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GENERAL INFORMATION

The journal aims to promote optimum drug therapy by providing original research articles and review articles covering important aspects of clinical and applied Pharmacology and Therapeutics. It also covers important areas in Medicine. The focus of the journal comprises drug evaluation reviews, which provide a detailed focus on different properties, i.e. dosage, toxicology, drugs interactions and a place in therapy of both newer and established drugs. Other review articles offer state-of-the-art literature surveys covering broader topics. Practical therapeutics articles and leading articles provide recommendations for specific situations of connections or emerging areas, respectively.

The *scientific standard* of the articles, which are accepted for publication, is controlled by the Editorial Board or by other experts in the various fields of Pharmacology, Pharmacokinetics, Therapeutics and Medicine.

Additionally, the journal has published, in special issues, articles on works presented at:

- *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry and Pharmacy of the University of Patras, Hellas*
- *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*
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- *1st Greek Scientific Society of Cosmetology Conference*

INSTRUCTIONS FOR AUTHORS

English is the preferred language for all articles. However, articles in French, can also be submitted, provided they are accompanied by an English summary. Drugs must be referred to by their generic or chemical name, but may be identified by trade name in parenthesis or footnote.

The journal requires that all authors have an ORCID at the time of publication.

All articles should be submitted to the Editor-in-Chief (Dr Eleni S. Plessa) *via* e-mail.

The following format should be used for the manuscripts:

On the first page, *the Title*, *Author(s)* and *the Name of the Institution* at which the work was done, the complete address of the corresponding author, including postal area code number, should be given under the rubric *Corresponding author* and the *Type of Article* (e.g. original paper, review paper).

Research Articles should follow the general form:
Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, Funding, Conflict of Interest, References.

Review Articles should follow the general form*:*
Abstract, Keywords, Introduction, Related Sections, Discussion, Conclusions, Future Directions, Acknowledgements, Funding, Conflict of Interest, References

Abstract: An Abstract in English (maximum length 250 words) must accompany all manuscripts.

Keywords: A list of keywords should be submitted after Abstract.

References: These should be numbered in the article and listed under References in order of their appearance in the text. The author(s) surname followed by the initials should be given first, then the complete title of the article, the name of the Journal or Magazine (abbreviated according to the Index Medicus), the volume number, page numbers and year of publication in parenthesis.

PROCEEDINGS OF THE 1ST INTERNATIONAL BABYLON CONFERENCE ON CLINICAL AND EXPERIMENTAL PHARMACOLOGICAL RESEARCH

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edited by

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and

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University of Babylon, Iraq


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1st International Babylon Conference on Clinical and Experimental Pharmacological Research
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1st International Babylon Conference on Clinical and Experimental Pharmacological Research

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CONTENTS

Since 1987
REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION | 38 (Sup2):1-2 (2024) Rcpp

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Open Access | **Editorial**

The 1st International Babylon Conference on Clinical and Experimental Pharmacological Research: mapping the Iraqi pharmacological research activity and priorities

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Abstract
The proceedings of the 1st International Babylon Conference on Clinical and Experimental Pharmacological Research (taking place at the College of Pharmacy of the University of Babylon, in Hillah, Iraq; May 4-5, 2024) are hereby hosted by the *Review of Clinical Pharmacology and Pharmacokinetics – International Edition*; a journal that has provided a significant service to the international pharmacological community since 1987. These proceedings include a total of 50 papers (including this editorial) and provide a representative overview of the conference's rich scientific programme. The latter has served two objectives: (i) to facilitate the mapping of the Iraqi pharmacological research and priorities, and (ii) to enable the establishment of international collaborations in the field. In that respect, the proceedings host the inspiring messages of several conference keynote speakers from abroad, and feature a variety of clinical and experimental studies that well relate to the priorities currently set by the Iraqi pharmacological community.

KEYWORDS
Iraq, pharmacological research, ethnopharmacology, antibiotic resistance, nanopharmacology

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MAIN MESSAGE

The 1st International Babylon Conference on Clinical and Experimental Pharmacological Research is an international scientific meeting taking place at the College of Pharmacy of the University of Babylon (Hillah, Iraq) on the 4th and the 5th of May 2024. This international conference has been a long pursuit and ambition of our dear colleague Prof. Hussam Al-Humadi (the Dean of the hosting institution) that has finally come to realisation after several years of postponing due to the restrictions imposed by the COVID-19 pandemic. The event is organized under the auspices of the University of Babylon and its College of Pharmacy, as well as the Al-Mustaqbal University and its College of Pharmacy; all based in Hillah, Iraq. The members of the Steering, the Organizing, and the Scientific Committees of the conference have worked tirelessly in order to organize the first of, hopefully,

many international gatherings of the medical, biomedical, pharmaceutical, and wider scientific communities that are working (either directly or indirectly) towards the improvement of clinical therapeutics in Iraq and abroad; a regular and truly international event in which the members of the local pharmacological community can present their clinical and experimental research work, and a forum from which, potentially, an Iraqi (national) pharmacological society will emerge.

The proceedings of the 1st International Babylon Conference on Clinical and Experimental Pharmacological Research are hereby hosted by the *Review of Clinical Pharmacology and Pharmacokinetics – International Edition*; a journal that has provided a significant service to the international pharmacological community since 1987. These proceedings include a total of 50 papers (including this editorial) and provide a representative overview of the conference's rich scientific programme. The latter has served two objectives: (i) to facilitate the mapping of the Iraqi pharmacological research and priorities, and (ii) to enable the establishment of international collaborations in the field. In that respect, the proceedings host the inspiring messages of several conference keynote speakers from abroad, and feature a variety of clinical and experimental studies that well relate to the priorities currently set by the Iraqi pharmacological community. Among these studies, readers will identify that popular themes include the pharmacological study and pharmaceutical analysis of medicinal herb extracts, the study of issues related to the emergence of antibiotic resistance, and the improvement of the pharmacokinetic performance of drugs through the adoption of nanotechnology. These themes align well with the abundance of recent studies on Iraqi ethnopharmacology [1,2], the worrying recent data regarding the antibiotic misuse in Iraq [3,4], and the challenges faced by the Iraqi pharmaceutical sector [5,6], respectively.

Our work as editors has focused on ensuring that the published works in these proceedings are presented with accuracy, clarity, and consistency. In this regard, we are grateful to all contributors for their fruitful collaboration in ensuring that their works are presented to the highest standard possible, despite the limitations imposed by the word count restrictions of the applied manuscript format. We hope that the readers of these proceedings will enjoy the informative nature of the papers included in them, and will benefit from the messages that they convey.

ACKNOWLEDGEMENTS

The proceedings editors are grateful to Dr Eleni Plessa, Prof. Falah H. Hussein, and Prof. Hussam W. Al-Humadi, for their enthusiastic help and continuous support. One of the proceedings editors (AZ) acknowledges acting as the British Pharmacological Society Ambassador to the University of Babylon.

CONFLICT OF INTEREST STATEMENT

Both of the proceedings editors (and authors of this editorial) serve as members of the Editorial Board of the hosting journal.

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Since 1987
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Open Access | Keynote Speech

Chronic nutritional deficit resulting in multiorgan disease presentations in remote Pakistan: case reviews from natural disaster settings

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Abstract
The aftermath of monsoon rains that fell in Pakistan from June to November 2022 were catastrophic as millions of people were affected, water systems were damaged, and agricultural production was affected. The floods aggravated the already existing nutrition crisis which largely increased children's vulnerability. Disaster Medicine and Dentistry (DMD), a UK-registered consultancy organization, provided support to the affected households in Dera Ismail Khan through the provision of water pumps, food rations, and climate-resistant houses. It also established a mobile medical unit that provided medical services to affected households. All the children that came for consultation were far below the expected height or weight range for their age, thus showing signs of chronic malnutrition. They also presented with multiorgan diseases as a result of the chronic malnutrition, and DMD's onsite and remote consultants managed them all. We took a wholistic approach to managing the nutrition crisis and the floods, which significantly improved the health outcomes of the affected individuals and households.

KEYWORDS
malnutrition, natural disaster, multiorgan disease, Pakistan, disease presentation

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1. INTRODUCTION

From mid-June to November 2022, Pakistan experienced extreme monsoon rainfall, which ultimately caused floods. The floods affected at least 33 million people [1] and killed approximately 2,000 people. Half of the people that were affected were children, making them more vulnerable [2]. Most water systems were damaged, forcing more than 5.4 million people to rely on contaminated water sources and exposing them to risk of disease outbreaks [1]. Making the situation even more difficult, the floods submerged one-third of the country [3], destroying approximately 4 million acres of agricultural land and more than 150,000 acres of land with standing crops, thereby causing significant shortages in food production and supplies [4]. About 13% of the health facilities were also destroyed [5], deepening pre-existing inequities and disparities in health whereby more than 50% of the

population do not have access to health services. On average, 40.2% of under-5 children are stunted, 28.9% are underweight, while 17.7% suffer from wasting [6]. The floods aggravated the nutrition crisis in the affected districts to an average malnutrition rate, thereby impacting over 3.5 million children. However, due to poor documentation and a large number of unreported cases, malnutrition is underreported.

Dera Ismail Khan (DI Khan; located in Khyber Pakhtunkhwa, in Pakistan) is one of the districts that were hugely affected by the 2022 floods. The district is one of the largest in Pakistan, spreading over 7,326 sq. km and bearing a population of 1,627,132 people [7]. The area's main economic stream is agriculture, which was heavily affected. Disaster Medicine and Dentistry (DMD), a UK-registered consultancy organization, provided support to the affected households.

We, herein, delve into the challenging reality of malnutrition in the remote villages of DI Khan, Pakistan. We highlight the struggles faced by malnourished children, and how DMD's onsite and remote consultants made an impact.

2. METHODS

Using our networks in the humanitarian field, we collaborated with independent volunteers in the grassroot sector in the area. The aim was to provide direct health care and support to affected households. In order to increase medical healthcare coverage in the area, we deployed a mobile medical service unit that reached out in different villages within DI Khan. We accentuated on history taking in our patients so as to help diagnosis. All these data were documented in patient files and retrospectively retrieved for analysis. We also provided climate resistant housing to some households (built on higher ground), enhanced access to clean water by installing water pumps, educated families on basic water purification methods, and delivered food rations, including ready-to-use therapeutic food (RUTF) sachets, to vulnerable families. We then ran six-month follow-up clinics to ensure proper management of the children. Finally, we conducted a retrospective data analysis of the data we collected from some of the cases we managed in DI Khan.

3. RESULTS

The average age of the under-5 children that presented with issues at our mobile health unit was 3.5 years, with an average height of 62.8 cm, and a weight of 9.6 kg. Individually, all the children were far below the expected height or weight range for their age. Through their history, all children showed signs of chronic malnutrition as they presented with sunken eyes, bowed legs, swollen belly, lethargy, osteoporosis, skin issues, and brittle nails among other sign of malnutrition, in addition to infections. Their guardians also showed signs of malnutrition as some of them came frail. The majority claimed to have lost their houses, their food, and crops, and that explained the micronutrient deficiencies, infections, insect bites, shock, and fear.

The climate-resilient houses we built for the households reduced the stress levels and shock that the families faced, as they now had a secure place to stay. It further demonstrated the possibility of resilient communities, and provided less exposure to contaminants and insects (therefore providing protection). Fortified rice, lentils, and oil provided the much needed nutrients and energy to the households, thereby reducing the risk of disease, while RUTF helped the children to recover from the acute malnutrition, promoted rapid weight gain, restored depleted nutrient stores, and supported their overall growth and development. In addition, the access to safe, clean water contributed to the prevention of waterborne diseases such as cholera, typhoid fever, and dysentery. This wholistic approach we took to managing the flood situation significantly improved the health outcomes of the affected individuals and households.

4. DISCUSSION

The findings of our intervention revealed signs of chronic malnutrition which have a profound effect on the body, leading to multiorgan diseases and complications. During the early stages of growth and development, chronic malnutrition can lead to cognitive deficits, developmental delays, and learning difficulties, which all are linked to neural damage. Some children that presented in our clinics with osteoporosis, weakness, and decreased mobility, all showed signs of musculoskeletal system impairment which is also a result of chronic malnutrition. The skin issues due to the lack of vitamins A and C, also revealed a linkage between the damaged integumentary system and chronic malnutrition.

5. CONCLUSION

Disasters have been associated with negative health outcomes that greatly affect the most vulnerable parts of a population (such as the children). Rapid response and collective actions to reduce the occurrence of outbreaks resulting from poor water, sanitation and hygiene, and lack of

food that comes along with natural disasters is essential. In children, malnutrition because of disasters is inevitable. However, addressing malnutrition needs a comprehensive approach that focuses on improving dietary intake, promoting nutritional education, and addressing underlying environmental, economic, and social factors, as it can also lead to multigenerational malnutrition. During pregnancy, inadequate maternal nutrition can lead to low birth weight, stunted growth, and increased risk to infections in newborns, which constitutes intergenerational malnutrition. In addition, it is believed that maternal malnutrition can induce epigenetic changes in the developing foetus, thereby creating a cycle of subsequent generational malnutrition.

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CONFLICT OF INTEREST STATEMENT
The author declares no conflicts of interest.

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Open Access | Keynote Speech

Inorganic polymers as drug carriers: opportunities and challenges

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Abstract

Innovative methods and significant developments in designing new synthetic inorganic materials have been used to overcome limitations of current drug delivery systems. Inorganic polymers are widely used in the field of biomedicine, imaging, tissue engineering and drug delivery because of their bioactivity, biocompatibility, and stability. A few of the more well-known wholly inorganic polymers are portland cement, silicon dioxide, polyanionic glasses (including titania- and aluminosilicate glasses), poly(sulphur nitride), polycrystalline diamond, graphite, poly(sulphur nitride), and aluminum-silicate materials. Inorganic polymers, especially those possessing significant porosity, are good potential candidates for the delivery of several drugs (anticancer, antibiotics, and anti-inflammatories), providing advantages such as encapsulation, controlled delivery, and improved targeting of drugs. Choosing a suitable drug carrier with a selective targeting potential also seems to be a very promising way for improving stability as well as selectivity. Despite all the advances, developing homogeneous inorganic polymers with narrow molecular weight distributions is a multidisciplinary challenge. The current keynote speech provides a review of the opportunities and challenges of using inorganic polymers as drug carriers.

KEYWORDS

inorganic polymers, drug delivery, controlled delivery, drug encapsulation, drug carriers

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MAIN MESSAGE

Inorganic polymers comprise a unique classification of polymers containing only inorganic atoms in their main chain, and have wide applications. The number and variety of elements as well as the tailoring of new structures of these polymers can offer them unique size- and shape-dependent physicochemical properties, high thermal stability due to strong bond formations, and high loading capacities; these properties along with the high abundance of inorganic materials in the earth's crust, are the main reasons to attend inorganic polymers as special materials with unique properties. Additionally, these structures can also be conjugated with targeting agents to improve their therapeutic effects. The different set of applications of inorganic polymers are: (i) tumour targeting, photothermal therapy, and immunotherapy mediators,

(ii) imaging agents [1], and (iii) the delivery of bioactive molecules such as drugs [2,3], genetic material, *etc*.

Polysiloxanes, polysilanes, polyphosphazenes, polyoxometalates, zeolites, other aluminumsilicates (including natural minerals and industrial wastes like kaolin, fly-ash, and slag from blast furnace), and polymeric carbons (such as diamonds and graphite) are some examples of inorganic polymers. Herein, the opportunities and challenges of using these polymers as drug delivery systems are discussed.

Polysiloxane and polysilane due to their biocompatibility, stability, optical transparency, and low toxicity are broadly used in the production of biomedical devices. Although, the hydrophobic structure of these types of polymers can be used as a hydrophobic drug carrier for molecules such as nifedipine or ibuprofen, this property barricades their application in drug delivery systems (such as those involving cell adhesion, long-term cell culture, or surgical implants). Moreover, they can be easily contaminated by pathogens and proteins. In order to improve hydrophilicity in these types of inorganic polymers, the functionalization of their surface has been attempted by using several physical and chemical treatments.

Polyphosphazenes with a phosphorus-nitrogen backbone, due to their biological performance, biocompatibility, and degradability, are used for antimicrobial and anticancer agents, drug and gene delivery, vaccine immunoadjuvants, and tissue engineering. It is known that macromolecules can selectively accumulate in malignant tissues due to a phenomenon known as the “enhanced permeation and retention effect” (“EPR effect”) [4]. This happens because the uncontrolled growth of tumours makes them permeable to macromolecules, due to defects on the surrounding vascular and lymphatic structures. Therefore, the selective targeting of tumours and cancer cells might be achieved by using several formulations of functionalized phosphazene.

Polyoxometalates (POMs) have been considered as novel, low-cost, transition metal oxide, nanosized inorganic drugs with anticancer, antibacterial, and antiviral properties [5]. Recently, POMs were found to be promising anticancer drug candidates [6]. Despite the advantages of using purely inorganic POMs, these compounds mostly suffer from side-effects like high and long-term toxicity, thereby impeding their clinical application. Therefore, the functionalization and encapsulation of POMs with organic moieties in order to synthesize modified organic-inorganic hybrids can not only reduce the toxicity of the POMs, but also increase their potential as chemotherapeutic agents.

The tunable structures, various pore sizes, chemical stability, high loading efficacy, and biocompatibility of zeolites make them attractive as drug delivery systems. The ion exchange capacity of zeolites is the most desirable function of a drug release mechanism. Various zeolites have been used as drug carriers for ibuprofen, 5-fluorouracil, diclofenac sodium, aspirin, doxorubicin hydrochloride, indomethacin, and levofloxacin. Despite the numerous advantages of zeolites as drug delivery systems, drug molecules can be released rapidly from the large pore size of zeolites, which is a serious challenge. Therefore, the modification of the zeolite is necessary so as to achieve a controlled release profile of the loaded drug [7].

Finally, nano-diamond-based drug delivery systems are excellent hydrophilic and hydrophobic drug carriers that can release drugs in response to light, pH, temperature, or enzymes. However, the major concern of nano-diamond carriers as drug delivery systems is their significant toxicity. In addition, they are characterized by a highly oxidized surface and carry numerous functional groups that directly affect the stability and agglomeration of nano-diamonds in a variety of media. Hence, mechanical and chemical surface modifications are used in order to reduce agglomeration and to enhance the stability and solubility in a variety of polar organic solvents, by substituting the functional groups on their surface [8].

Inorganic polymers are widely used in the field of biomedicine due to their biocompatibility, bioactivity, and stability; however, their use is limited by their nonspecific distribution throughout the body, leading to high doses, poor pharmacokinetics, rapid clearance, and major side-effects [9].

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Technology-enhanced learning in Pharmacology through non-linear storytelling

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Abstract

There is a growing body of literature highlighting the potential of game-based learning (GBL) in creating immersive, highly engaging, active learning experiences. Furthermore, advances in digital technology along with the demand for online learning during the COVID-19 pandemic has brought digital GBL to the forefront of innovative teaching practice. Here, I share some of the digital GBL strategies we have developed to support our pharmacology teaching. In collaboration with our students, we have co-created a series of "choose your own adventure" style games using Twine; an open-source storytelling game engine. Importantly, we have shown that adoption of these GBL approaches is highly effective in promoting student engagement, subject understanding, and learning community. Furthermore, I also discuss how AI tools can allow educators with little knowledge or experience of game development to create unique and engaging learning experiences for their students.

KEYWORDS

medical education, pharmacology, game-based learning, generative artificial intelligence, active learning

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MAIN MESSAGE

Game-based learning (GBL) has emerged as an innovative and engaging approach to teaching various subjects, and its application in medical education is gaining traction [1]. GBL allows students to apply their knowledge in an immersive, interactive, and yet low-stakes environment that promotes multimodal student engagement and participation [2]. Importantly, a student-centred approach through active GBL strategies in pharmacology has been shown to promote knowledge retention, and to deepen understanding and engagement when compared to traditional didactic modes of delivery such as lectures [3]. Educational games also provide immediate feedback, allowing students to learn from their mistakes in a risk-free environment; a particularly important feature for pharmacology, where accurate decision-making is vital. Importantly, games can replicate patient case studies, allowing students to explore clinical sce-

narios and to bridge the gap between theory and real-world application without compromising patient safety. Furthermore, GBL platforms can be readily tailored to the needs of the student, thereby providing a personalized learning experience. There is also growing evidence that GBL promotes key skill development beyond subject mastery, such as adaptability, critical thinking, and problem solving [4]. GBL has been shown to encourage teamwork and collaboration between students, effectively mirroring the interdisciplinary aspects of a typical workplace, enhancing employability, and preparing students for future teamworking. In addition, our work highlights the opportunity to build supportive learning communities and peer-to-peer learning through GBL.

Our work of developing GBL resources for pharmacology teaching has centred on the game engine Twine, which allows for the creation of "choose your own adventure" style games based on non-linear storytelling [5]. This game engine provides with its mechanics the flexibility to create bespoke resources that closely align to the course learning objectives and the difficulty level of the content, so as to balance entertainment with educational rigour. Educators must carefully align game-based activities with established learning objectives and outcomes. There may be resistance from traditionalists who are sceptical about the efficacy of GBL, or concerns about diverting resources from other essential educational components. A seamless integration plan, involving faculty collaboration and professional development, is necessary in order to address these challenges. Importantly, one of the biggest obstacles to the broad roll out of digital GBL is a lack of staff experience and training [6]. Upskilling programs and ongoing support are essential in order to equip educators with the skills and confidence needed to integrate GBL effectively into their teaching practice.

Our work also explores the opportunities for generative AI tools to empower educators to produce innovative and creative GBL experiences, regardless of technical capabilities or experience in utilizing game-based platforms. To address concerns about digital inequalities, we have developed our existing GBL resources using open-source tools that can be embedded in most virtual learning environments and can be integrated with learning analytics to monitor student engagement. The integration of GBL into pharmacology education represents a paradigm shift that holds immense promise for the future of medical education.

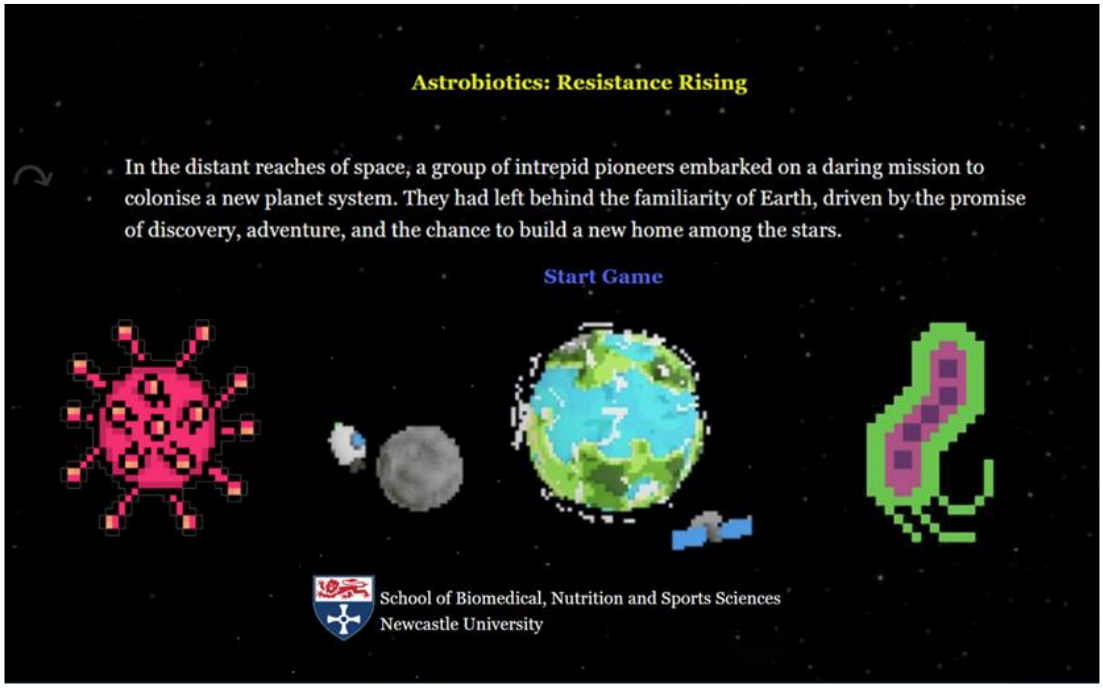

Figure 1. Screenshot from the homepage of "Astrobiotics: Resistance Rising"; a digital "choose your own adventure" style game built in Twine and developed in order to teach antibiotic resistance to first-year undergraduate pharmacology students at Newcastle University.

By addressing challenges and capitalising on the myriad benefits, educators can revolutionise the way pharmacology is taught. GBL engages students, enhances critical thinking, fosters collaboration, and provides a personalized learning experience. As technology advances and educational methodologies adapt, GBL stands as a powerful tool that can prepare graduates for the complexities of workplaces beyond the classroom.

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Tackling obesity from a nanomedicine perspective

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Abstract

Obesity is a worldwide issue that is progressively worsening. It can result in significant co-morbidities, including type 2 diabetes, cardiovascular disease, and obesity-related cancers. Current treatment options for obesity have several limitations, and the connection between obesity and cancer development is not well comprehended. We examine the current state and future prospects of obesity therapy, with a focus on the potential application of nanomedicine. The presentation emphasizes the necessity for further research in this field and how developments in cancer therapy using nanomedicines could be applied for the treatment of obesity, thereby providing a safe and effective treatment with reduced side-effects for those patients.

KEYWORDS

cancer, adiposopathy, magneto-mechanical stress, hyperthermia, nanomedicine

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MAIN MESSAGE

According to the World Health Organisation, obesity is defined as a body mass index (BMI) greater than 30 kg/m^2. Recent projections indicate that more than 1 billion people will be obese by 2030. Currently, there are approximately 500,000 deaths annually in the United States of America from obesity-related diseases. Obesity is a preventable and reversible chronic disease that predisposes patients to comorbidities due to underlying biological stresses such as chronic inflammation and the generation of reactive oxygen species (ROS) [1] that are associated with the disease. Common comorbidities include type 2 diabetes mellitus, dyslipidaemia, osteoarthritis, and coronary heart disease, but cancer is rarely mentioned or recognised as a serious comorbidity of obesity worldwide [2]. Obesity increases the likelihood of developing at least 13 different types of cancer (e.g. endometrial, pancreatic, breast, and colorectal), which equates to 200,000 new cases diagnosed each year [1]. In the US, approximately 14% of cancer deaths in men and 20% in women are attributed to patients who are overweight or obese [3]. Obesity constitutes an important determinant of cancer mortality, and intentional weight loss is the most effective way to reduce the risk of obesity-related cancers [3].

A balanced, low-calorie diet and an increased

physical activity are the cornerstones of obesity treatment and are essential to reduce energy intake and increase energy expenditure. However, lifestyle changes are challenging for patients due to genetic, biological, and environmental factors that affect long-term adherence. When lifestyle changes alone are not sufficient, adjunctive treatments are available. However, although a 5%-10% reduction in body mass can be achieved, those treatments are associated with unpleasant side-effects, and the failure rate of weight loss therapy is extremely high (90%), further reducing its prospects of reducing the prevalence of obesity and obesity-related comorbidities. Bariatric surgery and weight loss devices are highly invasive procedures that require intensive monitoring for life-threatening complications [4]. Therefore, new strategies are urgently needed to safely reduce the prevalence of obesity and its impact on obesity-related cancers. By integrating knowledge from already approved nano-based cancer therapies, anti-obesity nanomedicine can be developed and improved, thus reducing the future risk and mortality of obesity and obesity-associated cancer [5]. The similarities between adipocytes and cancer cells are characterised by their dysfunctional cellular behaviour [6]. During adipose tissue hypertrophy, adipocytes have high proliferation rates, and compromised vasculature and increased angiogenesis is developed, which are consistent with hallmarks of cancer growth [5]. Proinflammatory cytokines, chronic inflammation, and ROS generation from excess fatty acid influx are *bona fide* tumour promoters, and illustrate how increased weight gain contributes to an increased risk of tumour development [5]. In addition, the adipose tumour microenvironment enhances tumour survival and evasion of the host immune system [7]. This results in decreased survival and increased treatment complications in patients with cancer and obesity [6].

This presentation highlights the strong evidence for the use of nanomedicine in the treatment of obesity due to the improved safety and efficacy of advanced nanocarriers. For example, a nanoemulsion of orlistat developed by Chen *et al*. [8] showed a reduction in the incidence of adverse effects while reducing weight gain in a diet-induced obesity mouse model. Other organic nanocarriers have been investigated for the development of anti-obesity phytochemicals. The presentation focuses on the use of inorganic nanoparticles such as superparamagnetic iron oxide nanoparticles (SPIONs), which are FDA-approved inorganic nanomaterials with a good biodegradability, to combat obesity by providing examples from our research and the literature. We use a magneto-mechanical approach to induce oscillation of SPIONs by applying an alternating magnetic field to cause cell damage. Previous studies have shown that a high alternating magnetic field can cause significant delipidation of adipocytes through thermogenesis, with no signs of damage or mutation observed [9]. SPIONs can be administered orally or directly, and can be optimised for theranostic purposes. They can perform both magnetic resonance imaging and drug delivery.

Anti-obesity nanomedicine is still in its infancy, but as noted above, it has promising potential to reduce the development of co-morbidities, decrease patient mortality, and improve quality of life. The reduction in obesity prevalence could alleviate the financial burden on healthcare systems. Potential gaps for anti-obesity nanomedicine to reduce the incidence of obesity include managing drug-induced weight gain, improving the affordability, tolerability, and accessibility of treatment in a high-demand “rapid weight loss” market, or use in patients with morbid obesity.

Although there are several challenges to clinical translation, there is great promise for improving obesity therapy through nanomedicine developments in the near future. A more effective weight loss therapy could have a significant positive impact on obesity-related cancers, financial costs, and metabolic disorders, while it could also slow the increasing trend of obesity worldwide.

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The translational aspects of glucocorticoid biorhythmicity in modern therapeutics

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Abstract

Glucocorticoids are a class of steroid hormones, vital for mammalian life. They have a plethora of biological effects, mainly supporting metabolic, cognitive, and immunological functions. The pharmacological use of glucocorticoids makes them one of the most frequently prescribed drugs across all continents, and in all types of forms. Nevertheless, a number of serious adverse effects accompany the prolonged treatment with high doses of glucocorticoids. Research developments over the last 20 years have gradually reshaped the way we think about glucocorticoid-based therapeutics. Aside their circadian rhythm and their delayed regulatory influence over an extensive number of sensitive genes, glucocorticoids also possess an underlying, ultradian rhythm, and also exert rapid, non-genomic effects. The notion that chronicity of glucocorticoid stimulation may differentially modulate the type of biological effects of the hormone brings various chronopharmacological concepts on the table of modern glucocorticoid-based therapeutics.

KEYWORDS

glucocorticoids, biorhythmicity, chronopharmacology, pulsatile hormonal replacement, biosensor-assisted drug delivery systems

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MAIN MESSAGE

Glucocorticoids are a class of steroid hormones, vital for mammalian life. They have a plethora of biological effects, mainly supporting metabolic, cognitive, and immunological functions [1]. The fact that high concentrations of glucocorticoids show a strong immuno-suppressive activity was the key element for their wide use across medical specialties, in conditions where inflammatory responses of non-infectious origin constitute the principal pathophysiological mechanism, such as autoimmune disorders. The pharmacological use of (natural or mainly synthetic) glucocorticoids makes them one of the most frequently prescribed drugs across all continents, and in all types of forms (for example pills for oral use, liquid for intravenous administration or eyedrops, or particles released by pressurized inhalers). Nevertheless, a number of serious adverse effects accompany the prolonged treatment with high doses of glucocorti-

coids: patients may develop iatrogenic Cushing syndrome and/or suffer from various neuropsychiatric symptoms, such as fatigue, mood disorders or memory problems [2]. Given the fact that the levels of glucocorticoids increase in various physiological conditions (for instance, during stress) without causing the aforementioned adverse effects, it is crucial to elucidate the underlying dynamics that determine the biological effects of these hormones, to improve the efficacy and use of them in clinical therapeutics.

Research developments over the last 20 years have gradually reshaped the way we think about glucocorticoid-based therapeutics, although the new pieces of evidence have not been yet translated to a paradigm shift in the clinical setting. Traditionally, two features of glucocorticoid biology were considered as characteristic of these hormones [3]: (a) their circadian rhythm and (b) their regulatory influence over an extensive number of sensitive genes via the glucocorticoid receptor, acting as a transcription factor. Nowadays, these features have been complemented with many other, equally important, adding multiple layers to the complex biology (and pharmacology) of glucocorticoids.

For instance, the biorhythmicity of the natural glucocorticoid secretion by the adrenal glands does not just follow a circadian pattern, but a more complex one, combining circadian with ultradian components [4]. All of these components seem to have a biological significance and, thus, should not be disregarded when applying glucocorticoid-based treatments. Glucocorticoids are secreted periodically, in pulses, multiple times per day by the adrenal glands (ultradian rhythm), but the magnitude of the pulses is (under baseline conditions) determined by the time of the day (circadian rhythm). The ultradian rhythm of the hormonal secretion results from the continuous positive feedforward – negative feedback interplay with built-in delays between the anterior pituitary (stimulating the adrenal glands via corticotrophin (ACTH) to biosynthesize and release glucocorticoids) and adrenal glands (inhibiting the anterior pituitary via glucocorticoids to further release ACTH) [5]. The circadian rhythm superimposes the ultradian, originating from the hypothalamic (corticotrophin releasing hormone-mediated) influence on the anterior pituitary, which changes the sensitivity of the latter to the negative feedback control of glucocorticoids.

Moreover, various sources of evidence indicate that aside the genomic effects produced by the glucocorticoid receptors when activated by the hormone (which are mainly delayed effects), membranous variants of these receptors also trigger rapid, non-genomic processes, thereby significantly increasing the diversity of glucocorticoid functions [6]. When it comes to the brain, this diversity expands even more, since glucocorticoids target a second type of receptors, the mineralocorticoid receptors, with 10-fold greater affinity compared to the glucocorticoid receptors. Different variants of these receptors also produce rapid non-genomic as well as delayed genomic cellular events.

The notion that the chronicity of glucocorticoid stimulation may differentially modulate the type of biological effects of the hormone brings various chronopharmacological concepts on the table of modern glucocorticoid-based therapeutics, especially when it comes to neuropsychotherapeutics and managing brain-related adverse effects. For example, subcutaneous, non-pulsatile glucocorticoid administration correlates with poorer quality of sleep compared to a subcutaneous, pulsatile glucocorticoid delivery, mimicking the physiological complex pattern of the hormonal bioavailability. Similarly, oral treatment as well as subcutaneous non-pulsatile infusion were associated with poorer working memory performance under increased levels of cognitive demands, compared to the optimal, pulsatile treatment. These behavioural findings are coupled with brain imaging findings, showing that the functional connectivity of brain regions underlying emotional processing (amygdala, dorsal striatum, insula, orbitofrontal cortex) affecting attentional bias to and recognition accuracy of emotional cues change depending on the chronicity of glucocorticoid administration. Similarly, resting state networks and mood are also affected by the underlying mode of glucocorticoid biorhythmicity. These findings have been replicated in healthy volunteer studies as well as studies in patients with adrenocortical insufficiency [7-9].

The future of glucocorticoid therapeutics involves the use of biosensor-assisted drug delivery systems, in which dynamic detection of the hormonal biorhythm may determine the amount and temporal pattern of glucocorticoid release.

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The interaction of choline and one-carbon / folate metabolism derangements on the cardiac remodeling process with or without diabetes

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Abstract

Proper nutrition helps protect from illness and disease. Choline (Ch), an essential molecule of substantial importance for the optimal development and function of several biological systems, plays a crucial role in the pathway of one-carbon metabolism. On the other hand, Ch-deprivation (CD) has been linked with abnormal fat metabolism, insulin resistance, and myocardial dysfunction. The Ch-deficiency setting is an established experimental model of non-alcoholic steatohepatitis that resembles the human non-alcoholic fatty liver disease (NAFLD); a disease with constantly increasing incidence and prevalence. NAFLD, commonly associated with metabolic comorbidities such as obesity and type 2 diabetes mellitus, consists a high risk for cardiovascular disease. Experimental data of dietary CD through the administration of a Ch-deficient diet to rodents have revealed myocardial monocyte infiltration along with cardiac interstitial oedema and fibrosis, as well as a deleterious effect on cardiac valves that could lead to impaired heart mechanical properties which resemble to a restrictive pattern of cardiomyopathy characterised mainly by diastolic dysfunction. In a Ch-deprived diabetic experimental model, the diastolic heart failure has been characterized by a concentric hypertrophied myocardium, a left ventricular cavity with a thinner wall, and an increased left ventricular diastolic diameter, in addition to a left atrial dilatation that could also exert functional derangement and provoke arrhythmogenesis, thereby jeopardising cardiac output.

KEYWORDS

choline-deprivation, diabetes, diabetic cardiomyopathy, rat

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MAIN MESSAGE

Proper nutrition helps protect from illness and disease. Choline (Ch), a water soluble vitamin B co-factor, is an essential nutrient for which dietary intake recommendations have been established by the Institute of Medicine since 1998 and by the European Food Safety Authority since 2016. Ch is an intrinsic component of a number of important biomolecules, has a vital role in the one-carbon cycle by facilitating the metabolism of methyl groups,

and is also involved in cellular membrane integrity, in metabolic reactions (as methyl-donor), as well as in the biosynthesis of different macromolecules (including phospholipids and acetylcholine) [1].

On the other hand, Ch-deprivation (CD), observed either in physiological (pregnancy and lactation) or pathological (alcoholism and malnutrition) conditions, has attracted much consideration due to its association with various adverse health impacts that can occur across the lifespan. Prolonged CD causes decreased tissue S-adenosylmethionine levels, global DNA hypomethylation, and may lead to hepatocellular modifications and fatty liver induction [2,3]. The CD setting is an established experimental model of non-alcoholic steatohepatitis that resembles the human non-alcoholic fatty liver disease (NAFLD); a disease with constantly increasing incidence and prevalence. NAFLD, commonly associated with metabolic comorbidities such as obesity and type 2 diabetes mellitus, consists a high risk for cardiovascular disease [4].

Experimental data of dietary Ch-deprivation through the administration of a Ch-deficient diet to rodents have revealed myocardial monocyte infiltration along with cardiac interstitial oedema and fibrosis, as well as a deleterious effect on cardiac valves accompanied by concordant functional changes (i.e., impaired left ventricular contractility and diastolic function, probably due to reduced compliance of the myocardium) [5], and increased B-type natriuretic peptide levels that could be related to the impaired mitochondrial fatty acid oxidation. The dysregulation of matrix metalloproteinases (MMPs), more specifically of MMP-2 and MMP-9, and of their inhibitors that have been shown to be predominately responsible for cardiac matrix homeostasis [6], are probably among the factors contributing to myocardial dysfunction [7]. Furthermore, CD has been shown to modulate myocardial acetylcholinesterase (AChE) and important adenosine triphosphatase (ATPase; namely, Na^+,K^+-ATPase and Mg^{2+}-ATPase) activities [8]. Na^+,K^+-ATPase is an ion pump crucial for the electrochemical gradient maintenance and cardiomyocyte repolarization; it functions at the expense of cell energy expenditure, has the ability to alter multiple signalling cascades [9], and secondarily facilitates the transport of nutrients into the cell. On the other hand, Mg^{2+}-ATPase is believed to affect the activity of Ca^{2+}-ATPase in the membrane, thereby maintaining normal Mg^{2+} levels within the myocytes, which is vital for the contraction and relaxation cycles of the cardiac muscle. Furthermore, Ch is a precursor of acetylcholine; a basic neurotransmitter of the autonomous nervous system that regulates chronotropic and dromotropic responses of the heart. AChE, the enzyme responsible for acetylcholine degradation (as it terminates cholinergic neurotransmission), also plays a key role in the regulation of the parasympathetic tone of the heart, as one of the main compensatory mechanisms in heart failure is the shift of the sympathetic - parasympathetic equilibrium in favour of the sympathetic nervous system. Moreover, the local activity of AChE can influence the metabolic state of the cardiomyocytes.

In a Ch-deprived experimental model, the diabetic myocardium has been characterized by aggravation of inflammation and fibrosis, as well as by stiffness as a result of CD, which in turn has been associated with the up-regulation of the vascular endothelial growth factor expression in the myocardium and with an impaired structural morphology of the latter. More specifically, there has been evidence of a dilation of the left heart cavity as demonstrated by a concentric hypertrophied myocardium, a thinner left ventricular cavity wall and an increased left ventricular diastolic diameter. The significantly higher myocardial ejection velocity (associated with left ventricle's wall tension index) in addition to the left atrial dilatation could also exert functional derangement and provoke arrhythmogenesis, which, in turn, jeopardises cardiac output [10].

In conclusion, the Ch-deprived diabetic heart seems to arise as a new distinct phenotype of cardiomyopathy that simultaneously combines features of the restrictive and the dilated type.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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Network pharmacology speaking to ethnopharmacology: new data on an ancient remedy

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Abstract

Network pharmacology as a "green approach", predicting metabolite behaviours chemically and biologically and guiding biological experimental design, is a new strategy aiming to uncover the mechanism of action of natural products as drug candidates. It provides a powerful way to identify novel mechanisms of natural products with potential therapeutic effects. This approach has emerged as a powerful tool to overcome the limitations of traditional methods, such as the ability to predict the adverse effects of a drug and the likelihood of failure during clinical trials, by applying systems biology principles to the field of pharmacology. This method combines the multi-omics dataset, computer modeling, and chemical biology so as to reveal pharmaceutical actions and guide drug discovery. Therefore, computer-aided drug design combined with network pharmacology can be viewed as a novel *in silico* screening approach to drug discovery, by utilising chemoinformatics, bioinformatics, structure biology, and chemical biology. This strategy includes target-based virtual screening - molecular docking, ligand similarity-based virtual screening, and inverse screening (Inver-dock), providing a powerful tool for target identification of drug candidates, multitarget discovery, and natural bioactive product profiling. It can also be used for selectivity profiling of drugs, drug repositioning, safety profiling, and metabolism profiling prediction (ADMET).

KEYWORDS

network pharmacology, computer-aided drug design, molecular docking, virtual screening, ethnopharmacology

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MAIN MESSAGE

Network pharmacology is a predictive tool that utilizes computational methods to predict botanicals' chemical behavior and biological targets, thereby informing experimental lab activities [1]. This green approach combines network biology principles with pharmacology to uncover the natural products' mechanism of action (MOA) as potential drug candidates. By integrating multi-omics datasets, computer modeling, chemical biology, and computing chemistry, network pharmacology provides insights into the pharmaceutical actions of botanicals and guides drug discovery. Computer-aided drug design is a key component of network pharmacology, involving computational techniques such as molecular docking, ligand similarity-based virtual screening, and inverse screening. These

methods aid target identification, multitarget discovery, and the profiling of natural bioactive products. By utilizing computer-based predictions, researchers can prioritize and select botanicals for further investigation, thereby reducing the time, cost, and environmental impact of traditional experimental approaches. Traditional drug discovery and development processes often involve extensive laboratory experiments, which can be resource-intensive and time-consuming. In contrast, network pharmacology allows for the virtual screening and prediction of botanicals' chemical behavior and biological targets, thereby reducing the need for large-scale experimental testing [2,3]. This approach minimizes using laboratory resources, such as chemicals, reagents, and laboratory animals, leading to a more environmentally friendly and sustainable research process.

Moreover, network pharmacology enables researchers to explore a wide range of botanicals and their potential therapeutic effects without requiring extensive laboratory experiments. By utilizing computational models and databases, researchers can analyse and predict the pharmacological properties of botanicals, including their chemical behavior and potential biological targets. This approach allows for the identification of promising candidates for further experimental validation, reducing the number of unnecessary experiments and minimizing the overall environmental impact of the research process. In addition, network pharmacology can contribute to developing more sustainable and eco-friendly drug discovery strategies. By providing insights into the MOA and the potential therapeutic effects of botanicals, this approach can guide the selection and optimization of drug candidates with reduced toxicity and adverse effects. This can help develop safer and more environmentally friendly drugs, minimizing the negative impact on human health and the environment [4]. Network pharmacology adds value to experimental work by providing a predictive tool to inform and guide experimental lab activities. By integrating computational predictions with experimental validation, network pharmacology enhances drug discovery and development efficiency and success rate. Therefore, it serves as a green approach by reducing the reliance on extensive experimental testing and contributing to more sustainable research practices.

In addition, combining artificial intelligence (AI) and network pharmacology can also help elucidate the MOA of herbs or compounds used in treating diseases [5]. For example, graph neural networks can predict potential targets and infer the relationships between compounds and targets. At the same time, network pharmacology can provide information on the known targets and pathways associated with a disease. Combining these approaches makes identifying new targets and pathways involved in the disease condition and potential interactions between different compounds and targets possible. This can lead to the discovery of new drugs or the repurposing of existing drugs or therapies to treat the disease. This approach can help to reduce the time and cost associated with traditional therapy discovery methods. Generative adversarial networks are one of the most promising generative AI algorithms for generating new molecules with desired properties, such as high potency and low toxicity in recent years, which can significantly accelerate the therapy discovery process by developing potential candidates for testing and reducing the time and cost associated with traditional methods [6]. The framework based on an integrative strategy of network pharmacology to investigate pharmacological mechanisms is shown in the following steps: (i) pharmacokinetic parameters (ADMET, drug-likeness, *etc*.) and drug targets (PharmMapper, TargetNet, SwissPredicition, SEA, *etc*.) are predicted for the analysis, (ii) targets related to different diseases are selected from public gene databases (GEO, GeneCards, DisGeNET, *etc*.), (iii) the intersected gene list is obtained by overlapping drug targets and diseases targets, (iv) the mechanism of action analysis is performed using Metascape, STRING, and Cytoscape, and (v) the critical targets of medicinal herbs treating diseases are validated by molecular docking, molecular dynamics, and *in vitro* and *in vivo* experiments.

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CONFLICT OF INTEREST STATEMENT

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The off-target NHE1 inhibitory effect of SGLT2 inhibitors in cardiac remodeling

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Abstract
Sodium-glucose cotransporter-2 (SGLT-2) inhibitors, approved for the treatment of diabetes mellitus, have gained attention for their cardioprotective effect. The exact mechanism by which SGLT-2 inhibitors exert their cardioprotective effect remains unclear. Recent studies have suggested that empagliflozin (EMPA), an SGLT inhibitor, exerts its cardioprotective effect by inhibiting the Na^+/H^+ exchanger (NHE); a group of membrane proteins that regulate intracellular pH and cell volume. Increased activity and expression of NHE isoform 1 (NHE1), the predominant isoform expressed in the heart, leads to cardiac hypertrophy. Our research group investigates the indirect mechanisms by which SGLT inhibitors exert their cardioprotective effect and have demonstrated that angiotensin II (ANG)-induced hypertrophy of H9c2 cardiomyoblasts is accompanied with increased SGLT-1 and NHE1 protein expression; an effect which is reversed in the presence of EMPA. In addition, we demonstrated that dapagliflozin improved survival of transgenic mice expressing cardiac-specific NHE1.

KEYWORDS
cardiac remodeling, NHE1, SGLT1/2, cardiovascular disease

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1. INTRODUCTION

Sodium-glucose cotransporter-2 (SGLT-2) inhibitors, approved for the treatment of diabetes mellitus, have gained attention for their cardioprotective effect. The exact mechanism by which SGLT-2 inhibitors exert their cardioprotective effect remains unclear. Recent studies have suggested that empagliflozin (EMPA), an SGLT inhibitor, exerts its cardioprotective effect by inhibiting the Na^+/H^+ exchanger (NHE); a group of membrane proteins that regulate intracellular pH and cell volume. Increased activity and expression of NHE isoform 1 (NHE1), the predominant isoform expressed in the heart, leads to cardiac hypertrophy.

2. METHODS

In our study, H9c2 cardiomyoblasts were treated with angiotensin II (ANG) to activate NHE1 and gen-

erate a hypertrophic model. We aimed to understand whether EMPA reverses the ANG-induced hypertrophic response, and to elucidate the molecular pathway contributing to the cardioprotective effect of EMPA. *In vivo*, transgenic mice expressing cardiac specific NHE1were administered dapagliflozin, an SGLT inhibitor, and heart function was measured by echocardiography.

3. RESULTS

Our study demonstrated that ANG-induced hypertrophy of H9c2 cardiomyoblasts is accompanied with increased SGLT-1 and NHE1 protein expression; an effect which is prevented in the presence of EMPA. In addition, we demonstrated that dapagliflozin improved the survival and ejection fraction of transgenic mice expressing cardiac-specific NHE1.

4. CONCLUSION

EMPA reduces ANG-induced hypertrophy through the inhibition of SGLT-1 and NHE1 expression, while dapagliflozin improves the survival and ejection fraction of transgenic mice expressing cardiac-specific NHE1.

CONFLICT OF INTEREST STATEMENT
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Applications of quartz crystal microbalance with dissipation in nanomedicine (QCM-D): a personal experience

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Abstract
Due to their unique physical and chemical properties, the potential application of nanomaterials in medicine is particularly attractive. Despite the many advantages that nanomaterials can offer as diagnostic and therapeutic tools, their transition from the bench to clinical practice is extremely challenging. One of the many barriers that nanomedicines may encounter is their toxicological effect. In fact, the development of novel nanomaterials / nanoparticles must proceed always in tandem with the assessment of any potential toxicological effects associated to them. Once nanomaterials reach the systemic circulation, they interact with endothelial cells, plasma proteins, and other blood components. There is no doubt that the study of nanomaterials-blood interactions is crucial to warrant the biocompatibility of nanomaterials developed for human use.

KEYWORDS
QCM-D, nanomedicine, nanomaterials, nanotoxicology, blood platelets

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MAIN MESSAGE

Most of the current research in nanomedicine is devoted to the design of nanomaterials for diagnostic and therapeutic applications. However, nanomedicines, independently of their route of administration, will eventually reach the blood stream and interact with plasma proteins and, among other cells, with erythrocytes, platelets, and white blood cells [1]. Investigating the interactions of nanomaterials with biological systems, and particularly with blood components, is crucial for the development and optimisation of nanomaterials, as blood incompatibilities such as haemolysis, bleeding or thrombosis, among others, could result in serious side effects that may prevent their successful clinical translation. Therefore, the careful assessment and characterisation of nanomaterial interactions with blood is of critical importance as part of the development process of nanomedicines.

The principle of analysis of a quartz crystal microbalance (QCM) is based on changes of the resonance frequency of a quartz crystal when an alternating electric field is applied across the crystal. Material adsorption on the surface of the crystal results in a decrease in the frequency of vibration that is proportional to the mass that can be quantified using the Sauerbrey equation [2]. In fact, when the adsorbed layer is thin and rigid, the mass can be accurately calculated using this equation. However, the measurement of changes of the frequency alone does not offer any information about the viscoelastic characteristics of the deposited layer and, when the layer is soft and thick, the mass can be underestimated when the Sauerbrey equation is applied. The QCM with dissipation technology (QCM-D) however, measures, in addition to changes in frequency, the called "dissipation factor". This parameter is related to the viscoelastic properties of the adsorbed layer, and it can be used (i) to monitor its conformational and structural changes at real time and, together with the frequency, (ii) to calculate the mass and thickness of thick and viscoelastic layers using specific mathematical models [3]. We have applied this technology for looking at the interactions between nanoparticles developed for drug delivery and their targets [4], and the potential barriers that they must overcome to exert their action [5]. Our research team has developed a novel method for the quantification and characterization of platelet aggregation under flow conditions using a QCM-D that closely mimics the conditions encountered in the microvasculature [6]. We have demonstrated that this methodology, due to the sensitivity of the device, allows the detection of platelet microaggregates; the very initial process of thrombus formation. During the past years we have applied our approach for investigating the effect of different compounds, nanomaterials, and novel formulations on platelet function [7-10]. Our work supports the idea that ours is a unique approach for looking at platelet-nanomaterials interactions and for investigating the blood compatibility of nanomaterials.

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Preparation and characterization of nanoparticles loaded with dimethyl fumarate for the treatment of multiple sclerosis

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Abstract

When nanotechnology is used in medicine, it makes it easier to find and treat a wide range of diseases. One of the oral options for treating multiple sclerosis (MS) is dimethyl fumarate (DMF). DMF has been shown to be effective in lowering inflammatory diseases; nevertheless, it is characterized by several undesirable side effects that reduce patient compliance and add financial obstacles. The aim of this study was to use platelet membranes and platelet nanoparticles to generate a drug delivery system that works like a cell, so as to treat MS. During the experiments, there is a chance that the DMF solution might harden at room temperature. Therefore, in order to produce solid lipid nanoparticles (SLNs), DMF was combined with biocompatible lipids. The creation of SLNs involved the use of hot emulsion and ultrasonication. These DMF-SLNs were characterized by means of scanning electron microscopy, and Fourier-transform infrared spectroscopy. The herein demonstrated enhanced qualities of the devised SLNs suggest that the formulation may be a potential, longer-acting formulation for the improved management of MS. SLNs could change the way many illnesses are treated in a big way, if they are used for the delivery of medicines.

KEYWORDS

multiple sclerosis, dimethyl fumarate, immunomodulatory fumaric acid, solid lipid nanoparticles, microphotograph

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1. INTRODUCTION

Multiple sclerosis (MS) is an advanced illness of the central nervous system that is characterized by the collapse of the myelin sheath [1]. Dimethyl fumarate (DMF), also known as fumaric acid, exhibits antioxidant and immunomodulatory properties, and is used as an oral treatment for recurrent MS. In fact, DMF is a medication licensed for treating MS and psoriasis due to its antioxidative and anti-inflammatory properties. Moreover, its posi-

tive benefits have also been identified in other inflammatory disorders as well as in malignancies. Unfortunately, several disease-modifying treatments, including DMF for treating MS, have been linked to the development of progressive multifocal leukoencephalopathy [2].

Biocompatible lipids are used to create solid lipid nanoparticles (SLNs). SLNs are characterized by some desirable properties, such as a large surface area, an elevated drug capacity, and an interface point reaction. The delivery of nanoparticles to specific places is made possible by size-dependent targeting, as the altering of nanoparticles so as to achieve active targeting has been shown to be a successful approach.

2. MATERIALS AND METHODS

Materials: DMF ($C_6H_8O_4$) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Stearic acid ($C_{18}H_{36}O_7$) was purchased from Merck Schuchardt OHG (Hohenbrunn, Germany). Soya lecithin ($C_{35}H_{66}NO_7P$; palmitic acid 11.7%, stearic acid 4%, palmitoleic acid 8.6%, oleic acid 9.8%, linoleic acid 55%, and linolenic acid 4%) was obtained from Ever Gainful Enterprise Sdn Bhd (Petaling Jaya, Malaysia). Ethanol (C_2H_5OH) was purchased from Chemicals and Pharma Works (Selangor, Malaysia), while Tween 80 (polyoxyethylene sorbitan monooleate; $C_{64}H_{124}O_{26}$) was obtained from Chemsworth Chemicals (Selangor, Malaysia).

Preparation of SLNs: First, 1 g of DMF was sonicated with 30 mL of ethanol. This is the first of five steps. According to Ojha & Kumar [3], the mixture was subsequently boiled at 75°C with 2 g of stearic acid and 250 mg of soy lecithin. The third step involves creating a solution by dissolving 10 mL of Tween 80 in 150 mL of distilled water. In the fourth step, the resultant dispersion is dissolved in cold water at 2–5°C. This is done after 20 min of stirring at 10,000 rpm.

3. RESULTS AND DISCUSSION

Fourier-transform infrared (FTIR) spectroscopy analysis: When FTIR was used on scaffolds with various nanoparticle concentrations, the results did not significantly differ from those obtained with pristine cross-linked DMF resin. Figure 1A displays the FTIR spectra of our DMF nanoparticles. The coordination of unsaturated surface DMF atoms with hydroxyl ions or water molecules in the aqueous media results in the formation of OH groups, which alter the surface chemistry of magnetite nanoparticles. The aromatic C-H bending vibration that causes the peak for DMF at 990.22 cm^{-1} was seen in both films, but the adjacent H deformation at 1,159.64 cm^{-1} and the aromatic C=O stretching at 1,308 and at 1,438.90 cm^{-1}, as well as the C=O stretching for ester anhydride at 1,715.97 cm^{-1} and the O-H stretching at 3,428.77 cm^{-1} in the film samples did not match with what was seen in the pure drug sample, while the aromatic C-H stretching was noted at 3,076.50 cm^{-1}, as shown in Figure 1A. SLNs-1 displayed an aromatic C-H bending at 944.42 cm^{-1}, H deformation at 1,472.19 cm^{-1}, aromatic C=O stretching at 1,703.96 and at 2848.93 cm^{-1}, C=O stretching for ester anhydride at 2,916.94 cm^{-1}, O-H stretching at 3,649.46 cm^{-1}, and aromatic C-H stretching at 3,858.50 cm^{-1}, as shown in Figure 1B (teal colour). SLNs-2 displayed an aromatic C-H bending at 943.04 cm^{-1}, H deformation at 1,471.89 cm^{-1}, aromatic C=O stretching at 1,701.40 and at 2,848.38 cm^{-1}, C=O stretching for ester anhydride at 2,916.02 cm^{-1}, and O-H stretching at 3,505.05 cm^{-1}, as shown in Figure 1B (pink colour). Finally, SLNs-3 displayed the following characteristics: aromatic C-H bending at 944.11 cm^{-1}, H deformation at 1,472.22 cm^{-1}, aromatic C=O stretching at 1,703.32 and at 2,848.95 cm^{-1}, C=O stretching for ester anhydride at 2,917.15 cm^{-1}, and O-H stretching at 3,436.97 cm^{-1} (Figure 1B; red colour).

In the case of the C-H bend, the current result (944.42 cm^{-1}) is very close to the result presented by Ojha & Kumar [3] (948 cm^{-1}), while it is higher than the result measured by Sinha *et al*. [4] (775.38 cm^{-1}). A possible reason might be due to the shrinking angle between the bonds, as noted by Riaz *et al*. [5]. The current study's H bond (1,472.19 cm^{-1}) is larger than other studies' H bonds: 883.4 [4] and 1,099 cm^{-1} [3], respectively. As explained by Taib *et al*. [6], the change in bond angle between bonds with a typical atom may be a good reason for this result. The C=O bond has shown three values in the current study and the other two studies [3,4]. The sequence of the three stretching vibrations in the current study (1,703.96, 2,848.93, and 2,916.94 cm^{-1}) exhibited higher vibration than that of Sinha *et al*. [4] (1,161.15, 1,199.72, and 1,722.43 cm^{-1}), while it is almost identical to the sequence found by Ojha & Kumar [3] (1,129, 2,850, and 2,918 cm^{-1}). The C=O stretching is the most prominent peak due to the high polarity of single C-O bonds, as previously reported [6]. The values of the O-H bond in the current study (3,649.46 cm^{-1}) are slightly higher than those found by Sinha *et al*. [4] (3,429.43 cm^{-1}). Finally, the last stretching frequency (the C-H stretch) indicated that the C-H stretches can reach values greater than 3,000 cm^{-1}. However, the current study has shown a slightly higher frequency

(3,853.5 cm^{-1}) for this parameter than the value found by Ojha & Kumar [3] (3,400 cm^{-1}).

Field emission scanning electron microscopy (FE-SEM) analysis: The FE-SEM measurements of the surface topography of the SLNs provided a good explanation of their interior structure. The panels a–d of Figure 1C display the FE-SEM pictures of DMF, SLNs-1, SLNs-2, and SLNs-3, respectively. When viewed under a microscope, the prepared DMF films have a surface roughness that resembles nanofibers. These nanofibers included SLNs-1, SLNs-2, and SLNs-3. Nanoparticles were found to have an average size of 500–2,000 nm. Figure 1C reveals that the particles had an unsmooth surface and a spherical shape, and indicates that they were in the nanoscale range.

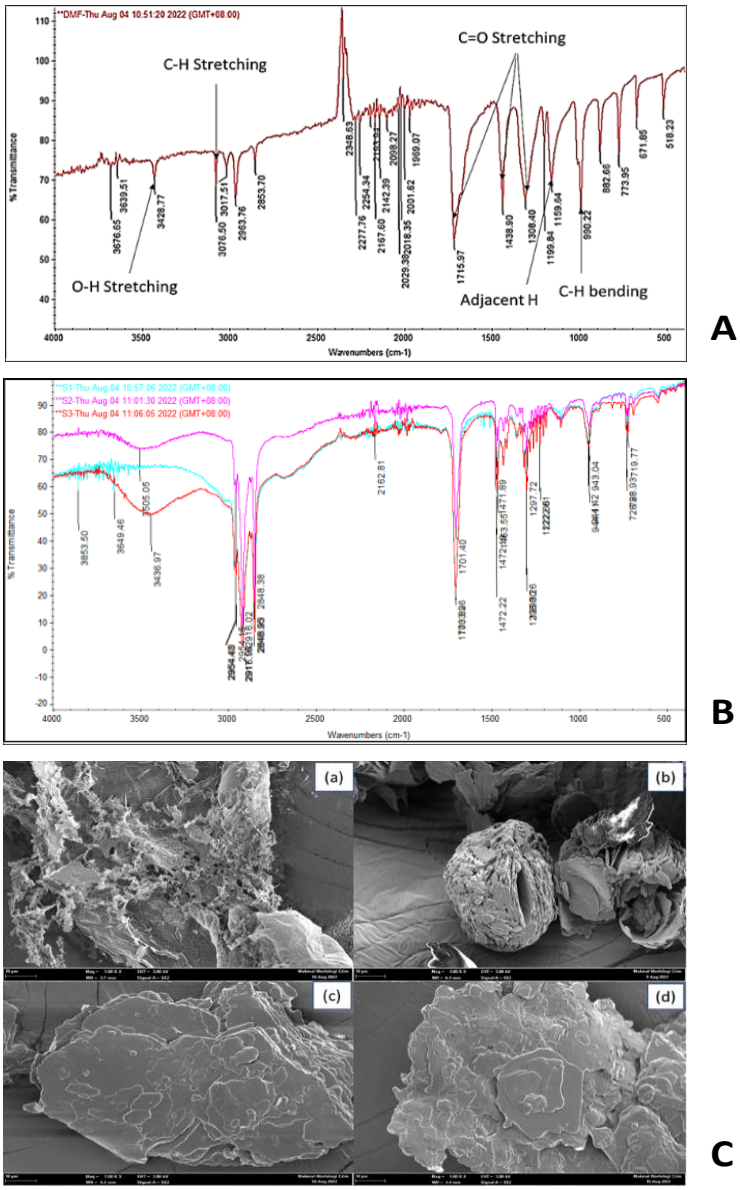

Figure 1. (**A**): Fourier-transform infrared (FTIR) spectrum of dimethyl fumarate (DMF); (**B**): FTIR spectra of three samples of solid lipid nanoparticles (SLNs), namely SLNs-1 (teal colour), SLNs-2 (pink colour), and SLNs-3 (red colour); (**C**): Field emission scanning electron microscopy (FE-SEM) of DMF (a), SLNs-1 (b), SLNs-2 (c), and SLNs-3 (d).

4. CONCLUSION

The present study discusses how to prepare and characterize nanoparticles depending on DMF. The nanoparticles were prepared and assessed for their size and zeta potential. The FTIR spectrum of nanoparticles in varying concentrations was examined. In an attempt to explore the cross-linked nanocomposites, FE-SEM was employed. Our findings suggest that DMF-controlled-loaded-release SLNs may be a good option for treating and managing MS. Meanwhile, the optimized SLNs had a mean particle size of 562, 1,997, and 849 nm. According to the results of this study, the current formulation is a promising, longer-acting formulation for the better management of MS. Moreover, SLNs hold promise as a medication delivery method that can revolutionize the treatment of many diseases.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Assessment of antifungal drugs' activity against some *Candida albicans* isolates in the presence or absence of human albumin: a study employing an *in vitro* pharmacokinetics / pharmacodynamics model

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Abstract

Invasive candidiasis associated with the dissemination of endogenous *Candida* species is a fatal condition linked to high rates of morbidity and mortality. Progressive drug resistance necessitates the need for prompt and effective therapy. Therefore, choosing a specific and effective treatment is crucial. A two-compartment *in vitro* pharmacokinetics (PK) / pharmacodynamics (PD) model has been used for this purpose, and the PD behaviours of amphotericin B (AMB; at 2.5 and 5 mg/L), voriconazole (VOR; at 1.5 and 3 mg/L), and itraconazole (ITR; at 1.5 and 3 mg/L) were assessed against two *Candida albicans* isolates (a sensitive and resistant one; ATCC-90028 and ATCC-10231, respectively) with or without the addition of human albumin (2%). PK were simulated as time-concentration profiles, while the PD susceptibility of all drug doses has been assessed through the minimum inhibitory concentration (MIC), the relative optical density of fungal growth, and the exposure - effect relationship ($fAUC_{0-24}$/MIC). A fungicidal activity without the presence of albumin was seen against both isolates of *C. albicans* at the highest dose of VOR, while the addition of albumin potentiated the efficacies of AMB and of VOR against both isolates, with no effect for ITR. Finally, human albumin exerted a variable and dose-dependent effect on the activities of some antifungal agents.

KEYWORDS
antifungal drugs, *Candida* species, *in vitro* model, pharmacokinetics, pharmacodynamics

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1. INTRODUCTION

Invasive candidiasis remains a significant clinical cause of high morbidity and mortality, especially when associated with a multi-drug-resistant strain in the intensive care unit (ICU). *Candida albicans* is the most common species causing candidaemia, and accounts for 56% of all *Candida* infections in ICU patients [1]. Amphotericin B (AMB) is

still, for decades, the treatment of choice for invasive candidiasis. *C. albicans* has acquired resistance to azole due to overexpression with cross-resistance from non-pathogenic *C. albicans* [2]. Ultimately, the clinical significance of the antifungal activity of azole drugs is decreased. Moreover, many patients have low serum drug exposure after oral administration; therefore, intravenous formulations are preferred due to their less variable pharmacokinetics (PK) and for their higher exposure that is ideal for the treatment and prevention of azole-resistant candida infections [3,4]. An *in vitro* PK / pharmacodynamics (PD) model has been used in this study in order to explore the details and the differences among AMB, voriconazole (VOR), and itraconazole (ITR) against two *C. albicans* strains, in the presence and absence of albumin.

2. MATERIALS AND METHODS

Candida isolates: Two *C. albicans* isolates (a sensitive and a resistant one; ATCC-90028 and ATCC-10231, respectively) were used in order to simulate different time-concentration profiles. The minimum inhibitory concentration (MIC) values were 0.06–2 and 0.12–4 mg/L, respectively, based on the median (range) of Clinical and Laboratory Standards Institute (CLSI). The isolates were stored in normal sterile saline with 10% glycerol at -70°C, and were revived by subculturing on Sabouraud dextrose agar (SDA) plates (SGC2; bioMérieux, Marcy l'Etoile, France) so as to ensure purity and viability. Inoculum suspensions were prepared in normal sterile saline from 24-h cultures, and were adjusted to a final inoculum of 2×10^4 colony-forming units (CFU)/mL in the *in vitro* model by using a counting chamber.

Antifungal drugs and medium: AMB (50-mg vial; BioLab, India), VOR (200-mg vial; Zydus Cadila, India) and ITR (200-mg tablet; Taj Pharmaceuticals, India). The medium used in the *in vitro* PK/PD model was RPMI-1640, buffered to pH 7.0 (Sigma Aldrich, Darmstadt, Germany), and supplemented with 100 mg/L chloramphenicol (AppliChem GmbH, Darmstadt, Germany). Moreover, a 2% albumin (Kedrion biopharma; Italy) was added as required.

In vitro PK/PD model: A two-compartment PK/PD model consists of a 500-mL beaker glass containing fresh RPMI-1640 medium to an initial volume of 5 mL from floating tubes with a dialytic membrane (20 kDa) for each isolate of *C. albicans* and antifungal dosing regimen. The central one was connected to a peristaltic pump (Minipuls Evolution; Gilson Inc., Middleton, WI, USA), adding fresh medium in order to dilute its content at a rate equal to the clearance of antifungal drugs in human plasma [5,6] (Figure 1K).

In vitro PK: The simulated targeting free (unbound) maximum plasma concentrations (fC_{max}) of 2.5 and 5.0 mg/L of AMB, 1.5 and 3.0 mg/L of VOR, and 1.5 and 3.0 mg/L of ITR, as well as a half-life ($t_{1/2}$) of 12–24 h were assessed in order to better describe the exposure - effect relationship. The simulated time - concentration profiles were chosen in order to simulate different 24-h drug exposures observed.

In vitro PD: In order to estimate the fungal load inside the floating dialytic tubes for each antifungal dosing regimen, 200-µL samples were collected at regular intervals up to 24 h, and fungal growth was assessed spectrophotometrically by measuring the relative optical density (ROD) at 405 nm, at each dilution. The ROD_{405} for each drug concentration at a specific timepoint, in relation to the control growth at same timepoint as well as over time, was plotted.

In vitro PK/PD analysis: The PK/PD index as the $fAUC_{0–24}$/MIC ratio was calculated for each simulated dose and isolate during the experiment. The drug exposure - response relationship, expressed as 24-h growth reduction for each dosing regimen and isolate, was compared with values at the start of dosing that were analysed by a nonlinear regression analysis using a sigmoidal model with variable slope. All data were analysed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. RESULTS

As shown in Figure 1, the nonlinear inhibition represented the mean of ROD values for each C_{max} of antifungal agents against both isolates, in the presence and absence of 2% human albumin. A fungicidal effect of VOR without addition of albumin was seen only with a C_{max} (3 mg/L) on both isolates (Figures 1D and 1E), while a similar picture was seen under other C_{max} conditions of other antifungal agents, but followed with a regrowth of *Candida* (Figures 1A, 1B, 1G, and 1H). The addition of 2% of human albumin leads to a complete inhibition of growth of both isolates treated with AMB or VOR (Figures 1A–1E). No significant growth inhibition was seen with ITR under both C_{max} conditions (Figures 1G and 1H). The $AUC_{0–24}$/MIC represented the exposure-effect relationships as the AUCs were determined for each simulated dose, and any deviation from the target values was adjusted. The 24-h change in the ROD *versus* $fAUC_{0–24}$/MIC relationship for both *C. albicans* isolates is displayed as a sigmoidal curve (Figures 1C, 1F, and 1I) for all antifungal agents in relation to control (Figure 1J).

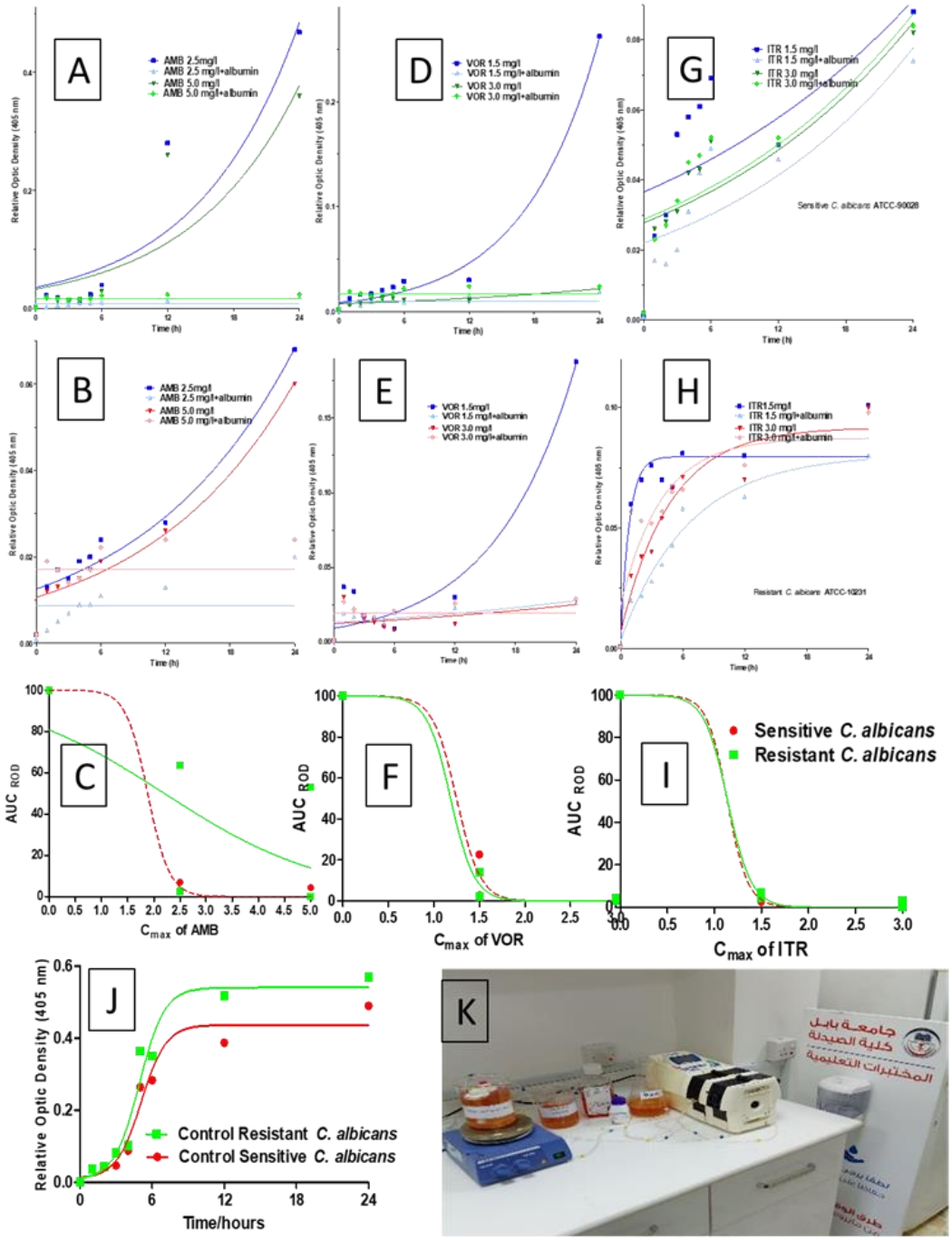

Figure 1. Data obtained from our *in vitro* pharmacokinetics / pharmacodynamics (PK/PD) model. Simulated human dosing with 2.5 and 5.0 mg/L of amphotericin B (AMB; **A** and **B**), 1.5 and 3.0 mg/L of voriconazole (VOR; **D** and **E**), or 1.5 and 3.0 mg/L of itraconazole (ITR; **G** and **H**) was used against two isolates of *C. albicans* (a sensitive and a resistant one; ATCC-90028 and ATCC-10231, respectively) in the presence and absence of 2% albumin for each maximum concentration (C_{max}). Panels **C**, **F**, and **I** represent the single-dose exposure-efficacy relationship of AMB, VOR, and ITR, respectively, against each isolate of *C. albicans*. Panel **J** presents the growth indices in control isolates, while panel **K** presents the *in vitro* PK/PD model used in our experiments.

4. DISCUSSION

Antifungal resistance is still a big challenge for physicians and researchers that needs to be addressed and managed effectively. Different antifungal agents that have been assessed by an *in vitro* PK/PD model have shown different drug efficacies. A fungicidal activity in the absence of albumin was observed against both isolates of *C. albicans* with the highest dose of VOR, and this finding might be due to the post-antifungal effects of the azole group [7]. The addition of albumin potentiates the efficacies of AMB and of VOR against both isolates, with no effect for ITR [8,10]. Finally, human albumin has a variable effect that is dose-dependent on the activities of some antifungal agents. Moreover, albumin itself may have a fungicidal activity against *C. albicans* [10].

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Assessment of the CO_2 water bath therapy effectiveness on diabetic foot ulcers through VEGF and TNF-α levels

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Abstract

Vasculopathy is typically seen in diabetic patients, and can lead to foot ulcerations. Carbon dioxide (CO_2) therapy was found to improve chronic wound healing in patients with vascular impairment. This type of therapy refers to the transcutaneous and subcutaneous application of CO_2 as well as CO_2 water baths for therapeutic purposes. In the method used herein, artificial CO_2-containing water for foot bathing was generated by dissolving Carbothera® (MRE-SPA-MD; Mitsubishi Rayon Engineering, Tokyo, Japan) and generating CO_2 (free CO_2 concentration: 1,000–1,200 mg/L; pH 4.6). The foot of each patient was immersed in CO_2-enriched water (depth of 20–30 cm, 37–38°C, 30-min duration) three times per week, for the next three months. Dramatic clinical improvement was observed in the CO_2 water bath therapy group before and after the treatment, while both the blood levels of the vascular endothelial growth factor and of the tumour necrosis factor-alpha in these patients exhibited significant changes. The advantages of this method are the absence of pain and the protection against infection, while the improved angiogenesis and oxygenation can result in healing of the chronic wound.

KEYWORDS

diabetic foot ulcer, carbon dioxide therapy, vascular endothelial growth factor, tumour necrosis factor-alpha.

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1. INTRODUCTION

Diabetic foot ulcers (DFUs) are a common consequence of longstanding and poorly managed diabetes. Of the estimated 537 million people worldwide who suffer from diabetes [1], 19–34% will develop a DFU in their lifetime [2]. Approximately 20% of the people who develop a DFU will require amputation that will be either minor (below the ankle) or major (above the ankle) [2], while 10% will die within one year of their first DFU diagnosis [3,4]. The treatment of DFUs is a global major health care problem, resulting in high care costs and mortality rate. The recognition of infection and ischaemia is very important, as it allows us to determine factors that predict the healing progress of the DFU and the risk of amputation. Ischaemia, the lack of blood circulation, develops due to chronic complications of diabetes. This can result in the

development of gangrene of the DFU, which may require amputation if not recognized and treated early. There are a number of DFU classification systems such as the Wagner, the University of Texas, and the SINBAD classification systems, which include require information regarding the site of the DFU, its area, its depth, the presence of neuropathy, the presence of ischaemia, and the presence of infection [5]. This study focuses on ischaemia and infection, which are defined by the presence of poor reperfusion to the foot [6] and the presence of bacterial soft tissue or bone infection in the DFU (as confirmed by blood testing) [7], respectively.

2. PATIENTS AND METHODS

A total of 95 patients with DFU (mean age: 55.6±11.6 years; 59 men, 36 women) were divided into two groups in this randomized double-blind study. The traditional therapy group was treated by using advanced dressings and antibiotics (control group), while the CO_2 therapy group (study group) was treated by using advanced dressings with antibiotics and CO_2 therapy. In brief, artificial CO_2 -containing water for foot bathing was generated by dissolving Carbothera® (MRE-SPA-MD; Mitsubishi Rayon Engineering, Tokyo, Japan) and generating CO_2 (free CO_2 concentration: 1,000–1,200 mg/L; pH 4.6). The foot of each patient was immersed in CO_2 -enriched water (depth of 20–30 cm, 37–38°C, 30-min duration) three times per week, for the next three months.

Written approvals for the undertaking of this study were obtained by the Research Ethics Board at the College of Medicine of Baghdad University and by the Hospital Research Review Committee, prior to the implementation of the study and the enrolment of any participants. Moreover, participants were given verbal information describing the nature of the study.

Blood samples (5 mL) were obtained from the two groups before and after the study. The blood levels of the vascular endothelial growth factor (VEGF) and of the tumour necrosis factor-alpha (TNF-α) were measured by the ELISA method. Moreover, the size, colour, and sensation of the ulcerative area were all evaluated and compared between the two groups.

3. RESULTS

According to the demographic data of the patients, there was no significant age difference between the study group and the control group, and the same applies with regard to their distribution based on their sex. A significant deference in the size, colour, and sensation of the ulcerative region was observed; these parameters were all improved in study group (Figure 1A). According to the undertaken laboratory investigation there was no significant deference between the study group and the control group with regard to their random blood sugar, glycated haemoglobin, renal function test, liver function test, and lipid profiles. However, the VEGF and TNF-α levels revealed highly significant differences when compared before and after the therapy in the study group ($p<0.001$; Figure 1B–C).

4. DISCUSSION

The findings of our study have confirmed that the application of CO_2 in a water bath can significantly improve the healing of DFUs. The wounds in the study group patients (that received CO_2 therapy) healed significantly faster compared with those of the control group. After CO_2 therapy, 66% of the wounds healed completely, as compared to 0% in the control group.

There is limited research on the specific effect of CO_2 therapy on bacterial infections in DFUs. CO_2 is a natural component of the atmosphere and is generally non-toxic to humans. The CO_2-enriched water bath therapy has been reported to accelerate the wound healing process of DFUs by lowering the pH levels of the wounds. As bacteria often thrive in neutral or slightly alkaline conditions, the lower pH inhibits their growth. Moreover, by enhancing the oxygen delivery, the CO_2-enriched water has the ability to enhance the oxygen-carrying capacity of red blood cells, thereby resulting in improved oxygen delivery to the wound. This increased oxygenation can be detrimental to anaerobic bacteria, as they prefer low oxygen or oxygen-deprived environments [8].

Shalan *et al.* [7] have published an observational study on 22 diabetic patients with chronic wounds. Their study had no control group, and they immersed their patients' feet into CO_2 −enriched water once daily, for 30 min, for 15 days. During this time, they noticed an improved blood flow (confirmed by Doppler flowmeter) and an improvement in the wound colour. There was no significant improvement observed in terms of the wound area reduction. The authors [7] rightfully assumed that the CO_2 treatment would have probably improved wound healing if the treatment lasted longer. Three years later, Abdulhamza *et al.* [8] conducted a study by using the same device as Shalan *et al.* [7].

Abdulhamza *et al.* [8] have performed a study similar to ours, with the exception that theirs was not double-blind. They included 100 diabetic pa-

tients with chronic wounds, divided into study and control groups. The study group received both standard treatment and CO_2 therapy. The latter was administered as gas in the water bath as in our study. The patients immersed their feet in CO_2-enriched water for 30 min, three times per week, for three months. After the treatment, a significant improvement was seen in the study group through Doppler imaging of the large leg arteries and veins, and a decrease in the wound size was also noted. The control group exhibited no differences in the observed parameters when assessed before and after treatment.

It is well-established that the CO_2 therapy exerts a vasodilatory effect, probably influenced by a NO-dependent pathway. Minamiyama and Yamamoto [9] have used intravital microscopy in order to confirm the subcutaneous vasodilatation occurring after CO_2 therapy in rats. Another mechanism that is involved immediately after the CO_2 application is the Bohr effect, which was confirmed by Sakai *et al.* [10]. The Bohr effect means that in increased pCO_2 changes the haemoglobin (Hb) – oxygen (O_2) dissociation curve shifts to the right, and so the Hb releases more O_2 and the tissue gets more oxygenized. It has already been shown that even 20 min of a foot's immersion to CO_2 can improve the tissue oxygenation. Sakai *et al.* [10] also verified this effect *in vivo* (in seven healthy volunteers) and confirmed the change between oxygenated and deoxygenated Hb during single transcutaneous applications of dry CO_2 therapy [10]. Therefore, a single treatment with CO_2 exerts vasodilatation and elevates O_2 release from Hb via the Bohr effect [10]. In order to enhance wound healing, the CO_2 application must be repeated so as to maintain tissue supply with O_2 ad to induce neo-angiogenesis; a delayed CO_2 effect of the CO_2 therapy. The importance of the repetition of the CO_2 therapy can be observed from the comparison of the findings of the two aforementioned studies on diabetic patients [7,8].

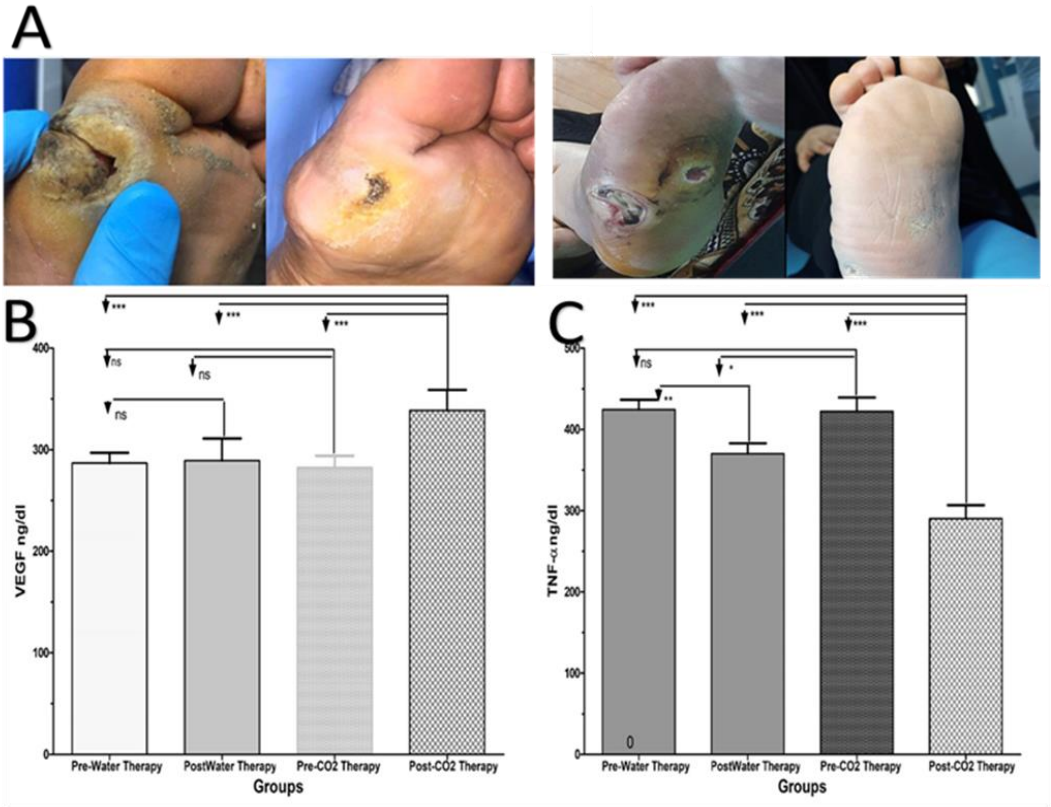

Figure 1. (A): Examples of diabetic foot ulcer (DFU) wounds before and after the CO_2 therapies in the study group. (B): Mean vascular endothelial growth factor (VEGF) blood levels before and after therapy in the two groups of the study. (C): Mean tumour necrosis factor-alpha (TNF-α) blood levels before and after therapy in the two groups of the study. Note: values in the graphs represent means ± standard deviations. Statistical significance: *, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$; ns: non-significant.

The strength of our study lies in its methodology, as it was designed as a controlled double-blind randomized study. The limitations of this study are the differences in the wound size and volume, as well as their response to the number of exposure sessions to CO_2 therapy, which may differ from patient to patient. However, the limitations were minimized by applying more sessions to those patients. After considering our results and the effects of the CO_2 therapy described in the literature, one can safely conclude that the CO_2-enriched water bath could be an effective adjuvant therapy in the treatment of chronic wounds. The latter are sometimes very difficult to treat; therefore, it is important to know what kind of adjuvant therapy is available and confirmed as effective. Since the mode of action of the CO_2 therapy involves the improvement of vascularization, it can be combined with previously described advanced therapeutic approaches in order to treat even the most persistent wounds.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Theoretical calculations and molecular design of novel dioxoisoindoline derivatives as anticonvulsant agents

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Abstract

Our study discusses the need for the development of alternative treatments for antiepileptic drugs. It proposes a theoretical chemical study using dioxoisoindoline derivatives and molecular docking in order to find potential alternative drugs. Three compounds (S1, S3, and S4) exhibited distinct activity against specific proteins related to epilepsy treatment. Our study also describes a DFT study that analysed the energy levels of the derivatives. Furthermore, we employed Lipinski's rule and drug likeness predictions in order to assess the suitability of the derivatives as medicines. The results indicate that the molecular mass, log P, hydrogen bonding donors, and acceptors of the compounds fall within acceptable ranges. Overall, our study emphasizes the importance of finding new treatments for epilepsy, and presents a preliminary investigation into the potential of dioxoisoindoline derivatives.

KEYWORDS

dioxoisoindoline derivatives, anticonvulsant, molecular docking, DFT study, ADME

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1. INTRODUCTION

Epilepsy is a neurological condition that is marked by rapid, irregular, or excessive neuronal excitation in the grey matter of the brain due to the brain's high excitability, and manifests symptomatically as seizures [1]. Epileptic seizures arise due to an imbalance between excitatory and inhibitory neurotransmitters in the brain. The excessive neuronal firing results from functional issues caused by macromolecules involved in excitatory and inhibitory communications, leading to the development of epilepsy [2]. Neuronal membrane and molecular channel alterations in ionic conduction are another mechanism [3]. The membrane potential is typically polarized and maintained by ion pumps and channels. When the membrane depolarizes, it creates an action potential that stimulates muscle cells. Neurotransmitters at the axon tip transmit this action potential to the next cell, thereby leading to neuronal activation. After depolarization, the membrane hyperpolarizes, reaching a voltage

lower than the resting potential [4]. In a healthy neural tissue, this is a reaction that prevents excessive excitability brought on by repeated firings, and the membrane quickly returns to the resting phase (polarization). As a result, repeated synchronized sub-threshold excitatory stimuli, increased excitatory synaptic neurotransmission, reduced inhibitory synaptic neurotransmission, a change in ion concentration on both sides of the membrane, or severe excitability occur [5].

While there are many effective drugs available for the treatment of epilepsy, some of them have toxic side effects and can interact with other medications. As a result, there is still a demand for new antiepileptic drugs that can better manage seizures without these drawbacks. Quantitative structure-activity relationship methods (QSAR and 3D-QSAR) have been widely employed in the design and effectiveness of novel compounds as well as in resolving the mechanisms of action of existing antiepileptic drugs. These methods support the identification of novel compounds with lower side-effect profiles as well as the prediction and enhancement of the activities of various compounds [6,7]. In this study, six dioxoisoindoline derivatives were designed and evaluated as potential alternative drugs for epilepsy. The study aimed to determine if these derivatives have a strong binding affinity (ΔG) with specific proteins in the brain. This research is part of an ongoing investigation into the synthesis of novel dioxoisoindolines with potential anticonvulsant activity.

2. MATERIALS AND METHODS

The online application SwissDock was utilized in order to predict the potential molecular interactions between a target protein and a small molecule. The proteins 1OHV (4-aminobutyrate-aminotransferase; from pig), 3F8E (coumarins as suicide carbonic anhydrase inhibitors), and 6KZP (calcium channel-ligand) were docked with six dioxoisoindoline derivatives proposed for the protein active site. In ChemOffice (ChemDraw version 20.0), the chemical structures were designed with the appropriate 2D orientation. MM2 energy minimization was performed for each structure so as to estimate the potential energy surface, including factors such as steric energy and thermal energy. The resulting conformations of the models were obtained [8]. Theory at the molecular level was employed. The energy-minimized ligand molecules were then treated with quantum mechanics using the B3LYP/6-31G++ (d,p) level of theory for frequency calculation and geometry optimization. The assessed compounds of residues ARG_{192}, GLY_{191}, HIS_{190}, PHE_{189}, ASN_{140}, GLU_{265}, *etc*. in the case of 1OHV, of residues PHE_{131}, THR_{200}, ASN_{62}, TRP_{5}, HIE_{64}, HIS_{94}, *etc*. in the case of 3F8E, and of residues ILE_{379}, LEU_{353}, SER_{383}, PHE_{384}, THR_{1777}, and GLN_{1816} in the case of 6KZP (Table 1). The three most prevalent interactions (between the assessed proteins and the assessed compounds) with residue involvement were chelation bonding, H-bonding, and pi–pi stacking. SwissDock was fed the density-functional theory (DFT)-optimized structures as input. The receptor molecule's crystal structures were obtained from the Protein Data Bank.

3. RESULTS AND DISCUSSION

Molecular docking: A molecular modelling theory called "docking" describes how two or more ligands and proteins fit into one another; it is determined by "ΔG". A greater negative ΔG indicates a better fit between the chemical compound and the protein [9]. Our study employed the molecular level theory and quantum mechanics calculations in order to analyse the interaction of various compounds with proteins involved in anticonvulsant activity. The compounds S3, S1, and S4 showed promising anticonvulsant action (Table 1). Compound S3 exhibited the highest affinity for the protein 1OHV, with a ΔG value of -4.833, while compound S1 had the highest association with the protein 3F8E (ΔG=-3.817) and compound S4 showed the strongest interaction with the protein 6KZP (ΔG=-6.665). Furthermore, it was found that ligand PLP (pyridoxal 5-phosphate) had the strongest affinity with the protein 1OHV (ΔG=-6.773), ligand TE1 had the highest association with the protein 3F8E (ΔG=-5.417), and ligand PLP showed the strongest interaction with the protein 6KZP (ΔG=-6.709). These ligands exhibited higher ΔG values than any of the compounds, thereby indicating their potential as effective drugs across a wider range of compounds. In conclusion, compound S3, with its interaction with protein 1OHV, showed the most promising anticonvulsant activity, while compounds S1 and S4, interacting with proteins 3F8E and 6KZP, respectively, also exhibited potential. Ligands PLP, TE1, and PLP were identified as the most effective ligands for the respective proteins. These findings suggest that these compounds and ligands may serve as potential candidates for the development of anticonvulsant drugs.

DFT analysis: Highest occupied molecular orbitals HOMOs) are the highest in DFT; an atomistic (simulation that calculates a variety of significant features. The least unoccupied molecular orbitals (LUMOs) are the next highest energy orbitals that are empty, while the HOMO–LUMO gap is their

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; 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.

Compound	ΔG	1OHV protein residues surrounding the compounds	Residues with interferences
S1	-4.402	ARG_{192}, GLY_{191}, GLY_{136}, HIS_{190}, PHE_{189}, ASP_{298}, GLU_{265}, GLU_{270}, VAL_{300}, GLN_{301}, ASN_{140}, SER_{137}, SER_{269}, SER_{328}, CYS_{135}, LYS_{329}	GLU_{265}, GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
S2	-3.703	ARG_{192}, GLY_{191}, GLY_{136}, HIS_{190}, PHE_{189}, GLN_{301}, VAL_{300}, ASP_{298}, GLU_{270}, GLU_{265}, SER_{269}, SER_{137}, SER_{328}, ASN_{140}, CYS_{135}, LYS_{329}	GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
S3	-4.833	ARG_{192}, GLY_{191}, GLY_{136}, HIS_{190}, PHE_{189}, ASN_{140}, SER_{137}, SER_{269}, GLY_{136}, CYS_{135}, GLU_{265}, ASP_{298}, VAL_{300}, GLN_{301}, LYS_{329}	SER_{137} (H-bonding); PHE_{189} (pi–pi stacking)
S4	-3.866	PRO_{76}, ILE_{75}, SER_{74}, SER_{328}, SER_{137}, LYS_{360}, LYS_{329}, MET_{332}, CYS_{135}, GLY_{136}, GLY_{191}, PHE_{189}, ARG_{192}, VAL_{300}	LYS_{329}, SER_{328}, GLY_{136} (H-bonding)
S5	-2.491	ARG_{192}, GLY_{191}, GLY_{136}, HIS_{190}, PHE_{189}, GLU_{270}, GLU_{265}, GLU_{299}, SER_{269}, SER_{328}, SER_{137}, ASP_{298}, VAL_{300}, GLN_{301}, THR_{302}, ASN_{140}, CYS_{135}, LYS_{329}	GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
S6	-4.164	ARG_{192}, GLY_{191}, GLY_{136}, HIS_{190}, PHE_{189}, GLN_{301}, VAL_{300}, GLU_{265}, GLU_{270}, ASP_{298}, ASN_{140}, SER_{137}, SER_{269}, SER_{328}, CYS_{135}, LYS_{329}	GLU_{265}, GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
Compound	**ΔG**	**3F8E protein residues surrounding the compounds**	**Residues with interferences**
S1	-3.817	ASP_{72}, GLU_{69}, ASN_{67}, ASN_{62}, ILE_{91}, GLN_{92}, HIS_{94}, HIE_{64}, TRP_{5}, THR_{200}, PHE_{131}	THR_{200}, HIE_{64} (H-bonding)
S2	-3.164	ARG_{58}, GLU_{69}, ASN_{67}, ASN_{62}, HIE_{64}, THR_{200}, PRO_{201}, TRP_{5}, HIS_{94}, GLN_{92}, ILE_{91}	GLU_{69}, H_2O (H-bonding)
S3	-3.15	PRO_{202}, PRO_{201}, THR_{200}, ASN_{62}, ASN_{67}, HIE_{64}, TRP_{5}, GLU_{69}, PHE_{70}, PHE_{131}, ILE_{91}, GLN_{92}, LEU_{57}	H_2O (H-bonding)
S4	-3.099	PRO_{202}, PRO_{201}, THR_{200}, TRP_{5}, ASN_{62}, ASN_{67}, HIE_{64}, ILE_{91}, GLN_{92}, GLU_{69}, PHE_{70}, PHE_{131}, ASP_{71}, ASP_{72}, LEU_{57}	GLU_{69}, H_2O (H-bonding)
S5	-3.422	LEU_{57}, ASP_{72}, ASP_{71}, PHE_{70}, GLU_{69}, ASN_{67}, ASN_{62}, HIE_{64}, TRP_{5}, THR_{200}, PRO_{201}, ILE_{91}, GLN_{92}	
S6	-3.586	ARG_{58}, GLU_{69}, ASN_{67}, ASN_{62}, GLN_{92}, ILE_{91}, HIE_{64}, TRP_{5}, THR_{200}, PRO_{201}	PRO_{201}, H_2O (H-bonding)
Compound	**ΔG**	**6KZP protein residues surrounding the compounds**	**Residues with interferences**
S1	-6.389	LEU_{1499}, LEU_{872}, LEU_{920}, PHE_{868}, PHE_{956}, PHE_{917}, ASN_{952}, THR_{921}, THR_{352}, GLN_{922}, LYS_{1462}	ASN_{952}, LEU_{920}, LYS_{1462}, GLN_{922} (H-bonding)
S2	-5.546	PHE_{917}, PHE_{956}, LEU_{920}, LEU_{1499}, LEU_{872}, LEU_{391}, ILE_{876}, ILE_{387}, THR_{921}, GLN_{922}, LYS_{1462}, ASN_{388}, ASN_{952}, TYR_{953}, GLY_{951}	LYS_{1462}, ASN_{952} (H-bonding)
S3	-5.963	PHE_{917}, PHE_{956}, LEU_{920}, LEU_{1499}, LEU_{872}, LEU_{391}, ILE_{876}, ILE_{387}, LYS_{1462}, THR_{921}, GLN_{922}, ASN_{388}, ASN_{957}, ASN_{952}, TYR_{953}, GLY_{951}	LYS_{1462}, ASN_{952} (H-bonding); PHE_{956} (pi–pi stacking)
S4	-6.665	ILE_{379}, LEU_{353}, LEU_{1819}, LEU_{391}, LEU_{1506}, SER_{383}, SER_{1776}, GLN_{1816}, VAL_{1820}, VAL_{1823}, VAL_{1505}, VAL_{960}, PHE_{1509}, PHE_{956}, PHE_{384}, ASN_{388}, THR_{1777}	ASN_{388}, GLN_{1816} (H-bonding); PHE_{384} (pi–pi stacking)
S5	-6.212	ILE_{387}, ILE_{876}, GLN_{922}, THR_{921}, LYS_{1462}, LEU_{920}, LEU_{1499}, LEU_{872}, ALA_{1502}, PHE_{917}, PHE_{956}, TYR_{953}, ASN_{952}	LYS_{1462}, LEU_{920}, ASN_{952} (H-bonding)
S6	-6.051	ILE_{876}, ILE_{387}, LEU_{872}, LEU_{1499}, LEU_{920}, PHE_{917}, PHE_{956}, LYS_{1462}, THR_{921}, GLN_{922}, TYR_{953}, ASN_{952}, GLY_{951}	LEU_{920}, LYS_{1462}, ASN_{952} (H-bonding); PHE_{956} (pi–pi stacking)

energy difference. According to the simulation, the LUMO, HOMO, and their gap values can define the inclination of molecules to act as bases as opposed to acids. Due to the molecules' high kinetic activity but low stability, the HOMO values of all compounds ranged from -0.227 to -0.199 eV, the LUMO values ranged from -0.092 to -0.087 eV, and the HOMO–LUMO gap values ranged from -0.135 to -0.112 eV. These features were employed in equations that allowed us to identify many molecular properties such the ionization potential (I) and the electron affinity (EA). The values of the studied compounds ranged from 0.199 to 0.227 in the case of their I, and from 0.087 to 0.092 in the case of their EA. Their electronegativity (μ) ranged from 0.143 to 0.159, their softness (S) ranged from 14.81 to 17.85, and their hardness (η) ranged from 0.056 to 0.067.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Preparation and characterization of azelnidipine-loaded D-α-tocopheryl polyethylene glycol succinate (TPGS) / solutol micelles

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Abstract

Azelnidipine is a calcium-channel antagonist classified as a “class 2” drug with high permeability and low aqueous solubility. It is used in the treatment of angina pectoris and hypertension without reflex tachycardia. Improvement of the solubility of azelnidipine and increasing drug’s bioavailability can be achieved through the drug encapsulation in solutol / D-α-tocopheryl polyethylene glycol succinate (TPGS) micelles. Six formulas were prepared by direct dissolution after using different amounts of solutol and TPGS. TPGS and solutol act as solubilizers, permeation enhancers, and P-glycoprotein inhibitors. The particle size, particle size distribution, zeta potential, and entrapment efficiency were determined. Depending on particle size and entrapment efficiency, formula #6 was selected and subjected to *in vitro* dilution stability and *in vitro* release studies. The results obtained showed that formula #6 was the best formula, with a high entrapment efficiency percentage equal to 86.5%±0.58% and a small particle size equal to 21.9±7.75 nm that did not change significantly after dilution up to 100-fold; a fact that reveals the high thermodynamic and kinetic stability of the optimum formula. The formula #6 release profile showed a controlled release of the drug from micelles when compared to plain drug release. Based on these results, polymeric nanomicelles are regarded as a promising delivery system for azelnidipine.

KEYWORDS
Azelnidipine, TPGS, solutol HS15, micelles, drug delivery

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1. INTRODUCTION

Polymeric micelles are spontaneously self-assembled structures composed of amphiphilic molecules, with two main types of segments: hydrophilic and hydrophobic. Their preparation, solubilizing capacity, and nano-size range made them interesting nanocarriers for oral administration routes [1].

Azelnidipine is a calcium channel antagonist with high permeability and low aqueous solubility, that is used in the treatment of angina pectoris and hypertension without causing reflex tachycardia [2].

D-α-tocopherol polyethylene glycol succinate (TPGS) is an amphiphilic copolymer composed of an hydrophilic polyethylene glycol head and a lipophilic tail of vitamin E that is used as a nanocarrier in order to increase drug solubility. Solutol HS15 (polyoxyethylene esters of 12-hydroxystearic acid) is a non-ionic polymer used for enhancing the solubility and stability of insoluble drugs, especially when combined with TPGS [1].

The aim of this study was the enhancement of the solubility of azelnidipine by using TPGS / solutol HS15 mixed micelles nanocarriers, leading to the enhancement of the drug bioavailability.

2. MATERIALS AND METHODS

Chemicals: Azelnidipine, TPGS, and solutol HS15 were purchased from HyperChem (Hangzhou, China). All other chemicals and solvents were of analytical grade.

Preparation of polymeric micelles: Different amounts of the two amphiphilic copolymers (solutol HS15 and TPGS) were used in order to prepare six formulas of azelnidipine-loaded mixed micelles as shown in Table 1. Solutol HS15 and TPGS were dissolved in 10 mL of distilled water in a glass vial, and then 8 mg of azelnidipine dissolved in the solutol HS15/TPGS solution by using a magnetic stirrer at 37°C with the stirring continuing until the complete dissolution of azelnidipine was achieved. After that, the sonication of this mixture was undertaken by using a bath sonicator. Finally, the filtration the mixture using 0.22-μm syringe filters was performed so as to obtain a clear and uniform micellar solution [1].

Particle size, polydispersity index (PDI), and zeta potential measurement: The particle size, the PDI, and the zeta potential of the prepared formulas were measured by a Zetasizer Nano (Malvern Pananalytical, UK) and was performed in triplicate at 25°C [3].

Entrapment efficiency percentage (%EE) measurement: In this study, each formula was centrifuged and then 1 mL of the supernatant was filtered through a 0.45-μm syringe filter and diluted, and the amount entrapped was measured directly by a UV-Vis spectrophotometer [4]. The %EE values were calculated by the following equation [5]:

$$\%EE = \frac{amount\ of\ drug\ in\ the\ micelles}{total\ amount\ of\ drug\ initially\ added} \times 100$$

Dilution stability: Formula #6 was diluted up to 100-fold by using a phosphate buffer at pH 7.4

The in vitro drug release study: The study was performed by using a USP dissolution test apparatus with a rotating paddle [6]. Briefly, capsules containing freeze-dried azelnidipine-loaded TPGS / solutol HS15 micelles (formula #6) or plain azelnidipine were immersed into the vessels of the dissolution testing apparatus containing 500 mL phosphate buffer at pH 6.8 with 0.5% SLS so as to maintain sink conditions, and the system was kept at 37±0.5°C with continuous stirring at 50 rpm. After that, samples equal to 5 mL were withdrawn from the release medium at predetermined time intervals, and were replaced immediately with a fresh buffer solution, and the amount of the released azelnidipine was determined by UV-Vis spectrophotometer [7]. Later, the percentage of the cumulative amount released was calculated and plotted against time [8]. The drug release profile from formula #6 was fitted by using several kinetic models such as zero-order, first-order, Higuchi's, Hixson-Crowell's, and Korsmeyer-Peppas's so as to determine the best-fit model and mechanism of azelnidipine release.

Statistical analysis: The results were expressed as the mean ± standard deviation (SD) of three independent measurements. One-way ANOVA was performed for the statistical analysis by using GraphPad Prism version 9; *P*-values <0.05 were considered as statistically significant [9].

3. RESULTS AND DISCUSSION

Method of polymeric micelles' preparation: The direct dissolution method is regarded as a suitable method for the preparation of these six formulas, because of the higher water solubility of the two copolymers (TPGS and solutol HS15). The use of two copolymers increases the solubility, %EE, and stability of the prepared micelles by the formation of hydrophobic bonds between the micelle's core and the hydrophobic drug (azelnidipine).

Particle size, PDI, and zeta potential determination: The small-size micelles were prepared by a combination of TPGS and solutol HS15 as shown in Table 1, and this increased the solubility, intestinal permeability, and bioavailability, and prolonged the *in vivo* circulation time. There is a significant decrease in particle size (P<0.05) when the concentration of TPGS increases and the concentration of solutol HS15 remains constant in the formulas (#1 compared to #4, #2 compared to #5, and #3 compared to #6) [1]. The narrow size distribution of the prepared formulas (as shown in Table 1) reveals the homogeneity and stability of the prepared formulas (*in vitro* and *in vivo* stability). The zeta potential of the prepared formulas was negatively charged and near neutral (Table 1), and this enhanced the mucus-penetrating prop-

erties of the micelles. Moreover, the presence of polyethylene glycol in the micelles shell generates an external crown that increases the stability of the micelles (steric stabilizer).

%EE determination: The %EE of the prepared formulas is shown in Table 1. There is a significant increase (P<0.05) in %EE when the concentration of TPGS increases and the concentration of solutol HS15 remains constant in the formulas (#1 compared to #4, #2 compared to #5, and #3 compared to #6) [1].

Selection of the optimum formula: Formula #6 which consists of 8 mg of azelnidipine, 100 mg of TPGS, and 100 mg solutol HS15 regarded as the best formula depending on particle size (21.9±7.75 nm), PDI, and %EE (86.5%±0.58%). It was, therefore, subjected to further characterization.

Dilution stability: There was no precipitation and turbidity observed, thereby suggesting a formula stability.

In vitro release study: Azelnidipine was released from the polymeric micelles at a much slower rate than from the plain drug. Within 1 h, almost all of the azelnidipine was released from the plain drug, while 86.66% of the drug was released from TPGS/solutol HS15 micelles at 2.5 h. This refers to the stability of the micelles core, which controls the release of the drug; it increases the contact time with the intestinal mucosa, prevents drug precipitation in the gastrointestinal tract, and increases the extent of the absorbed drug [10]. The formula #6 release profile was best fitted to the Hixon-Crowell's release kinetic model. In this study, the n value was equal to 0.789; it can, thus, be suggested that the mechanism of azelnidipine release from the TPGS/solutol HS15 micelles was through an anomalous non-Fickian diffusion, which indicates an azelnidipine release taking place as a combination of diffusion and erosion of the TPGS / solutol HS15 polymers.

Table 1. Formulas of the azelnidipine-loaded D-α-tocopheryl polyethylene glycol succinate (TPGS) / solutol HS15 micelles, and the results of the assessment of their particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency percentage (%EE). Note: Q.S., *quantum satis*.

Formulas of the TPGS/solutol HS15 micelles				
Formula code	**Azelnidipine** (mg)	**TPGS** (mg)	**Solutol HS15** (mg)	**Deionized water** (up to 10 mL)
Formula #1	8	50	50	Q.S.
Formula #2	8	50	75	Q.S.
Formula #3	8	50	100	Q.S.
Formula #4	8	100	50	Q.S.
Formula #5	8	100	75	Q.S.
Formula #6	8	100	100	Q.S.
Particle size, PDI, zeta potential, and %EE of the formulas devised				
Formula code	**Particle size** (nm)	**PDI**	**Zeta potential** (mV)	**%EE**
Formula #1	365.37±68.49	0.55±0.12	-12.76±6.06	41.64±1.24
Formula #2	166.57±19.27	0.31±0.08	-6.67±2.66	49.5±0.9
Formula #3	151.8±13.67	0.25±0.02	-5.67±5.5	54.07±0.37
Formula #4	36.52±5.21	0.24±0.18	-9.63±6.71	80.13±0.3
Formula #5	19.26±3.10	0.16±0.04	-4.13±5.76	83.78±0.66
Formula #6	21.9±7.75	0.27±0.13	-7.23±5.58	86.5±0.58

4. CONCLUSION

Based on our findings, the direct dissolution method is the proper method for the preparation of these formulas. Formula #6 is the best one, with small particle size, high %EE, and a controlled release profile that increases the azelnidipine bioavailability and is, therefore, regarded as a promising delivery system for azelnidipine.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Antibiotic resistance of *Bacillus* species isolated in foodstuff samples that were collected in Babylon (Iraq)

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Abstract

A total of 164 food samples were collected from various supermarkets and farmers in various areas of Babylon, were placed in plastic containers, and were transported to the laboratory. The isolation of *Bacillus* species was performed by culture in blood agar, chromogenic agar, and the absence of growth on McConkey agar, by staining with Gram stain, and through molecular identification of the species' 16SrRNA gene and sequencing. The antibiotic susceptibility test used eight types of antibiotics. The undertaken sequencing identified the *Bacillus* isolates to belong to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). The susceptibility test of the six *B. cereus* isolates revealed that 5 (83.3%), 4 (66.6%), 3 (50%), 2 (33.3%), 2 (33.3%), 2 (33.3%), 1 (16.6%), and 0 (0%) were resistant to rifampicin, clindamycin, erythromycin, tetracycline, trimethoprim, nitrofurantoin, gentamicin, and ciprofloxacin, while the respective resistance numbers for the two isolates of *B. subtilis* were 2 (100%), 2 (100%), 2 (100%), 0 (0%), 1 (50%), 0 (0%), 0 (0%), and 0 (0%). One isolate of *B. thuringiensis* presented resistance to erythromycin, erythromycin, trimethoprim, and rifampicin, while one isolate of *B. anthracis* was found to be resistant to gentamicin, erythromycin, nitrofurantoin, and rifampicin. The *B. spizizenii* isolate was resistant to all antibiotics except gentamicin and trimethoprim.

KEYWORDS

Bacillus spp., antibiotics, sequencing, foodstuff, antibiotic resistance

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1. INTRODUCTION

Bacterial infections transmitted through food have become one of the most prevalent global public health issues. The *Bacillus* species are Gram-positive, rod-shaped, motile (flagellate) bacteria that can be aerobic or anaerobic by choice. They are known for generating spores and biofilms, and are often found in nature, having been isolated from both fermented and unfermented diets and foodstuff [1,2].

Many *Bacillus* species, such as *B. cereus*, *B. subtilis*, and *B. licheniformis*, demonstrate resistance to antibiotics and can transmit genes for resistance to antibiotics. This suggests that residues of antibiotics could find their way into consumer food items and, subsequently, into the food chain that humans are part of [3]. This may result in the development of bacteria resistant to multiple

drugs, accompanied by the likelihood of transferring resistance genes to pathogenic and non-pathogenic bacteria [4]. The purpose of this study was to examine the antibiotic susceptibility of *Bacillus* species that have been isolated from food samples in Babylon (Iraq).

2. MATERIALS AND METHODS

Sample collection: In this study, a total of 164 food samples were collected; of these, 19 were of meat products (chicken, beef, minced meat), 77 were of dairy product (pasteurized milk, raw milk, cheese, and yogurt), and 68 were of other foods. The samples were collected from September 2023 to November 2023 from different supermarkets and farmers in various regions of Babylon (Iraq).

Bacterial identification: The food samples were measured, labelled distinctly, gathered individually in plastic containers, and then transported to the laboratory for suspension in brain heart infusion broth and incubation at 37°C for 24 h. The samples were then culture in blood agar, chromogenic agar, and MacConkey agar. The form, size, texture, and colony arrangement were then examined. Each colony was picked up, stained with Gram stain, and checked under the light microscope (100x) by using oil emersion.

DNA extraction and polymerase chain reaction (PCR): The DNA extraction was done according to the manufacturer's protocol (Geneaid, Taiwan). The PCR mixture for the 16SrRNA genes in this study was set up for each gene alone in a final volume of 25 µL. The PCR is an enzymatic reaction used for the *in vitro* amplification of target DNA with specific primers and a DNA polymerase. The extracted DNA, primers, and master mix were vortexed and centrifuged briefly to bring the contents to the bottom of the tubes, then placed in a PCR thermocycler in order to amplify the 16SrRNA genes (1,500 bp; F - AGA GTT TGA TCC TGG CTC AG; R - GGT TAC CTT GTT ACG ACT T [4].

Sequencing of 16SrRNA gene: The PCR products of 17 specimens of *Bacillus* species were stored at -20°C, and then the nucleotide sequences of their 16SrRNA genes were carried out by sending the specimens and primer to Macrogen Inc. (South Korea).

Antibiotic susceptibility test: The Kirby-Bauer method was used in order to carry out the antibiotic susceptibility test. The inhibition zones around the disks were measured in millimetres (mm) by using a metric ruler [5].

3. RESULTS AND DISCUSSION

A total of 164 different food samples were collected from September 2023 to November 2023 from various supermarkets and farmers in various areas of Babylon (Table 1). The prevalence of *Bacillus* species according to the undertaken cultures and the biochemical and molecular identification was 7.5% (11 samples). The *Bacillus* isolates belonged to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). Hornik *et al*. [6] have found that the percentage of *Bacillus* species is 10%. Another study has found that 35% of different food samples were positive for *B. cereus* [7]. In China, a recent study has found that 50% of all vegetable samples contained *B. cereus* [8], whereas in Thailand the percentage was approximately 21% [9]. The results differ depending on the origin and number of the sample collection.

The antibiotic susceptibility test used eight types of antibiotics. The undertaken sequencing identified the *Bacillus* isolates to belong to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). The susceptibility test of the six *B. cereus* isolates revealed that 5 (83.3%), 4 (66.6%), 3 (50%), 2 (33.3%), 2 (33.3%), 2 (33.3%), 1 (16.6%), and 0 (0%) were resistant to rifampicin, clindamycin, erythromycin, tetracycline, trimethoprim, nitrofurantoin, gentamicin, and ciprofloxacin, while the respective resistance numbers for the two isolates of *B. subtilis* were 2 (100%), 2 (100%), 2 (100%), 0 (0%), 1 (50%), 0 (0%), 0 (0%), and 0 (0%). One isolate of *B. thuringiensis* presented resistance to erythromycin, erythromycin, trimethoprim, and rifampicin, while one isolate of *B. anthracis* was found to be resistant to gentamicin, erythromycin, nitrofurantoin, and rifampicin. The *B. spizizenii* isolate was resistant to all antibiotics except gentamicin and trimethoprim.

Fiedler *et al*. [10] have found that all *Bacillus* species isolates were resistant to penicillin and cefotaxime, while the resistance to amoxicillin/clavulanic acid and ampicillin was 99.3%. They have also found that the percentage of sensitivity was 99.3%, 98.6%, 98.0%, 93.9%, 91.8%, 76.2%, 88.4%, and 52.4% against ciprofloxacin, chloramphenicol, amikacin, imipenem, erythromycin, gentamicin, tetracycline, and the trimethoprim / sulfamethoxazole combination, respectively [10].

Both public health officials and food processors are now concerned about the rising incidence of multiple antibiotic resistance. Since fresh food doesn't need to be heated further before consumption, there is worry that bacteria resistant to antibiotics might make it through the gastrointestinal tract and complicate therapy for elderly or very young patients, as well as for those with compromised immune systems [10].

Table 1. Overview of the foodstuff samples assessed and of the isolates identified.

Types of samples			**Positive samples** (number and %); **isolates**	**Negative samples** (number and %)	**Total samples** (number and %)
Milk products	yogurt		0 (0%)	11 (6.7%)	11 (6.7%)
	milk	raw	1 (0.6%); *B. cereus*	21 (14.3%)	22 (13.4%)
		canned	1 (0.6%); *B. cereus*	5 (3%)	6 (3.6%)
	cheese	raw	0 (0%)	11 (6.7%)	11 (6.7%)
		canned	0 (0%)	12 (7.3%)	12 (7.3%)
	cream	raw	1 (0.6%); *B. spizizenii*	4 (2.4%)	5 (3%)
		canned	0 (0%)	10 (6%)	10 (6%)
Meat products	beef meat	raw	0 (0%)	4 (2.4%)	4 (2.4%)
		cooked	0 (0%)	7 (4.2%)	7 (4.2%)
	chicken meat	raw	0 (0%)	4 (2.4%)	4 (2.4%)
		cooked	0 (0%)	4 (2.4%)	4 (2.4%)
	fish meat	raw	0 (0%)	1 (0.6%)	1 (0.6%)
		cooked	0 (0%)	2 (1.2%)	2 (1.2%)
Other products	vegetable		1 (0.6); *B. subtilis*	9 (5.4%)	10 (6%)
	rice	cooked	0 (0%)	7 (4.2%)	7 (4.2%)
		raw	1 (0.6); *B. subtilis*	3 (1.8%)	4 (2.4%)
	macaroni		0 (0%)	6 (3.6%)	6 (3.6%)
	bulgur		3 (1.8); *B. cereus* (2x), *B. thuringiensis*	5 (3%)	8 (4.8)
	flour		1 (0.6); *B. cereus*	4 (2.4%)	5 (3%)
	starch		1 (0.6); *B. anthracis*	3 (1.8%)	4 (2.4%)
	wheat		0 (0%)	2 (1.2%)	2 (1.2%)
	barley		0 (0%)	2 (1.2%)	2 (1.2%)
	honey		0 (0%)	5 (3%)	5 (3%)
	bread		1 (0.6); *B. cereus*	4 (2.4%)	5 (3%)
	juice		0 (0%)	7 (4.2%)	7 (4.2%)
Total samples (number and %)			**11 (7.5%)**	**153 (92.4%)**	**164 (100%)**

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Ceruloplasmin levels in β-thalassaemia major: therapeutic insights and implications for iron homeostasis

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Abstract

Ceruloplasmin (CP) is an enzyme that has ferroxidase activity and is important in maintaining iron homeostasis and serving as a copper-transporter in the bloodstream. Beta-thalassemia major (BTM), a common hereditary disorder in Iraq, can affect CP activity in patients with iron overload resulting from frequent blood transfusions in order to sustain haemoglobin levels. The current study is a single-center observational research. CP activity was measured in 304 patients (120 females and 184 males). Anthropometric indices were recorded and the iron load status was determined by measuring serum ferritin. Ninety-two healthy individuals were also included as a control group. Our findings revealed no relationship between age, body mass index, or plasma ferritin and CP activity in BTM patients. No significant influence of sex on CP activity was observed. The outcomes provide insight into assumed pathways regulating CP, and add to the growing body of research on CP's contribution to iron metabolism in BTM patients. To our knowledge, this work is the only study of its kind in Iraq, provides the groundwork for upcoming studies and potential therapeutic lines by generating insightful data on the multifaceted relationships between iron homeostasis, CP, and BTM.

KEYWORDS
thalassaemia, iron overload, ferritin, ceruloplasmin, ferroxidase

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1. INTRODUCTION

Ceruloplasmin (CP) is the main copper-transport protein with serum ferroxidase activity. Like several inflammatory biomarkers including C-reactive protein, tumour necrosis factors, interleukin-1, uric acid, and others, the blood levels of CP will rise in inflammation owing to its well-known positive hepatic acute-phase reactant [1]. CP adjusts the iron homeostasis of the body by its oxidizing ability of ferrous to ferric iron; a rather safer form. Additionally, the activity of ferroxidase has a major contribution in stimulating the loading of iron from body stores and blood toward transferrin. Moreover, CP

participates in several physiological functions like angiogenesis, coagulation, and acts as an antioxidant [2]. The existence of remnants of copper within the lysosomes and in the cytoplasm may be linked to the stronger premise of interaction of the metabolic pathways of the two metals (copper and iron), as CP is a copper-dependent oxidase protein. Thus, CP is essential for the effective release of iron from body cells.

Beta-thalassaemia major (BTM), the most commonly inherited disorder universally, affecting approximately 280 million individuals and causing 16,800 mortalities in 2015. BTM is defined by the lack or decreased synthesis of the β-globin chain, and constitutes a crucial health burden in Iraq [3]. Regular regimens of blood transfusions so as to preserve the haemoglobin levels are critical for the BTM patients' survival. Thus, persistent iron overload exceeding the capacity of the human body to bind free iron, causes its deposition in vital body organs, especially if improper iron chelators have been administered. Earlier articles from tertiary referral centers have demonstrated that the ferroxidase activity and CP levels were correlated with iron overload, thereby suggesting a modulatory influence of iron on the CP genetic expression [4]. The normal value of blood CP levels for adults is around 22 to 40 mg/dL for a male, or 25 to 60 mg/dL for a female [5]. Though the CP ferroxidase activity plays a main role in iron homeostasis, the data about its precise role concerning the status of ferritin in BTM patients are limited, and not well recognized. Hence, this study aimed at estimating the CP activity in patients with multi-transfused BTM, so as to assess the efficiency of the iron chelation therapy.

2. PATIENTS AND METHODS

Study design and sampling: During this single-center observational study, 304 BTM patients (184 males and 120 females) aged between 2.3 and 17.9 years were included throughout the period from May to December 2020. Patients were recruited from those documented and followed up at the Babylon Hereditary Blood Disorders Center in Babylon. The diagnosis of BTM was already established for registered patients. Those who were not fully-compliant with the blood transfusion regimens, or were severely incapacitated, were excluded from the study. A uniform and approved study formulary was filled by the interviewer so as to collect data from all BTM patients. Ninety-two healthy volunteers (60 males and 32 females) constituted the control group, and were included while performing a systematic medical examination in the Babylon Hospital for Maternity and Childhood. Demographic parameters, age at diagnosis, family history, rate of blood transfusions received per month, and types of chelating therapy received were recorded. Blood samples were collected for serum ferritin and CP analysis.

CP (ferroxidase) activity assay: The CP activity was measured by a spectrophotometric assay (*p*-phenylenediamine-method) [6].

Ethics approval: An ethics approval was obtained by the hospital's local ethical committee. A verbal consent was obtained from all participants.

3. RESULTS

The mean age was 14.6±7.3 years, with the dominance of males (n=184; 60.5%). Of the total contributors, 40% were on regular transfusions once monthly, and 47% were on bimonthly transfusions. Moreover, 194 (63.4%) patients were on deferasirox (Exjade®) oral chelators and 104 (34%) were on injectable chelators. There were significant variations in terms of the age, CP, ferritin, and body mass index (BMI) between the thalassaemia patients and the normal subjects. No significant alterations were observed in terms of the CP activity between those with serum ferritin levels above and those below 2.500 ng/mL, as well as between the serum CP levels and serum ferritin levels, age, or BMI of the patients.

Table 1. Comparison of the main study variables between the two groups assessed.

Variables	Group	Mean	Standard deviation	*P*-value
Age (y)	Patients	18.9	9.9	<0.001
	Control	29.6	8.1	
Ceruloplasmin levels (mg/dL)	Patients	42.3	12.6	<0.001
	Control	34.6	5.4	
Ferritin levels (ng/mL)	Patients	2,976.9	2,084.3	<0.001
	Control	180.0	34.7	
Body mass index (kg/m^2)	Patients	18.4	3.2	<0.001
	Control	24.1	3.9	

4. DISCUSSION

Isolating biomarkers involved in the pathogenesis of thalassemia can potentially have a contribution to the linking of the analytical gap and the formation of adapted management regimens. This work aimed to assess the blood CP levels through its ferroxidase activity rather than its concentration, in multi-transfused BTM patients, in an attempt to investigate the role of CP activity in BTM pathogenicity.

Our findings were inconsistent with those of previous reports that have shown that the CP activity was within the normal accepted ranges for both male and female BTM patients. Significantly high levels of CP were reported among thalassaemia patients with certain phenotypes of BTM in a Jordanian study that included 124 BTM patients. Along similar lines, a preceding Iraqi survey included 101 thalassaemic patients who revealed increased CP levels compared to the control group. Turkish researchers have reported significantly higher CP levels among thalassaemic patients aged 6-10 years in a series of 29 patients, whereas CP levels were found decreased in 10.3% of the patients. However, their population sample was small (29 patients) compared to the population of this study.

In the current study, a non-significant correlation between the serum levels of CP and the serum ferritin levels exists. Some authors have attributed the increased serum CP levels detected in thalassaemia patients to several mechanisms associated with iron overload rather than to inflammation. The observed higher CP levels in thalassaemia might be related to the antioxidative property of haptoglobin, which is primarily due to its high serum levels and its elevated affinity to free haemoglobin in the blood of thalassaemic patients. Finally, high CP serum levels might be due to hepatic insufficiency induced by thalassaemia and/or iron chelators.

The CP levels might increase in order to counteract reactive oxygen species through the Fenton pathway, which can be completed by the antioxidant ability of CP by the ferro-oxidation of iron, thereby inhibiting the oxidative injury of cellular proteins, lipids, and DNA [2]. CP is possibly elevated because of the tissue accumulation of iron, therefore increasing the iron efflux into transferrin in blood. Few researchers have delivered evidence to support the physiological ability of CP to mobilize iron molecules from cellular stores or plasma. Furthermore, experimental trials on subjects with haemochromatosis or aceruloplasminaemia have revealed marked iron multi-organ accumulation (including the liver and the brain) [7]. Iron accumulation can result from the inability of the CP ferroxidase capacity to mobilize iron from tissue stores or plasma. Experimental models of animals lacking CP have revealed that CP administration may restore iron homeostasis in these animals [8]. Finally, increased CP levels in thalassemia patients are likely secondary to prolonged hypoxia and defective erythropoiesis, which are shared characteristics among thalassaemia patients.

It is expected for thalassaemia patients to be under oxidative stress, and thus have disturbed iron metabolism besides a high iron-induced oxidative stress. Hence, it is likely that the functional activity of CP, as a main antioxidant, is changed in such a way so as to compete against the sequels of iron overload.

A significantly higher CP activity has been detected among females, although no changes have been observed in female BTM patients when compared with the control group in this regard; moreover, a lower activity in male control subjects has been observed compared to patients [4]. Moreover, serum CP levels have been found to be meaningfully higher in females [9]. To some extent, these incongruous outcomes can be explained from an endocrine perspective. In females, the lack of CP and/or reduced ferroxidase activity might be linked to oestrogen influence and could be responsible for the minor phenotypic expression observed. In any case, the gender differences in serum CP activity should be considered in future works.

To the best of our knowledge, the current study represents the first to evaluate CP activity in BTM patients in Iraq. The authors selected the method of Sunderman and Nomoto [6] employing a spectrophotometric *p*-phenylenediamine oxidase analysis, since it is simple, easy, and reproducible, and it allows us to distinguish the CP's actual activity from the activity of the total serum oxidase.

5. CONCLUSION

This study casts doubt on the idea that BTM patients have elevated CP levels, and emphasizes the necessity of further study into the intricate interactions between CP, iron overload, and BTM pathogenicity.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Comparative assessment of the effects of two intrauterine systems for long-term contraception on some haematological, biochemical, and immunological markers

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Abstract
An intrauterine system (IUS) is a type of contraception tool that is used in order to control fertility and prevent conception in women for a long period. The aim of this study was to assess the influence of copper- *versus* levonorgestrel-releasing IUSs on women's health. This is a descriptive cross-sectional study of 75 women that were randomly selected (50 women that used a copper-releasing IUS and the remaining 25 that used a levonorgestrel-releasing IUS) amongst those attending out patient's clinics at Hillah, Iraq, from March to July 2016. All women were between 18 to 46 years of age, and have had an IUS for at least three months. The measurement of serum ceruloplasmin (SCerP), haemoglobin, vitamin D (VD), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) levels was undertaken. Our results revealed that the copper-releasing IUS group has low haemoglobin and VD levels, along with high levels of SCerP and proinflammatory cytokines. One the other hand, the levonorgestrel-releasing IUS group displayed no significant changes on the above markers. We can conclude that the levonorgestrel-releasing IUS is free of any adverse effect when compared to a copper-releasing IUS, at least with regard to the parameters examined by our study.

KEYWORDS
intrauterine system, copper, levonorgestrel, vitamin D, IL-6, IFN-γ

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1. INTRODUCTION

An intrauterine system (IUS) is highly effective contraception, with less than a 1% failure rate. There are two main types: the copper-releasing

and the levonorgestrel-releasing IUS. While both types work similarly in preventing pregnancy, the levonorgestrel-releasing IUS also helps manage heavy menstrual bleeding and offers other health benefits like reducing the risk of endometrial cancer and pelvic inflammatory disease (PID) [1]. Side effects such as irregular bleeding, headaches, and mood changes are usually mild and improve over time with the levonorgestrel-releasing IUS. On the other hand, the copper-releasing IUS may cause heavier bleeding and cramping during periods, but does not affect hormones [2]. However, it may increase the risk of infection, PID, infertility, ectopic pregnancy, or even perforation during insertion. The suitability of the IUS depends on factors like the medical history, the reproductive goals, and the lifestyle, thereby necessitating a thorough discussion with a healthcare provider for proper decision-making [3].

There is an important role for vitamin D (VD) in the body's immune system: its receptors are found in significant concentrations in T-lymphocytes (mostly in the mature CD-8 T-lymphocytes). This suggests that the action of T-lymphocytes might be mediated by VD. In addition, VD is known to block the effects of adaptive immunity, thereby leading to the prevention of autoimmunity [4]. VD can also block the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), the tumour necrosis factor - alpha (TNF-α), and interferon-gamma (IFN-γ); a fact that may also be associated with the stimulation of the production of anti-inflammatory cytokines and the decrease in killer T-cell production [4,5]. On other hand, the deficiency of VD can inhibit the production of B-lymphocytes, thereby leading to the blocking of the production of immunoglobulins [5]. Our study has aimed at assessing the influence of copper- *versus* levonorgestrel-releasing IUSs on women's health, as reflected by the levels of VD and some important immunomodulatory markers (such as IL-6 and IFN-γ).

2. SUBJECTS AND METHODS

Study design: This is a descriptive cross-sectional study of 75 women that were randomly selected (50 women that used a copper-releasing IUS and the remaining 25 that used a levonorgestrel-releasing IUS) amongst those attending out patient's clinics of the Babylon Teaching Hospital for Maternity and Paediatrics and the Al-Hilla Teaching Hospital, at Hillah, Iraq, from March to July 2016. All women were between 18 to 46 years of age, and have had an IUS for at least three months.

Exclusion criteria: Eligible women ought not to document any known chronic diseases (such as diabetes, hypertension, chronic kidney disease, sexually transmitted diseases, or bleeding tendencies). We also excluded women with serious underlying diseases or any gynaecological diseases, and women who were smoking, as these conditions / habits could interfere with the results.

Ethics statement: The study was carried out in compliance with the Declaration of Helsinki principles, and was reviewed and approved by the local research ethics committees of the Faculty of Medicine of the University of Babylon. After gaining the verbal consent from our patients, the data were collected by a well-trained researcher using a standardized questionnaire. Participating women were told that their rejection or withdrawal from the study would have no impact on the medical care they received.

Demographic and clinical variables: These included age, educational level, employment status, pattern of menstrual period, any current vaginal infection, and body mass index (BMI).

Laboratory investigation: We measured the haemoglobin levels, the serum ceruloplasmin (SCerP; which reflected serum copper) levels by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (E-EL-H6026; Elabscience, USA), VD levels by using ELISA (DE1971; Demeditec Diagnostics, Germany), as well as IL-6 and IFN-γ levels *via* ELISA kits (E-EL-H0192 and E-EL-H0108, respectively; Elabscience, USA).

Statistical analysis: Wherever applicable, a *t*-test was utilized and the significance level was fixed at a $P<0.05$. GraphPad Prism 5.0 on Windows was used in order to conduct all analyses (GraphPad Software, San Diego, CA, USA).

3. RESULTS

The participants of copper-releasing IUS group were mainly between 20 to 30 years of age (52%), while those in the levonorgestrel-releasing group were mainly between 31 to 40 years of age (56%). Most participants in the levonorgestrel-releasing IUS group were higher education graduates (72%), while in the copper-releasing IUS group, the majority of the participants had completed only elementary studies (42%). Most of the levonorgestrel-releasing IUS group women were employed (68%), while in copper-releasing IUS group, the majority were unemployed (62%). The BMI of the levonorgestrel-releasing IUS group's participants was in most cases overweight (40%) or obese (40%), while in the copper-releasing IUS group, most participants were overweight (58%). The coloured vaginal discharge which might be associated with infection was reported by 32% of the

levonorgestrel-releasing IUS group women and by 70% of the copper-releasing IUS group women. Dyspareunia was present in 24% of the participants of the levonorgestrel-releasing IUS group and in 40% of the participants of the copper-releasing IUS group. Finally, menorrhagia was reported by 64% of the women belonging to the copper-releasing IUS group and only by 12% of the women belonging to the levonorgestrel-releasing IUS group (Table 1).

Table 1. General data and data corresponding to serological and immunological tests for the study's two groups of participants bearing an intrauterine system (IUS) in the Babylon Governorate.

General Data of the study's participants			
	Age (number of subjects and percentage)		
	<30 years	**30–40 years**	**>40 years**
Copper-releasing IUS (n=50)	26 (52.0%)	22 (44.0%)	2 (4.0%)
Levonorgestrel-releasing IUS (n=25)	7 (28.0%)	14 (56.0%)	4 (16.0%)
	Educational level (number of subjects and percentage)		
	Elementary	**Secondary**	**Higher education**
Copper-releasing IUS (n=50)	21 (42.0%)	17 (34.0 %)	12 (24.0%)
Levonorgestrel-releasing IUS (n=25)	2 (8.0 %)	5 (20.0 %)	18 (72.0%)
	Employment status (number of subjects and percentage)		
	Employed	**Unemployed**	
Copper-releasing IUS (n=50)	19 (38.0%)	31 (62.0%)	
Levonorgestrel-releasing IUS (n=25)	17 (68.0%)	8 (32.0%)	
	Duration of IUS insertion (number of subjects and percentage)		
	<1 year	**1–2 years**	**>2 years**
Copper-releasing IUS (n=50)	13 (26.0%)	29 (58.0%)	8 (16.0%)
Levonorgestrel-releasing IUS (n=25)	12 (48.0%)	11 (44.0%)	2 (8.0%)
	Body mass index (number of subjects and percentage)		
	Normal (18.5–24.9)	**Overweight (25–29.9)**	**Obese (>30)**
Copper-releasing IUS (n=50)	9 (18.0%)	29 (58.0%)	12 (24.0%)
Levonorgestrel-releasing IUS (n=25)	5 (20.0%)	10 (40.0%)	10 (40.0%)
	Vaginal discharge (number of subjects and percentage)		
	Whitish or transparent	**Coloured (purulent, brownish or bloody)**	
Copper-releasing IUS (n=50)	15 (30.0%)	35 (70.0%)	
Levonorgestrel-releasing IUS (n=25)	17 (68.0%)	8 (32.0%)	
	Dyspareunia (number of subjects and percentage)		
	Presence	**Absence**	
Copper-releasing IUS (n=50)	20 (40.0%)	30 (60.0%)	
Levonorgestrel-releasing IUS (n=25)	6 (24.0%)	19 (76.0%)	
	Menorrhagia (number of subjects and percentage)		
	Presence	**Absence**	
Copper-releasing IUS (n=50)	32 (64.0%)	18 (36.0%)	
Levonorgestrel-releasing IUS (n=25)	3 (12.0%)	22 (88.0%)	
	Haemoglobin levels		
	<12 g/dL	**≥12 g/dL**	
Copper-releasing IUS (n=50)	34 (68.0%)	16 (32.0%)	
Levonorgestrel-releasing IUS (n=25)	9 (36.0%)	16 (54%)	

Serological and immunological tests			
Levels	**Copper-releasing IUS** (n=50)	**Levonorgestrel-releasing IUS** (n=25)	***P*-value**
Serum ceruloplasmin (μg/dL)	121.28 ± 19.73	114.13 ± 11.69	>0.05
Vitamin D (ng/mL)	19.83 ± 3.79	24.65 ± 9.63	<0.01
Interleukin-6 (pg/mL)	314.51 ± 51.21	278.33 ± 38.19	<0.01
Interferon-gamma (pg/mL)	107.80 ± 18.16	91.16 ± 32.51	<0.01

Haemoglobin levels appeared to be mostly normal in the levonorgestrel-releasing IUS group (54%), while in the copper-releasing IUS group they were mostly <12 g/dL (68%). The SCerP levels in levonorgestrel-releasing IUS group were 114.13 ± 11.69 μg/dL, while in the copper-releasing IUS group the same levels were 121.8 ± 19.73 μg/dL (P>0.05). The serum VD levels ranged from 15.18 to 35.5 ng/mL, with the lower levels being observed in the copper-releasing IUS group (19.83 ± 3.79 ng/mL; P<0.01). The range of the IL-6 levels was 240.22 to 365.31 pg/mL, with the mean level being lower in the levonorgestrel-releasing IUS group (278.33 ± 38.19 pg/mL; P<0.01); similarly, the range of the IFN-γ levels was 95.22 to 125.93 pg/mL, with the mean level being lower in the levonorgestrel-releasing IUS group (91.16 ± 32.51 pg/mL; P<0.01) (Table 1)

4. DISCUSSION

Although contraceptive methods are commonly used, their reassessment is still an obligatory action aiming to decrease morbidity for women. This study has compared two commonly used birth control devices, aiming to determine their respective advantages for women and any accompanying conditions. We have identified differences in the impact of clinical and biochemical parameters between women using either levonorgestrel- or copper-releasing IUSs, independent of any underlying conditions that might affect their mode of action [6]. Monocausal theories were insufficient to explain the effects observed, such as the influence of age, BMI, clinical symptoms (such as vaginal discharge and dyspareunia), and duration of insertion; all of which negatively affect women using either type of IUD. Our study has also revealed a significant association between menstrual blood loss (menorrhagia) and the type of IUS used, thereby indicating that the copper-releasing IUS is more likely to cause menorrhagia and greater blood loss, leading to decreased haemoglobin levels and iron deficiency [7]. Additionally, levels of SCerP showed a significant difference, with the copper-releasing IUS having a greater (yet, non-significant) effect on women using it compared to the levonorgestrel-releasing IUS [8].

Our study also examined the physiological alterations associated with the use of IUS, by focusing on the immune system's role and its impact on women's health. The literature highlights the presence of various proteins, cytokines, and cells involved in maintaining immunity, with a particular emphasis on T-helper cells and their potential to trigger autoimmune and inflammatory diseases [9]. Studies suggest that high levels of proinflammatory cytokines (particularly IL-6) are associated with negative health outcomes in women, along with activated T-cells. While there is no significant evidence linking changes in IFN-γ levels with women's health, a recent study suggests a correlation between the VD status, IFN-γ secretion, and women's health and fertility [10]. Our findings suggest a nuanced role for the immune system in women's health beyond inflammation, thereby indicating that low VD levels in copper-releasing IUS users may lead to adverse outcomes and comorbidities. These findings are suggestive of the need for contraception with fewer side effects and greater efficacy.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Hepatotoxic effects of *Stevia rebaudiana* leaf extract and commercial stevia on rats: a comparative study

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Abstract

The popularity of stevia is high, especially among diabetics and those looking to reduce their calorie-intake. The aim of this study was to compare the effects of a commercially-available stevia and of a *Stevia rebaudiana* leaf extract on the liver function and histology of rats. After preparing the *Stevia rebaudiana* leaf extract, 60 healthy adult male rats were randomly separated into three groups: untreated control, commercial stevia treatment (25 mg/kg), and *Stevia rebaudiana* leaf extract treatment (25 mg/kg). Our results show that after 60 days of treatment (oral administration), a significant elevation of the alanine aminotransferase (ALT) levels was observed in the commercial stevia-treated group, suggesting potential effects on liver function. The *Stevia rebaudiana* leaf extract-treated group also exhibited increased ALT levels. Moreover, the aspartate aminotransferase (AST) levels were found significantly increased in both of these treatment groups (when compared to the control group). Alkaline phosphatase levels were not found altered between groups. Histological-examinations, in spite of the elevated ALT and AST levels, exhibited no abnormalities in the liver. Although stevia is generally regarded as safe, this study underlines the importance of considering the type and form of stevia when evaluating its effects on liver health. Further study is warranted so as to elucidate the specific components and mechanisms responsible for the observed variations in liver enzymes, and to confirm the overall safety of stevia products.

KEYWORDS

Stevia rebaudiana, steviol glycosides, rat model, liver enzymes, hepatotoxicity

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1. INTRODUCTION

Stevia has become popular in recent years as a natural, zero or low-calorie alternative to typical sweeteners like sugar and artificial-sweeteners. The leaves of *Stevia rebaudiana* are the main source of these pleasing chemicals [1]. They are

usually harvested, dried, and processed to produce steviol glycosides, which can be up to 300 times sweeter than table sugar (sucrose). Moreover, stevia has been suggested to possess anti-inflammatory, antioxidant, and antihypertensive properties [2]. However, it is necessary to note that, while stevia is typically considered harmless, individual-reactions to sweeteners might vary, and more research is required in order to properly understand its long-term effects.

It is important to note that many aspects can influence liver health, including an individual's overall diet, lifestyle, and any pre-existing medical issues. Few studies have explored the potential hepatoprotective effects of stevia in animal models, suggesting that it may exert antioxidant and anti-inflammatory effects [3]. The aim of this study was to compare the effects of a commercially-available stevia and of a *Stevia rebaudiana* leaf extract on the liver function and histology of rats. Our study tried to provide insights into the impact of different forms of stevia on liver function, shedding light on possible changes in liver enzyme responses and histological abnormalities.

2. MATERIALS AND METHODS

The *Stevia rebaudiana* leaf extract was prepared by drying the leaves in the dark, and then grinding them with a mortar. Subsequently, 500 g of the leaves were placed in a flask with 500 mL of distilled water, on a hot plate, and were left stirring for 24 h at 70°C. The *Stevia rebaudiana* extract was filtered through a Whatman No. 1 filter paper, and the supernatant was concentrated with a rotary vacuum evaporator [4].

This study involved 60 healthy adult male albino Wistar rats weighing between 240 and 280 g. Before beginning the experiment, all rats were acclimatized to the normal conditions of 12 h of light and 12 h of darkness, at 25°C±4°C. The rats were randomly separated into three groups; the first group (n=20) served as the untreated control group, the second-group (n=20) received a 25 mg/kg dose of commercially-available stevia (market stevia), and the third group was treated with a 25 mg/kg dose of the *Stevia rebaudiana* leaf extract. Stevia was liquefied in distilled water and was administered orally to the rats for 60 days. Following the 60-day treatment period, the animals received anaesthesia by using diethyl ether, and a heart puncture was performed in order to collect 1 mL of blood from each rat [4]. Blood test determined the alanine aminotransferase (ALT) levels based on the Wrobleski and Ladue methods, the aspartate aminotransferase (AST) levels on the method described by Karmen, and the alkaline phosphatase (ALP) levels based on the hydrolysis of *p*-nitrophenylphosphate [5]. The rat liver was removed in order to histologically assess it by staining its sections with haematoxylin and eosin. All statistical analyses were performed by using Microsoft Excel 2020 and the GraphPad Prism software (version 6).

3. RESULTS AND DISCUSSION

The weight of the extracted material from 500 g of *Stevia rebaudiana* leaves' powder was determined to be 411 mg, whereas the percentage weight corresponded to 0.0822% of the 500 g of the *Stevia rebaudiana* leaf extract. The extraction weight of the Stevia rebaudiana leaf powder is an important quantitative measure that shows the yield of the extraction process; it reveals the amount of the material extracted from the leaves during processing.

As far as the ALT levels are concerned, a statistically significant difference (P<0.0001) appears to exist between the control group (27.50±4.286 U/L) and the group treated with commercial stevia (68.80±8.205 U/L). Additionally, our results reveal a significant difference (P=0.0002) between the control group and the group treated with the *Stevia rebaudiana* leaf extract ALT levels (56.40±10.72 U/L), while a significant difference was also detected between the groups treated with commercial stevia and those treated with the *Stevia rebaudiana* leaf extract (P=0.0002). ALT is an enzyme found mainly in the liver, and variations in its levels frequently indicate liver health issues and functional disorders. The significant increase in ALT levels in the group treated with commercial stevia suggests that the material may have a negative effect on liver function. The significant difference observed between the two treatment groups suggests that the type or form of stevia used may also affect the ALT levels. This can be due to differences in the chemical compounds, the processing processes, or the concentrations of active components between the commercial stevia and the *Stevia rebaudiana* leaf extract [6].

The assessment of the AST levels revealed substantial differences between the study's groups. The rats treated with commercial stevia had significantly greater AST levels (50.05±12.23 U/L) than those of the control group (40.50±6.501 U/L; P=0.0038). Moreover, our study found that rats treated with the *Stevia rebaudiana* leaf extract also had significantly greater AST levels (50.55±14.71 U/L) when compared to those of the control group (P=0.0081). Remarkably, no significant difference was observed between the treatment groups. These findings reveal that both forms of stevia

cause a significant and similar increase in AST levels. These findings reveal the potential impact of stevia products on liver function, and underline the need for further research into the exact compounds and mechanisms affecting the AST levels [7].

The assessment of the ALP levels revealed no significant different among the studied groups. The ALP levels were 206.0±81.89, 208.3±73.54, and 217.9±82.51 U/L in the control, the commercial stevia-treated, and *Stevia rebaudiana* leaf extract-treated group, respectively. The absence of substantial differences in terms of the ALP levels between the studied groups implies a degree of safety from potential hepatotoxic consequences.

Finally, in spite of the significant differences in ALT and AST levels, the undertaken haematoxylin and eosin staining of liver sections revealed no significant changes in the cellular architecture and morphology of the liver in any of the studied rat groups. These histological findings could be attributed to the liver's remarkable ability to employ compensatory mechanisms. Even in the presence of elevated enzyme levels, the liver can sometimes maintain its structural integrity and functionality, thereby exhibiting a seemingly normal histological appearance [8].

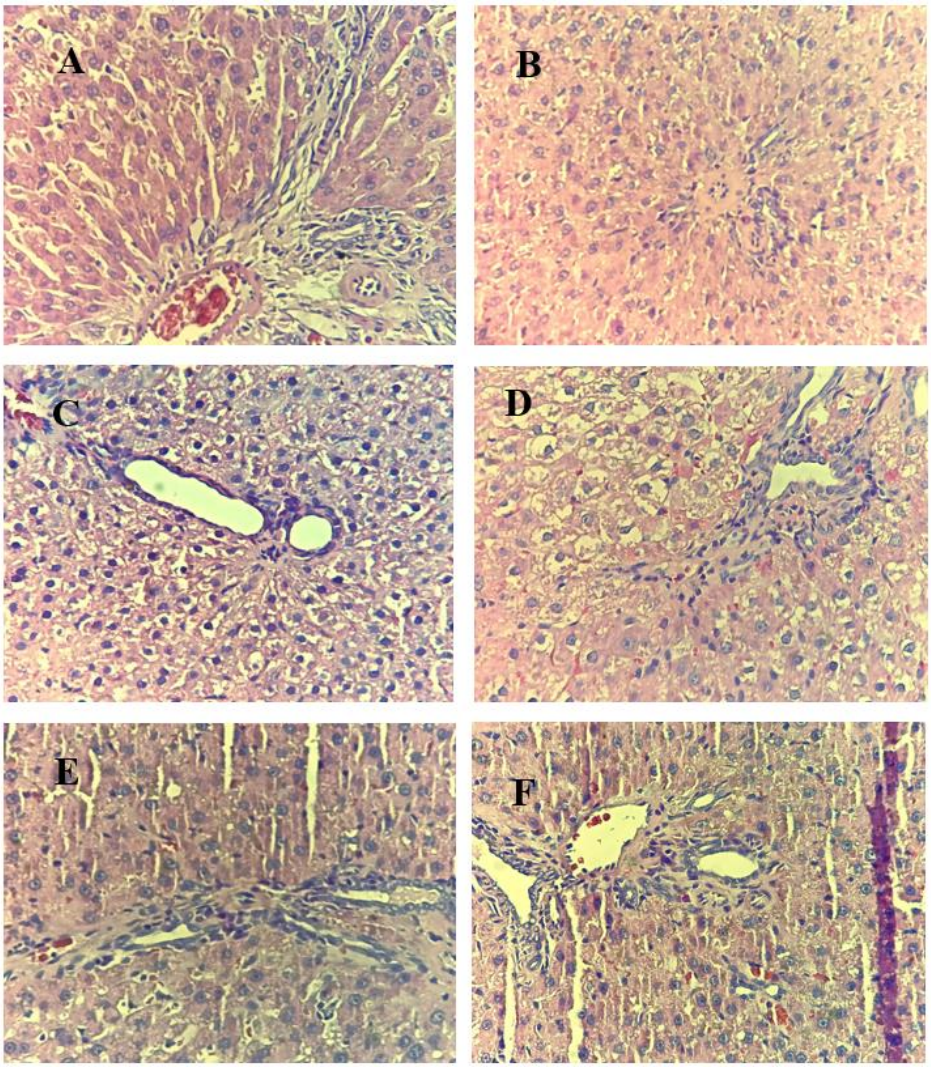

Figure 1. Representative light microscopy captions of the haematoxylin and eosin staining for the histological evaluation of the rat livers of the untreated control group (captions **A** and **B**), the commercial stevia-treated group (captions **C** and **D**), and the *Stevia rebaudiana* leaf extract group (captions **E** and **F**) showing no significant histopathological changes in all cases.

4. CONCLUSION

This study underlines the importance of considering the type and form of stevia when evaluating its effects on liver health. Further study is warranted so as to elucidate the specific components and mechanisms responsible for the herein observed variations in liver enzymes, and to confirm the overall safety of stevia products.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Molecular study of *KRAS* mutations in Iraqi patients with gastrointestinal tract cancer

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Abstract

Gastrointestinal cancers, including stomach, liver, oesophageal, pancreatic, and colorectal cancers, represent more than a quarter of all cancers. Many abnormal gene expressions and dysregulated signalling pathways have been found in human cancer. Cancer often has activating mutations of the KRAS (Kirsten rat sarcoma virus) oncogene. Fifty blood samples from gastrointestinal cancer patients were gathered from the Merjan Teaching Hospital in Babylon, Iraq, and were used for a case-control study in the Oncology Center. According to the results, the most common cancers were found in the colon (29%), followed by the liver (27%), pancreas (19%), stomach (13%), and other (12%). In this work, we evaluated the distribution of *KRAS* mutations across the gastrointestinal tract. Sequencing data revealed a significant regional difference in the frequency of *KRAS* mutations, while the alignment results revealed the presence of six variations in the analysed samples when compared with the referring reference DNA sequences. Six highly interesting nucleic acid polymorphisms were detected in the investigated samples. When combined with additional carcinogenic markers such as the patient sex, age, consistent molecular subtypes, and tumour stage, *KRAS* mutation is not the deterministic carcinogenic factor for gastrointestinal malignancies.

KEYWORDS
polymorphism, KRAS gene, gastrointestinal tract cancer, protein folding, mutation

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1. INTRODUCTION

Cancer is a leading cause of death worldwide, with gastrointestinal cancers such as colon, pancreatic, and gastric cancer posing significant health threats. Gastrointestinal cancers are a diverse group of malignancies that arise from different sites in the digestive system, each with its unique risk factors, symptoms, and molecular pathology [1]. Many risk factors or causes of cancer development contribute to the changes in a cell that can result in cancer. These risk factors may be intrinsic to an individual, such as sex, age, or genes. However, most risk factors are

external, in the individual's general environment. The interplay between the intrinsic and external factors is the major determinant of an individual's cancer risk [2].

A biomarker is an objectively measured characteristic that describes a normal or abnormal biological state in an organism by analysing biomolecules such as DNA, RNA, proteins, and peptides, as well as biomolecule chemical modifications. Biomarkers are useful in several ways, including measuring the progress of disease, evaluating the most effective therapeutic regimes for a particular cancer type, and establishing long-term susceptibility to cancer or its recurrence [3]. The three human RAS genes (*KRAS*, *NRAS*, and *HRAS*) are the most frequently mutated oncogenes in human cancer, appearing in 90% of pancreatic, 35% of lung, and in 45% of colon cancers. These high occurrences make RAS one of the most important targets in oncology for drug development, In particular, KRas is the isoform prevalently mutated in pancreas, lung, and colon cancer [4]. This case-control study aimed to analyse whether a relationship between the KRAS gene and gastrointestinal tract cancer patients exists.

2. PATIENTS AND METHODS

The current case-control study included 50 samples of patients with gastrointestinal tract cancer and 50 apparently healthy individuals as a control group. Samples were collected from the Merjan Teaching Hospital in Babylon (Iraq) by the Oncology Center. Each patient with cancer was confirmed and diagnosed by an oncologist; for all patients, a complete history was taken, which included age, sex, smoking habits, family history, duration of disease, record of chemotherapy, and type of cancer. This study was undertaken from February 2018 to May 2019. About 50 blood specimens were obtained from patients aged 30 years to 80 years, while the control cases were aged 33 years to 81 years. Genomic DNA was extracted and purified by using a G-spin™ Total DNA Extraction Mini Kit (Intron, Korea) according to the manufacturer's instructions. Conventional polymerase chain reaction tests were undertaken in order to detect the KRAS genome, while gene polymorphisms were genotyped by utilizing the single-strand conformation polymorphism method.

The study's protocol was reviewed and approved by the University of Babylon College of Science ethical committee.

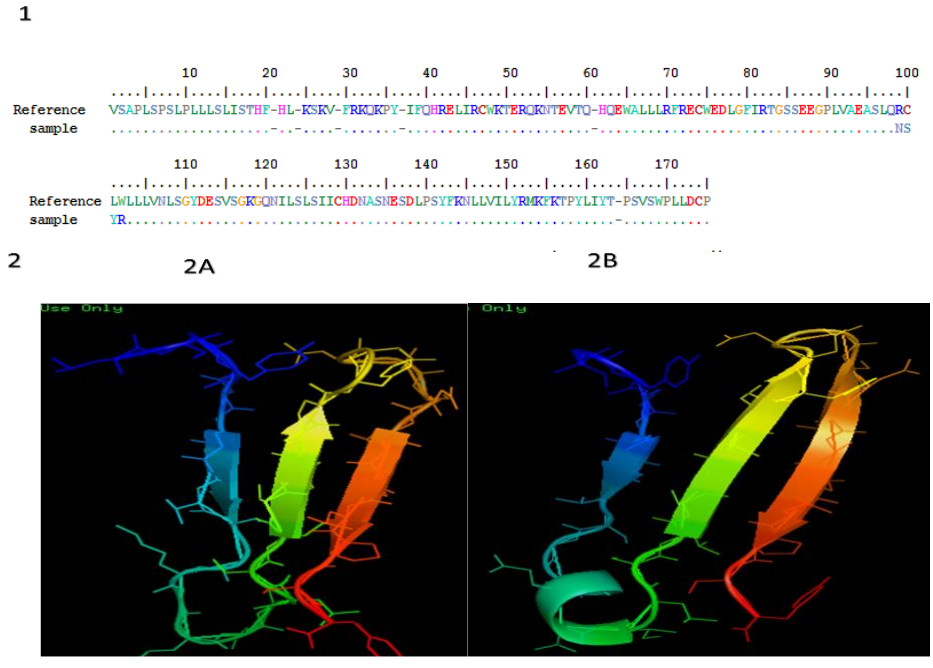

Figure 1. Top panel (1): sequence alignment of amino acids in the K-Ras protein drawn based on the sequence alignments obtained by the BioEdit program version 7.2.5. Bottom panel (2): 3D secondary structures of the K-Ras protein in patients with gastrointestinal cancer: normal (2A) and abnormal (2B)

3. RESULTS

A molecular study of the gastrointestinal tract cancer samples demonstrated that regarding the type of cancer, colon cancer represented 29% of the samples, with liver (27%), pancreas (19%), stomach (13%), and other types (12%) of cancer being represented at lower percentages. The results obtained from the sequenced 532 bp fragments as well as the detailed positions of the observed variations are described in the NCBI reference sequences. The KRAS gene polymorphism revealed six types of substitutions: guanine (G) to adenine (A; G>A) in position 301 of the sequence, G to thymine (T; G>T) in position 302 of the sequence, T to cytosine C; T>C) in positions 303 and 309 of the sequence, G to C (G>C) in all samples in position 304 of the sequence, C to T (C>T) in positions 305 and 306 of the sequence, and T to A (T>A) in positions 307 and 308 of the sequence. When translating the DNA sequence by using by the BioEdit program (version 7.2.5) according to the reference sequence alignment of the K-Ras protein, arginine changed to asparagine, cysteine to serine, leucine to tyrosine, and tryptophan to arginine. This result led to a change in the 3D secondary structure of the protein in patients with gastrointestinal cancer as shown in Figure 1.

4. DISCUSSION

The use of gene alterations in blood in order to track circulating tumour DNA has been attempted for clinical applications. For example, *KRAS* monitoring in colorectal cancer provides a valuable biomarker for diagnosis and the prediction of treatment outcomes. While half of colon cancer samples have a *KRAS* mutation, 90% of pancreatic cancer samples also showed a *KRAS* mutation, thereby suggesting that most pancreatic cancers can be a good candidate for *KRAS* monitoring [5].

KRAS mutations refer to a frequent G>A alteration. The K-Ras protein is a GTPase, which means it converts a molecule called GTP into another molecule called GDP. In this way the K-Ras protein acts like a switch that is turned on and off by the GTP and GDP molecules. To transmit signals, it must be turned on by attaching to a molecule of GTP. The K-Ras protein is turned off when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus [6].

The KRAS gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous [7]. The activation KRAS gene point mutations have been detected in many types of human tumours, as the oncogenic forms of the KRAS gene are prevalent in pancreatic (>80%), colon (40%–50%), and lung (30%–50%) cancers, but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, and myeloid leukaemia [8]; in fact, most studies support its early involvement in carcinogenesis. Current evidence correlates *KRAS* mutations with increased cell proliferation and apoptosis. Tumours positive for KRAS mutation can harbor hypermethylation-related changes in genome expression, and this can be the cause of concurrent loss of DNA repair proteins. Despite the existence of evidence that the *KRAS* mutation status affects cancer progression, the effects of these mutations on tumour sensitivity to cytotoxic chemotherapies and radiation have only been explored by a few studies [9]. The presence of oncogenic *KRAS* has been found to significantly increase the sensitivity of cells to a novel class of anticancer agents.

Finally, a protein is considered to be misfolded if it cannot achieve its normal native state. This can be due to mutations affecting its amino acid sequence. The misfolding of proteins can trigger the further misfolding and accumulation of other proteins into aggregates or oligomers [10].

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Protective effects of esomeprazole, curcumin, chitosan, and curcumin-chitosan mixture on ethanol-induced gastric mucosal injuries in female rats

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Abstract

Gastric ulcer is the most common health concern due to alcohol consumption, smoking, and physiological stress. An ethanol-induced gastric ulcer in an animal model resembles the pathophysiology of the human ulcer. The present study attempted to detect the protective effects of esomeprazole, curcumin, chitosan, and a mixture of curcumin and chitosan on ethanol-induced gastric ulcers in female rats. The present study included 60 rats with an average weight between 179.1 and 180.3 g, divided into two control groups and four treated groups (esomeprazole, curcumin, chitosan, and mixture), where each group included 10 rats. All groups were treated for 30 days. In order to induce a gastric ulcer, absolute ethanol (2 mL/rat) was given orally to all groups (except the negative control ones) after a period of fasting of 20 h. All animals were sacrificed 5 h later. The gastric ulceration was studied by comparing the volume and the pH of the gastric juice, the ulcer index as well as the protective index. Our results revealed a significant decrease (P<0.05) in the values of the ulcer index and the volume of gastric juice in the esomeprazole-, curcumin-, chitosan-, and mixture-treated rats as compared to those of the positive control group. The value of the gastric juice pH exhibited a significant increase (P<0.05) in these same groups.

KEYWORDS
gastric ulcer, esomeprazole, curcumin, chitosan, rat

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1. INTRODUCTION

Peptic ulcer is a common disorder of the entire gastrointestinal tract, although it occurs mainly in the stomach and the proximal duodenum [1]. Alcohol-induced gastric lesions cause a dysfunctionality of the gastric defence factors, the mucosal circulation, and the mucus secretion. The consumption of ethanol could lead to necrotic lesions in the gastric mucosa *via* various mechanisms, including the generation of a direct necrotic lesion, which in turn lowers the defence system, the secretion of bicarbonate, and the mucus formation.

The mucus gastric layers are considered a crucial factor in the protection against gastrointestinal injuries. Mucosal secretion has also been known as a key defensive factor in the prevention of gastric lesions. The produced gastric mucus is usually evaluated by the amount of gastric mucosal secreted [2].

The present study aimed at comparing the protective effects of esomeprazole, of curcumin, of chitosan, and of a curcumin-chitosan mixture on ethanol-induced gastric ulcers in female rats.

2. MATERIALS AND METHODS

Experimental Animals: The animals used in this study were adult female rats obtained from different sources in the city of Hillah.

Experimental design: Animals were randomly divided into six main groups: one control group and five treated groups (n=10 each) as follows: (i) control group (given distilled water at 2 mL orally through a stomach tube for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h), (ii) acetic acid group (given 2 mL of 0.1M / 0.06% acetic acid, orally through a stomach tube, for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h), (iii) esomeprazole-treated group (given esomeprazole at a dose of 40 mg, orally through a stomach tube, for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h), (iv) curcumin-treated group (given curcumin at a dose of 40 mg/kg, orally through a stomach tube, for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h), (v) chitosan-treated group (given chitosan at a dose of 150 mg/kg, orally through a stomach tube, for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h), and (vi) curcumin-chitosan mixture-treated group (given a curcumin-chitosan mixture containing 40 mg/kg of curcumin and 150 mg/kg of chitosan, orally through a stomach tube, for 30 days, then subdivided equally into subgroup 1 that was immediately sacrificed, and to subgroup 2 that was sacrificed after 19 h of fasting with access to water *ad libitum* and then receiving orally 2 mL of ethanol that was given for 5 h).

Measurement of the gastric ulcer index: The gastric ulcer index was estimated according to a previously described method [3].

Measurement of the pH and the volume of the gastric juice: The stomach of each euthanized rat was immediately dissected, and the gastric content was collected in sterilized tubes. The pH of the gastric juice was determined by a pH paper, and the gastric juice was then centrifuged for 10 min at 3,000 rpm so as to isolate the aqueous phase. The volume of the centrifuged gastric juice was measured by a graduated cylinder and was expressed as mL.

Statistical analysis: The Statistical Package for Social Science (SPSS) version 23.0 (SPSS, Chicago, USA) was used for the undertaking of the statistical analysis of the data.

3. RESULTS

An overview of the protective effects of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on the ulcer index, the protective index, as well as the volume and the pH of the gastric juice in female rats treated for 30 days, is provided in Table 1.

4. DISCUSSION

The exposure to ethanol produced gastric lesions by penetrating and digesting the gastric wall due to its proteolytic and hydrolytic action as well as due to endothelial cell damage as a result of the reduction in blood circulation [4]. The results of the present study showed that the oral administration of absolute ethanol (2 mL/rat) for 5 h can induce a gastric ulcer. A significant decrease (P<0.05) was noticed in the gastric ulcer index in the groups pretreated with esomeprazole, curcumin, chitosan, or the curcumin-chitosan mixture, with the protective index being equal to 64.58%, 45.83%, 62.5%, or 64.58%, respectively, as compared with that of the ulcer control group. This may reflect the gastroprotective effects of esomeprazole, curcumin, chitosan, and the curcumin-chitosan mixture on the gastric mucosa. This finding is in agreement with those of Xie *et al*. [5] who have shown that esomeprazole decreases the ulcer index in rats.

The significant decrease in the ulcer index in the group pretreated with curcumin in this study may be due to its antioxidant activity. The antioxi-

dant or free radical scavenging ability of curcumin arises from the phenolic OH group or from the CH_2 group of its b-diketone moiety. Free radical-mediated peroxidation of membrane lipids and oxidative damage of cellular molecules are believed to be associated with various chronic pathological complications such as cancer, ulcers, and other inflammatory diseases. Curcumin is assumed to play a vital role against these pathological conditions, and could be a potent antiulcer agent [6].

Table 1. Protective effects of esomeprazole, curcumin, chitosan and curcumin-chitosan mixture on the ulcer index, the protective index, as well as the volume and the pH of the gastric juice in female rats treated for 30 days. Notes: different letters indicate significant differences (P<0.05) among groups; n=5 for each group.

Groups	Ulcer index	Protective index (%)	Gastric juice volume (mL)	Gastric juice pH
distilled water (negative control group)	0.00±0.00 ^{a}	100	1.30±0.20 ^{ab}	5.40±0.25 ^{ab}
distilled water + ethanol (positive control group)	4.80±0.49 ^{b}	0.00	3.80±0.26 ^{e}	3.00±0.32 ^{c}
0.1 M acetic acid (negative control group)	0.00±0.00 ^{a}	100	1.60±0.19 ^{abc}	5.00±0.32 ^{ab}
0.1 M acetic acid + ethanol	3.00±0.45 ^{c}	37.5	3.60±0.25 ^{e}	3.60±0.25 ^{c}
esomeprazole 40 mg	0.00±0.00 ^{a}	100	1.00±0.16 ^{a}	5.60±0.5 ^{a}
esomeprazole 40 mg + ethanol	1.70±0.30 ^{d}	64.58	2.00±0.16 ^{bcd}	5.20±0.37 ^{ab}
curcumin 40 mg/kg	0.00±0.00 ^{a}	100	1.10±0.19 ^{a}	5.20±0.37 ^{ab}
curcumin 40 mg/kg + ethanol	2.60±0.51 ^{cd}	45.83	2.30±0.37 ^{cd}	4.60±0.51 ^{b}
chitosan 150 mg/kg	0.00±0.00 ^{a}	100	1.10±0.19 ^{a}	5.40±0.25 ^{ab}
chitosan 150 mg/kg + ethanol	1.80±0.37 ^{d}	62.5	2.50±0.27 ^{d}	5.00±0.32 ^{ab}
mixture (curcumin 40 mg/kg - chitosan 150 mg/kg)	0.00±0.00 ^{a}	100	1.00±0.27 ^{a}	5.80±0.20 ^{a}
mixture (curcumin 40 mg/kg - chitosan 150 mg/kg) + ethanol	1.70±0.30 ^{d}	64.58	1.90±0.33 ^{bcd}	5.20±0.37 ^{ab}

Wallace [7] has demonstrated that the mechanism by which chitosan prevents gastric mucosa damage may be due to its adhesion activity, which prevents direct contact of the injured mucosa with the physiological environment of the stomach and prevents the proliferation of microorganisms at the wound site. In addition, it inhibits the enzyme activities responsible for the synthesis of the microorganisms' cell wall at the injured site. The antiulcer action of the curcumin-chitosan mixture in this study may be related to the potent multi-target antioxidant, anti-inflammatory, gastroprotective, and ulcer-healing actions attributed to curcumin and chitosan [8,9].

The data presented in Table 1 clearly demonstrate a significant increase (P<0.05) of the gastric juice volume in the ethanol group, that may be due to the direct effects of ethanol on the gastric mucosa, as ethanol is known to cause gastric injuries *via* several pathways, including dehydration, which disrupts mucosal cell barriers and exerts cytotoxicity. This cytotoxicity contributes to the recruitment of reactive oxygen species-releasing leukocytes and inflammatory cytokines, all of which may contribute to cellular apoptosis. Interestingly, the nuclear factor-kappa B plays a key role in the relationship between these disparaging events [10].

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Beneficial effect of *Alhagi maurorum* on rats submitted to sulfadimidine-induced kidney injury

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Abstract

Alhagi maurorum is one of the many plants that have proven effectiveness in folklore medicine and that are still utilized to treat disease or disorders, thanks to their phytochemical compounds and other secondary metabolites. Sulfadimidine, chemical known as 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzene-sulfonamide, is an antibacterial drug that has side-effects on organs such as the kidney. In this study, the unwanted acute effect of this sulfonamide and of its metabolites was recorded in the form of rat interstitial nephritis and as an increase in creatinine and blood urea nitrogen (BUN) levels. Results showed a significant (P<0.05) decrease in BUN levels in rat groups treated with the ethanolic extract of *Alhagi maurorum* as a therapy, but there were no significant differences observed in terms of the creatinine levels in these groups. The undertaken histological study revealed an almost normal histological appearance of the kidneys in the two groups of rats that were treated with the plant extract as a therapy after the damage that occurred as a result of the drug injection (interstitial nephritis, infiltration lymphocytes, and mild tubular atrophy). Our study suggests a potential benefit from natural plants in the treatment of drug-related adverse effects.

KEYWORDS
Alhagi maurorum, sulfadimidine, kidney damage, creatinine, rat

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1. INTRODUCTION

Phytochemical analyses of medicinal plants like those belonging to the *Alhagi* species have revealed a content of alkaloids, phenol, tannin, steroids, carbohydrate, lipids, and terpenoids; these chemical components have been extensively studied for their biological activity [1]. In a study by Varshochi and Asadollahi [2], it was revealed that the phytochemical contents of *Alhagi* can be used as a remedy for the removal of urinary tract stones; moreover, *Alhagi maurorum* (AM) had the highest number of mentions related to the claim that the plant's components can reduce the concentration of oxalates and calcium oxalate stones. On the other hand, sulfonamides and their metabolites are known to be able to cause severe interstitial nephritis or tubular necrosis [3].

2. MATERIALS AND METHODS

Plant extract: The plant was harvested from Hillah (Iraq). The taxonomy of the plant was carried out in a herbarium affiliated with the College of Science of the University of Babylon. The AM extract was prepared, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay was carried out on it, and so was a screening for some major phytochemical substances (such as flavonoids, phenols, alkaloids, glycosides, and tannins), as previously described [4].

Animals used: A total of 48 male albino rats of the species of *Rattus*, weighing between 180 to 250 gr, and aged between two and three months were used. The animals were bred in the animal house that belongs to the Department of Biology of the College of Science of the University of Babylon. The animals were subjected to the same conditions throughout the experiment with standard feed and water. At the end of the experiment, the animals were sacrificed by using anesthetic chloroform, and their blood was collected through a heart puncture with a sterile syringe.

Experimental design: The rats in the experiment were divided into eight groups of six rats each (n=6) as follows: (i) group 1 (G1) as a control group (rats received distilled water), (ii) group 2 (G2) in which the rats were injected with 40 mg/kg of sulfadimidine (SDD), (iii) group 3 (G3) in which the rats were given 300 mg/kg of the AM extract, (iv) group 4 (G4) in which the rats were given 600 mg/kg of the AM extract, (v) group 5 (G5) in which the rats were given 300 mg/kg of the AM extract and were then injected with SDD (40 mg/kg), (vi) group 6 (G6) in which the rats were given 600 mg/kg of the AM extract and were then injected with SDD (40 mg/kg), (vii) group 7 (G7) in which the rats were injected with SDD (40 mg/kg) and were then given 300 mg/kg of the AM extract, and (viii) group 8 (G8) in which the rats were injected with SDD (40 mg/kg) and were then given 600 mg/kg of the AM extract.

Biochemical analyses and histological study: Blood samples were collected in order to determine the blood urea nitrogen (BUN) and creatinine levels based on the protocol of the Sunlong Biotech Co., Ltd ELISA kit. The histological processing was performed according to a previous study [5], and the tissues were stained with haematoxylin / eosin.

3. RESULTS

Biochemical analyses: Our study has found a significant increase (P<0.05) in the creatinine (11.16±1.1 nmol/mL) and BUN (827.30±10.2 nmol/mL) levels in the group that was injected with SDD. There were no significant differences observed in terms of the creatinine levels in all other groups. When comparing the BUN levels between groups G5, G6, G7, and G8, we noted a significant decrease (P<0.05) in the groups receiving the AM extract after the SDD injection (G7: 659.2±9.7 and G8: 687.4±8.2 nmol/mL, respectively) as compared to those receiving the AM extract after the SDD injection (G5: 733.7±15.2 and G6: 779.5±11.4 nmol/mL, respectively).

Phytochemical analysis and determination of antioxidant activity: The phytochemical screening of the ethanolic extract of the plant revealed the following compounds: phenols, tannins, alkaloids, glycosides, and glycosides. The plant extract inhibited 37.5% of the DPPH radicals.

Histological study: The G1 rat kidneys exhibited normal tissues with preserved glomerular and tubular structures that were covered by an epithelial layer and displayed no signs of congestion, haemorrhage, or interfacial damage. The kidneys of the G2 rats (that were injected with SDD) exhibited interstitial nephritis (infiltration by lymphocytes) with tubular atrophy. The kidneys of the G3 and G4 rats (that were treated with 300 and 600 mg/kg of the AM extract) exhibited normal glomerular and tubular structures, as in the control group. The kidneys of the G5 and G6 rats (that were treated with 300 and 600 mg/kg of the AM extract, respectively, before being injected with SDD) exhibited signs of interstitial nephritis (infiltration by lymphocytes) with mild tubular atrophy and frequent eosinophils. Finally, the kidneys of the G7 and G8 rats (that were treated with 300 and 600 mg/kg of the AM extract, respectively, after being injected with SDD) exhibited preserved glomerular and tubular structures (Figure 1).

4. DISCUSSION

It appears that the results of our biochemical analyses are supported by those of our histological study in terms of the kidney function. The observed atrophy of some renal tubules, along with the observed inflammation and the proliferation of lymphocytes and of some eosinophils, might be due to a state of chronic inflammation as a result of the injection of SDD. Mustafa *et al*. [6] suggest that sulfa drugs increase deposition in the kidneys, thereby impairing their function and secretion, and then worsening their accumulation in the urinary system and elevating the risk of developing severe interstitial nephritis and even necrosis of the urinary tubes. These changes could lead to the development of drug-induced acute interstitial nephritis [7].

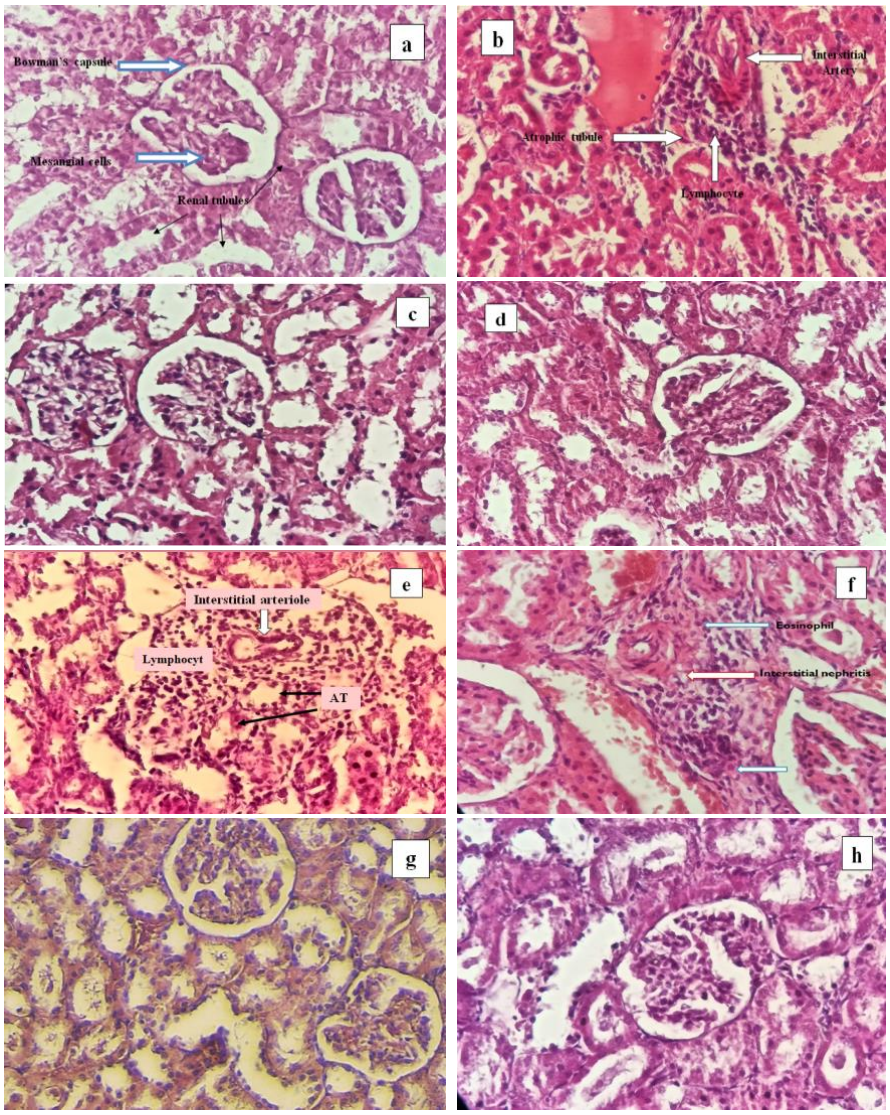

Figure 1. Kidney tissues of male rats stained with haematoxylin and eosin (×400): (**a**) the G1 rat kidneys exhibited normal tissues with preserved glomerular and tubular structures that were covered by an epithelial layer and displayed no signs of congestion, haemorrhage, or interfacial damage; (b) the kidneys of the G2 rats (that were injected with SDD) exhibited interstitial nephritis (infiltration by lymphocytes) with tubular atrophy; (**c** and **d**) the kidneys of the G3 and G4 rats (that were treated with 300 and 600 mg/kg of the AM extract) exhibited normal glomerular and tubular structures, as in the control group; (**e** and **f**) the kidneys of the G5 and G6 rats (that were treated with 300 and 600 mg/kg of the AM extract, respectively, before being injected with SDD) exhibited signs of interstitial nephritis (infiltration by lymphocytes) with mild tubular atrophy and frequent eosinophils; (**g** and **h**) the kidneys of the G7 and G8 rats (that were treated with 300 and 600 mg/kg of the AM extract, respectively, after being injected with SDD) exhibited preserved glomerular and tubular structures.

Our study confirmed through histopathological observations that in the rat groups treated with the AM extract after the SDD injection, the kidneys were nearly identical to those of the control group, and except for some inflammatory cells, the glomerulus and the tubules appeared normal. These findings might be due to phytochemical compounds identified in the AM extract (such as phenols and flavonoids) [8].

The antioxidant activity of the AM extract could work against the oxidative stress resulting from the drug administration, and this is in agreement with

the findings of a previous study [9]. Additionally, the herein studied plant has shown diuretic properties, which have led to the mitigation of the pH and of crystalluria, as well as to the excretion of sodium and potassium and the bulk of the volume of urine [10].

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Emergency department presentation and management of patients with acute decompensated heart failure at the Baghdad Teaching Hospital

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Abstract

Acute decompensated heart failure (ADHF) is a leading cause of hospital admission and many factors are known to precipitate decompensation. We aimed to assess the decompensating factors of heart failure and the management of patients admitted to the emergency department (ED). A total of 107 patients were examined, all diagnosed with ADHF in the ED of the Baghdad Teaching Hospital, from June 2017 to December 2017, and presenting with decompensation (pulmonary oedema, peripheral oedema, and fatigue). The mean patient age was 62.5 ± 9.8 years (range: 43–85 years); the majority of them were in their 7th decade (37.4%), and men were slightly more than women. Hypertension was the most commonly associated comorbidity (68.2%), followed by diabetes mellitus (57.9%), coronary artery disease (51.4%), dyslipidaemia (37.4%), arrhythmia (28%), and chronic obstructive pulmonary disease / asthma (23.4%). The most common presentation was pulmonary oedema (88.8%) followed by peripheral oedema (61.7%), and fatigue (26.2%). Uncontrolled hypertension was the most common precipitating condition for decompensation (58.9%), followed by infection (39.3%), acute coronary syndrome (31.8%), arrhythmia (27.1%), non-compliance (11.2%), and anaemia (2.8%). The majority of the admitted patients were managed with intravenously-administered (i.v.) diuretics (92.5%) that may have been combined with oxygen therapy (63.6%), antibiotics (58.9%), β-blockers (50.5%), nitroglycerin (40.2%), i.v. fluids (38.3%), and/or digoxin (19.6%).

KEYWORDS
acute decompensated heart failure, uncontrolled hypertension, pulmonary oedema, peripheral oedema, fatigue

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1. INTRODUCTION

Acute decompensated heart failure (ADHF) often occurs in patients with preexisting heart failure,

and presents with exacerbation of dyspnoea, oedema, and/or fatigue that requires urgent medical treatment at the emergency department (ED), thus mandating its management with intravenously-administered (i.v.) medication (e.g., diuretics) and respiratory support in the form of oxygen (O_2) therapy [1]. Several factors can contribute to the ADHF worsening, leading to decompensation. Excessive fluid accumulation in the body can strain the heart, leading to a worsening of the symptoms of heart failure. Monitoring fluid intake and output, assessing peripheral oedema, and evaluating the signs of pulmonary congestion are crucial in managing ADHF patients in the ED. Failure to adhere to prescribed medications, such as diuretics can exacerbate ADHF symptoms and contribute to decompensation [2]. It is essential to assess medication compliance and optimize medication regimens in the ED, as medication adherence may reduce the ADHF symptoms, high hospitalization, and mortality [3]. Conditions such as hypertension, diabetes, infections, and renal dysfunction can worsen ADHF and increase the risk of decompensation. Identifying and managing comorbidities are important aspects of the ADHF management in the ED. Infection triggers an immune response and systemic inflammation, which is associated with the worsening of the ADHF outcomes and can contribute to myocardial dysfunction [4]. Arrhythmias, such as atrial fibrillation (AF) or ventricular tachycardia, can precipitate or exacerbate ADHF episodes. Prompt recognition and treatment of arrhythmias are critical in preventing decompensation in ADHF patients presenting to the ED. Ischemia in the from of an acute coronary syndrome (CAS) or myocardial ischemia can also lead to ADHF decompensation. Evaluating for signs and symptoms of myocardial ischemia and initiating appropriate interventions, such as the administration of an antiplatelet therapy or revascularization, are crucial in the ADHF management in the ED [5]. Other non-cardiac conditions such as pulmonary embolism, chronic obstructive pulmonary disease (COPD), or asthma can also exacerbate the ADHF symptoms [6]. The aim of this study was to identify the decompensating factors of heart failure in patients with known history of heart failure that were admitted in the ED, and discuss the management of them with available therapies at the Baghdad Teaching Hospital (Iraq).

2. PATIENTS AND METHODS

General: This observational cross sectional study was conducted at the Baghdad Teaching Hospital, Baghdad Medical City, Baghdad, Iraq, from June 2017 to December 2017, and was carried on 107 patients that were admitted to the ED. Of them, 50 were women and 57 were men, with their age ranging from 43 to 85 years. The patients presented with ADHF and were interviewed after the management of the presenting symptoms with available therapies. All patients were subjected to full history, physical examination, and investigations at presentation with a direct interview inquiring about their age, sex, history of hypertension, history of diabetes mellitus, history of CAS, history of dyslipidaemia, history of stroke, history of AF, as well as history of COPD and/or asthma. Furthermore, we recorded the medication to the patient given at the ED (diuretics i.v., O_2 therapy, antibiotics, β-blockers, nitroglycerin, fluids i.v., and digoxin).

Inclusion criteria: Known case of heart failure in their medical history and previously confirming diagnosis through a chest X-ray or multiple attacks of decompensation symptoms.

Exclusion criteria: Unconfirmed diagnosis of heart failure.

Ethics statement: The study was carried out in compliance with the Declaration of Helsinki principles and was approved by the Scientific Ethics Committee of the Department of Emergency Medicine, Iraqi Board for Medical Specialities, Baghdad. The study was carried out after gaining their verbal consent from all participated patients; the latter were informed that their non-participation or withdrawal from the study would have no impact on the medical care they received.

Statistical analysis: All continuous data were presented as mean ± standard deviation, while categorical variables were presented in the form of numbers and percentages.

3. RESULTS

In the current study, the mean age of the patients was 62.5 ± 9.8 years (ranging from 43 to 85 years), with 37.4% of the patients' age being 60–69 years, and 31.8% of them being 50–59 years. Men were slightly more (53.3%) than women (46.7%), the mean duration of the heart failure was 8.2 ± 2.4 years, and 43.9% of the patients were smokers. Pulmonary oedema was the most common presentation (88.8%), followed by peripheral oedema (61.7%), and fatigue (26.2%). Uncontrolled hypertension was the most common precipitating condition for decompensation, followed by infection (respiratory tract infection or urinary tract infection), CAS, arrhythmia, non-compliance, and anaemia (Table 1). Regarding the treatment at ED, 92.5% of the patients were given diuretics (i.v.), 63.6% were administered an O_2 therapy, while 58.9% were treated with antibiotics, 50.5% with β-blockers, 40.2% with nitroglycerin, 38.3% with fluids (i.v.), and only 19.6% with digoxin (Table 1).

Table 1. Data collected from patients with acute decompensated heart failure (ADHF) presenting at the emergency department (ED) of the Baghdad Teaching Hospital (Iraq) between June 2017 and December 2017. Notes: *, uncontrolled hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg; **, non-compliance with medication, diet or fluid restrictions. Abbreviations used: CAS, coronary artery syndrome; COPD: chronic obstructive pulmonary disease; i.v., intravenously-administered; O_2, oxygen; RTI, respiratory tract infection; UTI, urinary tract infection.

General Data of Patients (n=107)					
Age (in years)					
<50	**50–59**	**60–69**	**70–79**	**≥80**	
5 (4.7%)	34 (31.8%)	40 (37.4%)	21 (19.6%)	7 (6.5%)	
Sex					
Male			**Female**		
57 (53.3%)			50 (46.7%)		
Co-morbid conditions associated with ADHF					
Hypertension	**CAS**	**Dyslipidaemia**	**Arrhythmia**	**Diabetes**	**COPD / Asthma**
73 (68.2%)	55 (51.4%)	40 (37.4%)	30 (28.0%)	62 (57.9%)	25 (23.4%)
Related to heart failure (mean ± SD; range)					
Duration of heart failure (in years)			**Number of attacks**		
8.2 ± 2.4 (1.0–12)			8.9 ± 5.6 (2–50)		
Clinical presentations to the ED					
Pulmonary oedema		**Peripheral oedema**		**Fatigue**	
95 (88.8%)		66 (61.7%)		28 (26.2%)	
Precipitating factors of ADHF					
Uncontrolled hypertension*	**Infection (RTI or UTI)**	**Acute coronary syndrome**	**Arrhythmia**	**Non-compliance****	**Anaemia**
63 (58.9%)	42 (39.3%)	34 (31.8%)	29 (27.1%)	12 (11.2%)	3 (2.8%)
Drugs used in treatment of ADHF patients at ED					
Diuretics (i.v.)	**O_2 therapy**	**Antibiotics (i.v.)**	**β-Blockers**	**Nitroglycerin**	**Fluids (i.v.)**	**Digoxin**
99 (92.5%)	68 (63.6%)	63 (58.9%)	54 (50.5%)	43 (40.2%)	41 (38.3%)	21 (19.6%)

4. DISCUSSION

Patients with ADHF resulting from uncontrolled hypertension can usually be readily stabilized in the hospital with blood pressure control within a relatively short length of stay, and a lower risk of adverse near-term outcomes; however it remains the most common cause of decompensation. Infections, such as pulmonary infections, increase metabolic demands which are common in patients with heart failure, may cause hypoxia, and are associated with worse outcomes [7]. Patient adherence to dietary restrictions and evidence-based medication is a cornerstone of heart failure management, while non-adherence to medications has been associated with increased risk of hospitalization and mortality in outpatients with chronic heart failure [8]. Patients with non-adherence to medications or diet are likely to be admitted with excessive sodium retention, which can be a leading decompensation factor [9]. These patients may more readily achieve compensation in response to salt restriction, adjustment of diuretics, and provision of medications during the inpatient hospitalization. It should be noted that patients with non-adherence to medications or diet as an admission precipitant were at high adjusted risk of a 60- to 90-day post-discharge mortality and of death/re-

hospitalisation similar to the overall heart failure population [10]. Patients identified as nonadherent to medications would be expected to be counselled during the index hospitalization regarding the importance of adherence to their medical regimen and, thus, may be less likely, at least in the short term, to repeat the medication nonadherence that precipitated a recent heart failure hospitalization. It should also be emphasized that the use of evidence-based heart failure medications in eligible patients at discharge is strongly associated with improved post-discharge outcomes [7]. Based on the above results, our study has shed more light on the demographics, common comorbidities, clinical presentations, and factors that contribute to the clinical deterioration of ADHF patients in Iraq.

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Evaluation of the active compound and antibacterial activity of a *Salvia rosmarinus* extract against pathogenic bacteria

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Abstract

When bacteria attach to the human body, they have many defense mechanisms. These mechanisms pose a global health risk. Medicinal plants can be used to control such bacterial attack mechanisms. The aim of this study was to assess the antimicrobial potential of an aqueous extract of *Salvia rosmarinus* and other plant extracts against some clinical bacterial isolates. The antimicrobial activity against Gram-positive and Gram-negative bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus* spp., and *Klebsiella* spp.) was determined by using the agar well diffusion method. When compared to an alcoholic extract, the aqueous extract of *Salvia rosmarinus* inhibited the studied bacterial isolates with greater efficiency.

KEYWORDS

antibacterial activity, herb extracts, *Salvia rosmarinus,* pathogenic bacteria, FTRI technique

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1. INTRODUCTION

A number of factors has hampered the production of antibacterial agents. Of them, bacterial resistance to antimicrobials has resulted in the latter losing their inhibitory effectiveness against bacteria [1]. The identification of the biologically active compounds responsible for the medicinal properties is a critical requirement for quality control [2]. Antimicrobial plant extracts are of interest to clinical microbiologists [3]. *Salvia rosmarinus* leaves and flowers have long been used in conventional medical treatments and beauty products. They are also used in food as flavoring agents [4]. The current study was designed so as to evaluate the antimicrobial activity of *Salvia rosmarinus* against clinical bacterial isolates. Moreover, Fourier-transform infrared (FTIR) spectroscopy was used in order to detect important compounds

of *Salvia rosmarinus*, as well as to detect some virulence factors that can be inhibited by the plant's extract.

2. MATERIALS AND METHODS

Bacterial isolates: The herein assessed bacterial isolates were provided by the Department of Biology of the University of Babylon (one sample for each isolate, collected from patients with severe urinary tract infection), and were identified by manual biochemical tests. The bacterial isolates were *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus* spp., and *Klebsiella* spp. isolates.

Plant extract: This experiment was conducted in 2023 at the University of Babylon's Environmental Research and Studies Center. Herbal samples were collected from local markets, and included *Laurus nobilis*, *Punica granatum*, and *Salvia rosmarinus*, while the parts used were the leaves, the peels, and leaves, respectively [5].

Agar diffusion assay: The standard agar diffusion assay was used for the undertaking of the antibacterial assay [6].

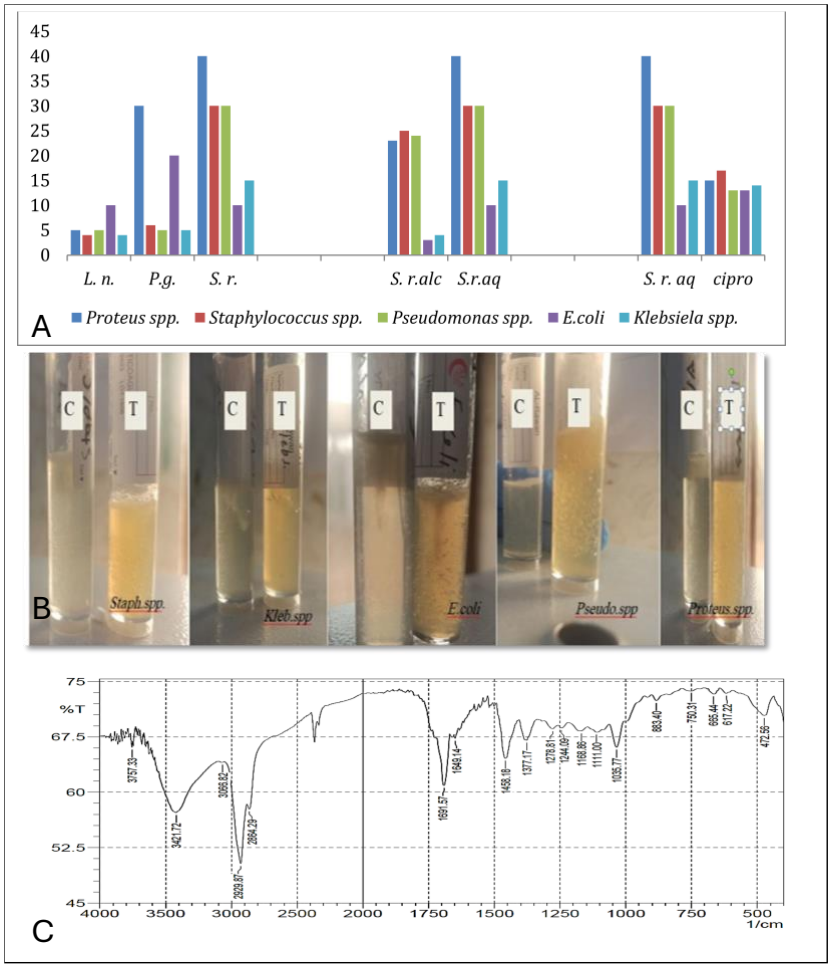

Figure 1. Antibacterial action of herbal plant extracts and antibiotics against clinical bacterial isolates (y-axis: zone of inhibition, in mm; abbreviations used: alc, alcoholic extract; aq, aqueous extract; cipro, ciprofloxacin; Ln: *Laurus nobilis*; Pg, *Punica granatum*; Sr, *Salvia rosmarinus*). (**B**): Activity of the aqueous extract of *Salvia rosmarinus* against the motility of bacteria. (**C**): Fourier-transform infrared spectrum of the rosemary extract.

3. RESULTS AND DISCUSSION

In the current study, after the preparation of the extracts of the studied herbals plants, a comparison was made so as to see which was more effective in inhibiting the pathogenic bacterial isolates caus-

ing urinary tract infection. Figure 1A shows how the aqueous extract of *Salvia rosmarinus* inhibited the studied isolates mentioned with high efficiency, and inhibited bacterial movement compared to the control treatment. The extract exhibited high efficiency in comparison to the antibiotic ciprofloxacin. Our results revealed that the alcoholic and aqueous extracts from specific plants can successfully inhibit the development of pathogenic organisms. Aqueous extracts of various plants have been shown to yield significantly more potency than alcoholic extracts of the same plants [7]. These findings contradict several previous studies that suggested that the alcohol extract has more efficacy against bacteria than the aqueous one. Thus, these chemical compounds can affect multiple target sites resistant to bacterial cells [8]. The *Salvia rosmarinus* extract inhibited more effectively the bacterial motility, while other extracts were less effective (Figure 1B). An FTIR scan of the herbal plant extract was conducted in the infrared region so as to detect the function groups present in the composition of the prepared extract. The band at 3,421.72 cm^{-1} was attributed to the stretching vibration of the phenolic hydroxyl group, while the aromatic C-H stretching exhibited a lane at about 3,066.82 cm^{-1}. The C-H stretching vibrations of the methoxyl group exhibited two bands at 2,929.87 and 2,864.29 cm^{-1}, while the bands at 1,691.57 and 1,649.14 cm^{-1} were attributed to the stretching vibration of the C=O group. Moreover, the asymmetrical stretching vibrations of the C-O-C connection in the C-O stretch, in addition to esters or phenolic hydroxyl, exhibited two bands at 1,278.81 and 1,244.09 cm^{-1}. Finally, three bands were attributed to ethoxyl: 1,168.6, 1,111.0, and 1,035.77cm^{-1} (Figure 1C).

4. CONCLUSION

Our study shows how the aqueous extract of *Salvia rosmarinus* can inhibit the studied bacterial isolates with great efficiency. In addition, it explains how this efficiency could be attributed to the influencing of one of the virulence factors, which is the motility factor.

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Phytochemical screening and antimicrobial activity of some medicinal plants

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Abstract

The antimicrobial activity of the aqueous and ethanolic extracts of *Myrtus communis*, *Ammi visnaga*, and *Equisetum arvense* was investigated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* by using the agar well diffusion method. Serial concentrations (15%, 30%, and 50%) of the extracts of each plant were tested and compared with gentamicin (10 µg) and fluconazole (25 µg). Most of the extract concentrations showed a relatively high antimicrobial activity against all the tested microbes, and the ethanolic extract was more effective than the aqueous extract. The activity of plant extracts increased with the increasing extract concentration of *Myrtus communis*, which appeared to possess a more antimicrobial activity than the other plants assessed; in fact, its ethanolic extract exhibited the highest inhibition zone against *S. aureus* (32 mm). The ethanolic plant extracts at a concentration of 50% displayed the maximum activity against the herein assessed isolates. Moreover, *E. coli* showed a higher sensitivity to most extracts, while the lowest effect being noticed on *C. albicans*.

KEYWORDS

Ammi visnaga, *Equisetum arvense*, *Myrtus communis*, screening, antimicrobial activity

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1. INTRODUCTION

Medicinal herbs have served as a valuable source of remedies in local communities across the globe for millennia. Despite the overwhelming influences and reliance on contemporary medicine along with remarkable advancements in synthetic pharmaceutics, *Myrtus communis*, a small evergreen shrub abundant in foliage and a member of the Myrtaceae family, stands as a significant representative of the medicinal and aromatic plants [1]. The effectiveness of myrtle can be attributed to its chemical constituents, likely alkaloids, tannins, flavonoids, phenol, organic acids, anthraquinones, saponin, essential oils, and fatty acids [2]. *Ammi visnaga*, a plant with a ring-like structure, is a member of the Apiaceae family. It possesses a slight aromatic scent and an exceedingly bitter fla-

vour. This plant contains diverse chemical components: coumarins, γ-pyrones, flavonoids, and essential oils. The quality and quantity of these ingredients are contingent upon the section of the plant, the environmental conditions, and the inclusion of any bioregulators [3]. Finally, *Equisetum arvense*, an ancient fern plant, is a member of the Equisetaceae family. This particular plant is renowned for its abundance of invaluable natural compounds, such as saponins, phytosterols, triterpenoids, flavonoids, alkaloids, and minerals. It is also recognized for its therapeutic properties, as well as its antimicrobial and antioxidant efficacy [4]. This *in vitro* study aimed at screening selected plants for their antimicrobial capacity and at evaluating their use in the management of infections.

2. MATERIALS AND METHODS

Preparation of extracts: Aqueous extracts were prepared from 20 g of each plant (air-dried powder) placed in a conical flask (500 mL), and then by adding distilled water, boiling for 2 h, filtering through a filter paper, and centrifuging at 5,000 rpm for 10 min. The filtrate was then collected and concentrated in an oven at 45°C until dry. Dried extracts were then stored at 4°C until use. The ethanolic extract was prepared in the same way as the aqueous extract, but the water was replaced by ethanol (70%) and the heating was skipped.

Qualitative tests of phytochemicals: We tested for phenols with lead acetate and for tannin with ferric chloride, we undertook the saponin test by foam, and we tested for alkaloids through the Fehling's test and the Mayer's test [5].

Antimicrobial activity assessment: The antimicrobial activity of the aqueous and alcoholic extracts of *Myrtus communis*, *Ammi visnaga*, and *Equisetum arvense* at concentrations 15%, 30%, and 50% was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* by using the disk diffusion test. In brief, 100 µL of different extracts at different concentrations for each plant were added to the wells of a plate that was previously streaked with microorganisms, then the plates were incubated at 37°C for 24 h. After that, the inhibition zone was evaluated for each plate (measured in mm). The test was performed in triplicates. Gentamicin (10 µg) and fluconazole (25 µg) were used as positive controls of antibacterial and antifungal activity, respectively [6].

3. RESULTS AND DISCUSSION

Phytochemical contents: According to the phytochemical tests undertaken for the aqueous and alcoholic extracts of *Ammi visnaga*, we detected the presence phenols (bulky white precipitates caused by lead acetate), tannin (black colour generated by ferric chloride 1%), and alkaloids (green colour), while saponin was not present. In *Myrtus communis* and *Equisetum arvense* the phytochemical tests detected the presence of phenols, tannin, alkaloids, and saponin. The knowledge of the phytochemical contents of medicinal plants is essential for researchers aiming to devise a new bioactive combination from natural sources that could be used for the synthesis of new drugs [7].

Antimicrobial activity: The effects of the various aqueous and alcoholic extract concentrations against the tested microbes are summarized in Table 1. The diameter of the inhibition zone increased along with the extract concentration, and at a concentration of 50% all microbes exhibited the highest zone of inhibition. *Myrtus communis* exhibited the maximum antimicrobial activity, while *E. coli* displayed a marked sensitivity towards most plant extracts. Aabed *et al*. [8] have shown that *Myrtus communis* extracts display antibacterial and antifungal activities. The myrtle extract activity has been linked to its chemical composition (e.g., flavanols, terpineol, acetate, linalyl, linalool, cineol, and tannins).

The present findings are similar to those of previous studies showing that the *Equisetum arvense* methanol extract exerts a higher antimicrobial activity than its aqueous extract against different bacteria, because the alcoholic extract contains more effective phytochemical compounds, such as essential oils that denature the bacterial adhesive proteins, prevent the transportation of proteins *via* the cell membrane, and disturb the cytoplasmic membrane [9].

In a study by Keddari *et al*. [10], the authors have found that *Ammi visnaga* has an antimicrobial activity; generally, this antimicrobial activity has been associated with khellin and visnagin, and these compounds are considered to possess antibacterial, antifungal, and antiviral activities. Different extracts exhibited different effects on the tested microorganisms, and these differences may be due to the differences in the structural nature of the microorganisms as well as to the different plant constituents.

4. CONCLUSION

The tested plant extracts exhibited different antimicrobial effects on the tested microbes and have a therapeutic promise for treating a number of diseases.

Table 1. The inhibitory zones (mm) of different microorganisms generated by the plant extracts assessed in our study.

Plant	Extract	Extract concentration	Inhibitory zone (in mm)		
			S. aureus	*E. coli*	*C. albicans*
M. communis	**aqueous**	15%	16	23	22
		30%	27	27	25
		50%	28	29	27
	alcoholic	15%	23	21	17
		30%	28	25	20
		50%	32	27	22
E. arvense	**aqueous**	15%	8	15	0
		30%	15	22	10
		50%	24	23	12
	alcoholic	15%	12	20	2
		30%	22	25	15
		50%	30	27	20
A. visnaga	**aqueous**	15%	10	14	0
		30%	13	15	5
		50%	20	18	6
	alcoholic	15%	15	20	0
		30%	19	22	5
		50%	24	24	15
Gentamicin (10 µg)			13	21	-
Fluconazole (25 µg)			-	-	12

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Cytotoxic effects of the *Crassula ovata* n-hexane fraction on human esophagus cancer KYSE-30 cells

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Abstract

The current study shows the cytotoxicity effect of the *Crassula ovata* n-hexane extract on esophagus cancer. *C. ovata* is a perennial succulent plant belonging to the Crassulaceae family. In Africa, the leaves were used medicinally to cure epilepsy and diarrhoea by boiling them in milk. The hexane fraction, which is obtained through the maceration method, demonstrates the presence of many compounds that have an anticancer effect, which are obtained by gas chromatography - mass spectroscopy. The phytosterol compound was isolated by a preparative thin layer chromatograph and was identified by liquid chromatography - mass spectroscopy. The hexane fraction was found to possess a strong anticancer effect against esophagus cancer. The obtained data from the human esophagus cancer KYSE-30 cell-line were analysed by one-way ANOVA, with a significance level of $p<0.05$.

KEYWORDS
b-sitosterol, *Crassula ovata*, cytotoxicity, esophagus cancer, phytosterol

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1. INTRODUCTION

Since ancient times, medicinal plants have been used to cure a wide range of diseases; in fact, in some places, the use of medicinal plants was thought to be witchcraft because people lacked the scientific concept at that time to explain and predict the plants' therapeutic effects [1]. About 70%-95% of the population depends on traditional medicines for primary care, and 70%-90% of the populations in developed countries use herbal medicines under the titles "competitive", "alternative", or "nonconventional" [2].

Crassula ovata is a plant belonging to the Crassulaceae family. The genus *Crassula* consists of nearly 200 species, most of which are found in southern Africa, which is considered their distribution center [3]. *Crassula ovata* is a perennial succulent plant with many branches and a bushy appearance, about 30-45 cm high, with thick, branching, succulent, and juicy stems. The leaves are

dark green, oval-shaped, and situated opposite one another on the stems. The white or light pink flowers are positioned at the apex of the stalks and only seldom bloom, especially in plants maintained indoors for ornamentation [4].

2. MATERIALS AND METHODS

Materials: This study used the human esophagus cancer KYSE-30 cell-line (Santa Cruz Biotechnology, USA). The sample consisted of whole plants (leaves, steam, and root) of *Crassula ovata* collected from the Babylon Nursery Plantation in March 2023. The plant was identified and authenticated by Dr Esraa Abdel-Al Razzaq Majeed (2023-1-29) at the Department of Biology, College of Sciences, University of Baghdad.

Extraction and isolation: About 350 gr of powdered plant were macerated for 48 h in n-hexane, and the plant material was then filtered. While the filtrate was evaporated and designated as the hexane extract, it was then submitted to gas chromatography - mass spectroscopy (GC-MS) as an identification step. The results indicated the presence of Y-sitosterol, and the isolation of phytosterol was performed by a preparative thin-layer chromatograph in acetone hexane (2.5 : 7.5) as the mobile solvent [5]. The sample was then sent for identification by liquid chromatography - mass spectroscopy (LC-MS).

Structural analysis: The structural analysis used for the n-hexane extract was GC-MS (7820A-5977E; Agilent Technologies, USA) and LC-MS (AB SCIEX 3200 QTRAP; Mashhad University of Medical Sciences).

MTT assay: The micro-culture tetrazolium assay (MTT assay) was used to evaluate the cytotoxicity of the n-hexane extract on the esophageal cell-line (KYSE-30 cells). The cells were grown inside the prepared media (10% fetal bovine serum, 100 g/mL streptomycin, and 100 units/mL penicillin), were transferred to 96-well plates (after trypsinization), and were incubated at 37°C. When a confluent monolayer was reached, the sample was applied. The sample was prepared by dissolving in dimethyl sulfoxide (DMSO; 11 mg/mL) as a stock solution, and by dilution, it was prepared into different concentrations (1,000, 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 µg/mL) and it was applied on the plate wells. After 48 h, the MTT dye was applied, and after 4 h, the media was removed and the formazan violet crystal was dissolved by DMSO. The measurement was undertaken by a microplate reader at 570 nm.

Statistical analysis: The data of the n-hexane extract were analysed by one-way ANOVA, at a significance level of $p<0.05$. The data means were compared with the control mean (which represents only cancer cells), after testing the data with a normality test and ensuring a normal distribution. We used the Levene test to assess variance homogeneity, and *post hoc* analysis so as to explain the significant difference between the means.

3. RESULTS

The undertaken GC-MS of the n-hexane extract demonstrated several compounds with a similarity index above 95%, including: hexadecane,2,6,10,14-tetramethyl-, nonadecane, hexadecanoic acid, methyl ester, hexadecanoic acid (palmitic acid), vitamin E (α-tocopherol), α-tocopheryl acetate, and γ-sitosterol, while the undertaken LC-MS (Figure 1) confirmed the structure of the isolated phytosterol compound as Y-sitosterol, TMS (trimethylsilyl), with the IUPAC name of 17(4-ethyl,5-methyl-hexane)-10,13-dimethyl cyclopentaphenanthr-5-ene-3-trimethylsiloxyl.

The results demonstrate a very noteworthy cytotoxic activity against the esophageal cancer cells, and the ability of the n-hexane extract to significantly curb the growth of the esophageal cancer cell-line, in a concentration-dependent manner. The data were tested using the Shapiro-Wilk tests, since the number of cases were fewer than 2,000. The null hypothesis of normality cannot be rejected for most concentrations, since their p values surpass 0.05; therefore, the data are normally distributed. The variance homogeneity assessed by the Levene test with adjusted degrees of freedom and the deviations of the experimental groups (7.5, 15, 31, 62, 125, 250, 500, 1,000, and Control) show significant differences. The undertaken *post hoc* analysis using the Games-Howell test revealed a significant difference ($F=33.556$, $p<0.05$) among groups, in particular, between the 500 and the 1,000 µg/mL treatment groups.

4. DISCUSSION

The observed cytotoxic effect is attributed to the presence of many compounds that have an anticancer effect, according to other studies focusing on compounds like vitamin E [6] or the n-hexadecanoic acid (palmitic acid) [7], which is indicated by our GC-MS and is known to act synergistically, especially with TMS sitosterol (which was isolated and confirmed by LC-MS). Phytosterols are plant sterol compounds synthesized by the isoprenoid biosynthesis pathway via squalene from acetyl coenzyme A, and are structurally similar to cholesterol except for an additional hydrocarbon chain at the C-24 position.

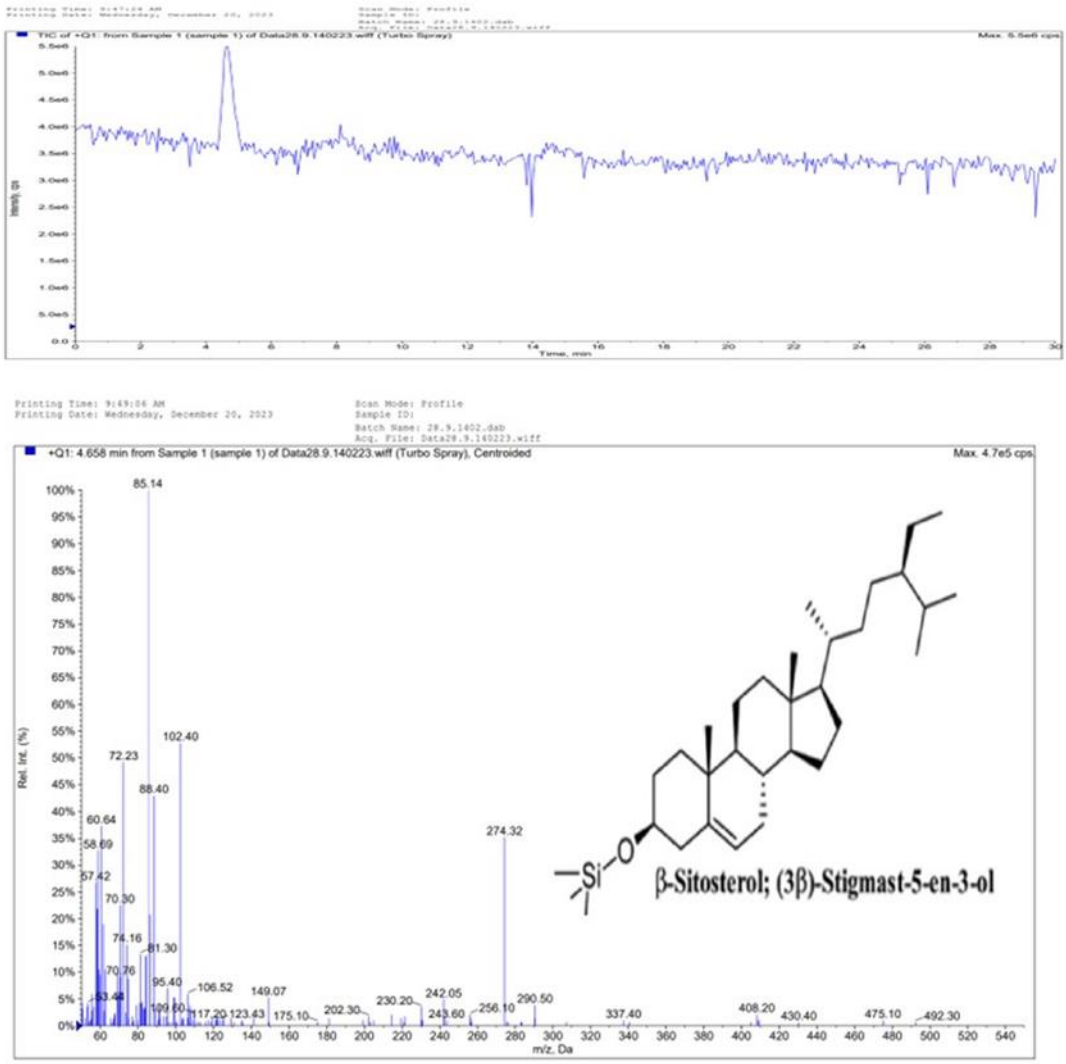

Figure 1. LC-MS chart of the isolated phytosterol compound (m/z of 486 $[M+H]^+$), where m/z=85 corresponds to the base peak result from the stable secondary carbocation fragment, m/z=274.32 corresponds to the steroid nucleus, m/z=72.23 corresponds to trimethylsilyl, and m/z=88.40 corresponds to trimethylsiloxyl.

Other studies have demonstrated that phytosterols could act through multiple mechanisms as anticancer agents and promote the inhibition of carcinogen production, cancer cell growth, angiogenesis, invasion, and metastasis, as well as cause the apoptosis of cancerous cells [8]. The apoptosis mechanism is known to be induced by the increased levels of the tumor suppressor protein p53 that interacts with kinase signalling pathways involving AMPK, PI3K/AKT/mTORR, RAS/RAF/MAPKK, and JAK/STAT [9], as well as the activation of procaspase-3 [10].

5. CONCLUSION

The n-hexane extract of *Crassula ovata* is found to have a strong anticancer effect against esophagus cancer cells, as it was observed from the application of different concentrations of the extract on a cell-line (KYSE-30).

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Isolation and characterization of a tetrahydroprotoberberine alkaloid from *Crassula ovata*

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Abstract

The presence of alkaloids in *Crassula ovata* is a topic that is still unexplored, as there are no published studies on the matter. This study demonstrates the presence of an alkaloid compound (and its class) for the first time in *Crassula ovata*. The plant material was defatted with n-hexane, and a Soxhlet apparatus was used for the extraction process, while the acid-base method was used for the isolation of alkaloids from the chloroform fractions. The quaternary alkaloid was precipitated from the aqueous layer spontaneously, in high quantity. By using standard spectroscopic methods (including liquid chromatography - mass spectroscopy) we were able to clarify the structure of the precipitated compound as a tetrahydroprotoberberine alkaloid based on the general fragmentation pattern of this class of alkaloids and the retro-Diels-Alder reaction; a characteristic fragmentation pathway of tetrahydroprotoberberine alkaloids.

KEYWORDS
protoberberine, *Crassula ovata*, alkaloid, solvent extraction

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1. INTRODUCTION

Recently, developed nations are increasingly turning to the employment of traditional medical practices that use herbal medications and remedies, leading to an increased reliance on these types of medicines. Several medications and other products have been developed and extracted from plants in industrialized countries [1]. *Crassula ovata* is a plant that belongs to the Crassulaceae family, and has formerly been utilized as a medicinal plant in South Africa and in other countries. In China, it is used in order to treat wounds and diarrhoea [2], while in North-East India (particularly in Manipur) *Crassula ovata* is primarily used for the treatment of diabetes (by consuming the plant's leaf juice) and various infections [3]. There is no previous study indicating the presence of alkaloids in *Crassula* plants, except for one study demonstrating a positive Mayer's test for *Crassula ovata*

[4]. However, the distribution of alkaloids has been studied in the Crassulaceae family, and only *Echeveria venezuelensis* has been found to contain piperidine alkaloids, while *Crassula multicava* does not [5]. This study focuses on the extraction of alkaloids from chloroform fractions of *Crassula ovata* by acid-base methods.

2. MATERIALS AND METHODS

Materials: The sample consisted of whole plants (leaves, steam, and root) of *Crassula ovata* collected from the Babylon Nursery Plantation in March 2023. The plant was identified and authenticated by Dr Esraa Abdel-Al Razzaq Majeed (2023-1-29) at the Department of Biology, College of Sciences, University of Baghdad.

Extraction and isolation: About 200 gr of the powdered plant were defatted for 48 h in n-hexane so as to remove any wax and fatty material. The plant material was then filtered and dried at room temperature, while 3 h after spraying the plant with ammonia, the extraction process began by using a Soxhlet apparatus for 9 h. A total of 300 mL of 85% aqueous ethanol was used as an extractor solvent, through simple filtration so as to remove any boiling chips and other residues, and to obtain the crud extract by rotary evaporation under reduced pressure. The crude extract (37.421 gr) was dissolved in 300 mL of water that was sequentially partitioned first with chloroform, then with ethyl acetate, and lastly with n-butanol (3 x 100 mL for each fraction). The chloroform fraction (crude extract) entered isolation and further purification through the acid-base method. It was first dissolved in acidified water and portioned with ethyl acetate, then after removing the aqueous layer, it was made basic by the addition of ammonia, and was further portioned in a separator funnel with a lipophilic organic solvent (chloroform) [6]. The compound precipitated from the aqueous layer as white crystals spontaneously, and this provided a mark that our alkaloid was a quaternary compound. The structure was clarified by liquid chromatography - mass spectroscopy (LC-MS).

Structural analysis: The standard spectroscopic methods for investigating the structure of natural products comprise of infrared spectroscopy (IR) and ultraviolet (UV) spectroscopy, which are both carried out in the Environmental and Water Research Department of the Ministry of Sciences and Technology; these methods are often combined with MS, including LC-MS (AB SCIEX 3200 QTRAP; Mashhad University of Medical Sciences).

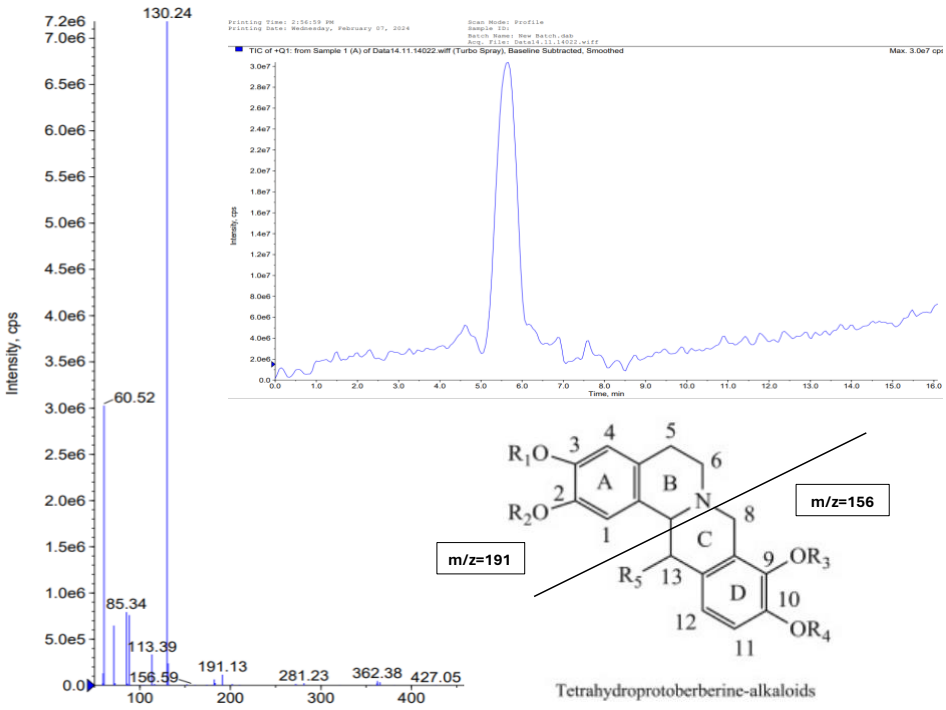

Figure 1. LC-MS chart of the isolated alkaloid compound (m/z of 362 $[M+H]^+$), where m/z=130 corresponds to the base peak result from the isoquinoline nucleus fragment, m/z=191 and m/z=156 correspond to the retro-Diels-Alder reaction, while m/z=113, m/z=85, and m/z=60 correspond to the isoquinoline nucleus fragmentation by loss (NH_3, CH_2=CH_2, and CH_2=CH_2, respectively).

3. RESULTS

As shown in Figure 1, LC-MS has clarified the structure of the isolated alkaloid compound as a tetrahydroprotoberberine compound, based on the general MS fragmentation pathways of protoberberine-type alkaloids [7], and a UV / visible spectrophotometer λ_{max} of 202.5. Moreover, the obtained Fourier transform IR (FT-IR) revealed a 3400 N-H bending, a 3119 C-H bending of CH_2=CH_2, a 2800 C-H bending of CH_2, a 1669 C=C bending, a 1387 C-N bending, and a 1042 C-O bending. The isolated compound started to melt at 180°C, then underwent decomposition at 220°C.

4. DISCUSSION

An alkaloid is a basic substance. When adding acidified water, it turns into salts and is present in the aqueous layer because it is dissolved in it. It is portioned with ethyl acetate so s to remove other substances and obtain the aqueous layer. Subsequently, ammonia is added and the alkaloid compound becomes free because the strong base releases the weak base from its salt and becomes dissolved in any lipophilic solvent (like chloroform), except if the alkaloid is quaternary; in the case of the latter, it remains in the aqueous layer. In this experiment, the compound precipitated from the aqueous layer automatically, and it was collected in high quantity from this plant. The most important spectroscopic method for investigating the structure of natural products is MS. The mass spectrum of a tetrahydroprotoberberine alkaloid like tetrahydropalmatine or corydaline shows the predominant ions at m/z of 192 and 165 or 150 after the loss of a methyl group from the retro-Diels-Alder reaction, which is a characteristic fragmentation pathway of tetrahydroprotoberberine, resulting in a C-ring opening so as to form tetrahydroisoquinoline fragment ions (m/z=130). It is significant that this tetrahydroisoquinoline fragment ion is not only a characteristic feature, but also gives structural information about this class of alkaloids. It is always the most abundant fragment in the mass spectra of these alkaloids, and most likely represents the substitution pattern in the A ring in Figure 1. For instance, the most prevalent ion in both corydaline and tetrahydropalmatine, the product ion with m/z of 192, is suggestive of two methoxy substituents in the A ring [8].

5. CONCLUSION

In conclusion, this study indicates the presence of alkaloids in *Crassula ovata*. The mass spectrum of the isolated crystals revealed that the possible compound is a tetrahydroprotoberberine alkaloid like corydaline and tetrahydropalmatine. Quaternary protoberberine alkaloids represent a very interesting and significant group of natural products with a broad range of biological activities, including antiparasitic, antitrypanosomal, and antileishmanial activities.

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CONFLICT OF INTEREST STATEMENT

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Protective effect of COVID-19 vaccination against a SARS-CoV-2 reinfection in the Babil Province

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Abstract

Reinfection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has been documented all over the world. Currently, limited evidence exists concerning the protection afforded by the COVID-19 vaccination against reinfection with SARS-CoV-2. This case-control study was performed in order to assess the association between COVID-19 vaccination and SARS-CoV-2 reinfection in the Babil Province; the study used an electronic questionnaire. The infected patients were 115 (aged ≥18 years) and were confirmed by a positive PCR and/or a CT scan, they were either fully vaccinated or not with a second dose of a (Pfizer, AstraZeneca, or Sinopharm) vaccine before the reinfection date, and they were compared with 300 control participants. The study's findings revealed that the unvaccinated individuals had 4.5 times the odds of reinfection compared to those who were fully vaccinated, without preference for the manufacturer of the vaccine. The conclusion suggests that getting fully vaccinated against COVID-19 can significantly reduce the likelihood of reinfection, can enhance overall protection, and can minimize the risk of future infections.

KEYWORDS
COVID-19, SARS-CoV-2, fully vaccinated, reinfection, case-control study

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1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a pandemic viral disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 broke out in Wuhan, China, in December 2019 and spread worldwide with a very high mortality rate. Infection with SARS-CoV-2 not only leads to the development of severe acute respiratory syndrome, but progressively develops into a systemic disease with relevant extrapulmonary manifestations (that increase the lethality of

COVID-19) that include vascular, cardiac, renal, gastrointestinal, and central nervous system complications [1]. Pathophysiologically, the entry of SARS-CoV-2 at the cellular level is accompanied by widespread endothelial damage, while the altered immune response contributes to the multiple organ involvement seen in COVID-19 [2]. The first time a person is infected with COVID-19, it can take days or weeks to get over the infection. After the infection, the person's immune system remembers what it learned about how to protect the body against that disease. The immune system produces lymphocytes, called "memory cells", that act quickly if the body gets infected with the same virus again. B-lymphocytes produce antibodies to attack the COVID-19 virus again in reinfected cases.

Different types of vaccines work in different ways in order to offer protection. However, with all types of vaccines, the body will have memory lymphocytes as well as B-lymphocytes that will fight the virus and kill it in the future. It typically takes a few weeks after vaccination for the body to produce memory lymphocytes and B-lymphocytes [3]. Therefore, it is possible that a person could be infected with the virus that causes COVID-19 just before or just after vaccination and then manifest a symptomatic infection; this would be because the vaccine did not have enough time to provide protection. Sometimes after vaccination, the process of building immunity can cause symptoms, such as fever. These symptoms are normal and are signs that the body is building immunity [4]. This case-control study was undertaken in order to study the association between vaccination and SARS-CoV-2 reinfection in the Babil Province.

2. PATIENTS AND METHODS

This case-control evaluation was undertaken in order to study the association between COVID-19 vaccination and SARS-CoV-2 reinfection in the Babil Province between December 2021 and January 2022. The study was conducted among individuals previously infected with SARS-CoV-2 (n=115) and recruited control participants (n=300) for comparison. Our electronic questionnaire was filled by patients (aged ≥18 years) with a SARS-CoV-2 infection confirmed by positive polymerase chain reaction (PCR) and/or computerized tomography (CT) scan. Control participants were residents with confirmed SARS-CoV-2 infection who were not reinfected, while a "case-patient" was defined as a resident with a history of a confirmed SARS-CoV-2 infection and a subsequent reinfection.

The vaccination status was determined, and case-patients were considered fully vaccinated if a second dose of a (Pfizer, AstraZeneca, or Sinopharm) vaccine was received ≥14 days before the reinfection date. Partial vaccination was defined as the receipt of one or more dose(s) of the vaccine, but either the vaccination series was not completed or the final dose was received in less than 14 days before the case-patient's reinfection date. For the controls' vaccination status, the same definition was applied. Age, sex, and occupation were also considered in this study. Excluded subjects included only the individuals whose infection was not confirmed by a PCR or a CT scan.

3. RESULTS

This study details the findings of a case-control assessment of the association between CCOVID-19 vaccination and SARS-CoV-2 reinfection in the Babil Province between December 2021 and January 2022, among people previously infected with SARS-CoV-2. We have found that among the Babil Province residents who were previously infected with SARS-CoV-2, the young age group (between 20 and 40 years of age) was the target group of infection with a high percentage of reinfection among the medical staff (53%). Obviously, those who were unvaccinated against COVID-19 had a significantly higher likelihood of reinfection (82.6%), with a decrease of the susceptibility for infection in both partially or fully vaccinated subjects. Table 1 provides a detailed overview of the results obtained from our electronic questionnaire.

4. DISCUSSION

The findings of our study suggest that among previously infected individuals, full vaccination is associated with a reduced likelihood of reinfection and, conversely, being unvaccinated is associated with a higher likelihood of being reinfected. The lower odds of reinfection among the partially vaccinated group (as compared with the unvaccinated group) is suggestive of a protective effect, and is consistent with findings from previous studies indicating higher titers after the first mRNA vaccine dose in people who have been previously infected [5]. The immunity resulting from natural infection, although not well understood, is suspected to persist for ≥90 days in most people. However, the emergence of new variants might affect the duration of the infection-acquired immunity, and laboratory studies have shown that sera from previously infected individuals might offer weak or inconsistent responses against several variants of concern [6]. The patient's occupation might play a role in reinfection because there is a difference in the amount of vi-

ral exposure; in our study, we discovered that the medical staff had the highest ratio of case-patients when compared to other occupations, which was 53.91%. Therefore, the evaluation of infection rates and associated features is necessary in order to improve and adjust the protective measures of this vulnerable and highly essential group. On other hand, the same occupational group had the higher rate of infection control (42%); a fact that might be due to their knowledge about protection and could also be attributed to them receiving vaccines [8].

Table 1. Demographic and vaccination data for the case-patient and the control group.

General population questionnaire results					
Age (in years)					
	18–29	**30–39**	**40–49**	**50–59**	**60–69**
Case-patients (n=115)	79 (68.69%)	24 (20.86%)	7 (6.08%)	3 (2.6%)	2 (1.73%)
Control (n=300)	180 (60%)	70 (23.33%)	23 (7.66%)	22 (7.33%)	5 (1.66%)
Sex					
	Male		**Female**		
Case-patients (n=115)	98 (85.21%)		17 (14.78%)		
Control (n=300)	248 (82.66%)		52 (17.33%)		
Type of employment					
	Student	**Medical staff**	**Other employee**	**Unemployed**	**Earner**
Case-patients (n=115)	23 (20%)	62 (53.91%)	18 (20%)	10 (20%)	2 (1.73%)
Control (n=300)	64 (21.33%)	126 (42%)	74 (21.33%)	27 (21.33%)	9 (3%)
State of vaccination					
	Manufacturer		**Case-patients** (n=115)	**Control** (n=300)	
Not vaccinated			**95 (82.6%)**	**51 (17%)**	
Partially vaccinated	Pfizer		2 (1.74%)	20 (6.66%)	
	AstraZeneca		1 (0.87%)	4 (1.33%)	
	Sinopharm		1 (0.87%)	2 (0.67%)	
	Total		**4 (3.46%)**	**26 (8%)**	
Fully vaccinated	Pfizer		12 (10.43%)	183 (61%)	
	AstraZeneca		3 (2.06%)	33 (11%)	
	Sinopharm		2 (1.74%)	9 (3%)	
	Total		**17 (14.23%)**	**225 (75%)**	

Vaccine manufacturers might also play a minor role regarding the protection against COVID-19, but no strong evidence supports these data. Our study has found that 80% of the vaccinated patients have received a Pfizer vaccine, 15% were vaccinated with an AstraZeneca vaccine, and only 5% have received a Sinopharm vaccine. These data might be related to the availability and distribution of these vaccines in the Babil Province. These data also reflect that Pfizer has provided the highest protection among the three companies, since 93.8% of the patients who received a full Pfizer vaccination were found to be protected from reinfection, while 91.7% of the patients who received a full AstraZeneca vaccination were found to be protected from reinfection and 81.8% of the patients who have received a full Sinopharm vaccination were found to be protected from reinfection; however, the differences between the three manufacturers were not significant [9].

All eligible individuals should be offered a COVID-19 vaccination, including those with a previous SARS-CoV-2 infection. This will reduce their risk for future morbidity and mortality. Our findings suggest that among individuals with a previous SARS-CoV-2 infection, full vaccination provides additional protection against reinfection, while partial vaccination provides lower levels of

protection. Moreover, the Pfizer vaccines can provide a higher protection than those of other manufacturers.

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Theoretical calculations and molecular modelling of isoindoline compounds as anticonvulsant agents

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Abstract

The lack of safe and effective antiepileptic drugs is a persistent issue that could be addressed through the repurposing or further development of commonly available drugs. Due to high accuracy, low effort, and high cost, it is best to begin the search for alternative treatments with a theoretical chemical study. Isoindoline derivatives, their ΔG, and their molecular docking were subjected to the molecular level theory. Having a ΔG of -4.9, compound A1 demonstrated a unique activity against protein 1OHV (4-aminobutyrate-aminotransferase; from pig), while the same compound demonstrated distinct activity against protein 3F8E (coumarins as suicide carbonic anhydrase inhibitors) with a ΔG of -4.533. Moreover, compound A3 exhibited a unique activity against protein 6KZP (calcium channel-ligand) with a ΔG of -7.597. The undertaken DFT analysis determined the highest occupied molecular orbital (HOMO), the least unoccupied molecular orbital (LUMO), and the HOMO-LUMO gap values for the studied derivatives (compound A1: -0.202, -0.091, and -0.111 eV; compound A3: -0.228, -0.102, and -0.126 eV, respectively). The ionization potential, the softness, the hardness, and other chemical properties of these compounds were subsequently computed. Drug likeness predictions were employed in order to show that the compounds adhered to Lipinski's rule. Our results indicate that the molecular mass, log P, as well as the hydrogen bonding donors and acceptors of the herein assessed isoindoline compounds fall within acceptable ranges.

KEYWORDS
isoindoline derivatives, anticonvulsant, molecular docking, DFT study, ADME

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1. INTRODUCTION

Behind Alzheimer disease and stroke, epilepsy is the third most prevalent neurological condition. During the previous three decades, a number of third-generation antiepileptic medications have improved the treatment of epilepsy. However, the need for novel medications or approaches to the treatment of epilepsy became urgent due to the patients' intolerance and resistance to antiepileptic medications in 20–30% of the cases [1]. Daily medication is typically used to treat epilepsy once a second seizure has manifested. In individuals who are at high risk of having more seizures, medication may even be initiated after the first seizure [2]. Moreover, several other treatment options, such as special diets, neurosurgery, or the implan-

tation of a neurostimulator, may be considered in drug-resistant cases [3].

The aim of this study was to identify the highest binding affinity (ΔG) value for isoindoline derivatives that have a significant association with specific proteins in the brain. When the ΔG value of isoindoline derivatives bound to the protein is higher than the ΔG value of the drug available as an anticonvulsant, this means that the derivative could be highly effective and reliable as an anticonvulsant drug.

2. MATERIALS AND METHODS

SwissDock is a protein ligand docking server that is based on the EADock dihedral space sampling (DSS). This server is intended to provide protein-ligand docking to a global scientific community [4]. The Swiss Institute of Bioinformatics offers SwissDock as a web service. The following proteins were docked using a standard protocol: 1OHV (4-aminobutyrate-aminotransferase; from pig), 3F8E (coumarins as suicide carbonic anhydrase inhibitors), and 6KZP (calcium channel-ligand). Five isoindoline derivatives have been suggested for the proteins' active sites. Every chemical structure was generated with the correct 2D orientation in ChemOffice (ChemDraw version 20.0). MM2 energy minimization was estimated for each structure, so as to estimate the potential energy surface (including factors such as steric energy and thermal energy).

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; 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.

Compound	ΔG	1OHV protein residues surrounding the compounds	Residues with interferences
A1	-4.9	CYS_{135}, GLY_{136}, GLY_{191}, SER_{137}, SER_{328}, SER_{269}, LYS_{329}, ASN_{140}, ASP_{298}, GLU_{265}, GLU_{270}, VAL_{300}, GLN_{301}, PHE_{189}, HIS_{190}, ARG_{192}	GLY_{136}, GLN_{301} (H-bonding); PHE_{189} (pi–pi stacking)
A2	-4.081	ARG_{192}, GLY_{191}, GLY_{136}, PHE_{189}, SER_{137}, SER_{328}, SER_{74}, CYS_{135}, ALA_{134}, VAL_{300}, LYS_{329}, MET_{332}	GLY_{136}, SER_{328}, CYS_{135} (H-bonding)
A3	-4.458	SER_{74}, SER_{328}, SER_{137}, MET_{332}, LYS_{329}, CYS_{135}, GLY_{136}, GLY_{191}, VAL_{300}, ASN_{140}, PHE_{189}, HIS_{190}, ARG_{192}	SER_{328}, GLY_{136} (H-bonding)
A4	-3.986	CYS_{135}, GLY_{136}, GLY_{191}, SER_{137}, SER_{328}, LYS_{329}, ASN_{140}, GLU_{265}, ASP_{298}, VAL_{300}, GLN_{301}, PHE_{189}, HIS_{190}, ARG_{192}	GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
A5	-0.275	CYS_{135}, GLY_{136}, GLY_{191}, SER_{137}, SER_{328}, SER_{269}, LYS_{329}, ASN_{140}, ASP_{298}, GLU_{299}, GLU_{265}, GLU_{270}, VAL_{300}, GLN_{301}, THR_{302}, PHE_{189}, HIS_{190}, ARG_{192}	GLY_{136} (H-bonding); PHE_{189} (pi–pi stacking)
Compound	**ΔG**	**3F8E protein residues surrounding the compounds**	**Residues with interferences**
A1	-4.533	ASN_{62}, ASN_{67}, HIE_{64}, GLN_{92}, HIS_{94}, TRP_{5}, THR_{200}, PRO_{201}, PRO_{202}	H_2O, ASN_{62} (H-bonding); HIE_{64} (pi–pi stacking)
A2	-3.92	TRP_{5}, PRO_{202}, PRO_{201}, THR_{200}, ASN_{62}, ASN_{67}, HIE_{64}, GLU_{69}, GLN_{92}, ILE_{91}, PHE_{131}	H_2O (H-bonding); PHE_{131} (pi–pi stacking)
A3	-3.907	PHE_{131}, PRO_{202}, PRO_{201}, THR_{200}, TRP_{5}, ASN_{62}, ASN_{67}, HIE_{64}, HIS_{94}, GLN_{92}, ILE_{91}	H_2O (H-bonding); PHE_{131} (pi–pi stacking)
A4	-3.27	PHE_{131}, ILE_{91}, GLN_{92}, ASN_{62}, ASN_{67}, HIE_{64}, THR_{200}	H_2O, HIE_{64} (H-bonding)
A5	-2.861	PRO_{202}, PRO_{201}, THR_{200}, TRP_{5}, ASN_{62}, ASN_{67}, HIE_{64}, GLU_{69}, PHE_{70}, ASP_{71}, ASP_{72}, LEU_{57}, PHE_{131}, ILE_{91}, GLN_{92}	H_2O (H-bonding)
Compound	**ΔG**	**6KZP protein residues surrounding the compounds**	**Residues with interferences**
A1	-6.524	PHE_{917}, PHE_{956}, LEU_{920}, LEU_{872}, LEU_{1499}, THR_{921}, GLN_{922}, LYS_{1462}, ILE_{876}, GLY_{951}, ASN_{952}	ASN_{952}, LYS_{1462} (H-bonding); PHE_{956} (pi–pi stacking)
A2	-5.49	GLN_{922}, THR_{921}, LEU_{920}, LEU_{872}, LEU_{1499}, ASN_{952}, GLY_{951}, PHE_{956}, PHE_{868}, ALA_{1502}, LYS_{1462}	ASN_{952}, LYS_{1462} (H-bonding)
A3	-7.597	THR_{921}, LEU_{920}, LEU_{872}, LEU_{959}, LEU_{1499}, LEU_{1506}, PHE_{917}, PHE_{868}, PHE_{956}, PHE_{1503}, ILE_{876}, CYS_{869}, VAL_{865}, ALA_{1502}, LYS_{1462}	H_2O (H-bonding); PHE_{131} (pi–pi stacking)
A4	-6.203	PHE_{917}, PHE_{956}, LEU_{920}, LEU_{1499}, LEU_{872}, THR_{921}, GLN_{922}, LYS_{1462}, TYR_{953}, ASN_{952}, GLY_{951}, ILE_{876}	ASN_{952}, LYS_{1462} (H-bonding); PHE_{956} (pi–pi stacking)
A5	-6.653	PHE_{917}, PHE_{956}, LEU_{920}, LEU_{1499}, LEU_{872}, LEU_{391}, THR_{921}, GLN_{922}, LYS_{1462}, TYR_{953}, ASN_{952}, GLY_{951}, ILE_{876}, ILE_{387}	ASN_{952}, LYS_{1462} (H-bonding); PHE_{956} (pi–pi stacking)

The resulting conformations of the models were obtained [5]. Subsequently, the molecular theory was employed. Following energy minimization, the ligand molecules with the participation of residues GLY_{136}, GLN_{301}, ASP_{298}, *etc.* in the case of 1OHV, of residues ASN_{67}, HIE_{64}, ASN_{62}, *etc.* in the case of 3F8E, and of residues LEU_{959}, VAL_{865}, ALA_{1502}, CYS_{869}, *etc.* in the case of 6KZP (Table 1) were subjected to quantum mechanics using the B3LYP/6-31G++ (d,p) level of theory for frequency calculation and geometry optimization. The three most prevalent interactions (between the assessed proteins and the assessed compounds) with residue involvement were found to be chelation bonding, H-bonding, and pi–pi stacking. The optimized by density-functional theory (DFT) structures were then fed into SwissDock. The receptor molecules' crystal structures were obtained from the Protein Data Bank.

3. RESULTS AND DISCUSSION

Molecular docking: A theory of molecular modelling known as "docking" explains the interactions between two or more proteins and ligands. The "ΔG" value makes that determination, where a better fit between the chemical and the protein is indicated by a larger negative ΔG [6]. The five compounds herein assessed were drug-like and could potentially exert an anticonvulsant effect, according to our ΔG calculations (Table 1). Through its interaction with the protein 1OHV, compound A1 exhibited the highest degree of association with the protein (ΔG=-4.9). Compound A1 also exhibited the highest degree of association (ΔG=-4.533) with the protein 3F8E, thereby exhibiting the most promising anticonvulsant activity through its interaction with coumarins; a novel class of suicide carbonic anhydrase inhibitors. Compound A3 exhibited the highest degree of association (ΔG=-7.597) with the protein 6KZP. Furthermore, it was found that ligand PLP (pyridoxal 5-phosphate) had the strongest affinity with the protein 1OHV (ΔG=-6.773), ligand TE1 had the highest association with the protein 3F8E (ΔG=-5.417), and ligand PLP had the strongest interaction with the protein 6KZP (ΔG=-6.709). Interestingly, compound A3 exhibited a smaller ΔG value than any of the ligands and compounds associated with 6KZP.

DFT analysis: In DFT, an atomistic simulation that computes a range of important features, the highest occupied molecular orbitals (HOMOs) are the highest. The least unoccupied molecular orbitals (LUMOs) are the next highest energy orbitals that are empty, while the HOMO–LUMO gap is their energy difference. According to the simulation, the LUMO, HOMO, and their gap values can define the inclination of molecules to act as bases as opposed to acids. In our study, the HOMO values of the assessed compounds ranged from -0.227 to -0.199 eV, their LUMO values ranged from -0.092 to -0.087 eV, while their HOMO–LUMO gap values ranged from -0.135 to -0.112 eV. These features were employed in equations that allowed us to identify many molecular properties such as the ionization potential (I) and the electron affinity (EA). The values of the studied compounds ranged from 0.197 to 0.228 in the case of their I, and from 0.081 to 0.102 in the case of their EA. Moreover, their electronegativity (μ) ranged from 0.139 to 0.165, their softness (S) ranged from 15.87 to 18.01, and their hardness (η) ranged from 0.055 to 0.063 [7].

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Metronidazole-loaded zinc oxide / graphene nanoparticles: synthesis, analysis, drug delivery, and antibacterial efficiency

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Abstract

In our study, zinc oxide (ZnO) nanoparticles (NPs) were prepared by precipitation (economically and in high quality) at a temperature range of 60°C to 80°C and at pH 8, and were then adorned with graphene (G) plates. To determine its antimicrobial potential, the ZnO/G complex was loaded with metronidazole. The morphology and diameter of the ZnO nanocomposite before and after the loading were validated by scanning electron microscopy. The average size of the ZnO NPs was found to be 20–40 nm, while X-ray diffraction examined how the physical features of these NPs varied from those of its individual components with an average size of 28.1 nm. The assessment of the ZnO/G complex's antibacterial efficacy against Gram-positive and Gram-negative bacteria was the main aim of our work. The agar well diffusion technique was used in order to assess the antibacterial activity of the ZnO/G complex with and without metronidazole. Our study demonstrates that the ZnO/G complex possesses antibacterial activity and might increase the antibiotic action by inhibiting Gram-positive bacteria (more than Gram-negative ones). It is, therefore, concluded that the ZnO/G NPs could be of use in formulating nano-drug conjugates that could act as antimicrobial agents.

KEYWORDS
ZnO, graphene, nanoparticles, instrumental analysis, drug delivery

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1. INTRODUCTION

Nanoparticles (NPs) are frequently called "the wonders of modern medicine" due to their remarkable abilities in many biological and physical aspects [1]. Microorganisms are omnipresent in the biosphere and always impact their surroundings; controlling their adverse consequences seems

most crucial. Kanamycin, spectinomycin, and penicillin are among the popular drugs used in order to fix this issue. However, their continuous use makes microorganisms resistant to these drugs [2]. Metal and metal oxide NPs have shown significant antibacterial action [3]. Of these, zinc oxide (ZnO; an n-type semiconductor) is used in solar cells, optical and antibacterial coatings, photocatalysts, electric devices, and gas sensors, due to its broad band gap (3.3 eV), its high excitation binding energy (60 mV), and its eco-friendliness [4]. When combined with graphene (G), this hybrid nanomaterial (ZnO/G) can integrate the exceptional electrical and antimicrobial characteristics of graphene, with the optical attributes and lethal efficacy of ZnO NPs against both Gram-positive and Gram-negative bacteria [5].

In our study, we used metronidazole-loaded ZnO/G nanocomposites in order to inhibit pathogens belonging to genera frequently associated to biodeterioration: Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. We also characterized some of the physical properties of these hybrid nanocomposites.

2. MATERIALS AND METHODS

Synthesis of ZnO NPs: ZnO NPs were prepared by direct thermal precipitation. Potassium hydroxide and zinc nitrate (0.4 M and 0.2 M, respectively) were mixed with deionized water at room temperature by slowly adding the aqueous solution while stirring. Controlling the temperature at 60°C for 120 min resulted in a white precipitation. Subsequently, we centrifuged the mixture at 500 rpm for 20 min and washed it three times with deionized water and 100% alcohol. A bespoke tubular muffle furnace facilitated the synthesis of ZnO at 500°C for 2 h [6]. The ZnO/G composites were produced as previously described [3], while the ZnO/G composite loading with metronidazole was performed according to Habeeb *et al.* [1].

Antibacterial activity: The agar well diffusion approach was used in order to cultivate *S. aureus* and *E. coli* in a Mueller-Hinton agar and to test their antibacterial properties. We swabbed 100 µL of old mature cultured media with the L-shaped rod after 24 h. The wells were created with a sterile 6-mm cork tool. The zone of inhibition was measured in mm. Each Petri dish included three wells with 50 µL each [1].

3. RESULTS

The scanning electron microscopy (SEM) images in Figures 1A–C present the morphologies of ZnO, of G, and of the ZnO/G-drug complex, respectively. Figure 1A shows the ZnO NPs' shape and formation, with a size ranging from 20 to 40 nm (as assessed with the use of the Image J software).

In order to gain information regarding the crystal structure of ZnO, of G, of ZnO/G, and of the ZnO/G-drug complex, we used X-ray diffraction (XRD) technique (Figure 1D). The following characteristic peaks at 2θ were observed: 31.7°, 34.4°, 36.2°, 47.5°, 56.7°, 63.0°, 66.4°, 68.1°, and 69.3°; corresponding to the planes 100, 002, 101, 102, 110, 103, 200, 112, and 201, respectively. The XRD patterns indicated the existence of ZnO NPs, and excluded the existence of other phases or impurities, thereby indicating the high purity of the herein assessed catalysts [1,5].

The antibacterial activity of ZnO/G NPs was tested against two bacterial strains at one concentration, with metronidazole as a standard. The highest antibacterial activity of ZnO/G with the drug was noted against *S. aureus* at 5 mm, while *E. coli* was not affected, as seen in (Figures 1E and F).

4. DISCUSSION

Figures 1A–C display the SEM images at a high magnification, and demonstrate the formation of NPs. They also provide a clearer idea about the particles' separation, without being highly affected by agglomeration. Figure 1A clearly shows that ZnO NPs appear as granule-like nanostructures, while Figure 1C exhibits the SEM imaging of the metronidazole-loaded ZnO/G NPs. The surface shape of metronidazole has been altered after being loaded on the ZnO/G complex. As one can see, the NPs manifested as granules with a light colour, and are evenly spread over the surface of metronidazole. When we compare Figures 1A and 1C, one can detect a considerable difference in the shape of metronidazole-loaded ZnO/G NPs.

The absence of the characteristic G diffraction peak at 2θ=24.6° in the XRD pattern of the ZnO/G complex is noteworthy. This is likely due to the fact that the ZnO crystals were obscured by a negligible amount of G, which has altered their structure [7]. Based on previous studies, the presence of grain boundaries in ZnO and ZnO/G nanocomposition is due to the existence of amorphous superficial and intergranular layers between ZnO and ZnO/G [8].

ZnO has an innate advantage of exerting broad antibacterial activities against bacteria, fungi, and viruses. The US Food and Drug Administration has recognized ZnO as a safe material. The release of Zn ions from ZnO has been suggested as one of the primary antibacterial mechanisms. Moreover the penetration of a bacterial membrane upon contact with ZnO can also contribute to the antibacterial ability of ZnO NPs [9].

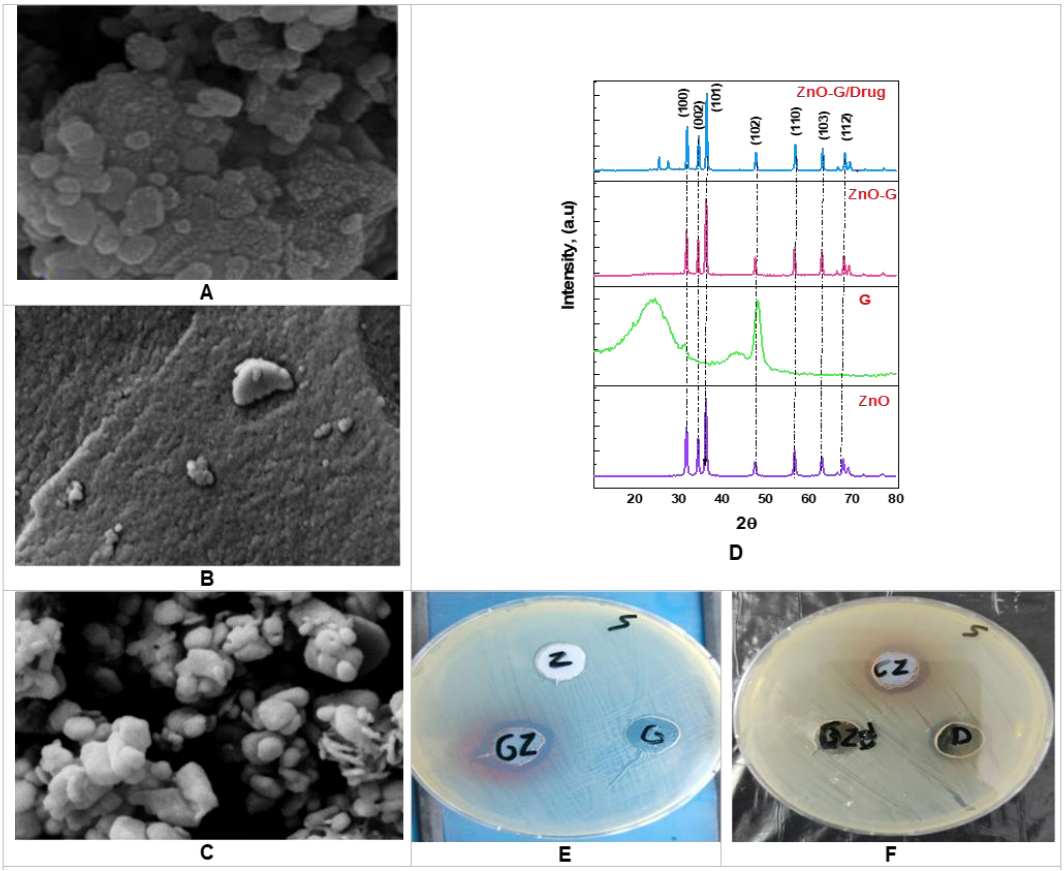

Figure 1. Instrumental analysis and antibacterial activity of zinc oxide (ZnO) / graphene (G) nanoparticles (NPs). **A–C:** Scanning electron microscopy images of ZnO, G, and the ZnO/G-drug complex, respectively. **D:** X-Ray diffraction patterns before and after decoration with graphene and loading with metronidazole. **E** and **F:** Zone of inhibition as a measure to establish the antibacterial potential of ZnO NPs and of ZnO-G-metronidazole NPs, respectively.

Several mechanisms have been proposed for the bactericidal activities of ZnO NPs, including the direct contact with cell membrane, the release of metallic ions, the generation of reactive oxygen species (ROS), and the internalization of ZnO NPs. For the direct contact killing mechanism, ZnO NPs tend to disrupt the cell membrane function and interfere the electron transport chain upon attachment on the cell wall, thereby leading to the production of ROS. Positively charged ZnO NPs can attach readily on the cell membranes of Gram-positive and Gram-negative bacteria due to the electrostatic interaction. This interaction disrupts the membrane structure and damages the cell integrity, leading to the leakage of intracellular contents. In particular, ZnO NPs with very small sizes (≤10 nm) can easily enter the cytoplasm, thereby inducing DNA damage. Due to their cell wall differences, Gram-positive and Gram-negative bacteria react differently to ZnO NPs. Gram-positive bacteria contain 20–80-nm peptidoglycan layers attached to the cytoplasmic membrane by lipoteichoic acid (LTA). As the peptidoglycan layers are porous and do not block tiny substrates, the cell walls of Gram-positive bacteria are highly charged anionic polymers with teichoic acid and LTA phosphate groups that facilitate the electrostatic attraction of positively-charged NPs. This is why ZnO NPs prefer Gram-positive bacteria over Gram-negative ones. However, the Gram-negative peptidoglycan is thinner (<10 nm) and wrapped with an outer membrane of lipopolysaccharide (LPS). LPS is a complex macromolecule that hydrophobic antibiotics cannot penetrate. Hydrophobic antibiotics and many hazardous chemicals are blocked by this outer membrane. This is why Gram-negative bacteria with thinner peptidoglycan layers and outer LPS membranes can withstand ZnO NPs

better than Gram-positive bacteria [10].

5. CONCLUSION

ZnO/G nanostructures with positive surface charge are able to adhere and attach on negatively-charged membranes *via* electrostatic interaction when they come in contact with bacteria. This effect disrupts the bacterial cell membrane function, interferes electron transport chain, and deactivates bacterial enzymes, thereby leading to cell death. Apart from the "contact" killing effect, other mechanisms such as the ROS production and the release of Zn ions have also been reported to be responsible for the bactericidal activity of ZnO-containing NPs. The antimicrobial activity of ZnO NPs is size-, shape-, and concentration-dependent.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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In vitro study of the antibacterial effects of the *Cydonia oblonga* extract

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Abstract

Cydonia oblonga is effective against many infections and has medicinal properties that are useful in the treatment of many other diseases. The aim of this study was to elucidate the antimicrobial activity (in the form of anti-adherence and anti-biofilm formation) of the aqueous *Cydonia oblonga* extract. The biological activity of the extract was compared to that of imipenem. All bacterial isolates of Gram-positive and Gram-negative bacteria assessed were found to be susceptible to the *Cydonia oblonga* extract and the zone of inhibition ranged from 24 to 34 mm. Most bacterial isolates were resistant to the antibiotic, and some bacterial isolates were sensitive to imipenem. The adherence and biofilm formation inhibitory activities in the presence of the aqueous extract of *Cydonia oblonga* were found to be moderate or elevated in most of the Gram-negative bacteria assessed. According to our findings, the aqueous *Cydonia oblonga* extract displays great effectiveness (and promise) against many pathogenic bacterial isolates.

KEYWORDS
Cydonia oblonga, antibacterial, biofilm formation, adherence inhibition, *in vitro*

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1. INTRODUCTION

Cydonia oblonga (quince) is a species of the family of Rosaceae that can be used as a whole or part for food and for the treatment for many diseases [1]. The seeds of *Cydonia oblonga* contain triterpenes, sterols, and tannins as active components, and can be used as an anti-diarrhoeal medication [2]. The presence of organic acids, phenolic component, and amino acids has been determined in the seeds of quince [3], while phenolic compounds, essential oils, organic acids, ionone glycosides, and tetracyclic sesterterpenes can be found in many plant parts of quince [4]. The aim of this study was to conduct a comprehensive *in vitro* characterization of the antibacterial activity of the aqueous *Cydonia oblonga* extract.

2. MATERIALS AND METHODS

The preparation of the aqueous extract of *Cydonia oblonga* at a concentration of 30% and that of imipenem were performed as previously described [5]. The bacterial isolates used in this study were *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Acinetobacter*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia* spp., *Salmonella typhi*, *Salmonella typhimurium*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus faecalis*. Isolates of bacteria were cultured three times on agar nutrient and were then kept at 4ºC as slants. The isolates were determined by many biochemical tests. A total of 14 types of Gram-negative bacteria and 8 types of Gram-positive bacteria were tested. The antimicrobial effectiveness was assessed through agar-well diffusion [6]. The antimicrobial activity was determined by using a disc-diffusion assay for the drug; a test performed in triplicate. The assessment of the bacterial adherence to the membrane of human epithelial cells (one of the principal factors of a microorganism's virulence) was documented on Gram-negative bacteria only. The biofilm formation assay was performed as previously described [6], and the output was determined as "none" (<0.120), "medium" (0.120–0.240), and "strong" (>0.240) based on the mean OD value obtained at 630 nm.

3. RESULTS AND DISCUSSION

The antibacterial effect of the aqueous *Cydonia oblonga* extract at a 30% concentration against a number of bacteria was studied (Table 1). All bacterial isolates were sensitive to this extract, and the range of the zone of inhibition was between 24 and 34 mm. We noted that the plant extract exerted potent antibacterial action against black-pigmented bacteria (i.e., *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*). On the other hand, the herein assessed Gram-negative bacteria showed similar responses to the Gram-positive ones (i.e., *Staphylococcus saprophyticus*, *Streptococcus mutans*, and *Streptococcus faecalis*) by obtaining the same result in terms of their zone of inhibition (34 mm); other Gram-positive bacteria recorded a 29–32-mm zone of inhibition, which can be attributed to the chemicals found in quince seeds. The effect of imipenem on Gram-positive and Gram-negative microorganisms, as shown by a disc diffusion assay, revealed that most bacterial isolates were resistant to this antibiotics and that some isolates were sensitive (Table 1).

Table 1. Effects of the *Cydonia oblonga* extract and of imipenem against bacterial isolates, and the inhibitory effects of the aqueous *Cydonia oblonga* extract on Gram-negative bacterial adherence and biofilm formation.

	Zone of inhibition (in mm)		Inhibitory effect of *Cydonia oblonga* extract	
Isolates	***Cydonia oblonga* extract**	**Imipenem**	**Adherence**	**Biofilm formation**
Aggregatibacter actinomycetemcomitans	33	18	moderate	elevated
Prevotella intermedia	33	18	elevated	moderate
Porphyromonas gingivalis	32	17	elevated	elevated
Pseudomonas fluorescens	29	13	moderate	moderate
Pseudomonas aeruginosa	28	12	moderate	moderate
Escherichia coli	30	14	moderate	elevated
Proteus mirabilis	30	15	moderate	moderate
Proteus vulgaris	30	15	moderate	moderate
Acinetobacter	30	14	elevated	moderate
Enterobacter aerogenes	30	13	moderate	moderate
Klebsiella pneumoniae	31	15	moderate	moderate
Serratia spp.	28	14	elevated	elevated
Salmonella typhi	27	13	moderate	moderate
Salmonella typhimurium	30	15	moderate	elevated
Staphylococcus saprophyticus	34	18	---	---
Staphylococcus epidermidis	32	19	---	---
Staphylococcus aureus	31	18	---	---
Streptococcus mutans	34	17	---	---
Streptococcus pneumoniae	30	15	---	---
Streptococcus pyogenes	29	16	---	---
Streptococcus agalactiae	31	15	---	---
Streptococcus faecalis	34	16	---	---

The adherence and biofilm formation inhibitory activities in the presence of the aqueous extract of *Cydonia oblonga* were found to be moderate or elevated in most of the Gram-negative bacteria assessed (Table 1).

Previous studies have found that the chemicals in *Cydonia oblonga* seeds are highly effective at inhibiting a variety of pathogenic bacteria. The tannins of quince are used as an antiseptic in respiratory infections because they kill bacteria, fungi, and viruses [7]. Furthermore, the extract of quince seeds can also be used in the treatment of coughs, diarrhoea, constipation, dysentery, and bronchitis. The analysis of the phytochemicals of the quince fruits has revealed numerous secondary metabolites [8].

Lipopolysaccharide, found in Gram-negative bacteria's outer membranes, prevents phenolic compounds from adherence to the surface of the bacteria [9], resulting in lower activity against Gram-negative bacteria. Moreover, the Gram-positive bacteria are highly sensitive to polyphenols [10]. On the other hand, the herein assessed extract was found to exert a potent anti-adherence and anti-biofilm formation activity, and to stop the formation of biofilm. Hindi *et al*. [6] have shown that plant extracts can inhibit the bacteria's ability to form biofilms and to adhere. The aqueous extract of *Cydonia oblonga* assessed in our study was found to be very effective against many pathogenic isolates, with an elevated inhibitory activity against biofilm formation and bacterial adherence.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Misuse of antibiotics in Iraq: Babylon Governorate as a model

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Abstract

The efficacy and easy access to antibiotics have led to their overuse, which allows for the development of resistance to them. This study took Babylon Governorate as a model to prove whether the above hypothesis is true or not. We aimed at exploring whether there are any restrictions for dealing with antibiotics by pharmacists in the Babylon Governorate, at identifying the distribution of pharmacies in Hillah, and at estimating the capability of the health authority to control them. A total of 165 pharmacies were visited in an attempt to purchase antibiotics without a prescription. Antibiotics were obtained from 164 pharmacies, and one pharmacy apologized because they did not have the antibiotic. Moreover, a survey was randomly distributed to different pharmacies and pharmacists asking them whether they sell antibiotics without prescriptions, and an online questionnaire was distributed to pharmacists and pharmacy students in the Babylon Governorate. The results revealed that the percentage of pharmacies that sell antibiotics without medical prescription is 98%; the remaining 2% belongs to the pharmacies that do not stock these antibiotics. We hypothesize that the main reason for this phenomenon is the chaotic distribution of pharmacies in the Governorate, and the lack of supervision by the authorities. Mapping has revealed a random distribution of pharmacies in Hillah, which is performed in an unscientific manner without taking into account whether these areas need pharmacies or not. Moreover, the lack of supervision by the Government and the health syndicates allows for antibiotics to be largely dispensed in the pharmacies without a medical prescription.

KEYWORDS
misuse, antibiotics, pharmacies, public health, Babylon Governorate

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1. INTRODUCTION

Antibiotics save lives; however, overusing or misusing prescription medication can have serious impacts, such as the development of antibiotic resistance, increasing risk for side effects, disruption of the gut microbiome, and inflated costs and use of healthcare resources. Unfortunately, the misuse and overuse of antibiotics has become a serious public health issue, threatening the great achievements of medicine. Antimicrobial resistance is a growing concern that threatens to compromise effective treatment of infectious diseases, especially in high-income countries [1]. Although the irrational use of antibiotics was once considered a problem only in developed countries, there has been a staggering rise in low- and middle-income countries [2].

Antibiotic resistance frequently occurs in hospitals due to the increasing number of patients, surgical procedures and interventions, that are associated with the increasing use of antibiotics in healthcare. Antibiotic resistance rates are increasing daily, not only with respect to the hospital community, but also in various other areas. Animals are given antibiotics to treat infections, but mostly for faster growth for commercial purposes.

Antibiotic resistance has been described as one of the biggest global threats of the 21st century [3]. Self-medication by consumers has been identified as one of the main causes of the development of antimicrobial resistance [4]. In an effort to control their disease, patients take the advice of false media sources and friends and family, which causes them to take antimicrobials unnecessarily or in excess. Many people resort to this out of necessity, when they have a limited amount of money to see a doctor, or in many developing countries where the economy is poorly developed and the lack of doctors is a reason for self-medication. In these developing countries, Governments are resorting to allowing antibiotics to be sold as over-the-counter medicines so that people can access them without having to find or pay to see a medical professional [5]. This increased access makes it extremely easy to obtain antibiotics without consulting a doctor and, as a result, many antibiotics are taken incorrectly, thereby resulting in resistant microbial strains. The main problem with self-medication is the public's lack of knowledge of the dangerous effects of antibiotic resistance, and how they can contribute to it through mistreatment or misdiagnosis of themselves. The risks of overuse or overprescribing of antibiotics include not only increases in antibiotic resistance, but increases in disease severity, length of illness, health complications and adverse effects, risk of death, healthcare costs, rehospitalization, and the need for medical treatment for health problems that may have resolved on their own [6].

2. MATERIALS AND METHODS

Our study included two parts. In the first part, the locations and numbers of pharmacies in the Babylon Governorate were identified for the purpose of drawing a map of their geographical distribution. The second part was an attempt to determine the extent of response to providing patients with antibiotics without requesting a prescription. The second part included two tasks. The first was a direct visit by the work team to 164 pharmacies in different areas of the Babylon Governorate centre, where we asked them to provide the patient with antibiotics (different types of antibiotics, including rifampicin) directly, without a prescription. The second was the distribution of a questionnaire that included 20 questions so as to determine the extent of knowledge of the dangers of the excessive use of antibiotics. This questionnaire was distributed to the people who were working in the pharmacies.

An official inquiry was also made to the Pharmacists' Syndicate in Babylon to provide researchers with a distribution plan for pharmacies in the Babylon Governorate, and the answer was that they do not have a map and that it was not possible to know the number of licensed pharmacies in the Hillah centre.

3. RESULTS

In direct visits to 164 pharmacies in the Babylon Governorate centre to request antibiotics without a prescription, the response rate was 100%, as they were obtained without any hesitation from the pharmacist and without requiring a prescription. One pharmacist out of the total number apologized for not providing the type of antibiotic that was asked about, as it is not available in his pharmacy.

By analysing the data from the questionnaires, we came to the conclusion that there is an obvious contradiction between what pharmacists practice and their answers to the items of the questionnaire. The other shocking result is that there are people working in the pharmacies who are not pharmacists. These people sell patients various drugs (including antibiotics) without complying with the national laws that govern drug sales.

Our results indicate that different types of antibiotics are easily dispended in private pharmacies (more than 55%) and are dispensed at lower levels in private clinics (about 20%), while the percentage of dispending in Government hospitals is about 25%. Antibiotics that are commonly used by patients without a prescription are as follows: amoxicillin (96.70%), metronidazole (44.40%), cefixime (31.10%), and cephalexin (27.80%). Other types of antibiotics used without prescription represent only 4.80% of the patients' choice. Results show a poor knowledge and massive misuse of antibiotics among the pharmacies who sell antibiotics and the users of theses antibiotics in the Babylon Governorate.

4. DISCUSSION

The dispensing of antibiotics without prescription is a common practice in the pharmacies of the Babylon Governorate. Community pharmacists tend to dispense antibiotics when these are requested by name, without asking for prescription.

This result is similar to those of previous studies conducted in Middle Eastern countries, thereby indicating that pharmacists appear irresponsible in dispensing antibiotics without a prescription by ignoring rules and regulations in many cases [7,8]. Antibiotics are largely dispensed to patients, even for cases that do not require them.

The lack of a supervisory role for antibiotics by the Government and the health syndicates allows pharmacists to dispense antibiotics without prescription. The increase in the number of pharmacies in the Babylon Governorate may encourage this phenomenon due to the competition among pharmacies for profit. On the other hand, patients prefer to buy antibiotics from the pharmacy so as to save time and the cost of consulting a doctor. Various studies have reported similar results, indicating that time and cost savings were associated with dispensing antibiotics without a prescription [7,9,10]. In fact, patients may spend a long time to see a doctor and remain waiting in long queues at primary healthcare centers or even at private clinics. However, it seems that the cost of consulting a doctor is high, which forces patients to buy antibiotics themselves.

Finally, distributing pharmacies randomly, in an unscientific manner, without knowing whether these areas need pharmacies or not, could be an important factor in this problem. These issues facilitate the increase in antibiotic resistance and, therefore, by addressing these issues one could regulate the process of dispensing antibiotics without prescription.

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Susceptibility to antibiotics and virulence profiling of *Proteus mirabilis* among foodstuff

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Abstract

Proteus mirabilis is a genus of bacteria that can cause foodborne diseases. We collected 100 canned food samples from local supermarkets in Hillah (Iraq), including juice (23%), milk products (22%), beef (20%), fish (14%), milk (12%), and tomato paste (9%) samples. We subsequently characterized some virulence factors of *P. mirabilis* found in these foodstuff, including biofilm formation, protease activity, motility, haemolysis, adhesion, urease production, lipase production, and antibiotic susceptibility. In a total of 32 foodstuff samples of positive culture, *P. mirabilis* was isolated in 15 (46.8%), including 3 juice samples, 4 milk product samples, 2 beef samples, 1 fish sample, 3 milk samples, and 2 tomato paste samples. All isolates exhibited swarming motility (100%) and urease production (100%), while none of the isolates was found to produce haemolysin. The results of the antibiotic susceptibility test revealed a higher resistance against ampicillin (86.6%).

KEYWORDS

Proteus mirabilis, canned foods, milk, virulence factors, antibiotic susceptibility

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1. INTRODUCTION

Food-borne infections are the most prevalent health issue in our country [1]. *Proteus mirabilis* is characterized by several virulence factors that enable it to invade, damage tissues, and evade immunity. The increased wrong use of most antibiotics has been accompanied by the emergence of strains characterized by high resistance, which is increasingly extended to include a wide range of antibiotics [2]. The present study aimed to characterize some virulence factors of *P. mirabilis* isolated from different canned foods collected from the supermarkets in the city of Hillah, Iraq.

2. MATERIALS AND METHODS

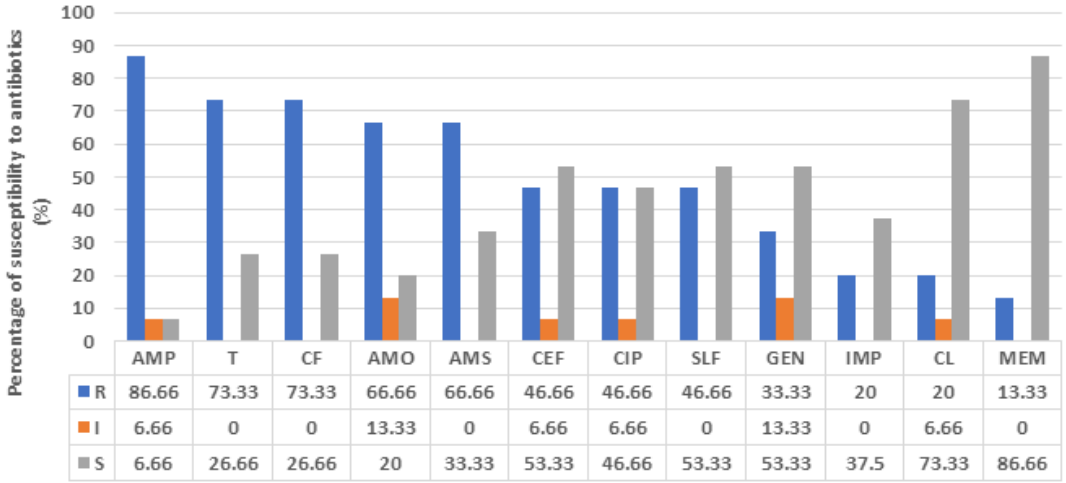
Samples' collection and bacterial isolates: A total of 100 canned food samples from local supermarkets in Hillah (Iraq), including juice (23%), milk products (22%), beef (20%), fish (14%), milk (12%), and to-

mato paste (9%) samples were randomly collected. The isolation and identification of bacterial isolates were carried out by culture and incubation at 37°C for 24 h.

Detection of some virulence factors: *Biofilm formation*: using a tube test, the optical density was measured at a wavelength of 600 nm in test tubes, and the visible line was interpreted as a positive for biofilm potential [2]. *Extracellular protease activity*: skim milk agar containing NaCl was used in order to test the ability of bacteria to produce protease after being incubated at 37°C for 48 h [2]. *Testing of swarming motility*: this test was carried out in Luria-Bertani medium with 0.7% agar, so as to test the swarming motility [3]. *Haemolytic activity*: the presence or absence of haemolysis was determined after the bacteria had been incubated on blood agar for 24 h [3]. *Adhesion of bacteria to epithelial cells*: the result is considered positive when bacteria are attached to the surface of epithelial cells in a single or combined form [4]. *Urease production*: *P. mirabilis* isolates were grown on urea agar medium by stabbing; the tubes were incubated at 37°C for 24–48 h, and the change in the colour from yellow to pink indicated a positive result [4]. *Lipase production*: bacteria cultured on egg yolk agar were inoculated with a bacterial suspension, and were incubated for 24 h at 37°C; the appearance of the decomposition around the colonies was positive [2]. *Antibiotic susceptibility test*: this was carried out by following the Kirby-Bauer method, and the diameter of the inhibition zone was measured [5].

3. RESULTS AND DISCUSSION

Several conventional biochemical tests were performed in order to characterize the suspected *Proteus* isolates, and the results indicated that these isolates were primarily identified as *P. mirabilis*. In a total of 32 foodstuff samples of positive culture, *P. mirabilis* was isolated in 15 (46.8%), including 3 juice samples, 4 milk product samples, 2 beef samples, 1 fish sample, 3 milk samples, and 2 tomato paste samples.



	AMP	T	CF	AMO	AMS	CEF	CIP	SLF	GEN	IMP	CL	MEM
R	86.66	73.33	73.33	66.66	66.66	46.66	46.66	46.66	33.33	20	20	13.33
I	6.66	0	0	13.33	0	6.66	6.66	0	13.33	0	6.66	0
S	6.66	26.66	26.66	20	33.33	53.33	46.66	53.33	53.33	37.5	73.33	86.66

Figure 1. Synopsis of the results of the undertaken antibiotic susceptibility test. Abbreviations used: AMO, amoxicillin / clavulanic acid; AMP, ampicillin; AMS, ampicillin / sulbactam; CEF, cefazolin; CF, cefepime; CIP, ciprofloxacin; CL, chloramphenicol; GEN, gentamicin; I: intermediate susceptibility; IMP, imipenem; MEM, meropenem; R: resistant; S: sensitive; SLF, sulfamethoxazole / trimethoprim; T, tetracycline.

Our results have shown that 9 of the isolates (60%) were able to produce a biofilm by the tube method. Foodborne bacteria develop biofilms in order to survive in a range of unfavourable environments, which are commonly a cause of continuing contamination and outbreaks of foodborne illness. Biofilms enhance the bacterial resistance to environmental stress in the food industry, including the resistance to washing, disinfection, and inhibition, thereby allowing microorganisms to continue to survive on the substrate during the industrial processes [6]. The *P. mirabilis* isolates were unable to produce protease and haemolysin, but they were able to adhere to epithelial cells and generate urease. Moreover, all isolates exhibited swarming motility. The latter is important because

it is coupled with the expression of virulence-associated genes and the ability to invade cells [7]. All 15 of the *P. mirabilis* isolates' strains were able to generate urease; an enzyme that hydrolyses urea so as to produce molecules of ammonia and carbonate, which automatically break down to produce another molecule of ammonia and carbonic acid. This raises the pH and increases the base of the urine, thereby resulting in the formation of stones. This finding was in agreement with those of previous studies [3,7,8]. The results of the undertaken antibiotic susceptibility test are presented in Figure 1. It is believed that the spreading of multi-drug resistance to antibiotics is related to the misuse of antibiotics in humans and animals, especially in countries where it is easy to purchase drugs without the need of pharmacies, as is the case in most developing countries.

4. CONCLUSION

It was found that the *P. mirabilis* isolated from canned food is characterized a number of virulence factors that increase the bacterium's ability to resist the conditions used in canning, in addition to its multi-drug resistance.

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The impact of human albumin on the activity of some anti-staphylococcal agents in an *in vitro* pharmacokinetics / pharmacodynamics model

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Abstract

The emergence of anti-staphylococcal drug resistance has significantly increased, thereby making it difficult to control the life-threatening staphylococcal infections. A validated two-compartment *in vitro* pharmacokinetics / pharmacodynamics (PK/PD) model has been used in order to estimate the efficacies of some anti-staphylococcal drugs - namely, vancomycin (maximum concentration or C_{max} of 3 and 5 mg/L), teicoplanin (C_{max} of 5 and 10 mg/L), and minocycline (C_{max} of 2 and 4 mg/L) – against a mixed staphylococcal infection (*S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228), with or without human albumin (2%). The PK profile for each drug was simulated as time-concentration depending on the drug's half-life. The minimum inhibitory concentration (MIC), the relative optical density for bacterial growth, and the exposure / effect relationship ($fAUC_{0-24}$/MIC) have also been assessed in this study. Our results revealed that minocycline has the best efficacy over other antibiotics against the assessed isolates (single or mixed). Moreover, the addition of albumin exhibited a negative effect on vancomycin and a positive effect on teicoplanin in both the single and the mixed infections. In conclusion, albumin drew a different antibiotic scenario in response to different pathogens.

KEYWORDS

anti-staphylococcal drugs, mixed staphylococcal infection, *in vitro* model, pharmacokinetics, pharmacodynamics

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1. INTRODUCTION

Antibiotic resistance is one of the big challenges in treating severe infections such as the mixed staphylococcal infections (MSIs), soft tissue infections, community-acquired and nosocomial pneumonia, and others [1]. *Staphylococci* are known gram positive pathogenic bacteria that play a crucial role in hospital-acquired infections and, especially, in immunocompromised patients, thereby exhibiting an increasing morbidity and mortality rate worldwide

[2]. On the other hand, trials to overcome these challenges are still running in parallel with the development of new drugs, with the aim to develop single or combined regimens of high efficacy agents characterized by low toxicity. Antibiotics can be classified according to their mechanism of action into those that act through the inhibition of the bacterial cell wall synthesis, the inhibition of nucleic acid replication and transcription, and the interference with bacterial protein synthesis [3]. An *in vitro* study of the efficacy of anti-staphylococcal drugs against MSIs through the use of a pharmacokinetics / pharmacodynamics (PK/PD) model involves the studying of the relationship between the concentration of a drug (PK) and its effect on the organisms (PD) [4]. This modelling helps us optimize the dosing regimens by predicting the drug's efficacy and toxicity.

2. MATERIALS AND METHODS

Bacterial strains and media: Both bacterial strains (*S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228) used in this study were grown in trypticase soy broth medium at 37°C. The simulated time-concentration profiles were performed according to the Clinical and Laboratory Standards Institute (CLSI), the minimum inhibitory concentrations (MICs) were 0.06–4 and 0.03–4 mg/L, respectively. Inoculum suspensions were prepared in normal sterile saline from 24-h cultures, and were adjusted to a final inoculum of 2×10^4 colony-forming units (CFU)/mL in the *in vitro* model by using a counting chamber.

Antibiotics used: Vancomycin (Vancolym; 1 g; Lyka, India), teicoplanin (Targocid; 200 mg / 3 mL; Sanofi, Italy), and minocycline (VULGA XR; 105 mg; Hikma Pharmaceuticals, Jordan) were used in our experiments. The medium used in the *in vitro* PK/PD model was trypticase soy broth (Bacto; dehydrated, 500 g; Fisher Scientific, UK), in which 2% albumin was added (Kedrion Biopharma, Italy).

In vitro PK/PD model: A two-compartment PK/PD model consists of a 500-mL beaker glass containing fresh trypticase soy broth to an initial volume of 5 mL as well as floating tubes with dialytic membranes (20-kDa) for each isolate of *Staphylococcus* alone or for mixtures of the latter under different antibiotic dosing regimens. The central one is connected to a peristaltic pump (MINIPULS Evolution; Gilson Inc., Middleton, WI, USA), thereby adding fresh broth in order to dilute its content at a rate equal to the clearance of antibiotics in the human plasma (Figure 1N) [4,5].

In vitro PK: The simulated in the *in vitro* PK/PD model targeting free (unbound) maximum plasma concentrations (fC_{max}) of vancomycin (3 and 5 mg/L), teicoplanin (5 and 10 mg/L), and minocycline (2 and 4 mg/L) and the half-life ($t_{1/2}$) of 12 to 24 h were evaluated in order to better describe the exposure / effect relationship. The simulated time-concentration profiles were chosen so as to simulate the different 24-h drug exposures observed.

In vitro PD: To estimate the bacterial growth inside the floating dialytic tubes for each antibiotic dosing regimen, 200-µL samples were collected at regular intervals up to 24 h, and bacterial growth was assessed spectrophotometrically by measuring the relative optical density (ROD) at 600 nm at each dilution. The ROD_{600} for each drug concentration at a specific time point, in relation to the control growth at the same time point as well as over time, was plotted.

In vitro PK/PD analysis: The PK/PD index as the $fAUC_{0\text{-}24}$/MIC ratio was calculated for each simulated dose and isolate during the experiment. The drug exposure / response relationship, expressed as 24-h growth reduction for each dosing regimen and isolate and compared with values at the start of therapy, was analysed with a non-linear regression analysis using a sigmoidal model with variable slope. All data were analysed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. RESULTS

As shown in Figure 1, depending on MIC, ROD, and $fAUC_{0\text{-}24}$/MIC, in the *S. aureus* isolate without the presence of albumin, minocycline had the highest anti-staphylococcal efficacy, followed by vancomycin and teicoplanin, while with the addition of albumin the efficacy of teicoplanin was enhanced, followed by minocycline and vancomycin. On other hand, in the *S. epidermidis* isolate without the presence of albumin, minocycline and teicoplanin had nearly the same higher activity with non-significant difference from vancomycin in both C_{max} values, while with the addition of albumin, the efficacies of teicoplanin and of vancomycin have been enhanced, with the lowest effect being that on vancomycin. As far as the mixed staphylococcal infection is concerned, minocycline exhibited the best anti-staphylococcal efficacy with or without albumin, followed by teicoplanin (that exhibited significant enhancement of its efficacy by albumin) and vancomycin (with little effect in response to the presence albumin). However, the $AUC_{0\text{-}24}$/MIC ratio (as a PK/PD index with or without the addition of albumin) gave the highest value in the case of teicoplanin, followed by vancomycin and minocycline. These PK/PD indices were displayed by a sigmoid curve.

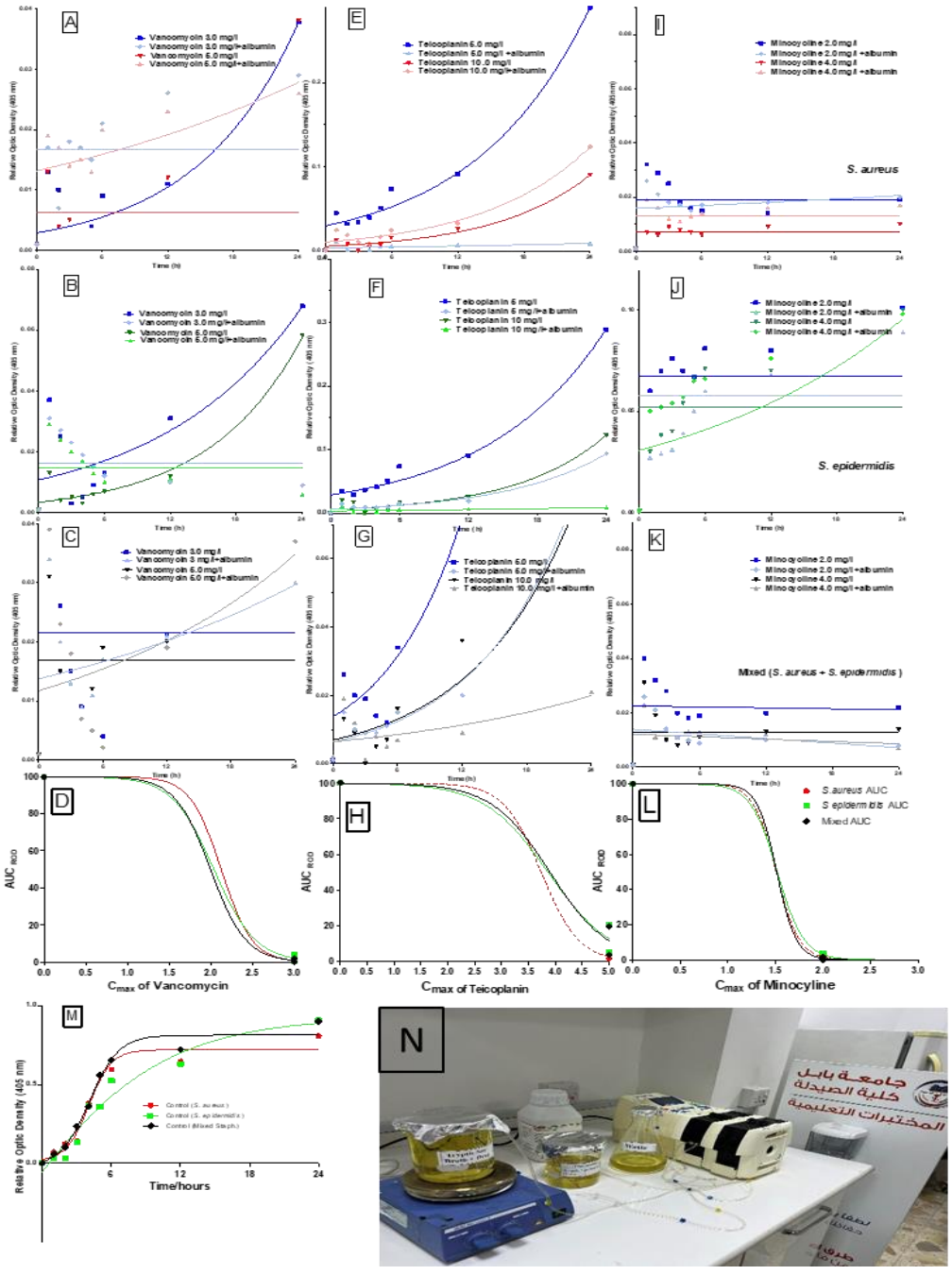

Figure 1. Data obtained from our *in vitro* pharmacokinetics / pharmacodynamics (PK/PD) model. Simulated human dosing with 3.0 and 5.0 mg/L of vancomycin (A-C), with 5.0 and 10.0 mg/L of teicoplanin (E-G), and with 2.0 and 4.0 mg/L of minocycline (I-K) was employed against two isolates of *Staphylococci* alone or combined (*S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228) with or without 2% albumin in each dose. Panels D, H, and L present the single-dose exposure-efficacy relationship of vancomycin, teicoplanin, and minocycline, respectively, against each isolate alone or combined (mixed infection) with a Clinical and Laboratory Standards Institute (CLSI) mode in the *in vitro* PK/PD model. Finally, panel M presents the growth indices in the control isolates (alone and as a mixed infection), while panel N depicts the herein employed *in vitro* PK/PD model set-up.

4. DISCUSSION

S. aureus and *S. epidermidis* are considered two of the most important pathogens in hospital infections that can simulate some cases in clinical practice and allow us to explore the efficacy of some anti-staphylococcal agents against specific isolates or mixed infections by using an *in vitro* model with or without the addition of human albumin [6]. Human albumin has an important role as an antibiotic-binding protein that can directly and indirectly affect the immune and inflammatory status, in addition to the duration of action and, ultimately, the antibiotic efficacy. At the same time, it can decrease the availability of the free / active drug. Moreover, hypoalbuminemia has been associated with the acquisition and severity of viral, bacterial, and fungal infections, and can predict infectious complications in non-infective diseases. Systemic inflammation in severe infection is known to alter the function and kinetics of albumin, which in turn can increase the risk of a worse clinical outcome [7]. Therefore, human albumin has been added in this study in order to assess the antibiotic activity; the latter can be summarized as minocycline having the best efficacy over the other antibiotics against the assessed isolates. Moreover, the addition of albumin revealed a negative effect on vancomycin and a positive effect on teicoplanin in both the single and the mixed infection conditions [8,9]. In conclusion, our study confirms that albumin can draw a different antibiotic scenario in response to different pathogens [6,10].

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Drug synthesis, separation, and identification of a di-stereoisomeric mixture of spiro-oxalidinonic derivatives of sorbinil by high-performance liquid chromatography

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Abstract

A straightforward and sensitive method for high-performance liquid chromatography (HPLC) was created in order to separate and identify the di-stereoisomeric mixture of a spiro-oxalidinonic derivative of sorbinil. HPLC was carried out using a C18 reversed-phase column. The mobile phase used in the isocratic elution process was a 70:30 v/v combination of methanol and acetonitrile flowed at a rate of 2 mL/min. The procedure delivered the best result among the various attempts made. Two peaks were identified on the chromatogram, attributable to the two di-stereoisomers of the mixture analysed. Analogues of sorbinil, a powerful inhibitor of aldose reductase, have been synthesized and further tested on aldose reductase as mixtures of di-stereoisomers, exhibiting an IC_{50} in the order of the micromolar. The structure of the compounds was checked with nuclear magnetic resonance spectroscopy. The chemical shifts were expressed in ppm scale δ.

KEYWORDS
di-stereoisomeric mixture, HPLC, sorbinil; synthesis, drug

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1. INTRODUCTION

Diabetes mellitus is a heterogeneous syndrome characterized by inadequate insulin synthesis or/and insulin resistance, in which there is an increase of the glucose concentration in the blood and chronic alterations in the carbohydrate, fat, and protein metabolism. As a result, long-term health effects such as the development of retinopathy, nephropathy, neuropathy, and circulatory system disorders can be observed [1]. Therapy

mainly includes the administration of insulin, but this type of treatment fails to prevent diabetes' complications, where high quantities of cellular glucose can be metabolized by aldose reductase (ALR2) to sorbitol [2,3]. The regulation of ALR2 is attributed to reactive oxygen species and nitric oxide (NO). The high sensitivity of the enzyme to these two species is caused by the existence of a residue of high-reactive cysteine (Cys) in its structure [4]. Oxidants like H_2O_2 can inactivate the enzyme as well as cause the glutathiolation of Cys-298. However, by changing the reaction conditions and the NO donor, ALR2 can be *S*-thiolated and, therefore, inactivated or *S*-nitrosated and activated [5]. Based on these observations, it has been believed that NO plays a regulatory role in the intracellular activity of ALR2 and, consequently, in glucose fluxing *via* the polyol pathway [6]. Given that in a hyperglycaemic state, NO release is markedly reduced compared to normoglycemia conditions, it has been proposed that the ALR2 function may be upregulated in the tissues of diabetic people.

Sorbitol cannot easily cross the plasma membrane and is gradually metabolized to fruit sugar (fructose); consequently, it tends to form high concentrations. Sorbitol causes a series of events that lead to irreversible morphological and functional changes. Therefore ALR2 inhibitors (ARIs) could be useful in blocking this metabolic pathway and preventing tissue damage. Since ARIs exert no effect on the plasma levels of glucose (as they do not cause hypoglycaemia), these drugs could be considered co-adjuvants in diabetes therapy [7]. Known ARIs are subdivided into two main types: (i) derivatives of carboxylic acid (zopolrestat, tolrestat, and epalrestat), and (ii) cyclic imides (sorbinil) (Figure 1A). Despite their structural differences, both classes of compounds possess common features: an aromatic planar portion and an acid function able to interact with the enzyme's active site.

Sorbinil is a well-known ARI; it is a spirohydantoin with limited therapeutic value because of its adverse effects, which are caused by the idantoinic group. Numerous analogous, with the idantoinic function substituted with bioisosteres such as thiazolidinedione, oxazolidinone, and succinimide group, have been developed [8]. In these molecules, the chromanonic moiety of sorbinil is substituted in position 2 with different aromatic groups. The same chromanonic moiety was also substituted in position 4 with a 5- or 6-terms spiro-like group linked to an acid function; typical of some ARIs [9]. Some of these compounds had shown a micromolar IC_{50}, but were tested as distereoisomeric mixtures. In an attempt to elucidate how this substitution in positions 2 and 4 of the chromanonic system influences the inhibitory activity of these compounds, it was necessary to determine an analytical method allowing the separation and, therefore, the evaluation of the components of the di-stereoisomeric mixture. We have, herein, carried out the preparation of two spiro-oxalidinonic derivatives, which have shown an IC_{50} of 1.60 and 6.30 μM, respectively, in biological tests.

2. MATERIALS AND METHODS

A solution of commercial 5'-bromo-2'-hydroxyacetophenone (7.90 g; 36.72 mmol) in CH_2CN (10 mL) was agitated at reflux for three days with the appropriate 4-substituted acetophenone (36.72 mmol) and pyrrolidine (0.78g; 11.61 mmol). While spending time to evaporate the solvent, AcOEt and NaOH/HCl removed the deposit. AcOEt was dried, filtered, and evaporated for unrefined oil. Oil was purified by column chromatography with a 95:5 hexane/AcOEt eluent combination. In the sequence of trimethylsilyl cyanide (290 mg; 2.92 mmol) and ZnI_2 (86.7 mg; 0.27 mmol), trimethylsilyl cyanide was added to a solution of appropriate chromanone (1.95 mmol) at ambient temperature, and the mixture was stirred for 24 h. Drying, filtering, and evaporating an organic phase yielded the compound after cleaning with water.

3. RESULTS AND DISCUSSION

In order to obtain maximum benefits from ALR2 inhibition, ARIs need to be highly specific and selective. Despite their strong affinity for the enzyme, the inhibitors that are currently on the market have a high level of nonspecific toxicity. Further studies must be conducted on ARIs so as to determine the extent and duration of the inhibition of the enzyme for a given dose and, more importantly, to study new ARIs that selectively reduce the enzymatic capacity so as to enhance the reduction of glutathione-conjugates (important for the transmission of intracellular signals) and glucose, without decreasing their detoxifying capacity towards aldehydes. The enzyme may have separate binding sites for glutathione and aldehydes, according to certain structure-activity studies using free aldehydes and their glutathione-conjugated analogues. Therefore, without compromising the enzyme's ability to detoxify aldehydes, specific alterations of the enzyme can stop it from recognizing and reducing glutathione-conjugates. These interesting findings suggest the likelihood of independently regulating the detoxifying role and the ability to transmit intracellular signals of ALR2.

By developing this important aspect, even more, selective inhibitors might be intended in the future, which could prevent cellular damage linked to diabetic complications without compromising the antioxidant defence. Numerous studies have established that ALR2 facilitates the aldehydes' reduction generated from lipids and their conjugates with glutathione, and protects against oxidative stress; therefore, a therapy based on the combination of ARIs and antioxidants could be effective. The beneficial and detoxifying effects of the enzyme decrease with the increase in the products of lipid peroxidation, and this can regulate the onset of long-term diabetic complications. The concomitant administration of ARIs and antioxidants, which can prevent peroxidation of lipids or enhance the antioxidant enzymes' expression, could recompense the reduced detoxifying action of ALR2. Antioxidant agents can also maintain the reduced enzyme form. Hence, one could avoid the development of typical ALR2 resistance towards drugs during hyperglycaemia, following its structural modifications [10].

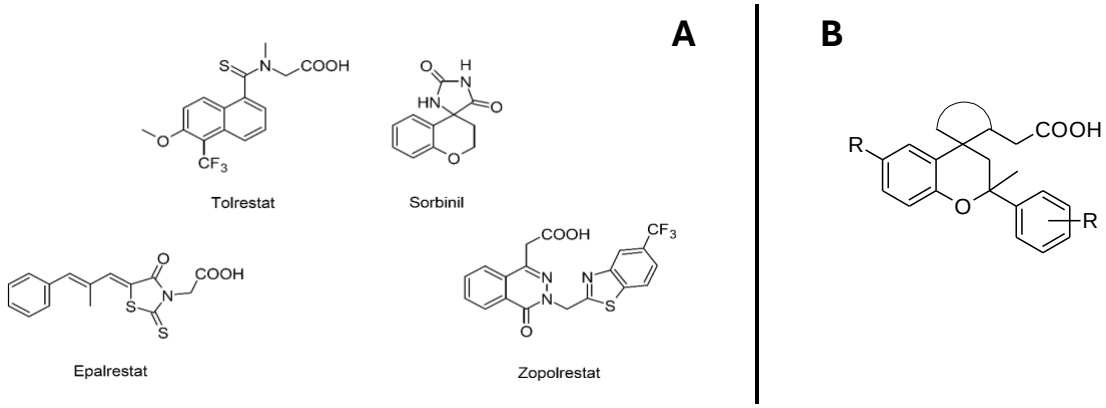

Figure 1. (**A**): Aldose reductase inhibitors: major carboxylic acid derivatives and cyclic imides. (**B**): Fusion of the classes of imides (sorbinil) and of the derivatives of acetic acid (tolrestat): possible resulting compounds

4. CONCLUSION

The chromatogram displays two peaks that can be attributed to the two di-stereoisomers of the spiro-oxalidinonic derivative analysed. The separation and evaluation of the components of the di-stereoisomeric mixture is important as it allows us to elucidate, in further studies, how the compounds' ability to inhibit ALR2 is affected by substitutions in positions 2 and 4 of the chromanonic system (Figure 1B).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Antibacterial activity of the alcoholic extract of berberine against *Staphylococcus aureus* isolated from burn and wound infections

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Abstract
Burns and wounds destroy the physical skin which is the body's natural barrier to the external environment. As a result the burned area is prone to infection and colonization by microorganisms. The most common pathogenic colonizing bacterium is *Staphylococcus aureus*. In order to solve the problem of bacterial resistance to antibiotics, some medicinal plants have been used so as to determine their effectiveness against *S. aureus*. Among these plants, we have herein used the alcoholic extract of berberine. Our results suggest that the alcoholic extract of berberine, at a concentration 250 mg/mL, can exert a high inhibitory efficacy against *S. aureus*.

KEYWORDS
Staphylococcus aureus, burns, wounds, berberine, infection

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1. INTRODUCTION

The incidence of burn wound infections appears to be correlated with both the depth and the size of wound. Moreover, the longer the wound remains open, the higher the chances on infection. Previous studies have shown that there are several bacterial species which are able to readily infect burn wounds. Among these, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been found to be the most common species. Interestingly, the variation in bacterial flora and the colonization rate changes over time after the initial infection [1].

Medicinal herbs could be the best source for the development of various medicines [2]. Due to their different therapeutic properties, medicinal herbs have been considered by many researchers worldwide [3]. In modern medicine, several studies have been conducted in order to identify the potential effects of various extracts of medicinal herbs that

have a pivotal role in the health of people and animals [4]. Berberine is an isoquinoline quaternary alkaloid (a 5,6-dihydrodibenzo(a,g)quinolizinium derivative) employed in traditional Chinese and Indian medicine for centuries. It has anti-inflammatory and antimicrobial properties, anti-diabetic and antioxidant effects, and multiple other pharmacological properties [5].

2. PATIENTS AND METHODS

All samples of the present study were collected after obtaining ethical clearance from the Ethics Committees of the Babylon Health Office and the agreement of the medical management of the Imam Al-Sadiq Hospital Specialized Burn Center in the Medical City of Baghdad and of Kufa. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and it was carried out with the patients' consent. The study's protocol as well as the subject information and consent form were reviewed and approved by a local ethics committee according to the document number M220109 (17-1-2022).

Different concentrations of the alcoholic extract of berberine were prepared by using the serial dilution method. Experiments were carried out by dissolving 1 g of berberine extract in 2 mL of 70% ethanol in four dilutions (500, 250, 125, and 62.5 mg/mL). Subsequently, 100 µL of test isolates were spread onto the surface of Mueller-Hinton agar plates by using a glass spreader, and the wells were filled with 50 µL of the berberine alcoholic extract. The test plates were incubated at 37°C for 24 h, while 70% ethanol was used as a negative control. The antibacterial activity of the alcoholic plant extracts was evaluated by measuring the zone of inhibition in mm [6].

3. RESULTS

Our study population included 230 participants with burn wound infections admitted to a burns' unit in the Imam Al-Sadiq Hospital, the Al-Hilla Teaching Hospital, and the Specialized Burn Center in the Medical City of Baghdad and of Kufa from February to September 2022. Burn wound swabs were taken from all patients registered in the study and were immersed in Stuart transport medium. Swabs were collected from infected wounds following cleansing of any remnant ointment. After collection, all swabs were inoculated on nutrient agar, blood agar, and manitol salt agar, and were incubated at 37ºC for 24 h prior to their morphological examination. The colonial morphology, the production of β-hemolysis on blood agar, and the production of pigmentation on the manitol salt agar revealed 63 isolates of *Staphylococcus* that were confirmed to be *S. aureus* by the Vitek 2 Advanced Expert System (bioMerieux, Marcy l'Etoile, France). In the well diffusion method, the alcoholic extract of berberine exhibited inhibitory effects on *S. aureus* isolates in different concentrations. It was found that out of 63 isolates, 15 isolates exposed to a berberine concentration of 250 mg/mL revealed the highest inhibition effectiveness for the alcoholic extract (Table 1).

Table 1. Antimicrobial activity of different concentrations of the alcoholic extract of berberine on *Staphylococcus aureus*.

Concentration of the alcoholic extract of berberine (mg/mL)	**N**	**Zone of inhibition** (mm; mean ± SD)	***P*-value**
62.5	15	1.000 ± 2.803	0.495
125	15	22.930 ± 2.120	0.181
250	15	31.600 ± 2.501	0.064
500	15	22.600 ± 2.414	0.180

4. DISCUSSION

Various clinical applications of berberine have been discovered, especially related to its antibacterial activity. Berberine, with a quaternary nitrogen, polycyclic, and planar system, could helpfully increase membrane permeability and strengthen the binding affinities with amino acids in biomolecules [7]. Previous studies have evaluated the antibacterial effects of berberine against clinical isolates of *S. aureus* [8], and have shown that berberine was effective in inhibiting the growth of *S. aureus* biofilms, which are communities of bacteria that can be particularly resistant to antibiotics [9]. The exact mechanism by which berberine exerts its antimicrobial effects on *S. aureus* is not fully understood, but several mechanisms have been proposed. In summary, it is believed that berberine can disrupt the bacterial cell membrane, interfere with DNA replication and protein synthesis, and inhibit enzymes necessary for bacterial survival [10].

In conclusion, our results suggest that the al-

coholic extract of berberine, at a concentration 250 mg/mL, can exert a high inhibitory efficacy against *S. aureus*.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Real-world data on the effectiveness of the meloxicam and pridinol combination for musculoskeletal pain

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Abstract

Musculoskeletal pain includes several types of discomfort associated with the skeletal system. Pharmaceutically, pridinol was developed in order to relax muscles. No empirical data exist to support the effectiveness of using meloxicam in combination therapy for the treatment of musculoskeletal pain. This study compared pridinol and meloxicam for musculoskeletal pain. The current observational study assessed a total of 82 patients. The study's participants were divided into three groups: the "meloxicam" group, the "pridinol" group, and the "meloxicam + pridinol" group. Pain levels were measured before and four weeks after giving the drug, by using a visual analogue scale (VAS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). We employed a Kruskal-Wallis test in order to evaluate the variations in pain measurement among the groups. The three groups' VAS and WOMAC scores did not differ before the drug administration. The "meloxicam + pridinol" treatment resulted in significant pain relief based on VAS and WOMAC scores at 1, 2, and 4 weeks (as compared to other groups; $p<0.05$). At 4 weeks, the VAS and WOMAC ratings exhibited no significant pain relief in the "meloxicam" group when compared to the "pridinol" group. The meloxicam-pridinol combination proved efficacious for musculoskeletal pain, and is recommended for its therapy.

KEYWORDS
muscle cramps, spasm, meloxicam, pridinol, combination therapy

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1. INTRODUCTION

The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience related to tissue damage or described as such [1]. Chronic musculoskeletal pain (CMP) is pain in bones, joints, and tissues that lasts more than three months. CMP affects a large portion of Western adults, with prevalence rates reaching up to 20% [2], and projections showing that it will rise by more than 50% by 2050 [3]. CMP includes many diseases, including osteoarthritis, discogenic back pain, spinal pain, fi-

bromyalgia, and chronic widespread pain.

Skeletal muscle relaxants treat central muscle spasms (like those after a stroke) and peripheral musculoskeletal spasms (like those associated with low back pain) [4]. Anticholinergic pridinol, a central nervous system muscle relaxant, weakens polysynaptic reflexes [5]. This molecule has long been used to relax skeletal muscles, and it is available as a standalone therapeutic drug in Germany and Italy. In January 2016, Strathmann's Hamburg-made "Myoson direct" tablets were pulled from the market. This decision was regulatory-compliant. The current study shows that Germany reauthorized pridinol-containing tablets in December 2017. Strathmann in Hamburg, Germany, manufactures "Myopridin" (3-mg tablets) to treat central and peripheral muscle spasms, torticollis, lumbago, and general muscle discomfort in adults. The user's text doesn't need rewriting [5]. Moreover, UK, Poland, and Spain approved pridinol tablets in 2020, using "Myopridin" as the reference product [5]. In Germany, pridinol is one of two muscle relaxants approved for peripheral muscle spasms linked to low back pain. Since re-approval, its prescriptions have increased: Germany prescribed 5.5 million daily defined doses in 2020; up 96.4% when compared to 2019 [6].

High doses of muscle relaxants and nonsteroidal anti-inflammatory drugs (NSAIDs) are needed for optimal efficacy. The European Medicines Agency (EMA) Committee on Medicinal Products for Human Use recommends administering selective and non-selective NSAIDs at the lowest effective doses, and for the shortest time needed to relieve disease symptoms. This strategy aims to lower cardiovascular disease risk [7]. Thus, choosing the right analgesic and muscle relaxant combination reduces the time needed for the analgesic and anti-inflammatory treatment to be effective [8]. One option is to give 500 mg of chlorzoxazone and 400 mg of ibuprofen. Indian authorities have approved this combination for short-term musculoskeletal pain treatment in 2010, as it reduces musculoskeletal disorder pain and spasms synergistically. Combining a skeletal muscle relaxant with an NSAID or paracetamol relieves pain better than giving the analgesic alone [9,10]. A clinical trial examined how well meloxicam and baclofen worked together and how well they were tolerated by 50 patients with a worsening CMP syndrome. The study found that by combining these medicines one can improve therapeutic outcomes and reduce pain intensity by over 50% in the first week. Moreover, meloxicam for chronic muscle and joint pain was found to be safe and effective [10].

This study examined the benefits of combining meloxicam and pridinol into one dose. In order to treat musculoskeletal disorders, a strong analgesic effect was accelerated this way, while in order to reduce nonsteroidal anti-inflammatory drug side-effects, the former is crucial. Recently named as "the best spasticity treatment", pridinol works centrally.

2. PATIENTS AND METHODS

The study protocol of this investigation has been approved by the ethical committee of our institution prior to study's start. Informed consent was diligently collected from each participant. The study sample consisted of individuals who were selected from a pool of outpatients who sought medical care at an orthopaedic clinic within the community. A total of 82 patients were chosen from a total of 120 individuals who had experienced various forms of musculoskeletal pain. These patients were selected based on the following inclusion criteria: (i) the existence of knee pain lasting for a duration exceeding one month, and (ii) the observation of muscle spasms during the testing process. The exclusion criteria included a documented medical history of knee pain, Parkinson disease, infection, or rheumatoid arthritis. The participants in the study were asked to complete a questionnaire that collected information on sociodemographic parameters (such as age and sex) as well as on the duration of their pain.

All participants underwent a visual analogue scale (VAS) assessment to evaluate pain during movement at various time points (before treatment as well as 1, 2, and 4 weeks after treatment initiation). Additionally, they completed the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire before the study and at the 4-week mark. Prior to the study, participants also underwent a painDETECT screening. The study also recorded every adverse event, including its level (mild, moderate, or severe) and the investigator's assessment of its relationship to each medicine. Patients received no antiemetics.

Data were analysed using statistical tests so as to compare pain values between the three classified groups. Specifically, a Kruskal-Wallis test was employed for this purpose. Additionally, a one-way ANOVA with *post hoc* comparisons was conducted in order to examine the relationship between age, symptom duration, and follow-up. Furthermore, a Fisher's test was utilised so as to assess the association between dichotomous or categorical variables. A significance level of $p<0.05$ was deemed to indicate statistical significance.

3. RESULTS

Our study included 82 patients (59 women and 23 men). Participants had an average age of 54.0 ± 4.0 years. The mean symptom duration was 23.7 ± 4.0 months. Osteoarthritis caused knee pain for at least a month. The VAS and WOMAC pain levels were found to be similar between groups (p>0.05). Table 1 presents the painDETECT score before and during the medication administration, indicating neuropathic pain likelihood as follows: "likely" (≥19), "possibly" (≥13 to ≤18), and "unlikely" (≤12). The study found 8 (9.8%) cases of "likely" neuropathic pain, while 57 participants (69.5% of the cohort) did not suspect any neuropathic pain, and 17 (20.7%) did. The three groups exhibited similar neuropathic pain scores (p>0.05). All three groups' pain scores improved during medication when compared to those before the treatment. The "meloxicam + pridinol" combination achieved significant pain reduction (VAS score at 1, 2, and 4 weeks; WOMAC score at 4 weeks) when compared to "meloxicam" or "pridinol" alone (p<0.05; Table 1).

Table 1. Overview of the assessment of the painDETECT score without and during medication. The painDETECT score had a distribution ranging from 0 to 38. The participants were categorised into three distinct categories based on their likelihood of experiencing neuropathic pain: "highly likely" (score of 19 or above), "somewhat likely" (scoring between 13 and 18), and "unlikely" (score of 12 or lower). Note: unless otherwise stated, values represent a mean ± SEM. Abbreviations used: VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Assessment of painDETECT score without medication					
Score	**Number of patients (%)** (n=82)	**Meloxicam (%)** (n=28)	**Pridinol (%)** (n=25)	**Meloxicam + Pridinol (%)** (n=29)	***p*-value**
0-12	57 (69.5%)	20 (71.4%)	17 (68%)	20 (69%)	0.52
13-18	17 (20.7%)	4 (14.3%)	5 (20%)	8 (27.6%)	0.44
19-38	8 (9.8%)	4 (14.3%)	3 (12%)	1 (3.4%)	0.61
Assessment of painDETECT score while receiving medication					
Pain score; VAS		**Meloxicam**	**Pridinol**	**Meloxicam + Pridinol**	***p*-value**
1 week		4.4 ± 2.1	4.2 ± 2.0	3.2 ± 2.0	0.024
2 weeks		3.7 ± 2.0	3.4 ± 1.9	2.4 ± 1.4	0.042
4 weeks		2.3 ± 1.5	2.1 ± 1.2	1.0 ± 1.1	0.02
WOMAC score (4 weeks)		**Meloxicam**	**Pridinol**	**Meloxicam + Pridinol**	***p*-value**
Pain		5.6 ± 2.2	5.8 ± 2.1	2.9 ± 1.5	0.41
Stiffness		4.6 ± 2.3	4.5 ± 2.1	4.6 ± 2.3	0.03
Physical function		32.0 ± 10.0	30.0 ± 11.0	19.2 ± 7.5	0.02
Total		42.2 ± 10.1	40.3 ± 10.5	26.7 ± 7.1	0.02

4. DISCUSSION

This study is a preliminary observational study aiming to evaluate the nonbenzodiazepine antispasmodic pridinol and the NSAID meloxicam in adults with acute muscle pain and osteoarthritis. Musculoskeletal pain can be relieved by combining skeletal muscle relaxants with NSAIDs or paracetamol. In this study, meloxicam and pridinol had significant analgesic effects at 1, 2, and 4 weeks, and increased the WOMAC score after 4 weeks.

Pridinol's efficacy and tolerability are examined using de-identified German Pain e-Registry data. The largest non-interventional pridinol study in the world involves 1,133 patients with acute musculoskeletal pain. Despite receiving mostly NSAIDs and non-opioid analgesics, these patients reported moderate-to-severe pain intensity and significant pain limitations in various life activities. In this group of patients, pridinol for 4 to 64 days was well received and improved pain intensity, pain-related impairments, and overall wellbeing in most cases. Only 6.4% of pridinol-receiving patients reported global treatment failures, which can be attributed to insufficient analgesic response or drug-related adverse events. A remarkable 58.8% of patients had a complete global response without drug-related adverse events, while 34.9% had a partial global response. Our study has found that the patients' real-world efficacy contradicts current antispasmodic guidelines.

5. CONCLUSION

Acute muscular pain often resolves without treatment, but many patients may need temporary medication to reduce pain and physical limitations. Current guidelines recommend starting with NSAIDs; however, their efficacy in noninflammatory muscular pain is limited, and their use is associated with serious side-effects. Our study has found that by combining meloxicam with pridinol, one can treat musculoskeletal pain well.

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The authors declare no conflicts of interest.

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Evaluation of serum cystatin C levels in multiple myeloma: diagnostic significance and clinical implications

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Abstract

Cystatin C (CysC) levels in patients with multiple myeloma (MM) have been linked to tumour load and outcomes' prediction. This study aimed at assessing the diagnostic utility of CysC in distinguishing MM patients from controls and advanced stages of MM. In total, 98 MM patients and 57 healthy controls participated in this cross-sectional case-control study. Demographic, clinical, and biochemical data were assessed. The study groups exhibited significantly diverse measures of urea, creatinine, CysC, β_2-microglobulin, and lactate dehydrogenase activity. β_2-Microglobulin was found to be a reliable predictor for both the MM staging and its diagnosis, but CysC was found to only possess a partial capacity of predicting advanced MM stages. Our results highlight the significance of taking into account many biomarkers in the therapy of MM, so as to achieve effective clinical evaluation. More research is required in order to clarify the CysC implication in the prognosis and management of MM.

KEYWORDS
multiple myeloma, LDH, β_2-microglobulin, staging, cystatin C

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1. INTRODUCTION

Multiple myeloma (MM) is characterized by a clonal plasma cell proliferation and is frequently complicated by renal insufficiency, which predicts an unfavourable prognosis [1]. Serum cystatin C (CystC) is considered as an accurate marker of the glomerular filtration rate and renal impairment [2]. Serum CystC levels, especially in patients with International Staging System "stage II" MM, have been linked to tumour load in MM and are predictive of patient outcomes [3], and tumour burden [4]. Nonetheless, there is still much to learn about the prognostic value of CystC in MM. This study aimed at assessing the CystC diagnostic utility in distinguishing MM patients from controls, and at comparing its levels between MM patients of different tumour stages.

2. PATIENTS AND METHODS

There were 155 participants in this case-control study, which was carried out in 2022 at the Babylon Main Hospital's Haematology Department. Ninety-eight patients with MM (49 men and 49 women) were chosen, and 57 healthy people served as the controls. Specific criteria, such as the presence of monoclonal proteins in the serum or the urine, osteolytic lesions in the bone, cytological examination of the bone marrow aspirate revealing at least 10% plasmacytes, and additional diagnostic tests (like complete blood counts, plasma protein electrophoresis, and renal function assessment) were used in order to establish the diagnosis of MM.

Our study included were 40 newly-diagnosed MM patients (15 on stage III and 25 on stage II) and 58 patients (35 on stage III and 23 stage II) undergoing first-line treatment with lenalidomide or bortezomib. Expert haematologists validated the diagnosis of MM by accepted protocols. The patient group was matched with healthy controls.

Patient demographics and medical histories were taken from hospital records and were among the data collected. Biochemical tests were conducted on blood samples at the hospital and at the College labs so as to measure creatinine, urea, β_2-microglobulin (β_2-M), CystC, and lactate dehydrogenase (LDH) levels.

All individuals provided written consent, and ethical approval was obtained. The SPSS and JASP software were used for statistical analysis.

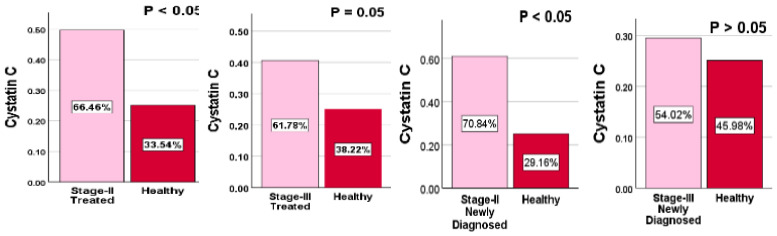

Figure 1-A

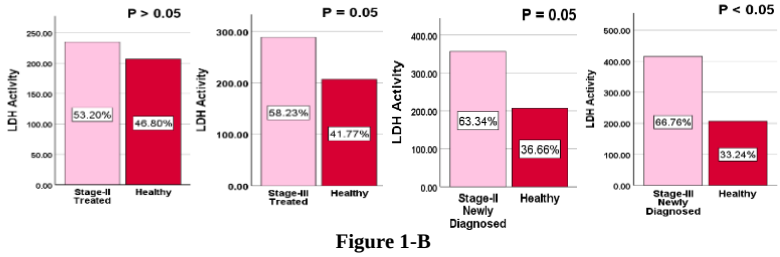

Figure 1-B

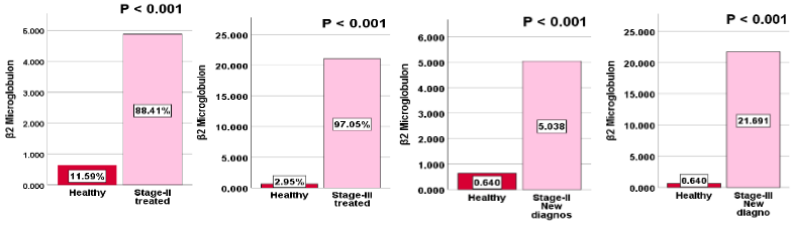

Figure 1-C

Figure 1. Multiple comparisons of the cystatin C levels (**A**), LDH activity (**B**), and β_2-microglobulin levels (**C**) among patients with different stages of multiple myeloma as compared with a healthy group.

3. RESULTS

Our study revealed no significant differences in age, body mass index, and sex distribution between MM patients and healthy controls. However, significant variations were noted in LDH activity,

β_2-M levels, CystC levels, creatinine levels, urea levels, and the prevalence of diabetes and hypertension. Age differences between sexes were significant, with men having a higher mean age. However, there were no significant differences in biomarker levels between men and women.

The β_2-M levels demonstrated excellent predictive ability (AUC=0.859) for differentiating MM patients from healthy controls, followed by LDH activity (AUC=0.817) and CystC levels (AUC=0.662). For differentiating between stage III and stage II MM cases, the β_2-M levels exhibited the perfect predictive ability (AUC=1.000). Moreover, β_2-M levels and LDH activity showed statistically significant variations when assessed as means between groups, thereby suggesting their significant role in discriminating between MM patients and controls. However, CystC levels did not exhibit significant differences in terms of group means (Figure 1).

4. DISCUSSION

The results of our investigation shed light on the potential diagnostic and prognostic value of several biomarkers in patients with MM. We understand more about the difficulties in managing and diagnosing MM by comparing MM patients with healthy controls, and by assessing the effectiveness of biomarkers in detecting different MM stages.

β_2-M confirms its long-standing function as a prognostic marker in the disease by revealing itself to be a reliable predictor in separating MM patients from healthy controls [5]. Meanwhile, CystC has little predictive capacity, indicating its limited utility in the MM analysis. Moreover, β_2-M exhibits effectiveness in differentiating various MM stages, underscoring its significance in predicting the disease progression. Nevertheless, LDH demonstrates a moderate predictive ability in identifying stage III MM, while CystC performs poorly in discriminating between MM stages. However, other researchers have found that the LDH is a biomarker of poor prognosis not only at diagnosis, but also at the first-relapse of MM patients [6].

Elevated CystC levels have been linked to higher levels of creatinine, LDH, and β_2-M according to recent studies [3], which is not different from what this study has found. In MM, higher CystC levels are linked to a considerably shorter progression-free survival and overall survival. Serum CystC serves as sensitive biomarker that successfully separates stage II survival characteristics, although it is not a stand-alone prognostic factor [7].

In MM, LDH emerges as a crucial prognostic factor. Poor prognosis, lower overall survival, progression-free survival, and aggressive disease are associated with elevated LDH activity at diagnosis [8]. LDH activity can be used to track the course of an illness and inform clinical decisions.

In individuals with MM, elevated levels of β_2-M are suggestive of a significant tumour load and are associated with either disease regression or progression after treatment. Elevations of β_2-M levels before therapy are linked to a less favourable outcome, rendering it a significant independent prognostic factor [9]. Similarly, studies have suggested that LDH could be used to monitor disease activity and directly shape clinical decision-making when combined with other indicators such as β_2-M and monoclonal immunoglobulin [10].

The LDH protein is a predictor of a poor outcome for MM patients, both at the onset and during their first episode of relapse [6]. Serum LDH levels have also been shown in several studies to be a helpful clinical measure for monitoring the progression of MM illnesses [8]. Furthermore, although elevated readings of LDH are uncommon at the beginning of MM, they frequently increase dramatically as the illness progresses. Compared to patients with normal LDH levels, those with higher concentrations have a shorter median life expectancy.

Finally, LDH and β_2-M are significant markers for the diagnosis, staging, and prediction of MM. These findings demonstrate how important it is to employ integrative diagnostic research in order to improve our approaches to the management of MM. Moreover, additional studies are required in order to fully appreciate the potential of β_2-M as a therapeutic target for MM, particularly when considering the application of antibody therapy in managing the symptoms of this type of cancer.

5. CONCLUSION

The current study exposes substantial variations in markers between MM patients and healthy subjects. β_2-M was found to be a robust predictor for both tumour staging and diagnosis, thereby contrasting the partial predictive capacity of CystC for later MM stages. This stresses β_2-M's significance as a reliable diagnostic and predictive marker in MM. Furthermore, the study shows links among variables, mainly connecting LDH activity with creatinine and urea levels.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Comparative evaluation of pharmacokinetic parameters between a pure nisoldipine suspension and a nisoldipine-loaded bilosome suspension

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Abstract

Bilosomes are nanocarriers that contain bile salts in their vesicular bilayer, thereby enhancing their flexibility and durability in the gastrointestinal tract. Unlike conventional vesicular systems they provide distinct advantages such as streamlined manufacturing procedures, cost efficiency, and improved stability. The main objective of this study was to attain a comparison of the pharmacokinetic parameters of nisoldipine (NSD) after administering an ordinary NSD suspension and an NSD-loaded bilosome suspension. The study used 60 Swiss albino rats weighing 200±15 g and divided into two groups (n=30 each). A dose of 2.2 mg/kg of NSD was administered from the ordinary NSD suspension to the rats of the first group and the same dose of NSD-loaded bilosome suspension was administered to the rats of the second group. NSD levels were determined in the rat plasma by using high-performance liquid chromatography. Our results showed that the C_{max}, the T_{max}, and the AUC_{0-36} were 51.47±0.94 ng/mL, 2±0.3 h, and 323.33±21 ng×h/mL for the pure suspension, and 116.41±1.22 ng/mL, 4±0.7 h, and 916±64.09 ng×h/mL for the bilosome suspension, respectively. The maximum concentration was significantly different between the pure and the bilosomal preparation (P<0.05), while the relative bioavailability of the pure suspension was 2.9 times that of the bilosomal suspension, 36 h after a single-dose NSD administration. In conclusion, the prepared bilosomal suspension enhanced the bioavailability of NSD, and could be considered as a vital delivery system.

KEYWORDS
bilosomes; nisoldipine, pharmacokinetics, animal study, bioavailability

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1. INTRODUCTION

The oral route is the most prevalent and favored method of administering drugs, due to its ease and high level of patient adherence. Nevertheless, administering the drug through this method frequently leads to a less than ideal therapeutic outcome due to the drug's limited ability to dissolve in gastrointestinal fluids, inadequate ability to pass through the gastrointestinal barrier, and significant initial metabolism [1].

Bilosomes are nanocarriers that contain bile

salts in their vesicular bilayer, thereby enhancing their flexibility and durability in the gastrointestinal tract (GIT) [2,3]. The resistance to enzymatic degradation in the GIT is attributed to the presence of bile salts in the vesicle membrane, which boost penetration and improve the effectiveness of oral delivery. Non-ionic surfactants, such as those in the Span family, are commonly employed in the creation of bilosomes.

Predicting plasma concentration-time curves is possible with the help of the drug's physicochemical properties in physiologically-based pharmacokinetic models. Validating the model with publicly-available clinical pharmacokinetic data is a must before implementation [4].

Nisoldipine (NSD) is a compound derived from dihydropyridine that functions as a blocker of calcium channels. NSD undergoes presystemic metabolism, resulting in just 5% of it being utilized by the body. Additionally, it exhibits a binding affinity to CYP3A and P-glycoprotein [5].

The main objective of this study was to create an enhanced formulation for NSD by using bilosomes, with the goal of improving its oral bioavailability as compared to a regular NSD suspension.

2. MATERIALS AND METHODS

Materials: The acquisition of NSD was made from Lee Chemicals, India. Cholesterol, surfactant Span 60, and bile salt sodium deoxycholate were obtained from HyperChem, India. All the chemicals and solvents used in this study were of analytical grade.

In vivo pharmacokinetic studies: This study employed 60 male Swiss albino rats, aged three months, with an average weight of 200±15 g. The rats were housed in the animal facility of the Research Centre for Cancer Research and Medical Genetics, Baghdad, Iraq, under controlled circumstances of a constant room temperature of 25°C±1°C and a 12-h light/dark cycle. The rats were provided unrestricted access to both food and water. The *in vivo* experiments performed on the rats were authorized by the Research Ethics Committee for Experimental Investigations, College of Pharmacy, Baghdad University, Iraq, under the protocol number RECAUBCP262022A.

Dosing protocol: Before administering the oral medication, two groups of rats (n=30 each) were subjected to an overnight fast lasting more than 12 h. Rats were orally administered the drug-loaded bilosome formulation (NSD-loaded bilosome suspension) or the control formulation (pure NSD powder suspension) using an oral gavage at a dose of 2.2 mg/kg. During administration, both groups were administered ketamine at a dose of 80 mg/kg, and each animal also received xylazine at a dose of 10 mg/kg [6].

Each sampling was timed, and a single dose was administered to both groups in order to assess the relative bioavailability of the pure NSD suspension and of the NSD-loaded bilosome suspension. A total of 5 mL of blood was acquired from the myocardium through piercing at predefined time intervals ranging from 0 to 36 h. EDTA-treated tubes were used in order to collect blood samples from the rats, which were then promptly separated. Plasma samples were obtained by centrifuging the blood samples (Hettich Zentrifugen EBA 20, Germany) at a speed of 4,000 rpm for a duration of 10 min. Plasma samples were collected from the liquid portion, transferred into Eppendorf tubes, and preserved in the freezer for subsequent investigation. A modified and confirmed approach was used in order to evaluate the samples of plasma by using reversed-phase high-performance liquid chromatography (RP-HPLC).

This study measured the maximum plasma concentration (C_{max}) of the medication as well as the time it took to reach C_{max} (T_{max}). The $AUC_{0\text{-}36}$ and $AUC_{0\text{-}\infty}$ were determined by calculating the integral of the plasma concentration-time curve from time 0 to 36 h and from 0 to infinity, respectively.

Statistical analysis: The results were presented as mean values with their standard deviation (±SD; n=3). A difference was deemed statistically significant if the *P*-value was less than 0.05. The pharmacokinetic parameters, namely C_{max}, T_{max}, and $AUC_{0\text{-}36}$, were subjected to statistical analysis by means of a Student's *t*-test [7]. The equation employed so as to evaluate the relative bioavailability (F) of NSD was as follows:

$$\frac{\text{AUC bilosomal suspension} \times \text{dose free drug suspension}}{\text{AUC free drug suspension} \times \text{dose bilosomal suspension}} \times 100$$

3. RESULTS

The calibration curve was generated by following the recommended protocol for the spiking plasma, by using a certain solution of NSD with a specified concentration. The HPLC analysis revealed the presence of endogenous components in the blank plasma chromatogram, with a retention time of 1.9. The spiked sample's chromatogram exhibited clear differentiation between NSD and nimodipine, with NSD having a retention time (Rt) of 5.79 min and nimodipine showing a signal at 3.57 min.

A validated HPLC method was employed to quantify the quantity of NSD in the plasma of the rats. All validation parameters met the conventional criteria. Six concentrations were employed in order to evaluate the linearity of the method and

determine the lower limit of detection, which existed at 1 ng/mL [8].

The oral NSD-loaded bilosome suspension was assessed for its relative bioavailability in comparison to the oral free NSD suspension. Figure 1 displays the average concentration of the drug in the rat plasma *versus* time after administering orally a free drug suspension and a drug-loaded bilosome suspension.

The statistical analysis employing a *t*-test indicated that the concentration (C_{max}) and the time (T_{max}) required in order to achieve the highest effect were 51.47±0.94 ng/mL and 2±0.3 h for the free NSD suspension and 116.41±1.22 ng/mL and 4±0.7 h for the NSD-loaded bilosome suspension, respectively. Our results indicate a statistically significant disparity between these values (P<0.05). Each of these numbers was tested at the significance level, and each relate to three separate measurements [9].

The $AUC_{0\text{-}36}$ (area under the curve from 0 to 36 h) for the free NSD suspension was 323.33±21 ng×h/mL, which was considerably lower (P<0.05) compared to the $AUC_{0\text{-}36}$ for the NSD-loaded bilosome suspension (916±64.09 ng×h/mL). The comparative bioavailability of the two preparations indicated that the bilosomal suspension can exert a 2.9-fold greater availability (P<0.05) than the pure drug suspension at 36 h after a single-dose administration.

4. DISCUSSION

The enhanced absorption of NSD from the bilosomal suspension can be related to the successful encapsulation of NSD within the bilosomes that are in the nano-size range and are primarily composed of surfactant (Span 60). This surfactant not only enhances solubility, but also acts as a penetration enhancer. In addition, bilosomal vesicles contain bile salts that confer elasticity to the vesicles, increase permeability, and provide resistance to physiological bile salts in the GIT. Moreover, the vesicles' negative charge facilitates the uptake of drugs by the M-cells of Peyer's patch in the intestine, and improves drug absorption through the pathway of intestinal lymphatic transport, thereby bypassing the initial drug processing in the liver [10].

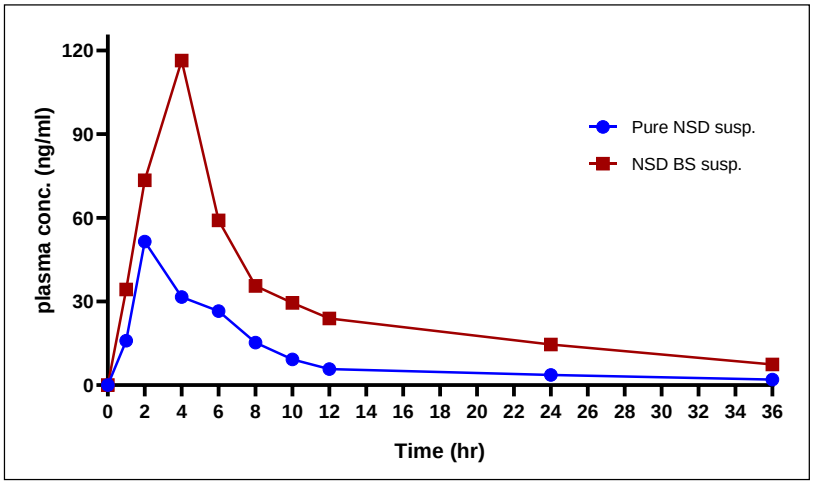

Figure 1. Rat plasma concentrations of nisoldipine (NSD) measured after administering the drug orally in the form of a free drug suspension and in the form of a NSD-loaded bilosome (BS) suspension.

5. CONCLUSION

Ultimately, the generated NSD-loaded bilosome suspension has a much higher relative bioavailability compared to the free NSD solution. Consequently, it is deemed as a more advantageous dosing form for the administration of NSD in the treatment of hypertension. In future research, the use of bilosomes as a delivery mechanism should be considered as a possible method to increase the bioavailability of NSD.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Immunomodulatory effects of *Stevia rebaudiana* leaves and commercial stevia on rats: a comparative study

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Abstract

Stevia is herbal native to South-America that is renowned for its natural sweet leaves containing stevia glycosides. Our study aimed at examining and comparing the immunomodulatory effects of the *Stevia rebaudiana* leaf extract and of commercially-available stevia products in rats. Our experiment involved the preparation of *Stevia rebaudiana* leaf extract and the use of market-available stevia obtained from the local-market. *Stevia rebaudiana* leaf extraction was meticulously performed, and 60 healthy adult male rats were randomly separated into three groups: untreated control, commercial stevia treatment (25 mg/kg), and *Stevia rebaudiana* leaf extract treatment (25 mg/kg). The rats were orally administered the treatments for 60 days, after which blood samples were collected for analysis. Our results revealed a significant rise in interleukin-1 beta (IL-1β) levels in rats treated with the *Stevia rebaudiana* leaf extract and the commercially-available stevia when compared to the control group. Additionally, immunoglobulin A (IgA) levels exhibited a notable increase in both stevia-treated groups, with the *Stevia rebaudiana* leaf extract-receiving group showing higher IgA levels than the commercially-available stevia-treated one. Our findings suggest that stevia may influence the immune response, particularly the regulation of the pro-inflammatory cytokine IL-1β and the IgA levels. This study contributes valuable insights into stevia's effects on the immune system.

KEYWORDS
cytokine, extraction, immunoglobulin A, interleukin-1 beta, stevia

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1. INTRODUCTION

Stevia rebaudiana belongs to the *Asteraceae* family, which includes sunflowers and daisies. These glycosides are responsible for stevia's heavy-duty sweetness, which makes it a popular sweetener. The stevia sweeteners are sugar alternatives with zero calories and widely used as sugar substitutes, high-intensity sweeteners, non-nutritive sweeteners, or low-calorie sweeteners, as they offer a satisfying taste of sweetness without the added calories [1]. The US Food and Drug

Administration (FDA) authorized stevia as a sweetener in January 2022. The FDA has also recognized as safe the use of high purity stevia glycosides, notably rebaudioside A (GRAS).

Some researchers suggest that stevia's components, such as stevioside and rebaudioside A, may have anti-inflammatory properties [2]. Inflammation is a component of the immune response, and drugs having anti-inflammatory properties could potentially affect the immune system's function. Stevia has been shown to exert antioxidant activity. Antioxidants help neutralizing free radicals that are responsible for causing oxidative-stress and inflammation, while antioxidants also contribute to overall health, and their specific impact on the immune function is nuanced and context-dependent [3]. This study aimed at investigating and comparing the potential differences in the immunomodulatory effects of the *Stevia rebaudiana* leaf extract and the commercially-available stevia products in rats.

2. MATERIALS AND METHODS

This study were conducted from April 2023 to January 2024 in Babylon city, Iraq. *Stevia rebaudiana* leaves and commercially-available stevia (market-available stevia) were obtained from the local market.

For the preparation of the extraction of the *Stevia rebaudiana* leaves, the leaves were ground into a fine powder by using a mortar. Subsequently, 500 g of this fine powder were placed in a beaker with warm water, and the mixture was continuously stirred for 24 h. The extraction was filtered by using a Whatman No.1 filter paper. The supernatant was then concentrated by using a rotary-evaporator under vacuum, was dried, and the percentage weight of the dried extraction was determined [4].

Our experiments included 60 rats that were randomly separated into three groups as follows: (i) the first group (n=20) served as the untreated "control", (ii) the second group (n=20) received a 25 mg/kg dose of the commercially-available stevia, and (iii) the third group was treated with a 25 mg/kg dose of the *Stevia rebaudiana* leaf extract. The rats were treated for 60 days, orally, by using stevia dissolved in distilled water. Following the 60-day treatment period, the animals underwent anesthesia using ether, and cardiac-puncture was performed in order to collect 1 mL of blood [5]. The assessment of the plasma levels of interleukin-1 beta (IL-1β) and immunoglobulin A (IgA) was performed by using an ELISA kit (Elabscience) as previously described [6].

All statistical analyses were done by using GraphPad Prism (version 6), including the performance of Student's *t*-test, analysis of variance (ANOVA), regression analysis, and non-parametric tests.

3. RESULTS AND DISCUSSION

The extraction weight was 329 mg our of 500 g of leaves of *Stevia rebaudiana* powered, and the percentage weight was 0.0658% of 500 g of the *Stevia rebaudiana* leaf powder. The amount of the extracted compounds is somewhat low, as indicated by the small percentage (0.0658%). Numerous factors can affect the extraction efficiency, including the extraction method used. Different extraction methods (e.g., solvent extraction, water extraction) can yield different results in relation to both the quantity and the quality of the extracted compounds.

The plasma IL-1β levels exhibited a significant increase (P<0.0001) in rats treated with the leaf extract when compared with those of the control group (Table 1). In addition, the rats treated with the commercial stevia also displayed a significant increase in their IL-1β levels (P=0.0036) when compared to those of the control group (Table 1). The significantly higher levels of IL-1β in the group treated with *Stevia rebaudiana* leaf extract when compared to those of the group treated with the market-available stevia may indicate that the extraction technique or specific components in the *Stevia rebaudiana* leaves may contribute to a more dramatic effect on IL-1β levels. *Stevia rebaudiana* or its components might stimulate the immune system, leading to an increase in IL-1β production that might be a reaction to perceived threats, even if those threats are not harmful [7].

The mean plasma IgA levels were 568.1 ± 55.92 ng/mL in the rats treated with market-available stevia, showing a significant difference (P=0.0022) when compared to those of the control group (Table 1). Rats treated with the *Stevia rebaudiana* leaf extract exhibited an extremely significant difference (P<0.0001) in terms of their IgA levels (605.4 ± 45.60 ng/mL) when compared to those of the control group (Table 1). Moreover, there was a significant difference observed (P=0.0263) in the IgA levels between the two stevia-treated groups (Table 1).

The rise in IgA levels, as observed in this study, could be influenced by various factors. IgA is an antibody that plays a crucial role in the immune-system, mainly in mucosal immunity and the defense against infections in mucosal membranes such as those in the digestive and the respiratory tracts. IgA is also essential for keeping a balance between the host and the gut microbiota [8]. Stevia

might influence the gut microbiota, leading to an increase in IgA as a response to the need of maintaining homeostasis in the gastrointestinal tract. These are immunomodulatory effects of stevia or of its components, as they stimulate the immune system, leading to an elevated IgA production.

Stevia has been reported to possess antioxidant properties [9]. If there is oxidative stress or inflammation in the body, the immune response, including IgA production, may be heightened so as to stabilize these phenomena. The dose and the period of treatment with stevia might also play a role in our findings. Different doses or extensive treatment periods could lead to varying immune responses.

Table 1. Comparison of interleukin-1 beta (IL-1β) and immunoglobulin A (IgA) levels between rats receiving different treatments. Notes: [a], comparison between the control group and the market-available stevia group; [b], comparison between the control group and the *Stevia rebaudiana* leaf extract group; [c], comparison between the market-available stevia group and the *Stevia rebaudiana* leaf extract group.

IL-1β levels in rats under different treatments			
Groups / treatments	**n**	**Mean ± SD (pg/mL)**	***P*-value**
Control	20	51.10 ± 7.953	--
Market-available stevia	20	77.70 ± 37.45	0.0036[a]
Stevia rebaudiana leaf extract	20	233.5 ± 81.93	< 0.0001[b,c]
IgA levels in rats under different treatments			
Groups / treatments	**n**	**Mean ± SD (ng/mL)**	***P*-value**
Control	20	568.1 ± 28.52	--
Market-available stevia	20	568.1 ± 55.92	0.0022[a]
Stevia rebaudiana leaf extract	20	605.4 ± 45.60	< 0.0001[b]; 0.0263[c]

4. CONCLUSION

This study contributes to the increasing research interest on stevia's potential health-effects, especially on the immune system. Both rat groups treated with *Stevia rebaudiana* leaf extract and market-available stevia exhibited a significantly increased IL-1β, and IgA levels compared to the control group. This finding suggests that stevia may influence the immune response, particularly the pro-inflammatory cytokine IL-1β and IgA.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Novel anticancer mechanisms of JinYingZi-derived oleanolic acid against renal cell carcinoma: an *in silico* analysis

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Abstract

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for 90% of all estimated cases. Advanced RCC often carries poor prognosis due to its high metastases rate, the lack of early-warning signs, as well as its complex clinical manifestations and its resistance to chemotherapy. Ethnopharmacologically, *Fructus Rosae laevigata* (JinYingZi) has been employed by Chinese medicine to treat various urinary tract and gastrointestinal disorders. This study aimed at performing a series of mechanistic analyses in order to unlock the anticancer potential of JinYingZi-derived bioactive components against RCC. Several network pharmacology tools were employed so as to analyse the drug-disease interactions. Our data revealed that more than 2,214 genes were dysregulated in RCC, whereas the JinYingZi-derived bioactive compounds modulated 347 genes. The intersecting between RCC and the bioactive compounds revealed 132 cross targets. Our results were further validated by conducting molecular docking, which revealed a stable association between oleanolic acid with each of the following targets: androgen receptor (*AR*), dipeptidyl peptidase (*DPP*), estradiol (*ESR1*), nitric oxide synthase 2 (*NOS2*), and cyclooxygenase-2 (*PTGS2*). Our approach is being used successfully in order to evaluate a panel of novel medicinal plant-derived bioactive compounds, and may lead to the identification of safe and effective chemical scaffolds that could act as templates for drug discovery or yield potential drug candidates.

KEYWORDS
renal cell carcinoma, JinYingZi, oleanolic acid, network pharmacology, molecular docking

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1. INTRODUCTION

Kidney cancers are highly prevalent malignant tumours worldwide, with an estimated 431,288 cases in 2020. In the United States, it is the sixth most common cancer among men and the ninth most prevalent malignant tumour among women, with an estimated 81,800 cases in 2023. Renal cell

carcinoma (RCC) is the most common type of kidney cancers, accounting for 90% of all estimated cases [1]. Advanced RCC often carries poor prognosis due to its high metastases rate, the lack of early-warning signs, as well as its complex clinical manifestations and its resistance to chemotherapy [2]. *Rosa laevigata* Michx. is one of the recognised ethnic medicinal plants that have been used in Chinese traditional medicine in order to treat different debilitating illnesses. Two main herbal remedies are derived from *R. laevigata*, namely *Fructus R. laevigata* and *Radix R. laevigata*. Ethnopharmacologically, *Fructus R. laevigata* (JinYingZi) has been employed by Chinese medicine to treat various urinary tract and gastrointestinal disorders [3]. This study aimed at performing a series of mechanistic analyses in order to unlock the anticancer potential of *Fructus R. laevigata*-derived bioactive components against RCC.

2. METHODS

This study was carried out in order to evaluate the anticancer activity of JinYingZi against RCC, by speculating the potential disease targets of the plant's bioactive components. Several network pharmacology tools were utilised throughout this study, including the Encyclopaedia of Traditional Chinese Medicine (ETCM), the Bioinformatics Analysis Tool for Molecular Mechanisms in Chinese Medicine (BATMAN-TCM), the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), DisGeNET, GenCards, SymMap, STRING, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, the DAVID platform, Cytoscape 3.6.0, and molecular docking.

3. RESULTS

Our analyses revealed that more than 2,214 genes were dysregulated in RCC, while the JinYingZi-derived bioactive components were found to be able to modulate 347 targets. The intersection between RCC and the bioactive compounds revealed 132 cross targets. The data were further analysed for the protein-protein interaction (PPI) network and GO and KEGG pathways. Among 14 bioactive components, the novel oleanolic acid seems to be one of the promising candidates to combat RCC *via* targeting several signalling pathways, including the advanced glycation end products / receptor for advanced glycation end products (AGE/RAGE) signalling, cellular senescence, the response to hormones, and apoptosis (Figure 1). The results were further validated by conducting molecular docking, which indicated stable associations between oleanolic acid with each of the following targets: androgen receptor (*AR*; 8.1 Kcal/mol), dipeptidyl peptidase (*DPP*; -7.5 Kcal/mol), estradiol (*ESR1*; -8.8 Kcal/mol), nitric oxide synthase 2 (*NOS2*; -6.6 Kcal/mol), and cyclooxygenase-2 (*PTGS2*; -8.8 Kcal/mol).

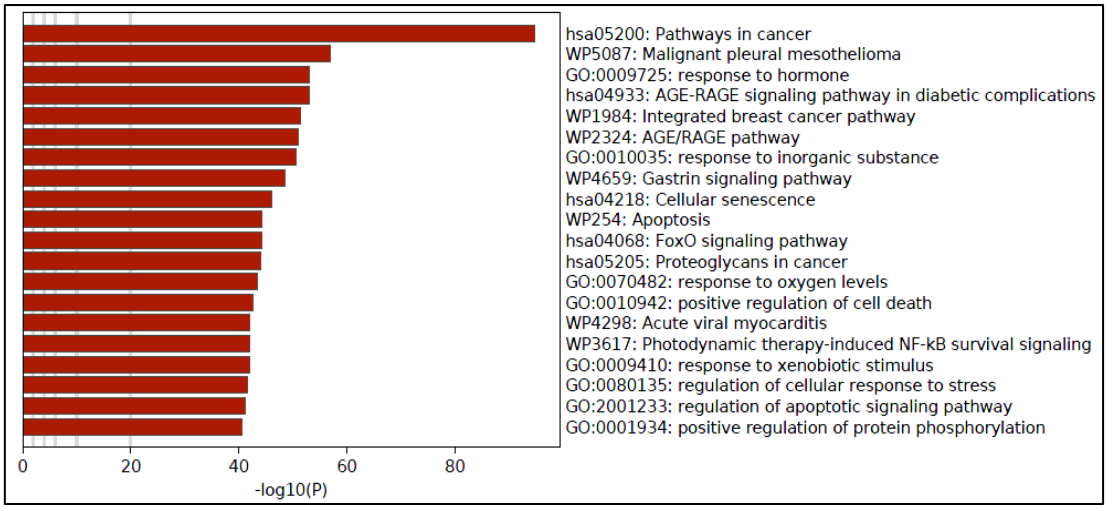

Figure 1. The heatmap of major Gene Ontology (GO) terms and disease prediction by Metascape shows common renal cell carcinoma - oleanolic acid target pathways.

4. DISCUSSION

Our analyses showed that the highest possible targets of oleanolic acid are hormones (*AR* and *ESR1*), inflammation (*NOS2* and *PTGS2*), and cancer metabolism (*DPP*). *AR* has been shown to play an integral role in developing tumour-initiating vasculogenesis and metastasis in RCC, *via* promoting and modulating the *Twist1*-associated long noncoding RNA regulated by *AR* (lncRNA-TANAR/TWIST1) signalling pathway [4]. Interestingly, the high cytoplasmic immunohistochemical expression of estrogen receptors is associated with poor prognosis as well as short overall survival and disease-free survival of RCC patients [5]. Likewise, high NOS expression is linked to bad prognosis and large tumour size in RCC patients [6]. The suppression of COX-2 is associated with the inhibition of RCC tumour progression and angiogenesis while improving patients' prognosis and survival rates [7]. Moreover, DPP (particularly DPP-4) has been identified as a cancer stemness related-protein and, therefore, targeting such a protein can enhance the sensitivity and overcome the RCC resistance to tyrosine kinase inhibitors; the main therapeutic line for treating advanced RCC [8].

Oleanolic acid has been shown to have substantial chemopreventive and antitumor activities against hepatic cancer cells, exerted *via* the inhibition of nuclear factor kappa-B (NF-κB) and the suppression of COX-2 [9]. Similarly, oleanolic acid is known to exert anti-tumour effects against breast and lung cancers by targeting the purine salvage pathway (PSP), thereby inducing metabolic perturbation *via* the activation of the superoxide dismutase 1 (SOD1) / reactive oxygen species (ROS) / AMP-activated protein kinase (AMPK) / mammalian target of rapamycin complex 1 (mTORC1) / macroautophagy / lysosomal pathway [10]. Taken together, oleanolic acid has shown a clear anticancer potential against different cancers by targeting the inflammation, the antioxidant system, the cellular senescence, and metabolic pathways. To our best knowledge, this is the first study to report the anticancer potential of oleanolic acid against RCC. The study also represents a theoretical basis for the undertaking of future experiments in order to test the cancer-supressing activities of oleanolic acid against RCC, and provides a strong foundation for further molecular analyses aiming to study its mechanism of action. Our approach is being used successfully in order to evaluate a panel of novel medicinal plant-derived bioactive compounds, and may lead to the identification of safe and effective chemical scaffolds that could act as templates for drug discovery or yield potential drug candidates.

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Study of some immunological indicators for COVID-19 patients in Babylon city

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Abstract

COVID-19 has been a global challenge caused by a coronavirus that infects the respiratory system and poses a high risk to life. This study dealt with some immunological indicators associated with this disease. A total of 150 samples was collected from COVID-19 patients (120 samples) and non-infected individuals (30 samples; control group) who were admitted to the Marjan Medical City Hospital in Al-Hilla, Babylon, Iraq for the period from March 1 to August 30, 2022. Of these participants, 65% were men and 35% were women, with ages ranging from 16 to 75 years. The samples were collected under the supervision of specialized doctors, according to the approved by the Iraqi Ministry of Health protocols. The parameters examined in this study included the complete blood count, the red blood cell (RBC) count, the packed cell volume, the haemoglobin levels, the platelet count, the white blood cell (WBC) count, and the levels of immunological indicators such as interleukin-1 beta (IL-1β), interleukin-17 (IL-17), the tumour necrosis factor-alpha (TNF-α), the C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR). Our results indicate a clear increase in the WBC count, the IL-1β levels, IL-17 levels, the TNF-α levels, the CRP levels, the ESR, and the RBC count, as well as a clear decrease in the lymphocyte count, as a result of COVID-19.

KEYWORDS
COVID-19, immunological indicators, Iraq, blood cells, cytokines

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1. INTRODUCTION

A coronavirus is a large and coated virus containing genetic material of the RNA type, and this new virus responsible for COVID-19 is now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The Australian scientist Wilson confirmed that COVID-19 patients can be divided into two categories: (i) those who carry the virus and do not show symptoms of the disease (and are the least harmful group), and (ii) those who carry the virus with the appearance of symptoms (such as fever and headache and other symptoms) [1,2].

2. PATIENTS AND METHODS

A total of 150 samples was collected from COVID-19 patients (120 samples) and non-infected indi-

viduals (30 samples; control group) who were admitted to the Marjan Medical City Hospital in Al-Hilla, Babylon, Iraq for the period from March 1 to August 30, 2022. Of these participants, 65% were men and 35% were women, with ages ranging from 16 to 75 years. The samples were collected under the supervision of specialized doctors, according to the approved by the Iraqi Ministry of Health protocols. The parameters examined in this study included the complete blood count, the red blood cell (RBC) count, the packed cell volume (PCV), the haemoglobin levels, the platelet count, the white blood cell (WBC) count, and the levels of immunological indicators such as interleukin-1 beta (IL-1β), interleukin-17 (IL-17), the tumour necrosis factor-alpha (TNF-α), the C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR). The complete blood count was conducted as previously described [3], while the determination of CRP and TNF-α levels was done through special kits, according to the manufacturer's instructions. The determination of the interleukin levels was done by ELISA. Statistical analysis was undertaken by using the Statistical Package for Social Sciences (SPSS) program.

Table 1. Comparative levels of immune variables, inflammation markers, and some common blood test parameters in the groups studied. Abrreviations used: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; IL-1β, interleukin-1 beta; IL-17, interleukin-17; PCV, packed cell volume; RBC, red blood cell; SD, standard deviation; TNF-α, tumour necrosis factor-alpha; WBC, white blood cell.

Parameter	Group	Mean	SD	Min	Max	*P*-value (control *vs.* patients)
IL-17 levels (pg/mL)	Control	123.27	18.9	99	155	0.01
	Patients	230.08	23.6	188	260	
	Total	208.71	48.5	99	260	
IL-1β levels (pg/mL)	Control	182.43	16.8	159	211	0.01
	Patients	276.02	21.3	242	310	
	Total	257.30	42.7	159	310	
TNF-α levels (pg/mL)	Control	202.60	21.3	175	235	0.01
	Patients	408.23	46.2	300	545	
	Total	367.11	92.7	175	545	
ESR (mm/h)	Control	8.03	3.8	5	20	0.01
	Patients	43.02	14.5	20	85	
	Total	36.02	19.1	5	85	
CRP levels (mg/L)	Control	3.35	1.4	0.4	6.1	0.01
	Patients	46.77	18.01	18.0	81.0	
	Total	38.09	23.73	0.4	81.0	
WBC count ($\times 10^3/\mu L$)	Control	5.95	1.8	4.20	7.44	0.001
	Patients	8.16	2.7	3.58	21.52	
	Total	7.72	2.6	3.58	21.52	
Lymphocytes (%)	Control	23.84	12.8	2.61	38.90	0.002
	Patients	12.55	3.8	4.90	19.70	
	Total	14.80	8.02	2.61	38.90	
RBC count ($\times 10^6/\mu L$)	Control	4.58	0.5	3.47	5.81	0.001
	Patients	5.12	0.5	3.81	6.73	
	Total	5.01	0.6	3.47	6.73	
Hb levels (g/dL)	Control	12.58	1.9	7.8	15.8	0.003
	Patients	14.41	1.7	9.5	19.0	
	Total	14.05	1.9	7.8	19.0	
PCV (%)	Control	39.06	5.2	25.7	48.0	0.002
	Patients	43.72	4.7	29.5	56.5	
	Total	42.79	5.1	25.7	56.5	
Platelet count ($\times 10^3/\mu L$)	Control	292.27	41.38	54	841	0.001
	Patients	265.52	75.13	89	445	
	Total	270.87	92.27	54	841	

3. RESULTS AND DISCUSSION

This study dealt with the secondary infections, as well as with the immune and inflammatory variables associated with COVID-19. In this study, out of the 120 COVID-19 patients included, 78 (65.0%) were male and 42 (35.0%) were female. Their average age was 40.7 years. Among the non-infected individuals (control group), 50% were men and 50% were women, with an average age of 39 years. These results contradict those of Morris *et al*. [4].

As detailed in Table 1, a clear increase in the levels of IL-1β and IL-17 was observed in COVID-19 patients. This significant rise may be attributed to the fact that IL-1β is one of the basic cytokines for the formation of inflammatory bodies as part of the immune response to the disease [5].

In COVID-19 patients, complete blood count blood tests have been associated with a lack of oxygen in the blood and body tissues, pneumonia, high cytokine storm, and increased lung secretions which lead to the closure of the alveoli and the bronchi with mucous pulmonary secretions thereby causing suffocation and respiratory failure that requires resorting to artificial respiration [6]. This coincides with a leak in the blood vessels, the clotting of the blood itself, the lowering of the blood pressure, and many systems beginning to fail to perform their functions. In fact, during a severe COVID-19 infection, the body believes that its tissues are extraneous and invasive bodies, thereby resulting in the generation of a cytokine storm.

Table 1 shows a clear increase in the TNF-α levels of the COVID-19 patients, and a clear difference with those of the control group. Moreover, a clear increase in CRP levels was recorded in COVID-19 patients; an indicator of bacterial infections. The latter finding is in agreement with that of Langford *et al*. [3], but in disagreement with that of Sun *et al*. [7].

The ESR values of COVID-19 patients were high compared to those of the control group, due to the rapid sedimentation of RBCs; these results are similar to those reported earlier [7]. The clear rise in WBCs observed may also be attributed to bacterial infections, although these results are also in disagreement with those of Sun *et al*. [7]. However, a clear decrease in the number of lymphocytes was observed in COVID-19 patients when compared with those of the control group (Table 1). Lymphocytes are target cells for the coronavirus, and their % may be affected by the presence of asymptomatic individuals among the control group [8].

As detailed in Table 1, there is a strong statistical significance (P=0.001) associated with the high number of RBCs in COVID-19 patients due to the lack of oxygen in the blood and tissues as well as the patients' need for oxygen carriers. These findings were somewhat similar to those of a previous study conducted on the complications of the coronavirus and the influenza virus [6].

A relative decrease in the PCV of COVID-19 patients, especially in the advanced stages of infection, was observed (Table 1). This may be attributed to anaemia as a result of poor appetite, especially at the peak of the disease. As far as the platelet count is concerned, Table 1 reveals a statistically significant drop of this parameter in COVID-19 patients. Platelets have a clear role in the formation of blood clotting in COVID-19 patients, forcing the patient to take anticoagulants so as to avoid blood clots due to the infection by this dreaded virus [9].

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Chemical analysis and antibacterial activity of the *Achillea millefolium* extract

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Abstract

Achillea millefolium L., a traditional medicinal plant from the Asteraceae family, is known for its anti-inflammatory, anti-ulcer, and anti-cancer properties, attributed to its rich content of phytochemicals (such as flavonoids, alkaloids, terpenes, tannins, and phenolic acids). This study has focused on the chemical analysis of the oil extracted from the aerial parts of *Achillea millefolium* subsp. *millefolium*, cultivated and harvested in Kurdistan, Iraq. The oil was extracted by using a Clevenger apparatus for hydro-distillation and was analysed by using gas chromatography with a flame ionization detector and an HP-5 MS capillary column. The analysis revealed the presence of 1,8-cineole (eucalyptus oil), Artemisia ketone, camphor, linalyl acetate, and D-limonene, with Artemisia ketone having the highest concentration at 15.04% and D-limonene the lowest at 7.51%. The extract of *Achillea millefolium* has been shown to aid in the treatment of oral mucositis (a common side effect of anticancer chemotherapy), likely due to the flavonoids and tannins present in the plant.

KEYWORDS
yarrow, gas chromatography - mass analysis, camphor, Artemisia ketone, 1,8-cineole

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1. INTRODUCTION

Many people use yarrow (*Achillea millefolium* L.), a Asteraceae family plant, as an ancient medicinal herbaceous plant [1]. Yarrow is native to Europe and Asia, but it has spread to North America. It is also known as “gordaldo”, “nosebleed plant”, “old man’s pepper”, “devil’s nettle”, “sanguinary”, “milfoil”, “soldier’s woundwort”, “thousand-leaf”, and “thousand-seal” [2]. Yarrow grows naturally in pastures, meadows, roadside ditches, and open forests. The herb has anti-bleeding qualities and promotes wound healing. The yarrow root, on the

other hand, has anaesthetic properties; it has been used in order to relieve toothaches, by applying fresh roots or leaves to the gums [3]. *Achillea millefolium* has been used in traditional medicine for centuries [4,5]. It possesses anti-inflammatory, anti-ulcer, and anti-cancer properties. Flavonoids, alkaloids, terpenes, tannins, phenolic acids, and other phytochemicals are abundant in *Achillea millefolium*, with flavonoids and phenolic acids being the most important components. The main component of the *Achillea millefolium* essential oil is chamazulene [6]. In terms of its beneficial effects in dental pathology, it has been demonstrated that the plant's extract assists in the treatment of oral mucositis; a typical side-effect of standard anticancer therapies [7].

2. MATERIALS AND METHODS

Achillea millefolium plants were cultivated and harvested from their natural habitats in Kurdistan, Iraq. The aerial parts of the plants were air-dried and stored in paper bags for 30 days. The dried plant materials (100 g) were then powdered and subjected to hydro-distillation by using Clevenger-type equipment for a duration of 3 h [8]. The extracted essential oil from *Achillea millefolium* was analysed using an HP 6890 gas chromatograph equipped with a flame ionization detector (FID) and an HP-5 MS capillary column (30 m; 0.25 mm i.d.; film thickness 0.25 µm). The same column and analytical conditions were used as in gas chromatography (GC) - mass spectrometry. The percentage composition of the essential oil was estimated by using GC-FID peak areas, without the use of adjustment factors. Each bioactive compound that was isolated and evaluated in the essential oil of *Achillea millefolium* has been studied in the literature. The potential bioactivity of these compounds against the common cavity-causing bacterium, *Streptococcus mutans*, was highlighted, particularly in the context of mouthwash production. The antibacterial activity of the yarrow oil extract was assessed against several bacterial species (*Streptococcus salivarius*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) by using the agar diffusion technique. A bacterial suspension (100 µL) containing approximately 1×10^8 cells/mL (McFarland solution) was uniformly spread on the surface of a Mueller-Hinton agar in a petri dish (90-mm diameter). Then, 0.1 mL of each concentration (0%, 0.1%, 0.3%, and 0.5%) of the yarrow extract was aseptically introduced into the wells (5-mm diameter). The plates were left for 30 min before their incubation at 37°C for 24 h. The inhibitory zones (in mm) were measured, indicating the antibacterial activity [9].

3. RESULTS AND DISCUSSION

Our GC-mass spectrometry analysis has shown that the yarrow oil contains numerous anti-inflammatory chemicals. The chemical analysis spectrum of the yarrow essential oil is influenced by intrinsic factors such as the number of chromosomes and whether the plant is diploid or tetraploid. Extrinsic factors, including the stage of harvest, the part of the plant, the harvesting season, the geographical origins, and the method used for the oil extraction and analysis, can also affect the chemical composition and yield of the oil obtained from the yarrow. Artemisia ketone, a member of the enone functional group with the structure RC(=O)CR', has been found to exist at 3.1% to 12.9%. Artemisia ketone is almost insoluble in water at 0.29 g/L and is a very weak basic (essentially neutral) molecule. This green, herbal chemical with a berry flavour is also found in the sunflower and in the tarragon. Linalyl acetate, which makes up 12.08% of the *Achillea millefolium* essential oil, is a phytochemical molecule found in many flowers, and has a lavender scent. Linalyl acetate is chemically composed of linalool acetate ester; it has a pleasant odour and a taste that is similar to its smell, and it can be used as a safe fragrance substance. 1,8-Cineole was found to be the third component at 11.08%, which varied from the oil recovered from commercial yarrow plants. Eucalyptol is another name for 1,8-cineole, which is naturally formed as a cyclic ether and monoterpenoid by several plants, most notably eucalyptus. Camphor and D-limonene, both terpenes, were found at 10.75% and 7.51%, respectively, in the extracted oil of the yarrow. Camphor was found to be a prominent ingredient of the yarrow oil, with a range of 0.6% to 17.6%. Camphor oil is primarily derived by steam distillation from the wood of camphor trees. D-limonene, a key ingredient in various citrus oils, is generally regarded as safe and may be found in popular food items. Camphor is often used in creams, ointments, and lotions so as to reduce skin irritation and itching, and may help enhance the skin's overall appearance.

Yarrow oil has been found to have antibacterial and antifungal properties, making it effective in treating infections. It is suggested that the yarrow extract could be used as a mouthwash to alleviate mucositis and ulcers following chemotherapy, and to reduce harmful bacterial dysbiosis. It could also be used in creams or lotions to prevent acne in young people, based on the bioactive ingredients identified in the oil. Oil extracted from *Achillea* spp. was tested against *Propionibacterium acnes* and *Staphylococcus epidermidis*, showing varying degrees of antibacterial activity. Yarrow oil, which is

rich in eucalyptol, could potentially be used in the manufacturing of mouthwash and cough suppressants. Eucalyptol has been shown to regulate airway mucus hypersecretion and asthma through the suppression of inflammatory cytokines. It can effectively treat nonpurulent rhinosinusitis and, when applied topically, can relieve inflammation and discomfort. The antibacterial efficacy of the oily yarrow extract against common cavity bacterial species has been discussed. The *Achillea millefolium* extract has demonstrated a dose-response antibacterial activity. All tested bacteria were susceptible to yarrow oil extract at its higher concentration of 0.5%. *Streptococcus salivarius* was less susceptible, with an inhibition zone reaching 11 mm, followed by *Staphylococcus epidermidis* with an inhibition zone of 13 mm. The yarrow oil extract showed higher antibacterial activity against *Staphylococcus aureus*, with an inhibition zone reaching 21 mm. An ethanol extract of the *Achillea millefolium* aerial parts was previously tested for antimicrobial activity against *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Candida albicans*. The highest MIC value of 62.50 mg/mL was observed against *Bacillus cereus* and *Salmonella enteritidis*, with no activity observed in the other three tested strains.

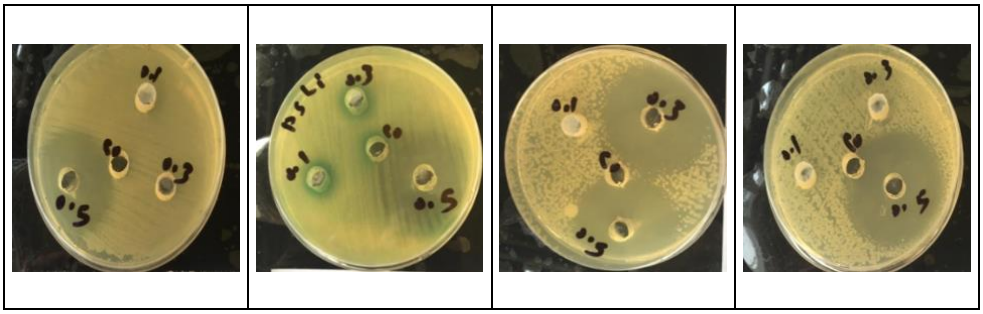

Figure 1. Antibacterial activity of the yarrow extract against various bacterial species at different concentrations.

4. CONCLUSION

A total of 19 compounds were discovered in our GC-mass spectrometry analysis of *Achillea millefolium*. The most common components were Artemisia ketone (15.04%), camphor (10.27%), linalyl acetate (12.08%), 1,8-cineole (11.08%), and D-limonene (7.51%).

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CONFLICT OF INTEREST STATEMENT

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Management of pharmaceutical waste in the Babylon Governorate

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Abstract

Globally, a massive amount of expired or leftover medications accumulates each year because of pharmaceutical overprescription, combined with overproduction. This pharmaceutical waste poses environmental, economic, and social/ethical challenges. The objective of this study is to understand societal behavior regarding the disposal of medications in the Babylon Governorate and develop a prototype of a knowledge-based system that promotes proper disposal of pharmaceutical waste. A two-phase cross-sectional study was carried out. The first phase involved interviewing pharmacists, while the second phase targeted the general population. A visit to Aljiumhori Hospital was made in order to assess the pharmaceutical waste disposal methods. The study found that most pharmacists (70%) and people (59.2%) prefer throwing expired medicine in the trash can. Moreover, 64.4% of the people participating in our study believe that placing unused drugs in special containers in each region is the best disposal method. Additionally, 48.2% of households are unaware of the environmental and health consequences of this waste. Pharmaceutical waste disposal in the Babylon Governorate is poorly managed. The absence of processes separating medical waste from general waste and the use of sanitary landfills as the sole method of disposal can pose serious environmental and public health risks. One can only address this issue with proper waste management, staff training, and protocol adherence.

KEYWORDS
waste management, pharmaceutical waste, disposal, public health, pharmacist

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1. INTRODUCTION

Pharmaceuticals are essential for human health, and their use continues to grow each year as new drugs are introduced into the market alongside existing products. This increasing volume of pharmaceutical usage raises concerns about its potential negative impact on healthcare workers, society, and public health. Research on the presence of pharmaceuticals in water has attracted significant attention over the past decade [1,2]. Pharmaceutical waste includes medications that have expired, have been unused (rejected by the patient), have been spilled, have become contaminated, or that are simply no longer needed. This category also includes prescribed and proprietary drugs, vaccines, and sera that require careful disposal due to their chemical or biological properties [3]. The way

pharmaceutical waste affects the environment depends on its toxicity, mobility, bioaccumulation, and persistence. Persistence includes factors such as transformation products, metabolites, solubility, and half-life. The adverse effects of toxicity can be seen at an individual level, in populations, or as a result of mixtures and additive effects. Pharmaceutical waste can end up in rivers, oceans, groundwater, soil, lakes, and sediments [4]. The main contributors of pharmaceutical chemicals in sewer systems are households and hospitals. Health facilities often dispose of items such as IV bags and syringes down the drain, while pharmaceutical waste discarded in landfills can leach into sewer systems and groundwater [5].

Various methods for the safe disposal of pharmaceuticals are outlined by the World Health Organization, focusing on the minimizing of risks to public health and the environment. These methods are suitable for countries with limited resources and equipment. If Ministries of Health, Environment, and other relevant authorities adopt and implement these guidelines, it will lead to a safe and cost-effective elimination of stockpiles of unusable pharmaceuticals. High-temperature incineration with proper flue gas cleaning is considered the most environmentally sound method for pharmaceutical destruction. However, many countries lack the infrastructure for this type of facility. As a result, these guidelines offer practical interim alternatives to aid those responsible for the safe disposal of unused pharmaceuticals. While the current guidelines suggest slightly less safe treatments and disposal methods, they are still acceptable when compared to the risks associated with improper or non-disposal. It's important to note that disposal options can differ widely depending on the circumstances, and the best solution may not always be feasible [6]. The global management of pharmaceutical waste has become a significant challenge due to the lack of take-back programs in many countries, leading to unsafe disposal practices. Additionally, public unawareness and improper use of medications by patients are major contributors to the accumulation of pharmaceutical waste [7]. Pharmacists, as trusted and accessible drug information resources, are well aware of the limited value of many medications. They should take on the responsibility of transforming the entire medication use process, seeking solutions, and minimizing the environmental impact of pharmaceuticals. Pharmacists are involved in every stage of the medication process, including prescribing, advising, dispensing, providing pharmaceutical care, and disposing of expired medicines, with the ultimate goal of reducing the discharge of metabolic waste into the environment. Therefore, pharmacists should identify and address any excessive or unnecessary prescribing so as to minimize leftover medications that may be improperly disposed of. They should also encourage and monitor patient adherence to ensure that all prescribed medications are used appropriately [8]. Pharmacists have a responsibility to prevent the disposal of pharmaceuticals through open dumpsites, burning, or insecure landfills, as these practices pose a significant risk to public health. They should educate relevant authorities about the financial implications of proper disposal, the various available options for disposal, and the potential for outsourcing disposal services [9]. Finally, pharmacists need to be well-informed about local drug disposal initiatives so as to educate the public, and contribute to minimizing the impact of pharmaceutical waste on the environment and well-being [10].

2. MATERIALS AND METHODS

This study aims to assess the pharmaceutical waste management in the Babylon Governorate, serving as a model for Iraq. It focuses on waste isolation, disposal methods, and environmental challenges faced by healthcare institutions. The study also aims to identify sustainable practices and innovative waste treatment technologies, and to promote awareness among healthcare professionals, consumers, and policymakers. To achieve this, a two-survey study was conducted. The first survey targeted the general public in order to assess their awareness of the risks associated with these types of waste and of the most common methods used to dispose of them. A total of 233 people participated in this questionnaire, with the majority completing it electronically, while some filled it out on paper. The second survey targeted pharmacists by assessing their awareness and determining the fate of expired and surplus medications in pharmacies. A total of 50 pharmacists participated in filling out the paper questionnaire. A visit to the Aljiumhori Hospital was also carried out in order to assess the hospital's pharmaceutical waste disposal methods.

3. RESULTS

The most common disposal method of expired medicines among pharmacists (70%) and the general population (59.2%) was to simply use a trash can. About 20.3% of the general population did not know that pharmaceutical waste disposal should be managed, while 44.8% knew it should be managed but were unaware of how to do it. We found that 44% of the pharmacists attributed the respon-

sibility of raising awareness about proper disposal of pharmaceutical waste to the Ministry of Health. Less than half (41%) of the general population's respondents did not have adequate information about the harmful effects of this waste on the environment. Most respondents (59%) of the general population kept the unused medication at their homes. The majority of pharmacists (92%) check the expiry date of the medicine before purchasing it. However, 74% of the pharmacists reported that some quantities of purchased medicine remains unused in the pharmacy. The study found that the Aljiumhori Hospital does not separate pharmaceutical waste from regular waste, leading to its disposal in landfills. Table 1 provides a detailed account of the general population's behavior, awareness, and attitude toward pharmaceutical waste.

Table 1. Synopsis of the respondents' knowledge of pharmaceutical waste management in the Babylon Governorate.

General Population Questionnaire					
Age (in years)					
18–25	26–30	31–39	40–49	50–59	>60
68%	11%	8%	3%	5%	5%
Sex					
Male			Female		
37%			63%		
Job					
Student	Employee		Housewife		Earner
55%	29%		5%		11%
Storage of medications					
Specific Space/ Home Pharmacy		Several Places		In Kitchen Cabinet	
54%		36%		10%	
Do you keep expired medications?					
Yes			No		
93%			7%		
Do you have medications that you no longer use?					
Yes			No		
59%			41%		
Do you have information that this medication should be disposed of?					
I know and I know how to manage it		I know, but I do not know how to manage it		I do not know at all	
35%		45%		20%	
How do you dispose of unused drugs?						
Throw them in the garbage	Throw them in the toilet or sink	Burn them	Return to pharmacy	Give it to a friend/ other people	keep them in-house to use when needed	Special containers in our region
59.2%	4.7%	4.3%	7.3%	2.6%	36.9%	1.7%
Does the pharmaceuticals unit in your region collect expired / unused medications?						
Yes			No			
83%			17%			
How well-informed do you consider you are about the hazards that these wastes represent?						
Pretty informed		Little informed		Not at all informed		
58%		41%		1%		
In your opinion, what is the most appropriate method to dispose of unused drugs?						
Do not know	Throw them in the garbage	Keep them in-house to use when needed	Burn them	Return them to the pharmacy	Use special containers in your region	
2.5%	2.4%	1.6%	7.7%	27%	64.4%	

4. DISCUSSION

Improper disposal of pharmaceutical waste can have several detrimental effects; therefore, waste management should be thoroughly planned, implemented, and maintained. Our study shows that the Babylon Governorate's population has inadequate information regarding the disposal of pharmaceutical waste. Improvement is required in both the knowledge and the practice of disposal of unused and expired medications. As a result, there is a need for information campaigns aiming to educate the population about the secure disposal of pharmaceutical waste. Unsurprisingly, 82.8% of the population reported that their local pharmaceutical unit does not collect expired or unused medications from citizens. The complex procedures, the lack of time, the absence of a legislation for collection, and high costs are the primary reasons for which some pharmacists decline to accept unused drugs from the population. Implementing special containers in pharmacies for the collection of pharmaceutical wastes is advisable, as 64.4% of the people who participated in our study believe that placing unused drugs in special containers in each region is the best disposal method. Furthermore, it is crucial to incorporate this crucial topic into the pharmacy syllabus, thereby taking a practical approach that reflects the current demand. Pharmacists (68%) in our study agreed that "drug take-back" programs should be mandatory. Returning leftover and expired medicine through such programs is the safest and most environmentally sound disposal method. Whenever possible, it is preferable to return the medicine to the manufacturer, as they are likely to have effective disposal methods in place.

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CONFLICT OF INTEREST STATEMENT

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Impact of residence on the association between benzo[*a*]pyrene-DNA adduct levels and CYP1B1 gene polymorphisms in breast cancer patients

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Abstract

Globally, breast cancer is the primary cause of cancer-related death, and rising incidence rates are anticipated. Improving illness prevention and treatment strategies requires a better understanding of the interactions occurring between genetic variables, environmental exposures, and disease pathogenesis. This study investigated the impact of residence on the association between benzo[*a*]pyrene-DNA adduct levels and CYP1B1 gene polymorphisms in breast cancer patients. In brief, 58 female breast cancer patients in Babylon, Iraq were recruited as subjects of this cross-sectional study. We gathered clinical information (including residency, age, age at diagnosis, and haematological markers), and by using molecular and biochemical methods, the *CYP1B1* polymorphisms and the benzo[*a*]pyrene-DNA adduct levels were assessed. Among the different types of breast cancer, there was no apparent association between the residence and *CYP1B1* polymorphisms. However, the amounts of benzo[*a*]pyrene-DNA adduct varied according to where a patient lived, with urban residents showing higher concentrations than rural residents. Benzo[*a*]pyrene-DNA adduct levels were shown to be correlated with specific polymorphisms in the CYP1B1 gene. Our study highlights the intricate connections between environmental exposures, genetic variables, and place of residency in the aetiology of breast cancer. Variations in quantities of benzo[*a*]pyrene-DNA adducts imply possible functions for environmental carcinogens, although no substantial correlation was found between genetic polymorphisms and the place of residence.

KEYWORDS

CYP1B1 gene polymorphisms, breast cancer, benzo[*a*]pyrene-DNA adduct concentrations, genetic susceptibility, residence

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1. INTRODUCTION

Breast cancer (BC) accounts for about one-third of cancers occurring in women that have been re-

ported to the Iraqi Cancer Registry, and represents the commonest cause of cancer-related fatalities worldwide (surpassing both prostate and lung cancers). It is anticipated that the incidence rates will rise globally [1,2]. New lifestyle and fertility patterns, particularly in less developed countries, impact this rise in addition to improved detection. In developed nations, the incidence of BC is notably higher. Significant differences in screening, treatment access, and supportive care still exist between nations, despite advances in medical care [1]. Genes such as *XRCC1*, *OGG1*, *CYP1A1*, and *MTHFR* have been associated with increased risk of developing BC; however, their mutation frequency in established BC risk genes is insignificant [3].

Cytochrome P450 enzymatic activity may be impacted by *CYP1B1* polymorphisms, especially in exons 2 and 3, that could alter sensitivity to environmental carcinogens [4]. Nevertheless, conflicting findings have been reported concerning the connection between *CYP1B1* polymorphisms and BC [5]. Taking residency status and genotype-allele frequency connections into account, this study aimed to examine the relationship between *CYP1B1* polymorphisms and BC in Babylon, Iraq.

2. PATIENTS AND METHODS

A cross-sectional study was carried out in 2023, and included 58 female BC patients, aged 25 to 60 years, mostly from the Marjan Hospital in Babylon. Medical history, clinical data, and participant demographics were gathered. According to accepted practices, the oncologist identified, managed, and tracked these patients. Various types and stages of BC were represented in the cohort. Those who were smoking, drinking alcohol, nursing a baby, or had a severe disease were among the excluded patients.

In order to extract DNA and to genotype *CYP1B1* polymorphisms, venous blood samples were obtained. An hematology analyzer and a human benzo[*a*]pyrene-DNA adduct detection kit for ELISA were used in our analyses. For the molecular genotyping of *CYP1B1*, 500 µL of frozen blood were subjected to DNA extraction. The polymerase chain reaction - restriction fragment length polymorphisms (PCR-RFLP) approach was employed in order to genotype rs1800440 polymorphisms of the CYP1B1 gene. PCR primers' design and restriction enzyme selection were carried out by the aid of NCBI-primer BLAST and Wat Cut online software, respectively. The selected primers (forward: 5-TCATTTTCGCAGGCTCATTTGG-3; reverse: 5-AGTGGCCTAACCCGGAGAA-3) were used to amplify 213 bp around the targeted polymorphisms. The PCR conditions were first optimized by gradient annealing temperature (55°C–65°C), and 10 µL of PCR product were subjected to overnight restriction analysis by 5 units of the BstMW I enzyme (SibEnzyme, Russia). The PCR product and the restriction reaction product were then resolved on 2% agarose and stained by ethidium bromide. The CC genotype produced 155- and 58-bp restriction fragments, the TC genotype produced 213-, 155-, and 58-bp restriction fragments, while the TT genotype produced the original amplicon of 213-bp fragments [6].

The statistical calculations for this study were carried out by using the IBM 2017 SPSS program (version 21.0). The data were displayed as mean ± standard error, and a statistically significant *P*-value was defined as being 0.05 or less. In order to determine whether there were any significant differences, the Student's *t*-test was employed.

The principles outlined in the Helsinki Declaration were strictly adhered to, and before beginning the study and after, patients were fully informed about it, and every participant provided verbal consent for her participation. Both the Marjan Hospital (Babylon Health Directorate) and the College of Pharmacy (University of Babylon) Institutional Review Board have authorized the study's protocol.

3. RESULTS

The characteristics and the benzo[*a*]pyrene-DNA adduct levels in female BC patients are presented in Table 1, according to their *CYP1B1* genotype and their place of residence (rural *versus* urban). White blood cells' count, platelets' count, packed cell volume percentage, haemoglobin levels, the concentration of benzo[*a*]pyrene-DNA adduct (ng/mL), and the age at diagnosis are among the variables assessed. Although statistical significance varied, there seem to be some overall differences in these variables across different categories. As an example, the concentration of benzo[*a*]pyrene-DNA adduct was found to be significantly higher in urban areas than in rural ones (2.7 ng/mL *versus* 1.9 ng/mL; *P*=0.015). Similarly, although statistical significance was not reached, the platelet count was found to be much greater in patients from urban than those from rural locations (283.8×10^9/L *versus* 250.4×10^9/L).

4. DISCUSSION

This study aimed at examining the relationship between benzo[*a*]pyrene-DNA adduct levels in BC patients and particular genetic polymorphisms in the CYP1B1 gene, after taking into account the residential location (rural *versus* urban) of the patients in order to understand the interaction be-

tween genetics, residency, and benzo[*a*]pyrene-DNA adduct levels in BC. The findings indicate that there is no significant relationship between residence and *CYP1B1* polymorphisms; however, urban residents exhibited greater quantities of benzo[*a*]pyrene-DNA adduct.

Table 1. Differences in benzo[*a*]pyrene-DNA adduct levels and other variables of clinical importance in Iraqi women with breast cancer according to their residence and *CYP1B1* genotype.

Variables	Residence / *CYP1B1* genotype	N	Mean	Standard error	*P*-value
Concentration of benzo[*a*]pyrene-DNA adduct (ng/mL)	Rural	21	1.9	0.03	0.015
	Urban	37	2.7	0.31	
	CC	27	2.5	0.4	>0.05
	CT	25	2.3	0.2	
	TT	6	2.3	0.2	
Haemoglobin (g/dL)	Rural	21	11.0	0.4	>0.05
	Urban	37	11.1	0.2	
	CC	27	10.9	0.2	>0.05
	CT	25	11.1	0.3	
	TT	6	11.6	0.6	
Packed cell volume (%)	Rural	21	33.2	0.9	>0.05
	Urban	37	34.3	0.6	
	CC	27	33.6	0.7	>0.05
	CT	25	34.1	0.9	
	TT	6	34.3	1.8	
White blood cells ($\times 10^9$/L)	Rural	21	6.3	0.7	>0.05
	Urban	37	6.9	0.5	
	CC	27	6.5	0.5	>0.05
	CT	25	6.9	0.7	
	TT	6	6.7	0.8	
Platelets ($\times 10^9$/L)	Rural	21	250.4	25.2	>0.05
	Urban	37	283.8	17.0	
	CC	27	247.1	17.8	>0.05
	CT	25	276.1	21.9	
	TT	6	364.2	54.7	
Age at diagnosis (years)	Rural	21	49.5	1.6	>0.05
	Urban	37	47.9	1.5	
	CC	27	46.9	1.9	>0.05
	CT	25	49.3	1.5	
	TT	6	52.8	3.0	

Elevated benzo[*a*]pyrene-DNA adduct levels have been associated with specific CYP1B1 gene variations, suggesting a possible genetic susceptibility to environmental carcinogens [3]. The cytochrome P450 superfamily, which includes CYP1B1, metabolizes a variety of drugs and affects the course of the development and of the chemotherapy of cancer [7]. For example, the metabolism of oestrogen by CYP1B1 increases the risk of BC [8].

Tumorigenesis is associated with dysregulated cellular proliferation, specifically in BC, which is caused by an overexpression of CDC20 [9]. Variations in disease susceptibility are influenced by genetic variants of *CYP1B1*, with varying outcomes noted in various groups and geographical areas [10]. Research indicates that in order to improve precision medicine and customize treatment plans, more research is required into the molecular mechanisms and epidemiological aspects of *CYP1B1* polymorphisms in BC [5]. Recognizing this function could increase the effectiveness of treatment and reduce side-effects, thereby highlighting the significance of tailored therapeutic approaches in the treatment of BC.

5. CONCLUSION

No considerable correlation between *CYP1B1* pol-

ymorphisms and place of residence (rural *versus* urban) was identified, despite examining a heterogeneous sample. On the other hand, the amounts of benzo[*a*]pyrene-DNA adduct varied according to where a person lived, with urban residents showing higher concentrations than rural ones. Moreover, benzo[*a*]pyrene-DNA adduct levels were correlated with specific polymorphisms in the CYP1B1 gene. These results highlight the intricate interactions that exist between environmental exposures, genetic variables, and the pathophysiology of BC. In order to clarify the underlying causes and consequences of customized treatment approaches, more research is necessary. However, our findings align with recent data from *in vitro* and animal studies suggesting that CYP1B1 may play a significant role in the pathogenesis of BC.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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Cytotoxic effect of silver nanoparticles biosynthesized from *Hirudo medicinalis* saliva on HepG2 cells

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Abstract

Primary hepatocellular carcinoma is a devastating type of liver cancer. Silver nanoparticles (AgNPs) have been assessed for a variety of purposes, including being tested as an anticancer agent. The aim of this study was to assess the cytotoxicity of AgNPs that were biosynthesized from leech saliva on HepG2 cells, through the undertaking of a simple MTT assay. HepG2 cells were obtained from the cell bank of the Pasteur Institute of Iran. In this study, AgNP-treated HepG2 cells were cultured at a density of 10^4 cells per well, and 100 µL of MTT at a concentration of 0.5 mg/mL were added to each well; the treated cells were then let to incubate for 4 h. Subsequently, a plate reader device operating at a wavelength of 570 nm was used in order to determine the concentration of the chemical dissolved in isopropanol. Representative images of the cells show remarkable changes in their morphology at AgNP concentrations of 25 and 50 µg/mL. At 48 h, the nanoparticle's IC_{50} value was 50 µg/mL. Our study shows that leech salivary extract-derived AgNPs are cytotoxic to HepG2 cells.

KEYWORDS

MTT assay, LSE-AgNPs, *Hirudo medicinalis*, HepG2 cells

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1. INTRODUCTION

The green synthesis of silver nanoparticles (AgNPs) has become one of the most promising methods of nanoparticle synthesis. Because of their strong antibacterial activity, AgNPs have been used in a wide range of applications, such as an anticancer agent and in wound dressing [1]. It is becoming necessary to obtain a better understanding of the toxicity of AgNPs and their potential mechanism of toxicity [2], as their biological method of synthesis is the least complicated, environmentally friendly, and commercially viable [3].

Hepatocellular carcinoma is the most common primary liver malignancy, with an increasing global incidence [4]. This is why we, herein, chose to assess the toxicity of AgNPs against HepG2 cells; a human hepatoma cell line widely used in hepatotoxicity studies. The toxicity mechanism of AgNPs is mainly attributed to the AgNPs' ability to release a large amount of reactive oxygen species (ROS)

that damage the cell membrane and lead to cell apoptosis [2].

2. MATERIALS AND METHODS

The production of AgNPs from leech salivary extract (LSE) was undertaken by using the method described by Jaganathan *et al*. [5]. Subsequently, different concentrations of LSE-AgNPs (0, 25, 50, and 100 µg/mL) were made using Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS), in order to study the toxicity of the nanoparticles and their impact on the growth and proliferation of the cells [6]. Finally, the MTT assay was performed as previous described in detail [7].

3. RESULTS

Characteristics of the produced LSE-AgNPs: The precipitates of these were dark brown in colour. The LSE-AgNPs' average size was measured by using a dynamic light scattering test and their zeta potential (measured at 649.1 nm) was found to be -0.060. Moreover, field emission scanning electron microscopy revealed that our samples included particles that were evenly scattered and nearly square in shape, ranging in size from about 20 to 720 nm with an average size of 600 nm.

Quantitative toxicity test (MTT assay): Cell death caused inhibition of the biochemical reaction and formation of purple formazan. The biochemical enzymatic cascade in live cells triggered a reduction of the substrate and changed the colour of the wells to purple (positive control). The MTT assay was performed on four determined nanoparticle concentrations (0, 25, 50, and 100 µg/mL) at three time different timepoints (24, 48, and 72 h). The viability of the HepG2 cells was found to be reduced after the interaction with various concentrations of the LSE-AgNPs. This reduction increased gradually with the increase of the concentration and the time of the cells' exposure to LSE-AgNPs. At 48 h, the nanoparticles' IC_{50} was 50 µg/mL (Table 1). These results reveal the significant differences in the % viability between non-treated HepG2 cells (control) and HepG2 cells treated with LSE-AgNPs, the significant differences in the % viability between the HepG2 cells that were exposed to the same concentration of LSE-AgNPs (25, 50, 100 µg/mL) between 24 and 48 h, and the absence of significant differences among the HepG2 cells between 48 and 72 h, and the absence of significant differences among HepG2 cells treated with different concentrations of LSE-AgNPs for the same duration.

Morphological changes in the HepG2 cells after an exposure to LSE-AgNPs: The HepG2 cells' morphology was observed under light microscopy after an incubation of 24, 48, and 72 h with the AgNPs at concentrations 0, 25, 50, and 100 µg/mL. Representative images reveal remarkable morphological changes, indicating the presence of unhealthy cells due to the exposure to AgNPs. As the AgNPs' concentration and exposure time increase, the exposed cells appeared to be clustered with a few cellular extensions, and cell spreading patterns were restricted with the observation of a few floating cells (as compared with the control cells). The morphology of cells between 24 and 48 h was greatly affected.

Table 1. The effect of different concentrations of leech salivary extract (LSE)-derived silver nanoparticles (AgNPs), that have been biosynthesized from *Hirudo medicinalis*, on the % viability of HepG2 cells.

	Period of exposure (B)		
Concentration of LSE-AgNPs (A)	**24 h**	**48 h**	**72 h**
Control	100±0.0	100±0.0	100±0.0
25 µg/mL	4.35±0.82	2.14±0.13	1.84±0.01
50 µg/mL	3.57±0.14	2.05±0.07	1.51±0.13
100 µg/mL	3.21±0.11	1.80±0.02	1.48±0.25

Note: values represent means ± standard deviations; LSD (p<0.05) (A*B)=1.217.

4. DISCUSSION

Hirudo medicinalis is one of the species that is most commonly used as a model in medicine. Although this organism secretes more than 100 different substances, only a small number of them exhibit potent anti-inflammatory, antibacterial, analgesic, anticoagulant, and anticancer activities [8]. The LSE is not pure and contains many factors [9]. The average size of the nanoparticles and par-

ticles, which ranged from 20 to 720 nm, was 600 nm. The leech saliva is abundant in protein, vitamins, amino acids, antioxidants, and other nutrients that have an important effect in lowering the amount of LSE-AgNPs produced [9].

The *in vitro* cytotoxic effects of the LSE-AgNPs on the HepG2 cells was assessed by the herein undertaken MTT assay. The IC_{50} of LSE-AgNPs was 50 µg/mL at 48 h. According to an earlier study [5], the earthworm-mediated AgNPs' IC_{50} was found to be 25.96 µg/mL, which is within the accepted range of activity. The active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, nitrogen bases, and phosphate groups in DNA is what causes AgNPs to exert cytotoxic effects, the production of ROS, and the increase of intracellular oxidative stress, which in turn triggers cell death processes like apoptosis and necrosis [10]. According to Supraja *et al*. [10] three concentrations of AgNPs over three times, resulted in somewhat greater morphological changes in AgNP-treated cells. Our results indicate good cytotoxic activity against cancer cells. Some of the chemotherapy drugs that were authorized had serious side effects. Therefore, there is a critical need to create alternative medications to combat this devastating illness. The use of LSE-AgNPs, which seem to be efficient cytotoxic agents against HepG2 cells, could be a promising step forward.

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Network pharmacology and molecular docking reveal the mechanisms of action of *Panax notoginseng* against post-COVID-19 thromboembolism

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Abstract

Panax notoginseng (PNGS) is a potent folk therapy for blood-related diseases. However, further research is required to fully elucidate the mechanisms of its pharmacological activities and to explore its therapeutic potential for treating thromboembolism (TE) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study aimed at analysing the molecular mechanisms of PNGS and at clarifying their potential role in treating TE induced by COVID-19, by employing network pharmacology and molecular docking. To this end, a network pharmacological approach was combined with expression profiling by high-throughput sequencing of GSE156701 so as to elucidate the compound constituents of PNGS for treating TE caused by SARS-CoV-2 at a systemic level. Protein-protein interaction network, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes analyses were employed in order to decipher the associated drug-target interactions. The integration of these results suggested that five targets, including the angiotensin-converting enzyme (ACE), the coagulation factor III (F3), interleukin-1 beta (IL-1β), the mitogen-activated protein kinase 1 (MAPK1), and the plasminogen activator inhibitor-1 (SERPINE1), represent major genes involved in thromboembolism. The data suggest that PNGS exerts collective therapeutic effects against TE caused by SARS-CoV-2, and provides a theoretical basis for further laboratory study of the active drug-like ingredients and the potential mechanisms of PNGS in TE treatment.

KEYWORDS

Panax notoginseng, SARS-CoV-2, post-acute COVID-19, thromboembolism, network pharmacology

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1. INTRODUCTION

While the containment of coronavirus disease 2019 (COVID-19) through the provision of various vaccines and the rise of herd immunity is ongoing, the understanding and management of the long-term sequelae of COVID-19 in survivors of acute disease has become a focal point of research. Many patients experience persistent or prolonged effects. Indeed, post-acute COVID-19 can be defined as persistent symptoms and delayed or long-term complications beyond four weeks from the onset of the symptoms. Following six months of the disease, the risk of thromboembolism (TE) in hospitalized COVID-19 survivors is sevenfold more significant than that of non-hospitalized COVID-19 patients. At the same time, those in the intensive care unit face an eighteen-fold higher substantial risk than all other survivors [1]. In addition, various adverse events following vaccination have been reported, ranging from mild symptoms to more specific organ failures. Post-vaccine thrombosis, including cerebral venous thrombosis, has become a major concern in patients, particularly women, who have received adenovirus-based vaccines [2]. *Panax notoginseng* (Burk) F. H. Chen (PNGS), as a traditional Chinese medicine, has been documented to possess anti-inflammatory, anti-oxidative, inhibitory of platelet aggregation, regulatory of blood glucose and blood pressure, inhibitory of neuronal apoptosis, and neuroprotective properties [3]. Ginseng and its major active constituents, ginsenosides and saponins, are known to improve the immune system and exert anti-inflammatory effects by targeting the inflammasome stimulation [4]. This study aimed at analysing the molecular mechanisms of action of PNGS and at clarifying their potential role in treating TE induced by COVID-19, by using network pharmacology and molecular dynamics.

2. MATERIALS AND METHODS

This study explores the molecular mechanisms underlying the effects of PNGS on COVID-19-associated TE, by employing network pharmacology and molecular docking. Furthermore, it provides a new strategy for developing a novel way to alleviate thromboembolism. To this end, a network pharmacological approach was combined with expression profiling by the high-throughput sequencing of GSE156701, so as to elucidate the compound constituents of PNGS for treating TE caused by SARS-CoV-2 at a systemic level.

3. RESULTS

Target fishing resulted in 508 targets related to 14 bioactive PNGS compounds. The overlapping targets between the PNGS-related and the TE-dysregulated genes resulted in a drug-disease target set with 51 genes (Figure 1). These overlapping genes were then used in order to perform herb-compounds-targets-disease network, protein-protein interaction network, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The integration of these results suggested that five targets, including the angiotensin-converting enzyme (ACE), the coagulation factor III (F3), interleukin-1 beta (IL-1β), the mitogen-activated protein kinase 1 (MAPK1), and the plasminogen activator inhibitor-1 (SERPINE1), represent major genes involved in TE. These targets involve more than four pathways, including the AGE/RAGE signalling pathway in diabetic complications, the fluid shear stress and atherosclerosis pathway, the complement and coagulation cascades, and COVID-19. Molecular docking and molecular dynamics simulation were applied so as to validate these results, indicating a stable combination between two compounds (quercetin and ginsenoside) and five targets (ACE, F3, IL-1β, MAPK1, and SERPINE1) with the following binding affinities being some examples: (i) ginsenoside-ACE: -9.7 kcal/mol, (ii) quercetin-SERPINE1: -8.7 kcal/mol, (iii) quercetin-MAPK1: -8.5 kcal/mol, and (iv) quercetin-ACE: -8.3 kcal/mol.

4. DISCUSSION

Based on our analysis, the major PNGS targets are ACE, MAPK1, and SERPINE1. ACE is part of the renin-angiotensin-aldosterone system, which regulates blood pressure. The S proteins of SARS-CoV2 allow the virus to enter the cells by interacting directly with ACE2. As such, ACE2 is arguably one of the primary targets for drugs aiming to exert therapeutic effects on COVID-19 [5]. MAPK1 participates in the MAPK and PI3-Akt signalling pathways. This might suggest that PNGS may target COVID-19-induced TE *via* a direct inhibition of MAPK1, thus suppressing the PI3K-Akt and MAPK signalling pathways [6]. This also suggests potential novel therapeutic targets for long COVID-19. SERPINE1, a serpin peptidase inhibitor, encodes for the plasminogen activator inhibitor 1 (PAI-1), which plays an integral role in COVID-19-induced endothelial dysfunction and thrombosis [7]. It has been reported that the PAI-1 levels are high in COVID-19 patients when compared to those of healthy individuals.

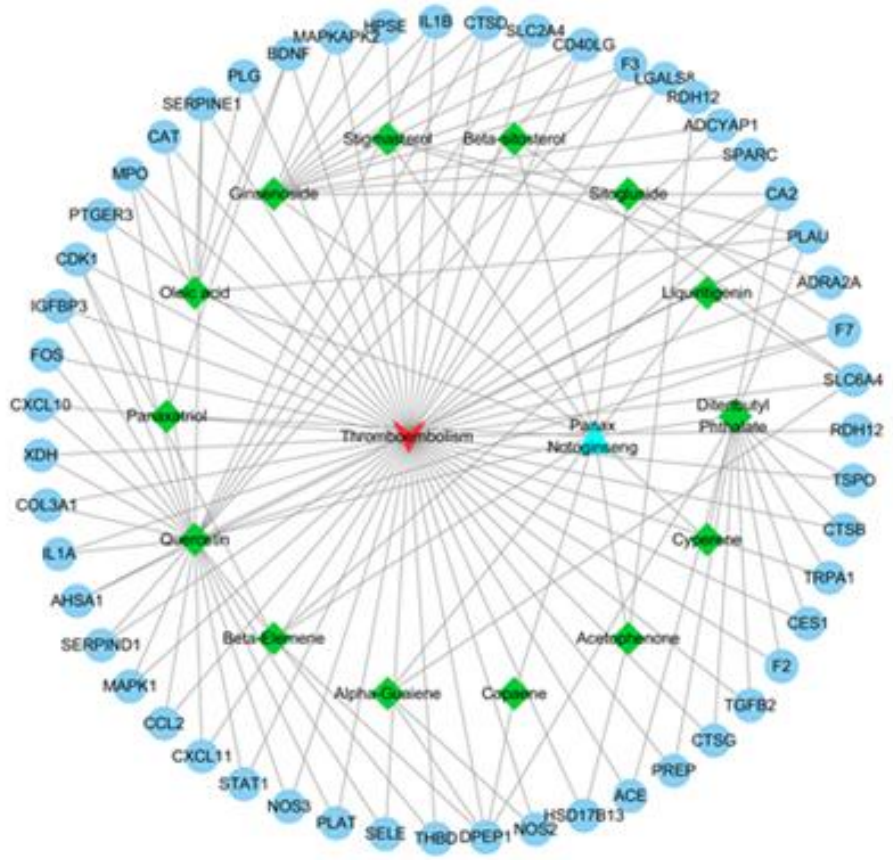

Figure 1. The overlapping gene symbols between the drug and the disease, and the herb-compounds-targets-disease (HCTD) network of *Panax notoginseng*. The HCTD visual network explained: green and blue nodes stand for bioactive components and related targets, respectively; azure nodes stand for targets; red nodes stand for disease.

Moreover, these patients have a strong correlation between their PAI-1 levels and their neutrophil activation, respiratory distress, and mortality rate. Therefore, both the efficacy and the safety of thromboprophylaxis by natural products in post-COVID-19 survivors depend on an enhanced understanding of the pathophysiology and the mechanisms of such complications. Our results have confirmed the top two out of 14 bioactive components in PNGS to be “quercetin” and “ginsenoside”. Ginsenoside demonstrates blood pressure-regulating activity with little or no effect on respiration. Ginseng saponin shows vasoactive results *via* the releasing of nitric oxide [8]. One proposed anti-COVID-19 mechanism of ginsenosides is *via* the suppression of the inflammasome activation. Thus, they facilitate the host’s immune status and exert anti-inflammatory activities [4]. Moreover, ginsenosides have been reported to reduce the cardiovascular insults associated with COVID-19. They maintain blood pressure, minimize the incidence of myocardial infarction, heart failure, and arrhythmia, and they also improve vascular and cardiac integrity [9]. Quercetin, on the other hand, has antiviral activity against many enveloped viruses, including the meningitis and the herpes simplex viruses. It can inhibit the H^+-ATPase of the lysosomal membrane, thereby preventing virus coat removal. In addition to its antioxidant and anti-inflammatory activities, it inhibits the expression of the human ACE2 receptors and SARS-CoV-2 enzymes, including MPro, PLPro, and RdRp [10].

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Regulatory B-cells: immunomodulating mechanisms and important cellular targets underlining immunotherapy by immunoregulation

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Abstract
Regulatory B-cells (Breg cells) represent as an important modulator of the immune system and their role is unique in autoimmunity, infection, tolerance to transplants, allergy, and cancer. Several regulatory mechanisms exist by which Breg cells can control the function of other immune cells through two main pathways: the secretion of soluble molecules and the use of cell surface-expressed molecules. Anti-inflammatory cytokine interleukin-10 acts as the hallmark of Breg cell function; other cytokines with a similar role include the transforming growth factor-beta and interleukin-35. Breg B cells also release the cytotoxic granzyme B that mediates cell apoptosis. Cell surface-expressed proteins include FasL, CD80, CD86, CD73, CD1d, and PD-L1. The present article reviews the immunosuppressive pathways in order to understand how they emerge and are induced to evoke their regulatory activities, and how we can benefit from them in the field of immunotherapy.

KEYWORDS
immunosuppressive mechanisms, regulatory B-cells, IL-10, TGF-β, PD-L1

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1. INTRODUCTION

B-cells do not only act as antibody producers and antigen-presenting cells (APCs), but also act as positive regulators of immune responses. Many previous studies have shown that certain subsets of B-cells have a regulatory function and that these subsets of B-cells could suppress the immune reaction independently of the antibody production. These cells can be divided into many subsets differing in phenotype, location, and functional style; all of them can inhibit the progression and/or enhance the recovery from acquired immune-mediated inflammatory responses and can induce immune tolerance [1]. The regulatory function of the regulatory B-cells (Breg cells) may be achieved through a variety of mechanisms that involve multiple soluble effectors: (i) through the production of immunoregulatory cytokines such as interleukin (IL)-10 (IL-10), the transforming growth

factor-beta (TGF-β), and IL-35, which have bidirectional effects on the immune cells with both pro-inflammatory and immunosuppressive activities, and (ii) through the secretion of immunoregulatory enzymes such as granzyme B and indoleamine 2,3-dioxygenase (IDO/IDO1). A direct cell-cell contact mechanism is also used by Breg cells in order to suppress effector immune cells and promote immune tolerance *via* the expression of membrane proteins and/or receptors such as CD80/CD86, FasL (CD95L), PD-L1, GITRL, CD1d, T2-MZP, TIM-1, the ectoenzyme CD73, and CD39. Important cellular targets include CD4+ T-cells, CD8+ T-cells, regulatory T-cells (Treg cells), monocytes, natural killer (NK) cells, Th17 cells, APCs, and even effector B-cells [2].

2. SOLUBLE EFFECTOR-DEPENDENT MECHANISMS

Immunoregulatory cytokines: IL-10 is an important anti-inflammatory and immunosuppressive cytokine, and the most secreted effector across Breg cell subsets. IL-10 inhibits T-cell activation by inhibiting the expression of CD54 (ICAM-1; the ligand for LFA-1), CD80, and CD86, which function as important costimulatory molecules for T-cell activation. This leads to a reduction of the antigen-presenting capacity of monocytes. IL-10 inhibits the secretion of proinflammatory cytokines (IL-1, IL-6, IL-8, and of the granulocyte-macrophage colony-stimulating factor) from type-1 helper T-cells, monocytes/macrophages, and polymorphonuclear leukocytes. Furthermore, IL-10 downregulates class II major histocompatibility complex expression on monocytes, dendritic cells, and Langerhans cells. IL-10, in the absence of professional APCs, has direct effects on CD4+ T-cells by suppressing IL-2 and tumor necrosis factor-alpha (TNF-α) secretion [3]. In addition to inhibiting antigen-specific responses, IL-10 induces long lasting antigen-specific anergy in human CD4+ T-cells. IL-10 is also responsible for the lack of cytotoxic activity by specific CD8+ T-cell clones, in which the blocking of endogenous IL-10 production can restore their cytotoxic activity. NK cells secreting interferon-gamma (IFN-γ) are also inhibited because of the IL-10 double action: by inhibiting IL-12 (a NK-cell-stimulating factor) production by APCs, and through its direct action on NK cells [4] (Figure 1).

TGF-β suppresses the immune system through many pathways. TGF-β downregulates the activating receptor NKG2D, which is expressed on NK cells and CD8+ T-cells and is considered as the master switch in determining the activation status of NK cells. Moreover, TGF-β promotes the apoptosis of CD8+ T-cells during the clonal expansions that occur during and after infection. Lipopolysaccharide (LPS)-activated B cells (LPS-B cells) express a significantly higher level of TGF-β1 on their surface. These regulatory cells failed to induce significant levels of proliferation, cytokine secretion (IFN-γ, TNF-α, IL-2, and IL-6), and cytotoxic ability of CD8+T cells. This hypo-responsiveness of CD8+ T-cells when activated by LPS-B cells was significantly rescued by anti-TGF-β1 antibodies. TGF-β-expressing Breg cells were reported to trigger anergy in CD4+ and CD8+ T-cells. In addition to that, TGF-β alone could drive naive T-cells into inducible-Treg (iTreg) cells and enhance the differentiation of Th17 from CD4+ T-cells through the synergistic effect of both TGF-β and IL-6. TGF-β appears to block the activation of lymphocytes and monocyte-derived phagocytes, and to mediate effective class switching to IgA upon CD40 activation and in the presence of IL-10 (Figure 1).

IL-35 secreted by Breg cells is an anti-inflammatory cytokine with immunosuppressive activities that stimulates the expansion of IL-10+ Breg cells and the generation of IL-35-secreting B cells. It can also promote tumor growth and metastasis, and block the development of Th1 and Th17 cells by limiting early T-cell proliferation. Moreover, IL-35 has a role in infection and autoimmunity, and can lower the expression of the vascular endothelial growth factor and of TNF-α [5] (Figure 1).

Immunoregulatory enzymes: Breg cells, in addition to secreting immunoregulatory cytokines, secrete immunoregulatory enzymes such as indoleamine 2,3-dioxygenase (IDO/IDO1) and granzyme B. Granzyme B is a member of the cytotoxic serine proteases group that mediates target cell apoptosis upon entering the cytoplasm after perforin-mediated membrane disruption. Granzyme B also plays a role in controlling the inflammatory process, and it has a role in tissue remodeling by cleaving a number of extracellular matrix components. Secretion of IDO dampens T-cell activation by catabolizing the essential amino-acid tryptophan, and the secretion of granzyme B kills T-cells, while Breg cells that secret granzyme B can inhibit CD4+ T-cell proliferation and the responses of both Th1 and Th17 cells [4] (Figure 1).

3. CELL CONTACT-DEPENDENT MECHANISMS

A cell-to-cell contact-dependent mechanism involving membrane proteins and/or receptors is known to promote immune tolerance by activating cell death markers or costimulatory molecules involving CD80, CD86, CD40-CD40L, and FasL. It is known that the glucocorticoid-induced TNFR-related protein (GITR) is activated by its ligand

(GITRL). GITRL+ Breg cells promote Treg cell expansion *via* GITRL. CD1d+T2-MZP Breg cells support the development and function of immunosuppressive invariant NK cells through the presentation of lipids on CD1d. Moreover, the binding of TIM-1 on TIM-1+ Breg cells induces their expansion [6]. Like Treg cells, CD73+CD39+ Breg cells can convert immunostimulatory ATP and ADP into immunosuppressive adenosine *via* the ectoenzymes CD73 and CD39.

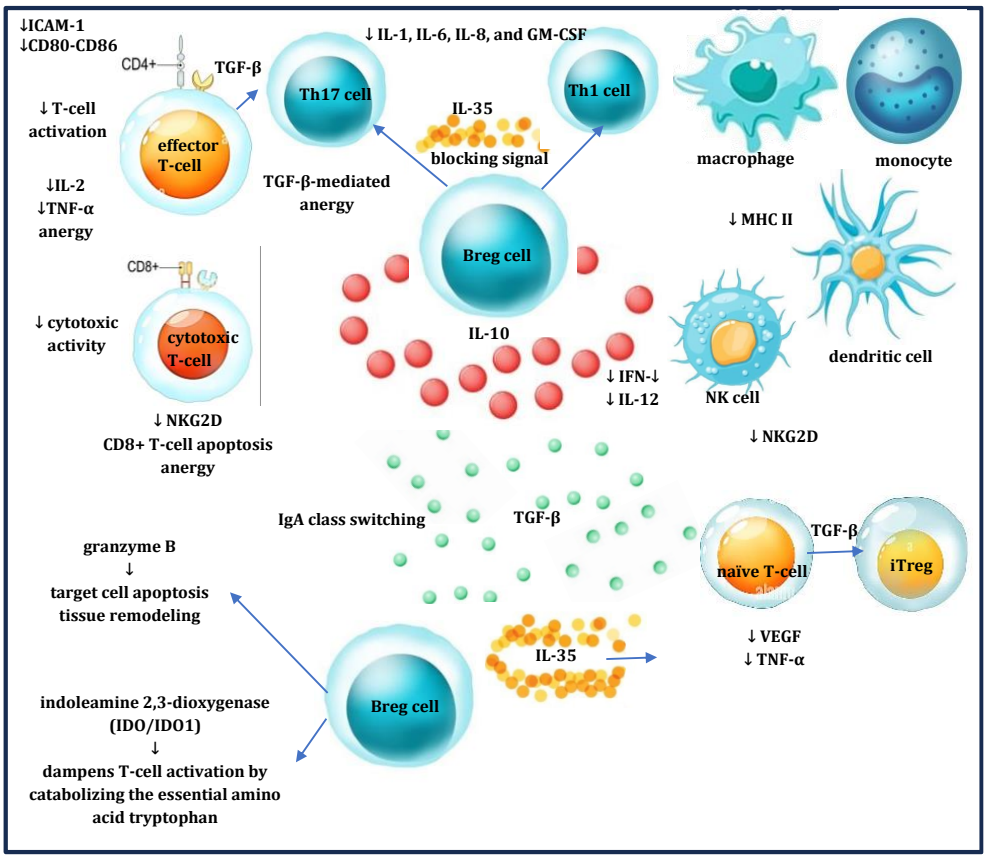

Figure 1. Common pathways of regulatory B-cell (Breg cell) soluble effector-mediated immune regulation. Abbreviations used: GM-CSF, granulocyte-monocyte colony stimulating factor; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MHC II, major histocompatibility complex type II; NK, natural killer; NKG2D, natural killer receptor group 2, member D; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

PD-1 is a type I transmembrane receptor expressed in activated monocytes, dendritic cells, NK cells, B-cells, and T-cells [7]. Through the binding of PD-1/PD-L1, PD-L2 delivers inhibitory signals that downregulate receptor-triggered cell survival, differentiation, and the secretion of proinflammatory cytokines. Studies have observed that PD-1+ and PD-L1+ Breg cells have a role in promoting IL-10 expression, suppress CD4+ T-cell activity and CD8+ T-cell cytotoxicity, induce Tr1 cells, dampen TNF production by macrophages and T-cells, and maintain peripheral tolerance [8].

4. REGULATORY B-CELL-BASED IMMUNOTHERAPY

The use of Breg cells in immunotherapy is still controversial and requires further research so as to realize a fully effectiveness of Breg cells in the field of immunotherapy. Breg cells can have multifaceted roles in tumor immunity, autoimmunity, and hypersensitivity. Breg cells help in clearing tumors by both direct and indirect mechanisms. The direct pathway is where B-cells differentiate into plasma cells and produce antibodies. Tumor-specific anti-

bodies against neoantigens are a powerful way to kill tumor cells in a specific manner, and they have considerably fewer side-effects. In the indirect pathway, B-cells help T-cells to carry out their anti-tumorigenic activity [9]. Infiltrating B-cells in a tumor can help CD4+ helper T-cells and CD8+ cytotoxic T-cells undergo activation and expansion. Breg cells acts as a form of cell immunotherapy for autoimmune disorders. What hasn't been clear to date, however, is how the Breg cell-induced IL-10 production and regulation of antigen-specific immune responses are controlled *in vivo* without inducing systemic immunosuppression. It has now been shown that the IL-10+ Breg cell expansion and maturation into functional IL-10-secreting effector cells that can inhibit autoimmune disease *in vivo* requires a stimulation by IL-21 as well as CD40-dependent cognate interactions with T-cells. These cells are produced in millions of copies and can be introduced back into someone with an autoimmune disease, thereby shutting down the disease [10]. In hypersensitivity, TGF-β+, IL-10+, and Foxp3+ Breg cell subsets seem to be able to negatively regulate allergic diseases, including contact dermatitis, asthma, anaphylaxis, and non-IgE-mediated food allergies related to atopic dermatitis.

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Characterization of promising smart nanopolymers that release drugs in response to illness-related signals: a review

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Abstract

Smart nanopolymers with the capability to release drugs on demand and in response to specific illness signals, represent a promising avenue in the field of drug delivery. Their synthesis and characterization process involves the careful design of nanopolymeric structures, incorporating stimuli-responsive elements. The responsiveness of these nanopolymers to specific illness signals is evaluated through *in vitro* studies that simulate physiological conditions. The potential of these nanopolymers is explored in the context of personalized medicine, where tailored drug delivery systems respond to individual patient needs. The characterization of these smart nanopolymers showcases their potential as a novel and effective approach for on-demand drug release in response to illness signals. The findings contribute to the advancement of precision medicine and the development of innovative drug delivery systems with enhanced therapeutic efficacy and reduced side-effects.

KEYWORDS

smart nanopolymers; drug release; personalized medicine; drug delivery systems; innovation

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1. INTRODUCTION

Smart nanopolymers offer a promising approach in personalized medicine by targeting specific triggers and by reducing side-effects. They can overcome challenges in chemotherapeutic drug delivery and treat tumours directly by enhancing the drug's solubility and effectiveness. Despite challenges in manufacturing, toxicity assessment, and regulatory approval, the future looks promising. The criteria for selecting biocompatible and biodegradable polymers are essential for drug delivery systems due to their sustainability, effectiveness, and potential for modification. These polymers can naturally break down in the body, thereby reducing environmental impact and improving therapeutic outcomes. Common polymers include collagen, alginate, chitosan, hyaluronic acid, and poly(vinyl alcohol). Some approved polymers can generate nanostructures and act as nanocarriers. Their

adaptability lowers environmental impact and allows for their use *via* multiple administration routes. Polymers are fundamental in drug conveyance frameworks, with regular hydrogels offering biocompatibility, biodegradability, and bioactivity. Manufactured hydrogels, such as poly(acrylic corrosive), offer tunable properties and can be changed for explicit functionalities. Polymeric nanoparticles and shrewd nanopolymers, such as poly lactic-*co*-glycolic acid copolymers, offer adaptability in surface functionalization and potential for designated helpful impacts on growths [1-5].

2. INCORPORATION OF SPECIFIC MOLECULES OR SIGNALING MECHANISMS

Smart nanopolymers are crucial for controlled drug delivery due to their ability to respond to external factors like pH, redox compounds, light, and temperature. Redox-responsive linkages and functionalization methods enhance their responsiveness to external influences [6,7].

3. CHARACTERIZATION TECHNIQUES

Analysing the physical and chemical properties of smart nanopolymers for drug delivery is essential for understanding their behavior and potential applications. Spectroscopy and microscopy are used in order to characterize their physical properties, while thermal analysis methods are used so as to investigate their thermal behavior and stability. These analytical techniques provide valuable insights into structure-function relationships and optimize their performance for personalized medicine applications [8].

4. DRUG RELEASE MECHANISMS EMPLOYED BY SMART NANOPOLYMERS

Smart nanopolymers for drug delivery have gained attention due to their capacity to react to different stimuli and release medications at specific sites in the body. Stimuli-responsive release involves materials responding to triggers such as light, temperature, pH, and enzyme concentration. Enzyme-based degradation involves polymers breaking down in response to specific enzymes found in pathological conditions. Targeted therapy strategies involve functionalized polymer nanocomposites targeting cancer cells with abnormal properties. These mechanisms enable precise drug delivery at specific times and locations while reducing side-effects and increasing effectiveness [8,9].

5. APPLICATIONS IN PERSONALIZED MEDICINE

Smart nanopolymers offer a range of advantages in personalized medicine, such as precise drug delivery with improved effectiveness and reduced side-effects. Smart nanopolymers have potential in tissue engineering, wound healing, biosensing, cancer therapy, and personalized medicine. They can respond to specific biological signals and achieve targeted drug delivery with enhanced efficacy, making them invaluable tools in revolutionizing disease treatment and prevention [9].

6. APPLICATIONS IN DISEASE TREATMENT AND PREVENTION

Smart nanopolymers are revolutionizing personalized medicine by enabling precise drug delivery, reducing side-effects, and enhancing effectiveness. They can be used in tissue engineering, wound healing, biosensing, tissue regeneration, and cancer therapies, potentially improving patient compliance and bioavailability [6].

7. CHALLENGES ASSOCIATED WITH SMART NANOPOLYMERS

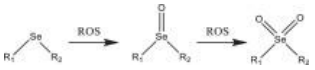
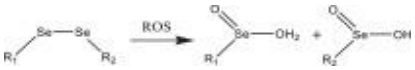
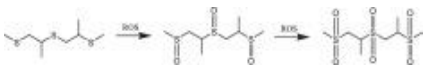
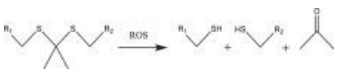
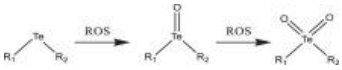
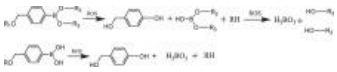
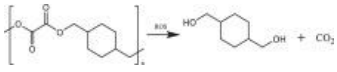
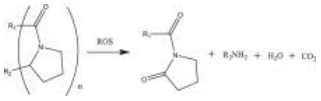
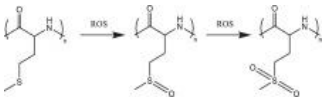
The use of smart nanopolymers for drug delivery presents with a number of difficulties, including toxicity and regulatory issues. Although responsive polymeric nanocarriers and smart nanoparticles are promising drug delivery methods, there are substantial obstacles to their practical use. Regulatory factors like toxicity evaluations and established manufacturing processes need to be taken into account in order to guarantee safe clinical use. To guarantee safe clinical use, biocompatibility and non-cytotoxicity must also be taken into consideration [7].

8. POTENTIAL FUTURE APPLICATIONS

Smart nanopolymers have the potential to transform cancer treatments, personalized medicine, and targeted drug delivery methods. They can help create safer and more efficient treatments, improve circulation half-life, reduce internalization, prevent denaturation, and deliver target agents in a precise manner. Additionally, they offer improved specificity and efficacy in treating and preventing diseases. Future trends include the further devel-

opment of biocompatible materials, the designing of reversible systems using low-energy light sources, the further characterization of *in vivo* biological conditions, the enhancement of stability, and the combining of independent photo-induced reactions into one unified system [8,10].

Table 1. Overview of reactive oxygen species (ROS)-responsive groups in drug delivery systems and of their activity and oxidation reaction mechanism. Abbreviations used: i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; NP, nanoparticle

Group	Chemical oxidation reaction	Mechanism of activity	Systems	Administration
selenium		hydrophobic to hydrophilic phase transition	micelle	*in vitro*
diselenide		Structural cleavage	micelle	--
thioether		hydrophobic to hydrophilic phase transition	nanocomplex, micellar NP, polymersome, hydrogel, microsphere	i.v., i.m.
vinyldithioether		Structural cleavage	micelle	*in vitro*
poly(thioketal)		Structural cleavage	fibrous patch, polyplex, microsphere, NP	transdermal, oral, i.v.
tellurium		hydrophobic to hydrophilic phase transition	micelle	
arylboronic acid/esters		Structural cleavage	polyplex, NP, microparticle, micelle	i.p., i.v.
polyoxalate		Structural cleavage	microparticle, NP	i.m.
poly(L-proline)		Structural cleavage	polymeric scaffolds	*in vitro*
poly(L-methionine)		hydrophobic to hydrophilic phase transition	vesicle	*in vitro*

9. CONCLUSION

Smart nanopolymers are revolutionizing drug delivery by improving targeting effectiveness, reducing side effects, and improving patient adherence. Despite challenges like the lack of standardized manufacturing methods and a clear connection between pre-clinical and clinical studies, they hold significant promise for personalized medicine.

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Novel *in silico* nano-drug design and delivery systems employing the density functional theory: a review

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Abstract

Nanoinformatics is a next-generation method for designing and simulating nanodrug candidates. It involves combining bioinformatics and quantum tools to predict and evaluate drugs. This approach addresses scientific problems in cheminformatics, configuration optimization, drug development, and administration. The integration of bioinformatics and quantum tools is crucial for the understanding of these advancements.

KEYWORDS

nanoinformatics, *in silico*, density functional theory, bioinformatics, nano-drugs

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1. INTRODUCTION

These methods help with the design and improvement of drug delivery systems in addition to providing important insights on drug behavior. The progress in computational drug design and genomic analysis has resulted in the rise of nanoinformatics as a cutting-edge tool for drug design and the undertaking of simulation studies for nanodrug candidates. Through computational tools, nanoparticle libraries, different modeling techniques, and simulation approaches, nanoinformatics plays an important role in enhancing the development of anticancer nanomedicines. The development of tailored nanomedicines in anticancer research might be accelerated by nanoinformatics, which is unquestionably important at this point. In addition, when compared to other *ab initio* methods that are currently in use, computational techniques like the density functional theory (DFT) offer very high levels of accuracy within a comparable computation timeframe, and are more economical in terms of processing resources [1-3].

2. NANOINFORMATICS IN DRUG DESIGN AND DELIVERY SYSTEMS

Nanotechnology enhances drug delivery, but challenges like tissue penetration and safety persist. Computational models aid in understanding biological processes and selecting effective anticancer

treatments. Professionals in nanoinformatics evaluate biotechnological data, predict nanoparticle structure, and manage biomedical data [3-5]. Some tools (like AutoDock Vina) can predict the binding affinities and orientations of ligands, are fast, accurate, and easy to use, but may not be as accurate for complex systems. While AutoDock and GOLD are used for ligand binding affinities and the prediction of orientations, particularly for flexible ligands, they require a license and can be expensive. Another tool is Glide, which can predict the binding affinities and orientations of ligands with accuracy, and can be integrated with other Schrödinger tools; however, the latter requires the Schrödinger suite, which can be expensive. Both LigandFit and SwissDock may not be as accurate for complex systems, but they are easy to use, accessible online, and integrated with other Schrödinger tools; these platforms are usually used for predicting the binding affinities and orientations of ligands (Table 1) [3-8].

DFT has significantly transformed drug delivery system design and development, particularly for biodegradable and bioabsorbable polymers. DFT simulations can accurately predict properties and structural characteristics, thereby providing insights into medication pharmacokinetics and metabolism parameters. As computing power increases, the emergence of multidisciplinary initiatives for nanomedicine from an atomic viewpoint, at a low cost, and with accurate quantum mechanics calculations are expected [3,7].

Table 1. List of companies using artificial intelligence (AI) and machine learning technologies in pharmaceutical research.

Domain	Technology and Outcome	Industry and Collaborations
Drug design	Innovative medicinal antibodies	Exscientia
Molecular drug discovery	Computing platform for structure-based drug creation powered by deep learning and AtomNet	AtomWise
Gene mutation related disease	Recursion operating system for biological and chemical datasets based on machine learning	Recursion
Drug design	*De novo* drug design using ligands and structures, particularly in multiparametric optimization	Iktos
Drug discovery	AI technology for generative modeling	Iktos and Galapagos
Drug development	Suitable candidates for preclinical research	Iktos and Ono Pharma
Drug design	Quick drug creation with “Makya” software	Iktos and Sygnature Discovery
Drug discovery and development	Pharma.AI, PandaMics, ALS.AI	Insilico Medicine
Drug target	Drug target selection for idiopathic pulmonary fibrosis and chronic kidney disease	BenevolentAI and AstraZeneca, GlaxoSmithKline, Pfizer
Clinical trials	AI in clinical trials	Pfizer and Vysioneer

3. ADAPTING PROFESSIONAL SKILLS FOR ANALYZING NANOBIOTECHNOLOGICAL DATA

Nanoinformatics is a rapidly evolving field that uses computational chemistry and bioinformatics to analyse data on nanobiotechnology. It plays a crucial role in drug design, advancing research in nanodrug delivery systems and regenerative medicine. Despite challenges like ethical considerations and computational modeling risks, successful case studies demonstrate the significant impact on the pharmaceutical industry, leading to efficient nanomedicines for better cures [6,7].

4. POTENTIAL BENEFITS AND IMPLICATIONS

The integration of nanoinformatics with computational methods like DFT and machine learning offers numerous benefits, including optimization, standardization, and understanding of the nanomaterials’ synthesis, characterization, and biological effects. This integration has potential to transform the pharmaceutical sector and improve patient outcomes [5].

5. THEORETICAL AND COMPUTATIONAL MODELING IN NANOINFORMATICS

Nanoinformatics and nanomodeling are crucial for computational nanodrug design and delivery systems. Theoretical and computational modeling can help solve problems in configuration, medication creation, and administration optimization. Combining traditional bioinformatics with chemical tools allows for accurate medication prediction and assessment. DNA computing information and artificial intelligence (AI) algorithms enable nanomedicine and regenerative medicine advancements. Computational modeling tools have shown promise in addressing empirical models and advancing nanomedicine by addressing shortcomings in existing models [3,5-7].

6. CONFIGURATION, MEDICATION CREATION, AND ADMINISTRATION OPTIMIZATION

Nanoinformatics and nanomodeling are crucial in computational nanodrug design and delivery systems. AI models can accurately forecast drug delivery systems by analysing physicochemical parameters and molecular features. They can also predict medication release and absorption characteristics, dissolution rates, and formulation properties. These features can advance computational nanodrug design and delivery systems by improving nanoparticle design, by predicting medication release and absorption, and by optimizing drug formulations [8].

7. POTENTIAL RISKS AND UNCERTAINTIES

Computational modeling in nanoinformatics for drug design and delivery systems has risks and uncertainties, including over-reliance on predictions without experimental confirmation, as well as challenges in representing molecular flexibility in biological molecules. Ethical considerations, patient privacy, and data security are crucial [3,4].

8. CASE STUDIES

Nanoinformatics can revolutionize drug design and delivery systems, optimizing site-specific drug delivery through deep learning and AI algorithms. This has already led to successful drug design, efficient interaction prediction, and personalized treatment regimens [1-9].

9. CONCLUSION AND FUTURE DIRECTIONS

The integration of data and information science has significantly impacted nanomedicine, thereby optimizing and standardizing nanomaterial synthesis and accelerating drug design. Traditional bioinformatics and computational chemistry tools have been adapted for data storage, analysis, and visualization. Nanoinformatics can enable personalized nanomedicines for cancer treatment.

The future of nanoinformatics and computational nanodrug design will involve quantum computing, AI integration, biomedical data standardization, and sustainability metrics. Future research should focus on flexible informatics systems and collaboration between computational scientists and pharmaceutical industry professionals.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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