. The solubility of EBS in natural polymers may be the cause of the observed increase in the oral absorption rate. The absorption across the mucosal membranes was improved by the released NSD. Together, these elements improved EBT's bioavailability. Previously, the NSD approach has been used for the improvement of the transdermal delivery of the therapeutic compounds^{9,10}.

4. Conclusion

The present study's findings demonstrate that the NSD method, which makes use of natural polymers such as SA and XG, is effective in improving the poor solubility of EBS. However, when compared to XG, the SA polymer with a drug to SA ratio of 1:1 ap-

peared to be the superior carrier. Using the solvent evaporation method, the optimized formula with the EBS:SA of 1:1 ratio appeared to be the best one for increasing the solubility, the dissolution, and the bioavailability of EBS.

Acknowledgements

The authors acknowledge the College of Pharmacy of the University of Thi-Qar for providing the required facilities for the undertaking of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdal-Hammid S.N., Alkufi H.K., Hussein Z.K., Salman A.H. Solubility enhancement of ebastine through natural solid dispersion: formulation and pharmacokinetics. *Pharmakeftiki* 37(2s), 34-38, 2025. <https://doi.org/10.60988/p.v37i2S.137>

Assessing the potential protective effects of tirzepatide against renal ischaemia-reperfusion injury in male rats: inhibition of *Foxo1* expression and pyroptosis

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KEY WORDS:

tirzepatide; renal ischaemia-reperfusion injury; FOXO1; KIM-1; pyroptosis

ARTICLE INFO:

Received: January 01, 2025

Revised: January 18, 2025

Accepted: February 06, 2025

Available online: October 10, 2025

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ABSTRACT

Renal ischaemia-reperfusion injury (RIRI) is a significant contributing factor to acute kidney injury, characterized by inflammation and oxidative stress formation that delay tissue repair. Our study has assessed the nephroprotective effects of tirzepatide on RIRI, through its effect on the forkhead box O1 gene (*Foxo1*) expression and pyroptosis in male rats. Our study included 24 Wistar rats divided to four groups (n=6 each): a sham group (without an induction of RIRI), a control group (with an induction of RIRI), a vehicle group (receiving an intraperitoneal injection of dimethyl sulfoxide at 1 mg/kg, 1 h before ischaemia), and a tirzepatide-treated group (receiving an intraperitoneal injection of tirzepatide at 1.35 mg/kg, 1 h before ischaemia). Our results revealed that tirzepatide can significantly reduce the kidney injury molecule-1 levels ($p=0.027$) and the levels of pyroptosis markers caspase-1 ($p<0.001$) and caspase-11 ($p=0.005$) in the studied rats' kidneys. Histopathological analysis of the latter revealed severe injury in the control group, while tirzepatide-treated rats exhibited injury scores under 40%. Additionally, the *Foxo1* expression was found to be significantly reduced as a result of the tirzepatide treatment ($p=0.031$). We concluded that tirzepatide may potentially relieve ischaemic renal damage through the modulation of pyroptosis and inflammatory pathways, thereby highlighting its potential as a targeted therapy for RIRI.

1. Introduction

Renal ischaemia-reperfusion injury (RIRI) constitutes a significant aetiological factor in the onset of acute kidney injury and has been associated with morbidity and mortality¹. The inflammatory responses and the overproduction of reactive oxygen species within the renal tissue after an RIRI can substantially impede the processes of tissue repair, thereby complicating efforts to effectively facilitate the regeneration of the RIRI-affected tissue².

Pyroptosis, a type of programmed cell death mediated by caspase-11 (which activates caspase-1), can trigger renal tubular cell death following renal ischaemia, leading to inflammatory cell damage³. The forkhead box O1 (FOXO1) gene (humans: *FOXO1*; rats: *Foxo1*) expression is up-regulated following ischaemic conditions, suggesting its critical role in triggering the expression of other genes implicated in programmed cell death as a result of RIRI⁴. At present, there are no targeted therapeutic interventions for RIRI. Nevertheless, several studies have demonstrated that the inhibition of pathways related to oxidative stress, inflammatory processes, and cellular apoptosis may facilitate the reduction of the severity of renal injury⁵.

Tirzepatide is a drug used in the management of type 2 diabetes through its binding to two receptors within the body, namely the glucagon-like peptide-1 receptor (GLP-1R) and the gastric inhibitory polypeptide receptor; in order to regulate blood glucose and obesity⁶. Tirzepatide may also improve ischaemic damage *via* its anti-inflammatory effects and the reduction of cell death⁷. Therefore, the aim of our study was to assess the potential nephroprotective effect of tirzepatide against RIRI in rats through *Foxo1* expression and pyroptosis.

2. Methodology

2.1. Study design and experiments

Twenty four adult male albino Wistar rats (weighting 200–250 g) were utilized in this study. The rats were randomly allocated into four groups (n=6 each): (i) a sham group (without an induction of RIRI), (ii) a control group (with an induction of RIRI), (iii) a vehicle group (receiving an intraperitoneal injection of dimethyl sulfoxide at 1 mg/kg, 1 h before ischaemia), and (iv) a tirzepatide-treat-

ed group (receiving an intraperitoneal injection of tirzepatide at 1.35 mg/kg, 1 h before ischaemia). Anaesthesia was administered *via* an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Ischaemia was induced through the clamping of both renal pedicles for 30 min, followed by 2 h of reperfusion.

Following reperfusion, the rats were euthanized under anaesthesia and kidney samples were collected for analysis. The right kidneys were imbedded in formalin for histological examination, while the left kidneys were kept at -80°C for the undertaking of ELISA and genetic analysis.

The undertaken histopathological assessment involved kidney samples processing, followed by staining with haematoxylin and eosin for microscopic examination. Quantitative and qualitative renal tissue damage assessment were achieved through the scoring of tissue degeneration.

Tissue homogenates from the frozen kidneys were washed, homogenized in phosphate buffered saline with protease inhibitors and Triton X-100, and they were subsequently centrifuged. Supernatants were aliquoted and frozen for the measurement of the levels of kidney injury molecule-1 (KIM-1) and of pyroptosis biomarkers (i.e., caspase-1 and caspase-11) through ELISA. In addition, total RNA extraction was undertaken for a real-time PCR analysis of the expression of *Foxo1*.

2.2. Statistical analysis

The SPSS v.26 software was utilized for the analysis of the current study's data. All values are expressed as mean $\pm$ standard deviation. One-way ANOVA (followed by a Tukey test) was used in order to assess the differences in the variables' means among the study groups, and a significance level was considered for *p*-values that were <0.05.

2.3. Ethical approval

Ethical approval for the present study was granted by the Central Committee for Bioethics of the University of Kufa (approval number: 20552; date: August 29, 2024).

3. Results and Discussion

The analysis of the kidney KIM-1 levels revealed sig-

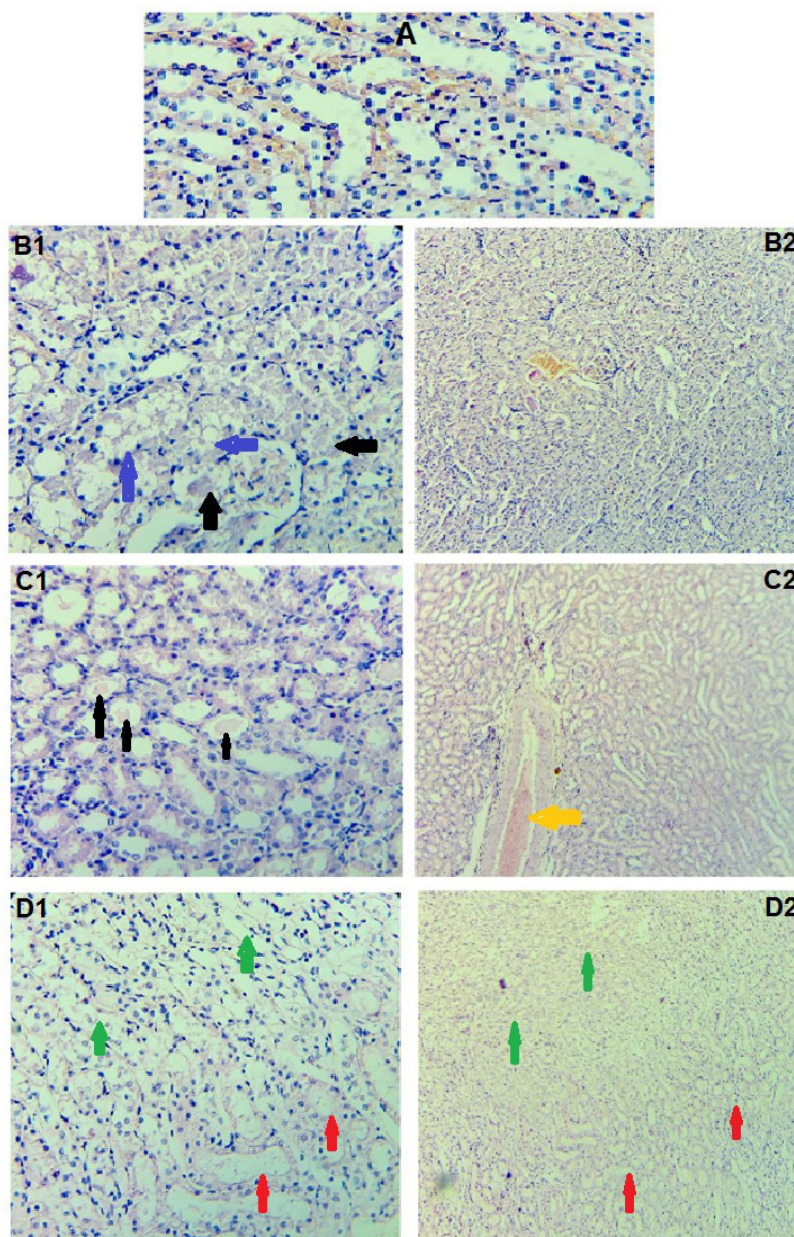


Figure 1. Representative microphotographs from the histopathological evaluation (using haematoxylin and eosin) of kidney tissues from the four herein studied rat groups. (A): The sham group tissue samples exhibited normal glomeruli and tubules. (B1,B2): The control group tissue samples exhibited significant alterations involving the development of cytoplasmic vacuoles (blue arrows) and eosinophilic casts (black arrows). (C1,C2): The vehicle group tissue samples exhibited significant alterations involving eosinophilic casts (black arrows) and vascular congestion (orange arrow). (D1,D2): The tirzepatide-treated group tissue samples exhibited a significant improvement of the renal tissue damage when compared to that observed in the control group; damaged tubules (red arrows) and normal tubules (green arrows) are highlighted. Caption magnification: x400 (A, B1, C1, D1); x100 (B2, C2, D2).

nificant elevations in both the control group and the vehicle group as compared to those of the sham group ($p<0.05$). Importantly, the treatment with tirzepatide led to a significant reduction in the kidney KIM-1 levels ($1,353.84 \pm 95.37$ pg/mL) compared to those of the control group ($1,546.88 \pm 71.72$ pg/mL; $p=0.027$). As far as the pyroptosis markers are concerned, the kidney caspase-1 levels were found to be notably elevated in the control and vehicle groups as compared to those of the sham group ($p<0.05$). The administration of tirzepatide resulted in a significant decrease in the kidney caspase-1 levels ($6,435.28 \pm 909.65$ pg/mL) when compared to those of the control group ($8,402.77 \pm 681.22$ pg/mL; $p<0.001$). Finally, the kidney caspase-11 levels also exhibited a significant increase in both the control and the vehicle groups as compared to those of the sham group ($p<0.05$). The treatment with tirzepatide effectively reduced the kidney caspase-11 levels ($2,479.15 \pm 144.60$ pg/mL) when compared to those of the control group ($2,941.24 \pm 203.60$ pg/mL; $p=0.005$).

Histopathological examination demonstrated severe renal tissue injury in both the control and vehicle groups, with score 4 damage characterized by cytoplasmic vacuoles, eosinophilic cast, oedema, and vascular congestion. On the other hand, the tirzepatide-treated group demonstrated a significant reduction in these pathological characteristics, with injury scores indicating a less than 40% involvement of the renal tubules (Figure 1).

The kidney *Foxo1* expression levels were found to be significantly lower in both the control and vehicle groups of our study as compared to those of the sham group ($p<0.001$). Tirzepatide treatment resulted in a further decrease in the kidney *Foxo1* expression (0.16 ± 0.00 mg/dL) when compared to that of the control group (0.30 ± 0.03 mg/dL; $p=0.031$).

RIRI is a recognized challenge in the clinical setting. Our study has shown the potential protective effect of tirzepatide in mitigating ischaemic damage through its effects on pyroptosis biomarkers and the tissue levels of *Foxo1* expression. Our results revealed a significant elevation in KIM-1 and pyroptosis marker (caspase-1 and caspase-11) levels as a result of the experimentally-induced RIRI in rats. These findings are consistent with previous studies highlighting

that renal ischaemia can trigger oxidative stress and inflammatory responses, which participate in the observed tubular cell death and kidney injury. In fact, the significant elevation in KIM-1 levels serves as a predictor biomarker for early tubular damage⁸. Although, to our knowledge, there are no known studies that have specifically examined the effect of tirzepatide on the herein examined parameters in rats undergoing RIRI, our data firmly suggest that tirzepatide can effectively reduce these biomarkers, thereby potentially inhibiting pyroptosis and ameliorating inflammation and renal tissue damage.

The herein presented histopathological evaluation has revealed a severe tissue injury in the ischaemic groups' tissues, characterized by the formation of cytoplasmic vacuoles, eosinophilic casts, and oedema. Previous studies have reported similar histopathological findings following ischaemic conditions in the rat kidneys². The significant reduction in the recorded injury score as a result of the treatment with tirzepatide suggests that the drug can enhance tissue repair and decrease the extent of cellular damage in the rat kidneys. Alathary and Kadhim have suggested that the agonistic effects of tirzepatide on GLP-1R might be implicated in its ability to mitigate renal injury through anti-inflammatory pathways⁶.

Finally, our results suggest that the rat kidney *Foxo1* expression is downregulated after renal ischaemia; this downregulation may be linked to the activation of the PI3K/AKT pathway that phosphorylates the FOXO1 transcription factor, leading to its translocation from the nucleus to the cytoplasm and the inhibition of its transcriptional activity on its own gene⁹. The herein assessed tirzepatide treatment was able to further reduce the *Foxo1* expression. As previous studies have reported that FOXO1 is involved in the regulation of various genes implicated in oxidative stress and cell death¹⁰, the downregulation of its gene expression by tirzepatide could underscore a potential protective response.

4. Conclusion

The downregulation of the rat kidney *Foxo1* expression, along with the reduction in the pyroptosis-associated caspases, suggests potential mechanisms for the observed tirzepatide-induced nephroprotective effects.

These findings suggest that tirzepatide could be a promising therapeutic option for mitigating RIRI.

Acknowledgements

We acknowledge the support of the University of Babylon College of Pharmacy, the University of Kufa College of Pharmacy, and colleagues.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdul-Husseein H.K., Alzubaidi F.A. Assessing the potential protective effects of tirzepatide against renal ischaemia-reperfusion injury in male rats: inhibition of *Foxo1* expression and pyroptosis. *Pharmakeftiki* 37(2s), 39-43, 2025. <https://doi.org/10.60988/p.v37i2S.138>

Evaluation of the nephroprotective effect of dapagliflozin in renal ischaemia-reperfusion injury in male rats: *Foxo1* modulation and pyroptosis

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KEY WORDS:

renal ischaemia-reperfusion; dapagliflozin; nephroprotection; pyroptosis; FOXO1

ARTICLE INFO:

Received: January 01, 2025

Revised: n/a

Accepted: January 14, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

Renal ischaemia-reperfusion injury (RRI) significantly contributes to acute kidney injury, leading to high morbidity and mortality rates. This study has investigated the nephroprotective effects of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, on RRI in male Wistar rats. Twenty four rats were divided into four groups (n=6 each): a sham group (without an induction of RRI), a control group (with an induction of RRI), a vehicle group (receiving an intraperitoneal injection of 10% v/v dimethyl sulfoxide in isotonic saline solution, 1 h before ischaemia), and a dapagliflozin-treated group (receiving an intraperitoneal injection of dapagliflozin at 1 mg/kg, 1 h before ischaemia). Ischaemia was induced by clamping the renal pedicles for 30 min, followed by 2 h of reperfusion. Histopathological analysis revealed marked renal tissue injury in the control and vehicle groups, while dapagliflozin administration reduced these injuries, suggesting that less than 50% of the renal tubules were affected as a result. Kidney injury molecule-1, caspase-1, and caspase-11 levels were found to be significantly elevated in the control and vehicle groups, but decreased in the dapagliflozin group. The forkhead box O1 gene (*Foxo1*) expression was also found significantly reduced as a result of the treatment. These findings suggest that dapagliflozin mitigates renal injury through the suppression of *Foxo1* expression and pyroptosis, thereby improving renal cell integrity. This study highlights dapagliflozin's potential as a nephroprotective agent against acute renal failure, as it can enhance renal cell survival and mitigate injury pathways.

1. Introduction

Renal ischaemia-reperfusion injury (RIRI) can cause acute kidney injury and is associated with high morbidity and mortality^{1,2}. During RIRI, oxidative stress (recognized by the production of reactive oxygen species) triggers inflammatory responses, which interfere with tissue repair and regeneration and disrupt renal function. Kidney injury molecule-1 (KIM-1), a proximal tubular protein, peaks following RIRI, acting as a vital biomarker for the early identification of the latter³. Pyroptosis, a programmed cell death, takes up a significant role in RIRI; it is principally mediated by caspase-1 and caspase-11, contributing to tubular epithelial cell death⁴.

Dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, is a novel therapeutic option exhibiting efficacy in glycaemic management and probable renal protection⁵. The forkhead box O1 (FOXO1) gene (humans: *FOXO1*; rats: *Foxo1*) expression is induced in RIRI, leading to mitochondrial dysfunction. Inhibition of the *Foxo1* expression is known to retard apoptosis, enhance mitochondrial function, and hasten renal function⁶. The aim of this study was to assess the ability of dapagliflozin in potentially mitigating RIRI in a rat model, by focusing on *Foxo1* expression and pyroptosis.

2. Methodology

2.1. Study design and experiments

Twenty four adult male albino Wistar rats (weighting 200–250 g) were utilized in this study. The rats were randomly allocated into four groups (n=6 each): (i) a sham group (without an induction of RIRI), (ii) a control group (with an induction of RIRI), (iii) a vehicle group (receiving an intraperitoneal injection of 10% v/v dimethyl sulfoxide in isotonic saline solution, 1 h before ischaemia), and (iv) a dapagliflozin-treated group (receiving an intraperitoneal injection of dapagliflozin at 1 mg/kg, 1 h before ischaemia). Anaesthesia was administered *via* an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Ischaemia was induced through the clamp-

ing of both renal pedicles for 30 min, followed by 2 h of reperfusion.

Following reperfusion, the rats were euthanized under anaesthesia and kidney samples were collected for analysis. The right kidneys were imbedded in formalin for histological examination, while the left kidneys were kept at -80°C for the undertaking of ELISA and genetic analysis.

The undertaken histopathological assessment involved kidney samples processing, followed by staining with haematoxylin and eosin for microscopic examination. Quantitative and qualitative renal tissue damage assessment were achieved through the scoring of tissue degeneration.

Tissue homogenates from the frozen kidneys were washed, homogenized in phosphate buffered saline with protease inhibitors and Triton X-100, and they were subsequently centrifuged. Supernatants were aliquoted and frozen for the measurement of the levels of KIM-1 and of pyroptosis biomarkers (i.e., caspase-1 and caspase-11) through ELISA. In addition, total RNA extraction was undertaken for a real-time PCR analysis of the expression of *Foxo1*.

2.2. Statistical analysis

The SPSS v.26 software was utilized for the analysis of the current study's data. All values are expressed as mean $\pm$ standard deviation. One-way ANOVA (followed by a Tukey test) was used in order to assess the differences in the variables' means among the study groups, and a significance level was considered for *p*-values that were <0.05.

2.3. Ethical approval

Ethical approval for the present study was granted by the Central Committee for Bioethics of the University of Kufa (approval number: 20552; date: August 29, 2024).

3. Results and Discussion

The histopathological assessment of the renal tissue injury in both the control and vehicle groups has rec-

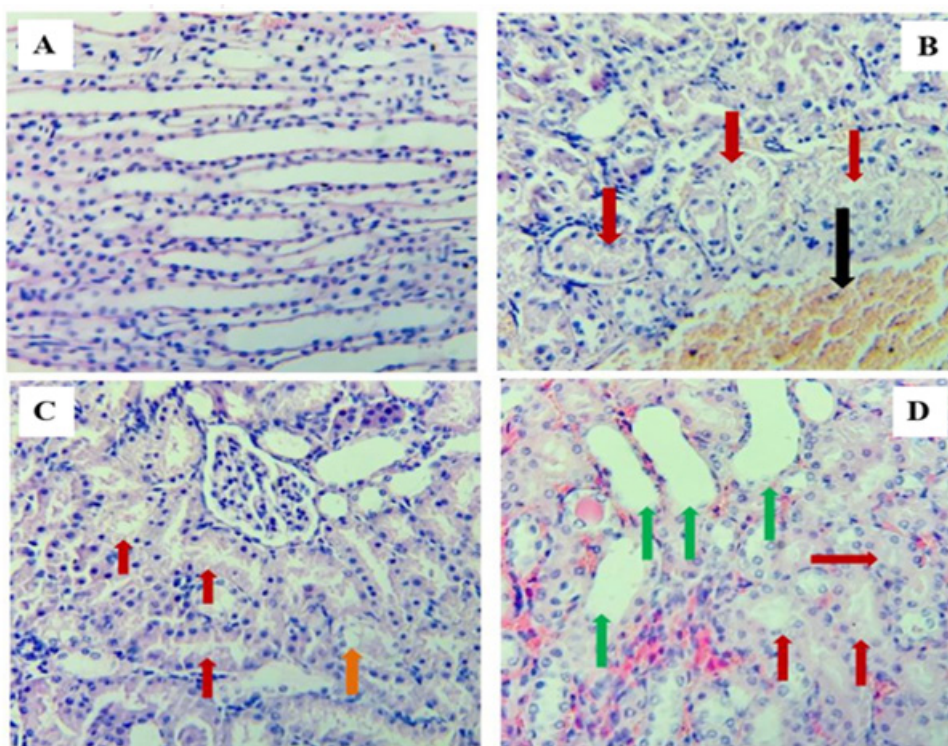


Figure 1. Representative microphotographs from the histopathological evaluation (using haematoxylin and eosin) of kidney tissues from the four herein studied rat groups. (A): The sham group tissue samples exhibited normal glomeruli and tubules. (B,C): The control (B) and the vehicle (C) group tissue samples exhibited significant alterations involving the development of cytoplasmic swelling and eosinophilia (red arrows), vascular congestion (black arrow), and cytoplasmic vacuoles (orange arrow). (D): The dapagliflozin-treated group tissue samples exhibited a significant improvement of the renal tissue damage when compared to that observed in the control group; damaged tubules (red arrows) and normal tubules (green arrows) are highlighted. Caption magnification: x400 (A–D).

ognized cytoplasmic swelling and eosinophilia, vascular congestion, and marked alterations in glomerular and tubular structures. In contrast, the dapagliflozin-treated group samples exhibited a reduction in the number of these pathological changes, with injury scores indicating that less than 50% of the renal tubules were affected by comparison (Figure 1).

KIM-1 levels were found to be significantly raised in the control ($1,546.87 \pm 71.71$ pg/mL) and vehicle ($1,488.52 \pm 54.93$ pg/mL) group kidney samples as compared to those of the sham group ($1,308.73 \pm 95.58$ pg/mL; $p=0.005$), while dapagliflozin significantly decreased the kidney KIM-1 levels ($1,185.98$

± 140.49 pg/mL) as compared to those of the control group ($p<0.001$).

The rat kidney caspase-1 levels were found to be significantly raised in the control ($8,402.76 \pm 681.21$ pg/mL) and the vehicle ($8,152.56 \pm 239.96$ pg/mL) groups as compared to those of the sham group ($6,998.98 \pm 229.23$ pg/mL; $p=0.005$), while dapagliflozin significantly decreased the kidney caspase-1 levels ($6,271.62 \pm 281.37$ pg/mL) as compared to those of the control group ($p<0.001$). The caspase-11 levels were found to be significantly raised in the control ($2,941.23 \pm 203.70$ pg/mL) and the vehicle ($2,929.38 \pm 235.67$ pg/mL) rat groups as compared

to those of the sham group ($2,542.00 \pm 229.70$ pg/mL; $p=0.020$), while dapagliflozin significantly decreased the kidney caspase-11 levels ($2,305.92 \pm 201.21$ pg/mL) as compared to those of the control group ($p<0.001$).

Finally, the rat kidney *Foxo1* expression was found to be significantly diminished in the control (0.30 ± 0.03 mg/dL) and the vehicle (0.18 ± 0.02 mg/dL) groups as compared to that of the sham group (1.01 ± 0.15 mg/dL; $p<0.001$). Dapagliflozin administration further reduced *Foxo1* expression (0.14 ± 0.02 mg/dL) as compared to that of the control group ($p=0.012$).

Our study has aimed at evaluating the effect of dapagliflozin on acute renal failure. Our results suggest that the pre-RIRI administration of dapagliflozin can alleviate renal tissue damage and minimize the mean tissue score damage to the moderate level of renal impairment. Furthermore, dapagliflozin has herein been found to suppress the expression of the *Foxo1* and prevent pyroptosis in the renal tubular epithelium. No studies exist that have assessed the effects of dapagliflozin on the same parameters in rats with RIRI. Nevertheless, Jallawee and Janabi (2024)³ have recently reported that a pretreatment with dapagliflozin can significantly mitigate the renal tubular injuries, as evidenced by histological evidence in rats with RIRI.

Our study has shown that the *Foxo1* expression was reduced following RIRI, which is consistent with previous studies reporting that the FOXO1 gene expression is regulated by FOXO proteins: following ischaemic conditions, the PI3K/Akt pathway is activated, triggering the nuclear export of FOXO and the transcriptional suppression of FOXO1 subsequently to the PI3K-dependent FOXO phosphorylation in the cytoplasm^{7,8}. Cheng⁶ has demonstrated that, FOXO1

regulates target genes implicated in oxidative stress and energy metabolism, thereby affecting mitochondrial function and integrity: it downregulates the antioxidant enzyme expression (which protects against oxidative damage) and plays a critical role in the deterioration of the mitochondrial function. Studies have also concluded that the FOXO1 overexpression can worsen ischaemic injury through the upregulation of gasdermin D; a pyroptosis-related protein⁹. Therefore, the further suppression of the FOXO1 gene expression may diminish pyroptosis and oxidative stress, and could mitigate RIRI by maintaining mitochondrial homeostasis in the renal tubular epithelial cells.

4. Conclusion

Dapagliflozin exhibits nephroprotective effects against RIRI through the suppression of *Foxo1* expression and pyroptosis in rats, thereby improving renal cell integrity and mitigating tissue damage.

Acknowledgements

We would like to acknowledge the University of Babylon College of Pharmacy, as well as the staff at the animal house and at the research center of the University of Kufa.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdul-Hussein H.K., Alzubaidi F.A. Evaluation of the nephroprotective effect of dapagliflozin in renal ischaemia-reperfusion injury in male rats: *Foxo1* modulation and pyroptosis. *Pharmakeftiki* 37(2s), 44–48, 2025. <https://doi.org/10.60988/p.v37i2S.139>

Design, ADME study, and molecular docking of serine derivatives of isatin – para-aminobenzoic acid conjugates

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ABSTRACT

A total of 20 novel isatin – para-aminobenzoic acid derivatives of serine have been designed in order to assess their potential effectiveness against some promising targets. Molecular docking was accomplished with the crystal structure of some of the predicted targets (i.e., 5UEN, 5FL4, 6QNG, 5SDB, and 6SUK), and we analysed the binding affinity between the compounds and each of the proteins of interest. Moreover, absorption, distribution, metabolism, and excretion (ADME) studies were conducted for the compounds with the best docking poses, thereby providing sufficient information about their pharmacokinetic, physicochemical, and drug-likeness properties.

KEY WORDS:

MOE software; ADME study; Molecular docking; isatin Schiff base; para-aminobenzoic acid

ARTICLE INFO:

Received: January 11, 2025

Revised: February 11, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

1. Introduction

Heterocyclic compounds are pivotal sets of organic molecules as they are characterized by sizable biological profiles; this makes these compounds continuously being employed by researchers dealing with drug design and development^{1,2}. Isatin (1H-indole-2,3-dione) is an invaluable aromatic heterocyclic organic compound; itself and its

corresponding compounds constitute an auspicious genre of heterocyclic molecules with many substantial activities and are benign in humans³. The isatin molecule is endowed with myriad pharmacological activities, including antimicrobial, antitubercular, antiviral, antitumour, antioxidant, anticonvulsant, anti-inflammatory, analgesic, antifungal, and central nervous system-depressant activities^{4,5}. Isatin and

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a primary amine undergo a condensation reaction to generate Schiff bases of isatin. Those Schiff bases are useful intermediates in the preparation of medicines, dyes, and other organic molecules due to their manifold chemical and biological characteristics⁶. Some clinically significant drugs comprise an isatin moiety, like the tyrosine kinases inhibitor nintedanib, the mRNA and protein synthesis inhibitor antiviral agent methisazone, and the receptor tyrosine kinase inhibitor anticancer drug sunitinib³.

2. Methodology

The present study utilized an HP system with 8 GB of RAM and Intel Core i7-8550U processors running at 2 GHz. Chem Draw Ultra 22.0.0 and MOE 2024.06 were both installed and configured. The molecular docking simulation was performed inside the active site of the following proteins (herein abbreviated as in the Protein Data Bank; PDB): (i) human carbonic anhydrase IX (5FL4), human carbonic anhydrase XII (6QNG), and human dihydrofolate reductase in complex with NADP and N10-formyl-tetrahydrofolate (5SDB) that were selected for the screening of anticancer activity, as well as (ii) human adenosine A1 receptor (5UEN) and neprilysin (6SUK) that were used for the screening of cardioprotective effects. The crystal structures of these proteins were downloaded from the PDB. The proteins were prepared by removing the solvent molecules, other associated molecules, and other chains of proteins of interest in order to facilitate the interaction of ligands with the complementary functional groups in the binding site of the selected receptors, followed by the addition of protons to the downloaded protein format from PDB, then the addition of broken bonds, and the fixation of the potential of the protein molecule. Finally, the active site of the receptors was selected in MOE depending on the site occupied by the co-crystallized ligands. The molecular structures of the ligands were precisely outlined using ChemDraw Professional (22.0.0). The ligands were subsequently protonated into a three-dimensional configuration, partial charges were incorporated, energy was minimized, and the outcomes were stored. The absorption, distribution, metabolism, and excretion (ADME) properties'

determination was accomplished by utilizing the SwissADME server: we evaluated the pharmacokinetic profiles of the compounds as well as their blood-brain barrier (BBB) penetration and bioavailability.

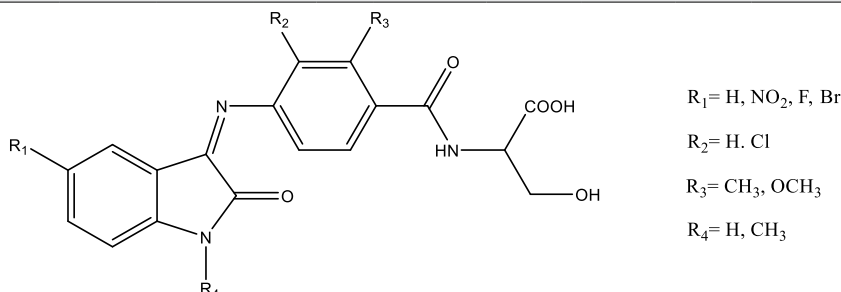
3. Results and Discussion

The docking and ADME outcomes offer valuable insights into the drug-likeness of the tested ligands (Table 1). Among them, ligands 1b, 1c, 1d, 3b, and 4b demonstrate strong binding affinities across multiple goal proteins, with docking scores as low as -10.34 kcal/mol (1b with 6SUK) and -10.05 kcal/mol (3b with 6SUK), thereby indicating robust interactions with their respective binding sites. These ligands also showcase high gastrointestinal (GI) absorption, suitable bioavailability (0.56), and no violations of Lipinski's Rule of Five, making them favourable candidates for oral drug development⁷. In our assessment, ligands 2a, 2b, 2c, and 2d display even more potent binding affinities, with 2c accomplishing a docking score of -10.23 kcal/mol with 6QNG and 2a attaining a -10.17 kcal/mol with 5FL4, thereby suggesting brilliant docking capacity. However, these ligands are afflicted by a low GI absorption and terrible bioavailability (0.11) due to their excessive hydrogen bond acceptor and topological polar floor area ($>140 \text{ \AA}^2$) performance, which limits their passive intestinal permeability⁸. The presence of extra polar practical corporations in those ligands in all likelihood contributes to their stronger protein-ligand interactions and reduces their absorption performance, suggesting an alternate-off between binding power and pharmacokinetic residences⁹. Importantly, none of the ligands are expected to penetrate the BBB, which may be beneficial only when targeting peripheral conditions.

4. Conclusion

All the designed compounds manifest vigorous binding affinities with the target proteins, highlighting their potential as therapeutic agents in cancer and cardiovascular diseases. Their interactions with proteins like neprilysin and adenosine A1 receptor suggest their function

Table 1. The molecular docking and ADME results of the designed compounds. Abbreviations used: 1a ($R_1=H, R_2=H, R_3=H, R_4=H$), 1b ($R_1=H, R_2=H, R_3=CH_3, R_4=H$), 1c ($R_1=H, R_2=H, R_3=OCH_3, R_4=H$), 1d ($R_1=H, R_2=Cl, R_3=H, R_4=H$), 2a ($R_1=NO_2, R_2=H, R_3=H, R_4=H$), 2b ($R_1=NO_2, R_2=H, R_3=CH_3, R_4=H$), 2c ($R_1=NO_2, R_2=H, R_3=OCH_3, R_4=H$), 2d ($R_1=NO_2, R_2=Cl, R_3=H, R_4=H$), 3a ($R_1=F, R_2=H, R_3=H, R_4=H$), 3b ($R_1=F, R_2=H, R_3=CH_3, R_4=H$), 3c ($R_1=F, R_2=H, R_3=OCH_3, R_4=H$), 3d ($R_1=F, R_2=Cl, R_3=H, R_4=H$), 4a ($R_1=Br, R_2=H, R_3=H, R_4=H$), 4b ($R_1=Br, R_2=H, R_3=CH_3, R_4=H$), 4c ($R_1=Br, R_2=H, R_3=OCH_3, R_4=H$), 4d ($R_1=Br, R_2=Cl, R_3=H, R_4=H$), 5a ($R_1=Br, R_2=H, R_3=H, R_4=CH_3$), 5b ($R_1=Br, R_2=H, R_3=CH_3, R_4=CH_3$), 5c ($R_1=Br, R_2=H, R_3=OCH_3, R_4=CH_3$), 5d ($R_1=Br, R_2=Cl, R_3=H, R_4=CH_3$), 5FL4 (human carbonic anhydrase IX), 5SDB (human dihydrofolate reductase in complex with NADP and N10-formyl-tetrahydrofolate), 5UEN (human adenosine A1 receptor), 6QNG (human carbonic anhydrase XII), 6SUK (nepriylsin), ADME (absorption, distribution, metabolism, and excretion), BBB (blood-brain barrier), F (absolute bioavailability), GI (gastrointestinal), TPSA (topological polar surface area).



Ligands	Molecular docking results (kcal/mol)					ADME results							
	5UEN	5FL4	6QNG	5SDB	6SUK	H-donor	H-acceptor	Molar refractivity	TPSA ($\text{\AA}^2$)	GI absorption	BBB permeability	F	Lipinski's Rule of Five
1a	-6.73	-9.44	-9.55	-7.98	-9.89	4	6	96.01	128.09	high	no	0.56	0
1b	-7.16	-9.58	-9.06	-8.20	-10.34	4	6	100.97	128.09	high	no	0.56	0
1c	-7.15	-9.54	-9.74	-8.30	-9.70	4	7	102.50	137.32	high	no	0.56	0
1d	-6.92	-9.66	-9.33	-8.14	-10.61	4	6	101.02	128.09	high	no	0.56	0
2a	-7.35	-10.17	-10.02	-8.92	-9.77	4	8	104.83	173.91	low	no	0.11	1 [N or O>10]
2b	-7.39	-9.73	-9.94	-8.73	-10.58	4	8	109.80	173.91	low	no	0.11	1 [N or O>10]
2c	-8.06	-9.60	-10.23	-8.95	-9.68	4	9	111.32	183.14	low	no	0.11	1 [N or O>10]
2d	-7.69	-9.64	-10.10	-8.56	-10.47	4	8	109.84	173.91	low	no	0.11	1 [N or O>10]
3a	-6.74	-9.99	-9.94	-8.02	-9.52	4	7	95.97	128.09	high	no	0.56	0
3b	-7.35	-9.70	-9.62	-8.13	-10.05	4	7	100.93	128.09	high	no	0.56	0
3c	-7.29	-9.78	-9.64	-8.56	-8.77	4	8	102.46	137.32	high	no	0.56	0
3d	-6.92	-9.35	-9.60	-8.08	-10.42	4	7	100.98	128.09	high	no	0.56	0
4a	-6.89	-9.21	-9.66	-8.03	-9.22	4	6	103.71	128.09	high	no	0.56	0
4b	-7.38	-9.40	-9.80	-8.45	-10.47	4	6	108.67	128.09	high	no	0.56	0
4c	-7.15	-9.68	-10.15	-8.56	-9.30	4	7	110.20	137.32	high	no	0.56	0
4d	-7.36	-9.32	-9.83	-8.35	-10.65	4	6	108.72	128.09	high	no	0.56	0
5a	-7.30	-9.79	-9.85	-8.74	-8.97	3	6	108.61	119.30	high	no	0.56	0
5b	-7.50	-9.39	-10.02	-8.82	-10.15	3	6	113.58	119.30	high	no	0.56	0
5c	-7.12	-9.37	-10.04	-9.12	-10.45	3	7	115.10	128.53	high	no	0.56	0
5d	-7.48	-9.14	-10.10	-8.85	-10.18	3	6	113.62	119.30	high	no	0.56	0

in modulating cardiovascular functions such as vasodilation, diuresis, and natriuresis, besides their potential in modulating the pH homeostasis maintained by carbonic anhydrases. Additionally, the deprivation of an important cofactor required for cancer cell multiplication (offered by the herein observed dihydrofolate reductase inhibition) warrants further research into their pharmacological applications.

Acknowledgements

We are deeply grateful to the College of Pharmacy of

the University of Babylon for giving us the opportunity and the facilities to accomplish this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdulsada A.H., Al-yassery H.K., Abdulkareem Z.R., Hasan S.H. Design, ADME study, and molecular docking of serine derivatives of isatin – para-aminobenzoic acid conjugates. *Pharmakeftiki* 37(2s), 49–52, 2025. <https://doi.org/10.60988/p.v37i2S.140>

Study of the serum levels of lipocalin-2 as well as of metabolic and oxidative stress markers in alopecia areata patients of the Babil Province

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KEY WORDS:

alopecia areata; lipocalin-2; Iraq; oxidative stress; metabolism

ARTICLE INFO:

Received: November 21, 2024

Revised: February 13, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

ABSTRACT

Rapid hair loss is caused by the chronic inflammatory disorder alopecia areata, which destroys the hair follicle. Alopecia areata often affects the scalp, although it can also affect other hairy areas such as the beard or the eyebrows. Metabolic abnormalities have been observed in patients with alopecia areata. The aim of this study was to investigate the potential roles of lipocalin-2, insulin, other associated metabolic markers, and oxidative stress in the pathophysiology of alopecia areata. In this case-control study, 90 individuals from the Babil Province of Iraq were recruited: 45 alopecia areata patients and 45 individuals with excellent hair health. Blood samples were collected from them between September 2023 and March 2024, in addition to the medical history and basic clinical measurements of all participants. In our study, alopecia areata patients presented with elevated serum levels of lipocalin-2, insulin, and C-peptide, together with elevated levels of important antioxidant enzymes and oxidative stress markers (as compared to those of age-matched healthy individuals), thereby suggesting a potential disease progression mechanism through metabolic dysregulation.

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1. Introduction

Alopecia areata is an autoimmune disease that manifests as a chronic inflammatory disease characterized by non-scarring patches of over-face hair loss, the scalp, and the

body. Different treatments can be administered for alopecia areata, but no curative ones exist and none can induce a state of remission. Approximately 1% to 2% of the general population may eventually be affected by the illness. According

Table 1. Levels of metabolic and oxidative stress-related markers in patients with alopecia areata (patient group) and healthy individuals (control group). Abbreviations used: CAT, catalase; GSH, glutathione; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.			
Variables	Patient group (mean ± SD)	Control group (mean ± SD)	Significance (p-value)
Lipocalin-2 (ng/mL)	308.0 ± 66.4	233.11 ± 28.08	<0.05
Total cholesterol (mg/dL)	185 ± 42	191 ± 23	0.08
LDL cholesterol (mg/dL)	112 ± 32	117 ± 27	0.09
HDL cholesterol (mg/dL)	69.0 ± 11.3	61.0 ± 13.7	0.07
Triglycerides (mg/dL)	89 ± 19	91 ± 13	0.11
Glucose (mg/dL)	91.0 ± 11.2	88.0 ± 13.9	0.06
Insulin (μIU/mL)	6.9 ± 1.9	4.2 ± 0.5	<0.05
HOMA-IR score	1.3 ± 0.5	0.9 ± 0.3	<0.05
C-peptide (ng/mL)	1.4 ± 0.4	1.3 ± 0.2	<0.05
CAT (ng/mL)	2.8 ± 1.1	1.5 ± 0.8	<0.05
SOD (μg/mL)	8.2 ± 3.1	5.2 ± 1.9	<0.05
GSH (μg/mL)	112.2 ± 59.2	84.7 ± 25.3	<0.05
MDA (nmol/mL)	1.7 ± 0.6	1.1 ± 0.5	<0.05

to skin biopsies from patients with alopecia areata, lymphocytes are present around the hair follicle bulb¹.

Currently, more than 600 adipokines with different biological properties within the human body have been identified; they are characterized by pro- and anti-inflammatory features². Pro-inflammatory adipokine upregulation stimulates a low-grade chronic inflammatory state, which is involved in the dysfunction of the metabolism. In addition, adipokines have been shown to be active regulators of cutaneous inflammation. Their contribution has been shown in several dermatological cases, such as acne, rosacea, psoriasis, and atopic dermatitis, in addition to alopecia areata. The disease pathogenesis of the latter has not been fully elucidated³. Previous studies have shown that alopecia areata is related to autoimmunity, including notably elevated serum levels of several cytokines⁴. A number of skin diseases has been associated with insulin resistance (i.e., acanthosis nigricans, acne, and psoriasis), while others have been suggested to be probably related to insulin resistance (i.e., acrochorda, androgenetic alopecia, hidradenitis suppurativa, and hirsutism). Insulin has been assumed to contribute to the

regulation of cutaneous androgen metabolism in addition to the hair growth cycle. However, the association between alopecia areata and insulin resistance remains controversial⁵.

Lipocalin-2 is an adipokine that contributes to glucose regulation, homeostasis, and systemic inflammation. Its contribution to several inflammatory diseases, such as psoriasis, rheumatoid arthritis, atopic dermatitis, and alopecia areata, is well-known. Moreover, oxidative stress has been reported to significantly contribute to alopecia areata pathogenesis; an imbalance takes place among oxidative and antioxidant mechanisms when the production of reactive oxygen species (ROS) rises or when the antioxidant activity is decreased⁶. The purpose of this study was to investigate the potential roles of lipocalin-2, insulin, other associated metabolic markers, and oxidative stress in the pathophysiology of alopecia areata.

2. Methodology

In this case-control study, 90 individuals from the Babil Province of Iraq were recruited: 45 alopecia areata patients and 45 individuals with

excellent hair health. Blood samples were collected from them between September 2023 and March 2024, in addition to the medical history and basic clinical measurements of all participants. Samples were obtained from patients who had visited dermatological clinics at the Hilla General Teaching Hospital, the Imam Al-Sadiq Teaching Hospital, and the Marjan Teaching Hospital. The patients were aged >25 years. Dermatologists assessed all participants and organised the study's groups. Individuals who were smokers, had received medication(s) within the previous 3 months, or were known to suffer from chronic conditions (such as diabetes, hypertension, and cancer) were excluded from the study.

After extracting the serum and placing it in Eppendorf tubes, the levels of lipocalin-2, insulin, and C-peptide were determined by using ELISA kits. The data were analysed by using the SPSS v. 26 software. Continuous variables are herein presented as mean $\pm$ standard deviation (SD), while group comparisons were performed using the independent *t*-test. Statistical significance was set at a *p*-value of <0.05. The ROC curve analysis was used in order to assess the diagnostic value of lipocalin-2.

This study was conducted following the ethical guidelines of the local health authorities, and its protocol was approved by the Institutional Review Board of the College of Pharmacy of the University of Babylon under protocol number 1345 (dated: 17-Jul-2023). Written informed consent was obtained from all participants prior to their inclusion in the study. This study adhered to the principles outlined in the Declaration of Helsinki.

3. Results and Discussion

The control and the alopecia areata groups did not differ significantly in terms of age and body mass index. However, alopecia areata patients presented with elevated serum levels of lipocalin-2, insulin, and C-peptide (Table 1). Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin, is a 25-kDa glycoprotein released by adipocytes in addition to immune cells such as neutrophils; its expression is promoted by several proinflammatory cytokines,

while studies have reported elevated serum levels of lipocalin-2 in inflammatory diseases such as psoriasis, atopic dermatitis, and rheumatoid arthritis⁷. In addition, it has been assumed that alopecia areata is probably related to an elevated risk of metabolic diseases, and that lipocalin-2 can significantly contribute to glucose homeostasis in addition to insulin sensitivity. In fact, alopecia areata patients have been previously shown to have significantly higher (*p*<0.05) fasting blood glucose serum levels, insulin levels, and C-peptide levels, besides a higher homeostatic model assessment of insulin resistance (HOMA-IR) score when compared to healthy controls⁸. Interestingly, serum glucose levels were also found increased in the alopecia areata patients of our study, but not to a statistically significant degree when compared to those of the control group (Table 1).

No significant variations were observed in terms of the serum levels of total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides among the alopecia areata patients and the healthy participants of our study (Table 1). At the same time, we observed notably (*p*<0.05) increased catalase (CAT) and superoxide dismutase (SOD) activities, in addition to increased malondialdehyde (MDA) and glutathione (GSH) levels in the serum of alopecia areata patients (Table 1). Our understanding of the correlation between oxidative stress and alopecia areata has been limited⁹. Intracellular ROS generation, which causes oxidative cellular damage, is known to increase as a result of the infiltrating inflammatory cells and the synthesized cytokines.

4. Conclusion

Our study has shown that Iraqi alopecia areata patients are presented with elevated serum levels of lipocalin-2, insulin, and C-peptide, together with elevated serum levels of important antioxidant enzymes (such as CAT and SOD) and oxidative stress markers (such as GSH and MDA), thereby suggesting a potential disease progression mechanism through metabolic dysregulation. The latter also affected the patients' HOMA-IR score, but

not their glucose, cholesterol (total, LDL, and HDL), and triglyceride levels (as compared to those of age-matched healthy individuals). Additional studies are required in order to determine the potential role of lipocalin-2 in alopecia areata diagnosis and disease progression tracking, while based on our findings, therapeutic strategies focusing on the management of metabolic derangements and oxidative stress show particular promise for the treatment of alopecia areata.

Acknowledgements

The authors wish to express their gratitude to the

College of Pharmacy of the University of Babylon for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Daami Q.J., Abdullah R.G., Al-Khakani M.F. Study of the serum levels of lipocalin-2 as well as of metabolic and oxidative stress markers in alopecia areata patients of the Babil Province. *Pharmakeftiki* 37(2s), 53-56, 2025. <https://doi.org/10.60988/p.v37i2S.141>

Impact of *CYP2C9*3* polymorphisms and clinical variables on the valproic acid levels of Iraqi patients suffering from grand mal epilepsy

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KEY WORDS:

valproic acid; grand mal epilepsy; monitoring; *CYP2C9*3*; *rs1057910*; polymorphism

ARTICLE INFO:

Received: January 30, 2025

Revised: February 11, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

Generalized tonic-clonic seizures form the key characteristic of severe epilepsy which neurologists call “grand mal” epilepsy. Targeting both epilepsy management and seizure control, valproic acid (VA) stands as one of the prevalent antiepileptic medications. *CYP2C9*3* is a mutation of the cytochrome P450 2C9 gene that encodes an enzyme in charge of VA metabolism. Variants of *CYP2C9*3* substantially influence drug metabolism, affecting therapeutic outcomes and toxicity. This study has evaluated how VA metabolism depends on *CYP2C9*3* polymorphisms while investigating clinical factors that impact drug levels in epilepsy. We have conducted a prospective cross-sectional study on 28 epileptic patients at the Merjan Medical City in Babylon (Iraq), from September 2024 to December 2024. VA levels were higher in epileptic patients bearing the C/C mutant genotype compared to those of patients with A/A or A/C genotypes. The dose of VA depends mainly on the age and the body mass index (BMI) of a patient. Those bearing mutant C/C genotypes displayed significantly higher VA concentrations than those with A/A or A/C variants. Moreover, age and BMI were recognized as crucial elements of VA dosing, highlighting the necessity of personalized styles for adjusting treatment efficacy while decreasing side effects. The study has shown that *CYP2C9*3* affects VA levels among grand mal epileptic patients, since patients expressing the C/C variant displayed higher blood levels of the drug. Future research should consider that optimum therapeutic outcomes require treatment approaches that account for the personal genetic constitution and clinical features.

1. Introduction

Grand mal epilepsy signifies a main epilepsy type that activates the total brain cortex. Its seizures trigger strong spasms while disturbing consciousness completely, and might substantially endanger both the safety and the life quality of the patients affected¹. The effective management of grand mal seizures remains serious since it decreases the risks of new fits taking place along with protective effects². An antiepileptic medication known as valproic acid (VA) shows excellent results in managing grand mal epilepsy as a first-choice treatment for controlling seizures³. However, therapeutic outcomes depend on genetic polymorphisms affecting the *CYP2C9* gene expression, as well as on clinical characteristics such as the patient age, gender, and body mass index (BMI)^{4,5}. The *CYP2C9* gene determines the concentration-to-dose (C-D) ratio of VA, yet the presence of the *CYP2C9*3* allele affects drug pharmacokinetic properties⁴.

Better treatment strategies require an understanding of the genetic and clinical factors that adjust VA blood concentrations. Studies have shown that people carrying the *CYP2C9*3* genotypes exhibit a lower drug metabolism, thereby causing raised blood VA levels⁶. Recent studies have shown that environmental factors and the general health well-being might also considerably influence VA safety and efficacy, signifying the importance of the adoption of a multifaceted therapeutic approach⁷.

This study examines how VA blood levels respond to the presence of *CYP2C9*3* pleiomorphisms, combined with different clinical features, among grand mal epileptic patients. These (combined) parameters may help identify how personalized dosing systems could improve therapeutic outcomes.

2. Methodology

The study included 28 patients with grand mal epilepsy that were diagnosed by specialist neurologists according to the Spanish Society of Neurology guidelines. The data collection timeline extended from September 2024 to December 2024, within

the consultation clinics of the Merjan Medical City in Babylon, Iraq. The Institutional Review Board (IRB: A0031/2024) at the College of Pharmacy of the University of Babylon has provided study approval; the study's protocol was also approved by local health authorities and each participant granted their informed consent before being involved in the study.

The current study uses a low participant count that stems from the low frequency of *CYP2C9* variations, combined with strict study selection criteria requirements. We intend to employ this preliminary study in order to develop a larger-scale future study. The study enrolled patients who had grand mal epilepsy diagnosis 6 months before the trial and required regular VA drug concentration assessments from their neurologists. The study excluded subjects who were receiving medications that could interfere with their VA treatment, had renal or hepatic dysfunction, were undergoing pregnancy, or had any medical condition that might influence the results.

The statistical analysis of the data was facilitated through the use of the SPSS v. 28 software. We performed descriptive statistical calculations on data from demographic and clinical variables by determining mean values, frequencies, standard deviations, and percentages. The independent *t*-test analysed the VA concentrations between subjects that expressed A/A genotypes and those with C/C genotypes. We utilized the Pearson correlation in order to analyse the relationship between the VA levels and age or BMI. The associations between the genotype groups and high VA levels were assessed through the Fisher's exact test. The chi-square test analysed whether patient genotypes differed according to their VA level classifications as high or low. Finally, a logistic regression model analysed the connection between genetic information and BMI values, and their impact on VA level elevation.

The serum levels of VA were measured *via* time-resolved immunofluorescence (Beijing Diagreat Biotechnology Co., Beijing, China). DNA fragments were extracted from venous samples and genetic studies of the *CYP2C9*3* polymorphism were completed through allele-specific PCR with real-time monitoring (SNP Biotechnology, Ankara, Turkey).

Table 1. Influence of CYP2C9*3 (rs1057910) polymorphisms and association of clinical variables on valproic acid (VA) levels among patients with grand mal epilepsy. The initial section reviews the demographic and clinical variables' statistics; the second part of the initial section uses analysis of variance (ANOVA) to compare VA levels across various genotypes. The second and third sections analyse correlations between VA levels and clinical factors for males and females, respectively. The fourth section presents the association of the genotypes with the VA levels, while the final section uses the predictive logistic regression analysis to present categorical genotypic associations with VA concentrations. Abbreviations used: BMI, body mass index; OR, odds ratio; *r*, correlation coefficient; SD, standard deviation.

Category	Variable	Mean	SD	p-value	r	OR
Descriptive statistics	age	13.2	15.2	-	-	-
	BMI	22.5	4.9	-	-	-
	male (%)	58.0	-	0.05	-	-
	female (%)	42.0				
Comparative ANOVA of VA levels (µg/mL) across the genotypes	wild genotype (A/A)	82.4	25.4	0.01	-	-
	heterogenous genotype (A/C)	93.3	32.3			
	mutant genotype (C/C)	165.0	20.5			
Correlation analysis for VA with age, BMI, and weight in males across the genotypes						
Wild genotype (A/A)	age	13.2	5.6	0.4	0.02	-
	BMI	22.5	4.3	0.5	0.01	-
	weight	53.4	11.2	0.3	0.04	-
Heterogenous genotype (A/C)	age	15.1	6.9	0.3	0.03	-
	BMI	23.6	5.0	0.4	0.02	-
	weight	57.2	12.0	0.2	0.05	-
Mutant genotype (C/C)	age	17.0	7.2	0.5	0.01	-
	BMI	24.7	5.4	0.6	0.008	-
	weight	61.5	12.8	0.4	0.02	-
Correlation analysis for VA with age, BMI, and weight in females across the genotypes						
Wild genotype (A/A)	age	12.7	5.4	0.3	0.03	-
	BMI	22.0	4.5	0.4	0.02	-
	weight	51.8	10.9	0.2	0.05	-
Heterozygous genotype (A/C)	age	14.8	6.6	0.2	0.04	-
	BMI	23.3	4.8	0.3	0.03	-
	weight	54.5	11.7	0.2	0.05	-
Mutant genotype (C/C)	age	16.2	7.3	0.4	0.02	-
	BMI	24.4	5.1	0.5	0.009	-
	weight	60.2	12.5	0.3	0.04	-
Association of the genotypes with the VA levels; i.e., high (>100 µg/mL) vs. low (<50 µg/mL)						
Fisher's exact test	wild genotype (A/A)	-	-	0.42	-	0.4
	heterogenous genotype (A/C)	-	-	0.13	-	4.0
	mutant genotype (C/C)	-	-	0.65	-	0.45
Predictive analytic association between genotypes and VA levels						
Logistic regression analysis	wild genotype (A/A)	-	-	0.43	0.12	0.9
	heterogenous genotype (A/C)	-	-	0.07	0.12	3.4
	mutant genotype (C/C)	-	-	0.32	0.12	1.5

3. Results and Discussion

The antiepileptic drug VA is widely used in treating patients with grand mal epilepsy³. The effectiveness and safety profile of VA strongly relates to CYP2C9 gene variations as well as three main clinical elements including age, gender, and BMI^{5,7}. This study of the VA levels in patients with grand mal epilepsy explored both *CYP2C9**3 polymorphisms at the 1075 (substitution of adenine to cytosine) A>C locus and clinical factors (Table 1).

The *CYP2C9* genetic factor plays a central role in producing VA; *CYP2C9**3 and other variants of this gene impact VA metabolism rates in patients. Our study has exposed substantial variations of VA based on genetic variations. Patients who carried the C/C mutant genotype demonstrated concentrated levels of VA compared to patients who possessed the wild-type (A/A) or the heterozygous (A/C) genotype. Studies have shown that people with the *CYP2C9**3 allele possess less enzyme activity, which causes elevated drug concentrations⁴.

The therapeutic levels of VA change with age, BMI, and gender^{3,5}. This study has revealed that clinical elements determine VA levels among distinct genetic groups. The C/C genotype displayed a powerful positive link between BMI and VA levels (Table 1). The levels of VA in participants with the C/C genotype increased linearly according to their age (Table 1). Older patients or those with higher BMIs should receive special attention when neurologists adjust their medications, as they might experience toxic effects according to earlier studies^{6,8}.

VA levels also depended on gender, and our study's population comprised a large number of males, which had a significant effect on VA concentrations by gender (Table 1). Although this was not fully elucidated by this study, there is an interaction between gender and genotype on VA levels. Previous research has shown that gender can affect the pharmacokinetics of some drugs on body composition and hormone bases, as females generally exhibit a higher C-D ratio than males⁵.

Associations between *CYP2C9* genotypes and high (>100 µg/mL) or low (<50 µg/mL) VA levels

were compared using the Fisher's exact test (Table 1). A small but consistent association was found with the A/C genotype (which was marginally higher in affected individuals), while no significant association was found for the A/A genotype. Moreover, there was a greater proportion of patients with high levels of VA among those with the C/C genotype, although this was not statistically significant (Table 1). Logistic regression analyses additionally corroborated these observations, identifying that patients with the A/C genotype were at increased odds of reaching higher VA levels than those bearing the A/A genotype.

Finally, patients bearing the C/C genotype may need lower VA doses in order to decrease toxicity, whereas having the A/A genotype may require larger doses in order to reach therapeutic thresholds. Likewise, monitoring individual age and/or BMI supports neurologists in prescribing precise doses more efficiently^{4,7,8}. Medical research must unite information from genetics with clinical findings in order to provide individualized treatment protocols in cases of epilepsy. Researchers should conduct bigger-scale trials that will assess the identified relationships and investigate additional genetic alterations related to VA pharmacokinetics. Pharmacogenomic guidelines for VA dose development would create substantial improvements in patient healthcare, by minimizing side effects and improving treatment outcomes⁹.

4. Conclusion

This study reveals that the *CYP2C9**3 pleomorphism can significantly impact VA concentrations in the serum of Iraqi patients with grand mal epilepsy. Subjects expressing the C/C genotype had elevated VA levels in their blood. Moreover, clinical variables such as the patient's BMI and age play a crucial role in defining VA levels, thereby emphasizing the necessity for dose modifications based on patient-specific features. These findings underscore the significance of embedding pharmacogenetic insights into epilepsy management, as a prerequisite for the improvement of therapeutic

efficacy and safety profiles.

None exist.

Acknowledgements

The authors would like to thank the patients for their participation in this study.

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Conflicts of interest

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HOW TO CITE:

Al-Hindy H.A.A.M., Mousa M.J., Hashim H.O., Hussien T.N., Mohammed M.A. Impact of CYP2C9*3 polymorphisms and clinical variables on the valproic acid levels of Iraqi patients suffering from grand mal epilepsy. *Pharmakeftiki* 37(2s), 57-61, 2025. <https://doi.org/10.60988/p.v37i2S.142>

Post-vaccination systemic adverse effects for three COVID-19 vaccines: a cross-sectional study in Iraq

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KEY WORDS:

**COVID-19; post-vaccination;
Pfizer-BioNTech; Sinopharm;
Oxford-AstraZeneca**

ARTICLE INFO:

Received: January 05, 2025

Revised: January 24, 2025

Accepted: February 07, 2025

Available online: October 10, 2025

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ABSTRACT

The general population can be enlightened and encouraged to get vaccinated by understanding the adverse effects of vaccination. The study aimed at assessing the risk factors and possible systematic side effects of the immunization against coronavirus disease 2019 (COVID-19) by the most used vaccines in Iraq, being the Oxford-AstraZeneca, the Pfizer-BioNTech, and the Sinopharm vaccines. In addition, the study aimed at comparing the side effects between the different vaccine groups. To this end, this cross-sectional retrospective survey-based study was done during August 2022, through a self-administered online-based multiple choice questionnaire that was distributed among the Iraqi population. In total, 215 participants answered the questionnaire from different regions of Iraq, with a response rate of 100%. Of these participants, 13.03% were men and 86.97% were women. A total of 148 (68.8%) of the participants had obvious adverse effects after vaccination, while 67 (31.2%) were asymptomatic. There was a statistically significant correlation ($p < 0.05$) between variables such as sex, age, vaccine dose, history of COVID-19 infection, and comorbidity with the herein recorded side effects. Localized injection pain, fever, fatigue, headache, localized swelling, and myalgia were the top side effects encountered by the present study, with a severity ranging from mild to moderate.

1. Introduction

Coronavirus disease 2019 (COVID-19) is brought on by SARS-CoV-2; a virus that causes a severe and acute respiratory illness. The first case of the illness was discovered in Wuhan (China) in December 2019, and it has since spread around the world¹. Vaccines aid in the prevention of illness from incurable conditions by stimulating the body's adaptive immunity and induce T-cell immunity in addition to neutralizing antibodies. Immunization or vaccination is the administration of certain antigens in order to activate the immune system against an infection². The majority of COVID-19 vaccines that have undergone and passed phase III trials have demonstrated efficiency in avoiding severe illness at about 90%. Ten vaccinations have currently been licensed; of these, the Pfizer-BioNTech, the Sinopharm, and the Oxford-AstraZeneca ones are the most widely used³. Most vaccine recipients experience immunological recognition of the vaccine antigens through the stimulation of local immune cells, the recruitment of circulating immune cells, and the induction of local inflammation by vasodilators and cytokines. For the induction of protective reactions without significant systemic consequences, a sufficient vaccination reactogenicity is required. Anti-SARS-CoV-2 antibodies bind to human antigens (like extractable nuclear antigens and myelin basic proteins) because the SARS-CoV-2 spike protein and some human proteins share antigenic similarities. Vasodilators and cytokines enter the bloodstream in the event of hyper-reactogenicity, triggering a state of systemic inflammation. Sometimes side effects are due to immune response after immunization⁴. Therefore, the evaluation of COVID-19 post-vaccination adverse effects evaluation is necessary in order to assess the different administered to the Iraqi population. Our study aimed at detecting the side effects occurring post-immunization, in addition to correlating the severity of these effects with study variables and the different vaccine types.

2. Methodology

This study is a cross-sectional retrospective survey-based study. All data were collected on August 8, 2022, among the Iraqi population. An online multiple-questionnaire was prepared, and the link was distributed randomly among different social media (such as Facebook, Telegram, and WhatsApp). The total number of participants was 215; they were all aged >18 years and have been administered different vaccine types.

The study was done after a detailed searching of the available literature in order to predict the most common systemic adverse effects after a COVID-19 vaccination. The questionnaire was distributed in the Arabic language to the participants. SPSS version 25.0 and Microsoft Excel were used for data entry and analysis. Descriptive statistics were used and statistical significance was determined using Fisher's exact test, with p -values <0.05 being considered as statistically significant. The study was conducted after obtaining approval from the Ethics Committee of the University of Kerbala College of Medicine (number: 1259; date: February 15, 2022), while written informed consent was obtained from the patients for their participation in the study.

3. Results

Of the 215 participants that have answered the questionnaire from different regions of Iraq with a response rate of 100%, 28 (13.03%) were men and 187 (86.97%) were women. The age of most of them was of a range of 20–29 years (71.8%). Kerbala recorded the highest participation rate (53.7%), followed by Baghdad (17.1%), Najaf (6.9%), and Babil (6%). The most administered vaccine was the Pfizer-BioNTech vaccine with 169 participants (78.2%) receiving it, of which 84.3% received two doses (Table 1). The Sinopharm vaccine was administered to 13.4% of the study's participants, while only 7.9% of the participants had received the Oxford-AstraZeneca vaccine (Table 1). The majority of the participants

Table 1. The frequency of systemic adverse effects associated with receiving three different COVID-19 vaccines.

Adverse effects reported	Pfizer-BioNTech vaccine recipients (N=169)	Sinopharm vaccine recipients (N=29)	Oxford-AstraZeneca vaccine recipients (N=17)	p-value
Fever	114 (53.0%)	9 (4.2%)	13 (6.0%)	<0.001
Chills	40 (18.6%)	3 (1.4%)	8 (3.7%)	0.129
Myalgia; joint pain	69 (32.1%)	1 (0.5%)	6 (2.8%)	0.001
Fatigue	117 (54.4%)	10 (4.7%)	13 (6.0%)	0.001
Headache	67 (31.2%)	4 (1.9%)	7 (3.3%)	0.025
Nausea; vomiting	15 (6.9%)	2 (0.9%)	1 (0.5%)	0.871
Diarrhoea	1 (0.5%)	3 (1.4%)	0	0.001
Anorexia	18 (8.4%)	1 (0.5%)	2 (0.9%)	0.463
Dizziness	29 (13.5%)	2 (0.9%)	4 (1.9%)	0.269
Abdominal pain	4 (1.9%)	1 (0.5%)	0	0.753
Chest pain	5 (2.3%)	1 (0.5%)	0	0.759
Cough; sneezing; other respiratory symptoms	18 (8.4%)	0	0	0.069
Sore throat	5 (2.3%)	2 (0.9%)	0	0.399
Lymph node enlargement	6 (2.8%)	0	0	0.432
Loss of smell or taste	4 (1.9%)	2 (0.9%)	1 (0.5%)	0.365
Increase or decrease of blood pressure	2 (0.9%)	0	0	0.760
Heart palpitations	9 (4.2%)	1 (0.5%)	2 (0.9%)	0.471
Loss of consciousness	2 (0.9%)	0	0	0.760
Rash in another site of the body	4 (1.9%)	0	0	0.574

(61.1%) had a history of COVID-19 infection. In the Iraqi recipients of the Pfizer-BioNTech vaccine, the most common systemic adverse effect was fatigue (corresponding to 54.4% of all participants), followed by fever (53.0%), myalgia (32.1%), headache (31.2%), chills (18.6%), dizziness (13.5%), anorexia (8.4%), respiratory symptoms such as cough (8.4%), nausea / vomiting (6.9%), heart palpitations (4.2%), lymph node enlargement (2.8%), chest pain (2.3%), and sore throat (2.3%). Table 1 provides details regarding the recorded adverse effects for the Sinopharm and the Oxford-AstraZeneca vaccines.

4. Discussion

In order to address the severe consequences of the COVID-19 pandemic on the global population, it is essential to implement safe and effective COVID-19

vaccinations and be prepared for potential side effects within our communities. Recent study outcomes reveal that side effects are a common occurrence with vaccination and can vary among the different vaccine types. A total of 148 (68.8%) out of the 215 participants of our study had obvious adverse effects after receiving a vaccine, while 67 (31.2%) were asymptomatic. Our results were in line with those of other studies; for example, a study conducted in Northern Iraq has identified fatigue, headache, myalgia, fever, and chills as the most common side effects⁵. A previous study in Wuhan has revealed that increased temperature, fatigue, headache, and myalgia have been reported post-vaccination at rates of 46%, 44%, 39%, and 17%, respectively⁶. The occurrence of injection site pain as a symptom has been reported in several studies⁷.

The findings of our study indicate that the Iraqi

individuals who have received the Pfizer-BioNTech vaccine were more susceptible to side effects (56.28%) when compared to the other two vaccines (7.44% for the Oxford-AstraZeneca vaccine and 5.12% for the Sinopharm one). In contrast, the frequency of side effects has been reported to be higher in the recipients of the Oxford-AstraZeneca vaccine than in those who have received the Pfizer-BioNTech or the Sinopharm vaccine⁸. Moreover, the Sinopharm vaccines recipients have reported a high rate of adverse reactions than the one recorded in our study⁹. Skin rash was rarely reported by our participants (1.9%) and, when it was, it was only associated with the Pfizer-BioNTech vaccine; this is also supported by another study in which the skin rash was reported by only 0.4% of the study's participants¹⁰.

5. Conclusion

Enhancing public trust in the vaccine and gaining a deeper insight into the potential side effects of the approved COVID-19 vaccines across different demographics are crucial goals. The occurrence of side effects following vaccination is not uncommon and serves as evidence of the immune system's response.

Our data support the safety and efficacy of the COVID-19 vaccines, and indicate that the most frequently reported adverse effects in the Iraqi population are fatigue, fever, headache, and myalgia.

Acknowledgements

We would like to thank the employees of the Department of Microbiology of the College of Medicine of the University of Kerbala for their help in sharing our e-questionnaire. We also greatly appreciate the help of the study's participants. Finally, we would like to thank the Department of Clinical and Laboratory Sciences of the College of Pharmacy of the University of Kerbala for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Ali M.M., Hussein R., Rayhaan S., Al-Hasnawi S.M.J. Post-vaccination systemic adverse effects for three COVID-19 vaccines: a cross-sectional study in Iraq. *Pharmakeftiki* 37(2s), 62-66, 2025.
<https://doi.org/10.60988/p.v37i2S.143>

Flavonoids from green tea reverse insulin resistance and inflammation: *in vitro* and *in vivo* evidence

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KEY WORDS:
flavonoids; molecular
mechanisms; diabetes
mellitus; phytochemicals;
green tea

ARTICLE INFO:

Received: January 09, 2025

Revised: March 03, 2025

Accepted: March 03, 2025

Available online: October 10, 2025

ABSTRACT

Flavonoids are a class of polyphenolic compounds that can be isolated from green tea (*Camellia sinensis*). This study has investigated the *in vitro* and *in vivo* effects of green tea flavonoids on insulin resistance and inflammation. Flavonoids were extracted from green tea using ethanol extraction followed by purification through the employment of HPLC. *In vitro* experiments were conducted on two cell lines in order to assess glucose uptake and inflammatory marker expression, while *in vivo* studies involved streptozotocin-induced diabetic mice treated with green tea-derived flavonoids (240 mg/kg) for 30 days, and with their blood glucose and glycated haemoglobin (HbA1c) levels being analysed. Our results revealed that the flavonoid treatment could significantly increase glucose uptake *in vitro* ($p < 0.05$), while diabetic mice exhibited lower fasting blood glucose ($p < 0.001$) and HbA1c ($p < 0.01$) levels compared to untreated controls. In conclusion, these findings suggest that flavonoids may serve as effective adjunct therapies for diabetes and inflammation.

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1. Introduction

Diabetes mellitus is a complex metabolic condition shaped through hereditary genetics, environmental influences, and lifestyle choices.

It is considered as a primary contributor to serious problems such as blindness, delayed wound healing, erectile dysfunction, kidney failure, and cardiovascular diseases, leading to extensive or-

gan damage and compromised cellular processes. It is a chronic condition marked by sustained hyperglycaemia, primarily resulting from defects in insulin secretion, insulin action, or a combination of both. Two primary forms for diabetes exist: type 1 (defined through the autoimmune destruction of pancreatic β -cells resulting to complete insulin deficiency that necessitates continuous exogenous insulin injection) and type 2 (which represents 90% of global cases of diabetes and is characterized by insulin resistance and relative insulin deficiency that needs to be controlled through lifestyle adjustment)¹.

Flavonoids, a variety of polyphenolic substances prevalent in plants, constitute an important class for phytochemicals with possible antidiabetic effects. These chemicals, comprising flavones, flavonols, anthocyanins, and isoflavones, demonstrate multifaceted functions that affect inflammation and metabolic signalling pathways^{2,3}. Flavonoids serve like powerful antioxidants and have been shown to exert anti-inflammatory, anticancer, antiviral, and cardioprotective effects⁴. Additionally, they are essential for the regulation of glucose and lipid metabolism, mitigating dyslipidaemia and insulin resistance, and altering stress-responsive signalling pathways⁵. Flavonoids also possess considerable potential as nutraceuticals and have been shown to exert their anti-diabetic effects through many pathways, such as the modulation for glucose metabolism, the attenuation for oxidative stress, and the enhancement of insulin sensitivity²⁻⁵.

The aim of this study was to investigate the *in vitro* and *in vivo* effects of green tea flavonoids on insulin resistance and inflammation.

2. Methodology

2.1. Flavonoid extraction and purification

Flavonoids were extracted from green tea (*Camellia sinensis*) using ethanol-water solvent extraction at high temperature (100°C) under controlled laboratory conditions. The extract was filtered, concentrated at 100°C, and lyophilized. HPLC was used in order

to purify the extracted flavonoids, ensuring a purity level above 95%.

2.2. *In vitro* experiments

To evaluate the effect of flavonoids on glucose metabolism, two cell lines were used: the INS-1E cell line (which is a rat insulinoma cell line that maintains notable differentiation to a stable β -cell phenotype achieved by selection for high insulin secretion) and the C2C12 cell line (which is an immortalized mouse myoblast cell line). Cells were treated with flavonoids at concentrations of 10, 50, and 100 μ M, and glucose uptake was measured using a glucose uptake assay method. The levels of inflammatory cytokines such as interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF- α) were measured by ELISA. Mechanistic analyses involving Western blotting were used in order to assess the AMP-activated protein kinase (AMPK) activation, the glucose transporter type 4 (GLUT4) translocation, and the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling.

2.3. *In vivo* experiments

The *in vivo* experiments of our study were approved by Kufa University (protocol number: 116; date: 20-Mar-2023). In brief, male BALB/c mice (weighting 30 g) were obtained from the market and were housed at suitable conditions (temperature: 25°C; 12:12 h light : dark cycle; free food and water access). For the induction of diabetes, mice were injected intraperitoneally with streptozotocin (at 240mg/kg). Mice were then randomly allocated into two groups (n=5 per group): (i) the control group (untreated diabetic mice) and (ii) the flavonoid-treated group (receiving flavonoids intraperitoneally at 240 mg/kg/day, for 30 days). Blood samples were collected through the heart on day 30 and the therapeutic effects of the flavonoids were evaluated through the measurement of fasting blood glucose, glycated haemoglobin (HbA1c), and low-density lipoprotein (LDL) cholesterol.

Table 1. The effect of a 30-day administration of green tea-derived flavonoids (240 mg/kg/day) on the levels of fasting blood sugar, glycated haemoglobin (HbA1c), and low-density lipoprotein (LDL) cholesterol of streptozotocin-induced diabetic mice.

Parameter	Control group (mean $\pm$ SD)	Flavonoid-treated group (mean $\pm$ SD)	p-value
Fasting blood glucose (mg/dL)	220 $\pm$ 15	135 $\pm$ 10	<0.001
HbA1c (%)	8.5 $\pm$ 0.5	6.2 $\pm$ 0.4	<0.01
LDL cholesterol (mg/dL)	130 $\pm$ 8	90 $\pm$ 6	<0.05

2.4. Statistical analysis

The statistical analysis was performed by using the SPSS software (version 25.0). One-way ANOVA and Tukey's *post hoc* test were used in order to evaluate the effects of the flavonoid treatments; differences with a *p*-value below 0.05 were deemed as statistically significant.

3. Results and Discussion

Green tea-derived flavonoid administration markedly decreased fasting blood glucose ($p < 0.001$) and HbA1c ($p < 0.01$) levels in diabetic mice as compared to those of the control group (Table 1). On the other hand, the exposure to green tea-derived flavonoids (15 μ M; 24 h) increased insulin secretion, insulin mRNA levels, and GLUT2 expression in INS-1E cells. An activation of the insulin signalling pathway was also observed in these cells, including an increased phosphorylation of IRS-1, PI3Kp85, and AKT, as well as an increased association between PI3K and IRS-1 levels. In the C2C12 cells, the exposure to green tea-derived flavonoids (40 μ M; 1 h) led to increased glucose uptake and GLUT4 translocation. Moreover, a significant phosphorylation of AMPK α and of ACC was observed, indicating an AMPK pathway activation. Co-treatment with insulin further enhanced p-AMPK α , p-ACC, and p-Akt protein levels.

Our findings indicate, that green tea-derived flavonoids can reduce blood glucose levels *in vivo*, and exert insulin-sensitizing activities *in vitro*, thereby qualifying them as a potential alternative or adjunct

option to traditional antidiabetic treatments⁶. The treatment with flavonoids resulted in a significant decrease in the fasting blood glucose levels of diabetic mice. This discovery confirms the capacity of flavonoids to stimulate essential metabolic pathways, including the promotion of GLUT4 translocation and the AMPK activation through substances such as epicatechin, consequently enhancing glucose uptake within skeletal muscles. The latter is known to enhance lipid metabolism and glucose absorption, directly targeting insulin resistance; a characteristic of type 2 diabetes. Catechins and epigallocatechin gallate (EGCG) have been found to augment glucose transport independently of insulin, while the inhibition of the vascular endothelial growth factor by flavonoids such as EGCG has exhibited potential in mitigating problems such like diabetic retinopathy through the obstruction of pathological neovascularization⁷.

The flavonoids' capacity to block enzymes such like α -glucosidase and α -amylase diminishes glucose absorption from the gastrointestinal tract, consequently effectively thought to regulate postprandial hyperglycaemia. In fact, flavonoids have shown similar efficiency to current antidiabetic therapies in regulating critical indicators, including fasting blood glucose levels, HbA1c levels, and lipid profiles. Quercetin and rutin have been shown to safeguard against diabetic nephropathy through the diminishing of advanced glycation end products and the enhancement of renal function indicators⁸. Moreover, the natural provenance for flavonoids and their negligible adverse effects qualify them as

a safer option to synthetic pharmaceuticals. However, the bioavailability of flavonoids presents a difficulty that necessitates the development of improved formulations⁹.

Notwithstanding the encouraging findings, this study possesses specific limitations. The bioavailability of flavonoids significantly varies based on their chemical structure and the host organism's metabolic processes. Future research should concentrate on optimizing flavonoid delivery modalities, including the use of nano-formulations or their co-administration alongside bioenhancers. Furthermore, one should note that although our *in vivo* and *in vitro* results turn out to be suggestive of the anti-diabetic potential of green tea-derived flavonoids, clinical trials are crucial in proving that these findings could be safe and useful for humans. Research must investigate the long-term effects of flavonoid supplementation and its combination with other drugs in order to guarantee safety and efficacy¹⁰.

4. Conclusion

Our findings reveal that the flavonoid treatment could significantly increase glucose uptake *in vitro*, while diabetic mice exhibited lower fasting blood

glucose and HbA1c levels compared to untreated controls. Our findings also indicate that green tea-derived flavonoids possess considerable potential as therapeutic agents for the management of diabetes and its complications. Flavonoids can improve glycaemic control through the targeting of critical associated molecular pathways, including the AMPK activation and the GLUT4 translocation; molecular steps that are pivotal for the pathogenesis of diabetes mellitus.

Acknowledgements

We are grateful to the College of Pharmacy of the University of Babylon for providing services and facilities for the undertaking of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Ali N.H., Abbas N.F., Abbas Z.M. Flavonoids from green tea reverse insulin resistance and inflammation: *in vitro* and *in vivo* evidence. *Pharmakeftiki* 37(2s), 67-71, 2025. <https://doi.org/10.60988/p.v37i2S.144>

Design, molecular docking, and DFT analysis of dioxoisindoline derivatives as potential anticonvulsant agents targeting epilepsy-associated proteins

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KEY WORDS:

dioxoisindoline derivatives; anticonvulsants; theoretical calculations; molecular docking; DFT study

ARTICLE INFO:

Received: October 14, 2024

Revised: November 01, 2024

Accepted: November 09, 2024

Available online: October 10, 2025

ABSTRACT

This study attempts to address the necessity of developing substitute therapies for antiepileptic medications. It suggests utilizing molecular docking and dioxoisindoline derivatives in a theoretical chemical investigation to identify possible substitute medications that could be used in the treatment of epilepsy. In this study five compounds show different activities against particular proteins related to epilepsy treatment; especially compound R3 shows high negative values when it is associated with the three studied proteins. A density functional theory (DFT) approach was employed in order to identify the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) for the five herein studied compounds; it was also used in order to calculate the gap between HOMO and LUMO, the ionization potential, the electron affinity, the electronegativity, as well as the softness and hardness of the molecules, among other chemical characteristics. Overall, this study highlights the need of developing efficient epilepsy treatments and provides a preliminary analysis of possible drugs.

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1. Introduction

Epilepsy is a neurological disease characterized by rapid, irregular, or excessive neuronal excitation in

the brain's grey matter. It is characterized by recurring episodes of uncontrollable movements, which can affect one or more parts of the body. In different areas of the brain,

a group of cells can be exposed to severe electrical discharge during epileptic seizures. These seizures may be mild (in the form of muscle spasms and loss of concentration) or severe (including spasms for long periods). The occurrence of these seizures ranges from once a year to many times per day¹. Treatment is carried out after the accurate diagnosis of the condition. Medications with regular follow-up can be used in order to control epilepsy in most of the cases; the surgical procedure is the last option allowing us to control seizures that cannot be treated with medications². Several methods of examination techniques are used in order to diagnose epileptic seizures, such as blood tests, computed tomography, clinical examination, and magnetic resonance imaging³. In the majority of the cases, these tests are not crucial for the diagnosis of epilepsy, but they reveal the key causes of these seizures and they define the most effective treatment approach for them⁴.

As far as the epilepsy treatment is concerned, the classification of epilepsy is one of the most important steps when assessing the patient's condition. It has an impact on every clinical consultation, but it also has a significant impact on basic and clinical epilepsy research as well as the development of new treatments. A framework for comprehending the type of seizures the patient has, the additional seizure types that are more likely to develop in that person, the potential triggers for their seizures as well as, frequently, their prognosis, are all defined by classification. In addition, classification provides information on the risks associated with comorbidities such as learning disabilities, intellectual disabilities, mental health conditions (such as the autism spectrum disorder), and mortality risks (such as sudden unexpected death in epilepsy). For the development of effective compounds in the treatment of epileptic seizures, many studies have employed quantitative structure-activity relationship (QSAR and 3D-QSAR) methodologies that have contributed to the anticipating that the activities of the new components would have fewer side effects⁵. Five dioxoisindoline derivatives were designed and assessed in this study as possible substitute medications for epilepsy. The purpose of the study was to ascertain wheth-

er these derivatives have a high binding affinity (ΔG) for particular brain proteins related to epilepsy. We should note that this study is a component of a larger research effort to synthesize new dioxoisindolines that may have anticonvulsant properties.

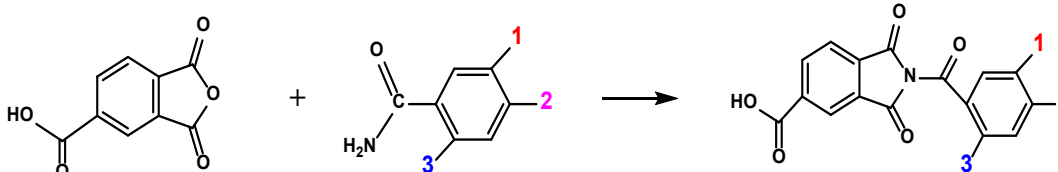
2. Methodology

CB-Dock was used in order to predict the possible molecular interactions between the target proteins and the small chemicals. We have, herein, docked five dioxoisindoline compounds (R1–R5) against the protein active sites of the proteins 10HV (4-aminobutyrate-aminotransferase; pig), 3F8E (coumarins as suicide carbonic anhydrase inhibitors), and 6KZP (calcium channel ligand), and have obtained the crystal structures of the receptor molecules from the Protein Data Bank. The chemical structures were built with the proper 2D-orientation in ChemOffice (ChemDraw version 20.0). For every structure, MM2 energy minimization was carried out in order to determine the potential energy surface, taking into account elements like thermal and steric energy. The models' final conformations were identified⁶. Moreover, by utilizing the Gaussian program and the density functional theory (DFT), the three most prevalent interactions (between the assessed proteins and the assessed compounds) with residue involvement were found to be the chelation bonding, the H-bonding, and the pi-pi stacking. The five compounds' efficacies were also determined (Table 1).

3. Results and Discussion

Molecular docking is a theory of molecular modeling that explains the fit between two or more proteins and ligands, which is dictated by " ΔG ". A better match between the chemical molecule and the protein is indicated by a larger negative ΔG value⁷. This study has used quantum mechanical computations and molecular level theory to examine how different substances interact with anticonvulsant-active proteins (Table 1). When comparing the obtained results with those of the antiepileptic medication phenytoin ($\Delta G = -8.3$ kJ/mol), it is evident that the five assessed

Table 1. Binding affinity (ΔG) and 1OHV, 3F8E, and 6KZP protein residues surrounding the assessed compounds. Amino-acid abbreviations used: ALA, alanine; ARG, arginine; ASN, asparagine; ASP, aspartic acid; CYS, cysteine; GLU, glutamic acid; GLN, glutamine; GLY, glycine; HG, mercury (Hg) atom; HIE, histidine with hydrogen on the epsilon nitrogen; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine.

				
Compounds		1	2	3
R1		CHOH	H	H
R2		H	H	OH
R3		CF ₃	H	H
R4		H	CN	H
R5		H	C ₄ H ₉	H
Compounds	ΔG (kJ/mol)	1OHV protein residues surrounding the compounds		Residues with interferences
R1	-8.7	ARG _{192'} , GLY _{191'} , GLY _{132'} , HIS _{190'} , PHE _{189'} , GLU _{265'} , GLU _{270'} , ASN _{140'} , SER _{137'} , SER _{328'} , SER _{269'} , ILE _{72'} , CYS _{135'} , LYS _{329'} , ASP _{298'} , VAL _{300'} , GLN _{301'} , THR _{302'}		PHE ₁₈₉ (pi-pi stacking); GLY _{136'} , GLU ₂₆₅ (H-bonding)
R2	-9	CYS _{135'} , GLY _{136'} , GLY _{191'} , SER _{137'} , SER _{269'} , SER _{328'} , ASN _{140'} , GLU _{265'} , GLU _{270'} , PHE _{189'} , HIS _{190'} , ARG _{192'} , ASB _{298'} , VAL _{300'} , GLN _{301'}		PHE ₁₈₉ (pi-pi stacking); GLY _{136'} , ASP ₂₉₈ (H-bonding)
R3	-9.3	ARG _{192'} , GLY _{191'} , GLY _{136'} , HIS _{190'} , PHE _{189'} , ASN _{140'} , SER _{137'} , SER _{328'} , SER _{74'} , CYS _{135'} , LYS _{329'} , ILE _{72'} , GLN _{71'} , VAL _{300'}		HIS _{190'} , GLY _{136'} , SER ₃₂₈ (H-bonding)
R4	-8.9	ARG _{192'} , GLY _{191'} , GLY _{136'} , HIS _{190'} , PHE _{189'} , GLN _{301'} , VAL _{300'} , ASP _{298'} , ASN _{140'} , SER _{137'} , SER _{269'} , SER _{328'} , CYS _{135'} , Glu _{270'} , Glu _{265'}		PHE ₁₈₉ (pi-pi stacking); GLY _{136'} (H-bonding)
R5	-8.9	CYS _{135'} , GLY _{136'} , GLY _{191'} , SER _{137'} , SER _{269'} , SER _{328'} , ASN _{140'} , GLN _{301'} , GLU _{270'} , GLU _{265'} , VAL _{300'} , ASP _{298'} , ARG _{192'} , ARG _{445'} , HIS _{190'} , PHE _{189'} , LYS _{329'} , MET _{332'} , THR _{333'}		PHE ₁₈₉ (pi-pi stacking); SER _{137'} , LYS _{329'} , MET ₃₃₂ (H-bonding)
Compounds	ΔG (kJ/mol)	3F8E protein residues surrounding the compounds		Residues with interferences
R1	-7.8	TRP _{5'} , HIE _{6'} , HIE _{64'} , HG _{264'} , LYS _{170'} , ASN _{62'} , ASN _{67'} , ALA _{65'} , GLN _{92'} , HIS _{94'} , THR _{200'} , PRO _{201'} , PRO _{202'}		TRP ₅ (pi-pi stacking); H ₂ O (H-bonding)
R2	-7.4	PRO _{201'} , PRO _{202'} , TRP _{5'} , ASN _{62'} , ASN _{67'} , HIE _{64'} , ILE _{91'} , GLN _{92'} , VAL _{121'} , PHE _{131'}		PRO _{201'} , HIE _{64'} , GLN _{92'} , H ₂ O (H-bonding)
R3	-8	PRO _{202'} , PRO _{201'} , THR _{200'} , TRP _{5'} , ASN _{62'} , ASN _{67'} , HIE _{64'} , GLU _{69'} , PHE _{131'} , ILE _{91'} , GLN _{92'} , VAL _{121'}		PRO _{201'} , HIE ₆₄ (H-bonding)
R4	-7.3	GLU _{69'} , ASN _{67'} , ASN _{62'} , HIE _{64'} , LEU _{60'} , ARG _{58'} , GLN _{92'} , ILE _{91'} , THR _{200'} , PRO _{201'} , TRP _{5'}		GLN _{92'} , GLU _{69'} , H ₂ O (H-bonding)
R5	-6.7	TRP _{5'} , PRO _{201'} , THR _{200'} , ASN _{62'} , ASN _{67'} , HIE _{64'} , GLU _{69'} , PHE _{70'} , PHE _{131'} , ASP _{71'} , ASP _{72'} , GLN _{92'} , ILE _{91'} , LEU _{57'}		HIE _{64'} , TRP ₅ (H-bonding)
Compounds	ΔG (kJ/mol)	6KZP protein residues surrounding the compounds		Residues with interferences
R1	-8	LEU _{391'} , LEU _{1819'} , LEU _{1506'} , LEU _{353'} , ASN _{388'} , ILE _{387'} , ILE _{351'} , PHE _{384'} , PHE _{956'} , PHE _{1509'} , SER _{383'} , THR _{352'} , GLN _{922'} , TYR _{953'} , VAL _{1505'} , VAL _{1823'} , VAL _{1820'}		ASN _{388'} , GLN ₉₂₂ (H-bonding)
R2	-8	GLN _{922'} , THR _{921'} , LEU _{920'} , LEU _{1499'} , LEU _{872'} , LEU _{955'} , PHE _{917'} , LEU _{868'} , PHE _{956'} , ASN _{952'} , GLY _{951'} , LYS _{1462'}		PHE ₉₅₆ (pi-pi stacking); ASN _{952'} , LYS ₁₄₆₂ (H-bonding)
R3	-9	PHE _{956'} , PHE _{868'} , LEU _{955'} , LEU _{872'} , LEU _{920'} , TYR _{953'} , ASN _{952'} , GLY _{951'} , ILE _{387'} , ILE _{876'} , LYS _{1462'} , GLN _{922'} , THR _{921'} , ALA _{1502'}		PHE ₉₅₆ (pi-pi stacking); ASN _{952'} , LYS ₁₄₆₂ (H-bonding)
R4	-8.2	ASN _{952'} , ASN _{957'} , TYR _{953'} , PHE _{956'} , PHE _{868'} , PHE _{917'} , LEU _{959'} , LEU _{920'} , LEU _{872'} , LEU _{1499'} , THR _{921'} , LYS _{1462'} , ILE _{876'} , ALA _{1502'}		ASN _{952'} , LYS ₁₄₆₂ (H-bonding)
R5	-8.4	GLN _{922'} , THR _{921'} , LEU _{920'} , LEU _{1499'} , LEU _{872'} , LEU _{959'} , PHE _{917'} , PHE _{956'} , PHE _{868'} , PHE _{1503'} , ALA _{1505'} , LYS _{1462'} , VAL _{865'} , CYS _{869'} , ILE _{876'} , ASN _{952'} , GLY _{951'}		GLN _{922'} , ASN ₉₅₂ (H-bonding)

compounds interact with the protein 1OHV and obtain a larger negative ΔG value; this means that they are more effective than phenytoin and, particularly, this is the case for compound R3 (which has a value of $\Delta G = -9.3$ kJ/mol). Compounds R1 and R3 have exhibited the highest affinity for the protein 3F8E, with a ΔG value of -7.8 and -8 kJ/mol, respectively; these are both higher than the ΔG value of the drug phenytoin with the protein, which is equal to -7.4 kJ/mol. Compound R2 has exhibited the same value ΔG as the drug, while compounds R4 and R5 have scored a lower value than the drug. The binding with the final protein 6KZP has shown that compound R3 has a ΔG value similar to that of the drug (equal to -9 kJ/mol), while the other compounds have a lower ΔG value. Compounds that exhibited more negative values than the drug have the potential to be effective antiepileptic medications. Based on our results, it is clear that compound R3 has the highest negative values for ΔG when interacting with the three proteins, thereby indicating that it is more active and could be used as an anticonvulsant drug. In DFT, an atomistic simulation has computed a range of important features, such as the highest occupied molecular orbital (HOMO) and the lowest lying unoccupied molecular orbital (LUMO). The HOMO values of all compound ranged from -0.268 to -0.232 eV, while the LUMO values were between -0.125 and -0.110 eV; therefore their gap ranged from 0.121 to 0.146 eV. These features were employed in equations in an attempt to identify many molecular properties such as the ionization potential (I) and the electron affinity (EA).

The values of the studied compounds ranged from 0.146 to 0.121 for I, from 0.110 to 0.125 for EA, from 0.171 to 0.196 for electronegativity, from 13.612 to 16.467 for softness, and from 0.060 to 0.073 for hardness⁸.

4. Conclusion

Our results reveal that most of the assessed compounds (being dioxoisindoline derivatives) have the potential to display anticonvulsant activity, with the most promising finding resulting from the association of 1OHV with compound R3 ($\Delta G = -9.3$ kJ/mol). In addition, this same compound has also exhibited a high ΔG value with the other two proteins: -8 kJ/mol with 3F8E and -9 kJ/mol with 6KZP.

Acknowledgements

The authors express their deep appreciation to the University of Anbar for providing the necessary support and resources, and which significantly contributed to the completion of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Ali R.S., Ezzat M.O. Design, molecular docking, and DFT analysis of dioxoisoindoline derivatives as potential anticonvulsant agents targeting epilepsy-associated proteins. *Pharmakeftiki* 37(2s), 72-76, 2025. <https://doi.org/10.60988/p.v37i2S.145>

Molecular docking of 1,3,4-oxadiazoles: a step toward novel tuberculosis therapies

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KEY WORDS:
tuberculosis; antitubercular
agents; protein-
tyrosine-phosphatase;
1,3,4-oxadiazole; molecular
docking

ARTICLE INFO:

Received: January 02, 2025

Revised: n/a

Accepted: January 17, 2025

Available online: October 10, 2025

ABSTRACT

Tuberculosis remains a major global health challenge, especially with the rise of multidrug-resistant strains that demand new treatment strategies. One promising target is the *Mycobacterium* protein-tyrosine-phosphatase B (MptpB), which helps *Mycobacterium tuberculosis* evade the immune system. This study explores the potential of 1,3,4-oxadiazole derivatives as inhibitors of MptpB. Using advanced molecular docking techniques, four derivatives of 5-(thiophen-2-yl)-1,3,4-oxadiazole-2-amine were designed and evaluated. These compounds were assessed for their ability to bind to the active site of MptpB. Among them, two showed significant potential, with compound #1 achieving a docking score of -8.8 kcal/mol; the highest in the study. Detailed interactions, including multiple hydrogen bonds and hydrophobic contacts, suggest strong binding affinity and stability within the active site. The findings highlight these compounds as promising candidates for new tuberculosis treatments, addressing the urgent need for innovative approaches to combat resistant strains. Further experimental validation and biological studies are necessary in order to translate these computational insights into practical therapeutic applications.

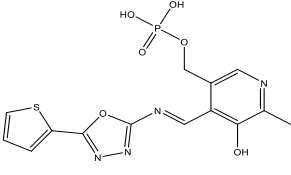
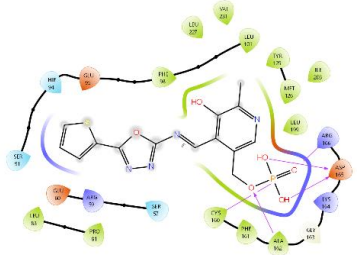
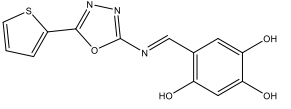
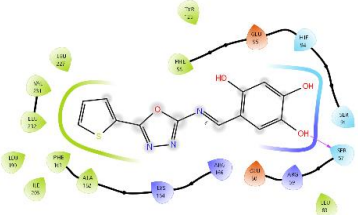
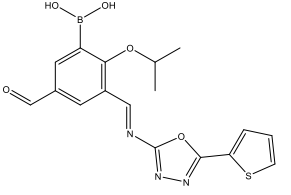
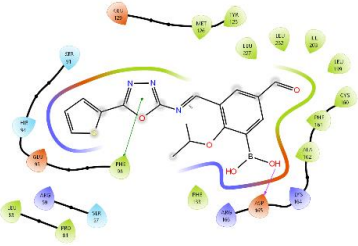
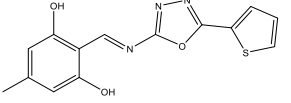
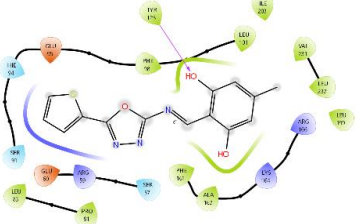
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1. Introduction

Mycobacterium tuberculosis is estimated to infect a quarter of the global population. The life cycle of *M. tuberculosis* relies on its capacity to engage with the immune

system in various manners: it circumvents the innate immune response, endures the adaptive immune response without inducing symptomatic disease, and provokes a vigorous inflammatory response¹. Comprehending the

Compound	Chemical structure of the compound	Docking score (kcal/mol)	Binding interactions	Two-dimensional structure within the binding active site
#1		-8.8	five H-bonds with CYS ₁₆₀ , ALA ₁₆₂ , ASP ₁₆₅ (2x), and ARG ₁₆₆ ; hydrophobic interactions with SER ₅₇ , ARG ₅₉ , GLU ₆₀ , SER ₉₁ , HIE ₉₄ , GLU ₉₅ , PHE ₉₈ , LEU ₁₀₁ , TYR ₁₂₅ , MET ₁₂₆ , CYS ₁₆₀ , PHE ₁₆₁ , ALA ₁₆₂ , GLY ₁₆₃ , LYS ₁₆₄ , ASP ₁₆₅ , ARG ₁₆₆ , and LEU ₁₉₉	
#2		-8.584	single H-bond with SER ₅₇ ; hydrophobic interactions with SER ₅₇ , ARG ₅₉ , GLU ₆₀ , SER ₉₁ , HIE ₉₄ , GLU ₉₅ , PHE ₉₈ , PHE ₁₆₁ , ALA ₁₆₂ , LYS ₁₆₄ , ARG ₁₆₆ , LEU ₂₂₇ , VAL ₂₃₁ , and LEU ₂₃₂	
#3		-8.265	single H-bond with ASP ₁₆₅ ; pi-pi interaction with PHE ₉₈ ; hydrophobic interactions with SER ₉₁ , HIE ₉₄ , GLU ₉₅ , PHE ₉₈ , PHE ₁₃₃ , CYS ₁₆₀ , PHE ₁₆₁ , ALA ₁₆₂ , LYS ₁₆₄ , ASP ₁₆₅ , ARG ₁₆₆ , LEU ₁₉₉ , ILE ₂₀₃ , LEU ₂₂₇ , and LEU ₂₃₂	
#4		-8.183	single H-bond with TYR ₁₂₅ ; hydrophobic interactions with SER ₅₇ , ARG ₅₉ , GLU ₆₀ , SER ₉₁ , HIE ₉₄ , GLU ₉₅ , PHE ₉₈ , LEU ₁₀₁ , PHE ₁₆₁ , ALA ₁₆₂ , LYS ₁₆₄ , ARG ₁₆₆ , VAL ₂₃₁ , and LEU ₂₃₂	

mechanisms by which *M. tuberculosis* regulates its life cycle is essential for the development of preventive and innovative therapeutic treatments.

The emergence of extensively multidrug-resistant tuberculosis necessitates the creation of drugs with innovative modes of action. Alternative therapeutic strategies, including antivirulence approaches, are increasingly attracting the attention of researchers aiming to develop more effective treatments for tuberculosis and to address antibi-

otic resistance².

Protein-tyrosine-phosphatases (PTPs) dephosphorylate substrate proteins by removing phosphoryl groups. PTPs can modulate various physiological activities in conjunction with protein tyrosine kinases, including cell growth, differentiation, proliferation, and immune responses³. Pathogenic organisms use PTPs in order to avoid host immune clearance; a fact emphasizing their importance in human diseases. Interestingly, *M. tuberculo-*

sis releases two protein-tyrosine-phosphatases, MtpA and MtpB, into the macrophages of the host; these enzymes affect host signalling directly, thereby allowing *M. tuberculosis* to survive in the macrophages⁴. Thus, MtpA and MtpB are interesting antitubercular drug targets.

Macrophages are the first immune defence against tuberculosis. Macrophages encapsulate bacteria or foreign particles into phagosomes, which engage with the endocytic pathway and modify membranes to recruit V-ATPase and hydrolases⁵. V-ATPase (located on the phagosomal membrane) develops an acidic environment with a pH of 4.5–5.0, indicating maturation. The invader is then destroyed by acid-activated hydrolases. MtpB inhibits phagosomal acidification and macrophage phagocytosis⁶.

The 1,3,4-oxadiazole is a five-membered heterocycle and an excellent bioisostere of amide and ester functional groups, contributing significantly to essential physicochemical properties and facilitating bond dynamics through participation in hydrogen bonding interactions with various receptors. Moreover, the 1,3,4-oxadiazoles are heterocyclic molecules including nitrogen and oxygen, recognized for their beneficial effects and regarded as important pharmacophores in drug development. The 1,3,4-oxadiazole compounds show a wide array of activities, including cytotoxic effects such as antibacterial, anticonvulsant, antiepileptic, antiallergic, anticancer, antitubercular, and insecticidal activity⁷.

2. Methodology

The structures of four 5-(thiophen-2-yl)-1,3,4-oxadiazole-2-amine derivatives were developed following a comprehensive study conducted by our team. The selection of 2oz5 from the deposited crystallographic structures of MtpB in the Protein Data Bank was based on its crystallization with an inhibitor (7XY); the latter exhibits well-defined binding interactions with the MtpB protein⁸.

The 3D similar conformations of four deriv-

atives were generated using ChemDraw 16.0 (ChemOffice, 2016). The SDF format was used in order to save these compounds' lowest energy conformation. With 200 optimizations and 1,500 interactions, the Spartan 14.0 and the Monte-Carlo approach aided the optimization process. Glide (Maestro software 11.4), a component of the Schrödinger software, was used for molecular drug design and docking evaluation on a Windows 7 workstation with an Intel Core i7 CPU 895 3.4 GHz, 32 GB RAM, and 1 TB hard drive. The crystal structure of MtpB had a 2.0-Å resolution. This protein was minimized and optimized using Pro-Prep. LigPrep was used in order to prepare the ligand structures before docking, which involved adding hydrogens and determining the orientation and ionization state so as to obtain the lowest energy conformation of all derivatives utilizing the OPLS force field. With a partial atomic value of 0.27, the grid box was calibrated to 1.20 Å. This arrangement allowed for each component of the studied compounds to rotate freely, identifying optimal conformations for binding free energy⁹.

3. Results and Discussion

This study considered binding affinity approaches and theoretical design in order to identify pioneer molecules as inhibitors with enhanced binding at the active site of the MtpB protein. The newly developed ligands are Schiff bases derived from a 1,3,4-oxadiazole core, demonstrating excellent affinity for binding the MtpB active site (Table 1). The linkage of various aldehydes to the core moiety 5-(thiophen-2-yl)-1,3,4-oxadiazole-2-amine leads to the development of multiple ligands exhibiting different docking scores within the MtpB protein 2oz5. In virtual screening, computational techniques identify specific molecules for the assessment of their binding ability to the target receptor.

The screening results for the binding of these molecules ranged from -8.8 to -8.183 kcal/mol for the 2oz5 protein (Table 1). In comparison, the binding affinity of the reference ligand 7XY, which

has been crystallized with the receptor protein, was -8.443 kcal/mol.

Within the active site of the target protein, compound #1 engages with and binds to various amino acids; the interactions primarily consist of hydrogen bonds and hydrophobic interactions, specifically totalling five hydrogen bonds (two between ASP₁₆₅ with two hydroxyls of the phosphate group, ALA₁₆₂ with an oxygen atom, ARG₁₆₆ with another oxygen atom, and CYS₁₆₀ with the phosphate group) alongside the hydrophobic interactions that transpire with adjacent amino acids (SER₅₇, ARG₅₉, GLU₆₀, SER₉₁, HIE₉₄, GLU₉₅, PHE₉₈, LEU₁₀₁, TYR₁₂₅, MET₁₂₆, CYS₁₆₀, PHE₁₆₁, ALA₁₆₂, GLY₁₆₃, LYS₁₆₄, ASP₁₆₅, ARG₁₆₆, and LEU₁₉₉). Compound #2, which has the second highest docking score, exhibits the following interactions at the MptpB active site: one hydrogen bond between SER₅₇ and a hydroxyl group, along with hydrophobic interactions with the surrounding amino acids (SER₅₇, ARG₅₉, GLU₆₀, SER₉₁, HIE₉₄, GLU₉₅, PHE₉₈, PHE₁₆₁, ALA₁₆₂, LYS₁₆₄, ARG₁₆₆, LEU₂₂₇, VAL₂₃₁, and LEU₂₃₂).

4. Conclusion

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Our molecular docking study has identified several 1,3,4-oxadiazole derivatives as promising inhibitors for the MptpB protein of *M. tuberculosis*. Compounds #1 and #2 have shown strong binding affinities and significant interactions with critical active site residues, indicating their potential as lead compounds. These findings pave the way for the undertaking of further biological evaluations in order to validate their efficacy and contribute to the development of innovative antitubercular agents.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Ali Y., Abd Razik B., Obaid A. Molecular docking of 1,3,4-oxadiazoles: a step toward novel tuberculosis therapies. *Pharmakeftiki* 37(2s), 77-81, 2025. <https://doi.org/10.60988/p.v37i2S.146>

Antibiotic susceptibility patterns of pathogenic bacteria isolated from the operating room of the Al Sader Teaching Hospital (Iraq)

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KEY WORDS:

operating rooms;
contamination; nosocomial
infections; antibiotic
resistance; susceptibility

ARTICLE INFO:

Received: January 09, 2025

Revised: February 14, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

ABSTRACT

In hospital settings, microbial contamination plays a major role in the transmission of healthcare-associated illnesses. This study aimed at evaluating the level of bacterial contamination in operating rooms. A cross-sectional study was carried out in the operating theatres and delivery rooms of Al Sader Teaching Hospital in Iraq, between July and November 2023. Samples were collected and sent to the laboratory for standard diagnostic analysis and the undertaking of antimicrobial sensitivity testing. The analysis revealed varying contamination rates across different operating rooms. The most frequently detected bacterial isolates belonged to *Escherichia coli* (12%), *Klebsiella pneumoniae* (17%), *Pseudomonas aeruginosa* (20%), and *Staphylococcus aureus* (25%). The antibiotic sensitivity testing showed that different bacterial strains exhibited varied responses to antibiotics, underscoring the challenges in treating infections caused by these pathogens. These findings highlight the importance of monitoring antibiotic resistance patterns in these bacterial strains in order to guide treatment decisions and enhance patient outcomes.

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1. Introduction

Healthcare-associated infections (HAIs) refer to nosocomial infections that are not present in the patient at the time of admission to the

healthcare facility¹. HAIs are most commonly associated with hospitalization in intensive care units (ICUs), although they can occur in various wards during treatment. Patients in ICUs are five to ten times more likely

to acquire an HAI due to both extrinsic factors (such as the use of medical devices) and intrinsic factors (such as immunodeficiency). Furthermore, ICUs often serve as breeding grounds for multidrug-resistant microorganisms². Surgical site infections (SSIs) account for 36% of nosocomial infections in surgical patients, making them the most common type of hospital-acquired infection. SSIs are associated with elevated morbidity and mortality rates, as well as increased costs³. The failure of antibiotic therapy may be attributed to the presence of multidrug-resistant strains, which are defined as isolates resistant to three or more classes of antimicrobials. Additionally, the polymicrobial nature of these infections and the involvement of anaerobic bacteria in SSIs further complicate treatment outcomes⁴. Thus, identifying the causative bacteria and their resistance to antimicrobial therapies is essential for achieving effective treatment outcomes for these infections. In addition to identifying and detecting bacteria from surgical sites, the current study sought to explore the isolates' antibiotic susceptibility profiles.

2. Methodology

A cross-sectional study with a laboratory investigation focus was conducted at the operating theatres of the Al Sader Teaching Hospital in Iraq, between July and November 2023. There are three operating theatres in the hospital for surgical services (general, private wings, as well as delivery and emergency wards). A total of 100 cotton swab samples were collected from the surgical instruments and surfaces in three operating theatres inside the hospital's surgical services' department. In order to enrich the samples, they were inoculated into peptone water and subsequently subjected to subculture into mannitol salt agar, MacConkey agar, and blood agar. Moreover, microorganism identification was performed by biochemical testing. The disc diffusion method was used, with one disc placed in the centre of the plate and eight discs evenly spaced, each around 15 mm from the plate's edge. A gentle press was applied to each disc in order to guarantee even contact with the media. Following an overnight incubation period at 37°C,

the size of the inhibition zone was measured and was compared to the standard diameter of the inhibition zones for each antibiotic.

3. Results and Discussion

A total of 100 swab samples from various surfaces of surgical personnel and equipment within three distinct operating rooms, were collected and processed; of these, 52% were negative. Four bacterial species were isolated and identified in this study, including: *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (20%), *Klebsiella pneumoniae* (17%), and *Escherichia coli* (12%).

Previous studies have revealed varying percentages of contaminating bacteria in Iraq (Gram-positive and -negative). *P. aeruginosa*, *E. coli*, and other bacteria have been isolated within three years at the Al-Yarmouk Teaching Hospital in Baghdad, Iraq and, of these, *P. aeruginosa* was the most prevalent (35.8%), followed by *E. coli* (25.9%)⁵. A study carried out in two surgical units at the Abu-Bakr Tafawa Balewa University Teaching Hospital, Bauchi Nigeria has reported high isolated *S. aureus* levels (28.2%), followed by those of *Bacillus* spp., *Acinetobacter* spp., *Klebsiella pneumoniae*, *Enterobacter* spp., and *Enterococcus* spp.⁶. Moreover, according to Abd-Elmonsef *et al.*⁷, *P. aeruginosa* and *S. aureus* are the two most common isolates in ICUs.

Antimicrobial resistance is a rising health concern. Success in the correct treatment of patients depends on identifying the resistance pattern of these pathogens in every hospital. The most often isolated bacteria in our study included *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, and this is why we have herein also assessed their susceptibility to antibiotics (Table 1).

In relation to the antimicrobial susceptibility pattern of the bacteria isolated in our study, numerous published studies have documented the prevalence of resistant Gram-negative bacteria among patients hospitalized in various regions of Iraq. The most common organisms isolated from trauma patients with ventilator-associated pneumonias at a Lebanese tertiary care facility, HAIs in Turkey, and ICUs in Kuwait were found to include *K. pneumoniae*,

Table 1. Antibiotic susceptibility patterns of pathogenic bacteria isolated from the operating room of the Al Sader Teaching Hospital (Iraq). Abbreviations used: MS, moderately sensitive; R, resistant; S, sensitive.

Antibiotics	Sensitivity to antibiotics			
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
amoxicillin and clavulanate	R	R	R	R
cefotaxime	S	R	S	R
imipenem	S	R	S	S
levofloxacin	R	S	S	S
cefixime	R	R	R	R
meropenem	R	R	S	S
tobramycin	R	S	R	S
netilmicin	S	R	S	S
ampicillin and cloxacillin	R	R	R	R
cefdinir	R	R	R	R
nitrofurantoin	R	R	R	R
nalidixic acid	R	R	R	S
norfloxacin	R	R	S	S
gentamycin	R	S	S	R
ofloxacin	R	R	R	R
ceftazidime	R	R	R	R
doxycycline	R	R	MS	R
piperacillin and tazobactam	S	R	S	S

Acinetobacter spp., *P. aeruginosa*, and *E. coli*^{8,9}. The factors contributing to the elevated occurrence of these antibiotic-resistant bacteria remain undefined; however, potential associations may exist with the regional infection control protocols, the utilization of antibiotics in inpatient or community settings, or various climate and environmental factors.

4. Conclusion

Our study shows that bacterial contamination from the operating rooms can have a direct and visible impact on hospital infection rates, especially those brought on by HAIs. One of the riskiest places for patients to contract a nosocomial infection is the operation room.

Acknowledgements

For its assistance and resources during the undertaking of this study, the authors would like to thank the University of Babylon's College of Pharmacy.

Conflicts of interest

None exist.

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HOW TO CITE:

Aljaryan H.B., Al-Shamari A.K., Nayyef A.A., Al-Ameedy T.H., Rabeea H., Ali F. Antibiotic susceptibility patterns of pathogenic bacteria isolated from the operating room of the Al Sader Teaching Hospital (Iraq). *Pharmakeftiki* 37(2s), 82-85, 2025. <https://doi.org/10.60988/p.v37i2S.147>

Comparison of various *Helicobacter pylori* diagnostic techniques in samples isolated from Iraqi patients

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KEY WORDS:

***Helicobacter pylori*; urea breath test; S-Ag test; serology; endoscopy; non-invasive test**

ARTICLE INFO:

Received: January 10, 2025

Revised: February 08, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

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ABSTRACT

The *Helicobacter pylori* infection is a major global health concern, contributing to various gastrointestinal disorders, including gastritis, peptic ulcers, and gastric cancer. Disease management relies on the availability of both accurate and reliable diagnostic methods. This study evaluates and compares the diagnostic accuracy of three widely used non-invasive tests (the ¹³C-urea breath test or ¹³C-UBT, the stool antigen test, and serological testing) against endoscopy (as the gold standard) in Jood Specialist Lab in Hillah (Iraq) between February and December 2024. A total of 100 patients was included in this study, and specimens were inspected using each of the aforementioned tests. Results showed that the ¹³C-UBT had the highest sensitivity (98.9%) and specificity (76.9%), which makes it the best non-invasive test for the diagnosis of an *H. pylori* infection. Meanwhile, the stool antigen test exhibited a slight lower performance in both sensitivity (94.8%) and specificity (65.2%), while the serological testing exhibited the lowest specificity (24.5%), resulting in a greater frequency of false positives. These findings underscore the limitations of serological testing and reinforce ¹³C-UBT's position as the method of choice for the non-invasive diagnosis of *H. pylori* infections in Iraqi patients.

1. Introduction

A microaerophilic spiral-shaped gram-negative bacterium, *Helico-*

bacter pylori, colonises the stomach and causes most duodenal ulcers, gastritis, mucosa-associated lymphoid tissue lymphoma, and

stomach cancer cases. Poor countries have 70% to 90% *H. pylori* prevalence, while wealthy ones have 25% to 50%. Person-to-person transmission is most likely. The diagnostic methods for *H. pylori* include invasive and non-invasive methods. Histology, the rapid urease test (RUT), microbiological culture, and PCR are endoscopic biopsy tests. On the other hand, the ^{13}C -urea breath test (^{13}C -UBT), the stool antigen test, and serological testing are non-invasive methods for the diagnosis of an *H. pylori* infection. Clinical circumstances, sensitivity, specificity, and cost-effectiveness influence diagnostic approaches¹. All approaches have downsides. Countries with lots of endoscopes chose histopathology. Histopathology demands skilled pathologists and appropriate samples, as misleading results derive from poor biopsies and depend on observer factors, stomach topography, *H. pylori* density and patchy distribution, and the *H. pylori* stain type. Stomach biopsies cultured for bacteria can reveal an *H. pylori* infection. However, technical approaches can affect the lab culturing process and the test sensitivity².

RUT is the dominant approach in clinical practice, but its sensitivity requires at least 105 bacteria in the sample. Since this amount may not be present after 4 weeks of an eradication treatment failure, the test is not recommended for post-eradication follow-up. Serum antibodies are easiest to perform in non-gastroscopist settings in order to diagnose an *H. pylori* infection. Finally, ^{13}C -UBT is more sensitive and specific than other non-invasive tests, but gut urease-producing bacteria limit it³. Moreover, it needs expensive and advanced hardware.

The aim of this study was to evaluate and compare the diagnostic accuracy of three widely used non-invasive tests (i.e., the ^{13}C -UBT, the stool antigen test, and serological testing) against endoscopy (as the gold standard) in Iraqi patients.

2. Methodology

This study was undertaken from February 2024 to December 2024 at the Jood Specialist Lab and the Merjan Medical City in Hillah, Iraq. Ethical approval was obtained from the Ethics Committee of the Mer-

jan Medical City Hospital (July 15, 2024). Written informed consent was obtained from all participants before enrolment.

Extensive testing for *H. pylori* was performed on 100 patients, with a mean age of 43 years (range: 17–70 years). Three rigorous non-invasive testing methods were applied in this evaluation: the ^{13}C -UBT, the stool antigen test, and serological testing. Subsequently, the results were correlated to traditional endoscopy results being done at the time. Just as importantly, the consumption history of antibiotics as well as the use of H_2 -receptor antagonists, proton pump inhibitors, and nonsteroidal anti-inflammatory drugs during the 30 days prior to the evaluation were among the exclusion criteria for this study.

The SPSS v.25 software was used in order to analyse the data collected during the investigation and to determine sensitivity, specificity, and accuracy. Moreover, the chi-squared and the McNemar's tests were used in order to note the statistical significance of the observed differences in the results.

3. Results and Discussion

The ^{13}C -UBT and the stool antigen test yielded high sensitivity percentages, as both tests demonstrated a sensitivity of 98.9% for the ^{13}C -UBT and 94.8% for the stool antigen test, respectively (Table 1). The serological testing's sensitivity was lower, at 91.5% (Table 1). Our results also showed that the ^{13}C -UBT had the highest specificity (76.9%), which makes it the best non-invasive test for the diagnosis of an *H. pylori* infection (Table 1). Meanwhile, the stool antigen test exhibited a lower performance in terms of specificity (65.2%), while the serological testing exhibited the lowest specificity (24.5%), resulting in a greater frequency of false positives (Table 1).

Patients with peptic ulcers or *H. pylori* infections are often diagnosed using endoscopic procedures. Yet, these procedures are costly, riskier, and more uncomfortable than other non-invasive options. Typically, serological testing, stool antigen tests, and ^{13}C -UBTs are less expensive. The results of the herein assessed *H. pylori* tests have been confirmed by endoscopic examination, which is considered

Table 1. The results of three non-invasive diagnostic methods for H. pylori infections are compared to the gold standard (standard endoscopy).						
Diagnostic methods	Standard endoscopy		Sensitivity	Specificity	Accuracy	McNemar's test <i>p</i> -value
	positive	negative				
¹³ C-urea breath test						
positive	86	3	98.9%	76.9%	90.7%	0.625
negative	1	10				
stool antigen test						
positive	73	8	94.8%	65.2%	82.8%	0.388
negative	4	15				
serological testing						
positive	43	40	91.5%	24.5%	56.0%	<0.001
negative	4	13				

the gold standard for the diagnosis of *H. pylori* infections⁴⁻⁶. While serological testing is overall less sensitive than other testing platforms for the identification of an *H. pylori* infection^{7,8}, its relative low cost and ease of use make it ideal for wide availability (e.g. in a general practitioner's office). On the other hand, numerous stool antigen tests have been developed so far, but despite the high sensitivity performance of these tests, their specificity is relatively low, thereby indicating that there is still room for improvement⁹. To an extent, one might consider that in the same way that surgery and medical management can be combined in order to produce the best clinical outcomes for a number of diseases, the same principles could be applied to the diagnosis of *H. pylori*¹⁰.

4. Conclusion

The ¹³C-UBT surpassed both the stool antigen test and serological testing in terms of sensitivity and

accuracy in diagnosing an infection due to *H. pylori*. However, the stool antigen test was the more rapid and cost-effective option for the Iraqi patients.

Acknowledgements

The successful completion of this project was made possible through the invaluable support of Benin Walid Tamkeen Shanin, Heba Karim Mohsen, as well as the resources and expertise provided by Jood Specialist Lab and Merjan Medical City in Hillah.

Conflicts of interest

None exist.

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HOW TO CITE:

Alkawaz A.J.A.A.H., Naser M.S., Obaid A.J. Comparison of various *Helicobacter pylori* diagnostic techniques in samples isolated from Iraqi patients. *Pharmakeftiki* 37(2s), 86-89, 2025.
<https://doi.org/10.60988/p.v37i2S.148>

Immunohistochemical investigation of vascular endothelial growth factor during the healing of gastric ulcer in female rats: experimental study

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KEY WORDS:

curcumin; esomeprazole;
stomach; ulcer; VEGF

ARTICLE INFO:

Received: November 07, 2024

Revised: December 27, 2024

Accepted: January 06, 2025

Available online: October 10, 2025

ABSTRACT

The current study attempts to characterize the effects of esomeprazole, curcumin, chitosan, as well as of a mixture of curcumin and chitosan on the expression of the vascular endothelial growth factor (VEGF) in gastric ulcers induced by ethanol in female rats. This study included 30 rats; their average weight ranged between 195.6 and 198.6 g, and the rats were separated into two control groups (negative and positive control groups) and four treatment groups (n=5 rats per group). All groups were treated for 5 days after last administration of distilled water or ethanol. The immunohistochemical staining for VEGF in the cells of gastric mucosa revealed a positive staining in both the control groups and all treated groups. The H-score for VEGF was found to exhibit no significant change ($p>0.05$) in any of the assessed groups.

1. Introduction

Increasing intragastric pH and reducing daily gastric secretion output are two of the most effective ways to decrease gastric acid secretion. Proton pump inhibitors (PPIs) act by inhibiting the stomach H^+,K^+ -ATPase (proton pump)¹. Clinically available PPIs include pantoprazole, rabeprazole, lansoprazole,

omeprazole, and esomeprazole. In addition to these PPIs, tenatoprazole has a 5- to 7-fold longer elimination half-life than the aforementioned PPIs². Esomeprazole, the S-isomer of omeprazole (which is a racemic mixture of S- and R- optical isomers), is the first PPIs to be produced as a single optical isomer and has a good pharmacokinetic profile³.

Curcumin has a long history of

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use in the traditional medicine of India, Iran, and China, and it has been used for the treatment of many diseases (including liver diseases, diabetes, rheumatoid diseases, cancers, infectious diseases, as well as atherosclerosis)⁴. On the other hand, chitosan is regarded as a natural and most promising bio-polymer; it has been included in the GRAS (Generally Recognized as Safe) category by the FDA due to its excellent biodegradability, non-toxic antimicrobial activity, and biocompatibility. It has been known to exert bactericidal or bacteriostatic effects against a wide range of microorganisms⁵.

The current study's objective was to evaluate the effects of esomeprazole, curcumin, chitosan, and of the combination of curcumin and chitosan on the expression of the vascular endothelial growth factor (VEGF) in stomach ulcers induced by ethanol in female rats.

2. Methodology

2.1. Experimental animals

We used 30 adult female rats with an average weight of 195.6 to 198.6 g. The rats were kept in specially designed cages, were given unlimited food and water. Before the experiments began, the rats were allowed around two weeks to get used to the new environment. Ethical approval was received for the herein described experiments (7-17-7922; November 3, 2022).

2.2. Drug and chemicals

Esomeprazole (manufactured by Ajanta Pharma Ltd, India) was used in this study. It was obtained from a local pharmacy in Hillah (Iraq); each tablet containing 40 mg of the drug. Curcumin (98%) and chitosan were purchased from Shanghai Macklin Biochemical Co Ltd (China). All other chemicals were bought from local commercial suppliers.

2.3. Curcumin–chitosan mixture preparation

After slowly adding 150 mg of chitosan (dissolved in 10 mL of 0.1-M acetic acid) to 40 mg of curcumin,

the mixture was triturated in order to produce a uniformly yellow-coloured liquid⁶.

2.4. Experimental design

Rats were randomly separated into six groups: two control groups and four treated groups (5 rats per group) as follows: (i) negative control group (distilled water; all rats in this group were given distilled water at a dose of 2 mL per rat, by oral gavage, during the experimental period), (ii) positive control group (ethanol; all rats in this group were given ethanol at a dose of 2 mL per rat, by oral gavage, in double dose after fasting for 19 h before the administration of each ethanol dose), (iii) esomeprazole-treated group (same as the positive control group, but after 1 h from the last ethanol dose, esomeprazole was administered in a single oral daily dose of 3.54 mg/kg and dissolved in 2 mL of distilled water, for 5 successive days), (iv) curcumin-treated group (same as the positive control group, but after 1 h from the last ethanol dose, curcumin was administered in a single oral daily dose of 40 mg/kg and dissolved in 2 mL of 0.06% (0.1 M) acetic acid, for 5 successive days), (v) chitosan-treated group (same as the positive control group, but after 1 h from the last ethanol dose, chitosan was administered in a single oral daily dose of 150 mg/kg and dissolved in 2 mL of distilled water, for 5 successive days), and (vi) chitosan–curcumin mixture-treated group (same as the positive control group, but after 1 h from the last ethanol dose, the chitosan–curcumin mixture was administered in a single oral daily dose of 2 mL, for 5 successive days).

2.5. Immunohistochemical staining

The sliced gastric tissues (5-µm thickness) were stained by using an Animal Research Kit in order to identify the VEGF protein expression. The standard proteins were obtained from MyBioSource (San Diego, CA, USA). The staining intensity for VEGF expression was evaluated by using scores from 0 to 3 as follows: 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining. Data conversion to the numeric total immunoreactive score was established by using semi-quantitative anal-

Table 1. Quantification of the intensity of the vascular endothelial growth factor (VEGF) staining and of the percentage of VEGF-stained cells in the gastric mucosa tissue of rats. Different letters indicate significant differences ($p<0.05$) among the studied experimental groups.			
Experimental groups	Staining intensity (mean $\pm$ SE)	Percentage of stained cells (mean $\pm$ SE)	Histochemical scoring (mean $\pm$ SE)
Negative control group (distilled water)	2.750 $\pm$ 0.164 ^a	48.125 $\pm$ 3.399 ^{a,b}	135.625 $\pm$ 15.190 ^a
Positive control group (ethanol)	2.000 $\pm$ 0.258 ^b	41.313 $\pm$ 7.040 ^{a,b}	108.188 $\pm$ 22.595 ^a
Esomeprazole-treated group	2.583 $\pm$ 0.149 ^{a,b}	56.250 $\pm$ 6.885 ^a	156.250 $\pm$ 24.819 ^a
Curcumin-treated group	2.000 $\pm$ 0.162 ^b	36.250 $\pm$ 5.212 ^{a,b}	87.350 $\pm$ 16.526 ^a
Chitosan-treated group	2.208 $\pm$ 0.170 ^{a,b}	43.500 $\pm$ 7.288 ^{a,b}	122.833 $\pm$ 23.139 ^a
Chitosan–curcumin mixture-treated group	2.000 $\pm$ 0.204 ^b	32.063 $\pm$ 6.035 ^b	81.125 $\pm$ 20.121 ^a

ysis, in which the percentage of stained cells was multiplied by the staining intensity scores⁷.

2.6. Statistical analysis

The data underwent statistical analysis with the use of SPSS version 23.0. The data are presented in the format of arithmetic mean $\pm$ standard error (SE). A statistical analysis was conducted by employing analysis of variance (ANOVA).

3. Results

The undertaken immunohistochemical staining of the rat gastric mucosa for VEGF showed positive staining in both control groups as well as in all treated groups (Table 1). However, the H-score for VEGF was found to show no significant change ($p>0.05$) in any of the studied groups.

4. Discussion

The present study reveals that the administration of esomeprazole, curcumin, chitosan, and of a mixture of chitosan and curcumin in rats with ethanol-induced gastric ulcers does not involve the gastric mucosal VEGF production. This result is not in agreement with previous studies that shown that VEGF expression is

regarded as one of the main growth factors involved in the healing mechanisms of wounded tissues, through the stimulation of neovascularization processes^{8,9}. In fact, the expression of VEGF is considered essential for the maintenance of gastrointestinal mucosal integrity, and angiogenesis is a pivotal mechanism contributing to the healing of gastrointestinal ulcers¹⁰.

5. Conclusion

After attempting to characterize the effects of esomeprazole, curcumin, chitosan, as well as of a mixture of curcumin and chitosan on the expression of VEGF in gastric ulcers induced by ethanol in female rats, we have found no significant differences in terms of the H-score of this protein among the studied groups under the examined experimental conditions.

Acknowledgements

We want to express our deep gratitude to the Biology Department of the College of Science of the University of Babylon for providing the necessary facilities for the undertaking of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Kawaz J.M.J., Al-Zubaidy F.M., Al-Harbi H.J.O. Immunohistochemical investigation of vascular endothelial growth factor during the healing of gastric ulcer in female rats: experimental study. *Pharmakeftiki* 37(2s), 90–93, 2025. <https://doi.org/10.60988/p.v37i2S.150>

The role of treatment patterns and body mass index in asthma management: a case-control study

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KEY WORDS:

asthma; treatment modalities; obesity; pulmonary function; case-control study

ARTICLE INFO:

Received: January 11, 2025

Revised: February 07, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

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ABSTRACT

This study aimed at comparing the effects of different treatment approaches on asthma, by also focusing on the impact of obesity on these treatment approaches. A total of 100 asthma patients were recruited (along with a control group of 100 individuals) in order to determine the effect of various therapies on pulmonary function parameters, including forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), their ratio (FEV1/FVC), and peak expiratory flow. All study participants were recruited from Marjan Medical City (Iraq) by the haematology department, from March to December 2023. The majority (95%) of the obese patients used a budesonide / formoterol Turbuhaler, which had better efficacy than montelukast and steroids in terms of enhancing their pulmonary function. A lower FEV1/FVC was inversely associated with increased body mass index (BMI), thereby indicating that an increased BMI worsens airway obstruction. Moreover, the adequacy of the prescribed treatments varied considerably regarding the obesity of the patients. However, based on this measurement, only 27% of the study's patients were considered to be well-controlled. These findings underline the need to individualize asthma treatment plans with a view of targeting both respiratory disorders and obesity as vital elements of a strategy to both enhance the management of the condition and the lung functions. Therefore, obesity and asthma cross-modification strategies should adopt a more encompassing system of treatment and management.

1. Introduction

Asthma is a chronic disease characterized by inflammation and airway obstruction^{1,2}. There has been growing concern in recent years about obesity and its impact on the management of asthma. Obesity, as associated with asthma, has emerged as a critical health issue because it is a determinant of severe asthma and is known to exacerbate asthma-related symptoms^{3,4}. Other complications related to obesity can negatively affect airway inflammation and reduce lung function, making it difficult to manage asthma⁵. Recent findings indicate that different medications used for asthma may produce different outcomes in obese patients than in non-obese patients⁶. In particular, research on safer drugs (such as the combination of budesonide and formoterol) has highlighted the enhancement of lung function among those diagnosed with obesity⁷. Knowledge of the pattern of the treatment response and of the patient's body mass index (BMI) is essential for the effective management of asthma. This study aimed at evaluating the effects of several therapeutic modalities on asthmatic patients while considering their BMI, thereby estimating the impact of these factors on pulmonary function.

2. Methodology

This cross-sectional comparative study compared 100 asthmatic patients attending a tertiary health-care facility and 100 healthy controls. The patients were recruited from Marjan Medical City (Iraq) by the haematology department, from March to December 2023, under appropriate institutional ethical consent (IRB: A0035, 2023) and written consent from each participant. The participants' basic demographics were obtained and then, each of them underwent specific spirometry (in duplicate; at the hospital, by experts) in order to evaluate pulmonary function metrics such as forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), their ratio (FEV1/FVC), and peak expiratory flow (PEF). The BMI of each participant was computed according to standard approaches for categorizing

obese and non-obese subjects. Treatment modalities were documented (i.e., treatment with budesonide / formoterol Turbuhaler, systemic steroids, short-acting β -agonists, and montelukast), and statistical analyses were performed by using the SPSS software so as to evaluate correlations between BMI, treatment type, and pulmonary function outcomes.

3. Results and Discussion

The mean patients' age was 31.71 ($\pm$ 15.02) years, while the mean age of the controls was 36.91 ($\pm$ 10.99) years ($p=0.013$). The patients had a mean FEV1 of 77.9 ($\pm$ 21.1) L, which was significantly lower than the control mean of 97.1 ($\pm$ 0.3) L ($p<0.001$). Moreover, the mean FVC for patients was recorded at 1.1 ($\pm$ 0.4) L, with a non-significant difference compared to the control mean FVC of 1.2 ($\pm$ 0.2) L ($p=0.265$). Finally, the mean PEF for asthma patients was found to be significantly lower at 65.8 ($\pm$ 22.1) L/min, compared to the mean PEF of the control group (89.9 $\pm$ 18.8 L/min; $p<0.001$) (Table 1).

The FEV1/FVC for the patients was measured at 0.85 ($\pm$ 0.1), which was significantly lower than the control group's ratio of 0.9 ($\pm$ 0.1; $p=0.001$). Treatment modalities varied significantly between obese and non-obese groups; notably, the Turbuhaler-utilising therapy was administered to an overwhelming majority of obese patients (95%), while only a small percentage received short-acting β -agonists (5%). Montelukast and systemic steroids were prescribed to 31% and 27% of obese patients, respectively (Table 1).

Only 27% of the obese patients were classified as well-controlled in terms of asthma control levels, while moderately controlled patients and poorly controlled patients accounted for 41% and 32% of the study's asthmatic patients, respectively. Correlation analysis indicated a negative correlation between BMI and FEV1/FVC ($r=-0.30$, $p=0.01$), while a positive correlation was noted between BMI and FVC ($r=0.15$), but it was non-significant (Table 1).

The interplay between obesity and asthma management is a critical area of investigation, particularly given the rising global prevalence of obesity.

Table 1. Therapeutic modalities, pulmonary functions, and their correlations with BMI and disease duration in the asthma and control groups of the study. Abbreviations used: BMI, body mass index; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow.				
Category	Variable / treatment	Asthma patients (N=100) (mean $\pm$ SD, N (%), or <i>r</i> -value)	Control group (N=100) (mean $\pm$ SD)	<i>p</i> -value
Demographics	Age (years)	31.71 $\pm$ 15.02	36.91 $\pm$ 10.99	0.013
Pulmonary function tests	FEV1 (L)	77.92 $\pm$ 21.06	97.07 $\pm$ 10.32	<0.001
	FVC (L)	1.12 $\pm$ 0.36	1.17 $\pm$ 0.16	0.265
	PEF (L/min)	65.77 $\pm$ 22.10	89.88 $\pm$ 18.84	<0.001
	FEV1/FVC	0.85 $\pm$ 0.08	0.90 $\pm$ 0.07	0.001
Asthma control by gender	well controlled	13 (13%) male 12 (12%) female	-	0.768
	moderately controlled	23 (23%) male 18 (18%) female	-	0.796
	poorly controlled	24 (24%) male 14 (14%) female	-	0.560
Treatment modalities by obesity status	Turbuhaler	42 (95%) obese 40 (90%) non-obese	-	0.192
	short-acting β -agonist	2 (5%) obese 4 (10%) non-obese	-	0.678
	montelukast	14 (32%) obese 10 (25%) non-obese	-	0.114
	systemic steroids	12 (27%) obese 8 (20%) non-obese	-	0.186
Asthma control by obesity status	well controlled	12 (12%) obese 10 (10%) non-obese	-	0.380
	moderately controlled	18 (18%) obese 14 (14%) non-obese	-	0.888
	poorly controlled	14 (14%) obese 10 (10%) non-obese	-	0.559
Correlations with BMI	BMI vs. FEV1	0.12	-	0.230
	BMI vs. FVC	0.15	-	0.130
	BMI vs. PEF	-0.22	-	0.040
	BMI vs. FEV1/FVC	-0.30	-	0.010
Correlations with disease duration	duration vs. FEV1	-0.00	-	0.970
	duration vs. FVC	0.10	-	0.350
	duration vs. PEF	-0.10	-	0.360
	duration vs. FEV1/FVC	-0.20	-	0.060

This case-control study suggests that treatment modalities significantly affect asthma management outcomes depending on the obesity status. Obese patients (primarily receiving a Turbuhaler therapy) have demonstrated improved pulmonary function metrics compared to those receiving alternative treatments such as montelukast or systemic steroids. The observed negative correlation between BMI

and FEV1/FVC highlights the detrimental impact of obesity on airway function as BMI increases. These findings suggest that the ability to maintain normal airflow diminishes in asthmatic patients, leading to greater obstruction⁷. Indeed, obesity leads to decreased static and dynamic pulmonary volumes due to mechanical loading from excess fat⁵, particularly affecting expiratory reserve volume^{7,8}. Furthermore,

the active management of obesity is vital for improving asthma manifestations and overall respiratory wellbeing^{8,9}. Therefore, weight loss interventions could improve anti-asthmatic efficacy and decrease asthma burden in obese patients¹.

In the current study, treatment approaches differed between the two study groups, with the obese patients receiving mostly Turbuhaler therapy (95%). This agrees with the current literature, which indicates that combination therapies are effective in controlling asthma in the obese population. The higher use of a Turbuhaler therapy observed in obese subjects may signify that practitioners prefer this type of combination inhaler because both its components act synergistically in order to effectively control inflammation and bronchoconstriction in obese patients¹⁰. The positive outcomes associated with the Symbicort use for obese patients with asthma specifically demonstrate which pulmonary function metrics provide additional support for patient-tailored approaches that consider the patients' body weight and asthma severity.

Although Symbicort was our study's most prevalent prescribed treatment, 27% of obese patients were well-controlled. This reveals the effectiveness of current therapeutic modalities in promoting optimal asthma control in obese individuals. Moderate control was noted in 40% of the patients and poor control in 31%; these scores remain a concern for clinicians. The study has also confirmed that combined medications with the use of montelukast and systemic steroids were used in only 31% and 27% of the obese patients, respectively. These results indicate a gap between the availability of medications and their utilization, and warrant the investigation of the efficacy of these drugs when combined with weight loss programs.

The correlation analysis revealed positive non-significant associations between BMI and FVC ($r=0.15$), indicating that obesity could also influence the other domains of lung function, although its intensity is not equal to that of FEV1. This underlines the challenging effects of obesity on the lungs and emphasizes a multimodal approach to treating obesity asthmatics. Another noteworthy fact is the absence of

sex or disease duration differences in the ranges of the treatment effect that can be seen while claiming that these factors are important for treatment outcomes in many patients. Nevertheless, it is crucial to consider specific patient characteristics when developing treatment strategies.

Patients with asthma displayed significantly reduced pulmonary functions compared to the controls. In particular, the FEV1 was 77.9% for patients *versus* 97.1% for the controls ($p<0.001$). This stark alteration underscores the influence of asthma on pulmonary function, which is worsened by obesity. Numerous comparable reports have reported identical outcomes^{1,3,4}.

Based on these findings, it is imperative to urge clinicians to adopt a holistic approach to asthma management by including aspects of weight loss alongside the pharmacological approach to treatment. Recent research has confirmed that there are cases in which weight reduction results in massive positive changes in the manner in which the ailment and the bodily system in question manifest themselves. Hence, the use of therapeutic approaches such as lifestyle modifications or behavioural interventions may improve general results for obese asthma patients.

4. Conclusion

The current study underscores the significance of tailored therapeutic modalities for treating asthma in obese individuals. The large differences in pulmonary function measures suggest clinicians must consider BMI when advising medications. Future studies should emphasize long-term outcomes related to various therapeutic plans and their combined influence on weight management and asthma control, so as to develop comprehensive protocols that enhance respiratory health outcomes and patient quality of life.

Acknowledgements

The authors would like to thank the patients and their guardians for their participation in the current

study. In addition, the authors are grateful to the volunteers who have participated in this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Alkelabi A.A., Al-Mumin A.S., Al-Hindy H.A.A.M., Salih A.M., Obaid S.R., Abd Ali M.H., Makki M.M., Hadi F.H., Dawood N.A.T. The role of treatment patterns and body mass index in asthma management: a case-control study. *Pharmakeftiki* 37(2s), 94-98, 2025. <https://doi.org/10.60988/p.v37i2S.151>

A novel approach to the synthesis of acetylsalicylic acid *via* H/D substitution

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KEY WORDS:

aspirin; deuteration; H/D exchange; platinum catalyst; hydrothermal autoclave

ARTICLE INFO:

Received: November 07, 2024

Revised: December 27, 2024

Accepted: January 07, 2025

Available online: October 10, 2025

ABSTRACT

This study introduces a unique technique for synthesizing acetylsalicylic acid (aspirin) with a hydrogen-deuterium (H/D) exchange, by using a hydrothermal autoclave device, for the first time. Aspirin was subjected to deuterium substitution by dissolving it in deuterium oxide and allowing it to react with K_2PtCl_4 in a hydrothermal autoclave. The reaction conditions (temperature, stress, and time) were optimized in order to facilitate the H/D exchange. The characterization of the synthesized deuterium-substituted aspirin was performed through the employment of ^1H -nuclear magnetic resonance (NMR) spectroscopy and ^{13}C -NMR spectroscopy, confirming a hit substitution at particular hydrogen sites. The findings were validated after full-size adjustments in NMR spectra, indicating a powerful incorporation of deuterium, which enhances aspirin's pharmacokinetic properties and decreases its metabolic liabilities. This study highlights the ability of H/D change to improve drug efficacy.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used globally. These drugs include NSAIDs containing a carboxylic acid moiety, those with enolic acid systems, and compounds that lack carboxylic acid or different acidic corporations. Aspirin is a famous NSAID, celebrated for its ability to relieve pain and inflammation. Introduced in 1899, aspirin became

the first effective remedy for rheumatic illnesses¹, and is also used as an anticoagulant so as to treat headaches related to the hyperviscosity of the blood. Aspirin is one of the earliest drugs to be categorized inside identified therapeutic classes, with its use dating back to the Sumerians and the Egyptians that used to apply willow bark as a medication².

Prostaglandins (PGs), inclusive of PGG_2 and PGH_2 , are bioactive mole-

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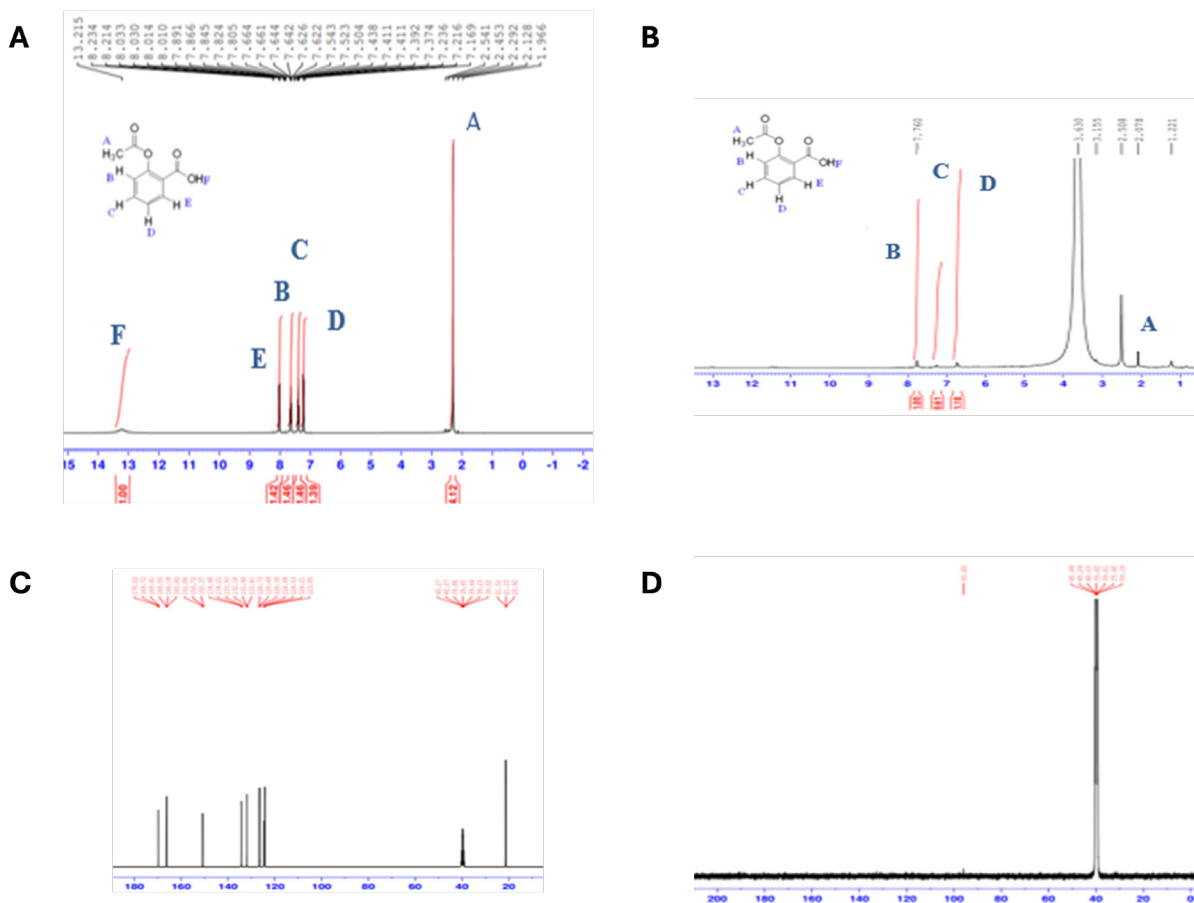


Figure 1. (A): The ^1H -nuclear magnetic resonance (NMR) spectroscopy chart for aspirin before deuteration. (B): The ^1H -NMR spectroscopy chart for aspirin after deuteration. (C): The ^{13}C -NMR spectroscopy chart for aspirin before deuteration. (D): The ^{13}C -NMR spectroscopy chart for aspirin after deuteration.

cules synthesized through cyclooxygenase (COX), in particular COX-1 and COX-2, in the course of inflammatory responses. Aspirin exerts its consequences by inhibiting COX-1, thereby influencing platelet activation. Moreover, high doses of aspirin also suppress COX-2 by covalently binding to the Ser516 residue at its active site³.

The hydrogen-deuterium exchange (H/D exchange) refers back to the chemical method in which a hydrogen atom in a covalent bond is changed by means of a deuterium atom. This trade takes place with the participation of hydrogen and deuterium compounds, and is normally catalysed by way of plat-

inum (Pt) or palladium. The response charge is stimulated through factors which include temperature and pressure⁴. The H/D exchange is a crucial method in chemical research, providing insights into molecular interactions. It is an essential tool in pharmaceutical research, particularly for the identification of precise drug binding sites and the assessment of their distribution and effects. Additionally, the H/D exchange is employed in order to investigate the stability, folding, and characteristics of proteins and nucleic acids⁵. The approach also aids at studying metabolic tactics and changes in various factors, thereby contributing to a higher knowledge of biomolecular

dynamics⁶. This technique is particularly beneficial for analysing the steadiness and conformational modifications of biomolecules, shedding light on their shape and function. By substituting hydrogen with deuterium, one can influence drug excretion and oxidative metabolism, as enzymes within the cytochrome P450 (CYP) alter many drugs⁷. However, oxidative metabolism can cause several unwanted outcomes, inclusive of the production of poisonous, reactive metabolites, variations in bioavailability because of genetic variations, and drug-drug interactions. Therefore, the substitution of hydrogen with deuterium by using the kinetic isotope effect (KIE) has been employed by pharmaceutical developers in order to lessen the CYP-mediated metabolism and its related disadvantages⁸.

2. Methodology

For our study, we have used deuterium oxide (Merck, USA), aspirin (Sigma-Aldrich, USA), potassium tetrachloroplatinate(II) (Fisher Scientific, UK), dichloromethane (BDH, UK), magnesium sulfate (BDH, UK), and ethanol (Sigma-Aldrich, USA) of the purest grade. Deuterium-substituted aspirin was synthesized in a new way for the first time by using a hydrothermal autoclave device, by way of dissolving 1.5 g of aspirin in 25 mL of deuterium oxide (D₂O). The solution was stirred magnetically, and 0.1 g of the catalyst (K₂PtCl₄) were added whilst preserving continuous stirring. The solution was then transferred into a hydrothermal autoclave reactor, which was securely sealed before being placed in the autoclave. The reactor was heated for 120 min at 300 W power, 150 psi pressure, and a temperature variety of 170°C to 180°C. After this step, the reactor was allowed to cool completely. Subsequently, the contents were transferred gradually to a separation funnel, wherein the organic phase was extracted. The natural segment was washed three times with 10 mL of dichloromethane (CH₂Cl₂; three times × 10 mL). After discarding the aqueous layer, the organic segment was obtained, was dried with 0.05 grams of magnesium sulfate (MgSO₄), was filtered, and then collected with the use of a rotary evaporator (Büchi

R-205 Rotavapor).

The characterization of both aspirin and deuterium-substituted aspirin was performed by using ¹H-nuclear magnetic resonance (NMR) spectroscopy (Bruker Avance Neo 400, Germany) at a frequency of 400 MHz, and DMSO-d₆ as the solvent. The pulse program used was zg30, with the probehead identifier z163739-0420. The FID resolution (FIDRES) was 0.250144 Hz. Additionally, the ¹³C-NMR spectra were recorded, at a frequency of 100 MHz.

3. Results and Discussion

When comparing the ¹H-NMR spectra of aspirin before and after deuterium substitution (Figures 1A and 1B), a marked decrease in peak intensity at 7–8 ppm was observed in spectra B, C, and D. In spectrum E, this peak is completely absent, suggesting that protons at more sterically hindered sites were replaced by deuterium, facilitated by the platinum catalyst (K₂PtCl₄) under elevated pressure and temperature. Additionally, a reduction in peak intensity at 2 ppm is noted in spectrum A, with the corresponding signal disappearing entirely in spectrum F, which represents the deuterium-substituted aspirin. These observations further support the occurrence of H/D exchange, promoted by the platinum catalyst, high pressure, and thermal activation. The findings are consistent with those reported in previous studies⁹.

Significant differences are also evident when comparing the ¹³C-NMR spectra of standard aspirin and deuterium-substituted aspirin. In the spectrum of unmodified aspirin, carbon atoms C1–C9 exhibit chemical shifts at 170, 168, 150, 134, 132, 126, 123, 122, and 21 ppm, respectively (Figure 1C). In contrast, the spectrum of deuterium-treated aspirin (Figure 1D) shows the merging and disappearance of several peaks, indicating the replacement of hydrogen atoms bound to carbon with deuterium. This substitution alters the local magnetic environment, leading to the attenuation or loss of specific carbon signals. The observed spectral changes are consistent with the expected effects of deuterium incorporation and align with those of previous reports¹⁰.

4. Conclusion

The comparative analysis of the ^1H -NMR and ^{13}C -NMR spectra of aspirin before and after deuterium substitution provides compelling evidence for the successful incorporation of deuterium atoms. The identified spectral alterations are consistent with those reported by previous studies, thereby reinforcing the reliability of the applied methodology. Collectively, our findings highlight the critical role of catalytic and environmental conditions in enabling efficient H/D exchange in aspirin, representing a significant advancement in isotopic labelling techniques for organic compounds.

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Acknowledgements

We acknowledge the continuous support and encouragement received by the laboratory staff of the Chemistry Department at the College of Science of Mustansiriyah University, and the assistance of the supervisors.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Kinany S.Q., Inayah H., Al-Kazazz F.F.M. A novel approach to the synthesis of acetylsalicylic acid via H/D substitution. *Pharmakeftiki* 37(2s), 99-103, 2025. <https://doi.org/10.60988/p.v37i2S.152>

A prospective cohort study on the association between ear drop use and otomycosis: prevalence, treatment outcomes, and recurrence

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KEY WORDS:
otomycosis; ear drops;
ototopical agents; fungal
infection; otitis externa

ARTICLE INFO:
Received: December 04, 2024
Revised: January 02, 2025
Accepted: January 17, 2025
Available online: October 10, 2025

ABSTRACT

This study investigates the incidence of otomycosis associated with ear drop usage and evaluates treatment outcomes and recurrence rates in Iraqi patients with and without a recent history of ear drop application. Conducted at the ENT Clinic of Almahawil Hospital (Iraq) from April 1, 2022, to March 31, 2024, the study involved 280 patients diagnosed with otomycosis. Participants were divided into two groups: group 1 (N=72) comprised individuals who had used ear drops within 1–2 weeks prior to diagnosis, while group 2 (N=208) comprised individuals who had not recently used ear drops. Our findings revealed that 26% of otomycosis cases were linked to recent ear drop use, with ciprofloxacin being the most commonly used agent (61.1%). Both groups demonstrated high rates of symptom resolution following treatment; however, the recurrence rate was notably higher among ear drop users (11.1%) compared to non-users (7.8%). These results suggest that ear drop usage may elevate the risk of developing otomycosis and increase the likelihood of recurrence, thereby highlighting the need for judicious prescribing practices.

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1. Introduction

Otomycosis, commonly referred to as fungal otitis externa, is a superficial fungal infection affecting the external auditory canal. It is typically characterized by symptoms such as itching, pain, otorrhea, and hearing impairment. The primary causative

agents are species of *Aspergillus* and *Candida*, with the infection being particularly prevalent in tropical and subtropical climates, where warm and humid conditions promote fungal proliferation^{1,2}. Several risk factors have been identified in relation to otomycosis, notably the use of topical ear drops, which can

disrupt the normal microbial flora and create an environment conducive to fungal growth^{3,4}. In particular, the application of antibiotic ear drops has been associated with an increased risk of otomycosis, as these agents may disturb the natural microbial balance and facilitate fungal overgrowth⁵.

This prospective cohort study has investigated the prevalence of ear drop-induced otomycosis, treatment outcomes, and recurrence rates among Iraqi patients with and without recent ear drop use. Conducted at the ENT Clinic of Almahawil Hospital, Iraq, from April 1, 2022, to March 31, 2024, this study aimed at enhancing our understanding of the clinical implications of ear drop usage in relation to otomycosis in Iraqi patients.

2. Methodology

This prospective cohort study was conducted at the ENT Clinic of Almahawil Hospital in the Babylon Governorate of Iraq, from April 1, 2022, to March 31, 2024. The study protocol was approved by the Ethics Committee of the Almahawil Hospital (approval number: AHEC-2022-045) on March 15, 2022. Written informed consent was obtained from all participants prior to their inclusion in the study.

A total of 280 patients diagnosed with otomycosis were enrolled, comprising 188 men and 92 women. Upon enrolment, patients were divided into two groups based on their history of ear drop usage. Group 1 included patients who reported using ear drops within 1–2 weeks prior to their clinic visit. Group 2 included patients who denied any ear drop use within the same timeframe. For group 1, the type of ear drops used was documented, and the duration of use prior to the clinic visit was recorded as either less than one week or more than one week. All patients underwent standard clinical management and assessments, including otoscopy and mycological testing, in order to verify treatment outcomes. Treatment responses were categorized as complete resolution of symptoms (absence of itching, pain, otorrhea, and hearing loss), partial improvement, or treatment failure. Recurrence of otomycosis was monitored, and recurrence rates were compared be-

tween group 1 and group 2.

Data were analysed using appropriate statistical methods in order to estimate the prevalence of ear drop-induced otomycosis. In order to assess the significance of differences in treatment outcomes and recurrence rates, a *p*-value lower than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

The study included a total of 280 patients diagnosed with otomycosis, comprising 188 men (67.1%) and 92 women (32.9%). The age range for male patients was 19 to 74 years, with a mean age of 35 years, while the age range for female patients was 20 to 58 years, with a mean age of 34 years. Seventy-two patients (26%) reported using ear drops within 1–2 weeks prior to their clinic visit for otomycosis (group 1), while 208 patients (74%) denied recent ear drop usage (group 2). This indicates that over a quarter of the diagnosed otomycosis cases were potentially associated with recent ear drop use. Among the 72 patients in group 1, 44 (61.1%) had used ciprofloxacin ear drops, 4 (5.6%) gentamicin ear drops, 13 (18.1%) dewaxing solutions, 5 (6.9%) betamethasone ear drops, and 6 (8.3%) other / unidentified ear drops (Figure 1). The duration of ear drop use prior to the clinic visit varied, with 62 patients (86.1%) reporting use for less than 1 week and 10 patients (13.9%) for more than 1 week.

Following standard management and clinical assessments (i.e., otoscopy and mycological testing), a complete resolution of symptoms was observed in 65 patients (90.3%) of group 1 and 190 patients (91.3%) of group 2. Partial improvement was noted in 7 patients (9.7%) of group 1 and 18 patients (8.7%) of group 2. There were no cases of treatment failure in either of the studied groups. The recurrence rates of otomycosis were 11.1% (8 patients) in group 1 and 7.8% (12 patients) in group 2.

The finding that 26% of patients diagnosed with otomycosis had recently used ear drops suggests that certain formulations may predispose individu-

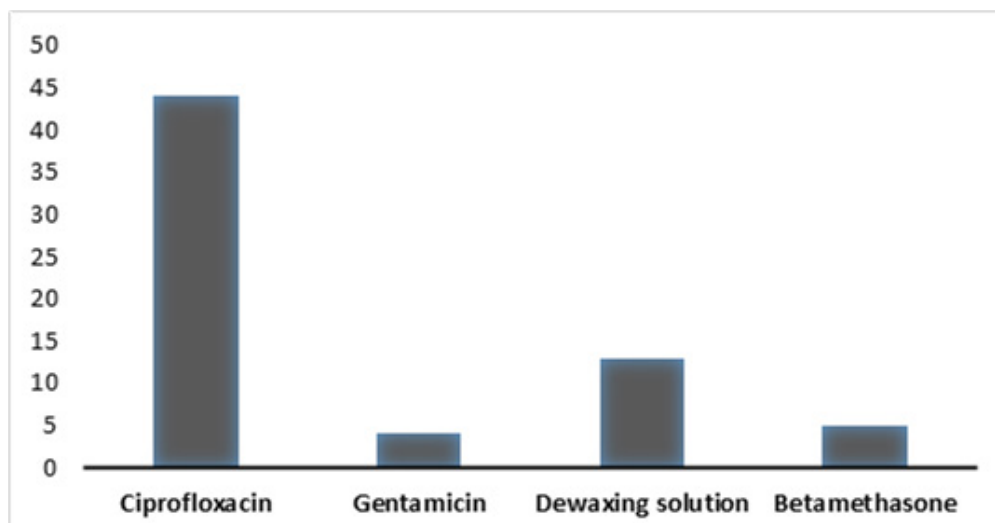


Figure 1. Substances in the ear drops that were used by the study participants belonging to group 1. Note: the y axis denotes the number of the participants, while six participants in this group (not shown) have reported using other / unidentified ear drops.

als to fungal infections of the ear. The predominance of ciprofloxacin use among these patients (61.1%) aligns with previous research indicating that antibiotic ear drops can disrupt the natural microbial balance of the ear, creating an environment conducive to fungal proliferation^{5,6}.

Notably, the high rate of complete symptom resolution in both groups (90.3% in group 1 and 91.3% in group 2) suggests that standard clinical management is generally effective in treating otomycosis, regardless of recent ear drop use. However, the slightly higher recurrence rate in the ear drop user group (11.1%) compared to the non-user group (7.8%) indicates a potential association between ear drop usage and recurrent otomycosis.

Several studies have examined the factors contributing to the development and recurrence of otomycosis. Ho *et al.*⁵ have identified the use of ototopical antibiotics as a significant risk factor due to their suppression of bacterial flora, which normally inhibit fungal colonization. Similarly, Munguia and Daniel³ have noted that inappropriate or prolonged use of ear drops may promote fungal growth.

Finally, the demographic data from this study,

showing a higher prevalence of otomycosis in males, may reflect occupational or behavioural factors that increase exposure to fungal pathogens. The mean age of patients (35 years for men and 34 years for women) suggests that otomycosis primarily affects middle-aged adults, consistent with findings from other epidemiological studies^{7,8}. Further research is warranted in order to explore alternative treatments and preventive strategies aimed at reducing the incidence and recurrence of otomycosis in patients using ear drops.

4. Conclusion

This study highlights a notable association between recent ear drop usage and the occurrence of otomycosis, with ciprofloxacin identified as the most frequently implicated agent. Although both ear drop users and non-users demonstrated favourable treatment outcomes, the recurrence rate was higher among those with recent ear drop use. These findings underscore the need for heightened clinical awareness regarding the potential risk of otomycosis associated with topical ear drop application.

Acknowledgements

The author gratefully acknowledges the support provided by Al Mustaqbal University for the completion of this study (grant number: MUC-M-0222).

Conflicts of interest

None exist.

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HOW TO CITE:

Almaamuri A.M. A prospective cohort study on the association between ear drop use and otomycosis: prevalence, treatment outcomes, and recurrence. *Pharmakeftiki* 37(2s), 104-107, 2025. <https://doi.org/10.60988/p.v37i2S.153>

Evaluation of the serum levels of tumor necrosis factor-alpha in Iraqi patients with autoimmune thyroid disorders: a study from the Najaf Governorate

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KEY WORDS:

autoimmune thyroid disease;
Hashimoto's thyroiditis;
Graves' disease; TNF- α ; Iraq

ARTICLE INFO:

Received: January 12, 2025

Revised: February 28, 2025

Accepted: February 28, 2025

Available online: October 10, 2025

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ABSTRACT

Thyroid disorders frequently arise due to iodine deficiency or autoimmune conditions. Autoimmune thyroid disorders, such as Graves' disease (GD) and Hashimoto's thyroiditis (HT), result from dysregulation of the immune system. In this study, a total of 140 samples were analysed using immunodiagnostic methods. Participants were divided into two groups: 70 healthy controls and 70 patients (35 diagnosed with GD and 35 with HT), all recruited from medical facilities in the Najaf Governorate (Iraq) between July and October 2023. Participant ages ranged from 20 to over 50 years. Thyroid hormone levels – triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) – were measured in order to classify cases as hypothyroid or hyperthyroid. Results showed significantly reduced levels of T3, T4, and TSH in patients with hypothyroidism. On the other hand, the levels of anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase (anti-TPO) antibodies were markedly elevated in patients with HT or with GD compared to the control group. HT was more prevalent among women aged 30 to 50 years. Smoking was found to be significantly associated with the presence of thyroid disorders. A notable difference in mean tumor

necrosis factor-alpha (TNF- α) concentrations was observed between patients with GD and the control group, whereas no significant difference was found for patients with HT. The study concludes that TNF- α levels vary significantly between autoimmune thyroid conditions, underscoring its potential role in disease pathogenesis.

1. Introduction

Autoimmune diseases affect approximately 5% of the global population and remain challenging to treat, causing significant distress for patients and imposing a substantial economic burden¹. These conditions arise when the immune system fails to recognize self-antigens, leading to chronic inflammation and tissue damage². Autoimmune disorders may target specific organs or affect multiple systems. They are more prevalent in middle-aged women and represent a major cause of morbidity and mortality in this demographic. Moreover, the prevalence of autoimmune diseases increases with age and is consistently higher in women than in men³.

The occurrence of multiple autoimmune disorders in a single individual ("polyautoimmunity") or within a family ("familial autoimmunity") supports the hypothesis that these diseases share common environmental, epigenetic, and genetic triggers⁴. Autoimmune thyroid diseases are the most common organ-specific autoimmune disorders, affecting 2%–5% of the population. Their prevalence is significantly higher in women (5%–15%) than in men (up to 5%)⁵. Autoimmune thyroid diseases include Graves' disease (GD; which causes hyperthyroidism) and Hashimoto's thyroiditis (HT; which leads to hypothyroidism). Thyroid hormones – triiodothyronine (T3) and thyroxine (T4) – regulate metabolism and are synthesized by the thyroid gland. T3, the more active form, is derived from T4 as needed. Disruptions in thyroid function can result in iodine deficiency, thyroid enlargement, or even thyroid cancer, and they are among the most common endocrine disorders globally⁶. Hyperthyroidism is

characterized by suppressed thyroid-stimulating hormone (TSH) levels and elevated T3 and / or T4 concentrations. GD is marked by the presence of thyroid-stimulating autoantibodies that activate the TSH receptor⁷. In contrast, HT is a leading cause of hypothyroidism and is associated with chronic inflammation and the presence of anti-thyroid peroxidase (anti-TPO) antibodies that damage thyroid cells⁸.

Cytokines such as tumor necrosis factor-alpha (TNF- α) play a critical role in regulating inflammation and immune responses. TNF- α has been implicated in the development and progression of autoimmune and inflammatory diseases⁹. This study's aim was to explore the relationship between serum TNF- α levels and autoimmune thyroid disorders in Iraqi patients.

2. Methodology

This study included 140 participants, divided into two groups: 70 healthy controls and 70 patients with autoimmune thyroid disorders (35 with GD and 35 with HT). Participants were recruited from the Al-Sadr Medical City (Diabetes and Endocrinology Center) and the Al-Zahraa Teaching Hospital in Najaf Governorate between July and October 2023. Ages ranged from 20 to over 50 years. Thyroid hormone levels (T3, T4, and TSH) were measured in order to classify the study's participants as hypothyroid or hyperthyroid. Anti-thyroglobulin (anti-TG) and anti-TPO antibody levels were also assessed. TNF- α concentrations were measured in order to evaluate their association with thyroid dysfunction. Statistical analysis was conducted using SPSS version 26. A *p*-value lower than 0.05 was considered statistically

Table 1. Serum levels of biomarkers in patients with autoimmune thyroid diseases and healthy controls. Abbreviations used: anti-TG, anti-thyroglobulin (antibody); anti-TPO, anti-thyroid peroxidase (antibody); GD, Graves' disease; HT, Hashimoto's thyroiditis; SD, standard deviation; T3, triiodothyronine; T4, thyroxine; TNF- α , tumor necrosis factor-alpha; TSH, thyroid-stimulating hormone.

Serum biomarkers	Groups	N	Mean	SD	p-value	95% confidence interval	
						Lower bound	Upper bound
TSH (μ UI/mL)	HT group	35	23.27	2.68	<0.001	15.48	31.05
	GD group	35	0.34	0.13	<0.001	0.01	0.24
	Control group	70	2.43	1.67	<0.001	2.03	2.82
T3 (nmol/L)	HT group	35	0.67	0.80	0.330	0.40	0.95
	GD group	35	1.53	1.13	<0.001	1.14	1.92
	Control group	70	1.18	0.50	0.330	1.06	1.30
T4 (nmol/L)	HT group	35	5.01	2.27	<0.001	4.23	5.79
	GD group	35	12.72	9.53	0.002	9.45	15.99
	Control group	70	31.62	40.99	<0.001	21.85	41.39
anti-TPO (pg/mL)	HT group	35	928.76	685.75	0.031	693.20	1,164.32
	GD group	35	1,297.85	2,350.91	0.002	490.28	2,105.42
	Control group	70	159.37	240.77	0.031	101.96	216.78
anti-TG (pg/mL)	HT group	35	722.89	798.73	0.276	448.52	997.26
	GD group	35	608.76	361.69	<0.001	484.52	733.01
	Control group	70	123.92	87.72	0.276	103.01	144.84
TNF-α (pg/mL)	HT group	35	27.21	25.86	0.330	18.33	36.09
	GD group	35	22.37	18.20	<0.001	16.12	28.62
	Control group	70	25.28	13.94	0.330	21.96	28.60

significant. Ethical approval was granted by the Ethics Committee of the College of Medicine, University of Kufa (19-Sep-2023).

3. Results and Discussion

The distribution of age groups revealed a higher prevalence of thyroid disorders among participants aged 30 to 50 years. Among those with hyperthyroidism, 48.6% fell within this age range, while 68.5% of hypothyroid patients were also in the 30–50 age group. However, this difference in age distribution was not statistically significant ($p=0.49$). Gender analysis showed that thyroid disorders were more common in women, who comprised 75.7% of all participants. Within the patient groups, 82.8% of individuals with HT and 80% of those with GD were women, compared to 70% in the control group.

This difference in gender distribution was also not statistically significant ($p=0.277$).

Body mass index (BMI) analysis categorized participants as normal weight (18–24.9 kg/m²), overweight (25–29.9 kg/m²), or obese (≥ 30 kg/m²). Significant differences in BMI were observed between patients and healthy controls ($p=0.0163$), with obesity being more common among those with thyroid disorders: 40% of HT patients and 25.7% of GD patients were obese, compared to 14.3% of the control group. Smoking was also significantly associated with thyroid disorders ($p=0.0133$); 82.8% of hyperthyroid patients and 77.1% of hypothyroid patients were smokers, compared to only 21.6% of healthy controls. The analysis of the serum thyroid hormone levels revealed significant differences between the herein assessed groups. HT patients had markedly

elevated mean TSH levels of 23.27 $\mu\text{IU/mL}$, compared to 2.43 $\mu\text{IU/mL}$ in controls ($p<0.05$), while GD patients had significantly lower mean TSH levels of 0.34 $\mu\text{IU/mL}$ (Table 1). T3 and T4 levels were found to be significantly elevated in GD patients compared to both HT patients and controls ($p<0.05$) (Table 1). The levels of anti-TPO and anti-TG antibodies were also found to be significantly higher in patients with autoimmune thyroid disorders than in controls (Table 1). Finally, the mean serum concentration of TNF- α differed significantly across groups: GD patients had a mean level of 22.369 pg/mL, HT patients had a higher mean of 27.209 pg/mL, and the control group had a mean of 25.280 pg/mL (Table 1). A statistically significant difference ($p=0.0001$) was observed between the GD and the control group in terms of the TNF- α levels, although the difference between HT and controls was not statistically significant.

4. Conclusion

Our study has identified significant differences in serum TNF- α levels among patients with autoimmune thyroid disorders, underscoring the biomarker's potential role in the pathogenesis of these conditions. Notably, TNF- α concentrations were higher in patients with HT compared to those

with GD, suggesting that pro-inflammatory cytokines may contribute differently to the development and progression of these disorders. These findings highlight the importance of further research into the role of inflammatory mediators such as TNF- α in autoimmune thyroid diseases.

Acknowledgements

We thank the University of Kufa, College of Medicine, for their support. We extend our sincere gratitude to Prof. Dr Sabah Al-Fatlawi (Medical Consultant) and Prof. Dr Abdulkareem Abdullah Mahmood (Statistical Consultant). We also express our heartfelt appreciation to the staff of Al-Sadder Medical City and Al-Sadiq Specialized Laboratory. Finally, we are deeply grateful to the patients who participated in this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Almayahi T.A.M., Al-Timimi T.G.Y., Al-Muhanna E.H., Alzamly A.H.O., Al-Juaifari A.N.A. Evaluation of the serum levels of tumor necrosis factor-alpha in Iraqi patients with autoimmune thyroid disorders: a study from the Najaf Governorate. *Pharmakeftiki* 37(2s), 108-112, 2025. <https://doi.org/10.60988/p.v37i2S.154>

Serum dehydroepiandrosterone sulfate and anti-Müllerian hormone levels as predictors of polycystic ovarian syndrome and premature ovarian failure in Iraqi women of reproductive age

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KEY WORDS:

anti-Müllerian hormone;
dehydroepiandrosterone
sulfate; polycystic ovary
syndrome; premature ovarian
failure; Iraq

ARTICLE INFO:

Received: January 09, 2025

Revised: February 04, 2025

Accepted: February 10, 2025

Available online: October 10, 2025

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ABSTRACT

Infertility is defined as the inability of a couple to achieve pregnancy after one year of regular, unprotected sexual intercourse. Among its many causes are ovulatory disorders such as polycystic ovary syndrome (PCOS), primary ovarian insufficiency (POI; formerly known as "premature ovarian failure"), and anatomical abnormalities of the reproductive system. This case-control study included 120 women aged 20–40 years, divided into two groups: 60 healthy controls and 60 infertile patients, comprising 47 with PCOS and 13 with POI. Data were collected between November 2023 and May 2024 at the Gynaecology Hospital in Kerbala (Iraq). The serum levels of dehydroepiandrosterone sulfate (DHEA-S), anti-Müllerian hormone (AMH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol (E2), prolactin, and testosterone were measured using enzyme-linked immunosorbent assay (ELISA). In the PCOS group, serum AMH and DHEA-S levels were found to be significantly elevated ($p < 0.0001$), while in the POI group, both markers were found to be significantly reduced ($p < 0.0001$), compared to those of the control group. Moreover, in the PCOS group, the serum FSH levels were found to be significantly decreased ($p < 0.0001$), whereas the prolactin, tes-

tosterone, E2, and LH levels were found to be elevated. Conversely, in the POI group, the serum FSH, prolactin, testosterone, and LH levels were found to be significantly increased ($p<0.0001$), while the E2 levels were found to be decreased when compared to those of the control group. These findings suggest that AMH and DHEA-S are reliable biomarkers for assessing ovarian function, and could serve as sensitive indicators for the early diagnosis of PCOS and POI in women of reproductive age.

1. Introduction

Infertility refers to the inability to achieve pregnancy after one year of frequent, unprotected, and regular sexual intercourse¹. It is now recognized as a global health concern by the World Health Organization, affecting approximately 48.5 million couples worldwide. Among the causes of female infertility are ovulatory disorders (25%), tubal factors (20%), and cervical abnormalities (approximately 3%)². Ovarian disorders represent a significant clinical challenge and encompass several conditions. One of the most prevalent is polycystic ovary syndrome (PCOS); an endocrine disorder affecting women of reproductive age. PCOS is considered the most common cause of anovulatory infertility³. Although the precise pathophysiology of PCOS remains unclear, it is believed to involve hormonal imbalances, particularly elevated levels of androgens and other reproductive hormones⁴. Primary ovarian insufficiency (POI), previously referred to as "premature ovarian failure", is another complex reproductive endocrine disorder, with a global prevalence of approximately 3.5%⁵. POI is characterized by impaired ovarian function, leading to reduced oestrogen production, altered gonadotropin hormone levels, and amenorrhea, ultimately resulting in infertility⁶.

Dehydroepiandrosterone sulfate (DHEA-S) is the most abundant circulating steroid hormone and serves as a key precursor of adrenal androgens⁷. It is secreted primarily by the zona reticularis of the adrenal cortex, as well as by the ovarian theca cells, the brain, and the gonads. DHEA-S plays a central role in the biosynthesis of oestradiol (E2) and testosterone.

A diminished ovarian reserve (defined as a reduced quantity and quality of oocytes) has been associated with low DHEA-S levels. Conversely, elevated DHEA-S levels are often observed in women with PCOS; the leading cause of female infertility⁸.

The anti-Müllerian hormone (AMH) is a glycoprotein secreted by granulosa cells of preantral and small antral follicles in the ovaries. It plays a critical role in folliculogenesis and growth differentiation. AMH is considered a highly specific and reliable marker of ovarian reserve, as it reflects the size of the growing follicular pool. Studies have shown that infertile women tend to have higher AMH levels than healthy controls, underscoring its utility in assessing ovarian health, infertility risk, and miscarriage potential. Moreover, aging significantly influences AMH levels, with women over the age of 35 typically experiencing reduced fertility and oocyte production⁹.

The primary objective of this study was to investigate the relationship between ovulatory infertility and the serum levels of AMH and DHEA-S in Iraqi women.

2. Methodology

A case-control study was conducted in order to evaluate the fertility status and the hormonal profiles associated with POI and PCOS among women in Karbala, Iraq. Data were collected between November 2023 and May 2024 from the Fertility Unit at the Womens Obstetrics and Gynaecology Hospital in Karbala (Iraq). Participants were divided into two main groups: group A (consisted of 60 healthy women with no history of infertility, serving as the control group) and group B (consisted of 60 infertile wom-

Table 1. Comparison of the serum levels of the herein studied biomarkers in polycystic ovary syndrome (PCOS), and primary ovarian insufficiency (POI) infertile patients, as compared to healthy control women. Abbreviations used: AMH, anti-Müllerian hormone; DHEA-S, dehydroepiandrosterone sulfate; E2, oestradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SD, standard deviation.

Parameters	Groups	Mean	SD	p-value
AMH (ng/mL)	PCOS group	10.99	2.37	0.0002
	POI group	0.6	0.39	
	Control group	3.11	0.60	
DHEA-S (µL)	PCOS group	2.79	0.53	<0.0001
	POI group	0.54	0.23	
	Control group	0.89	0.12	
FSH (mIU/mL)	PCOS group	5.83	2.30	0.0002
	POI group	33.51	3.61	
	Control group	8.10	2.15	
Prolactin (ng/mL)	PCOS group	29.24	4.99	0.0007
	POI group	26.06	3.04	
	Control group	13.44	3.30	
Testosterone (pg/mL)	PCOS group	58.53	17.39	0.0004
	POI group	7.24	2.01	
	Control group	5.34	0.90	
E2 (pg/mL)	PCOS group	186.25	48.23	<0.0001
	POI group	10.07	1.43	
	Control group	24.93	7.23	
LH (mIU/mL)	PCOS group	17.15	5.81	<0.0001
	POI group	19.77	2.88	
	Control group	2.93	1.28	

en diagnosed with ovarian disorders). This patient group was further subdivided into group I (consisting of 47 women diagnosed with PCOS) and group II (consisting of 13 women diagnosed with POI). Diagnoses were confirmed by consultant gynaecologists using transvaginal pelvic ultrasound (Toshiba Xario prime; UK), while PCOS was diagnosed according to the Rotterdam criteria.

The serum levels of AMH, DHEA-S, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, E2, and testosterone were measured by using an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (ELISA Kit; Cloud-Clone Corp., USA).

All participants provided informed consent prior to enrolment, in accordance with the Declaration of

Helsinki. The study protocol and data collection procedures were reviewed and approved by the Ethics Committee of the Department of Clinical Laboratories of the College of Applied Medical Sciences of the University of Kerbala (approval number: IQ.UOK.CAMS.DCL.REC.4; date: 1/9/2024).

Statistical analysis was performed by using SPSS version 23. A *p*-value lower than 0.05 was considered as statistically significant. The homogeneity of variance was assessed using Levene's test, while data normality was evaluated using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used for comparisons across groups, followed by Scheffé's and Duncan's *post hoc* tests for within-group comparisons. Graphs were generated using GraphPad Prism version 9.

3. Results and Discussion

In the PCOS group, serum AMH and DHEA-S levels were found to be significantly elevated ($p < 0.0001$), while in the POI group, both markers were found to be significantly reduced ($p < 0.0001$), compared to those of the control group (Table 1). Moreover, in the PCOS group, the serum FSH levels were found to be significantly decreased ($p < 0.0001$), whereas the prolactin, testosterone, E2, and LH levels were found to be elevated (Table 1). Conversely, in the POI group, the serum FSH, prolactin, testosterone, and LH levels were found to be significantly increased ($p < 0.0001$), while the E2 levels were found to be decreased when compared to those of the control group (Table 1).

The elevated serum AMH levels observed in PCOS patients may be attributed to the increased number of small antral and preantral follicles, which are known to secrete AMH. It has been reported that granulosa cells in PCOS follicles produce up to 75 times more AMH than those in normal follicles. In contrast, the markedly reduced AMH levels in POI patients reflect diminished ovarian reserve and declining follicular activity, thereby reinforcing AMH's role as a sensitive biomarker for the early detection of POI.

The elevated serum DHEA-S levels observed in our PCOS patients may result from increased adrenal androgen production, possibly triggered by stress-related activation of the hypothalamic–pituitary–adrenal axis. This elevation contributes to the endocrine and metabolic disturbances characteristic of PCOS. Conversely, the reduced serum DHEA-S levels observed in our POI patients are consistent with impaired steroidogenesis and diminished ovarian reserve, as DHEA-S is a precursor in oestrogen biosynthesis.

On the other hand, the significant increase in the serum FSH levels among POI patients is likely due to reduced oestrogen feedback on the hypothalamic–pituitary axis, prompting compensatory gonadotropin release. This elevation correlates with the severity of ovarian failure. In PCOS, FSH levels were found decreased, likely due to disrupted gonadotropin-releasing hormone pulsatility, which favours LH over FSH secretion, thereby contributing to follicular arrest and anovulation.

Serum prolactin levels were found to be elevated in both PCOS and POI groups. In PCOS, hyperprolactinaemia may contribute to anovulation and polycystic ovarian morphology, although the underlying mechanisms remain unclear. In POI, the elevated prolactin may interfere with the synthesis of oestrogen and progesterone, further disrupting ovulatory function and menstrual regularity.

Serum testosterone levels were found to be markedly elevated in PCOS patients; a finding that is consistent with the hyperandrogenic profile of the syndrome. This may be the result of an increased LH-induced stimulation of the theca cells or insulin resistance, both of which enhance androgen production. In POI, serum testosterone levels were only found to be mildly elevated, likely due to residual adrenal or ovarian androgen secretion despite diminished ovarian function.

Serum LH levels were significantly elevated in both patient groups. In PCOS, this may reflect reduced hypothalamic sensitivity to negative feedback from E2 and progesterone, contributing to the hyperandrogenic state. In POI, elevated LH may result from diminished ovarian hormone production and impaired feedback regulation.

Finally, serum E2 levels were found to be significantly elevated in PCOS patients, likely due to an increased aromatization of androgens in the ovarian theca cells under elevated LH-induced stimulation. This hormonal imbalance contributes to follicular arrest and oligo-ovulation. In contrast, POI patients exhibited significantly reduced serum E2 levels, reflecting impaired follicular development and oestrogen synthesis due to ovarian insufficiency.

At this point, one should admit that a key limitation of this study is the relatively small sample size of POI patients. Future research should aim to include a larger cohort in order to enhance the statistical power and generalizability of the findings.

4. Conclusion

Our study has concluded that the serum AMH and DHEA-S were significantly elevated in women with PCOS compared to those of healthy controls, where-

as these hormone levels were markedly reduced in women with POI. These hormonal alterations may play a role in the pathophysiology of both the reproductive and metabolic features of PCOS and POI. Furthermore, AMH and DHEA-S may serve not only as sensitive biomarkers for ovarian reserve and early diagnosis, but also as potential surrogate indicators of cardiovascular risk; an area warranting further investigation.

Acknowledgements

The authors gratefully acknowledge the support of

the College of Applied Medical Sciences of the University of Kerbala, as well as of the Infertility Unit of the Womens Obstetrics and Gynaecology Hospital.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Mousawie Z.A.A.A.J., Al-Fatlawi A.C.Y., Al-Tamimi M.N.A. Serum dehydroepiandrosterone sulfate and anti-Müllerian hormone levels as predictors of polycystic ovarian syndrome and premature ovarian failure in Iraqi women of reproductive age. *Pharmakeftiki* 37(2s), 113-117, 2025.
<https://doi.org/10.60988/p.v37i2S.155>

Histological changes in the brain and liver of high-fat diet-fed male rats as a result of orlistat administration

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KEY WORDS:

histological changes; orlistat;
rat; brain; liver

ARTICLE INFO:

Received: December 28, 2024

Revised: January 13, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

ABSTRACT

Obesity is a disorder characterized by excessive body weight gain. Orlistat is one of the medications used in the treatment of obesity, and acts by inhibiting gastric and pancreatic lipases. The objective of this study was to examine the short-term effects of orlistat on liver and brain tissues in rats fed a high-fat diet. In this study, 20 male albino rats (weighing approximately 193–212 g) were divided into two groups: group I (control group; consisting of five rats administered distilled water daily) and group II (orlistat group; comprising 15 rats given a daily oral dose of orlistat at a dose of 32 mg/kg body weight per day, *via* gastric tube, for 10 weeks). At the end of the experimental period, the animals were sacrificed, and the liver and brain tissues were prepared for histological analysis. Rats treated with orlistat exhibited degenerative changes in both liver and brain tissues compared to the control group. In the liver, these changes included dilation, blood vessel congestion, and lymphocyte infiltration. In the brain, the observed changes were characterized by the infiltration of neutrophils or mononuclear leukocytes. In conclusion, orlistat exerts harmful effects on liver and brain tissues; therefore, its clinical use should be closely monitored.

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1. Introduction

Obesity is one of the leading causes of mortality worldwide. It is considered by health professionals

to be one of the most significant public health challenges of the twenty-first century¹. In order to address weight-related disorders, a lipophilic medication known as

orlistat (Xenical) is commonly used. Orlistat is a gastrointestinal lipase inhibitor that treats obesity by preventing the absorption of dietary fat. By inhibiting pancreatic lipase, it allows approximately 30% of dietary lipids to pass through the intestine undigested². It is available in 120-mg capsule form. Xenical is prescribed for individuals with a body mass index (BMI) of 30 or higher, or for overweight individuals (BMI $\geq$ 28) who are at risk of obesity-related diseases. It should not be used if a meal is skipped or contains no fat³. Severe adverse effects of Xenical include intense abdominal pain, haematuria, shortness of breath, loss of appetite, and jaundice. It should not be used by individuals allergic to its components⁴. The aim of this study was to examine the short-term effects of orlistat on liver and brain tissues in rats fed a high-fat diet.

2. Methodology

Twenty male albino rats (weighing approximately 193–212 g) were used in this study, conducted between December 2022 and March 2023 at the Animal House of the College of Pharmacy of the University of Babylon. The animals were fed a high-fat diet obtained from Research Diets (New Brunswick, USA), were given tap water, and were maintained under controlled environmental conditions. All procedures adhered to the guidelines of the institutional ethics committee (approval date: 11/03/2022; protocol no.: A-0014). After a 1-week acclimation period, the rats were divided into two groups: group I (consisting of five healthy control rats that received distilled water daily) and group II (comprising fifteen rats administered orlistat dissolved in distilled water, *via* gastric tube, at a dose of 32 mg/kg body weight per day, for 10 weeks). Orlistat was supplied by the Hikma Pharmaceutical Company (Jordan). At the end of the experimental period, the animals were sacrificed. Liver and brain tissue samples were collected from each rat and were prepared for histological analysis following the methodology described by Suvarna *et al.*⁵. Tissue sections were examined and photographed using a light microscope.

3. Results

As shown in Figures 1A–1C, the liver tissue of control rats appeared normal, whereas orlistat-treated rats exhibited pathological changes such as dilation, vascular congestion, and lymphocyte infiltration. Similarly, while the brain tissue of control rats maintained normal architecture (Figure 1D), the brain tissue of orlistat-treated rats showed signs of inflammation (Figure 1E).

4. Discussion

In the present study, orlistat administration induced histological alterations in the liver and brain tissues of male rats that were fed a high-fat diet. The lymphocyte infiltration observed in the liver tissues is consistent with previous findings^{6,7}, suggesting that reactive oxygen species and lipid peroxidation products may cause mitochondrial dysfunction leading to apoptosis and necrosis, and initiating a cascade that results in fibrosis and collagen deposition. Filippatos *et al.*⁸ have also reported mononuclear cell infiltration associated with the release of inflammatory mediators (such as tumour necrosis factor- α), which are likely contributors to liver damage. In our study, the orlistat-treated group exhibited significantly dilated and congested hepatic vessels, aligning with previous reports of congested portal and central veins⁷. The observed vascular dilation may reflect metabolic disturbances in the liver, thereby suggesting a potential hepatotoxic effect of orlistat⁷.

Histological abnormalities in brain tissue were noted in group II, including infiltration by neutrophils and mononuclear leukocytes. Immune cell-mediated inflammation is a key mechanism linking obesity to insulin resistance, and may underlie the observed brain damage⁹. Additionally, orlistat has been shown to alter the histological structure of the hypothalamus, thereby affecting levels of various neuropeptides and central monoamine neurotransmitters⁹.

5. Conclusion

Our study concludes that orlistat can induce patho-

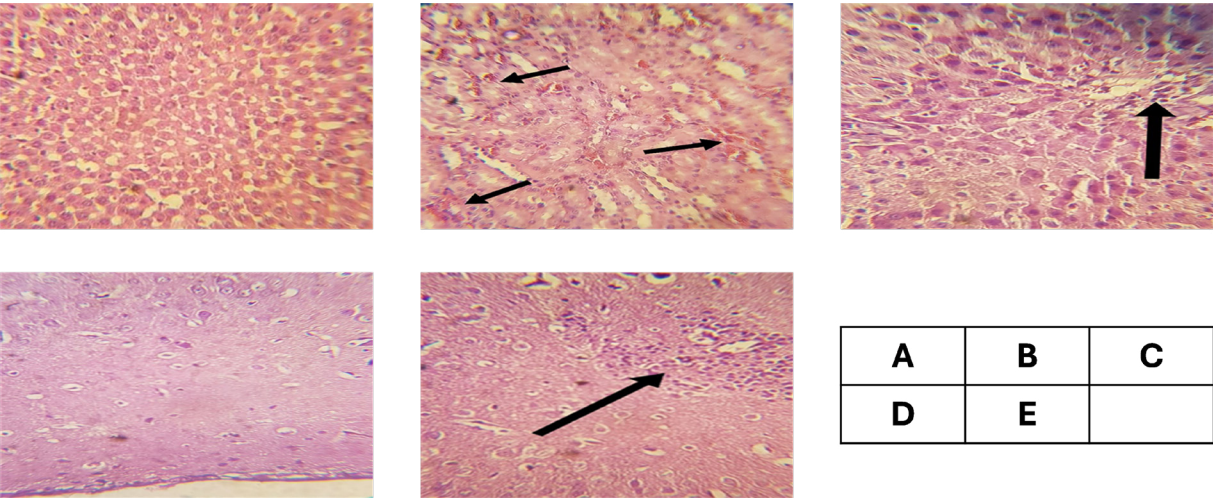


Figure 1. Microphotographs of rat liver and brain tissue sections, stained with haematoxylin and eosin ($\times 400$). (A): Liver tissue section from a group I (control) rat showing normal histological architecture. (B): Liver tissue section from a group II (orlistat-treated) rat displaying dilated and congested blood vessels (black arrows). (C): Liver tissue section from a group II (orlistat-treated) rat showing lymphocyte infiltration (black arrow). (D): Brain tissue section from a group I (control) rat exhibiting normal histological structure. (E): Brain tissue section from a group II (orlistat-treated) rat showing infiltration by neutrophils or mononuclear leukocytes (black arrow).

logical changes in liver and brain tissues. Therefore, its use should be closely monitored by healthcare professionals, and patients should be observed for any unusual symptoms.

Acknowledgements

We express our sincere gratitude to the College of Pharmacy of the University of Babylon, for providing access to its research laboratories.

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Conflicts of interest

None exist.

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HOW TO CITE:

Almuttairi R.S., Alzubaidy F.M., Al-Taei A.H.O., Abdulhakeem Z.R. Histological changes in the brain and liver of high-fat diet-fed male rats as a result of orlistat administration. *Pharmakeftiki* 37(2s), 118-121, 2025. <https://doi.org/10.60988/p.v37i2S.156>

Protective effect of a combined glutathione and prednisolone administration on mice subjected to a lipopolysaccharide-induced cytokine storm

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KEY WORDS:

cytokine storm;
glutathione; lung injury;
lipopolysaccharide; mice

ARTICLE INFO:

Received: January 31, 2025

Revised: February 24, 2025

Accepted: February 25, 2025

Available online: October 10, 2025

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ABSTRACT

Cytokine storm is a life-threatening hyperinflammatory condition that can result from infectious diseases, including COVID-19, as well as from non-infectious autoimmune disorders. This study has investigated the potential of glutathione to mitigate a cytokine storm in mice, particularly when administered in combination with prednisolone; a synthetic glucocorticoid. Fifty male albino mice were randomly assigned into five groups. The negative control group received 1% dimethyl sulfoxide (DMSO) intraperitoneally (i.p.), while the positive control group received a single i.p. dose of lipopolysaccharide (LPS) at 5 mg/kg. The treatment groups received either glutathione (200 mg/kg i.p.), prednisolone (5 mg/kg i.p.), or a combination of both drugs, each administered as a single dose, 1 h prior to the LPS injection. After 24 h, blood samples were collected in order to assess the serum interleukin-6 (IL-6) levels, and lung tissues were harvested for histopathological analysis. The results demonstrated that glutathione, prednisolone, and their

combination can significantly reduce the LPS-induced elevation of IL-6 levels ($p<0.05$) and can ameliorate the LPS-induced histopathological damage in the lung tissues of mice. In conclusion, pre-treatment with glutathione, prednisolone, or their combination can effectively attenuate both the systemic IL-6 elevation and the pulmonary histopathological alterations associated with cytokine storm. Notably, the combined administration of glutathione and prednisolone exhibited synergistic protective effects, suggesting their potential utility as prophylactic agents against cytokine storm syndromes.

1. Introduction

The term “cytokine storm” encompasses a group of immune-mediated dysregulated conditions characterized by systemic inflammation and widespread tissue damage, which, if left unmanaged, may culminate in multi-organ failure¹. Among the pro-inflammatory mediators, interleukin-6 (IL-6) plays a central role; its serum concentration remains elevated during sustained inflammatory responses, whereas levels of other cytokines typically decline more rapidly².

Lipopolysaccharides (LPS), large molecules composed of lipid and polysaccharide components, are found in the outer membrane of Gram-negative bacteria and are known to trigger potent inflammatory responses³. Exposure to LPS has been implicated in the pathogenesis of various inflammatory diseases.

Glutathione is a naturally occurring tripeptide with flavanol-like properties, deriving from plant sources. It exhibits potent antioxidant, anti-inflammatory, and immunomodulatory activities, with a favourable safety profile⁴. In contrast, corticosteroids – long used for their immunosuppressive and anti-inflammatory effects – are associated with significant adverse effects, particularly at high doses or with prolonged use⁵. Given these considerations, the present study aimed at evaluating the anti-cytokine storm potential of glutathione, both alone and in combination with prednisolone; a synthetic corticosteroid.

2. Methodology

2.1. Drugs and chemicals

Glutathione and prednisolone (dry powder form) were obtained from Hangzhou Hyper Chem Ltd (China). LPS was purchased from Sigma-Aldrich (Germany). All biochemical, haematological, and IL-6 assay kits were sourced from Roche (Germany) and CUSABIO (USA).

2.2. Preparation of treatment solutions

Fresh stock solutions of glutathione were prepared at concentrations of 200 and 50 mg/kg by dissolving the compound in 1% dimethyl sulfoxide (DMSO), followed by dilution with distilled water. Prednisolone stock solutions (5 and 1.25 mg/kg) were similarly prepared using 1% DMSO.

2.3. Induction of cytokine storm

Cytokine storm was induced by an intraperitoneal (i.p.) injection of (*Escherichia coli*-derived) LPS at 5 mg/kg, administered in a 300-μL volume. The LPS stock solution was prepared at a concentration of 1 mg/mL, following the manufacturer's instructions⁶.

2.4. Animal groups and experimental design

Fifty male albino Swiss mice (25–35 g) were randomly divided into five groups (n=10 per group): (i) negative control group (received 1% DMSO i.p.), (ii) positive control (LPS) group (received LPS at 5 mg/kg i.p.), (iii) glutathione group (received glutathione at 200 mg/kg i.p., 1 h before LPS), (iv) prednisolone group (received prednisolone at 5 mg/kg i.p., 1 h be-

Table 1. Injury scores conferred by the histopathological assessment of murine tissues stained with haematoxylin and eosin. Abbreviations used: LPS, lipopolysaccharide; SD, standard deviation.					
Groups (n=10 mice each)	Injury score in tissues (mean ± SD)				p-value
	liver	kidney	heart	lung	
negative control	0	0	0	0	--
positive control (LPS)	8 ± 2.7	4 ± 1.5	2 ± 1.3	5 ± 1.4	<0.001 vs. negative control group
glutathione + LPS	1 ± 1.5	1 ± 1.3	1 ± 1.4	2 ± 1.3	<0.05 vs. positive control (LPS) group
prednisolone + LPS	3 ± 1.2	1 ± 0.5	1 ± 1.9	2 ± 1.6	<0.01 vs. positive control (LPS) group
glutathione + prednisolone + LPS	1 ± 0.7	1 ± 0.4	1 ± 1.2	1 ± 0.7	<0.001 vs. positive control (LPS) group

fore LPS), and (v) drug combination group (received a 25% of the aforementioned doses of glutathione and of prednisolone, i.p. 1 h before LPS).

2.5. IL-6 levels' measurement

At 24 h post-induction, blood samples (1–1.5 mL) were collected from the jugular vein under chloroform anaesthesia into Eppendorf tubes, and were allowed to clot for 15 min. Samples were centrifuged at 3,000 rpm for 10 min, and serum IL-6 levels were quantified using an enzyme-linked immunosorbent assay (ELISA). Following blood collection, all animals were euthanized *via* cervical dislocation for the collection of lung tissue samples and the undertaking of histopathological analysis.

2.6. Histopathological evaluation

Tissues were fixed, embedded in paraffin, and sectioned at an average thickness of 5 µm using a rotary microtome. Sections were deparaffinized using heat, xylene, and graded ethanol, were mounted on clean slides, and were stained with haematoxylin and eosin for microscopic examination.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using the SPSS software (version 20). A *p*-value below 0.05 was considered statistically significant.

2.8. Ethics approval

This study was approved by the Institutional Review Board of the College of Pharmacy of Al-Nahrain University, under document IRB/204 and approval code UNCOPIRB202402242 (dated: 24 February 2024). The protocol was reviewed in accordance with the latest ethical guidelines and research standards.

3. Results and Discussion

Serum IL-6 levels were quantified using ELISA and expressed in pg/mL. The positive control group (LPS-treated) exhibited a statistically significant elevation in IL-6 levels compared to the negative control group (*p*<0.001). Pre-treatment with glutathione, prednisolone, and their combination resulted in a significant (*p*<0.05), highly significant (*p*<0.01), and very highly significant (*p*<0.001) reduction in IL-6 levels, respectively, when compared to those of the LPS-only-treated group.

LPS is known to activate the expression of multiple pro-inflammatory cytokines and to increase the production of reactive oxygen species (ROS), thereby contributing to the development of a cytokine storm⁶. Previous studies have shown that both glutathione and prednisolone can attenuate IL-6 levels in LPS-induced cytokine storm models⁷. Notably, in the present study, a combination of reduced doses (25%) of glutathione and prednisolone was as effective as the full-dose monotherapies in significantly lowering the serum IL-6 levels in mice exposed to LPS, thereby suggesting a synergistic interaction.

The histopathological examination of lung tissue sections stained with haematoxylin and eosin has further supported these findings. The negative control group displayed normal pulmonary architecture, with intact alveoli and alveolar spaces (injury score = 0). In contrast, the LPS-treated group exhibited marked pathological changes, including vascular congestion, dense inflammatory cell infiltration, and destruction of the alveolar septa (mean injury score = 5). The glutathione-treated group showed congestion with haemorrhage and mild thickening of interalveolar septa, along with modest inflammatory cell infiltration (mean injury score = 2). On the other hand, the prednisolone-treated group exhibited slight congestion and localized septal thinning, with mild inflammatory cell infiltration (mean injury score = 2). Finally, the group treated with the drug combination demonstrated minimal inflammatory cell infiltration and slight congestion in the interalveolar septa (mean injury score = 1), thereby indicating the most pronounced histological improvement (Table 1).

The histopathological changes observed in the LPS group were very highly significant compared to those of the control group. The observed tissue damage is consistent with oxidative stress-induced inflammation, which can perpetuate a cycle of cellular injury and immune activation⁸. LPS is known to interact with toll-like receptors, activate the nuclear factor kappa B (NF- κ B) pathway, and promote the release of pro-inflammatory cytokines, nitric oxide, and ROS, leading to macrophage activation and inflammatory cell recruitment⁹. The lungs, due to their extensive surface area and vascular exposure, are particularly susceptible to inflammatory and oxidative insults. Additionally, the pulmonary capillary bed may receive cytokine-rich blood from the liver following hepatic injury, potentially explaining the lung's vulnerability to secondary damage. This aligns with previous studies reporting significant histopathological alterations in the lungs following LPS administration in murine models¹⁰.

Importantly, the pre-treatment groups (i.e., those receiving glutathione, prednisolone, or their combination) have exhibited statistically significant improvements ($p < 0.05$) in their lung histology com-

pared to that of the LPS-only-treated group. The protective mechanism likely involves glutathione's ability to downregulate NF- κ B expression and suppress oxidative stress, thereby reducing IL-6 production^{7,10}. Overall, the combined assessment of IL-6 levels and histopathological findings confirms that a pre-treatment with glutathione, prednisolone, or their combination through a single i.p. dose can confer substantial protection against the LPS-induced cytokine storm in mice.

4. Conclusion

A pre-treatment with glutathione and prednisolone can effectively mitigate the LPS-induced cytokine storm in mice, by reducing serum IL-6 levels and preserving lung tissue integrity. Notably, the combination of glutathione and prednisolone exhibits a synergistic protective effect, suggesting its potential as a corticosteroid-sparing strategy for managing cytokine storm-related pathologies.

Acknowledgements

The authors gratefully acknowledge the College of Pharmacy of Al-Nahrain University, for providing the facilities and institutional support necessary to carry out this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Rajhi S.H., Hamood H.M., Shihab E.M., Shareef S.M., Ridha-Salman H., Shahooth S.S., Attarbashee R.K., Abbas A.H., Adham L.S., Al-Bairmani R.J.H., Mohammed Z.S., Hajwal S.K. Protective effect of a combined glutathione and prednisolone administration on mice subjected to a lipopolysaccharide-induced cytokine storm. *Pharmakeftiki* 37(2s), 122-126, 2025. <https://doi.org/10.60988/p.v37i2S.157>

Apoptosis-mediated antitumor activity of the ethanolic extract of *Calotropis procera* flowers: *in vitro* testing on the MG63 human osteosarcoma cell line

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KEY WORDS:

apoptosis; cancer;
osteosarcoma; MG63;
Calotropis procera

ARTICLE INFO:

Received: January 24, 2025

Revised: February 08, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

ABSTRACT

Developing cancer therapeutics that selectively target malignant cells without harming healthy tissue or encountering resistance remains a central challenge in oncology. This study aimed at evaluating the effects of an ethanol (70%) extract of *Calotropis procera* L. flowers on the osteosarcoma MG63 cell line. Following serial dilution, the extract was assessed by using a multi-parameter cytotoxicity assay in order to measure cell viability (valid cell count), total nuclear intensity, mitochondrial membrane potential, cell membrane permeability, and cytochrome c release. These parameters were analysed via high-content screening (HCS) in order to determine the extract's therapeutic potential. The HCS results demonstrated that treatment with 200 µg/mL of the *Calotropis procera* flower extract can produce a highly significant effect across all five of the aforementioned parameters in MG63 cells.

1. Introduction

Cancer is characterized by the uncontrolled proliferation of cells that invade surrounding tissues and spread to distant sites through the lymphatic system and the bloodstream; a process known as "me-

tastasis"¹. Effective anticancer therapies must minimize harm to normal cells while targeting multiple oncogenic pathways². Conventional treatments such as chemotherapy and radiotherapy often impose significant physiological stress and may further compromise patient

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Table 1. High-content screening (HCS) assay of MG63 cells treated with various concentrations of an ethanolic <i>Calotropis procera</i> flower extract. Results are presented as mean $\pm$ standard deviation, in fluorescence intensity units. Identical superscript letters indicate no statistically significant difference ($p>0.05$) between the groups, while different superscript letters denote significant differences ($p<0.05$).					
<i>Calotropis procera</i> flower extract ($\mu\text{g/mL}$)	Valid cell count	Total nuclear intensity	Cell membrane permeability	Mitochondrial membrane potential	Cytochrome c release
untreated (control)	$3,539.5 \pm 159.09^a$	404 ± 24.04^c	204.5 ± 27.57^b	827 ± 8.48^a	463 ± 15.55^b
200	$2,070.5 \pm 119.50^c$	599.5 ± 16.26^a	294.5 ± 24.74^a	658.5 ± 20.50^c	600.5 ± 30.40^a
100	$2,222 \pm 60.81^c$	463.5 ± 17.67^b	183.5 ± 6.36^b	740.5 ± 2.12^b	577.5 ± 6.36^a
50	$3,024 \pm 212.13^b$	418.5 ± 3.53^c	188.5 ± 9.19^b	826.5 ± 7.77^a	454.5 ± 10.60^b
25	$3,593 \pm 63.64^a$	406.5 ± 30.40^c	192 ± 22.62^b	811 ± 11.31^a	444 ± 18.38^b

health. Consequently, there is growing interest in alternative and complementary therapies for cancer management.

Complementary medicine frequently incorporates crude plant materials in the form of extracts, powders, or tablets³. *Calotropis procera* L. belongs to the family Apocynaceae and is widely distributed across central and southern Iraq. Phytochemical analyses of its flower extract have revealed a rich profile of pharmacologically active compounds, including sterols (stigmasterol, sitosterol, campesterol), proceragenin, α -amyrin, β -amyrin, rosmarinic acid, and cinnamic acid^{4,5}. These bioactive compounds exhibit cytotoxic effects against various cancer cell types through diverse mechanisms, such as by inducing apoptosis, by inhibiting cell proliferation, by suppressing angiogenesis, and by modulating oxidative stress. As such, *Calotropis procera* represents a promising candidate for the development of novel anticancer agents with minimal toxicity to normal cells². Given the lack of prior research on the anticancer potential of *Calotropis procera* flower extracts, this study investigates their potential antitumor activity against the osteosarcoma cell line MG63.

2. Methodology

2.1. Preparation of a raw powder and ethanolic extract of *Calotropis procera* flowers

Fresh, fully bloomed, and healthy flowers of

Calotropis procera were collected in March 2024 from Hillah, Iraq. The plant material was identified and authenticated by Dr Shaimaa Mohi (Department of Biology, College of Science, University of Babylon). The dry powder of the *Calotropis procera* flowers was prepared according to Al Ghezil *et al.*⁶, while the ethanolic extraction was done according to Ekenyong *et al.*⁷, with modifications.

2.2. Cell line used

The osteosarcoma MG63 cell line was stored in the vapor phase of liquid nitrogen at temperatures below -130°C , in cryogenic vials. These vials were maintained at the Tissue Culture Laboratory of the Centre for Natural Product Research and Drug Discovery of the Department of Pharmacology of the Faculty of Medicine of the University of Malaya (Kuala Lumpur).

2.3. High-content screening (HCS)

HCS was performed by using the Cellomics Multiparameter Cytotoxicity Kit (Thermo Fisher Scientific, USA) in order to simultaneously evaluate multiple cellular parameters in the MG63 cell line. These parameters included valid cell count, total nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome c levels. The fluorescence distribution and intensity within the MG63 cells were captured and analysed using the ArrayScan HCS Reader, thereby enabling a quantita-

tive assessment of the cytotoxic effects induced by the *Calotropis procera* flower extract.

2.4. Statical analysis

Data were analysed using SPSS version 23. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used in order to determine significant differences between groups. A p -value lower than 0.05 was considered as statistically significant.

3. Results and Discussion

The HCS technology enables precise characterization of complex cellular behaviours. By combining automated high-throughput microscopy with advanced image analysis, HCS facilitates the exploration of intricate phenotypes and yields rich cellular data⁸. In this study, HCS was employed in order to assess apoptosis-related changes in the MG63 osteosarcoma cell line following treatment with four concentrations of an ethanolic *Calotropis procera* flower extract (200, 100, 50, and 25 µg/mL). The assay evaluated five key cellular parameters: valid cell count, total nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome c release.

As shown in Table 1, the 200 µg/mL concentration produced a highly significant effect across all parameters compared to untreated control cells. The 100 µg/mL concentration exhibited a statistically significant effect ($p < 0.05$) on valid cell count and cytochrome c release, with a less pronounced effect on nuclear intensity and mitochondrial membrane potential. Lower concentrations (50 and 25 µg/mL) yielded results comparable to those of the control group ($p > 0.05$).

The *Calotropis procera* flower extract has reduced valid cell counts and has suppressed cancer cell viability and proliferation, thereby suggesting cell cycle arrest at the G0/G1 phase. Morphological changes (such as cell rounding and size reduction) were indicative of apoptosis. The observed increase in nuclear intensity may reflect chromatin condensation;

a hallmark of apoptotic progression. Additionally, the extract has enhanced membrane permeability in MG63 cells, which typically exhibit rigid membranes and multidrug resistance due to elevated cholesterol content⁹. At higher concentrations, the extract has significantly decreased mitochondrial membrane potential and increased cytochrome c release. These effects suggest mitochondrial involvement in the apoptotic response. The phytochemicals in *Calotropis procera* flowers may act through multiple mitochondrial mechanisms, including oxidative stress induction, inhibition of the electron transport chain, modulation of apoptotic proteins, and disruption of energy metabolism¹⁰. Such mechanisms are desirable in the context of anticancer therapy. Future work should focus on evaluating gene expression profiles in cancer cells treated with the *Calotropis procera* flower extract in order to further elucidate its molecular mechanisms of action.

4. Conclusion

Based on our findings, the ethanolic extract of *Calotropis procera* flowers demonstrates the ability to induce apoptosis in cancer cells, highlighting its potential as a promising candidate for anticancer therapy.

Acknowledgements

The authors express their sincere gratitude to the staff of the Biotechnology Centre of the Al-Nahrain University, Baghdad, Iraq, for their assistance in conducting the assays, and to the reviewers for their valuable feedback and constructive suggestions.

Conflicts of interest

None exist.

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HOW TO CITE:

Alryahi I.A., Mohammed F.H., Al-Saily H.M.N. Apoptosis-mediated antitumor activity of the ethanolic extract of *Calotropis procera* flowers: *in vitro* testing on the MG63 human osteosarcoma cell line. *Pharmakeftiki* 37(2s), 127-130, 2025. <https://doi.org/10.60988/p.v37i2S.158>

Cytotoxic effect of the ethanolic extract of *Calotropis procera* flowers on human cancer cell lines

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KEY WORDS:
cancer; cytotoxicity;
osteosarcoma; glioblastoma;
Calotropis procera

ARTICLE INFO:

Received: January 25, 2025

Revised: February 08, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

ABSTRACT

Conventional cancer treatments are often associated with adverse effects, prompting researchers to investigate alternative anticancer agents deriving from natural sources, including traditional medicinal plants. One such plant, *Calotropis procera* L., is rich in phytochemicals with broad pharmacological potential. This study has evaluated the cytotoxic effects of its flower's extract on two cancer cell lines – human osteosarcoma (MG63) and human glioblastoma (A172) – using normal human dermal fibroblasts (neonatal; HdFn) as a control. The MTT assay was employed in order to assess cytotoxicity and apoptosis induction. Our results demonstrate a dose-dependent inhibition of cell growth. The most effective concentrations of the *Calotropis procera* flower extract were 400, 200, and 100 µg/mL, with MG63 cells showing greater sensitivity than A172 cells. No cytotoxicity was observed in HdFn cells. The IC₅₀ values were 213.7 µg/mL for MG63, 282.66 µg/mL for A172, and 844.08 µg/mL for HdFn. The selective cytotoxicity of the extract is attributed to the presence of potent phytochemicals that induce apoptosis in cancer cells without harming healthy cells. These findings suggest that the flower extract of *Calotropis procera* may serve as a promising and safe anticancer agent.

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1. Introduction

Cancer remains a major global health challenge, significantly

impacting human populations. There is a need for novel therapies that can treat and prevent this life-threatening disease without

Table 1. Viability of MG63, A172, and HdFn cells following a 24-h treatment with varying concentrations of a <i>Calotropis procera</i> flower extract. Identical superscript letters indicate no statistically significant difference ($p>0.05$) between the groups, while different superscript letters denote significant differences ($p<0.05$).					
Cell viability (%) (mean $\pm$ standard deviation)					
Cell lines	<i>Calotropis procera</i> flower (ethanolic) extract ($\mu\text{g/mL}$)				
	400	200	100	50	25
MG63	37.80 $\pm$ 1.88 ^c	43.17 $\pm$ 1.14 ^c	54.89 $\pm$ 1.09 ^c	64.04 $\pm$ 2.15 ^c	77.08 $\pm$ 2.23 ^c
A172	42.97 $\pm$ 4.46 ^c	51.65 $\pm$ 2.98 ^c	59.68 $\pm$ 1.54 ^c	73.95 $\pm$ 1.72 ^b	86.72 $\pm$ 1.30 ^b
HdFn	74.65 $\pm$ 2.57 ^a	86.34 $\pm$ 1.53 ^a	93.59 $\pm$ 2.10 ^a	94.17 $\pm$ 1.57 ^a	95.21 $\pm$ 0.82 ^a

causing adverse effects. The plant kingdom offers a vast reservoir of naturally-occurring secondary metabolites, many of which are under investigation for their anticancer properties and have contributed to the development of new drugs¹. *Calotropis procera* L., a wild-growing perennial flowering plant of the Apocynaceae family, is considered a rich source of diverse phytochemicals. Each part of the plant contains bioactive compounds with distinct therapeutic significance, including triterpenoids, anthocyanins, flavonoids, cardiac glycosides, cardenolides, α -amyrin, β -amyrin, lupeol, β -sitosterol, flavanols, resin, the potent bacteriolytic enzyme calactin, and the non-toxic proteolytic enzyme calotropin. The plant has demonstrated a wide range of pharmacological activities, including antimicrobial, anthelmintic, anti-inflammatory, analgesic, antipyretic, anticancer, antiangiogenic, immunomodulatory, antidiabetic, cardiovascular, hypolipidaemic, gastroprotective, hepatoprotective, nephroprotective, antioxidant, anticonvulsant, and wound-healing effects². Despite its broad therapeutic potential, the anticancer efficacy of *Calotropis procera* flowers has not been previously explored. This study investigates the cytotoxic activity of the flower extract of *Calotropis procera* on MG63 (human osteosarcoma) and A172 (human glioblastoma) cells.

2. Methodology

2.1. Preparation of a raw powder of *Calotropis procera* flowers

Fresh, fully bloomed, and healthy flowers of

Calotropis procera were collected in March 2024 from Hillah, Iraq. The plant material was identified and authenticated by Dr Shaimaa Mohi (Department of Biology, College of Science, University of Babylon). The flowers were cleaned so as to remove dust and were air-dried in the shade at room temperature. Once dried, they were ground into a fine powder using an electric mill, and stored in a sterile, airtight container for later use³.

2.2. Preparation of a *Calotropis procera* flower (ethanolic) extract

The powdered flowers were made into an extract using 70% ethanol in a 1:10 (w/v) ratio. The mixture was shaken at 100 rpm for 2 h, followed by incubation in a water bath at 40°C for an additional 2 h. The extract was then filtered through filter paper, and the filtrate was dried in an oven at 45°C. The resulting solid extract was ground using an electric grinder, sterilized under UV light for 20 min, and stored in a sterile, dark, and tightly sealed vial⁴.

2.3. MTT assay

The MTT assay is a colorimetric method used in order to assess cell viability by measuring the reduction of yellow tetrazolium bromide to dark purple formazan crystals by the mitochondrial succinate dehydrogenase in metabolically active cells. The intensity of the colour, measured at 570 nm, correlates with the number of viable cells. The assay was performed using an MTT Kit (Intron Biotech, Korea), which includes MTT reagent and a solubilization

solution. A total of 100 µL of cell suspension (MG63, A172, or HdFn) was seeded into flat-bottomed microculture plate wells and incubated at 37°C in a 5% CO₂ atmosphere for 24 h. After incubation, 100 µL of the *Calotropis procera* flower extract at concentrations of 25, 50, 100, 200, or 400 µg/mL was added to the wells. Absorbance was measured using an ELISA reader at 570 nm. The cell viability (%) was calculated using the formula:

$$\text{cell viability (\%)} = (\text{absorbance of treated cells} / \text{absorbance of control}) \times 100$$

The control consisted of growth medium with MTT dye only, representing 100% viability. IC₅₀ values were calculated by using the GraphPad Prism software (multiple parameter analysis).

2.4. Statical analysis

Data were analysed using SPSS version 23. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used in order to determine significant differences between groups. A *p*-value lower than 0.05 was considered as statistically significant.

3. Results and Discussion

The cytotoxic effects of the *Calotropis procera* flower extract were evaluated against two human cancer cell lines, namely MG63 (osteosarcoma) and A172 (glioblastoma), using the MTT assay. This colorimetric method is based on the reduction of pale yellow tetrazolium salt (MTT) by mitochondrial dehydrogenase enzymes in viable cells, resulting in the formation of dark purple formazan crystals. These crystals accumulate within metabolically active cells, and their quantity is directly proportional to cell viability⁵.

To assess cytotoxicity, various concentrations (25, 50, 100, 200, and 400 µg/mL) of the 70% ethanolic extract of the *Calotropis procera* flowers were applied to MG63, A172, and normal human dermal fibroblast (HdFn) cells. After 24 h of incubation, a dose-dependent reduction in cell viability was observed, as shown in Table 1. The most pronounced

effects were seen at concentrations of 100, 200, and 400 µg/mL, with MG63 cells exhibiting greater sensitivity than A172 cells. Specifically, the MG63 cell viability decreased to 77.08%, 64.04%, 54.89%, 43.17%, and 37.80% across the five assessed concentrations, with an IC₅₀ value of 213.7 µg/mL. On the other hand, A172 cell viability was recorded at 86.72%, 73.95%, 59.68%, 51.65%, and 42.97%, with an IC₅₀ of 282.66 µg/mL. In contrast, HdFn cells maintained higher viability levels of 95.21%, 94.17%, 93.59%, 86.34%, and 74.65%, with an IC₅₀ of 844.08 µg/mL.

The extract demonstrated selective cytotoxicity, significantly reducing the viability of cancer cells while sparing normal cells (Table 1). MG63 cells were more susceptible to the extract than A172 cells. This selective cytotoxicity is likely attributed to the presence of bioactive phytochemicals in the *Calotropis procera* flower extract, such as cinnamic acid derivatives, rosmarinic acid, and β-amyrin, which have been shown to reduce cancer cell viability without affecting normal cells⁶⁻⁸. Supporting studies have reported similar findings, attributing the cytotoxic effects of *Calotropis procera* flower extract to its high polyphenolic content^{6,9} and its ability to elevate intracellular reactive oxygen species, thereby inducing cytotoxicity, apoptosis, and autophagy in cancer cells such as MCF-7¹⁰.

4. Conclusion

The ethanolic extract of *Calotropis procera* flowers exhibits potent cytotoxic activity against cancer cells (MG63 and A172), while sparing normal human dermal fibroblasts. This selective action suggests that the *Calotropis procera* flower extract might serve as a promising and safe anticancer agent, with minimal adverse effects on healthy human tissues.

Acknowledgements

The authors express their sincere gratitude to the staff of the Biotechnology Centre of the Al-Nahrain University, Baghdad, Iraq, for their assistance in con-

ducting the assays, and to the reviewers for their valuable feedback and constructive suggestions.

Conflicts of interest

None exist.

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HOW TO CITE:

Alryahi I.A., Mohammed F.H., Al-Saily H.M.N. Cytotoxic effect of the ethanolic extract of *Calotropis procera* flowers on human cancer cell lines. *Pharmakeftiki* 37(2s), 131-134, 2025.
<https://doi.org/10.60988/p.v37i2S.159>

Adverse effects of Herceptin treatment in women with breast cancer in Hillah (Iraq)

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KEY WORDS:

adverse effects; Herceptin;
trastuzumab; breast cancer;
Iraq

ARTICLE INFO:

Received: January 31, 2025

Revised: February 19, 2025

Accepted: February 20, 2025

Available online: TBC

ABSTRACT

Herceptin (trastuzumab) is a targeted anticancer therapy that has significantly improved outcomes in the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer since its introduction in 1998. This study has employed a retrospective cross-sectional design using a paper-based questionnaire administered to women with breast cancer and receiving Herceptin at the Oncology Center of the Imam Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq. The study period spanned from 1 November 2022 to 31 March 2023. A total of 50 Iraqi women aged 21 to 71 years participated in the study. The most common age groups were 41–50 and 51–60 years. Of the participants, 49 (98%) had undergone breast surgery. Seventeen distinct adverse effects associated with Herceptin use were reported, with all patients (100%) experiencing at least one. The most frequently reported effects included fatigue, exhaustion, and loss of energy, bone and muscle pain, headache, anorexia, gastrointestinal pain (cramping), sleep disturbances, visual disturbances, as well as bloating and increased abdominal size. These were followed by drowsiness, prolonged sleep, cardiac issues, anaemia, itching and skin allergies, as well as immunodeficiency. Less common adverse effects included hypertension, hyperglycaemia, kidney problems, and hypercholesterolaemia. Eighteen women (36%) experienced infusion-related reactions, including allergic-like responses and anaphylaxis. Other reported infusion-related reactions included vomiting, severe chills, muscle tremors, high fever, loss of motor control, coughing and choking, shortness of breath, jaw tremors, and temporary loss of speech.

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1. Introduction

Patients with HER2-positive breast cancer overexpress the human epidermal growth factor receptor 2 (HER2), which promotes cancer cell proliferation and is associated with a more aggressive disease course compared to other breast cancer subtypes. Herceptin (trastuzumab), a monoclonal antibody, is used as a targeted anticancer therapy. Since its introduction in 1998, Herceptin has significantly improved clinical outcomes in HER2-positive breast cancer. It functions by binding to HER2 on cancer cells, thereby inhibiting its growth-promoting activity^{1,2}. Despite its therapeutic benefits, Herceptin is associated with several notable adverse effects. These may include anaphylaxis upon initial administration, dermatological and nail changes, and haematological toxicities (particularly when combined with chemotherapy) which can lead to neutropenia or lymphopenia, thereby increasing susceptibility to infections³. Cardiac and pulmonary complications have also been reported³. Given the limited data from Iraq on this subject, the present study aimed at documenting the adverse effects of Herceptin treatment in women with breast cancer in a realistic clinical setting.

2. Methodology

This retrospective cross-sectional study employed a paper-based questionnaire targeting women with breast cancer who were receiving Herceptin (as immunotherapy) at the Oncology Centre of Imam Al-Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq. The study was conducted from 1 November 2022 to 31 March 2023. The chi-squared test was used in order to assess statistically significant relationships between variables. Participants who provided oral consent were interviewed prior to the administration of their Herceptin dose, with sensitivity to their psychological state. Patients receiving concurrent anticancer therapies alongside Herceptin were excluded in order to minimize confounding effects from overlapping treatment-related side effects. The study received ethical approval

from the Ethics Committee of the College of Pharmacy of the University of Babylon (approval number: A-0046; date: 9 February 2025).

3. Results and Discussion

Immunotherapy is among the most promising cancer treatments, developed to enhance the immune system's ability to combat malignancies. Cancer cells often exploit immune regulatory mechanisms in order to suppress immune responses within the tumor microenvironment during disease progression^{2,3}.

This study included 50 Iraqi women with HER2-positive breast cancer receiving Herceptin, aged between 21 and 71 years. The most common age group was 41–50 years (23 patients; 46%), followed by 51–60 years (18 patients; 36%). Published studies indicate that in developed countries, most women diagnosed with breast cancer are over 63 years old, whereas in developing countries, the majority are under 50 years old^{4,5}. Of the participants, 31 (62%) resided in urban areas. Educational attainment varied: 20 (40%) had completed primary education, 10 (20%) had attended middle or high school, 9 (18%) held undergraduate degrees, 3 (6%) had postgraduate qualifications, and 8 (16%) reported other educational backgrounds. Among the 50 women, 49 (98%) had undergone breast surgery: 37 (75.5%) have undergone mastectomy and 12 (24.5%) lumpectomy (Table 1). Most surgeries were performed on women over 40 years of age. Forty patients (80%) began Herceptin treatment postoperatively, while 10 (20%) received it preoperatively (Table 1). A common malpractice in Iraq involves breast cancer surgeries being performed by non-specialist surgeons, contributing to the high prevalence of total mastectomies, which can have profound psychological consequences for patients.

Seventeen distinct adverse effects of Herceptin were recorded, with all patients (100%) experiencing at least one (Table 1). However, a direct comparison of incidence rates with those reported in other studies is not appropriate due to differences in patient populations, study periods, drug manufacturers, and clinical settings. As shown in Table 1, the

Table 1. Adverse effects and infusion reactions associated with Herceptin treatment in the participants of the current study. Notes: *, signifies a statistically significant relationship; **, the total number of women who experienced an infusion reaction was 18 out of 50 (36%).

Variables		Total (%)	Age		Total	
			1-40 years	>40 years		
Did you have breast surgery?	yes	49 (98%)	5 (10%)	44 (90%)	50	
	no	1 (2%)	0 (0%)	1 (2%)		
Breast surgery	mastectomy	37 (75.5%)	2 (5%)	35 (95%)	49	
	lumpectomy	12 (24.5%)	3 (25%)	9 (75%)		
When did you start taking Herceptin?	before surgery	10 (20%)	1 (10%)	9 (90%)	50	
	after surgery	40 (80%)	4 (10%)	36 (90%)		
Number of doses given to patients up to the date of the study	1-10 doses	30 (60%)	4 (13%)	26 (87%)	50	
	11-20 doses	15 (30%)	3 (20%)	12 (80%)		
	≥21 doses	5 (10%)	0 (0%)	5 (100%)		
Adverse effects associated with Herceptin and their significant relationship with the age						
Adverse effects		Total (%)	Age		OR	p-value
			1-40 years	>40 years		
Fatigue, exhaustion, and loss of energy		43 (86%)	7	36	2.243	0.01 *
Bone and muscle aches		35 (70%)	2	33	0.005	0.012 *
Headaches		32 (64%)	3	29	1.911	0.011 *
Anorexia		29 (58%)	2	27	0.396	0.022 *
Gastrointestinal pain (cramping)		27 (54%)	5	25	1.189	0.03 *
Sleep disturbances		26 (52%)	2	24	0.015	0.022 *
Visual disturbances		25 (50%)	0	25	0.601	0.031 *
Bloating and increased abdominal size		25 (50%)	0	25	0.807	0.04 *
Drowsiness and prolonged sleep		22 (44%)	0	22	0.165	0.032 *
Heart problems		21 (42%)	3	18	1.624	0.203
Anaemia		14 (28%)	0	14	5.828	0.016 *
Itching and skin allergies		8 (16%)	0	8	3.935	0.047 *
Immunodeficiency		8 (16%)	1	7	0.557	0.809
Hypertension		7 (14%)	1	6	0.092	0.762
Hyperglycaemia		3 (6%)	1	2	0.018	0.893
Kidney problems		3 (6%)	1	2	0.002	0.962
Hypercholesterolaemia		2 (4%)	0	2	0.713	0.398
Infusion reactions **						
Reactions during Herceptin infusion		Of N=18 (%)	Reactions during Herceptin infusion		Of N=18 (%)	
Vomiting		12 (66.7%)	Loss of motor control		7 (38.9%)	
Severe chills		15 (83.4%)	Coughing and choking		7 (38.9%)	
Muscle tremors		10 (55.6%)	Loss of consciousness		5 (27.7%)	
Fever		12 (66.7%)	Jaw tremors and loss of speech control		3 (16.6%)	

most frequently reported side effects included fatigue, exhaustion, and loss of energy, bone and mus-

cle pain, headache, anorexia, gastrointestinal pain (cramping), sleep disturbances, visual disturbances,

as well as bloating and increased abdominal size. These were followed by drowsiness and prolonged sleep, cardiac issues, anaemia, itching and skin allergies, as well as immunodeficiency. Less common effects included hypertension, hyperglycaemia, kidney problems, and hypercholesterolaemia (Table 1). These findings are largely consistent with Herceptin's summary of product characteristics published by Roche.

A statistically significant relationship was observed between patient age and the majority of adverse effects, including fatigue, exhaustion, and loss of energy, musculoskeletal pain, headache, anorexia, gastrointestinal pain (cramping), sleep and visual disturbances, bloating, drowsiness, prolonged sleep, anaemia, itching and skin allergies (Table 1). Patients over 40 years of age were more likely to experience these effects. In contrast, no significant association was found between age and cardiac complications, immunodeficiency, or other less common adverse effects (Table 1).

Cardiomyopathy is a serious adverse effect of Herceptin, particularly when used alone or in combination with paclitaxel following an anthracycline-based chemotherapy. It can range from moderate to life-threatening severity and has led to treatment discontinuation in some patients. For early-stage breast cancer, cardiac function should be assessed at baseline, monitored every three months during treatment, and re-evaluated every 6 months for up to 24 months after treatment cessation^{1,2}.

Neutropenia is another serious concern, reported more frequently in patients receiving Herceptin in combination with chemotherapy than in those receiving chemotherapy alone. This underscores the importance of vigilant monitoring for signs of infection during Herceptin therapy⁶.

Despite Herceptin's clinical success, many of its mechanisms of action and long-term implications remain poorly understood. In severe cases, adverse effects such as congestive heart failure, significant reductions in left ventricular function, severe infusion reactions, and pulmonary toxicity may necessitate the discontinuation of therapy. Additionally,

therapeutic resistance to Herceptin has emerged as a major clinical challenge, emphasizing the need for further research into its mechanisms and potential new applications in order to enhance its clinical efficacy^{3,7}.

Of the 50 participants of our study, 18 (36%) experienced infusion-related reactions, including allergic-like responses and anaphylaxis. Symptoms included vomiting, severe chills, muscle tremors, fever, loss of motor control, coughing and choking, jaw tremors, and loss of speech control (Table 1). Infusion reactions typically occur within minutes to hours during or after administration. Herceptin can cause serious pulmonary toxicity, particularly in patients with pre-existing lung disease or metastatic lung tumours, leading to dyspnoea at rest. Early recognition and immediate clinical intervention are essential so as to ensure patient safety.

The absence of standardized protocols for the prevention, management, and reporting of infusion reactions represents a significant quality and safety gap in oncology care^{7,8}. Beyond the medical implications, Herceptin also imposes a substantial economic burden due to its high cost, placing significant strain on national healthcare budgets; particularly in resource-limited countries such as Iraq.

4. Conclusion

Although Herceptin represents a promising and effective treatment for HER2-positive breast cancer, it is associated with a broad spectrum of adverse effects. This survey-based study has documented these effects in a realistic clinical context, thereby contributing to a better understanding of patient experiences and treatment outcomes in Iraq.

Acknowledgements

We extend our sincere gratitude to the patients who generously provided their consent and cooperation, making this study possible. We also thank the dedicated staff at the participating oncology centers for their invaluable support throughout the research process.

Conflicts of interest

None exist.

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HOW TO CITE:

Al Sa'ady A.T., Alisawi L., Al-Shareefy D.M.H., Kadhum N.H., Mutar H.K., Khlaif G.T. Adverse effects of Herceptin treatment in women with breast cancer in Hillah (Iraq). *Pharmakeftiki* 37(2s), 135-139, 2025. <https://doi.org/10.60988/p.v37i2S.160>

Adverse effects associated with chemotherapy in cancer patients: a survey from Hillah (Iraq)

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KEY WORDS:
adverse effects;
chemotherapy; cancer
patients; Hillah; Iraq

ARTICLE INFO:

Received: January 12, 2025

Revised: February 22, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

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ABSTRACT

Most cancer patients undergo at least one type of chemotherapy, which is often accompanied by a wide range of adverse effects, from mild symptoms such as nausea, vomiting, and hair loss to life-threatening complications. This retrospective cross-sectional study utilized a paper-based questionnaire targeting cancer patients receiving chemotherapy at the Oncology Center of the Imam Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq. The study was conducted between December 6, 2022, and April 30, 2023. A total of 64 patients, aged 1 to 80 years, participated in the study. The most represented age groups were 61–70 years (26.56%), 41–50 years (25%), and 51–60 years (23.43%). The sample included 59.37% females and 40.62% males. Various cancer types were reported, with breast cancer being the most prevalent (40.62%). Both adjuvant and neoadjuvant chemotherapy protocols were documented. Twenty-three distinct adverse effects were identified; the most common included fatigue, exhaustion, and loss of energy (76.56%), anorexia (62.5%), bone and muscle pain (62.5%), vision disorders (57.81%), drowsiness and prolonged sleep (54.69%), sleep disorders (51.56%), as well as nausea and vomiting (50%). All cancer patients (100%) experienced at least one adverse effect as a results of the chemotherapy they received.

1. Introduction

Although chemotherapy improves

cancer patient survival and enhances quality of life, its toxicity remains a significant concern. Chemotherapy

is associated with a wide spectrum of adverse effects, ranging from mild symptoms such as nausea, vomiting, and hair loss to more severe and potentially life-threatening complications, including febrile neutropenia, bone marrow suppression, acute oral mucositis, and sepsis. These adverse effects may arise due to the resistance of cancer cells to chemotherapy and their structural and functional similarities to normal cells. The severity and nature of chemotherapy-induced adverse effects vary depending on the specific agents used. These effects may be short-term, long-term, or delayed, while not all chemotherapeutic agents produce the same side effects. Alarming, some adverse effects may manifest late and pose serious health risks^{1,2}. Given the limited number of Iraqi studies in this field, the present study aimed at assessing the adverse effects of chemotherapy among cancer patients during treatment.

2. Methodology

This retrospective cross-sectional study was based on a paper-based questionnaire administered to cancer patients receiving chemotherapy at the Oncology Center of the Imam Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq. The study was conducted from December 6, 2022, to April 30, 2023. A chi-squared test was used in order to identify statistically significant relationships between the assessed variables. Patients who provided oral consent were interviewed prior to the administration of their chemotherapy dose, with consideration given to their psychological state. Patients undergoing concurrent radiotherapy and chemotherapy were excluded so as to avoid confounding effects due to overlapping side effects. Ethical approval for the study was granted by the Ethics Committee of the College of Pharmacy of the University of Babylon (approval number: A-0045; date: February 9, 2025).

3. Results and Discussion

In recent years, surveys of cancer patients have provided more accurate and realistic insights into the adverse effects of chemotherapy than those obtained solely from clinical trials³. A total of 64 patients, aged 1 to 80

years, participated in the study. The most represented age groups were 61–70 years (26.56%), 41–50 years (25%), and 51–60 years (23.43%). The sample included 59.37% females and 40.62% males.

A variety of cancer types were recorded, with breast cancer being the most prevalent (26 patients; 40.62%), followed by lung and bladder cancer (8 patients each; 12.5% each), brain cancer (6 patients; 9.37%), and liver, ureteral, and shoulder cancers (2 patients each, 3.12% each). Other cancers included colon, eye, small bowel, kidney, throat, lymph node, hand, and uterine cancers (1 patient each; 1.56% each). Notably, the Iraqi Cancer Council's 2022 annual report has also identified breast cancer as the most common cancer type in Iraq⁴.

As far as the timing of chemotherapy initiation is concerned, 42 patients (65.6%) received chemotherapy after cancer resection surgery, while 22 patients (34.4%) received it beforehand. Chemotherapy is often administered in conjunction with surgery or other treatments such as radiotherapy, targeted therapy, or immunotherapy. Neoadjuvant chemotherapy, given before surgery, aims at shrinking tumours and facilitating less invasive, more effective surgical procedures. In contrast, adjuvant chemotherapy, administered post-operatively, targets residual cancer cells in order to reduce the risk of recurrence or metastasis¹. No anti-cancer therapy is entirely free of toxicity. In this study, 23 distinct adverse effects were reported, and all patients (100%) experienced at least one, which often hindered treatment adherence and increased the likelihood of discontinuation. Due to differences in study conditions, timeframes, and populations, a direct comparison of adverse effect incidence rates with those of other studies is challenging.

Table 1 presents the most frequently reported adverse effects of chemotherapy identified by our study. Previous studies have shown that females are more susceptible to chemotherapy-related fatigue, influenced by factors such as inflammation, neuroticism, insomnia, pain, and depression^{3,5}. A loss of appetite may result from nausea, vomiting, oral ulcers, altered taste, or psychological distress. Chemotherapy-induced alopecia typically begins at 2–3 weeks after treatment initiation, as rapidly dividing hair follicle cells are dam-

Table 1. Adverse effects associated with chemotherapy among the patients participating in our study.			
Adverse effects	Frequency (%)	Association with sex (<i>p</i>-value)	Association with age (<i>p</i>-value)
Fatigue, exhaustion, and feeling of loss of energy	49 (76.56%)	0.702	0.603
Anorexia	40 (62.50%)	0.6	0.72
Bone and muscle aches	40 (62.50%)	0.501	0.561
Vision disorders	37 (57.81%)	0.6	0.79
Drowsiness and prolonged sleep	35 (54.69%)	0.609	0.54
Sleep disorders	33 (51.56%)	0.59	0.6
Nausea and vomiting	32 (50.00%)	0.55	0.61
Immunodeficiency	30 (46.88%)	0.7	0.78
Stomach and intestinal ache (spasms)	28 (43.75%)	0.59	0.71
Feeling of bloating and an increase in the size of the abdomen	26 (40.63%)	0.7	0.77
Vertigo	25 (39.06%)	0.901	0.56
Nail pigmentation	25 (39.06%)	0.8	0.81
Itching and skin sensitivity	25 (39.06%)	0.5	0.89
Headaches	24 (37.50%)	0.95	0.59
Hyperglycaemia	17 (26.56%)	0.508	0.76
Ulcers and pain in the mouth	16 (25.00%)	0.8	0.88
Loss of hair, eyelashes, and eyebrows	15 (23.44%)	0.605	0.78
Anaemia	12 (18.75%)	0.508	0.66
Hypertension	11 (17.19%)	0.94	0.9
Kidney problems	10 (15.63%)	0.55	0.9
Nail loss	9 (14.06%)	0.507	0.93
Heart problems	7 (10.94%)	0.803	0.57
High blood cholesterol	6 (9.38%)	0.501	0.915
How long do adverse effects last?			
Duration of adverse effects	Frequency (%)		
More than a week	35 (54.68%)		
A week	22 (34.38%)		
Less than a week	7 (10.94%)		

aged by cytotoxic agents⁶.

As shown in Table 1, no statistically significant relationships were found between the reported adverse effect rates and the patients' sex or age. However, other studies have reported significant associations between adverse effects and the type or mechanism of chemotherapy used².

Explanations for the toxicity exerted by chemotherapy vary. Commonly cited mechanisms include systemic DNA damage, inflammation in healthy tissues, and direct cytotoxicity to normal cells. Recent studies have proposed that chemotherapy may induces double-strand DNA breaks and activate inflammatory and

apoptotic pathways, thereby contributing to its toxic effects⁷.

Finally, as far as the duration of adverse effects is concerned, patients were categorized into three groups: 54.68% of them experienced symptoms lasting more than one week, 34.38% of them experienced symptoms that lasted for one week, and 10.94% of them experienced symptoms that lasted for less than one week (Table 1). Although the half-life of most chemotherapeutic agents is approximately 24 h, their toxic effects may persist for 2–3 weeks, depending on the patient and the specific chemotherapy regimen followed.

4. Conclusion

This survey-based study has documented chemotherapy-associated adverse effects in a realistic clinical context, thereby contributing to a better understanding of patient experiences and treatment outcomes in Iraq. Incorporating routine surveys of chemotherapy-related adverse effects into standard clinical practice has become essential. Such assessments can support the timely identification and management of side effects, thereby improving patients' emotional well-being and enhancing adherence to chemotherapy regimens.

Acknowledgements

We extend our sincere gratitude to the patients who generously provided their consent and cooperation,

making this study possible. We also thank the dedicated staff at the oncology centers involved in this study for their invaluable support.

Conflicts of interest

None exist.

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HOW TO CITE:

Al Sa'ady A.T., Al-Kremy N.A.R., Khdhair O.A., Al-Ameedy T.H., Eidan B.A.K., Rabeea H., Emad S.S., Hussien T.H. Adverse effects associated with chemotherapy in cancer patients: a survey from Hillah (Iraq). *Pharmakeftiki* 37(2s), 140-143, 2025. <https://doi.org/10.60988/p.v37i2S.161>

Adverse effects associated with radiotherapy: a study on cancer patients from Hillah (Iraq)

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KEY WORDS:

**adverse effects; radiotherapy;
cancer patients; Hillah; Iraq**

ARTICLE INFO:

Received: January 31, 2025

Revised: February 27, 2025

Accepted: February 28, 2025

Available online: October 10, 2025

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ABSTRACT

Radiopharmaceuticals play a critical role in the treatment of both malignant and benign tumours. The present study is a retrospective cross-sectional analysis based on a paper-based questionnaire administered to cancer patients undergoing radiotherapy at the Oncology Center of the Imam Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq, between November 15, 2022 and March 15, 2023. Fifty patients aged 1–80 years participated in the study, with the most common age group being 51–60 years (26.56%). Females constituted the majority of the participants (N=38; 76%). A range of cancer types was documented, including breast cancer, brain cancer, lymphoma, uterine cancer, cancers of the mouth and pharynx, stomach cancer, hip cancer, bone cancer, rectal cancer, shoulder cancer, skin cancer, and pilonidal carcinoma. Twenty-one adverse effects were reported, the most common being fatigue, exhaustion, and loss of energy (80%), followed by drowsiness and prolonged sleep (74%), as well as muscle and bone pain (74%). Several adverse effects of radiotherapy demonstrated a statistically significant association with age and sex, occurring more frequently in patients over 40 years of age and in females. Among breast cancer patients who had undergone surgery, neck pain and cramps, breast pain, and chest pain were the most commonly reported adverse effects associated with radiotherapy.

1. Introduction

Radiotherapy constitutes a vital component of comprehensive can-

cer treatment protocols, employed for localized management, symptom palliation, and disease control in refractory malignancies. Its inte-

gration with breast-conserving surgery significantly reduces the risk of local recurrence over a ten-year period, irrespective of tumour size, histological grade, margin width, or patient age^{1,2}. Despite efforts to maximize precision and minimize collateral damage, healthy tissues adjacent to the tumour may be affected by radiotherapy, resulting in unpleasant and sometimes complex adverse effects that can be both acute (early-onset) and delayed^{2,3}. Improving clinical outcomes requires optimization of the therapeutic ratio: increasing the likelihood of tumour control while minimizing complications in normal tissues. Although radiation-induced side effects vary depending on the cancer type and anatomical site, certain common reactions are frequently observed^{3,4}.

Ionizing radiation generates free radicals that induce DNA breaks, chromosomal aberrations, and potentially apoptosis due to damaged genetic material or inadequate chromosomal repair. Additionally, radiation may trigger signalling pathways that activate pro-inflammatory and pro-fibrogenic cytokines, initiate the coagulation cascade, and inflict vascular injury. It may also elevate the risk of secondary malignancies within the irradiated field³⁻⁵. In light of the paucity of published Iraqi data on the adverse effects of radiotherapy, this study aimed at documenting the most frequently reported side effects in cancer patients undergoing radiotherapy in Hillah, Iraq.

2. Methodology

A retrospective cross-sectional study was conducted by using a paper-based questionnaire targeting cancer patients receiving radiotherapy at the Oncology Centers of the Imam Sadiq Teaching Hospital and at the Marjan Medical City Hospital in Hillah, Iraq, between November 15, 2022 and March 15, 2023. The chi-squared test was applied in order to examine any statistically significant associations among the variables. Questionnaire data were collected prior to the initiation of the radiotherapy doses, from patients who provided oral consent. Particular attention was paid to the patients' psychological state during data collection. Individuals receiving concurrent anticancer treatments (e.g., immunotherapy or chemother-

apy) were excluded in order to eliminate potential confounding due to overlapping side effects. Ethical approval was obtained from the Ethics Committee of the College of Pharmacy of the University of Babylon (approval number: A-0047; date: February 9, 2025).

3. Results and Discussion

Although most cancer patients worldwide receive radiotherapy as a core component of their treatment, it is associated with a range of side effects. These adverse effects can be reduced in both severity and frequency through the use of newer, more precise techniques that lower the dose of radiation administered. These techniques may also allow for dose escalation in cases of radioresistant cancers without increasing the risk of additional side effects^{6,7}.

The current study included 50 patients undergoing radiotherapy, ranging in age from 1 to 80 years. The most common age group was the 51–60-years one, accounting for 20 patients (40%), while females constituted the majority of the participants of the study (38 patients; 76%). The number of radiotherapy sessions ranged from 10 to 40, with 27 patients (54%) receiving between 11 and 20 sessions.

A wide range of cancer types was recorded: breast cancer (27 patients; 54%), brain cancer (4 patients; 8%), lymphoma (3 patients; 6%), uterine cancer (3 patients; 6%), mouth and pharyngeal cancer (3 patients; 6%), stomach cancer (3 patients; 6%), hip cancer (2 patients; 4%), and one case (2%) each of bone cancer, rectal cancer, shoulder cancer, skin cancer, and pilonidal carcinoma.

Twenty-one adverse effects related to radiotherapy were documented and are presented in Table 1. The most frequently reported were fatigue, exhaustion, and loss of energy (80%), followed by drowsiness and prolonged sleep (74%), as well as muscle and bone pain (74%). Some adverse effects showed statistically significant correlations with patient age, occurring more frequently in individuals over 40 (Table 1). Moreover, most adverse effects manifested directly at the site of radiation exposure, primarily as dermatological changes, including discoloration, itching, peeling, redness, swelling, ulceration, irrita-

Table 1. Adverse effects associated with radiotherapy among the patients participating in our study. Notes: *, signifies a statistically significant relationship; #, the total number of the participating patients in the current study was 50.

Adverse effects of radiotherapy	Frequency (%) [#]	Age		Sex					
		1–40 years	>40 years	male	female				
Fatigue, exhaustion, and loss of energy	40 (80%)	11	29 *	10	30				
Drowsiness and prolonged sleep	37 (74%)	12	25 *	8	29 *				
Muscle and bone pain	37 (74%)	11	26 *	9	28 *				
Dry mouth	32 (64%)	10	22	9	23				
Skin discoloration at the treatment site (dark)	32 (64%)	11	21	7	25 *				
Sleep disturbances	30 (60%)	11	19	5	25 *				
Fever and chills	30 (60%)	9	21	6	24				
Nervousness and agitation	29 (58%)	9	20 *	6	23				
Pain at the treatment site	28 (56%)	8	20	6	22 *				
Headache	28 (56%)	8	20	6	22 *				
Change in the sense of taste	26 (52%)	10	16	7	19				
Hair loss	26 (52%)	9	17	6	20 *				
Itching and peeling of the skin at the treatment site	25 (50%)	7	18 *	5	20				
Blurred vision	25 (50%)	10	15	6	19				
Redness of the skin at the treatment site	23 (46%)	9	14	2	21 *				
Swelling at the treatment site	22 (44%)	6	16 *	3	19				
Depression	22 (44%)	9	13 *	6	16 *				
Ulceration and irritation of the skin at the site of treatment	16 (32%)	7	9	4	12				
Ulceration of the mouth and gums	14 (28%)	6	8	2	12				
Tooth decay	13 (26%)	2	11	4	9				
Nail loss	5 (10%)	3	2	1	4 *				
Breast cancer patients (N=27)									
Number of women who underwent breast surgery		lumpectomy		14 (51.9%)					
		mastectomy		13 (48.1%)					
Adverse effects of radiotherapy and their relationship with age in breast cancer patients that have undergone surgery									
Adverse effects	Total	Age		p-value	Adverse effects	Total	Age		p-value
		1–40 years	>40 years				1–40 years	>40 years	
Neck pain and cramps	25	9	16	0.77	Shoulder pain and stiffness	14	4	10	0.01 *
Breast pain	25	8	17	0.02 *	Cough with phlegm	13	3	10	0.21
Chest pain	24	7	17	0.06	Stiffness of the jaw	10	4	6	0.01 *
Rib pain	20	5	15	0.04 *	Swelling in the upper arm	10	2	8	0.05 *
Swelling under the armpit	17	3	14	0.05 *	Shortness of breath	10	2	8	0.05 *
Difficulty in swallowing	14	4	10	0.01 *	Earache	8	3	5	0.02 *

tion, and pain (Table 1).

Given the predominance of breast cancer cases in this cohort, the study included a dedicated section on this group (Table 1). All 27 breast cancer patients (100%) in our study underwent breast surgery: 14 (51.9%) had lumpectomies and 13 (48.1%) underwent mastectomies. Twelve adverse effects associated with radiotherapy were reported among breast cancer patients who had undergone surgery, as presented in Table 1; most of these effects were significantly more prevalent in patients aged over 40.

Adverse effects in normal tissues may appear either early or late. Early (acute) effects occur during or shortly after radiotherapy and can develop within weeks, whereas late effects tend to be progressive and chronic, emerging months or years after treatment^{3,8}. Research indicates that the severity and probability of acute side effects depend on the total radiation dose, duration of exposure, the specific body parts irradiated, and other contributing factors. While early adverse effects often resolve over time, some may progress to complications that impede treatment and pose considerable challenges for both patients and clinicians. The timely identification and management of these effects are critical to improving patient outcomes and quality of life, especially in paediatric patients who may live for decades with long-term consequences of radiotherapy and its associated morbidities^{7,9}. Finally, although advances in radiotherapy have led to better long-term survival rates, cancer survivors remain at elevated risk for late-onset radiotherapy-associated

side effects, including cardiovascular, neurological, and reproductive complications, as well as the potential development of secondary radiation-induced malignancies^{9,10}.

4. Conclusion

Radiotherapy-related side effects exhibit considerable variation among Iraqi patients and are significantly influenced by individual factors such as age and sex. The majority of these effects are localized to the irradiated site.

Acknowledgements

The authors extend their sincere gratitude to the patients who generously participated in this study, and to the dedicated staff at the Oncology Centers of the Imam Sadiq Teaching Hospital and of the Marjan Medical City Hospital for their invaluable cooperation and support throughout the research process.

Conflicts of interest

None exist.

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HOW TO CITE:

Al Sa'ady A.T., Alyassiry M.R.S., Al-Shareefy D.M.H., Alisawi L. Adverse effects associated with radiotherapy: a study on cancer patients from Hillah (Iraq). *Pharmakeftiki* 37(2s), 144-148, 2025. <https://doi.org/10.60988/p.v37i2S.162>

Cytotoxic and molecular effects of captopril on a colon cancer cell-line: an *in vitro* study

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KEY WORDS:

cisplatin; captopril; colon cancer cell-line; PIK3CA gene; cytotoxicity

ARTICLE INFO:

Received: December 07, 2024

Revised: February 21, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Colorectal carcinoma originates from aberrant cellular proliferation within the colonic or rectal mucosa, culminating in malignant neoplastic growth. As of 2018, cancer accounted for approximately 9.6 million deaths worldwide, ranking as the second leading cause of mortality. This study investigates the antiproliferative effects of captopril, an angiotensin-converting enzyme inhibitor, on the SW480 colorectal adenocarcinoma cell line. SW480 cells were treated with increasing concentrations of cisplatin (15.6–500 µg/mL) and captopril (31.25–1,000 µg/mL), administered both individually and in combination. Cisplatin achieved maximal cytotoxicity at 62.5 µg/mL, whereas captopril required 2,000 µg/mL in order to elicit a comparable effect. The most pronounced anticancer synergy was observed with a captopril / cisplatin combination at 31.25 / 15.6 µg/mL, thereby indicating enhanced chemotherapeutic efficacy through drug interaction. Real-time PCR analysis of PIK3CA gene expression revealed a concentration-dependent transcriptional response. The highest levels of PIK3CA upregulation occurred at 250 µg/mL for cisplatin, 62.5 µg/mL for captopril, and 20 / 1000 µg/mL for their combination. PIK3CA, a key oncogene implicated in cellular proliferation and survival, exhibited significant modulation across the herein examined treatment conditions. These findings underscore captopril's potential as a novel adjuvant in colorectal cancer therapy, capable of influencing critical oncogenic pathways and amplifying the cytotoxic effects of standard chemotherapeutic agents.

1. Introduction

Colorectal cancer is among the most frequently diagnosed cancers worldwide, ranking as the third most common malignancy across all cancer types. It is a leading cause of cancer-related morbidity and mortality, with over one million new cases reported annually. Despite advances in screening and treatment, colorectal cancer remains the third leading cause of cancer-related deaths in both men and women, particularly in the United States^{1,2}. The disease originates in the epithelial cells lining the colon or the rectum, which are characterized by a high rate of proliferation and turnover. Colorectal carcinoma develops when these cells undergo genetic and epigenetic alterations, leading to uncontrolled proliferation and the formation of an abnormal mass. These tumours may be benign or malignant^{2,3}.

Multiple approaches have been employed for the assessment of colorectal cancer. In Iraq, research has examined associations among gender, age, tumor recurrence, tumor characteristics, and medical treatment outcomes^{1,2}. Globally, colorectal cancer remains a major public health concern, affecting over one million individuals each year⁴. In Iraq, colorectal cancer predominantly affects the elderly, with rising incidence and mortality observed across all age groups. These trends necessitate a re-evaluation of public health policy concerning colorectal cancer, including improved public awareness, screening protocols, and management strategies⁵. Males aged 50 to 60 years are more likely to develop colorectal cancer than females, while right-sided colon cancer is more prevalent among Iraqi patients⁶.

Angiotensin-converting enzyme (ACE) inhibitors, such as captopril, are primarily prescribed for the management of hypertension and cardiovascular diseases. Captopril inhibits the conversion of angiotensin I to angiotensin II and deactivates bradykinin; a vasodilatory peptide. Emerging research indicates that captopril possesses anti-angiogenic properties that can inhibit the formation of new blood vessels required for tumor growth and metastasis⁷. Angiogenesis plays a critical role in tumour progression by supplying cancer cells with oxygen and essential

nutrients. Tumours activate the “angiogenic switch” in order to promote the release of pro-angiogenic factors, such as vascular endothelial growth factor, which correlates with tumour aggressiveness and metastatic potential⁸. Inhibiting angiogenesis – by using agents like captopril – may suppress tumour progression by restricting nutrient delivery to cancer cells. Interestingly, observational data suggest that patients treated with captopril for hypertension exhibit a lower risk of developing cancer, prompting further investigation into its potential anticancer effects⁹.

This study evaluates the impact of captopril on the SW480 colorectal cancer cell line, examining its influence on cell proliferation and its potential as an adjunctive therapy for colorectal cancer.

2. Methodology

2.1. Cell lines

The SW480 colorectal adenocarcinoma cell line was obtained from Rwafid Alelom Limited. Treatment concentrations were as follows: cisplatin at 15.6, 31.25, 62.5, 125, 250, and 500 µg/mL and captopril at 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. Cell viability was assessed following exposure to captopril, cisplatin, and their combination. SW480 cells were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum, and 100 U/mL penicillin and streptomycin. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

2.2. MTT assay

The MTT assay was employed in order to assess the cytotoxic effects of captopril and its combination with cisplatin on tumor cell viability. The MTT assay measures mitochondrial metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. In this colorimetric, non-radioactive method, the intensity of the resulting formazan dye reflects the number of metabolically active cells. The Cell Proliferation Kit I (MTT) provides a quantitative and scalable platform suitable for rapid analysis of multiple samples.

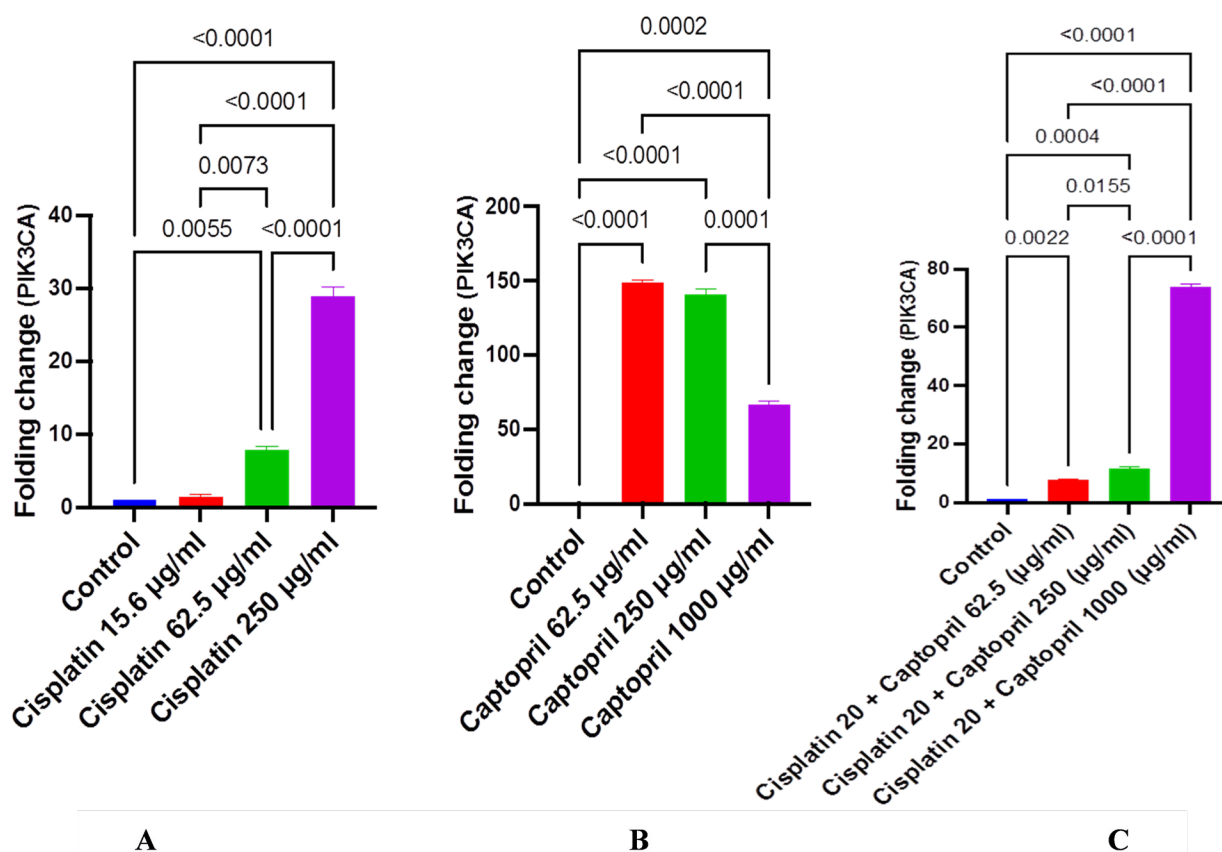


Figure 1. Effects of cisplatin, captopril, and their combination on *PIK3CA* gene expression in SW480 cells. (A): Cisplatin treatment resulted in the highest *PIK3CA* expression at 250 µg/mL. (B): Captopril treatment yielded peak *PIK3CA* expression at 62.5 µg/mL. (C): Combination therapy (captopril / cisplatin) induced maximal *PIK3CA* expression at 20 / 1,000 µg/mL, thereby demonstrating a synergistic dose-dependent effect.

2.3. Gene expression analysis of *PIK3CA*

Total RNA was extracted using the Easy-spin™ (DNA-free) RNA extraction kit. Lyophilized primers were reconstituted in nuclease-free ddH₂O to a final concentration of 100 pM/µL, according to the supplier's protocol. The working concentration for primer use was adjusted to 10 pM/µL. Primers were stored at -20°C until use.

2.4. Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 5.0 (GraphPad Software Inc., USA).

The Wilcoxon signed-rank test was applied in order to assess differences in gene expression for cisplatin, captopril, and their combinations. Mean gene expression levels in SW480 cells were compared using two-way ANOVA. All experiments were repeated at least three times in triplicate. A *p*-value below 0.05 was considered as statistically significant.

3. Results and Discussion

The cytotoxic effects of cisplatin, captopril, and their combination on SW480 colorectal cancer cells were evaluated using cell counting and MTT assays. Cisplatin demonstrated effective cytotoxicity at a con-

centration of 62.5 µg/mL, while the maximal lethal concentration of captopril was 2,000 µg/mL. Combined treatment exhibited the highest cytotoxic effect at 31.25 / 15.6 µg/mL for captopril / cisplatin, thereby indicating a synergistic interaction.

The impact of cisplatin, captopril, and their combination on *PIK3CA* expression was also assessed. Peak expression levels were observed at 250 µg/mL for cisplatin, 62.5 µg/mL for captopril, and 20 / 1,000 µg/mL for the combination therapy, revealing dose-dependent modulation of gene regulation (Figure 1).

Captopril, a widely prescribed ACE inhibitor, has exhibited immunomodulatory and anti-angiogenic properties that may contribute to cancer management by attenuating tumor progression and influencing immune pathways. These findings are consistent with those of previous reports confirming captopril's cytotoxic activity against colorectal cancer cells and its role in tumor reduction. The PI3K signalling pathway and, specifically, the *PIK3CA* oncogene, plays a central role in colorectal carcinogenesis by promoting cellular proliferation and inhibiting apoptosis. Mutations in *PIK3CA*, frequently identified in colorectal cancer, can activate downstream targets such as AKT, thereby driving tumor growth and resistance to programmed cell death. The herein reported results suggest that captopril may modulate *PIK3CA* expression, thereby offering a promising target for therapeutic intervention.

4. Conclusion

This study demonstrates the potential of captopril as an adjuvant therapeutic agent in the treatment of colorectal cancer, with observable effects in reducing SW480 cell proliferation and modulating *PIK3CA* expression. These findings support the hypothesis that captopril may enhance the efficacy of conventional chemotherapy by targeting critical molecular pathways involved in tumor growth and survival.

Acknowledgements

The authors express their sincere appreciation to the College of Pharmacy of the University of Hilla, for its continuous support and encouragement of ongoing research efforts.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Saady T.A., Ibrahim O.M.S., Al-Hussaniy H.A. Cytotoxic and molecular effects of captopril on a colon cancer cell-line: an *in vitro* study. *Pharmakeftiki* 37(2s), 149-153, 2025. <https://doi.org/10.60988/p.v37i2S.163>

***In vitro* cytotoxic and molecular effects of metoprolol, alone or combined with cisplatin, on colon cancer cells**

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KEY WORDS:

metoprolol; cisplatin; colon cancer cells; cell viability; cytotoxicity

ARTICLE INFO:

Received: January 06, 2025

Revised: February 20, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

ABSTRACT

Colorectal cancer ranks among the most prevalent malignancies worldwide, with surgical interventions frequently associated with substantial postoperative mortality and complications. Contemporary therapies often target molecular pathways such as the epidermal growth factor receptor and vascular endothelial growth factor. Recently, antihypertensive agents have been investigated for their potential anticancer properties, though results remain inconclusive. This study has assessed the cytotoxic effects of metoprolol, both as a monotherapy and in combination with cisplatin, on the SW480 colon cancer cell line using the MTT assay. Cisplatin achieved the highest cytotoxic activity at a concentration of 62.5 µg/mL, while metoprolol alone exhibited maximal cell-killing effects at 1,000 µg/mL. Notably, the combination of metoprolol (1,000 µg/mL) with cisplatin (15.6 µg/mL) demonstrated the most pronounced cytotoxic effect, thereby indicating potential synergistic activity. The findings suggest the potential of repurposing metoprolol as an adjunctive agent in combination chemotherapy, in order to enhance therapeutic outcomes in colorectal cancer treatment.

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1. Introduction

Colorectal cancer remains a significant global health concern, with surgical intervention serving

as the primary curative modality. However, colorectal cancer surgery has historically been associated with considerable postoperative morbidity and mortality¹. Despite

advances in perioperative care and postoperative surveillance, the rate of postoperative mortality has remained relatively stable over the past decade, highlighting the ongoing complexity of surgical outcomes in colorectal cancer patients². Recent therapeutic developments have concentrated on targeted approaches – particularly those inhibiting the vascular endothelial growth factor and the epidermal growth factor receptor pathways – which are critical for tumour angiogenesis and cellular proliferation³. Colorectal cancer patients often present with multiple comorbid conditions requiring continuous pharmacological management. This overlap stems from shared risk factors between colorectal cancer and chronic diseases such as metabolic syndrome and cardiovascular disorders⁴.

Hypertension, a widespread chronic condition, affects approximately 25% of the adult population in Canada⁵. Owing to its high prevalence, antihypertensive medications represent the most commonly prescribed drug class in the country, with over four million prescriptions dispensed monthly⁶. Emerging research suggests that antihypertensive agents may exert dual influences on carcinogenesis, either by promoting or by inhibiting tumour progression through distinct cellular pathways. This duality has prompted focused investigation into the oncological implications of the five major classes of antihypertensive drugs, especially in the context of colorectal cancer. Although many studies have examined the relationship between antihypertensive drug use and colorectal cancer risk, results remain inconclusive, warranting further inquiry into the underlying mechanisms and clinical outcomes⁷.

2. Methodology

2.1. Cell lines

The SW480 colon adenocarcinoma cell line was obtained from Rwafid Alelom Limited. Treatment concentrations for cisplatin were 15.6, 31.25, 62.5, 125, 250, and 500 µg/mL, while for metoprolol were 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. Cell viability was subsequently assessed following exposure

to cisplatin, metoprolol, and their combination. Cells were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum, along with 100 U/mL penicillin and streptomycin. All cultures were maintained at 37°C in a humidified 5% CO₂ atmosphere.

2.2. MTT assay

SW480 cells were seeded into 96-well plates and were incubated overnight until reaching approximately 80% confluence. Subsequently, 200 µL of complete growth medium was replaced with medium containing varying concentrations of cisplatin, metoprolol, and their combination, followed by 24-h incubation at 37°C, 5% CO₂. Each concentration was tested in five replicates. Cell viability was evaluated using the MTT cytotoxicity assay. The assay measures mitochondrial metabolic activity as a surrogate for cell proliferation, survival, and cytotoxicity. In this non-radioactive colorimetric method, darker solutions indicate a higher number of metabolically active cells. The Cell Proliferation Kit I (MTT) enables optimized quantitative analysis, facilitating rapid processing of large sample sets⁸.

2.3. Statistical analysis

Data analysis was performed using the SPSS software (version 23/2021; IBM, Armonk, NY, USA). One-way ANOVA was utilized in order to assess differences across treatment conditions. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

The effects of cisplatin on SW480 colorectal cancer cells were evaluated at varying concentrations (15.6, 31.25, 62.5, 125, 250, and 500 µg/mL), with untreated cells serving as the control. After 24 h of treatment, a statistically significant reduction in cell viability was observed ($p < 0.001$), demonstrating a clear dose-dependent cytotoxic effect. The concentration of 62.5 µg/mL produced the most pronounced cytotoxicity, thereby indicating its potency in inducing cell death in SW480 cells (Figure 1A).

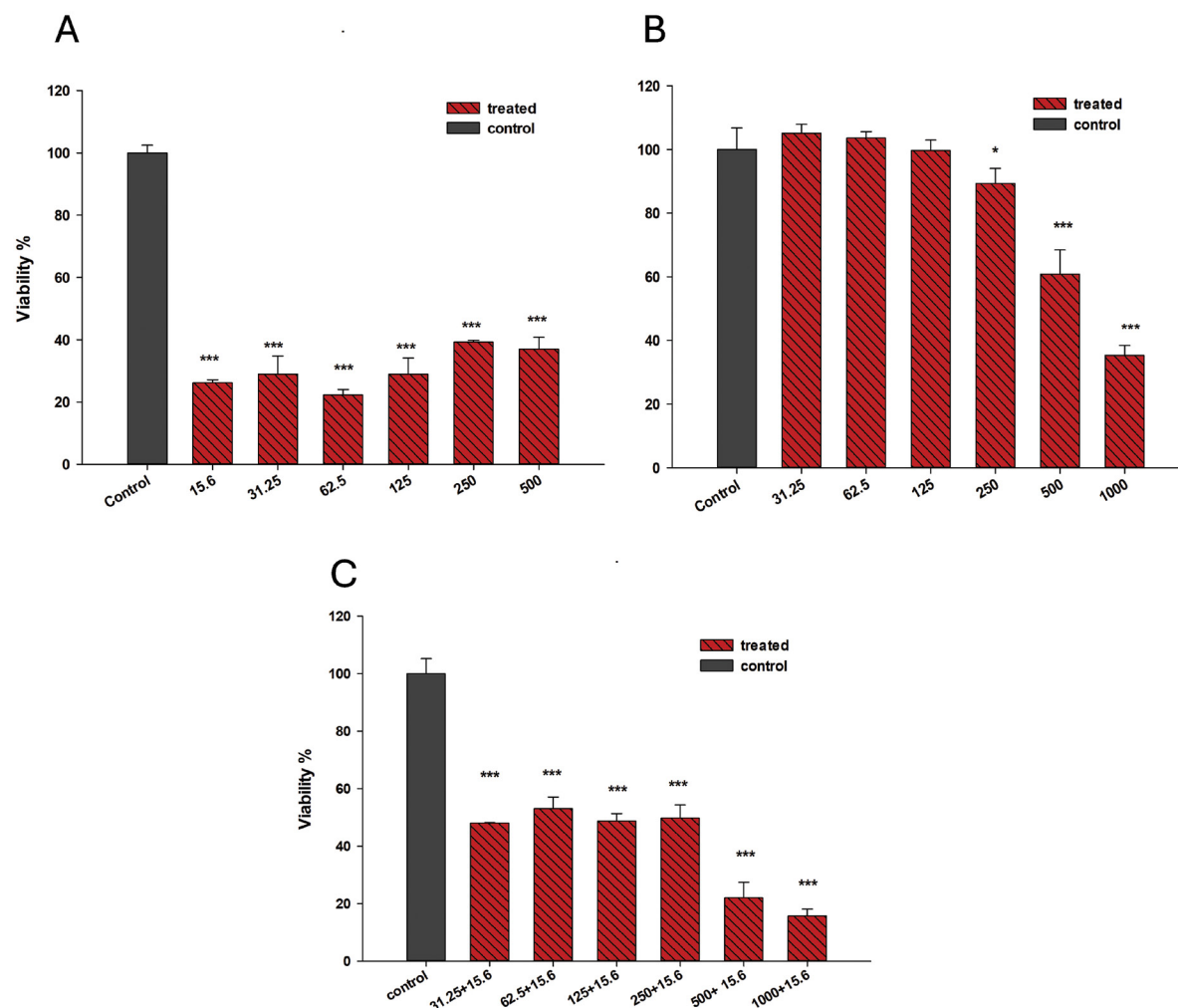


Figure 1. Cytotoxic effects of cisplatin, metoprolol, and their combination on SW480 colon cancer cells. (A): Treatment with cisplatin demonstrated maximal cytotoxicity at 62.5 µg/mL, representing the most potent concentration for inducing cell death. (B): Exposure to metoprolol revealed that 1,000 µg/mL produced the highest reduction in cell viability. (C): Combined administration of metoprolol (1,000 µg/mL) and cisplatin (15.6 µg/mL) yielded the greatest cytotoxic effect, thereby indicating a synergistic enhancement of cell-killing activity. Notes: *, $p < 0.05$; ***, $p < 0.001$.

The impact of metoprolol on SW480 cell viability was examined across concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. A significant concentration-dependent decrease in viability was detected ($p < 0.001$), with 1000 µg/mL yielding the highest cytotoxic effect (Figure 1B).

In order to assess the potential synergistic inter-

action between metoprolol and cisplatin, multiple concentration combinations were tested (Figure 1C). The combination of 1,000 µg/mL metoprolol with 15.6 µg/mL cisplatin resulted in the greatest reduction in cell viability ($p < 0.001$), suggesting a potentiation of cell-killing efficacy when both agents are co-administered. These findings offer valuable

insights into the individual and combined cytotoxic effects of cisplatin and metoprolol on SW480 colon cancer cells, underscoring their potential therapeutic relevance.

Our results corroborate earlier work by Hajatbeigi and Hajighasemi⁹, which has demonstrated the cytotoxic effects of both cisplatin and metoprolol on SW480 cells. Cisplatin, a well-characterized alkylating agent, induces apoptosis by forming intrastrand cross-links between adjacent guanine bases, and subsequently interacting with neighbouring guanine and adenine residues. These DNA modifications disrupt replication and transcription, culminating in programmed cell death. The cytotoxic activity of metoprolol has also been reported by Caldwell *et al.*¹⁰, who have utilized the MTT assay in order to evaluate the drug's impact on cell viability. Their observations align with those of the present study, reinforcing the notion that metoprolol contributes to cancer cell death.

As a β -adrenergic receptor antagonist, metoprolol has attracted attention for its putative anticancer properties, primarily through modulation of pathways involved in carcinogenesis, angiogenesis, and tumor progression. The oncology community has shown increasing interest in the therapeutic potential of β -blockers, particularly given their affordability, widespread availability, and favourable safety profile. Considering the financial burdens and limited survival rates associated with current oncologic treatments, the repurposing of β -blockers offers a

compelling avenue for future research in both pre-clinical and clinical contexts. These findings contribute meaningfully to the growing body of literature supporting the anticancer potential of β -blockers, and they advocate for deeper investigation into the mechanistic foundations and translational relevance of these agents.

4. Conclusion

This study demonstrates the potential of metoprolol as an adjuvant therapeutic agent in the treatment of colorectal cancer, with observable *in vitro* effects in reducing SW480 cell proliferation.

Acknowledgements

The authors express their sincere appreciation to the College of Pharmacy of the University of Hilla, for its continuous support and encouragement of ongoing research efforts.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Saady T.A., Ibrahim O.M.S., Al-Hussaniy H.A. *In vitro* cytotoxic and molecular effects of metoprolol, alone or combined with cisplatin, on colon cancer cells. *Pharmakeftiki* 37(2s), 154-158, 2025. <https://doi.org/10.60988/p.v37i2S.164>

Synthesis of new derivatives of hydrochlorothiazide containing diazonium groups and study of their biological activity

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KEY WORDS:

azo compounds; diazonium
salt; pyrazole; biological
activity; physical
characteristics

ARTICLE INFO:

Received: November 03, 2024

Revised: February 27, 2025

Accepted: February 27, 2025

Available online: October 10, 2025

ABSTRACT

Diazonium salts are highly reactive compounds that serve as intermediates in numerous chemical reactions. In this study, they were synthesized and subsequently allowed to react with phenolic drugs, ethyl acetoacetate, and acetylacetone in order to yield compounds M1–M5. Following further processing in order to produce compounds M6 and M7, compounds M4 and M5 interacted with hydrazine hydrate, resulting in an 80% conversion. The synthesized compounds (M1–M7) were characterized through an analysis of their physical properties, nuclear magnetic resonance spectra, elemental composition, and infrared spectra. The biological activities of the same compounds were also evaluated against various bacterial strains (i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, *Morganella*, *Acinetobacter*, *Salmonella*, and *Streptococcus pneumoniae*) and a yeast species (*Candida albicans*), using the agar well diffusion method.

1. Introduction

Hydrochlorothiazide is a widely prescribed thiazide diuretic for managing hypertension¹. Its diuretic efficacy is mediated through the modulation of electrolyte reabsorption in the distal renal tubules, resulting in a nearly equivalent in-

crease in sodium and chloride excretion. While the exact mechanism of action remains unclear, redistribution and elimination of systemic sodium may contribute to its therapeutic effect. Aromatic diazonium salts, typically represented as Ar-N₂⁺X⁻, serve as versatile intermediates in the synthesis of diverse or-

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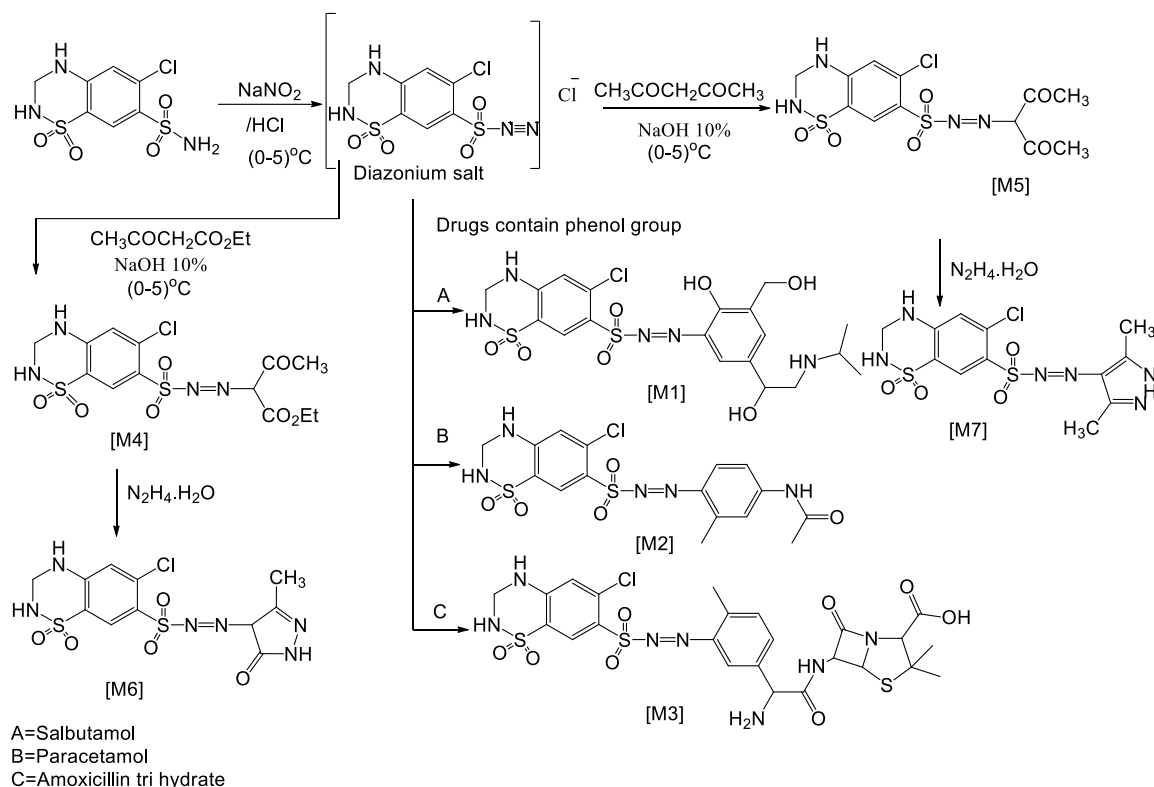


Figure 1. Pathway of the synthesis of compounds M1–M7.

ganic aromatic compounds². These salts may incorporate various inorganic and organic anions; due to the low hydrolytic tendency of aryl diazonium ions, strong acid salts dissociate fully in neutral aqueous media, thereby enabling the biological activity observed in certain derivatives. Azo compounds are pharmacologically significant and have been employed for their anti-diabetic, antimicrobial, antibacterial, anticancer, antiseptic, antioxidant, antifungal, anti-HIV, and electroconductive properties. Pyrazole, a five-membered heteroaromatic ring with two nitrogen atoms, is a fundamental scaffold for many drugs exhibiting a broad spectrum of therapeutic effects (including antifungal, anticancer, antigenic, and antimicrobial activity)³. This study describes the preparation of novel heterocyclic derivatives and the evaluation of their antibacterial properties. Structural elucidation of the synthesized compounds

was performed by using Fourier-transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (¹H-NMR).

2. Methodology

2.1. Chemicals

Reagents were procured from Thomas Baker, Merck, BDH, GCC, and Scharlau, and were used without further purification. Melting points were determined using a Gallenkamp capillary melting point apparatus. FT-IR spectra were obtained using a Shimadzu spectrometer (Japan), while the ¹H-NMR spectra of the products were recorded on a Bruker DMX-500 spectrophotometer.

2.2. Synthesis of azo compounds M1–M5 (Figure 1)

As detailed previously^{4,5}, the aromatic primary amine (1 g; 0.003 mol) was dissolved in 2.5 mL concentrated HCl and 7 mL H₂O under heating (60°C, 10 min) until complete dissolution and salt formation. Upon cooling (0°C–5°C), diazotization was carried out using 0.2 g sodium nitrite in 2 mL H₂O, added dropwise with vigorous stirring. The temperature was maintained at 0°C–5°C for 30 min. The resulting diazonium salt was added to aqueous solutions of phenolic drugs, ethyl acetoacetate, and acetylacetone (0.003 mol each) at 0°C–5°C and pH adjusted to 8–9 using 10% NaOH. After stirring for 60 min, the precipitate was filtered and repeatedly washed with 1:1 ethanol to water ratio. The process produced the following compounds: (i) compound M1 (light orange precipitate; yield: 85%; MP: 217°C–219°C; formula: C₁₃H₁₅ClN₄O₇S₂; MW: 438.86 mg/mol; elemental analysis (%) calculated / found: C = 65.50/65.55, H = 3.76/3.72, N = 22.23/22.26), (ii) compound M2 (yellow precipitate; yield: 66%; MP: 220°C–222°C; formula: C₁₆H₁₆ClN₅O₅S₂; MW: 457.91 mg/mol; elemental analysis (%) calculated / found: C = 57.3/57.9, H = 3.76/3.72, N = 17.07/17.25), (iii) compound M3 (white precipitate; yield: 54%; MP: 218°C–220°C; formula: C₁₉H₂₄ClN₅O₇S₂; MW: 457.91 mg/mol; elemental analysis (%) calculated / found: C = 65.50/65.55, H = 3.76/3.72, N = 13.82/13.90), (iv) compound M4 (light yellow precipitate; yield: 62%; MP: 208°C–210°C; formula: C₂₄H₂₆ClN₇O₈S₃; MW: 672.15 mg/mol; elemental analysis (%) calculated / found: C = 60.39/60.45, H = 4.12/4.23, N = 25.92/25.35), and (v) compound M5 (yellow precipitate; yield: 75%; MP: 185°C–187°C; formula: C₁₂H₁₃ClN₄O₆S₂; MW: 408.84 mg/mol; elemental analysis (%) calculated / found: C = 66.70/66.78, H = 5.08/5.15, N = 18.33/18.46).

2.3. Synthesis of pyrazole compounds M6 and M7 (Figure 1)

Following prior methodologies⁴, compounds M4 and M5 (0.001 mol each) were dissolved in 7 mL absolute methanol. After stirring for 10 min at room temperature, 0.001 mol of 80% hydrazine hydrate was added gradually. The mixture was refluxed for 7–8

h, monitored *via* thin layer chromatography, and the resulting product was filtered, dried, and purified using methanol. The process produced the following compounds: (i) compound M6 (orange precipitate; yield: 50%; MP: 175°C–177°C; formula: C₁₁H₁₁ClN₆O₅S₂; MW: 406.83 mg/mol; elemental analysis (%) calculated / found: C = 65.54/65.65, H = 5.58/5.26, N = 13.57/13.67) and (ii) compound M7 (dark yellow precipitate; yield: 77%; MP: 185°C–187°C; formula: C₁₂H₁₃ClN₆O₄S₂; MW: 404.85 mg/mol; elemental analysis (%) calculated / found: C = 58.66/58.70, H = 4.45/4.22, N = 15.11/15.24).

2.4. Biological activity testing

Compounds M1–M7 were assessed for antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, *Morganella*, *Acinetobacter*, *Salmonella*, *Streptococcus pneumoniae*, and *Candida albicans* at a concentration of 100 mg/mL, using the agar well diffusion method⁶. Nutrient agar was prepared with beef extract (3 g), agar (15 g), peptone (5 g), NaCl (5 g), and distilled water (up to 1,000 mL). All glassware, culture tubes, pipettes, and broth media were autoclaved and sterilized prior to incubation at 25°C ± 2°C for 72 h.

3. Results and Discussion

Hydrochlorothiazide, an aromatic primary amine, reacts with freshly generated nitrous acid (HONO) – formed by sodium nitrite in the presence of excess mineral acid – to produce a diazonium salt; a well-characterized intermediate in organic synthesis. Due to its instability, the diazonium salt must be prepared in cold solution. The synthesis of azo dyes involves two main steps: (i) the formation of the diazonium salt and (ii) the subsequent coupling with a phenolic drug, which acts as a highly reactive nucleophile because of its strong electron-donating hydroxyl group. Further coupling of the diazonium salt with ethyl acetoacetate and acetylacetone under alkaline conditions has yielded azo compounds M4 and M5.

The compounds were characterized using physical property evaluation, elemental analysis, FT-IR

spectroscopy, and ^1H -NMR spectroscopy. The FT-IR spectra of compounds M1–M5 revealed the disappearance of NH_2 stretching vibrations ($3453\text{--}3341\text{ cm}^{-1}$), consistent with diazotization, and the appearance of $\text{N}=\text{N}$ stretches at 1458 , 1450 , 1462 , 1404 , and 1455 cm^{-1} , respectively. In compound M4, ester and carbonyl bands appeared at 1766 and 1710 cm^{-1} , while compound M5 exhibited a ketonic carbonyl stretch at 1735 cm^{-1} . The ^1H -NMR spectrum of compound M3 showed characteristic signals at δ 1.19 (NH-amine), δ 1.80 (s, 2CH_3), δ 2.42 (s, CH-methine), δ 2.49 (s, CH-methane), δ 2.95 (d, CH-propiolactam), δ 3.31 (s, CH_2 -methylene), δ 4.26 (s, NH_2 -amine), δ 4.67 (d, CH-propiolactam), δ 4.83 (s, NH-aromatic), δ 5.39 (s, OH-phenol), δ 6.88– 8.40 (m, aromatic protons), δ 8.88 (s, NH-secondary amide), and δ 12.50 (s, OH-carboxylic)⁷.

Reaction of compound M3 with 80% hydrazine hydrate produced compound M6. FT-IR analysis showed loss of the ester and carbonyl bands of compound M4 (1766 and 1710 cm^{-1}), and emergence of amide-related bands at 1689 cm^{-1} ($\text{C}=\text{O}$) and 3232 cm^{-1} (NH stretch). The ^1H -NMR spectrum of compound M6 showed signals at δ 1.19 (NH-amine), δ 2.40 (s, $3\text{H}-\text{CH}_3$), δ 3.12 (s, CH-methylene), δ 4.67 (s, NH-aromatic), δ 6.93– 7.67 (m, aromatic protons), and δ 8.21 (s, NH-pyrazole).

Similarly, the treatment of compound M5 with 80% hydrazine hydrate yielded compound M7. The FT-IR spectrum of M7 displayed the disappearance of the 1735 cm^{-1} ketonic carbonyl band, and new absorptions at 3381 cm^{-1} (NH stretch) and 1639 cm^{-1} ($\text{C}=\text{N}$ stretch). The ^1H -NMR spectrum of compound M7 revealed signals at δ 2.18 (s, NH-S), δ 2.33 (s, $6\text{H}-\text{CH}_3$), δ 4.66 (s, CH-methylene), δ 5.31 (s, NH-aromatic), δ 7.04– 7.97 (m, aromatic protons), and δ

10.98 (s, NH-pyrazole)^{8,9}.

These data collectively confirm the successful transformation of hydrochlorothiazide into the desired azo and pyrazole derivatives. The synthesized compounds exhibited promising antimicrobial activity when compared to ciprofloxacin, particularly against *Escherichia coli*, *Morganella*, *Salmonella*, and *Candida albicans* (results not shown).

4. Conclusion

This study successfully synthesized and characterized a series of novel pyrazole and azo derivatives. Structures were confirmed *via* FT-IR, elemental analysis, and ^1H -NMR spectroscopy. These compounds hold considerable relevance in medicinal and pharmaceutical contexts, and several of them have demonstrated moderate antibacterial activity.

Acknowledgements

The authors gratefully acknowledge the College of Science for Women of the University of Baghdad, for providing the chemical reagents and research facilities necessary to conduct this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Sahib S.A., Hussein I.A., Abdulridha M.Q., Albanaa M.A. Synthesis of new derivatives of hydrochlorothiazide containing diazonium groups and study of their biological activity. *Pharmakeftiki* 37(2s), 159-163, 2025. <https://doi.org/10.60988/p.v37i2S.165>

Synthesis, characterization, and biological evaluation of new cyclic oxazepine derivatives as potential antibacterial and antifungal agents

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KEY WORDS:

Schiff bases; oxazepine;
antibacterial; antifungal;
synthesis

ARTICLE INFO:

Received: November 09, 2024

Revised: January 17, 2025

Accepted: February 06, 2025

Available online: October 10, 2025

ABSTRACT

In this study, Bis(4,4'-diaminophenoxy)ethane (compound C1) was synthesized *via* the reaction of *p*-hydroxyaniline with 1,2-dibromoethane. Schiff bases (compounds C2–C4) were subsequently obtained by condensing compound C1 with various aromatic aldehydes. These intermediates were further reacted with different anhydrides – namely phthalic anhydride and maleic anhydride – in order to yield the final derivatives (compounds C5–C10). All obtained compounds were characterized by using infrared spectroscopy and proton nuclear magnetic resonance, as well as through an assessment of their physical properties. Antimicrobial evaluation was conducted on some of the generated compounds using two bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and one fungal strain (*Candida albicans*), selected for their clinical relevance as causative agents of diverse human diseases.

1. Introduction

The development of synthetic routes to widely used organic compounds using readily available reagents remains a central goal of organic synthesis. Schiff bases constitute an important class of organic compounds due to their broad spectrum of biological activities, including antima-

larial, anticancer, antiviral, antifungal, antibacterial, antioxidant, antiproliferative, and anti-inflammatory effects¹. Schiff bases are condensation products formed from primary amines and carbonyl compounds (aldehydes or ketones)². First reported by the German chemist Hugo Schiff in 1864, these compounds are also known as azomethines or

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imines³. Structurally, they consist of an imine group (R-CH=N-R'), where the substituents R and R' may be cycloalkyl, alkyl, heterocyclic, or aryl groups⁴.

Heterocyclic compounds play a prominent role in drug synthesis. One notable example is the 1,3-oxazepine; a seven-membered ring containing two heteroatoms: oxygen and nitrogen⁵. Oxazepines exhibit diverse pharmacological activities and have been employed as antibiotics, enzyme inhibitors, and antidepressants. Over time, their synthesis has been extensively documented and investigated. These compounds are typically prepared *via* the reaction of Schiff bases or hydrazones with various anhydrides⁶.

In this study, we sought to convert selected Schiff bases into oxazepine derivatives through cyclization of the azomethine bond using maleic anhydride and phthalic anhydride⁷. Oxazepine derivatives are known to exhibit significant variations in biological activities, including antibacterial, anticonvulsant, antiviral, and antifungal properties, among other applications⁹.

2. Methodology

2.1. Chemicals and equipment

All chemicals were obtained from various suppliers (e.g., Thomas Baker, Merck, BDH, GCC, and Scharlau) and were used without further purification. Melting points were determined using an electrothermal melting point apparatus (Stuart, Germany) and are reported uncorrected. Purity and reaction completion of all compounds were assessed *via* thin-layer chromatography (TLC) on aluminum-coated silica plates (TLC 60 F₂₅₄, E. Merck), with absolute ethanol as the mobile phase and visualization under iodine vapor.

Infrared spectra were recorded as KBr disks within the range of 400–4000 cm⁻¹ using a Shimadzu FTIR spectrometer (Japan). Proton nuclear magnetic resonance (¹H-NMR) spectra were recorded for the synthesized compounds on a Bruker DMX-500 spectrophotometer (500 MHz) using DMSO-d₆ as the solvent.

2.2. Synthesis of Bis(4,4'-diaminophenoxy)ethane (compound C1)

Alcoholic sodium hydroxide (8 g, 0.2 mol) was added to 20 mL of absolute ethanol containing *p*-hydroxyaniline (0.02 mol, 2.91 g). The mixture was stirred until complete dissolution of the solids, followed by the addition of 1,2-dibromoethane (0.01 mol, 0.64 mL). The reaction mixture was refluxed for 4 h, and progress was monitored by TLC. Upon completion, the solution was poured into ice-cold distilled water, precipitating a violet solid. The crude product was filtered and purified using absolute ethanol⁹. Compound C1 was obtained as a violet precipitate (MP: 200°C–202°C).

2.3. Synthesis of Schiff bases (compounds C2–C4)

A mixture of compound C1 (2 g, 0.008 mol) and various aldehydes (benzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde) (0.016 mol) was dissolved in 20 mL of absolute ethanol along with a few drops of glacial acetic acid. The reaction mixture was refluxed for 8–10 h. The resulting solids were filtered and purified using absolute ethanol⁸. Compound C2 was obtained as a dark red precipitate (MP: 184°C–186°C), compound C3 was obtained as a violet precipitate (MP: 118°C–120°C), and compound C4 was obtained as a violet precipitate (MP: 166°C–168°C).

2.4. Synthesis of (oxazepine) compounds C5–C10

Compounds C2–C4 (0.001 mol) were individually dissolved in 10 mL of dry benzene, then reacted with either phthalic anhydride or maleic anhydride (0.002 mol) under reflux for 5 h. TLC was used in order to monitor the reaction completion. Upon cooling, the reaction mixture was filtered and the crude products were purified using ethanol⁶. Compound C5 was obtained as a red precipitate (MP: 190°C–192°C), compound C6 was obtained as a brown precipitate (MP: 173°C–175°C), compound C7 was obtained as a pale brown precipitate (MP: 181°C–182°C), compound C8 was obtained as a red precipitate (MP: 175°C–177°C), compound C9 was obtained as a brown precipitate (MP: 160°C–162°C), and compound C10 was obtained as a dark brown precipitate (MP: 130°C–132°C).

2.5. Antibacterial and antifungal activity

Antimicrobial activities of selected compounds (i.e., compounds C2, C3, C4, C5, C8, and C10) and of amoxicillin were assessed *via* the agar well diffusion method¹⁰. Tests were conducted against two bacterial strains – *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) – and one fungal strain (*Candida albicans*). Wells of 6-mm diameter were created on agar plates using a sterile cork borer. Each well was filled with 0.2 mL of microbial suspension, evenly spread using a sterile cotton swab. Test compounds and amoxicillin were introduced at a concentration of 0.2 mL per well *via* a micropipette. Plates were incubated at 37°C for 24 h, after which inhibition zone diameters were measured with a ruler.

3. Results and Discussion

Compound C1 was synthesized by reacting *p*-hydroxyaniline with 1,2-dibromoethane in the presence of sodium hydroxide in ethanol. Its structure was confirmed by Fourier transform infrared (FT-IR) spectroscopy, which showed the disappearance of the characteristic stretching vibration of the hydroxyl (-OH) group. A new band corresponding to the C-O-C stretching vibration was observed at 1234 cm⁻¹. Additionally, absorption bands at 3417 and 3375 cm⁻¹ were attributed to asymmetric and symmetric N-H₂ stretching vibrations, respectively.

Refluxing compound C1 with equimolar amounts of various carbonyl compounds in absolute ethanol, in the presence of a few drops of glacial acetic acid, resulted in the formation of a new series of Schiff bases (compounds C2–C4). The FT-IR spectra of these compounds indicated the disappearance of N-H₂ bands at 3417 and 3375 cm⁻¹, alongside the appearance of a new band corresponding to the imine (-CH=N) group, thereby confirming Schiff base formation. The resulting Schiff bases were then reacted with different anhydrides in order to yield oxazepine derivatives (compounds C5–C10). The FT-IR characterization of compound C5 revealed key vibrational bands associated with the oxazepine ring: a signal at 1739 cm⁻¹ in-

dicating ring vibration, and a band at 1651 cm⁻¹ attributed to the carbonyl stretching of lactone and lactam functionalities.

Further structural confirmation was obtained for compound C7 *via* ¹H-NMR spectroscopy, which showed: (i) δ 8.9 ppm (s, 2H, -CH=N), (ii) δ 6.5–7.7 ppm (m, 16H, aromatic protons), (iii) δ 3.69–4.42 ppm (t, 4H, methylene -CH₂-), and (iv) δ 2.97–3.06 ppm (multiplet, 2H, additional -CH₂- units).

The antimicrobial efficacy of the synthesized compounds was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the agar well diffusion method. Bacterial strains were incubated at 37°C for 24 h, while fungal cultures were maintained at 28°C for 3–5 days. Multiple compounds demonstrated significant inhibitory activity when compared to the control antibiotic amoxicillin. Compound C10 exhibited the most pronounced activity, with inhibition zones measuring: (i) 24 mm against *Staphylococcus aureus*, (ii) 22 mm against *Escherichia coli*, and (iii) 20 mm against *Candida albicans*. Notably, compound C10 outperformed amoxicillin in terms of antifungal activity, thereby suggesting a broad-spectrum antimicrobial effect and underscoring its potential as a lead candidate for further development.

4. Conclusion

This study provides meaningful contributions to antibacterial and antifungal research through the synthesis and evaluation of novel heterocyclic compounds. Structural characterization has offered key insights into the chemical profiles of the synthesized derivatives, while biological assays have highlighted their potential antimicrobial activity. Although most compounds displayed moderate antibacterial efficacy relative to amoxicillin, compound C10 exhibited notably strong inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. These findings suggest that compound C10 possesses broad-spectrum antimicrobial properties and merits further investigation for its potential as an alternative therapeutic agent, particularly in the fight against drug-resistant infections.

Acknowledgements

The authors gratefully acknowledge the College of Science for Women of the University of Baghdad, for generously providing the chemicals and research facilities necessary to conduct this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Alsahib S.A., Kadhim E.A., Mahmood G.H., Abdulghani R.H. Synthesis, characterization, and biological evaluation of new cyclic oxazepine derivatives as potential antibacterial and antifungal agents. *Pharmakeftiki* 37(2s), 164-167, 2025. <https://doi.org/10.60988/p.v37i2S.166>

Pain management strategies for acute abdominal pain in the emergency department: a study from the Al-Hilla Teaching Hospital (Iraq)

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KEY WORDS:

acute abdominal pain;
emergency department; pain
management; analgesics;
multimodal approach

ARTICLE INFO:

Received: January 25, 2025

Revised: February 11, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

Acute abdominal pain is a frequent cause of emergency department (ED) visits, yet the management of pain in patients with acute abdominal pain remains controversial. Concerns about masking symptoms and delaying diagnosis have traditionally led to the withholding of analgesics prior to diagnosis. However, recent evidence suggests that early pain management does not compromise diagnostic accuracy or outcomes. This study evaluates the demographic and clinical characteristics of 97 patients presenting with acute abdominal pain at the Al-Hilla Teaching Hospital in Babylon, Iraq, from June 2023 to September 2023. The study focuses on their medication intake and pain management strategies, highlighting the effectiveness of a multimodal approach, including the use of esomeprazole, ketorolac, hyoscine butylbromide, ondansetron, and budesonide, in alleviating pain and associated symptoms. The results show that majority of the patients (90.7%) presented to the ED within 6 h of pain onset. The most frequently administered medications were esomeprazole (95.8%), ketorolac (70.1%), and antibiotics (64.9%). Pain subsided within 30–49 min in 60.8% of patients, demonstrating the effectiveness of early analgesia. The study supports the safe and timely administration of analgesics in the ED, challenging traditional practices and emphasizing the need for updated guidelines in order to improve patient care in emergency settings.

1. Introduction

Acute abdominal pain is one of the most common reasons for emergency department (ED) visits, accounting for a significant proportion of cases worldwide. It is a symptom that can arise from a wide range of underlying conditions, including gastrointestinal, genitourinary, and gynaecological disorders, as well as systemic illnesses. Despite its prevalence, the management of pain in acute abdominal pain patients remains a subject of debate¹. The challenge lies in balancing the need for prompt pain relief with the necessity of accurate diagnosis, as the underlying cause of abdominal pain can vary from benign conditions to life-threatening emergencies such as perforated ulcers, appendicitis, or ectopic pregnancies. Historically, clinicians have been reluctant to administer analgesics prior to diagnosis, fearing that pain relief might obscure clinical signs and lead to diagnostic errors. This traditional approach was based on the belief that masking pain could interfere with the ability to localize the source of the problem or assess the severity of the condition. For example, a reduction in pain might make it difficult to detect peritoneal signs, such as rebound tenderness, which are critical for diagnosing conditions such as peritonitis. However, emerging evidence suggests that early analgesia does not impair diagnostic accuracy and may, in fact, improve patient comfort and outcomes². Studies have shown that patients who receive timely pain relief are better able to cooperate during physical examinations and diagnostic procedures, leading to more accurate assessments.

In Iraq, where healthcare resources are often strained, the management of acute abdominal pain in the ED presents unique challenges³. Limited access to advanced diagnostic tools (such as computed tomography scans or magnetic resonance imaging) and a high patient load can delay both diagnosis and treatment, making clinicians less precise on clinical judgment; in such cases, the effective management of pain is even more critical, as it can help stabilize patients while diagnostic evaluations are underway. This study aimed at evaluating the demographic and clinical characteristics of patients presenting with

acute abdominal pain at the Al-Hilla Teaching Hospital (Iraq), with a focus on their medication intake and pain management strategies. By examining the effectiveness of a multimodal approach to pain management, the study sought to provide evidence-based recommendations for improving patient care in resource-limited settings. The study also aimed at challenging the traditional dogma of withholding analgesics prior to diagnosis, particularly in settings where diagnostic delays are common.

2. Methodology

A retrospective analysis was conducted on 97 patients who presented with acute abdominal pain to the ED of the Al-Hilla Teaching Hospital, Babylon, Iraq, from June 2023 to September 2023. Ethical approval for this study was obtained from the Institutional Review Board of the Al-Hilla Teaching Hospital (protocol number: AHT-2023-ED-01; date: May 15, 2023). Data were collected on demographic characteristics, associated symptoms, duration of pain before presentation, and medications administered in the ED. The medications evaluated included esomeprazole, ketorolac, antibiotics (intravenous), hyoscine butylbromide, ondansetron, intravenous fluids, and budesonide. The duration of the pain subsidence after treatment was also recorded. Statistical analysis was performed in order to determine the frequency and distribution of medication use and its association with pain relief. All categorical variables were presented in the form of numbers and percentages.

3. Results

The study included 97 patients, with a mean age of 39.6 years. The majority of the patients were female (60.8%) and the most common age group was 39–48 years (28.9%). The most frequently reported symptoms associated with acute abdominal pain were nausea and vomiting (95.8%), followed by loose stools (52.5%), and fatigue (38.1%). Most patients (90.7%) presented to the ED within 6 h of pain onset (Table 1).

Table 1. Demographic data of the study's 97 patients that entered the emergency department (ED) with acute abdominal pain and an overview of their associated symptoms, duration of pain before presentation, and medications administered. Abbreviations used: i.v., intravenous; SOB, shortness of breath.

Demographic data of patients (N=97)						
Age (in years)						
18–28	29–38	39–48	49–58	59–68	69–78	≥79
21 (21.6 %)	23 (23.7 %)	28 (28.9 %)	12 (12.4%)	7 (7.2 %)	5 (5.2 %)	1 (1 %)
Sex						
female			male			
59 (60.8 %)			38 (39.2%)			
Symptoms associated with acute abdominal pain						
nausea, vomiting	loose stool	fatigue	vertigo	fever	SOB	
93 (95.8%)	51 (52.5%)	37 (38.1%)	27 (27.8%)	32 (33.0%)	7 (7.2%)	
Duration of the pain before the presentations to the ED						
≤6 h		6–12 h		≥12 h		
88 (90.7%)		6 (6.2%)		3 (3.1%)		
Drugs used in treatment of patients acute abdominal pain at ED						
esomeprazole	ketorolac	antibiotics (i.v.)	hyoscine butylbromide	ondansetron	fluids (i.v.)	budesonide
93 (95.8%)	68 (70.1%)	63 (64.9%)	54 (55.6%)	43 (44.3%)	48 (49.4%)	21 (21.6%)
Duration of subsiding from acute abdominal pain after treatment						
20–29 min	30–39 min	40–49 min		50–59 min	≥60 min	
12 (12.4%)	27 (27.8 %)	32 (33.0 %)		20 (20.6 %)	6 (6.2 %)	

The most commonly administered medications were esomeprazole (95.8%), ketorolac (70.1%), and antibiotics (64.9%). Hyoscine butylbromide was used in 55.6% of cases, while ondansetron and intravenous fluids were administered to 44.3% and 49.4% of the patients, respectively. Budesonide was the least frequently used medication (21.6%). Pain subsided within 20–29 min in 12.4% of patients, while the majority experienced relief within 30–49 min (60.8%) (Table 1).

4. Discussion

The findings of this study demonstrate that a multi-modal approach to pain management is effective in alleviating acute abdominal pain in the ED. A multi-modal strategy involves the use of multiple medications with different mechanisms of action in order to target various aspects of pain and its associated symptoms. This approach not only provides more

comprehensive pain relief, but also minimizes the side effects associated with high doses of single medications.

Esomeprazole, a proton pump inhibitor, was the most frequently administered medication (95.8%), reflecting its role in reducing gastric acid secretion and alleviating gastrointestinal discomfort, particularly in patients with suspected peptic ulcers or gastritis⁴. Ketorolac, a nonsteroidal anti-inflammatory drug, was the second most commonly used medication (70.1%); its anti-inflammatory and analgesic properties make it effective in managing pain caused by inflammation, such as in cases of appendicitis or diverticulitis⁵. The combination of esomeprazole and ketorolac addresses both the underlying gastrointestinal issues and the inflammatory component of pain, providing a dual approach to symptom relief.

Hyoscine butylbromide, an antispasmodic agent, was used in 55.6% of cases; this medication targets smooth muscle spasms in the gastrointestinal tract,

which are a common source of colicky abdominal pain, particularly in conditions like irritable bowel syndrome or biliary colic⁶. Ondansetron, an antiemetic, was administered to 44.3% of patients to manage nausea and vomiting, which were present in 95.8% of cases; by controlling these symptoms, ondansetron not only improves patient comfort, but also reduces the risk of dehydration and electrolyte imbalances, which can complicate the clinical picture⁷. Finally, the relatively low use of budesonide (21.6%) suggests that it may be reserved for specific cases, such as inflammatory bowel disease. Budesonide is a corticosteroid with potent anti-inflammatory effects, particularly in the gastrointestinal tract; its targeted action makes it suitable for managing conditions like Crohn's disease or ulcerative colitis, where localized inflammation is the primary concern⁸.

The rapid subsidence of pain in most patients (within 30–49 min) underscores the effectiveness of early analgesia⁹. This finding is particularly significant in the context of acute abdominal pain, where timely pain relief can improve patient cooperation during diagnostic procedures and reduce overall distress. These results align with recent evidence challenging the traditional practice of withholding analgesics prior to diagnosis¹⁰. Historically, there was concern that pain relief might mask clinical signs and lead to diagnostic errors. However, contemporary studies have shown that early analgesia does not impair diagnostic accuracy and may, in fact, enhance it by allowing patients to better communicate their symptoms and tolerate physical examinations.

Our study has significant implications for emergency care in Iraq and similar resource-limited settings. It also highlights the importance of addressing associated symptoms, such as nausea and vomiting, which were present in the majority of patients. By incorporating medications such as ondansetron into the pain management strategy, clinicians can pro-

vide more holistic care, addressing not only the pain, but also the secondary symptoms that contribute to patient discomfort. This comprehensive approach is particularly important in resource-limited settings such as Iraq, where delayed or inadequate treatment can lead to worse outcomes. Future research should focus on the long-term outcomes of early analgesia and its impact on diagnostic accuracy and patient satisfaction.

5. Conclusion

This study supports the safe and timely administration of analgesics in patients presenting with acute abdominal pain in the ED. A multimodal approach, incorporating esomeprazole, ketorolac, hyoscine butylbromide, ondansetron, and other medications, is effective in alleviating pain and associated symptoms. These findings challenge the traditional dogma of withholding analgesics prior to diagnosis and emphasize the need for updated guidelines in order to improve patient care in emergency settings.

Acknowledgements

We would like to express our gratitude to the Administration of the Al-Hilla Teaching Hospital for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Saigh A.J., Abdalwahd N., Kadhun R.W., Al-Saigh R.J., Al-Humadi S.H., Alsaigh N.J. Pain management strategies for acute abdominal pain in the emergency department: a study from the Al-Hilla Teaching Hospital (Iraq). *Pharmakeftiki* 37(2s), 168-172, 2025. <https://doi.org/10.60988/p.v37i2S.167>

Quality assurance for school health service providers at primary healthcare centers in Hillah (Iraq)

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KEY WORDS:

evaluation; quality assurance;
school health providers;
primary health; care centers

ARTICLE INFO:

Received: January 07, 2025

Revised: February 20, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

This study aimed at evaluating the performance of school health providers at primary health centers in Al-Hilla City (Hillah) through the application of a quality assurance framework. A descriptive evaluation study was conducted from October 5, 2024 to March 25, 2025. A purposive sample of 63 individuals was selected using a non-probability sampling technique from 15 primary healthcare centers, drawn from two health sectors within the Al-Hilla City Center under the Babylon Health Directorate in Iraq. The sample comprised 20 members of the medical staff and 43 members of the nursing staff. A panel of thirteen experts participated in a pilot study in order to assess the reliability of the evaluation instrument through content validity and internal consistency metrics. The findings indicate a growing demand and a diminishing supply of workforce in primary health care. The quality assurance assessment of school health activities performed by medical and nursing staff revealed a fair overall performance. However, health education services provided to teaching staff and students were found to be suboptimal.

1. Introduction

Quality assurance is a continuous

process aimed at improving and maintaining optimal standards in healthcare services; it involves de-

Table 1. Quality assurance score distribution for the performance of school health providers in Hillah (Iraq).								
Quality assurance	Poor (10–19)		Fair (20–26)		Good (27+)		Total	
medical services	N	%	N	%	N	%	N	%
	6	30%	11	55%	3	15%	20	100%
Quality assurance	Poor (15–27)		Fair (28–41)		Good (42+)		Total	
nursing services	N	%	N	%	N	%	N	%
	13	30%	21	49%	9	21%	43	100%

veloping performance indicators, monitoring adherence to standards, and addressing systemic challenges through a team-based approach¹. In the context of school health services, quality assurance plays a pivotal role in confidential event reporting and data analysis systems. These mechanisms form the foundation for evidence-based practices and ongoing systemic improvements. In order to meet the evolving and complex health needs of today’s student populations, quality assurance must be integral to service delivery. Beyond facilitating appropriate referrals to healthcare providers, robust school healthcare systems safeguard and enhance the health and safety of students under their care².

Quality assurance in school health services is also recognized as a structured framework for promoting excellence at both individual and team levels; it equips health personnel with tools allowing them to assess current performance and foster continuous improvement. In doing so, students receive care from skilled providers. Conversely, failure to deliver quality services undermines trust and may lead students to engage with the health system only in urgent circumstances requiring preventive, curative, or promotive care³. Furthermore, quality assurance initiatives offer opportunities for professional development, elevating healthcare workers’ standing in their communities and improving job satisfaction, since service providers are ultimately the cornerstone of quality in healthcare delivery⁴.

In Iraq, data from the year 2000 indicated that 51% of the population was under the age of 15. Given that school health services address individuals from early childhood (i.e., age of 4 years) through university graduation, they serve a demo-

graphic spanning of over two decades, amounting to approximately one-third of the Iraqi population. School health services play a critical role in correcting health misconceptions and disseminating clear, practical health messages. As schools can be vectors for disease transmission, health education and intervention within school environments are essential for curbing the spread of communicable diseases⁵. The present study proposes a pathway for evaluating and enhancing school health services by assessing the performance of medical and nursing staff; the primary providers of these services.

2. Methodology

A descriptive evaluation study was conducted across primary healthcare centers in Al-Hilla City, Iraq, from October 5, 2024 to March 25, 2025. The objective was to assess the quality assurance performance of school health service providers operating within these centers. A multistage cluster sampling method was employed, resulting in a probability-based selection of 63 staff members, comprising 20 members of the medical staff and 43 members of the nursing personnel.

An evaluation instrument was designed based on the updated guidelines from the Quality Assurance Manual for School Health Services⁴, with contextual modifications tailored to the local healthcare setting. The tool consisted of three sections: (i) part I (examining the demographic characteristics of the care providers), (ii) part II (examining the training and professional development of the care providers), and (iii) part III (examining the activities and responsibilities of the medical and nursing staff).

In total, 43 items were included in the instrument. Reliability was verified through internal consistency measures, while validity was established via expert review by a panel of 13 professionals.

Quality assurance scores were computed using a quartile deviation test applied to the checklist assessing process standards, specifically staff activities and duties. Quartiles were categorized as follows: (i) first and second quartiles ($\leq 25\%$) as "poor", (ii) third quartile ($\approx 50\%$) as "fair", and (iii) fourth quartile ($\geq 75\%$) as "good"⁵. For the medical staff, performance thresholds were classified as "poor" (scores 10–19), "fair" (20–26), and "good" (≥ 27). For the nursing staff, thresholds were classified as "poor" (15–27), "fair" (28–41), and "good" (≥ 42). Data collection involved structured interviews guided by the evaluation tool, alongside documentation of contact records in order to facilitate sample access. Each interview lasted approximately 5 to 10 min. Data were gathered, coded, and analysed using the SPSS version 20.0 software for Windows.

Ethical approval was granted by the reviewing board of the College of Nursing of the Al-Mustaqbal University, following a full review of the research protocol, informed consent documentation, and supporting materials. The study adhered to the ethical principles of the Declaration of Helsinki, with formal approval recorded under document 1650 on October 2, 2024.

3. Results and Discussion

Analysis of the quality assurance data for school health providers' activities reveals that both medical services (55%) and nursing services (49%) were rated at a fair performance level (Table 1). These figures suggest that the primary health centers in Hillah (Iraq) fall short of meeting the desired standards for delivering acceptable school health services,

primarily due to deficiencies in one key component of quality assurance: the process dimension⁶. The underperformance of school health professionals appears to be a major contributor to this shortfall. Many providers operate across multiple roles within the primary healthcare centers, which may compromise the effectiveness of their school health service delivery⁷. This pattern of underperformance may also be attributed to insufficient oversight and follow-up by the Babylon Health Directorate and the Iraqi Ministry of Health. Without robust monitoring systems, staff performance has shown signs of deterioration.

4. Conclusion

Our study's overall evaluation indicates that the quality assurance performance of school health providers in the primary healthcare centers of Hillah (Iraq) is at a fair level.

Acknowledgements

The authors would like to express their gratitude to the faculty members of the College of Nursing of Al-Mustaqbal University for their support.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Taee M.H., Hajwal S.K., Ayad Z.M., Muhaibes F.J., Khaleel M.A., Hassan A.A.H., Abbas A.H., Ridha-Salman H., Abbas Z.H. Quality assurance for school health service providers at primary healthcare centers in Hillah (Iraq). *Pharmakeftiki* 37(2s), 173-176, 2025. <https://doi.org/10.60988/p.v37i2S.168>

The correlation between some immunological parameters and causative agent in patients with otitis media

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KEY WORDS:

otitis media; bacteria; CRP;
IL-6; IL-10

ARTICLE INFO:

Received: October 14, 2024

Revised: January 01, 2025

Accepted: January 14, 2025

Available online: October 10, 2025

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ABSTRACT

Otitis media, an inflammation of the middle ear, is a common illness in both childhood and adulthood. It arises due to various factors, primarily microbiological and immunological. This study aimed at investigating the microorganisms and fungi associated with otitis media in Iraqi patients, as well as to examine the correlation between causative agents (bacteria and fungi). The study was conducted between November 2017 and April 2018 at the Al-Hilla Teaching Hospital in Babylon City. A total of 90 pus and blood samples were collected from patients aged 1–80 years with otitis media. Samples were taken from discharging ears using sterile transport medium swabs. Of the 90 samples, 86 (95.6%) tested positive for microbial agents, while 4 cases (4.4%) had no identifiable causative agent. Among the infected individuals, 57 were male (63.3%) and 33 were female (36.7%). Samples were analysed in the microbiology laboratory in order to identify microbial species responsible for otitis media. The highest infection rates were observed in the age groups 1–20 years (34.4%) and 41–60 years (32.2%). Immunological parameters from the patients' sera revealed significant differences compared to those of the control group (N=20). While C-reactive protein levels showed no significant difference between patients and controls, serum interleukin-10 concentrations were found to be significantly elevated in patients with bacterial infections.

1. Introduction

Otitis media (OM) is an inflammation of the middle ear, with acute OM representing its acute manifestation. The diagnosis of acute OM should be based on the presence of acute symptoms and middle ear effusion. It is a multifactorial disease, influenced by environmental, bacteriological, host, immunological, and genetic factors¹. OM is characterized as an inflammatory response to either acute or persistent stimuli, typified by the accumulation of both cellular and chemical mediators in the middle ear. These mediators (glycoproteins, peptides, cytokines, prostaglandins, leukotrienes, and others) play roles in the inflammatory process through mechanisms such as changes in vascular permeability and chemotaxis. OM is frequently caused by bacteria; however, sterile cases are not uncommon. Pathogens typically enter the middle ear from the nasopharynx *via* the Eustachian tube.

In bacterial OM, keratinocytes can produce various soluble inflammatory mediators in response to injury, independent of immune cells. These include interleukin-1 (IL-1), -6 (IL-6), and -8 (IL-8)². IL-6 is a proinflammatory cytokine that plays a key role in the acute phase reaction, including the induction of C-reactive protein (CRP), which is predominantly synthesized by hepatocytes under the transcriptional control of IL-6. IL-6 is considered a sensitive and reliable marker of neonatal bacterial infection³. Interleukin-10 (IL-10), a critical immunoregulatory cytokine, is also involved in modulating the middle ear's inflammatory response to bacterial infection⁴.

This study aimed at investigating the microorganisms and fungi associated with OM in Iraqi patients, as well as to examine the correlation between causative agents (bacteria and fungi).

2. Methodology

Sterile cotton swabs and gel tubes were used in order to collect ear exudate samples from a total of 90 patients diagnosed with OM. These patients attended private surgical clinics during the five-month pe-

riod from November 2017 to the end of April 2018, at the ENT Department of the Al-Hilla Teaching Hospital in Babylon City. Our study's control group consisted of 20 healthy and randomly selected volunteers. All collected samples were directly cultured on the following media: (i) blood agar, MacConkey agar, chocolate agar, and mannitol salt agar (in which the cultures were incubated at 37°C, for 24–48 h), and (ii) potato dextrose agar (in which the cultures were incubated at 25°C, for 5–7 days). Direct smears were prepared from samples before and after culturing. Gram staining was performed in order to differentiate Gram-positive and Gram-negative bacteria. The serum levels of CRP, IL-6, and IL-10 were determined using ELISA kits. Data were analysed using Microsoft Excel (2010) and SPSS version 23.

3. Results and Discussion

Out of 90 samples, 86 yielded positive microbial cultures, while 4 showed no growth after 48 h. These 4 cases may involve other types of causative agents (such as viruses, parasites, *Chlamydia*, or *Mycoplasma*), which require more specialized detection techniques⁵.

Bacterial infection was the predominant cause (62.2%) of OM, followed by mixed infections involving both fungi and bacteria (28.9%). Cases attributed solely to fungi or unknown agents accounted for 4.4%. A high proportion of the causative organisms of OM were Gram-positive bacteria (69 isolates; 46%); a finding consistent with previous findings⁶.

Among the positive OM cases, 57 were male (63.3%) and 33 female (36.7%). The age groups 1–20 years and 41–60 years exhibited the highest infection rates, 34.4% and 32.2% respectively, while individuals aged 61–80 years exhibited the lowest infection rate (10%).

Serum CRP levels (measured in mg/L) were compared between the OM infection and the control groups by using one-way ANOVA with a significance threshold of $p < 0.05$. The mean CRP levels were significantly higher in the bacterial (1.61 ± 3.88 mg/L), fungal (1.5 ± 3.0 mg/L), and unknown (1.5 ± 3.0 mg/L) cases compared to those of the control group

Table 1. Correlations among the serum C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-10 (IL-10) levels in Iraqi otitis media patients. Note: *, signifies a statistically significant correlation at $p < 0.05$.

Pearson correlation coefficient (<i>r</i>)	IL-6 levels	IL-10 levels	CRP levels
IL-6 levels	1		
IL-10 levels	-0.078	1	
CRP levels	0.256 *	-0.129	1

(0.10 ± 0.00 mg/L). However, the mixed OM infection group did not exhibit a significant increase in CRP levels (0.92 ± 2.20 mg/L) when compared to those of the control group. The least significant difference (LSD) value was 0.97.

Serum IL-6 levels were found to be significantly elevated ($p < 0.05$) in both the unknown cause case (157.73 ± 46.32 pg/mL) and the bacterial case (144.63 ± 148.78 pg/mL) groups, compared to those of the control group (53.53 ± 11.01 pg/mL). No significant increase was noted in the mixed OM infection (119.69 ± 145.82 pg/mL) or fungal case (101.02 ± 27.67 pg/mL) groups. The LSD value was 70.57.

As far as the serum IL-10 levels are concerned, only the bacterial case group exhibited a statistically significant increase (72.1 ± 22.89 pg/mL) compared to those of the control group (57.69 ± 2.97 pg/mL). Other groups – fungal case (71.35 ± 14.03 pg/mL), mixed case (67.07 ± 11.08 pg/mL), and unknown cause case (67.31 ± 4.97 pg/mL) groups – did not exhibit significant differences in terms of their IL-10 levels when compared to those of the control group. The LSD value was 13.70.

The correlations between the serum levels of the aforementioned parameters are presented in Table 1. Our findings suggest that serum CRP levels have a strong positive correlation with those of IL-6 in OM, thereby reinforcing their diagnostic value in distinguishing OM patients from healthy individuals.

Variations in infection type may be influenced by environmental conditions, geographic factors, and individual health status. Mixed infections likely re-

sult from a primary bacterial infection, followed by secondary fungal colonization. Diagnosing the initial *versus* the secondary agent in such cases remains complex, as supported by previous findings⁶.

The present study has revealed significant age-related variations in OM incidence, with highest infection rates observed in the 1–20 and the 41–60 year groups (34.4% and 32.2%, respectively). This may be attributed to several predisposing factors in these age ranges, including smoking, diet, environmental exposures, immature immune systems (particularly in children), and weaker physical constitutions⁷.

In contrast to a previous study⁸ that has identified the highest OM infection rates in children aged 6–12 months, the current study has found a marked sex-based difference, with males (63.3%) being more affected than females (36.7%). This diverges from earlier studies that have reported no significant sex differences, largely because their cohorts focused on children. The higher male incidence in this study may reflect greater exposure to environmental stressors⁹.

Finally, the acute stage of an OM infection appears to correlate with elevated CRP levels, especially in the case of bacterial OM. This supports the view that bacterial pathogens are more potent in inducing CRP production than fungal or other / unidentified causes¹⁰.

4. Conclusion

The immunological assessment of Iraqi OM patients has demonstrated significant differences compared to that of controls. Although the serum CRP levels did not reveal significant differences across all OM infection

types, the serum IL-6 levels were found to be markedly elevated in OM patients, and the serum IL-10 levels were found to be significantly increased in those OM patients with bacterial infections, as compared to the respective markers' levels of the control group.

Acknowledgements

The authors wish to express their sincere gratitude to the College of Science of the University of Babylon and to its Advanced Microbiology Laboratory for

their valuable support.

Conflicts of interest

None exist.

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HOW TO CITE:

Althahab A., Abdul-Majeed K., Hameed W., Hathal W. The correlation between some immunological parameters and causative agent in patients with otitis media. *Pharmakeftiki* 37(2s), 177-180, 2025. <https://doi.org/10.60988/p.v37i2s.169>

In silico* docking and target prediction of stigmasterol extracted from *Chrysanthemum hortorum

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KEY WORDS:

**MOE software; *in silico* study;
hPXR; 7N4V; 7AXK**

ARTICLE INFO:

Received: December 28, 2024

Revised: January 09, 2025

Accepted: January 31, 2025

Available online: October 10, 2025

ABSTRACT

This *in silico* study explores the therapeutic potential of stigmasterol (a phytosterol identified in *Chrysanthemum hortorum* via gas chromatography mass spectrometry; GC-MS) through computational docking against 14 proteins predicted using SwissTargetPrediction. These proteins, implicated in various cancers and metabolic pathways, were retrieved from the Protein Data Bank (PDB) and were analysed using the Molecular Operating Environment (MOE) software. Stigmasterol exhibited high binding affinities with key targets, notably 7N4V (Niemann-Pick C1-like 1; NPC1L1) and 7AXK (the ligand-binding domain of the human pregnane X receptor; hPXR), with ΔG values of -9.2442 and -9.0119 kcal/mol, respectively. Hydrophobic interactions involving residues such as TRP, TYR, PHE, and ILE enhanced ligand stability, while water-mediated contacts (particularly those observed in 4EY7 and 2ZNN) contributed to binding specificity. These findings suggest stigmasterol's potential to modulate cancer-associated pathways, especially those of hormone-sensitive cancers, by influencing cholesterol metabolism and nuclear receptor signalling.

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1. Introduction

Developed nations are increasingly integrating conventional clinical practices with the use of herbal medicines and remedies, resulting in growing reliance on these treatment strategies. Numerous phar-

maceuticals have been developed and derived from plants in industrialized countries. Approximately 70% to 95% of the global population relies on traditional medicine as their primary form of health-care. *Chrysanthemum hortorum*, a member of the Asteraceae family, is

Table 1. Molecular docking results obtained using the Molecular Operating Environment (MOE) software. Target proteins include: 1Q1M (protein tyrosine phosphatase 1B), 2ZNN (human PPAR alpha ligand-binding domain), 2ZNQ (human PPAR delta ligand-binding domain), 4C49 (reactive loop-cleaved human corticosteroid-binding globulin; CBG), 4EY7 (recombinant human acetylcholinesterase), 4YVX (aldo-keto reductase family 1 member C3), 6ESY (human butyrylcholinesterase), 6HJ2 (human pregnane X receptor; hPXR), 6IIU (human thromboxane A₂ receptor), 6K9H (human liver X receptor beta; LXR-β), 6Q2W (human retinoic acid receptor-related orphan receptor gamma; RORγ LBD), 7AXK (ligand-binding domain of the human pregnane X receptor; hPXR), 7CX3 (taprenepag), and 7N4V (cholesterol-bound human Niemann-Pick C1-like 1; NPC1L1). Other abbreviations used: ALA, alanine; ARG, arginine; CYS, cysteine; GLY, glycine; HIS, histidine; HOH, water; IEU, placeholder for ILE or LEU; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; RMSD, root mean square deviation in binding; TRP, tryptophan; TYR, tyrosine; VAL, valine.

Proteins	ΔG (kcal/mol)	RMSD (Å)	HOH	Amino acids included in H-bonding	Amino acids included in short contacts
4EY7	-6.2767	2.0025	HOH ₉₅₂ (2x)	-	TRP ₂₈₆ , PHE ₃₃₈ , TYR ₃₄₁ , TYR ₃₃₇ , TYR ₁₂₄ , TRP ₈₆
4YVX	-7.3928	1.0548	-	-	PHE ₃₀₆ , HIS ₁₁₇ , PHE ₃₁₁ , TRP ₈₆ , TRP ₂₂₇ , TYR ₂₄ , TYR ₅₅ , LEU ₂₆₈ , TYR ₂₁₆
6ESY	-7.1311	1.5331	-	GLY ₁₉₇	TYR ₃₃₂ , GLY ₄₃₉ , TRP ₈₂ , HIS ₄₃₈
7N4V	-9.2442	1.3738	-	-	VAL ₆₉₇ , ILE ₁₂₃₄ , VAL ₇₀₁ , PHE ₁₂₃₉ , LEU ₂₃₄ , ILE ₆₉₈ , TRP ₃₈₃ , PHE ₇₇₂ , IEU ₈₇₁ , TYR ₁₁₀₂ , LEU ₆₂₁ , ALA ₈₇₆ , ILE ₆₂₅ , VAL ₁₁₆₆
4C49	-6.7286	1.5322	-	ARG ₂₆₀	ARG ₂₆₀ , TRP ₃₇₁ , HIS ₃₆₈ , PHE ₃₆₆ , ILE ₂₆₃
7AXK	-9.0119	1.5027	-	ARG ₄₁₀ (2x)	ARG ₄₁₀ (2x)
6K9H	-6.4266	1.4434	-	-	PHE ₃₄₉ , IEU ₃₁₃ , ILE ₃₅₃ , MET ₃₁₂ , PHE ₃₄₀ , ILE ₃₂₇ , PHE ₃₅₄ , IEU ₃₄₅ , ILE ₃₅₀ , ILE ₃₀₉ , HIS ₄₃₅ , LEU ₄₄₂ , VAL ₄₃₉ , ALA ₂₇₅ , TRP ₄₅₇ , PHE ₃₄₉
6Q2W	-7.3919	1.3449	-	-	MET ₃₆₅ , VAL ₃₇₆ , ILE ₃₉₇ , PHE ₃₈₈ , LEU ₃₉₁ , ILE ₄₀₀ , TRP ₃₁₇ , MET ₃₅₈₈ , LEU ₃₉₆ , CYS ₃₂₀ , PHE ₃₇₈ , LEU ₃₂₄
6HJ2	-7.5185	1.4055	-	-	ILE ₄₁₄ , LEU ₄₁₁ , LEU ₂₀₉ , MET ₂₄₃ , VAL ₂₁₁ , MET ₂₄₆ , TYR ₃₀₆ , MET ₃₂₃ , TRP ₂₉₉ , PHE ₂₈₈ , HIS ₄₀₇ , PHE ₂₅₁ , PHE ₄₂₉ , PHE ₄₂₉ , PHE ₂₈₁ , CYS ₂₈₄
2ZNN	-7.6006	1.8405	HOH ₁₀₀₄	-	VAL ₂₅₅ , CYS ₂₇₅ , ILE ₃₅₄ , PHE ₃₁₈ , LEU ₃₂₁ , MET ₃₅₅ , MET ₃₃₀ , CYS ₂₇₆ , VAL ₃₃₂ , ILE ₃₃₉ , ILE ₂₇₂ , ALA ₃₃₃ , LEU ₂₅₀ , LEU ₂₅₄ , LEU ₂₄₇ , ALA ₂₅₀ , ILE ₂₄₁
2ZNQ	-5.6081	1.6253	-	CYS ₂₈₅	VAL ₃₄₁ , LEU ₃₅₃ , VAL ₃₄₈ , VAL ₂₈₁ , ARG ₂₈₄ , LYS ₃₆₇ , PHE ₃₂₇ , HIS ₄₄₉
7CX3	-8.4296	1.4498	-	-	MET ₁₁₆ , ILE ₃₀₉ , ILE ₈₅ , MET ₃₁ , ARG ₃₀₂ , VAL ₈₉
1Q1M	-6.43447	1.7784	-	ARG ₂₄ , GLY ₂₅₉	VAL ₄₉ , LYS ₁₂₀ , TYR ₄₆ , PHE ₁₈₂ , ALA ₂₁₇
6IIU	-5.3068	1.4327	-	-	ALA ₁₅₈ , LEU ₁₆₁ , LEU ₇₆ , TRP ₁₅₇

widely cultivated as a cut flower and potted plant¹. The genus *Chrysanthemum* comprises approximately 37 species, predominantly distributed across East Asia, including China, Korea, Japan, and Siberian Russia². *Chrysanthemum* L. has long been valued in

traditional medicine for its associations with health and longevity. Decoctions of *Chrysanthemum* are known to reduce blood pressure, improve coronary circulation, regulate myocardial function, lower low-density lipoprotein (LDL) cholesterol, exert an-

titoxic and anticancer effects, and support the autonomic nervous system. Phytochemical evaluation of *Chrysanthemum hortorum* Bailey has revealed the presence of essential oils, organic acids, and phenolic compounds such as flavonoids, hydroxycinnamic acids, and tannins, which contribute to its medicinal profile³. Additional studies have confirmed the presence of phytosterols (including sitosterol, stigmasterol, and campesterol), thereby further substantiating its therapeutic potential^{4,5}. The present study investigates the molecular docking of stigmasterol with 14 selected proteins identified through SwissTargetPrediction.

2. Methodology

Whole plants of *Chrysanthemum hortorum* (including leaves, stems, and roots) were collected from the Babylon Nursery Plantation. A total of 100 g of powdered plant material was macerated in n-hexane for 48 h, and were subsequently filtered. The filtrate was evaporated in order to obtain the hexane extract, which was then analysed using gas chromatography mass spectrometry (GC-MS) for compound identification⁶.

The study focused on the molecular docking of stigmasterol with 14 proteins (4EY7, 4YVX, 6ESY, 7N4V, 4C49, 7AXK, 6K9H, 6Q2W, 6HJ2, 2ZNN, 2ZNQ, 7CX3, 1Q1M, and 6IIU) selected based on their relevance to stigmasterol's anticipated biological activity, particularly in cancer and metabolic pathways. The three-dimensional structure of stigmasterol was retrieved from the PubChem database, was geometrically optimized, and was assigned atomic charges in order to comply with the docking protocol requirements.

Crystal structures of the target proteins were obtained from the Protein Data Bank (PDB). Proteins were protonated, missing hydrogen atoms were added, bond orders were corrected, and suitable protonation states were assigned in order to simulate physiological conditions. Docking studies were conducted by using the Molecular Operating Environment (MOE) software, with attention given to binding interactions between stigmasterol and the active sites of the target proteins. Binding affinities and interaction types

(such as hydrogen bonding and hydrophobic contacts) were analysed in order to assess the stability and energetics of the ligand-protein complexes. This methodological approach enabled a systematic evaluation of stigmasterol's interactions with target proteins, providing insights into its potential therapeutic mechanisms across disease pathways.

3. Results and Discussion

The undertaken structural analysis of the n-hexane extract confirmed the presence of stigmasterol. Molecular docking revealed significant interactions between stigmasterol and the selected proteins from SwissTargetPrediction, underscoring its therapeutic potential. Notably, proteins such as 7N4V (Niemann-Pick C1-like 1; NPC1L1) and 7AXK (ligand-binding domain of the human pregnane X receptor; hPXR) demonstrated high binding affinities, with ΔG values of -9.2442 and -9.0119 kcal/mol, respectively. These results suggest robust and stable ligand-protein interactions, which may be critical in modulating cancer-associated pathways.

Hydrophobic interactions involving residues such as TRP, TYR, PHE, and ILE were observed; these seem to enhance ligand stability, especially in targets such as 6HJ2 (hPXR) and 7CX3 (Taprenepag). Additionally, water-mediated interactions – particularly in 4EY7 (acetylcholinesterase) – contributed to binding specificity. Proteins such as NPC1L1 and hPXR were emphasized for their roles in cholesterol metabolism and nuclear receptor signaling, both of which are commonly dysregulated in cancer^{7,8}.

These findings are consistent with earlier studies indicating that stigmasterol may influence cholesterol uptake and nuclear receptor pathways, thereby suggesting promising therapeutic avenues for hormone-sensitive malignancies.

4. Conclusion

Stigmasterol demonstrated strong binding affinities with key protein targets, highlighting its potential as a therapeutic agent in cancer and metabolic dis-

orders. Its interactions with proteins such as hPXR and NPC1L1 suggest a role in modulating cholesterol metabolism and hormone-sensitive signalling pathways, warranting further pharmacological investigation.

Acknowledgements

We extend our sincere gratitude to the College of Pharmacy of the University of Babylon for providing

the facilities and support that enabled this research.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-yassery H.K., Abdulsada A.H. *In silico* docking and target prediction of stigmasterol extracted from *Chrysanthemum hortorum*. *Pharmakeftiki* 37(2s), 181-184, 2025. <https://doi.org/10.60988/p.v37i2S.183>

Transdermal anastrozole patch formulation and *in vitro* release study

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KEY WORDS:

anastrozole; breast cancer; transdermal patches; sustained release; chemotherapy

ARTICLE INFO:

Received: January 29, 2025

Revised: February 22, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

ABSTRACT

Anastrozole is commonly prescribed to postmenopausal women with hormone-sensitive breast cancer; however, its severe side effects – often dependent on dosage – are frequently linked to the standard oral formulation. A sustained-release transdermal delivery system may offer a means of mitigating these adverse effects. In this study, transdermal patches containing anastrozole were developed, and the influence of polymer composition on the drug's *in vitro* release profile was evaluated. Polyethylene glycol 400 (PEG 400) served as a plasticizer, with varying ratios of hydrophobic to hydrophilic polymers: Eudragit L-100 and hydroxypropyl methylcellulose (HPMC), respectively. The formulations were assessed for their physicochemical properties and their *in vitro* drug release in phosphate-buffered solution (pH 7.4) at 32.0°C ± 1.0°C. Among these, the formulation containing a 7:3 Eudragit L-100 to HPMC ratio was selected, as its release kinetics conformed to the Higuchi model, thereby suggesting diffusion as the predominant mechanism of sustained drug release.

1. Introduction

Breast cancer remains the leading cause of mortality and the most pressing reproductive health concern among women aged over 15. Third-generation nonsteroidal aromatase inhibitors, such as anastrozole, are approved for the treatment of breast cancer. Currently, the only

available form of anastrozole is an orally administered, immediate-release formulation, which is frequently associated with serious systemic side effects¹. In contrast, a controlled-release transdermal formulation of anastrozole could offer several potential advantages, including prolonged pharmacological action due to sustained release, maintenance of

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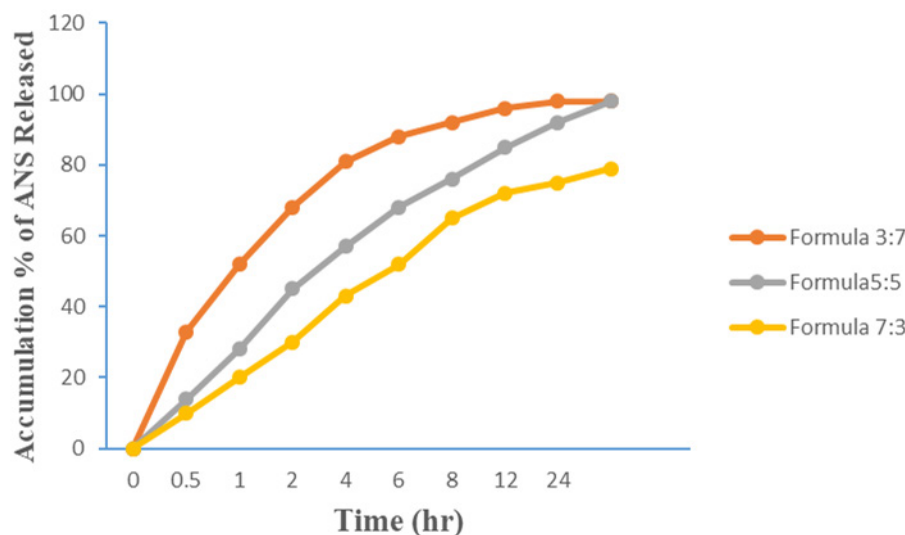


Figure 1. *In vitro* drug release (%) from anastrozole-loaded transdermal patches prepared with varying ratios of Eudragit L-100 to hydroxypropyl methylcellulose (HPMC): 7:3, 5:5, and 3:7. Notes: results are presented as mean $\pm$ standard deviation (SD); $n=3$.

stable, therapeutically effective drug concentrations, avoidance of undesirable plasma peak fluctuations, and improved patient compliance². Accordingly, the present study aimed at developing transdermal anastrozole patches and at assessing the influence of polymer ratios on the drug's *in vitro* release profile.

2. Methodology

2.1. Materials and transdermal adhesive patch preparation

Anastrozole was procured from HyperChem® (Zhejiang, China), and all polymers and solvents were supplied by the Pioneer Company. The transdermal patches were prepared using a solvent-casting technique. A polymeric solution was created by dissolving varying ratios of Eudragit L-100 to hydroxypropyl methylcellulose (HPMC) at ratios 7:3, 5:5, and 3:7, in 15 mL of ethanol and water, followed by stirring for 30 min at 250 rpm using a hot plate magnetic stirrer. Polyethylene glycol 400 (PEG 400; 30% w/w) was incorporated as a plasticizer. Separately, 1 mg of anastrozole was dissolved in 5 mL of methanol and was added to the poly-

mer solution. The combined mixture was sonicated for 20 min at 35°C in order to remove residual air bubbles. The resulting solution was poured onto a petri dish lined with a 5% w/v polyvinyl alcohol (PVA) backing layer. An inverted funnel was placed above the dish so as to regulate evaporation, and the solvent was allowed to evaporate at ambient temperature over 24 h².

2.2. Assessment of the transdermal patches

2.2.1. Weight and thickness measurement

Each of the three formulations was weighed using a digital scale, and both mean weight and standard deviation (SD) were calculated³. Patch thickness was measured using a vernier caliper ($n=3$), and the mean thickness with SD was recorded².

2.2.2. Swelling index and percentage of weight change⁴

Three 1×1-cm film samples were pre-weighed and placed in petri dishes containing distilled water. Weights were recorded at predetermined intervals. Swelling behavior was quantified using the formulas:

$$\text{swelling index} = (W_2 - W_1) / W_1$$

$$\text{swelling-induced weight gain (\%)} = [(W_2 - W_1) / W_1] \times 100$$
where W_1 is the initial weight of the film, and W_2 is the weight at time t . After 60 min, the erosion was calculated as:

$$\text{erosion-induced weight loss (\%)} = [(W_1 - W_2) / W_1] \times 100$$

2.2.3. Content uniformity test⁵

A 2×2-cm film sample (in triplicate for each formulation) was dissolved in 100 mL of phosphate buffer (pH 7.4) using a magnetic stirrer at 32°C. A 3-mL aliquot was filtered through a syringe filter and diluted with an equal volume of fresh buffer. Drug concentration was measured by UV spectrophotometry at 215 nm, and quantified using a calibration curve.

2.2.4. Drug release study⁵

In vitro release studies (n=3) were performed using a USP apparatus containing 200 mL of phosphate buffer (pH 7.4), maintained at 32.0°C ± 1.0°C with constant agitation at 50 rpm. Samples of 3 mL were withdrawn at regular intervals over 24 h and were analysed spectrophotometrically at 215 nm.

2.2.5. Drug release kinetic modelling⁵

The release kinetics of anastrozole from the patches were assessed using mathematical models following the *in vitro* release experiments.

3. Results and Discussion

The transdermal patches developed in this study exhibited weights ranging from 1.50 ± 0.011 to 1.60 ± 0.013 g. Weight variation within each formulation was minimal and consistent, as evidenced by the low standard deviation values. The relatively higher patch weight is attributable to the presence of a backing layer⁶. On the other hand, patch thickness ranged from 0.66 ± 0.013 cm to 0.69 ± 0.012 cm; the narrow range and low standard deviations suggest that the fabrication method produced reproducible films⁶.

Among the formulations, the 7:3 Eudragit L-100 to

HPMC ratio displayed the lowest swelling index and percentage weight gain (0.91 and 80.69%, respectively), while the 3:7 ratio yielded the highest values (1.70 and 125.74%, respectively), consistent with its elevated hydrophilic polymer content. When immersed in water, the swelling index reflects the extent of hydration within the film. Excess hydration may create voids within the polymer matrix, which could affect the sustained-release profile. Additionally, excessive swelling can compromise the patch's mechanical stability⁶.

Erosion and disintegration profiles following 60 min of water immersion revealed that the increased Eudragit L-100 content – owing to its hydrophobic nature – was associated with reduced disintegration⁷. The 7:3 formulation exhibited the lowest erosion rate at 2.25%.

Drug content across all assessed formulations was highly consistent (100.06% ± 0.02%, 98.20% ± 0.08%, and 99.78% ± 0.04%), as indicated by minimal standard deviation values. Uniform drug distribution within the matrix is a critical feature of sustained-release systems, supporting predictable pharmacokinetics⁸.

Figure 1 illustrates that patches with higher HPMC content exhibited a pronounced burst release effect, whereas the 7:3 formulation had the lowest burst release among all tested variants. This trend reflects the role of the hydrophobic polymer composition in moderating drug release. A reduction in the initial burst and an enhancement of the sustained-release behavior were achieved by increasing the hydrophobic fraction and by reducing the hydrophilic polymer content in the matrix⁹. Finally, the release kinetics of the 7:3 formulation demonstrated optimal fit with the Higuchi model (correlation coefficient $R^2 = 0.9821$), thereby indicating that diffusion was the dominant mechanism governing drug release from the matrix¹⁰.

4. Conclusion

The findings of this study support the development of a sustained-release transdermal patch containing anastrozole using a 7:3 Eudragit L-100 to HPMC ratio. As the proportion of HPMC increases, drug release is accelerated, whereas Eudragit L-100 demonstrates retardant properties at higher concentrations. This polymer combination facilitates a sustained release of

anastrozole *via* diffusion-driven kinetics, and may offer a viable alternative to existing oral formulations.

Acknowledgements

The authors thank the Department of Pharmaceutics of the College of Pharmacy of the University of Baghdad, for the support received.

Conflicts of interest

None exist.

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HOW TO CITE:

Alwan L.A., Abd Alhammid S.N. Transdermal anastrozole patch formulation and *in vitro* release study. *Pharmakeftiki* 37(2s), 185-188, 2025. <https://doi.org/10.60988/p.v37i2S.186>

Suppressive effect of rutin on methotrexate-induced salivary gland injury in rats

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KEY WORDS:

rutin; methotrexate; salivary gland damage; oxidative stress; inflammation

ARTICLE INFO:

Received: January 17, 2025

Revised: February 20, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Methotrexate is an antimetabolite used in the treatment of various cancers and autoinflammatory disorders. However, it can adversely affect oral tissues, particularly impairing salivary gland function. The antioxidant and anti-inflammatory effects of rutin may counteract these toxic effects. This study aimed at examining whether rutin confers protective effects on the salivary glands of rats exposed to methotrexate. Twenty-four male rats were randomly assigned to three groups. The control group received normal saline intraperitoneally for 10 days. On day six of the experiment, the methotrexate group was administered methotrexate intraperitoneally at a dose of 20 mg/kg. The methotrexate + rutin group also received rutin intraperitoneally at 50 mg/kg once daily for 10 days. On day 11, the animals were euthanized, and their salivary gland tissues were harvested for histological and biochemical analyses. Rutin markedly ameliorated the methotrexate-induced histopathological changes and biochemical alterations, as indicated by reduced levels of the tumour necrosis factor- α and malondialdehyde, alongside elevated levels of interleukin-10 and superoxide dismutase. These findings suggest that the antioxidant and anti-inflammatory properties of rutin may offer a promising strategy for mitigating the methotrexate-associated toxicity in submandibular gland tissues.

1. Introduction

Methotrexate is a folic acid analogue widely employed in the treatment

of various malignancies and autoinflammatory conditions. Despite its antitumor and anti-inflammatory properties, methotrexate may ex-

ert harmful effects on certain tissues. Experimental studies have demonstrated its detrimental impact on secretory glands, particularly targeting the submandibular glands¹. Clinical manifestations of salivary gland hypofunction include extensive dental caries, periodontal disease, pharyngeal infections, and disturbances in swallowing, taste, and speech. Rutin, a flavonoid glycoside derived from *Ruta graveolens* and other botanical sources (including citrus peels, buckwheat, apples, and strawberries), has been associated with multiple potential health benefits, notably antioxidant, anti-inflammatory, and antiproliferative activities². The objective of this study was to investigate whether rutin attenuates histological changes, oxidative stress, and inflammatory biomarkers in the submandibular glands of rats administered 20 mg/kg of methotrexate.

2. Methodology

2.1. Drugs and chemicals

Methotrexate was obtained from Ebewe, and so was rutin; both compounds were prepared in normal saline prior to administration.

2.2. Experimental design

The experiment was conducted between April 2023 and December 2024. A total of 24 male albino rats, aged 6 to 9 weeks and weighing between 190 and 210 g, were randomly assigned to three groups (n=8 per group): (i) group I (control) that received intraperitoneal injections of normal saline once daily for 10 days, (ii) group II (methotrexate) that received a single intraperitoneal dose of methotrexate (20 mg/kg) on day six, and (iii) group III (methotrexate + rutin) that, apart from methotrexate (as in group II), also received rutin (50 mg/kg) *via* oral gavage once daily for 10 days.

2.3. Animal preparation and tissue sampling

On day 11, rats were euthanized using a combination of ketamine and xylazine (80 mg/kg). Submandibular gland tissues were subsequently dissected. Tissue samples were collected for histopathological evalua-

tion and some were processed into homogenates for biomarker analysis.

2.4. Determination of inflammation and oxidative stress biomarkers

Levels of tumour necrosis factor-alpha (TNF- α), interleukin-10 (IL-10), malondialdehyde (MDA), and superoxide dismutase (SOD) were quantified in the obtained rat glandular tissues using standardized ELISA kits and following the manufacturer's instructions.

2.5. Histopathological evaluation

Tissues were embedded in paraffin blocks using stainless-steel molds. Once solidified, blocks were trimmed and sectioned into 5- μ m slices using a rotary microtome. Paraffin was removed by sequential treatment with heat, xylol, and ethanol. Specimens were mounted on clean glass slides and stained with haematoxylin and eosin³.

2.6. Statistical analysis

Data were analysed using SPSS version 24. Descriptive statistics included means and standard deviations (SD). One-way analysis of variance (ANOVA) was employed in order to compare multiple group means, followed by Tukey's HSD *post hoc* test for pairwise comparisons. A *p*-value below 0.05 was considered as statistically significant.

2.7. Ethics approval

This study was approved by the Institutional Review Board of the College of Dentistry of the University of Mosul on April 20, 2024 (approval number: UoM. Dent.24/42), following review of the final protocol, topic outline, and research design.

3. Results and Discussion

The methotrexate group exhibited significantly elevated levels of oxidative stress markers – specifically, MDA (9.2 ± 1.5 nmol/mg protein) – and markedly reduced levels of antioxidative enzymes such as SOD

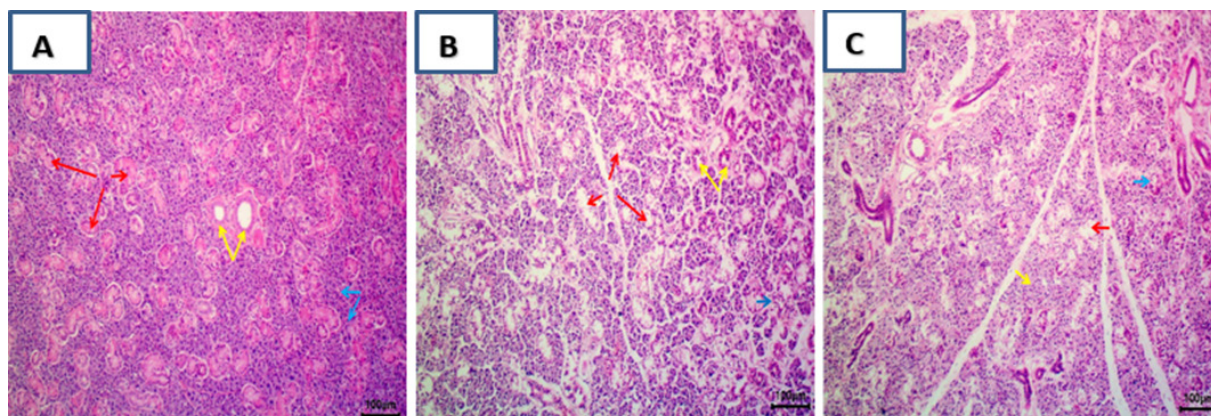


Figure 1. Histopathological evaluation of the rat salivary glandular tissues. (A): Representative section from the control group showing normal histoarchitecture, including intact mucous acini (yellow arrows), granular convoluted tubules (red arrows), and striated ducts (blue arrows). (B): Representative section from the methotrexate-treated group exhibiting severe degenerative and necrotic changes, particularly in the granular convoluted tubules (red arrows), along with disorganized mucous acini (yellow arrows) and distorted striated ducts (blue arrow). (C): Representative section from the methotrexate + rutin group showing preserved mucous acini (yellow arrow), mildly degenerated granular convoluted tubules (red arrow), and relatively intact striated ducts (blue arrow), indicating partial restoration of the tissue's integrity. Notes: all sections stained with haematoxylin and eosin; magnification: $\times 100$; scale bar: $100\ \mu\text{m}$.

(18.5 ± 3.9 U/g protein), compared to the control group (MDA: 1.6 ± 0.4 nmol/mg protein; SOD: 54.6 ± 5.1 U/g protein; $p < 0.05$). On the other hand, rats in the methotrexate + rutin group exhibited significantly lower levels of MDA (2.8 ± 0.8 nmol/mg protein) and significantly higher levels of SOD (42.6 ± 6.8 U/g protein), thereby demonstrating a protective effect of rutin against methotrexate-induced oxidative damage ($p < 0.05$).

Similarly, the methotrexate group exhibited substantially elevated concentrations of the pro-inflammatory cytokine TNF- α (428.5 ± 21.6 pg/g) and decreased levels of the anti-inflammatory cytokine IL-10 (149.1 ± 5.4 pg/g), relatively to controls (TNF- α : 145.7 ± 9.3 pg/g; IL-10: 298.2 ± 14.6 pg/g; $p < 0.05$). Rutin administration in the methotrexate + rutin group resulted in significantly reduced TNF- α levels (185.1 ± 15.2 pg/g) and increased IL-10 levels (268.8 ± 10.5 pg/g) compared to methotrexate alone ($p < 0.05$).

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the availability of endogenous antioxidants. This imbalance provokes inflammatory cascades and sustains a destructive feedback loop that exac-

erbates tissue degeneration. Methotrexate is known to stimulate ROS formation, promote lipid peroxidation, and elevate MDA levels. It also inhibits key antioxidative components, including SOD and glutathione peroxidase, and increases the expression of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6¹. Further studies indicate that methotrexate-induced damage to salivary gland tissues is associated with elevated MDA and TNF- α levels and diminished levels of glutathione and SOD⁴. Rutin, by contrast, appears to mitigate methotrexate toxicity through its ability to enhance endogenous antioxidant defences (e.g., SOD), lower MDA, and downregulate pro-inflammatory mediators; this dual action helps reverse degenerative changes in submandibular tissues.

Targeting oxidative and inflammatory pathways may reduce lipid peroxidation and free radical generation, restoring glandular integrity. Rutin's antioxidant effect is primarily mediated *via* the suppression of oxidative stress and the inhibition of lipid peroxidation². Its anti-inflammatory role is further supported by its ability to modulate nuclear factor kappa B signalling, thereby reducing tissue levels of MDA and

cytokines such as TNF- α , IL-1 β , and IL-17⁵.

Histological examination revealed that the salivary glands obtained from the control group displayed normal architecture, with intact glandular ducts and densely packed serous acini (Figure 1A). The methotrexate group exhibited pronounced degenerative changes, including granular convoluted tubule necrosis, periductal fibrosis, vascular congestion, and distorted pyknotic nuclei (Figure 1B). In contrast, the methotrexate + rutin group exhibited milder histopathological features: minor ductal expansion, moderate vascular congestion, and limited acinar degeneration, with only occasional pyknotic nuclei (Figure 1C). These observations further underscore the protective effect of rutin ($p < 0.05$).

4. Conclusion

Rutin demonstrates significant protective effects against methotrexate-induced salivary gland damage, primarily through its potent antioxidative and anti-inflammatory properties.

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Acknowledgements

We extend our sincere gratitude to the College of Dentistry at the University of Mosul for its generous support and provision of resources essential for the successful completion of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Attarbashee R.K., Al-Athari A.J.H., Shihab E.M., Ridha-Salman H., Zigam Q.A., Abbas W.J., Abbas A.H., Raheem A.K., AbdulAemah M.A., Hassan Z.M., Abbas S.N., Shareef S.M. Suppressing effect of rutin on methotrexate-induced salivary gland injury in rats. *Pharmakeftiki* 37(2s), 189-192, 2025. <https://doi.org/10.60988/p.v37i2S.187>

The relationship between paediatric antibiotic misuse and recurrent acute respiratory tract infection

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KEY WORDS: antibiotics;
bacterial infections; antibiotic
misuse; antibiotic resistance;
paediatrics

ARTICLE INFO:

Received: January 07, 2025

Revised: February 17, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Antimicrobial resistance (AMR) represents one of the most pressing challenges in contemporary public health. Neonates and children are among the most vulnerable populations, not only by being at heightened risk of developing AMR, but also for being among the ones most frequently being prescribed antibiotics, particularly for respiratory tract infections (RTIs). This study aimed at examining the relationship between antibiotic overuse and resistance in paediatric patients with recurrent RTIs. A descriptive cross-sectional design was employed from 10 December 2023 to 10 March 2024, targeting children in the Babil Province (Iraq) that were selected through a non-probability convenience sampling method. Findings revealed a high prevalence of antibiotic prescription errors: 62% of the cases involved inadequate course duration, 69% of the patients received duplicate therapy, and 61% were prescribed antibiotics with insufficient dosing frequency. A significant association was observed between these prescription errors and a history of recurrent RTIs. Such errors appear to contribute to the development of AMR, not only in children but potentially extending to adult populations. Given the potentially fatal consequences of antibiotic misuse, both paediatricians and parents must exercise rigorous caution when administering antibiotics to children.

1. Introduction

Acute respiratory infections are

conditions affecting the respiratory tract that impair normal breathing, posing significant health risks,

Table 1. Baseline characteristics and antibiotic usage patterns in Iraqi paediatric patients with respiratory tract infections.

Characteristics		Frequency	Percentage
Age	1-24 months	42	42%
	2-6 years	33	33%
	6-11 years	15	15%
	11-16 years	10	10%
Sex	male	61	61%
	female	39	39%
Weight	3-10 kg	46	46%
	11-18 kg	44	44%
	≤19 kg	10	10%
Number of antibiotics prescribed per prescription	1	28	28%
	2 or more	72	72%
Type of antibiotic	amoxicillin	25	25%
	gentamycin	3	3%
	augmentin	11	11%
	azithromycin	18	18%
	cefixime	15	15%
	ceftriaxone	20	20%
	meropenem	4	4%
	vancomycin	4	4%
Length of stay	short (<5 days)	73	73%
	long (≥5 days)	27	27%
Duration of course	short (5-7 days)	74	74%
	long (7-30 days)	26	26%
Acute respiratory tract infections	upper	69	69%
	lower	31	31%
Recurrent infection	yes	62	62%
	no	38	38%
Diseases	pneumonia	5	5%
	bronchitis	24	24%
	total lower respiratory tract infections	2	2%
	tonsillitis and pharyngitis	22	22%
	common cold	20	20%
	whooping cough	12	12%
	laryngitis and croup	8	8%
	total upper respiratory tract infections	7	7%
Total		100	100%

especially among children under 5 years of age in developing countries. According to Global Burden of Disease Study data (2019), lower respiratory infections ranked as the second leading cause of death in this age group.

When administered appropriately, antibiotics can eradicate or suppress bacterial growth. However, they can also trigger adverse outcomes, most notably antimicrobial resistance (AMR); the latter arises

when bacteria evade antibiotic-induced stress through mutation or modified gene expression¹. Saeedi *et al.*² note that resistance is particularly concerning when bacteria harbour multiple resistance genes. The escalating global crisis of multidrug-resistant bacteria – defined as resistance to at least one agent in three or more antimicrobial classes – has intensified morbidity and mortality, prolonged hospital stays, and raised healthcare costs³. Multid-

rug-resistant organisms (or “superbugs”) jeopardize socioeconomic and environmental sustainability and threaten global health initiatives. Projections suggest multidrug resistance-associated mortality could surge from 700,000 to 10 million annually by 2050, with cumulative global costs exceeding \$100 trillion, thereby surpassing cancer-related deaths⁴.

This study investigates the association between antibiotic overuse and resistance in Iraqi children with recurrent respiratory tract infections (RTIs).

2. Methodology

2.1. Study design

A descriptive cross-sectional design was employed in order to assess antibiotic misuse among paediatric acute RTI patients and its correlation with recurrent RTIs. The study was conducted from 10 December 2023 to 10 March 2024 in Babil Province (Iraq). A purposive (non-probability) sample of 100 children of both sexes was recruited from the Al-Sadiq Teaching Hospital.

2.2 Study instruments

A structured questionnaire was developed following a comprehensive literature review. It comprised sections on baseline characteristics, types of antibiotics prescribed, prescription errors, and respiratory disease classification.

2.3. Data collection

Data were collected *via* a well-prepared cross-sectional questionnaire.

2.4. Statistical analysis

Data were coded and analysed using SPSS (version 26.0 for Windows).

2.5. Ethical considerations

The study received ethical approval from the insti-

tutional review board of Al-Mustaqbal University's College of Nursing. All procedures complied with the Declaration of Helsinki. Approval documentation (ref.: 1751) was issued on 15 November 2023 after formal review of the research protocol, participant data, and informed consent forms.

3. Results and Discussion

Baseline characteristics revealed that 42% of the participants were aged 1–24 months, 46% weighed between 3–10 kg, and 61% were male (Table 1). Notably, 72.0% of the participants received two or more antibiotic prescriptions. Amoxicillin (25%), ceftriaxone (20%), azithromycin (18%), and cefixime (15%) were the most frequently prescribed antibiotics. A total of 73% of patients were hospitalized for fewer than five days, and 74% underwent a documented course of antibiotic therapy (Table 1). Upper RTIs accounted for 69% of cases, with 62% of the total cases experiencing recurrent infections. Diagnoses included bronchitis, tonsillitis, pharyngitis, and the common cold (Table 1).

The findings underscore how widespread antibiotic use – spanning human medicine, agriculture, and food production – has accelerated AMR. The peak incidence of acute RTIs occurred in patients aged 1–24 months, diverging from results reported by Walke *et al.*⁵. Overprescribing for viral acute RTIs has likely contributed to the dissemination of resistant bacterial strains.

A national survey in Pakistan has found that irrational prescribing practices are common, driven by resistance concerns and lack of diagnostic clarity⁶. Given that bronchitis and most acute RTIs are viral in origin, antibiotics are often prescribed due to inadequate diagnostic training, parental pressure, desire for rapid symptom resolution, and concern over secondary infections. In this cohort, 69% of children had RTIs, and 62% met criteria for recurrent RTIs according to Schaad⁷: ≥8 episodes / year in children <3 years, ≥6 episodes / year in those aged ≥3 years, or ≥3 episodes in one fall–winter season for 2 years in a row.

Antibiotic overuse perpetuates negative outcomes

and therapeutic failures due to AMR emergence. A strategic management framework for recurrent RTIs is therefore essential, not only in order to alleviate symptoms, but to disrupt the pathogenic cycle of mucosal colonization.

Prescription errors were primarily linked to incorrect dosage, insufficient frequency, inappropriate duration, and duplication therapy. These errors appear to stem from nonadherence to paediatric dosing guidelines and national / international formularies. Incorrect frequency and duration have been strongly associated with recurrent RTI history. Antibiotic overuse for viral upper RTIs remains entrenched in outpatient care⁸, and broad-spectrum prescriptions continue to rise, even when clinically unwarranted⁹. These practices contribute to avoidable drug-related adverse events, AMR development, and economic burden.

4. Conclusion

Prescription errors are strongly correlated with recurrent RTIs and may drive the emergence of antimicrobial resistance in children and, by extension, in adults. Given the potentially fatal consequences

of antibiotic misuse, paediatricians and caregivers must exercise utmost caution when prescribing and administering antibiotics.

Acknowledgements

The authors gratefully acknowledge the staff of the Departments of Physiology and Pharmacology of the College of Veterinary Medicine of Al-Qasim Green University, for their invaluable support.

Conflicts of interest

None exist.

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HOW TO CITE:

Ayad Z.M., Al-Ameedi A.I., Hajwal S.K., Al-Taei M.H., Hassan A.A.H., Khaleel M.A., Muhaibes F.J., Abbas A.H., Ridha-Salman H. The relationship between paediatric antibiotic misuse and recurrent acute respiratory tract infection. *Pharmakeftiki* 37(2s), 193-197, 2025. <https://doi.org/10.60988/p.v37i2s.188>

Aromatase inhibitors: assessment of knowledge, adherence, and associated factors among Iraqi women with breast cancer

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KEY WORDS:

breast cancer; aromatase inhibitors; knowledge; beliefs; adherence

ARTICLE INFO:

Received: January 26, 2025

Revised: February 09, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

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ABSTRACT

Adherence to aromatase inhibitors (AIs) as oral anticancer therapy remains a significant challenge in the management of hormone receptor-positive breast cancer. Despite their proven efficacy in reducing disease recurrence, many patients struggle to maintain long-term adherence. This study aimed at evaluating the patients' knowledge and beliefs regarding AIs and their influence on non-adherence. An observational cross-sectional study was conducted in order to assess adherence and its association with patients' beliefs, knowledge, and sociodemographic and clinical characteristics. Eligible breast cancer patients receiving AIs (either anastrozole or letrozole) on a monthly basis at the Babylon Oncology Center were recruited between 18 February and 1 October 2024 (N=70). Validated, tailored questionnaires were administered through structured interviews. The mean age of the participants was 56.1 ± 9.6 years. With respect to educational attainment, 48.6% had completed primary school, 25.7% had attained secondary education, 12.9% were illiterate, and 12.9% held a bachelor's degree. Most patients (98.6%) were married. A majority (62.9%) resided in rural areas, while 37.1% lived in urban settings. Adherence showed a strong positive correlation with the patients' knowledge of AIs ($R=0.638$, $p<0.001$). It was also positively associated with the perceived necessity of therapy ($R=0.64$, $p=0.025$), and negatively associated with concerns regarding adverse effects ($R=-0.74$, $p<0.001$). These findings suggest that breast cancer patients who exhibit greater awareness and a stronger sense of personal responsibility toward their treatment regimen are more likely to adhere to AI therapy. The study underscores the importance of addressing both perceived benefits and concerns in order to enhance adherence outcomes.

1. Introduction

Approximately 70% of breast cancer (BC) cases are hormone receptor-positive, and aromatase inhibitors (AIs) form the basis of therapy to reduce BC recurrence and improve overall survival (OS) among postmenopausal women¹. Despite the convenience and flexibility offered by oral anticancer agents such as AIs, adherence to treatment is critical for maximizing therapeutic effectiveness and enhancing OS². Several factors (such as regimen complexity, prolonged treatment duration, adverse effects, drug-drug and drug-food interactions, medication cost, and forgetfulness) significantly influence adherence to oral anticancer therapies^{3,4}.

Additionally, patients' beliefs and knowledge regarding the medication regimen and its potential side effects are modifiable determinants that affect adherence⁵. This study aimed at assessing the knowledge and beliefs of women with BC regarding AIs and their relationship to non-adherence.

2. Methodology

An observational cross-sectional study was conducted in order to evaluate adherence and its association with beliefs, knowledge, and sociodemographic and clinical characteristics among women with BC receiving monthly AI therapy (either anastrozole or letrozole) at the Babylon Oncology Center, between 18 February and 1 October 2024. Seventy patients meeting the inclusion criteria were recruited. Eligibility required women aged 18 years or older with a confirmed BC diagnosis, irrespective of stage or grade. Exclusion criteria comprised patients who had been on AIs for less than one month, individuals under 18, and those with psychological disorders impairing their ability to understand and accurately complete the questionnaire.

Tailored, validated questionnaires were administered through structured interviews. Data collection included sociodemographic and clinical variables, as well as assessments of AI-related knowledge and treatment adherence. To evaluate beliefs surrounding AIs, the Beliefs about Medicines Questionnaire - Specific (BMQ-Specific), encompassing Specific-

ic-Necessity and Specific-Concerns subscales, was employed⁶. The instrument was adapted from previously validated studies^{7,8}.

Ethical approval was obtained from the Scientific and Ethical Committee of the Faculty of Medicine of the University of Kufa (ref.: MEC-18; date: 14 February 2024). Verbal informed consent was obtained from all eligible participants.

Statistical analysis was performed using SPSS version 24 and Microsoft Excel 2019. Knowledge levels were classified as high (71%–100%), moderate (51%–70%), or low (0%–50%)⁹. Categorical variables were presented as frequencies and percentages, while numerical variables were reported as means, standard deviations, medians, and ranges. Distribution normality was assessed using the Kolmogorov-Smirnov test. Linear regression analysis, including analysis of variance (ANOVA), was conducted in order to evaluate correlations between demographic / clinical variables, knowledge, beliefs, and adherence. A *p*-value below 0.05 was considered as statistically significant.

3. Results and Discussion

The mean age of women receiving adjuvant hormonal therapy with AIs was 56.1 ± 9.6 years. Educational attainment varied: 48.6% had completed primary school, 25.7% had attained secondary education, 12.9% were illiterate, and 12.9% held a bachelor's degree. Marital status analysis revealed that 98.6% were married, with only 1.4% single. A majority (62.9%) resided in rural areas, while 37.1% lived in urban settings.

A first-degree family history of BC was reported by 37.1% of patients, whereas 42.9% had no such history. Most participants (62.9%) reported no history of infertility. In terms of disease stage, 51.4% were diagnosed at stage II and 24.3% at stage III. Regarding molecular subtype, 68.6% had luminal A BC, while 14.3% and 11.4% had luminal B BC with HER2-positive and HER2-negative profiles, respectively. Treatment durations of one, three, and four years were each reported by 14.3% of participants. Hypertension (43.3%) and diabetes mellitus (21.7%) were the most prevalent comorbidities.

Table 1. Association between adherence scores and sociodemographic characteristics, clinical variables, knowledge levels, BMQ-Necessity scores, and BMQ-Concerns scores, based on the data of the current study.					
Variable	Adherence scores				
	Unstandardized coefficients		Standardized coefficients		p-value
	B	SE	Beta	t	
Age	-0.033	0.012	-0.314	-2.711	0.009 *
Marital status	-1.222	0.968	-0.143	-1.262	0.211
Educational level	0.294	0.131	0.252	2.240	0.029 *
Family history	-0.169	0.128	-0.149	-1.318	0.192
Stage of breast cancer	0.264	.1200	0.248	2.209	0.031 *
Number of medications (excluding anticancer ones)	-0.116	0.148	-0.095	-0.784	0.436
Duration of treatment / months	-0.001	0.008	-0.012	-0.095	0.925
Knowledge scores	0.611	0.090	0.638	6.828	<0.001 *
BMQ-Necessity scores	0.062	0.027	0.249	2.300	0.025 *
BMQ-Concerns scores	-0.117	0.022	-0.572	-5.279	<0.001 *

AI-related knowledge was low in 57.1% of the study’s participants and moderate in 42.9% of them. Adherence levels were categorized as high ($\geq 80\%$) in 17.1%, low (0%–59%) in 18.1%, and moderate (60%–79%) in 64.3%. Analysis of adherence scores provided further insight into influencing factors (Table 1).

Adherence was positively correlated with knowledge ($R=0.638$, $p<0.001$). Previous studies support that patients with a stronger understanding of treatment use, regimen structure, side effects, and AI interactions tend to be more compliant with prescribed therapy^{8,10}.

Beliefs significantly influenced adherence: specifically, a positive association was observed with belief in the necessity of AIs ($R=0.64$, $p=0.025$), while concerns about adverse effects were negatively associated ($R=-0.74$, $p<0.001$), as shown in Table 1. Comparable findings indicate that patients who perceive treatment as beneficial are more likely to adhere, whereas apprehensions about side effects or long-term risks may reduce adherence^{5,7}.

4. Conclusion

The study demonstrates that women with BC who

exhibit higher levels of treatment-related knowledge and personal accountability are more likely to adhere to AI therapy. Enhancing educational outreach may therefore be a key strategy for promoting adherence. Furthermore, patients’ beliefs about the benefits of medication were positively associated with adherence, while their concerns about adverse effects showed a negative correlation; addressing both domains (i.e., perceived necessity and concerns) is critical to improving adherence outcomes.

Acknowledgements

The authors wish to express their sincere gratitude to the staff of the Babylon Oncology Center for their invaluable support throughout the undertaking of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Bash H.S., Rabeea I.S. Aromatase inhibitors: assessment of knowledge, adherence, and associated factors among Iraqi women with breast cancer. *Pharmakeftiki* 37(2s), 198-201, 2025.
<https://doi.org/10.60988/p.v37i2S.189>

Impact of clinical pharmacist intervention on tamoxifen-related problems among breast cancer women in Babil Province

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KEY WORDS:

**breast cancer; tamoxifen;
clinical pharmacist
intervention; drug-related
problems; Iraq**

ARTICLE INFO:

Received: December 11, 2024

Revised: December 31, 2024

Accepted: January 13, 2025

Available online: October 10, 2025

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ABSTRACT

Patients with breast cancer (BC) are frequently exposed to drug-related problems (DRPs) due to the long-term administration of highly toxic chemotherapeutic agents such as tamoxifen. Clinical pharmacists play a vital role in monitoring these issues and mitigating their frequency and severity. This study has aimed at evaluating the impact of clinical pharmacy interventions on the incidence and intensity of DRPs, including drug interactions, adverse effects, and inappropriate tamoxifen dosing. A pre-post interventional study was conducted at the Babylon Oncology Center, involving 70 women diagnosed with BC who were receiving a monthly tamoxifen treatment. The clinical pharmacist identified a total of 94 DRPs; an average of 1.34 per patient. The most frequent adverse effects attributed to tamoxifen were grade-2 hot flashes (16.9%), grade-2 osteoporosis (13.1%), grade-2 musculoskeletal pain (12.3%), weight gain (10%), and hand / foot swelling (6.9%). Three months following the pharmacist-led interventions, the number of DRPs was reduced to 48. A statistically significant difference was observed between the pre- and post-intervention DRP counts ($p < 0.05$). These findings underscore the pivotal role of clinical pharmacists in improving treatment outcomes for BC patients through the effective reduction of DRPs.

1. Introduction

Breast cancer (BC) ranks as the second-leading cause of cancer-related

mortality globally, with an estimated prevalence of 2.3 million cases in 2022. Worldwide, BC accounts for approximately 6.9% of all deaths¹.

Patients with BC are exposed to numerous drug-related problems (DRPs), including adverse drug reactions and drug interactions, largely due to the long-term administration of highly toxic chemotherapeutic agents. Consequently, clinical pharmacists play an essential role in monitoring and mitigating the frequency and severity of DRPs².

Tamoxifen's pharmacologic activity is curtailed by its adverse effect profile. Pulmonary embolism, uterine cancer, and stroke are serious complications associated with its use; these have formed the basis for its FDA black box warning issued in 2002. Additional side effects include vaginal discharge, irregular menstruation, oedema, hypertension, hot flashes, nausea, and vomiting³. Through the improvement of medication adherence, the management of adverse effects, and the optimization of therapy based on patient age, comorbidities, and polypharmacy, clinical pharmacists contribute significantly to the reduction of DRPs and the enhancement of treatment outcomes for BC patients⁴. This study aimed at assessing the impact of clinical pharmacist interventions on the frequency and severity of DRPs related to tamoxifen, including drug interactions, adverse effects, and inappropriate dosing.

2. Methodology

A pre-post interventional study was conducted at the Babylon Oncology Center of the Merjan Teaching Hospital, in the Babil Province of Iraq, between 18 February 2024 and 1 October 2024. Ethical approval was obtained from the Scientific and Ethical Committee of the Faculty of Medicine of the University of Kufa (ref.: MEC-18; date: 14 February 2024). Written informed consent was obtained from all participants following a detailed explanation of the study's purpose.

The study enrolled 70 women diagnosed with BC who were receiving monthly tamoxifen therapy. Eligibility criteria included an age ≥ 18 years, a confirmed histopathological diagnosis of BC at any stage or grade, and a signed informed consent. During the observational phase, DRPs were identified in the 70 enrolled patients through face-to-face evaluation by a clinical pharmacist. Documented DRPs included drug to drug interactions involving tamoxifen, adverse ef-

fects, and dosing errors. A structured intervention followed, in which the clinical pharmacist provided patient-specific education and therapeutic guidance aimed at preventing or mitigating DRPs.

Outcomes were reassessed 12 weeks post-intervention in order to evaluate changes in the frequency and severity of DRPs. Statistical analysis was performed using SPSS version 24 and Microsoft Excel 2019. The Shapiro-Wilk test was applied in order to assess data normality. Descriptive statistics for categorical variables were reported as frequency and percentage, while numeric variables were presented as mean, standard error of the mean, median, and interquartile range (IQR=Q1-Q3). The Wilcoxon signed-rank test was employed in order to compare DRP frequency before and after the intervention. A *p*-value below 0.05 was considered as statistically significant.

3. Results and Discussion

Seventy female BC patients (median age: 45 years; IQR: 40–49 years) receiving monthly tamoxifen therapy at the Babylon Oncology Center were included in this study. Each patient underwent a detailed review and interview by a clinical pharmacist, resulting in the identification of 94 DRPs; an average of 1.34 per patient.

Tamoxifen's therapeutic efficacy is largely dependent on its active metabolite, endoxifen, which is generated *via* cytochrome P450 enzymes, particularly CYP2D6. However, this metabolic pathway is subject to inhibition and induction by various co-medications, thereby increasing the risk of DRPs⁵.

The incidence of DRPs in this cohort exceeded that reported in a prospective observational study conducted in Thailand⁶, likely due to differences in patient population: the Thai study assessed general oncology patients, whereas the present study focused exclusively on BC patients receiving tamoxifen. Among the 94 DRPs identified, adverse effects of tamoxifen represented the most frequent category (68 cases; 73.9%), followed by drug-drug interactions (16 cases; 17.0%) and dosing errors or other issues (8 cases; 8.5%). This profile is modestly higher than that observed in a retrospective cross-sectional study by Degu and Kebede in Ethiopia⁷, where adverse drug

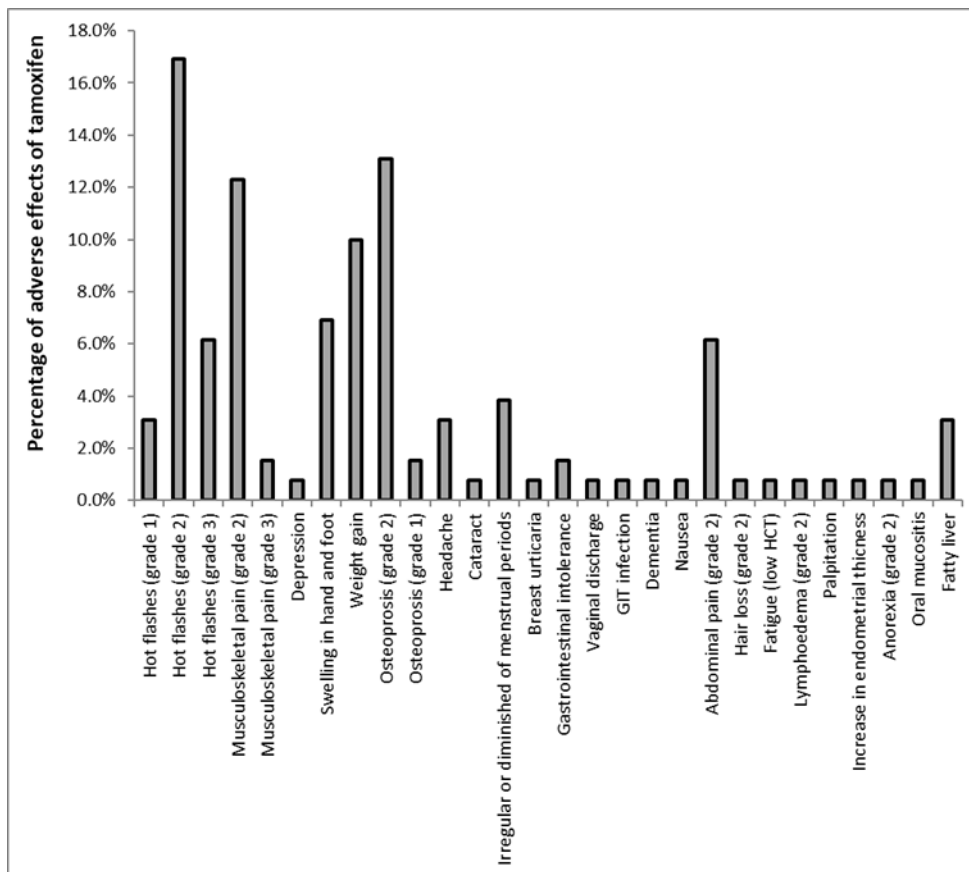


Figure 1. The frequency of the different types of adverse effects of tamoxifen recorded in this study.

reactions accounted for 48.6% of the recorded DRPs.

Figure 1 displays the distribution of adverse effects observed. The most common were grade-2 hot flashes (16.9%), grade-2 osteoporosis (13.1%), grade-2 musculoskeletal pain (12.3%), weight gain (10.0%), and hand / foot swelling (6.9%). These findings align with the symptom profile documented by Rosso *et al.*⁸ in their assessment of tamoxifen-associated adverse effects.

Following targeted pharmacist-led interventions (including focused review, education, and clinical guidance), the number of DRPs decreased to 48 (mean: 0.69 DRPs per patient). Among these, adverse effects of tamoxifen remained predominant (33 cases; 63.5%), followed by drug-drug interactions (12 cases; 23.1%) and inappropriate dosing (7 cases; 13.5%). The reduction in DRP frequency was found to be statistically

significant ($p < 0.05$). These findings corroborate prior reports highlighting the critical role of oncology pharmacists in the partial or full resolution of DRPs^{9,10}.

4. Conclusion

Clinical pharmacists play an indispensable role in oncology settings by improving treatment outcomes for BC patients. Their therapeutic interventions effectively reduce the frequency and severity of DRPs, thereby enhancing patient safety and medication efficacy.

Acknowledgements

The authors wish to express their sincere gratitude to the staff of the Babylon Oncology Center for their invaluable support throughout the undertaking of

this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Bash H.S., Rabea I.S. Impact of clinical pharmacist intervention on tamoxifen-related problems among breast cancer women in Babil Province. *Pharmakeftiki* 37(2s), 202-205, 2025.
<https://doi.org/10.60988/p.v37i2S.190>

Selenium and total antioxidant levels in patients with intra-bronchial and extra-bronchial lung cancer

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KEY WORDS:

antioxidants; selenium;
bronchoscopy; biomarkers;
oxidative stress

ARTICLE INFO:

Received: January 02, 2025

Revised: February 20, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

This study investigates the levels of total antioxidant capacity (TAC) and selenium (Se) in patients with lung cancer treated at the Taiba Hospital for Surgical Specialties. Fifty-seven patients aged 40 to 80 years were included in the study, with samples collected through bronchoscopy between December 19, 2021, and April 11, 2022. Patients were classified into two groups based on tumour location: 24 patients with extra-bronchial tumours (EXO; group A) and 33 patients with intra-bronchial tumours (ENDO; group B). All diagnoses were confirmed by histological and cytological tests. Significant differences in TAC and Se levels were observed between the groups. The EXO group exhibited lower TAC levels (1.783 ± 4.391 units/mL) compared to the ENDO group (5.616 ± 4.915 units/mL; $p=0.004$). In contrast, Se levels were higher in the EXO group (95.954 ± 3.233 ppb) than in the ENDO group (84.200 ± 3.824 ppb; $p<0.001$). These findings reveal a strong association between tumour type and the levels of TAC and Se, suggesting their potential utility as biomarkers for the diagnosis and prognosis of lung cancer. Further research is warranted in order to explore the clinical implications of these findings for treatment and prevention strategies.

1. Introduction

Lung cancer has remained the most common type of cancer worldwide

in recent decades. In 2012, an estimated 1.8 million new cases were reported (representing 12.9% of all cancers), with 58% occurring

in less developed countries. The prevalence of lung cancer is typically lower in women, reflecting gender differences in smoking behavior. Globally, lung cancer is also the leading cause of cancer-related death. Mortality rates (per 100,000) in 2012 were 47.6 and 44.8 among men in Central and Eastern Europe and Eastern Asia, respectively, and 23.5 and 19.1 among women in Northern America and Northern Europe, respectively¹.

Selenium (Se) is an essential micronutrient implicated in numerous biological processes; it plays a critical role in the function of several organs, including the thyroid, brain, muscles, prostate, and testis. Se exists in both organic and inorganic forms, with distinct biochemical properties. The margin between its deficient and toxic intake levels is notably narrow: both extremes are associated with adverse health outcomes. Moderate Se deficiency has been linked to increased risks of male infertility, nephropathy, prostate cancer, neurological disorders, ischaemic heart disease, and endemic osteoarthritis². The anticancer properties of Se have been proposed for over a century, beginning with early *in vivo* observations of tumour regression. It has been shown to inhibit DNA damage *in vitro* and to reduce pulmonary metastasis and radiation-induced carcinogenesis *in vivo*. A meta-analysis of observational studies has further associated Se levels with decreased lung cancer risk³.

Due to their physiological role, the lungs are highly exposed to oxidative damage. Elevated oxygen pressure (comparable to atmospheric levels) promotes oxidation, especially in the presence of reactive oxygen species (ROS) from tobacco smoke and air pollution. Oxidative stress is a major factor in lung cancer pathogenesis; consequently, protection against ROS is considered a key preventative strategy.

Systemic antioxidant defence comprises both exogenous and endogenous elements, the total activity of which defines the total antioxidant capacity (TAC)⁴. ROS generated in inflamed tissues – including superoxide anion, hydrogen peroxide, and hydroxyl radical – can damage cellular structures, denature proteins, and injure DNA, thereby promoting tumour development. These species affect physiologi-

cal metabolism, activate oncogenic pathways, and contribute to both the initiation and the progression of a carcinoma. Moreover, the body continually consumes antioxidant reserves in order to counteract the ROS-induced damage, thereby exacerbating oxidative stress⁵.

Lung cancer encompasses a heterogeneous group of tumours, broadly classified into non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC accounts for approximately 85% of all lung cancer cases and includes over 50 histological subtypes. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors are considered first-line therapy for NSCLC patients with EGFR mutations, owing to significantly improved objective response rates and progression-free survival compared to conventional chemotherapy⁶.

2. Methodology

Fluid samples were collected from patients diagnosed with lung cancer at the Taiba Hospital for Surgical Specialties. Sampling was performed by expert physicians through bronchoscopy between December 19, 2021, and April 11, 2022. Over this five-month period, 57 patients (male and female, aged 40–80 years) underwent chest radiography and computed tomography (CT) scanning prior to diagnostic bronchoscopy. All cases were confirmed histologically and cytologically.

Patients were categorized into two groups based on tumour location: group A and group B. In group A, extra-bronchial tumour (EXO) bronchoalveolar lavage fluid (BALF) samples were obtained by washing the bronchial area, although tumours were located outside the bronchial lumen and were not visible through bronchoscopy. These tumours were detected *via* CT scanning and X-ray imaging. This group consisted of 24 patients of both sexes. In group B, intra-bronchial tumour (ENDO) BALF samples were obtained from the tumour site itself, located within the bronchial lumen and being visible through bronchoscopy. This group included 33 patients of both sexes.

Se concentrations were measured *via* atomic ab-

Table 1. Levels of total antioxidant capacity and selenium in the bronchoalveolar lavage fluid (BALF) obtained from patients with extra-bronchial (group A; N=24) and intra-bronchial (group B; N=33) lung tumours.			
Parameters	Group A (mean ± SD; N=24)	Group B (mean ± SD; N=33)	p-value
Total antioxidant capacity (units/mL)	1.783 ± 4.391	5.616 ± 4.915	0.004
Selenium (ppb)	95.954 ± 3.233	84.200 ± 3.824	<0.001

sorption spectroscopy. Total antioxidant capacity (TAC) levels (in units/mL) were quantified using spectrophotometry at the laboratories of the Ministry of Science and Technology. These assays were used in order to evaluate correlations with lung cancer pathology.

Ethical approval for this study was obtained in accordance with the Declaration of Helsinki. The study protocol was confirmed by the Ethical Committee of the University of Babylon's College of Pharmacy (document #5; date: December 25, 2024). Informed consent was obtained from all participating women.

Statistical analyses were performed using the SPSS software (version 23). Data were expressed as mean ± standard deviation (SD). Group comparisons were assessed using independent *t*-tests and Mann–Whitney U tests for parametric and nonparametric variables, respectively. Age and body mass index (BMI) were compared using one-way analysis of variance (ANOVA). A *p*-value below 0.05 was considered as statistically significant.

3. Results and Discussion

The total of 24 patients with extra-bronchial tumours (group A) exhibited a mean TAC of 1.783 ± 4.391 units/mL, whereas the 33 patients with intra-bronchial tumours (group B) showed a significantly higher mean TAC of 5.616 ± 4.915 units/mL (*p*=0.004). Similarly, mean Se levels were 95.954 ± 3.233 ppb in group A and 84.200 ± 3.824 ppb in group B, again reflecting a statistically significant difference (*p*<0.001), as summarized in Table 1.

These findings reveal a clear divergence in TAC and Se profiles between patients with extra-bron-

chial (EXO) *versus* intra-bronchial (ENDO) lung tumours. Group A's lower TAC levels may point to impaired antioxidant defence mechanisms, while the elevated TAC in Group B suggests a compensatory response to increased oxidative stress; an inference supported by heightened levels of oxidative stress markers observed in ENDO cases⁷. It is well established that antioxidant systems are disrupted during carcinogenesis. Nevertheless, the specific contributions of endogenous and exogenous factors to TAC remain poorly defined in lung cancer. The antioxidant status in such patients likely reflects disease stage and individual physiological variability. Further research is needed in order to identify the dominant drivers of TAC alterations in this population⁴.

Group A also exhibited significantly higher Se levels than Group B. The role of Se in lung cancer appears complex and may involve a biphasic effect, whereby both deficient and excessive levels influence risk. Some studies have linked low Se concentrations to increased incidence of lung cancer, though consensus on causality remains elusive^{3,8}.

The statistical significance of these intergroup differences supports a strong association between tumour type and both TAC and Se levels. These parameters may have diagnostic or prognostic utility, and their further exploration could refine the biochemical understanding of lung cancer pathophysiology.

To validate these findings, larger and longitudinal studies are warranted. Such studies could elucidate whether TAC and Se levels function as reliable biomarkers for disease progression or outcome prediction. If confirmed, they might inform therapeutic strategies aimed at optimizing antioxidant and micronutrient status in affected patients.

4. Conclusion

This study demonstrates a statistically significant relationship between bronchial tumour type and both TAC and Se levels. These findings merit further research in order to clarify mechanistic pathways and to evaluate potential implications for treatment and preventative care in lung cancer.

Acknowledgements

The authors extend their sincere gratitude to the University of Babylon for its moral and material sup-

port, and to the Dean of the College of Pharmacy of the University of Babylon, for his continued encouragement.

Conflicts of interest

None exist.

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HOW TO CITE:

Ejam Z.Y., Rafied E., Wahhab D.N., Radhi S.S. Selenium and total antioxidant levels in patients with intra-bronchial and extra-bronchial lung cancer. *Pharmakeftiki* 37(2s), 206-209, 2025.
<https://doi.org/10.60988/p.v37i2S.191>

Soluble urokinase plasminogen activator receptor as a biomarker of antibiotic-resistant patterns of Gram-negative bacterial infections: a case-control study

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KEY WORDS:

Gram-negative bacteria; soluble urokinase plasminogen activator receptor; extensively drug-resistant bacteria; multidrug-resistant bacteria

ARTICLE INFO:

Received: January 06, 2025

Revised: February 17, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

The soluble urokinase plasminogen activator receptor (SuPAR) is an emerging biomarker associated with various clinical disorders; however, its utility in identifying antimicrobial-resistant (AMR) bacterial infections remains underexplored. AMR bacteria pose a significant global health challenge, with certain Gram-negative bacteria exhibiting considerable resistance to multiple antibiotics. This case-control study aimed at evaluating the diagnostic accuracy of SuPAR in detecting severe Gram-negative AMR bacterial infections. A total of 100 samples were analysed, comprising 60 clinically diagnosed cases of Gram-negative bacterial infections and 40 control samples. Pathogens were identified by using conventional laboratory techniques (including Gram staining, culture, colony morphology, and biochemical assays), supplemented by automated methods such as the VITEK2 Compact system for bacterial identification and antimicrobial susceptibility testing. Serum SuPAR levels were quantified via an enzyme-linked immunosorbent assay (ELISA). SuPAR levels were found to be significantly elevated in infected patients compared to controls ($p=0.0006$). Among the resistance profiles, extensively drug-resistant (XDR) infection cases exhibited the highest SuPAR levels, followed by multi-drug resistant (MDR) cases ($p=0.0003$). Non-MDR infection cases exhibited moderately elevated levels relative to controls. Across pathogen types, *Acinetobacter baumannii* was associated with the highest SuPAR levels, while *Burkholderia cepacia* exhibited the lowest. Notably, elevated SuPAR levels were also observed

in cases of skin infection and sepsis. In conclusion, SuPAR levels were found to be markedly increased in patients with Gram-negative bacterial infections, particularly in those involving XDR and MDR strains, thereby suggesting its potential role as a biomarker in assessing antimicrobial resistance severity.

1. Introduction

Antimicrobial resistance represents a major global health challenge, with specific bacteria exhibiting resistance to multiple antibiotics¹. Gram-negative bacteria are among the most concerning pathogens due to their high levels of antibiotic resistance². According to the World Health Organization (WHO), drug-resistant infections currently account for at least 700,000 deaths annually, with projections estimating up to 10 million deaths per year by 2050¹. *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* are prominent Gram-negative bacteria known for their multidrug resistance³.

Antibiotic resistance can be categorized into three types: (i) innate resistance (conferred by genes encoding efflux pumps and reduced membrane permeability), (ii) acquired resistance (resulting from chromosomal mutations or horizontal gene transfer *via* transformation, transposition, or conjugation), and (iii) environmentally-induced resistance (occurring *via* genetic or metabolic adaptations)³. Resistance profiles are further classified as follows: (i) multidrug-resistant (MDR) bacteria (displaying resistance to at least one agent in three or more antimicrobial groups), (ii) extensively drug-resistant (XDR) bacteria (displaying resistance to all but one or two antimicrobial groups), and (iii) pan-drug-resistant (PDR) bacteria (displaying resistance to all available antibiotics)⁴.

While culture-based assays remain the gold standard for assessing antimicrobial resistance in Gram-negative bacilli, results typically require 24–48 h, potentially delaying treatment in critically ill patients. Such delays may exacerbate illness and increase mortality risk. Therefore, rapid diagnostic

methods – including those that assess resistance profiles directly from clinical specimens – are increasingly critical⁵. Rapid diagnostic tests are now integrated into many laboratories and emergency departments in order to accelerate diagnosis, enhance infection control, guide initial therapeutic decisions, and support antimicrobial stewardship in high-throughput environments⁶. Additionally, evaluating inflammatory biomarkers may offer indirect, yet timely evidence of bacterial infection and may help optimize antimicrobial therapy in high-risk populations⁷.

The soluble urokinase plasminogen activator receptor (SuPAR) is an inflammatory biomarker released after cleavage of its glycosylphosphatidylinositol anchor during innate immune activation. SuPAR is thought to play a chemotactic role in guiding leukocyte migration, and it is expressed in multiple cell types including leukocytes, macrophages, fibroblasts, cardiomyocytes, and renal tubular cells. SuPAR levels can be quantified in plasma by using an enzyme-linked immunosorbent assay (ELISA)⁸.

This study has assessed the utility of SuPAR as part of a diagnostic panel for identifying MDR Gram-negative bacterial infections. Our goal was to contribute to the development of more rapid and accurate diagnostic approaches for these difficult-to-treat infections.

2. Methodology

2.1. Patients

We conducted a descriptive case-control study between October and December 2024 across three hospitals: the Al-Husseini Teaching Hospital, the Al-Kafeel Specialized Hospital, and the Imam Hassan

Table 1. Comparison of the soluble urokinase plasminogen activator receptor (SuPAR) levels (in ng\L) between control and patient groups, as stratified by pathogen type, antimicrobial resistance profile, and infection site. Note: different letters as superscripts after the mean values indicate statistically significant differences between these values. Abbreviations used: MDR, multidrug-resistant; XDR: extensively drug-resistant.				
Groups	Subgroup	Mean	SD	p-value
Pathogen types	control	59.41 ^a	11.10	0.0006
	<i>Klebsiella pneumonia</i>	126.75 ^{b,c}	103.16	
	<i>Escherichia coli</i>	174.61 ^{b,c}	74.63	
	<i>Acinetobacter baumannii</i>	216.27 ^c	81.86	
	<i>Pseudomonas aeruginosa</i>	156.60 ^{b,c}	72.92	
	<i>Burkholderia cepacia</i>	103.35 ^{a,b}	6.53	
Antimicrobial resistance profile	XDR	190.98 ^c	94.37	0.0003
	MDR	169.72 ^{b,c}	76.02	
	non-MDR	128.12 ^b	45.53	
Type of infection	urinary tract infection	151.66 ^{b,c}	68.61	0.0005
	respiratory tract infection	191.87 ^{b,c}	101.16	
	sepsis	172.76 ^{b,c}	106.92	
	skin infection	206.51 ^c	106.92	
	meningitis	123.34 ^{a,b}	21.74	

Al-Mujtaba Hospital. The study included 60 cases of Gram-negative bacterial infection from various infection sites and 40 healthy controls.

2.2. Inclusion and exclusion criteria

Bacterial infections were clinically diagnosed by attending physicians. Inclusion criteria: clinical suspicion of Gram-negative bacterial infection, elevated white blood cell count and C-reactive protein levels, and site-specific symptoms consistent with infection. Exclusion criteria: age <18 years, diagnosis of an autoimmune disease, pregnancy, history of malignancy, and prostate disorders in male patients.

2.3. Collection of the samples

Samples were collected from infection sites and were inoculated onto blood agar, nutrient agar, and Mac-Conkey agar plates. Blood cultures were processed using brain heart infusion broth. Urine specimens were cultured using semi-quantitative methods. All inoculated plates were incubated aerobically at 37°C for 16–18 h. Gram stains were prepared for provisional identification, followed by subculturing and analysis

using the VITEK-2 Compact system for both bacterial identification and antimicrobial susceptibility testing.

2.4. Ethical approval

Ethical approval was obtained from the Ethics Committees of the Kerbela Health Office and from the College of Medicine of the University of Kerbala (approval number: 1259; date: 1 October 2024). Written informed consent was obtained from all participants.

2.5. Statistical analysis

Statistical analyses were performed using the IBM SPSS version 23 software. Mean values and standard deviations were calculated, while significance was determined using *p*-values. Comparisons among multiple groups were conducted using analysis of variance (ANOVA), followed by Duncan's *post hoc* test for pairwise comparisons.

3. Results

The study included 60 infected patients (26 fe-

males, 43.33%; 34 males, 56.66%) and 40 healthy controls. The infection site distribution was as follows: urinary tract infections (28.33%), respiratory infections (28.33%), sepsis (20%), skin infections (20%), and meningitis (3.33%). As shown in Table 1, the SuPAR levels varied significantly across infection sites and between infected patients and controls ($p=0.0005$). All infection groups exhibited markedly elevated SuPAR levels compared to controls (59.41 ± 11.10 ng/L). Skin infections had the highest mean SuPAR levels (206.51 ± 106.92 ng/L), followed by respiratory tract infections (191.87 ± 101.16 ng/L), sepsis (172.76 ± 106.92 ng/L), urinary tract infections (151.66 ± 68.61 ng/L), and meningitis (123.34 ± 21.74 ng/L). As seen in Table 1, significant differences were also observed across different antimicrobial resistance profiles ($p=0.0003$), and across different pathogens ($p=0.0006$).

4. Discussion

This study yielded three main findings. Firstly, SuPAR levels at admission varied significantly by infection site, with the highest levels observed in skin infections and the lowest in meningitis. Secondly, SuPAR levels were found to be significantly higher in cases of a resistant infection, peaking in XDR cases, followed by MDR and then non-MDR bacterial infections, thereby, suggesting a relationship between SuPAR levels and the severity of antimicrobial resistance. Finally, among the identified infections, those caused by *Acinetobacter baumannii* were associated with the highest SuPAR levels, potentially reflecting infection severity.

Our results echo earlier findings demonstrating elevated SuPAR in MDR infections (mean: 15.4 ng/L

vs. 8.2 ng/L in susceptible strains)⁹. They also align with reports identifying *K. pneumoniae* as having the highest proportion of XDR isolates, followed by *P. aeruginosa* and *E. coli*⁴. However, our data differ from those of *in vitro* studies suggesting a strong presence of XDR *P. aeruginosa*¹⁰.

One should note that this study was retrospective, with SuPAR levels measured only at hospital admission, and involving a relatively small sample size. Future research should consider multicenter, prospective designs with larger cohorts and serial SuPAR measurements in order to validate these findings.

5. Conclusion

We strongly advocate for the use of novel biomarkers (such as SuPAR) in order to aid clinicians in assessing the severity of bacterial infections and guiding appropriate antibiotic therapy. A panel-based biomarker approach can deliver valuable diagnostic insights in high-risk populations, support antimicrobial stewardship, and reduce inappropriate antibiotic use.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Eliwi S.H., Ali M.M., Al-Hasnawi S. Soluble urokinase plasminogen activator receptor as a biomarker of antibiotic-resistant patterns of Gram-negative bacterial infections: a case-control study. *Pharmakeftiki* 37(2s), 210-214, 2025. <https://doi.org/10.60988/p.v37i2S.192>

Alterations of basic blood parameters in Iraqi COVID-19 patients with hyperglycaemia and acute kidney injury

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KEY WORDS:

COVID-19; diabetes; acute kidney injury; D-dimer; urea

ARTICLE INFO:

Received: December 29, 2024

Revised: February 26, 2025

Accepted: February 26, 2025

Available online: October 10, 2025

ABSTRACT

Emerging evidence indicates that coronavirus disease 2019 (COVID-19) can exert direct effects on the kidneys, leading to acute kidney injury (AKI). This is particularly concerning for individuals with diabetic nephropathy, who may already exhibit compromised renal function. The pathophysiology of COVID-19-related AKI may involve mechanisms such as a direct viral invasion of renal cells, systemic inflammation, and microvascular damage. Our study has included a total of 250 patients with confirmed COVID-19 that were treated between February and April 2022 in designated COVID-19 centres throughout the Babil Province (Iraq). Clinical and laboratory data were retrospectively obtained from the patients' medical records. Across all COVID-19 severity categories, our study has observed notable elevations in blood markers, including urea, creatinine, D-dimer, and blood glucose levels. Moreover, male patients exhibited significantly higher levels of urea, creatinine, D-dimer, C-reactive protein, and blood glucose compared to female patients ($p < 0.05$). Our study's findings suggest that diabetes mellitus and AKI can both be associated with heightened systemic inflammation and greater disease severity, and could be key predictors of adverse COVID-19 outcomes.

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1. Introduction

Coronavirus disease (COVID-19), caused by the novel RNA virus

SARS-CoV-2, was first identified in late 2019 in China. Clinical presentations range from asymptomatic infection to severe respiratory

Table 1. Selected blood variables were measured in 250 COVID-19 patients. One-way ANOVA was used in order to assess significant differences across patient groups based on disease severity (mild to moderate vs. severe) and gender.

Parameters	Mild to moderate COVID-19 patients		Severe COVID-19 patients		p-value
	Male (N=70)	Female (N=55)	Male (N=60)	Female (N=65)	
Glucose (mmol/L)	10.50 ± 0.64	9.00 ± 0.40	21.00 ± 0.57	19.75 ± 0.85	<0.001
Serum creatinine (Umol/L)	79.50 ± 2.10	77.75 ± 0.85	168.75 ± 3.72	155.00 ± 6.45	<0.001
Blood urea (mmol/L)	8.00 ± 0.40	6.50 ± 0.28	18.00 ± 0.40	17.00 ± 0.44	<0.0001
D-dimer (ng/mL)	562.50 ± 55.43	512.50 ± 31.45	7,550.00 ± 212.00	4,750.00 ± 220.02	<0.05
C-reactive protein (mg/L)	8.50 ± 0.64	9.80 ± 0.50	14.00 ± 0.57	12.75 ± 0.85	<0.001

illness, including acute respiratory distress syndrome (ARDS). Recurrent respiratory infections are a common clinical complaint and can progress to pneumonia¹. While the majority of individuals experience mild to moderate symptoms, a subset may rapidly deteriorate, developing life-threatening complications such as ARDS, severe respiratory failure, metabolic acidosis, coagulopathies, and septic shock. Reported rates of intensive care unit (ICU) admission range from 5% to 16%, depending on the demographic and regional characteristics of the studied population.

The widespread transmission of SARS-CoV-2 overwhelmed public health infrastructure in numerous countries, with ICUs becoming particularly strained². Although respiratory symptoms predominate, SARS-CoV-2 has systemic effects. Among the hospitalised patients, 60%–70% present with hypercoagulability, thrombocytopenia, venous thrombosis, or disseminated intravascular coagulation (DIC). Elevated D-dimer levels are now used not only in order to diagnose deep vein thrombosis, but also to anticipate recurrence and signal early DIC. Bode *et al.*³ have reported that 36% of the COVID-19 patients exhibit tachycardia, with an average D-dimer level of 0.9 mg/L.

Diabetes mellitus and hyperglycaemia are critical prognostic factors in hospitalised patients with acute medical conditions, including COVID-19. Recent data confirm that diabetes is one of the most prevalent comorbidities among individuals infected with SARS-CoV-2. Initial studies have identified diabetes in 7.4% to 19% of COVID-19 cases, with no-

tably higher prevalence (16.2% to 26.9%) among patients with severe disease².

Up to 25% of patients in retrospective cohorts have shown evidence of acute kidney injury (AKI); a known contributor to mortality in individuals with ARDS. The hallmark features of AKI include endothelial dysfunction, microangiopathy, and tubular injury, frequently associated with elevated inflammatory markers. The present study aimed at identifying urinary parameters predictive of AKI development in hospitalised COVID-19 patients.

2. Methodology

Clinical and demographic data were obtained from medical records at hospitals across the Babil Province, Iraq. Data collection included patient symptoms, medical history, comorbid conditions, chest computed tomography (CT) findings, and laboratory results from February 20 to April 25, 2022. Samples were extracted from confirmed COVID-19 cases treated in designated COVID-19 centres throughout the Babil Province.

Blood samples were collected on the same day from hospitalised individuals aged 20–80 years (mean age: 36 years). The cohort included 250 participants. Informed consent was obtained from all individuals, and the study was conducted under ethical approval (number: A-0028) granted by the Institutional Ethics Committee for Human Research at the Babylon Health Directorate (Babil, Iraq) in January 2021. The study complies with the principles of the Declaration of Helsinki, and all participants were

Iraqi nationals.

Serum levels of D-dimer, C-reactive protein (CRP), urea, creatinine, and blood glucose were measured. Statistical analyses were conducted using the SPSS software. Results were deemed as statistically significant at $p < 0.05$.

3. Results and Discussion

Our study has found that male patients exhibited significantly higher levels of urea, creatinine, D-dimer, CRP, and blood glucose compared to female patients ($p < 0.05$).

Due to their low cost, automated processing, and non-invasive sampling, urinary biochemical markers are frequently employed to detect urinary tract infections, monitor kidney disease, and assess treatment efficacy. However, evidence remains limited regarding their prognostic value in critically ill COVID-19 patients. A marked difference in the frequency of haematuria was observed between healthy controls and individuals with COVID-19. Prior research has demonstrated that urine sediment analysis may aid in predicting in-hospital mortality among admitted COVID-19 patients⁵, particularly in those presenting with elevated serum creatinine and urea levels.

The majority of COVID-19 patients in this cohort exhibited increased blood urea and serum creatinine concentrations, with statistically significant findings detailed in Table 1. These results are consistent with observations reported by Bonetti *et al.*⁵, who have noted similar elevations in renal biomarkers among patients with a SARS-CoV-2 infection. The subgroup with the highest frequency of urea and creatinine elevation comprised male patients. Multiple mechanisms may contribute to renal impairment in patients with SARS-CoV-2 pneumonia, leading to elevated levels of these markers⁶.

Hyperglycaemia was also prevalent, affecting between 42.4% and 51.1% of patients whose blood glucose exceeded 7.77 mmol/L⁶. Discrepancies in disease severity across studies – some focusing on moderate cases, others on severe – may account for the variability observed in the reported rates. Indi-

viduals with chronic kidney disease appear more vulnerable to SARS-CoV-2-associated pneumonia, potentially due to inflammation-induced immunosuppression affecting both innate and adaptive immunity. This immunocompromised state is linked to increased risk of upper respiratory infections and pneumonia-related mortality⁷.

Research from Indonesia, Japan, Greece, and Wuhan (China), has consistently associated elevated D-dimer levels with severe SARS-CoV-2 infection. Thrombocytopenia in affected patients may exacerbate this pattern, with elevated D-dimer contributing to delayed coagulation and a heightened risk of DIC; a potentially fatal complication⁸.

Further analysis revealed that creatinine and CRP were robust predictors of mortality in COVID-19 patients with diabetes. Chronic hyperglycaemia and systemic inflammation impair immune function, and diabetes itself may accelerate progression to severe or fatal COVID-19 outcomes. The present findings highlight an increased susceptibility in diabetic individuals, particularly those with poor glycaemic control. These insights underscore the need to prioritize high-risk patients for access to safe and effective COVID-19 vaccination⁹.

4. Conclusion

Our study's findings suggest that diabetes mellitus and AKI can both be associated with heightened systemic inflammation and greater disease severity, and could be key predictors of adverse COVID-19 outcomes.

Acknowledgements

The authors gratefully acknowledge the College of Pharmacy of the University of Babylon, for their support throughout the research process. Special thanks are extended to Dr Mohammed Obies for his ongoing guidance and critical input in the preparation and refinement of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Ewadh R.M.J., Kadhum S.A., Omran A.M., Jameel Z.I., Altaie N.H.A. Alterations of basic blood parameters in Iraqi COVID-19 patients with hyperglycaemia and acute kidney injury. *Pharmakeftiki* 37(2s), 215–218, 2025. <https://doi.org/10.60988/p.v37i2S.193>

New diazo coupling of metoclopramide with 2,6-dimethyl phenol after using cloud point extraction method

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KEY WORDS:

MCP; dimethyl phenol; cloud point; nitro phenol; Triton X-114

ARTICLE INFO:

Received: November 16, 2024

Revised: February 13, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

ABSTRACT

An environmentally safe spectroscopic technique has been developed for the determination of metoclopramide (MCP) in pharmaceutical samples. This method is rapid, practical, and suitable for the analysis of MCP both in its pure form and within pharmaceutical preparations, with or without the application of point cloud extraction technology. The procedure involves the nitration of metoclopramide at 5°C using sodium nitrite, followed by its reaction with 4-nitrophenol in an alkaline medium in order to yield a purple-coloured product. All experimental variables were optimized *a priori* so as to achieve robust and reproducible results. After stabilization of the chromophore, absorbance measurements were performed at 440 nm. Within the concentration range of 1–12 µg×mL⁻¹, the method adhered to Beer's law.

1. Introduction

Metoclopramide (MCP), a benzamide alternative, is widely regarded as an antiemetic agent that supports gastrointestinal function and alleviates certain forms of nausea and vomiting. Its therapeutic action is attributed to the strengthening of the lower oesophageal sphincter and the acceleration of gastric emptying – both for liquids and solids – into the small intestine. MCP

also reduces gastroesophageal reflux and mitigates regurgitation. Notably, it is prescribed in order to manage chemotherapy-induced emesis. To date, diverse analytical supports for MCP estimation have been documented, including voltage calibration techniques and UV-visible spectrophotometry^{1,2}.

2. Methodology

2.1. Instrumentation

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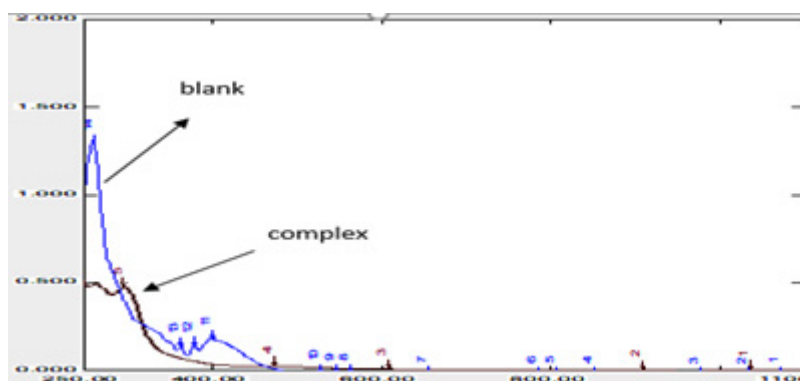


Figure 1. Spectra of metoclopramide (MCP) and 2,6-dimethylphenol. Notes: the y axis represents absorbance, while the x axis indicates wavelength (in nm).

Key laboratory equipment included a pH meter, a Korean-made centrifuge, a German-manufactured electronic balance, a German-made water bath, and a SHIMADZU UV-visible spectrophotometer.

2.2. Preparation of standard solutions

High-purity reagents and drug samples were dissolved in distilled water. Sodium nitrite and sulfamic acid solutions (1,000 ppm) were prepared by dissolving 1 g of each substance in a 100-mL volumetric flask. Other acidic and basic solutions were prepared *via* standard dilution protocols. Surfactants were concentrated to 10% by adding 10 mL of surfactant to a 100-mL volumetric flask.

2.3. General Procedure for Azo Coupling

Diazonium salt formation was performed in a 10-mL volumetric flask maintained in an ice bath. The sequential additions were: 1 mL MCP, 1 mL hydrochloric acid, 1 mL sodium nitrite (while chilled), 1 mL sulfamic acid, 1 mL of the coupling reagent, 1 mL sodium hydroxide, and 1 mL distilled water. The optimal wavelength for the absorbance measurement of the resulting complex was determined from its spectral profile (see Figure 1).

3. Results and Discussion

3.1. First method (azo complex formation)

Absorbance was measured at 420 nm, and the in-

fluence of various parameters was examined. Using fixed additions and varying HCl volumes (0.1–1 mL), maximum absorbance was achieved at 0.4 mL. Among the different bases used, KOH yielded optimal absorbance at a volume of 0.5 mL. Absorbance increased with a NaNO_2 volume up to 1 mL, beyond which impurity effects (including diazonium instability due to excess nitrate) led to diminished absorbance³. After varying the 2,6-dimethylphenol reagent volume (0.2–0.6 mL), it was observed that 1 mL provided the highest absorbance⁴. Finally, a calibration curve for the MCP–4-nitrophenol complex was constructed by measuring the absorbance for MCP concentrations ranging from 1–12 $\mu\text{g}\times\text{mL}^{-1}$, thereby demonstrating compliance with Beer's law⁵.

3.2. Second method (cloud point extraction)

The surfactant Triton X-114 was selected based on prior findings⁶, with micelle-mediated separation enhanced by ethanol addition in order to improve both efficiency and absorbance. Extraction efficiency increased with the surfactant volume, peaking at 1.2 mL after a 20-min incubation at 60°C. Finally, solutions of MCP (1–12 $\mu\text{g}\times\text{mL}^{-1}$) were incubated at 50°C for 20 min. After cloud point formation and ethanol-mediated separation, absorbance was measured⁷.

4. Conclusion

The combined cloud point extraction – spectrophotometric method proved effective for the MCP esti-

mation, offering high sensitivity, excellent extraction efficiency, and environmental compatibility. The technique is simple, requires minimal volumes, and facilitates reliable preconcentration. MCP's affordability and reagent-grade availability allow its repurposing as a coupling agent in other reactions. Additionally, the method yields a strong relative standard deviation and a commendable detection limit, making it well-suited for broader analytical applications.

Acknowledgements

The author expresses sincere gratitude to all who

contributed to this work. Special thanks are extended to the College of Science for Women of the University of Baghdad for providing the raw materials, laboratory facilities, and technical equipment essential to the research.

Conflicts of interest

None exist.

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HOW TO CITE:

Fadhel S.N. New diazo coupling of metoclopramide with 2,6-dimethyl phenol after using cloud point extraction method. *Pharmakeftiki* 37(2s), 219-221, 2025. <https://doi.org/10.60988/p.v37i2S.194>

Assessing the impact of Span 60 and cholesterol on mupirocin niosomes developed for topical delivery

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KEY WORDS:

mupirocin; niosome; topical delivery; vesicle size; entrapment efficiency

ARTICLE INFO:

Received: January 10, 2025

Revised: February 06, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

ABSTRACT

Mupirocin (MUP), a broad-spectrum antibacterial agent, is commonly used in order to treat superficial skin infections. However, its therapeutic efficacy is limited by poor skin permeability and the impracticality of prolonged topical administration. This study reports the development of a niosomal delivery system engineered so as to enhance topical MUP delivery by optimizing vesicle size and drug entrapment efficiency. Niosomes were prepared using the thin-film hydration method, and a 2² full factorial design was employed in order to optimize formulations based on Span 60 and cholesterol concentrations. Vesicle size and entrapment efficiency served as dependent variables. Both models were significant, thereby indicating that formulation variables and their interactions substantially influenced vesicle characteristics. The findings demonstrate that a full factorial design is an effective approach to elucidate and optimize the impact of formulation variables on MUP-loaded niosomes.

1. Introduction

The broad-spectrum antimicrobial activity and antibiofilm properties of mupirocin (MUP) make it a widely used treatment for superficial topical infections. MUP inhibits bacterial protein synthesis by

reversibly blocking isoleucyl-tRNA synthetase. Its distinctive mechanism of action may explain its lack of cross-resistance with other antibiotics¹. Crystalline MUP has a molecular weight of 500.6 g/mol and appears white to off-white. Its maximum aqueous solubility is

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0.0265 g/L. Exhibiting lipid-soluble behavior, it has a partition coefficient (Log P) of 2.45. Clinically, MUP is used for topical skin infections and nasal decolonization of *Staphylococcus aureus*. However, its limited skin permeability and the absence of sustained drug release hamper its topical effectiveness².

Niosomes are vesicular carriers composed of non-ionic surfactants and cholesterol, which enhance membrane stability³. These carriers primarily localize within the stratum corneum, increasing drug residence time and enhancing skin permeation while minimizing systemic absorption. Studies suggest that niosomes improve intercellular lipid fluidity and reduce the barrier function of the epidermis, thereby increasing drug permeability⁴. This study aimed at formulating MUP-loaded niosomes for topical delivery, using a 2² full-factorial experimental design in order to optimize vesicle size and entrapment efficiency (EE%).

2. Methodology

2.1. Materials

MUP was sourced from Hangzhou Hyper Chemicals Limited (China). Span 60 and cholesterol were procured from Xi'an Sonwu Biotech Co., Limited (China). Methanol and chloroform were supplied by Alpha Chemicals.

2.2. Experimental design

The effects of Span 60 and cholesterol concentrations on MUP niosomal EE% and particle size (PS) were examined using a 2² full-factorial design comprising 4 base runs and 8 total runs, performed in duplicate (see Table 1). The experimental framework was developed using the Design Expert software. Blocking was implemented in order to minimize confounding variables, enhance precision, and address operational constraints. Data analysis was conducted with the Design Expert software (version 13), employing analysis of variance (ANOVA) in order to compare formula means. Statistical significance was set at $p < 0.05$.

2.3. Preparation of MUP niosomes

Niosomal formulations of MUP were prepared by the thin-film hydration method. Table 1 outlines the formulation components. In brief, MUP, Span 60, and cholesterol were dissolved in 15 mL of a methanol-chloroform mixture (2:1, v/v). The solution was transferred to a 250-mL round-bottom flask equipped with a rotary evaporator (IKA RV8, USA). Solvent evaporation under reduced pressure at 60°C and 100 rpm over 30 min yielded a translucent thin film on the flask walls. Subsequently, 10 mL of distilled water were added in order to hydrate the film, followed by rotation at 150 rpm in a 70°C water bath for 1 h. The resulting dispersion was sonicated in a bath sonicator for 30 min in order to reduce PS, then refrigerated overnight at 4°C so as to stabilize the vesicular membranes.

2.4. Characterization of MUP niosomes

Vesicle size was measured by dynamic light scattering using the Zetasizer Nano ZS (Malvern Instruments, UK). For optimal scattering intensity, the niosomal dispersion was diluted (1:10) in distilled water⁵. Ultrafiltration was employed in order to determine the EE%, based on the quantification of unencapsulated MUP in the supernatant, measured spectrophotometrically at 226 nm. The EE% was calculated by using the following formula⁵:

$$EE\% = \left[\frac{\text{total amount of drug added} - \text{free drug}}{\text{total amount of drug added}} \right] \times 100\%$$

3. Results and Discussion

Characterization data are presented in Table 1. The full-factorial ANOVA has revealed that the particle size model was statistically significant ($p < 0.0001$), thereby indicating that the component concentrations and their interactions substantially influenced PS. Span 60 exerted a significant main effect ($p < 0.0001$), while cholesterol did not ($p = 0.2319$); however, their interaction was found to be highly significant ($p = 0.0012$).

Effective vesicular systems for topical delivery

Table 1. Preparation and characterization parameters for the herein assessed mupirocin (MUP) niosomes. Abbreviations used: EE%, entrapment efficiency; PS, particle size.

Run order	Blocks	MUP (mg)	Span 60 (mg)	Cholesterol (mg)	PS (nm)	EE%
1	1	5	86	67.6	180	30%
2	1	5	86	33.8	130	50%
3	1	5	172	67.6	210	67%
4	1	5	172	33.8	260	75%
5	2	5	172	67.6	213	68%
6	2	5	86	67.6	185	32%
7	2	5	172	33.8	255	75%
8	2	5	86	33.8	130	50%

require optimization of PS, as smaller vesicles penetrate skin layers more efficiently⁶. Increasing surfactant concentration markedly enlarged the vesicle size, likely due to the long alkyl chain of Span 60 facilitating large vesicle formation⁷.

ANOVA has also indicated a significant model for EE% ($p<0.0001$), thereby confirming that the component concentrations and their interaction influence drug encapsulation. Both Span 60 ($p<0.0001$) and cholesterol ($p<0.0001$) had significant individual effects, and their interaction was also significant ($p=0.0012$).

High drug loading is critical for effective topical delivery. MUP, a lipophilic compound with a Log P of 2.45, preferentially partitions into the lipid bilayers of niosomes⁸. Span 60 was selected due to its advantageous physicochemical characteristics, being a solid state at ambient temperature (melting point, T_m : 53°C), long alkyl chain, and high lipophilicity (low hydrophilic-lipophilic balance), which promote stable vesicle formation and high MUP entrapment⁹.

Balancing cholesterol and non-ionic surfactant concentrations is essential for maximizing the EE%. Insufficient cholesterol may cause drug leakage and vesicle fusion, while excess surfactant can enhance the lipophilic environment, thereby improving drug entrapment. Conversely, low surfactant levels produce fewer vesicles, which limit drug loading capacity¹⁰.

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4. Conclusion

The findings of this study support the use of vesicular carriers in order to enhance topical delivery of MUP and improve clinical efficacy. Niosomes prepared *via* thin-film hydration, using Span 60 as a surface-active agent and cholesterol as a membrane stabilizer, have herein demonstrated promising attributes for dermal drug delivery.

Acknowledgements

The authors extend their gratitude to Dr Nawar M. Toma from the College of Pharmacy of the University of Baghdad, for assisting with particle size measurements. Acknowledgement is also due to the College of Pharmacy of the University of Basrah, for providing essential instrumentation.

Conflicts of interest

None exist.

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HOW TO CITE:

Fareed N.Y., Naji G.H., Al-Zheery W.H. Assessing the impact of Span 60 and cholesterol on mupirocin niosomes developed for topical delivery. *Pharmakeftiki* 37(2s), 222-225, 2025. <https://doi.org/10.60988/p.v37i2S.195>

Effects of rosuvastatin on post-thrombotic syndrome following deep vein thrombosis: a randomized clinical trial

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KEY WORDS:

post-thrombotic syndrome;
venous thrombosis;
rosuvastatin; rivaroxaban;
warfarin

ARTICLE INFO:

Received: January 06, 2025

Revised: January 29, 2025

Accepted: February 11, 2025

Available online: October 10, 2025

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ABSTRACT

This study evaluates the effectiveness of rosuvastatin as an adjunct to standard anticoagulant therapy in reducing the incidence of post-thrombotic syndrome (PTS) in patients with deep vein thrombosis (DVT) treated at the Al-Imamain Al-Kadhimain Medical City between October 2022 and September 2024. A total of 465 patients were randomized into two groups: a control group receiving standard anticoagulants (warfarin or rivaroxaban) and an intervention group receiving the same anticoagulants with the addition of rosuvastatin. Over the subsequent three months, the authors assessed the development of PTS and measured the serum concentrations of D-dimer and C-reactive protein (CRP). The occurrence of PTS was found to be significantly lower in the rosuvastatin group compared to the control group ($p=0.021$), with no severe adverse events reported. Furthermore, the intervention group exhibited marked reductions in CRP and D-dimer levels, suggesting that rosuvastatin may contribute to improved clinical outcomes in the management of DVT. These findings support the incorporation of rosuvastatin into conventional anticoagulant regimens as a means of mitigating PTS risk and enhancing overall recovery. The study underscores the value of adjunct therapies in reducing long-term complications associated with venous thromboembolism.

1. Introduction

Deep vein thrombosis (DVT) is a se-

rious clinical condition characterized by the formation of a thrombus in major deep veins, most common-

ly of the lower limbs. It poses considerable risk by predisposing patients to pulmonary embolism and post-thrombotic syndrome (PTS), both of which can severely impair mobility and quality of life¹. PTS is a chronic sequela of DVT caused by venous outflow obstruction due to valvular dysfunction, venous hypertension, and persistent insufficiency. It manifests with pain, oedema, and dermatological changes. The primary treatment for DVT is anticoagulant therapy²; however, the optimal strategy for PTS prevention remains undefined.

Recent research suggests that statins – particularly, rosuvastatin – may confer therapeutic benefits beyond cholesterol reduction by exerting anticoagulant and anti-inflammatory effects³. The JUPITER12 trial has demonstrated that rosuvastatin can decrease levels of high-sensitivity C-reactive protein (hs-CRP), which is implicated in inflammatory and thrombotic processes⁴. This study aimed at investigating the impact of rosuvastatin as an adjunct to standard anticoagulants in reducing the incidence of PTS among patients diagnosed with DVT, thereby improving treatment outcomes and quality of life.

2. Methodology

This randomized clinical trial was conducted in order to evaluate the effect of rosuvastatin on patients presenting with unilateral DVT at the Al-Imamain Al-Kadhimain Medical City between October 2022 and September 2024. A total of 465 participants were randomly allocated to four treatment arms: (i) rivaroxaban alone, (ii) warfarin alone, (iii) warfarin combined with rosuvastatin, and (iv) rivaroxaban combined with rosuvastatin. Socio-demographic and medical history data were collected *via* face-to-face interviews at baseline. Treatment was administered over a three-month period.

Thrombus size and location were assessed clinically and through Doppler ultrasonography by a radiologist blinded to the group assignment. Exclusion criteria included serious comorbidities, pregnancy or breastfeeding, and inability to provide informed consent.

Serum samples for hs-CRP and D-dimer testing

were collected at baseline and at three months. hs-CRP was quantified using an enzyme-linked immunosorbent assay (ELISA); a sensitive method for detecting inflammatory markers. D-dimer levels were measured using a quantitative immunoassay in order to assess fibrin degradation products that are reflecting active thrombosis.

Calf circumference was measured at 4 cm below the tibial tuberosity in order to evaluate oedema. At the three-month follow-up, hs-CRP and D-dimer levels, changes in calf circumference, and PTS occurrence were reassessed using the Brandjes criteria⁵. DVT resolution was also re-evaluated.

Ethical approval was obtained from the Baghdad Al-Karkh Health Directorate and the Al-Imamain Al-Kadhimain Medical City (protocol number: KHD, BA: 7-2022; date: July 2022). Informed consent was secured from all participants in compliance with international standards for ethical research.

Statistical analyses were performed using IBM SPSS v25 software. Comparisons between groups employed analysis of variance (ANOVA) and descriptive statistics (mean $\pm$ SD as well as absolute / relative frequencies), focusing on inflammatory markers and PTS incidence.

3. Results and Discussion

Rosuvastatin, when added to standard anticoagulant therapy, was associated with a significantly lower incidence of PTS. Among patients treated with rivaroxaban plus rosuvastatin, only 12 (9.2%) developed PTS, compared to 42 (32.3%) in the warfarin group, 39 (30.0%) in the warfarin plus rosuvastatin group, and 26 (20.0%) in the rivaroxaban group ($p=0.021$). Severe PTS cases were reported in just one patient (0.8%) from the rivaroxaban plus rosuvastatin group *versus* five patients (3.8%) in the warfarin group (Table 1).

The hs-CRP and D-dimer levels were found to be markedly lower in the statin groups, thereby indicating a potential anti-inflammatory effect that could contribute to improved vascular outcomes. These findings support the hypothesis that rosuvastatin may reduce both the incidence and severity of PTS

Table 1. Comparative outcomes of rosuvastatin and standard anticoagulant therapy in reducing post-thrombotic syndrome (PTS) incidence among patients with deep vein thrombosis (DVT). Abbreviations used: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; PTS, post-thrombotic syndrome; SD, standard deviation.

Variable	Group I: warfarin (N=111)	Group II: rosuvastatin / warfarin (N=120)	Group III: rivaroxaban (N=116)	Group IV: rivaroxaban / rosuvastatin (N=118)	p-value
Gender (male %)	40.8%	59.2%	43.8%	40.0%	0.81
Age (years, mean ± SD)	47.0 ± 10.1	58.0 ± 1.3	49.9 ± 8.1	51.8 ± 7.3	0.09
BMI (kg/m ² , mean ± SD)	27.8 ± 4.9	30.4 ± 4.9	33.3 ± 0.1	29.8 ± 4.1	0.59
hs-CRP (mg/L, mean ± SD)	13.9 ± 4.5	14.3 ± 4.7	13.7 ± 4.6	14.1 ± 4.3	0.850
D-dimer (ng/mL, mean ± SD)	3,200.2 ± 2,300.1	3,150.7 ± 2,400.4	3,300.9 ± 2,450.3	3,250.8 ± 2,350.2	0.690
Calf size difference >3 cm (N (%))	110 (84.6%)	89 (68.6%)	87 (66.9%)	80 (61.5%)	0.29
Thrombosis location above knee (N (%))	40 (30.8%)	36 (27.7%)	33 (25.4%)	38 (29.2%)	0.11
Post 3-month change in hs-CRP (mg/L, mean ± SD)	13.9 ± 4.5 → 7.9 ± 3.2	14.3 ± 4.7 → 3.9 ± 2.9	13.7 ± 4.6 → 9.1 ± 1.8	14.1 ± 4.3 → 2.9 ± 0.9	0.003
Post 3-month change in D-dimer (ng/mL, mean ± SD)	3,200.2 ± 2,300.1 → 459.9 ± 99.6	3,150.7 ± 2,400.4 → 201.3 ± 109.2	3,300.9 ± 2,450.3 → 411.7 ± 333.	3,250.8 ± 2,350.2 → 317.8 ± 223.9	0.007
PTS occurrence (N (%))	42 (32.3%)	39 (30.0%)	26 (20.0%)	12 (9.2%)	0.021
Mild PTS severity (N (%))	22 (16.9%)	19 (14.6%)	11 (8.5%)	5 (3.8%)	0.001
Moderate PTS severity (N (%))	15 (11.5%)	14 (10.8%)	11 (8.5%)	6 (4.6%)	0.019
Severe PTS severity (N (%))	5 (3.8%)	6 (4.6%)	4 (3.1%)	1 (0.8%)	0.021

following DVT.

PTS results from chronic venous insufficiency, characterized by pain, oedema, and skin discoloration. Pathophysiological mechanisms include thrombus-induced inflammation, venous hypertension, and valvular incompetence¹. Elevated inflammatory markers, such as hs-CRP and D-dimer, are strongly associated with increased PTS risk. This study aligns with prior research suggesting statins may enhance venous recovery through multiple pathways, including modulation of lipid metabolism and suppression of cytokines and adhesion molecules⁶.

Statins' anti-inflammatory properties may protect endothelial integrity and preserve venous valve function, thereby reducing PTS development potential⁷. The significant reduction in CRP observed in this trial reinforces the link between statins and reduced inflammation in DVT.

The study design demonstrated methodological rigor through precise population definition and

thoughtful selection of the control arms. Randomization minimized bias and enhanced internal validity. Nevertheless, certain limitations should be noted. For example, the trial's duration limits conclusions about long-term outcomes, including potential drug interactions and sustained efficacy. Furthermore, medication adherence, monitored *via* phone calls, may not accurately reflect actual compliance.

4. Conclusion

This randomized clinical trial demonstrates that rosuvastatin, when combined with standard anticoagulant therapy, effectively reduces the incidence of PTS in patients with DVT. The associated decrease in inflammatory markers such as CRP and D-dimer underscores the drug's potential role in improving vascular outcomes. Future studies should explore long-term benefits, broader patient demographics, and alternative clinical settings.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Fayadh N.A.H., Al-Anbari A.J.K., Al-Hindy H.A.A.M. Effects of rosuvastatin on post-thrombotic syndrome following deep vein thrombosis: a randomized clinical trial. *Pharmakeftiki* 37(2s), 226-229, 2025. <https://doi.org/10.60988/p.v37i2S.196>

Synthesis, characterization, and antimicrobial activity of copper oxide nanorods

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KEY WORDS:

co-precipitation; X-ray diffraction; crystallinity; sol-gel process; nanomedicine

ARTICLE INFO:

Received: January 15, 2025

Revised: February 27, 2025

Accepted: February 27, 2025

Available online: October 10, 2025

ABSTRACT

This study focuses on the synthesis and characterization of copper oxide (CuO) nanorods, which have attracted considerable interest due to their diverse applications in catalysis, electronics, and biomedicine. CuO nanorods were synthesized *via* a co-precipitation method, employing copper sulfate and sodium hydroxide as precursor materials. The synthesis was optimized by maintaining a pH of 12 and by carefully regulating the calcination temperature, yielding nanoparticles with an average crystallite size of 10.538 nm and a rod-like morphology with a diameter of approximately 25 nm. Structural and morphological features of the synthesized nanoparticles were confirmed through X-ray diffraction and field emission scanning electron microscopy. The antibacterial efficacy of CuO nanorods against *Staphylococcus aureus* was also assessed, revealing pronounced activity attributed to their nanoscale dimensions and positive surface charge, which facilitate interactions with bacterial membranes. Although the synthesized CuO nanorods exhibit strong potential across multiple domains, limitations related to scalability and particle size uniformity must be addressed in future studies. Overall, this study underscores the antimicrobial promise of CuO nanorods and calls for further research in order to improve their applicability.

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1. Introduction

Copper oxide nanoparticles (CuO) are increasingly important materials across industry, envi-

ronmental science, and medicine, owing to their distinctive physicochemical properties (including excellent electrical conductivity, notable antibacterial activity,

and efficient light absorption). CuO nanoparticles can be synthesized through various methods, encompassing both chemical and biological approaches. For example, chemical precipitation allows for a precise control over particle size and physicochemical characteristics, while green synthesis using plant extracts has been explored in order to reduce copper ion release and mitigate the environmental impact¹. Due to their nanoscale dimensionality (1–100 nm), CuO nanoparticles exhibit unique structural, optical, electrical, magnetic, and mechanical properties not observed in bulk materials, placing nanotechnology at the forefront of scientific innovation in the twenty-first century². Among the key parameters influencing nanoparticle performance are particle size, morphology, and crystallinity. In order to optimize these attributes, several synthesis methods have been developed, including the sonochemical method, sol-gel processing, laser ablation, electrochemical techniques, chemical precipitation, and surfactant-mediated approaches.

Despite their biomedical potential, CuO nanoparticles pose toxicity concerns. CuO nanoparticles may exhibit cytotoxic effects on mammalian cells and various vertebrate and invertebrate species. Their primary mechanism of toxicity involves the generation of reactive oxygen species (ROS), which induce oxidative stress in human pulmonary epithelial cells, potentially damaging DNA and mitochondria. Transition metal oxide nanoparticles, including CuO nanoparticles, are now widely applied in catalysis, biosensing, cosmetics, pharmacy, food and agriculture, electronics, dentistry, energy, and environmental science; domains where their popularity has markedly grown over the past two decades³.

Chemical precipitation is commonly employed so as to control particle size and crystallinity, with optimal outcomes achieved at specific calcination temperatures⁴. Green synthesis approaches using natural biopolymers such as chitosan and alginate have also gained traction for their environmental benefits and enhancement of antibacterial properties. Co-precipitation has been used to fabricate

Cu-doped nanoparticles exhibiting improved antibacterial and photocatalytic efficacy. CuO nanoparticles have demonstrated utility in antimicrobial, antifungal, anti-inflammatory, anticancer, and antioxidant applications⁵. Their antibacterial mechanism is primarily attributed to the generation of ROS, which induces lipid peroxidation, protein oxidation, and DNA degradation, ultimately leading to cell death⁶.

This study aimed at synthesizing CuO nanorods by employing the co-precipitation method and at evaluating their antibacterial efficacy against *Staphylococcus aureus* by determining the inhibition percentage across varying nanoparticle concentrations.

2. Methodology

CuO nanorods were synthesized *via* a co-precipitation method using copper sulfate and sodium hydroxide as precursor compounds. Specifically, copper sulfate (0.5 M) was dissolved in distilled water, and upon complete dissolution, sodium hydroxide solution (1 M) was added dropwise under constant stirring, with drops directed along the vessel walls in order to maintain a pH of 12. The reaction mixture was allowed to proceed for 2 h. The resulting suspension was left to settle overnight; the supernatant was carefully discarded, and the precipitate was thoroughly washed with distilled water. The washed precipitate was dried at 80°C overnight and was subsequently calcined at 200°C for 1 h in order to obtain the final product⁷.

The antibacterial activities of the synthesized CuO nanorods against *Staphylococcus aureus* were evaluated *in vitro* by using the total bacterial count method. CuO nanorods (0.1, 0.15, 0.2, 0.25, and 0.3 g) were suspended in 5 ml deionized water and subjected to ultrasonic treatment for 45 min. Subsequently, 100 µl of bacterial suspension was introduced into each nanoparticle solution, which was agitated in a shaking incubator for 1 h. Samples were then cultured on a Mueller-Hinton agar and incubated at 37°C for 24 h.

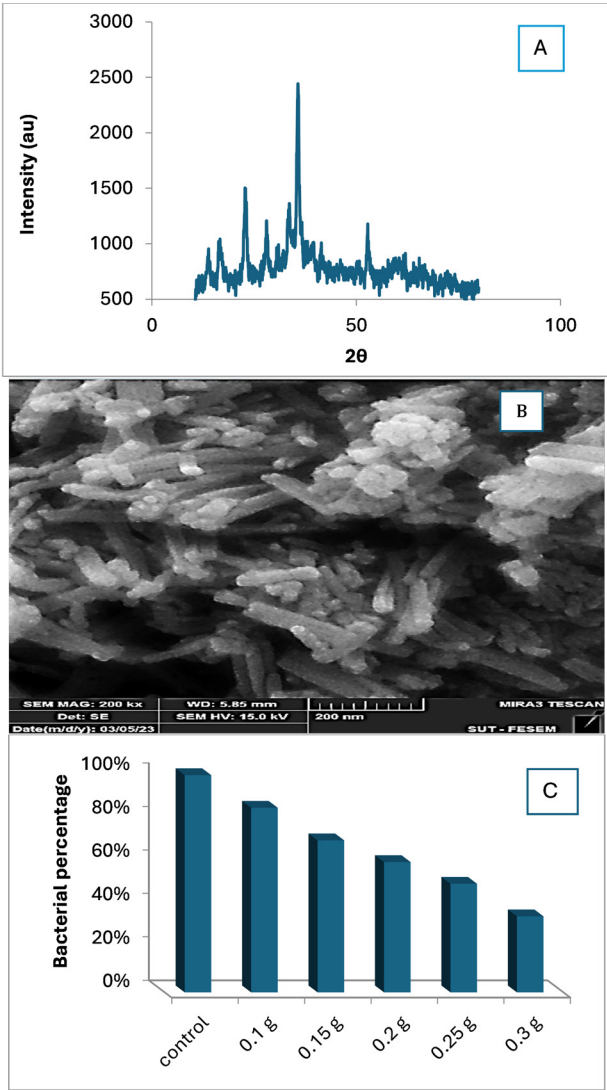


Figure 1. Instrumental analysis and antibacterial activity of copper oxide (CuO) nanorods (NRs). (A): X-ray diffraction pattern of CuO NRs. (B): Field emission scanning electron microscopy image of CuO NRs. (C): Percentage of *Staphylococcus aureus* in response to varying concentrations of CuO NRs.

3. Results and Discussion

X-ray diffraction (XRD) was employed in order to determine the crystal structure and size of the synthesized nanoparticles. XRD analysis provided information on crystalline phase, lattice parameters, and average crystallite size (Figure 1.A). By using the Scherrer's formula, the average crystal-

lite size was calculated as 10.538 nm⁸. Field emission scanning electron microscopy (FE-SEM) has enabled high-resolution morphological analysis, revealing well-defined rod-shaped CuO nanorods with diameters of approximately 25 nm⁹, as shown in Figure 1.B. Both particle size and concentration of CuO nanorods were found to be critical determinants of antibacterial activity. As indicated in Fig-

ure 1.C, smaller CuO nanorods demonstrated enhanced penetration across bacterial membranes due to their larger surface area and positive surface charge. These properties facilitated interactions with bacterial cell membranes and promoted the disruption of essential cellular components (including lipids, DNA, and proteins), leading to effective bactericidal action against *S. aureus*¹⁰.

4. Conclusion

In conclusion, CuO nanorods possess notable physicochemical properties and promising antibacterial capabilities. Structural characterization via XRD confirmed a crystalline architecture with an average crystallite size of approximately 10.538 nm, while FE-SEM analysis validated the rod-like morphology, with diameters near 25 nm. The observed antibacterial potency against *Staphylococcus aureus* is largely attributable to the small size and positive surface charge of CuO nanorods, ena-

bling disruptive interactions with bacterial membranes and internal cellular structures.

Acknowledgements

The authors would like to thank the University of Babylon for its moral and material support, and the Dean of the College of Pharmacy of the University of Babylon for his support and encouragement.

Conflicts of interest

None exist.

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HOW TO CITE:

Gassim F.A.Z.G., Mohammed G.J., Abass E.R., Rahi A.K., Yas H.A., Kadhim Z.A. Synthesis, characterization, and antimicrobial activity of copper oxide nanorods. *Pharmakeftiki* 37(2s), 230-234, 2025. <https://doi.org/10.60988/p.v37i2S.197>

Antibacterial activity and structure properties of $\alpha\text{Fe}_2\text{O}_3$ nanoparticles synthesized by thermal precipitation method

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KEY WORDS:

$\alpha\text{Fe}_2\text{O}_3$ nanoparticles; thermal precipitation method; structural properties; antibacterial activity; *Staphylococcus aureus*

ARTICLE INFO:

Received: December 27, 2024

Revised: n/a

Accepted: January 06, 2025

Available online: October 10, 2025

ABSTRACT

In this study, alpha iron oxide ($\alpha\text{Fe}_2\text{O}_3$) nanoparticles (NPs) were successfully synthesized via a simple, cost-effective thermal precipitation method, with reaction parameters (such as temperature and alkalinity) systematically controlled in order to ensure high-quality outcomes. The structural and morphological properties of $\alpha\text{Fe}_2\text{O}_3$ NPs were characterized using Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). XRD analysis confirmed a hematite phase with an average crystallite size of 24 nm, while SEM imaging revealed NPs of defined morphology with an average particle size of approximately 57 nm. The antibacterial activity of $\alpha\text{Fe}_2\text{O}_3$ NPs against the Gram-positive bacterium *Staphylococcus aureus* was assessed using the agar well diffusion method. The NPs were tested at concentrations of 10, 15, 20, and 25 mg/mL, and their efficacy was compared to that of a standard antibiotic. The results demonstrated notable antibacterial activity at concentrations of 15 mg/mL and above, with clear zones of inhibition observed against *S. aureus*.

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1. Introduction

Nanoparticles (NPs) are often regarded as “the wonders of modern

medicine” due to their extraordinary biological and physical capabilities¹. Iron oxides exhibit various crystalline phases, including those of hema-

tite ($\alpha\text{Fe}_2\text{O}_3$), akaganeite ($\beta\text{Fe}_2\text{O}_3$), maghemite ($\gamma\text{Fe}_2\text{O}_3$), and magnetite (Fe_3O_4)². Hematite, a magnetic, nontoxic, and corrosion-resistant compound, is especially valued for its wide range of applications in gas sensors, photocatalysis, lithium- and sodium-ion batteries, magnetic materials, supercapacitors, optical devices, and biomedical technologies³.

Metal oxide NPs play a critical role across chemistry, materials science, and biology. Owing to their electronic properties, they are well-suited for use in sensors and conductors. Their suitability for medical and pharmaceutical applications (including controlled drug release, diagnostics, and wound healing) is attributed to their distinctive physicochemical properties and antibacterial activity. These features suggest that metal NPs may serve as effective carriers for targeting bacterial biofilms and multidrug-resistant infections when formulated with appropriate antimicrobial payloads.

Metal oxide NPs exhibit multiple mechanisms of antibacterial action. Key among them are their size and morphology, which offer a high surface-to-volume ratio and thereby enhance interaction with bacterial cells. Mechanisms include disruption of bacterial cell walls, induction of oxidative stress, and release of metal ions. These features make metal oxide NPs particularly effective at reducing the likelihood of bacterial resistance. Moreover, their ability to generate reactive oxygen species (ROS) under specific conditions enhances their antimicrobial activity. An additional antibacterial mechanism involves the release of heavy metal ions⁴.

This study evaluates the antibacterial activity of $\alpha\text{Fe}_2\text{O}_3$ NPs against *Staphylococcus aureus* over a concentration range of 5, 10, 15, and 25 mg/L. The optimum inhibitory concentration was found to be 25 mg/L.

2. Methodology

2.1. Synthesis of $\alpha\text{Fe}_2\text{O}_3$ NPs

Pure $\alpha\text{Fe}_2\text{O}_3$ NPs were synthesized using ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). Specifically, 4 g (0.05 M) of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in 100 mL of distilled water and stirred magnetically for 30 min at 70°C.

To adjust the pH to 11, a precipitating agent – 50 mL of NaOH (2 M) – was gradually added under continuous stirring for 3 h. The resultant precipitate was collected and centrifuged at 6,000 rpm, followed by repeated rinsing with distilled water to eliminate residual chlorides. The precipitate was dried at 80°C, and the sample – designated as “ Fe_2O_3 500°C” – was calcined for 4 h at 500°C⁵.

2.2. Antibacterial activity assay

The antibacterial efficacy of $\alpha\text{Fe}_2\text{O}_3$ NPs against Gram-positive *Staphylococcus aureus* was assessed via the agar well diffusion method. Comparative analysis was performed against established antibiotic controls. Test organisms were cultured in nutrient broth at 37°C for 24 h. Post incubation, the inoculum was evenly spread onto Mueller-Hinton agar plates using sterile swabs. Wells of 6-mm diameter were created using a sterile cork borer, and 100 μL of NP suspensions (10, 15, 20, and 25 mg/mL in distilled water) were added to the wells. Plates were incubated at 37°C for 24 h, and antibacterial activity was evaluated by measuring the diameter of the inhibition zones (in mm).

3. Results and Discussion

X-ray diffraction (XRD) analysis confirmed the crystal structure and phase composition of the $\alpha\text{Fe}_2\text{O}_3$ NPs. As shown in Figure 1A, all peaks corresponded well to the hexagonal hematite phase of $\alpha\text{Fe}_2\text{O}_3$ (JCPDS card no. 33-0664). The average crystallite size of 24 nm was calculated using the Debye-Scherrer formula⁷, and powder formation may reflect grain coalescence.

Scanning electron microscopy analysis (Figure 1B) revealed particles of uneven size and morphology, largely tending toward distorted spherical forms with an average diameter of approximately 57 nm. Fourier-transform infrared (FT-IR) spectroscopy (Figure 1C) exhibited prominent absorption bands at 530 cm^{-1} and 4850 cm^{-1} . A weaker Fe–O stretching vibration peak appeared at 1515.5 cm^{-1} , consistent with commercial $\alpha\text{Fe}_2\text{O}_3$ spectra⁸.

Figure 1D illustrates the inhibition zones produced

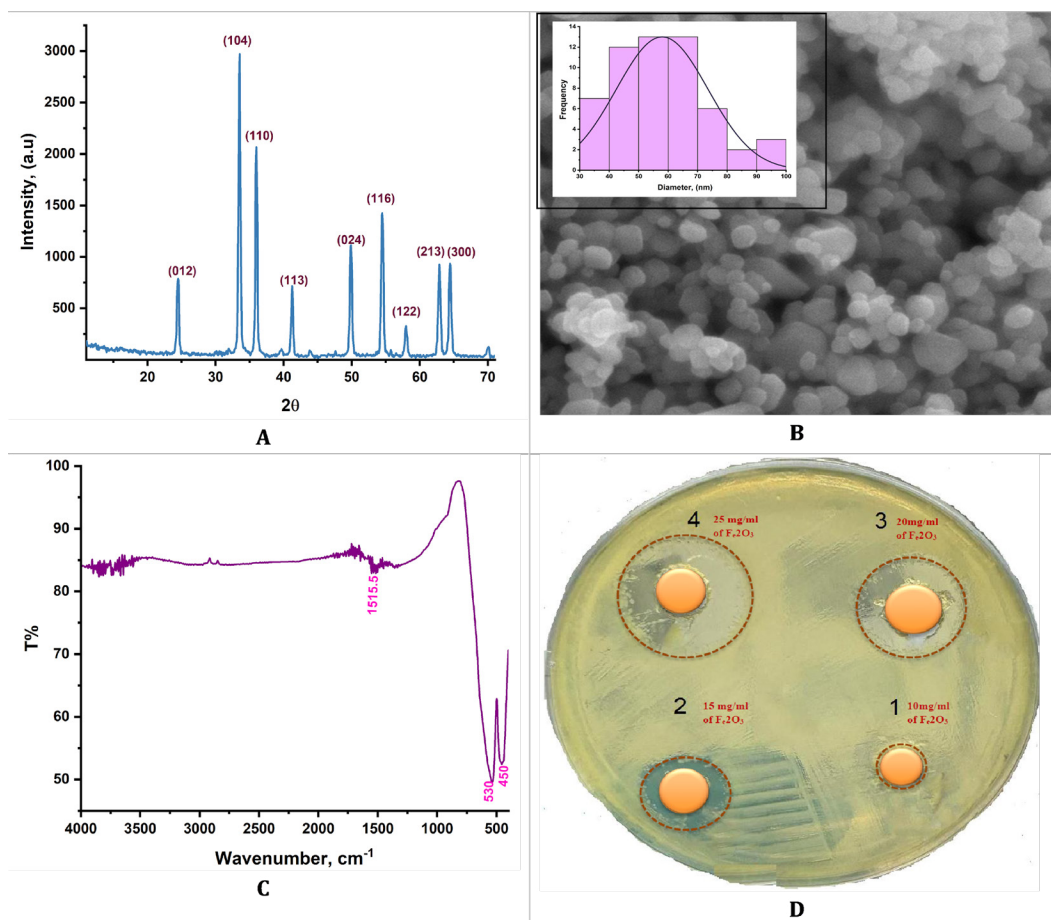


Figure 1. Structural characterization and antibacterial assessment of alpha iron oxide ($\alpha\text{Fe}_2\text{O}_3$) nanoparticles (NPs). (A): X-ray diffraction patterns confirming the hematite phase of $\alpha\text{Fe}_2\text{O}_3$ NPs. (B): Scanning electron microscopy image depicting the morphology and particle size distribution of $\alpha\text{Fe}_2\text{O}_3$ NPs. (C): Fourier-transform infrared spectra of $\alpha\text{Fe}_2\text{O}_3$ NPs highlighting key vibrational bands. (D): Zones of inhibition illustrating the antibacterial efficacy of $\alpha\text{Fe}_2\text{O}_3$ NPs against *Staphylococcus aureus* via the agar well diffusion method.

by increasing concentrations (10, 15, 20, and 25 mg/mL) of $\alpha\text{Fe}_2\text{O}_3$ NPs against *S. aureus*. Antibacterial activity increased proportionally with concentration, with significant inhibition observed at 15 mg/mL and above.

The antimicrobial mechanism of $\alpha\text{Fe}_2\text{O}_3$ NPs primarily involves the generation of ROS under oxidative stress. These ROS induce biomolecular damage (including DNA fragmentation, protein oxidation, and lipid peroxidation), ultimately leading to bacterial cell death. Additionally, the release of positively charged metal ions may disrupt membrane integrity and hinder

DNA replication *via* electrostatic interactions with negatively charged bacterial surfaces.

Zones of inhibition reflect the NPs' antibacterial efficacy, yet several factors influence these measurements. These include NP concentration, incubation time, bacterial strain, diffusion rate through agar, and particle-cell wall interaction. Synergistic or antagonistic effects within the agar medium also modulate antimicrobial performance. Thus, inhibition zone measurements alone should not be used in order to quantify antibacterial potency; rather, they must be interpreted in the context of these complex factors^{8,9}.

4. Conclusion

The herein assessed $\alpha\text{Fe}_2\text{O}_3$ NPs were successfully synthesized *via* a thermal precipitation method. XRD confirmed a hexagonal hematite phase, with an average particle size of 57 nm. Antibacterial testing demonstrated pronounced activity against *Staphylococcus aureus*, with efficacy increasing in a dose-dependent manner. These findings suggest that $\alpha\text{Fe}_2\text{O}_3$ NPs may serve as effective nanocarriers for enhancing the performance of antimicrobial agents.

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Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Habeeb S.A., Hammadi A.H., Abed D.A., Hamza L.F., Kbah N.Z. Antibacterial activity and structure properties of $\alpha\text{Fe}_2\text{O}_3$ nanoparticles synthesized by thermal precipitation method. *Pharmakeftiki* 37(2s), 235-238, 2025. <https://doi.org/10.60988/p.v37i2S.198>

Formulation and evaluation of flunarizine-loaded spanlastic nanovesicles for treating migraine

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KEY WORDS:

flunarizine; spanlastic nanovesicles; edge activator; ethanol injection method; migraine

ARTICLE INFO:

Received: December 30, 2024

Revised: January 14, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

ABSTRACT

This study investigates the use of spanlastic nanovesicles in order to enhance the solubility and bioavailability of flunarizine. Flunarizine-loaded spanlastics were prepared using the ethanol injection method, and were subsequently evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, and deformability index. The optimized formulation exhibited a particle size of 185 nm, a polydispersity index of 0.2134, a zeta potential of -17 mV, an entrapment efficiency of 68.01%, and a relative deformability of 8.32 g. The findings support spanlastic nanovesicles as a viable delivery system for improving the solubility and enhancing the bioavailability of flunarizine.

1. Introduction

Migraine is a major, yet under-recognized, public health concern that imposes substantial financial burdens on individuals and society. Affecting over one billion people across geographical, cultural, and socioeconomic boundaries, migraine is a chronic condition with a high prevalence and a demonstrable impact on quality of life. The migraine pathophysiology involves dysregulation of brainstem neu-

rons that mediate vascular control, in conjunction with the trigemino-vascular nociceptive system^{1,2}.

Spanlastics are surfactant-based nanovesicular elastic carriers composed of Span 60 and an edge activator (EA). These flexible vesicles have been employed in order to improve the solubility and bioavailability of both lipophilic and hydrophilic pharmaceutical agents^{3,4}. Flunarizine is effective in migraine therapy by mitigating the intracellular Ca²⁺ overload induced

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Table 1. Overview of the findings of the undertaken experimental trials of the developed flunarizine-loaded spanlastic nanoformulations. Abbreviations used: DI, deformability index; EA, edge activator; EE%, entrapment efficiency percentage; PDI, polydispersity index; PS, particle size.							
Formulation	Type of EA	Span : EA ratio	Sonication time (min)	PS (nm)	PDI	EE%	DI
F1	Tween 20	9:1	0	487.7	0.5317	64.51%	1.02
F2			3	436.9	0.4534	61.21%	1.36
F3			6	358.8	0.392	57.78%	1.79
F4	Tween 20	7:3	0	342.1	0.4224	70.76%	4.89
F5			3	185.8	0.2134	68.01%	8.32
F6			6	149.3	0.3024	60.82%	10.7
F7	Tween 20	5:5	0	199.4	0.413	62.31%	9.24
F8			3	151	0.2767	54.92%	11.97
F9			6	92.65	0.1857	51.61%	13.84

by cerebral hypoxia. However, flunarizine is practically insoluble in water, necessitating the use of cosolvents for injectable formulations; an approach associated with an increased risk of adverse effects. Consequently, existing formulations are suboptimal for severely ill patients due to poor bioavailability and absorption. This study aimed at developing a novel nanovesicular system in order to enhance the solubility and bioavailability of flunarizine.

2. Methodology

2.1. Materials

Flunarizine dihydrochloride was obtained from Hyperchem, China. Span 60 and Tween 20 were also sourced from Hyperchem, China. All additional reagents and solvents were of analytical grade and used without further purification.

2.2. Preparation of flunarizine-loaded spanlastic nanovesicles

Flunarizine-loaded spanlastic nanovesicles were prepared *via* the ethanol injection method, using Span 60 and Tween 20 in varying ratios. Briefly, the vesicle-forming agent and flunarizine were dissolved in ethanol. This ethanolic solution was then slowly injected, with stirring, into an aqueous phase containing the EA, preheated to 70°C. The resulting mixture was continuously stirred at 70°C, cooled to

5°C, and stored pending further analysis^{4,5}.

3. Results and Discussion

3.1. Particle size (PS), polydispersity index (PDI), and zeta potential (ZP)

Table 1 presents the particle size measurements of the formulated spanlastics, which ranged from 92.65 to 487.7 nm. The selected formulation, sonicated for 3 min with a Span 60 to Tween 20 ratio of 70:30, exhibited a notably reduced particle size. It was evident that by lowering the Span 60 and by increasing the EA concentrations, one yielded smaller vesicles, likely due to reduced interfacial tension that facilitates particle division and formation of smaller nanostructures⁶. All formulations demonstrated a substantial decrease in particle size following sonication, regardless of the sonication duration⁷. The recorded zeta potential (ZP) was -20 mV, thereby suggesting low aggregation propensity among the resulting spanlastics.

3.2. Vesicle elasticity measurement

Table 1 indicates that the deformability index (DI) of the nano-spanlastics ranged from 1.02 to 13.84 g. Increasing the EA percentage improved the DI relative to the Span 60 to EA ratio, potentially due to a bilayer fluidization induced by the EA enrichment. This enhancement likely reflects the EA in-

corporation into the vesicular membrane structure. Moreover, longer sonication times corresponded with greater DI values⁸.

3.3. The impact of formulation variables on the EE% of flunarizine-loaded spanlastic nanovesicles

As shown in Table 1, flunarizine entrapment within the spanlastics varied between 51.61% and 70.76%. The data clearly indicate that a 30% drug loading yielded the highest EE%, influenced by the Span 60 to EA ratio. A reduced bilayer fluidization at optimal EA levels may improve drug retention and minimize leakage. However, the excessive EA compromised the EE%, likely due to increased membrane fluidity leading to drug leakage. Additionally, the extended sonication times significantly diminished the EE% in flunarizine-loaded spanlastics⁹.

4. Conclusion

The developed flunarizine-loaded spanlastic nanovesicles effectively enhanced drug solubility, there-

by addressing limitations stemming from poor aqueous solubility and extensive first-pass metabolism. Our study's findings suggest that an encapsulation in spanlastic carriers presents a viable nanoscale delivery approach for improving flunarizine bioavailability.

Acknowledgements

The authors express their sincere gratitude to the College of Pharmacy of the University of Baghdad, and to its Department of Pharmaceutics, for generously providing all necessary resources to complete this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Haiss M.A., Abd Alhammid S.N. Formulation and evaluation of flunarizine-loaded spanlastic nanovesicles for treating migraine. *Pharmakeftiki* 37(2s), 239-242, 2025. <https://doi.org/10.60988/p.v37i2S.199>

Nurses' knowledge of the medical triage at the emergency unit of an Iraqi teaching hospital

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KEY WORDS:

**emergency unit;
life-threatening situations;
knowledge; nurses; triage**

ARTICLE INFO:

Received: January 07, 2025

Revised: February 21, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Triage refers to the placement of patients in the appropriate setting at the appropriate time in order for them to receive the appropriate level of care, along with the allocation of resources tailored to their medical needs. A descriptive study was conducted in order to assess nurses' knowledge of medical triage between October 15, 2023, and April 1, 2024. A non-probability convenience sample comprising 50 nurses working in the Emergency Unit of the Al Hillah Teaching Hospital was included. The study utilized a questionnaire developed through a comprehensive review of the relevant literature and modified as necessary. Its content validity was established through evaluation by a panel of five experts. Data were collected using a researcher-developed, self-administered questionnaire in Arabic, employing both interview and self-report formats. Data analysis was performed using the SPSS software, version 24. The general level of nurses' knowledge regarding medical triage, as assessed in this study, was found to be fair to good. It is recommended to appoint qualified registered nurses so as to enhance the quality of care provided in medical triage settings.

1. Introduction

Triage is a method used to prioritize patient treatment. In accident and emergency departments, it involves

assessing and categorizing patients – typically by an experienced nurse – in order to determine the order in which care is provided. This process may also be employed at the scene

Table 1. Overall assessment level of the nurses' knowledge of the medical triage in the emergency unit of the Al Hillah Teaching Hospital (N=50).		
Overall assessment	Frequency	Percentage
Poor (40 – 66.6)	13	26%
Fair (66.7 – 93.3)	19	38%
Good (93.4 – 120)	18	36%
Total	50	100%

of major incidents so as to guide immediate medical interventions¹. As a specialized technique of prioritization, triage aims to ensure timely and appropriate medical care. Within the emergency department (ED), it serves to enhance patient safety by guaranteeing that care is delivered based on clinical urgency. Triage systems also contribute to departmental organization, monitoring, and evaluation, thereby promoting clinical fairness and operational efficiency².

The triage area is typically the ED's initial point of contact for patients. Assessment includes a synthesis of the patient's presenting complaint, overall appearance, and relevant physiological indicators. Vital signs should be recorded at triage when required in order to assess urgency or when time permits. Patients classified as Australian Triage Scale (ATS) category 1 or 2 must be promptly transferred to an appropriate care area. The receiving nurse should then conduct a comprehensive nursing evaluation³. Several validated triage scales have emerged internationally since the late 1980s, including the Manchester Triage Scale (MTS), the Canadian Triage and Acuity Scale (CTAS), the Emergency Severity Index (ESI), the Australasian Triage Scale (ATS), and the Rapid Emergency Triage and Treatment System (RETTS)⁴.

Triage decisions are inherently complex and are often made under uncertainty with limited or ambiguous information. The triage nurse must swiftly identify life-threatening conditions and evaluate the probability of their occurrence. These decisions are critical in initiating emergency care and significantly impact patient outcomes⁵. Triage techniques are adapted for use in both hospital and prehospital settings in order to manage requests for acute medical care. Systems have been designed to rank urgency levels among patients presenting to EDs. Although numerous triage scales are employed, their validation processes vary, especially across European EDs⁶.

This study was conducted in order to assess nurses' knowledge of medical triage at the ED of an Iraqi teaching hospital.

2. Methodology

This descriptive study was conducted at the Al Hillah Teaching Hospital (Iraq) between October 15, 2023, and April 1, 2024. It aimed at evaluating the nurses' knowledge of medical triage in the ED of the Al Hillah Teaching Hospital. A non-probability convenience sample of 50 nurses working in the hospital's ED was recruited. The data collection tool was a questionnaire developed after an extensive literature review and modified as needed. It comprised two parts: (i) part I (demographic characteristics, including age, gender, educational level, total nursing experience, and experience in the ED), and (ii) part II (nurses' knowledge of medical triage in the ED). Content validity was reviewed by a panel of five experts, who assessed the questionnaire's form and relevance to the study objectives. Feedback was respectfully considered and incorporated. The instrument was deployed in Arabic via a self-administered format. The researcher introduced the study, obtained informed consent, and facilitated participation. Each self-report took approximately 15 to 20 min to complete. Responses were coded and analysed using the SPSS software, version 24. The research was reviewed and approved by the ethics committee of the Al-Mustaqbal University's College of Nursing. Ethical compliance was ensured in accordance with the Declaration of Helsinki. Approval was granted under document 950 on October 11, 2023.

3. Results and Discussion

The majority of the participating nurses exhibited a

fair level of knowledge regarding medical triage in the ED of the Al Hillah Teaching Hospital (Table 1). These findings are consistent with those reported by Duko *et al.*⁷ in Ethiopia, where 51.5% of nurses demonstrated fair knowledge levels. The similarity may be attributed to limited access to comprehensive triage information and training.

In our study, the lowest mean score was 1.64 (items 3 and 37), and the highest was 2.88 (item 12). These findings contrast with those of Firouzkouhi *et al.*⁸, where the overall mean knowledge score was also categorized as fair.

4. Conclusion

The study has found that the nurses working in the ED at the Al Hillah Teaching Hospital generally possess a fair to good level of knowledge regarding the medical triage.

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Acknowledgements

The authors gratefully acknowledge the faculty of the College of Nursing at the Al-Mustaqbal University, Iraq, for their support and collaboration.

Conflicts of interest

None exist.

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HOW TO CITE:

Hajwal S.K., Al-Taei M.H., Ayad Z.M., Hassan A.A.H., Khaleel M.A., Muhaibes F.J., Abbas A.H., Ridha-Salman H., Abbas Z.H. Nurses' knowledge of the medical triage at the emergency unit of an Iraqi teaching hospital. *Pharmakeftiki* 37(2s), 243-245, 2025. <https://doi.org/10.60988/p.v37i2S.200>

Male breast cancer in Iraq: exploring the potential of chitotriosidase as a key diagnostic and prognostic biomarker

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KEY WORDS:

male; breast cancer; chitotriosidase; obesity; genetic predisposition

ARTICLE INFO:

Received: January 30, 2025

Revised: February 15, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Male breast cancer (MBC) is rare (accounting for less than 1% of all breast cancer cases worldwide), but remains a significant public health concern. The prevalence of MBC is rising, particularly in regions with limited epidemiological data, such as Iraq, where delayed diagnosis and low awareness exacerbate outcomes. This retrospective observational study was conducted at the Specialized Oncology Center in Tikrit (Iraq), between December 2020 and September 2024. A total of 24 patients were included, and key risk factors (such as hormone misuse, family history of cancer, and obesity) were assessed. Patients were stratified into four age groups for prevalence analysis. Descriptive and inferential statistical methods revealed that the highest incidence of MBC occurred in the 35–44 year group (N=7; 29.2%). Hormone use was most prevalent in the 25–34 year group (80%) and declined with advancing age. Obesity rates increased progressively from 40% in the 25–34 year group to 83% in the 55–70 year group. Similarly, the proportion of individuals reporting a family history of cancer rose from 20% to 67% across these respective age groups. Odds ratio (OR) calculations indicated that both obesity (OR: 2.10) and hormone use (OR: 1.93) were positively associated with increased MBC risk.

1. Introduction

Breast cancer remains a globally

prevalent disease, primarily diagnosed in women, and is rare among men; less than 1% of cases involve

male patients. Its rarity contributes to frequent misdiagnosis and delayed treatment¹. A lack of qualified medical practitioners constitutes a principal challenge, often leading to substandard management and increased recurrence rates. Additionally, immunological and pathological treatment options are often complex due to limited early-stage medical attention². Risk factors for male breast cancer (MBC) include obesity, hormone misuse, and genetic predisposition³.

Chitotriosidase involvement in immune reactions suggests its potential utility in predicting MBC outcomes. Elevated levels of chitotriosidase in other cancers imply possible diagnostic relevance for MBC. This enzyme, associated with immune response and inflammation, has been reported to increase in various malignancies, supporting its putative role in MBC identification⁴. Growing evidence on BRCA1 and BRCA2 gene mutations in MBC underscores the importance of genetic screening and adjunctive early detection strategies⁵. The use of anabolic hormones has also been implicated in MBC, as exogenous androgens can disrupt endocrine balance, elevate oestrogen levels, and stimulate the development of breast tissue and tumorigenesis, particularly in younger males. Obesity, a well-established risk factor across multiple cancer types, contributes to a chronic inflammatory state. Adipose tissue-derived mediators, coupled with excess androgens, may facilitate oestrogen formation and promote carcinogenesis. These findings support the need for obesity prevention efforts in high-risk populations⁶.

Given the current paucity of epidemiological data on MBC in Iraq, this study aimed at generating evidence that informs prevention, detection, and treatment strategies for Iraqi MBC patients.

2. Methodology

This retrospective observational study has analysed data from 24 MBC cases reported at the Specialized Oncology Center in Tikrit, Iraq, between December 2020 and September 2024. Patients were stratified into four age groups (i.e., 25–34, 35–44, 45–54, and 55–70 years), comprising: 5 cases (25–34 years), 7

cases (35–44 years), 6 cases (45–54 years), and 6 cases (55–70 years). Data on hormone use, obesity, and family history of cancer were collected. The self-reported use of anabolic steroids was defined as “hormone use”. Obesity was identified as a body mass index (BMI) $>30 \text{ kg/m}^2$. A family history was recorded if first-degree relatives had been diagnosed with cancer.

This study has received ethical approval from the Scientific Research Ethical Committee (SREC) of the College of Pharmacy, Tikrit University (approval number: SREC202012; December 2020). All participants have provided written informed consent, ensuring compliance with ethical standards for human research. Descriptive and inferential statistical methods were employed. Frequencies and percentages were calculated for the three risk factors across all age groups. Given the small sample size ($N=24$), the Kruskal-Wallis test was used in order to compare prevalence rates. Odds ratios (ORs) with 95% confidence intervals were calculated in order to assess associations with MBC. Statistical significance was set at $p<0.05$. All analyses were performed using the SPSS version 22 software.

3. Results and Discussion

The 35–44 year group exhibited the highest incidence of MBC ($N=7$; 29.2%), followed by the 45–54 and 55–70 year groups. Hormone use was most prevalent in the 25–34 year group (80%) and decreased with age, reaching 17% in the 55–70 year group. Obesity rates rose progressively with age, from 40% in the youngest group to 83% among the oldest. Similarly, the proportion of patients with a family history of cancer increased from 20% in the youngest group to 67% in the oldest (Table 1).

Kruskal-Wallis testing revealed statistically significant differences in hormone use, obesity, and family history across age groups ($p<0.05$). The undertaken OR calculations suggested that obesity (OR: 2.10) and hormone use (OR: 1.93) were associated with elevated MBC risk (Table 1).

These findings align with those of prior studies by Khan and Tirona² and by Bhardwaj *et al.*³, which

Table 1. Overview of the study’s male breast cancer (MBC) cases, risk factor distribution, and statistical analysis. The latter was conducted using the Kruskal–Wallis test, with $p < 0.05$ considered as statistically significant. Approximate odds ratios (ORs) are provided in order to assess the relative likelihood of MBC development associated with each risk factor across age categories. These data illustrate age-related patterns in risk factor prevalence and their potential contribution to disease occurrence.

Age group	Cases	Hormone use	Obesity	Family history	p-value	OR (approx.)
25–34 years	5	4 (80%)	2 (40%)	1 (20%)	0.028 *	1.93
35–44 years	7	5 (71%)	3 (43%)	2 (29%)	0.028 *	2.10
45–54 years	6	3 (50%)	4 (67%)	3 (50%)	0.020 *	0.91
55–70 years	6	1 (17%)	5 (83%)	4 (67%)	—	—

have reported increased MBC risk with age, life-style factors, and hormone exposure. The pronounced hormone use in the 25–34 year group affirms earlier observations linking anabolic steroid misuse in younger males to MBC. Similarly, the obesity’s rising prevalence with age is consistent with studies by Seiler *et al.*⁶ and Fox *et al.*⁷, which have underscored its role in oestrogen elevation and carcinogenesis. Moreover, Lei *et al.*⁸ have reported a positive correlation between family history and MBC, thereby supporting our findings. Collectively, our statistical analysis reveals robust associations between MBC and all three investigated risk factors.

4. Conclusion

Our study demonstrates that MBC among Iraqi males is strongly associated with hormone misuse, obesity, and family history of cancer. These find-

ings advocate for targeted public health interventions, including nationwide awareness campaigns and preventive measures, particularly those addressing hormone use among younger males.

Acknowledgements

We express our sincere gratitude to Tikrit University for its support in conducting this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Hasan A.K., Ali H.M., Ismail A.M., Abbood M.K., Tahseen N.J. Male breast cancer in Iraq: exploring the potential of chitotriosidase as a key diagnostic and prognostic biomarker. *Pharmakeftiki* 37(2s), 246-249, 2025. <https://doi.org/10.60988/p.v37i2S.201>

The impact of the rs887829 C>T genetic polymorphism of *UGT1A1* on the response to deferasirox in Iraqi thalassaemia patients

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KEY WORDS:

thalassaemia; rs887829; C>T; *UGT1A1*; deferasirox; polymorphism

ARTICLE INFO:

Received: January 26, 2025

Revised: February 13, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

Beta thalassaemia major, also known as transfusion-dependent thalassaemia (TDT), is an autosomal recessive genetic disorder necessitating lifelong blood transfusions. Due to the absence of physiological iron excretion, patients require iron chelation therapy. Despite treatment with the oral iron chelator deferasirox (DFX), many patients exhibit elevated serum ferritin (SF) levels. UDP-glucuronosyl-transferase 1A1 (*UGT1A1*) – the principal gene of the UGT1 enzyme family – plays a central role in DFX metabolism. This cross-sectional study was conducted at the Karbala Hereditary Blood Diseases Center between November 2023 and April 2024. A total of 96 Iraqi patients with TDT (aged 8–39 years) were treated with DFX at a daily dose of 30–40 mg/kg for a minimum of three months. Their SF levels, serum erythroferrone levels, and liver and kidney functions were assessed. Genotyping of the rs887829 C>T single nucleotide polymorphism (SNP) in *UGT1A1* was performed using allele-specific polymerase chain reaction. The genotype frequencies for rs887829 were: CC (79.2%), CT (13.5%), and TT (7.3%). The wild-type (C) allele frequency was 0.86, compared to 0.14 for the mutant (T) allele. Patients with the TT genotype had significantly higher total serum bilirubin (TSB) levels compared to those with CC or CT genotypes ($p=0.03$). The rs887829 SNP was associated with increased TSB levels and may contribute to the development of hyperbilirubinaemia in Iraqi patients with TDT.

1. Introduction

Thalassaemias are autosomal recessive genetic disorders characterized by a reduced production of either α - or β -globin chains. Approximately 1% of the global population is affected by β -thalassaemia, which is particularly prevalent in the Middle East¹. Regular blood transfusions can lead to progressive iron overload and associated clinical complications, including hypogonadism (35%–55%), hypothyroidism (9%–11%), hypoparathyroidism (4%), diabetes mellitus (6%–10%), liver fibrosis, and heart failure (33%)².

Iron chelation therapy is typically initiated after 10–20 transfusions or when serum ferritin (SF) levels exceed 1,000 ng/mL. Available chelators include deferoxamine, deferiprone, and deferasirox (DFX). DFX was approved as a first-line treatment for transfusion-dependent thalassaemia (TDT) in over 70 countries, including the United States (2005) and throughout the European Union (2006)³. It is a potent, orally administered, selective iron chelator that forms a 2:1 stable complex with iron. DFX and its metabolites are primarily excreted *via* the faeces (approximately 84%) following extensive glucuronidation by the uridine glucuronosyltransferase (UGT) enzymes⁴. DFX administered at 10–40 mg/kg/day has been shown to reduce liver iron and SF levels and increase iron excretion. Major adverse effects include hepatic and renal failure, gastrointestinal bleeding, and bone marrow suppression³.

The UGT1A1 gene, located on chromosome 2q37, encodes the UGT1A1 enzyme; a key component of the UGT1 family. The rs887829 C>T single nucleotide polymorphism (SNP) resides in the main promoter region of *UGT1A1*. Previous research has demonstrated that promoter region polymorphisms in *UGT1A1* are linked to variability in drug response and disease risk⁵. This study aimed at investigating the impact of the rs887829 C>T SNP on the therapeutic response to DFX.

2. Methodology

2.1. Study subject recruitment

This cross-sectional study was approved by the Scientific and Ethical Committee of Karbala University College of Pharmacy (reference number: 2023HU7; approved on August 2, 2023). A total of 96 patients with TDT were recruited from the Karbala Hereditary Blood Diseases Center between November 2023 and April 2024, from a pool of 650 patients. Participant ages ranged from 8 to 39 years. All patients had received DFX monotherapy for at least 3 months at the recommended dose of 30–40 mg/kg/day. Patients with liver disease, those who consumed alcohol, individuals with sickle cell anaemia, and patients with other forms of thalassaemia were excluded.

Each subject provided 5 mL of venous blood *via* a disposable syringe. Of these, 2.5 mL were transferred to a plain tube and left to coagulate for 30 min. Samples were centrifuged at 4,000×g in order to isolate serum for erythroferrone (ERFE) and biomarker analysis, and stored at -20°C. The remaining 2.5 mL were placed in evacuated ethylenediaminetetraacetic acid (EDTA) tubes for haematological testing and DNA extraction.

2.2. Genetic analysis

Genomic DNA was extracted by using the Geneaid DNA Extraction/Genomic DNA Purification Prep Mini Kit (Taiwan), following the manufacturer's protocol. Allele-specific polymerase chain reaction (PCR) was used in order to detect the SNP. Lyophilized primers, designed through the Primer-BLAST program, were obtained from Macrogen-Korea. Each 25- μ L PCR reaction included: 12 μ L master mix (Promega, USA), 2 μ L of extracted DNA (100 ng/ μ L), 1 μ L each of forward and reverse primers, and 9 μ L nuclease-free water. PCR conditions involved an initial denaturation at 95°C for 3 min, followed by 30 amplification cycles at an annealing temperature of 62°C and extension at 72°C for 40 sec. PCR products were resolved using 2% (w/v) agarose gel electrophoresis.

2.3. Biochemical analysis

Red blood cell (RBC) and haemoglobin (Hb) lev-

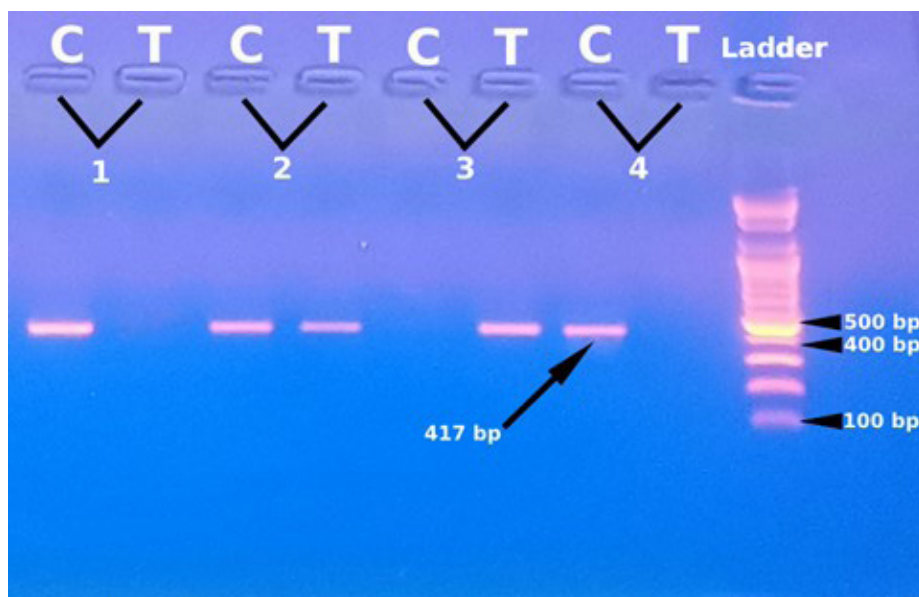


Figure 1. Agarose gel electrophoresis using allele-specific polymerase chain reaction in order to detect the rs887829 C>T single nucleotide polymorphism in the UGT1A1 gene. The wild-type genotype (CC) is represented in lanes 1 and 4, the heterozygous genotype (CT) in lane 2, and the homozygous mutant genotype (TT) in lane 3.

els were measured using the Swelab Alfa Analyzer. ERFE levels were quantified by using a sandwich-method enzyme-linked immunosorbent assay (ELISA) with a Sunlong Biotech kit. SF levels were measured by using the Cobas e411 analyser (Germany), while liver and kidney function tests were performed by using the Mindray BS 240 system.

2.4. Statistical analysis

Data were analysed using the SPSS v. 22 (SPSS Inc., Chicago, US). Chi-squared testing was applied to categorical data. Group means for haematological and biochemical parameters were compared using *t*-tests (two groups) and one-way analysis of variance (ANOVA) ($\geq$ three groups). A *p*-value <0.05 was considered as statistically significant.

3. Results and Discussion

Ninety-six patients (43% male) with TDT were included in our study. Indicative PCR results are shown in Figure 1. The age distribution ranged

from 8 to 39 years, with 67% of the patients being younger than 19 years; only two participants were older than 30 years. Parental consanguinity or marital relativity was reported in 88% of patients. Genotype distribution for rs887829 C>T SNP was as follows: CC (79%), CT (14%), and TT (7%). Significant differences were noted in total serum bilirubin (TSB) levels among genotypes, with the TT group showing elevated TSB ($p=0.03$), while other parameters did not differ significantly.

Consanguinity has been suggested to increase the risk of TDT⁴. In the present study, 88.5% of patients were born to first- or second-degree consanguineous unions.

Elevated TSB levels were significantly associated with the homozygous TT genotype ($p=0.03$). UGT1A1 is the primary UGT1 gene product and plays a critical role in bilirubin conjugation. Unconjugated bilirubin is the principal cause of hyperbilirubinaemia. Functional or structural disruptions in the UGT1A1 enzyme, often due to promoter or exon mutations, may impair glucuronidation⁶.

Because both bilirubin and DFX undergo hepatic

glucuronidation⁷ – primarily *via* UGT1A1 – polymorphisms such as rs887829 C>T may reduce *UGT1A1* expression⁸, leading to elevated DFX serum concentrations⁹. Higher DFX levels may enhance iron chelation efficiency, thereby lowering SF levels over time. In this study, the SF levels in TT carriers ($2,321.14 \pm 1,071.80$ ng/mL) were lower than those in CC ($3,333.48 \pm 1,050.18$ ng/mL) and CT ($3,739.15 \pm 1,113.79$ ng/mL) carriers, but the differences were not statistically significant.

Finally, ERFE levels, which rise in thalassaemia due to ineffective erythropoiesis and influence hepcidin regulation¹⁰, were lowest in the TT group (38.16 ± 20.13 ng/L), compared to those in the CC (43.74 ± 28.56 ng/L) and the CT (47.87 ± 20.89 ng/L) groups, though the differences did not reach statistical significance. Lower ERFE levels may partly explain the reduced SF levels observed in the TT group.

4. Conclusion

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The rs887829 C>T SNP in the UGT1A1 gene is significantly associated with elevated TSB levels, thereby suggesting a potential role in the development of hyperbilirubinaemia in patients with TDT. No significant associations were observed for other haematological or biochemical parameters.

Acknowledgements

We would like to extend our sincere appreciation to the Thalassaemia Center in Karbala for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Hasan H.S., Ismael A.H., Jubair S. The impact of the rs887829 C>T genetic polymorphism of *UGT1A1* on the response to deferasirox in Iraqi thalassaemia patients. *Pharmakeftiki* 37(2s), 250-254, 2025. <https://doi.org/10.60988/p.v37i2S.202>

Fabrication and evaluation of poloxamer-facilitated, glyceryl monooleate-based tizanidine cubosomes

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KEY WORDS:

cubosomes; muscular spasm; tizanidine; transdermal delivery; vesicular system

ARTICLE INFO:

Received: January 09, 2025

Revised: n/a

Accepted: January 31, 2025

Available online: October 10, 2025

ABSTRACT

Cubosomes are lipid-based, nanostructured aqueous dispersions formed through a highly organized, spontaneous self-assembly process. These dosage forms have demonstrated potential as effective drug carriers, enhancing therapeutic efficacy. In this study, cubosomes were developed in order to evaluate their suitability as nanocarriers for the transdermal delivery of tizanidine (TZN); a skeletal muscle relaxant with low oral bioavailability. Cubosomal vesicles were prepared through the thin-film hydration technique, by using glyceryl monooleate (GMO) as the lipid component and poloxamer 407 (P407) as the stabilizer. Vesicle properties were modulated by varying the surfactant-to-lipid ratio. The developed TZN-loaded cubosomes were characterized for vesicle size (113.5–182.6 nm), polydispersity index (0.049–0.301), and drug entrapment efficiency (41.39%–79.20%). The results indicate that TZN-loaded cubosomal dispersions may represent a promising approach to transdermal drug delivery.

1. Introduction

Cubosomes are advanced drug delivery systems composed of hydrated surfactant structures capable of forming a bicontinuous liquid cubic crystal phase, thus achieving thermodynamic stability *via* self-association. These nanocarriers feature two internal aqueous channels

and a three-dimensional honeycomb-like architecture built from curved, bicontinuous lipid bilayers. Cubosomes are typically synthesized through the self-assembly of amphiphilic lipids, such as glyceryl monooleate (GMO) and phytantriol, using poloxamer 407 (P407) as a stabilizing agent¹.

Tizanidine (TZN) is a centrally

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Table 1. Physicochemical characteristics of different tizanidine (TZN)-loaded cubosomes. In all formulations, TZN was 10 mg. Abbreviations used: EE%, encapsulation efficiency; GMO, glyceryl monooleate; P407, poloxamer 407; PDI, polydispersity index; PS, particle size.					
Formulation	GMO (mg)	P407 (mg)	PS (nm)	PDI	EE%
F1	100	6	165.0 ± 9.4	0.301 ± 0.061	69.04% ± 1.30%
F2	150	6	171.2 ± 7.0	0.135 ± 6.966	73.40% ± 4.35%
F3	200	6	182.6 ± 3.6	0.178 ± 0.007	79.20% ± 6.74%
F4	100	12	134.1 ± 5.0	0.119 ± 0.008	41.39% ± 2.50%
F5	150	12	141.7 ± 5.5	0.130 ± 0.530	59.56% ± 0.81%
F6	200	12	173.2 ± 0.4	0.141 ± 0.100	63.47% ± 2.56%
F7	100	24	113.5 ± 9.4	0.109 ± 0.022	67.09% ± 1.02%
F8	150	24	150.3 ± 7.0	0.049 ± 0.049	72.94% ± 3.41%
F9	200	24	160.9 ± 3.6	0.113 ± 0.006	76.80% ± 2.02%

acting muscle relaxant classified as a myotonolytic agent and a selective α_2 -adrenoceptor agonist, indicated for the treatment of spasticity in patients with cerebral or spinal pathology. TZN exhibits antispastic efficacy comparable to baclofen, with a more favourable tolerance profile. Its reported half-life ranges from 2.1 to 4.2 h, and its oral bioavailability is approximately 34%–40% due to extensive first-pass metabolism^{2,3}. Owing to its short half-life, TZN necessitates frequent administration. Numerous studies have explored formulation strategies aimed at enhancing bioavailability and reducing dosing frequency. Among these, novel drug delivery technologies have emerged in order to address pharmacokinetic limitations. Recent investigations suggest that the skin offers a viable route for systemic drug administration⁴.

Vesicular carriers (including cubosomes) have garnered attention for their potential to facilitate transdermal drug delivery by improving permeation across the stratum corneum. Compared to oral delivery, transdermal administration offers several advantages: sustained release at a uniform rate, ease of termination by device removal, self-administration, and circumvention of hepatic first-pass metabolism, thereby reducing systemic side effects⁵.

This study aimed at formulating TZN-loaded cubosomal nanovesicles stabilized with P407 and characterized by small particle size (PS), low polydispersity index (PDI), and high entrapment efficiency (EE%).

2. Methodology

The materials used included TZN, GMO, and P407 (Hyper Chem, China), polyvinyl alcohol (PVA; HiMedia, India), ethanol (HPLC grade), chloroform (AR grade; Chem-Lab, Belgium), potassium dihydrogen phosphate, and sodium hydroxide.

Cubosomes were prepared through the thin-film hydration method. Briefly, 10 mg of TZN were dissolved with variable amounts of GMO (100–200 mg) and P407 (6–24 mg), as detailed in Table 1, in a mixture of ethanol and chloroform (3 mL each). The organic solvent was removed by rotary evaporation under reduced pressure (Heidolph, Germany), yielding a thin film of GMO and P407. This film was hydrated with 10 mL of phosphate buffer containing 1.25% w/v PVA at 70°C ± 2°C. The resulting dispersion was subjected to probe sonication (Bandelin Electronic, Germany) in pulse mode (2-sec off, 2-sec on) for 2 min. The dispersion was then stirred for 2 h and was cooled to ambient temperature. Samples were transferred into glass vials and stored at room temperature for further analysis⁶.

Cubosomal PS and PDI were assessed *via* dynamic light scattering by using a Zetasizer (Malvern, UK). Prior to measurement, samples were diluted tenfold with deionized water⁷. EE% was determined by ultrafiltration, quantifying the unencapsulated drug in the supernatant. Specifically, 2 mL of the dispersion were placed into a microcentrifuge filter tube (Am-

icon Ultra, Ireland; 10-kDa cutoff) and were centrifuged at 6,000 rpm for 10 min. The filtrate was diluted appropriately with ethanol⁸, and absorbance was measured at 319 nm using a Shimadzu VR ultraviolet spectrophotometer. Blank cubosomes (without TZN) served as controls. EE% was calculated using the following equation:

$$EE\% = [(total\ drug\ added - free\ drug) / total\ drug\ added] \times 100$$

Data were expressed as mean $\pm$ standard deviation. Statistical analysis employed one-way ANOVA, with $p < 0.05$ considered as significant.

3. Results and Discussion

PS was significantly influenced by the concentrations of both GMO and P407 ($p < 0.05$). Increasing the levels of P407 led to reduced size distribution of cubosomes. GMO was positively correlated with higher EE%, whereas P407 exerted a negative impact. Notably, the P407 concentration significantly reduced PS ($p < 0.05$), thereby corroborating with the findings by Alkawak *et al.*⁹. This reduction likely stems from P407's surfactant capacity to lower surface tension, thereby diminishing the surface energy and inhibiting particle aggregation.

Surfactant concentration plays a dual role: facilitating emulsification and preventing droplet coalescence; the observed effects of P407 on PS distribution likely follow similar reasoning. Additionally, by increasing the lipid phase concentration one can elevate the dispersion viscosity, which may hinder a fragmentation of bicontinuous structures into smaller particles¹⁰.

The decrease in EE% with elevated P407 concentrations may reflect an enhanced solubility of TZN in the external medium, leading to drug leakage. Conversely, by reducing the P407 concentration while increasing lipid concentration appeared to accelerate solidification of cubosomal nanoparticles, potentially attributable to increased viscosity. This phenomenon may restrict drug migration to the external phase, thereby enhancing the EE%⁹.

4. Conclusion

The study demonstrates that TZN-loaded cubosomal dispersions, formulated with GMO and stabilized by P407, possess favourable physicochemical characteristics for transdermal delivery. These nanocarriers may serve as promising vehicles for the improvement of the bioavailability and the reduction of the dosing frequency of TZN.

Acknowledgements

We extend our appreciation to the College of Pharmacy of the University of Baghdad, for granting this research project.

Conflicts of interest

None exist.

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HOW TO CITE:

Hasan M.J., Rajab N.A. Fabrication and evaluation of poloxamer-facilitated, glyceryl monooleate-based tizanidine cubosomes. *Pharmakeftiki* 37(2s), 255-258, 2025. <https://doi.org/10.60988/p.v37i2S.203>

***In silico* screening of FDA-approved drugs for antiviral activity against the influenza virus: a novel approach to the repurposing of existing medications**

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KEY WORDS:

***in silico*; influenza; lysine; FDA-approved drugs; antiviral**

ARTICLE INFO:

Received: February 20, 2025

Revised: February 21, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

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ABSTRACT

This study investigated the efficacy of FDA-recognized compounds in preventing influenza virus infections, using *in silico* analysis. By focusing on the structural characteristics of neuraminidase (a key target in antiviral therapy), the study identified 12 critical regions amenable to drug interaction. Among the evaluated compounds, lysine emerged as the most promising candidate, exhibiting an XP GScore of -9.912 and relatively high binding affinity. Molecular docking simulations revealed that lysine engages essential amino acid residues within the neuraminidase active site through hydrogen bonding and salt-bridge formation, thereby supporting its potential to inhibit viral replication. Other compounds, including iohexol and capastat, also demonstrated notably high binding constants. These findings underscore the feasibility of drug repurposing, particularly involving known dietary supplements such as lysine, as a strategic response to emerging antiviral resistance, severe influenza cases, and seasonal outbreaks. The results provide a preliminary experimental framework to guide future studies and support the clinical deployment of these agents against influenza and related infections.

1. Introduction

Influenza viruses are a major cause

of respiratory illness, affecting an estimated 1 billion individuals annually worldwide. According to the

World Health Organization (WHO), influenza-related respiratory infections result in approximately 3–5 million cases of severe illness and 290,000 to 650,000 deaths each year¹. The disease burden is especially pronounced among vulnerable populations – namely young children and the elderly – where complications often lead to significant morbidity and mortality. The rapid transmission of the virus in densely populated environments underscores the urgent need for effective antiviral therapies².

Current therapeutic options primarily consist of neuraminidase and M2 protein inhibitors, which have demonstrated efficacy, but are increasingly compromised by the emergence of drug-resistant viral strains³. In recent years, *in silico* approaches have gained recognition as powerful tools in drug discovery and repurposing. These computational strategies enable researchers to simulate molecular interactions and assess the antiviral potential of FDA-approved compounds against influenza viruses. Crucially, by leveraging existing safety and pharmacokinetic profiles, such methods can accelerate the identification of viable treatment candidates⁴.

Among these candidates is lysine, an essential amino acid with known antiviral activity. Preliminary studies suggest that lysine may disrupt viral replication by interfering with the functional role of arginine in viral propagation⁵. This study aimed at evaluating the antiviral properties of FDA-approved drugs through *in silico* screening, with particular emphasis on lysine's potential as a repurposed therapeutic agent against influenza virus infection.

2. Methodology

2.1. Ligand preparation

A total of 1,379 FDA-approved drugs were included in the analysis. Three-dimensional (3D) molecular structures were retrieved from the ZINC15 database (<https://zinc20.docking.org/substances/subsets/fda/>). Ligand preparation was executed using the GLIDE suite within the Schrödinger software (Maestro v. 12.8.117). Partial atomic charges

were assigned using the Optimized Potentials for Liquid Simulation 4 (OPLS4) force field. For each compound, the default number of low-energy conformers was generated at pH 7.0, while compounds containing more than 1,000 atoms were excluded in order to ensure computational tractability.

2.2. Protein preparation

The neuraminidase protein structure (PDB ID: 3CLO) was obtained from the Protein Data Bank. Structural refinement was performed using the Protein Preparation Wizard in GLIDE, which involved bond correction, addition of hydrogen atoms, and energy minimization under standard conditions (pH 7.0), utilizing the OPLS4 force field with a root-mean-square deviation (RMSD) cutoff of 0.30 Å.

2.3. Docking protocol

Molecular docking simulations were carried out with GLIDE in order to evaluate the binding interactions between the ligands and neuraminidase protein. The active site was defined based on previously characterized residues essential for ligand binding: Arg118, Arg152, Arg156, Arg224, Arg292, Asp151, Glu425, Glu276, Glu277, Arg371, Glu119, and Glu22. The docking protocol aimed at predicting the binding compatibility of each ligand with these target residues.

2.4. Binding affinity prediction

Binding affinity was quantified using the XP GScore metric provided by GLIDE. This score integrates various molecular interaction parameters (including hydrogen bonding, electrostatic forces, and steric fit) in order to estimate ligand-protein binding strength. Lower GScores correspond to stronger predicted affinities.

2.5. Analysis of docking results

Docking outcomes were assessed in order to identify lead compounds demonstrating high-affinity

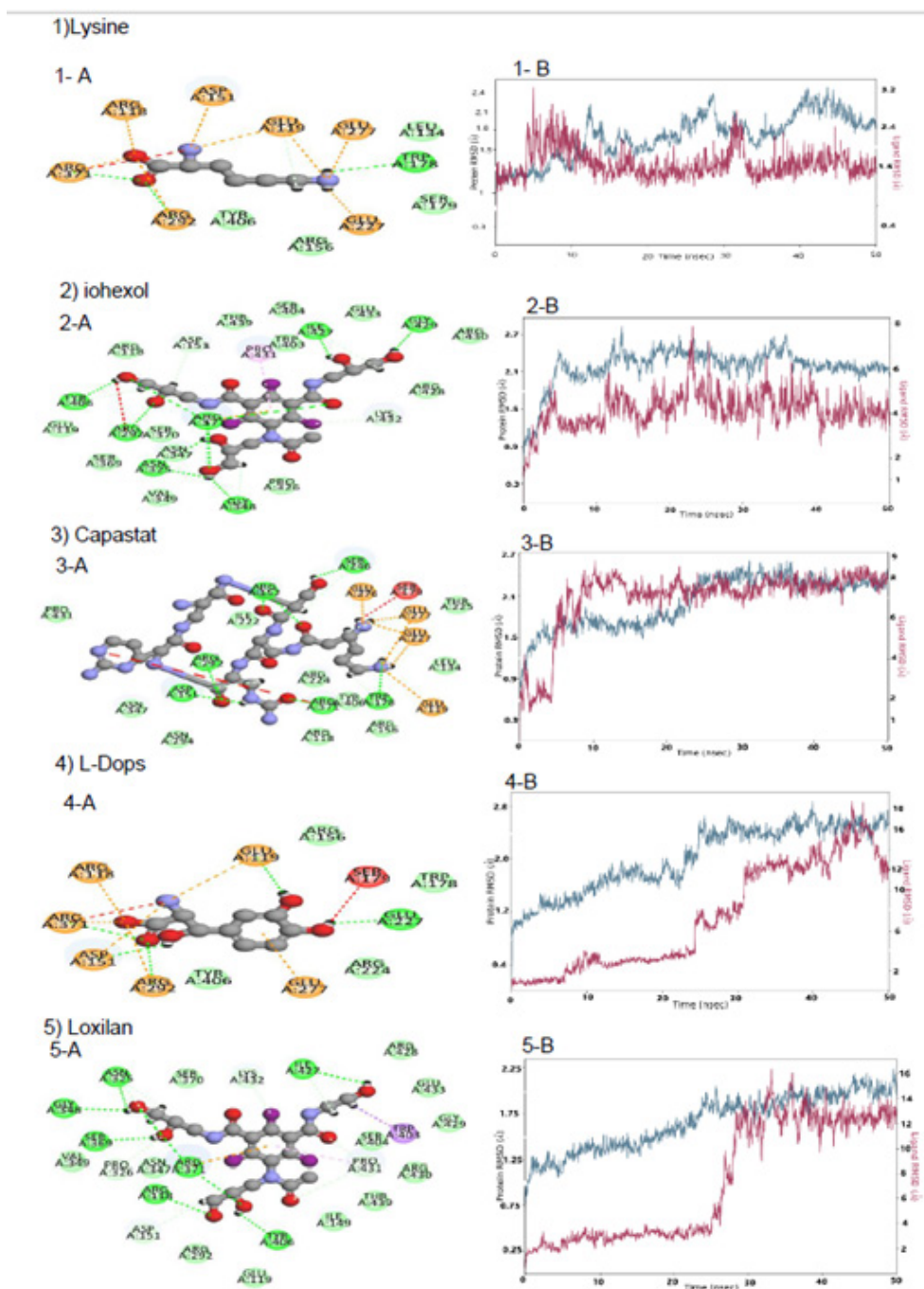


Figure 1. Molecular docking and binding affinity analysis of five selected compounds: lysine (1), iohexol (2), capastat (3), L-DOPS (4), and loxilan (5). The left panels (A) present 2D diagrams of ligand–protein interactions, highlighting key hydrogen bonds and salt bridges. The right panels (B) displays root-mean-square deviation (RMSD) trajectories from molecular dynamics simulations, reflecting the structural stability of each ligand–protein complex over time.

interactions with neuraminidase. Ligand-protein interactions were visualized by using Discovery Studio V20.1 (BIOVIA, Dassault Systèmes), focusing on conventional hydrogen bonds, salt bridges, and unfavourable contacts for each complex.

3. Results and Discussion

The *in silico* screening of FDA-approved compounds for anti-influenza activity has identified several promising candidates, most notably lysine⁵. Docking simulations have revealed robust binding affinities between these compounds and neuraminidase, specifically for those targeting its catalytic site³.

Lysine has ranked highest among candidates, with an XP GScore of -9.912, thereby signifying strong predicted affinity for neuraminidase (PDB ID: 3CL0). It has formed conventional hydrogen bonds with Trp178, Arg292, and Arg371, as well as salt bridges with Glu227 and Arg118 (Figure 1.1)⁶. Additional attractive interactions with Glu119 and Asp151 have further reinforced lysine's therapeutic potential. Lysine's historical role as a dietary supplement in managing the herpes simplex virus (HSV) suggests a mechanistic crossover relevant to influenza⁷. By antagonizing the arginine-dependent replication pathways, lysine may disrupt the influenza viral life cycle; a strategy compounded by its established safety profile and clinical accessibility.

Beyond lysine, several other drugs have demonstrated strong binding affinities: (i) iohexol has exhibited an XP GScore of -10.908 and extensive hydrogen-bonding interactions with Gly429 and Arg371 (10 conventional hydrogen bonds; Figure 1.2)⁸, (ii) capastat has yielded an XP GScore of -10.271, forming hydrogen bonds and salt bridges with Ser246 and Arg152, thereby potentially interfering with neuraminidase enzymatic activity and reducing viral release (Figure 1.3)⁹, and (iii) L-DOPS and loxilan have scored -10.326 and -10.345, respectively, by engaging key residues including Arg292 and Arg371 *via* both hydrogen bonds and salt bridges (Figures 1.4–1.5)¹⁰. These compounds appear to exert antiviral effects by mimicking the

binding of sialic acid to neuraminidase, thereby blocking its enzymatic function. Given neuraminidase's role in facilitating the release of progeny virions from infected cells, its inhibition presents a compelling strategy to curtail viral dissemination⁹.

Collectively, these findings support the clinical repurposing of FDA-approved agents to combat influenza. The use of pharmacologically characterized compounds expedites therapeutic deployment, especially in the face of seasonal outbreaks and evolving viral strains². Coupled with computational screening, this approach offers a scalable and responsive framework for antiviral drug development⁴. Targeting vulnerable populations remains an urgent priority, and this study contributes a rationale for broadening the therapeutic arsenal against influenza. Further experimental validation (including the undertaking of *in vitro* and *in vivo* assays) is essential in order to substantiate these predictions. Moreover, investigating combination therapies may reveal synergistic effects capable of enhancing antiviral efficacy.

4. Conclusion

The herein undertaken *in silico* docking analysis of FDA-approved compounds identified lysine and several other agents as promising inhibitors of influenza neuraminidase. These findings support the strategic repurposing of well-characterized drugs in order to mitigate influenza viral replication and transmission.

Acknowledgements

The authors thank all contributors and affiliated institutions for their support throughout this study. Special appreciation is extended to those involved in the computational analyses and interpretative evaluation of the antiviral potential of FDA-approved compounds against influenza viruses.

Conflicts of interest

None exist.

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HOW TO CITE:

Hashim H.O., Al-Hindy H.A.A.M., Bakr A.M., Hayif Q.A., Humadi Y.T. *In silico* screening of FDA-approved drugs for antiviral activity against the influenza virus: a novel approach to the repurposing of existing medications. *Pharmakeftiki* 37(2s), 259-263, 2025. <https://doi.org/10.60988/p.v37i2S.204>

Targeting the Crimean-Congo haemorrhagic fever virus with natural compounds: an *in silico* study

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KEY WORDS:

in silico; Crimean-Congo haemorrhagic fever virus; natural compounds; FDA-approved drugs; antiviral drugs

ARTICLE INFO:

Received: January 16, 2025

Revised: February 20, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

The Crimean-Congo haemorrhagic fever virus (CCHFV) is a highly transmissible pathogen capable of inducing severe haemorrhagic manifestations with high fatality rates. This study has aimed at demonstrating a virtual screening workflow and compound selection strategy to evaluate the potential of natural compounds to inhibit CCHFV through its ovarian tumor domain protease (vOTU); a key mediator of immune evasion. From a curated library of 5,749 natural compounds, five candidates met the selection criteria. Among these, 1,3,6-trigalloyl glucose exhibited the highest binding affinity (XP GScore: -9.351 kcal/mol) and geometric stability, as evidenced by a root mean square deviation (RMSD) of <4.5 Å in molecular dynamics simulations. The second most promising compound, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-O-(6-deoxy-α-L-gulopyranosyl)-β-D-talopyranoside, demonstrated an XP GScore of -7.909 kcal/mol and an RMSD <2.8 Å. These findings suggest that the identified natural compounds may serve as promising antiviral candidates against CCHFV. Further experimental validation is warranted in order to assess their therapeutic potential. Overall, this study underscores the utility of *in silico* approaches for the identification of novel antiviral agents derived from natural sources.

1. Introduction

The Crimean-Congo haemorrhagic

fever (CCHF) is a severe zoonotic viral disease caused by infection with the Crimean-Congo haemorrhagic

fever virus (CCHFV)¹. Transmission occurs primarily through bites from infected *Hyalomma* ticks or *via* direct contact with the blood, tissues, or bodily fluids of infected animals². First documented in the mid-twentieth century, CCHF is endemic across Africa, Asia, and Eastern Europe, posing a significant public health threat due to its high case fatality rate, which may exceed 30% in severe outbreaks¹.

The pathogenesis of CCHF involves complex interactions between CCHFV and the host immune system³. Viral infection activates the endothelial cells and the macrophages, triggering an excessive release of pro-inflammatory cytokines. This dysregulated immune response contributes to hallmark clinical features, including haemorrhage and multi-organ dysfunction³. Currently, the therapeutic options are limited; ribavirin remains the only antiviral agent with reported efficacy, though its clinical effectiveness remains controversial². In response to the urgent need for novel antiviral strategies, researchers have increasingly turned to computational (*in silico*) drug discovery platforms⁴.

This study has investigated the potential of natural compounds to inhibit CCHFV by targeting the viral ovarian tumor domain protease (vOTU); a deubiquitinating enzyme implicated in immune suppression⁵. Using molecular docking and molecular dynamics (MD) simulations, we have herein aimed to identify promising lead compounds for further experimental validation.

2. Methodology

This study employed an *in silico* approach to identify natural compounds capable of inhibiting CCHFV by targeting vOTU. The workflow included ligand preprocessing, protein optimization, molecular docking, and MD simulations. A phytochemical database was compiled from 200 Middle Eastern plant species, yielding 12,971 natural compounds. After deduplication, 5,749 unique ligand conformers were retained for analysis. Ligand preparation was performed using the Glide suite (Schrödinger), with optimization via the OPLS4 force field at physiological pH (7.0). Compounds exceeding 1,000 atoms were excluded

in order to reduce computational overhead. Low-energy conformers were selected for virtual screening.

The crystal structure of CCHFV vOTU protease (Protein Data Bank ID: 5V5H) was prepared using the Protein Preparation Wizard in Glide. This included correction of bond orders, addition of hydrogen atoms, and energy minimization using the OPLS4 force field at pH 7. Structural integrity was confirmed *via* a root mean square deviation (RMSD) analysis, with a cutoff of 0.30 Å.

Molecular docking was performed using Glide's extra precision (XP) mode. The active site was defined based on key residues known to mediate ligand binding: Lys27, Asp39, Glu51, Asp52, Arg80, Leu81, and Val82. The XP GScores were used in order to rank ligand binding affinities. MD simulations were conducted in order to assess the stability of ligand-protein complexes and to characterize dynamic interactions. RMSD values were used for the evaluation of conformational stability. Binding modes and interaction types were analysed using Discovery Studio v20.1 (BIOVIA).

3. Results and Discussion

The virtual screening of 5,749 natural compounds identified five candidates with high binding affinities to vOTU, as indicated by XP GScores ranging from -9.351 to -7.909 kcal/mol (Figure 1). Among these, 1,3,6-trigalloyl glucose emerged as the top-performing ligand, with an XP GScore of -9.351 kcal/mol. This compound formed eight conventional hydrogen bonds, two carbon-hydrogen (C-H) bonds, one π -alkyl interaction, and one donor-donor interaction with vOTU.

MD simulations confirmed the stability of the 1,3,6-trigalloyl glucose-vOTU complex, with RMSD values consistently being below 4.5 Å. The second-ranked compound, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-O-(6-deoxy- α -L-gulopyranosyl)- β -D-talopyranoside, has exhibited an XP GScore of -7.909 kcal/mol and RMSD values below 2.8 Å. This compound formed three hydrogen bonds and ten C-H interactions with vOTU, thereby suggesting a strong inhibitory potential.

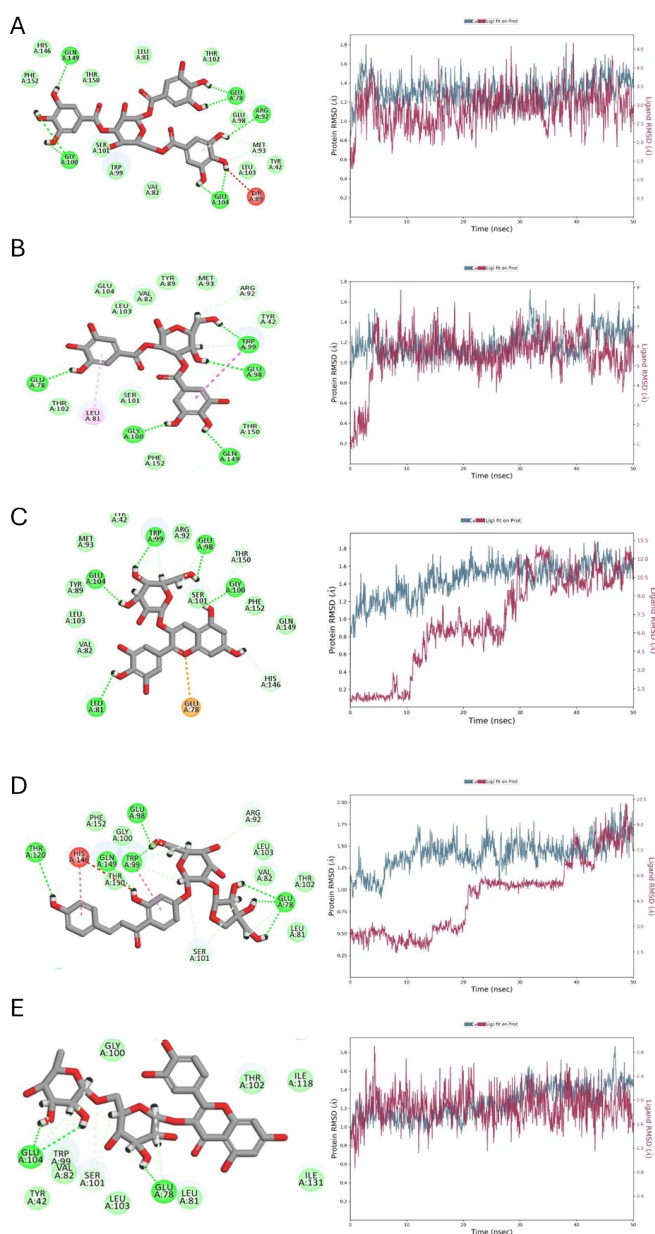


Figure 1. Molecular docking and molecular dynamics (MD) analysis of natural compounds targeting the Crimean-Congo haemorrhagic fever virus (CCHFV) ovarian tumor domain protease (vOTU). (A): 1,3,6-Trigalloyl glucose exhibited the highest binding affinity (-9.351 kcal/mol), forming strong hydrogen bonds and hydrophobic interactions with key catalytic residues (Lys27, Asp39, Glu51), while RMSD analysis confirmed stable binding (<4.5 Å) over a 50-nsec simulation. (B–D): Other candidate compounds demonstrated binding affinities ranging from -7.986 to -7.909 kcal/mol, with diverse interaction profiles and comparatively lower conformational stability. (E): 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-O-(6-deoxy-α-L-gulopyranosyl)-β-D-talopyranoside exhibited a favourable binding score (-8.556 kcal/mol), forming multiple hydrogen bonds and maintaining an RMSD >4 Å; indicative of high structural stability. The right panel illustrates RMSD fluctuations of ligand–protein complexes over time, confirming sustained ligand occupancy within the binding pocket. These findings suggest that compounds (A) and (E) are promising inhibitors of CCHFV vOTU and merit further experimental validation.

The observed ligand-vOTU interactions are significant, as vOTU plays a critical role in immune evasion by deubiquitinating and deISGylating host proteins⁶. Inhibiting vOTU may restore host antiviral responses and mitigate CCHFV pathogenesis. Given the limited efficacy of ribavirin², the identification of natural inhibitors targeting viral proteases represents a promising adjunct or alternative therapeutic strategy⁷.

Natural products offer structural diversity and bioactivity, making them attractive candidates for antiviral drug development. Several phytochemicals have demonstrated inhibitory effects against the main protease (Mpro) of SARS-CoV-2, suggesting potential cross-viral applicability⁸. Further investigation into structure-activity relationships may enhance the potency and specificity of these compounds against CCHFV.

4. Conclusion

This study highlights the utility of *in silico* drug discovery for identifying natural compounds with inhibitory activity against CCHFV vOTU. Molecular docking and MD simulations revealed two lead compounds, namely 1,3,6-trigalloyl glucose and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-

4H-chromen-3-yl 6-O-(6-deoxy- α -L-gulopyranosyl)- β -D-talopyranoside, with strong binding affinities and stable complex formation. These findings warrant further *in vitro* and *in vivo* validation in order to assess their therapeutic potential and safety profiles.

Acknowledgements

The authors gratefully acknowledge the support of all contributors and affiliated institutions involved in this research. Their assistance was instrumental in advancing the computational exploration of natural compounds as potential inhibitors of CCHFV.

Conflicts of interest

None exist.

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HOW TO CITE:

Hashim H.O., Al-Hindy H.A.A.M., Razzaq M.R., Badr S.H., Ali Z.A., Abdulwahid T.R. Targeting the Crimean-Congo haemorrhagic fever virus with natural compounds: an *in silico* study. *Pharmakeftiki* 37(2s), 264-268, 2025. <https://doi.org/10.60988/p.v37i2S.206>

***In silico* screening of natural compounds for antiviral activity against the influenza virus**

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KEY WORDS:

***in silico*; influenza virus;
natural compounds;
neuraminidase; antiviral**

ARTICLE INFO:

Received: January 16, 2025

Revised: February 20, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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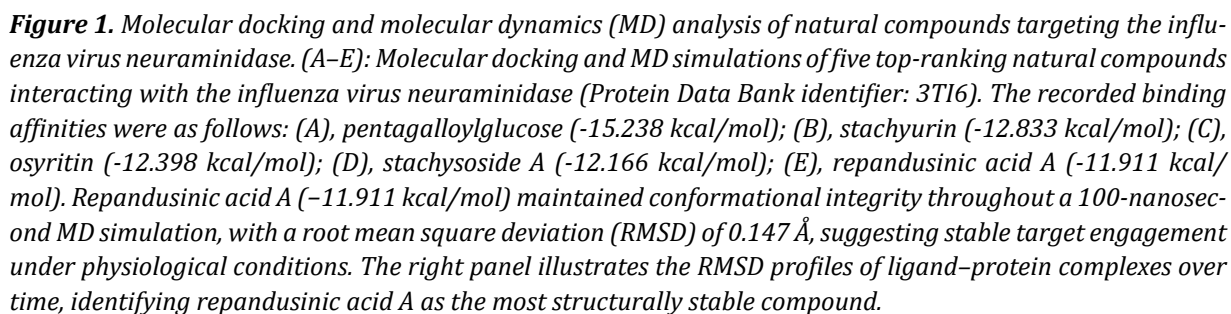
ABSTRACT

Influenza A viruses are major pathogens responsible for significant global morbidity and mortality, necessitating the development of novel antiviral agents targeting drug-resistant strains. This study has assessed the antiviral potential of various natural compounds against influenza A by performing molecular docking analyses in order to evaluate their binding affinities with neuraminidase (Protein Data Bank identifier: 3TI6); a critical viral enzyme that facilitates progeny virion release and dissemination. A total of 5,749 unique phytoconstituents were computationally screened, yielding five candidates with high binding affinities: pentagalloylglucose (-15.238 kcal/mol), stachyurin (-12.833 kcal/mol), osyritin (-12.398 kcal/mol), stachyoside A (-12.166 kcal/mol), and repandusinic acid A (-11.911 kcal/mol). Molecular dynamics simulations of repandusinic acid A corroborated the docking results and geometry optimization data, demonstrating enhanced structural stability and conformational flexibility relative to analogous compounds, with a root mean square deviation of 0.147 Å. These findings indicate that the identified bioactive compounds, particularly repandusinic acid A, hold promise as antiviral agents targeting neuraminidase. However, extensive experimental validation is required in order to elucidate their therapeutic efficacy, toxicity profiles, and pharmacokinetic parameters in clinical contexts.

1. Introduction

Influenza viruses primarily com-

prise types A and B, which are responsible for seasonal epidemics and recurring global pandemics.



Their high mutation rates and propensity for genetic reassortment pose persistent threats to the efficacy of preventive and therapeutic measures. Transmission occurs *via* respiratory droplets expelled from infected individuals, with acute-onset symptoms including fever, cough, and myalgia¹.

The causative agent of influenza is an enveloped virus classified within the family *Orthomyxoviridae*, characterized by a segmented RNA genome enclosed within a lipid bilayer and studded with glycoproteins such as neuraminidase and hemagglutinin². Existing antiviral therapies target neuraminidase and the M2 protein; however, the emergence of drug-resistant strains has compromised their clinical utility. For example, the prevalence of resistance to oseltamivir among influenza A(H1N1) variants rose markedly within a year of emergence³. This landscape highlights the critical need for novel antiviral agents with alternative mechanisms of action.

Natural products have gained attention as promising sources of structurally diverse antiviral candidates⁴. The present study aimed at evaluating the antiviral potential of selected natural compounds against influenza virus through *in silico* methods, specifically molecular docking, in order to determine their binding affinities to neuraminidase and nominate viable leads for further development.

2. Methodology

This study has employed *in silico* approaches for the characterization of the interactions between natural compounds and influenza viral proteins. A total of 12,971 phytochemicals were initially sourced from 200 medicinal plants prevalent in the Middle East. After excluding redundant molecular structures, 5,749 unique ligand conformations remained. Ligand structures were optimized by using the Ligand Preparation (LigPrep) module within the Glide suite. Calculations were performed at physiological pH (7.0), incorporating partial atomic charges and low-energy conformations *via* the Optimized Potentials for Liquid Simulations version 4 (OPLS4) force field. The extensive atomic content of these compounds typically precludes their routine application

in docking workflows, but their inclusion was justified by the exploratory nature of the study.

The crystal structure of the influenza virus neuraminidase (PDB ID: 3TI6) was retrieved from the Protein Data Bank. Protein structure optimization was executed by using the Protein Preparation Wizard in Glide, involving bond order validation, addition of missing hydrogen atoms, and energy minimization at pH 7 using OPLS4, yielding a root mean square deviation (RMSD) of 0.30 Å. This step ensured compatibility of the receptor model for accurate docking simulations.

Docking studies have estimated the protein–ligand binding affinities by using Glide software. The neuraminidase active site was defined by seven functionally critical residues: Arg118, Arg152, Arg292, Glu119, Glu227, Asp151, and Tyr402. Residue assignments reflected their catalytic and interaction roles.

In an attempt to validate docking outcomes and to assess complex stability, molecular dynamics (MD) simulations were conducted on the top-scoring ligand. A 100-nsec MD run was performed in order to monitor temporal fluctuations of the ligand–protein complex. RMSD values were tracked for both protein backbone and ligand positions, thereby providing insights into conformational integrity under simulated physiological conditions.

3. Results and Discussion

From the 5,749 compounds screened, five emerged as potent neuraminidase inhibitors, exhibiting binding affinities ranging from -15.238 to -11.911 kcal/mol (Figure 1). Repandusinic acid A exhibited a strong binding affinity (-11.911 kcal/mol) and demonstrated the highest structural stability during the undertaken MD simulation, as reflected by its lowest RMSD values among the tested compounds. As an ellagitannin, its polyphenolic scaffold and abundance of hydroxyl moieties contribute to its enhanced bioactivity⁵. Other notable ligands included pentagalloylglucose, stachyurin, osyritin, and stachysoside A; all of which interacted favourably with key neuraminidase residues – specifical-

ly, Arg118, Asp151, and Glu227⁶. These interactions are essential for the enzymatic function of neuraminidase and imply a potential to inhibit viral replication⁷.

MD simulations corroborated the docking predictions, thereby confirming the stability of repandusinic acid A over 100 nsec, with minimal deviation from its native conformation. Stability under such conditions is imperative for therapeutic viability, as unstable compounds may degrade or lose efficacy in biological systems. Natural products such as repandusinic acid A offer intrinsic advantages over synthetic molecules, including diverse chemical frameworks and reduced likelihood of resistance development. Their mechanisms may diverge from conventional antiviral pathways, making them particularly suited for combating drug-resistant influenza strains⁴.

Notably, strains such as influenza A(H1N1) have exhibited rapid resistance to existing antivirals, reinforcing the need for alternative therapies. Literature further supports the antiviral promise of natural compounds with novel modes of action⁷; for instance, alkaloids have been shown to inhibit viral enzymes implicated in replication⁸. Recent reports affirm the broad-spectrum antiviral activity of phytochemicals – such as alkaloids, flavonoids, phenolic acids, lignans, coumarins, terpenes, and proteinaceous agents – against respiratory viruses including influenza A and SARS-CoV-2. In fact, their demonstrated efficacy against SARS-CoV-2 under-

scores the possibility of dual utility for future outbreaks⁹.

4. Conclusion

This study has identified five natural compounds with strong affinity for influenza virus neuraminidase through *in silico* screening, notably highlighting the stability and therapeutic promise of repandusinic acid A through molecular dynamics simulation. Further empirical studies are warranted in order to establish clinical efficacy, safety profiles, and pharmacokinetic properties.

Acknowledgements

The authors thank the College of Pharmacy for its support and for providing the facilities required to conduct this research.

Conflicts of interest

None exist.

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HOW TO CITE:

Hashim H.O., Al-Hindy H.A.A.M., Zenad A.H., Jafar A.F., Sahib B.A., Jasim M.M. *In silico* screening of natural compounds for antiviral activity against the influenza virus. *Pharmakeftiki* 37(2s), 269-273, 2025. <https://doi.org/10.60988/p.v37i2S.207>

Repurposing FDA-approved drugs against the Crimean-Congo haemorrhagic fever virus: an *in silico* study

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KEY WORDS:

in silico; Crimean-Congo haemorrhagic fever; paromomycin; FDA-approved drugs; antiviral

ARTICLE INFO:

Received: January 16, 2025

Revised: February 20, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

The Crimean-Congo haemorrhagic fever (CCHF) is a severe viral illness with a case fatality rate of up to 30%, typically transmitted to humans through bites from infected soft ticks. In the absence of US Food and Drug Administration (FDA)-approved antiviral therapies, novel therapeutic strategies are urgently required. This study has employed an *in silico* drug repurposing approach targeting the ovarian tumor domain (OTU) protease of the Crimean-Congo haemorrhagic fever virus (CCHFV), by utilizing FDA-approved pharmacological agents. A total of 1,379 compounds were virtually screened *via* molecular docking and molecular dynamics (MD) simulations in order to assess their binding affinities and structural stabilities. Paromomycin exhibited the strongest binding profile, with an extra precision Glide score (XP GScore) of -11.156 kcal/mol and a root mean square deviation (RMSD) consistently below 5 Å over the 50-nsec MD trajectory. Mapping against the enzymatic region of the CCHFV OTU protease has revealed multiple interactions with key catalytic residues, thereby suggesting that paromomycin may act as a reversible inhibitor of this viral protease. These findings support the hypothesis that computational techniques can identify promising candidates for CCHF treatment and underscore the need for further experimental validation of these predictions.

1. Introduction

The Crimean-Congo haemorrhagic

fever (CCHF) is a viral illness caused by the Crimean-Congo haemorrhagic fever virus (CCHFV). First docu-

mented in the Crimean region in 1944, CCHF remains a pressing global health concern due to its high case fatality rate, ranging from 10% to 30%. The disease affects an estimated 10,000–15,000 individuals annually, resulting in approximately 500 deaths worldwide¹. Currently, no antiviral therapy for CCHF is approved by the US Food and Drug Administration (FDA). Although the World Health Organization (WHO) has proposed ribavirin as a potential treatment, its efficacy remains unverified and may depend on prompt administration². The virus's high transmissibility and lethality – compounded by limited management strategies and the absence of preventive tools – underscore the need for innovative therapeutic interventions³.

Advancements in computational biology have introduced *in silico* approaches that accelerate the identification of antiviral drug candidates⁴. This study harnesses molecular docking and molecular dynamics (MD) simulations in order to repurpose FDA-approved drugs targeting the CCHFV ovarian tumor domain-like protease (OTU protease); an enzyme implicated in the virus's evasion of host immune responses.

2. Methodology

The three-dimensional structure of the CCHFV OTU protease was retrieved from the Protein Data Bank (PDB; ID: 5V5H). Protein preparation was performed using by the Glide suite. Structural refinement included bond correction and hydrogen atom addition *via* the Protein Preparation Wizard. Energy minimization was executed at physiological pH (7.0) using the Optimized Potentials for Liquid Simulations force field, version 4 (OPLS4), with a root mean square deviation (RMSD) cut-off of 0.30 Å.

A random set of 1,379 FDA-approved compounds was selected for virtual screening. Ligand structures were obtained from the ZINC20 database (<https://zinc20.docking.org/substances/subsets/fda/>). Each ligand was optimized using OPLS4 with atom-specific partial charges and conformer generation at pH 7.0. Molecules containing more than 1,000 atoms were excluded.

Molecular docking was conducted *via* Glide, focusing on interactions at the OTU protease active site. The cat-

alytic triad – cysteine (Cys40), aspartate (Asp37), and histidine (His151) – was prioritized based on prior enzymatic studies. Ligand affinity rankings were determined by extra precision Glide score (XP GScore).

Subsequent MD simulations of the top five ligands were executed by using the Desmond module in Schrödinger for a duration of 50 nsec. Trajectory analysis included RMSD evaluation to assess ligand–protein complex stability. Further docking validation was performed with the Discovery Studio version 20.1 software, where the ligand–residue interactions were visualized and quantified.

3. Results and Discussion

The undertaken virtual screening of 1,379 FDA-approved drugs has identified five lead candidates exhibiting XP GScore values between -11.156 and -7.21 kcal/mol (Figure 1). Of these, paromomycin demonstrated the strongest binding (Figure 1A), supported by superior MD stability.

Paromomycin, an aminoglycoside antibiotic, achieved the most negative binding affinity (XP GScore: -11.156 kcal/mol), confirming its status as the top-ranked ligand for the OTU protease. MD analysis revealed consistent RMSD values below 5 Å, thereby indicating robust binding over the simulation period. These data suggest that paromomycin can effectively associate with the protease active site, potentially inhibiting CCHFV's capacity to evade immune detection. Interaction mapping has highlighted several key residue engagements that likely contribute to paromomycin's specificity and stability (Figure 1A).

Previous literature supports the involvement of Cys40, His151, and Asp37 as catalytic residues within the OTU protease⁵. In addition, paromomycin has shown a binding affinity for an adjacent pocket spanning Y89–W99⁶, suggesting a dual inhibitory mechanism and targeting both catalytic and modulatory sites. Beyond CCHF, paromomycin has demonstrated efficacy against parasitic infections and antiviral activity against SARS-CoV-2⁷, thereby reinforcing its repurposing potential. This cross-utility advocates for the adoption of broader *in silico* screening frameworks in order to address emergent viral threats.

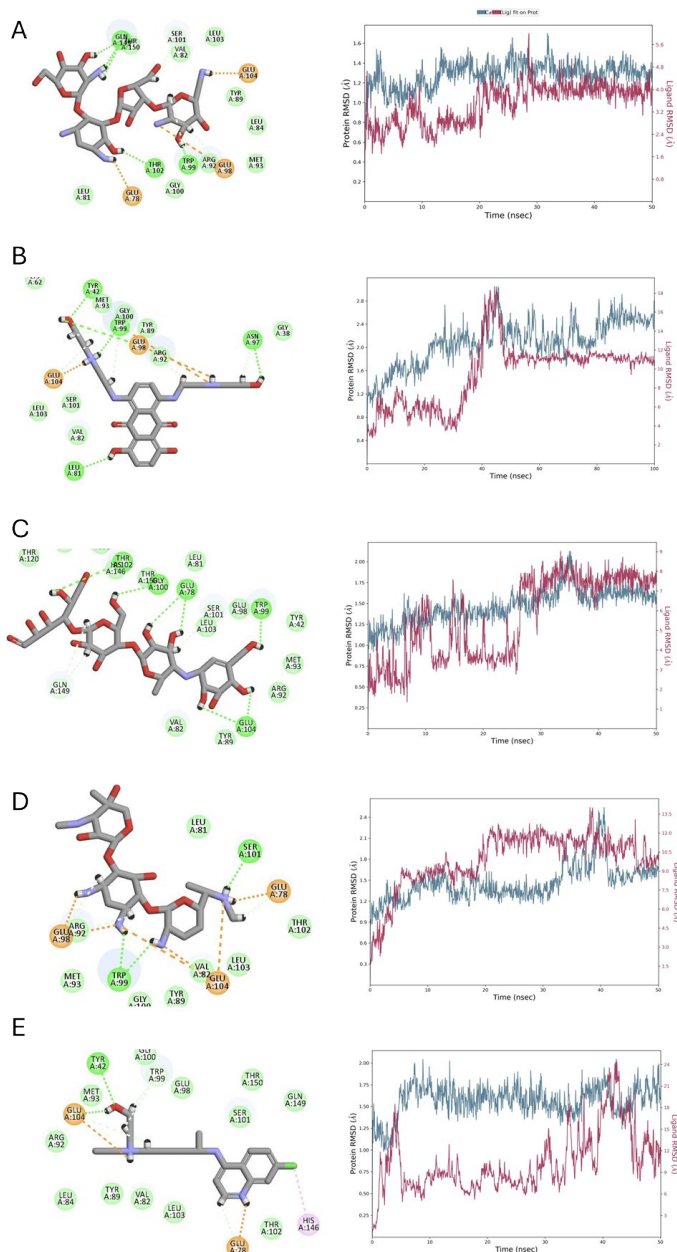


Figure 1. Molecular docking and dynamics analysis of US Food and Drug Administration (FDA)-approved drugs targeting the Crimean-Congo haemorrhagic fever virus (CCHFV) ovarian tumor domain (OTU) protease. Panels (A–E) present the molecular docking and molecular dynamics (MD) simulation results of five drugs approved by the FDA targeting the CCHFV OTU protease: paromomycin (A), mitoxantrone (B), acarbose (C), gentamicin (D), and hydroxychloroquine (E). The left sub-panels depict two-dimensional (2D) ligand–residue interaction maps, focusing on catalytically and structurally relevant residues: cysteine (Cys40), histidine (His151), aspartate (Asp37), and the pocket region Y89–W99. The right sub-panels show root mean square deviation (RMSD) plots across 50-nsec MD trajectories, highlighting paromomycin’s superior conformational stability (RMSD < 5 Å). The obtained XP GScore values were as follows: A, -11.156 kcal/mol; B, -8.931 kcal/mol; C, -8.941 kcal/mol; D, -7.577 kcal/mol; E, -7.210 kcal/mol.

Our findings validate computational drug repurposing as a powerful approach for identifying viable inhibitors of CCHFV OTU protease, warranting further *in vitro* and *in vivo* evaluation^{4,8}. Finally, this workflow may be applicable to other high-mortality pathogens lacking approved treatments.

4. Conclusion

Our study reinforces the utility of *in silico* screening of FDA-approved compounds as a viable strategy for antiviral drug discovery. The obtained binding affinities and residue-level interactions underscore the method's potential to accelerate therapeutic development for emerging viral diseases.

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Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Hashim H.O., Omran A.M., Al-Hindy H.A.A.M., Abdulkareem Y.S.H., Abd Z.S.J., Rebat E.A., Hadi R.K. Repurposing FDA-approved drugs against the Crimean-Congo haemorrhagic fever virus: an *in silico* study. *Pharmakeftiki* 37(2s), 274-277, 2025. <https://doi.org/10.60988/p.v37i2S.208>

Molecular design and theoretical calculations of new 1,3-dioxoisindoline derivatives as potential antiepileptic drugs

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KEY WORDS:

**dioxoisindoline derivatives;
molecular docking; DFT
study; epilepsy; antiepileptic
drugs**

ARTICLE INFO:

Received: December 03, 2024

Revised: January 22, 2025

Accepted: February 07, 2025

Available online: October 10, 2025

ABSTRACT

This study aimed at identifying alternative therapeutic agents for epilepsy syndromes, with a focus on compounds that may attenuate seizure activity. A theoretical chemical approach was employed in order to assess the potential of dioxoisindoline derivatives, by utilizing molecular docking techniques so as to explore ligand–protein interactions relevant to epileptogenesis. Several derivatives have demonstrated notable binding affinities toward proteins implicated in the pathophysiology of the disorder. Density functional theory (DFT) calculations were conducted in order to evaluate the electronic properties of the candidate molecules, particularly those derivatives subject to structural constraints or regulatory bans. Additionally, the suitability of these compounds as drug candidates was assessed by using Lipinski's Rule of Five and predictive similarity metrics. The resulting data indicate that the molecular weight, the partition coefficient (log P), the hydrogen bond donors, and the hydrogen bond acceptors of the evaluated molecules fall within acceptable pharmacokinetic thresholds. Taken together, these findings underscore the need for novel antiepileptic therapies and offer a preliminary appraisal of dioxoisindoline derivatives as promising candidates for further investigation.

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1. Introduction

For more than fifty years, epilepsy syndromes have been recognized as distinct electroclinical phenotypes

with implications for prognosis and therapeutic strategy. However, there is no formally established classification system for epilepsy syndromes endorsed by the Inter-

national League Against Epilepsy (ILAE)¹. Approximately one-third of individuals with epilepsy are considered drug-resistant, although prevalence varies based on the syndrome type, underlying aetiology, age at seizure onset, and presence of comorbid neurological impairments². Nonetheless, treatment outcomes and quality of life have improved, driven by an expanded understanding of the syndrome-specific drug efficacy and an increased caution regarding medications with severe cognitive side effects or polytherapy burdens³. Epileptic seizures result from a dysregulation in the balance between excitatory and inhibitory neurotransmitters⁴. Abnormal neuronal hyperexcitability may arise from dysfunctional macromolecules involved in neural signalling, contributing to epileptogenesis.

In the present study, eight dioxoisindoline derivatives were synthesized and evaluated as candidate antiepileptic compounds. The objective was to investigate their binding affinity (ΔG) toward selected brain proteins implicated in epilepsy⁵. This study is part of an ongoing effort to develop dioxoisindoline-based molecules with potential anticonvulsant properties.

2. Methodology

In an attempt to predict molecular interactions between candidate compounds and target proteins, blind docking simulations were performed by using the Cavity Detection Guide Blind Docking platform (CB-DOCK). The following proteins were selected for docking studies: 1OHV (4-aminobutyrate aminotransferase; *Sus scrofa*), 3F8E (coumarins acting as suicide inhibitors of carbonic anhydrase), and 6KZP (calcium channel-ligand complex). Each of the eight dioxoisindoline compounds was docked into the predicted active sites of these proteins.

Chemical structures were generated in ChemOffice (ChemDraw version 23.1.1) and optimized using MM2 energy minimization, considering steric and thermal parameters. The final conformations of the molecules were identified⁶. Quantum mechanical optimization of these energy-minimized ligands was subsequently performed using the B3LY-

P/6-31G++(d,p) level of theory for vibrational analysis and geometry refinement.

The residue-level interactions for each protein were as follows: (i) 1OHV (GLY₁₃₆, GLY₁₉₁, ASN₁₄₀, VAL₃₀₀, PHE₁₈₉, HIS₁₉₀, ARG₁₉₂), (ii) 3F8E (HIE₆₄, TRP₅, PRO₂₀₁, PRO₂₀₂, THR₂₀₀, GLN₉₂, ILE₉₁), and (iii) 6KZP (THR₉₂₁, GLN₉₂₂, ASN₉₅₂, GLY₉₅₁, LYS₁₄₆₂, ALA₁₅₀₂). The predominant interaction modes observed were π - π stacking, hydrogen bonding, and chelation bonding. All density functional theory (DFT)-optimized ligand structures were used as input for CB-DOCK. Protein crystal structures were obtained from the Protein Data Bank (PDB).

3. Results and Discussion

Molecular docking theory describes a ligand-protein fit by using the Gibbs free energy of binding (ΔG), where greater negative values indicate a stronger binding affinity⁷. In this study, docking simulations and quantum mechanical modelling were used in order to assess compound interactions with proteins of known anticonvulsant relevance⁸. The inhibitory potential of compounds A4, A1, and A3 appeared promising⁹.

Compound A4 exhibited the strongest affinities: $\Delta G = -8.6$ with 1OHV and $\Delta G = -7.2$ with 3F8E. Compounds A1 and A3 showed strong binding to 6KZP ($\Delta G = -8.2$). Phenytoin (5,5-diphenylimidazolidine-2,4-dione) exhibited a $\Delta G = -7.9$ for 1OHV, a $\Delta G = -7.4$ for 3F8E, and a $\Delta G = -7.8$ for 6KZP. Phenytoin consistently displayed higher binding affinities than other compounds, thereby suggesting a broad potential as a reference or benchmark compound. In summary, compound A4 demonstrated the most robust potential anticonvulsant activity *via* interactions with 1OHV and 3F8E. Compounds A1 and A3 showed promising interactions with 6KZP. Phenytoin was validated as the optimal ligand across all protein targets. These data highlight the inhibitory potential of the studied compounds and support their further evaluation as anticonvulsant drug candidates¹⁰.

In the DFT analysis undertaken, several key electronic properties were computed, including: (i) the highest occupied molecular orbital (HOMO; -0.211

Table 1. Selected chemical properties of the synthesized compounds. Abbreviations: EA, electron affinity; HOMO, highest occupied molecular orbital; IP, ionization potential; LUMO, lowest unoccupied molecular orbital; S, softness; η , chemical hardness; μ , electronegativity; ω , electrophilicity index.								
Properties	A1	A2	A3	A4	A5	A6	A7	A8
LUMO	-0.114	-0.113	-0.112	-0.112	-0.113	-0.107	-0.111	-0.104
HOMO	-0.211	-0.223	-0.218	-0.217	-0.211	-0.181	-0.219	-0.187
HOMO-LUMO gap	0.097	0.11	0.106	0.105	0.098	0.074	0.108	0.083
IP	0.211	0.223	0.218	0.217	0.211	0.181	0.219	0.187
EA	0.114	0.113	0.112	0.112	0.113	0.107	0.111	0.104
μ	0.163	0.168	0.165	0.165	0.162	0.144	0.165	0.146
η	0.049	0.055	0.053	0.052	0.049	0.037	0.054	0.042
S	20.62	18.18	18.87	19.05	20.41	27.03	18.52	24.09
ω	0.271	0.257	0.257	0.262	0.268	0.280	0.252	0.254

to -0.187 eV), (ii) the lowest unoccupied molecular orbital (LUMO; -0.114 to -0.104 eV), and (iii) the HOMO-LUMO gap (0.097 to 0.083 eV). These ranges reflect high kinetic activity and moderate molecular instability. Additional quantum descriptors were calculated: (i) the ionization potential (I; 0.211 to 0.187), (ii) the electron affinity (EA; 0.114 to 0.104), (iii) the chemical hardness (η ; 0.049 to 0.042), (iv) the electronegativity (μ ; 0.163 to 0.146), and (v) the softness (S; 20.62 to 24.09). These values support the energetic plausibility and reactivity profiles of the candidate compounds (Table 1).

4. Conclusion

Preclinical evidence supports the potential anticonvulsant activity of several studied compounds: compound A4 yielded the most promising ΔG values (-8.6 and -7.2), while compounds A1 and A3 also

demonstrated favourable interactions ($\Delta G = -8.2$). Phenytoin was identified as the most effective ligand across all evaluated protein targets. These findings suggest that the tested compounds and ligands hold promise as future anticonvulsant agents.

Acknowledgements

The authors gratefully acknowledge the University of Anbar for providing the facilities necessary to conduct this research.

Conflicts of interest

None exist.

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HOW TO CITE:

Hashim K.M., Ezzat M.O. Molecular design and theoretical calculations of new 1,3-dioxoisindoline derivatives as potential antiepileptic drugs. *Pharmakeftiki* 37(2s), 278-281, 2025.
<https://doi.org/10.60988/p.v37i2S.209>

Incidence and management of hypotension in spinal anaesthesia in urological pelvic surgery

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KEY WORDS:

hypotension; spinal
anaesthesia; urological
surgery; ephedrine;
intravenous administration

ARTICLE INFO:

Received: December 30, 2024

Revised: January 13, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

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ABSTRACT

The management of hypotension in specific clinical contexts frequently involves the administration of ephedrine; a vasopressor with demonstrated efficacy across multiple scenarios: spinal anaesthesia for uro-pelvic surgery, caesarean section, renal transplantation, and select cases of autonomic dysfunction. Recent evidence suggests that ephedrine use may delay the onset of hypotension. This prospective study has evaluated the incidence and management of hypotension in 80 adult patients (aged 17–45 years) undergoing urological pelvic surgery under spinal anaesthesia. Participants were recruited from the Hilla Teaching Hospital and the Al-Imam Al-Sadeq Teaching Hospital (Iraq) between June 2023 and December 2024, and were randomly assigned to receive either intravenous fluids alone or in combination with ephedrine. Systemic blood pressure was monitored at four time points: preoperatively, following intervention, after anaesthesia induction, and postoperatively. Compared to fluid administration alone, the ephedrine-treated group exhibited significant changes in both systolic and diastolic blood pressure values ($p < 0.05$). These findings suggest that ephedrine enhances haemodynamic stability during spinal anaesthesia and may play a clinically significant role in the intraoperative management of hypotension.

1. Introduction

Hypotension is a common compli-

cation associated with spinal anaesthesia, particularly in urological pelvic (uro-pelvic) procedures¹. If

inadequately managed, hypotension can result in significant morbidity². Its incidence during spinal anaesthesia may range from 30% to 70%, depending on several variables, including patient positioning, age¹, and the administration of fluids or vasopressors^{3,4}. Physiologically, hypotension during spinal anaesthesia is largely attributed to sympathetic blockade, which induces vasodilation and reduces venous return, ultimately leading to decreased cardiac output⁵. Additionally, the Bezold-Jarisch reflex (mediated by serotonin receptors) has been implicated as a contributing mechanism⁵. Given the potential risks associated with intraoperative hypotension, active management is essential. While intravenous fluid (IVF) therapy has traditionally served as the first-line treatment, increasing attention has been directed toward the adjunctive use of vasopressors such as ephedrine for a more effective control of blood pressure⁶.

This prospective study aimed at comparing the efficacy of IVF alone *versus* IVF combined with ephedrine in the management of hypotension during spinal anaesthesia in elective uro-pelvic procedures. The study has investigated the incidence of hypotension and evaluates blood pressure (BP) responses across two therapeutic modalities, with the goal of informing optimized anaesthetic care and improving patient outcomes.

2. Methodology

A total of 80 adult patients (aged 17–45 years) scheduled for elective uro-pelvic surgeries under spinal anaesthesia were prospectively enrolled. Participants were recruited from the Hilla Teaching Hospital and the Al-Imam Al-Sadeq Teaching Hospital (Iraq) between June 2023 and December 2024. Subjects were randomly allocated to two groups: group 1 (N=38) received IVF alone, while group 2 (N=42) received IVF combined with 6 mg of injectable ephedrine. Inclusion criteria comprised adults aged 17 to 45 years who provided informed consent. Exclusion criteria included contraindications to spinal anaesthesia or ephedrine, as well as the presence of chronic or advanced cardiovascular disease.

Spinal anaesthesia was administered using a 25-gauge Whitacre needle introduced into the suba-

rachnoid space at either the L3/L4 or the L4/L5 interspace. Procedures were performed with patients in either the lateral or sitting position, adhering to institutional protocols for local anaesthetic administration. Following anaesthesia induction, patients were monitored for BP, heart rate, and oxygen saturation. Treatment was initiated immediately after the spinal anaesthesia onset. BP readings were recorded at four key intervals: preoperative baseline, post-anaesthesia, post-treatment, and upon surgery completion. Demographic variables (age, gender, weight, and height) were collected. Hypotension was defined as a $\geq 20\%$ reduction in systolic BP from baseline or an absolute systolic BP being < 90 mmHg. Incidence rates and supplementary interventions were documented.

Statistical analysis included descriptive statistics for demographic data. The Shapiro–Wilk test was used in order to assess the normality of the obtained BP data. Between-group comparisons were performed by using independent samples *t*-tests for normally distributed data and Mann–Whitney U tests otherwise. Categorical variables were analysed *via* the chi-square test. Statistical significance was defined as $p < 0.05$.

Ethical approval was obtained from the Hammurabi College of Medicine's Institutional Ethics Committee (reference: HCM-CR-1-20-2024). Written informed consent was secured from all participants in accordance with established ethical guidelines.

3. Results and Discussion

Table 1 summarizes the descriptive statistics for systolic BP (SBP), diastolic BP (DBP), and age distribution across both treatment groups. No statistically significant differences were observed between the groups at baseline (preoperative SBP and DBP), following anaesthesia, or immediately post-treatment. However, significant differences ($p < 0.05$) were noted in SBP and DBP after ephedrine administration and at the end of surgery, thereby indicating that ephedrine may confer a haemodynamic advantage during critical procedural phases.

Haemodynamic stability is vital during spinal anaesthesia for uro-pelvic procedures. This study demonstrates that ephedrine, when used adjunct-

Table 1				
Variable	Category	Fluids (N=38)	Fluids + Ephedrine (N=42)	p-value
Age distribution	<20 years	2	1	0.07
	21–30 years	9	3	
	31–40 years	10	9	
	41–45 years	17	29	
Preoperative blood pressure	systolic blood pressure	133.3 ± 16.4	134.5 ± 13.6	0.79
	diastolic blood pressure	88.9 ± 11.7	86.6 ± 8.2	0.46
Blood pressure after spinal anaesthesia	systolic blood pressure	83.7 ± 9.8	85.0 ± 10.9	0.71
	diastolic blood pressure	44.5 ± 9.4	45.3 ± 7.4	0.77
Blood pressure after treatment	systolic blood pressure	128.7 ± 20.0	122.5 ± 18.9	0.01
	diastolic blood pressure	84.0 ± 14.1	82.0 ± 9.9	0.02
Blood pressure at the end of the surgery	systolic blood pressure	119.6 ± 19.0	111.3 ± 17.9	0.04
	diastolic blood pressure	77.5 ± 14.6	75.2 ± 11.2	0.04

tively with IVF, significantly improves BP parameters compared to IVF alone. These findings corroborate those of the literature indicating that ephedrine increases heart rate and peripheral vascular resistance, thereby optimizing cardiovascular performance during acute hypotensive episodes^{6,7}.

Despite its benefits, ephedrine may cause adverse effects such as hypertension, tachycardia, and cardiac arrhythmias, which can complicate intraoperative monitoring. In select patients (particularly those with pre-existing cardiovascular conditions), alternative agents such as noradrenaline may be preferred for sustained hypotensive management⁸.

A systematic review of ephedrine’s safety profile concluded that most adverse events are transient and mild, although vigilant perioperative monitoring remains essential⁹. Clinicians must carefully weigh the haemodynamic benefits of ephedrine against its potential risks¹⁰. The incidence of hypotension also appears to vary with surgical complexity. Procedures involving extensive pelvic manipulation or longer durations, such as radical prostatectomy, are associated with higher rates of hypotension than more minor interventions like cystoscopy^{5,10}. This necessitates individualized anaesthetic strategies tailored to both patient-specific and procedural variables.

The age distribution between groups was com-

parable ($p=0.07$), thereby supporting the internal validity of treatment comparisons. Although acute hypotension may result in postoperative complications – including organ hypoperfusion and nausea – there remains a paucity of data on its long-term consequences⁷. Some studies suggest a correlation between recurrent intraoperative hypotensive episodes and higher postoperative morbidity, but definitive conclusions require further longitudinal research⁵.

4. Conclusion

This study affirms the clinical relevance of targeted therapeutic strategies for managing hypotension during spinal anaesthesia in uro-pelvic surgery. The combination of IVF with ephedrine demonstrates promise as an effective modality for the enhancement of haemodynamic stability. Larger-scale studies are warranted to validate these findings and to establish optimal dosing regimens for ephedrine and alternative vasopressors across various surgical contexts.

Acknowledgements

The authors extend their gratitude to all study participants and acknowledge the contributions of volunteers involved in this research.

Conflicts of interest

None exist.

ORCIDs

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HOW TO CITE:

Ibraheem A., Alzubaidi M.A., Muhsen M.I., Almusawi A.A.H., Al-Hindy H.A.A.M. Incidence and management of hypotension in spinal anaesthesia in urological pelvic surgery. *Pharmakeftiki* 37(2s), 282-285, 2025. <https://doi.org/10.60988/p.v37i2S.210>

Molecular detection of *Lactobacillus acidophilus* in the dental plaque of Iraqi patients

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KEY WORDS:

***Lactobacillus acidophilus*;
polymerase chain reaction;
dental plaque; periodontal
health; culturing method**

ARTICLE INFO:

Received: January 27, 2025

Revised: February 27, 2025

Accepted: February 27, 2025

Available online: October 10, 2025

ABSTRACT

Microbial colonization of both hard and soft oral surfaces constitutes the primary component of dental plaque. The probiotic strain *Lactobacillus acidophilus* has been implicated in the formation of dental plaque, with its heightened acidogenicity considered a potential adverse effect. This study aimed to investigate the presence of *L. acidophilus* within the oral biofilm of periodontally healthy individuals. A total of 90 subjects aged 20–40 years were enrolled. Specimens were collected from patients attending a private dental clinic in Hillah, Iraq, seeking comprehensive dental and periodontal evaluation between October 2022 and February 2023. Both male and female participants were included. The detection of *L. acidophilus* was performed using polymerase chain reaction (PCR) and conventional culturing methods. Among the 42 samples analysed via culture, only 2 (4.8%) tested positive. In contrast, PCR identified *L. acidophilus* in 4 out of 48 samples (8.3%). Although PCR is widely regarded as the most sensitive and specific method for bacterial identification, its accuracy may be compromised by technical variables such as excessive sample dilution.

1. Introduction

Lactobacillus acidophilus is a non-motile, Gram-positive bacterium that exhibits either rod-shaped or coccoid morphology. As a terminal product of its metabolic and fermentative activity, *L. acidophilus*

produces lactic acid. This species can survive in environments with a pH as low as 4–5, or even lower¹. *L. acidophilus* was the first bacterial species to be classified as a probiotic. Among oral probiotics, *Lactobacillus* spp. are potent inhibitors of key periodontal pathogens, in-

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cluding *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Prevotella intermedia*. Due to their highly aciduric and acidogenic nature, *Lactobacillus* spp. are predominantly found in deep carious lesions².

Compared to the diverse array of pathogenic and commensal bacteria that colonize oral surfaces in biofilm form, *Lactobacillus* spp. play a secondary role in lesion progression and biofilm composition, rather than in lesion initiation. Notably, the population of *Streptococcus mutans* declines as pH levels drop, while *Lactobacillus* spp. increase in number under acidic conditions^{3,4}. Like *S. mutans*, *L. acidophilus* is considered part of the normal oral flora. However, its abundance increases with the advancement of carious lesions⁵. In highly acidic environments (such as deep cavities) *L. acidophilus* reaches peak concentrations and is thus regarded as a secondary invader⁶.

In the context of periodontal health, *L. acidophilus* exhibits inhibitory effects against *P. gingivalis* by downregulating proinflammatory interleukins, including interleukin (IL)-6, IL-1, and IL-8, which are induced by this pathogen⁷. Additionally, *L. acidophilus* can co-aggregate with *Fusobacterium nucleatum*, thereby preventing its adhesion and invasion of host tissues⁸. *L. acidophilus* also demonstrates bactericidal activity against *A. actinomycetemcomitans* through the production of specific enzymes, such as lipase⁹. Given these properties, *L. acidophilus* is considered a promising periodontal probiotic with potential future applications in the prevention of periodontal diseases¹⁰.

The present study aimed at detecting *L. acidophilus* using both conventional culturing and polymerase chain reaction (PCR) techniques. It also sought to evaluate the effects of chlorhexidine (CHX) and green tea oil on *L. acidophilus* viability.

2. Methodology

Ninety dental plaque samples were collected from patients attending a private dental clinic in Hillah, Iraq. Swabs were taken from plaque deposits of individuals aged 20–40 years. Subjects undergoing

pharmacological treatment were excluded. Sampling occurred between October 2022 and February 2023. Ethical approval was obtained from the College of Dentistry of the University of Babylon (approval number: 70; date: 26/2/2025).

Growth and colonization of *Lactobacillus* spp. were supported using the de Man, Rogosa and Sharpe (MRS) agar. After 24 h of anaerobic incubation at 37°C, the characteristic colony morphology was assessed in order to identify the target bacteria.

For molecular detection, *L. acidophilus* was identified via PCR. Approximately 780 base pairs (bp) of *Lactobacillus* DNA were amplified by using primers targeting the 16S ribosomal RNA (rRNA) gene. DNA was extracted from biofilm samples that were collected via sterile swabs, which were immediately placed in anaerobic transport vials or sterile tubes. PCR products were analysed by gel electrophoresis.

3. Results and Discussion

Of the 90 dental plaque samples analysed, only 2 (4.8%) out of 42 were found positive for *L. acidophilus* using the culturing method. In contrast, PCR detected *L. acidophilus* in 4 (8.3%) out of 48 samples, as illustrated in Figure 1. *L. acidophilus* was identified by PCR through amplification of the 16S rRNA gene using specific primers. The presence of two distinct bands confirmed the detection of *L. acidophilus*.

Several factors may account for the low detection rate, including low bacterial concentration in highly diluted samples and potential mutations in the amplified DNA fragment due to polymerase error. Primer non-specificity may also lead to off-target amplification, compromising PCR specificity. Furthermore, intra-species variability in colonization capacity has been documented for *Lactobacillus* spp. Despite these limitations, 16S rRNA gene sequencing remains a powerful tool for microbial identification, including novel species. PCR continues to be a relevant and sensitive technique for routine microbial diagnostics.

The present study has also demonstrated that CHX (0.2%) exhibited the highest inhibitory activity against *L. acidophilus*. This effect is attributed to

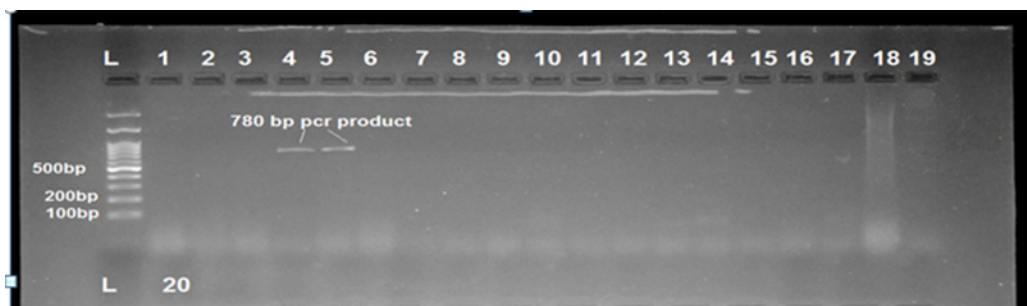


Figure 1. Electrophoresis of 1.5% agarose gel for 60 min at 72 V, showing the polymerase chain reaction (PCR) amplification of *Lactobacillus acidophilus* (780 bp). Lanes 4–5 represent positive isolates; lane M denotes the 100-bp DNA marker.

CHX's potent antimicrobial properties, which disrupt microbial cell membranes and increase intracellular leakage. Conversely, green tea oil showed comparatively lower inhibition of *L. acidophilus*. Although antimicrobial oils can affect microbial activity, their efficacy varies depending on the chemical composition and ability to penetrate bacterial structures.

4. Conclusion

PCR is regarded as the most accurate method for bacterial detection due to its high sensitivity and specificity. However, its performance may be affected by factors such as excessive sample dilution and cross-reactivity.

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Acknowledgements

The authors gratefully acknowledge the College of Dentistry of the University of Babylon, for its support in conducting this study.

Conflicts of interest

None exist.

ORCIDs

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HOW TO CITE:

Jasim A.I., Alwarid R.J., Jassim S.D. Molecular detection of *Lactobacillus acidophilus* in the dental plaque of Iraqi patients. *Pharmakeftiki* 37(2s), 286-289, 2025. <https://doi.org/10.60988/p.v37i2S.211>

The effect of the aqueous and alcoholic extracts of *Tamarindus indica* in suppressing hyperglycaemia in alloxan-induced diabetic rats

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KEY WORDS:

T. indica; alloxan monohydrate; diabetes mellitus; insulin; blood glucose levels

ARTICLE INFO:

Received: January 11, 2025

Revised: February 23, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

ABSTRACT

This study has investigated the antihyperglycaemic effects of aqueous and alcoholic extracts of *Tamarindus indica* (*T. indica*) in adult male Sprague Dawley rats with alloxan-induced diabetes. Forty-nine rats (mean body weight: 230 ± 20 g) were randomly assigned to seven groups (n=7 per group): group 1 comprised healthy control rats, group 2 included diabetic control rats, groups 3 and 4 included diabetic rats that received 500 mg/kg of aqueous or alcoholic *T. indica* extract (orally, via gavage), groups 5 and 6 included non-diabetic (normal) rats that received the same doses of aqueous or alcoholic *T. indica* extract as the previous two groups; and group 7 included diabetic rats that were treated subcutaneously with insulin (6 IU/kg body weight). Diabetic rats (group 2) exhibited a significant elevation in blood glucose levels ($p < 0.05$), whereas all treatments with *T. indica* extracts (groups 3–6) resulted in a statistically significant reduction ($p < 0.05$) of the rat blood glucose levels, with the alcoholic extract demonstrating the most pronounced effect. These findings provide evidence of the antihyperglycaemic activity of *T. indica*, the extracts of which possess potent antioxidant properties and may offer therapeutic benefit in the management of diabetes.

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1. Introduction

Plants offer considerable potential as alternative therapies for blood glucose regulation due to their accessibility, cost-effectiveness, ef-

ficacy, and safety. They produce secondary metabolites (such as terpenoids, flavonoids, alkaloids, tannins, and ferulic acid) that exhibit glucose-lowering properties¹. Moreover, herbal remedies are gen-

erally more affordable, safer, and associated with fewer adverse effects than synthetic pharmaceuticals. It has been proposed that using the whole plant enhances synergistic effects, as the constituent compounds interact to yield more beneficial outcomes. Herbalists argue that this synergy among plant components may also mitigate toxicity².

Each plant species possesses a distinct profile of bioactive compounds, offering unique nutritional and therapeutic benefits. A notable example is *Tamarindus indica* (commonly known as tamarind)³; an evergreen ornamental tree native to tropical Central Africa. It can reach heights of 20–30 m and is characterized by a broad, spreading crown. Traditionally, tamarind has been employed – either alone or in combination with other herbs – to treat a wide array of ailments, including cardiovascular disease, pain, diabetes, constipation, fever, jaundice, parasitic infections, and respiratory disorders. It is also renowned for its laxative and aphrodisiac properties.

Numerous studies have demonstrated that various parts of the tamarind plant possess anti-diabetic and hypoglycaemic effects. Additionally, ethanolic extracts of *T. indica* fruits have exhibited hepatoprotective activity when administered orally to rats with alloxan-induced diabetes⁴.

2. Methodology

Both aqueous and alcoholic extracts of *T. indica* were obtained from the Al-Amin Centre for Scientific Research in Najaf. Stock solutions were prepared by dissolving 500 mg of each extract powder in 5 mL of distilled water. Alloxan monohydrate was sourced from the Sigma-Aldrich Chemical Company (St. Louis, MO, USA), while glucose assay kits were procured from Bio Diagnostic Co. (USA).

Forty-nine adult male Sprague Dawley rats (average weight: 230 ± 20 g) were acquired from the Animal House of the Department of Life Sciences of the College of Science of the University of Kufa. The animals were housed in a well-ventilated laboratory under a 12-h light/dark cycle, at 24°C and 50%–52% relative humidity. Ethical approval for the study was granted by the Central Committee at the

University of Kufa (approval number: 23793; date: 7 August 2024). The rats were randomly assigned to seven groups (n=7 per group; see Table 1): (i) group 1 (healthy control rats, orally administered normal saline for 30 days), (ii) group 2 (diabetic control rats, fed a basal diet without treatment for 30 days), (iii) group 3 (diabetic rats, orally administered aqueous *T. indica* extract at 500 mg/kg b.w. for 30 days), (iv) group 4 (diabetic rats, orally administered alcoholic *T. indica* extract at 500 mg/kg b.w. for 30 days), (v) group 5 (non-diabetic rats, orally administered aqueous *T. indica* extract at 500 mg/kg b.w. for 30 days), (vi) group 6 (non-diabetic rats, orally administered alcoholic *T. indica* extract at 500 mg/kg b.w. for 30 days), and (vii) group 7 (diabetic rats, subcutaneously injected with insulin at 6 IU/kg b.w. for 30 days). Diabetes was induced in the rats of groups 2, 3, 4, and 7 via a single intraperitoneal injection of freshly prepared alloxan (150 mg/kg b.w.) diluted in 10 mL of normal saline⁵. In order to prevent hypoglycaemic shock and mortality, rats were provided with a 5% glucose solution overnight post-injection.

At 72 h after the alloxan administration, blood samples were collected from the orbital plexus vein using capillary tubes. Rats with fasting blood glucose levels exceeding 200 mg/dL were considered diabetic; non-diabetic rats were excluded from treatment analysis. After a 30-day treatment period and a 12-h fast, rats were euthanized. Venous blood was collected in sterile centrifuge tubes for serum separation. Glucose levels were quantified using a glucose oxidase kit. This assay involves the oxidation of glucose to gluconic acid, with the resultant hydrogen peroxide reacting with phenol and 4-aminoantipyrine in the presence of peroxidase to form a violet-red quinonimine dye. The intensity of the dye correlates with glucose concentration, calculated using the formula:

$$\text{glucose concentration (mg/dL)} = 100 \times (\Delta A_{\text{sample}} / \Delta A_{\text{standard}})$$

Statistical analysis was performed using the SPSS software. Descriptive statistics (mean $\pm$ standard error) were computed, and intergroup comparisons were conducted using one-way analysis of variance (ANOVA) followed by the least significant difference

Table 1. Effects of alloxan, the aqueous and alcoholic extracts of <i>Tamarindus indica</i> , and insulin on the blood glucose levels of male rats. Values represent the mean $\pm$ standard error (SE) for each group (n=7 rats per group). Means followed by different superscript letters within the same column differ significantly at $p<0.05$, as determined by one-way analysis of variance (ANOVA) and the least significant difference (LSD) test. The value of the latter was found to be 21.208.	
Groups	Blood glucose levels (mg/dL; mean $\pm$ SE)
Group 1: control	109.8 $\pm$ 4.28 ^{c d e}
Group 2: diabetes (150 mg/kg alloxan)	424.0 $\pm$ 6.11 ^a
Group 3: diabetes + aqueous extract of <i>T. indica</i> (500 mg/kg)	126.0 $\pm$ 8.61 ^c
Group 4: diabetes + alcoholic extract of <i>T. indica</i> (500 mg/kg)	112.6 $\pm$ 4.92 ^{c d}
Group 5: aqueous extract of <i>T. indica</i> (500 mg/kg)	92.8 $\pm$ 6.82 ^{d e}
Group 6: alcoholic extract of <i>T. indica</i> (500 mg/kg)	89.2 $\pm$ 7.37 ^e
Group 7: diabetes + insulin (6 units/kg)	180.60 $\pm$ 9.98 ^b

(LSD) test at a significance level of $p<0.05$.

3. Results and Discussion

As shown in Table 1, statistically significant differences ($p<0.05$) were observed among the groups. Group 2 (alloxan-induced diabetic rats) exhibited a marked elevation in blood glucose levels (424.0 $\pm$ 6.11 mg/dL) compared to group 1 (healthy controls; 109.8 $\pm$ 4.28 mg/dL) and all other experimental groups. This finding corroborates previous studies^{5,6}, which have reported that alloxan at 150 mg/kg induces diabetes by damaging pancreatic β -cells responsible for insulin secretion⁷. Elevated glucose levels may also result from enhanced hepatic gluconeogenesis and impaired insulin synthesis.

Conversely, diabetic rats treated with *T. indica* extracts (groups 3 and 4) exhibited significant reductions in their blood glucose levels (126.0 $\pm$ 8.61 mg/dL and 112.6 $\pm$ 4.92 mg/dL, respectively) compared to untreated diabetic rats (group 2). These results are consistent with those of prior studies^{8,9}, which have employed similar extract concentrations and have demonstrated hypoglycaemic effects. The observed glucose-lowering activity may be attributed to the antioxidant properties of the bioactive compounds in *T. indica*, which potentially reduce oxidative stress, preserve β -cell integrity, and restore insulin levels.

Finally, the group 7 rats that were treated with insulin (6 IU/kg) have also shown a reduction in their blood glucose levels (180.60 $\pm$ 9.98 mg/dL), aligning with previous findings demonstrating that exogenous insulin promotes glycolysis and glycogen synthesis while inhibiting hepatic glucose output¹⁰.

4. Conclusion

Both of the herein assessed extracts of *T. indica* have effectively reduced blood glucose levels in diabetic rats. The alcoholic extract appeared more potent, likely due to its superior antioxidant activity.

Acknowledgements

The authors express their gratitude to Dr Ruqaya Munther Ewadh for her valuable guidance.

Conflicts of interest

None exist.

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HOW TO CITE:

Jassim S.M., Jwad S.M. The effect of the aqueous and alcoholic extracts of *Tamarindus indica* in suppressing hyperglycaemia in alloxan-induced diabetic rats. *Pharmakeftiki* 37(2s), 290-293, 2025.
<https://doi.org/10.60988/p.v37i2S.212>

Fenugreek extract as a natural therapy for dyslipidaemia: an *in vivo* study on rats

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KEY WORDS:

atorvastatin; dyslipidaemia;
fenugreek extract; lipid
profile; natural therapy

ARTICLE INFO:

Received: January 30, 2025

Revised: February 13, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

Dyslipidaemia is a major risk factor for cardiovascular disease, necessitating effective lipid-lowering interventions. This study investigates the lipid-modulating effects of fenugreek (*Trigonella foenum-graecum*) extract in comparison to atorvastatin, on rats, with a focus on dose-dependent responses. Male rats (n=150) were randomly assigned to five groups (n=30 per group): negative control (NC), positive control treated with atorvastatin (5–20 mg/kg), and three experimental groups receiving low (100 mg/kg), medium (250 mg/kg), or high (500 mg/kg) doses of fenugreek extract. Lipid profiles – including total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides (TG) – were assessed post-treatment using standard biochemical assays. Atorvastatin significantly reduced TC (75.33 ± 19.49 mg/dL, $p < 0.05$) and LDL-cholesterol (26.93 ± 10.36 mg/dL, $p < 0.0001$), while elevating HDL-cholesterol (55.20 ± 13.19 mg/dL, $p < 0.0001$). Similarly, high-dose fenugreek extract significantly lowered TC (75.97 ± 32.58 mg/dL, $p < 0.05$) and LDL-cholesterol (22.17 ± 10.11 mg/dL, $p < 0.0001$), with a concomitant increase in HDL-cholesterol (57.90 ± 20.45 mg/dL, $p < 0.0001$). No significant changes in TG levels were observed across the assessed groups. These findings suggest that the fenugreek extract, particularly at higher doses, exerts lipid-lowering effects comparable to atorvastatin, supporting its potential as a natural alternative or adjunctive therapy in the management of dyslipidaemia.

1. Introduction

Fenugreek (*Trigonella foenum-graecum*), a plant native to the Mediterranean, South Asia, and North Africa, is renowned for its diverse health-promoting properties, attributed to its exceptional nutritional and bioactive composition. The seeds and leaves of fenugreek have long been utilized in both traditional medicine and modern therapeutic applications. Rich in proteins, dietary fibre, vitamins (notably B₆ and C), and phytochemicals such as saponins, alkaloids, and flavonoids, fenugreek contributes significantly to overall health maintenance¹.

This study aimed at evaluating the effects of fenugreek extract on the lipid profiles of rats and to compare its dose-dependent lipid-modulating effects with those of atorvastatin (a widely used lipid-lowering agent), in order to assess its potential as a natural alternative for improving lipid metabolism.

2. Methodology

Fresh fenugreek was procured from a local market. Extraction was performed on 100 g of finely crushed seed powder using a Soxhlet apparatus at 70°C for 4 h, in order to isolate bioactive constituents.

A total of 150 healthy male Wistar rats (weight range: 350–500 g) were housed in ventilated cages under controlled environmental conditions: 12-h light/dark cycle, temperature of 22°C ± 2°C, and relative humidity of 50%–60%. All experimental procedures adhered to ethical standards for animal research and were approved by the Ethical Research Committee of the College of Dentistry of Al-Iraqia University (ESA & HER 09-12-08-2024). The rats were randomly assigned to five groups (n=30 per group): (i) healthy control (HC) group (no treatment administered), (ii) positive control (PC) group (treated with atorvastatin at 10 mg/kg body weight), (iii) low-dose fenugreek (LD-F) group (treated with fenugreek extract at 100 mg/kg body weight), (iv) medium-dose fenugreek (MD-F) group (treated with fenugreek extract at 250 mg/kg body weight), and (v) high-dose fenugreek (HD-F) group (treated with fenugreek extract at 500 mg/kg body weight). Treat-

ments were administered orally once daily for one month. Rats were monitored for adverse effects and treatment responses. Blood samples (~3 mL) were collected *via* cardiac puncture under anaesthesia and centrifuged at 1,600 rpm for 20 min so as to obtain serum.

Serum lipid levels – including those of total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol (LDL-cholesterol), high-density lipoprotein (HDL) cholesterol (HDL-cholesterol), and triglycerides (TG) – were analysed using the Biolyzer 300 (Analyticon Biotechnologies AG, Germany), employing automated enzymatic and colorimetric techniques. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by *t*-tests in order to identify significant differences among groups. A *p*-value below 0.05 was considered as statistically significant.

3. Results and Discussion

The lipid-modulating effects of atorvastatin and of the fenugreek extract were evaluated across all experimental groups (Table 1). ANOVA revealed no statistically significant differences in TG levels (*p*=0.1491), but significant differences were observed for TC (*p*=0.0072), LDL-cholesterol (*p*<0.0001), and HDL-cholesterol (*p*<0.0001).

As shown in Table 1, the fenugreek extract exhibited dose-dependent effects on TC. The LD-F and MD-F rat groups did not show significant reductions compared to the HC group, suggesting that these doses may be insufficient to elicit a substantial hypocholesterolaemic effect. These findings are consistent with those of previous studies indicating that bioactive compounds in fenugreek, such as saponins and galactomannan, require adequate concentrations to meaningfully influence lipid metabolism. In contrast, the HD-F group demonstrated a significant reduction in TC, achieving mean values comparable to those observed in the atorvastatin-treated group. This suggests that high-dose fenugreek extract may confer therapeutic benefits similar to conventional lipid-lowering agents. The hypocholesterolaemic effect of fenugreek is likely mediated through multiple

Table 1. Lipid profile parameters (mg/dL) across the herein assessed experimental groups. Abbreviations used: CI, confidence interval; HD-F, high-dose fenugreek group (500 mg/kg); HDL, high-density lipoprotein; LD-F, low-dose fenugreek group (100 mg/kg); LDL, low-density lipoprotein; MD-F, medium-dose fenugreek group (250 mg/kg); NC, negative control group (untreated); PC, positive control group (treated with atorvastatin); SD, standard deviation.					
Group	N	Mean ± SD	Range	Significant difference	95% CI of difference
Total cholesterol					
NC	30	101.4 ± 38.19	52–187	-	-
PC	30	75.33 ± 19.49	36–110	yes	1.616 to 50.52
LD- F	30	97.40 ± 41.11	40–193	no	-20.45 to 28.45
MD-F	30	86.83 ± 35.86	39–210	no	-9.884 to 39.02
HD-F	30	75.97 ± 32.58	42–185	yes	0.9824 to 49.88
Triglycerides					
NC	30	71.70 ± 24.43	38–105	-	-
PC	30	59.47 ± 20.04	24–102	no	-4.440 to 28.91
LD- F	30	68.03 ± 29.12	33–121	no	-13.34 to 20.01
MD-F	30	67.67 ± 20.39	44–108	no	-12.64 to 20.71
HD-F	30	57.33 ± 21.33	32–102	no	-2.307 to 31.04
LDL-cholesterol					
NC	30	39.17± 11.88	14–62	-	-
PC	30	26.93 ± 10.36	10–48	yes	4.027 to 20.44
LD- F	30	34.43 ± 13.43	12–58	no	-3.473 to 12.94
MD-F	30	32.60 ± 11.45	11–59	no	-1.640 to 14.77
HD-F	30	22.17 ± 10.11	10–51	yes	8.793 to 25.21
HDL-cholesterol					
NC	30	35.47 ± 13.60	19–78	-	-
PC	30	55.20 ± 13.19	31–88	yes	-31.50 to -7.963
LD- F	30	44.80 ± 16.35	22–84	no	-21.10 to 2.437
MD-F	30	47.03 ± 17.71	23–91	no	-23.34 to 0.2041
HD-F	30	57.90 ± 20.45	25–89	yes	-34.20 to -10.66

mechanisms, including the inhibition of intestinal cholesterol absorption, an enhanced faecal excretion of bile acids, and the modulation of hepatic lipid metabolism by bioactive phytochemicals².

Table 1 also summarizes TG values across groups. Although atorvastatin is known to moderately reduce TG by inhibiting hepatic production of very-low-density lipoprotein, the observed reductions were not statistically significant. This may be attributable to the NC group's normal baseline TG levels, the atorvastatin dosage, or sample size limitations. Fenugreek has demonstrated a potential to

lower TG levels, particularly at higher concentrations. Bioactive constituents such as dietary fibre and saponins may contribute to this effect. However, the absence of statistically significant TG reductions in the fenugreek-treated groups suggests that its TG-lowering capacity may be modest, requiring higher doses or extended treatment duration in order to achieve clinically meaningful outcomes³.

Table 1 presents the recorded LDL-cholesterol levels across groups. The lack of significant reductions in the LD-F and MD-F groups may reflect insufficient concentrations of active compounds such as sapon-

ins, flavonoids, and alkaloids. Additionally, inter-individual variability in absorption and metabolism may influence treatment efficacy⁴. A larger sample size or longer treatment duration may be necessary to detect subtle changes at lower doses. Notably, the HD-F group exhibited LDL-cholesterol reductions comparable to those achieved with atorvastatin. This effect may result from synergistic actions of fenugreek's bioactive constituents. Saponins inhibit intestinal cholesterol absorption, flavonoids modulate hepatic lipid metabolism, and enhanced bile acid excretion promotes conversion of cholesterol into bile salts, collectively reducing circulating LDL-cholesterol levels. These findings support the potential of high-dose fenugreek extract as an alternative or adjunctive strategy for managing hyperlipidaemia. While statins such as atorvastatin remain the cornerstone of dyslipidaemia management, they are associated with adverse effects including myopathy, hepatotoxicity, and increased diabetes risk in some patients⁵. The fenugreek extract may offer a safer, plant-based alternative with comparable efficacy at appropriate doses.

Finally, the recorded HDL-cholesterol levels for all rat groups are summarized in Table 1. The dose-dependent increase in HDL-cholesterol observed in fenugreek-treated groups is likely attributable to its complex phytochemical profile, including alkaloids, flavonoids, and saponins. These compounds possess lipid-lowering, anti-inflammatory, and antioxidant properties. Fenugreek may enhance HDL-cholesterol through multiple mechanisms, including an up-regulation of HDL-associated proteins and enzymes

involved in cholesterol efflux. It may also stimulate apolipoprotein A-I synthesis, thereby promoting HDL particle formation and improving reverse cholesterol transport⁶.

4. Conclusion

Both atorvastatin and the fenugreek extract significantly modulated lipid parameters in rats, with the exception of TG levels, where effects were not statistically significant. Atorvastatin effectively increased HDL-cholesterol and reduced TC and LDL-cholesterol levels. The fenugreek extract demonstrated a dose-dependent efficacy, with the HD-F group achieving lipid profile improvements comparable to those of the atorvastatin-treated group.

Acknowledgements

The authors extend their sincere gratitude to the Al-Hussein General Teaching Hospital, Al-Muthanna, for providing all necessary resources for the conduct of this research.

Conflicts of interest

None exist.

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HOW TO CITE:

Jawad D., Khalifa A.Y., Mones M.H., Abed H.H. Fenugreek extract as a natural therapy for dyslipidaemia: an *in vivo* study on rats. *Pharmakeftiki* 37(2s), 294-298, 2025. <https://doi.org/10.60988/p.v37i2S.213>

Assessment of the prognostic value of serum beta-2 microglobulin and transforming growth factor beta levels for multiple myeloma patients

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KEY WORDS:

plasma cell tumor; beta-2 microglobulin; transforming growth factor beta; multiple myeloma; Iraq

ARTICLE INFO:

Received: December 31, 2024

Revised: February 13, 2025

Accepted: February 13, 2025

Available online: October 10, 2025

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ABSTRACT

This study has evaluated the joint prognostic utility of circulating beta-2 microglobulin (β_2 -microglobulin) and transforming growth factor beta (TGF- β) levels in 98 patients with multiple myeloma (MM) and 31 healthy volunteers (controls). Participants were recruited from the Haematology Department of the Marjan Medical Hospital, Babil Province, Iraq, between March 2021 and December 2022. The combined biomarker model yielded an overall diagnostic accuracy of 0.862, with a sensitivity of 93.2% and specificity of 90.1% for distinguishing MM cases from healthy individuals. Notably, β_2 -microglobulin alone achieved an area under the curve (AUC) of 0.961 in differentiating stage III from stage II MM. These findings support the potential of integrated β_2 -microglobulin and TGF- β profiling to enhance diagnostic and staging precision in MM, warranting further investigation into their clinical and translational applicability.

1. Introduction

Multiple myeloma (MM) is a haem

atologic malignancy characterized by uncontrolled proliferation of terminally differentiated B cells

Table 1. Comparative analysis of biomarker levels across different multiple myeloma (MM) stage groups. Statistical significance levels indicate the strength of group differences, highlighting the progressive elevation of transforming growth factor beta (TGF-β) and β ₂ -microglobulin levels with increasing disease severity in MM. In the stage II-treated group, TGF-β and β ₂ -microglobulin levels were marked with *** (p<0.001), denoting highly significant elevations compared to the healthy control group and within-group comparisons. In the stage III-treated group, both biomarkers were marked with ** (p<0.01), reflecting a slightly lower but still statistically significant difference relative to baseline values.								
Group	Mean ± standard deviation			Combined effects of both biomarkers				Effect size
	Age (years)	TGF-β (pg/mL)	β ₂ -microglobulin (mg/L)	AUC	Sensitivity	Specificity	95% CI	
Healthy volunteers (N=31)	56.8 ± 6.2	90.7 ± 56.4	0.67 ± 0.4	N/A	N/A	N/A	N/A	N/A
Stage II treated (N=41)	61.4 ± 6.3	521.8 ± 338.1 ***	4.87 ± 0.5 ***	0.502	0.4	0.439	[60.5, 62.3]	0.6
Stage III treated (N=25)	61.8 ± 8.9	519.6 ± 300.3 **	20.88 ± 6.2 **	0.502	0.4	0.439	[61.0, 62.6]	1.2
Stage II newly diagnosed (N=14)	61.1 ± 3.9	551.2 ± 149.3 ***	5.01 ± 0.4 ***	1.0	0.444	0.615	[60.9, 61.3]	0.45
Stage III newly diagnosed (N=18)	62.1 ± 5.9	180.1 ± 113.7 ****	22.0 ± 5.5 ****	1.0	0.444	0.615	[61.8, 62.4]	1.35

(plasma cells) within the bone marrow, leading to excessive production of monoclonal immunoglobulins¹. The disease typically evolves from a precursor condition known as monoclonal gammopathy of undetermined significance (MGUS), wherein aberrant plasma cells secrete monoclonal (M) proteins instead of functional antibodies. With the accumulation of oncogenic mutations, MGUS may progress to smoldering myeloma; a clinically indolent phase marked by elevated serum M-protein levels and an increased proportion of clonal plasma cells. Approximately 50% of individuals with smoldering myeloma eventually develop symptomatic MM, often accompanied by persistently rising M-protein concentrations². Although MGUS and smoldering myeloma are generally asymptomatic, both can result in renal impairment due to protein deposition, affecting 20%–40% of patients at the time of MM

diagnosis³.

Beta-2 microglobulin (β₂-microglobulin), a low-molecular-weight polypeptide derived from all nucleated cells and associated with major histocompatibility complex class I molecules, has emerged as a key biomarker in MM. Elevated serum β₂-microglobulin levels reflect increased cellular turnover and are strongly correlated with disease burden, prognosis, and treatment response. Although its predictive value remains under investigation, accumulating evidence supports its utility in prognostic stratification for MM⁴. Transforming growth factor beta (TGF-β), a multifunctional cytokine involved in immune regulation and cellular differentiation, has also been implicated in myelomagenesis and therapeutic resistance. TGF-β exhibits context-dependent tumor-suppressive and tumor-promoting effects within the bone marrow

microenvironment⁵. This study aimed at evaluating a predictive model integrating serum β_2 -microglobulin and TGF- β concentrations in order to enhance clinical decision-making in the management of MM.

2. Methodology

This case-control study was conducted at the Haematology Department of Marjan Medical Hospital, Babylon Province, Iraq, between March 2021 and December 2022. A total of 121 patients with confirmed MM were initially screened, of whom 98 (52 females and 46 males; mean age: 61.6 years; range: 41–76 years) were selected based on diagnostic criteria established by board-certified haematologists. Inclusion criteria comprised the detection of monoclonal M-proteins in the serum or urine, radiologically-confirmed osteolytic lesions, and $\geq 10\%$ plasma cells in bone marrow aspirates. Among the selected patients, 31 had a confirmed diagnosis of MM, while 67 were undergoing first-line therapy with either lenalidomide or bortezomib. The control group consisted of 31 healthy individuals (16 males and 15 females; mean age: 54.96 years) with no significant medical history. Participants with comorbidities such as type 1 diabetes mellitus, hepatitis, or renal insufficiency were excluded. Demographic and clinical data were collected *via* structured questionnaires linked to patient records. Venous blood samples were obtained from all participants for biochemical analysis. Serum concentrations of β_2 -microglobulin and TGF- β were quantified using enzyme-linked immunosorbent assay (ELISA) kits.

Ethical approval was granted by the local health authorities and the College of Pharmacy of the University of Babylon (IRB: A0030/2021). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Statistical analyses were performed using SPSS (version 28.0) and Python (version 3.10). Group comparisons for biomarker levels and age were conducted by employing independent *t*-tests or one-way analysis of variance (ANOVA) with

Tukey's *post hoc* correction. Effect sizes were calculated using Cohen's *d*. Receiver operating characteristic (ROC) curve analysis and logistic regression were employed in order to determine the area under the curve (AUC), sensitivity, and specificity of the combined biomarkers. Cross-validation was applied in order to assess model robustness. Confidence intervals were estimated *via* bootstrapping, and statistical significance was set at $p < 0.05$.

3. Results and Discussion

Table 1 presents the progressive elevation of serum β_2 -microglobulin and TGF- β levels across MM disease stages. Healthy controls served as the baseline reference, while newly diagnosed and treated patients exhibited distinct biomarker profiles, with higher effect sizes (Cohen's *d*) indicating clinical relevance. The combined biomarker model demonstrated moderate diagnostic performance in treated patients (AUC: 0.502) and perfect discrimination in newly diagnosed cases (AUC: 1.0), with sensitivity and specificity improving incrementally across disease stages.

The study confirmed the prognostic value of serum β_2 -microglobulin and TGF- β in MM. These biomarkers effectively distinguished MM patients from healthy controls and differentiated stage II from stage III disease. Elevated β_2 -microglobulin levels were strongly associated with advanced disease and poorer prognosis, consistent with prior findings that link high serum β_2 -microglobulin to increased tumor burden and reduced remission duration^{6,7}. While β_2 -microglobulin has been extensively validated as a prognostic marker, the role of TGF- β remains less well defined. Nonetheless, its involvement in tumorigenesis and immune modulation suggests potential relevance^{5,8}.

The present findings indicate that although TGF- β alone may have limited utility in disease staging, its receptor expression profile could offer greater prognostic insight^{9,10}. Importantly, the combined use of multiple biomarkers may enhance diagnostic precision. The AUC for the integrated model was 0.862 when comparing MM

patients to healthy controls, underscoring its predictive strength. Despite these promising results, limitations related to sample size should be acknowledged. Larger, multicenter studies are warranted in order to validate these findings and explore additional biomarker combinations across diverse patient populations.

4. Conclusion

This study underscores the prognostic potential of serum β_2 -microglobulin and TGF- β in the clinical management of MM. Both markers exhibited stage-dependent elevation, supporting their role in guiding therapeutic decisions. Future research should focus on developing predictive algorithms incorporating these biomarkers, so as to facilitate personalized treatment strategies and improve patient outcomes.

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Acknowledgements

The authors express their sincere gratitude to the patients and healthy volunteers who participated in this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Joudah M.M., Maarroof R.J.A., Al-Rikabi E.H., Al-Hindy H.A.A.M., Mousa M.J., Ramadan G.M., Al-Anbari A.J.K. Assessment of the prognostic value of serum beta-2 microglobulin and transforming growth factor beta levels for multiple myeloma patients. *Pharmakeftiki* 37(2s), 299-303, 2025. <https://doi.org/10.60988/p.v37i2S.214>

Investigating DNA methylation patterns in hair samples of patients with COVID-19-related acute telogen effluvium

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KEY WORDS:

acute telogen effluvium;
COVID-19; DNA methylation;
hair; Iraq

ARTICLE INFO:

Received: December 31, 2024

Revised: January 14, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

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ABSTRACT

This study has investigated the association between acute telogen effluvium and global DNA methylation in female patients recovering from coronavirus disease 2019 (COVID-19). In a cross-sectional case-control design, 100 participants were enrolled: 50 women presenting with post-COVID-19 hair loss and 50 age-matched healthy controls. Samples were obtained from patients attending consultant physician clinics at the Hilla Teaching Hospital and the Al-Imam Al-Sadeq Teaching Hospital between November 2022 and May 2023. Hair specimens were collected, DNA was extracted, and global methylation levels were quantified using liquid chromatography-mass spectrometry. A correlation was observed between age and the likelihood of hair loss; however, no statistically significant differences in DNA methylation levels were detected between the study groups. These findings suggest that while age may influence susceptibility to post-COVID-19 telogen effluvium, global DNA methylation does not appear to play a major role. Further research is warranted in order to elucidate the contribution of epigenetic mechanisms in post-viral hair loss syndromes.

1. Introduction

The outbreak of the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),

has led to a range of chronic sequelae^{1,2}, including hair loss – specifically, acute telogen effluvium (ATE). ATE has been observed in individuals exposed to multiple stressors, including viral infections, and is

frequently reported to occur following COVID-19. Emerging evidence implicates epigenetic modifications, particularly DNA methylation (DNAm), in the pathophysiology of ATE. DNAm refers to the enzymatic addition of a methyl group to cytosine residues within DNA, thereby modulating gene expression without altering the underlying nucleotide sequence³.

Epigenetics encompass heritable changes in gene expression that occur independently of alterations in DNA sequence. DNAm is a key epigenetic mechanism, wherein DNA methyltransferases (DNMTs) catalyse the covalent attachment of a methyl group to the 5'-carbon of cytosine residues, typically resulting in transcriptional silencing⁴. The viral perturbation of DNAm may disrupt immune regulation and contribute to host pathology⁵. For example, aberrant DNAm patterns in the angiotensin-converting enzyme 2 (ACE2) promoter have been shown to influence ACE2 expression; a critical determinant of SARS-CoV-2 cellular entry⁶. Notably, SARS-CoV-2 has been identified as a potent modulator of DNAm, with methylation changes persisting well beyond the acute phase of infection³. DNAm alterations in leukocytes have been shown to remain dysregulated for up to 12 months post-recovery, suggesting a role in the sustained immune dysregulation observed in post-acute COVID-19 syndrome.

This study aimed at investigating the relationship between hair loss and DNAm, specifically the ratio of 5-methylcytosine to adenine, in female Iraqi patients recovering from COVID-19.

2. Methodology

This study was conducted at the Department of Biochemistry, College of Pharmacy, University of Babylon, Hillah, Iraq. A total of 100 female participants aged 17–45 years were enrolled, comprising two groups: 50 women diagnosed with ATE following COVID-19 and 50 healthy volunteers (controls) without a history of hair loss. Participants were recruited from consultant physician clinics at the Hilla Teaching Hospital and the Al-Imam Al-Sadeq Teaching Hospital between November 2022 and May 2023.

Hair samples were collected for DNA extraction, and global DNAm levels were quantified by using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The analysis focused on the ratio of 5-methylcytosine to adenine as a proxy for DNAm activity. Spectrometric measurements were performed using the AB Sciex Triple Quadrupole 4500 System.

Ethical approval was obtained from the Babil Health Directorate (protocol number: BHD, CR-BC, 11-5-2022; approval date: 22 May 2022). Written informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki. Statistical analyses were conducted using SPSS version 24.0. Data normality was assessed by using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Normally distributed continuous variables are presented as mean $\pm$ standard deviation. Group comparisons were performed by using independent-samples *t*-tests or Mann–Whitney U tests, as appropriate. Statistical significance was defined as $p < 0.05$.

3. Results and Discussion

A significant correlation was observed between age and the likelihood of post-COVID-19 hair loss (Table 1). The mean $\pm$ standard deviation of the recorded DNAm levels was 0.22 ± 0.02 in the patient group and 0.27 ± 0.03 in the control group ($p = 0.35$; Table 1). Although variations in DNAm were noted, the difference was not statistically significant, thereby underscoring the complexity of epigenetic contributions to ATE in post-COVID-19 female patients. These findings suggest that while age may influence susceptibility to ATE, global DNAm does not appear to be a primary driver. The lack of statistical significance may reflect limitations in sample size or variability in DNA yield from hair specimens. Moreover, differences in sample integrity and DNA quality could have influenced methylation quantification.

Previous studies have demonstrated that COVID-19 can induce DNAm changes at CpG sites involved in immune regulation⁷. Inflammatory cytokines released during infection may alter DNMT

Table 1. Characteristics of the study participants; values expressed as mean ± standard deviation.			
Variable	Control	COVID-19 patients	p-value
Age (years)	31.9 ± 8.3	27.6 ± 8.6	0.011
5-methylcytosine intensity / adenine intensity ratio	0.27 ± 0.3	0.22 ± 0.2	0.35

activity, particularly during myeloid cell differentiation. COVID-19 patients exhibit hypomethylation of interferon-inducible genes compared to healthy individuals⁸, potentially contributing to immune dysregulation and exacerbation of conditions such as ATE. It is important to note that most DNAm studies in COVID-19 patients rely on bulk tissue analyses, which may obscure cell type-specific methylation patterns due to limitations in single-cell sequencing technologies and associated costs⁹. Understanding these cellular-level alterations is essential for elucidating the mechanisms underlying ATE and other post-COVID-19 sequelae.

This study reinforces the need for further investigation into epigenetic factors that may influence long-term health outcomes following COVID-19. Insights into DNAm-mediated immune memory could inform novel therapeutic and vaccination strategies against SARS-CoV-2¹⁰. Epigenetic interventions may help restore immune homeostasis, mitigate hair loss symptoms, and enhance vaccine efficacy. Future research should explore the clinical utility of epigenetic modulators. Agents targeting DNAm pathways may support immune recovery and improve responses to COVID-19 vaccines¹⁰. Moreover, differential DNAm patterns between acute and convalescent phases may guide the development of immune-based therapies.

Finally, targeting inflammation may also alleviate ATE symptoms in recovered COVID-19 patients, who appear particularly susceptible⁸. An integrated approach combining immunomodulation and epigenetic therapy warrants urgent exploration.

Although this study advances understanding of

DNAm and hair loss in post-COVID-19 females, further research is needed in order to identify specific genes and pathways implicated in ATE, with the goal of developing targeted interventions to reverse post-viral hair loss.

4. Conclusion

This study underscores the multifaceted relationship between DNAm and ATE in women recovering from COVID-19. While age was significantly associated with hair loss, no significant differences in global DNAm levels were observed between COVID-19 patients and controls. Although epigenetic mechanisms remain a promising avenue for therapeutic intervention, further studies are required in order to delineate their role in post-COVID-19 hair loss.

Acknowledgements

The authors express their gratitude to the patients for their participation in this study. Appreciation is also extended to the volunteers who participated in the study.

Conflicts of interest

None exist.

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HOW TO CITE:

Jubair A.H., Hashim H.O., Al-Hindy H.A.A.M., Baloh Y.H. Investigating DNA methylation patterns in hair samples of patients with COVID-19-related acute telogen effluvium. *Pharmakeftiki* 37(2s), 304-307, 2025. <https://doi.org/10.60988/p.v37i2S.215>

***In silico* study of four new phenoxybenzoic acid - isatin derivatives: targeting VEGFR and cancer**

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KEY WORDS:

***in silico*; cancer; isatin derivatives; phenoxybenzoic acid; VEGFR**

ARTICLE INFO:

Received: January 30, 2025

Revised: February 16, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

ABSTRACT

Cancer remains a major global health challenge, necessitating the development of novel therapeutic agents. This study hypothesizes that phenoxybenzoic acid derivatives can effectively inhibit the vascular endothelial growth factor receptor (VEGFR; Protein Data Bank code: 4ASE); a key molecular target in cancer therapy. Four compounds – designated F1, F2, F3, and F4 – were designed and synthesized to test this hypothesis. Their molecular structures were sketched using ChemDraw Ultra version 12.0, and their binding affinities were evaluated through molecular docking simulations conducted with the Molecular Operating Environment (MOE) program. Docking scores and root-mean-square deviation values were analysed in order to assess the compounds' potential effectiveness. Compared to sorafenib, a clinically approved VEGFR inhibitor, the synthesized compounds exhibited significant binding affinities within the receptor's active site, supporting their potential as anticancer agents. These findings provide a rationale for further biological evaluation and optimization of these compounds for targeted cancer therapy.

1. Introduction

Cancer remains one of the most life-threatening diseases due to its hallmark feature of uncontrolled cellular proliferation. Consequently, the development of high-quality anticancer agents – particularly those exhibiting potent biological activity, enzyme inhibitory prop-

erties, and minimal toxicity – is of paramount importance. Designing effective and safe chemotherapeutic agents for malignant conditions poses a significant challenge for medicinal chemists, whose efforts aim to elucidate the biochemical mechanisms underlying tumorigenesis and metastasis^{1,2}.

Among the most promising strat-

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egies in oncology is targeted therapy, which enhances therapeutic efficacy while minimizing systemic toxicity. Multi-kinase inhibitors, especially tyrosine kinase inhibitors, represent a highly promising class of targeted anticancer agents. Kinases are enzymes that catalyse the phosphorylation of biomolecules, thereby regulating complex cellular processes such as motility, growth, proliferation, differentiation, and programmed cell death (apoptosis)³.

A key angiogenic regulator implicated in tumour progression is the vascular endothelial growth factor (VEGF). VEGF exerts its pro-angiogenic effects by binding to specific kinase domains on vascular endothelial growth factor receptors (i.e., VEGFR1, VEGFR2, and VEGFR3). Among these, VEGFR2 – a receptor tyrosine kinase subtype – is primarily responsible for mediating angiogenesis⁴. This physiological process is essential for meeting the nutritional and oxygen demands of cells, tissues, and organs. However, dysregulated angiogenesis – whether excessive or insufficient – is associated with various pathological conditions, including malignancies, inflammatory disorders, and autoimmune diseases⁵.

In cancer, aberrant angiogenesis leads to the formation of atypical vascular networks, which compromise the efficacy of conventional therapies and facilitate tumour expansion⁶. Antiangiogenic therapy has emerged as a compelling approach in oncology, as increased vascularity in malignant tissues correlates with tumour growth and proliferation⁷. The U.S. Food and Drug Administration (FDA) has approved several small-molecule inhibitors, including sunitinib and sorafenib, for the treatment of angiogenesis-dependent malignancies.

Benzoic acid and its derivatives exhibit a broad spectrum of biological activities, including flavouring, antioxidant, antibacterial, anticancer, diuretic, analgesic, anti-inflammatory, and local anaesthetic effects. Derivatives belonging to the diaryl / diphenyl ether class demonstrate antimicrobial, antifouling, and antiproliferative properties⁸. Diphenyl derivative bioisosteres interact with diverse biotarget families, and their unique electronic and spatial characteristics – modulated by structural substituents – enhance target binding and pharmacological

versatility⁹.

This study aims at evaluating the binding affinities of four synthesized phenoxybenzoic acid derivatives (designated F1, F2, F3, and F4) against VEGFR (Protein Data Bank code: 4ASE) by using molecular docking simulations. By comparing their docking scores and root-mean-square deviation (RMSD) values with those of sorafenib, we seek to assess their potential as anticancer agents.

2. Methodology

The computational analyses were performed on an MSI system equipped with an Intel Core™ i7-1355U central processing unit (CPU) operating at 1.70 GHz and 8.00 GB of random-access memory (RAM). The Molecular Operating Environment (MOE) 2015 software and ChemDraw Ultra version 12.0 were installed for molecular modelling and visualization. The docking protocol comprised two primary steps: (i) ligand preparation and (ii) protein preparation.

3. Results and Discussion

Molecular docking was employed in order to determine the optimal binding conformations of ligands within the active site of the target protein. The MOE software was selected for its capacity to generate detailed graphical representations of ligand-receptor interactions, thereby facilitating the visualization and characterization of binding events¹⁰.

The docking simulations revealed that all four synthesized compounds selectively bound to VEGFR, occupying the same principal active site as sorafenib. A comparative analysis of the inhibitory effects and the amino acid interactions at the active site was conducted. Figure 1 provides a comprehensive overview of the interaction parameters for compounds F1 through F4, including docking scores (S-scores), RMSD values, and the key amino acid residues involved. Docking scores and RMSD values serve as indicators of binding affinity and structural alignment. The S-score reflects binding energy, while the RMSD quantifies the average interatomic distance between ligand atoms and the target site. Optimal binding is

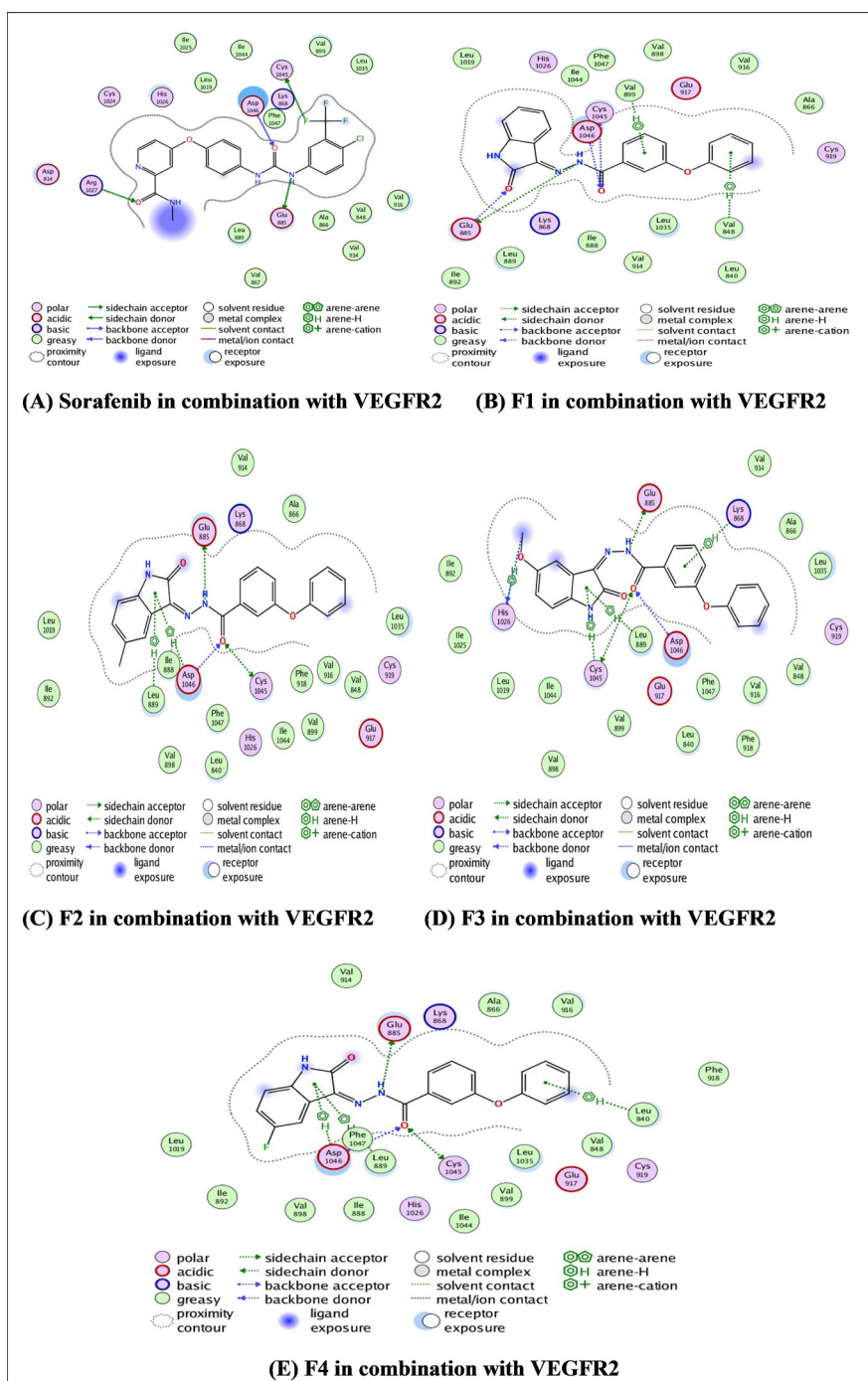


Figure 1. Two-dimensional representations of the four designed compounds and of sorafenib bound to the vascular endothelial growth factor receptor-2 (VEGFR2). This figure illustrates the binding interactions of the four synthesized compounds and the reference drug sorafenib within the VEGFR2 active site. Images generated using the Molecular Operating Environment (MOE) software depict key molecular interactions (including hydrogen bonding and hydrophobic contacts), highlighting structural differences and similarities. This visual comparison provides insight into the inhibitory potential of the designed compounds, supporting the *in silico* predictions of their anticancer activity.

indicated by lower RMSD values and more favourable (i.e., lower) S-scores.

All synthesized compounds (F1–F4) exhibited S-scores comparable to that of sorafenib, thereby suggesting similar inhibitory potential. The respective S-scores were -8.54 (F1), -7.82 (F2), -7.16 (F3), and -8.20 (F4), with corresponding RMSD values of 1.96, 1.57, 1.80, and 1.07.

The compound N'-(2-oxoindolin-3-ylidene)-3-phenoxybenzohydrazide incorporates an indolinone (isatin) core (which is a well-established scaffold in kinase inhibitors) alongside a phenoxybenzohydrazide moiety. The indolinone core mimics adenosine triphosphate (ATP) binding, rendering it suitable for targeting kinases such as VEGFR2, while the phenoxy group enhances hydrophobic interactions within the receptor pocket.

Docking simulations conducted using MOE demonstrated that all ligands adopted similar orientations within the VEGFR2 active site, which is flanked by a membrane-binding domain that facilitates substrate entry. The results confirmed the anticancer (antiangiogenic) potential of the synthesized compounds, with most ligands exhibiting strong binding affinities relative to sorafenib.

Compound F1 yielded the highest S-score (-8.54), indicating that the absence of substitution (hydrogen) may enhance its orientation within the receptor pocket. The F4 derivative, featuring fluoro-substitution, formed stabilizing hydrogen bonds with key amino acid residues, resulting in a lower RMSD (1.07) and a strong docking score (-8.20). In con-

trast, the unsubstituted inhibitor displayed a higher RMSD (1.96) but retained a favourable docking score (-8.54). These findings underscore the potential of the synthesized compounds as VEGFR2 inhibitors and warrant further experimental validation.

4. Conclusion

This *in silico* study, conducted using the MOE program, successfully designed and evaluated VEGFR2 tyrosine kinase inhibitors incorporating diphenyl ether and isatin moieties. The obtained molecular docking results confirmed strong binding affinities, with compound F1 demonstrating the highest S-score (-8.54) and compound F4 forming stabilizing hydrogen bonds due to fluoro-substitution. These findings highlight the promise of these compounds as VEGFR2 inhibitors for anticancer therapy and support their further biological evaluation.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Kamalaldeen F.Y., Alibeg A.A.A. *In silico* study of four new phenoxybenzoic acid - isatin derivatives: targeting VEGFR and cancer. *Pharmakeftiki* 37(2s), 308-312, 2025. <https://doi.org/10.60988/p.v37i2S.216>

Synthesis, characterization, immunological, and biological evaluation of several heterocyclic rings obtained from antipyrine derivatives

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KEY WORDS:

heterocyclic compounds;
antipyrine derivatives;
synthesis; antimicrobial
activity; immunological effect

ARTICLE INFO:

Received: December 27, 2024

Revised: January 06, 2025

Accepted: January 17, 2025

Available online: October 10, 2025

* CORRESPONDING

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ABSTRACT

The structures of newly synthesized heterocyclic compounds incorporating 4-aminoantipyrine (a key intermediate for the development of diverse derivatives) were elucidated through melting point analysis, Fourier-transform infrared spectroscopy (FTIR), and evaluation of physical properties. These compounds were subsequently tested for antibacterial activity against both Gram-positive and Gram-negative bacterial strains, specifically: *Staphylococcus aureus* and *Streptococcus mutans* (Gram-positive); *Shigella flexneri* and *Pseudomonas aeruginosa* (Gram-negative). Among heterocycles bearing three nitrogen atoms, antipyrine-based derivatives represent a widely studied structural class with significant relevance in academic research and medical applications. Although not naturally occurring, these compounds exhibit versatile utility across organic synthesis, chemical biology, polymer chemistry, supramolecular chemistry, and pharmaceutical development. Accordingly, the establishment of a straightforward and efficient synthetic route for antipyrine derivatives remains a priority. All tested compounds demonstrated notable antibacterial efficacy when benchmarked against conventional antibiotics. Moreover, *in vivo* studies revealed their capacity to induce pro-inflammatory cytokines such as interferon-gamma (IFN- γ), secreted by CD4⁺ T helper 1 (Th1) cells, thereby suggesting an immunomodulatory role.

1. Introduction

Antipyrine and its derivatives have garnered significant attention due to their roles as analgesic, antipyretic, and anti-inflammatory agents. These compounds exhibit a broad spectrum of biological activities, including anti-inflammatory, analgesic, immunomodulatory, anthelmintic, and insecticidal properties^{1,2}. Notable derivatives include β -lactams and imidazolidines, which have been evaluated for their antioxidant potential. Additionally, antipyrine derivatives serve as valuable precursors in the synthesis of biologically active Schiff bases and metal complexes³, as well as macrocyclic compounds and corrosion inhibitors for copper in acidic media.

4-Aminoantipyrine, a key intermediate, was here-in employed in the synthesis of Schiff bases and poly-functionally substituted heterocyclic rings with antibacterial and immunomodulatory properties^{1,3}. In this study, novel drug candidates were synthesized and their antibacterial efficacy was assessed alongside their ability to elicit a cellular immune response in an experimental rabbit model.

2. Methodology

2.1. Synthesis of compound I

Compound I, (E)-1,5-dimethyl-4-((4-nitrobenzylidene)amino)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was synthesized by dissolving 0.03 mol of 4-aminoantipyrine in 25 mL of ethanol, followed by the addition of 0.03 mol of 2-nitrobenzaldehyde. A few drops of glacial acetic acid were added, and the mixture was stirred continuously for 20–25 min using a magnetic stirrer. Subsequently, 0.02 mol of compound I were gradually introduced, and the reaction was completed over 8 h at 60°C–65°C. The product was isolated with an 85% yield, a melting point of 134°C–137°C, and Rf of 0.80. The dry residue was recrystallized from absolute ethanol.

2.2. Synthesis of compound II

Compound II, (2-chloro-4-(4-nitrophenyl)-3-ox-

oazetidin-1-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was obtained by dissolving 0.03 mol of compound I in 0.01 mol of chloroacetyl chloride and 1 mL of triethylamine. The reaction was carried out in 10 mL of dioxane at 0°C–5°C for 10 h. The mixture was evaporated and dried, and the product was recrystallized from ethanol. Thin-layer chromatography confirmed purity: Rf: 0.75; melting point: 187°C–193°C; red solid; yield: 75%.

2.3. Synthesis of compound III

Compound III, 1,5-dimethyl-4-(2-(4-nitrophenyl)-5-oximidazolidin-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was synthesized by combining 0.03 mol of compound I with 25 mL of tetrahydrofuran, followed by continuous shaking. Then, 0.03 mol of aminoacetic acid were added gradually, and the reaction was completed over 14 h at 50°C. The product was dried and recrystallized from absolute ethanol: Rf: 0.61; melting point: 147°C–150°C; brown solid; yield: 87%.

2.4. Antibacterial and immunological evaluation

The antibacterial activity of compounds I–III was assessed *in vitro* using the agar-well diffusion method against two Gram-positive bacterial strains (*Staphylococcus aureus* and *Streptococcus mutans*) and two Gram-negative strains (*Shigella flexneri* and *Pseudomonas aeruginosa*). Compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 10, 20, and 30 μ g/mL⁴.

Eighteen male rabbits (*Oryctolagus cuniculus*) were immunized with 1 mL of each compound at the aforementioned concentrations. Two rabbits received sterile normal saline as controls. A multisite injection protocol was used (0.25 mL per site). Cellular immune responses were evaluated by measuring systemic levels of interferon-gamma (IFN- γ) using an enzyme-linked immunosorbent assay (ELISA) kit (Beckman Coulter), with results compared to the control group⁵.

3. Results and Discussion

Compound I was characterized by melting point and

Table 1. Inhibition zone diameters (in mm) of the synthesized chemical compounds against bacterial growth at varying concentrations.

Chemical compound	Concentration (µg/mL)	Mean of inhibition zone (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		<i>S. aureus</i>	<i>S. mutans</i>	<i>S. flexneri</i>	<i>P. aeruginosa</i>
I	10	9	12	10	11
	20	13	15	17	20
	30	19	18	20	23
II	10	9	13	10	11
	20	16	16	15	18
	30	23	24	28	30
III	10	11	12	12	14
	20	15	18	25	23
	30	27	29	34	35

FTIR spectroscopy. The FTIR spectrum showed a new absorption band at 1649 cm^{-1} , corresponding to the azomethine (C=N) group, and the disappearance of bands associated with NH_2 and C=O groups of the starting materials. A peak at 1595 cm^{-1} was attributed to C=C stretching in the pyrazole ring, while a band at 1718 cm^{-1} corresponded to the ring's C=O group. A broad band near 3392 cm^{-1} indicated intermolecular hydrogen bonding *via* the hydroxyl (O-H) group.

Compound II exhibited a characteristic absorption band at $1651\text{--}1660\text{ cm}^{-1}$, attributed to the C=O stretch of the azetidine ring. Compound III showed NH symmetric and asymmetric stretching bands in the range $3226\text{--}3294\text{ cm}^{-1}$, along with a tautomeric O-H band and a strong carbonyl stretch at 1710 cm^{-1} , indicative of the imidazolidinone ring.

All compounds demonstrated antibacterial activity, with compounds [II] and [III] showing the most potent effects across all tested bacterial strains (Table 1). This activity is likely due to disruption of bacterial cell wall permeability, protein denaturation, and interference with metabolic pathways, ultimately leading to cell death⁶.

Finally, a statistically significant increase ($p<0.05$) in IFN- γ levels was observed, suggesting activation

of Th1 cells and induction of cell-mediated immunity.

4. Conclusion

Our *in vivo* experiments have confirmed that the herein assessed novel antipyrene derivatives can stimulate cell-mediated immunity. These compounds have also exhibit marked antibacterial activity against the tested microorganisms.

Acknowledgements

The authors gratefully acknowledge the College of Pharmacy of the University of Babylon, for providing the necessary equipment and support for this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Kareem A.F., Mahdi D., Alhamdani H.S., Kadhum S.A. Synthesis, characterization, immunological, and biological evaluation of several heterocyclic rings obtained from antipyrine derivatives. *Pharmakeftiki* 37(2s), 313-316, 2025. <https://doi.org/10.60988/p.v37i2S.217>

Harmfulness awareness and smoking status determinants among university students in Baghdad (Iraq)

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KEY WORDS:

cigarette smoking;
questionnaire; smoking
status; university students;
tobacco

ARTICLE INFO:

Received: January 30, 2025

Revised: February 16, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Tobacco use remains one of the most pressing global health challenges. Approximately 80% of smokers worldwide reside in low- and middle-income countries, where the burden of tobacco-related morbidity and mortality is disproportionately high. Cigarette smoking is notably prevalent among university students. This study aimed at investigating and evaluating the awareness and determinants of smoking status within a university student sample, and at examining the association between awareness levels and smoking-related determinants. A descriptive-analytical cross-sectional study was conducted involving 230 university students who smoke, recruited from Baghdad City. Data were collected using a structured questionnaire designed to assess participants' awareness of smoking-related harms and the factors influencing their smoking behavior. The majority of the participants were male and aged between 18 and 23 years. Most reported smoking cigarettes and hookah, and demonstrated awareness of the harmful effects of smoking, including its role as a major cause of lung cancer, heart disease, and respiratory conditions. The lowest level of agreement was recorded for the statement linking smoking to road traffic accidents. Nearly half of the participants acknowledged that smoking negatively affects family income. Statistical analysis revealed a significant association between students' awareness and the determinants of smoking status, as well as with their sociodemographic characteristics.

1. Introduction

Although smoking prevalence has declined overall – with current smoking rates decreasing from 18.2% in 2011/2012 to 14.9% in 2017/2018, and daily smoking from 16.3% to 13.1% over the same period – rates remain disproportionately high among individuals aged 18–24 years (20%), 25–34 years (22%), Pasifika peoples (23%), and Māori (34%), compared with the general population¹.

Many college health professionals report that students often respond to smoking status questions with statements such as “*I only smoke when I go out*” or “*I only smoke socially*”. Focus group discussions with college students have revealed that some identify as “social smokers” and perceive themselves as distinct from habitual smokers².

Tobacco use continues to pose a major global health concern. Approximately 80% of the world’s smokers reside in low- and middle-income countries, where tobacco-related morbidity and mortality rates are highest. Tobacco consumption is increasing at an estimated annual rate of 3.4% in developing countries, particularly in regions such as Africa and the Eastern Mediterranean, where health systems are under-resourced³. Given that smoking is responsible for approximately 440,000 deaths annually and incurs an estimated \$157 billion in health-related economic costs each year, this burden is projected to rise. Without adequate and comprehensive health education, student smokers may face escalating health risks and complications⁴.

University students undergo significant social and identity transitions as they move away from home (in most cases) and form new peer networks⁵. Increased independence and exposure to new social circles may encourage smoking among occasional users and facilitate progression to daily smoking⁶.

This study aimed at investigating the awareness and determinants of smoking status among university students, and at examining the association between awareness levels and smoking-related determinants in relation to selected variables.

2. Methodology

A quantitative, descriptive-analytical cross-sectional study was conducted in order to assess

awareness and determinants of smoking status among university students who smoke, from 14 October 2022 to 25 April 2023. The study included a purposive sample of 230 smoking students, distributed equally across five colleges in Baghdad City: College of Nursing (N=46), College of Pharmacy (N=46), College of Business Administration (N=46), College of Dentistry (N=46), and College of Law (N=46). In order to achieve the study objectives, a structured questionnaire was developed and refined following an extensive review of relevant literature. The final instrument comprised two sections: (i) Part One addressed socio-demographic and personal characteristics, while (ii) Part Two assessed determinants and awareness related to smoking status.

Data were analysed using version 21 of the SPSS software. Ethical approval was obtained from the institutional review board of Al-Mustaqbal University’s College of Nursing. The study adhered to the ethical principles outlined in the Declaration of Helsinki. According to document 1015, the local ethics committee reviewed and approved the research protocol, data collection instruments, and informed consent documentation on 1 December 2024.

3. Results and Discussion

The majority of participants were male (72%) and aged 18–23 years (55%). Most were first-year students (27%). Smoking prevalence was higher among individuals from low to moderate socioeconomic status backgrounds. All participants were smokers, as per the purposive sampling criteria. Among them, 44% smoked cigarettes, 42% smoked hookah, and 14% used electronic cigarettes. Notably, 84% reported being unaffected by the smell of cigarettes, likely due to olfactory desensitization from habitual smoking. Awareness of the harmful effects of passive smoking was reported by 80% of the sample (Table 1).

While general awareness of smoking-related health risks was high (including recognition of its role in lung cancer, heart disease, bladder cancer,

Table 1. General determinants and awareness of smoking status among the studied sample.

#	Questions	Yes		No	
		N	%	N	%
#1	Do you work after study hours?	177	77%	53	23%
#2	If the answer to #1 is "yes", what is the work?	employee: 27 (15%); free business: 150 (85%)			
#3	Is there any of your family a smoker?	204	89%	26	11%
#4	Is there any of your friends a smoker?	169	73%	61	27%
#5	Do you get upset and bothered with cigarette smoking?	37	16%	193	84%
#6	Do you know the harmful effects of passive smoking?	185	80%	45	20%
#7	Are you with a project without smoking?	129	56%	101	44%
#8	Are there laws that prevent smoking in public?	170	74%	60	26%
#9	The number of cigarettes that can you consume in a day.	less than a pack: 55 (24%); one pack: 141 (61%); more than a pack: 34 (15%)			
#10	Smoking is harmful to health; smoking is a major cause of lung cancer, heart disease, bladder cancer, oral cancer, and respiratory diseases.	215	93%	15	7%
#11	Smoking is a cause of road accidents.	3	1%	227	99%
#12	Smoking affects family income.	110	48%	120	52%
#13	Awareness of the harms of smoking helps stopping it.	75	33%	155	67%
#14	The lack of awareness of the harms of smoking is one of the reasons for its use.	133	58%	97	42%
#15	The cultural level of the family affects the use of smoking.	29	13%	201	87%

oral cancer, and respiratory illnesses), awareness of less commonly discussed risks was limited. For instance, only 1% (N=3) agreed with the statement that smoking contributes to road traffic accidents. Approximately half of the participants acknowledged the negative impact of smoking on family income. These findings align with recent studies indicating that most student smokers are aware of major health risks associated with smoking. However, the results diverge from those of an earlier study⁷, which has reported low awareness among smokers regarding certain health consequences.

Finally, statistical analysis revealed significant associations between awareness levels and selected socio-demographic variables, particularly age and sex.

4. Conclusion

While general awareness of smoking-related health risks was found to be high, awareness of its contri-

bution to road accidents was notably low. Half of the participants recognized the economic burden of smoking on their families. Statistically significant associations were observed between students' awareness and their socio-demographic characteristics, especially age and sex.

Acknowledgements

The authors extend sincere gratitude to the College of Pharmacy of the Al-Mustaqbal University, for providing the necessary facilities and support to complete this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Khaleel M.A., Hassan A.A.H., Ayad Z.M., Hajwal S.K., Al-Taeae M.H., Muhaibes F.J., Abbas A.H., Ridha-Salman H. Harmfulness awareness and smoking status determinants among university students in Baghdad (Iraq). *Pharmakeftiki* 37(2s), 317-320, 2025. <https://doi.org/10.60988/p.v37i2S.218>

Diagnostic evaluation of LTA and suPAR as combined biomarkers for multidrug-resistant Gram-positive infections: a case-control study in Iraq

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KEY WORDS:

antimicrobial resistance;
multidrug-resistant; Gram-
positive bacteria; lipoteichoic
acid; soluble urokinase-
type plasminogen activator
receptor

ARTICLE INFO:

Received: January 06, 2025

Revised: February 01, 2025

Accepted: February 12, 2025

Available online: October 10, 2025

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ABSTRACT

Antimicrobial resistance (AMR) has emerged as a critical threat to global public health. Timely and accurate identification of resistant pathogens (followed by appropriate antimicrobial therapy and robust stewardship) is essential in order to curb the emergence and spread of AMR. Although current diagnostic modalities offer clinical value, they are constrained by time requirements and limited capacity to differentiate colonization from active infection. Lipoteichoic acid (LTA), specific to Gram-positive bacteria, serves as a direct indicator of bacterial presence. In parallel, the soluble urokinase-type plasminogen activator receptor (suPAR) reflects host immune response, providing insight into infection severity and progression. This study investigates the diagnostic and prognostic utility of a dual biomarker panel comprising LTA and suPAR for the rapid assessment of bacterial infections. In a case-control design, we have quantified the serum levels of LTA and suPAR by ELISA in 62 patients with confirmed bacterial infections and 38 matched controls. Statistically significant elevations were observed in patients with extensively drug-resistant (XDR) infections: LTA levels reached 54.47 ng/L ($p=0.0111$), and suPAR levels reached 284.74 ng/mL ($p=0.0019$), compared to the multidrug resistant (MDR) infection and the non-MDR infection groups. These findings underscore the potential of LTA and suPAR as complementary biomarkers for the early detection and stratification of bacterial infections.

1. Introduction

Major antimicrobial resistance (AMR) represents a critical global health challenge, driven largely by the overuse and misuse of antibiotics. Inappropriate antibiotic application across agricultural, medical, and veterinary practice fosters the emergence of resistance genes, contributing to a “silent pandemic” projected to surpass other leading causes of mortality by 2050¹. The proliferation of antibiotic-resistant microorganisms poses a substantial threat to global morbidity and mortality. Given its profound public health and socioeconomic implications, AMR has long been recognized by the World Health Organization (WHO) as a priority area for pharmaceutical innovation. In 2016, WHO member states requested the development of a priority list of antibiotic-resistant bacteria to guide research and drug development efforts².

In the intensive care unit (ICU), *Staphylococcus aureus* is frequently implicated in sepsis, ventilator-associated pneumonia, surgical site infections, and infections related to indwelling medical devices. The widespread emergence of methicillin-resistant *S. aureus* (MRSA) has led to the classification of many ICU staphylococcal infections as drug-resistant. Additionally, coagulase-negative staphylococci (CoNS), including *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and more recently *S. lugdunensis*, have become prominent pathogens in healthcare-associated infections³. Across hospitals and urban centers, the treatment of bacterial infections caused by antibiotic-resistant strains presents an escalating clinical challenge⁴.

Antimicrobial susceptibility testing remains essential for selecting appropriate antimicrobial agents in the management of infectious diseases. Diagnostic microbiology laboratories have seen continuous methodological evolution. Traditional phenotypic approaches such as disc diffusion and broth microdilution, though labour-intensive and time-consuming, are still regarded as gold standards. In response to clinical demands, research efforts increasingly focus on developing rapid susceptibility testing methods suitable for routine laboratory use. These innova-

tions often incorporate automation and leverage genotypic or micro- / nanotechnological platforms⁵.

Terminology such as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) is commonly employed in order to describe the spectrum of resistance patterns observed in healthcare-associated bacterial infections. Notably, infections caused by multidrug-resistant organisms often present with clinical features indistinguishable from those caused by susceptible strains⁶.

Lipoteichoic acid (LTA), a surface-associated amphiphilic molecule involved in regulating autolytic wall enzymes (muramidases), is produced by Gram-positive bacteria. Upon bacteriolysis (induced by β -lactam antibiotics, lysozyme, or leukocyte-derived cationic peptides), LTA is released and may persist within macrophages, contributing to chronic inflammation⁷. The soluble urokinase-type plasminogen activator receptor (suPAR), the bioactive form of the membrane-bound glycoprotein uPAR, is predominantly expressed on immunologically active cells. As a marker of local inflammation and immune activation, suPAR has gained recognition as a predictive biomarker in various inflammatory conditions⁸.

This study aimed at evaluating a biomarker panel comprising LTA and suPAR for the detection of Gram-positive MDR bacterial infections, with the goal of advancing rapid and accurate diagnostic strategies for these clinically challenging pathogens.

2. Methodology

A case-control study was conducted between October and December 2024 at the Al-Husseini Teaching Hospital and the Al-Kafeel Specialized Hospital, Iraq. The study included 62 patients with confirmed bacterial infections and 38 healthy controls. Diagnoses were based on clinical signs and symptoms observed by physicians at the Al-Imam Al-Hussain Hospital, under the Karbala Health Directorate.

Among the 62 cases of Gram-positive bacterial infections, the distribution was as follows: sepsis (N=27), diabetic foot ulcers (N=8), urinary tract infections (N=9), wound infections (N=8), vaginitis (N=3), abscesses (N=3), and pneumonia (N=4). In-

Table 1. Comparison of biomarker levels of lipoteichoic acid (LTA) and soluble urokinase-type plasminogen activator receptor (suPAR), measured in ng/L and ng/mL, respectively, across control and patient groups or stratified by antimicrobial resistance patterns. Abbreviations used: MDR, multidrug resistant; SD, standard deviation; UTI, urinary tract infection; XDR, extensively drug resistant.

Variable		LTA		suPAR	
		Mean	SD	Mean	SD
Bacterial infection types	Control	15.27	7.17	120.67	23.68
	Sepsis	47.68	9.98	231.97	84.18
	UTI	47.32	8.44	250.49	82.32
	Diabetic foot ulcer	45.86	4.66	243.53	55.15
	Pneumonia	44.59	12.98	197.77	50.67
	Vaginitis	43.12	4.39	300.47	113.35
	Wound infection	60.62	9.44	257.05	107.77
	Abscess	29.94	4.54	191.8	39.46
p-value		0.0002		0.0001	
Antimicrobial resistance patterns	Non-MDR	43.86	11.32	199.38	47.25
	MDR	48.91	9.09	260.44	87.60
	XDR	54.47	8.57	284.74	96.63
p-value		0.0111		0.0019	

clusion criteria were based on physician-confirmed bacterial infections in patients aged 18 years or older, of either gender. Exclusion criteria encompassed individuals under 18 years of age, those with autoimmune diseases, pregnant women, patients with catheters, and males with prostate conditions.

Specimen collection included urine, blood, and swab samples for microbial isolation and identification. A loopful of each sample was inoculated onto MacConkey and blood agar plates, followed by overnight incubation at 37°C under aerobic conditions. Blood samples were also collected so as to quantify LTA and suPAR levels in both patient and control groups.

Microbial identification and antimicrobial susceptibility testing were performed using the VITEK 2 system (bioMérieux), employing the ID-GPC identification card and AST modules. Ethical approval was obtained from the Ethics Committees of the Kerbela Health Office, with institutional consent from the Imam Al-Hussain Hospital and the Al-Kafeel Specialized Hospital.

Statistical analysis was conducted by using the SPSS version 23 software. Mean and standard deviation values were calculated, and statistical significance was assessed using a threshold of $p < 0.05$. Analysis of variance (ANOVA) was applied for group comparisons, with Duncan's *post hoc* test being used in order to evaluate multiple comparisons at the same significance level.

3. Results and Discussion

All infection groups demonstrated significantly elevated LTA levels compared to controls (15.27 ± 7.17 ng/L; $p = 0.0002$). Among the infection types, wound infections exhibited the highest mean LTA concentration (60.62 ± 9.44 ng/L), followed by sepsis (47.68 ± 9.98 ng/L), urinary tract infections (47.32 ± 8.44 ng/L), diabetic foot ulcers (45.86 ± 4.66 ng/L), pneumonia (44.59 ± 12.98 ng/L), and vaginitis (43.12 ± 4.39 ng/L), with abscesses showing the lowest, yet still elevated levels (29.94 ± 4.54 ng/L) (Table 1).

Similarly, suPAR levels were markedly elevated

ed across all infection groups compared to controls (120.67 ± 23.68 ng/mL; $p=0.0001$). Vaginitis showed the highest mean suPAR concentration, followed by wound infections (257.05 ± 107.77 ng/mL), urinary tract infections (250.49 ± 82.32 ng/mL), diabetic foot ulcers (243.53 ± 55.15 ng/mL), and sepsis (231.97 ± 84.18 ng/mL), while pneumonia (197.77 ± 50.67 ng/mL) and abscesses (191.85 ± 39.46 ng/mL) exhibited the lowest, yet still elevated levels (Table 1).

In terms of resistance patterns, LTA levels were significantly higher in XDR cases (54.47 ng/L; $p=0.0111$) compared to non-MDR (43.86 ng/L) and MDR (48.91 ng/L) groups. suPAR levels were also significantly ($p=0.0019$) elevated in XDR (284.74 ng/mL) and MDR (260.44 ng/mL) cases relative to non-MDR infections (199.38 ng/mL) (Table 1).

These findings corroborate previous studies. For instance, elevated LTA levels have been demonstrated in bacterial infections, even in surgically treated mice without bacterial inoculation⁸. Elevated suPAR levels across all infection groups align with the findings of a previous study⁹, which has reported that high plasma suPAR concentrations may predict case fatality and severe sepsis in emergency department patients with suspected infection. Furthermore, our results support the diagnostic and prognostic utility of LTA and suPAR in bacterial infections and antimicrobial resistance profiling¹⁰.

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4. Conclusion

In conclusion, the significant elevation of LTA and suPAR in patients with bacterial infections underscores their potential as diagnostic and prognostic biomarkers. These findings contribute to the growing body of evidence supporting the role of these molecules in the host immune response to bacterial pathogens. Future research should aim at elucidating the underlying mechanisms of these biomarker elevations and explore their therapeutic implications in clinical practice.

Acknowledgements

The authors express their sincere gratitude to the staff of the Department of Microbiology for their invaluable support in facilitating this research within the Master of Microbiology program at the College of Medicine of the University of Kerbala.

Conflicts of interest

None exist.

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HOW TO CITE:

Khalife M.S., Ali M.M., Al-Hasnawi S.M.J., Hawas B.K. Diagnostic evaluation of LTA and suPAR as combined biomarkers for multidrug-resistant Gram-positive infections: a case-control study in Iraq. *Pharmakeftiki* 37(2s), 321-325, 2025. <https://doi.org/10.60988/p.v37i2S.219>

Pregabalin prescription patterns in private clinics in Iraq: a cross-sectional study

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KEY WORDS:

**pregabalin; private clinics;
neuralgia; neuropathic pain;
misuse**

ARTICLE INFO:

Received: January 18, 2025

Revised: February 09, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

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ABSTRACT

Pregabalin, despite its widespread use across neurology, psychiatry, and primary care, is increasingly recognized for its potential to be misused. Reports have documented rising prescription volumes alongside anecdotal evidence of an expanding illicit market. This study, conducted at private clinics in Diyala, Iraq, between January and December 2024, aimed at evaluating the pregabalin prescribing patterns in Iraqi general practice and at assessing whether its use warrants concern. Pharmacologically, pregabalin exhibits GABA-mimetic properties and may exert direct or indirect effects on the dopaminergic reward pathway. Although its addiction liability at therapeutic doses is considered low, the perception among misusers that pregabalin constitutes a legitimate substitute for illicit substances raises public health concerns. Our study has found that 45% of pregabalin users were aged 60–69 years. Prolonged use (>4 weeks) was observed in 75% of cases and was statistically significant ($p<0.001$). Insomnia accounted for 43% of prescriptions, also reaching statistical significance ($p<0.001$). These findings underscore the importance of thorough clinical assessment prior to pregabalin initiation, including evaluation of any history of substance misuse and vigilance for signs of abuse during treatment.

1. Introduction

Pregabalin is an anticonvulsant medication primarily used in the treat-

ment of epilepsy. Although it is a structural analogue of γ -aminobutyric acid (GABA), pregabalin does not directly interact with either GABA

or benzodiazepine receptors. Instead, it inhibits the release of several central neurotransmitters, including substance P, glutamate, and norepinephrine¹. In cases where patients with epilepsy exhibit poor response to conventional anticonvulsant therapy, pregabalin is often prescribed as a second-line agent.

Currently, pregabalin is licensed for the treatment of neuropathic pain (particularly diabetic neuropathy), fibromyalgia, postherpetic neuralgia, seizures, and generalized anxiety disorder. It is also frequently used off-label for conditions such as migraine, alcohol withdrawal syndrome, restless legs syndrome, and menopausal vasomotor symptoms^{2,3}.

Recent global publications have highlighted the emerging risk of pregabalin misuse. In Munich, Germany, the number of individuals abusing pregabalin increased from zero in 2008 to five in 2011, and then to 105 by 2015, according to Zellner *et al.*⁴. Zhou *et al.*⁵ have reported that pregabalin-related ambulatory care visits in the United States have doubled between 2003 and 2016, with 50.0% of the cases involving a co-abuse of benzodiazepines and/or opioids without a prescription.

Concerns regarding both patient and clinician awareness of pregabalin's abuse potential have been raised, particularly in light of findings that 45.8% of pregabalin prescriptions were issued by clinicians⁶. Many patients who misused pregabalin after receiving it legally were unaware of its addictive properties, underscoring the need for increased public education regarding its potential for abuse⁷. Recent studies have shown that pregabalin carries a similar risk of misuse to gabapentin⁸. In Iraq, the patients most likely to misuse pregabalin include those with spinal disc herniation, chronic pain, and neuropathic pain.

2. Methodology

This cross-sectional observational study was conducted at private clinics in Diyala, Iraq, between January and December 2024. Data on pregabalin prescriptions were collected, with particular emphasis on therapeutic indications, identification of misuse, and associated factors. Ethical approval was granted by the Research Ethical Committee of the College of Pharmacy of the Bi-

lad Alrafidain University (10 December 2023).

The dataset comprised 400 pregabalin prescriptions issued during the study period. Eligibility criteria included all prescriptions involving pregabalin, irrespective of indication. Prescriptions with incomplete documentation or unrelated to pregabalin were excluded. Data were systematically extracted and categorized into key variables: patient demographics (age and gender), prescription characteristics (dosage, duration of therapy, and indication), co-prescriptions (e.g., non-steroidal anti-inflammatory drugs / NSAIDs, opioids, or antidepressants), prescription validity (distinguishing valid from invalid prescriptions), and adverse effects or withdrawal symptoms (as reported by patients). Primary outcomes included patterns of prescription validity, usage trends, and prevalence of misuse indicators. Secondary outcomes assessed the incidence of adverse effects and withdrawal symptoms.

Statistical analysis was performed by using the SPSS version 22 software. Descriptive statistics summarized both the demographic and the prescription data. Chi-square tests and other inferential methods were applied in order to evaluate associations, with p -values <0.05 considered statistically significant.

3. Results and Discussion

The majority of patients (45%) were aged 60–69 years, followed by those aged 50–59 years (24%) and 40–49 years (16%). Prescriptions were relatively balanced by gender, with 55% issued to males and 45% to females. Dosage distribution showed that 55% of prescriptions were for 75 mg and 45% for 150 mg; however, the preference for 75 mg was not statistically significant ($p=0.317$). Prolonged use (>4 weeks) was observed in 75% of cases, a statistically significant finding ($p<0.001$). Insomnia was the most common indication (43%), followed by peripheral neuropathy (23%), chronic back pain (18%), and mood stabilization or rest (10%).

NSAIDs were co-prescribed in 65% of cases, followed by antidepressants (60%) and opioids (56%). Table 1 summarizes key findings, including significant trends in prescribing practices and associations between dosage, duration, and co-prescription patterns.

Table 1. Summary of the identified pregabalin prescription patterns across demographic categories, dosage preferences, treatment durations, co-administration patterns, and therapeutic indications. Key statistical outcomes are reported in order to highlight significant associations (denoted by *). Abbreviations used: NSAIDs, nonsteroidal anti-inflammatory drugs.				
Variable	Results		Chi-square value	p-value
Age distribution	40–49 years	16%	23.28	<0.001
	50–59 years	24%		
	60–69 years	45% *		
	70–79 years	15%		
Gender distribution	Male	55%	1.00	0.317
	Female	45%		
Drug dosage	75 mg	55%	1.00	0.317
	150 mg	45%		
Duration of use	<2 weeks	10%	78.50	<0.001
	2–4 weeks	15%		
	>4 weeks	75% *		
Co-administration	Antidepressants	60%	3.84	0.279
	NSAIDs	65%		
	Opioid drugs	56%		
	Cardiovascular drugs	45%		
Indications	Peripheral neuropathy	23%	41.90	<0.001
	Insomnia	43% *		
	Chronic back pain	18%		
	Mood / rest	10%		
	Others	6%		

This study provides insight into pregabalin prescribing and potential misuse within private clinics in Diyala, Iraq. The findings reflect growing concerns regarding misuse in clinical settings. Although pregabalin has low addictive potential at therapeutic doses, the high prevalence of prolonged use and co-prescription with central nervous system (CNS) depressants such as opioids and benzodiazepines necessitates caution.

Our results align with broader trends reported in the literature. A decade-long analysis of pregabalin prescribing patterns revealed a consistent increase in its use, particularly among older adults, with prolonged therapy identified as a potential misuse indicator⁸. This is consistent with our observation of extended treatment durations in an older patient cohort.

Further research in forensic settings has emphasized pregabalin’s misuse potential, especially among individuals with prior substance use disorders⁹. Although our study did not specifically assess substance use his-

tories, the high rate of co-prescriptions with opioids and antidepressants raises concern about misuse risk in vulnerable populations.

Finally, qualitative studies have shown that some patients self-medicate with pregabalin for anxiety and chronic pain, often without medical oversight^{3,7}. This resonates with our data, where insomnia – a condition frequently associated with anxiety – was the leading indication. A study by Mikhael *et al.*¹⁰, conducted in Iraqi private clinics, has reported similar patterns of pregabalin misuse alongside CNS depressant prescriptions, corroborating our findings. These co-prescription trends highlight the need for targeted interventions by clinicians and pharmacists in order to mitigate associated risks.

4. Conclusion

The findings of this study are consistent with those of

previous studies indicating an increase in pregabalin prescriptions and highlighting its potential for misuse. These parallels underscore the importance of cautious prescribing, particularly when pregabalin is co-administered with CNS depressants or used off-label. Comprehensive patient assessment, education, and monitoring are essential in order to enhance safety and reduce the risk of misuse.

Acknowledgements

We extend our sincere gratitude to the University of Bi-

lad Alrafidain, Tikrit University, and Al-Bayan University for their support in facilitating this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Kurji H.A., Hasan A.K., Abbood M.K., Ali H.M. Pregabalin prescription patterns in private clinics in Iraq: a cross-sectional study. *Pharmakeftiki* 37(2s), 326-329, 2025. <https://doi.org/10.60988/p.v37i2S.220>

Low serum vitamin D levels as a risk factor for peptic ulcer development in Iraqi patients

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KEY WORDS:

peptic ulcer; vitamin D; *H. pylori*; duodenum endoscopy; gastric ulcer

ARTICLE INFO:

Received: January 09, 2025

Revised: February 01, 2025

Accepted: February 12, 2025

Available online: October 10, 2025

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ABSTRACT

The pathophysiology of peptic ulcer (PU) involves a multifactorial interplay between gastric acid secretion, mucosal barrier integrity, *Helicobacter pylori* (*H. pylori*) infection, and the modulatory effects of various nutrients on the ulcer onset and severity. This cross-sectional study aimed at investigating whether PU pathophysiology and severity are associated with serum vitamin D levels. A total of 110 individuals aged 20–65 years were enrolled. Blood samples were collected between July and October 2024 from patients attending the Morgan Teaching Hospital and the Imam Sadiq Hospital in the Babil Province, Iraq. Following upper gastrointestinal endoscopy, participants were stratified into two groups: those without PU (N=40) and those diagnosed with PU (N=70). Each participant provided 5 mL of venous blood for vitamin D quantification and biochemical profiling. Among the PU patients, females were more prevalent (55.7%). Body mass index (BMI) was lower in the PU group. Vitamin D deficiency was significantly more frequent in PU patients (75.7%) compared to controls (32.5%), with mean serum vitamin D levels markedly reduced (14.61 ± 6.74 ng/mL vs. 26.22 ± 9.00 ng/mL). *H. pylori* infection was more common in PU patients (58.6%). Additionally, PU patients exhibited elevated lipid profile parameters. No statistically significant sex-based differences were observed within either group. In conclusion, serum vitamin D levels appear to be a strong predictor of PU severity, suggesting a potential role for vitamin D status in PU pathogenesis and clinical management.

1. Introduction

Peptic ulcers (PUs), defined as open

sores occurring in the stomach or duodenum, continue to pose a substantial global health burden. Their

pathogenesis involves a multifaceted interaction among gastric acid secretion, mucosal barrier integrity, and *Helicobacter pylori* (*H. pylori*) infection. Recent studies have also highlighted the potential influence of various nutrients and biomarkers on the development and severity of PUs¹.

Vitamin D, traditionally recognized for its role in calcium homeostasis and bone metabolism, is increasingly acknowledged for its immunomodulatory functions. It is synthesized in the skin upon exposure to ultraviolet radiation and subsequently converted into its active form, calcitriol, *via* hepatic and renal hydroxylation. Calcitriol exerts its biological effects through the vitamin D receptor, which is expressed in multiple tissues, including the gastrointestinal tract. Emerging evidence suggests that vitamin D deficiency may elevate the risk of gastrointestinal disorders such as inflammatory bowel disease and PUs. One proposed mechanism by which vitamin D may influence the PU pathogenesis is through the modulation of the immune response². Vitamin D regulates the activity of immune cells including macrophages and T lymphocytes, which are integral to inflammation control and mucosal repair. This is particularly relevant in the context of *H. pylori* infection; a primary etiological factor in PU disease. Studies have demonstrated that vitamin D can inhibit *H. pylori* growth and attenuate its virulence.

On the other end, vitamin D deficiency has been associated with increased mucosal inflammation, delayed ulcer healing, and heightened risk of complications. It may contribute to PU development and exacerbation by impairing immune defences and compromising mucosal integrity³. The present study aimed at evaluating the serum vitamin D levels as a predictive marker associated with increased risk and severity of PUs.

2. Methodology

This cross-sectional study included 110 patients (both male and female), aged 20–65 years, diagnosed with PUs. Samples were collected between July and October 2024 from the Morgan Teaching Hospital and the Imam Sadiq Hospital in the Babil Province

of Iraq. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Verbal and analytical consent was obtained from all participants prior to sample collection. The study protocol, participant information sheet, and consent form were reviewed and approved by the local ethics committee under document number 2541, dated 11 May 2023.

Each participant underwent upper gastrointestinal endoscopy for diagnosis and evaluation of PUs. During the procedure, an endoscope was inserted orally in order to visualize the stomach and the duodenum. Venous blood samples (5 mL) were collected post-endoscopy for biochemical screening. Blood was placed in gel tubes, centrifuged at 3,000 rpm for 5 min in order to separate the serum, which was then aliquoted into Eppendorf tubes and stored at -20°C until the vitamin D quantification by enzyme-linked immunosorbent assay (ELISA), using a kit supplied by Bioassay Technology Laboratory Company.

Statistical analyses were performed using IBM SPSS version 28. Nominal data were reported as frequencies and percentages, with comparisons made using the chi-square test. Numerical data were expressed as mean $\pm$ standard deviation (SD), and group comparisons were conducted using independent *t*-tests, analysis of variance (ANOVA), and *post hoc* tests. A *p*-value <0.05 was considered statistically significant.

3. Results and Discussion

Table 1 presents the demographic and clinicopathological characteristics of participants with and without a PU. No significant difference was observed in age distribution between PU and non-PU groups ($p=0.970$). Females comprised a higher proportion of PU patients (55.7%) than males (44.3%), although this difference was not statistically significant ($p=0.113$). Body mass index (BMI) was significantly lower in PU patients ($p<0.001$). Notably, 72.9% of the PU patients were underweight or of normal weight, compared to 30.0% in the non-PU group, whereas overweight and obesity were more prevalent among individuals without PU (70.0%) than those with PU

Table 1. Demographic and clinicopathological characteristics of all studied participants with and without peptic ulcer (PU). Other abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation; VLDL, very-low-density lipoprotein.				
Demographic and clinical characteristics		Groups		p-value (chi-square)
		PU	Without PU	
Age	mean ± SD (in years)	40.54 ± 16.19	37.3 ± 11.81	0.270
	≤ 40 years (in N (%))	44 (62.9%)	25 (62.5%)	0.970 (0.001)
	> 40 years (in N (%))	26 (37.1%)	15 (37.5%)	
Sex	male (in N (%))	31 (44.3%)	24 (60.0%)	0.113 (2.51)
	female (in N (%))	39 (55.7%)	16 (40.0%)	
Body max index (BMI)	mean ± SD (in kg/m ²)	21.84 ± 3.39	26.61 ± 3.86	<0.001
	<25 kg/m ² (in N (%))	51 (72.9%)	12 (30.0%)	<0.001 (19.11)
	>25 kg/m ² (in N (%))	19 (27.1%)	28 (70.0%)	
Vitamin D status	<20 ng/mL (in N (%))	53 (75.7%)	13 (32.5%)	<0.001 (19.81)
	≥20 ng/mL (in N (%))	17 (24.3%)	27 (67.5%)	
<i>H. pylori</i>	Ve+ (in N (%))	41 (58.6%)	10 (25.0%)	<0.001 (11.54)
	Ve- (in N (%))	29 (41.4%)	30 (75.0%)	
Type of PU	none (in N (%))	0 (0%)	40 (100%)	0.137 (3.97)
	duodenal ulcer (in N (%))	18 (25.7%)	0 (0%)	
	gastric ulcer (in N (%))	31 (44.3%)	0 (0%)	
	duodenal and gastric (in N (%))	21 (30.0%)	0 (0%)	
Cholesterol levels	mean ± SD (in mg/dL)	179.84 ± 44.74	157.96 ± 33.59	0.008
Triglyceride levels	mean ± SD (in mg/dL)	184.87 ± 70.63	147.71 ± 52.6	0.005
LDL-cholesterol levels	mean ± SD (in mg/dL)	110.32 ± 39.08	91.78 ± 31.2	0.012
VLDL-cholesterol levels	mean ± SD (in mg/dL)	36.97 ± 14.13	29.54 ± 10.54	0.004
HDL-cholesterol levels	mean ± SD (in mg/dL)	32.55 ± 6.92	36.64 ± 10.96	0.018
Vitamin D levels among studied categories				
Vitamin D levels	mean ± SD (in ng/mL)	14.61 ± 6.74	26.22 ± 9.00	<0.001
Age	≤40 years	17.56 ± 6.34	27.39 ± 10.38	<0.001
	>40 years	9.61 ± 3.83	24.29 ± 5.89	
p-value		<0.001	0.177	
Sex	male	15.51 ± 7.2	26.3 ± 9.09	<0.001
	female	13.89 ± 6.34	26.11 ± 9.16	
p-value		0.381	0.938	
<i>H. pylori</i>	Ve+	12.65 ± 5.65	24.37 ± 12.5	<0.001
	Ve-	17.37 ± 7.26	26.84 ± 7.67	
p-value		0.010	0.366	
Vitamin D status	<20 ng/mL	11.65 ± 4.6	16.77 ± 1.54	<0.001
	≥20 ng/mL	23.8 ± 2.84	30.78 ± 7.36	
p-value		<0.001	<0.001	

(27.1%) ($p<0.001$).

Vitamin D deficiency, defined as serum levels <20

ng/mL, was significantly more prevalent in PU patients (75.7%) than in controls (32.5%) ($p<0.001$).

Mean serum vitamin D levels were markedly lower in PU patients (14.61 ± 6.74 ng/mL) compared to non-PU individuals (26.22 ± 9.00 ng/mL) ($p < 0.001$). *H. pylori* infection was significantly more common among PU patients (58.6%) than in controls (25.0%) ($p < 0.001$). Among PU patients, 44.3% had gastric ulcers, 25.7% had duodenal ulcers, and 30.0% had both types, though these differences were not statistically significant ($p = 0.137$).

The undertaken lipid profile analysis revealed significantly elevated levels of total cholesterol ($p = 0.008$), triglycerides ($p = 0.005$), low-density lipoprotein cholesterol (LDL-cholesterol; $p = 0.012$), and very-low-density lipoprotein cholesterol (VLDL-cholesterol; $p = 0.004$) in PU patients. High-density lipoprotein cholesterol (HDL-cholesterol) levels were significantly lower in PU patients compared to controls ($p = 0.018$).

Vitamin D levels were significantly reduced in PU patients aged ≤ 40 years (9.61 ± 3.83 ng/mL) compared to those aged > 40 years (17.56 ± 6.34 ng/mL) ($p < 0.001$). No significant age-related differences were observed in the non-PU group (27.39 ± 10.38 vs. 24.29 ± 5.89 ng/mL). Sex-based comparisons revealed no significant differences in vitamin D levels within either group: PU males (15.51 ± 7.20 ng/mL) vs. PU females (13.89 ± 6.34 ng/mL), and non-PU males (26.30 ± 9.09 ng/mL) vs. non-PU females (26.11 ± 9.16 ng/mL).

Among the PU patients, those positive for *H. pylori* had significantly lower vitamin D levels (12.65 ± 5.65 ng/mL) compared to *H. pylori*-negative individuals (17.37 ± 7.26 ng/mL; $p = 0.010$). No significant difference in vitamin D levels was observed between *H. pylori*-positive and -negative individuals in the non-PU group (24.37 ± 12.50 vs. 26.84 ± 7.67 ng/mL).

These findings reinforce the association between vitamin D deficiency and PU, particularly in the context of *H. pylori* infection. Vitamin D deficiency may impair immune responses, increase susceptibility to *H. pylori*, and influence treatment outcomes. Prior

studies have corroborated this relationship, suggesting that adequate vitamin D levels may confer protection against *H. pylori* infection^{4,5}.

Age-stratified analyses have revealed that vitamin D deficiency was more pronounced in PU patients under 40 years of age, which is consistent with previous findings⁶. Although recent trends suggest an increasing prevalence of PUs among women, this study has found no significant sex-based differences in *H. pylori* infection rates, thereby contrasting with reports indicating higher infection rates among men⁷. Additionally, the PU patients in this study exhibited elevated cholesterol levels, in disagreement with findings that suggest reduced cholesterol levels in PU patients⁸.

4. Conclusion

PU patients – particularly those who are younger, vitamin D-deficient, and *H. pylori*-positive – appear to be at increased risk of disease severity. Age, triglycerides, VLDL-cholesterol, and BMI were inversely associated with vitamin D levels. These findings support the role of vitamin D as a predictive biomarker for PU severity.

Acknowledgements

The authors extend sincere gratitude to the consultant physician and the staff of the Digestive System Center, and to all individuals who participated in this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Majid Z., Sharba I.R. Low serum vitamin D levels as a risk factor for peptic ulcer development in Iraqi patients. *Pharmakeftiki* 37(2s), 330-334, 2025. <https://doi.org/10.60988/p.v37i2S.221>

Molecular docking studies of new thiazolidin-4-one derivatives as combretastatin A4 analogues

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KEY WORDS:

anti-cancer;
combretastatin A4; tubulin;
thiazolidin-4-one; molecular
docking

ARTICLE INFO:

Received: January 12, 2025

Revised: February 25, 2025

Accepted: February 26, 2025

Available online: October 10, 2025

ABSTRACT

In the present study, a series of thiazolidin-4-one derivatives were designed as analogues of combretastatin A4 (CA4); a known anti-tubulin agent. The molecular structures incorporate either a 3,4,5-trisubstituted phenyl ring or a 3-alkoxy-4-methoxyphenyl ring, with the two aromatic systems connected *via* an amide linkage and a thiazolidin-4-one scaffold. Structural variation was introduced through different substituents (R groups) on the 4-methoxyphenyl ring. All compounds, along with CA4 as the reference molecule, were subjected to molecular docking studies targeting the tubulin β -2B chain (Protein Data Bank code: 8QEA). The docking results revealed that the newly synthesized molecules exhibit binding affinities and inhibition constant (K_i) values comparable to those of CA4. These findings suggest that the designed thiazolidin-4-one derivatives warrant further experimental investigation for their potential as anti-tubulin (i.e., anti-cancer) agents.

1. Introduction

Chemotherapeutic agents remain among the most effective strategies for cancer treatment. Several anti-cancer compounds exert their effects through distinct mechanisms, including interaction with structural

proteins such as tubulin; a key component in the mitotic process. Tubulin polymerizes to form microtubules, which are essential for cell division^{1,2}. Accordingly, the inhibition of tubulin polymerization by specific molecules can induce apoptosis and arrest the cell cycle

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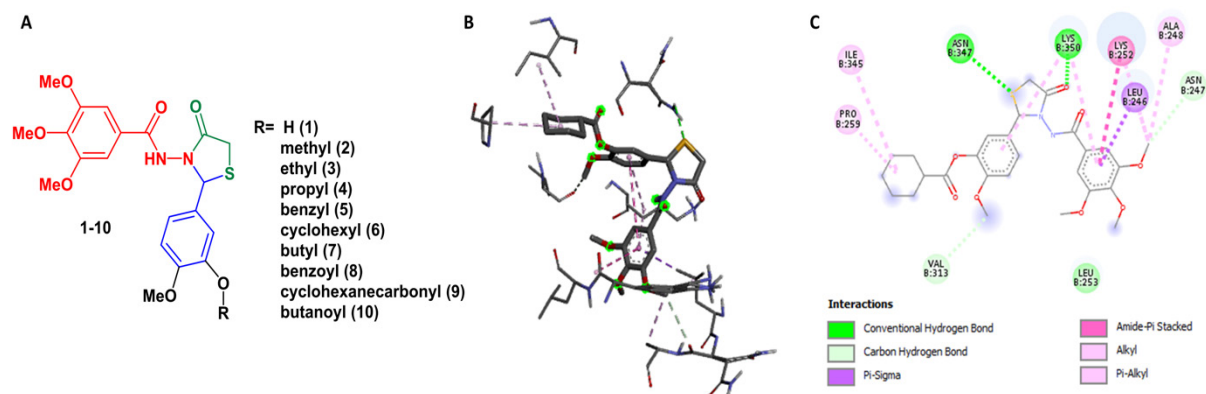


Figure 1. (A): General structure of the designed compounds 1–10. (B): The best pose of compound 9 at the combretastatin A4 binding site, in 3D. (C): The best pose of compound 9 at the combretastatin A4 binding site, in 2D.

at the G2/M phase, thereby suppressing cancer cell proliferation^{3,4}.

Taxol and Vinca alkaloids are well-known inhibitors of tubulin polymerization; however, their clinical utility is limited by adverse effects and the emergence of drug resistance⁵. Combretastatin A4 (CA4), a natural compound, is recognized as a potent tubulin inhibitor. Its structure comprises a 3,4,5-trimethoxyphenyl ring and a 3-hydroxy-4-methoxyphenyl ring connected *via* a cis-configured double bond (C=C), which facilitates an interaction with the β -tubulin subunit at the colchicine-binding site, thereby inhibiting tubulin polymerization⁶. This mechanism not only disrupts microtubule formation, but also selectively inhibits angiogenesis by obstructing tumor-associated blood supply.

Moreover, CA4 and its analogues exhibit promising activity against multidrug-resistant cancer phenotypes. Nonetheless, the compound's therapeutic potential is hindered by the isomerization of its active *cis*-isomer to the biologically inactive *trans*-isomer⁷. Consequently, there is growing interest in the development of structurally related molecules that retain the pharmacophoric features of CA4 while offering enhanced stability, potency, and selectivity.

Molecular docking is a widely employed computational technique for evaluating the interaction of

designed compounds with biological targets. In the present study, we report a series of 1,3-thiazolidin-4-one derivatives as structural analogues of CA4, and investigate their binding interactions with the CA4 site on the tubulin β -2B chain using molecular docking simulations.

2. Methodology

The three-dimensional structure of the tubulin β -2B chain (PDB code: 8QEA) was retrieved from the Protein Data Bank in PDB format. Ligands, water molecules, and chains A and C were removed by using the BIOVIA Discovery Studio Visualizer. Polar hydrogen atoms and Kollman charges were added by using AutoDock Tools. Docking parameters were set as follows: exhaustiveness: 50; grid box center coordinates (x, y, z): 11.39, -16.58, and 17.42; grid box dimensions (x, y, z): 16.22, 18.72, and 18.18, respectively.

Density Functional Theory (DFT) calculations were performed by using the ORCA 5.0.3 software package⁸. Ligands were fully optimized by using the BP86 functional in conjunction with the def2-TZVP basis set for all atoms, and the auxiliary basis set of Weigend (def2/J)^{9,10}.

3. Results and Discussion

A series of thiazolidin-4-one derivatives were de-

signed as structural mimics of CA4. Each molecule features either a 3,4,5-trimethoxyphenyl or a 3-alkoxy-4-methoxyphenyl ring, connected *via* an amide group and a 1,3-thiazolidin-4-one scaffold.

Molecular docking simulations were conducted for ligands 1–10 against the CA4 (*cis*) binding site of the tubulin β -2B chain (PDB code: 8QEA). The calculated binding affinities (in kcal/mol) for the ligands 1–10 were -7.3, -7.2, -7.2, -7.3, -7.6, -7.7, -7.1, -7.9, -8.2, and -7.6, respectively. For CA4, the binding affinity was -7.4 kcal/mol. These results indicate that all designed compounds exhibit favourable binding interactions comparable to CA4.

Ligand 9 demonstrated the most favourable binding pose, with a binding affinity of -8.2 kcal/mol (Figure 1). Its interaction with the target protein (8QEA) involved two hydrogen bonds: ASN₃₇₄ with the sulfur atom (2.69 Å) and LYS₃₅₀ with the oxygen atom (2.49 Å). Additionally, two carbon-hydrogen interactions were observed: ASN₂₇₄ with the methoxy group's carbon atom (3.77 Å) and VAL₃₁₃ with another methoxy carbon (3.55 Å).

The inhibition constant (K_i) values were calculated using the equation $K_i = \exp(\Delta G/RT)$, yielding the following results (in μ M) for ligands 1–10: 4.457, 5.276, 5.276, 4.457, 2.686, 2.269, 6.247, 1.619, 0.976, and 2.686, respectively. Ligand 9 exhibited the lowest K_i value, thereby indicating the highest predicted efficacy.

4. Conclusion

A series of 1,3-thiazolidin-4-one derivatives were developed as structural analogues of CA4 and were evaluated through molecular docking against the CA4 (*cis*) binding site of the tubulin β -2B chain (PDB code: 8QEA). All compounds demonstrated favourable binding affinities and inhibition constants, with ligand 9 (featuring a cyclohexanecarbonyl substituent) exhibiting the most potent interaction profile. These findings support the potential of these compounds for further experimental validation as anti-cancer agents.

Acknowledgements

The authors gratefully acknowledge the support of the College of Pharmacy of the University of Babylon.

Conflicts of interest

None exist.

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HOW TO CITE:

Makki S., Abd Al-Hussein B., Abd Al-Zahra S., Razak A., Obies M., Balakit A., Makki T. Molecular docking studies of new thiazolidin-4-one derivatives as combretastatin A4 analogues. *Pharmakeftiki* 37(2s), 335-338, 2025. <https://doi.org/10.60988/p.v37i2S.222>

Association between ABO blood groups and Müllerian anomalies: a single-center cohort study of 16,690 infertile women

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KEY WORDS:

Müllerian anomaly; ABO blood groups; Rh blood group; uterus; congenital

ARTICLE INFO:

Received: January 25, 2025

Revised: February 07, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

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ABSTRACT

This study investigated the relationship between ABO blood groups and Müllerian anomalies in infertile women evaluated at the Teba Infertility Center (TIC) in Babylon, Iraq. Conducted over a three-month period beginning in August 2024, the study retrospectively included infertile couples who attended TIC between 2016 and 2022. A total of 1,215 archived records were reviewed from among 16,690 infertile females diagnosed with Müllerian uterine anomalies. Comprehensive patient reports were compiled, and three-dimensional (3D) sonographic examinations were performed in order to classify uterine morphology according to the standards of the European Society of Human Reproduction and Embryology / European Society for Gynaecological Endoscopy (ESHRE/ESGE). All participants were assessed for ABO blood group and Rhesus (Rh) factor positivity in order to explore potential associations with specific uterine anomalies. Statistical analyses were conducted using the SPSS software, applying frequency and percentage distributions, mean values with standard deviations, and comparative tests for non-normally distributed and categorical variables. The findings revealed significant associations between ABO blood groups and the presence of Müllerian anomalies. Notably, individuals with blood group O exhibited a higher prevalence

lence of these abnormalities. These results underscore the relevance of ABO typing in reproductive health assessments and suggest that women with specific blood group profiles may benefit from targeted diagnostic and clinical interventions. Further research is warranted in order to validate these associations and elucidate the underlying biological mechanisms.

1. Introduction

Müllerian anomalies arise from the abnormal development of the female reproductive tract. These congenital conditions affect approximately 7% of the general population, with prevalence rising to 25% among women experiencing infertility and recurrent miscarriage¹. A deeper understanding of Müllerian anomalies and their implications for fertility is essential in order to elucidate their aetiologies and associated risk factors.

The ABO blood group system is determined by specific antigens on the surface of red blood cells and is encoded on chromosome 9 at locus 9q34. Research has demonstrated that ABO blood types influence physiological processes and disease susceptibility, including both oncologic and non-oncologic conditions². A large-scale retrospective study involving 482,914 individuals found significant associations between ABO blood groups and Rhesus (Rh) factor status with various disease risks across the population³.

Despite these findings, few studies have explored the relationship between ABO blood groups and Müllerian anomalies, and comprehensive investigations remain scarce. It is known, for example, that parental blood group AB may influence the outcomes of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in Chinese women⁴. However, the impact of ABO blood groups on congenital uterine anomalies has not been thoroughly examined. Given the potential implications for reproductive health, further research is warranted to investigate the association between ABO blood types and Müllerian anomalies. The present study aims at

evaluating this relationship among female infertility patients attending a tertiary care clinic in Babylon, Iraq, with the goal of identifying novel risk factors and informing more effective diagnostic and therapeutic strategies.

2. Methodology

Building upon prior investigations into infertility^{5,6}, this cross-sectional descriptive study was conducted at the Teba Infertility Center (TIC) in Babylon, Iraq. The study spanned a three-month period beginning in August 2024 and included infertile couples who attended TIC between 2016 and 2022. From a total of 16,690 patients, 1,215 infertile women diagnosed with Müllerian anomalies were selected for analysis. Each participant's medical, gynaecological, and obstetric history was reviewed in detail. Uterine morphology was assessed using two-dimensional (2D) and three-dimensional (3D) transvaginal sonography so as to identify Müllerian anomalies, which were classified according to the standards of the European Society of Human Reproduction and Embryology / European Society for Gynaecological Endoscopy (ESHRE/ESGE)⁵. Blood group and Rh factor status were determined for all patients with diagnosed uterine anomalies. The evaluation protocol included 2D/3D vaginal ultrasonography, complete blood count, coagulation profile, electrolyte panel, and serum human chorionic gonadotropin (hCG) testing. All clinical data were meticulously documented.

Data extraction was performed by using Microsoft Excel, and statistical analyses were conducted by using the SPSS software. Mann-Whitney U tests were applied to non-normally distributed variables,

Table 1. Distribution of the characteristics of congenital uterine anomalies (age, ABO, Rh, types of anomalies) and their correlations among women attending the Teba Infertility Center.

Variable	Categories	N	%	R or (B)	p-value
Age groups	<20 years	5	0.4%	-	-
	20–30 years	237	19.5%	-	-
	30–40 years	594	48.9%	-	-
	40–50 years	355	29.2%	-	-
	≥50 years	24	2.0%	-	-
Unicornuate uterus	Rh-positive	23	1.87%	0.18	0.045
	Rh-negative	4	0.33%	-0.12	0.067
Bicornuate uterus	Rh-positive	12	1.02%	0.22	0.012
	Rh-negative	2	0.18%	-0.08	0.091
T-shape	Rh-positive	70	5.78%	0.15	0.033
	Rh-negative	12	1.02%	-0.1	0.056
Septum	Rh-positive	926	76.33%	0.3	<0.001
	Rh-negative	164	13.47%	-0.05	0.045
Correlation of Rh groups with uterine anomalies	Rh-positive	1,033	85.0%	0.25	<0.001
	Rh-negative	182	15.0%	-0.18	0.045
Correlation of detailed blood groups with uterine anomalies	O	450	37.0%	0.12	0.045
	B	328	27.0%	-0.05	0.22
	A	340	28.0%	0.08	0.13
	AB	97	8.0%	0.02	0.57
Detailed association of uterine anomalies with blood group	Unicornuate	24	2.0%	0.21	0.012
	Bicornuate	12	1.0%	0.14	0.034
	T-shape	85	7.0%	0.18	0.025
	Septum	1,094	90.0%	0.15	0.045

while Fisher's exact and chi-square tests were used in order to assess associations between categorical variables, with statistical significance set at $p < 0.05$. Categorical data were expressed as frequencies and percentages. The study protocol was approved by the Institutional Review Committee of the College of Pharmacy of the University of Babylon (approval number: A0040 / 2023).

3. Results and Discussion

This study evaluated the association between ABO blood groups and congenital Müllerian anomalies among women attending a tertiary infertility center. The data revealed significant correlations between specific blood groups and types of uterine anomalies, with particular emphasis on Rh factor status.

Age distribution analysis showed that the majority of participants were aged 30–40 years (48.9%), followed by those aged 40–50 years (29.2%). These findings are consistent with previous studies indicating that reproductive tract anomalies are more prevalent in these age groups. Among 200 infertile women aged 15–45 years, the incidence of uterine anomalies was 6%, with a mean age of approximately 30 years⁷. However, the incidence was not significantly higher in the 30–40-year age group compared to other age ranges. Congenital uterine anomalies affect an estimated 5% of the general population, with prevalence rates of 0.5% among fertile women and 3.5% among infertile women⁸. Notably, women under 20 years of age represented only 0.4% of cases, thereby suggesting that Müllerian anomalies are infrequently diagnosed in this demographic (Table 1).

Rh-positive individuals exhibited a markedly higher prevalence of septate uterus (76.33%) compared to Rh-negative individuals (13.47%; $p < 0.001$). This finding aligns with prior research linking Rh positivity to adverse pregnancy outcomes. Rh-negative women have been reported to experience increased rates of preeclampsia and restricted foetal growth⁹, although a larger cohort study found no significant differences in pregnancy outcomes between Rh-positive and Rh-negative women¹⁰.

In terms of ABO distribution, uterine anomalies were most prevalent among patients with blood group O (37%), followed by groups A (28%) and B (27%). Group AB accounted for 8% of cases (Table 1). These results suggest a higher susceptibility to Müllerian anomalies among individuals with blood group O².

The most common anomaly identified was a septate uterus (90%), followed by a T-shaped uterus (7%) and an unicornuate uterus (2%). A septate uterus was disproportionately observed in Rh-positive patients, prompting further investigation into the underlying mechanisms of susceptibility.

Statistical analyses revealed strong associations between specific blood groups and distinct types of uterine anomalies. An unicornuate uterus was consistently associated with blood group O, while a T-shaped uterus was more frequently observed in Rh-positive individuals. These findings support the hypothesis that ABO and Rh blood group systems may contribute to the pathogenesis of Müllerian anomalies, although further research is needed in order to elucidate the biological pathways involved.

Understanding the relationship between blood group profiles and Müllerian anomalies may enhance clinical decision-making in reproductive medicine. Identifying blood type-related risk factors can facilitate personalized diagnostic protocols and early therapeutic interventions for women with suspected reproductive tract anomalies. Healthcare providers

should consider genetic and haematologic profiles when evaluating patients for uterine anomalies. Expanding research to include diverse populations will be essential in order to validate these associations and to potentially inform blood group-based therapeutic approaches for reproductive disorders.

4. Conclusion

The present study highlights significant associations between ABO blood groups and Müllerian anomalies, with implications for reproductive health. Incorporating blood group information into the diagnostic and therapeutic framework may improve outcomes for women with congenital uterine anomalies. Identifying individuals at elevated risk enables clinicians to implement targeted testing and treatment strategies aimed at enhancing fertility and pregnancy success. Further research is warranted in order to clarify the mechanisms underlying these associations and to develop evidence-based interventions.

Acknowledgements

The authors express their gratitude to the administrative staff of the Teba Infertility Center for their support.

Conflicts of interest

None exist.

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HOW TO CITE:

Meghil S., Al-Bdairi A.A., Al-Hindy H.A.A.M., Khedhair S.H., Shawki O., Alkhalidi B., Kareem H.K. Association between ABO blood groups and Müllerian anomalies: a single-center cohort study of 16,690 infertile women. *Pharmakeftiki* 37(2s), 339-343, 2025. <https://doi.org/10.60988/p.v37i2S.223>

Exploring the antibacterial potential of novel fenamate-based derivatives: insights from molecular docking studies

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KEY WORDS:

**antibacterial; fenamate;
antimicrobial resistance;
fatty acids; docking**

ARTICLE INFO:

Received: January 11, 2025

Revised: February 12, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

The rapid emergence of antibiotic-resistant bacteria poses a critical global health threat, highlighting the urgent need for novel antimicrobial agents. In this context, fenamate-based drugs (non-steroidal anti-inflammatory compounds containing a fenamic acid scaffold) have recently gained attention as promising antibacterial candidates. These agents have demonstrated potent activity against a spectrum of pathogenic bacteria, including multidrug-resistant (MDR) strains. In the present study, several newly synthesized derivatives incorporating a fenamate moiety were evaluated for their antibacterial properties against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* by using the agar diffusion technique. Most of the fenamate derivatives exhibited notable antibacterial activity, particularly against *E. coli*. Compounds 1, 3, and 5 showed the highest inhibitory effects, with compound 3 demonstrating the most potent activity, effectively suppressing all tested bacterial strains at concentrations as low as 250 µg/mL. These findings underscore the therapeutic potential of fenamate-based compounds in combating antibiotic resistance. Their capacity to overcome MDR mechanisms, possible synergistic interactions with existing antibiotics, and additional immunomodulatory properties render them compelling candidates for further investigation as next-generation antimicrobial therapies.

1. Introduction

The rise of antibiotic-resistant bacteria has become a major global health concern in recent decades. Infections caused by multidrug-resistant (MDR) pathogens are increasingly common and pose a significant threat to public health¹. Certain bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae* (CRE), and *Clostridioides difficile*, have developed resistance to many (or in some cases all) available antibiotic treatments, making them extremely difficult to eradicate².

The World Health Organization has warned that without the development of new antibacterial agents, we risk entering a “post-antibiotic era” in which common infections and minor injuries could become life-threatening. This underscores the urgent need for innovative strategies to combat the growing threat of antibiotic resistance.

The rapid evolution of antibiotic resistance is driven by several factors, including the overuse and misuse of antibiotics, inadequate infection control practices, and a stagnation in antibiotic development within the pharmaceutical industry³. To address this critical issue, substantial investment and collaborative research efforts are required to stimulate the discovery of new antibacterial agents. Promising approaches include targeting bacterial virulence factors, exploiting metabolic pathways, and leveraging the human microbiome.

At the forefront of this search is β -ketoacyl-acyl carrier protein synthase III (FabH); a pivotal enzyme in *Escherichia coli* that catalyses the initial condensation reaction in fatty acid biosynthesis⁴. FabH is essential for the production of fatty acids that maintain bacterial membrane integrity and overall cellular function, making it a compelling target for antibiotic development. The inhibition of FabH could disrupt fatty acid synthesis and thereby offer a strategic route to novel antibacterial agents⁵.

In this context, tolfenamic acid and other fenamate-based drugs have emerged as promising candidates for antibacterial development. Tolfenamic acid is a nonsteroidal anti-inflammatory drug (NSAID) be-

longing to the fenamate class. Recent studies have shown that tolfenamic acid and its structural analogues exhibit potent antibacterial activity against a range of pathogenic bacteria, including MDR strains⁶. Proposed mechanisms of action include disruption of bacterial membranes, inhibition of key enzymes, and interference with quorum sensing; a signalling system critical for bacterial communication and virulence⁶.

Fenamate-based drugs have demonstrated activity against resistant strains such as MRSA and CRE⁷, suggesting their potential utility in treating infections caused by drug-resistant pathogens. Furthermore, tolfenamic acid and its derivatives have shown synergistic effects when combined with conventional antibiotics, enhancing the efficacy of these agents⁷. Such combination therapies may help revitalize existing antibiotics and delay the emergence of further resistance. In addition to their direct antibacterial properties, fenamate-based drugs possess anti-inflammatory and immunomodulatory effects, which may enhance the management of bacterial infections. By reducing inflammation and modulating host immune responses, these compounds could contribute to the overall effectiveness of antimicrobial treatment.

Although the development of tolfenamic acid and related fenamate-based antibacterial agents remains in its early stages, current evidence indicates significant promise. Continued research (including mechanistic studies, optimization of drug candidates, and clinical evaluation) is essential in order to fully assess their potential as novel antibacterial therapies.

2. Methodology

The titled compounds (Figure 1) were synthesized previously according to established procedures⁸. Newly synthesized derivatives were screened for antibacterial activity against selected bacterial strains: Gram-negative *E. coli* and *Pseudomonas aeruginosa*, and Gram-positive *S. aureus*, using the agar diffusion technique. Various concentrations of the derivatives, dissolved in dimethyl sulfoxide (DMSO), were placed individually into wells in the

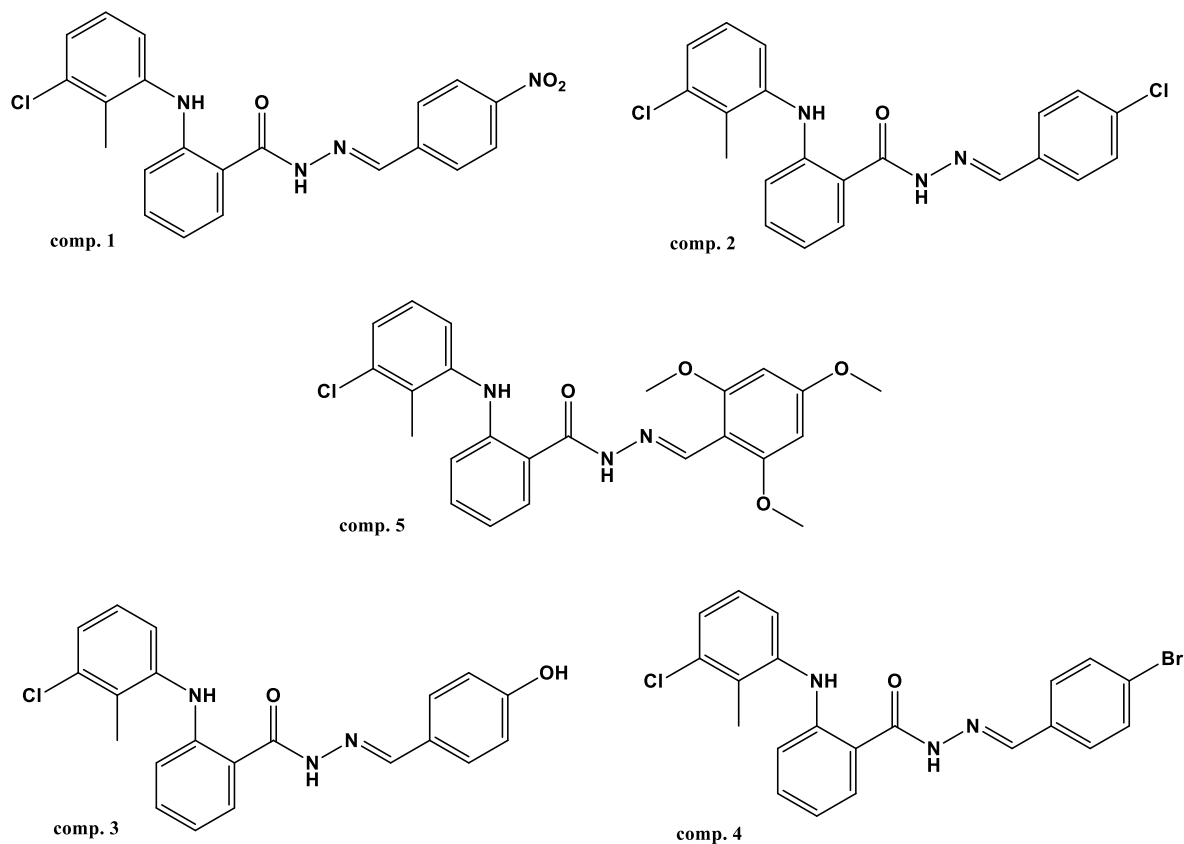


Figure 1. The synthesized compounds 1–5.

agar medium. All plates were incubated overnight at 37°C, and inhibition zones were measured after 24 h.

Chemical structures of the compounds were drawn using ChemDraw Office Suite 20.01 and converted to MOL format for dataset inclusion. Ligands were imported into Molecular Operating Environment (MOE) docking software and subjected to energy minimization using the AMBER force field in order to optimize atomic interactions within the compounds and between the compounds and the receptor.

The protein structure of FabH from *E. coli* (Protein Data Bank / PDB ID: 5BNS, complexed with a small-molecule inhibitor) was retrieved from the Research Collaboratory for Structural Bioin-

formatics PDB and prepared using MOE's default QuikPrep function. Selection criteria included a resolution of 2 Å or better and an R-value below 0.5 to ensure structural quality. Geometric parameters such as bond lengths and angles were verified, confirming that protein 5BNS met the necessary standards for docking studies⁹.

The active site was identified using MOE's Site Finder tool, which delineated the region of ligand-receptor interaction. This defined the docking area and isolated relevant atoms and backbone structures to facilitate rigid receptor docking. Docking simulations were performed by using default parameters, evaluating both visual interactions and ligand fit. A lower docking score indicates a more favourable interaction, reflecting

reduced energy requirements for ligand binding to the receptor's active site⁹.

3. Results and Discussion

Antibacterial activity assays revealed that most synthesized compounds exhibited substantial inhibitory effects against all tested microorganisms. At a concentration of 250 µg/mL, compounds 1 and 5 demonstrated the highest activity against *P. aeruginosa*, with inhibition zones of 2.4 cm and 1.4 cm, respectively, comparable to the reference antibiotic azithromycin (2.4 cm). Compound 3 strongly inhibited all tested bacteria at the same concentration, with inhibition zones of 2.0 cm (*E. coli*), 1.0 cm (*S. aureus*), and 1.9 cm (*P. aeruginosa*).

Docking analyses of compounds 1–5 against the active site of FabH (PDB ID: 5BNS) revealed favourable binding interactions. Visual inspection of two-dimensional receptor-ligand interactions and docking scores (representing binding free energy) indicated that compounds 4 and 3 exhibited strong receptor affinity, with scores of -7.094 kcal/mol and -6.874 kcal/mol, respectively. Compound 4 formed two hydrogen bonds, one hydrophobic interaction, and one water bridge with the receptor, while compound 3 formed two hydrogen bonds and two hydrophobic interactions. These docking results highlight the influence of aromatic side-chain substitutions on receptor binding affinity.

4. Conclusion

This study underscores the potential of novel antibacterial agents targeting FabH in *E. coli* as effective alternatives against resistant bacterial strains. Molecular docking analyses elucidate their binding modes, offering a foundation for further optimization. These promising findings suggest that continued development and synthesis of fenamate-containing compounds may represent a valuable direction for future antimicrobial research.

Acknowledgements

The authors gratefully acknowledge the Department of Pharmaceutical Chemistry of the College of Pharmacy of the University of Al-Ameed, for providing facilities for the present work.

Conflicts of interest

None exist.

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HOW TO CITE:

Mehihi A.A., Abdul Hussein S.A., Kubba A.A.R.M., Aswed A.S., Saeed A.M. Exploring the antibacterial potential of novel fenamate-based derivatives: insights from molecular docking studies. *Pharmakeftiki* 37(2s), 344-348, 2025. <https://doi.org/10.60988/p.v37i2S.224>

Efficacy of a *Cyperus rotundus* extract and antibiotics on bacteria isolated from urinary tract infections

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KEY WORDS:

***Cyperus rotundus*; extract; antibiotic sensitivity; urinary tract infection; antibiotics**

ARTICLE INFO:

Received: January 10, 2025

Revised: February 07, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

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ABSTRACT

Urinary tract infection (UTI), an inflammatory condition resulting from pathogenic invasion of the urinary tract, remains a prevalent clinical concern. This study aimed at isolating common uropathogenic bacterial strains from urine samples collected from patients attending consultation clinics at the Al-Imam Sadeq and the Al-Hilla Teaching Hospitals (Iraq) between October and December 2024. The objectives were twofold: (i) to evaluate the antimicrobial susceptibility profiles of the isolated strains, and (ii) to assess the antibacterial efficacy of *Cyperus rotundus* extract (CRE). The findings demonstrated that CRE at a concentration of 1,000 µg/mL inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*, alongside other locally isolated bacterial species. The minimum inhibitory concentration (MIC) of CRE was determined to be 62.5 µg/mL. Notably, *E. coli* and *P. aeruginosa* exhibited susceptibility to all tested antibiotics, whereas *Proteus mirabilis* showed resistance to all agents. Additional isolates, including *Staphylococcus* spp. and *Enterococcus faecalis*, displayed variable susceptibility patterns, with *E. faecalis* being sensitive to certain antibiotics and resistant to others.

1. Introduction

The emergence and proliferation of antibiotic-resistant bacteria have been significantly driven by the

misuse of antimicrobial agents in developed countries. Among the most frequent bacterial pathogens affecting humans is the Gram-negative bacterium *Escherichia coli*,

which is widely recognized as the principal etiological agent of urinary tract infections (UTIs). UTIs rank among the most prevalent health concerns globally, second only to respiratory tract infections, and encompass both hospital-acquired and community-acquired cases. Resistance to antimicrobial drugs is rising markedly, with recent studies reporting an increased incidence of antibiotic-resistant UTIs. This resistance complicates treatment, particularly in pregnant women, where therapeutic options are limited and the consequences may affect both maternal and foetal health¹.

Antibiotic resistance during pregnancy can lead to prolonged infections, elevated healthcare costs, and potential complications for the developing foetus and the mother. This underscores the critical need for judicious antibiotic use and the exploration of alternative therapeutic strategies². Accordingly, the investigation of novel antimicrobial agents derived from natural sources is increasingly viewed as essential in order to address the multifaceted health and socioeconomic challenges posed by drug-resistant bacteria³.

Research has increasingly focused on medicinal plants due to their notable antibacterial and bioactive properties. Unlike synthetic drugs, which are frequently misused and contribute to resistance, plant-based therapies offer a safer alternative for both human health and the environment⁴. *Cyperus rotundus*, also known as nut grass and belonging to the Cyperaceae family, is widely distributed in India and referred to locally as "Nagarmotha." *Cyperus rotundus* contains a rich array of bioactive compounds, including vitamin C, polyphenols, glycosides, flavonoids, tannins, alkaloids, saponins, terpenoids, essential oils, carbohydrates, and proteins⁵.

Traditionally, *Cyperus rotundus* has been employed for its pharmacological properties, including its anti-inflammatory, anti-adiposity, antidiabetic, and neuroprotective effects. It has also exhibited potent antioxidant and free-radical scavenging activities in various *in vitro* assays. *In vivo* studies have further revealed its anxiolytic and cognitive-enhancing effects in murine models of anxiety and hypobaric hypoxia^{6,7}. Given these therapeutic potentials, the

present study was designed in order to isolate uropathogenic bacteria and evaluate the antimicrobial efficacy of the *Cyperus rotundus* extract (CRE).

2. Methodology

Bacterial isolates were obtained from urine samples of patients attending urology consultation clinics at the Al-Imam Sadeq and the Al-Hilla Teaching Hospitals (Iraq) between October and December 2024. All patients were clinically diagnosed with UTIs and referred for urine culture and antimicrobial susceptibility testing.

Rhizomes of *Cyperus rotundus* were collected from a medicinal plant source in Najaf, Iraq, and authenticated by a qualified botanist. The rhizomes were washed, dried, and ground into a fine powder. Ethanol (300 mL) was employed in order to macerate 100 mg of the powdered material at room temperature (RT) for 24 h so as to ensure a thorough extraction of the bioactive constituents. The resulting extract was cooled, filtered through sterile paper, and oven-dried at 40°C for 24 h.

The ethanolic CRE was tested against four bacterial species: two Gram-positive strains (*Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC BBA-335) and two Gram-negative strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). All strains were obtained from UTI cases and commercially sourced.

Inocula were prepared from ten single colonies grown on Mueller-Hinton agar (MHA) for 24 h. Six serial dilutions of CRE (1,000, 500, 250, 125, 62.5, and 31.25 µg/mL) were tested using the broth microdilution method in 96-well microtiter plates. Colonies were suspended in 2 mL of sterile water, and the optical density at 600 nm (OD₆₀₀) was measured using a Biotech 800ST plate reader (Biotech, USA), yielding a final OD₆₀₀ of 0.236 (equivalent to the McFarland standard 0.67). The bacterial suspension (1 × 10⁶ CFU/mL) was diluted 100-fold, and 50 µL was inoculated into each well, excluding blank controls.

The half-maximal inhibitory concentration (IC₅₀) and minimum inhibitory concentration (MIC) values were determined in triplicate for both positive

Table 1. Antibiotic susceptibility for the herein assessed Gram-positive and Gram-negative bacteria. Abbreviations used: I, intermediate (susceptibility); R, resistant; S, sensitive.

Antibiotic	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Streptococcus spp.</i>
Cefoxitin screen	+	-	-
Benzylpenicillin	R	-	-
Ampicillin	-	R	R
Oxacillin	R	-	-
Imipenem	-	-	-
Gentamicin	-	R	S
Streptomycin	-	R	R
Gentamicin	S		-
Ciprofloxacin	R	S	S
Moxifloxacin	R	-	-
Inducible clindamycin resistance	-	-	-
Erythromycin	R	R	R
Clindamycin	S	-	-
Teicoplanin	S	R	R
Vancomycin	S	R	R
Tetracycline	R	R	R
Tigecycline	S	S	S
Fusidic acid	-	-	-
Rifampicin	S	-	-
Trimethoprim	R	-	-
Antibiotic	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ticarcillin	R	S	S
Ticarcillin and clavulanic acid	R	S	S
Piperacillin / tazobactam	R	S	S
Ceftazidime	R	S	S
Cefepime	R	S	S
Aztreonam	R	-	-
Imipenem	R	S	R
Meropenem	R	S	R
Amikacin	R	S	S
Gentamicin	R	S	S
Tobramycin	R	S	S
Ciprofloxacin	R	S	S
Pefloxacin	-	-	-
Minocycline	R	-	-
Colistin	-	S	I
Rifampicin	-	-	-
Trimethoprim / sulfamethoxazole	R	-	-

and negative controls. Each 96-well plate had a final volume of 225 μ L per well. Prior to adding the CRE stock solution (100 mg/mL), 100 μ L of broth medium was dispensed. MIC was defined as the lowest concentration at which no visible bacterial growth was observed following overnight incubation.

3. Results and Discussion

Bacterial strains isolated from UTI patients included *P. aeruginosa* (ATCC PAO1 and ATCC 27853), *S. pneumoniae* (ATCC BAA-334), and *E. coli* (ATCC 25922). The CRE at 1,000 μ g/mL inhibited the growth of *E. coli* and *P. aeruginosa*, as well as other locally isolated strains. The MIC of CRE was determined to be 62.5 μ g/mL, effectively suppressing the growth of *S. pneumoniae* and *Staphylococcus* spp. Previous studies corroborate these findings, reporting high antimicrobial activity of CRE against *S. aureus* and *Bacillus subtilis* (31 and 30 mm inhibition zones, respectively), and moderate activity against *Aspergillus niger* and *Candida albicans* (20 and 26 mm)⁸. Inhibition zones of 19 mm and 20 mm were observed against *E. coli* and *P. aeruginosa*, respectively. Additionally, aqueous leaf extracts and ethanolic rhizome-tuber extracts demonstrated antibacterial activity against *S. aureus* and *E. coli*, consistent with prior research⁹. However, the *Cyperus* oil showed no inhibitory effect on bacteria or fungi, including *C. albicans*.

Antibiotic susceptibility testing (Table 1) revealed that *Enterococcus faecalis* was sensitive to ciprofloxacin and tigecycline, but resistant to other agents. *Streptococcus* spp. were susceptible only to gentamicin, ciprofloxacin, and tigecycline. These results

align with earlier studies identifying ciprofloxacin as the most effective agent against *E. coli*, which exhibited universal susceptibility¹⁰. In contrast, *Proteus mirabilis* was resistant to all tested antibiotics, likely due to indiscriminate drug use or the presence of resistance-conferring genes. Further investigation is warranted in order to explore the clinical applicability of CRE in UTI management.

4. Conclusion

This study demonstrated that CRE possesses notable antibacterial activity against both Gram-positive and Gram-negative bacteria, with distinct MIC values. The findings support the potential of CRE as a natural therapeutic agent for UTIs, particularly in the context of rising antibiotic resistance.

Acknowledgements

The authors extend their gratitude to the staff of the Department of Pharmacology and Toxicology of the College of Pharmacy of the University of Babylon, for their invaluable support in completing this project.

Conflicts of interest

None exist.

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HOW TO CITE:

Mohammed H.R., Sabbar R., Jawad Y., Al-Hindy H.A.A.M. Efficacy of a *Cyperus rotundus* extract and antibiotics on bacteria isolated from urinary tract infections. *Pharmakeftiki* 37(2s), 349-353, 2025. <https://doi.org/10.60988/p.v37i2S.225>

Serum CA 19-9 as a potential biomarker for diabetic neuropathy: a comparative study among diabetic patients and healthy controls

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KEY WORDS:

vitamin D; CA 19-9; neuropathy; diabetes; outcomes

ARTICLE INFO:

Received: January 28, 2025

Revised: February 11, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

This study investigated the potential of serum carbohydrate antigen 19-9 (CA 19-9) as a biomarker for diabetic neuropathy (NP) by comparing its levels among patients with diabetes mellitus (DM) and NP, patients with DM without NP, and healthy controls. Participants were recruited between February and August 2022 at the Center of Diabetes, Merjan Medical City, Babil Province, Iraq. A total of 220 subjects were analysed. Serum CA 19-9 concentrations were significantly higher in the DM with NP group (21.99 ± 5.23 U/mL) compared to the DM without NP group (20.29 ± 4.67 U/mL) and healthy controls (9.65 ± 3.12 U/mL). Correlation analysis revealed a strong positive association between CA 19-9 and glycated haemoglobin (HbA1c) levels ($r=0.72$, $p<0.01$). Receiver operating characteristic curve analysis demonstrated high diagnostic accuracy, with an area under the curve of 0.95 for distinguishing DM with NP from healthy subjects. Logistic regression analysis suggested a positive, though statistically non-significant, association between CA 19-9 and NP. These findings support the potential role of CA 19-9 as a biomarker for the early detection of diabetic NP, underscoring its relevance in clinical practice to enhance DM management and patient outcomes. Further research is warranted in order to elucidate the underlying mechanisms and validate these findings across diverse populations.

1. Introduction

Diabetic neuropathy (NP) is a com-

mon complication of diabetes mellitus (DM), characterized by neuronal damage resulting from prolonged

hyperglycaemia. Diabetic NP affects approximately half of individuals with DM, contributing to significant morbidity and reduced quality of life¹. Early diagnosis and intervention are essential to prevent irreversible tissue damage and associated complications, such as foot ulcers and amputations². Current diagnostic approaches primarily rely on clinical assessments and nerve conduction studies; however, these methods can be invasive, time-consuming, and may not consistently yield prompt results¹.

Recent research has highlighted the potential utility of biomarkers in the early detection of DM-related complications. Among these, carbohydrate antigen 19-9 (CA 19-9) – a well-established marker of pancreatic malignancy³ – has emerged as a promising candidate due to its association with various metabolic disorders linked to DM^{2,4}. Elevated CA 19-9 levels have been observed in patients with DM, suggesting a connection to pancreatic inflammation or tissue injury, both of which are implicated in the pathophysiology of diabetic NP⁵.

This study aimed to evaluate serum CA 19-9 concentrations in DM patients with NP compared to healthy controls, thereby assessing its potential as a biomarker for early diabetic NP detection. By elucidating the relationship between serum CA 19-9 levels and diabetic NP, the findings may inform improved screening strategies and therapeutic interventions in DM. These results could also support further investigation into CA 19-9 as a non-invasive biomarker to enhance clinical management of diabetic complications.

2. Methodology

This case-control study was conducted between February and August 2022 at the Center of Diabetes, Merjan Medical City, Babil Province, Iraq. Biochemical analyses were performed at the College of Pharmacy of the University of Babylon. A total of 220 participants were enrolled, comprising 160 patients with type II DM and 60 healthy individuals. Diabetic patients were divided into two groups: group 1 (N=80) included those with NP, and group 2 (N=80) included those without NP.

Participants aged 30–70 years were selected based on medical history, clinical presentation, and nerve conduction study results. Exclusion criteria included type I DM, gestational DM, hepatic disease, chronic cardiac conditions, endocrine disorders, and chronic kidney disease. All participants were sex-matched to ensure group comparability.

Demographic data – including age, sex, duration of DM (months), weight (kg), height (m), and body mass index (BMI) – were recorded. Blood samples were collected for biochemical evaluation, including measurements of glycated haemoglobin (HbA1c), CA 19-9, and vitamin D₂. HbA1c was quantified using high-performance liquid chromatography (HPLC), while vitamin D₂ levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Elabsience® Biotechnology Ltd, USA). The CA 19-9 concentrations were determined using a chemiluminescence autoanalyzer (Snibe® Maglumi 800, China).

Ethical approval was granted by the local ethics committees of the Merjan Hospital and the Babylon Health Directorate (IRB A0030) in January 2022. Informed verbal consent was obtained from all participants prior to sample collection.

Statistical analyses were performed using Microsoft Excel (2017) and the IBM SPSS Statistics for Windows (version 28) software. Data are presented as mean ± standard deviation, with statistical significance set at $p < 0.05$. Analysis of variance (ANOVA) was used in order to detect differences among groups, while regression analysis assessed correlations. The Chi-square test evaluated categorical variables, and receiver operating characteristic (ROC) curve analysis was employed in order to further explore diagnostic performance.

3. Results and Discussion

This study assessed serum CA 19-9 concentrations as a potential biomarker for diabetic NP by comparing levels among patients with DM and NP, patients with DM without NP, and healthy controls. The data revealed significant differences in CA 19-9 levels across groups, as summarized in Table 1.

The DM with NP group exhibited a mean CA 19-9

Table 1. Comprehensive analysis of metabolic and diagnostic insights regarding the carbohydrate antigen 19-9 (CA 19-9) levels in diabetic neuropathy. Abbreviations used: AUC, area-under-the-curve; BMI, body mass index; DM, diabetes mellitus; HbA1c, glycated haemoglobin; NP, neuropathy.	
Category	Key insights
Descriptive statistics	CA 19-9 measures significantly higher in the subgroup of DM with NP (21.9 ± 5.2 U/mL), compared to the DM group without NP (20.3 ± 4.7 U/mL); the healthy group exhibited much lower concentrations (9.7 ± 3.1 U/mL)
Correlation analysis	CA 19-9 exhibited a robust direct correlation with HbA1c ($r=0.72$, $p<0.01$), signifying that increased CA 19-9 levels are related to poorer glycemic control; the correlation with BMI was weak but significant ($r=0.15$, $p=0.02$); additionally, CA 19-9 exhibited a moderate inverse correlation with vitamin D ₂ ($r=-0.601$, $p<0.01$), highlighting a negative relationship
Comparative statistics	Analysis of variance revealed statistically significant differences in the CA 19-9 levels across the three groups ($p<0.01$); <i>post-hoc</i> testing showed that the most pronounced difference was between the DM with NP group and the control group
Receiver operating characteristic analysis	High diagnostic accuracy of CA 19-9 for DM with NP vs. the control: the AUC was 0.95 (95% CI: 0.92–0.98), with a cut-off value of 15.3 U/mL, giving a 91% sensitivity and a 93% specificity; for DM without NP vs. healthy control, the AUC was 0.85 (95% CI: 0.80–0.90) with a 14.8 U/mL value cut off, providing a 78% sensitivity and a 81% specificity; for all DM vs. the control, the AUC was 0.95 (95% CI: 0.92–0.98), with a cut-off value of 14.9 U/mL, yielding a sensitivity of 89% and a specificity of 90%
Logistic regression analysis	CA 19-9 had a positive coefficient (1.34), indicating a potential association with increased odds of neuropathy; however, the result was not statistically significant ($p=0.329$); other variables like HbA1c ($p=0.364$), BMI ($p=0.423$), and vitamin D ₂ ($p=0.657$) also showed no significant associations with neuropathy in this analysis

level of 21.9 ± 5.2 U/mL, significantly higher than the DM without NP group (20.3 ± 4.7 U/mL). Healthy controls demonstrated markedly lower levels (9.7 ± 3.1 U/mL). Previous studies have reported elevated CA 19-9 levels in approximately 14% of DM patients with poor glycaemic control⁴. Diabetic NP is associated with increased oxidative stress and inflammatory markers, which may influence CA 19-9 concentrations².

Elevated CA 19-9 levels have been linked to the severity of diabetic complications, independent of malignancy⁶. The biomarker has also shown correlations with risk factors for diabetic retinopathy, suggesting a role in microvascular complications⁷. Additionally, high-normal serum carcinoembryonic antigen levels have been proposed as indicators of diabetic peripheral neuropathy, implying that CA 19-9 may similarly reflect neuropathic severity⁸. While advanced glycation end products are associated with diabetic complications, their relationship with diabetic NP appears distinct; CA 19-9 may offer

clearer insights into the NP severity⁹.

Correlation analyses confirmed a strong positive association between CA 19-9 and HbA1c levels ($r=0.72$, $p<0.01$), indicating that poorer glycaemic control is linked to elevated CA 19-9. This aligns with prior findings that inflammatory markers in DM are associated with reduced glycaemic control^{1,4}. A weak but statistically significant correlation was observed between BMI and CA 19-9 ($r=0.15$, $p=0.02$).

A moderate inverse correlation was found between CA 19-9 and vitamin D₂ levels ($r=-0.60$, $p<0.01$), suggesting that vitamin D deficiency may be associated with metabolic dysregulation and heightened systemic inflammation^{1,4,10}.

ANOVA revealed significant differences in CA 19-9 levels among the three groups ($p<0.01$). *Post hoc* analysis confirmed a statistically significant difference between DM with NP and healthy controls, reinforcing the association between elevated CA 19-9 and diabetic NP. Prior studies have similarly reported increased inflammatory markers in patients with

diabetic NP and diabetic retinopathy⁷.

ROC curve analysis demonstrated high diagnostic accuracy for CA 19-9 in identifying diabetic NP. The area-under-the-curve (AUC) was 0.95 (95% CI: 0.92–0.98) for distinguishing DM with NP from healthy controls. A cutoff value of 15.3 U/mL yielded 91% sensitivity and 93% specificity. For DM without NP *versus* healthy controls, the AUC was 0.85 (95% CI: 0.80–0.90), with 78% sensitivity and 81% specificity at a cutoff of 14.8 U/mL. These findings affirm the diagnostic potential of CA 19-9 in diabetic NP, consistent with earlier research^{6,7}.

Logistic regression analysis yielded a coefficient of 1.34, suggesting a possible association between CA 19-9 and diabetic NP risk; however, this was not statistically significant ($p=0.3$). Elevated CA 19-9 levels were positively correlated with poor glycaemic control, including HbA1c and fasting plasma glucose⁶. Prior studies have linked increased CA 19-9 to diabetic retinopathy and nephropathy, supporting its role in risk stratification^{1,7}. No significant associations were found between diabetic NP and HbA1c ($p=0.4$), BMI ($p=0.4$), or vitamin D₂ ($p=0.7$), possibly due to sample size limitations or residual confounding.

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4. Conclusion

This study demonstrated elevated serum CA 19-9 levels in DM patients with NP, with a strong positive correlation to HbA1c, thereby suggesting its utility in identifying poorly controlled DM. ROC analysis confirmed CA 19-9 as a promising biomarker for early diabetic NP detection. Further research is warranted in order to clarify its role in screening and managing DM-related complications.

Acknowledgements

The authors express their gratitude to the patients and volunteers who participated in this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Mousa M.J., Al-Hindy H.A.A.M., Alsaffar Y. Serum CA 19-9 as a potential biomarker for diabetic neuropathy: a comparative study among diabetic patients and healthy controls. *Pharmakeftiki* 37(2s), 354-358, 2025. <https://doi.org/10.60988/p.v37i2S.227>

Risk factors associated with peptic ulcer disease in patients attending two Iraqi teaching hospitals

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KEY WORDS:

risk factors; NSAIDs; smoking; peptic ulcer; patients

ARTICLE INFO:

Received: January 07, 2025

Revised: February 20, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Peptic ulcer disease (PUD) is a chronic condition of the digestive system that can lead to serious complications, increasing both morbidity and mortality and adversely affecting patients' quality of life. This study aimed to assess the sociodemographic characteristics of the sample and to evaluate patients' responses to known risk factors associated with PUD. A descriptive study was conducted in hospitals located in Al Hilla, Iraq. A purposive sample of 100 patients diagnosed with PUD was selected based on predefined inclusion criteria. Data were collected using a structured questionnaire and were analysed using descriptive statistics. Frequencies and percentages were used in order to categorize responses, while the mean and standard deviation were calculated in order to assess central tendency and variability. The findings revealed that patients reported high levels of exposure to several risk factors, including psychological stress, consumption of spicy foods, and intake of caffeinated beverages such as tea and coffee. The highest mean score was observed for the use of non-steroidal anti-inflammatory drugs, whereas the lowest scores were recorded for alcohol consumption, tobacco smoking, and rapid ingestion of food. In conclusion, the study highlights that dietary habits and lifestyle factors play a significant role in the development and exacerbation of PUD.

1. Introduction

Peptic ulcer disease (PUD) is among the most prevalent gastrointestinal disorders worldwide. It refers to an erosive lesion in the gastric or intestinal mucosa, typically affecting the upper gastrointestinal tract (including the oesophagus, stomach, and duodenum), and contributing significantly to global morbidity and mortality¹. PUD can occur at any age and affects both males and females. It may result in work absenteeism and reduced productivity. The disease is particularly widespread in developing countries, where prevalence rates are estimated to reach 70%, compared to a maximum of 40% in developed nations^{2,3}. Multiple risk factors contribute to the development of PUD, including the use of non-steroidal anti-inflammatory drugs (NSAIDs; e.g., diclofenac, aspirin, ibuprofen), tobacco smoking, and consumption of caffeinated beverages such as tea and coffee. Additional contributors include alcohol intake, psychological stress, socioeconomic status, and dietary habits¹. Najm⁴ has reported that over 90% of PUD patients were infected with *Helicobacter pylori*, alongside other contributing factors such as caffeinated beverages, stress, and pharmacological agents.

Despite advances in medical science, PUD remains a common condition among adults and older individuals, particularly those with chronic comorbidities. Enhancing public health knowledge and educating patients on preventive measures – especially regarding dietary modifications – may reduce the incidence and complications of PUD. This study was conducted in order to (i) identify the sociodemographic characteristics of patients diagnosed with PUD, (ii) determine the most common risk factors associated with the disease, and (iii) explore the relationship between dietary patterns and PUD.

2. Methodology

This study was conducted at the Marjan Hospital and the Al Hilla Teaching Hospital in Iraq. A non-probability purposive sample of 100 patients diagnosed with PUD was selected. Inclusion criteria comprised patients aged over 20 years, without complications of PUD, and willing to participate. Exclusion criteria included preg-

nancy, confirmed psychiatric conditions, age over 60 years, and serious illnesses such as renal failure and cancer.

Data collection involved structured questionnaires addressing sociodemographic variables (age, gender, education level, marital status) and risk factors. The questionnaire was developed and adapted from relevant literature and prior studies. Ethical approval was obtained from Al-Mustaqbal University's College of Nursing, with institutional permissions granted by the Al Hilla Teaching Hospital and the Marjan Hospital. The study adhered to the ethical principles of the Declaration of Helsinki. According to Document 1650, the local ethics committee reviewed and approved the study protocol, data instruments, and consent forms on November 14, 2024.

Each patient was interviewed individually in order to allow for the assessment of risk factors associated with PUD. To ensure content validity, the questionnaire was reviewed by 10 academic experts in the field. Their feedback was used in order to refine the instrument for clarity and applicability. Reliability was assessed using Cronbach's alpha coefficient, yielding a value of 0.80, indicating acceptable internal consistency. Finally, data were coded and analysed using the SPSS version 25.0 software for Windows.

3. Results and Discussion

Peptic ulcer disease is characterized by erosion of the mucosal lining of the upper digestive tract and presents as a chronic condition with diverse clinical manifestations. It has posed a significant global health challenge over the past two centuries^{1,2}.

Table 1 shows that the mean age of participants was 45.2 years, with most falling between 40 and 60 years. These findings align with those reported by Rajan⁵. In the present study, most patients reported sufficient income, consistent with findings from Egypt⁶.

Regarding education, the majority of participants had only basic schooling, with just 10% holding a Bachelor of Science degree or diploma. Most participants resided in rural areas, likely reflecting the demographic composition of Al Hilla city. This finding contrasts with Shamseya *et al.*⁷, who have reported that urban resi-

Table 1. Sociodemographic characteristics of the study's sample.			
Variables	Categories	N	%
Age	20–30 years	5	5%
	31–40 years	22	22%
	41–50 years	36	36%
	51–60 years	37	37%
	mean $\pm$ SD: 45.2 $\pm$ 6.1 years		
Gender	male	40	40%
	female	60	60%
Marital status	single	4	4%
	married	88	88%
	divorced	8	8%
	widowed	0	0%
Economic status	sufficient	45	45%
	somewhat adequate	23	23%
	insufficient	32	32%
Educational level	cannot read and write	4	4%
	can read and write	28	28%
	primary school	28	28%
	secondary school	30	30%
	institute or college graduate	10	10%
Residence	rural	57	57%
	urban	43	43%
Occupation	employee	40	40%
	unemployed	28	28%
	housewife	30	30%
	retired	2	2%
Family history of peptic ulcer	yes	30	30%
	no	70	70%

dents had better disease-related knowledge.

Notably, 70% of participants had no family history of PUD, corroborating findings from other research⁵. Only 10% reported hypertension, and most had no chronic illnesses, contrary to findings by a previous study⁵ in which the chronic disease prevalence was higher.

In terms of lifestyle and dietary factors, most participants did not associate alcohol consumption, smoking, or rapid eating with PUD. The majority were non-smokers, consistent with findings from West Bengal⁸, which have reported a low incidence of PUD among smokers. Shamseya *et al.*⁷ have noted that PUD affects both smokers and non-smokers. The low prevalence of smoking and alcohol use in the present study may be attributed

to the predominance of female participants and cultural norms in Muslim-majority countries, where such behaviours are more common among males³. Social, religious, and community factors may further discourage these habits among women in Arab societies.

Conversely, high mean scores were observed for tea and coffee consumption and psychological stress. The highest mean score was recorded for NSAID use (2.93). This may reflect cultural practices in Iraq, where frequent tea consumption is common and analgesics are often taken without medical supervision. These findings are consistent with a previous study⁹ that has identified stress and NSAID use as key risk factors for PUD. Additional contributors include dietary habits,

spicy foods, caffeine, and alcohol⁹.

4. Conclusion

This study has identified several factors associated with PUD, including female predominance, age range between 20 and 60 years, adequate economic status, and absence of chronic disease or family history. High response rates were observed for stress, NSAID use, and consumption of spicy foods. Conversely, alcohol and smoking were associated with the lowest mean scores.

Acknowledgements

The authors gratefully acknowledge the nursing staff at the Al Hilla Teaching Hospital for their valuable con-

tributions to this study. Appreciation is also extended to Al-Mustaqbal University for supporting the research and providing ethical approval.

Conflicts of interest

None exist.

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HOW TO CITE:

Muhaibes F.J., Dhaidan K.J., Al-Tae M.H., Hajwal S.K., Ayad Z.M., Hassan A.A.H., Khaleel M.A., Abbas A.H., Ridha-Salman H. Risk factors associated with peptic ulcer disease in patients attending two Iraqi teaching hospitals. *Pharmakeftiki* 37(2s), 359-362, 2025. <https://doi.org/10.60988/p.v37i2S.228>

Esculetin improves gut dysbiosis in isoproterenol-induced myocardial infarction in male rats

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KEY WORDS:

**esculetin; isoproterenol;
myocardial infarction;
intestinal dysbiosis; rat**

ARTICLE INFO:

Received: December 29, 2024

Revised: February 21, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Myocardial infarction (MI), a prevalent form of cardiovascular disease, has been associated with alterations in gut microbiota composition. Esculetin, a coumarin derivative, possesses multiple therapeutic properties. This study aimed at investigating the potential protective role of esculetin against isoproterenol (ISO)-induced myocardial toxicity in male rats, with a particular focus on intestinal dysbiosis and histopathological changes. The experimental protocol spanned 21 days, during which rats were randomly assigned to five groups (n=6 per group): (i) group 1 (normal control) received normal saline, (ii) group 2 (positive control) was administered ISO at 100 mg/kg, subcutaneously, every 24 h for two consecutive days, and (iii) groups 3, 4, and 5 were similarly administered ISO (100 mg/kg, subcutaneously, every 24 h for two days), followed from day 3 by oral esculetin at doses of 20, 40, and 60 mg/kg once daily, respectively. ISO administration significantly elevated serum creatine kinase-MB (CK-MB) levels, indicating myocardial injury. Esculetin treatment markedly reduced CK-MB levels in a dose-dependent manner. ISO also significantly decreased sodium-potassium adenosine triphosphatase (Na⁺,K⁺-ATPase) activity (a key marker of cellular membrane integrity and ion transport) while esculetin administration restored its activity. Histopathological examination of the small intestine in ISO-treated rats revealed severe enteritis characterized by villous damage, epithelial sloughing, vascular congestion, and pronounced leukocyte infiltration. Esculetin treatment ameliorated these changes, with the highest dose (60 mg/kg) yielding the most notable improvements, including restoration of normal villous architecture, enterocyte integrity, and intestinal gland morphology. These findings suggest that esculetin exerts protective effects against ISO-induced MI by mitigating cardiac toxicity, reversing intestinal dysbiosis, and improving histopathological outcomes.

1. Introduction

Cardiovascular diseases encompass a range of disorders affecting the circulatory system, with myocardial infarction (MI) remaining a leading cause of global mortality and morbidity. Obstruction of the coronary arteries compromises myocardial perfusion, resulting in ischemic necrosis of cardiac tissue.

The induction of MI using isoproterenol (ISO), a synthetic catecholamine, is a widely accepted, non-invasive method in experimental models. ISO induces myocardial stress primarily through free radical generation, leading to irreversible damage to cardiac membranes. Its administration elevates heart rate, oxygen demand, intracellular calcium load, and cyclic adenosine monophosphate levels in myocardial cells¹.

Recent studies underscore the role of gut microbiota in modulating the pathogenesis of coronary artery disease. Notably, Emoto *et al.*² have demonstrated a correlation between gut microbiota composition and the severity of MI in rats². Gut dysbiosis – defined as an imbalance in the composition, diversity, and abundance of intestinal microorganisms – has been strongly linked to increased cardiovascular risk. Alterations in microbial populations can compromise the intestinal barrier, suggesting a mechanistic connection between cardiac injury and gut microbiota integrity³.

Esculetin (6,7-dihydroxycoumarin), a plant-derived coumarin isolated from *Artemisia capillaris* and *Citrus limonia*, is a low molecular weight phenolic compound with diverse pharmacological properties. It has demonstrated efficacy in managing thromboembolic events, including stroke and MI. Given its safety profile and therapeutic potential, esculetin is a promising candidate for mitigating MI and associated intestinal dysbiosis.

This study investigates the protective effects of esculetin against ISO-induced MI and gut dysbiosis, with particular emphasis on its influence on myocardial lysosomal and membrane-bound enzymes and associated inflammatory responses in rats.

2. Methodology

Thirty untreated male albino Wistar rats (200–250 g)

were procured from the Animal House of the College of Pharmacy, Mustansiriyah University. The animals were housed in large cages with unrestricted access to food and water, and acclimatized for 3 weeks under controlled environmental conditions, including air vacuum systems. MI was induced by a subcutaneous injection of ISO at a dose of 100 mg/kg, dissolved in normal saline, administered over two consecutive days. The total study duration was 3 months, encompassing biochemical and histological analyses.

The treatment phase lasted 21 days, during which the rats were randomly assigned to five groups (n=6 per group): (i) group 1 (negative control) received water and a standard diet, (ii) group 2 (positive control) received ISO (100 mg/kg) subcutaneously, every 24 h for two days, (iii) group 3 received ISO as above, followed from day 3 by esculetin (20 mg/kg in 1 mL of normal saline) administered orally once daily, (iv) group 4 received ISO followed by esculetin (40 mg/kg in 1 mL of normal saline) orally once daily, and (v) group 5 received ISO followed by esculetin (60 mg/kg in 1 mL of normal saline) orally once daily.

On day 22, rats were fasted and sedated using ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were collected from the right ventricle and centrifuged for biomarker analysis. Cardiac biomarkers, including creatine kinase-MB (CK-MB) and sodium–potassium adenosine triphosphatase ($\text{Na}^+\text{K}^+\text{-ATPase}$), were quantified using enzyme-linked immunosorbent assay (ELISA). Intestinal tissues underwent histopathological processing: fixation, dehydration, clearing, embedding, and haematoxylin and eosin staining.

Ethical approval was granted by the Mustansiriyah University College of Pharmacy Ethics Committee (approval number: 49; date: 12/2/2025). Statistical analysis was performed using the GraphPad Prism 5.0 software. Results were expressed as mean $\pm$ standard error of the mean (SEM), and analysed *via* analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. A *p*-value <0.05 was considered statistically significant.

3. Results and Discussion

Serum CK-MB activity was significantly elevated

Table 1. The effect of esculetin on serum cardiac enzymes sodium–potassium adenosine triphosphatase (Na⁺,K⁺-AT-Pase) and creatine kinase-MB (CK-MB). Statistical analysis was evaluated using the least significant difference test; statistical significance between tested means is represented by the letters a, b, c, and d, with “a” denoting the highest level of significance and “d” the lowest. Identical letters indicate no statistically significant difference between the corresponding means.

Groups	CK-MB activity (IU/L)	Na ⁺ ,K ⁺ -ATPase activity (IU/L)
Group 1 (negative control)	1.302 ± 0.323 ^a	5.336 ± 1.356 ^d
Group 2 (positive control; isoproterenol)	7.177 ± 1.409 ^c	0.463 ± 0.178 ^a
Group 3 (esculetin; 20 mg/kg)	3.914 ± 1.499 ^b	2.790 ± 0.963 ^b
Group 4 (esculetin; 40 mg/kg)	2.563 ± 0.581 ^b	3.147 ± 0.694 ^b
Group 5 (esculetin; 60 mg/kg)	1.318 ± 0.532 ^a	4.116 ± 0.481 ^c

($p < 0.05$) in the ISO-induced group (7.177 ± 1.409 IU/L) compared to the control group (1.302 ± 0.323 IU/L). Esculetin treatment at 20 mg/kg reduced CK-MB activity levels to 3.914 ± 1.499 IU/L ($p < 0.05$), with further reductions observed at 40 mg/kg (2.563 ± 0.581 IU/L) and 60 mg/kg (1.318 ± 0.532 IU/L). The highest dose showed no significant difference from the control group (Table 1).

Similarly, Na⁺,K⁺-ATPase activity was significantly suppressed ($p < 0.05$) in the ISO group (0.463 ± 0.178 IU/L) relative to controls (5.336 ± 1.356 IU/L). Esculetin restored enzyme activity in a dose-dependent manner: 20 mg/kg (2.790 ± 0.963 IU/L), 40 mg/kg (3.147 ± 0.694 IU/L), and 60 mg/kg (4.116 ± 0.481 IU/L), with near-complete recovery at the highest dose (Table 1).

Histopathological findings were as follows: group 1 exhibited normal intestinal villi, enterocytes, and glands; group 2 showed severe enteritis with villous damage, epithelial sloughing, vascular congestion, and leukocyte infiltration; group 3 displayed thickened villi, increased cellularity in the villus core, enterocyte and goblet cell hyperplasia, and intact glands; group 4 demonstrated normal intestinal architecture; group 5 showed predominantly normal villi and glandular structures.

ISO-induced MI was associated with significant cardiac injury and intestinal dysbiosis, primarily mediated by inflammatory mechanisms. CK-MB, a reliable marker of myocardial necrosis, was markedly elevated in ISO-treated rats, consistent

with prior findings⁴. ISO mimics infarct-like cardiac damage comparable to human MI. Esculetin reduced CK-MB levels in a dose-dependent fashion, with the 60 mg/kg dose restoring levels to near-control values.

ISO-induced oxidative stress impaired Na⁺,K⁺-ATPase activity, compromising membrane integrity. Esculetin stabilized myocardial and lysosomal membranes, preventing enzyme leakage and reducing necrosis. All esculetin-treated groups showed significant recovery of Na⁺,K⁺-ATPase activity⁵.

Histopathological analysis confirmed dose-dependent improvements in intestinal morphology. At 60 mg/kg, esculetin nearly resolved cardiac injury and restored normal villous architecture. ISO-treated rats exhibited severe intestinal damage, including villous thickening, epithelial sloughing, leukocyte infiltration, and hyperplasia; findings consistent with those of Zhao *et al.*⁶ who have reported deterioration of the intestinal barrier in MI models. Collectively, these results affirm the dual therapeutic potential of esculetin in ameliorating cardiac and intestinal damage induced by ISO.

4. Conclusion

Esculetin significantly attenuated ISO-induced MI and gut dysbiosis by enhancing cardiac enzyme activity and restoring intestinal architecture in a

dose-dependent manner. The 60 mg/kg dose yielded the most pronounced improvements, suggesting that esculetin is a promising candidate for the treatment of MI and its associated gastrointestinal complications.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Mujbel Z.J., Jasim G.A., Waheed H.J. Esculetin improves gut dysbiosis in isoproterenol-induced myocardial infarction in male rats. *Pharmakeftiki* 37(2s), 363-366, 2025. <https://doi.org/10.60988/p.v37i2S.230>

Protective effects of *Mentha* leaves' extract against ethanol-induced liver damage and inflammatory response in mice

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KEY WORDS:

mint extract; anti-inflammatory effects; TNF- α ; IL-6; IL-1 β

ARTICLE INFO:

Received: January 30, 2025

Revised: February 17, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

ABSTRACT

The mint (*Mentha* spp.) leaf extract plays a pivotal role in enhancing immunological functions, notably by stimulating the activity of macrophages and natural killer cells; key components in pathogen recognition and elimination. This study aimed to investigate the anti-inflammatory properties of mint leaf extract in a murine model of ethanol-induced inflammation, with particular emphasis on its modulatory effects on major inflammatory cytokines: tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β). Male mice were randomly assigned to four groups: control, ethanol-only, and two experimental groups receiving mint extract at doses of 100 mg/kg and 200 mg/kg, respectively, in combination with ethanol for 30 consecutive days. Serum cytokine concentrations were quantified using ELISA. Ethanol administration resulted in a significant elevation of the TNF- α , IL-6, and IL-1 β levels. Co-treatment with the mint extract at both 100 mg/kg and 200 mg/kg attenuated these cytokine levels, with the higher dose demonstrating a more pronounced effect. These findings indicate that the mint extract exerts a dose-dependent anti-inflammatory response and may hold therapeutic promise for mitigating ethanol-induced inflammation and other cytokine-mediated inflammatory conditions.

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1. Introduction

Mint (*Mentha* spp.) is a medicinal

plant belonging to the Lamiaceae family, widely recognized for its aromatic properties and diverse

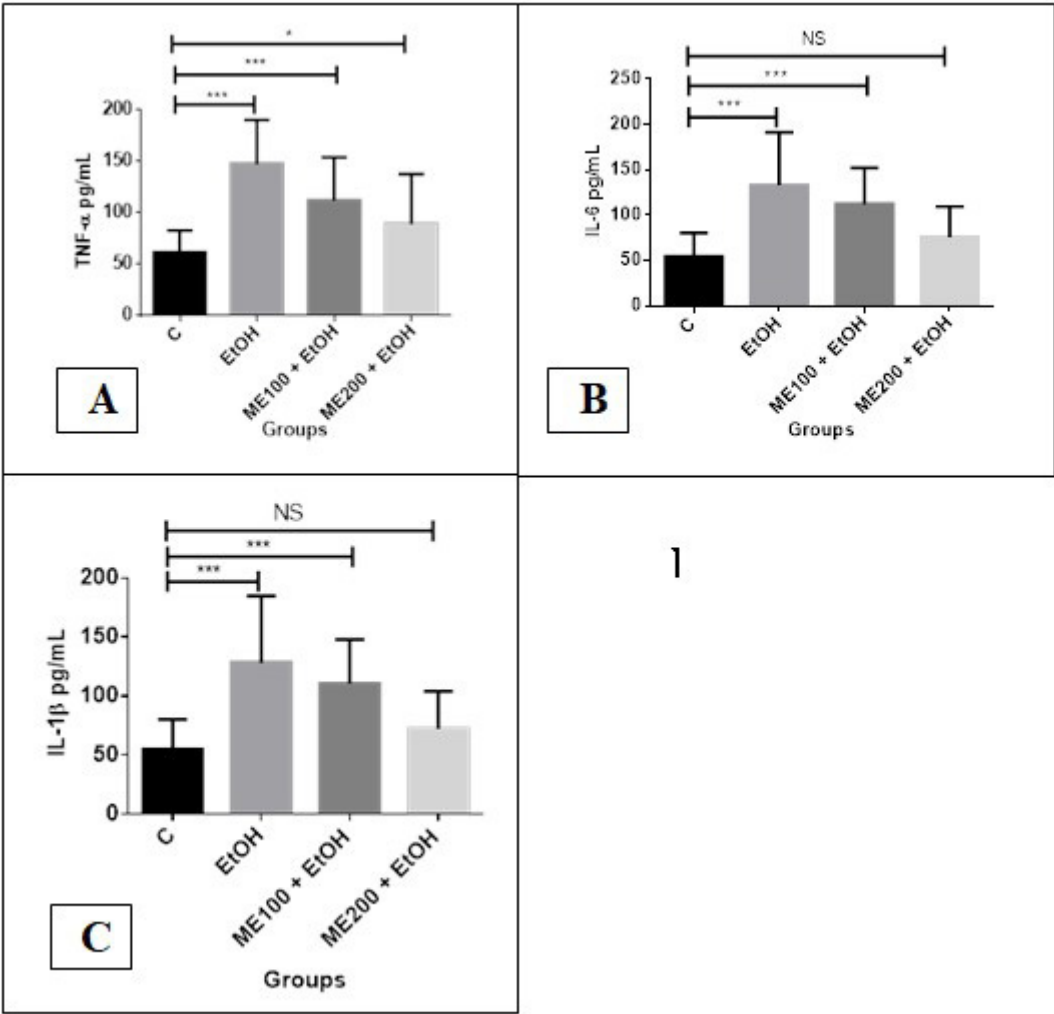


Figure 1. Cytokine levels across experimental groups: TNF- α (A), IL-6 (B), and IL-1 β (C). Statistical significance was determined by one-way ANOVA: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, non-significant. Group definitions: EtOH, ethanol (20%) only; ME100 + EtOH, mint extract (100 mg/kg) with ethanol (20%); ME200 + EtOH, mint extract (200 mg/kg) with ethanol (20%). Other abbreviations used: IL-1 β , interleukin 1 beta; IL-6, interleukin-6; TNF- α , tumor necrosis factor alpha.

health benefits. Mint leaves are extensively used for both culinary and therapeutic purposes¹. They contain bioactive compounds such as rosmarinic acid and flavonoids, which exhibit antioxidant and antibacterial activities². These constituents are particularly effective against respiratory infections, as mint inhibits bacterial proliferation within the body.

Moreover, the anti-inflammatory components of

mint – especially rosmarinic acid – help attenuate inflammation, promote a balanced immune response, and prevent chronic inflammatory disorders³. Mint also contains antioxidant vitamins, notably vitamin C and vitamin A, which protect immune cells from oxidative damage, thereby preserving their function and enhancing overall immunological efficacy⁴.

Mint modulates immune cell activity, including the

activation of macrophages and natural killer cells, both of which are essential for pathogen recognition and clearance⁵. Additionally, mint promotes the production of immune-enhancing cytokines, thereby strengthening the host's defence against infections⁶.

This study investigates the anti-inflammatory effects of mint leaf extract in a murine model of ethanol-induced hepatic inflammation, with a focus on its regulatory impact on key pro-inflammatory cytokines: tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β).

2. Methodology

Dried mint leaves were pulverized into a fine powder using a blender. A total of 250 g of powdered leaves was added to a flask containing 250 mL of ethanol. The mixture was heated to 50°C and stirred continuously for 18 h. The solution was then filtered through a Büchner funnel, and the filtrate was concentrated using a rotary evaporator.

A total of 120 male mice (weighing 30–40 g) were housed under controlled environmental conditions. The study protocol was approved by the College of Pharmacy of the University of Babylon, under ethical clearance number A-0048 (April 2024).

Mice were randomly allocated into four experimental groups (n=30 per group): (i) group 1 (C) acting as a negative control group (standard feeding), (ii) group 2 (EtOH) receiving ethanol (20% v/v) in the drinking water for 30 days in order to induce hepatic and systemic inflammation, (iii) group 3 (ME100 + EtOH) receiving the mint Extract (at 100 mg/kg) co-administered with ethanol, and (iv) group 4 (ME200 + EtOH) receiving the mint extract (at 200 mg/kg) co-administered with ethanol. All treatments were administered orally once daily for 30 consecutive days.

Serum levels of TNF- α , IL-6, and IL-1 β were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabsience), following the manufacturer's protocol. Cytokine concentrations were expressed in pg/mL⁷.

Statistical analyses were performed using GraphPad Prism (version 6) and Microsoft Excel 2020. In-

tergroup differences were assessed using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons.

3. Results and Discussion

This study evaluated the anti-inflammatory efficacy of mint extract in a murine model of ethanol-induced hepatic inflammation, focusing on its impact on the serum TNF- α , IL-6, and IL-1 β levels (Figure 1).

Analysis of TNF- α levels revealed significant intergroup differences ($p < 0.001$; Figure 1.A). The control group (C) exhibited a mean serum TNF- α concentration of 61.77 ± 20.94 pg/mL. The ethanol group (EtOH) showed a marked elevation to 147.6 ± 42.72 pg/mL ($p < 0.001$ vs. control). A co-treatment with mint extract at 100 mg/kg (ME100 + EtOH) reduced the serum TNF- α levels to 112.1 ± 41.76 pg/mL ($p < 0.001$ vs. ethanol group), while the 200 mg/kg dose (ME200 + EtOH) further reduced the serum TNF- α levels to 89.07 ± 48.50 pg/mL ($p < 0.05$ vs. ethanol group). These findings suggest a dose-dependent anti-inflammatory effect of the mint extract, with the higher dose offering greater protection against ethanol-induced TNF- α elevation.

The bioactive constituents of the mint extract – particularly terpenoids, flavonoids, and phenolic acids – are likely responsible for these effects. These compounds possess antioxidant properties that neutralize reactive oxygen species, thereby mitigating oxidative stress. Previous studies have demonstrated that phenolic and flavonoid compounds in *Mentha piperita* suppress pro-inflammatory cytokine production and reduce oxidative damage. The present findings support the potential of the *Mentha* extract as a therapeutic agent for ethanol-induced hepatic inflammation, with a clear dose-response relationship^{5,8}.

IL-6 levels were also significantly affected ($p < 0.001$; Figure 1.B). The control group (C) exhibited an average serum IL-6 concentration of 54.50 ± 25.71 pg/mL. Ethanol exposure elevated the serum IL-6 levels to 132.8 ± 58.52 pg/mL ($p < 0.001$ vs. control). Treatment with ME100 + EtOH reduced the serum IL-6 levels to 112.1 ± 40.06 pg/mL ($p < 0.001$ vs.

ethanol group), while ME200 + EtOH yielded a mean of 75.83 ± 33.47 pg/mL, though this reduction was not statistically significant.

The undertaken IL-1 β analysis revealed significant intergroup differences ($p < 0.001$; Figure 1.C). The Control Group (C) exhibited mean serum IL-1 β levels of 55.03 ± 25.25 pg/mL. Ethanol administration increased the serum IL-1 β levels to 128.4 ± 56.51 pg/mL ($p < 0.001$ vs. control). The ME100 + EtOH co-administration reduced the serum IL-1 β levels to 110.8 ± 37.28 pg/mL ($p < 0.001$ vs. ethanol group), while the ME200 + EtOH co-administration achieved a further reduction to 72.17 ± 31.71 pg/mL, though this was not statistically significant.

The observed reductions in IL-6 and IL-1 β may be attributed to the antioxidant and anti-inflammatory properties of phenolic acids and flavonoids in *Mentha piperita*. These compounds likely modulate immune responses, attenuate oxidative damage, and interfere with inflammatory signalling pathways.

4. Conclusion

This study demonstrates the anti-inflammatory po-

tential of the mint extract in a murine model of ethanol-induced hepatic inflammation. Administration of mint extract at 100 mg/kg significantly reduced the serum levels of TNF- α , IL-6, and IL-1 β , indicating its capacity to modulate inflammatory responses. These findings suggest that the mint extract may serve as a natural therapeutic agent for ethanol-induced inflammation, although further research is warranted to optimize dosing and evaluate long-term efficacy.

Acknowledgements

The authors gratefully acknowledge Dr Gaffir for his assistance with animal housing and care.

Conflicts of interest

None exist.

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HOW TO CITE:

Nayif E.M., Abass H.A., Ali A.M., Abed H.H. Protective effects of *Mentha* leaves' extract against ethanol-induced liver damage and inflammatory response in mice. *Pharmakeftiki* 37(2s), 367-371, 2025. <https://doi.org/10.60988/p.v37i2S.231>

Antibiotic susceptibility and efflux pump activity in uncommon bacterial species associated with conjunctivitis

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KEY WORDS:

conjunctivitis; antibiotic susceptibility; efflux pumps; uncommon bacteria; common bacteria

ARTICLE INFO:

Received: January 30, 2025

Revised: February 16, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

This study investigates the antibiotic susceptibility and phenotypic characteristics of uncommon bacterial species associated with conjunctivitis. A total of 150 conjunctival swab samples were collected from patients presenting with symptoms of conjunctivitis at the Al-Nasiriyah Teaching Hospital and the Al-Haboubi General Hospital (Ophthalmology Consultant Unit; Dhi Qar Governorate, Iraq) between August and December 2024. Bacterial isolates were identified based on cultural and microscopic characteristics, supplemented by the Vitek® 2 Compact System (bioMérieux, France). Gram-positive bacteria predominated, with *Staphylococcus aureus* accounting for 40% of isolates. Several uncommon species were also identified, including *Kocuria kristinae*, *Staphylococcus hominis* subsp. *hominis*, *Escherichia coli*, *Brucella melitensis*, *Gemella bergeri*, *Staphylococcus pseudintermedius*, and *Leuconostoc citreum*. Antibiotic resistance profiles were determined using the Kirby-Bauer disk diffusion method, revealing notable resistance to commonly prescribed agents such as erythromycin and ciprofloxacin. Efflux pump activity (a key mechanism contributing to multidrug resistance) was detected in 37.5% of isolates using the ethidium bromide cartwheel method. These findings highlight the importance of robust antibiotic stewardship and underscore the need for continuous surveillance of bacterial resistance patterns in order to optimize therapeutic strategies for conjunctivitis.

1. Introduction

Conjunctivitis, commonly referred

to as “pink eye”, is an ocular infection characterized by inflammation of the conjunctiva (a thin mucous

membrane that may appear pink or crimson), typically caused by bacterial, viral, or allergic agents^{1,2}. The most frequently implicated bacterial species include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Chlamydia* spp., *Moraxella* spp., *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. In addition to these, several uncommon bacterial species have been associated with conjunctivitis, such as *Kocuria kristinae*, *Staphylococcus hominis* subsp. *hominis*, *Escherichia coli*, *Brucella melitensis*, *Gemella bergeri*, *Staphylococcus pseudintermedius*, and *Leuconostoc citreum*².

These uncommon bacteria are increasingly recognized for their capacity to develop resistance to multiple antibiotics, largely attributed to the indiscriminate use of antimicrobial agents in hospital settings, which complicates treatment protocols³. Efflux pumps (protein transporters embedded in bacterial cell membranes) play a pivotal role in expelling toxic compounds, including antibiotics, thereby contributing to antimicrobial resistance⁴. Accordingly, the present study aimed at isolating and identifying uncommon bacterial pathogens associated with conjunctivitis, assessing their antibiotic susceptibility profiles, and detecting efflux pump activity among the isolates.

2. Methodology

A total of 150 conjunctival swab samples were collected from paediatric patients (aged birth to 7 years) diagnosed with conjunctivitis in the Dhi Qar Governorate (Nasiriyah), Iraq, between August and December 2024. Sampling was conducted at the Ophthalmology Consultant Unit of the Nasiriyah Teaching Hospital and the Al-Haboubi General Hospital. Following clinical examination by a specialist, purulent material from the conjunctiva was collected by using sterile swabs and inoculated onto three types of culture media: blood agar, chocolate agar, and MacConkey agar. The inoculated plates were incubated at 37°C for 24 h.

Phenotypic identification of bacterial isolates was performed by using the Vitek® 2 Compact System (bioMérieux, France), which integrates cultural and microscopic characteristics with biochemical profiling. The system demonstrates high diagnostic accuracy and specificity (95%–99%). Antimicrobial susceptibility testing was conducted in accordance with established

protocols⁵. Detection of active efflux pump activity was carried out using the ethidium bromide cartwheel method⁶.

Institutional review board approval was obtained from the College of Medicine of the University of Kufa, Iraq (approval number: 5628/2024), in compliance with the Declaration of Helsinki and international guidelines for human research protection. Informed consent was secured from adult participants or the legal guardians of paediatric patients. Data analysis was performed using the SPSS software, version 24.

3. Results and Discussion

Of the 150 specimens analysed, 85 (56.67%) exhibited positive bacterial growth on culture media after 24 h of incubation at 37°C, while 65 (43.33%) showed no growth even after 48 h. Among the 85 culture-positive specimens, 74 (87.06%) were identified as Gram-positive and 11 (12.94%) as Gram-negative.

The most frequently isolated species was *Staphylococcus aureus* (34 isolates; 40%), followed by *Staphylococcus hominis* (7; 8.24%), *Staphylococcus pseudintermedius* (5; 5.88%), *Staphylococcus epidermidis* (4; 4.71%), *Kocuria kristinae* (2; 2.35%), *Gemella bergeri* (1; 1.18%), *Leuconostoc citreum* (1; 1.18%), *Escherichia coli* (9; 10.59%), and *Brucella melitensis* (2; 2.35%). Additionally, 20 isolates (23.52%) were classified as “unidentified” organisms.

Antibiotic resistance profiles varied across species. *S. aureus* exhibited high resistance to erythromycin and doxycycline, with moderate resistance to vancomycin and the highest recorded resistance to ciprofloxacin (maximum: 36). *S. hominis* showed notable resistance to erythromycin and tetracycline, though overall resistance was lower than that of *S. aureus*. *S. pseudintermedius* demonstrated extensive resistance, particularly to vancomycin and ciprofloxacin (maximum: 46). *S. epidermidis* displayed relatively lower resistance, with erythromycin and doxycycline being the most affected. *K. kristinae* showed generally low resistance, although some isolates were significantly resistant to ciprofloxacin. *G. bergeri* and *L. citreum* had limited data points, but appeared to exhibit low resistance across tested antibiotics. *E. coli* presented variable resistance, especially to ciprofloxacin and amoxicillin–clavulanate, with

Table 1. Results of phenotypic detection of efflux pump activity in uncommon bacterial isolates associated with conjunctivitis, using varying concentrations of ethidium bromide (EtBr) dye in tryptic soy agar.				
EtBr (mg/L) and degree of fluorescence produced				
Isolate (and its number)	1 mg/L	2 mg/L	3 mg/L	Efflux activity
<i>Staphylococcus aureus</i> (1)	-	-	-	Negative
<i>Staphylococcus aureus</i> (4)	-	-	-	Negative
<i>Kocuria kristinae</i>	+	+	+	Positive
<i>Staphylococcus hominis</i>	-	-	-	Negative
<i>Escherichia coli</i>	+	+	+	Positive
<i>Brucella melitensis</i>	-	-	-	Negative
<i>Gemella bergeri</i>	-	-	-	Negative
<i>Staphylococcus epidermidis</i> (10)	-	-	-	Negative
<i>Staphylococcus aureus</i> (13)	-	-	-	Negative
<i>Staphylococcus aureus</i> (14)	-	-	-	Negative
<i>Staphylococcus epidermidis</i> (15)	-	-	-	Negative
<i>Staphylococcus pseudintermedius</i> (16)	-	+	+	Intermediate
<i>Staphylococcus hominis</i> (17)	+	+	+	Positive
<i>Leuconostoc citreum</i> (19)	+	+	+	Positive
<i>Staphylococcus pseudintermedius</i> (20)	+	+	+	Positive
<i>Staphylococcus pseudintermedius</i> (22)	+	+	+	Positive

some isolates fully resistant to ampicillin. Finally, *B. melitensis* showed inconsistent resistance, with some isolates highly resistant to imipenem and cefotaxime. Overall, erythromycin, ciprofloxacin, and doxycycline were associated with higher resistance rates across multiple species, whereas vancomycin and imipenem retained relatively lower resistance levels.

The efflux pump activity was assessed in 16 isolates of uncommon bacterial species using the ethidium bromide–agar cartwheel method. Six isolates (37.5%) tested positive for efflux pump activity, as determined by the lowest concentration at which fluorescence was absent under ultraviolet light exposure (Table 1).

The findings suggest that bacterial pathogens are the predominant cause of conjunctivitis, potentially exacerbated by prior exposure to antibiotics and corticosteroids. A comparative study conducted in the Babil Governorate, Iraq, has reported that 52.5% of the conjunctival specimens were culture-positive, while 47.5% were culture-negative⁷.

Resistance trends were particularly concerning for *S. pseudintermedius*, which demonstrated the highest resistance levels, especially to ciprofloxacin and

vancomycin, posing a significant clinical challenge. In contrast, *S. epidermidis* and *K. kristinae* exhibited lower resistance rates, particularly to vancomycin and ciprofloxacin, suggesting a more favourable susceptibility profile⁸. *S. aureus* remains a global concern due to its high resistance to erythromycin and doxycycline. Conversely, *E. coli* and *B. melitensis* exhibited relatively low resistance to critical antibiotics such as imipenem, thereby indicating that standard treatment protocols may remain effective.

The observed resistance to ciprofloxacin and amoxicillin underscores a pressing public health issue, necessitating revised treatment guidelines and alternative therapeutic options for resistant pathogens such as *S. pseudintermedius* and *S. aureus*⁹. These data reinforce the importance of surveillance programs and antimicrobial stewardship to track resistance patterns and inform clinical decision-making. Further research is warranted to elucidate the genetic and environmental factors driving resistance¹⁰.

4. Conclusion

This study confirms that bacterial pathogens

– particularly Gram-positive species – are the primary aetiological agents of conjunctivitis. Notably, several Gram-negative isolates exhibited efflux pump activity, which contributes to antibiotic resistance and complicates treatment outcomes.

Acknowledgements

The authors extend their gratitude and appreciation to the health institutions in the Dhi Qar Gov-

ernorate, the Al-Hussein Teaching Hospital, and the Al-Nasiriya Teaching Hospital.

Conflicts of interest

None exist.

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HOW TO CITE:

Obaid H.A.J., Shaltagh S.M.M. Antibiotic susceptibility and efflux pump activity in uncommon bacterial species associated with conjunctivitis. *Pharmakeftiki* 37(2s), 372-375, 2025. <https://doi.org/10.60988/p.v37i2S.233>

Antifungal effects of silver nanoparticles produced by *Cinnamomum verum* bark extract against fungi isolated from toothbrushes

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KEY WORDS:

toothbrush; green synthesis; antifungal activity; silver nanoparticles; *Cinnamomum verum*

ARTICLE INFO:

Received: December 28, 2024

Revised: February 28, 2025

Accepted: March 01, 2025

Available online: October 10, 2025

ABSTRACT

The findings revealed that 50 toothbrush samples used by individuals aged 20–50 years were contaminated with fungi. The infection rate was higher among females (40%), and fungal contamination increased when toothbrushes were used for more than three months. The most frequently isolated fungal species were *Aspergillus niger* (45%) and *Penicillium citrinum* (18%), while *Fusarium oxysporum* was the least common (2%). Silver nanoparticles (Ag NPs) were synthesized via a green method using *Cinnamomum verum* bark extract. The Ag NPs inhibited the growth of *A. niger*, *A. flavus*, *A. terreus*, and *P. citrinum* by 44.44%, 75.11%, 84.83%, and 98.66%, respectively, at a concentration of 200 µg/mL. Complete fungal inhibition was observed at 500 µg/mL, with statistically significant differences ($p < 0.05$) compared to the untreated control.

1. Introduction

The toothbrush remains the most effective tool for cleaning the surfaces of the teeth and tongue. It also serves as a conduit between the external environment and the body's digestive and respiratory tracts, thereby creating a favourable setting for microbial colonization¹. Al-

though findings remain limited and subject to debate, studies investigating the antibacterial activity of nanoparticles (NPs) have demonstrated their potential to mitigate harmful microbial effects².

Silver NPs (Ag NPs) exhibit notable antibacterial properties even at low concentrations³. In the present study, *Cinnamomum verum* bark

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extract was employed for the green synthesis of Ag NPs, owing to its broad biological potential. Antibacterial activity was evaluated using both the disc diffusion and broth microdilution methods, with results corroborated by fluorescence microscopy observations⁴.

2. Methodology

This study was conducted on 50 toothbrush samples collected from students and staff at the University of Kufa (Iraq), aged between 20 and 50 years, representing both sexes. All samples were submitted to the mycology laboratory during the period of September to October 2024. Toothbrush heads were sectioned and transferred using sterile forceps onto culture dishes containing Sabouraud dextrose agar (SDA) for fungal cultivation. The inoculated plates were then incubated for three days⁵.

3. Results and Discussion

Fifty toothbrush samples from individuals of varying ages and genders were processed in the mycology laboratory. The toothbrush heads were dissected and cultured on SDA, followed by a three-day incubation period⁵. Twelve SDA flasks were divided into four experimental groups, each treated with Ag NPs at varying concentrations. The distribution of fungal contamination by age revealed the lowest infection rate in the 20–30-year group (28%) and the highest in the 41–50-year group (40%). Females exhibited a higher infection rate (76%) compared to males (24%), with a 2:1 female-to-male ratio, particularly pronounced in the 41–50-year group. This trend may be attributed to age-related hormonal changes and diminished immune function.

Fungal and bacterial contamination was more prevalent in toothbrushes used for longer than three months. Accordingly, it is recommended that toothbrushes be replaced every three to four months so as to preserve their efficacy in dental plaque removal⁶.

The most frequently isolated fungal species were *Aspergillus niger* (45%) and *Penicillium citrinum* (18%), while *Fusarium oxysporum* was the least

common (2%). These findings are consistent with previous reports⁷.

Transmission electron microscopy analysis of the synthesized Ag NPs revealed an average particle diameter of 18–20 nm, confirming monodispersity. The spherical morphology and minimal agglomeration of the particles suggest that *C. verum* bark extract was highly effective as both a reducing and stabilizing agent.

The Ag NPs synthesized from *C. verum* demonstrated *in vitro* antifungal activity against *A. niger*, *A. flavus*, *A. terreus*, and *P. citrinum*, with inhibition rates of 33.5%, 11.1%, 44.4%, and 66.7%, respectively, at a concentration of 100 µg/mL. Inhibition increased with higher concentrations, culminating in complete fungal growth suppression at 500 µg/mL, consistent with prior findings⁸.

Studies have shown that Ag NPs synthesized from biological sources such as plants, algae, and bacteria possess antimicrobial, antioxidant, and anticancer properties. The formation of Ag NPs begins when the biological extract is mixed with silver nitrate, resulting in a colour change from light to dark brown, which confirms NP synthesis⁷. In green synthesis, the extract functions as both a reducing and stabilizing agent, with 60°C identified as the optimal reaction temperature. This method yields stable, spherical Ag NPs⁹.

4. Conclusion

Fungal contamination was observed in toothbrushes used by both adult males and females, with a higher infection rate among women. The most prevalent fungal species were *Aspergillus niger* and *Penicillium citrinum*, while *Fusarium oxysporum* was the least frequently detected. The environmentally friendly synthesis of Ag NPs using *C. verum* bark extract proved effective.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Sahib R.A., Obaid R.F., Ali H.A. Antifungal effects of silver nanoparticles produced by *Cinnamomum verum* bark extract against fungi isolated from toothbrushes. *Pharmakeftiki* 37(2s), 376-378, 2025. <https://doi.org/10.60988/p.v37i2S.234>

The effects of *Stevia rebaudiana* consumption on male reproductive parameters: a cross-sectional study

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KEY WORDS:

fertility; male reproductive system; semen; stevia; testosterone

ARTICLE INFO:

Received: January 31, 2025

Revised: February 14, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

Stevia, a natural sweetener deriving from the leaves of the plant *Stevia rebaudiana*, has gained widespread popularity as a sugar substitute. This study aimed at evaluating the effects of stevia consumption on male reproductive parameters, with particular emphasis on serum testosterone concentration and sperm count. A cross-sectional study was conducted in Samawah City, Iraq, between February 2023 and April 2024, involving 80 male participants. Subjects were categorized into two groups: a stevia-treated group (N=40), which consumed stevia as a dietary sugar substitute, and a control group (N=40), which maintained their habitual dietary intake. Serum testosterone levels ($\mu\text{g/L}$) and semen samples were analysed in order to assess reproductive parameters, including sperm count ($\times 10^6/\text{mL}$), sperm motility (%), and sperm morphology. The mean age of participants did not differ significantly between the control group (33.65 ± 7.16 years) and the stevia-treated group (35.95 ± 5.72 years). Stevia consumption had no statistically significant effect on serum testosterone levels, with mean values of $21.10 \pm 11.84 \mu\text{g/L}$ in the control group and $20.03 \pm 10.84 \mu\text{g/L}$ in the stevia-treated group. Although a non-significant increase in sperm morphological abnormalities was observed in the stevia-treated group, sperm motility remained unaffected. Notably, a statistically significant reduction in sperm count was detected in the ste-

via-treated group ($18.03 \pm 8.938 \times 10^6/\text{mL}$) compared to controls. In conclusion, dietary stevia intake did not alter serum testosterone levels or sperm motility. However, it was associated with a significant decrease in sperm count and a slight, non-significant increase in sperm deformities.

1. Introduction

Stevia is a natural sweetener extracted from the leaves of *Stevia rebaudiana*. It has gained popularity as an alternative to conventional sweeteners, primarily due to its potential to reduce caloric and sugar intake. As a plant-derived product, stevia appeals to individuals seeking a more natural dietary option. It is widely incorporated into various food and beverage products, including confectionery and soft drinks¹. In addition to its sweetening properties, stevia has been investigated for potential health benefits, particularly its role in blood glucose regulation and weight management.

The US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) generally consider stevia-derived compounds to be safe when consumed within recommended limits. However, as of January 2022, studies examining the effects of stevia on male fertility remain limited and inconclusive. Some animal studies have reported that high doses of steviol (a bioactive compound found in stevia) may negatively affect male reproductive parameters, including sperm count and motility². It is important to note that such studies often utilize doses far exceeding those typically consumed through regular dietary intake.

This study aimed at investigating the potential impact of stevia consumption on male reproductive health. Specifically, it has evaluated serum testosterone concentration, sperm morphology (deformities), sperm motility, and sperm count in individuals who regularly consumed stevia as a sweetener, compared to a control group with no stevia intake.

2. Methodology

This cross-sectional study was conducted in Samawah City, Iraq, between February 2023 and April 2024. Ethical approval was obtained from the Ethical Research Committee at the College of Dentistry, Al-Iraqia University (HAS & HER 08-05-07-2024). All participants provided written informed consent. Study procedures were carried out at the Al-Hussein Teaching Hospital in Samawah City and at the Kamal Alsamurai Specialized Infertility Hospital in Baghdad City, Iraq.

A total of 80 male participants, aged 18–45 years, were enrolled. Subjects were divided into two groups: the control group comprised healthy individuals who did not consume stevia or any other low-calorie sweetener (e.g., saccharin), while the stevia-treated group included individuals who had consumed stevia products as a sweetener for more than three months. Exclusion criteria included a history of infertility, diabetes mellitus, current medication use, abnormal body mass index ($\text{BMI} < 18.5$ or > 24.9), smoking, alcohol consumption, or other lifestyle factors known to affect serum testosterone levels or semen quality.

Semen and serum samples were collected from all participants. Serum testosterone levels were measured using the Cobas e 411 immunoassay analyser (Roche Diagnostics International Ltd., Germany), based on the competitive binding principle. Semen samples were visually assessed by an experienced operator for colour, density, and general appearance. Microscopic and physical evaluations of sperm were performed using an automated sperm analyser (SCA Scope, Spain).

Statistical analyses were conducted using Micro-

Table 1. Statistical comparison of control and stevia-treated groups with respect to age, serum testosterone levels, sperm deformity rate, sperm motility, and sperm count.

Variable	Group	N	Mean $\pm$ SD	p-value
Age (years)	control	40	33.65 $\pm$ 7.16	0.1167
	stevia-treated	40	35.95 $\pm$ 5.72	
Serum testosterone levels ($\mu\text{g/L}$)	control	40	21.10 $\pm$ 11.84	0.6745
	stevia-treated	40	20.03 $\pm$ 10.84	
Rate of deformities (%)	control	40	14.12 $\pm$ 8.06	0.2810
	stevia-treated	40	16.22 $\pm$ 9.23	
Sperm motility (%)	control	40	37.19 $\pm$ 16.36	0.3321
	stevia-treated	40	33.44 $\pm$ 17.93	
Sperm count ($\times 10^6/\text{mL}$)	control	40	23.25 $\pm$ 9.02	0.0111
	stevia-treated	40	18.03 $\pm$ 8.94	

soft Excel 2020 and GraphPad Prism (version 16). Data normality was assessed using the Shapiro-Wilk test. Parametric analyses were performed using independent *t*-tests and analysis of variance (ANOVA), with a *p*-value <0.05 considered as statistically significant.

3. Results and Discussion

As shown in Table 1, there was no statistically significant difference in age between the control and the stevia-treated groups. This finding is critical for interpreting the study outcomes, as age is known to influence several reproductive parameters, including serum testosterone concentration, sperm count, and motility³. The absence of age-related variation enhances the internal validity and reproducibility of the study.

Testosterone levels, as also presented in Table 1, did not differ significantly between the two groups. Although a slight reduction was observed in the stevia-treated group, this change was not statistically significant and likely falls within the range of normal physiological variation. Factors such as the duration of stevia intake, the dosage, and the individual variability may contribute to this outcome⁴. Importantly, serum testosterone concentrations in both groups remained within the normal reference range for adult males, and no clinically meaningful reduction was observed in the stevia-treated group.

Sperm morphology analysis revealed a non-significant difference in the rate of deformities between the groups (Table 1). Although the stevia-treated group exhibited a numerically higher rate of morphological abnormalities, this variation was not statistically significant and remained within the normal range. Sperm morphology is a key parameter in assessing male fertility, and alterations may have reproductive implications. However, within the context of this study, stevia consumption did not appear to affect sperm morphology in a clinically significant manner. These findings provide preliminary evidence that short-term stevia intake does not increase sperm deformities⁵. Further research with larger sample sizes and longer exposure durations is warranted in order to explore potential long-term effects.

Sperm motility, another critical determinant of male reproductive potential, showed no statistically significant difference between the control and the stevia-treated groups (Table 1). Both groups maintained motility values within the normal range. Sperm motility can be influenced by numerous factors, including lifestyle habits, environmental exposures, and general health status⁶. The absence of significant variation suggests that stevia consumption does not impair sperm motility under the conditions studied.

Sperm count (sperm concentration), a fundamental indicator of male fertility, demonstrated a statistically significant reduction in the stevia-treated

group compared to controls (Table 1). Despite this reduction, the mean sperm count in the stevia group remained within the reference range for healthy males. This finding warrants cautious interpretation, as sperm production is a dynamic process subject to short-term fluctuations. Although many confounding factors were excluded during data collection, residual influences such as environmental exposures and dietary habits may still play a role⁷. Longitudinal studies are needed in order to determine whether the observed reduction in sperm count is transient or persistent. Given that sperm motility and morphology were unaffected, the clinical relevance of the reduced sperm count remains uncertain.

4. Conclusion

This study exhibited no significant difference in age between the control and stevia-treated groups, thereby reinforcing the reliability of the comparative analysis. Serum testosterone levels and sperm motility were not significantly affected by stevia consumption, and although a slight increase in sperm morphological abnormalities was observed in the stevia-treated group, this change was not statisti-

cally significant. In contrast, a significant reduction in sperm count was detected in the stevia-treated group, though values remained within the normal range. These findings suggest that while stevia consumption may influence sperm concentration, its short-term use does not appear to impair other key reproductive parameters.

Acknowledgements

The authors extend their gratitude to the Kamal Al-samarai Specialized Infertility Hospital, Baghdad, Iraq, for providing the facilities and support necessary to conduct this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Oliwi A.A., Al-Hamdani H.A., Khalifa A.Y., Abed H.H., Hussein T.A., Al Salihi K.A. The effects of *Stevia rebaudiana* consumption on male reproductive parameters: a cross-sectional study. *Pharmakeftiki* 37(2s), 379-383, 2025. <https://doi.org/10.60988/p.v37i2S.235>

Patient satisfaction in the public hospitals of Hillah (Iraq): a survey

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KEY WORDS:

patient satisfaction; health services; healthcare quality; patient experience; service delivery

ARTICLE INFO:

Received: January 06, 2025

Revised: February 07, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

ABSTRACT

Patient satisfaction is a critical metric for healthcare systems, prompting hospitals and clinical practices to prioritize it as a strategic objective. This study, conducted in Al Hilla City, Iraq, assessed patient satisfaction with public hospital services and examined associated sociodemographic factors. The survey was carried out in primary healthcare facilities across Al Hilla City between February 5 and February 21, 2024, focusing on four governmental hospitals. Findings revealed that 40% of respondents considered specialized services to be accessible, while 15.3% expressed overall dissatisfaction. Regarding treatment adequacy, 36.7% strongly agreed and 46.7% agreed that their treatment was appropriate. Patient perceptions also highlighted healthcare personnel attentiveness (46.7% agreement), wait times (36% agreement, 16.7% strong agreement), and dissatisfaction with prolonged waiting periods (54%). Furthermore, 52.7% agreed and 12% strongly agreed that treatments were delivered in a rushed manner. Timely assessment of patient satisfaction is essential for enhancing care quality and guiding targeted improvements in healthcare facilities.

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1. Introduction

Patient satisfaction constitutes a subjective evaluation of service quality relative to established expectations, typically quantified by soliciting direct feedback from pa-

tients regarding their levels of contentment or dissatisfaction¹. The analysis of patient perceptions concerning healthcare services, personnel, and resources is essential for the comprehensive assessment of healthcare quality². Primary

health care (PHC), the initial interface connecting individuals to the healthcare system, facilitates essential services at the community level and is vital for public health outcomes. PHC was identified as the principal strategy for achieving the goal of "Health for All"³, underscoring the importance of providing accessible and inclusive healthcare services to the entire population⁴.

Patient satisfaction is a significant metric within the healthcare domain, particularly in the context of PHC in Western societies. It encompasses dimensions such as medical guidance, service utilization, and the doctor-patient relationship, thereby serving as an indicator of service quality⁵. The evaluation of patient satisfaction fulfils three essential roles: first, as a measure of care quality; second, as an independent metric of progress; and third, as an indicator of service inadequacies within a dynamic healthcare system⁶.

Concerns regarding the quality of healthcare services in Iraq have eroded public trust in both public and private hospitals, prompting an increasing number of Iraqi patients to seek medical care abroad. Given the scarcity of studies analysing healthcare quality in Iraq, it is imperative to evaluate the performance of healthcare services nationwide, with particular emphasis on integrating patient feedback into the evaluative framework. This study aims to determine the level of satisfaction among patients with the healthcare services provided by public hospitals in Al Hilla City. Furthermore, it investigates the sociodemographic variables associated with varying levels of patient satisfaction.

2. Methodology

This cross-sectional study assessed patient satisfaction with healthcare services provided by government hospitals in Babylon City between February 5 and 21, 2024. The study included patients attending four hospitals: Imam Al-Sadiq Hospital, Marjan Teaching Hospital, Al-Hilla General Teaching Hospital, and Babil Teaching Hospital for Maternity and Children. Both inpatients and outpatients from various departments were eligible to participate, provided they gave informed consent.

ed they gave informed consent.

A structured questionnaire was developed in order to collect data with. The first section captured sociodemographic variables, including gender, age, marital status, income, employment status, education level, type of residence, and purpose of visit. The second section evaluated key aspects of the healthcare experience, including waiting times, cleanliness, service comprehensiveness, staff availability, adequacy of equipment and supplies, drug availability, affordability, physician behavior, responsiveness to complaints, privacy, availability of health education materials, and ease of appointment scheduling. Patient satisfaction was assessed using a three-point Likert scale (good, not good, somewhat).

Ethical approval was obtained from the Institutional Review Board of the College of Pharmacy of the University of Babylon (protocol number: A-0017; approval date: February 1, 2024). Confidentiality was maintained throughout the study. Data analysis was performed using the SPSS version 16 software. Categorical variables were expressed as frequencies and percentages and analysed using Chi-square tests. Statistical significance was defined as a p -value < 0.05 .

Inclusion criteria: (i) patients who received medical treatment at public hospitals in Hillah, Iraq and (ii) patients capable of providing informed consent. Exclusion criteria: (i) patients who did not receive medical treatment at public hospitals in Hillah, Iraq and (ii) patients unable to provide informed consent due to cognitive impairment.

3. Results and Discussion

The results of this study indicate that marital status, age, educational level, type of consultation, and occupational status significantly influenced patient satisfaction scores ($p < 0.05$). In contrast, gender, residence, and economic status exhibited minimal impact ($p > 0.05$; Table 1). These findings suggest that healthcare providers should tailor their services to accommodate contextual and demographic factors in order to enhance patient satisfaction.

Satisfied patients are more likely to establish pos-

Table 1. Statistical correlation between the overall patient satisfaction and the patients' demographic data (N=150) in the Babylon Governorate.

Variables	Categories	Total satisfaction score						p-value
		unsatisfied		somewhat satisfied		satisfied		
		N	%	N	%	N	%	
Age	<20 years	2	1.33%	3	2%	4	2.66%	0.015
	20–29 years	31	20.66%	31	20.66%	22	14.66%	
	30–39 years	2	1.33%	4	2.66%	7	4.66%	
	40–49 years	7	4.66%	3	2%	7	4.66%	
	50–60 years	3	2%	4	2.66%	4	2.66%	
	>60 years	0	0%	4	2.66%	12	8%	
Gender	male	15	10%	13	8.66%	15	10%	0.72
	female	30	20%	36	24%	41	27.33%	
Residence	rural	22	14.66%	25	16.66%	35	23.33%	0.33
	urban	23	15.33%	24	16%	21	14%	
Marital status	single	31	20.66%	24	16%	15	10%	<0.001
	married	14	9.33%	23	15.33%	39	26%	
	widowed	0	0%	1	0.66%	2	1.33%	
	separated	0	0%	1	0.66%	0	0%	
Educational level	illiterate	0	0%	1	0.66%	10	6.66%	<0.001
	primary	0	0%	9	6%	17	11.33%	
	intermediate	4	2.66%	2	1.33%	4	2.66%	
	secondary	1	0.66%	3	2%	2	1.33%	
	college	40	26.66%	34	22.66%	23	15.33%	
Occupational status	not working	0	0%	2	1.33%	3	2%	<0.001
	student	27	18%	20	13.33%	13	8.66%	
	housewife	5	3.33%	9	6%	25	16.66%	
	self-employed / private	1	0.66%	6	4%	7	4.66%	
	governmental employee	12	8%	11	7.33%	4	2.66%	
	retired	0	0%	1	0.66%	4	2.66%	
Economic status	insufficient	9	6%	8	5.33%	18	12%	0.16
	somewhat sufficient	25	16.66%	22	14.66%	26	17.33%	
	sufficient	11	7.33%	19	12.66%	12	8%	
Number of visits	few	34	22.66%	35	23.33%	26	17.33%	0.025
	1–3 monthly	7	4.66%	11	7.33%	21	14%	
	4–6 monthly	1	0.66%	1	0.66%	6	4%	
	>6 monthly	3	2%	2	1.33%	3	2%	
Type of consultation	internal medicine	8	5.33%	11	7.33%	18	12%	0.01
	surgery	4	2.66%	3	20%	10	6.66%	
	gynaecology / obstetrics	5	3.33%	9	6%	7	4.66%	
	emergency	16	10.66%	8	5.33%	2	1.33%	
	dentistry	0	0%	1	0.66%	2	1.33%	
	medication refill	7	4.66%	5	3.33%	8	5.33%	
	other	5	3.33%	12	8%	9	6%	

itive relationships with healthcare providers, which in turn promotes adherence, continuity of care, and improved health outcomes. Among the 150 participants, individuals aged 20–29 years constituted the largest subgroup and reported notably lower satisfaction levels compared to other age groups, reflecting trends observed in a study conducted in Riyadh⁶. Moreover, lower educational attainment was associated with higher satisfaction, consistent with findings from Saudi Arabia and Iran^{6,7}. In contrast, individuals with higher education levels in the Kingdom tend to prefer private hospitals, with education emerging as a key determinant of patient satisfaction^{7,8}.

Gender did not influence satisfaction levels in the present study. However, other research has shown that women report higher satisfaction than men with PHC services, likely due to their roles as patients or caregivers. This contrasts with findings from Saudi Arabia, where men reported greater satisfaction than women⁹. Consistent with studies by Weber *et al.*¹⁰, our findings underscore that hospital cleanliness is a major concern for patients and significantly affects satisfaction, alongside the competence and demeanour of healthcare personnel.

This study was limited to 150 participants due to constraints such as sample size, recruitment challenges, and occasional lack of cooperation from patients. These limitations may affect the generaliza-

bility of the findings to the broader population.

4. Conclusion

Assessing patient satisfaction is essential for ensuring quality healthcare. While patients expressed satisfaction with staff behavior, they have reported dissatisfaction with medication availability, equipment adequacy, waiting area comfort, and cleanliness.

Acknowledgements

The authors wish to express their gratitude to the College of Pharmacy of the University of Babylon for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Oubaid E.N., Abdulameer N.A., Watife A.T., Alzubaidi F.A., Abdul-Hussein H.K., Al Jubouri F.M., Al-Shuai-bi H.F., Azzam A.A.H. Patient satisfaction in the public hospitals of Hillah (Iraq): a survey. *Pharmakeftiki* 37(2s), 384-388, 2025. <https://doi.org/10.60988/p.v37i2S.236>

Synthesis and *in vitro* anticancer activity assessment of bis-1,3,4-thiadiazol-2-amino flavone derivatives

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KEY WORDS:

bis-thiadiazol-2-amino flavone; imine flavone ethyl acetate; FT-IR; ¹H-NMR; MCF-7

ARTICLE INFO:

Received: January 10, 2025

Revised: February 28, 2025

Accepted: March 01, 2025

Available online: October 10, 2025

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ABSTRACT

2-Amino-1,3,4-thiadiazole is a heterocyclic scaffold associated with a wide range of pharmacological activities, including anticancer, antibacterial, antifungal, antiviral, antiparasitic, anticonvulsant, anticoagulant, antidiabetic, and antitumor effects. This structural motif has attracted considerable interest due to its therapeutic potential. Although several synthetic approaches have been proposed for this class of compounds, most are neither straightforward, economical, nor environmentally friendly. Given the established biosynthetic pathway of pyrimidines, it is reasonable to hypothesize that thiadiazoles may possess significant therapeutic value. The presence of a sulfur atom in the thiadiazole ring enhances liposolubility, while the mesoionic nature of these compounds facilitates cellular membrane penetration. In this study, novel 1,3,4-thiadiazole derivatives of bis-flavone imine were synthesized from bis-flavone imine ethyl acetate derivatives using a convergent synthetic strategy, yielding satisfactory product quantities. The chemical structures of the synthesized compounds were characterized using Fourier-transform infrared (FT-IR) spectroscopy and proton nuclear magnetic resonance (¹H-NMR) spectroscopy. *In vitro* anticancer assays on MCF-7 human breast cancer cells have revealed that compound T8 can achieve a 50% inhibition of cell viability (IC₅₀) at a concentration of 29.23 µg/mL. Compound T4 has exhibited a comparable anticancer effect, with an IC₅₀ value of 30.02 µg/mL.

1. Introduction

Breast cancer remains a major global health concern, affecting approx-

imately 14% of women worldwide and ranking as the most frequently diagnosed malignancy among females¹. Standard treatment modalities

Table 1. A comparison of the <i>in vitro</i> cytotoxic effects of the synthesized compounds T1-T8 on the MCF-7 human breast cancer cell-line.		
Synthesized compounds		IC ₅₀ (µg/mL)
T1	5,5'-((((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(methylene))bis(oxy))bis(1,3,4-thiadiazol-2-amine)	113.29
T2	5,5'-((((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(methylene))bis(oxy))bis(1,3,4-thiadiazol-2-amine)	102.04
T3	5,5'-((((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	145.45
T4	5,5'-((((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	30.02
T5	5,5'-((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	119.10
T6	5,5'-((((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	154.66
T7	5,5'-((((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	48.91
T8	5,5'-((((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	29.23

ties include surgery, radiotherapy, and chemotherapy, each tailored to the individual patient. However, a persistent challenge in the pharmacological management of breast cancer is the rapid emergence of drug resistance. This underscores the urgent need for the development of novel anticancer agents².

A series of new glucoside derivatives incorporating the 1,3,4-thiadiazole moiety were synthesized from D-glucose and 5-amino-1,3,4-thiadiazole-2-thiol using a convergent synthetic strategy, yielding promising results³. The anticancer potential of amide derivatives of imidazo[2,1b][1,3,4]thiadiazoles has been evaluated *in vitro* against four human cancer cell lines, including MCF-7 and MDA-MB-231; both associated with breast cancer⁴.

Additional 1,3,4-thiadiazole derivatives were synthesized from α-methyl cinnamic acid⁵. Thiadiazoles are well-documented for their antitumor activity.

Four isomeric forms of thiadiazole exist: 1,3,4-thiadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, and 1,2,5-thiadiazole; among these, 1,3,4-thiadiazole derivatives are considered the most promising for therapeutic applications⁶.

The antitumor activity of 1,3,4-thiadiazole derivatives includes both fused-ring systems and simple 2,5-disubstituted structures⁷. These compounds exert their effects through various molecular targets, such as carbonic anhydrase IX, Src and Abl kinases, and topoisomerase II⁸. Under diverse reaction conditions, substituted carboxylic acids condense with thiosemicarbazide to yield 2-amino-5-substituted 1,3,4-thiadiazoles⁹.

2. Methodology

The melting point (m.p.) of each compound was determined using an electrothermal melting point

apparatus (Model 200D / MP-200D-HR). Fourier-transform infrared (FT-IR) spectra of all synthesized compounds were recorded using a Bruker spectrophotometer. Proton nuclear magnetic resonance (^1H -NMR) spectra were obtained at the University of Tehran, Iran, using a Bruker Avance III 500 spectrometer (499 MHz for ^1H -NMR), with dimethyl sulfoxide (DMSO) as the solvent. All reagents were purchased from Sigma-Aldrich (Munich, Germany) and used without further purification.

The MCF-7 breast cancer cell line (ATCC HTB-22) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Bis-1,3,4-thiadiazol-2-amino flavones were synthesized by gradually adding imine flavone ethyl acetate (1 mmol), phosphoryl chloride (3 mmol), and thiosemicarbazide (2 mmol) to a reaction flask under stirring at room temperature for 2–3 hours. The mixture was then refluxed at 90°C for 8 h. Upon completion, the reaction mixture was poured into ice-cold water and neutralized with ammonium hydroxide solution.

Cell viability was assessed using the MTT assay (Sigma-Aldrich). Cells were harvested, trypsinized, and seeded at a density of 1×10^4 cells/well in 96-well plates containing 200 μL of fresh medium per well. After 24 h of incubation, a monolayer was established and cells were treated with the test compounds for 48 h at 37°C in a humidified atmosphere containing 5% CO_2 . Five serial dilutions were tested, ranging from 100 to 6.25 $\mu\text{g/mL}$. After 24 h of treatment, the supernatant was removed and 200 μL /well of MTT solution (0.5 mg/mL in phosphate-buffered saline) was added. Plates were incubated for an additional 4 h at 37°C. The supernatant was then discarded, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm using an ELISA reader (Model Wave XS2, BioTek, USA) in order to quantify cell viability¹⁰.

3. Results and Discussion

The chemical structures of the synthesized compounds were confirmed by FT-IR and ^1H -NMR spec-

troscopy. Detailed compound names are presented in Table 1, and key data are summarized below.

Compound T1: orange solid; yield: 68.7%; m. p.: 331°C–337.2°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.50 (s, 1H), 7.23 (m, 10H), 6.78 (m, 8H), 6.54 (s, 1H) proton at (-NH₂), 6.24 (s, 1H), 5.87 (s, 1H), 5.42 (s, 4H) (-CH₂).

Compound T2: green solid; yield: 68.7%; m. p.: 381°C–385.5°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.95 (s, 1H), 7.61 (m, 10H), 7.20 (m, 8H), 6.83 (s, 1H) proton at (-NH₂), 6.41 (s, 1H), 5.95 (s, 1H), 5.18 (s, 4H) (-CH₂).

Compound T3: white solid; yield: 77%; m. p.: 387.2°C–389.5°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.75 (s, 1H), 7.43 (m, 10H), 7.16 (m, 8H), 6.76 (s, 1H) proton at (-NH₂), 6.38 (s, 1H), 5.86 (s, 1H), 5.53 (s, 4H) (-CH₂), and 3.30 (s, 12H) proton of [-N(CH₃)₂].

Compound T4: dark yellow solid; yield: 74%; m. p.: 367.2°C–380.5°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.75 (s, 1H), 7.36 (m, 10H), 7.00 (m, 8H), 6.62 (s, 1H) proton at (-NH₂), 6.33 (s, 1H), 5.88 (s, 1H), 5.42 (s, 4H) (-CH₂).

Compound T5: violet solid; yield: 78.4%; m. p.: 392.4°C–395.7°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.88 (s, 1H), 7.63 (m, 10H), 7.14 (m, 8H), 6.70 (s, 1H) proton at (-NH₂), 6.28 (s, 1H), 5.92 (s, 1H), 5.57 (s, 4H) (-CH₂).

Compound T6: brownish-yellow solid; yield: 58.7%; m. p.: 395.3°C–397.7°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.95 (s, 1H), 7.41 (m, 10H), 7.08 (m, 8H), 6.75 (s, 1H) proton at (-NH₂), 6.47 (s, 1H), 5.96 (s, 1H), 5.52 (s, 4H) (-CH₂).

Compound T7: dark brown crystals; yield: 54.3%; m. p.: 388.3°C–392.8°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.65 (s, 1H), 7.34 (m, 10H), 6.85 (m, 8H), 6.52 (s, 1H) proton at (-NH₂), 6.26 (s, 1H), 5.94 (s, 1H), 5.43 (s, 4H) (-CH₂).

Compound T8: faint red solid; yield: 61.7%; m. p.: 348°C–353.7°C; ^1H -NMR spectrum (499 MHz, DMSO) peaks δ 7.84 (s, 1H), 7.40 (m, 10H), 7.08 (m, 8H), 6.74 (s, 1H) proton at (-NH₂), 5.35 (s, 1H), 5.03 (s, 1H), 4.43 (s, 4H) (-CH₂).

The FT-IR spectra of the bis-1,3,4-thiadiazol-2-amino flavones (T1–T8) revealed character-

istic peaks: symmetric and asymmetric stretching of the amino group ($-\text{NH}_2$) at $3416.92\text{--}3471.32\text{ cm}^{-1}$, stretching of the imine group ($-\text{C}=\text{N}$) at $1612.08\text{--}1692.68\text{ cm}^{-1}$, aromatic $\text{C}=\text{C}$ stretching at $1484.50\text{--}1629.18\text{ cm}^{-1}$, ether $\text{C}-\text{O}$ stretching at $1164.10\text{--}1251.87\text{ cm}^{-1}$, and cyclic ether $\text{C}-\text{O}$ stretching at $1034.77\text{--}1177.20\text{ cm}^{-1}$.

The ^1H -NMR spectra (499 MHz, DMSO) have revealed multiple signals: aromatic protons at δ 7.23–7.63 ppm; substituted aromatic protons at δ 7.00–7.85 ppm; singlet signals for amine protons at δ 6.52–6.83 ppm; cyclic $\text{C}=\text{C}$ protons at δ 5.03–6.38 ppm; and methylene ($-\text{CH}_2$) protons of ether groups at δ 4.43–5.57 ppm.

The assessment of the *in vitro* anticancer activity has revealed that compound T8 inhibited 50% of cell viability (IC_{50}) at a concentration of $29.23\text{ }\mu\text{g/mL}$. Compound T4 has exhibited comparable activity with an IC_{50} of $30.02\text{ }\mu\text{g/mL}$, compound T7 has exhibited a moderate activity (IC_{50} : $48.91\text{ }\mu\text{g/mL}$), while the remaining compounds had IC_{50} values exceeding $100\text{ }\mu\text{g/mL}$, as summarized in Table 1.

4. Conclusion

Novel thiosemicarbazide and 1,3,4-thiadiazole de-

rivatives were successfully synthesized and evaluated for their *in vitro* anticancer activity against a human breast cancer cell line (MCF-7). Structural characterization was performed using FT-IR and ^1H -NMR spectroscopy. The IC_{50} values of these compounds against the MCF-7 cells ranged from 29.23 to $154.66\text{ }\mu\text{g/mL}$; notably, compounds T4 and T8 demonstrated significant cytostatic activity against the MCF-7 cells.

Acknowledgements

The authors gratefully acknowledge Prof. Dr S. A. Aowda and Prof. Dr H. W. Al-Humadi for providing the necessary facilities at the College of Pharmacy.

Conflicts of interest

None exist.

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HOW TO CITE:

Radhi S.S., Alwan H.A., Alsadoon A.H., Abdul Hussein S.A. Synthesis and *in vitro* anticancer activity assessment of bis-1,3,4-thiadiazol-2-amino flavone derivatives. *Pharmakeftiki* 37(2s), 389-393, 2025. <https://doi.org/10.60988/p.v37i2S.237>

Polyvinylpyrrolidone-loaded Zn and ZnO nanoparticles influence *Escherichia coli* isolated from urinary tract infection patients

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KEY WORDS:

Escherichia coli; urinary tract infections; Zn-NP; ZnO-NP; *phoA*

ARTICLE INFO:

Received: November 13, 2024

Revised: December 29, 2024

Accepted: January 13, 2025

Available online: October 10, 2025

ABSTRACT

The biofilm formed by *Escherichia coli* contributes to pathogenesis through the intercellular contact within the biofilm, thereby facilitating bacterial proliferation and infection. This underscores the need for alternative antimicrobial agents with enhanced efficacy compared to traditional antibiotics. In this study, urinary tract infection (UTI)-derived *E. coli* isolates were assessed for susceptibility to zinc (Zn) and zinc oxide (ZnO) nanoparticles stabilised with the use of polyvinylpyrrolidone. Thirty isolates were treated with Zn and ZnO nanoparticles, and conventional polymerase chain reaction (PCR) was employed in order to detect the presence of virulence genes (*phoA*, *VTL*, and *LT1*). The *phoA* gene was detected in the majority of isolates, whereas the *VTL* and *LT1* genes were not observed. Bacterial counts were higher in untreated controls than in nanoparticle-treated samples, with this difference being particularly pronounced in *phoA*-positive isolates. These findings suggest that Zn and ZnO nanoparticles hold therapeutic potential against UTI-causing *E. coli* strains.

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1. Introduction

The global prevalence of urinary tract infections (UTIs) caused by *Escherichia coli* is largely attributed to increasing antibiotic resistance¹.

Zinc oxide (ZnO) nanoparticles and zinc (Zn) have demonstrated promising antibacterial properties. ZnO is particularly effective due to its ability to generate reactive oxygen species (ROS) and disrupt bacterial

membranes. Polyvinylpyrrolidone (PVP) serves as a stabilizing agent for these nanoparticles, enhancing dispersibility and modulating release profiles; features that make the Zn/ZnO-PVP nanocomposite suitable for treating persistent infections². Pathogenic *E. coli* strains associated with UTIs require the alkaline phosphatase-encoding alkaline phosphatase (*phoA*) gene for survival³. This study investigates the bactericidal efficacy of the Zn/ZnO-PVP nanocomposite against UTI-associated *E. coli*, and examines the *phoA* expression as a potential biomarker for alternative therapeutic approaches.

2. Methodology

2.1. Sample collection

Thirty urine specimens were collected from patients with symptomatic UTIs attending the Hillah Teaching Hospital (Iraq). Diagnostic criteria included dysuria and urinary frequency. Samples were processed in order to isolate *E. coli*, which were compared with control strains not associated with UTIs. Clean-catch midstream samples were used for microbiological analysis. The urine microbiome was evaluated in order to confirm the *E. coli* presence and assess antibiotic resistance.

2.2. Primer preparation

Primers targeting the *phoA*, verotoxin (VT1), and heat-labile toxin (LT1) genes were designed and synthesized by an industrial oligonucleotide supplier following standard protocols. Bioinformatics validation confirmed primer specificity to *E. coli* genomic sequences. Upon receipt, primers were reconstituted to 100 µM in nuclease-free water and were subsequently diluted to 10 µM for polymerase chain reaction (PCR) assays. Sequence alignments demonstrated exclusive (to *E. coli*) target matches^{4,5}.

2.3. PCR assay

Multiplex PCR was performed in order to amplify the *phoA*, VT1, and LT1 genes from *E. coli* isolates.

Each 50-µL reaction contained 10 µL of PCR buffer, 1 µL of each primer (forward and reverse: *phoA*, 5'-GTGACAAAAGCCCGGACACCATAAATGCCT-3', 5'-TACACTGTCATTACGTTGCGGATTTGGCGT-3'; VT1, 5'-GAACGAAATAATTTATATGTG-3', 5'-CCTGATGATGGCAATTCAGTA-3'; LT1, 5'-TTACGGCGTTACTATCCTCTCTA-3', 5'-GGTCTCGGTCAGATATGTGATTC-3'), 0.5 µL of DNA polymerase, 1–5 µL of template DNA, and nuclease-free water. Thermal cycling involved an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation (94°C, 1 min), annealing (56°C, 1 min), and extension (72°C, 1 min), with a final extension at 72°C for 10 min. Agarose gel electrophoresis confirmed amplification of the target genes (*phoA*, VT1, and LT1)⁶.

2.4. Nanoparticle preparation

Nanoparticles were synthesized *via* laser ablation in liquids using 99%+ purity Zn targets sourced from Sigma-Aldrich (Germany). A 1% PVP solution in deionised water was prepared as a stabilizing medium. Prior to ablation, Zn targets were cleansed with ethanol and deionised water. Ablation was performed using a switched Nd:YAG laser at 1064 nm, 5-nsec pulse width, 6-Hz repetition rate, and 160 mJ per pulse⁷.

2.5. Transmission electron microscopy

For the precise morphological characterization of core/shell Zn/ZnO nanoparticles, transmission electron microscopy (TEM) was conducted using the Zeiss EM10C system operated at 100 kV. This upgraded method was selected over previous field emission scanning electron microscopy (FE-SEM) for improved dimensional accuracy.

3. Results and Discussion

Out of 30 *E. coli* isolates, 19 were found to be *phoA*-positive; VT1 and LT1 genes were not detected (Figure 1). The undertaken UV-Vis spectral analysis revealed distinct absorption edges at 400 nm and 300 nm for Zn and ZnO nanoparticles, respectively, consistent with electronic band gap characteristics. The PVP polymer exhibited a peak at 279 nm, which is indicative of func-

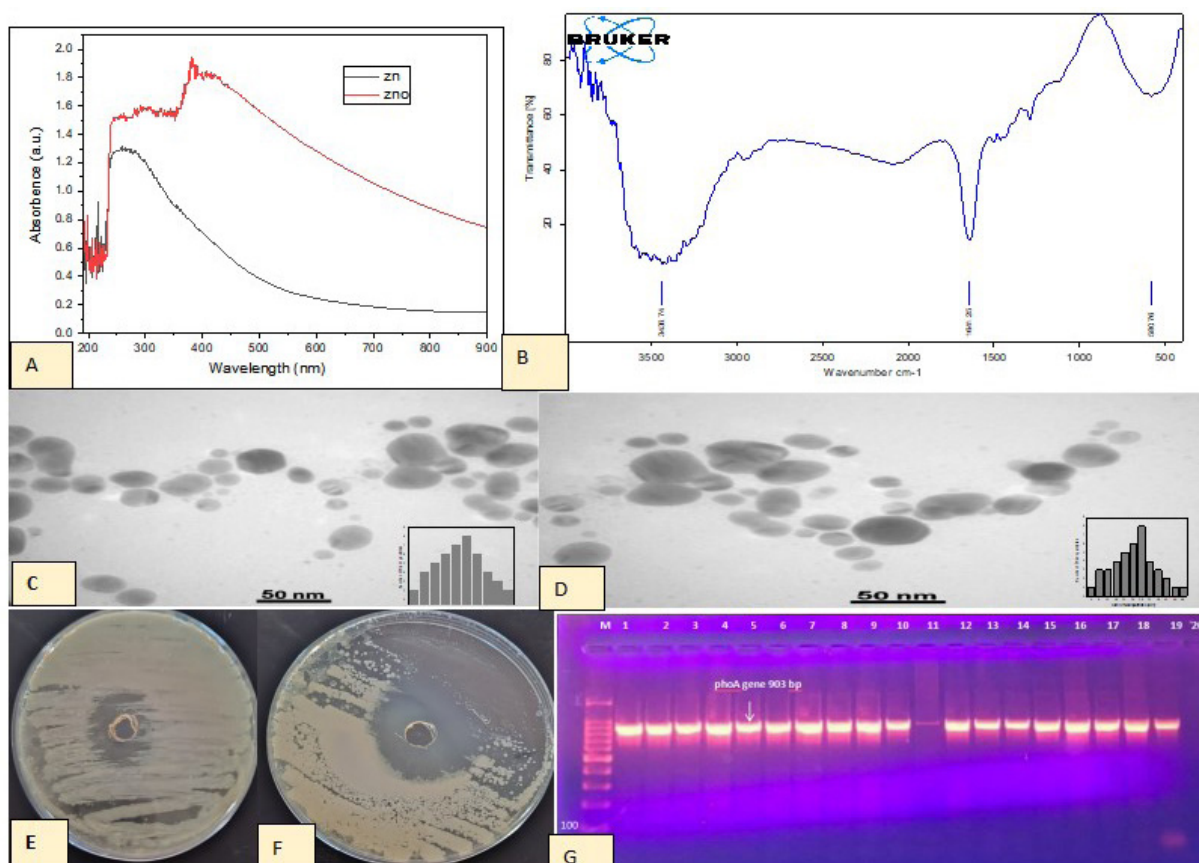


Figure 1. (A): UV-Vis spectra of zinc (Zn) and zinc oxide (ZnO) nanoparticles stabilized with a polyvinylpyrrolidone (PVP) polymer. (B): Fourier transform infrared spectroscopy (FTIR) spectra indicating absorption peaks at 3436, 1641, and 580 cm^{-1} . (C): Transmission electron microscopy (TEM) micrograph of Zn nanoparticles. (D): TEM micrograph of ZnO nanoparticles. (E–F): Zones of inhibition and minimum inhibitory concentrations (MIC) of ZnO (15 mm) and Zn (30 mm) nanoparticles against *E. coli* on Mueller-Hinton agar. (G): Gel electrophoresis of the 903 bp *phoA* amplicon from urinary tract infection-derived *E. coli*; 1.5% agarose gel run at 75 V for 45 min; lanes 1–19 show positive amplification; M denotes the molecular marker.

tional groups. Fourier-transform infrared spectroscopy (FTIR) confirmed the chemical identity of the synthesized nanoparticles: absorption bands at 3400 cm^{-1} corresponded to O–H stretching (hydroxyl groups), at 1630 cm^{-1} to C=O stretching (carbonyl or amide groups), and at 590 cm^{-1} to metal–oxygen bonds of Zn/ZnO (Figure 1).

TEM imaging illustrated uniform spherical morphology in Zn and ZnO nanoparticles (Figure 1), with PVP stabilization limiting particle sizes to 30 nm and 28 nm, respectively. This controlled nanoparticle architecture enhances their biomedical utility. Given that biofilms

contribute significantly to *E. coli* resistance and survival, chemical agents and nanoparticles such as those synthesized in this study are critical in disrupting biofilm formation⁸.

In the undertaken direct bacterial inhibition assays, Zn nanoparticles exhibited superior antibacterial activity compared to ZnO nanoparticles. Mechanistically, nanoparticle-induced ROS production and Zn^{2+} ion release underpin bactericidal effects. The *phoA* gene product (alkaline phosphatase) facilitates phosphorus mineralization, but also contributes to virulence *via* the hydrolysis of phosphate monoesters⁹.

4. Conclusion

The herein assessed Zn nanoparticles effectively inhibited *E. coli* proliferation, indicating their potential as safe, non-antibiotic alternatives in UTI management. This is particularly relevant in the context of antimicrobial resistance driven by genetic mutations.

Acknowledgements

The authors would like to thank the College of Science for Women of the University of Babylon for al-

lowing them to undertake this work in their laboratories.

Conflicts of interest

None exist.

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HOW TO CITE:

Radi N.K., Mohammed H.A., Alwarid R.J., Mohammed N.A., Ewadh R.M.J. Polyvinylpyrrolidone-loaded Zn and ZnO nanoparticles influence *Escherichia coli* isolated from urinary tract infection patients. *Pharmakeftiki* 37(2s), 394-397, 2025. <https://doi.org/10.60988/p.v37i2S.238>

Platelet-derived growth factor levels in Iraqi multiple myeloma patients: a case-control study

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KEY WORDS:

PDGF; multiple myeloma; bone marrow neoplasm; plasma cells; Iraq

ARTICLE INFO:

Received: January 09, 2025

Revised: February 12, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

Multiple myeloma (MM), the second most prevalent haematological malignancy, is characterized by monoclonal plasmacytosis within the bone marrow. Platelet-derived growth factor (PDGF), a key mediator of angiogenesis and tumorigenesis, has been implicated in the pathophysiology of MM. This study aimed at investigating whether pre-treatment serum levels of PDGF correlate with MM diagnosis, disease stage, and therapeutic response. A case-control study was conducted involving 55 Iraqi patients diagnosed with MM and 25 healthy controls. Participants were recruited from the Department of Haematology at the Marjan Medical Hospital, Babil Province, Iraq, between March 2021 and December 2022. Serum PDGF concentrations were quantified using enzyme-linked immunosorbent assay (ELISA). MM patients exhibited higher mean PDGF levels than controls (mean difference: 187.3 pg/mL), although this difference did not reach statistical significance ($p=0.077$). Among MM patients, those receiv-

ing treatment demonstrated significantly lower platelet counts ($p=0.012$) and elevated serum urea levels ($p=0.045$) compared to untreated counterparts. Receiver operating characteristic (ROC) curve analysis revealed poor diagnostic accuracy of PDGF for MM (area-under-the-curve; AUC: 0.575; $p=0.030$), as well as limited discriminatory power between stage II and stage III disease (AUC: 0.586; $p=0.059$). In conclusion, although serum PDGF levels alone do not constitute a statistically significant or specific diagnostic biomarker for MM, the findings underscore the need for further investigation into the combined utility of PDGF and other molecular markers to improve diagnostic and prognostic stratification in MM.

1. Introduction

Plasma cell myeloma (multiple myeloma; MM) is a malignancy characterized by the uncontrolled proliferation of plasma cells within the bone marrow, with an incidence rate of 4.5–6 per 100,000 individuals¹. It ranks second only to non-Hodgkin lymphoma among common haematological neoplasms. MM arises from dysregulated bone remodelling, wherein bone formation is diminished relative to accelerated bone resorption. Diagnostic criteria for MM include the detection of monoclonal proteins (M-proteins) in plasma or urine. Although MM remains incurable, recent therapeutic advances have significantly improved patient outcomes².

Myeloproliferative disorders, including MM, are marked by pathological angiogenesis. It is hypothesized that imbalances between pro-angiogenic and anti-angiogenic factors influence tumor perfusion and progression³. Several cytokines with tumorigenic potential – such as the fibroblast growth factor-2², the vascular endothelial growth factor⁴, and the platelet-derived growth factor (PDGF) – have been implicated in MM. PDGF, in particular, regulates key cellular functions in MM, including proliferation and migration. Consequently, PDGF is considered a candidate biomarker for MM diagnosis and staging. This study aims to evaluate the clinical relevance of serum PDGF levels and to determine their association with MM diagnosis, disease stage, and comparison to healthy controls.

2. Methodology

A case-control study was conducted at the Oncology Center of Merjan Medical City, Babylon, Iraq. The study included 55 patients diagnosed with MM and 25 healthy volunteers aged 38–72 years. Patient recruitment occurred at the Department of Haematology of the Marjan Medical Hospital, Babil Province, Iraq, from March 2021 to December 2022. MM diagnoses were confirmed by specialist haematologists. Control subjects were age-matched and free from acute or chronic illnesses.

Patients were undergoing first-line cytotoxic chemotherapy with either lenalidomide or bortezomib. They were stratified into four groups: group I comprised 26 patients with stage II MM; group II included 16 patients with stage III MM; group III consisted of 6 newly diagnosed stage II patients; and group IV included 7 newly diagnosed stage III patients. Groups III and IV were classified as untreated for analytical purposes.

Exclusion criteria included type 1 diabetes mellitus, renal or hepatic failure, prior bone marrow transplantation, and use of alternative MM therapies. Venous blood samples (5 mL) were collected from each participant for biochemical analysis. Socio-demographic data and haematological / biochemical test results were extracted from the patients' records.

Table 1. Diagnostic potential of serum and clinical markers in differentiating stages of multiple myeloma (MM). Abbreviations used: AUC, area-under-the-curve; CI, confidence interval; PDGF, platelet-derived growth factor; ROC, receiver operating characteristic.						
Variable	Group comparison	p-value	ROC analysis			
			AUC	Specificity, sensitivity	95% CI	p-value
Age (years)	Control vs MM Patients	0.088	0.575	0.625, 0.56	0.485–0.665	0.03
Platelets ($\times 10^3$)	Control vs MM Patients	0.001	0.575	0.625, 0.56	0.485–0.665	0.03
Blood urea (mg/dL)	Control vs MM Patients	0.059	0.575	0.625, 0.56	0.485–0.665	0.03
Serum creatinine (mg/dL)	Control vs MM Patients	0.082	0.575	0.625, 0.56	0.485–0.665	0.03
Serum PDGF (pg/mL)	Control vs MM Patients	0.077	0.575	0.625, 0.56	0.444–0.707	0.03
Age (years)	Stage II vs Stage III MM	0.061	0.586	0.619, 0.67	0.480–0.692	0.059
Platelets ($\times 10^3$)	Stage II vs Stage III MM	0.012	0.600	0.650, 0.68	0.490–0.710	0.050
Blood urea (mg/dL)	Stage II vs Stage III MM	0.045	0.590	0.630, 0.66	0.475–0.705	0.047
Serum creatinine (mg/dL)	Stage II vs Stage III MM	0.092	0.570	0.600, 0.65	0.465–0.675	0.089
Serum PDGF (pg/mL)	Stage II vs Stage III MM	0.059	0.586	0.619, 0.67	0.480–0.692	0.059

Serum PDGF concentrations were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience®) at the College of Pharmacy research laboratory. Data were analysed using the Statistical Package for the Social Sciences (SPSS, IBM). Receiver operating characteristic (ROC) curve analysis and logistic regression were employed in order to calculate the area-under-the-curve (AUC), sensitivity, and specificity for combined biomarkers. Cross-validation was performed in order to ensure analytical robustness, with statistical significance set at $p < 0.05$.

Ethical approval was granted by local health authorities and the Institutional Review Board (IRB) of the College of Pharmacy, University of Babylon (IRB number: A0032; date: 1-2022). Informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

3. Results and Discussion

Age demonstrated limited discriminative capacity between MM stages, with an AUC of 0.586 ($p = 0.059$), indicating no statistically significant difference. Among all evaluated parameters, platelet count ex-

hibited the highest diagnostic accuracy (AUC: 0.600, specificity: 0.650, sensitivity: 0.680; $p = 0.012$). Blood urea levels also showed reasonable diagnostic utility (AUC: 0.590; $p = 0.045$), while serum creatinine was less effective in stage differentiation (AUC: 0.570; $p = 0.092$). Serum PDGF yielded a moderate AUC of 0.586 ($p = 0.059$), suggesting limited standalone diagnostic value (Table 1).

Although MM patients exhibited elevated serum PDGF levels compared to healthy individuals, the difference was not statistically significant. PDGF also demonstrated poor performance in distinguishing between stage II and stage III MM. The role of angiogenesis in tumor pathogenesis is well established², and PDGF is recognized as a key mediator in this process. Previous studies have explored PDGF receptor expression in MM and reported associations with disease prognosis^{2,5}. However, the immunological implications of monoclonal immunoglobulin gammopathy in MM remain uncertain, particularly regarding cytokine-mediated modulation of myeloma cell intermediates¹.

PDGF is known to enhance endothelial cell migration and has recently been identified as a major cytokine involved in MM pathogenesis, affecting

both neoplastic and normal cellular environments⁶. Contrasting findings from other studies suggest that elevated PDGF receptor expression correlates with advanced disease stages⁷, while others report reduced plasma PDGF-BB levels in MM patients relative to healthy controls⁸.

Platelets are implicated in tumor growth, invasion, and chemoresistance². The heightened platelet reactivity observed in MM may have confounded PDGF measurements, as MM patients in this study exhibited significant differences in platelet counts compared to controls.

Although patients were not stratified by specific chemotherapeutic agents, all received treatment at a single center to minimize confounding variables. Existing literature indicates that PDGF contributes to the stabilization of stressed microvascular phenotypes via pericytes and vascular smooth muscle cells^{5,8}. Some researchers propose that PDGF- β may function as a latent angiogenic factor in haematological malignancies, with microvascular density positively correlating with MM progression^{1,5,8}.

Despite the absence of treatment-based stratification, uniform therapeutic protocols were applied to all participants to reduce bias. The observed discrepancies in PDGF levels before and after chemotherapy suggest that PDGF may serve as a marker of immune status in advanced MM. PDGF appears to play a multifaceted role in cancer biology, acting as an antitumor factor in tumours with high PDGF production, and as a potent growth factor in those with lower expression.

The age distribution of MM patients in this study aligns with trends reported in other populations, indicating broad demographic representation. Ongoing research continues to identify molecular mechanisms and therapeutic targets that drive MM progression, underscoring the need for novel treatment strategies². This study does not advocate for PDGF as a definitive biomarker; rather, it highlights the potential of molecular genetic analyses to elucidate PDGF-related oncogenic pathways and inform

drug development targeting MM-specific PDGF signalling. Further research is warranted in order to clarify the functional role of PDGF in malignancy and to enhance therapeutic precision.

4. Conclusion

Serum PDGF demonstrates limited diagnostic utility in distinguishing stage II from stage III MM. Although PDGF is involved in angiogenesis and tumor progression, its low sensitivity precludes its use as a standalone biomarker. Platelet count and blood urea levels showed stronger staging performance, with statistically significant *p*-values, whereas age and serum creatinine were only moderately predictive. These findings support the need for multi-marker approaches so as to improve MM diagnostic accuracy and refine staging and treatment strategies.

Acknowledgements

The authors express their sincere gratitude to the patients and their families for participating in this study. Appreciation is also extended to the healthy volunteers who contributed to the study.

Conflicts of interest

None exist.

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HOW TO CITE:

Ramadan G.M., Al-Sadi H.I., Alkafaji S.I., Al-Hindy H.A.A.M., Mousa M.J., Jawad H.S., Chabuk S.K., Abbas R.J., Abd Ali M.H., Makki M.M., Hadi F.H., Dawood N.A.T. Platelet-derived growth factor levels in Iraqi multiple myeloma patients: a case-control study. *Pharmakeftiki* 37(2s), 398-402, 2025. <https://doi.org/10.60988/p.v37i2S.239>

Formulation and *in vitro* assessment of indomethacin submitted to solid dispersion for the purpose of enhancing its dissolution

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KEY WORDS:

dissolution enhancement;
indomethacin; amorphous
solid dispersion; solvent
evaporation; Soluplus

ARTICLE INFO:

Received: January 02, 2025

Revised: January 17, 2025

Accepted: February 06, 2025

Available online: October 10, 2025

ABSTRACT

Indomethacin (IND), a nonsteroidal anti-inflammatory drug, is widely employed in the pharmaceutical sector for its analgesic, anti-inflammatory, and antipyretic properties. However, IND exhibits poor dissolution in gastrointestinal tract fluids, leading to limited bioavailability. Solid dispersion (SD) is a well-established and widely adopted strategy for enhancing drug dissolution. This study aimed to formulate and evaluate, *in vitro*, the SD technique for improving IND dissolution. IND-based SDs were prepared using the solvent evaporation method and three distinct polymers: Soluplus®, polyvinylpyrrolidone K30 (PVP K30), and hydroxypropyl methylcellulose E5 (HPMC E5). The resulting SDs were assessed for *in vitro* drug release and subjected to solid-state characterization. Among the formulations, the Soluplus®-based SD demonstrated superior performance, achieving 100% drug release within 2 h, compared to 74.75% and 53.00% release from PVP K30 and HPMC E5-based SDs, respectively. Solid-state analyses confirmed complete amorphization of IND within the SD matrix, with no evidence of physicochemical incompatibility. In conclusion, the Soluplus®-based SD prepared *via* solvent evaporation represents an effective approach for enhancing the dissolution profile of IND.

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1. Introduction

Poor aqueous solubility is widely recognized as a major challenge for formulation scientists during the

early stages of modern drug development¹. This limitation is directly associated with reduced oral bioavailability and suboptimal therapeutic outcomes¹.

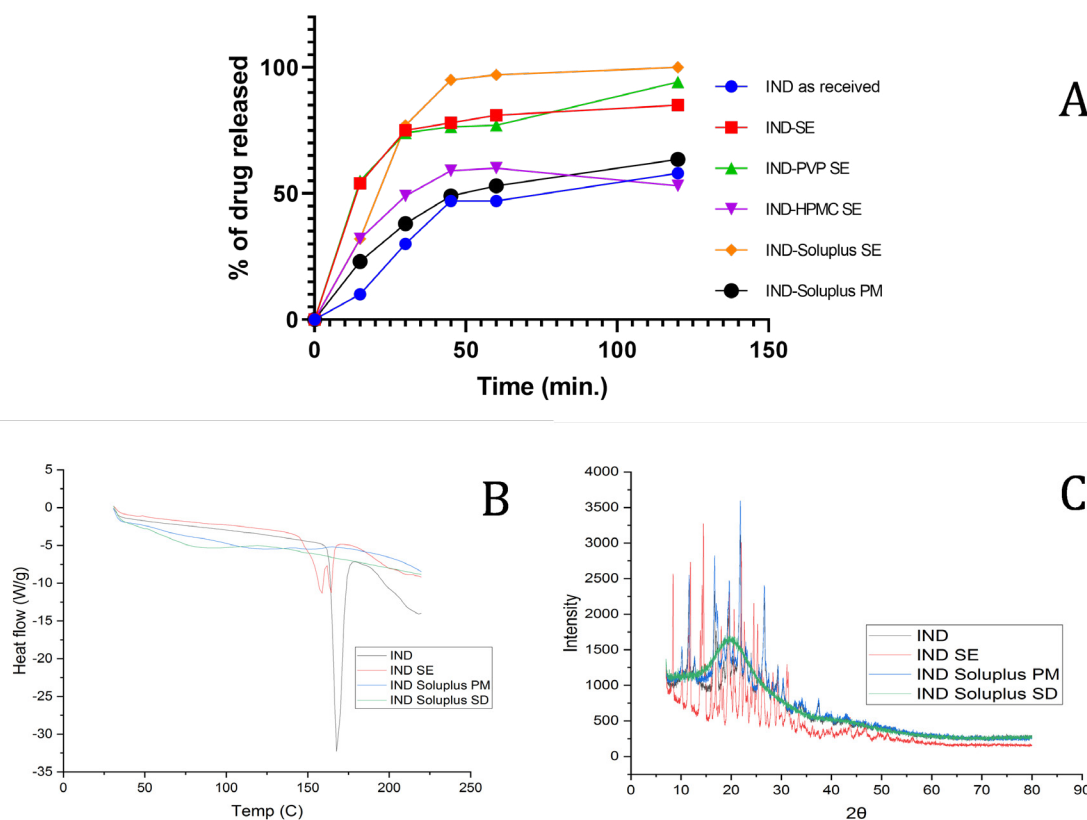


Figure 1. *In vitro* characterization of indomethacin (IND) solid dispersion (SD) formulations: dissolution profiling (A); differential scanning calorimetry (B); powder X-ray diffraction (C).

Solid dispersion (SD) is a widely adopted technique for enhancing drug dissolution². SDs are formed by dispersing hydrophobic active pharmaceutical ingredients within a hydrophilic polymeric matrix in the solid state³. The mechanism underlying dissolution enhancement in SD formulations involves several key factors, including improved wettability, reduced particle size, prevention of aggregation, and conversion of the drug from a crystalline to an amorphous phase^{2,4}.

Indomethacin (IND), a nonsteroidal anti-inflammatory drug, is extensively used for its analgesic, anti-inflammatory, and antipyretic properties⁵. IND is a weakly acidic compound with a pKa ranging from 3.0 to 4.5. Upon oral administration, IND exhibits poor dissolution in gastrointestinal tract fluids, resulting in limited bioavailability. It is classified under the Biopharmaceutics Classification System⁵. The present study

aims to evaluate the effectiveness of the SD technique in enhancing the dissolution of IND.

2. Methodology

IND and hydroxypropyl methylcellulose E6 (HPMC E6) were obtained from Macklin Co., Ltd. (China). Polyvinylpyrrolidone K30 (PVP K30) was purchased from HiMedia Laboratories (India) and Soluplus® from the BASF Pharmaceutical Industries (Germany).

IND-based SDs were prepared using the solvent evaporation method. A quantity of IND (25 mg) was mixed with 50 mg of each carrier polymer (PVP K30, HPMC E6, and Soluplus®) in a 1:2 ratio in a porcelain dish. Ethanol was used to dissolve the components, and the solvent was subsequently removed under reduced pressure for 90 min at 55°C using a rotary vacu-

um evaporator. The resulting SDs were pulverized in a mortar, passed through a sieve, dried in an oven for 2 h, and stored in a desiccator containing silica gel in order to minimize moisture prior to characterization.

Samples including unprocessed IND, IND solvent evaporate (SE), IND SDs, and a physical mixture (PM) of IND with Soluplus® were subjected to *in vitro* dissolution testing (Figure 1.A). These experiments were conducted in parallel using a United States Pharmacopeia Type I dissolution apparatus. Each sample, equivalent to 25 mg of IND, was dissolved in 900 mL of phosphate buffer (pH 6.8) maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, with a paddle rotation speed of 75 revolutions per min. At predetermined intervals, 5-mL aliquots were withdrawn and replaced with fresh buffer. The filtered samples were analysed spectrophotometrically at 319.5 nm. All measurements were performed in triplicate².

Differential scanning calorimetry (DSC) was employed in order to determine the melting points of IND, IND SE, the PM, and the SD of IND with Soluplus® (Figure 1.B). Analyses were conducted using a Shimadzu DSC-60 (Japan). Approximately 3 mg of each sample was accurately weighed and sealed in aluminum pans (5–6-mg capacity). Thermal scans were performed from 35°C to 280°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ ⁶.

Powder X-ray diffraction (PXRD) analysis was conducted on IND, IND SE, the PM, and the SD of IND with Soluplus® using a diffractometer manufactured by Aires (Netherlands). Operational parameters included a current of 30 mA, voltage of 40 kV, scan rate of $1^{\circ}/\text{min}$, and a 2θ range of 10° to 90° ⁶.

Statistical analysis was performed using GraphPad Prism version 9. Data were expressed as mean $\pm$ standard deviation (SD). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's or Dunnett's multiple comparison *post hoc* tests. A *p*-value <0.05 was considered statistically significant.

3. Results and Discussion

The dissolution profile of pure IND revealed limited solubility, with only 58% of the drug released after 2 h under the specified conditions. This poor performance is attributed to IND's hydrophobic nature, which causes it to float on the medium surface and hinder disso-

lution. In contrast, IND SE and SDs prepared with PVP K30 and Soluplus® exhibited significantly improved drug release compared to the unprocessed form (Figure 1.A). Enhanced solubility in SD systems is primarily due to particle size reduction and increased surface area². The hydrophilic environment further promotes drug wettability and solubility^{2,4}. Among the tested formulations, the Soluplus®-based SD demonstrated superior dissolution performance, supporting its selection for further evaluation. Previous studies have highlighted the efficacy of Soluplus® in improving the dissolution of poorly soluble drugs when used as a carrier in SD systems⁷. Soluplus® is an amorphous, amphiphilic copolymer composed of polyvinyl caprolactam, polyvinyl acetate, and polyethylene glycol. It forms micelles and serves as a polymeric matrix that enhances dissolution rates⁸. Additionally, Soluplus® prevents drug precipitation or recrystallization during dissolution, improves wettability, and stabilizes SDs during storage by forming hydrogen bonds with the drug⁸. Conversely, the SD of IND with HPMC E6 resulted in lower drug release after 2 h compared to the pure drug. Only the Soluplus®-based SD achieved significantly higher IND release than its SE counterpart (Figure 1.A).

The DSC analysis of pure IND revealed a sharp endothermic peak at 169.9°C , corresponding to its melting point (Figure 1.B). This observation aligns with previously published data and confirms the crystalline nature and purity of the drug⁹. The thermogram of the PM showed no distinct peak, likely due to dilution effects from mortar mixing. In the selected SD formulation, the absence of the endothermic peak indicates complete amorphization of IND (Figure 1.B)³.

The PXRD analysis of pure IND displayed multiple sharp Bragg peaks at specific 2θ angles, confirming its crystalline structure (Figure 1.C). In contrast, the binary SD system of IND with Soluplus® exhibited a halo pattern with no sharp peaks, indicating a transition to the amorphous state (Figure 1.C)¹⁰. This transformation supports the successful formation of SD³.

4. Conclusion

The Soluplus®-based SD of IND, prepared *via* the solvent evaporation method, proved to be an effective

strategy for enhancing the drug's dissolution profile.

Conflicts of interest

Acknowledgements

None exist.

The authors extend their gratitude to Assistant Professor Dr Omar Saeb Salih of the College of Pharmacy of the University of Baghdad, for conducting DSC analyses. The College of Pharmacy at Mustansiriyah University provided essential equipment and support for this study.

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HOW TO CITE:

Razooqi K.D., Hameed G.S., Albakaa A.R.M. Formulation and *in vitro* assessment of indomethacin submitted to solid dispersion for the purpose of enhancing its dissolution. *Pharmakeftiki* 37(2s), 403-406, 2025. <https://doi.org/10.60988/p.v37i2S.240>

Antibacterial activity of phenylboronic acid on pathogenic isolates of *Escherichia coli* and *Staphylococcus aureus*

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KEY WORDS:
phenylboronic acid; *E. coli*; *S. aureus*; agar diffusion; anti-bacterial

ARTICLE INFO:

Received: December 28, 2024

Revised: n/a

Accepted: January 06, 2025

Available online: October 10, 2025

ABSTRACT

Boron, a bioactive trace element present in humans, plays a critical role in bone formation and maintenance, and exhibits both antibacterial and anti-inflammatory properties. This study investigated the antibacterial activity of boronic acid at varying concentrations in aqueous solution. Phenylboronic acid (PBA) was prepared at concentrations of 3 mg/mL, 6 mg/mL, and 9 mg/mL, and its antibacterial efficacy was assessed using the agar diffusion method against clinically isolated strains of *Escherichia coli* and *Staphylococcus aureus*. The results demonstrated a dose-dependent antibacterial effect of PBA on both bacterial species.

1. Introduction

Arylboronic acids represent a prominent chemical class due to their broad spectrum of applications. Although these compounds have been known for over a century, their properties and uses continue to expand. Key areas of interest include the synthesis of biaryl compounds, molecular receptors, covalent chemical frameworks, and bioactivity profiles¹.

The nature and position of substituents on the phenyl ring significantly influence acidity, receptor binding, and biological activity. Another major area of investigation is optics, with numerous studies exploring the optical properties of boron-containing compounds. Phenylboronic acid (PBA) has garnered attention in drug delivery research. Kitano *et al.* described a glucose-responsive polymer complex incorporating a PBA moiety, which

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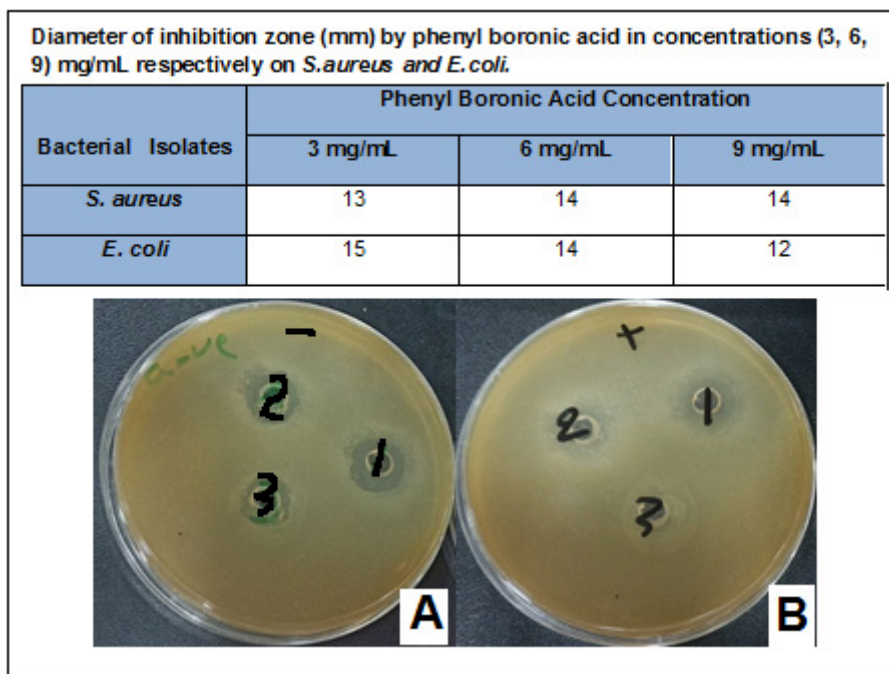


Figure 1. Agar well diffusion assay to test the antibacterial activity of phenylboronic acid in concentrations 3, 6, and 9 mg/mL, respectively, against *E. coli* (A) and *S. aureus* (B).

functioned as a novel drug delivery system. Recent advances have highlighted the therapeutic potential of boronic acids in antiviral, antibacterial, and anticancer applications².

Many antimicrobial agents incorporate halogen atoms, which play a critical and expanding role due to their electronegativity and ability to form complexes with hydrogen bond acceptors. This behaviour has been characterized at the molecular level through electrostatic potential surface analysis. Extensive research has examined the effects of halogenation – specifically chlorination, bromination, and iodination – on antibacterial activity³. Halogenation remains a powerful strategy for modulating the properties of bioactive compounds, including antibacterial agents.

Boronic acids are both chemically reactive and stable, with low toxicity. As bioisosteres of carboxylic acids, they share the same periodic group as carbon. Their ability to bind saccharides makes them useful in studying biological systems and identifying me-

tabolites implicated in the aetiology of diabetes. As mild Lewis acids, boronic acids are indispensable in organic synthesis and cross-coupling reactions due to their stability and operational simplicity. They have also been employed as functional groups in anticancer, antiviral, and antibacterial agents. Consequently, halogenated boronic acids have been predicted to exhibit antimicrobial and antibiofilm activity against *Vibrio* species⁴.

Boron derivatives have been utilized in the design of novel therapeutic agents, with their biological activity largely dependent on the chemical behavior of the boron atom⁵. Many boron-containing compounds are promising candidates for antimicrobial drug development. A clinically approved cyclic boronic acid derivative has been used to treat urinary tract infections, with its mechanism of action involving inhibition of β -lactamase enzymes; key mediators of bacterial resistance⁶.

Accordingly, the aim of this study was to investigate the *in vitro* antibacterial activity of PBA com-

pounds against *Escherichia coli* and *Staphylococcus aureus*.

2. Methodology

In vitro antibacterial activity was assessed using the agar well diffusion assay. Loopful growths from bacterial isolates were inoculated into nutrient broth and incubated at 37°C for 18 h. The resulting bacterial suspensions were diluted with normal saline, and turbidity was adjusted to match the 0.5 McFarland standard, yielding a uniform suspension containing 1.5×10^8 colony-forming units per milliliter (CFU/mL). A sterile cotton swab was dipped into the adjusted suspension and used in order to streak the entire surface of Mueller–Hinton agar plates for each tested bacterium. Plates were allowed to dry at room temperature for 5–15 min. Using a cork borer, three wells (5-mm diameter) were cut into each plate. Into each well, 20 µL of PBA solution at concentrations of 3, 6, and 9 mg/mL were added. All tests were performed in triplicate. Plates were incubated overnight at 37°C. Following incubation, zones of inhibition were recorded for each concentration and bacterial isolate. The diameters of the inhibition zones were measured in millimeters using a calibrated measuring scale.

3. Results and Discussion

Bacterial antibiotic resistance has emerged as a critical global public health concern. Boron-based organic synthesis has gained prominence due to the unique coordination chemistry of boron, which facilitates the development of novel functionalized compounds. The design and application of boron derivatives have been extensively explored across pharmaceutical domains⁷.

In the present study, results illustrated in Figure 1 demonstrate that PBA exhibited antibacterial activity against both *E. coli* and *S. aureus*, as determined by the agar well diffusion assay.

Gram-positive bacteria such as *S. aureus* lack an outer membrane, rendering Gram-negative bacteria more susceptible to PBA complexes than their

Gram-positive counterparts⁸. Boronic acid derivatives have also been identified as promising inhibitors of the chromosomal NorA efflux pump in *S. aureus*⁹.

The clinical relevance of these compounds is underscored by the approval of five boronic acid or boronic ester-based drugs by the US Food and Drug Administration. Over the past decades, boronic acids have been investigated as inhibitors of penicillin-binding proteins (PBPs), with recent studies focusing on their ability to covalently interact with serine residues. Notably, branched boronic acids have demonstrated inhibitory activity against PBP1b and exhibited measurable antimicrobial effects, including minimum inhibitory concentration values¹⁰.

4. Conclusion

Given the escalating threat of bacterial antibiotic resistance in both Gram-positive and Gram-negative pathogens, the development of novel agents such as PBA represents a promising therapeutic strategy. In this study, PBA demonstrated antibacterial activity against *E. coli* and *S. aureus*, suggesting its potential utility in future antimicrobial therapies. Further research is warranted in order to evaluate the biological activity of PBA against other resistant bacterial strains.

Acknowledgements

The authors express their sincere appreciation to the College of Pharmacy of the University of Babylon, for the financial support of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Salman H.D., Hammadi A.H., Habeeb S.A., Mahdi Dahadh O.A. Antibacterial activity of phenylboronic acid on pathogenic isolates of *Escherichia coli* and *Staphylococcus aureus*. *Pharmakeftiki* 37(2s), 407-410, 2025. <https://doi.org/10.60988/p.v37i2S.241>

Assessing the effects of a DPP IV inhibitor and risperidone on behavioural interactions and lipid peroxidation in a mouse model of induced autism

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KEY WORDS:

sitagliptin; open field test; risperidone; DPP-4; autism

ARTICLE INFO:

Received: December 18, 2024

Revised: February 06, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

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ABSTRACT

The autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by early-onset deficits in communication and social interaction. This study investigated the effects of a dipeptidyl peptidase-4 (DPP IV) inhibitor, sitagliptin, in a murine model of ASD induced by prenatal exposure to sodium valproate (VPA). In order to evaluate ASD-like behaviours, an open field test (OFT) was conducted so as to assess locomotor activity and anxiety-related responses. The antioxidant and anti-inflammatory properties of sitagliptin were examined by quantifying levels of reduced glutathione, malondialdehyde (MDA), and interleukin-6. VPA-exposed mice were allocated into four experimental groups: (i) saline control, (ii) VPA + sitagliptin (DPP IV inhibitor), (iii) VPA + risperidone, and (iv) VPA-only. Behavioural assessments *via* OFT were performed on postnatal day 65, followed by biochemical analyses of oxidative stress and inflammatory markers on postnatal day 66. Sitagliptin treatment significantly attenuated oxidative stress and neuroinflammation, leading to marked improvements in aberrant behavioural phenotypes. It exhibited pronounced anxiolytic effects, ameliorating ASD-associated symptoms such as anxiety, hyperactivity, and fearfulness in the OFT. Furthermore, sitagliptin demonstrated superior antioxidant efficacy compared to risperidone, notably in reducing lipid peroxidation. A dose of 10 mg/kg of sitagliptin yielded significant reductions in MDA levels, underscoring its free radical scavenging potential. These findings suggest that sitagliptin may offer a promising therapeutic strategy for mitigating core and associated symptoms of ASD.

1. Introduction

The autism spectrum disorder (ASD) arises from a complex interplay of environmental and genetic factors during the prenatal and early postnatal periods^{1,2}. Prenatal contributors associated with increased ASD incidence include maternal illness and exposure to toxins. Notably, a meta-analysis of over 40,000 ASD cases has identified a correlation between elevated ASD risk and viral infections occurring during labor³. A successful ASD therapy is characterized by individualized, integrative strategies that address both cognitive and behavioural dimensions⁴. Currently, risperidone and aripiprazole are the only medications approved by the US Food and Drug Administration for the treatment of ASD-associated symptoms, particularly irritability and aggression⁵.

Sitagliptin, a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4 or DPP IV), exerts its effects through competitive inhibition, resulting in a notable 95% reduction in enzymatic activity over a 12-h period. This inhibition improves glycaemic control by reducing both postprandial and fasting hyperglycaemia, enhancing insulin secretion, and suppressing glucagon levels. As monotherapy, sitagliptin has been shown to reduce glycated haemoglobin levels by approximately 0.8% in patients with type 2 diabetes.

The study of ASD frequently relies on animal models that adopt a multimodal approach encompassing behavioural, neuropathological, physiological, and genetic parameters. While mice carrying mutations in ASD-associated genes are commonly used, comparative studies involving both mice and rats are gaining traction, particularly given rats' more pronounced social behaviours^{7,8}.

2. Methodology

This study employed 60 healthy adult albino mice weighing between 27 and 40 g. The cohort consisted of 40 females and 20 males, donated by the Ministry of Science and Technology in Baghdad, Iraq. Animals were housed in standard plastic cages at the College of Medicine of the University of Babylon, under controlled conditions: a 12-h light/dark cycle, ambient

temperature of $24^{\circ}\text{C} \pm 5^{\circ}\text{C}$, and relative humidity of $65\% \pm 5\%$. Food pellets and water were provided *ad libitum*. All experimental procedures adhered to institutional guidelines for laboratory animal care and were approved by the Department of Pharmacology of the College of Pharmacy of the University of Babylon (approval number: A-0041; date: 5/11/2023).

After a 12-day acclimatization period, pregnant female mice were randomly assigned to two primary groups for comparative analysis. Control groups received intraperitoneal (i.p.) injections of saline. In total, ten groups were formed, each comprising two pregnant females. In order to induce the ASD model, a single i.p. injection of sodium valproate (VPA) at a dose of 600 mg/kg was administered.

On postnatal day 40, offspring were weaned and distributed into eight groups of ten animals each: (i) control groups: group 1 (received oral normal saline), groups 2 and 3 (received oral sitagliptin at doses of 10 mg/kg and 15 mg/kg, respectively), and group 4 (received oral risperidone at a dose of 1 mg/kg), and (ii) VPA-induced groups: group 5 (received oral normal saline), groups 6 and 7 (received oral sitagliptin at doses of 10 mg/kg and 15 mg/kg, respectively), and group 8 (received oral risperidone at a dose of 1 mg/kg).

Hyperactivity was assessed using line crossings and reproductive behaviours in an open field test (OFT). Line crossings, defined as the number of squares traversed along grid lines, served as a measure of locomotor activity. All behaviours were recorded using a Redmi Xiaomi camera (China) for subsequent analysis.

Brain tissue concentrations of interleukin-6 (IL-6), a pro-inflammatory cytokine, were quantified using an enzyme-linked immunosorbent assay (ELISA) kit. Brain tissue levels of malondialdehyde (MDA) were evaluated by the thiobarbituric acid assay method on a spectrophotometer. Finally, reduced glutathione (GSH) levels in the brain tissues were determined according to the Murat Aliskit's colorimetric method. Data were processed and analysed using the SPSS version 26 software. One-way analysis of variance (ANOVA) followed by *post hoc* least significant difference (LSD) testing was employed. Statistical significance was set at $p < 0.05$.

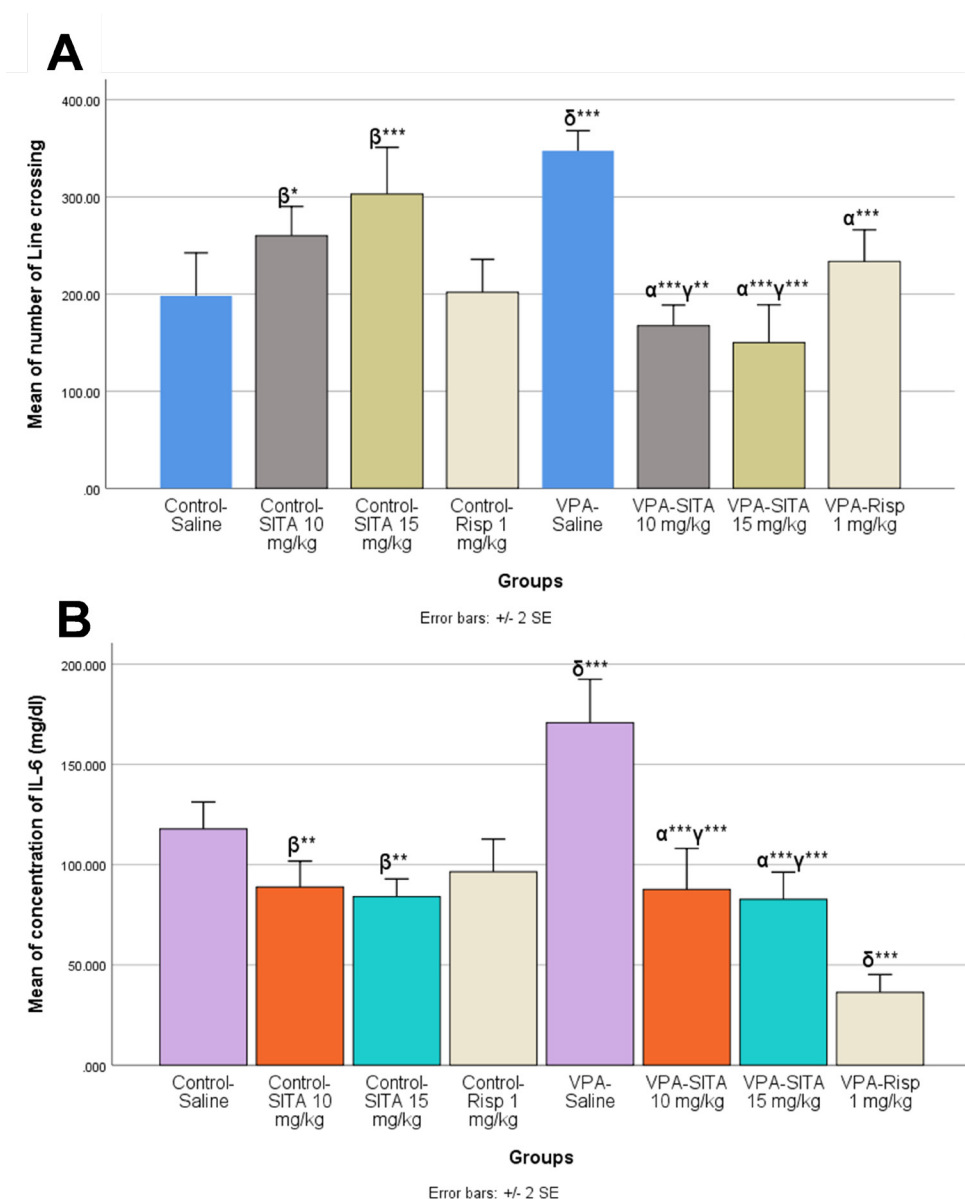


Figure 1. (A): Effect of sitagliptin (SITA) and risperidone (Risp) on the number of line crossings in the open field test. (B): Effect of SITA and Risp on tissue concentrations of interleukin-6 (IL-6). Notes: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; α : versus the sodium valproate (VPA)-saline group; β : versus the control-saline group; γ : versus the VPA-risperidone group. δ : versus other groups.

3. Results and Discussion

The VPA-saline group exhibited significantly elevated line crossings ($p < 0.001$) compared to all other groups. In contrast, both low- and high-dose VPA-si-

tagliptin and VPA-risperidone groups demonstrated significantly reduced line crossings ($p < 0.001$) relative to the VPA-saline group. Within the control groups, the high-dose sitagliptin group showed a marked increase in line crossings ($p < 0.001$), while

the low-dose sitagliptin group also exhibited a significant increase ($p=0.014$) compared to the control-saline group. Among the VPA-induced groups, the high-dose VPA-sitagliptin group showed a pronounced reduction in line crossings ($p<0.001$), and the low-dose VPA-sitagliptin group exhibited a highly significant decrease ($p=0.009$) when compared to the VPA-risperidone group. These findings are illustrated in Figure 1.A.

IL-6 levels were found to be significantly reduced ($p<0.001$) in both low- and high-dose VPA-sitagliptin and VPA-risperidone groups compared to the VPA-saline group. Furthermore, IL-6 concentrations were significantly lower in the VPA-risperidone group than in either VPA-sitagliptin group ($p<0.001$). Within the control groups, IL-6 levels were significantly diminished in both the low-dose ($p=0.009$) and high-dose ($p=0.002$) sitagliptin groups compared to the control-saline group. These data are presented in Figure 1.B.

The levels of GSH were found to be significantly increased in the 15 mg/kg VPA sitagliptin group as compared with the VPA-risperidone group. Moreover, the levels of MDA were found to be significantly decreased in both dose groups of sitagliptin as compared with the VPA-risperidone group. The dose of 10 mg/kg sitagliptin yielded significant decrease in MDA levels as compared with the dose of 15 mg/kg sitagliptin.

The objective of this study was to establish an ASD model by administering VPA intraperitoneally to pregnant mice on gestational day 12. Offspring exposed to VPA during foetal development exhibited behavioural alterations including reduced social interaction and creativity, increased locomotor activity with diminished exploratory behavior, elevated anxiety scores, and heightened oxidative stress. Treatment with sitagliptin and risperidone mitigated oxidative stress and improved behavioural outcomes in the offspring.

OFT results revealed elevated anxiety in VPA-saline offspring, as evidenced by increased speed, grooming, and rearing behaviours compared to controls. These findings align with previous studies^{1,9}. The VPA-sitagliptin group demonstrated a significant reduction in line crossings compared

to the VPA-risperidone group. Conversely, the control-sitagliptin group showed a marked increase in line crossings relative to both the control-saline and VPA-risperidone groups. These results corroborate earlier findings indicating that sitagliptin exerts anxiolytic effects in OFT paradigms¹⁰.

This study also evaluated the impact of sitagliptin on IL-6 levels in VPA-exposed mice. Both sitagliptin and risperidone treatments significantly reduced IL-6 concentrations. Notably, IL-6 levels were lower in the VPA-risperidone group than in the VPA-sitagliptin groups. Additionally, IL-6 concentrations were significantly reduced in the 15 mg/kg sitagliptin group compared to the 10 mg/kg group ($p<0.001$). These findings are consistent with prior research on dementia models demonstrating cytokine modulation by sitagliptin¹⁰.

Reactive oxygen species cause cellular damage (including lipid peroxidation) and interfere with the fluidity of cell membrane. The imbalance between the production and the removing of reactive oxygen species resulted in oxidative stress. Increased levels of GSH and decreased levels of MDA in the VPA-sitagliptin and the VPA-risperidone groups (as compared with VPA-saline group) suggest that both drugs exerted antioxidant activity. Based on these results, sitagliptin proved to have strong antioxidant and free radical scavenging activities. Indirect mechanisms for sitagliptin antioxidant activity upregulated the expression of the nuclear factor erythroid 2-related factor 2^{9,10}.

4. Conclusion

Our findings indicate that sitagliptin effectively alleviates ASD-like symptoms in mice, demonstrating potent anxiolytic activity in OFT assessments. Sitagliptin also exerted a significant anti-inflammatory effect by reducing IL-6 levels, although its efficacy was lower than that of risperidone. Overall, sitagliptin emerges as a promising candidate for future pharmacological intervention in ASD.

Acknowledgements

We extend our heartfelt gratitude to the Depart-

ment of Pharmacology of the College of Pharmacy at the University of Babylon for graciously providing us with the necessary facilities to conduct this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Sudan A, Ali Y., Jihad S., Howaidy S. Assessing the effects of a DPP IV inhibitor and risperidone on behavioural interactions and lipid peroxidation in a mouse model of induced autism. *Pharmakeftiki* 37(2s), 411-415, 2025. <https://doi.org/10.60988/p.v37i2S.242>

Development and assessment of rhodamine B-loaded nanostructured lipid carrier-based *in situ* gel for enhanced brain targeting

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KEY WORDS:

BBB; *in situ* gel; *in vivo*; NLC; rhodamine B

ARTICLE INFO:

Received: January 08, 2025

Revised: February 06, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

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ABSTRACT

The development of effective drug delivery systems for brain targeting remains a significant challenge due to the restrictive nature of the blood-brain barrier. The intranasal route has emerged as a promising alternative, enabling direct drug transport to brain tissue via the nasal cavity. The primary objective of the present study was to investigate the incorporation of rhodamine B (a model fluorescent marker) into *in situ* gel-based nanostructured lipid carriers (NLCs) for potential brain targeting *via* the trigeminal or olfactory pathways. The formulated NLCs were characterized for particle size and polydispersity index (PDI). *In vivo* biodistribution studies were conducted in rats, which were divided into a control group and a treatment group receiving a single intranasal administration of rhodamine B-loaded NLC *in situ* gel. The distribution of the nanocarriers within brain tissues was assessed using fluorescence microscopy. The optimized formulation exhibited a mean particle size of 109.3 ± 9.8 nm and a PDI of 0.35 ± 0.07 . Biodistribution analysis revealed a time-dependent accumulation of rhodamine B within brain tissues, with peak fluorescence intensity observed at 2 h post-administration. These findings suggest that rhodamine B-loaded NLC *in situ* gel enhances brain-targeting efficiency and may serve as a viable strategy for intranasal delivery of therapeutics intended for the management of central nervous system disorders.

1. Introduction

Delivering drugs effectively to the brain remains a major challenge in

pharmaceutical research due to the selective permeability of the blood-brain barrier, which restricts the entry of most therapeutic agents. The

nose-to-brain delivery route has emerged as a promising strategy for the direct transport of various compounds from the nasal cavity to distinct brain regions *via* the olfactory or trigeminal pathways¹. This approach necessitates the use of nanocarriers to facilitate the efficient transport of therapeutic agents across the nasal mucosa and into the brain. Nanocarriers enhance bioavailability, improve molecular stability, and enable targeted delivery, thereby optimizing navigation through the olfactory and trigeminal routes.

The development of robust nanosystems has become a priority in drug delivery research. Lipid-based nanocarriers – including microemulsions, spanlastics, and nanostructured lipid carriers (NLCs) – have been extensively investigated for their ability to deliver both lipophilic and hydrophilic drugs, while protecting them from enzymatic degradation and mucociliary clearance within the nasal cavity. Concurrently, the exploration of thermosensitive polymers with mucoadhesive properties for the formulation of *in situ* gel matrices is ongoing, with the aim of enhancing the therapeutic efficacy of lipid-based nanocarriers and minimizing adverse effects²⁻⁴.

Rhodamine B, a fluorescent dye, is widely employed as a model compound for the assessment of the performance of novel drug delivery systems. Its incorporation into an NLC-based *in situ* gel enables real-time tracking of biodistribution and facilitates validation of brain-targeting potential⁵. The present study aimed at developing and evaluating a rhodamine B-incorporated NLC *in situ* gel for brain-targeted delivery in rats.

2. Methodology

Rhodamine B was procured from Central Drug House (CDH®). Glyceryl monostearate and glycerol trioleate were obtained from Shanghai Macklin Biochemical Co., Ltd. Tween 40 and other analytical-grade reagents were purchased from Sisco Research Laboratories (India). Soluplus® and Kolliphor® P 407 were sourced from D-BASF.

Glyceryl monostearate (used as the solid lipid) and glycerol trioleate (serving as the oil phase) were combined and melted at 85°C. During lipid phase preparation, 5 mg of rhodamine B were incorporated. A hot

aqueous surfactant solution containing Tween 40 and Soluplus® was added dropwise to the lipid phase under continuous stirring for 30 min. The resulting emulsion underwent sonication in a water bath for 15 min, followed by probe sonication for 2.5 min using an ultra-probe sonicator (Q Sonica Q500, USA) at 30% amplitude with a 2-sec on/off pulse cycle, performed over an ice bath to achieve reduced particle size⁶.

The optimized rhodamine B-loaded NLC was subsequently incorporated into a gel matrix containing 19% Kolliphor® P 407 and 0.1% carbopol. The final formulation was stored at 4°C for further analysis. Particle size and polydispersity index (PDI) were measured using a Malvern Zetasizer Ultra (Hungary); each sample was diluted with double-distilled water and analysed in triplicate.

In vivo studies were conducted on rats under approval from the Research Ethics Committee for Experimental Investigations at the College of Pharmacy of the University of Baghdad (protocol number: REC02202507A). To assess brain-targeting efficiency, a small-scale study was designed using ten adult female Wistar rats (190–220 g). The animals were divided into a control group (n=2), which received no treatment, and a treatment group (n=8), which received 40 µL of the rhodamine B-loaded NLC *in situ* gel intranasally *via* a micropipette. At designated time points (0, 0.5, 1, 2, and 5 h), two rats were euthanized using an overdose of diethyl ether followed by cervical dislocation.

Brain tissues were immediately harvested, rinsed with isotonic saline, and fixed in 10% formaldehyde solution for histopathological analysis. Tissue slide preparation involved three sequential steps: fixation, embedding, and sectioning. Fixation stabilized tissue architecture using formalin or alcohol-based fixatives. Embedding was performed in paraffin wax following dehydration and clearing, providing structural support for slicing. Sectioning was carried out using a microtome to produce slices of 3–5-µm thickness, which were mounted on slides for staining and microscopic evaluation.

Fluorescence imaging was conducted using a fluorescence microscope (Zeiss, Germany) equipped with rhodamine-specific filters (excitation wavelength: 530–570 nm). Images were captured using a digital

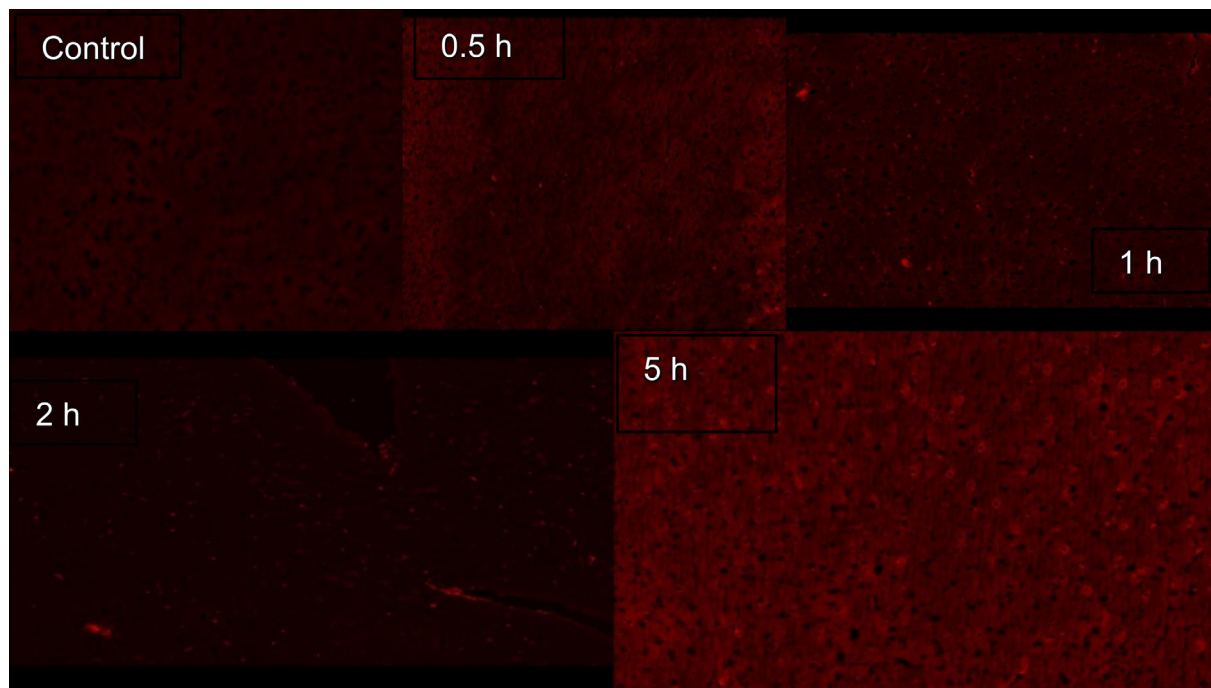


Figure 1. Fluorescence images of brain tissues from the control group (no treatment) and from the groups administered rhodamine B-loaded nanostructured lipid carrier (NLC) *in situ* gel via the intranasal route.

camera (Axiocam 202 mono) and processed in Adobe Photoshop to enhance contrast while preserving data integrity⁷.

3. Results and Discussion

The rhodamine B-loaded NLC *in situ* gel exhibited a particle size of 109 nm and a PDI of 0.35. Numerous studies have identified particle size as a critical determinant for successful nose-to-brain delivery^{8,9}. Smaller particles are known to traverse the mucosal layer more rapidly and are more readily absorbed by the olfactory epithelium compared to particles exceeding 200 nm. Additionally, PDI influences the *in vivo* behavior of lipid-based nanocarriers, affecting their circulation time, absorption, biodistribution, and clearance. A PDI below 0.3 is generally preferred for intranasal formulations, as lower values – combined with smaller particle sizes – support uniform drug absorption across the nasal mucosa¹⁰.

The biodistribution study revealed a time-depend-

ent accumulation of rhodamine B in brain tissues. As shown in Figure 1, weak fluorescence signals were detected at 30 and 60 min post-administration, indicating initial uptake. The signal was localized to the olfactory bulb, suggesting successful intranasal transport. Fluorescence intensity peaked at 2 h, reflecting maximal brain uptake. Although the signal diminished by the 5-h mark, it remained detectable, indicating sustained retention of the compound. Early detection of fluorescence in multiple brain regions following intranasal administration of the optimized *in situ* gel underscores the rapid transport of rhodamine B from the nasal cavity to the brain *via* the olfactory pathway, involving both neuronal and extraneuronal mechanisms.

4. Conclusion

This study demonstrates that rhodamine B-loaded NLC *in situ* gel achieves peak brain distribution at 2 h following intranasal administration, with gradual clearance thereafter. These findings highlight the potential of this deliv-

ery system for brain-targeted therapies, offering both rapid uptake and sustained release of therapeutic agents.

Acknowledgements

The authors would like to express their thanks to the College of Pharmacy of the University of Baghdad, for its support and the resources provided.

Conflicts of interest

None exist.

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HOW TO CITE:

Taher S.S., Al-Kinani K.K. Development and assessment of rhodamine B-loaded nanostructured lipid carrier-based *in situ* gel for enhanced brain targeting. *Pharmakeftiki* 37(2s), 416-419, 2025. <https://doi.org/10.60988/p.v37i2S.243>

Huperzine A *versus* epicatechin: a comparative study of their potential protective effect on lipopolysaccharide-induced lung injury in mice

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KEY WORDS:

huperzine A; epicatechin;
methylprednisolone;
lipopolysaccharide; cytokine
storm

ARTICLE INFO:

Received: January 15, 2025

Revised: February 14, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

A cytokine storm is a severe and potentially fatal condition resulting from an excessive immune response. Epicatechin and huperzine A have demonstrated anti-inflammatory and antioxidant properties, suggesting their potential utility in mitigating tissue damage and cytokine storm severity. This study aimed to compare the protective effects of huperzine A and epicatechin in a cytokine storm-like murine model. Sixty male Swiss albino mice were randomly allocated into six groups. Except for the control group, all animals received a single intraperitoneal injection of lipopolysaccharide (LPS; 5 mg/kg) in order to induce a cytokine storm. The induction group received LPS without further intervention. The remaining groups were pre-treated for three consecutive days prior to LPS administration as follows: vehicle group (1% dimethyl sulfoxide), methylprednisolone group (50 mg/kg/day methylprednisolone), huperzine A group (0.2 mg/kg/day huperzine A), and epicatechin group (25 mg/kg/day epicatechin). The histological analysis of lung tissues and the quantification of serum cytokines – interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) – revealed that all pre-treated groups exhibited significant anti-inflammatory effects. Notably, epicatechin conferred a more pronounced protective effect than either

methylprednisolone or huperzine A, as evidenced by reduced pulmonary histopathological alterations and lower serum cytokine concentrations. In conclusion, both huperzine A and epicatechin demonstrated protective efficacy against the LPS-induced cytokine storm, with epicatechin showing superior performance in attenuating systemic inflammation and lung injury.

1. Introduction

A cytokine storm is an acute, life-threatening inflammatory response characterized by an excessive activation of the immune system and the uncontrolled release of pro-inflammatory cytokines. This phenomenon is a hallmark of severe inflammatory conditions, including sepsis, autoimmune disorders, and viral infections such as coronavirus disease 2019 (COVID-19). Among the key mediators, interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) play essential roles in immune regulation, but can drive hyperinflammation during cytokine storms, leading to extensive tissue damage, multi-organ failure, and increased mortality¹.

Huperzine A, an alkaloid extracted from the Chinese herb *Huperzia serrata*, is a potent acetylcholinesterase inhibitor known for its neuroprotective properties. It modulates the cholinergic anti-inflammatory pathway, reduces oxidative stress, and inhibits D-galactose-induced expression of pro-inflammatory cytokines².

Epicatechin, a flavonoid found abundantly in cocoa, green tea, and various fruits, has demonstrated notable anti-inflammatory potential. It exerts its effects by modulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and nuclear factor erythroid 2-related factor 2 signalling pathways, both of which are central to the regulation of inflammatory and oxidative stress responses³.

This study investigates and compares the protective effects of epicatechin and huperzine A against cytokine storm-induced lung injury in mice, focusing on their capacity to prevent histopathological dam-

age and modulate serum cytokine levels.

2. Methodology

Huperzine A, epicatechin, and methylprednisolone were procured from Haibo Chemicals, Inc. (China). Lipopolysaccharide (LPS) was obtained from Sigma Aldrich Chemical Co., Ltd. (USA). Dimethyl sulfoxide (DMSO) was sourced from Chemlab NV (Belgium), and chloroform (99%) was manufactured by LobaChemie Pvt. Ltd. (India). Enzyme-linked immunosorbent assay (ELISA) kits for TNF- α , IL-1 β , and IL-6 (mouse-specific) were supplied by Xinlong Technology Co., Ltd. (China).

In order to prepare the LPS solution, 10 mg of freeze-dried LPS powder were dissolved in 10 mL of normal saline and mixed for 15 min. Solutions of huperzine A, epicatechin, and methylprednisolone were prepared by dissolving the compounds in 1% DMSO, followed by dilution with distilled water so as to achieve the required volume.

The experimental work was conducted at the Al-Nahrain University between January and June 2023. Sixty healthy male Swiss albino mice (aged 7–8 weeks; weight 25–30 g) were acclimatized for one week in a well-ventilated, pathogen-free environment with ad libitum access to food and water.

Mice were randomly assigned to six groups (n=10 per group): (i) control group (healthy, untreated mice), (ii) induction group (received a single intraperitoneal injection of LPS at 5 mg/kg, without further treatment), (iii) vehicle group (received 1% DMSO intraperitoneally once daily for three days, followed by an LPS injection), (iv) methylprednisolone group (pre-treated with methylprednisolone

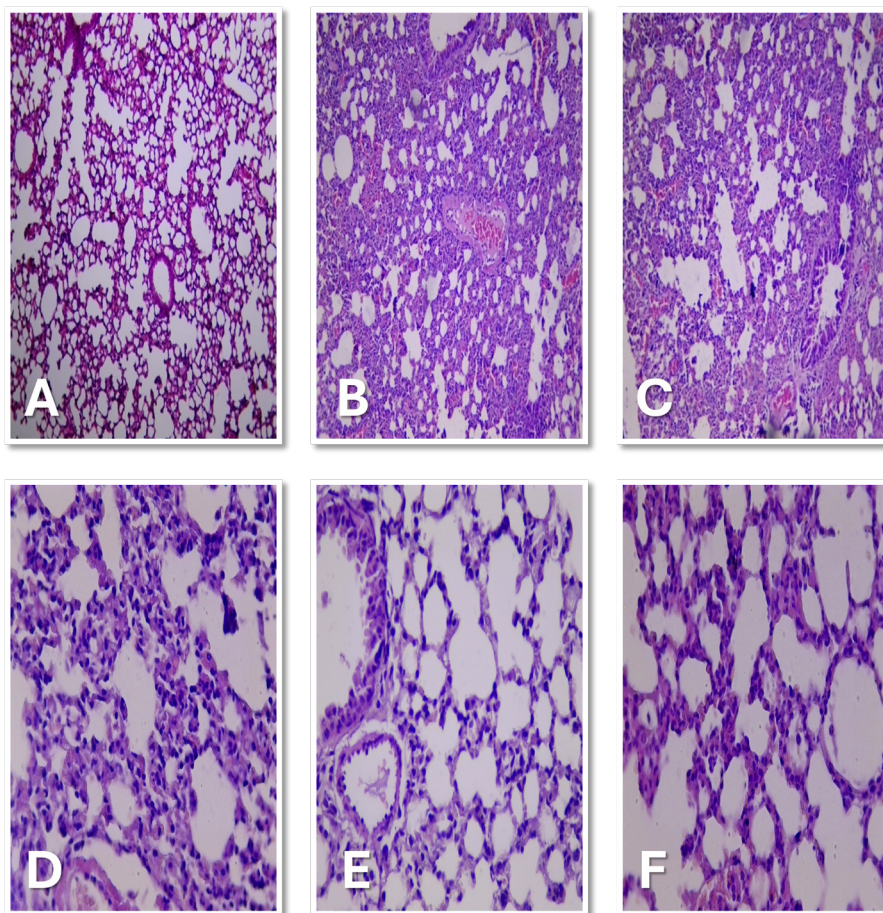


Figure 1. Representative haematoxylin-eosin sections of lung tissues from the mice of this study. (A): The lung sections for the mice of the control group show normal alveoli surrounded by septa, no congestion or inflammatory cell infiltration (20x). (B): The lung sections for the mice of the induction group show severe interstitial inflammatory cell infiltration, congestion of alveolar capillary, diffuse alveolar damage, with thickening of the alveolar wall with hyaline membrane (20x). (C): The lung sections for the mice of the vehicle group show severe interstitial inflammatory cell infiltration, congestion and haemorrhage of the alveolar capillary, with diffuse alveolar destruction (20x). (D): The lung sections for the mice of the methylprednisolone group show moderate inflammatory cell infiltration with focal destruction of the alveolar septa and emphysematous changes, (40x). (E): The lung sections for the mice of the huperzine A group show moderate inflammatory cell infiltration with mild destruction of the alveoli (40x). (F): The lung sections for the mice of the epicatechin group show mild inflammatory cell infiltration with mild destruction of the alveoli (40x).

at 50 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection), (v) huperzine A group (pre-treated with huperzine A at 0.2 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection), and (vi) epicatechin group (pre-treated

with epicatechin at 25 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection).

All animals were sacrificed on day six. Blood samples were collected *via* jugular venipuncture under light chloroform anaesthesia and centrifuged at

3,000 rpm for 20 min. Serum was stored in Eppendorf tubes at -20°C. Lung tissues were preserved in 10% formalin for histological examination. Serum cytokine levels (IL-1 β , IL-6, TNF- α) were quantified using ELISA kits according to the manufacturer's instructions. Histological slides were evaluated blindly by a histopathologist using a semi-quantitative scoring system (0: normal; 1: mild; 2: moderate; 3: severe).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS). Results were expressed as mean $\pm$ standard deviation. Group comparisons were conducted using two-tailed *t*-tests and analysis of variance (ANOVA). Statistical significance was defined at a *p*-value <0.05.

Ethical approval was granted by the Institutional Review Board of the Al-Nahrain University College of Medicine on 3 August 2022 (document: IRB/170; approval number: UNCOMIRB202206161), following review of the study protocol and supporting documentation.

3. Results and Discussion

The administration of LPS (5 mg/kg) successfully induced a cytokine storm, as evidenced by elevated serum levels of TNF- α , IL-1 β , and IL-6 compared to the control group. These findings are consistent with prior studies demonstrating similar immunostimulatory effects of LPS⁴. Treatment with methylprednisolone, huperzine A, or epicatechin resulted in a significant reduction in serum cytokine levels relative to the induction group. Among these, epicatechin exhibited the most pronounced anti-inflammatory effect.

Huperzine A demonstrated robust anti-inflammatory and antioxidant activity. It attenuated levels of IL-1 β , IL-6, and TNF- α and reduced oxidative stress by activating the cholinergic anti-inflammatory pathway *via* α 7 nicotinic acetylcholine receptors. This activation suppressed NF- κ B signalling and enhanced interleukin-10 expression. Additionally, huperzine A inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), reduced reactive oxygen species (ROS)-mediated NF- κ B activation, and up-

regulated antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase. These mechanisms collectively prevented activation of the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome and mitigated inflammation⁵.

Epicatechin counteracted the LPS-induced inflammation by downregulating Toll-like receptor 4 expression, inhibiting mitogen-activated protein kinase (MAPK) and NF- κ B signalling, and neutralizing ROS. It also suppressed COX-2 and iNOS activity, thereby reducing nitric oxide and prostaglandin E2 production⁶.

Histological analysis revealed marked lung damage in the induction and vehicle groups, including proteinaceous exudates, severe inflammatory cell infiltration, alveolar congestion and haemorrhage, and destruction of the alveolar septa. Diffuse alveolar damage with emphysematous changes was evident compared to the control group (Figure 1). In contrast, the methylprednisolone, huperzine A, and epicatechin groups exhibited significantly improved histopathological scores (*p*<0.05). As illustrated in Figure 1, these treatment groups exhibited reduced histological alterations, with only mild interstitial inflammatory cell infiltration and alveolar capillary obstruction. Epicatechin conferred superior protection, yielding the lowest histology scores among the treatment groups.

Huperzine A has also been shown to mitigate LPS-induced lung injury by decreasing levels of high mobility group box 1 protein in the airways and circulation, thereby reducing inflammation and tissue damage⁷. Epicatechin has further suppressed the macrophage migration inhibitory factor and directly interacted with phosphorylated p38 within the MAPK pathway, thereby modulating neutrophil chemotaxis and limiting tissue injury⁸.

4. Conclusion

Both huperzine A and epicatechin demonstrated protective effects against the LPS-induced cytokine storm induced in mice, as evidenced by the reduced serum levels of IL-1 β , IL-6, and TNF- α and the improved lung histopathology. Notably, epicatechin

exhibited superior efficacy in attenuating systemic inflammation and preserving pulmonary architecture.

Acknowledgements

The authors would like to express their appreciation to the College of Pharmacy of the Al-Nahrain University for providing all the facilities needed for the completion of this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Tariq Z.T., Abu-Raghif A.R., Raheem A.K., Ridha-Salman H., Abbas A.H., Zigam Q.A., Abbas W.J., AbdulAemah M.A., Abbas H.A.M., Al-Athari A.J.H., Shihab E.M., Hajwal S.K., Al-Khafaji A.A., Al-Tae M.H. Huperzine A versus epicatechin: a comparative study of their potential protective effect on lipopolysaccharide-induced lung injury in mice. *Pharmakeftiki* 37(2s), 420-424, 2025. <https://doi.org/10.60988/p.v37i2S.244>

Effects of dapagliflozin on inflammatory and apoptotic markers in rats exposed to cyclophosphamide-induced hepatotoxicity: comparison with silymarin

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KEY WORDS:

**cyclophosphamide;
dapagliflozin; silymarin;
inflammation; apoptosis**

ARTICLE INFO:

Received: December 20, 2024

Revised: February 22, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

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ABSTRACT

Cyclophosphamide (CPA) is a widely used chemotherapeutic agent; however, its clinical utility is constrained by dose-dependent hepatotoxicity. This study evaluated the hepatoprotective effects of dapagliflozin (DAPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor with known anti-inflammatory properties, in comparison to silymarin (SILY); a well-established hepatoprotective flavonoid. Fifty Wistar rats were randomly assigned to five groups: (i) negative control, (ii) vehicle control receiving 2% sodium carboxymethyl cellulose, (iii) CPA-treated (30 mg/kg/day), (iv) CPA + DAPA (3 mg/kg/day), and (v) CPA + SILY (200 mg/kg/day). All treatments were administered for ten consecutive days. CPA administration significantly elevated hepatic levels of tumor necrosis factor alpha (TNF- α ; a pro-inflammatory cytokine), while reducing interleukin-10 (IL-10; an anti-inflammatory mediator), thereby aggravating liver injury. DAPA was more effective than SILY in attenuating inflammation and restoring IL-10 levels. Furthermore, CPA markedly increased expression of caspase-3 (a pro-apoptotic marker) and decreased B-cell lymphoma 2 (BCL-2; an anti-apoptotic protein), indicating enhanced apoptotic activity. Both DAPA and SILY ameliorated these apoptotic changes, with DAPA exerting a more pronounced anti-apoptotic effect. In conclusion, DAPA demonstrated superior hepatoprotective efficacy compared to SILY, characterized by stronger anti-inflammatory and anti-apoptotic actions. These findings support the potential of DAPA as a therapeutic candidate for CPA-induced hepatotoxicity, warranting further investigation into its clinical applicability and possible synergistic use in combination therapies.

1. Introduction

Cyclophosphamide (CPA; an alkylating agent) is extensively employed in the treatment of various malignancies, including solid tumours, lymphomas, and leukaemia, as well as in non-neoplastic conditions such as rheumatoid arthritis and systemic lupus erythematosus¹. Despite its therapeutic efficacy, CPA's clinical application is frequently limited by hepatotoxicity associated with standard dosing regimens². Evidence suggests that the CPA-induced liver injury is primarily mediated by oxidative stress, which contributes to mitochondrial damage and impaired cellular respiration. This cascade involves nucleic acid alterations, mitochondrial dysfunction, and activation of multiple cytotoxic signalling pathways, ultimately provoking inflammatory responses³.

CPA undergoes hepatic metabolism to yield active intermediates, including aldophosphamide and phosphoramidate mustard. These metabolites interact with DNA, inhibiting replication and promoting apoptosis^{4,5}. Dapagliflozin (DAPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor, has been approved by the European Medicines Agency for the management of type 2 diabetes mellitus. Notably, DAPA confers nephroprotective effects against cyclosporine A-induced renal toxicity in mice⁶. However, its potential hepatoprotective properties in rat models remain uninvestigated.

Milk thistle (*Silybum marianum*) seeds are a natural source of silymarin (SILY), a complex of flavonolignans widely used in the treatment of both acute and chronic hepatic disorders. In several countries, SILY is also marketed as a dietary supplement. Its hepatoprotective activity is primarily attributed to antioxidant mechanisms, including free radical scavenging and iron chelation *via* phenolic hydroxyl groups. Numerous studies have demonstrated that SILY effectively mitigates experimental liver injury induced by carbon tetrachloride, acetaminophen, ethanol, and CPA⁶⁻⁸.

This study was designed in order to examine the protective effects of DAPA and SILY against CPA-induced hepatotoxicity in rats, with particular emphasis on biomarkers of inflammation, oxidative stress, and apoptosis.

2. Methodology

Adult male rats were procured from the animal facility of the College of Pharmacy of the University of Baghdad. Ethical approval for the study was granted by the College of Pharmacy (REC062463A / 2-3-2024). Animals were randomly allocated into five experimental groups.

The negative control group received distilled water orally along with a standard diet. The vehicle control group was administered a 2% aqueous solution of sodium carboxymethylcellulose (Na⁺-CMC) *via* oral gavage for ten consecutive days, with dosage adjusted according to body weight. The induction group received intraperitoneal (i.p.) injections of CPA at 30 mg/kg/day for ten days. The DAPA-treated group received oral DAPA at 3 mg/kg/day, dissolved in Na⁺-CMC, concurrently with CPA (30 mg/kg/day, i.p.) for ten days. The SILY-treated group received oral SILY at 200 mg/kg/day, also dissolved in Na⁺-CMC, alongside CPA (30 mg/kg/day, i.p.) for ten days. All dosages were adjusted based on individual body weight.

At 24 h after the final treatment (day 11), all animals were euthanized using diethyl ether and sacrificed *via* cervical dislocation. The liver of each animal was promptly excised, rinsed with phosphate-buffered saline (PBS; pH 7.4) at 4°C, and processed for subsequent analyses.

Inflammatory and anti-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10), were quantified using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Apoptotic and anti-apoptotic markers, specifically caspase-3 and B-cell lymphoma 2 (BCL-2), were assessed *via* Western blot analysis.

Statistical analyses were performed using GraphPad Prism version 8.3. Data are presented as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied. A *p*-value <0.05 was considered statistically significant.

3. Results and Discussion

CPA administration significantly elevated hepatic

TNF- α levels compared to both the negative control (4.316 ± 0.494 pg/mL vs. 1.649 ± 0.336 pg/mL; $p < 0.001$) and the Na⁺-CMC-treated group (4.316 ± 0.494 pg/mL vs. 1.453 ± 0.248 pg/mL; $p < 0.001$), indicating pronounced inflammation. Co-treatment with either DAPA or SILY significantly reduced TNF- α levels to 1.479 ± 0.120 pg/mL and 1.751 ± 0.249 pg/mL, respectively ($p < 0.001$), with no statistically significant difference between the two interventions.

CPA exposure also led to a marked reduction in IL-10 levels relative to the negative control (0.18 ± 0.069 pg/mL vs. 1.45 ± 0.417 pg/mL; $p < 0.001$) and the Na⁺-CMC group (0.18 ± 0.069 pg/mL vs. 1.416 ± 0.391 pg/mL, $p < 0.001$). DAPA treatment significantly increased IL-10 levels to 2.41 ± 0.318 pg/mL ($p < 0.001$), while SILY elevated them to 1.869 ± 0.621 pg/mL ($p < 0.001$), with DAPA showing a significantly greater effect than SILY ($p < 0.05$).

CPA administration resulted in a substantial increase in caspase-3 levels compared to the negative control (9.123 ± 0.791 ng/mL vs. 2.5 ± 0.577 ng/mL, $p < 0.001$), indicating enhanced apoptotic activity. Both DAPA and SILY significantly reduced caspase-3 levels to 1.31 ± 0.546 ng/mL and 1.44 ± 0.309 ng/mL, respectively ($p < 0.001$), with no significant difference between the two treatments or relative to the negative control (DAPA: 1.31 ± 0.546 ng/mL vs. 2.5 ± 0.577 ng/mL; SILY: 1.44 ± 0.309 ng/mL vs. 2.5 ± 0.577 ng/mL).

Moreover, CPA exposure significantly decreased BCL-2 levels compared to the negative control (0.4 ± 0.058 ng/mL vs. 2.5 ± 0.577 ng/mL, $p < 0.01$), suggesting increased susceptibility to apoptosis. DAPA and SILY treatments restored BCL-2 levels to 3.825 ± 0.848 ng/mL and 2.91 ± 0.933 ng/mL, respectively ($p < 0.001$). No significant difference was observed between the DAPA and SILY groups (3.825 ± 0.848 ng/mL vs. 2.91 ± 0.933 ng/mL), nor when compared to the negative control (DAPA: 3.825 ± 0.848 ng/mL vs. 2.5 ± 0.577 ng/mL; SILY: 2.911 ± 0.933 ng/mL vs. 2.5 ± 0.577 ng/mL).

These findings indicate that both SILY and DAPA possess substantial therapeutic potential in mitigating CPA-induced hepatotoxicity by modulating inflammatory responses, restoring cytokine equilibrium,

and inhibiting apoptosis. DAPA appears to confer slightly superior anti-inflammatory benefits, particularly in enhancing IL-10 expression. Its hepatoprotective effects may be attributed to SGLT2 inhibition and modulation of oxidative stress-related pathways, contributing to both anti-inflammatory and anti-apoptotic outcomes⁹. In contrast, SILY likely exerts its protective effects through membrane stabilization and potent antioxidant activity, thereby reducing hepatic inflammation and apoptosis¹⁰. Collectively, these results suggest that both agents may serve as effective adjuvant therapies for chemotherapy-associated liver injury, with potential applications extending beyond CPA-induced hepatotoxicity.

4. Conclusion

Silymarin and dapagliflozin exhibit promising therapeutic efficacy in attenuating CPA-induced hepatotoxicity: both compounds reduce hepatic inflammation, restore cytokine homeostasis, and protect hepatocytes from apoptotic damage. SILY primarily acts through antioxidant and membrane-stabilizing mechanisms, whereas DAPA demonstrates more pronounced anti-inflammatory effects, particularly in upregulating IL-10. The hepatoprotective benefits of DAPA may be linked to its regulation of oxidative stress and inhibition of SGLT2.

Acknowledgements

We want to express our deep gratitude to the Department of Pharmacology and Toxicology of the College of Pharmacy of the University of Baghdad for providing the necessary facilities for the undertaking of this study.

Conflicts of interest

None exist.

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HOW TO CITE:

Thiab Hlail A., Naji Al-Shawi N. Effects of dapagliflozin on inflammatory and apoptotic markers in rats exposed to cyclophosphamide-induced hepatotoxicity: comparison with silymarin. *Pharmakeftiki* 37(2s), 425-428, 2025. <https://doi.org/10.60988/p.v37i2S.245>

Promoting health in the Babylon Governorate: public awareness regarding a healthier lifestyle

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KEY WORDS:

health promotion; public awareness; healthy lifestyle; community health; lifestyle interventions

ARTICLE INFO:

Received: January 08, 2025

Revised: February 04, 2025

Accepted: February 13, 2025

Available online: October 10, 2025

ABSTRACT

This study explores public awareness of healthy lifestyle practices in the Babylon Governorate (Iraq), with a particular focus on Hillah city and its surrounding districts. The research investigates perceptions and engagement in behaviours that promote well-being and mitigate the risk of chronic disease. Data were collected from 391 participants between December 23, 2022, and February 9, 2023. Socio-demographic variation was minimal, except in relation to marital status, age, body mass index (BMI), family size, and occupation. Notably, female participants demonstrated significantly greater familiarity with the concept of the food pyramid compared to their male counterparts ($p < 0.05$). While many respondents reported adherence to healthy habits – such as adequate sleep, stress management, hydration, and general nutritional awareness – only a small proportion engaged in regular physical activity or consistently applied food pyramid principles to their dietary choices. This discrepancy suggests a gap between nutritional knowledge and actual dietary behavior. Overall, awareness of healthy living did not differ significantly between urban and rural populations within the Babylon Governorate.

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1. Introduction

Fostering well-being requires the preservation of physical and mental health, the promotion of well-being, and the alleviation of stress. Numerous organizations advocate

for the adoption of health-promoting behaviours, emphasizing the importance of balanced nutrition, regular physical activity, weight management, and stress reduction. Wellness is a continuous pursuit, centered on a varied diet that incor-

Table 1. Sociodemographic characteristics, lifestyle behaviours, and health status of the study's participants (Hillah city center: N=254; Babil Province districts: N=137; total: N=391).				
Variable	Categories	Hillah city center (N (%))	Districts (N (%))	p-value
Gender	male	71 (28.0%)	42 (30.7%)	0.574
	female	183 (72.0%)	95 (69.3%)	
Age	15–19 years	27 (10.6%)	10 (7.3%)	0.015
	20–39 years	201 (79.1%)	124 (90.5%)	
	40–59 years	21 (8.3%)	3 (2.2%)	
	60–79 years	5 (2%)	0 (0%)	
Body mass index (BMI)	underweight (<18.5)	12 (4.7%)	6 (4.4%)	0.017
	normal weight (18.5–24.9)	103 (40.6%)	67 (48.9%)	
	overweight (25–29.9)	80 (31.5%)	50 (36.5%)	
	obesity (30–39.9)	59 (23.2%)	14 (10.2%)	
Marital status	single	173 (68.1%)	114 (83.2%)	0.001
	married	75 (29.5%)	21 (15.3%)	
	separated / divorced	6 (2.4%)	0 (0%)	
	widow(er)	0 (0%)	2 (1.5%)	
Smoking	1–10 cigarettes daily	10 (3.9%)	8 (5.8%)	0.289
	11–20 cigarettes daily	5 (2.0%)	0 (0.0%)	
	>20 cigarettes daily	5 (2.0%)	4 (2.9%)	
	no smoking	234 (92.1%)	125 (91.2%)	
Fruit consumed	daily	86 (33.9%)	41 (29.9%)	0.676
	<3 times a week	68 (26.8%)	39 (28.5%)	
	< 3 times a week	82 (32.3%)	50 (36.5%)	
	never	18 (7.1%)	7 (5.1%)	
Do you drink 8 cups of water daily?	yes	118 (46.5%)	52 (38.0%)	0.106
	no	136 (53.5%)	85 (62.0%)	
Do you sleep 8 h daily?	yes	135 (53.1%)	78 (56.9%)	0.473
	no	119 (46.9%)	59 (43.1%)	
Regular exercise	yes	74 (29.1%)	34 (24.8%)	0.362
	no	180 (70.9%)	103 (75.2%)	

porates all essential food groups without imposing rigid caloric restrictions¹.

Global campaigns have long sought to advance public awareness of the benefits associated with a health-conscious lifestyle. Despite these extensive efforts, some individuals remain resistant to change and persist in unhealthy behaviours. Although relinquishing familiar dietary and social habits may pose challenges, it is achievable. A range of strategies exists to support individuals in transitioning toward healthier living. Foundational steps include recog-

nizing the importance of prioritizing health, regulating dietary intake, engaging in consistent physical activity, and managing stress effectively¹.

Maintaining a nutritionally balanced diet – comprising meats, dairy products, fruits, and vegetables – is essential for sustaining optimal health. Adopting a lifestyle rooted in health-conscious choices markedly reduces the risk of serious medical conditions and premature mortality. While not all diseases are preventable, a substantial proportion of deaths, particularly those linked to cardiovascular disease and

pulmonary malignancies, can be avoided. Scientific evidence has identified behaviours such as tobacco use and poor dietary habits as major contributors to chronic illness and early death. Health encompasses more than the absence of disease; it includes physical, psychological, and social well-being. By cultivating healthy habits, individuals not only enhance their own health but also serve as positive role models for their families, especially children. This guide seeks to assist readers in transforming their habits, improving their health outcomes, and extending their lifespan^{2,3}. Accordingly, the present study aims to assess public awareness of healthy lifestyle practices among residents of Babylon Governorate (Babil Province), including the Hillah city center and its surrounding districts and sub-districts.

2. Methodology

This cross-sectional study was conducted between December 23, 2022, and February 9, 2023. A total of 391 participants from Hillah city and adjacent areas in Babil Province voluntarily completed a structured questionnaire and provided informed consent. The instrument, previously validated for use among Iraqi populations, was disseminated via social media using a Google Form. Responses were compiled into an online spreadsheet for analysis. In order to ensure linguistic consistency and cultural relevance, the survey was translated into Arabic.

The questionnaire captured data on lifestyle behaviours, health practices, and demographic characteristics. Collected sociodemographic variables included gender, age, marital status, occupation, education level, household size, and income. Physical attributes such as weight and height were recorded to calculate body mass index (BMI), defined as weight (in kilograms) divided by height (in meters squared). BMI categories were classified as follows: normal weight ($18.5 \leq \text{BMI} < 24.9$), overweight ($25 \leq \text{BMI} < 29.9$), and obese ($\text{BMI}: 30\text{--}39.9$)⁴. Additional variables included alcohol consumption, smoking status, dietary habits, exercise frequency, health knowledge, and self-reported health status. Participants residing outside Babylon Governorate were

excluded. Ethical approval for the study was granted by the College of Pharmacy of the University of Babylon, in December 2023 (protocol number: A-0017).

Data were analysed using the SPSS version 23 software. Descriptive statistics were presented as frequencies and percentages, while associations between categorical variables were examined using the Chi-square test, with the statistical significance set at $p < 0.05$.

3. Results and Discussion

Table 1 summarizes the sociodemographic characteristics, lifestyle behaviours, and health status of the 391 participants in our study. No significant differences were observed in healthy living awareness between urban (Hillah city center) and rural (Babylon Governorate district) populations. While positive habits such as adequate sleep and stress management were commonly reported, fewer participants engaged in regular physical activity or adhered to the food pyramid guidelines. This suggests a disconnection between nutritional knowledge and actual dietary behavior.

Sociodemographic variation between urban and rural respondents was minimal, except for age, marital status, BMI, family size, and occupation. Lifestyle and health behaviours did not differ significantly across these groups. Female participants demonstrated significantly greater familiarity with the food pyramid compared to males ($p < 0.05$). Smoking status was not significantly associated with comorbidity ($p > 0.05$); only 8.2% of respondents were smokers, while 91.8% were non-smokers. The majority reported no chronic illnesses. Similarly, no significant association was found between BMI and comorbidity ($p > 0.05$), with most participants across all BMI categories reporting no chronic conditions.

Approximately 30.5% of respondents from the Babil Province were classified as overweight, with prevalence rising to nearly 50% among those in peripheral areas. Obesity rates were higher in the urban center than in the periphery. Chronic diseases and common illnesses were more prevalent among overweight and obese individuals than among those

with normal BMI. These findings align with prior studies linking obesity to hypertension and diabetes^{5,6}, although some evidence suggests that overweight individuals may exhibit lower mortality risk⁶.

A Malaysian study has reported that 45% of adults failed to adopt healthy lifestyles due to poor dietary choices and sedentary behavior, resulting in various physical ailments⁸. In the present study, only 21.7% of the respondents actively applied food pyramid principles to their dietary choices, indicating limited translation of nutritional awareness into practice. Nevertheless, 60.4% of participants reported being free from illness, suggesting that informed dietary decisions may positively influence overall health. Despite 43.5% falling within the normal weight range, many participants demonstrated food pyramid awareness, preferred home-cooked meals, and rarely consumed fast food.

Cai Lian *et al.*⁹ have identified a high prevalence of obesity and non-communicable diseases strongly correlated with sedentary lifestyles, advocating for increased physical activity among the general population. Behavioural interventions have been proposed to promote healthy living and address overweight and obesity¹⁰. Although most participants were aware of the food pyramid, only 21.7% adhered to its guidelines. Many favoured homemade meals, limited junk food intake, and consumed three meals daily. However, just 21.7% engaged in regular exercise.

4. Conclusion

This study found no significant difference in healthy lifestyle awareness between residents of the Hillah city center and those in the Babylon Governorate districts. While many participants reported positive habits (including adequate sleep, stress management, and food pyramid knowledge), consistent physical activity and dietary adherence were lacking. These findings underscore the gap between health knowledge and behavioural implementation.

Acknowledgements

The authors wish to express their gratitude to the College of Pharmacy of the University of Babylon for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Watife A.T., Abdulameer N.A., Alzubaidi F.A., Abdul-Husseein H.K., Al-Shuaibi H.F., Al Jubouri F.M., Owadh H.K.H. Promoting health in the Babylon Governorate: public awareness regarding a healthier lifestyle. *Pharmakeftiki* 37(2s), 429-433, 2025. <https://doi.org/10.60988/p.v37i2S.246>

Structural alerts for the prediction of drug toxicity: a mini-review

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KEY WORDS:

structural alerts; toxicity prediction; drug discovery; machine learning; molecular reactivity

ARTICLE INFO:

Received: January 11, 2025

Revised: February 28, 2025

Accepted: March 01, 2025

Available online: October 10, 2025

ABSTRACT

An essential part of drug research and development is the prediction of drug toxicity, which aims at recognizing possible hazards early on. Toxicophores, another name for structural alerts, are functional groups or molecular substructures linked to harmful biological effects. In computational toxicology, these signals are frequently used to forecast the possible toxicity of drug candidates. This mini-review explores structural alerts and their relevance in forecasting drug toxicity. We discuss the design and implementation of structural alert systems, their integration with machine learning and quantitative structure-activity relationship models, and their limitations, including the risk of over-prediction and the requirement for contextual interpretation. In addition, we discuss recent advances in the field, such as the incorporation of mechanistic insights and the use of large-scale toxicity databases. While structural alerts are still a useful technique for predicting toxicity, their effectiveness is increased when supplemented with other computational and experimental methodologies in order to provide a more thorough assessment of medication safety. This mini-review emphasizes the significance of structural alerts in guiding drug design and reducing the risk of toxicity within the preclinical development.

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1. Introduction

Structural alerts are key substructures that indicate specific toxicity in compounds. They are identified through frequency analysis and statistical parameters, allowing for

the prediction of toxicity by matching compounds against predefined SMARTS (SMiles ARbitrary Target Specification) patterns in the SA-predictor web-server¹. Identifying structural alerts for toxicity involves using computational approaches like

frequency analysis and interpretable machine learning models. These methods enhance predictive capability and can explain quantitative structure-activity relationships, aiding in both environmental toxicology and drug discovery applications².

Common structural alerts for toxicity include high chemical reactivity molecular fragments or those that can be bioactivated by human enzymes into reactive fragments. Approximately 78%–86% of drugs with residual toxicity contain such structural alerts, indicating their significance in drug design³. Structural identification faces limitations such as potential pitfalls that can lead to misleading results, scepticism about its value, and challenges in accurately correlating analytical models with measured data, which can hinder effective decision-making and performance forecasting. A limitation of structural alerts is their potential to significantly reduce the chemistry space for drug discovery, as many marketed drugs match these alerts. Additionally, strict application may overlook compounds with acceptable safety profiles, leading to missed opportunities in drug development⁴.

Toxicity prediction is crucial in drug discovery as it helps filter out molecules likely to fail early in development, thereby saving time and resources. Accurate predictions enhance the efficiency of *de novo* drug design and improve overall safety in pharmaceuticals⁵. Structural alerts are key substructures predicting molecular initiating events in toxicity. They help identify chemicals' interactions with biological targets, enhancing mechanistic understanding and enabling better activity predictions through models that incorporate receptor-binding interactions and pharmacophore features⁶.

Structural alerts are molecular substructures linked to mutagenic and carcinogenic properties, aiding in the classification of potential carcinogens. They help us to understand genotoxicity mechanisms, including DNA mutations, replication blockage, and interference with DNA repair processes leading to various genetic alterations. Applying structural alerts in drug discovery involves identifying substructures in drug candidates that may form reactive metabolites, which can lead to toxicity. Recognizing these alerts early can minimize the risk of adverse reactions and potential withdrawal during drug development.

Structural alerts remain a critical component of the drug discovery process, with over 50 years of established use. As research and technology continue to evolve, new techniques, such as machine learning and systems biology, will further enhance our ability to predict and mitigate toxicity, ensuring the development of safer and more effective therapeutics. An overview of the function of structural alerts in drug toxicity prediction is the goal of this mini-review. It will focus on their use in identifying molecular characteristics linked to harmful toxicological effects, such as genotoxicity, cardiotoxicity, and hepatotoxicity. This mini-review will also discuss the use of structural alerts in screening methods and computational models aiming to evaluate drug candidates' safety at an early stage of the drug discovery process. It will also point out the difficulties and restrictions associated with employing structural alerts for toxicity prediction.

2. The purposes of structural alerts

A main purpose of structural alerts is toxicity prediction by using known toxicophores (toxic structural motifs), where the structural alerts assist in identifying substances that may have mutagenic, carcinogenic, or other harmful consequences. When employed during the early screening, structural alerts lower the chances of late-stage failures by identifying potentially dangerous chemicals in the early stages of drug development. Finally, as part of the regulatory compliance, structural alerts are essential in regulating frameworks that demand toxicity evaluations for chemicals and pharmaceuticals, such as REACH and ICH M7.

3. Benefits of structural alerts

Thanks to their simplicity, structural alerts are accessible to non-experts because of their ease of interpretation and application, while due to their cost-effectiveness they offer a rapid and affordable method of screening vast chemical libraries. Moreover, the transparency of structural alerts provides understandable explanations for why a substance might be harmful, in contrast to certain machine learning algorithms⁷. Reduction of animal testing in accordance with the

principles of 3Rs (Replacement, Reduction, and Refinement) in animal research is an additional benefit of the use of structural alerts, as the latter can lessen the need for animal testing in the early phases of drug development by providing a view of the anticipated toxicity. Structural alerts also help to advance the science of predictive toxicology as well as the standardization and knowledge-sharing by promoting cooperation and by facilitating the development of standardized toxicity databases and knowledge-sharing platforms. Finally, withstanding these advantages, it is critical to understand that structural alerts are not perfect and should be combined with additional experimental and computational methods in order to guarantee a thorough evaluation of drug toxicity.

4. Current developments

Integration with machine learning (developed by adding more chemical characteristics and context) and the combining of structural alerts with machine learning models can increase prediction accuracy. In addition to the Alert Library Expansion impart, new structural alerts for emerging toxins and underrepresented hazardous endpoints are being currently sought after. Moreover, within the Metabolism-Based Alerts, the predictive potential of structural alert frameworks is increased by integrating metabolic activation pathways⁸.

5. Challenges and limitations

Using structural alerts in toxicity prediction is a common approach in computational toxicology, where specific molecular substructures or functional groups are associated with toxic effects. While this method is useful, it is characterized by several challenges and limitations.

5.1. Restricted predictive power

Although structural alerts frequently rely on well-known toxicophores or toxic functional groups, they might not fully account for the intricacies of toxicity mechanisms. Not every molecule with a structural alert is harmful, and some harmful substances might

not have any known signals.

5.2. High percentage of false positives and negatives

Structural alerts may result in false negatives (in which harmful compounds are overlooked because there are no warnings) and false positives (in which non-toxic compounds are marked as toxic).

5.3. Absence of contextual information

Certain detoxification and metabolic activation processes are not taken into consideration by structural alerts. Only after undergoing metabolic change can a substance turn hazardous, something that structural alerts cannot foretell. Moreover, by ignoring biological pathways, structural alerts do not directly address toxicity, which frequently depends on interactions with biological targets (such as proteins or DNA).

5.4. Unpredictability of new toxicity mechanisms

Structural alerts are restricted to known alerts, because the structural alerts rely on past data. As a result, they might not be able to forecast toxicity for new substances or mechanisms that have not been seen before. Moreover, structural alerts might prove inadequate for complex mechanisms in which simple structural rules are insufficient to predict toxicity that results from intricate interactions or multi-step processes.

5.5. Chemical complexity and diversity

One must note that it is challenging to develop an extensive set of structural alerts due to the great diversity of chemical structures. Moreover, certain substances interact with several biological targets, thereby resulting in intricate toxicity profiles that are difficult for structural alerts to fully identify⁹.

5.6. Limitations in quantitative terms

Major limitations are the absence of dose-response data (the potency and the dose-response relationship of harmful effects are not disclosed) and the binary classification undertaken without taking into account

toxicity gradations (leading structural alerts to usually categorize chemicals as either “toxic” or “non-toxic”).

6. Conclusion

By highlighting possible toxicity hazards linked to particular chemical moieties, structural alerts are useful tools that help researchers pick safer compounds and lower the possibility of adverse reactions later within the drug development process. For a more thorough and accurate evaluation of drug toxicity, structural alerts must be integrated with sophisticated computational models, *in vitro* tests, and *in vivo* studies. It is anticipated that future developments in cheminformatics, systems biology, and machine learning will improve the predictive ability of structural alerts, thereby increasing their usefulness in creating safer and more

efficient treatments.

Acknowledgements

The authors would like to express their sincere gratitude to the Dean and the staff of the University of Babylon's Pharmacy College for their constant support and direction during the writing of this mini-review.

Conflicts of interest

None exist.

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HOW TO CITE:

Abbood G.S., Abdul Hussein S.A. Structural alerts for the prediction of drug toxicity: a mini-review. *Pharmakeftiki* 37(2s), 434-437, 2025. <https://doi.org/10.60988/p.v37i2S.249>

Enoxaparin thromboprophylaxis during pregnancy: a review of pharmacist-led interventions

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KEY WORDS:

**enoxaparin;
thromboprophylaxis;
venous thromboembolism;
pregnancy; pharmacist-led
interventions**

ARTICLE INFO:

Received: December 04, 2024

Revised: December 30, 2024

Accepted: January 13, 2025

Available online: October 10, 2025

ABSTRACT

Enoxaparin, a low-molecular-weight heparin, is a critical agent for anticoagulation during pregnancy due to its efficacy and safety. It is generally used as a prophylactic agent against venous thromboembolism; a particularly serious and dangerous situation when occurring during pregnancy. However, the enoxaparin administration during pregnancy requires accurate dosing and monitoring. Complications as a result of prescribing mistakes highlight the need for competitive control strategies. This review attempts to examine the effects of selected pharmacist-led interventions on enoxaparin-induced thromboprophylaxis in pregnant patients. Based on the findings of studies from the literature, we herein emphasize the crucial role of pharmacists in improving anticoagulation treatment goals, correcting dosing errors, ensuring patient adherence, and protecting from the development of adverse events during antenatal thromboprophylaxis.

1. Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis and pulmonary embolism, presents a significant risk during pregnancy. Pregnancy induces a hyper-coagulable state characterized by increased pro-coagulant factor levels and decreased levels of natu-

ral anticoagulants, thereby elevating the risk of thrombosis. Additionally, the increased blood volume during pregnancy raises venous pressure, further predisposing pregnant women to VTE. The incidence of VTE in pregnant women is approximately 1 in 1,000 pregnancies, with the highest risk occurring during the third trimester and the immediate post-

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partum period. The serious consequences of VTE highlight the critical need for effective thromboprophylaxis in high-risk pregnant populations, as it plays a pivotal role in safeguarding maternal and foetal health. Enoxaparin, a low-molecular-weight heparin, is commonly used as a prophylactic agent due to its predictable pharmacokinetic profile and favourable safety characteristics¹.

Enoxaparin is the preferred anticoagulant for the induction of thromboprophylaxis in pregnant women, favoured over unfractionated heparin due to its predictable pharmacokinetics, its lower risk of heparin-induced thrombocytopenia, and its limited placental transfer, which together enhance its safety profile. It is widely used as a prophylactic agent to prevent VTE in women with a history of VTE, thrombophilia, or other risk factors (including obesity and prolonged immobility)¹. Despite the benefits of enoxaparin therapy during pregnancy, several challenges must be considered. Precise dosing is of paramount importance: underdosing can lead to therapeutic failure, while overdosing increases the risk of haemorrhage. Ensuring patient adherence to prescribed regimens, especially for self-administered treatments, is often challenging. Moreover, poor adherence can significantly impact treatment efficacy¹.

The inherent risks associated with prescribing errors and the complex nature of managing enoxaparin therapy during pregnancy necessitate a robust approach to prescribing practices. Pharmacists, with their specialized expertise in pharmacotherapy, are uniquely positioned to address these challenges and enhance patient safety. The pharmacists' in-depth knowledge of drug interactions and patient-specific factors allows them to ensure accurate dosing of enoxaparin, thereby minimizing the risk of underdosing or overdosing. Pharmacists are skilled at providing clear and concise patient education regarding medication administration, potential side-effects, and the importance of adherence to prescribed regimens. Their specialized expertise in pharmacotherapy is invaluable in navigating the complexities of this medication and ensuring its safe and effective use².

The aim of this review was to summarize key findings of interventions led by pharmacists in order to

optimize enoxaparin thromboprophylaxis during pregnancy. This review was conducted by systematically searching key databases, including PubMed, Scopus, and the Cochrane Library, for research articles published between 2000 and 2024 that assessed the role of pharmacists in improving enoxaparin thromboprophylaxis during pregnancy. The search method employed a variety of terms, including "pharmacist intervention", "enoxaparin", "thromboprophylaxis", "pregnancy", and "venous thromboembolism". In order to be included in the evaluation, studies had to meet a number of criteria. Firstly, the studies had to include a pharmacist-led intervention. Secondly, the studies had to report on one or more of the following outcomes: prescribing accuracy, dosing, or patient protection.

2. Interventions in Europe and a review from the USA

In France, Bedouch *et al.*³ have evaluated the impact of clinical pharmacists' interventions in hospital settings. Their study suggests that pharmacist-led interventions ended in superior medication control³. Similarly, Löfner *et al.*⁴ have conducted a qualitative study in Germany with the goal of analysing the perceptions regarding interprofessional collaboration among general practitioners and community pharmacists. Their study emphasizes the significance of effective collaboration in optimizing patient care, even though it does not focus on specific remedy-associated interventions⁴.

In a different context, Daughety *et al.*⁵ have conducted a literature review and assessment of the control of anticoagulation in pregnant women with mechanical heart valves. Their findings provide precious insights into the broader context of anticoagulation management and highlight the need of improving anticoagulation control practices across heterogeneous patient populations⁵.

3. Interventions in Arab countries

In Arab countries, the contribution of pharmacists to optimal enoxaparin-induced thromboprophylaxis is increasingly recognized, although the degree of pharmacist involvement varies. In Saudi Arabia, a study

Table 1. Outline of the studies discussed in this review.			
Study	Location	Intervention	Outcome
Bedouch <i>et al.</i> (2008) ³	France	clinical pharmacist-led interventions in hospitals	improved medication management and patient outcomes
Löffler <i>et al.</i> (2017) ⁴	Germany	interprofessional collaboration between general practitioners and community pharmacists	enhanced understanding of collaborative care benefits
Daughety <i>et al.</i> (2020) ⁵	USA; global	management of anticoagulation in pregnant women with mechanical heart valves	relevant for broader context of anticoagulation management; not specific to enoxaparin
Shilbayeh (2020) ⁶	Saudi Arabia	pharmacist-led educational video on warfarin	demonstrated the role of educational interventions in improving medication management
Ahmed <i>et al.</i> (2017) ⁷	Sudan	clinical pharmacist-led interventions in an anticoagulation clinic	improved warfarin dosing accuracy and enhanced patient outcomes
Obaid <i>et al.</i> (2022) ⁸	Middle East	review of pharmacy practice and clinical pharmacy research	emphasizes the potential of pharmacist-led interventions in addressing healthcare challenges
Mikhael <i>et al.</i> (2024) ⁹	Iraq	pharmacist-led nurse education on enoxaparin injection technique	improved injection technique accuracy and reduced complications
Suker <i>et al.</i> (2021) ¹⁰	Iraq	pharmacist-led intervention for venous thromboembolism prophylaxis after caesarean section	clinical pharmacist-led intervention has resulted in encouraging guideline implementations, thereby improving the accuracy of the thromboprophylaxis duration and dosing

by Shilbayeh⁶ has demonstrated the impact of pharmacist-led educational videos on warfarin utilization. Although not specific to enoxaparin, this study provides evidence of the effectiveness of pharmacist-led educational programs⁶. The same is true in the case of Ahmed *et al.*⁷, which have investigated the impact of an intervention by hospital pharmacists in an anticoagulation clinic in Sudan. The study has found that the intervention led to marked improvements in anticoagulation management, including warfarin dosing accuracy and patient outcomes⁷. Furthermore, a study of the scope of pharmaceutical practice and clinical pharmaceutical research throughout the Middle East that has been conducted by Obaid *et al.*⁸ has highlighted the power of pharmacist-led interventions in addressing a variety of healthcare challenges, including those related to anticoagulation. The study emphasizes the wider context of pharmacist-led involvement in the development of healthcare practices, as well as its capability for ensuring a better anticoagulation control⁸.

In Iraq, pharmacist-led interventions have had a

vast effect on enoxaparin administration, thereby highlighting the potential role of pharmacists in securing the enhancement of medication control. A study conducted by Mikhael *et al.*⁹ at the Baghdad Teaching Hospital has evaluated the efficacy of pharmacist-led nurse training on enoxaparin injection strategies. The study reports an improved accuracy of the injection method and a decrease in associated complications, thereby demonstrating the effectiveness of specialized academic programmes⁹. Similarly, Suker *et al.*¹⁰ have examined the impact of a pharmacist-led intervention on the practice of enoxaparin thromboprophylaxis after caesarean delivery. This study reports the promising potential for the pharmacist involvement in the post-caesarean healthcare, with respect to improving enoxaparin dosing accuracy¹⁰. The aforementioned studies emphasize the growing importance of integrating pharmacists into multidisciplinary healthcare groups as a way of improving patient care.

4. Conclusion

The existing literature regarding pharmacist-led interventions in the context of enoxaparin-induced thromboprophylaxis during pregnancy is remarkably poor and in need of urgent enrichment. However, the existing studies suggest a critical potential of pharmacists in optimizing enoxaparin thromboprophylaxis during pregnancy. The implementation of pharmacist-led interventions can reduce dosing errors and improve outcomes.

Acknowledgements

We are grateful for the support received from the Faculty of Pharmacy of the University of Kufa.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdulridha G.M., Salah I. Enoxaparin thromboprophylaxis during pregnancy: a review of pharmacist-led interventions. *Pharmakeftiki* 37(2s), 438-441, 2025. <https://doi.org/10.60988/p.v37i2S.250>

Psoriasis: a short review of its pathophysiology and causes

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KEY WORDS:

psoriasis; plaque psoriasis;
types of psoriasis; topical
treatments; systemic
treatment

ARTICLE INFO:

Received: January 11, 2025

Revised: February 18, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Psoriasis is a chronic autoimmune inflammatory condition characterized by abnormal keratinocyte metabolism. Its development is closely associated with immune system dysfunction and aberrant activation. This common skin disorder is marked by the formation of clearly defined, scaly, erythematous plaques, typically affecting the knees, elbows, scalp, and trunk. While there is no cure for psoriasis, it is believed to be an immune-mediated disease in which skin cells proliferate more rapidly than normal. The pathogenesis of psoriasis involves multiple factors, including genetic predisposition, lipoprotein-2, galectin-3, fractalkine, vaspin, human neutrophilic peptides, T-cells, dendritic cells, and antimicrobial peptides. Four forms of psoriasis exist, each with distinct clinical manifestations and pathological features: erythrodermic, pustular, chronic plaque, and articular psoriasis. Plaque psoriasis is the most common type, characterized by dry, itchy scales covering raised areas of skin. Although currently incurable, psoriasis treatment should focus on minimizing patients' physical and psychological distress through early intervention, identification and prevention of associated multimorbidity, lifestyle modifications, and a personalized treatment approach. This short review summarizes recent progress in understanding psoriasis pathophysiology and its causes, aiming to improve our comprehension of the disease.

1. Introduction

Psoriasis is a prevalent, chronic,

and multifaceted inflammatory skin condition that is associated with various comorbidities. It is charac-

terized by immune cell infiltration into the dermis and epidermal hyperplasia¹. Psoriasis is a systemic disorder that affects not just the skin but also the liver, joints, and kidneys. It has been demonstrated that psoriasis can raise the incidence of various inflammatory conditions. Treatment for psoriasis may lessen the occurrence of these comorbidities, as the psoriasis-associated high levels of pro-inflammatory cytokines can be carried by the blood to other systems, thereby resulting in inflammation and subclinical inflammation². Moreover, psoriasis lesions are known to be triggered or made worse by a variety of conditions.

An Iraqi study, conducted at a private dermatology clinic in Samawa City and the Al Hussein Teaching Hospital, has found that the most common type of psoriasis among children aged 6 months to 14 years is the plaque psoriasis, followed by guttate and scalp psoriasis³. The study has also indicated a higher prevalence of psoriasis in females than in males³. Childhood psoriasis differs from adult psoriasis, necessitating specific consideration in order to address potential long-term psychological issues related to the disease. This short review provides an overview of recent progress in our understanding of the psoriasis pathophysiology and its causes, aiming to enhance our comprehension of the disease processes involved.

2. Epidemiology of psoriasis

Patients with psoriasis have been found to have greater rates of mental health issues, such as anxiety, depression, and suicidal thoughts. Psoriasis can affect individuals of any age. However, its onset appears to follow a bimodal distribution, with peaks between the ages of 20 and 30 and again between 50 and 60. A retrospective study conducted in the outpatient clinic of the Baquba Teaching Hospital in Diyala has examined the prevalence of psoriasis in Iraq⁴. Of the 10,964 outpatients with skin conditions, 220 (2%) were diagnosed with psoriasis, of which 102 (46%) were female and 118 (54%) were male⁴. An earlier study has also indicated that the IL20RA gene contributes to genetic susceptibility to psoria-

sis within the Iraqi population⁵.

3. Causes of psoriasis

Several risk factors contribute to the development of psoriasis, including genetic predisposition, age, sex, stress, obesity, and bacterial infections.

4. Pathophysiology of psoriasis

Psoriasis is a complex, chronic inflammatory skin condition primarily driven by immune system dysfunction. It is characterized by abnormal differentiation, hyperproliferation of keratinocytes, and angiogenesis. The development of psoriasis involves multiple interacting factors, including immune system dysfunction, genetic predisposition, and environmental triggers. Increased DNA synthesis and a markedly lower rate of epidermal turnover are indicators of hyperproliferation. Reduced expression of some keratins (1 and 10) and increased expression of others (6 and 16), which are typically expressed in differentiating skin, are characteristics of abnormal keratinocyte differentiation^{1,6}.

4.1. Immune system participation

Psoriasis is mostly a T lymphocyte-mediated condition, in which skin inflammation and keratinocyte hyperproliferation are caused by the activation of pathogenic T lymphocytes and innate immune cells. Although B lymphocytes have long been underappreciated in the pathogenesis of psoriasis, new research has shown their significance in inflammatory skin disorders⁶.

4.2. Genetic factors

Psoriasis more commonly follows a polygenic inheritance pattern. Research on affected families has identified chromosomal regions, known as "PSORS" (psoriasis susceptibility loci), that are associated with the disease's development⁷. While twelve such regions have been identified, the *PSORS1* region is the most significant, accounting for 35%–50% of

hereditary psoriasis cases. This region also contains *HLA-Cw6* (human leukocyte antigen C); the first gene linked to psoriasis, which has been detected in 10.5% to 77.2% of patients⁸. Monozygotic twins are two to three times more likely to have psoriasis than dizygotic twins, and first- and second-degree relatives of those who have the condition are more likely to get it themselves. *TNFAIP3*, *IL23R*, and *HLA-Cw6* are among the many genes that raise an individual's risk of psoriasis⁸.

5. Symptoms, clinical presentation, and diagnosis

Common symptoms of psoriasis include skin scaling, erythema (redness), itching, fatigue, burning, bleeding, and swelling. Psoriasis presents in five clinical types: (i) plaque psoriasis (also known as “psoriasis vulgaris”, is the most prevalent kind, making up between 80% and 90% of all cases), (ii) flexural psoriasis (also known as “inverse psoriasis”, is characterized by intertriginous areas that are affected by mildly erosive erythematous plaques and patches), (iii) pustular psoriasis (the occurrence of many sterile pustules that coalesce is a characteristic of this type of psoriasis; it may be widespread or isolated; palmoplantar pustular psoriasis and acrodermatitis continua of Hallopeau are two different localized subtypes), (iv) erythrodermic psoriasis (1% to 2.5% of psoriasis patients have erythrodermic psoriasis, which is one of the rarest and most severe types; it is characterized by significant morbidity), and (v) psoriatic arthritis (20% to 30% of people suffering from psoriasis also have psoriatic arthritis; a chronic, genetically-determined musculoskeletal condition that frequently appears a few years after the onset of psoriasis)^{2,9}.

The majority of psoriasis diagnoses are clinical; 80% to 90% of people with psoriasis have chronic plaque psoriasis, which is the most prevalent clinical type. Erythematous, symmetrical, well-defined plaques covered in silvery scales are the hallmark of classic plaque psoriasis. Although plaques can develop anywhere on the body, the scalp, trunk, buttocks, and extremities are where they are most commonly

encountered. There may also be nail involvement, occasionally without plaques present. Itchy or painful lesions are possible. Clinical presentation is used in order to make the diagnosis⁹.

6. Treatment and management of psoriasis

The UK National Institute for Health and Care Excellence recommendations list topical therapy, phototherapy, and systemic treatment as possibilities for treating psoriasis. Topical, systemic, and phototherapeutic are the basic categories into which these treatments can be divided. When psoriasis is mild, topical treatment is usually advised. Psoriasis is typically treated with corticosteroids, biologic medicines, methotrexate, cyclosporine, acitretin, calcineurin inhibitors, vitamin D3 analogs, and phototherapy⁹.

6.1. Topical therapies

The first-line treatment for mild, localized psoriasis is topical therapy. It can lower the percentage of the body surface area that is impacted by plaque lesions, as well as their quantity and thickness. Despite the current availability of biologic medications and apremilast for the treatment of psoriasis, the ongoing need for new therapies remains, particularly in order to tailor treatment approaches based on individual patient clinical characteristics and comorbidities, and to achieve a sustained therapeutic response⁹.

6.2. Systemic therapies

Poor tolerability and cumulative toxicity are the main drawbacks of using traditional systemic psoriasis therapies for an extended period of time. Examples include liver damage from methotrexate, kidney toxicity from cyclosporine, and skin carcinogenesis from phototherapy (especially psoralens with ultraviolet A). A biological drug called “etanercept” has been authorized for the treatment of psoriasis¹⁰.

7. Conclusion

Psoriasis is a skin condition that can reduce life expectancy due to its significant negative impact on patients' quality of life and its increased risk of various comorbidities. Psoriasis also has a substantial socioeconomic impact on society. A better understanding of its pathophysiology could lead to the development of more effective treatments.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Abdulridha G.A.O., Al-Zamali S.K.S., Otaiwi M.S., Ali H.A., Mohammed M.S. Psoriasis: a short review of its pathophysiology and causes. *Pharmakeftiki* 37(2s), 442-445, 2025. <https://doi.org/10.60988/p.v37i2S.251>

Nose-to-brain drug delivery: a short review of the available systems and their applications

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KEY WORDS:

**nose-to-brain delivery;
central nervous system;
blood-brain barrier; non-
invasive drug delivery;
olfactory nerve**

ARTICLE INFO:

Received: January 11, 2025

Revised: February 11, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

ABSTRACT

The brain is a crucial organ that controls most bodily functions; however, its protection by the blood-brain barrier (BBB) makes drug delivery to the brain difficult. The BBB prevents most drugs from entering the brain, which is a challenge for treating neurological diseases such as Parkinson disease, Alzheimer disease, multiple sclerosis, and stroke. The nasal-to-brain delivery route is a promising, non-invasive method to bypass the BBB, offering a direct connection between the nasal cavity and the central nervous system. This short review is focusing on ways through which drugs can reach the brain *via* the olfactory nerve, and the strategies to improve drug delivery through the intranasal route. In addition, it discusses the advantages and limitations of this type of drug administration and the recent formulations and applications of intranasal drug delivery with examples. Intranasal drug delivery holds great promise for treating neurological diseases and other conditions such as pain and cancer, and also provides a mean for reusing drugs that otherwise fail to reach the brain through the BBB. However, further developments in terms of the used formulations and the overcoming of challenges relating to absorption and mucosal irritation are essential for its widespread use in clinical settings.

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1. Introduction

The brain, a highly complex and vital organ, processes sensory information and controls body functions¹. The blood-brain barrier (BBB) is a unique microvascular

system in the brain that controls the movement of ions, molecules, and cells between the blood and the brain. The BBB restricts the passage of toxins and pathogens, but is also a major barrier to the delivery of therapeutic drugs, with

98% of small molecules and almost all macromolecules being unable to do so². There are many neurological diseases that require treatment where drugs must reach the brain, such as neuroinfections, Parkinson disease, Alzheimer disease, multiple sclerosis, age-related neurodegenerative diseases, and cerebral ischaemia. The nasal-to-brain route offers a non-invasive method to bypass the BBB, leveraging the direct anatomical link between the nasal cavity and the central nervous system (CNS). This makes nasal formulations a promising strategy for targeted brain drug delivery. Indeed, it is well known that the nasal cavity and the CNS are anatomically directly linked, suggesting the development of nasal formulations for brain targeting³.

The aim of this short review is to discuss how drugs can reach the brain *via* the olfactory nerve, and the strategies to improve drug delivery through the intranasal route. In addition, it discusses the advantages and limitations of this type of drug administration as well as the recent formulations and applications of intranasal drug delivery systems.

2. Pathways for the achievement of nose-to-brain drug delivery

The nasal cavity is divided by the nasal septum into two halves, each containing three zones: the nasal vestibule, the respiratory zone, and the olfactory zone. The nasal vestibule is the nose's entrance, while the respiratory zone covers most of the nasal surface. The olfactory zone, located on the nasal cavity's roof about 7 cm from the nostrils, contains olfactory nerves that bypass the BBB, providing direct access to the CNS. One route is systemic absorption from the respiratory zone, whereby drugs are taken into the systemic circulation and then pass through the BBB to the brain (especially lipophilic drugs). The other route is through the olfactory and trigeminal pathways, where drugs are transported directly from the nasal cavity to the CNS (cerebrospinal fluid and brain tissue)⁴. The mechanisms by which drugs that pass through the olfactory membrane reach the CNS are different. The first mechanism involves the drug migrating directly to the

primary neurons in the olfactory epithelium, being transported to the olfactory bulb *via* an intracellular axonal transport, and then being distributed to more distant brain tissues. A second mechanism is through the penetration of drugs into olfactory epithelial cells by transcellular or paracellular mechanisms and, subsequently, through uptake by the CNS⁵.

3. Strategies to improve drug delivery to the brain

Two main approaches can be used in order to bypass the BBB: invasive and non-invasive. Invasive techniques involve direct injection of the drug into the brain parenchyma or the cerebrospinal fluid or the therapeutic opening of the BBB. The direct injection or the implantation of drugs into the brain parenchyma has been studied in order to treat neurological and mental disorders as well as stroke. However, a direct injection can be less efficient because of limited diffusion between the cerebrospinal and the extracellular fluids. In addition, there can be potential risks of brain tissue damage and significant fluctuations in intracranial pressure as a result of such invasive techniques⁶. Non-invasive methods utilize endogenous cellular mechanisms in order to facilitate the transport of drugs into the CNS through transcellular pathways. Non-invasive strategies include the nose-to-brain route of administration, the inhibition of efflux transporters, the development of prodrugs and chemical drug delivery systems, and the application of nanocarriers⁷. The intranasal drug delivery approach offers more advantages over the other methods.

4. Advantages and limitations of the intranasal drug delivery approach

Some intranasal drug delivery systems can bypass the BBB, offering direct drug delivery to the brain with several advantages, such as a large surface area, the ease of self-administration, the avoidance of hepatic first-pass metabolism, the utilization of a highly vascular mucosa, and increased absorption⁸. However, challenges include the limited drug absorption

Table 1. Different formulations for nose-to-brain drug delivery, with specific examples and advantages.			
Formulation strategy	Description	Preclinical applications	Advantages
Solution	drug dissolved in an aqueous solution and administered <i>via</i> nasal delivery devices	insulin delivery to the brain; oxytocin delivered with a C_{max} of 0.003% of a 10-g dose	simple and effective for small molecules; allows direct brain targeting
<i>In situ</i> gelling systems	systems that transition from fluid to gel upon exposure to environmental stimuli (e.g., temperature, pH)	amantadine, levodopa, and ropinirole have been delivered <i>via</i> intranasal <i>in situ</i> gelling systems	high compatibility with a wide range of drugs; controlled release
Nanoparticles	colloidal solid particles ranging from 1 to 1000 nm that enhance mucosal penetration	nanoparticles used for immune delivery <i>via</i> the nasal route	small size enables transport through tight junctions in the mucosa, thereby enhancing delivery efficiency
Lipid-based nanoparticles	colloidal systems composed of lipids, oils, surfactants, and additives like nanoemulsions and liposomes	used for efficient nose-to-brain drug delivery	improved stability and enhanced brain targeting for a variety of drugs, including soluble and insoluble ones

and penetration, a variability in absorption due to the nasal anatomy and mucosal differences, and a short duration of action requiring frequent dosing. Additionally, nasal irritation, dryness, and mucosal damage may occur, thereby affecting absorption and patient comfort. These limitations must be addressed in order to optimize therapeutic efficacy and patient tolerance⁹.

5. Recent formulations and applications

Generally, the most frequently used formulation for the administration of drug molecules through the nose to the brain is the solution. The drug is usually dissolved in an aqueous solution and administered through the nose. The solution is usually given through a nasal delivery device. One of the first studies on peptide delivery to the brain has attempted the delivery of insulin through the nose to the brain. Oxytocin has also been delivered to the brain *via* the nose in solution form⁴.

In situ gelling systems are undergoing a left-to-gel transition at the site of administration. They are fluid prior to administration and with the help of environmental stimuli (such as temperature, pH,

ionic shift, magnetic field or other biological back-grounds) undergo a left-to-gel transition. Therefore, they are highly compatible with a wide range of drugs, including soluble and insoluble ones as well as low and high molecular weight ones.

Nanoparticles are colloidal solid particles that offer advantages over other delivery techniques due to their small size. Furthermore, only the smallest nanoparticles can pass through the mucosa by extracellular transport through tight junctions. As a result, nanoparticles are considered the most suitable technology for nasal antigen delivery.

Finally, lipid-based nanoparticles are colloidal systems composed of lipids, oils, surfactants, and other additives (including nanoemulsions, liposomes, and solid lipid nanoparticles). They have attracted great interest as a promising approach for nasal-to-brain drug delivery¹⁰. Table 1 provides an overview of different formulations assessed for nose-to-brain drug delivery, with specific examples and advantages.

6. Conclusion

The nose-to-brain drug delivery holds significant

potential for treating neurological diseases by bypassing the BBB. However ongoing research and development are needed in order to optimize formulation strategies, improve absorption efficiency, and address challenges such as mucosal irritation. With continued innovation, this method could become a valuable tool in the treatment of brain-related conditions.

Acknowledgements

We express our gratitude to the College of Phar-

macy staff at the University of Babylon, represented by its Dean, Professor Dr Hussam Al-Humadi.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Hasnawi D., Al-Azzawi S. Nose-to-brain drug delivery: a short review of the available systems and their applications. *Pharmakeftiki* 37(2s), 446-449, 2025. <https://doi.org/10.60988/p.v37i2S.252>



Emerging trends in nanopharmacology: new therapeutic approaches to paediatric parasitology

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KEY WORDS:

**nanopharmacology;
nanotechnology;
nanoparticles; parasite;
paediatric**

ARTICLE INFO:

Received: January 01, 2025

Revised: February 13, 2025

Accepted: February 13, 2025

Available online: October 10, 2025

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ABSTRACT

New and emerging trends, particularly in the employment of nanotechnology for the treatment of paediatric parasitic infections, are transforming the therapeutic modalities of parasitic diseases that largely affect children. This review outlines how nanopharmacological methods, especially liposomal and polymeric nanoparticles, boost drug delivery systems and transform treatment options for parasitic infections in children. Emerging nanopharmacological methods can be combined with recent discoveries with practical applications in the diagnosis, vaccine development, and fighting of drug-resistant organisms. We, herein, also briefly present the novel horizons of nanopharmacology and how the advancements in the field that must overcome existing limitations (such as the development of drug resistance and the low bioavailability of several therapeutic agents) by refining the effectiveness and the selection of therapeutic solutions. The combined application of nanotechnology and paediatric parasitology could be the ultimate approach to improving the quality of paediatric health, especially in developing countries. Finally, this review highlights current and future directions for paediatric parasitology, as it handles drug resistance and enhances therapeutic effectiveness while developing better vaccine technologies.

1. Introduction

Parasitic infections are the leading aetiology of paediatric morbidity and remain a public medical problem in low-income countries. Diseases, such as malaria, schistosomiasis, and leishmaniasis, may result in severe morbidity and death among children. Conventional pharmacological interventions face problems such as poor solubility, drug resistance, and adverse effects¹. Nanotechnology exploits the structure of nanoparticles in an attempt to enhance therapeutic efficacy². This article reviews various aspects of nanotechnology as a dynamic field in contemporary science, and how its progress can affect the field of paediatric parasitology.

2. Understanding nanoparticles in the context of drug delivery

Nanoparticles are molecules that are less than or equal to 1 µm in size and greater than or equal to 1 nm. Their small size enables them to possess certain physico-chemical characteristics that can be exploited for medical applications³. Many types of nanoparticles have been employed in the field of nanopharmacology, including: (i) liposomes (spherical vesicles that can encapsulate hydrophilic and hydrophobic drugs in order to enhance their delivery and/or decrease their toxicity)²⁻⁴, (ii) polymeric nanoparticles (deriving from biocompatible polymers and designed to deliver drugs under certain conditions, such as changes in pH or temperature)^{2,3}, (iii) solid lipid nanoparticles (SLNs; although having features that resemble both liposomes and polymeric nanoparticles, SLNs simultaneously offer extended drug release with biocompatibility)^{2,3}, and (iv) dendrimers (branched macromolecules that can be synthesized with a high degree of controlled-drug delivery applications because of the clear structure of the branched macromolecules)^{2,3}.

Nanoparticles can improve drug delivery through several mechanisms: (i) targeted delivery (through functionalization with ligands that interact with specific parasitic receptors, the drug concentration at the site of parasitic infection can be increased, while the overall toxicity is reduced), (ii) controlled release (some nanoparticles have been developed to stimulate drug release

over time, thereby maintaining circulatory therapeutic concentrations), and (iii) enhanced permeability (nanoparticles can increase the absorption of expensive drugs and reduce the impact of biological barriers because of their molecular structure)^{3,5}.

3. Applications of nanotechnology in paediatric parasitology

Another area where growth is observed almost as revolutionary is the drug delivery techniques introduced in the nanotechnology field when working with children with parasitic diseases. Liposomal formulations have been shown to be potential treatments for parasitic diseases. For instance, liposomal amphotericin B is used for the treatment of visceral leishmaniasis^{3,4}. They increase the bioavailability of drugs and decrease nephrotoxicity when compared with classical preparations. Randomized controlled trials have confirmed that liposomal formulations are beneficial in such subjects^{2,3}.

Polymeric nanoparticles can be used to encapsulate and deliver antiparasitic drugs, such as antimalarial artemisinin. Through sustained release kinetics, systemic therapeutically-relevant concentrations are achieved and sustained for prolonged durations, thereby enhancing treatment compliance and long-term outcomes^{2,3}.

On the other hand, parasitic infections must be diagnosed at an early stage for the achievement of a better treatment. Traditional diagnostic approaches are insensitive and nonspecific, resulting in delayed treatment and a poor prognosis¹. Nanotechnology has been proven to provide good results in the development of biosensors with improved sensitivity and speed compared to traditional biosensors, including: (i) gold nanoparticles (such nanoparticles can enhance diagnostic signalling assays because of their unique optical characteristics; they are used as conjugates in lateral flow assays for the rapid diagnosis of malaria and other parasitic infections) and (ii) quantum dots (semiconductor nanoparticles have peculiar fluorescence ability that can be employed in the imaging and detection processes; quantum dots labelled with parasitic antigen antibodies provide a highly sensitive detection technique)^{2,5}.

A major goal of disease control is vaccination, and vaccines have been developed for a multitude of viral

and bacterial diseases. The development of effective vaccines to immunize against parasitic diseases has been difficult due to the parasites' complicated life cycles and their high rate of antigenic variation⁶. The use of nanoparticles in vaccines could provoke a very effective immune system reaction against parasitic diseases, particularly through: (i) their adjuvant properties (nanoparticles possess the property of an immune adjuvant when conjugated to antigens; this approach has been proposed for vaccines against malaria and schistosomiasis)⁶ and (ii) targeted antigen delivery (using nanoparticles that have a preference for certain immune cells, such as dendritic cells, can improve antigen uptake and presentation, thereby improving the immune response)^{6,7}.

Drug-resistant parasites present a substantial challenge for treatment efficacy. Nanoparticles can offer novel solutions to this issue^{1,2,7}. Ordered drug delivery using nanotechnologies can enhance drug efficacy because parasites are targeted using multiple modalities^{2,7}. Antimalarial agents can be combined with secondary drugs in order to improve the chance of eradicating the parasites with a lower possibility of inducing drug resistance⁸. Finally, scientists can achieve targeted delivery, minimize off-target effects, and improve drug effectiveness by altering the nanomolecular surface to ligands that distinguish targeted parasitic receptors⁷.

4. Perspectives of nanopharmacology for paediatric parasitology

Despite optimistic improvements, the nanotechnological applications for the treatment of paediatric parasitic diseases are still limited. Future studies should emphasize on the following: (i) optimizing formulations (adapting nanopharmacological formulations to certain parasites is vital for the optimization of therapeutic benefits with fewer adverse effects)⁷, (ii) securing prolonged safety profiles (evaluating the long-term safety profiles of nanoparticle-based treatments is vital before their deployment in clinical settings)⁸, and (iii) conducting clinical trials (nanotherapies, predominantly in the paediatric field, will need to be assessed for their efficacy and safety through the undertaking of additional clinical trials).

Integrating nanotechnology with future health strat-

egies could substantially impact worldwide efforts to fight parasitic diseases in children. Where parasitic diseases are most predominant in poor populations, health outcomes can be improved by refining access to actual management through affordable nanopharmacological solutions. In fact, emergent economic nanoparticle-based medications could facilitate extensive adoption in low-resource areas^{2,8}. Funding programs and infrastructure for research should be initiated in order to ensure the practical application of nanotechnology developments in the public welfare system^{2,9}.

In order to optimize the possible benefits of nanopharmacology in combating paediatric parasitic diseases, educational initiatives must be employed at several levels: (i) training healthcare professionals (strengthening training programs for nanopharmacology will ensure that healthcare providers have adequate knowledge in applying emerging enhanced technologies) and (ii) community awareness programs (educating communities about parasitic diseases and nanotechnology treatments for diseases will help families obtain proper early treatment)^{1,2,9}. Moreover, the collaboration between researchers, industry partners, and government agencies will drive innovation and the fast-track development of satisfactory new nanotechnology solutions specifically designed for use in paediatrics⁹.

Similar to any evolving biotechnology, ethical considerations should be addressed when implementing nanopharmacological methods in the treatment of paediatric patients. It is also fundamental to guarantee that parents or guardians are fully informed of the pros and cons of new therapies for their children's conditions. Finally, equity in the access of these medications should be ensured, while the generation and elimination of nanoparticles should also be evaluated for their environmental impacts, thereby ensuring sustainability in their use¹⁰.

5. Conclusion

Nanopharmacology is a promising model for pharmacotherapy in children with parasitic infections. These applications mean that nanoparticles can improve the effectiveness of drug delivery systems, boost the accuracy of diagnostic tests, and create vaccines against parasitic diseases that affect children worldwide. Addi-

tional research and funding in this regard are desirable in order to allow us to understand the potential of nanotechnology to positively impact the health of deprived children worldwide. As we build concepts and directions for these technologies, we ensure a ready solution to a core set of problems related to parasitic infections in children.

Acknowledgements

None.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Hindy H.A.A.M., Hassnawi F.S.M., Obaid S.R., Al-Anbari A.J.K. Emerging trends in nanopharmacology: new therapeutic approaches to paediatric parasitology. *Pharmakeftiki* 37(2s), 450-453, 2025. <https://doi.org/10.60988/p.v37i2S.253>

Oncogenic potential of *Salmonella* infection: a short review

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KEY WORDS:

***Salmonella* infection; colon cancer; gallbladder cancer; gut microbiome; toxin-anti-toxin modules**

ARTICLE INFO:

Received: January 31, 2025

Revised: n/a

Accepted: February 11, 2025

Available online: October 10, 2025

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ABSTRACT

Salmonella infections, whether typhoidal or non-typhoidal, represent a major global health burden. While acute infections primarily manifest as gastroenteritis, chronic infections have been increasingly associated with severe long-term complications, including inflammatory bowel disease, gallbladder cancer, and colorectal cancer. Emerging evidence indicates that *Salmonella* infections may promote tumorigenesis through multiple mechanisms, such as chronic inflammation, DNA damage, and the manipulation of host cell signalling pathways. Specifically, *Salmonella* effector proteins, including AvrA, SopE, SopE2, and SopB, have been shown to play a critical role in activating oncogenic pathways such as Wnt/ β -catenin, PI3K/Akt, and MAPK, which drive cellular transformation and tumour progression. Additionally, *Salmonella* infection can disrupt the gut microbiome, leading to dysbiosis, which may further contribute to cancer risk. Environmental factors, lifestyle choices, and genetic predispositions also modulate the risk of developing these cancers. This short review highlights the urgent need for further research in order to unravel the complex interactions between *Salmonella* infections, host factors, and cancer development, with the goal of improving prevention, early detection, and therapeutic strategies.

1. Introduction

Salmonella enterica, a species that includes both typhoidal and non-typhoidal subspecies which

can be responsible for typhoid fever and severe gastroenteritis. The primary mode of transmission is through the consumption of contaminated food or water. *Salmonel-*

la infections are a significant global health burden, causing millions of cases of gastroenteritis annually, with high mortality rate¹. Beyond acute gastroenteritis, chronic *Salmonella* infections have been linked to inflammatory bowel disease, colon cancer, and gallbladder cancer; they can contribute to tumorigenesis through mechanisms such as chronic inflammation and DNA damage². This short review focuses on the role of *Salmonella* infection in the development of colorectal and gallbladder cancer, by discussing the interplay between the gut microbiome, host factors, and environmental influences.

2. Experimental models of *Salmonella* infection-linked tumorigenesis

A study by van Elsland *et al.*³ has revealed that a low repetitive exposure to *Salmonella* infection can increase the risk of colon cancer, and so can a single high-dose exposure to the bacterium. These findings suggest that even subclinical or asymptomatic *Salmonella* infections could predispose individuals to colon cancer³. The same study has also found that invasive *Salmonella* bacteria could be recovered from colonic tumours of mice exposed to azoxymethane and dextran sodium sulfate; an experimental model used for the induction of inflammatory colorectal cancer³.

In addition to chemically-induced models of cancer, researchers have used a mouse model with a conditional deficiency in the adenomatous polyposis coli (APC) gene, which is critical for suppressing tumour growth in the colon⁴. While *Salmonella* infection did not increase the number of tumours in those mice, it accelerated tumor growth, likely through the activation of the β -catenin pathway which promotes the hyperproliferation of intestinal epithelial cells⁴.

As bile juice is secreted from the liver and transferred to the gallbladder for storage during a chronic *Salmonella* infection, it releases various types of toxins in the gallbladder. These toxins are carcinogenic⁵.

Salmonella infection employs various effector proteins in order to manipulate the host cell processes, thereby facilitating the bacterial survival and replication. Two key pathogenicity islands, *SPI-1* and *SPI-*

2, encode type III secretion system (T3SS). One such effector, AvrA, activates the Wnt/ β -catenin pathway, leading to hyperproliferation and tumorigenesis in the colon. AvrA also inhibits autophagy by regulating the conversion of LC3-I to LC3-II, thereby reducing the levels of Beclin-1; a protein essential for autophagy⁶. This inhibition of autophagy promotes *Salmonella*'s intracellular survival, as suggested by observations in AvrA-deficient *Salmonella* strains⁶. Other effector proteins such as SopE, SopE2, and SopB, can activate oncogenic signalling pathways such as the PI3K/Akt and the MAPK, leading to enhanced cellular proliferation and survival⁷. The *Salmonella* infection can also induce DNA damage through the release of carcinogenic toxins, such as the cytolethal distending toxin, which creates double-stranded DNA breaks in the host cells⁷. This DNA damage, combined with chronic inflammation, creates a favourable environment for the development of gallbladder cancer⁷.

3. Gut microbiome after a *Salmonella* infection

The gut microbiome plays a critical role in human health, and its composition is dynamic, responding rapidly to environmental changes. *Salmonella* infections alter the gut microbiome, affecting the metabolome and increasing inflammation, which may contribute to tumorigenesis. Short-chain fatty acids, produced by the microbiota, are influenced by the ecosystem's structure, available nutrients, and infection. Restoring the microbiome after a *Salmonella* infection has been shown to improve barrier function and suppress inflammation, thereby highlighting the importance of understanding how *Salmonella* infections alter the microbiome and increase cancer risk⁸. Another study has shown that younger patients (under 60 years of age) with serological evidence of a *Salmonella* infection are more likely to develop colon cancer⁹. Additionally, the role of antibiotics in colon cancer risk is unclear. Some studies suggest that antibiotic exposure may increase cancer risk, possibly due to dysbiosis caused by antibiotic-induced changes in the gut microbiome. Fur-

Table 1. Overview of key studies on <i>Salmonella</i> infections and their association with cancer development. Abbreviations used: NTS, non-typhoidal <i>Salmonella</i> .	
Studies	Key findings
van Elsland <i>et al.</i> (2022) ³	repetitive exposure to NTS increases colon cancer risk in mice; low-dose NTS exposure leads to the development of colonic tumours; invasive <i>Salmonella</i> was found in the tumours
Cheng <i>et al.</i> (2020) ⁴	a mouse model with a conditional deficiency in the adenomatous polyposis coli (APC) gene shows accelerated tumour growth with NTS infection; NTS infection accelerates tumor growth <i>via</i> the β -catenin pathway
Scanu <i>et al.</i> (2015) ⁵	<i>Salmonella</i> -induced gallbladder cancer; chronic <i>Salmonella</i> infection is linked to gallbladder cancer
Jiao <i>et al.</i> (2020) ⁶ ; Stender <i>et al.</i> (2000) ⁷	<i>Salmonella</i> effector proteins manipulate host pathways; AvrA activates the Wnt/ β -catenin pathway; SopE/SopE2 facilitate intracellular replication
He <i>et al.</i> (2023) ⁸	environmental factors influence <i>Salmonella</i> infection outcomes; younger patients with a history of a <i>Salmonella</i> infection are more likely to develop colon cancer
Sun (2022) ⁹	gut microbiome alterations take place after a <i>Salmonella</i> infection; the latter disrupts the microbiome, thereby increasing inflammation and cancer risk
McDowell <i>et al.</i> (2022) ¹⁰	exposure to antibiotics may increase cancer risk through gut dysbiosis

ther research is needed in order to elucidate the relationship between *Salmonella* infection, antibiotic use, and colon cancer development¹⁰.

4. Conclusion

Salmonella infections are a significant global health concern, with emerging evidence suggesting a link to increased colon cancer risk. Multiple factors, including host genetics, gut microbiome composition, and environmental exposures, contribute to this risk. A better understanding of the mechanisms through which a *Salmonella* infection can contribute to tumorigenesis could lead to new strategies for cancer prevention.

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Acknowledgements

We express our gratitude to the College of Pharmacy of the University of Babylon for its invaluable support and assistance.

Conflicts of interest

None exist.

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HOW TO CITE:

Alhussainy A., Al-Humadi H.W., Al-Saigh R.J. Oncogenic potential of *Salmonella* infection: a short review. *Pharmakeftiki* 37(2s), 454-457, 2025. <https://doi.org/10.60988/p.v37i2S.254>

Microneedles for advanced drug delivery: a short review

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KEY WORDS:

**microneedles; drug delivery
systems; manufacturing
techniques; vaccines;
advancements**

ARTICLE INFO:

Received: January 10, 2025

Revised: February 05, 2025

Accepted: February 14, 2025

Available online: October 10, 2025

ABSTRACT

Microneedles represent an innovative and minimally invasive modality for drug administration, effectively addressing the limitations of conventional techniques such as hypodermic injections and topical applications. These instruments penetrate the stratum corneum without activating nociceptive nerve endings, thereby enabling painless delivery of both low- and high-molecular-weight pharmacological agents. Fabricated from diverse materials (including polymers, metals, and ceramics), microneedles can be engineered in various forms: solid, hollow, coated, and dissolvable. Each type offers distinct advantages for systemic drug delivery and targeted localized treatments. The clinical applications of microneedles span multiple disciplines, including dermatology, vaccination, wound healing, ocular therapy, oncology, and cosmetic procedures. Despite their promise, challenges remain in material selection, drug encapsulation, and manufacturing scalability. Continued advancements in microneedle technology signal substantial potential for enhancing patient adherence, improving therapeutic precision, and reducing adverse effects. This review outlines recent developments in microneedle systems, highlighting design classifications, fabrication methods, constituent materials, and pharmaceutical applications.

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1. Introduction

The oral route remains the oldest and most convenient method of drug administration, offering acceptable ease for patients. Howev-

er, its long-term use is associated with systemic side effects, particularly impacting vital organs such as the liver and the kidneys¹. Microneedles significantly enhance formulation permeability – includ-

ing ointments, creams, lotions, gels, liniments, matrix systems, reservoir systems, and foams – thus improving transdermal drug delivery². Conventional skin-based delivery methods, such as hypodermic needles and topical creams, present limitations: needles often cause patient discomfort, while topical formulations suffer from low bioavailability. The skin's structure acts as a robust barrier, permitting only lipophilic and low-molecular-weight drugs to penetrate it. This restricts formulation efficacy and presents considerable challenges in topical drug design. Among emerging solutions, microneedles stand out by overcoming these intrinsic barriers and formulation challenges³. Transdermal delivery offers additional advantages, such as reducing systemic organ involvement and enabling sustained drug release¹. This review examines the rising interest in microneedles as novel transdermal delivery systems, with particular attention to their potential in vaccine administration.

2. Characteristics of microneedles

Microneedles are designed to penetrate the epidermis at depths ranging from 70 to 200 μm . Their short, slender geometry prevents contact with dermal nerves, thereby enabling painless administration of pharmaceutical agents². These devices are fabricated from various materials, including silicon, titanium, stainless steel, and biocompatible polymers. Microneedle systems support the delivery of both small and large therapeutic agents, ranging from conventional drugs and proteins to genetic materials and nanomedicines. Owing to their structural adaptability and minimal invasiveness, microneedles are regarded as highly versatile tools that are less painful, safer, and less damaging than traditional needle-based technologies³.

3. Categorization of microneedles

3.1. Solid microneedles

Solid microneedles facilitate skin pre-treatment by forming micron-sized pores. Their pointed tips pen-

etrate the epidermis in order to create microchannels, thereby enabling subsequent drug delivery *via* patches and improving drug permeation. Once absorbed into capillaries, the drug may exert systemic or localized effects. Solid microneedles, commonly used in dermatology for collagen induction therapy, enhance bioavailability and mechanical strength through passive diffusion. Stainless steel variants have demonstrated improved efficacy in delivering drugs such as captopril and metoprolol tartrate³.

3.2. Hollow microneedles

Hollow microneedles deliver liquid formulations through a central bore and are typically fabricated from stainless steel, silicon, or glass. With dimensions ranging from 27 to 35 gauge and lengths starting at 150 μm , they are significantly smaller than conventional hypodermic needles (2 mm or longer; 26–30 gauge)⁴. These microneedles are primarily used in order to administer high-molecular-weight substances, including proteins, vaccines, and oligonucleotides³.

3.3. Coated or layered microneedles

Coated microneedles use the “coat and poke” technique, in which a drug formulation is applied to the surface of solid microneedles. Upon insertion into the skin, the coating dissolves, rapidly releasing the therapeutic agent. This method is suitable for the delivery of macromolecules such as vaccines, proteins, peptides, and DNA. Despite its simplicity and efficiency, the approach is limited by the small quantity of drug that can be coated, thereby restricting its use to potent molecules⁵.

3.4. Dissolving microneedles

Dissolving microneedles are fabricated from biodegradable polymers that encapsulate the drug within the microneedle structure. Upon skin insertion, the microneedles dissolve, releasing the drug without the need for removal. Controlled degradation of the polymer matrix enables sustained drug delivery, making this design particularly advantageous for long-term

therapies. The use of non-toxic, biocompatible polymers also supports improved patient adherence³.

4. Materials for microneedles

4.1. Metal materials

Metals are extensively used in the production of solid, coated, and hollow microneedles. Commonly employed metals include stainless steel, gold, platinum, titanium, nickel, and iron². Stainless steel and titanium are especially prevalent due to their favourable mechanical properties. However, one major concern is the potential for microneedle fracture within the dermis, which poses safety risks and necessitates the exploration of alternative materials. Additionally, metal microneedles may trigger allergic responses in susceptible individuals¹.

4.2. Inorganic materials

Inorganic substances such as silicon, glass, and ceramics are often used in order to manufacture solid, coated, and hollow microneedles. While these materials offer precision in fabrication, limitations exist (including incompatibility with biological membranes and the potential for fragment breakage, particularly with silicon), thus narrowing their practical applicability².

4.3. Polymeric materials

Polymers present highly attractive alternatives for microneedle fabrication due to their excellent biocompatibility, biodegradability, and minimal toxicity⁶. Biodegradable polymers such as polylactic acid and polyglycolic acid enable safe drug release and environmental breakdown, while non-degradable polymers like photolithographic epoxy offer structural rigidity for specific applications⁷.

5. Applications of microneedles

5.1. Disease diagnosis

Microneedles enhance diagnostic capabilities by accessing serum inhibitory factors through epidermal

penetration. Metabolites derived from the serum interstitial fluid serve as potential biomarkers for the detection of health conditions, including cancer and cardiovascular diseases³.

5.2. Drug and vaccine delivery

Microneedles enable direct drug administration into the bloodstream, thereby supporting controlled release and improved therapeutic efficacy⁸. Extensive research has explored microneedle-based techniques for combating pathogens such as influenza, measles, poliovirus, rotavirus, adenovirus, the Calmette-Guérin bacillus, botulinum toxin, as well as hepatitis B and C⁹.

5.3. Ocular therapy

In retinal disease treatment, microneedles allow precise drug delivery to the sclera or suprachoroidal space, thereby minimizing systemic exposure. They are employed in order to administer anti-inflammatory and anti-glaucoma agents for the management of ocular inflammation and intraocular pressure⁴.

5.4. Cancer therapy

Microneedles facilitate the administration of chemotherapeutic agents such as paclitaxel and gemcitabine in breast cancer therapy. Various microneedle systems have been employed in treating superficial malignancies⁸.

5.5. Cosmetic applications

Widely used in dermatological and cosmetic procedures, microneedles promote skin rejuvenation and hair growth. Notable examples include dissolvable patches delivering ascorbic acid and retinyl retinoate¹. Their growing significance in dermatology addresses conditions ranging from skin blemishes to aesthetic enhancement⁹.

6. Advantages of microneedles

Microneedle arrays enable targeted skin delivery by penetrating the stratum corneum and accessing the

dermis⁶. Their ease of use permits self-administration with minimal training, while minimizing pain and enhancing patient compliance. The reduced invasiveness supports faster recovery, mitigates infection risk, and facilitates efficient wound healing. Microneedles inflict minimal skin damage, thereby lowering the chance of bacterial entry and adverse cutaneous responses³.

7. Challenges of microneedle fabrication

Although polymeric microneedles are increasingly favoured, they present fabrication challenges such as mechanical instability under stress and difficulties in achieving precise geometries¹. Silicon microneedles exhibit brittleness, which may cause fractures during skin insertion and provoke foreign body reactions like abscesses or granuloma formation. Conventional manufacturing techniques (including molding and etching) are often labour-intensive, costly, and offer limited scalability⁷.

Drug delivery *via* microneedles primarily relies on passive diffusion into the dermis. Administering large drug volumes remains problematic, as considerable loss may occur at the epidermal surface. In vaccine delivery, dosage inconsistencies can reduce bioavailability and impair immune response¹⁰. Moreover, safety evaluation is essential in clinical trials, focusing on variables such as skin irritation, sensitization, and immune response. Erythema is a noted adverse effect. Improper microneedle application may result in the retention of foreign

materials, thereby increasing the risk of infection. Additional concerns include allergic reactions, cutaneous irritation, and restricted drug-loading capacities³.

8. Conclusion

Microneedles represent a pivotal innovation in drug delivery, offering numerous advantages including pain-free administration, improved patient adherence, and enhanced therapeutic precision. Their versatility in material composition and manufacturing techniques enables customizable designs tailored to diverse clinical needs. Nonetheless, critical challenges (such as material refinement, safety assessment, and drug loading efficacy) must be addressed in order for them to realize their full potential.

Acknowledgements

The authors would like to express their gratitude toward the College of Pharmacy of the University of Babylon for supporting this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Aljubouri N.A., Masheta D. Microneedles for advanced drug delivery: a short review. *Pharmakeftiki* 37(2s), 458-462, 2025. <https://doi.org/10.60988/p.v37i2S.255>

Immunopathogenesis pathways of primary immune thrombocytopenia: a comprehensive overview

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KEY WORDS:

anti-platelet antibodies; CTLs; platelets apoptosis; TFH cells; megakaryocytes; B cells

ARTICLE INFO:

Received: January 11, 2025

Revised: February 14, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

ABSTRACT

Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by both the destruction and impaired production of platelets. Mucocutaneous bleeding is the predominant symptom in patients with severely reduced platelet counts. The pathogenesis of ITP involves complex immune mechanisms initiated by aberrant T cell responses and the action of splenic T follicular helper cells, which activate autoreactive B cells. These B cells produce anti-platelet autoantibodies that promote platelet phagocytosis by macrophages; the primary antigen-presenting cells in ITP. Additionally, enhanced cytotoxic T-cell activity induces platelet apoptosis, further contributing to thrombocytopenia. Immune-mediated targeting of megakaryocytes, along with reduced levels of thrombopoietin (the principal growth factor for megakaryocyte maturation) results in dysfunctional bone marrow platelet production. This review outlines the immune mechanisms driving ITP and elucidates the roles of immune cells in both platelet destruction and impaired production.

1. Introduction

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder affecting both adults and children; it presents with haematological manifestations characterized by bleeding and a platelet count of less than $100 \times 10^9/L$, due to underlying mechanisms that increase platelet clearance and impair megakaryo-

poiesis and thrombopoiesis. ITP is categorized into primary and secondary forms. Approximately 80% of newly diagnosed adult cases are primary ITP, with no identifiable predisposing factors. However, autoimmune conditions such as Graves' disease, Hashimoto's thyroiditis, autoimmune haemolytic anaemia, antiphospholipid syndrome, rheumatoid arthritis, and

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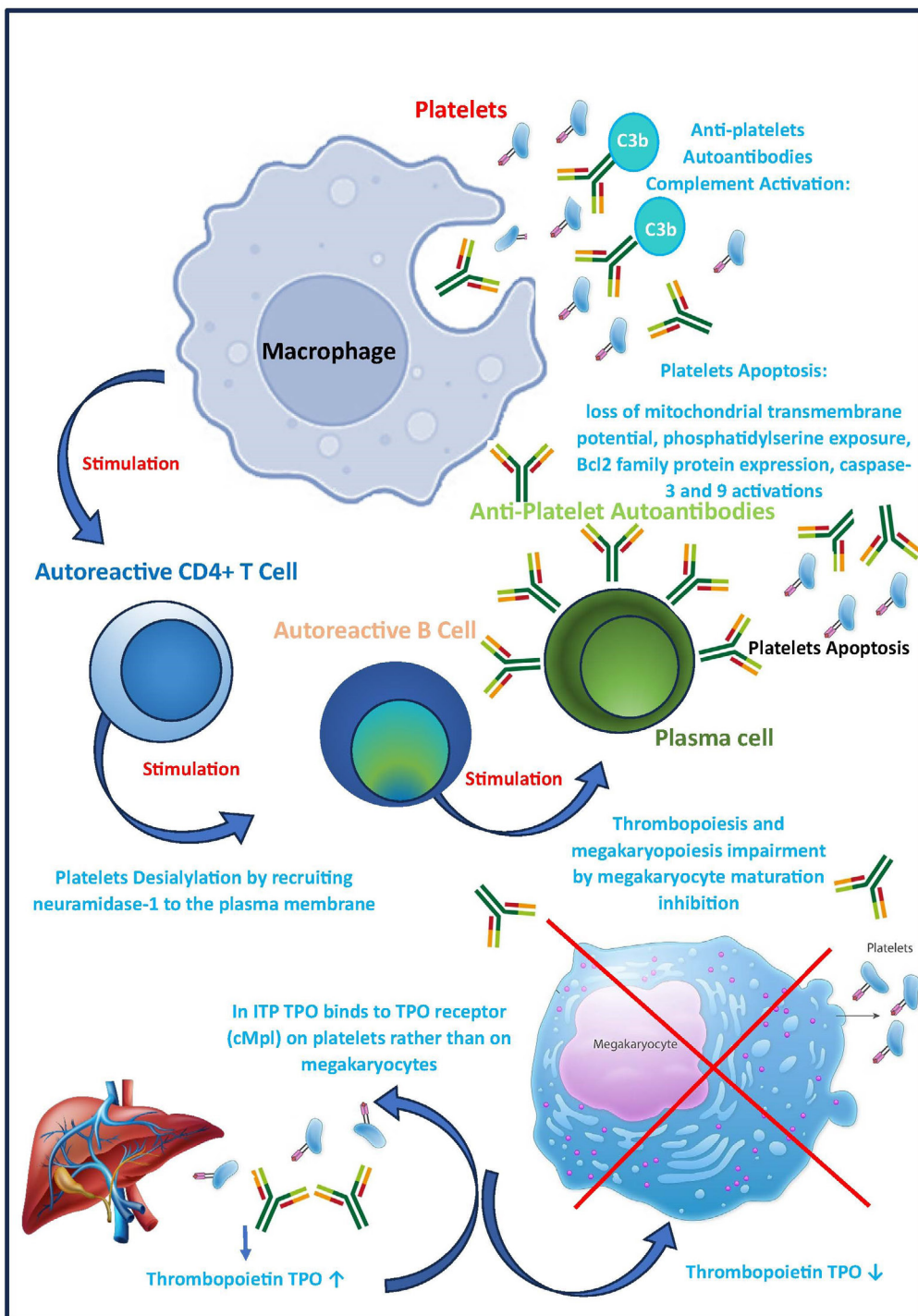


Figure 1. Overview of the immunopathogenic mechanisms underlying primary immune thrombocytopenia (ITP). Anti-platelet autoantigens lead to platelet destruction and impairment of their production via a number of pathways (including complement activation, platelet apoptosis, platelet desialylation, and inhibition of the megakaryocyte maturation). Abbreviations used: C3b; complement C3b protein; TPO, thrombopoietin.

systemic lupus erythematosus may act as risk factors or co-occur with secondary ITP. Secondary ITP can also be triggered by infections, malignancies, or certain medications¹. A diagnosis of primary ITP is established after excluding other causes of thrombocytopenia typically associated with secondary ITP². This review aims at elucidating the immunopathogenic pathways of primary ITP, focusing on the roles of autoantibodies and immune cells in disease development, in order to better inform optimized therapeutic strategies and improve patient outcomes.

2. Mechanisms of platelet destruction and megakaryopoiesis dysregulation

2.1. Autoantibody-related mechanisms

A dysregulated immune response and the loss of tolerance to platelet surface autoantigens lead to the formation of autoantibodies, which play multiple roles in the pathophysiology of primary ITP. These autoantibodies, predominantly IgG, target platelet surface glycoproteins. Antibody-coated platelets are cleared from circulation *via* a macrophage-mediated phagocytosis. Macrophages serve a dual role, as effector cells and as principal antigen-presenting cells, thereby stimulating autoreactive CD4⁺ T cells (including follicular helper T cells; TFHs), which in turn activate autoreactive B cells in order to differentiate them into antibody-producing plasma cells².

Autoantibodies can also activate the complement system, leading to C3b deposition on antibody-coated platelets. This enhances their phagocytosis by splenic macrophages. Complement involvement is observed in approximately half of the ITP cases and is associated with autoantibody specificity; in fact, anti-GPIIb/IIIa antibodies are more likely to activate complement than anti-GPIb/IX antibodies³.

Platelet apoptosis represents another pathogenic mechanism triggered by anti-platelet autoantibodies; it involves a mitochondrial depolarization, phosphatidylserine exposure, altered expression of Bcl-2 family proteins, and an activation of caspases (particularly caspase-3 and -9), which are significantly implicated in ITP-associated platelet apoptosis².

Autoantibodies also impair thrombopoiesis and megakaryopoiesis by inhibiting megakaryocyte maturation, resulting in decreased platelet production². An additional pathway of platelet destruction involves antibody-mediated desialylation. In the latter case, autoantibodies recruit neuraminidase-1 to the platelet membrane, thereby accelerating the removal of sialic acid residues. Desialylated platelets are then recognized by the Ashwell-Morell receptor on hepatocytes, leading to their clearance and the induction of thrombopoietin (TPO) production; a key growth factor for megakaryocytes³. In ITP, TPO levels are inappropriately regulated. TPO activates megakaryocytes *via* its receptor, c-Mpl, which is also expressed on platelets. The circulating platelet pool influences TPO availability (Figure 1). Under normal conditions, thrombocytopenia increases free TPO, thereby enhancing platelet production. Conversely, thrombocytosis depletes free TPO by increasing platelet binding. In ITP, circulating platelet levels may appear normal, yet platelets are rapidly destroyed, binding excessive TPO and reducing its bio-availability. Consequently, TPO concentrations in ITP patients are lower than in those with aplastic anaemia, despite similar platelet counts⁴.

2.2 Immune cell-related mechanisms

The immunopathogenesis of primary ITP involves several immune cell types, including CD4⁺ T cells, antigen-presenting cells (macrophages and dendritic cells), B cells, plasma cells, TFHs, cytotoxic T lymphocytes (CTLs), Th17 cells, and regulatory T cells (Tregs). The latter play a crucial role in maintaining immune tolerance by modulating T and B cell-mediated autoimmunity and by inducing a tolerogenic phenotype through interactions with antigen-presenting cells. A loss of peripheral tolerance arises from impaired Treg activity – whether due to reduced generation or altered function – and is associated with disease severity. This breakdown of self-tolerance contributes to T-cell dysregulation and initiates aberrant antigen presentation. Consequently, abnormal Th cell production occurs, along with Th-cell anergy and an imbalance in the Th1/

Th2 ratio, which inversely correlates with disease severity⁵.

Antigen-presenting cells (APCs) present platelet autoantigens to autoreactive CD4⁺ T cells, triggering a cascade that culminates in the production of anti-platelet autoantibodies by autoreactive B cells (that aberrantly differentiate into plasma cells) (Figure 1). These autoantibodies target platelets and megakaryocytes, thereby promoting their dysfunction and clearance in the spleen and liver².

Dendritic cells contribute to the immune activation as monocyte-derived APCs capable of phagocytosing apoptotic platelets and stimulating T cells. They possess tolerogenic properties and are instrumental in converting naïve T cells into Tregs, while also guiding T-cell polarization toward a Th2 phenotype⁵. In primary ITP, dendritic cells exhibit increased expression of co-stimulatory molecules (CD80 and CD86) and elevated production of interleukin-12 (IL-12); changes correlated with Treg deficiency and heightened pathogenic T cell activation⁶.

TFHs are central to ITP pathogenesis; they express the surface receptor CD154 and are major producers of IL-21, thereby facilitating autoreactive B cell differentiation and promoting anti-platelet antibody production⁷. At the same time, CTLs also play a significant role. CD8⁺ CTLs mediate platelet destruction through direct, contact-dependent cytotoxicity (likely occurring in the spleen). CTLs are also recruited to the bone marrow, where they target megakaryocytes. Evidence supports that both humoral and T cell-mediated autoimmunity contribute to megakaryocyte dysfunction and destruction in patients with ITP, representing an additional pathogenic mechanism

alongside peripheral platelet clearance⁸.

Finally, Th17 cells contribute to autoimmunity by secreting proinflammatory cytokines, notably IL-17, which is found at elevated levels in ITP patients (when compared to those of healthy individuals). IL-17 promotes the expression of other inflammatory mediators (including IL-6, transforming growth factor beta, matrix metalloproteinases, and intracellular adhesion molecule-1) across various cell types (such as bone marrow stromal cells), thereby sustaining the inflammatory cytokine milieu that is characteristic of ITP⁹.

3. Conclusion

Multiple immunological pathways contribute to platelet destruction and impaired production in ITP, driven by a loss of self-tolerance to platelet surface autoantigens. Understanding these mechanisms is essential for the development of targeted immunotherapies aimed at improving outcomes in patients with primary immune thrombocytopenia.

Acknowledgements

I wish to express my gratitude to the College of Pharmacy of the University of Babylon for its support.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Kaabi Z.I.L. Immunopathogenesis pathways of primary immune thrombocytopenia: a comprehensive overview. *Pharmakeftiki* 37(2s), 463-467, 2025. <https://doi.org/10.60988/p.v37i2S.256>

Toll-like receptor 4 signalling pathway: a therapeutic target for multiple diseases

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KEY WORDS:

**Toll-like receptor 4;
inflammatory pathologies;
cardiovascular disorders;
neoplastic conditions;
Alzheimer's disease**

ARTICLE INFO:

Received: January 05, 2025

Revised: February 18, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

ABSTRACT

The Toll-like receptor 4 (TLR4) signalling pathway is integral to the innate immune response and plays a pivotal role in numerous disease states, including infections, autoimmune disorders, metabolic conditions, and cancers. The activation of TLR4 initiates a cascade of signalling events that modulate key cellular processes and immune functions. The dysregulation of TLR4 signalling has been implicated in several pathophysiological conditions, particularly those involving chronic inflammation and immune dysfunction. This short review examines the mechanisms by which TLR4 signal transduction pathways influence cellular behaviour, highlights TLR4-mediated disease processes, and surveys current therapeutic strategies targeting TLR4. Evidence suggests that TLR4 inhibition can improve clinical outcomes in various settings, underscoring its therapeutic potential. We, herein, provide a succinct synthesis of recent advances in TLR4 research, emphasizing its viability as a clinical target. Finally, we consider the future implications of TLR4-focused therapies, and how such approaches might be integrated into routine practice in order to treat a broad spectrum of diseases.

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1. Introduction

The Toll-like receptor 4 (TLR4) is a critical component of the innate immune system, playing a central role in pathogen recognition and the coordination of immune responses.

As a member of the Toll-like receptor family, TLR4 detects molecular patterns associated with microbial infections; most notably, lipopolysaccharides (LPS) found in the outer membranes of Gram-negative bacteria. Upon activation, TLR4

initiates a cascade of intracellular signalling events that lead to the production of pro-inflammatory cytokines, thereby amplifying immune responses against invading pathogens¹.

Aberrant or dysregulated TLR4 signalling is increasingly implicated in the pathogenesis of various diseases, including chronic inflammatory disorders, autoimmune conditions, and certain malignancies. For example, TLR4-mediated signalling has been linked to heightened inflammatory responses and exacerbated tissue damage in diseases such as rheumatoid arthritis (RA) and type 2 diabetes. Emerging evidence also suggests that the modulation of TLR4 activity offers a promising avenue for therapeutic intervention across a wide range of pathological contexts. For example, preclinical studies indicate that TLR4 antagonists may attenuate inflammatory processes and improve clinical outcomes in chronic disease states, thereby reinforcing the receptor's appeal as a therapeutic target. Furthermore, deeper insight into the regulatory mechanisms governing TLR4 signalling could inform the development of novel therapeutic strategies, either to potentiate immune responses during infection or to dampen them in inflammatory disorders².

TLR4 is a membrane-associated protein with an estimated molecular weight of approximately 95 kDa. It features a leucine-rich extracellular domain and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain, which enables interaction with a diverse range of ligands³. TLR4 primarily recognizes pathogen-associated molecular patterns (PAMPs), such as bacterial LPS, as well as damage-associated molecular patterns (DAMPs) released during tissue injury. Upon engagement, the receptor triggers signalling cascades that culminate in the production of pro-inflammatory cytokines; key mediators of the immune defence against pathogenic challenges⁴.

TLR4-mediated signalling proceeds *via* two distinct pathways: the myeloid differentiation primary response 88 (MyD88)-dependent pathway and the TIR-domain-containing adapter-inducing interferon-beta (TRIF)-dependent pathway. The MyD88-dependent signalling cascade is initiated at the cell surface and involves the adaptor protein MyD88, ultimately

leading to the production of cytokines such as tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6). In contrast, the TRIF-dependent pathway is triggered following receptor internalization into endosomes, where TLR4 interacts with TRIF and the TRIF-related adaptor molecule (TRAM). This second pathway induces the production of type I interferons, which enhance antiviral immunity⁵.

TLR4 is predominantly expressed in immune cells, including macrophages and dendritic cells, but it is also detectable in various non-immune cell types, such as epithelial and endothelial cells. Its expression is modulated by numerous environmental cues and inflammatory mediators, ultimately influencing the breadth and magnitude of immune responses³. This short review aims at examining the role of TLR4 in disease progression and to explore strategies for targeting this receptor in the context of diverse clinical conditions.

2. Role of TLR4 in disease progression

2.1. Cardiovascular diseases

TLR4 plays a significant role in inflammatory signalling pathways that aggravate heart failure. Experimental models deficient in TLR4 demonstrate improved cardiac function and reduced inflammatory marker expression, thereby suggesting that TLR4 inhibition holds promise as a therapeutic strategy for heart failure⁶.

TLR4 is also centrally involved in mediating post-ischaemic inflammation following stroke. Its activation contributes to neurological deterioration *via* the upregulation of pro-inflammatory cytokines, including TNF- α and IL-6; key drivers in cardiovascular and cerebrovascular pathology. Accordingly, TLR4 is considered a viable therapeutic target in stroke management. Recent studies are increasingly focused on the development of targeted TLR4 inhibitors in order to attenuate inflammation and improve clinical outcomes in cardiovascular and cerebrovascular diseases. In fact, by blocking TLR4, one may yield protective effects by modulating the inflammatory cascade³.

2.2. Metabolic disorders; obesity

TLR4 activation has been linked to chronic low-grade inflammation in obesity. This inflammatory milieu promotes the release of cytokines that exacerbate insulin resistance and accelerate the progression of metabolic syndrome⁵.

2.3. Autoimmune diseases; RA

RA is a chronic autoimmune condition characterized by persistent joint inflammation, progressive joint destruction, and functional impairment. TLR4 is increasingly recognized as a key factor in RA pathophysiology. As a pattern recognition receptor, TLR4 detects both PAMPs and DAMPs, with its activation being implicated in the initiation and perpetuation of inflammation in RA⁷.

2.4. Infectious diseases and sepsis

TLR4 plays an essential role in initiating immune responses to bacterial infections. However, excessive stimulation can lead to a hyper-inflammatory state, thereby increasing the risk of sepsis and septic shock⁸.

2.5. Neurological disorders and Alzheimer's disease

TLR4 is involved in neuroinflammatory mechanisms relevant to Alzheimer's disease. Its activation is associated with the deposition of amyloid- β plaques and subsequent neuronal degeneration⁹.

2.6. Cancer / tumour progression

TLR4 contributes to tumour progression by amplifying inflammation within the tumour microenvironment. Its activation is correlated with enhanced tumour growth and metastasis across multiple cancer types. Chronic inflammation induced by TLR4 plays a documented role in oncogenesis. Cytokines

released following TLR4 activation promote cell survival, proliferation, and angiogenesis. Moreover, neoplastic tissues may exploit TLR4 signalling in order to evade immune surveillance. The activation of this receptor can drive the production of immunosuppressive factors that hinder anti-tumour immunity and support unchecked tumour expansion¹⁰. Recent research has explored combination therapies involving TLR4 inhibitors alongside immunotherapeutic or chemotherapeutic agents; these synergistic approaches may increase treatment efficacy by re-sensitizing tumours to immune-mediated eradication.

3. Conclusion

The TLR4 signalling pathway presents a compelling target for therapeutic development across a wide range of diseases. Its involvement in the pathogenesis of infectious, autoimmune, metabolic, oncologic, and neurodegenerative conditions underscores its relevance to translational medicine. Continued investigation is required in order to unravel the complexity of TLR4-mediated pathways and to design precise interventions capable of modulating immune activity and improving clinical outcomes.

Acknowledgements

The authors gratefully acknowledge the support provided by the College of Pharmacy of the University of Babylon.

Conflicts of interest

None exist.

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HOW TO CITE:

Alnoih L.F.A., Alzubaidi F.A., Oubaid E.N. Toll-like receptor 4 signalling pathway: a therapeutic target for multiple diseases. *Pharmakeftiki* 37(2s), 468-471, 2025. <https://doi.org/10.60988/p.v37i2S.257>

Improving mass transfer efficiency for penicillin production by using a continuous stirred tank reactor: a review

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KEY WORDS:

antibiotic; penicillin;
volumetric mass transfer
coefficient; continuous
stirred tank reactor; drug
production

ARTICLE INFO:

Received: January 12, 2025

Revised: February 24, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

ABSTRACT

Continuous stirred tank reactors (CSTRs) play a pivotal role in the industrial production of chemicals, including antibiotics such as penicillin, due to their ability to enhance mass transfer efficiency. The CSTR's design facilitates precise control over aeration and agitation; two critical parameters that influence the volumetric mass transfer coefficient. Aeration supports cellular metabolism by supplying oxygen, while agitation promotes the dispersion of gas bubbles, thereby improving phase contact. This review explores the historical development of penicillin, its mechanism of action, the principles underlying bioreactor operation, and the specific ways in which aeration and agitation affect mass transfer dynamics. By optimizing reactor configuration, operating conditions, and associated process parameters, producers can significantly enhance the efficiency of penicillin biosynthesis.

1. Introduction

Since Alexander Fleming's discovery that certain *Penicillium* fungi produce penicillin, β -lactam antibiotics have significantly impacted global public health and quality of life. These fungi belong to a broader class of microorganisms capable of synthesizing antibiotic compounds that either kill competing micro-

organisms or inhibit their growth. In 2021, global expenditure on antibiotics reached \$40.70 billion; a figure projected to rise to \$61.29 billion by 2029¹. This increasing demand underscores the need to enhance antibiotic production. The present review discusses the discovery of penicillin, its mechanism of action, and the role of aeration and agitation in optimizing its yield.

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2. History of penicillin

Penicillin, an antibiotic produced by the fungus *Penicillium chrysogenum*, is widely used in the treatment of bacterial infections. Chemically, it comprises a six-aminopenicillanic acid nucleus – a fusion of a β -lactam ring and a thiazolidine ring – with various cyclic side chains. In 1928, Fleming serendipitously observed that a culture of *Staphylococcus aureus* at St. Mary's Hospital in London had been contaminated by a *Penicillium* species². The fungus secreted a substance that inhibited bacterial growth, which Fleming named “penicillin”. Initially sceptical of its therapeutic potential in living organisms, Fleming did not pursue its clinical use. It was not until 1945 that a team from Oxford University – comprising Ernst Chain, Norman Heatley, and Howard Florey – successfully demonstrated penicillin's efficacy through clinical trials.

3. Mechanism of action of penicillin

Bacterial cells are typically enclosed by a peptidoglycan cell wall, which provides mechanical stability and prevents osmotic lysis. During growth and division, this structure undergoes continuous remodelling. Penicillin disrupts the biosynthesis of the peptidoglycan layer by irreversibly inhibiting transpeptidation. It binds covalently to penicillin-binding proteins, including the enzyme DD-transpeptidase, which catalyses the cross-linking of the peptidoglycan chains³. The strained β -lactam ring of penicillin facilitates this interaction, rendering the enzyme inactive. As a result, the bacterial cell cannot maintain its structural integrity, leading to cell death.

4. Penicillin production

Following the initial discovery, Fleming produced penicillin in shallow petri dishes (1- to 2-cm thick), yielding only minute quantities⁴. These limited yields posed challenges for the Oxford team's therapeutic trials, which coincided with the onset of World War II. Faced with the urgency of treating infected war wounds, the team developed a scalable production

method. They engineered a compact, space-efficient vessel that offered optimal conditions for fungal growth and significantly increased penicillin output. This apparatus known as a “bioreactor” or “fermenter” was foundational to modern large-scale antibiotic manufacturing.

5. Bioreactors

Bioreactors are specialized cylindrical vessels engineered so as to create optimal conditions for the microbial fermentation required to produce desired biochemical products. Depending on how nutrients and substrates are supplied to the microbial culture, bioreactors are categorized as batch, continuous, or fed-batch bioreactors⁵. An effective bioreactor design enhances productivity while complying with operational standards, thereby ensuring consistent, high-quality output in a cost-efficient manner. Key design features typically include: (i) the working and headspace volumes, (ii) an integrated agitation mechanism, (iii) oxygen delivery and foam control systems, (iv) temperature and pH regulation units, (v) sampling ports, (vi) cleaning and sterilization functions, and (vii) material loading / unloading lines.

5.1. Improving penicillin production

In order to meet growing pharmaceutical demand, researchers continually seek to increase the efficiency of penicillin biosynthesis. Multiple factors influence production levels, including fermentation conditions (e.g., temperature, pH), the fungal strain employed, and the reactor configuration. Among various reactor designs, the continuous stirred tank reactor (CSTR) is considered optimal in over 70% of bioprocess applications. Its widespread adoption stems from its capacity to finely regulate reaction parameters (including temperature, pH, agitation intensity, and aeration rate) and thereby enhance penicillin yields by improving the mass transfer coefficient⁶. The latter quantifies the movement of solutes from regions of higher concentration to those of lower concentration⁷. In penicillin fermentation,

the effective delivery of oxygen and nutrients is critical. As oxygen diffuses slowly in aqueous media, it is typically introduced in the form of air bubbles *via* filtered inlets or vacuum systems. These bubbles are then dispersed throughout the reactor through agitation, which plays a crucial role in increasing the surface area available for oxygen transfer from the gas phase to the liquid medium.

This process is governed by the volumetric mass transfer coefficient ($K_L a$), which reflects the rate at which oxygen transitions between phases. Enhanced aeration and agitation lead to higher $K_L a$ values, and thus to improved oxygen availability, thereby resulting in increased penicillin concentrations being produced.

5.2. Methods of calculating the volumetric mass transfer coefficient

One of the earliest methods used in order to estimate $K_L a$ is sulphite oxidation⁸. In this technique, a sodium sulphite solution is stirred at controlled speeds and exposed to variable air flow rates. At regular intervals, samples are collected and titrated with sodium thiosulphate in order to measure the sulphite concentration. Plotting these values over time yields a linear graph, whose slope corresponds to the oxygen transfer rate.

The static gassing-out method⁹ offers an alternative approach. Initially, oxygen is purged from the medium, commonly by introducing nitrogen. Once the dissolved oxygen level approaches zero, nitrogen flow is halted, and ambient air is introduced at a constant rate. The increase in dissolved oxygen concentration (C_L) over time can be described by the

equation: $dC_L/dt = K_L a (C^* - C_L)$.

A similar principle underlies the dynamic gassing-out method¹⁰. In the latter method, aeration is temporarily discontinued until the dissolved oxygen falls below a threshold. Once aeration resumes, real-time data on dissolved oxygen levels are collected. The rate of gas-liquid oxygen transfer is modelled using the logarithmic form: $\ln((C^* - C)/(C^* - C_0)) = K_L a \cdot t$, where the slope of the linear relationship corresponds to $-K_L a$.

6. Conclusion

Optimizing mass transfer efficiency between *Penicillium chrysogenum* and the liquid medium is central to improving penicillin synthesis in CSTR systems. This requires stringent control of operational variables (particularly mixing and aeration) so as to maintain ideal fermentation conditions.

Acknowledgements

The authors are grateful to the Dean of the College of Engineering, Dr Ali Abdul-Hussein Jazea, and to the College Librarian for their assistance in obtaining reference materials.

Conflicts of interest

None exist.

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HOW TO CITE:

Al-Rekabi D., Rushdi S. Improving mass transfer efficiency for penicillin production by using a continuous stirred tank reactor: a review. *Pharmakeftiki* 37(2s), 472-475, 2025. <https://doi.org/10.60988/p.v37i2S.258>

Sustained release of medications in liquid form: utility, advantages, and disadvantages

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KEY WORDS:

sustained release; modified release; controlled release; liquid dosage form; drug delivery systems

ARTICLE INFO:

Received: January 11, 2025

Revised: February 12, 2025

Accepted: February 18, 2025

Available online: October 10, 2025

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ABSTRACT

Dosage forms function as vehicles for delivering drugs into the body, with oral formulations typically classified as solid, liquid, or gaseous. Among these, liquid oral dosage forms are often preferred due to their superior bioavailability relatively to solids ones, as well as their enhanced absorption and ease of administration. These formulations are prepared by dissolving or suspending active pharmaceutical ingredients in suitable solvents. Key challenges in liquid formulations – such as taste masking, dose uniformity, and excipient optimization (e.g. sweeteners and viscosity modifiers) – are critical for ensuring patient acceptability and effective drug delivery. Modified-release dosage forms (including controlled-release, extended-release, delayed-release, and sustained-release systems) are designed to improve therapeutic outcomes by enabling prolonged drug delivery, reducing dosing frequency, and minimizing side effects. Sustained-release drug delivery systems are particularly beneficial in chronic conditions such as hypertension, where continuous medication is required. These systems aim at maintaining consistent plasma drug concentrations while attenuating peak levels to reduce the risk of toxicity. The ideal sustained-release formulation would support a single-dose regimen that delivers therapeutic effects over days, weeks, or longer. Several systems (such as diffusion-, dissolution-, and osmotic pressure-controlled systems) have been developed in order to achieve sustained release. However, challenges remain, including incomplete drug release, the risk of dose dumping, and the need to maintain therapeutic drug concentrations in a consistent manner.

1. Introduction

Dosage forms (including oral solids,

liquids, gases, inhalers, granules, and tablets) are employed in order to administer drugs *via* routes

such as oral, topical, rectal, and ophthalmic. These forms vary between infants and adults, with mechanisms of action tailored to individual physiological and therapeutic needs. Sustained-release systems, including controlled-release and depot formulations, aim at prolonging therapeutic effects, reducing dosing frequency, and minimizing side effects by delivering drugs uniformly over time. Liquid preparations, which typically offer superior bioavailability, face challenges such as taste masking and dose uniformity. These issues are often addressed using excipients including sweeteners and viscosity modifiers. Recent advancements (e.g. the development of nanoparticles, microparticles, and transdermal patches) have improved drug bioavailability and reduced adverse effects, with polymeric microparticles emerging as a promising delivery platform^{1,2}.

The development of sustained-release drug delivery systems seeks to enhance drug efficacy by reducing the number of doses or by localizing delivery at the site of action, thereby minimizing the required dosage while ensuring uniform distribution. An ideal drug delivery system meets two key conditions: it provides a single dose with a treatment period ranging from several days to a week, as in infectious diseases, or it enables lifelong therapy, as in chronic conditions such as hypertension and diabetes. This short review highlights the importance of sustained-release drug systems in improving drug efficacy and patient compliance compared to immediate-release formulations.

2. Problems that occur during multiple dosing

If the dosing interval does not correspond to the biological half-life of the drug, plasma concentrations may fluctuate outside the therapeutic range, potentially resulting in toxicity. Such variability is inconvenient for patients and may contribute to missed doses and poor adherence to the prescribed regimen³.

3. Modified-release dosage forms

Modified-release formulations are designed to alter

the release profile of a drug compared to conventional dosage forms. The drug release may be delayed or extended, depending on the desired therapeutic effect⁴.

4. Categories of modified-release dosage forms

Controlled-release formulations release the drug at a constant rate, thereby maintaining relatively stable plasma concentrations over time. Extended-release drugs are taken less frequently than their immediate-release counterparts. For example, controlled-release metformin (Glucophage XR), used in the treatment of type 2 diabetes, provides a gradual release of metformin throughout the day, helping maintain stable blood glucose levels. Controlled-release medications (including sustained-release and long-acting formulations) are designed to provide a continuous drug supply over prolonged periods. In order to avoid rapid absorption, the design must mitigate excessively high peak plasma concentrations. An example is prolonged-release diltiazem (Cardizem CD); a calcium channel blocker that delivers the drug slowly for extended blood pressure control.

Delayed-release preparations postpone the release of the active ingredient for a specific time after administration. Examples include repeat-acting tablets and capsules, as well as enteric-coated tablets that utilize barrier coatings in order to delay release. Delayed-release omeprazole (Prilosec), a proton pump inhibitor used for the treatment of gastro-esophageal reflux disease, exemplifies this category by releasing the drug in the small intestine, thereby avoiding gastric degradation.

Sustained-release drugs maintain steady plasma levels by releasing the drug at a controlled rate over an extended period, thus minimizing side effects. The primary goal of sustained-release systems is to improve patient compliance while enhancing bioavailability and therapeutic efficacy^{1,5}.

5. Advantages of the sustained-release drug delivery system

Sustained-release systems enhance patient conven-

ience and adherence by lowering dosing frequency. They also minimize fluctuations in drug plasma levels, resulting in improved disease control and fewer adverse effects. For high-potency drugs, precise management of plasma concentrations increases safety and optimizes drug utilization, potentially reducing total dosage requirements. Furthermore, these systems help contain healthcare costs by shortening treatment durations, limiting the need for frequent dosing, and reducing the time and the resources required for administration and monitoring⁶.

6. Disadvantages of the sustained-release drug delivery system

Limitations of sustained-release systems include incomplete drug release, increased first-pass metabolism, chemical instability, and insufficient residence time in the gastrointestinal tract for the achievement of full absorption. Additionally, discrepancies between *in vitro* and *in vivo* behaviour can affect predictability. Patient behaviours (such as chewing oral drugs or altering food intake) may cause dose dumping, thereby increasing the risk of toxicity. Moreover, in overdose situations, the recovery of sustained-release drugs can be particularly difficult^{7,8}.

7. Approaches to a sustained-release drug delivery system

Various advanced strategies have been developed in order to improve therapeutic performance and patient compliance. Diffusion-controlled systems regulate drug release through polymer barriers, enabling consistent and predictable delivery. Dissolution-controlled systems base release on the rate at which the drug dissolves. Combining both approaches results in hybrid systems that optimize release profiles through simultaneous dissolution and diffusion mechanisms. Furthermore, ion exchange resin-drug complexes facilitate tailored drug interactions, thereby enhancing release characteristics. Finally,

pH-dependent formulations⁹ exploit gastrointestinal pH gradients for targeted drug release, while osmotic pressure-controlled systems⁴ rely on osmotic principles in order to ensure regulated medication delivery over time.

In controlled diffusion-release systems, the diffusion of dissolved drug molecules through a polymer barrier is the rate-limiting step. As the insoluble matrix gradually depletes, the diffusion path lengthens, preventing zero-order release kinetics. Controlled-release technologies typically depend on diffusion through polymeric membranes⁶, and can achieve programmable delivery by fine-tuning formulation and processing parameters¹⁰.

8. Conclusion

Sustained-release liquid dosage forms represent a significant advancement in drug delivery, offering solutions to critical challenges related to patient compliance and therapeutic reliability. Modified-release systems, including sustained-release formulations, improve clinical outcomes by maintaining consistent drug levels in the body over extended durations, thereby minimizing dosing frequency, and reducing the risk of side effects or toxicity associated with peak plasma concentrations.

Acknowledgements

The authors would like to express their appreciation to the staff of the College of Pharmacy of the University of Babylon, represented by its Dean, Professor Dr Hussam W. Al-Humadi, for supporting this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Alsahy S., Masheta D. Sustained release of medications in liquid form: utility, advantages, and disadvantages. *Pharmakeftiki* 37(2s), 476-479, 2025. <https://doi.org/10.60988/p.v37i2S.269>

Novel therapeutic targets in the treatment of obesity: a review

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KEY WORDS:

obesity; ghrelin; gut peptides;
weight loss; appetite

ARTICLE INFO:

Received: January 10, 2025

Revised: February 07, 2025

Accepted: February 17, 2025

Available online: October 10, 2025

ABSTRACT

Obesity is a chronic condition that increases the risk of both complications and mortality. The development of effective and safe entero-pancreatic peptide-based pharmacotherapies (such as those targeting ghrelin, leptin, peptide YY, and related molecules) requires a nuanced understanding of the gut-brain axis and its role in regulating appetite and body weight. This brief review places particular emphasis on novel incretin-based agents co-formulated with glucagon and neuropeptide Y, which have demonstrated weight-reduction outcomes approaching those of bariatric surgery, yet without the invasiveness of surgical intervention. In cases where obesity-related comorbidities (such as type 2 diabetes mellitus) are present, such substantial weight loss may contribute to clinical improvement, especially given that many of these agents exert independent effects on glycaemic control. Additionally, we herein evaluate the therapeutic potential of ghrelin inhibition *via* ghrelin-O-acyltransferase blockade, concluding that this approach does not yield meaningful effects on satiety, body weight, or caloric intake. Finally, we discuss emerging compounds currently under clinical investigation that may offer future avenues for obesity treatment.

1. Introduction

Excess body fat is a defining feature of obesity, which increases the risk of multiple conditions, including dyslipidaemia, hypertension, type 2 diabetes mellitus, osteoarthritis, nonalcoholic fatty liver disease,

sleep apnoea, and infertility. Obesity may reduce life expectancy by 5 to 20 years and significantly elevate healthcare costs¹. The body mass index (BMI), calculated as weight in kilograms divided by height in meters squared, is the standard metric for assessing obesity; a BMI

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of 18.5–24.9 kg/m² is considered normal, 25.0–29.9 kg/m² is classified as overweight, and ≥30.0 kg/m² is defined as obese¹.

Obesity arises from multifactorial causes, including excessive caloric intake, genetic predisposition, insufficient sleep, sedentary lifestyle, medical conditions, pharmacotherapy, ethnic background, psychosocial stressors, socioeconomic disparities, and exposure to endocrine-disrupting chemicals². Although lifestyle interventions remain foundational in obesity management, even intensive programs typically yield only 5%–10% weight loss, complicating long-term maintenance. Approved pharmacotherapies for chronic weight management are limited by cost-effectiveness, clinical efficacy, and long-term cardiovascular safety concerns¹.

Several gastrointestinal peptides such as ghrelin, leptin, cholecystokinin (CCK), peptide YY (PYY), and neuropeptide Y (NPY) have been investigated as potential anti-obesity agents due to their roles in appetite regulation *via* the central nervous system². This review provides a brief overview of novel therapeutic targets with potential for safe and effective anti-obesity treatment.

2. Ghrelin

Ghrelin is an orexigenic hormone that stimulates appetite and promotes adiposity through mechanisms such as the activation of orexigenic neurons, the enhancement of triglyceride uptake in white adipose tissue, and increased lipogenesis³. BI1356225, a ghrelin-O-acyltransferase antagonist currently in phase 1 trials, was administered to adult subjects with a BMI of 27.0–39.9 kg/m² for 28 days at doses of 0.2, 1.0, 2.5, or 10.0 mg. It significantly reduced the acyl-to-des-acyl ghrelin ratio (>80%), although no changes in satiety, body weight, or caloric intake were observed. The findings suggest that elevated des-acyl ghrelin may contribute to weight regulation⁴.

3. PYY

PYY, secreted by intestinal endocrine cells, inhibits gastric acid secretion and gallbladder contraction.

In obese individuals, elevated PYY levels suppress ghrelin release and prolong gastrointestinal (GI) transit time⁵. PYY analogs investigated in phase 1 trials (NCT02568306 and NCT03574584), were administered to overweight / obese volunteers subcutaneously (s.c.) every 7 to 14 days. These analogs demonstrated safety and potential efficacy, with weight reductions of 2.9 and 3.6 kg at 31 days and a 38%–55% decrease in food consumption compared to placebo⁶.

4. Leptin

Leptin, a hormone correlated with BMI, is often elevated in obese individuals, leading to hypothalamic receptor saturation and impaired energy balance and satiety signalling. Hyperleptinaemia also affects GI function, reducing oesophageal motility. Animal studies show that exogenous leptin inhibits small intestinal motility and delays gastric emptying⁵. Mibavademab, a leptin analog in phase 2 trials, was administered to participants intravenously at doses of 0.3, 1, 3, 10, or 30 mg/kg or s.c. at 300 or 600 mg for 12 weeks; its impact on body weight varied depending on baseline leptin concentrations⁷.

5. NPY

NPY, a neurotransmitter secreted by the adipose tissue, is elevated in obesity and contributes to increased appetite and delayed gastric emptying⁵. Velneperit, an NPY receptor subtype 5 inhibitor, was evaluated in a phase 2a trial involving 342 obese participants. Daily doses of 1,600 mg and 400 mg resulted in weight reductions of 5.3 kg (5.6%) and 2.5 kg (2.7%), respectively, with favourable tolerability and dose-dependent efficacy⁵.

6. CCK

CCK is a gut-derived peptide that modulates appetite *via* the activation of the cholecystokinin type 1 receptor (CCK1R), making it a promising target for obesity therapy⁸. In obese Göttingen minipigs, NN9056 – a CCK1R analog – was administered once

daily s.c. at doses of 10, 20, 40, or 80 nmol/kg for 13 weeks. Food intake decreased for two days at 20 and 40 nmol/kg, and for four days at 80 nmol/kg, with concurrent reductions in body weight⁸.

7. Growth-differentiation factor 15 (GDF-15)

GDF-15, a stress-responsive cytokine secreted by adipocytes, cardiomyocytes, and macrophages, is associated with weight loss and represents a potential therapeutic target⁶. In murine models, GDF-15 enhances satiety and reduces food intake. The GDF-15 agonist LY3463251, administered to mice s.c. once weekly at doses of 1–9 mg for 12 weeks, resulted in a 3% reduction in body weight. Early-phase clinical trials also include GDF-15 agonists JNJ-9090/CIN-109 and NNC0247-0829⁶.

8. Theobromine

Theobromine exhibits anti-obesity properties *in vitro* and *in vivo* by activating brown adipose tissue, inducing browning of white adipose tissue, reducing inflammation, and mitigating oxidative stress. Administration of 200 µM theobromine to 3T3-L1 preadipocytes for 6–8 days decreased adipogenesis and enhanced lipolysis⁹.

9. Glucagon-like peptide 1 (GLP-1) and glucagon

Glucagon analogs may increase energy expenditure *via* hypoaminoacidaemia, but can also lead to lean mass loss. Glucagon improves lipid metabolism and promotes hepatic fatty acid oxidation. GLP-1 reduces appetite, delays gastric emptying, and enhances satiety. Dual agonists targeting both glucagon and GLP-1 may optimize weight loss while minimizing hyperglycaemia risk¹⁰.

Survodutide, in phase 2 trials, achieved up to 18.7% weight reduction *versus* 2% with placebo at weekly doses of 0.6–4.8 mg over 46 weeks and is progressing to phase 3. Mazdutide's phase 2 trial yielded an 11.3% weight reduction after 24 weeks at weekly doses of 3, 4.5, and 6 mg; phase 3 trials (4–9 mg) are ongoing in Chinese populations. Finally, efinopegdutide, evaluat-

ed in phase 2 trials at weekly doses of 5, 7.4, and 10 mg s.c. for 26 weeks, demonstrated weight reductions of 8.5%–11.8% *versus* 1.8%–7.5% in 295 *versus* 119 participants, respectively⁶.

10. Glucose-dependent insulinotropic polypeptide (GIP) and GLP-1

GIP, an endogenous incretin, regulates weight *via* lipid storage and metabolic activation. GIP antagonism improves metabolic parameters and suppresses food intake¹⁰. AMG133 (maridebart cafraglutide), a biphasic molecule combining GIP antagonism and GLP-1 agonism, produced a dose-dependent weight reduction of approximately 14.5% *versus* 1.5% with placebo in a phase 1 trial, after being administered s.c. every 4 weeks. AMG133 is currently undergoing a 52-week phase 2 trial in obese and overweight individuals⁶.

11. Conclusion

Obesity, a global health challenge with numerous comorbidities, necessitates innovative therapeutic strategies. Targeting appetite-regulating peptides such as ghrelin, PYY, CCK, and GDF-15 offers promising avenues. Emerging treatments (including PYY analogs, CCK-positive allosteric modulators, and ghrelin receptor modulators) may provide more effective and safer options for long-term weight management.

Acknowledgements

We gratefully acknowledge Prof. Dr Hussam Al-Humadi, Dean of the College of Pharmacy of the University of Babylon, for his encouragement.

Conflicts of interest

None exist.

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HOW TO CITE:

Alsharifi Z.H., Bash A.A. Novel therapeutic targets in the treatment of obesity: a review. *Pharmakeftiki* 37(2s), 480-483, 2025. <https://doi.org/10.60988/p.v37i2S.270>

Applications of nanoparticles in the transdermal delivery of chemotherapy against breast cancer: a mini-review

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KEY WORDS:

**application; breast cancer;
chemotherapeutics;
nanoparticles; transdermal**

ARTICLE INFO:

Received: January 24, 2025

Revised: February 21, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

ABSTRACT

Transdermal administration represents a promising drug delivery strategy due to its non-invasive nature and potential for controlled, sustained release. By circumventing hepatic first-pass metabolism, it may reduce the need for excessive dosing and enhance therapeutic efficacy. However, the transdermal delivery of chemotherapeutic agents for breast cancer remains challenging, primarily due to the poor skin permeability of most anticancer drugs, which typically exhibit high molecular weight and limited lipid solubility. This mini-review critically examines recent advances in nanoparticle-based systems designed to facilitate transdermal chemotherapy for breast cancer. We categorize the types of nanoparticles employed, discuss their mechanisms of action and therapeutic advantages, and outline future directions for research and clinical translation.

1. Introduction

Most transdermal formulations currently available utilize small molecules with a molecular weight below 500 Da and moderate lipophilicity. Transdermal drug delivery offers several advantages, including ease of administration and discontinuation, avoidance of injections and hospitalizations, and protection against drug degradation caused by

gastric pH, enzymatic activity, or hepatic metabolism¹. A strategy to enhance transdermal drug delivery involves the use of nanoparticles and their derivatives. The morphology, size, and surface characteristics of nanoparticles are key determinants of their functionalization with targeting ligands².

Recent studies into the transdermal chemotherapy for breast cancer have yielded promising outcomes,

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owing to the unique physiological architecture of the breast tissue. The presence of subcutaneous and retromammary adipose tissue, along with internal lymphatic circulation, may facilitate broader distribution of transdermally administered agents. Moreover, the concept of localized transdermal delivery suggests a reduction in systemic adverse effects and an increase in site-specific drug concentration. Such formulations may also prove beneficial in post-radiation and post-surgical contexts³.

2. Types of nanoparticles used in transdermal delivery (Table 1)

2.1. Vesicular nanocarriers

Vesicular nanocarriers (including liposomes, niosomes, transferosomes, spanlastics, and ethosomes) have emerged as preferred platforms for medicinal delivery. Numerous liposome-based pharmaceuticals are currently approved for clinical use. Liposomes have been proposed as drug delivery systems that enhance therapeutic efficacy while minimizing toxicity. Their biocompatibility and prolonged circulation half-life make them attractive candidates for cancer therapy. However, liposomes are structurally fragile; upon traversing the skin, they tend to adhere to intracellular membranes, leading to phospholipid-associated body collapse and leakage of encapsulated agents. This limits their ability to deliver active compounds to deeper skin layers⁴. Consequently, the use of flexible liposomes – also referred to as “transformable” liposomes – has become a pivotal strategy for achieving effective transdermal drug delivery⁵.

2.2. Lipid nanoparticles

Lipid nanoparticles (including solid lipid nanoparticles and nanostructured lipid carriers) are well-suited for transdermal applications. These systems can be engineered in various sizes, and their surface polarity can be modulated to enhance skin penetration. The mechanical flexibility of lipid nanoparticles enables deeper dermal penetration from the epidermal layer^{6,7}.

2.3. Polymeric nanoparticles

Polymeric nanoparticles (including polymeric micelles and dendrimers) can modulate drug activity, regulate release kinetics, and extend drug residence time within the skin. These nanoparticles serve as reservoirs for lipophilic drugs, thereby facilitating their deposition in the stratum corneum and controlling their transdermal diffusion^{8,9}.

2.4. Inorganic nanoparticles

Inorganic nanoparticles (including metallic nanoparticles, metal oxide nanoparticles, silica nanoparticles, and quantum dots) exhibit higher cytotoxicity in malignant cell lines compared to healthy cells. Proposed mechanisms of cytotoxicity include reactive oxygen species generation, mitochondrial membrane permeabilization, and targeted DNA cleavage, culminating in cancer cell death *via* necrosis, autophagy, or apoptosis¹⁰. Chemotherapeutic formulations incorporating these nanoparticles can be topically applied using appropriate excipients and techniques in order to enhance skin permeability.

3. Mechanism of transdermal delivery

Nanoparticles are engineered in order to improve drug penetration through the skin *via* mechanisms such as passive diffusion, follicular transport, and active targeting. Key strategies include: (i) microneedle arrays (which create microchannels in the stratum corneum so as to facilitate deeper nanoparticle penetration), (ii) chemical penetration enhancers (such as surfactants and fatty acids, which disrupt the skin barrier in order to promote absorption), and (iii) sonophoresis and electroporation (which employ ultrasound or electrical pulses in order to transiently increase skin permeability and enhance nanoparticle delivery)³.

4. Advantages of nanoparticle-based transdermal delivery

The benefits of nanoparticle-mediated transdermal delivery include: (i) enhanced permeability (as nano-

Table 1. Examples of studies on nanoparticle-based transdermal chemotherapeutic systems for the treatment of breast cancer.			
Nanoparticle type	Chemotherapeutic agent	Mechanism / features	Reference
Spanlastics	Letrozole and quercetin	Enhanced skin permeation and sustained drug release; decreased drug resistance	Mekkawy <i>et al.</i> ⁵
Solid lipid nanoparticles	Paclitaxel	Enhanced transdermal delivery of paclitaxel, improving drug stability and controlled release through the skin	Jiang <i>et al.</i> ⁶
Nanostructured lipid carriers	Tamoxifen	Improved transdermal permeation of tamoxifen, providing a controlled release system for breast cancer treatment	Sani <i>et al.</i> ⁷
Polymeric nanoparticles	5-Fluorouracil	Increased skin permeation of 5-fluorouracil, allowing for sustained and controlled drug release in breast cancer therapy	Dongsar <i>et al.</i> ⁸
Polymeric nanoparticles	Anastrozole	Facilitated transdermal delivery and controlled release through the skin	Altameemi and Abd-Alhammid ⁹
Gold nanoparticles	Methotrexate	Enhanced transdermal delivery of methotrexate, increasing drug penetration and accumulation in breast cancer cells	Mukhtar <i>et al.</i> ¹⁰
Superparamagnetic iron oxide nanoparticles	Doxorubicin	Facilitated transdermal delivery of doxorubicin, enhancing skin penetration and targeting breast cancer cells	Jiang <i>et al.</i> ⁶

particles improve drug solubility and diffusion across the skin), (ii) sustained release (enabling controlled drug delivery and reduced systemic toxicity), (iii) targeted therapy (wherein functionalized nanoparticles bearing ligands or antibodies selectively accumulate in cancerous tissues *via* active and passive targeting mechanisms), (iv) reduced side effects (due to the avoidance of hepatic first-pass metabolism), and (v) non-invasive administration (which improves patient compliance relative to intravenous chemotherapy)⁶.

5. Limitations and challenges

Despite its promise, nanoparticle-based transdermal drug delivery faces several obstacles⁴: (i) the skin's barrier function, particularly the stratum corneum, limits drug permeability, (ii) formulation stability, as maintaining nanoparticle integrity within transdermal systems is essential, (iii) scalability and cost, with manufacturing and commercialization presenting significant challenges, and (iv) long-term safety, as potential toxicity and immunogenicity require further investigation.

6. Anti-cancer properties of nanoparticles: recent innovations

Nanoparticles offer site-specific drug delivery capabilities, and recent innovations include³: (i) hybrid nanoparticles, which integrate multiple therapeutic functionalities into a single platform, (ii) smart response systems, which release drugs in response to biological stimuli for precise dosing, and (iii) enhanced imaging modalities, wherein nanoparticles serve as contrast agents to improve cancer diagnosis and treatment monitoring.

7. Future perspectives

Ongoing research aims to optimize nanoparticle formulations for improved efficacy and reduced limitations. Emerging modalities (such as microneedle-assisted delivery and bio-responsive nanocarriers) show potential in overcoming the skin's barrier properties. Further clinical trials are essential in order to be able to translate these advances into practical therapeutic solutions.

8. Conclusion

Nanoparticles represent a promising modality for

transdermal chemotherapy in breast cancer, offering improved therapeutic outcomes and reduced systemic toxicity. Nonetheless, comprehensive research is needed in order to address current limitations and ensure a safe and effective clinical implementation.

Acknowledgements

The authors gratefully acknowledge the Department of Pharmaceutics of the College of Pharmacy of the Uni-

versity of Baghdad.

Conflicts of interest

None exist.

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HOW TO CITE:

Alwan L.A., Abd Alhammid S.N. Applications of nanoparticles in the transdermal delivery of chemotherapy against breast cancer: a mini-review. *Pharmakeftiki* 37(2s), 484-487, 2025. <https://doi.org/10.60988/p.v37i2S.271>

Micro- and nano-emulsion drug delivery systems: a review

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KEY WORDS:

**emulsion; micro-emulsion;
nano-emulsion; drug delivery
system; dispersion**

ARTICLE INFO:

Received: January 07, 2025

Revised: February 02, 2025

Accepted: February 13, 2025

Available online: October 10, 2025

ABSTRACT

Interest in micro- and nano-emulsions has steadily grown over recent decades, owing to their distinctive physicochemical properties and expanding utility in the medical field. These nano-scaled formulations – typically ranging from tens to several hundreds of nanometres – offer considerable potential for pharmaceutical applications. This review provides a structured overview of recent advances in micro- and nano-emulsion-based drug delivery systems. It begins by defining emulsions and their classifications, then delineates the distinctions between conventional emulsions and their micro/nano counterparts, with particular emphasis on the differences between micro- and nano-emulsions. The review further examines the principal advantages and limitations of these systems relative to other pharmaceutical formulations. Finally, it highlights their emerging roles in targeted drug delivery, underscoring their promise as versatile platforms for tissue-specific therapeutic interventions.

1. Introduction

Emulsions are defined as mixtures of two immiscible liquids, wherein spherical droplets constitute the dispersed phase and the surrounding liquid forms the continuous phase¹. Notable advancements have emerged in nanotechnology, particularly in the development of particles smaller than 1 µm. Nanoparticles (comparable in size to cellular organelles)

have garnered significant attention². Micro- and nano-emulsions are liquid-based formulations that offer considerable promise for enhancing drug bioavailability across diverse therapeutic domains. Their ability to improve solubility, stability, and targeted delivery renders them valuable tools in the design of novel pharmaceutical products³.

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2. Emulsion

An emulsion is a biphasic system in which one liquid phase is dispersed as fine droplets within another immiscible liquid phase¹. These droplets are typically invisible to the naked eye, and emulsions are often classified as colloidal systems. As the droplet size decreases, the emulsion's appearance transitions from milky white to translucent². Although thermodynamically unstable, emulsions can be stabilized by emulsifying agents. The dispersed phase – also termed the “internal” or “discontinuous” phase – is suspended within the dispersion medium, known as the “external” or “continuous” phase. The emulsifying agent occupies the interfacial region and is referred to as the “intermediate” phase or simply “interphase”¹.

3. Micro- and nano-emulsion

The conceptual origins of nano-emulsions trace back to early 20th-century studies into colloidal systems. Initial research focused on macro- and micro-emulsions, laying the groundwork for the nano-emulsion development³. Micro- and nano-emulsions are nanometric dispersions comprising an oil phase, a water phase, and surfactants. Despite their compositional similarities, they exhibit distinct physicochemical properties that facilitate differentiation and classification⁴.

These systems typically contain droplets smaller than 1 μm . Micro-emulsions are thermodynamically stable dispersions with droplet sizes ranging from 100 to 400 nm, whereas nano-emulsions are thermodynamically unstable but kinetically stable, with droplet sizes between 1 and 100 nm. Nano-emulsions often require cosurfactants to overcome their elevated free energy and achieve stability². Unlike micro-emulsions, nano-emulsions maintain prolonged physical consistency and resist aggregation and flocculation³.

Nano-emulsion technology has found applications in various sectors, including food science, biomedicine, and cosmetics. In skin bioengineering, nano-emulsions have emerged as effective carriers

for therapeutic agents and cosmetic compounds, addressing challenges related to penetration and absorption².

4. Differences between micro- and nano-emulsions

Although micro- and nano-emulsions share similar components, they differ significantly in droplet size and thermodynamic behavior. As already stated above, micro-emulsions typically exhibit droplet sizes of 100–400 nm, while nano-emulsions range from 1 to 100 nm². The critical differentiator is system free energy, which influences both formulation and stability. Micro-emulsions are thermodynamically stable and can form spontaneously; however, external energy inputs – such as heating or magnetic stirring – are often employed in order to facilitate the mixing of water, oil, and surfactant. In contrast, nano-emulsions are energetically unfavourable and require substantial external energy so as to overcome the free energy barrier between immiscible phases and achieve colloidal dispersion. This disparity in free energy also impacts long-term stability⁴.

5. Advantage and disadvantage of micro- and nano-emulsions

Micro- and nano-emulsions offer several advantages over conventional drug delivery systems. They enhance the bioavailability of hydrophobic pharmaceuticals¹, protect active compounds from degradation, and extend the shelf life of medications³. Their ability to safeguard drugs from oxidation and hydrolysis contributes to formulation stability, while their liquid dosage form improves patient adherence⁵. These systems are relatively easy to prepare and exhibit notable physicochemical stability⁶. Owing to their non-toxic and non-irritating nature, they are suitable for application to mucous membranes and skin⁷. Additionally, they can mask metallic and acrid flavours³, and are amenable to oral administration when formulated with biocompatible surfactants. Micro- and nano-emulsions also serve as precursors in the fabrication of nanocapsules and nanospheres, thereby

facilitating efficient drug transport⁷. Their versatility allows for formulation into various dosage forms (including foams, creams, liquids, and sprays)⁷, and they are applicable in both veterinary and human medicine⁷. Importantly, these systems can help circumvent hepatic first-pass metabolism, thereby improving systemic drug availability³.

Despite these benefits, micro- and nano-emulsions present certain limitations. The production of small droplets necessitates specialized equipment and techniques, often involving costly procedures. The configuration of homogenizers – essential tools in emulsion formulation – is expensive, and production methods such as ultrasonication and microfluidization require substantial financial investment. Storage stability remains a challenge, and the high concentration of emulsifiers required for cosmetic applications further escalates production costs.

6. Applications

Micro- and nano-emulsions can be economically formulated into various pharmaceutical dosage forms, including creams, gels, foams, sprays, and aerosols. They support multiple routes of administration: oral, topical, intravenous, intrapulmonary, transnasal, and intraocular. Nano-emulsions are particularly effective in enhancing bioavailability for a range of therapeutic indications³.

Micro-emulsions have demonstrated utility across diverse fields, including food science, coatings, lubricants, antimicrobial agents, drug delivery, nanoparticle synthesis, detergents, pesticides, biotechnology, and extraction processes. They also show promise in clinical diagnostics and disease management⁶.

Numerous nano-emulsion-based drug delivery systems have been developed for medications such as anticancer, antimalarial, antipsychotic, antiglau-

coma, and lipid-lowering agents⁷. For instance, nano-emulsion formulations of flurbiprofen (a poorly water-soluble drug) have improved its dissolution, solubility, stability, and bioavailability⁵. Similarly, nano-emulsions of piroxicam have enhanced its stability, release profile, and analgesic efficacy⁸. Mupirocin, a topical antibacterial agent limited by *in vivo* inactivation and plasma protein binding, has shown improved skin delivery when formulated as a nano-emulsion⁹. Finally, among the available nano-emulsion administration routes, topical and ocular delivery are gaining particular interest, alongside oral, nasal, and intravenous options¹⁰.

7. Conclusion

Micro- and nano-emulsions exhibit superior pharmacokinetic profiles and targeting capabilities compared to conventional emulsions and other dosage forms. Their nanometric scale and favourable physicochemical properties support their expanding role in pharmaceutical applications, positioning them as promising platforms for advanced drug delivery.

Acknowledgements

We gratefully acknowledge the College of Pharmacy of the University of Babylon, and its Dean, Professor Dr Hussam W. Al-Humadi, for their support and for providing the opportunity to pursue this work.

Conflicts of interest

None exist.

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HOW TO CITE:

Alzubaidy S.H., Al-Azzawi S. Micro- and nano-emulsion drug delivery systems: a review. *Pharmakeftiki* 37(2s), 488-491, 2025. <https://doi.org/10.60988/p.v37i2S.272>

Challenges in the modelling of the pharmacokinetics and pharmacodynamics of an anti-cancer drug combination on urinary bladder cancer T24 cells: a technical review

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KEY WORDS:

transitional cell carcinoma of the bladder; T24 cell line; *in vitro* pharmacokinetics / pharmacodynamics model; drug combination; chemotherapy

ARTICLE INFO:

Received: January 31, 2025

Revised: February 13, 2025

Accepted: February 19, 2025

Available online: October 10, 2025

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ABSTRACT

The development of novel anticancer therapies requires rigorous preclinical evaluation in order to ensure both efficacy and safety prior to clinical translation. The T24 cell line, derived from human transitional bladder carcinoma, is a widely employed *in vitro* model owing to its aggressive phenotype and strong relevance to human disease pathology. It offers a valuable platform for investigating bladder cancer progression, drug responsiveness, and mechanisms of resistance. However, replicating the complex tumour microenvironment and accurately predicting *in vivo* therapeutic outcomes still pose significant challenges *in vitro*. The pharmacokinetics (PK) and pharmacodynamics (PD) of candidate agents must be carefully assessed, as these parameters often diverge markedly between *in vitro* and *in vivo* contexts. *In vitro* PK/PD modelling – linking drug concentration to biological effect – facilitates dose optimization, particularly when integrated with pulsatile drug administration and advanced methodologies that simulate the three-dimensional tumour microenvironment. Such approaches enhance the translational relevance of bladder cancer research and may improve therapeutic outcomes. This review underscores the critical importance of optimizing the experimental design of *in vitro* PK/PD models in an attempt to address prevailing limitations and advance bladder cancer therapy.

1. Introduction

Bladder cancer is among the most prevalent urological malignancies, marked by high recurrence rates and considerable morbidity, particularly in advanced stages. The T24 bladder cancer cell line, derived from a high-grade transitional cell carcinoma, is a widely used *in vitro* model for investigating tumour biology, mechanisms of drug resistance, and therapeutic responses¹. Owing to its aggressive phenotype, it serves as a valuable platform for evaluating the efficacy of novel anticancer agents, including chemotherapeutics, targeted therapies, and combination regimens². However, preclinical assessments using the T24 cell line present distinct challenges, especially in the integration of pharmacokinetics (PK) and pharmacodynamics (PD), both of which are essential for predicting clinical outcomes¹.

In vitro PK/PD modelling seeks to establish a quantitative relationship between drug concentration and biological effect, thereby supporting dose optimization and refinement of therapeutic strategies. Nonetheless, the application of conventional *in vitro* models is inherently limited. A major constraint is the inability of traditional two-dimensional (2D) monolayer cultures to replicate the structural and functional complexity of the three-dimensional (3D) tumour microenvironment (TME), which critically influences drug penetration, cell-cell interactions, and resistance mechanisms³. Moreover, standard *in vitro* systems lack dynamic physiological conditions such as blood flow, immune surveillance, and metabolic clearance, thereby impeding the translation of preclinical findings to clinical settings.

In an attempt to enhance the predictive value of *in vitro* PK/PD studies involving the T24 cell line, researchers must adopt innovative approaches that address these limitations¹. Optimizing drug exposure conditions – such as pulsatile rather than continuous administration – alongside refined dose-response analyses and combination therapy evaluations, can yield a more comprehensive understanding of drug action and resistance. By closely mimicking the *in vivo* tumour environment through advanced experimental methodologies, *in vitro* PK/PD modelling can accelerate the development of more effective bladder cancer

therapies, ultimately improving patient survival and quality of life⁴. This review underscores the importance of optimizing the experimental design of *in vitro* PK/PD models in order to overcome prevailing therapeutic challenges.

2. Replicating the TME *in vitro*

The TME plays a central role in cancer progression, therapeutic resistance, and drug responsiveness. It comprises a dynamic and heterogeneous network of cellular and extracellular components, including fibroblasts, immune cells, endothelial cells, and matrix proteins. Conventional 2D cell cultures fail to replicate the structural and functional intricacies of the TME. To address these limitations, 3D cell culture models have emerged as superior *in vitro* systems that more accurately simulate the *in vivo* tumour context. For example, co-culturing T24 bladder cancer cells with fibroblasts enables detailed investigation of stromal-mediated drug resistance, particularly through monitoring transforming growth factor-beta (TGF- β) and hepatocyte growth factor signalling pathways⁵.

In vitro PK/PD modelling remains a cornerstone of preclinical drug development, aiming to quantify the temporal relationship between drug concentration and biological effect. *In vivo*, drug behaviour is modulated by physiological factors such as perfusion, tissue permeability, enzymatic metabolism, and clearance; features that are difficult to replicate under static *in vitro* conditions. Nevertheless, *in vitro* PK/PD systems have been engineered to simulate these pharmacokinetic processes, allowing for controlled assessment of drug exposure and efficacy⁶.

The integration of 3D culture platforms with PK/PD modelling offers a more physiologically relevant framework for evaluating drug combinations and optimizing treatment protocols. By refining both 3D culture techniques and *in vitro* PK/PD methodologies, researchers can bridge the translational gap between preclinical testing and clinical application, thereby advancing personalized therapeutic strategies for bladder cancer⁷ (Table 1).

3. Interpretation of *in vitro* PK/PD data regarding drug combination

Table 1. Key studies that (directly or indirectly) inform our understanding of the challenges and advances in the <i>in vitro</i> pharmacokinetics / pharmacodynamics (PK/PD) modelling for bladder cancer using the T24 cell line.		
Aspect	Details	Reference
Preclinical evaluation for anticancer therapies	Using the T24 cell line (derived from human transitional bladder carcinoma); strong relevance to human disease pathology; predicting <i>in vivo</i> therapeutic responses	Pinto-Leite <i>et al.</i> (2009) ¹
Limitations of conventional 2D models	Employing a 3D tumor microenvironment, leading to good predicting of <i>in vitro</i> drug responses (cell–cell interactions)	Barbosa <i>et al.</i> (2021) ²
<i>In vitro</i> PK/PD model development	<i>In vitro</i> PK/PD model development achieves high accuracy of drug effects	Jihad <i>et al.</i> (2024) ⁴
Cancer-associated fibroblasts in bladder cancer	The use of fibroblasts with the T24 cell line provides deeper insights for the study of drug resistance	Caramelo <i>et al.</i> (2023) ⁵
<i>In vitro</i> PK/PD modelling	The employed <i>in vitro</i> PK/PD modelling sheds light on the relationship between drug concentration and biological effects over time, thereby aiding dose optimization	Abdalwahd <i>et al.</i> (2024) ⁶
Bridge between <i>in vitro</i> and <i>in vivo</i> models by 3D cell cultures	Integration of a 3D culture with an <i>in vitro</i> PK/PD model gives good interpretation between <i>in vitro</i> and clinical data	Urzi <i>et al.</i> (2023) ⁷
Intra-tumoral heterogeneity of bladder cancer	Intra- and inter-tumoral heterogeneity might complicate the evaluation of anti-cancer therapies	Warrick <i>et al.</i> (2019) ⁸
Quantitative systems to predict <i>in vivo</i> efficacy from <i>in vitro</i> data	<i>In vitro</i> PK/PD model drug levels fluctuate with time, leading to well estimation of drug efficacy (simulating time-dependent drug exposure)	Bouhaddou <i>et al.</i> (2020) ⁹
Mechanisms and challenges of cancer drug resistance	Drug resistance is a major obstacle in cancer treatment, often arising from genetic mutations, epigenetic changes, or adaptive responses	Khan <i>et al.</i> (2024) ¹⁰

Bladder cancer exhibits pronounced intra- and inter-tumoral heterogeneity, complicating the evaluation of anticancer therapies. Although the T24 cell line is a valuable model, it represents only a single subtype of bladder cancer and may not fully capture the disease's biological diversity. Interpreting *in vitro* PK/PD data in the context of *in vivo* and clinical settings remains a significant challenge⁸. Conventional *in vitro* models often employ static drug concentrations, whereas both *in vivo* and advanced *in vitro* PK/PD systems simulate fluctuating drug levels over time; an approach that more accurately reflects clinical pharmacodynamics and improves the estimation of therapeutic efficacy.

By simulating time-dependent drug exposure in T24 cells, one can yield more reliable predictions of treatment outcomes, particularly for combination chemotherapy regimens⁹. Furthermore, drug re-

sistance remains a major barrier to effective cancer therapy, frequently arising from genetic mutations, epigenetic alterations, or adaptive responses within the TME. Long-term exposure of T24 cells to sub-lethal drug concentrations can induce resistance, thereby enabling the study of underlying mechanisms and the evaluation of strategies to overcome them¹⁰ (Table 1).

4. Conclusion

The evaluation of novel anticancer combinations using *in vitro* PK/PD modelling in the T24 bladder cancer cell line holds considerable promise, yet is accompanied by methodological and translational challenges. The standardization of these models, their integration into drug development pipelines, and the validation

of their clinical relevance are essential next steps. By addressing these issues, researchers can enhance the interpretability of *in vitro* PK/PD data in relation to *in vivo* and clinical studies, thereby accelerating the development of effective bladder cancer therapies.

Acknowledgements

We sincerely thank Prof. Dr Rafal J. Al-Saigh of the College of Pharmacy of the University of Babylon, for her

invaluable support and assistance.

Conflicts of interest

None exist.

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HOW TO CITE:

Atshan S.H., Al-Humadi H.W. Challenges in the modelling of the pharmacokinetics and pharmacodynamics of an anti-cancer drug combination on urinary bladder cancer T24 cells: a technical review. *Pharmakeftiki* 37(2s), 492-495, 2025. <https://doi.org/10.60988/p.v37i2S.273>

Physical characteristics of novel nanomagnetic radiopharmaceuticals: a review

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KEY WORDS:
nanomagnetic
radiopharmaceuticals; drugs;
cancer; nuclear medicine;
nanoparticles

ARTICLE INFO:

Received: December 27, 2024

Revised: January 06, 2025

Accepted: January 29, 2025

Available online: October 10, 2025

ABSTRACT

Hybrid nanomagnetic radiopharmaceuticals represent a frontier in targeted therapy and diagnostic imaging. These compounds synergistically combine magnetic nanoparticles with radiopharmaceutical agents in order to enhance the precision, delivery, and therapeutic efficacy of nuclear medicine interventions. This review summarizes their physicochemical properties, presents their mechanisms of action, and discusses their translational potential across a range of clinical applications.

1. Introduction

Hybrid nanomagnetic radiopharmaceuticals integrate nanotechnology with radiolabelled compounds in order to enable targeted therapies, particularly for oncological applications. These agents employ externally guided magnetic nanoparticles (MNPs) for site-specific drug delivery, and incorporate radioisotopes for both therapeutic irradiation and diagnostic imaging. Their nanoscale dimensions enhance biological interactions and biodistribution, thereby establishing a theranostic paradigm essential for personalized medicine and early disease detection¹.

By expanding the diagnostic and therapeutic capabilities of nuclear medicine, hybrid nanomagnetic radiopharmaceuticals offer precise targeting of malignant tissues while minimizing collateral damage to healthy cells. The integration of MNPs with radiopharmaceuticals facilitates simultaneous diagnosis and treatment, thereby advancing individualized therapeutic strategies. Moreover, their compatibility with imaging modalities such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) strengthens tumor visualization and monitoring, which is pivotal for future innovations in nuclear medicine^{2,3}.

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2. Nanoparticles in radiopharmaceuticals

Nanoparticles (NPs) are central to the development of hybrid nanomagnetic radiopharmaceuticals, serving both therapeutic and diagnostic functions. Their unique physicochemical properties (particularly size and magnetism) enhance targeted drug delivery, imaging resolution, and therapeutic efficacy, thereby supporting precision medicine. Radiopharmaceuticals, which contain radionuclides, function by emitting detectable gamma rays or particulate radiation for diagnostic or therapeutic purposes. Common diagnostic radionuclides include technetium-99m and gallium-68, typically used in conjunction with PET and SPECT imaging. Therapeutic radionuclides such as iodine-131 deliver localized radiation for cancer treatment. The selection of radionuclides must consider energy emission profiles and half-lives so as to optimize safety and clinical effectiveness^{1,4}.

3. Mechanisms of targeted delivery

Targeted delivery mechanisms in hybrid nanomagnetic radiopharmaceuticals include both passive and active strategies. Passive targeting exploits the enhanced permeability and retention effect, whereby NPs preferentially accumulate in tumor tissues due to aberrant vascular architecture⁴. Active targeting involves functionalizing NPs with ligands or biomolecules that selectively bind to overexpressed receptors on diseased cells, thereby increasing binding specificity and promoting cellular internalization⁵. Advanced imaging techniques facilitate real-time assessment of these delivery mechanisms, thereby enabling visualization of radiopharmaceutical distribution and accumulation. The combined use of passive and active targeting may enhance therapeutic efficacy while minimizing systemic toxicity by concentrating therapeutic agents at pathological sites³.

4. Potential applications in therapy and imaging

Hybrid nanomagnetic radiopharmaceuticals offer transformative potential in oncology by merging targeted therapy with advanced imaging capabili-

ties. The advent of theranostic agents (i.e., NPs engineered for both imaging and treatment) permits real-time monitoring of drug biodistribution and therapeutic response *via* modalities such as magnetic resonance imaging (MRI) and PET⁶. This dual functionality supports the development of personalized treatment regimens tailored to the molecular and anatomical characteristics of individual tumours, thereby improving clinical outcomes⁷.

5. Enhanced efficacy and precision

These hybrid agents are redefining diagnostic imaging by improving the sensitivity and specificity of cancer detection. PET utilizes radiolabelled ligands in order to identify tumor-specific biomarkers, enabling precise localization and informed therapeutic decisions⁸. Concurrently, MRI benefits from hybrid NPs serving as contrast enhancers, which improve image resolution and delineation of tumor margins and adjacent tissues⁷⁻¹⁰. The incorporation of multifunctional NPs into imaging platforms allows for the integration of diverse modalities, thereby enhancing diagnostic accuracy and clinical utility.

6. Reduced side-effects and toxicity profiles

Hybrid nanomagnetic radiopharmaceuticals contribute to safer cancer therapies by localizing drug activity at tumor sites and minimizing exposure to non-target tissues. This targeted approach reduces systemic toxicity and permits lower therapeutic dosages¹⁰. MNPs also stabilize therapeutic agents against degradation and sustain drug release over extended periods. Surface modifications of NPs improve biocompatibility, attenuate immunogenic responses, and enhance cellular uptake, collectively reducing adverse effects and improving patient tolerability in radiopharmaceutical regimens.

7. Challenges and limitations

Despite their promise, hybrid nanomagnetic radiopharmaceuticals face significant regulatory and manufacturing challenges. Regulatory agen-

Table 1. Examples of novel hybrid nanomagnetic radiopharmaceutical drugs, including their key components, properties, applications, and clinical significance.					
Hybrid nanomagnetic radiopharmaceutical	Nanoparticle type	Radioisotope	Targeting ligand	Application	Clinical significance
Iron oxide nanoparticles + iodine-131	Iron oxide (Fe ₃ O ₄)	Iodine-131	Antibodies / peptides	Treatment of thyroid cancers	Selective targeting of thyroid tissues for therapy
Gold nanoparticles + technetium-99m	Gold nanoparticles	Technetium-99m	Folate-targeted ligands	Imaging of tumors	Improved imaging contrast for cancer detection
Silica nanoparticles + lutetium-177	Silica nanoparticles	Lutetium-177	Peptide ligands	Targeted radiation therapy	Effective treatment for neuroendocrine tumors
Magnetic hybrid nanoparticles + gallium-68	Magnetic iron oxide	Gallium-68	Monoclonal antibodies	PET imaging of cancer	Enhanced specificity for tumor localization
Polymeric nanoparticles + samarium-153	Polymeric nanospheres	Samarium-153	Tumor-specific peptides	Palliative treatment for bone pain	Pain relief from bone metastases
Liposomal nanoparticles + radium-223	Lipid-based nanocarriers	Radium-223	None or amino acid ligands	Treatment of metastatic prostate cancer	Targeted radiation therapy with minimal side effects

cies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) impose stringent requirements for complex nanotheranostic systems. Ambiguities in regulatory frameworks – particularly concerning the intersection of chemical and nanotechnological domains – necessitate comprehensive characterization data, which can delay clinical translation⁹. The rapid evolution of nanotechnology may outpace existing regulatory mechanisms, complicating the assessment and approval of novel agents. Nonetheless, regulatory oversight remains essential for ensuring patient safety. Personalized radiopharmaceuticals, enabled by MRI and PET monitoring, offer tailored therapeutic options, but their reproducible manufacture is technically demanding. Each production step must be tightly controlled to prevent variability that could compromise efficacy or safety. Scalable production methods, including emerging technologies such as three-dimensional (3D) printing, require further refinement in order to ensure batch consistency.

8. Future perspectives and innovations in the field

Advances in nanomedicine are reshaping disease diagnosis, monitoring, and treatment. Multifunctional NPs (termed “nanotheranostics”) enable simultaneous imaging and therapy, enhancing medical precision through integration with established modalities such as PET and MRI. Immunofluorescent sensors, functioning as biosensors, facilitate early disease detection. The convergence of artificial intelligence and machine learning with precision medicine accelerates diagnostic workflows and supports the development of individualized treatment strategies⁷⁻⁹.

9. Conclusion

Hybrid nanomagnetic radiopharmaceuticals are catalysing significant progress in medical imaging and cancer therapy. Their unique magnetic and radiolabelling properties enhance diagnostic accuracy and therapeutic precision. The fusion of nanotechnolo-

gy with radiopharmaceutical science underpins the evolution of personalized medicine, offering novel avenues for patient-centered care.

Acknowledgements

This work was supported by the College of Pharmacy of the University of Babylon. The authors gratefully acknowledge Professor Hussam W. Al-Humadi for his assistance in revising the manuscript.

Conflicts of interest

None exist.

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HOW TO CITE:

Aysa N.H., Aziz S.W., Saleh R.H., Hamza L.F., Al-Assaly R. Physical characteristics of novel nanomagnetic radiopharmaceuticals: a review. *Pharmakeftiki* 37(2s), 496-499, 2025. <https://doi.org/10.60988/p.v37i2S.274>

Proteolysis-targeting chimeras (PROTACs): a review of their development and potential pharmacological applications

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KEY WORDS:

**PROTAC; protein of interest;
small molecule inhibitor;
drug discovery; therapeutic
applications**

ARTICLE INFO:

Received: January 11, 2025

Revised: February 24, 2025

Accepted: February 25, 2025

Available online: October 10, 2025

ABSTRACT

Beyond conventional small-molecule inhibitors, proteolysis-targeting chimeras (PROTACs) represent a novel therapeutic strategy for targeted protein degradation. These heterobifunctional molecules are engineered in order to recruit an E3 ubiquitin ligase to a specific protein of interest, thereby facilitating its ubiquitination and subsequent degradation by the proteasome. This approach has garnered significant attention due to its ability to target proteins traditionally considered “undruggable”, including those lacking enzymatic activity or accessible binding pockets. This mini-review outlines the mechanistic basis of PROTACs, key design principles, and recent advances in the field. Particular emphasis is placed on the potential of PROTACs to address persistent challenges in drug discovery, notably the need for selective elimination of pathogenic proteins. The review also explores strategies for optimizing PROTAC-based therapies and discusses current limitations, such as pharmacokinetic constraints and the need for improved E3 ligase engagement. Owing to their distinctive mode of action and expanding therapeutic scope, PROTACs hold promise for transforming the treatment landscape of cancer, neurological disorders, and other diseases driven by aberrant protein expression.

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1. Introduction

Proteolysis-targeting chimeras (PROTACs) represent a novel therapeutic modality that harnesses

the ubiquitin–proteasome system (UPS) in order to selectively mark intracellular proteins for degradation. Physiologically, the UPS eliminates misfolded, mutated, or other-

wise deleterious proteins from the cellular environment; PROTACs exploit this endogenous degradation machinery so as to direct the removal of specific proteins of interest¹.

Compared to conventional small-molecule inhibitors (SMIs), PROTACs offer several advantages. Firstly, PROTACs can induce the degradation of a wide array of target proteins, often requiring lower concentrations than SMIs to achieve therapeutic efficacy, thereby potentially reducing SMI-associated toxicity. Secondly, PROTACs can engage proteins traditionally considered “undruggable”, such as those lacking enzymatic activity or accessible binding pockets. Thirdly, PROTAC-based therapies may overcome drug resistance arising from mutations in the target protein. Fourthly, PROTACs can counteract SMI resistance due to target upregulation by promoting auto-degradation of the target protein itself². Finally, PROTACs may enhance drug selectivity and specificity; for instance, non-selective inhibitors have been converted into selective degraders through PROTAC-mediated targeting, offering promising avenues for the rational design of selective SMIs³.

The therapeutic potential of PROTACs is particularly compelling in the context of neurodegenerative diseases, where protein-specific degradation is critical. Aging is a major risk factor for these disorders, with Alzheimer disease being the most prevalent. In this condition, aggregation of tau and β -amyloid proteins initiates and sustains neurodegeneration. Parkinson disease, another common neurodegenerative disorder, is associated with the progressive loss of dopaminergic neurons. Huntington disease, although less frequent, belongs to a group of genetically determined disorders characterized by irreversible deposition of misfolded huntingtin protein⁴.

Importantly, tumour development is often accompanied by aberrant protein synthesis, providing a rationale for PROTAC-based interventions. Current targets include tumour expansion factors, which can be effectively inhibited *via* PROTAC-mediated degradation. Oncogenes and tumour suppressor genes also represent viable targets, where selective protein degradation may significantly impede tumour progression⁵.

2. Strategies of developing PROTACs

The first PROTAC prototype, Protac-1, was introduced in 2001. This molecule validated the concept that engineered compounds could selectively bind and degrade cellular proteins *via* the UPS. Protac-1 was designed to target methionyl aminopeptidase 2; a protein implicated in angiogenesis and various pathophysiological processes, including oncogenesis. In 2003, the same research group developed a PROTAC incorporating oestradiol, an oestrogen derivative, to induce the degradation of oestrogen receptor alpha, which promotes the growth of certain breast carcinoma cells upon oestrogen activation. They also constructed a PROTAC containing dihydrotestosterone in order to target the androgen receptor, which is known to drive proliferation in prostate cancer cell lines following androgen stimulation.

A critical consideration in PROTAC design is the linker connecting the ligand for the protein of interest and the ligand for the E3 ubiquitin ligase. Both linker length and composition are pivotal for ternary complex formation. If the linker is too short, steric hindrance may prevent simultaneous binding to both targets. Conversely, an excessively long linker may impair the spatial proximity required for efficient ubiquitination. Current structural optimization efforts focus on the structure-activity relationship analyses across various linker lengths, typically beginning with longer variants and systematically shortening them until activity is lost⁶.

Traditional small-molecule PROTACs often exhibit poor pharmacokinetic profiles and lack cell or tissue selectivity, potentially leading to systemic toxicity. Recent advances in ligand modification have yielded tumour-targeted PROTACs, including conjugates with folate, antibodies, and aptamers. These modified PROTACs demonstrate enhanced tumour accumulation, improved antitumor efficacy *in vivo*, and increased cellular uptake.

2.1. Aptamer-based PROTACs

Aptamers are single-stranded DNA or RNA oligonucleotides comprising fewer than 100 nucleotides.

They exhibit high affinity and specificity, low toxicity, superior tissue penetration, ease of chemical synthesis, and notable stability, making them attractive candidates for targeted PROTAC delivery.

2.2. Pre-PROTACs

Pre-PROTACs are pharmacologically redesigned agents that become activated upon specific molecular triggers within designated signalling pathways. These constructs aim at reducing systemic toxicity while preserving therapeutic potency. Reactive oxygen species play a key role in modulating the activation cascades of these compounds⁷.

2.3. Reactive-triggered (RT)-PROTACs

RT-PROTACs are designed to mitigate systemic toxicity associated with conventional PROTACs, by minimizing off-target protein degradation⁸.

2.4. POLY-PROTACs

POLY-PROTAC 13 comprises a ligand for von Hippel-Lindau and a ligand for bromodomain-containing protein 4, connected *via* tunable linkers. Its advantages include: (i) activation by multiple stimuli, leading to enhanced tumour accumulation, improved

protein degradation, and deeper tumour penetration; (ii) tumour-specific delivery; and (iii) compatibility with photodynamic therapy⁹.

3. Conclusion

PROTACs have emerged as a transformative platform within the UPS framework, offering unprecedented precision in protein-selective targeting. Their capacity to dismantle neoplastic processes, circumvent resistance mechanisms, and enhance therapeutic efficacy and specificity positions them as a promising frontier in drug discovery.

Acknowledgements

We sincerely thank the Deanship of Scientific Research at the College of Pharmacy of the University of Babylon.

Conflicts of interest

None exist.

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HOW TO CITE:

Chfat K.N., Abdul Hussein S.A. Proteolysis-targeting chimeras (PROTACs): a review of their development and potential pharmacological applications. *Pharmakeftiki* 37(2s), 500-503, 2025.
<https://doi.org/10.60988/p.v37i2S.275>

Recent treatment technologies for Alzheimer disease: a review

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KEY WORDS:

**Alzheimer disease;
nanotechnology; gene-
editing; therapeutic
techniques; monoclonal
antibody therapies**

ARTICLE INFO:

Received: January 08, 2025

Revised: February 19, 2025

Accepted: February 20, 2025

Available online: October 10, 2025

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ABSTRACT

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, confusion, and behavioural changes. It primarily impairs brain function, leading to a gradual decline in cognitive abilities such as thinking, reasoning, and problem-solving. As the most common cause of dementia, AD reflects a deterioration in mental faculties severe enough to disrupt daily activities. The disease advances slowly and typically worsens over time. Currently available pharmacological treatments address only the symptoms of AD, largely due to the restrictive nature of the blood-brain barrier (BBB), which impedes the entry of therapeutic agents into the central nervous system. This review summarizes recent technological advances in AD treatment, focusing on monoclonal antibody (mAb) therapies, nanotechnology-based delivery systems, and gene-editing approaches. mAb therapies encompass passive immunotherapy and tau-targeting interventions aimed at mitigating pathological protein accumulation. Nanotechnology-based strategies for AD include the use of nano-carriers, nano-polymers, dendritic nanoparticles, liposomes, and exosomes. These platforms have demonstrated enhanced drug delivery across the BBB with minimal adverse effects. Furthermore, gene-editing techniques target specific genetic variants, such as apolipoprotein E4 (*ApoE4*), which are implicated in both early- and late-onset forms of AD. Gene-editing is emerging as a promising therapeutic modality for correcting dominant mutations associated with AD pathogenesis. Despite these advances, further research and clinical trials are essential in order to develop and validate novel treatment strategies for AD.

1. Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder that advances through preclinical, early, moderate, and ultimately late stages. Initial symptoms typically involve general cognitive impairment, most notably memory loss. As cognitive decline progresses, physical disabilities (such as difficulties with walking, eating, and sitting) signal the transition to the advanced stages of the disease. The aetiology of AD is multifactorial and not yet fully understood. While the accumulation of amyloid-beta ($A\beta$) and tau proteins is considered central to AD pathogenesis, numerous additional factors may contribute to it, including neuroinflammation, acetylcholine deficiency, glutamate imbalance, autophagy dysfunction, mitochondrial impairment, disrupted cholesterol homeostasis, oxidative stress, and insulin resistance. Although several theories have been proposed to explain the pathophysiology of AD, a unified hypothesis remains elusive.

AD is currently incurable, and available treatments primarily aim at alleviating symptoms and at modestly improving cognitive function. Therapeutic limitations stem from two major challenges: first, the disease progresses *via* multiple pathogenic pathways that are difficult to delineate; second, the blood-brain barrier (BBB) significantly impedes drug delivery to the central nervous system, thereby restricting therapeutic efficacy.

2. Therapeutic techniques of AD

Various therapeutic approaches have been explored over the past decades, yet existing pharmacological agents predominantly target symptoms rather than underlying disease mechanisms. These drugs have not demonstrated consistent efficacy in improving cognition, quality of life, functional capacity, or overall behavioural outcomes. The development of novel therapeutic modalities is essential for the advancement of the understanding of AD pathophysiology and enhancing treatment outcomes. To date, only two classes of drugs have been approved by the US Food and Drug Administration (FDA) for symp-

tomatic treatment: cholinesterase inhibitors (ChEIs) and N-methyl-D-aspartate receptor modulators. ChEIs such as galantamine, donepezil, and rivastigmine are indicated for mild to moderate AD and offer temporary symptomatic relief. More recently, two monoclonal antibodies (mAbs) – lecanemab and aducanumab – have been approved for targeting $A\beta$ plaque accumulation in the AD brain. Conventional therapies are limited by low efficacy, hepatotoxicity, gastrointestinal side effects, and poor BBB penetration¹. This review aims to highlight recent technological advances in AD treatment.

3. Monoclonal antibody therapies

Several passive immunotherapy agents have entered clinical trials for AD. Anti-amyloid mAbs represent the first disease-modifying treatments that slow clinical decline by targeting core biological mechanisms. Cummings *et al.*¹ have reported trials demonstrating a 15%–25% reduction in measurable $A\beta$ plaques and an approximately 30% decrease in cognitive decline, which is considered clinically meaningful for preserving cognitive function and delaying disease progression. The approval of these agents marks a paradigm shift in AD treatment. Hong² has evaluated the efficacy, safety profiles, and adverse effects of aducanumab and lecanemab across different stages of AD, reporting improved therapeutic outcomes. Further research³ has introduced a chimeric peptide, D20, which selectively promotes tau dephosphorylation. A single dose of D20 significantly reduced phosphorylated tau at multiple AD-relevant sites and lowered total tau levels, suggesting its potential as a therapeutic candidate for AD and related tauopathies.

4. Nanotechnology techniques for AD treatment

Ongoing research explores the use of nanotechnology and nanoparticles (NPs) for targeted drug delivery in AD, aiming to minimize adverse effects. Nanotechnology has emerged as a promising strategy for enhancing therapeutic efficacy.

A prior study⁴ has reported the development of

biodegradable and biocompatible dendrimeric delivery systems to transport flurbiprofen (FP; a drug with limited brain penetrability) across the BBB and release it at targeted sites. The conjugation of FP to dendrimeric poly(epsilon-lysine) macromolecules significantly improved BBB permeability.

In another study⁵ researchers have synthesized a nanoscale cobalt/niacin metal-organic framework (Co/Niacin-MOF). *In vitro* and molecular docking analyses of acetylcholinesterase inhibitors (AChE-Is) identified two compounds – 4-(1H-benzo[d]imidazol-2-yl)phenyl 4-methylbenzene sulfonate and 4-(benzo[d]thiazol-2-yl)phenyl benzoate – as promising candidates compared to donepezil.

Further research⁶ has investigated FP-peptide delivery systems featuring an apolipoprotein E (ApoE)-mimicking peptide in order to facilitate receptor-mediated transcytosis across the BBB. The successful synthesis and attachment of FP to lysine-terminal molecular branches has demonstrated enhanced delivery without cytotoxicity.

Khope *et al.*⁷ have emphasized that nanocarriers can deliver therapeutic agents directly to targeted regions of the central nervous system; a strategy particularly relevant for neurodegenerative and malignant conditions. *In vivo* studies have shown promising results for AD nanomedicines, including curcumin-loaded solid NPs, quercetin-loaded exosomes, and resveratrol delivered *via* non-metal inorganic NPs⁸.

While nanomedicine holds substantial promise for AD treatment, further research is needed in order to evaluate the safety and efficacy of these emerging therapies.

5. Gene-editing technology for AD treatment

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology offers a transformative approach to AD therapy by targeting specific genes, such as apolipoprotein E4 (*ApoE4*), which are implicated in both early- and late-onset AD. This method also addresses challenges related to therapeutic delivery.

One study⁹ has employed the CRISPR-Cas9 system

derived from *Streptococcus pyogenes* to deactivate the *PSEN1M146L* allele in human fibroblasts, resulting in reduced extracellular A β levels and decreased presenilin (PSEN) expression. These findings underscore the potential of CRISPR-Cas9 to selectively mitigate AD-associated phenotypes. Additionally, Chen *et al.*¹⁰ have introduced a gene-clustering framework using convolutional neural networks, termed Dual-Stream Subspace Clustering Network (DS-SCNet). This method has effectively identified biologically meaningful gene clusters from large-scale single-cell RNA sequencing data and has outperformed conventional clustering techniques across multiple evaluation metrics.

6. Conclusion

This review has outlined recent technological innovations in AD treatment, including monoclonal antibody therapies, gene-editing strategies, and nanotechnology-based delivery systems. Monoclonal antibody therapies encompass passive immunotherapy and tau-targeting approaches, while nanotechnology has demonstrated enhanced drug transport across the BBB with minimal adverse effects. Gene-editing techniques targeting specific genes, offer promising avenues for addressing dominant mutations associated with AD onset. Nonetheless, continued research and clinical validation are essential for the development of effective and safe therapeutic strategies.

Acknowledgements

We sincerely thank the Deanship of Scientific Research at the College of Pharmacy of the University of Babylon.

Conflicts of interest

None exist.

ORCIDs

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HOW TO CITE:

Dhaidan A.S., Al-Azzawi S. Recent treatment technologies for Alzheimer disease: a review. *Pharmakeft-iki* 37(2s), 504-507, 2025. <https://doi.org/10.60988/p.v37i2S.276>

Self-medication practices and perceived associated health implications: a review

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KEY WORDS:

self-medication; over-the-counter medications; antibiotic resistance; drug-drug interactions; health risks

ARTICLE INFO:

Received: January 10, 2025

Revised: February 21, 2025

Accepted: February 21, 2025

Available online: October 10, 2025

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ABSTRACT

Self-medication – defined as the use of pharmaceutical products without a formal prescription from a licensed healthcare provider – is a globally prevalent practice, particularly in developing countries where medications are more readily accessible and healthcare costs remain prohibitive. This behaviour includes the consumption of over-the-counter (OTC) drugs, the reuse of previously prescribed medications, and the sharing of pharmaceuticals among family members. Although self-medication offers certain advantages, such as economic feasibility, ease of access, and perceived autonomy, it simultaneously poses significant health risks. These include misdiagnosis, potential drug-drug interactions (DDIs), and the development of antimicrobial resistance (AMR), especially in the context of inappropriate antibiotic use. The prevalence of self-medication is notably high in South Asian countries, driven by factors such as prior personal health experiences and pervasive pharmaceutical marketing strategies. Commonly used medications in this context include analgesics and antibiotics, often employed in order to treat minor ailments such as upper respiratory tract infections and cephalalgia. Despite its utility in managing non-serious health conditions, improper self-medication – particularly involving antibiotics – can lead to severe consequences, including AMR and adverse drug reactions. DDIs may further exacerbate these risks, potentially resulting in critical outcomes such as renal impairment. In order to mitigate these threats, it is imperative to strengthen patient education, advocate for the judicious use of OTC medications, and enhance public awareness regarding the risks associated with unsupervised pharmaceutical consumption.

1. Introduction

Self-medication refers to the administration of pharmacological agents in order to address health conditions without confirmation or guidance from a licensed medical professional. Common ailments managed through self-medication include fever, bodily discomfort, dyspepsia, and diarrhoea¹. The World Health Organization (WHO) defines self-medication as the use of over-the-counter (OTC) pharmaceuticals for self-diagnosed symptoms or the continued consumption of previously prescribed medications for recurrent health issues. This definition highlights the widespread nature of self-medication practices globally².

Beyond the procurement of pharmaceuticals without a valid prescription, self-medication is often embedded in cultural norms, such as the reuse of previously issued prescriptions to obtain medications or the sharing of medicinal products among family members, relatives, and acquaintances³. The phenomenon is prevalent in both developed and developing countries, with a notably higher incidence in the latter, largely due to the increased availability of pharmaceuticals without prescription requirements¹.

At Damascus Hospital, self-medication is commonly practiced, with a reported prevalence of 67.3% over the preceding three-month period. Table 1 presents prevalence data across different countries / regions. Variations in recall periods, access to healthcare services, economic conditions, and sociocultural factors may account for differences in prevalence rates⁴. This review aims to provide a comprehensive overview of self-medication practices, including prevalence, underlying motivations, commonly used medications, and associated risks.

2. Reasons for self-medication

The utilization of self-medication is influenced by multiple factors, including rising healthcare costs and the increasing burden of chronic illnesses. Key determinants contributing to its prevalence include enhanced accessibility to pharmaceutical products,

prior personal experiences with similar health conditions, aggressive marketing of medicinal products, and the belief that minor ailments do not warrant professional medical intervention. These factors are particularly pronounced in South Asian countries².

3. Benefits of self-medication

Self-medication offers several potential benefits to healthcare systems. It facilitates a more efficient use of clinical resources, improves access to pharmaceuticals, and may contribute to reducing expenditures associated with prescribed medications under publicly funded health programs⁵.

4. Risks of self-medication

The risk of inappropriate self-medication is elevated among individuals with limited health literacy. Accordingly, it is essential to assess community-level knowledge regarding the use of OTC medications⁶. During the COVID-19 pandemic, self-medication practices surged, partly due to the widespread distribution of the so-called "COVID-kit"; a collection of pharmaceuticals promoted as early treatment options.

Health education initiatives are critical to informing the public about the dangers of self-medication. Although OTC medications are intended for self-administration and have demonstrated efficacy and safety, misuse stemming from inadequate understanding of dosage, side effects, and drug interactions can lead to serious consequences. Vulnerable populations (including children, older adults, and individuals in special physiological states such as pregnancy) are particularly at risk⁶.

Globally, the rise in self-medication is concerning and represents a major contributor to the development of antimicrobial resistance in microbial populations. Public education must address the misuse of antibiotics, including their use for minor conditions such as pharyngitis, premature discontinuation of treatment upon symptom relief, and unsupervised modification of antibiotic regimens⁷.

Table 1. Prevalence of self-medication among different countries / regions.	
Country / region	Self-medication prevalence
Jordan	42.5%
Egypt	73%
Lebanon	79.1%
Brazil	16.1%
Vietnam	83.3%
Pakistan	84.4%
Ethiopia	50.2%
Palestine	87%

Self-medication also poses significant health risks through drug-drug interactions (DDIs), which may result in adverse drug reactions (ADRs). A detailed analysis identified 153 DDIs, with 59.5% classified as pharmacodynamic interactions. Of the 119 pharmaceuticals involved, 51% were prescription-only medications. Frequently used drug categories included analgesics, anti-inflammatory agents, dietary supplements, and antibiotics. The most commonly reported ADRs involved haemostatic disorders and renal dysfunction. These findings underscore the potential severity of self-medication-related complications, exacerbated by insufficient information provided in medication package leaflets⁸.

Headache, influenza, and cough were the three most frequently cited reasons for self-medication. Survey data have revealed that 40% of students believed self-medication could be recommended to or received from others. Furthermore, 94% agreed that self-medication requires monitoring, and nearly half expressed comfort with its use⁹.

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5. Conclusion

Self-medication is a complex phenomenon encompassing both beneficial and harmful dimensions. While it may serve as a convenient and cost-effective alternative to formal healthcare, its improper use can lead to serious health consequences.

Acknowledgements

We sincerely thank the Deanship of Scientific Research at the University of Babylon College of Pharmacy.

Conflicts of interest

None exist.

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HOW TO CITE:

Hamza B.A., Fadheel Q.J. Self-medication practices and perceived associated health implications: a review. *Pharmakeftiki* 37(2s), 508-511, 2025. <https://doi.org/10.60988/p.v37i2s.277>

Crohn's disease and smoking: a review of the effects of the latter on inflammatory markers and gene expression

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KEY WORDS:

Crohn's disease; smoking; inflammatory markers; upregulating; downregulating

ARTICLE INFO:

Received: January 01, 2025

Revised: January 18, 2025

Accepted: February 06, 2025

Available online: October 10, 2025

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ABSTRACT

Crohn's disease (CD), a chronic form of inflammatory bowel disease, affects the gastrointestinal tract and is characterized by a multifactorial aetiology involving both genetic predisposition and environmental influences. Among environmental modifiers, cigarette smoking plays a pivotal role in exacerbating the progression and severity of CD. Individuals with CD who smoke exhibit a heightened risk of disease flare-ups, increased surgical intervention rates, and diminished responsiveness to pharmacological therapies. Smoking intensifies intestinal inflammation by elevating key biomarkers, including C-reactive protein, erythrocyte sedimentation rate, and faecal calprotectin, while simultaneously dysregulating pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-6, interleukin-1 beta, and interleukin-17. Furthermore, smoking alters gene expression by upregulating cytokine and matrix metalloproteinase pathways, and downregulating tight junction proteins essential for maintaining intestinal epithelial barrier integrity. These molecular disruptions compromise epithelial cohesion, exacerbate mucosal injury, and sustain chronic inflammation. Epigenetic modifications – most notably DNA methylation – further contribute to immune dysregulation in smokers with CD. Notably, smoking cessation has been associated with downregulation of pro-inflammatory gene expression and attenuation of disease activity, underscoring its therapeutic relevance in CD management. A deeper understanding of the mechanisms by which smoking modulates inflammatory biomarkers and gene expression may inform the development of targeted interventions aimed at improving clinical outcomes in patients with CD.

1. Introduction

Crohn's disease (CD) is a chronic, idiopathic form of inflammatory bowel disease that can affect any segment of the gastrointestinal (GI) tract, from the oral cavity to the anus. The etiopathogenesis of CD is multifactorial, involving both genetic predisposition and environmental influences. The management of CD typically requires long-term pharmacological treatment, which may be associated with adverse effects, and in some cases necessitates surgical intervention and recurrent hospital admissions. These burdens significantly impact patients' social, professional, and psychological well-being. CD affects males and females equally and is most commonly diagnosed in young, active adults, thereby imposing a substantial societal and economic burden¹.

Cigarette smoking is a well-established environmental risk factor for CD. The prevalence of current smokers is significantly higher among individuals with CD compared to control populations. A systematic review and meta-analysis of 33 studies has demonstrated that smokers with CD are at increased risk of spontaneous disease exacerbation, postoperative recurrence, and a higher likelihood of requiring both initial and repeat surgical procedures². Tobacco smoking is the most extensively studied environmental modifier in CD and has been shown to increase the need for additional immunosuppressive agents while reducing both the efficacy and durability of pharmacological treatments. In CD, smoking elevates the risk of disease flares, increases reliance on glucocorticoids and immunosuppressants, and diminishes therapeutic response to agents such as infliximab. Smokers also exhibit altered T cell profiles, with heavy smoking associated with elevated levels of suppressor CD8⁺ cells, suggesting a link between smoking and immune suppression.

Cigarette smoke may influence the gut microbiota by upregulating oxidative stress-related enzymes in intestinal immune tissues, disrupting the mucin layer, altering the expression of tight junction proteins, and inducing acid-base imbalance in the colon. These effects may result from direct toxicity of

tobacco constituents or from bacterial transmission *via* cigarette exposure³. The present review aims to summarize how smoking modulates inflammatory markers and gene expression in patients with CD, thereby contributing to disease severity (Table 1).

2. Impact of smoking on inflammatory markers in CD

C-reactive protein (CRP) is a non-specific biomarker of systemic inflammation and is frequently elevated in individuals with CD. Smoking has been shown to further increase CRP levels, thereby amplifying systemic inflammatory responses and potentially worsening clinical outcomes. The erythrocyte sedimentation rate (ESR), another non-specific marker of inflammation, is commonly elevated in CD and correlates with disease activity. Smoking exacerbates ESR elevation, reinforcing its role in intensifying systemic inflammation in CD⁴.

Faecal calprotectin (FC) is a specific marker of intestinal inflammation and is widely used in order to monitor disease progression in CD. Elevated FC levels reflect active mucosal inflammation, and smoking has been shown to significantly increase FC concentrations, indicating a worsening of localized GI inflammation. Additionally, pro-inflammatory cytokines – including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 beta (IL-1 β), and interleukin-17 (IL-17) – are frequently dysregulated in CD. These cytokines drive the inflammatory cascade characteristic of the disease, and smoking further disrupts their regulation, thereby intensifying the inflammatory milieu and contributing to disease severity.

Finally, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) facilitate leukocyte migration to inflamed tissues. Elevated ICAM-1 levels in CD enhance leukocyte adhesion and promote tissue inflammation. Smoking has been associated with increased activity of adhesion molecules, thereby aggravating mucosal damage and perpetuating inflammation⁵.

3. Impact of smoking on gene regulation in CD

Table 1. Major studies exploring the association of smoking with gene expression with Crohn's disease.		
Study	Methodology	Main findings
Boronat-Toscano <i>et al.</i> ¹⁰	genome-wide epigenetic analysis; quantitative gene expression evaluated by qPCR	smoking alters adipose stem cells' immune regulatory properties; ex-smokers retain pro-inflammatory epigenetic changes in stem cells
Wang <i>et al.</i> ⁶	sure independence screening (SIS) for mediator CpGs selection; LASSO regression for variable selection in mediation analysis	smoking is significantly associated with Crohn's disease; changes in DNA methylation mediate the effect of smoking on Crohn's disease
Papoutsopoulou <i>et al.</i> ³	Medline search for relevant smoking and intestinal inflammation terms; assessment of epidemiological, experimental, and clinical evidence	smoking causes oxidative damage and impairs intestinal barrier function; it alters immune cell function and microbiota composition
Allais <i>et al.</i> ⁸	examined cytokine profiles in human subjects and mouse models; investigated TRPV1 expression and cytokine production effects	cigarette smoke increases IL-8 in healthy ileum; TRPV1 levels decrease in Crohn's disease patients and smoking controls
Yan Ang <i>et al.</i> ⁹	prospective cohort study in UK Biobank participants; two-sample Mendelian randomization and genome-wide methylation analysis	current smoking is associated with higher risk of Crohn's disease; epigenetic alterations in DNMT3A, TNF/LTA, and AHRR genes are linked to inflammatory bowel disease risk
Nicolaides <i>et al.</i> ⁴	comprehensive review of English literature from 1946 to April 2019; search conducted using Medline, Embase, and Cochrane central library	smoking affects treatment choice and efficacy in inflammatory bowel disease; impact of smoking on disease pathways and therapy efficacy
Lee <i>et al.</i> ⁵	systematic review and meta-analysis conducted; searched databases for smoking effect on anti-TNF therapy in Crohn's disease	smoking reduces effectiveness of anti-TNF therapies in Crohn's disease; current smokers less likely to achieve clinical response and remission

Smoking influences the expression of multiple gene families that govern inflammation and immune responses in CD. It upregulates genes encoding pro-inflammatory cytokines, thereby exacerbating inflammatory signalling. TNF- α is a central mediator of inflammation and contributes to CD progression, IL-6 is associated with systemic inflammation and adverse clinical outcomes, and IL-1 β plays a key role in tissue injury and inflammatory amplification⁵.

The expression of genes involved in innate immunity, such as that of nucleotide-binding oligomerization domain-containing 2 (*NOD2*), may also be altered by smoking. The dysregulation of *NOD2* increases the susceptibility to intestinal inflammation and is implicated in CD pathogenesis³. Smoking further affects the expression of microRNAs (small non-coding RNAs that regulate gene expression related to immune function and inflammation): altered

microRNA profiles disrupt intestinal homeostasis and contribute to inflammatory dysregulation⁶.

Moreover, smoking elevates the expression of matrix metalloproteinases (MMPs), enzymes responsible for extracellular matrix remodelling. Increased MMP activity leads to mucosal degradation and sustained inflammation, thereby exacerbating tissue injury in CD⁷. Smoking also downregulates genes encoding tight junction proteins such as occludin and claudins, which are essential for maintaining epithelial barrier integrity. This downregulation results in increased intestinal permeability ("leaky gut"), further fuelling inflammation⁸.

Finally, smoking induces the expression of genes associated with oxidative stress, activating pro-inflammatory pathways. Elevated oxidative stress contributes to mucosal injury and accelerates disease progression in CD⁹. The regulation of apopto-

sis-related genes is also affected by smoking, potentially prolonging the survival of inflammatory cells within the intestinal mucosa; this dysregulation sustains chronic inflammation and worsens CD symptoms¹⁰.

4. Conclusion

Smoking alters the expression of the NOD2 gene, and individuals with such dysregulation exhibit more severe disease progression compared to non-smokers. Moreover, smoking increases the levels of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-17, and induces epigenetic modifications (including DNA methylation) that contribute to immune dysregulation in CD. Smoking also significantly elevates inflammatory markers such as CRP. Conversely, smoking cessation has been associated with a downregulation of pro-in-

flammatory gene expression and an attenuation of disease activity, underscoring its therapeutic importance in CD management.

Acknowledgements

We would like to extend our heartfelt thanks to the College of Pharmacy of the University of Babylon, for its support and the assistance provided.

Conflicts of interest

None exist.

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HOW TO CITE:

Hasan B., Al-Saigh R.J., Al-Humadi H.W. Crohn's disease and smoking: a review of the effects of the latter on inflammatory markers and gene expression. *Pharmakeftiki* 37(2s), 512-516, 2025. <https://doi.org/10.60988/p.v37i2S.278>

Update on the pathophysiology and therapeutics of chorea disease

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KEY WORDS:
movement disorders; chorea;
basal ganglia; neurochemical
abnormalities; glutamate

ARTICLE INFO:

Received: January 05, 2025

Revised: January 25, 2025

Accepted: February 07, 2025

Available online: October 10, 2025

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ABSTRACT

Chorea is a primary category of involuntary movement disorders arising from aberrant neural circuitry connecting the frontal cortical motor regions and the basal ganglia. Clinically, the syndrome manifests as a high frequency of brief, erratic, and involuntary muscle contractions. Although chorea may result from various aetiologies, the most common contributors include dysregulation of glucose metabolism, thyroid dysfunction, and renal impairment. Neurochemical abnormalities have been implicated in its pathophysiology, notably dysfunction of the presynaptic nigrostriatal dopaminergic pathway, which may lead to increased dopamine turnover. The authors emphasize that the current pharmaceutical armamentarium for chorea remains limited, offering few therapeutic options to clinicians. Tetrabenazine and its derivative, deutetabenazine, are the principal agents approved by the US Food and Drug Administration specifically for the treatment of chorea associated with Huntington's disease. This case underscores the persistent and substantial challenges faced by healthcare professionals in managing this complex neurological condition. Further research is urgently warranted in order to expand the therapeutic landscape and to elucidate the pharmacodynamics and pharmacokinetics of existing and emerging agents across diverse patient populations affected by chorea. The objective of this article was to provide a concise overview of the biochemical alterations and pharmacological interventions relevant to chorea.

1. Introduction

Chorea is a primary category of involuntary movement disorders resulting from aberrant neural networks linking the frontal cortical motor regions and the basal ganglia. The condition is characterized by a frequent succession of brief, erratic, involuntary muscle contractions, which may arise from a variety of aetiologies¹. The World Federation of Neurology Committee on Classification defines chorea as “*a state of excessive, spontaneous movements, irregularly timed, non-repetitive, randomly distributed, and abrupt in character*”. The severity of these movements ranges from subtle, intermittent exaggerations in gesture and facial expression to restlessness involving hand twitching, an unstable dance-like gait, or a continuous stream of violent, incapacitating motions. The term “chorea”, akin to choreography, reflects the dance-like quality of these movements. Chorea is classified as primary when idiopathic or genetic in origin, and secondary when associated with infectious, immunological, or other systemic causes².

2. Biochemical alterations in patients with chorea disease

Glutamate, the principal excitatory neurotransmitter in the brain, has been found to be significantly reduced in the striatum of individuals with chorea compared to controls. This reduction is hypothesized to reflect decreased neuronal volume in the affected region. Notably, elevated levels of spermidine were observed in the striatum. Spermidine, along with putrescine, spermine, and agmatine, belongs to the class of polyamines (PAs), whose biosynthesis is tightly regulated. Dysregulated PA metabolism has been implicated in Alzheimer's disease and other neurodegenerative conditions³. PAs play essential roles in the central nervous system, where they initiate the polyamine stress response in reaction to various transient stressors, including heat, ultraviolet radiation, reactive oxygen species, and ageing. As such, PAs are considered primordial stress inducers⁴.

3. Pharmacological targets in chorea disease

Investigating pharmacological targets in chorea requires a comprehensive understanding of the underlying neurobiological mechanisms and potential therapeutic pathways. The literature provides valuable insights into the pathophysiology of chorea, particularly through the lens of motor control and the intricate interplay among striatal neuronal populations. Liu⁵ emphasizes the role of striatal circuitry – specifically of large aspiny cholinergic interneurons – in maintaining dopaminergic transmission balance and regulating movement, thereby underscoring the importance of the cholinergic system in Parkinson's disease. This perspective suggests that analogous pathways may be leveraged in the treatment of chorea, where motor control is similarly disrupted.

Research addressing both inherited and acquired forms of chorea has broadened the therapeutic scope. Detailed analyses underscore the importance of timely and accurate diagnosis, particularly in identifying treatable causes of acquired chorea, which may stem from autoimmune, metabolic, or other systemic disorders. The integration of advanced next-generation sequencing technologies has significantly enhanced the ability of researchers and clinicians to detect genetic underpinnings associated with these heterogeneous conditions. In parallel with discoveries concerning the striatal cholinergic system's role in chorea pathogenesis, the expanding genetic landscape and growing recognition of reversible causes underscore the potential for targeted pharmacological interventions. These developments hold promise for improving patient outcomes and refining therapeutic strategies⁶.

The recognition of the striatum's central role in both Parkinson's disease and chorea provides a compelling framework for future research aimed at identifying and precisely targeting pharmacological interventions to alleviate motor symptoms in these complex disorders. Elucidating the connections between neurophysiological processes and clinical manifestations enhances our understanding of chorea's pathophysiology and paves the way for innovative treatment modalities. Ultimately, as novel

therapies are developed and implemented, this integrative approach may significantly improve patients' quality of life⁷.

4. Current pharmaceutical interventions for chorea disease

Current data on pharmaceutical interventions for chorea reveal a nuanced interplay between underlying aetiologies and symptomatic treatment strategies. This evolving field includes a focused analysis of levodopa's role in chorea management, highlighting both its therapeutic potential and limitations. Although levodopa has occasionally yielded symptomatic improvement – particularly in patients with brain-thyroid-lung syndrome – the overall evidence base remains limited and insufficiently documented. Only 18 cases have been reported demonstrating a response to levodopa across diverse aetiologies, raising critical questions about its generalizability and efficacy in broader chorea populations. In fact, levodopa's effectiveness may be closely tied to the specific underlying disorder being treated, reinforcing the need for rigorous research in order to delineate its role in chorea associated with various movement-impairing conditions⁸.

Importantly, the pharmaceutical landscape for chorea remains narrow, with few therapeutic options available to clinicians. Tetrabenazine and its derivative, deutetrabenazine, continue to be the primary medications approved by the US Food and Drug Administration for the treatment of chorea associated with Huntington's disease. This case exemplifies the persistent and formidable challenges faced by healthcare providers in managing this complex neurological disorder. As the field advances, further research is urgently needed in order to explore additional treatment modalities and to deepen our understanding of the pharmacodynamics and the pharmacokinetics of these agents across diverse chorea patient populations⁹.

In contrast, Ortigoza-Escobar¹⁰ addresses the di-

agnostic and therapeutic challenges of chorea as a manifestation of inherited metabolic disorders. In this regard, the importance of prompt genetic testing and disease-specific interventions is emphasized, particularly in acute presentations where chorea is prominent. A holistic treatment approach is advocated, encompassing not only motor symptoms, but also comorbidities such as mental health conditions. The discussion of dietary therapies (including zinc supplementation for Wilson disease and low-lysine diets for glutaric aciduria) highlights the relevance of metabolic considerations in chorea management.

4. Conclusion

While current models attribute choreic disorders to neural imbalances, emerging evidence suggests that biochemical alterations in brain activity may also contribute to symptomatology. This review has examined the biochemical factors implicated in chorea pathogenesis and has emphasized the importance of understanding neurobiological mechanisms and therapeutic pathways. Continued investigation is essential in order to unravel these complexities and to advance the clinical management of chorea.

Acknowledgements

None.

Conflicts of interest

None exist.

ORCIDs

0000-0003-0919-3219 (R. Hussein); 0009-0004-9715-2082 (S. Rayhaan); 0009-0008-1375-3331 (H.F. Abbas); 0704-2843-0004-0009 (A.A. Jasim); 0009-0002-2133-5179 (L.A. Hasan); 0000-0002-6575-2735 (R.M. Hameed); 0009-0000-5715-9088 (Z.R. Al-Mousawi)

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HOW TO CITE:

Hussein R., Rayhaan S., Abbas H.F., Jasim A.A., Hasan L.A., Hameed R.M., Al-Mousawi Z.R. Update on the pathophysiology and therapeutics of chorea disease. *Pharmakeftiki* 37(2s), 517-520, 2025. <https://doi.org/10.60988/p.v37i2S.279>

Targeting the IL-33/ST2 signalling pathway as a therapeutic strategy for the treatment of multiple inflammatory diseases

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KEY WORDS:

interleukin-33; ST2 receptor;
IL-33/ST2 pathway;
therapeutic strategy;
inflammatory diseases

ARTICLE INFO:

Received: January 06, 2025

Revised: February 04, 2025

Accepted: February 13, 2025

Available online: October 10, 2025

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ABSTRACT

Interleukin-33 (IL-33) is a cytokine belonging to the interleukin-1 (IL-1) superfamily. It is expressed and upregulated following pro-inflammatory stimulation, and plays a key role in inducing the synthesis of T-helper type 2 (Th2) cytokines and in promoting the pathogenesis of Th2-related diseases. IL-33 exerts its biological effects by binding to its receptor, ST2; a member of the IL-1 receptor family that exists in two isoforms: the transmembrane form (ST2L) and the soluble form (sST2). Through ST2L, IL-33 signals surrounding immune cells in response to tissue injury. In the respiratory system, IL-33 overexpression can exacerbate chronic respiratory diseases. In the cardiovascular system, cardiomyocytes and cardiac fibroblasts increase the expression of both IL-33 and ST2. Within the central nervous system, IL-33 promotes M2 macrophage polarization and/or regulatory T cell (Treg) activation, thereby contributing to an anti-inflammatory response in various disease contexts. In the renal system, IL-33/ST2 signalling has been implicated in both pathogenic and tissue-protective processes across multiple renal disorders. In the gastrointestinal tract, targeting the IL-33 signalling pathway presents a potential therapeutic strategy. In type 2 diabetes mellitus, metformin treatment has revealed a key role for IL-33 and ST2 in disease modulation. In skin diseases such as psoriasis and vitiligo, increased IL-33 expression has been observed in lesional tissues. Finally, in rheumatological diseases, the IL-33/ST2 pathway appears to exert a detrimental influence during both early and advanced disease stages.

1. Introduction

Interleukin-33 (IL-33) is a cytokine belonging to the interleukin-1 (IL-1) superfamily. It is produced primarily by stromal cells, including endothelial and epithelial cells, and is upregulated following pro-inflammatory stimulation. IL-33 can function both as a traditional cytokine and as a nuclear transcriptional regulator. It is thought to act as an “alarmin”, released upon cell necrosis to signal tissue injury to the immune system. Its biological activity is mediated through interaction with the ST2 (IL-1RL1) receptor and the IL-1 receptor accessory protein (IL-1RAcP), both of which are highly expressed by T helper 2 (Th2) cells and innate immune cells¹. IL-33 promotes Th2 cytokine synthesis and contributes to the pathogenesis of Th2-mediated diseases such as asthma and atopic dermatitis. Conversely, it exhibits protective effects in conditions such as atherosclerosis, cardiac remodelling, type 2 diabetes (T2D), and obesity. The functional role of IL-33 – either pro-inflammatory or anti-inflammatory – depends on disease context and experimental model².

ST2 exists in two isoforms: transmembrane (ST2L) and soluble (sST2). ST2L serves as the signalling receptor for IL-33, triggering immune responses and the production of inflammatory cytokines. In contrast, sST2 acts as a decoy receptor, neutralizing IL-33 and inhibiting ST2L-mediated signalling. This review summarizes the role of IL-33/ST2 signalling in inflammatory disease pathogenesis and its potential as a therapeutic target.

2. Respiratory system

Type II inflammation is a chronic inflammatory response characterized by prolonged activation and dysregulation of immune cells. It underpins several respiratory disorders, notably asthma, chronic obstructive pulmonary disease (COPD), allergic rhinitis, and pulmonary fibrosis. The process involves a complex crosstalk among pro-inflammatory cytokines and regulatory pathways, with key contributions from Th2 cells, IL-4, IL-5, IL-9, IL-13, IL-33, and thymic stromal lymphopoietin. IL-33 is a central me-

diator of type II inflammation, particularly in asthma and COPD. Its overexpression correlates with increased disease severity *via* sustained inflammation and airway remodelling. IL-33 also plays a role in allergic rhinitis by inducing Th2 cytokines associated with nasal congestion and irritation. Several ongoing randomized controlled trials are investigating anti-IL-33 agents (itepekimab, tozorakimab) and anti-ST2 antibodies (astegolimab) in patients with asthma and COPD³.

3. Cardiovascular system

The IL-33/ST2 pathway plays a critical role in cardiovascular physiology, particularly in response to myocardial stretch and injury. Following myocardial infarction or heart failure, IL-33 modulates cardiac hypertrophy, remodelling, and fibrosis. However, endothelial cells and fibroblasts release sST2, which antagonizes IL-33 by functioning as a decoy receptor. Elevated sST2 levels are associated with inadequate cardioprotection and heightened risk of ventricular dysfunction.

The IL-33/ST2 signalling is implicated in several cardiovascular conditions, including ischaemic heart disease, cardiomyopathy, and myocarditis. sST2 is also recognized as a biomarker in systemic and pulmonary hypertension and as a diagnostic indicator in acute aortic dissection. In 2013, the American College of Cardiology Foundation / American Heart Association classified sST2 as an independent biomarker for heart failure and myocardial infarction⁴.

4. Central nervous system

In the central nervous system, IL-33 is primarily expressed by astrocytes and oligodendrocytes. Beyond its role in promoting brain tumorigenesis, IL-33 facilitates M2 macrophage polarization and regulatory T cell (Treg) activation and expansion. These mechanisms support anti-inflammatory responses in conditions such as multiple sclerosis, Alzheimer's disease, traumatic brain injury, and ischaemic stroke. These findings suggest IL-33 administration may offer therapeutic potential in CNS disorders⁵.

5. Renal system

Numerous studies have examined IL-33 activity in renal injury models, including ischemia–reperfusion injury (IRI), ovalbumin-induced nephrotoxicity, and cisplatin-induced acute kidney injury. Notably, an engineered IL-2/IL-33 fusion protein has demonstrated renoprotective effects in IRI models. Moreover, across various renal pathologies, the IL-33/ST2 signalling exhibits a dual role, contributing to both injury and tissue repair, thereby emphasizing its context-dependent function².

6. Gastrointestinal system

The IL-33/ST2 complex interacts with intestinal epithelial cells during injury and activates innate immune responses. Elevated IL-33 levels have been linked to exacerbated inflammation and tissue damage in ulcerative colitis, and to recurring inflammation with Th1 skewing in Crohn's disease. In colorectal cancer, IL-33 promotes tumor progression through pro-angiogenic signalling and the activation of subepithelial myofibroblasts. Thus, targeting the IL-33 pathway holds promise for the development of therapeutic interventions in gastrointestinal diseases⁶.

7. Diabetes mellitus

Pancreatic β -cell dysfunction is central to both type 1 diabetes (T1D) and T2D, resulting from autoimmune destruction in T1D and multifactorial stressors (such as glucolipotoxicity, mitochondrial dysfunction, endoplasmic reticulum stress, and inflammation) in T2D. Emerging evidence suggests a protective role for IL-33 in diabetes. IL-33 suppresses insulinitis, inhibits autoimmune diabetes progression, and enhances glucose homeostasis *via* the stimulation of islet-resident group 2 innate lymphoid cells. Interestingly, metformin treatment in T2D patients has been associated with elevated IL-33 and reduced ST2 expression, which correlates with improved glycaemic control and highlights the cytokine's inflammatory and therapeutic significance⁷.

8. Skin diseases

Atopic dermatitis (AD) is a Th2-driven condition mediated *via* the IL-33/ST2 signalling. Patients with AD exhibit elevated IL-33 levels in lesional skin, although serum sST2 levels remain unchanged. Clinical trials are underway in order to evaluate novel anti-IL-33 antibodies in this context. By contrast, psoriasis is governed by Th1 and Th17 cytokines, yet psoriatic lesions still show increased IL-33 expression. In vitiligo, skin biopsies reveal concurrent elevation of IL-33 and ST2, with corresponding increases in serum IL-33 levels⁸.

9. Rheumatological system

The IL-33/ST2 pathway plays a deleterious role in the pathogenesis of several rheumatological conditions, including rheumatoid arthritis, scleroderma, systemic lupus erythematosus, psoriasis, and gout. In early stages, IL-33 released from injured epithelium activates neighbouring tissues and recruits immune cells. In later stages, IL-33 functions as a pro-fibrogenic factor that may also support barrier maintenance. These findings underscore the pleiotropic and context-dependent nature of IL-33 signalling⁹.

10. Conclusion

IL-33 exhibits multifaceted immunomodulatory effects across a broad spectrum of inflammatory diseases. It promotes Th2 cytokine synthesis and contributes to Th2-related pathologies such as asthma and atopic dermatitis. Conversely, IL-33 confers protection in cardiovascular and metabolic disorders, including atherosclerosis, obesity, cardiac remodelling, and T2D. Understanding the optimal timing and context for modulating the IL-33/ST2 signalling might prove essential for the development of effective therapeutic strategies.

Acknowledgements

The authors gratefully acknowledge the support of

the College of Pharmacy of the University of Babylon.

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Conflicts of interest

None exist.

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HOW TO CITE:

Ismail H., Alzubaidi F.A., Oubaid E.N. Targeting the IL-33/ST2 signalling pathway as a therapeutic strategy for the treatment of multiple inflammatory diseases. *Pharmakeftiki* 37(2s), 521-524, 2025. <https://doi.org/10.60988/p.v37i2S.280>

Non-pharmacological pain management strategies: a mini-review

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KEY WORDS:

non-pharmacological intervention; transcutaneous electrical nerve stimulation; cognitive behavioural therapy; Quran therapy; strategies

ARTICLE INFO:

Received: January 20, 2025

Revised: February 22, 2025

Accepted: February 24, 2025

Available online: October 10, 2025

ABSTRACT

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Non-pharmacological interventions offer several advantages, including reduced medical costs, increased accessibility, broader applicability, ease of use, and greater patient satisfaction. These strategies may also reduce the patients' pain levels while minimizing the adverse effects commonly associated with pharmacological treatments. However, non-pharmacological techniques have limitations concerning their applicability and efficacy. They are classified according to their mechanisms of action into four main categories: mind-body interventions, biological treatments, manipulative and body-based practices, and energy-based strategies. Common examples include the transcutaneous electrical nerve stimulation, heat therapy, massage therapy, light therapy, cognitive behavioural therapy, acupuncture, yoga, virtual reality therapy, Quran therapy, and aromatherapy. This mini-review provides an overview of the mechanisms through which these strategies alleviate pain, and highlights the advantages that contribute to their growing global appeal.

1. Introduction

According to the International Association for the Study of Pain, pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage¹. Pain negatively affects quality of life, economic status, and family

well-being, often due to lost work-days, increased healthcare costs, and physical incapacity². Millions of individuals worldwide suffer from pain both in hospital settings and at home. In acutely ill patients, pain is linked to adverse physiological and psychological outcomes³.

Pain mechanisms involve three

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fundamental components: nociceptors (which identify pain stimuli), the spinal cord (which transmits and modulates signals), and the brain (which interprets and regulates the entire pain experience)⁴. Pain pathways are complex and dynamic, encompassing sensory, cognitive, and behavioural elements. These systems have evolved in order to coordinate appropriate reactions to noxious stimuli, ranging from primitive spinal reflexes to consciously experienced emotional responses⁵.

Non-pharmacological treatments (including behavioural, cognitive, rehabilitative, and complementary therapies) can alleviate the stress associated with hospitalization, enhance the quality of life, and prevent physiological and behavioural changes in neonates. Techniques such as oral sweet solutions, sucking, positioning, skin-to-skin contact, and environmental adjustments reduce stress and may enhance medication efficacy or reduce reliance on pharmacological agents, thus mitigating drug-related side effects⁶.

Non-pharmacological interventions are categorized by their mechanisms of action into four modalities: mind-body interventions, biologically based treatments, manipulative and body-based practices, and energy therapies. Examples include meditation, herbal and essential oil therapies, massage, and energy-based approaches such as reiki and therapeutic touch⁷.

This mini-review aimed at describing the mechanisms of non-pharmacological pain management strategies across different types of pain, along with their limitations, their advantages over pharmacological treatments, and their potential for global implementation – not merely as adjunct therapies, but as viable primary options.

2. Advantages of non-pharmacological pain management strategies

Non-pharmacological strategies have demonstrated beneficial effects in reducing pain intensity and minimizing the side effects associated with pharmacological treatments³. These approaches can reduce or even replace the need for pharmacological interven-

tion, thereby lowering harm and improving patients' quality of life. Major advantages include ease of application, increased safety, accessibility for millions of people, and ease of learning, making them globally implementable⁸.

3. Some limitations of non-pharmacological pain management strategies

Some limitations include the need for a time-intensive application, the need for a long-term follow-up, and the reduced efficacy when used concurrently with pharmacological treatments for severe pain. Additionally, certain interventions may be culturally specific or context-dependent⁷.

4. Non-pharmacological strategies for pain management and their underlying mechanisms

4.1. Transcutaneous electrical nerve stimulation

Studies suggest that this strategy influences the expression and activity of pain-related ion channels in the peripheral nervous system (including voltage-gated sodium channels and transient receptor potential, TRP, channels), thereby modulating nociceptive excitability and neurotransmission. It also increases endogenous opioid and serotonin levels in the brain, contributing to descending pain modulation.

4.2. Heat therapy and cryotherapy

These involve applying heat or cold to the affected body areas for pain relief. Heat sources include hot packs, paraffin wax, and infrared radiation for superficial or deep heating. Heat therapy improves blood flow, relaxes muscles, and activates thermosensitive TRP channels, thereby modulating peripheral sensitivity and central thermal regulation. Clinical trials suggest its efficacy in reducing neuropathic pain in conditions such as diabetic neuropathy, chemotherapy-induced peripheral neuropathy, and post-spinal cord injury pain.

Cryotherapy uses agents such as ice packs, cold

compresses, and refrigerant sprays to lower tissue temperature. It inhibits inflammation and slows nerve conduction velocity *via* vasoconstriction and reduced release of serotonin and glutamate.

4.3. Massage therapy

Massage is increasingly recognized as effective in managing chronic pain. Although its mechanisms are not fully understood, the gating control theory posits that massage activates spinal interneuron inhibition, thereby suppressing pain signal transmission. Massage may also normalize sensory input, improve circulation, reduce muscle tension and trigger points, and restore descending inhibition, thereby preventing central sensitization.

4.4. Light therapy

Infrared light and laser-based approaches may enhance nitric oxide production and mitochondrial function, thereby modulating neuronal pathways relevant to analgesia. Specific wavelengths – such as far infrared and therapeutic laser – promote tissue healing and pain reduction. Applied either transcranially or to painful skin regions, light therapy operates through mechanisms involving retinal photoreceptors, mitochondrial activation, anti-inflammatory effects, and neural modulation.

4.5. Cognitive behavioural therapy

Cognitive behavioural therapy enhances the expression of endogenous opioid β -endorphin and reduces pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α), yielding both analgesic and anti-inflammatory effects.

4.6. Acupuncture

Traditional stimulation of acupoints *via* manual or electrical methods enhances activity of A δ and C fibers and stimulates release of enkephalins, endorphins, and dynorphins. These bind to opioid recep-

tors in the peripheral and the central nervous systems in order to suppress pain perception.

4.7. Yoga and tai chi

These modalities increase anti-inflammatory cytokines such as IL-10 and IL-4, while reducing pro-inflammatory mediators such as TNF- α , IL-6, and IL-1 β .

4.8. Virtual reality therapy

Virtual reality-based approaches modulate neuronal plasticity and affect brain regions related to anxiety and pain. They may interrupt or reverse central sensitization; a key factor in the transition from acute to chronic pain.

4.9. Mindfulness strategies

These employ top-down regulation from the prefrontal cortex and anterior cingulate cortex in order to inhibit pain signalling. Studies suggest mindfulness enhances modulatory neurotransmitters (such as endogenous opioids, cannabinoids, and serotonin) while downregulating inflammatory gene and protein expression. This dual effect reduces both peripheral and central sensitization⁴.

4.10. Quran therapy

This method reduces labor pain *via* three interrelated mechanisms. First, the rhythmic recitation of murottal Quran stimulates the neuroendocrine system, inducing a calming haemodynamic effect similar to music therapy. Second, murottal Quran-listening provides a distraction from pain, allowing patients to focus and relax. Third, the spiritual component supports emotional resilience and addresses psychosocial challenges associated with illness⁹.

4.11. Aromatherapy

Aromatherapy activates the limbic system *via* inhalation and olfactory pathways, modulates neuro-

transmitter levels, and provides anti-inflammatory benefits. It also diverts attention from pain, thereby promoting relaxation and pleasant sensory experiences¹⁰.

5. Conclusion

Non-pharmacological pain management strategies encompass a broad range of approaches and are classified based on their physiological mechanisms. These strategies offer considerable benefits over pharmacological treatments by reducing analgesic dependence and providing cost-effective, culturally adaptable alternatives. Their growing potential as primary pain management tools underscores the importance of ongoing research to fully realize their

benefits and minimize their limitations.

Acknowledgements

We gratefully acknowledge the College of Pharmacy of the University of Babylon, for its invaluable support and assistance.

Conflicts of interest

None exist.

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HOW TO CITE:

Jawad R.H., Fadheel Q.J. Non-pharmacological pain management strategies: a mini-review. *Pharmakeftiki* 37(2s), 525-529, 2025. <https://doi.org/10.60988/p.v37i2S.281>

Genetic polymorphisms and adverse effects that affect the natalizumab clinical response: a review

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KEY WORDS:
natalizumab; genetic
polymorphisms; multiple
sclerosis; mutations;
response

ARTICLE INFO:

Received: November 12, 2024

Revised: January 12, 2025

Accepted: February 05, 2025

Available online: October 10, 2025

ABSTRACT

The clinical response to natalizumab in patients with multiple sclerosis (MS) may be significantly influenced by genetic variation. Mutations in genes related to the drug's mechanism of action or the pathological milieu of MS can contribute substantially to interindividual differences in treatment outcomes. This review aims to provide an overview of previous studies that have examined genetic polymorphisms associated with the clinical efficacy of natalizumab. A systematic literature search was conducted across the PubMed, Google Scholar, and ResearchGate databases using targeted keywords relevant to the subject matter. Several genetic loci were found to be linked to natalizumab responsiveness, including the integrin subunit alpha 4 (*ITGA4*), the nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase 1 (*NQO1*), the glutathione S-transferase pi 1 (*GSTP1*), the glycoprotein VI platelet (*GP6*), and the alpha serine/threonine-protein kinase (*AKT1*) genes. Further research is warranted in order to explore the influence of genetic factors on treatment response across diverse populations. By synthesizing existing evidence, this review underscores the role of pharmacogenomics in optimizing the use of natalizumab and highlights its efficacy and safety in improving clinical outcomes.

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1. Introduction

Natalizumab, a recombinant humanized monoclonal antibody targeting the $\alpha 4$ -integrin (*ITGA4*) component of the cell adhesion molecule, is among the most ef-

fective therapies for active relapsing-remitting multiple sclerosis (RRMS)¹ and has also been approved for the treatment of inflammatory bowel disease². In addition to previously identified factors, emerging research indicates that

genetic variation may significantly influence therapeutic response in patients with multiple sclerosis (MS). Mutations in genes associated with natalizumab's mechanism of action or the pathological features of MS can substantially modulate treatment efficacy. This review aimed at synthesizing prior research investigating genetic polymorphisms that affect the clinical responsiveness to natalizumab.

2. Methodology

A comprehensive and systematic literature search was conducted using the PubMed, Google Scholar, and ResearchGate databases. Search keywords included "natalizumab", "genetic polymorphism", "multiple sclerosis", and "response". The initial query identified fifteen distinct publications. Two of the authors independently reviewed the titles and the abstracts of these studies, excluding those deemed irrelevant. Search results were subsequently imported into Mendeley, and articles were selected based on the following inclusion criteria: (i) studies examining associations between clinical response to natalizumab and genetic variation, (ii) availability of sufficient extractable data, and (iii) reporting of genotype or allele frequencies in patient cohorts. Exclusion criteria included duplicate publications, case studies, book chapters, letters to the editor, and conference proceedings. A standardized data extraction checklist was used in order to compile relevant information, including the first author's name, journal and year of publication, location, age range or mean age, genotyping method, and genotype frequencies in case and control groups. To ensure reliability, the three authors independently extracted the data. Discrepancies were resolved by consensus.

3. Response to natalizumab in MS

The integration of pharmacogenetics and pharmacogenomics into MS therapy aims to identify genetic markers that can predict drug toxicity and treatment response. Recent studies suggest that genetic diversity may play a critical role in interindividual variability in natalizumab efficacy. Variants affecting ei-

ther the drug's mechanism of action or MS pathology are of particular relevance.

A point mutation in exon 24 of the *ITGA4* gene results in a substitution of arginine (CGG) with glutamine (CAG) at position 3061. This 3061G variant may alter the conformation of the subunit, thereby increasing its affinity for vascular cell adhesion molecule 1 in MS patients³.

Genetic polymorphisms have also been associated with differential drug response and increased risk of adverse reactions (Table 1). A study from Iraq has linked natalizumab response to the presence of the +3061 (G>A) missense mutation in *ITGA4*. The AG genotype was identified as a potential biomarker for non-responsiveness among Iraqi MS patients, thereby predicting ineffectiveness prior to treatment initiation⁴.

In contrast, a different study involving 60 healthy individuals and 66 Iraqi MS patients has found no significant association between natalizumab responsiveness and the *ITGA4* polymorphism rs113276800 (-269C/A)⁵. Residues Gln-152, Lys-201, and Lys-256 of the *ITGA4*-encoded subunit are considered critical for natalizumab binding⁶. Moreover, in a cohort of 62 MS patients, no associations were found between natalizumab response and the rs200000911 (A/T) missense mutation, intronic mutation rs936587744 (T/C), and rs2305588 (T/C)⁷.

Another potential mechanism by which natalizumab exerts its effects is through the reduction of oxidative stress. Antioxidant activity may correlate with improved clinical outcomes following treatment. The detoxifying enzyme nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase 1 (NQO1), which inhibits reactive oxygen species generation, was studied for its rs1800566 C/A polymorphism. Similarly, the rs1695 A/G polymorphism in the gene of the phase II detoxification enzyme glutathione S-transferase pi 1 (*GSTP1*) was examined for its role in treatment response⁸. Alexoudi *et al.*⁸ have analysed the impact of *NQO1* and *GSTP1* genotypes on natalizumab efficacy in 130 MS patients. The study also examined correlations with clinical subtypes and evaluated the combined effects of the *GSTP1* 313 A/G (rs1695) and *NQO1* 609

Table 1. Overview of studies that assess the clinical response of multiple sclerosis (MS) patients to natalizumab, based on genetic variations. Abbreviations used: AKT1, alpha serine/threonine-protein kinase; GP6, glycoprotein VI platelet; GSTP1, glutathione S-transferase pi 1; ITGA4, integrin subunit alpha 4; NQO1, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase 1.

Study	Country and year of the study	Study design	Studied polymorphisms	Genotype proportion		Study outcome
				Responders	Non-responders	
Ahmed <i>et al.</i> ⁴	Iraq (2023)	single-centered, prospective, cross-sectional study	<i>ITGA4</i> rs1143676 (+3061, G/A) (N=59)	GG: 0 AG: 8 AA: 21 (N=29)	GG: 0 AG: 17 AA: 13 (N=30)	the AG genotype has been shown to significantly raise the likelihood of not responding to natalizumab
Khaleil <i>et al.</i> ⁵	Iraq (2022)	single-centered, prospective, case-controlled study	<i>ITGA4</i> rs113276800 (-269, C/A) (N=66)	CC: 16 CA: 14 CC: 2 (N=32)	CC: 18 CA: 14 CC: 2 (N=34)	the rs113276800 polymorphism does not affect how MS patients respond to natalizumab
Ahmed <i>et al.</i> ⁷	Iraq (2023)	single-centered prospective cross sectional study	<i>ITGA4</i> rs200000911 (256, A/T) (N=62) <i>ITGA4</i> rs936587744 (intronic, T/C) (N=62) <i>ITGA4</i> rs2305588 (intronic, T/C) (N=62)	AA: 23 AT: 9 TT: 0 (N=32) TT: 18 TC: 14 CC: 0 (N=32) TT: 22 TC: 10 CC: 0 (N=32)	AA: 22 AT: 8 TT: 0 (N=30) TT: 17 TC: 13 CC: 0 (N=30) TT: 23 TC: 4 CC: 3 (N=30)	no correlation was found between the missense mutation rs200000911 (A/T), rs936587744 (T/C), and rs2305588 (T/C) intronic mutations with the response to natalizumab
Alexoudi <i>et al.</i> ⁸	Greece (2016)	single-centered, prospective study	<i>GSTP1</i> rs1695 (313, A/G) (N=129) <i>NQO1</i> rs1800566 (609, C/T) (N=124)	AA: 54 AG: 48 GG: 12 (N=114) CC: 63 CT: 41 TT: 5 (N=109)	AA: 6 AG: 9 GG: 0 (N=15) CC: 5 CT: 9 TT: 1 (N=15)	a significantly greater frequency of double <i>GSTP1</i> and <i>NQO1</i> mutant polymorphisms was observed when comparing non-responders to responders
Al-Mojel <i>et al.</i> ⁹	Kuwait (2019)	single-centered, prospective study	<i>GP6</i> rs2304166 (940, C/G) exome cohort (N=33) replicate cohort (N=86)	GG: 7 GC: 12 CC: 4 (N=23) GG: 20 GC: 37 CC: 10 (N=67)	GG: 0 GC: 3 CC: 7 (N=10) GG: 1 GC: 4 CC: 14 (N=19)	a poor response to natalizumab is linked to the CC genotype; rs2304166 in <i>GP6</i> may be used as a genetic predictor of how MS patients respond to natalizumab
Rossi <i>et al.</i> ¹⁰	Italy (2013)	prospective study	<i>AKT1</i> rs2498804 (G/T) (N=67)	GG: 29 GT/TT: 38 (N=67)	none	T allele carriers were shown to have a lower lymphocyte count and a lower risk of recurrence after therapy

C/T (rs1800566) polymorphisms⁸. Given the role of these enzymes in mitigating inflammation-induced oxidative stress, genotype variation may influence disease progression. Notably, double mutant genotypes in *GSTP1* and *NQO1* were more prevalent among non-responders, while wild-type or singly mutated variants correlated with better clinical outcomes⁸.

A missense mutation (rs2304166) in the glycoprotein VI platelet (GP6) gene, which is involved in collagen-induced platelet aggregation and thrombus formation, has been identified *via* exome sequencing. In MS patients, the homozygous CC genotype has been associated with poor response to natalizumab⁹. The study evaluated 33 patients *via* sequencing, and has subsequently validated findings in an expanded cohort of 86 MS patients, thereby confirming the association⁹.

Finally, the rs2498804 G/T polymorphism in the AKT1 gene (encoding a serine/threonine protein kinase involved in cell proliferation) has been analysed by Rossi *et al.*¹⁰ in 67 Italian RRMS patients. The T allele was linked to reduced lymphocyte counts and lower relapse risk following natalizumab therapy¹⁰.

4. Conclusion

Poor responsiveness to natalizumab has been linked to the homozygous CC genotype of the rs2304166 variant in *GP6*, which may be associated with progressive MS disability. Continued research is needed in order to elucidate the relationship between specific genetic variants and natalizumab responsiveness, with a focus on identifying the most influential genes.

Acknowledgements

The authors gratefully acknowledge the Scientific Committee of the University of Baghdad's College of Pharmacy.

Conflicts of interest

None exist.

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HOW TO CITE:

Yawuz M., Thamir F., Imad S. Genetic polymorphisms and adverse effects that affect the natalizumab clinical response: a review. *Pharmakeftiki* 37(2s), 530-534, 2025. <https://doi.org/10.60988/p.v37i2S.282>