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INTRODUCTION

The Jordan Journal of Pharmaceutical Sciences (JJPS) is a peer-reviewed Journal, which publishes original research work that contributes significantly to further the scientific knowledge in pharmaceutical sciences' fields including pharmaceutical/medicinal chemistry, drug design and microbiology, biotechnology and industrial pharmacy, instrumental analysis, phytochemistry, biopharmaceutics and Pharmacokinetics, clinical pharmacy and pharmaceutical care, pharmacogenomics, bioinformatics, and also JJPS is welcoming submissions in pharmaceutical business domain such as PharmacoEconomics, Pharmaceutical Marketing, and Management. Intellectual property rights for pharmaceuticals, regulations and legislations are also interesting topics welcomed from our colleagues in Schools of Law.

On a current topic in Pharmaceutical Sciences are also considered for publication by the Journal. JJPS is indexed in SCOPUS (Q3). It’s a journal that publishes 4 issues per year since 2021 in (March, June, September, December). The Editorial Team wishes to thank all colleagues who have submitted their work to JJPS). If you have any comments or constructive criticism, please do not hesitate to contact us at jjps@ju.edu.jo. We hope that your comments will help us to constantly develop JJPS as it would be appealing to all our readers.

Prof Ibrahim Alabbadi
Editor-in-Chief
School of Pharmacy- The University of Jordan
Amman 11942- Jordan

Letter from the Editor-in-Chief

Another year went by. It was an extraordinary year that none of us will soon forget, not only because of hard health times, but also because of the bad economic crisis. However, after every dusk comes the light, hoping that 2021 would be the start of the dawn. Jordan Journal of pharmaceutical Sciences (JJPS) completed 2020 publishing 4 issues on regular times; one issue per quarter (achieving an extra issue than the years before), besides having 10 articles per issue (instead of 5) in order to decrease the waiting time for the accepted articles to be published; trying to serve as much researchers as we can.

One of the achievements is the diversity areas of submissions to JJPS, the latter makes JJPS distinguished with an added value of a different taste that hopefully matches the journal readers' desires in Jordan as well as in the region. Nowadays we have submissions not only in the pharmaceutical chemistry, pharmacognosy and pharmacology, but also in pharmacy practice, clinical pharmacy, pharmaceutical care and behavioral areas related to humans and patients such as psychological considerations during the COVID-19 pandemic. Furthermore, JJPS received submissions from all around the world; giving readers the opportunity to be exposed more to different scientific research patterns worldwide with an increase in number of submissions by 62% in 2020 compared to 2019. Moreover, citations increased in 2020.

The new members in the editorial board are distinguished professors representing almost all fields of pharmaceutical sciences from different backgrounds coming from diversified research schools from USA, Canada, Europe, Australia, and Jordan. Also, they came from different work environments: governmental and private higher education institutions. The latter started smart and hard work toward becoming one of your choices to submit your article in any of the pharmaceutical fields.

In the new issues of JJPS in 2021, we will see an editorial commentary written by one of our colleagues in JJPS expressing one of the interests and thoughts related to the status que in general from their point of view.

Finally, it is really a great honor to have a new advisory board consisting of well-known scientists from different regional and international countries representing almost all pharmaceutical fields; the JJPS family is sure that the respected scientists will have a positive impact and will add value particularly in the quality of manuscripts accepted for publication. Looking forward to more achievements in 2021.

Prof Ibrahim Alabbadi
Editor-in-Chief

Editorial Commentary

Dear researchers,

We would like to welcome all our prominent readers and authors in this new issue of JJPS. As we all know that vaccines provided the ultimate solution for corona epidemic worldwide late 2020, we would like to shed some light on pharmaceutical biotechnology as futuristic definitive science providing new hopes and solutions for the humanity.

The most distinctive area of biotechnology was the production of therapeutic proteins and vaccines via genetic engineering. A fledgling "biotech" industry began to coalesce in the mid to late 1970s in which the first recombinant DNA technology product was commercialized. Many multinational companies began manufacturing genetically engineered entities primarily for a wide range of medical and environmental uses. Following the FDA approved recombinant human insulin in 1982, biopharmaceutical medications such as human growth hormone, clotting factors for hemophiliacs, fertility drugs, erythropoietin, interferons, clot-dissolving agents, and other drugs have been commercialized around the world. Late 1990's witnessed a new era of biopharmaceutical Monoclonal Antibodies (MABs) dominating the bio drugs industry e.g. the possibility to treat breast cancer. MABs applications further intensified to a distinctly unbeatable level with the development of fully human MAB e.g., adalimumab. The discovery of cancer monoclonal immunotherapy via immune checkpoint inhibition represented a recent shift in cancer therapy. A major biotransformation phase preludes both antisense technology (antisense RNA) and RNA interference (RNAi) in regulating the gene expression of any disease. Antisense technology was pioneered in 2013 for familial hypercholesterolemia. RNAi technology has manifested to clinical application by FDA approval in 2019. In the late 20th and early 21st centuries, biotechnology has expanded to include new and diverse therapeutic vaccines such as RNA and living vaccines. They are administered after the disease or infection has already occurred. They work by activating immune system of the patient to fight against infection. Historically, they were developed against cancer, but recently they are mostly active against viral infections. The first therapeutic vaccine was approved by FDA in 2010 against prostate cancer.

A messenger RNA (mRNA) or RNA vaccine is a type of viral vector vaccine that uses a copy of mRNA to produce an immune response. Once inside the immune cells, the vaccine's RNA functions as mRNA, causing the cells to build the foreign protein that would normally be produced by a pathogen or by a cancer cell. These protein molecules stimulate an adaptive immune response. The RNA vaccines have superior design and production speed, lower cost of production, and the induction of both cellular as well as humoral immunity. Exceptionally viral vectors cannot cause COVID-19 infection or integrate genetic material delivered into person's genome. By December 2020, there were two novel mRNA vaccines for COVID-19. They were granted emergency use authorization: the Moderna COVID-19 vaccine (mRNA-1273) and the Pfizer–BioNTech COVID-19 vaccine (BNT162b2).Hopefully, a spotlight shed on new and bright futuristic terminologies and translational applications such as live virus vaccines, personalized medicine, genetic polymorphisms, gene therapy and genetics related ethics. Finally, anticipations of expanding earnestly and relentlessly the scope and horizons of our topics may culminate into attracting novel manuscripts within this substantially and rapidly innovative area.

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CONTENTS

Online self-medication practices among university students in Palestine: a questionnaire-based study

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ABSTRACT

Background: Advertisement of medical products on social media has become increasingly common, and is associated with increased online shopping in pursuit of self-medication. Such practice highlights the influence of social media advertising on individual use of medicinal products without consultation with health care professionals.

Objectives: To better understand the practice of online self-medication and investigate its prevalence in Palestine, this study specifically assessed the probable reasons, extent of use, and source of advice for online self-medication among university students in Palestine. In addition, the study evaluated factors that influence online self-medication in this population, such as gender, age, knowledge in medical specialty, and perception of online products.

Methods: This study was conducted using a "paper pretested questionnaire" prepared in the Arabic language and self-administered to 700 students from three public universities in Palestine (Al-Najah, Al-Quds, and Bethlehem Universities). The study was conducted over three months (Nov. 2019 – Jan. 2020) and included university students of all years from both medical and nonmedical faculties. Data were collected, coded, entered, analyzed, and summarized using Statistical Package for Social Sciences version 25. Descriptive results were expressed as frequency, percentage, and mean±SD.

Results: Female (87.6%), younger (20-29 years), and medical (57.4%) students tended to use online self-medication more than their peers. Respondents practiced online self-medication to save time (50.4%) and money (49.8%), and a majority (65.7%) reported using online products without consulting physician or pharmacist.

Nearly a third of respondents (29.6%) reported that they did not have any instructions on how to use products, and a significant number experienced side effects from the products they used (p-value <0.001). The internet was the most commonly reported source for self-medication (45.3%), particularly sponsored advertising campaigns on websites (16.7%). In terms of product type, skin care products (76.7%) were the most commonly used, followed by hair products (72.2%), and vitamins (58.8%). In addition, cream and ointments were the most frequently used dosage forms (71.3%). The majority of respondents (64.1%) described their experience as "bad" and "not healthy"; half (50.9%) reported having side-effects and a third (33.6%) stopped using the products because of side effects. Statistical analysis showed that the difference in usage between genders was significant for vitamins, traditional herbs, weight loss products, hair products, skin products, nail products, and food supplements (p-value <0.05). In addition, the relation between specialty domain and product use was significant for hair products, food supplements, traditional herbs, and herbal mixtures (p-value <0.05). The relationship between reading information and experiencing side effects was significant with a p-value of 0.000. Finally, the relationship between side effects and product re-use was significant (p-value <0.05)

Conclusions: Online self-medication is a common practice of young Palestinian university students; this constitutes a health problem, and intervention is needed to minimize risk. We emphasize the important role of health care professionals in educating the community, especially the youth (<30 years), regarding online medication practices that may have harmful side effects.

Keywords: online self-medication, university students, practices, Palestine.

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INTRODUCTION

Self-medication has been defined as patient use of medication (modern and/or traditional) for self-treatment on his/her own initiative or according to the advice of another without consulting either a physician or pharmacist for diagnosis, prescription, or surveillance of treatment (1). Online self-medication has become quite common due to the pervasive importance of social media in modern lives, which features excessive advertisements for medicinal products. It is much easier these days for any profit-seeking companies to sell their products (for skin care, hair, obesity, etc...) by calling on a social media influencer (a person that has an immense number of followers on social media) to advertise those products while pretending to be a user. Then, based on the influencer's exaggerated descriptions, followers take up and use the product themselves.

These online advertisements have substantial effects on individuals, especially among the youth (<30 years); their prevalence raises the potential for incorrect self-diagnosis, and encourages people to use these products without consulting a physician. Furthermore, online orders allow high-pressure sales practices to be used, which may provide a favorable price to the customer and enable customizing offers, product presentation, and various gifts; an account manager; and priority in processing and delivering orders. In addition, the potential for counterfeit products is high, keeping in mind that the proportion of counterfeit medicinal products is estimated to be 10% worldwide (3). Here, we refer to these medications as products because we cannot be sure what these products are: whether they are really medications that have useful active ingredients, or just profit-seeking advertising that aims to convince people to buy these products.

The practice of misusing online products without medical advice is a serious problem. Online self-medication and excessive drug consumption present high risks of developing a condition such as drug poisoning, functional disorders, or serious allergic reactions. At the same time, the overuse of drugs without medical advice presents the risk of extant or new diseases not receiving timely diagnosis (i.e. at the first symptoms of a disease). Furthermore, patients administrate the drug they think they need, which may not be what a doctor or pharmacist would advise. Ultimately, the dangers behind self-medicating online orders are profound. (2)

This study aimed to better understand the practice of online self-medication (medicinal products and food supplements) and to investigate its prevalence. The study specifically assessed the probable reasons, extent of use, and source of advice for online self-medication practice among university students in Palestine. In addition, the study evaluated factors that influence online self-medication in this population, such as gender, age, medical knowledge (of medical specialty students), and perceptions of online products.

Methods:

Study design and setting

A questionnaire study was conducted over a three-month period from November 2019 to January 2020. Participants consisted of university students from the West Bank universities of Al-Quds University, Al-Najah University, and Bethlehem University who were enrolled at different medical and nonmedical faculties and of all years of study.

Permission to carry out this study was obtained from the Faculty of Pharmacy, Al-Quds University. Ethical approval was obtained from the Al-Quds University Ethical Committee.

The questionnaire was prepared in the Arabic language to make it clear for participants, and it was self-administered. Students from the faculties of Public Health, Pharmacy, Dentistry, and Medicine were designated as "medical students," while the remainder were considered to be "nonmedical students."

Students who accepted participation in this survey were given the questionnaire during their breaks; the approximate time taken to complete the questionnaire

was 3 minutes. A total of 700 questionnaires were distributed, of which 651 were used in the analysis; 49 questionnaires were excluded due to being incomplete or not returned when the students went back to their lectures.

University students were chosen for this study for several reasons: they are young, believed to spend time on social media on a regular basis, and can be influenced by social media celebrities. Nothing has been previously published regarding online self-medication practices among university students in Palestine. Moreover, this population was easily accessible and more likely to respond than others.

Questionnaire development

The questionnaire consisted of thirty-one open- and closed-ended questions grouped into four parts.

Demographic information:

The first part consisted of questions regarding demographic information such as gender, age, specialty (medical or non-medical), and geographical location. (Seven questions with 4- or 3-point multiple choice responses.)

Source of information about different products used:

The second part of the questionnaire focused on the type of products the respondent used, sources of information about the products, their reasons for online self□medication, whether they have been asked about their health status to determine the appropriate product, and whether they are satisfied with their experience. (Six questions with different numbers of point according to the nature of the question, except one yes/no question.)

Self-medication practices of students:

These questions related to the respondent's extent of internet use, if they see advertisements while using their social media accounts, if they ever ordered products online for self-medication, if they received instructions before using the product, if they understood the instructions, whether they have ever experienced adverse events, and what they did if side effects happened. (This section included a total of 12 multiple choice and yes/no questions.)

The last section consisted of a series of questions to which respondents could agree or disagree, such as whether they support online product shopping or prefer physician diagnosis and pharmacist advice. (Five questions with agree or disagree response options.)

Statistical analysis:

The data was entered, cleaned, and coded using the Statistical Package for Social Sciences (SPSS, version 25), then analyzed according to the research questions. Analysis included generating a set of descriptive statistics from the essential variables, represented by counts and percentages (tables and graphs), which describes the practices of the sampled population regarding online self-medication. Inferential statistics were also used in order to examine the significance of a set of hypothesized relationships at $p < 0.05$. These statistics consisted of the chi-square test when examining a relation between two categorical variables, and the ANOVA test when examining the relationship between scaled and polytomous categorical variables.

Reliability

To validate the questionnaire, a pilot study was carried out on 35 students and Cronbach's alpha coefficient was calculated to assess internal consistency. The questionnaire was found to be reliable and valid, with a Cronbach's alpha coefficient of (α =0.541). Pilot study participants were not included in the final analysis (22).

Results:

Sample characteristics

The study analyzed data from 651 students out of 700

(93%), predominantly aged below 29 years (98.2%). The socio-demographic characteristics of the respondents are presented in Table 1. Most participants were female (87.6%), and the majority were studying medical specialties (57.4%). Just under one-fifth were employed (19.2%). Half of the respondents lived in a city (50.1%), while slightly less than half lived in a village.

Self-medication practices

The proportion of respondents using each of seven types of internet-bought products—which product is not specifically mentioned—is given in Figure 1. It is clear that skin and hair products were the types most commonly used, with percentages exceeding 70%. A slight majority of respondents bought vitamins, and about a quarter bought weight loss products, while less than 20% of respondents used food supplements, herbal mixtures, or traditional herbs purchased via the internet. Concerning overall internet usage, 32.9% reported using the internet for more than six hours per day,

Regarding sources of information for these online products, the most common response was that the students knew about the product from the internet (Figure 2). About a quarter learned about it from friends, and less than a fifth from sponsored advertisements, while almost none had previous experience with the product.

Figure 3 illustrates the numbers of online self-medication products used by participating students. The majority of students (80.6%) used 2-4 such products. The most frequently ordered dosage form was as creams and ointments (71.3%).

Slightly less than half of respondents (45.3%) indicated that they are willing to re-try products purchased via the internet. Most respondents considered the country of manufacture, type, and price of the online self-medication product (81.3%), while about two-thirds read the product contents (67.6%).

However, for those that stopped using a product, the most common reason was that they did not get the benefit expected (about 30%). A slightly smaller percentage stopped because they completed the product regime (28.1%), while about a quarter stopped because they received the expected benefit (24.3%), and a small proportion (15.4%) stopped because of side effects. Half of respondents (50.8%) reported experiencing side effects after using a self-medication product. Of those who experienced side effects, most stopped using the product (38.1%), about a third gave the product to someone else (33.6%), and the remainder looked on the internet for a solution (28.3%).

The majority of students reported knowing the therapeutic dose due to internet searching, while 14.7% learned from the advice of relatives and friends and 12% predicted the dose by themselves. However, 54.1% of students reported poor adherence to the recommended dose, and changing the therapeutic dose was very common, as 53.5% reported taking less or more than recommended. The most common reasons for deviating from the recommended dose were to solve their problem more quickly (34.9%) and due to not getting the perceived benefit (31.4%). About a fifth (20.6%) changed their doses to reduce side effects, while about a tenth (10.2%) reported finding the product unsuitable.

In terms of their satisfaction concerning using self-medication products obtained through the internet, participants were nearly equally divided in considering their experience good, bad, or unhealthy. A good experience was reported by 35.9%, while 34.1% reported a bad experience and 30% an unhealthy experience.

Responses to agreement prompts

In the last section of the questionnaire, a majority (70%) responded yes to the statement "Do you believe that every individual has the right to buy any product they want?" A greater proportion (79.4%) agreed that "Shopping through the internet is better." However, 38% did not agree that "Consulting a beauty expert is better than consulting doctors because the results are faster." A small

proportion of respondents (17.8%) would recommend online medical shopping over doctor and pharmacist consulting, while more than half (55.6%) would like to sell products online themselves.

Statistical differences

Statistical analysis showed that a greater percentage of females used online products than did males, and the difference in usage between genders was significant (at 95% confidence level) for vitamins (*p*-value< 0.001), traditional herbs (*p*-value 0.029), weight loss products (*p*-value< 0.001), hair products (*p*-value 0.024), skin products (*p*-value< 0.001), nail products (*p*-value< 0.001), and food supplements (*p*-value <0.001). The only product that did not show significant gender difference in usage was herbal mixtures.

Another statistical relationship that was evaluated was the difference in product purchases by type between students with medical specialties and those having non-medical specialties. A higher percent of students with medical specialties used internet-purchased products than did those of non-medical specialties. The relation between specialty domain and product use was significant (at 95% confidence) for hair products (*p*-value 0.023), food supplements (*p*-value 0.001), traditional herbs (*p*-value 0.003), and herbal mixtures (*p*-value 0.006).

More than half of participants that used soap, spray, injection, cream, or tablet products reported side effects. In particular, 49% of those that used creams experienced side effects. However, no statistical relationship was observed (at 95% confidence) between dosage form and side effects (*p*-value 0.288).

In addition, about 63% of those who never read information about the product experienced side effects, while over 40% of those who sometimes or always read information experienced side effects. Of those who reported no information being provided with the product, 83.1% experienced side effects. The relationship between reading information and experiencing side effects was significant (at the 95% confidence level), with *p*-value 0.000.

The mean number of products purchased via the internet was highest for those students who reported being attracted by online advertisements. However, this relationship did not reach significance at 95% confidence (*p*-value 0.625).

Finally, as shown in Table 8, of those who experienced a side effect, less than half re-used the product, while more than half did not. With a *p*-value< 0.049, the relationship between side effects and product re-use is significant (at 95% confidence).

Discussion:

Self-medication is a common practice in developing countries like Palestine due to the easy availability of non-prescription drugs. Self-medication reduces the treatment burden on health care facilities and saves time for the patient by increasing access to medications. However, using the internet to buy pharmaceutical products means the safe and effective use of these medicines includes some uncertainty. Self-medication increases risks such as incorrect diagnosis, polypharmacy, and excessive use of medication (4).

Studies on self-medication have reported different prevalence rates in different parts of the world, ranging from 26.2% to as high as 98% (5, 6, 7, 8). These studies highlight self-medication without specifically considering the use of the internet for self-medication. This study was carried out to examine online self-medication practices in Palestine, specifically among university students. With a response rate of 93%, the results show that online self-medication is very common among university students in Palestine.

The majority of the participants were undergraduate students. In terms of gender, the majority of students who have practiced online self-medication were female, which emphasizes that this population is highly influenced by social media and internet promotion of online self-medication. This finding is similar to a study from Kuwait,

which showed that females practiced self-medication significantly more often than males (9). However, a study in Abbottabad, Pakistan found no significant difference in self-medication between the two genders of university students (11, 12).

The prevalence of online self-medication in this study was 65.7%. About (57.1%) of the respondents belonged to medical specialties, and medical students were generally more interested in online self-medication. However, non-medical students also had notably high odds of using online self-medication; this is not a healthy finding at all, because such students lack medical backgrounds and could be deceived by the attractive promises of advertisements.

In contrast, a study in Karachi (10) found no significant difference between self-medication practices of medical and nonmedical students, of males and females, or by respondent year of study. This could be explained by the fact that non-medical students consider themselves to be just as knowledgeable about medicines as medical students, or it may simply be that university students both medical and nonmedical do not care much about the implications of such behavior and thus do not hesitate to indulge in such activities. Notably, in the present study, self-medication rates were not significantly lower in students aware of its harmful effects ($p = 0.21$). It was also noticed in this study that students who use the internet more are the ones exposed to ads while perusing their social network accounts.

In our study, the most reported reasons for ordering drugs via the internet were interest in the ads and saving time by forgoing a consultation. This is similar to reports from a 2018 study of pharmacy students in Jordan.

In this study, the therapeutic classes most commonly consumed through online self-medication were skin products and hair products in cream and ointment forms. This is likely due to common skin problems among females such as unwanted pigmentation, acne, and dryness. Accordingly, females were both the most targeted population and the most consuming population of these products. The same could hold for hair products. Moreover, online companies selling products did not ask consumers about the health condition they are trying to treat when ordering the product. Hence, there is potential for some companies to take advantage of vulnerable customers and used them to make a profit.

Surprisingly, nearly two-thirds of participants reported that their experience was “bad” or “not healthy,” while about a third reported their experience was “good.” Furthermore, when asked if they gave the product another shot, more than half reported not using it again after their first trial. Of course, there are many factors that would make for a bad experience with a product, including a lack of instructions for its use or partial or negligible understanding of the instructions.

Students that had good experiences with the products were asked if they read the directions for use. The most frequently reported answer was “yes” (41.2%); however, a majority (65.3%) reported not trusting what the seller said about the product and searching about it on the internet before use.

The majority of respondents (65.7%) reported using online products without consulting a physician or a pharmacist, and more than a third of the students (32.4%) in this study reported not reading the product ingredients before use, as a strong point for companies making the purchasing process easier. Which raises the questions of what really are the products they used, and what were the active ingredients? Are these products licensed for use?

A majority of respondents (59.1%) reported that the product they used did not contain any forbidden ingredients, such as alcohol or pig fats, while 36.4% reported not knowing if there were forbidden ingredients, and 4.1% reported the product did contain a forbidden ingredient. So, what did these products really contain? Does the Ministry of Health know that these products, which are available everywhere and can be ordered and used by anyone, contain pig fats and alcohol?

Similar concerns exist regarding the doses of these products. The majority of students reported knowing the

therapeutic dose due to internet searching, while 14.7% learned from the advice of relatives and friends and 12% selected the dose by themselves. However, 54.1% of students reported poor adherence to the recommended dose, and changing the therapeutic dose was very common; 53.5% were taking less or more than recommended. The change in doses as well as differences in sample size caused differences in the prevalence of side effects.

The most common reason reported for changing dosage was to get faster results (18.4%) , followed by not getting the desired result (18%) and reducing the dose to decrease side effects (11.6%). By this, we can emphasize the uselessness of these products. These results are similar to other studies that found changes in doses during the course of self-medication for one reason or another. Such a change was reported by 30.4% of the respondents in the current study, and by a higher percentage (57.1%) in another study (15). Meanwhile, adverse drug reactions because of self-medication practice were reported by a small percentage (9.2%) of subjects in another study (16).

While 50.8% of participants in the current study reported suffering from side effects, a study conducted in Kuwait found that only 14.8% of 900 undergraduate medical students reported adverse effects. Similarly, a cross-sectional study carried out at Suez Canal University in Egypt found that self-medication caused side effects among 28.9% of students (26 of 90) (17). Surprisingly, in our study, 38.1% of participants reported giving the product to one of their family members after suffering from side effects. This is similar to the finding of a study conducted in Germany, in which 83.6% of participants frequently advised other people about self-medication (18).

Interestingly, 79.4% of participants agreed that purchasing these products through the internet is better than consulting a doctor or a pharmacist. This relatively high percentage might be due to the saving of time and money with online self-medication, even though the results of self-medication ranged from no response to the occurrence of side effects. However, 38.9% affirmed that consulting a beauty expert is better than consulting a doctor or pharmacist because the results are faster. This is likely due to the lack of strict rules to limit consultations by beauty experts; in some cases, respondents stated that "The beauty expert prescribed some drugs such as antibiotics for acne."

Overall, the results from this study draw attention to the attitudes and patterns of online self-medication among university students in Palestine. Unsafe patterns of self-medication could lead to the occurrence of adverse drug reactions (19, 20), and this needs to be addressed in future studies. Self-medication is believed to do more harm than good and to lead to wastage of resources, the development of adverse drug reactions, and prolonged illness and suffering (21). This is likely due to the free access to medicines and lack of strict rules for obtaining them that holds in developing countries like Palestine.

This study showed that online self-medication practices are very common among university students in Palestine; therefore, students should be educated on the consequences of self-medication practices. Moreover, national guidelines on medicine access should be developed, and strong measures should be implemented to halt the selling of medications without a proper prescription. Further studies should also be done to assess self-medication practice in the community. Unfortunately, there are no data available regarding self-medication practices among the general public in Palestine, which makes it difficult to compare the extent of self-medication among university students with that among the general public.

Study limitations:

Most participants were female students, and the study was done with university students and may not apply to the rest of the population. In addition، online self-medication practices of Palestinian university students may particularly be different from those of the general population of Palestine in terms of the use of herbal mixtures. On the other hand، a lot of students denied their online self-medication practice، as

they refused to participate and talk about their own experiences. Finally، there was "recall bias" in the answered questionnaire for one reason or another.

Recommendations:

Efficient communication within the physician - patient - pharmacist triangle is necessary in order to educate people on the possible dangers of self-medication. Contraindications, drug interactions, and possible side effects must be written and pointed out for the patient receiving an online order. In addition، awareness and public health education must be improved all over Palestine by using social media networks and by publishing awareness leaflets in Ministry of Health offices and clinics in order to make clear how dangerous these products are and to stop people from purchasing them. Furthermore، strict policies from health authorities need to be implemented concerning the advertising and selling of products that make medical claims, the better to prevent this problem from expanding.

Conclusion:

Online self-medication is a common practice of young Palestinian University students; this constitutes a health problem that requires intervention to minimize risk. We emphasize the important role of pharmacists in educating the community, especially the youth (<30 years), regarding online self-medication practices and in alerting them to the seriousness of consequences such as harmful side effects of these products and sharing medications among family members and friends.

The practice of online self-medication is alarming. Our research focused on the types of drugs, their use, their safety, and reasons for the practice. Self-medication would be safe if the person has sufficient knowledge about the dose and other related instructions along with the expected side effects. Unfortunately, the present pervasive lack of information can cause serious effects such as skin problems, hypersensitivity and allergy, hair loss, and so on.

Patients can easily purchase online products that carry potential health risks, and also counterfeit medicines. As a result, patients who self-medicate will often take products of uncertain origin and quality.

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Table 1: socio-demographic characteristics

Variable		Count	Percentage
Gender	Male	81	12.4%
	Female	570	87.6%
Age	Less than 20 years	265	40.7%
	20-29	374	57.5%
	30-50	12	1.8%
Educational level	Higher studies	16	2.5%
	University	635	97.5%
Working status	Employed	125	19.2%
	Not employed	526	80.8%
Living area	City	326	50.1%
	Village	295	45.3%
	Camp	30	4.6%
Study field	Medical	372	57.4%

Variable		Count	Percentage
	Non-medical	276	42.6%
Directorate	North	160	24.6%
	Middle	295	45.3%
	South	196	30.1%

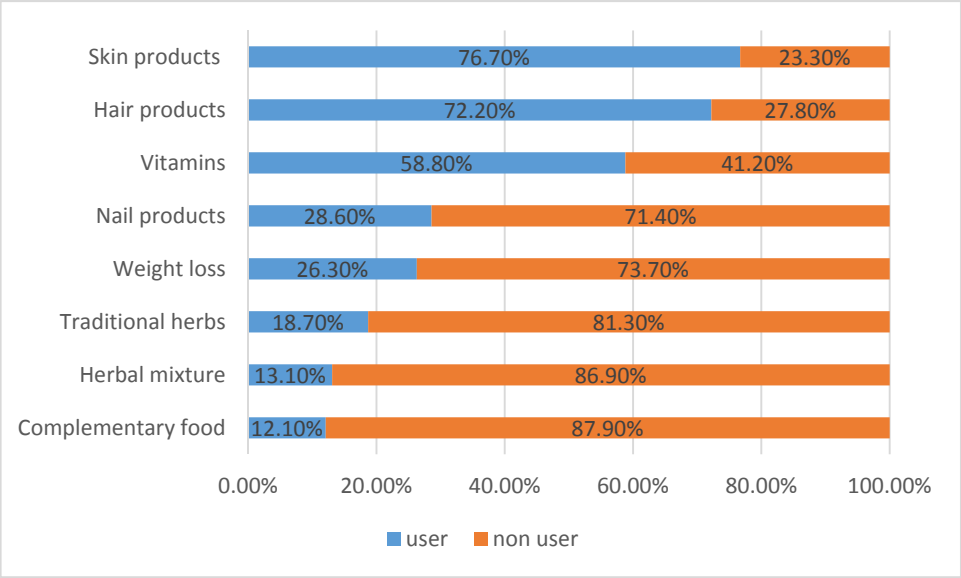

Figure 1: Distribution of online product use by product type.

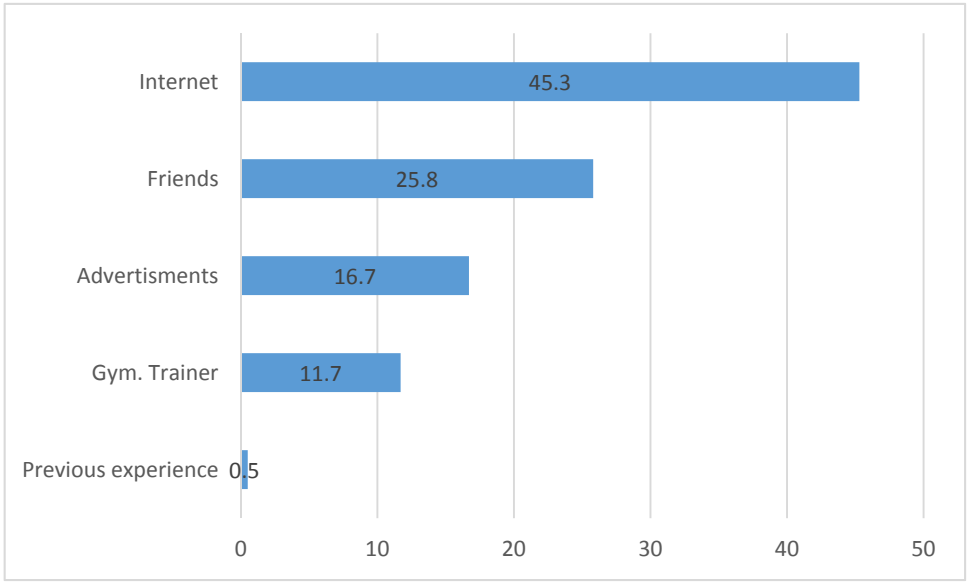

Figure 2: How respondents became aware of products.

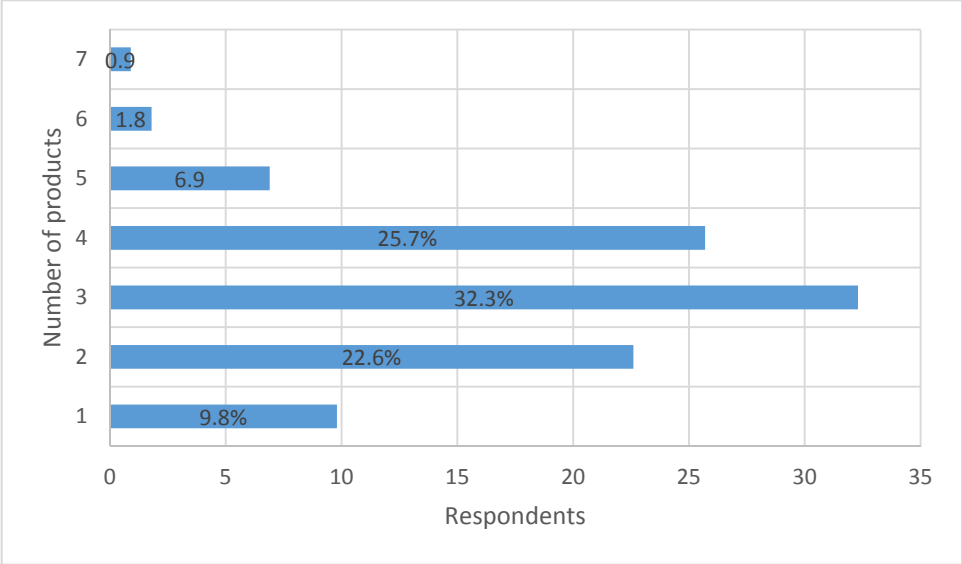

Figure 3: Distribution of respondents by number of products used.

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مدى استخدام الانترنت ووسائل أخرى فى استعمال الأدوية والمكملات الغذائية فى فلسطين

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ملخص

خلفية البحث: تزايد الاعلانات التجارية الممولة والغير ممولة للمنتجات الطبية أدى الى تزايد شراء المنتجات الطبية هذه دون اللجوء الى استشارة طبيب أو صيدلاني والاعتماد على ما ذكر في هذه الاعلانات، وتركز هذه الدراسة على مدى تأثير هذه الاعلانات على الأفراد في استخدام هذه المنتجات دون استشارة أهل الاختصاص.

الهدف من الدراسة: تركز هذه الدراسة على تحسين الفهم وزيادة الوعي حول استخدام المنتجات الطبية بالاعتماد على الدعايات لها ولمعرفة نسبة انتشار هذه الظاهرة في فلسطين. ولمعرفة الأسباب التي تدفع الأفراد لاستخدام منتجات طبية عبر الانترنت، مدى الاستخدام، ومصادر الحصول على نصيحة لتجربة المنتجات عبر الانترنت بالإضافة لدراسة العوامل التي تدفع الأفراد لممارسة هذه السلوكيات مثل الجنس، العمر، المعرفة الطبية الدوائية من عدمها.

طريقة البحث: تمت الدراسة باستخدام استبيان تم تحضيره باللغة العربية وتم توزيعه على 700 طالب وطالبة من مختلف التخصصات وجميع السنوات الدراسية من جامعات فلسطين، جامعة النجاح الوطنية، جامعة القدس–أبو ديس وجامعة بيت لحم. تم توزيع وتجميع العينات خلال فترة 3 شهور متتالية من شهر تشرين الثاني لعام 2019 حتى شهر كانون الثاني لعام 2020. تم جمع البيانات واحصائها وتحليلها وتلخيصها باستخدام البرنامج الاحصائي نسخة25.

النتائج: نسبة المشاركين بتعبئة الاستبيان من أصل 700 هم 93%. 57.5% من فئة الشباب (20–29 عام)، (87.6%) من الاناث، (57.4%) من دارسي التخصصات الطبية، ومن أهم الأسباب التي دفعتهم لاستخدام الانترنت، (50.4%) بهدف توفير الوقت، (49.8%) لتوفير المال –بدل استشارة طبيب (65.7%) .-من المشاركين بالبحث استخدموا المنتجات الطبية دون استشارة طبية، (29.6%) من مستخدمي المنتجات الطبية عبر الانترنت لم تصلهم تعليمات لكيفية استخدام المنتجات وحصول مضاعفات جانبية اثر استخدامها، (35.3%) من المستخدمين استخدموا المنتجات على ثقة تامة بما قيل لهم بالإعلان، (65.3%) لجأوا الى لاانترنت للبحث عن هذه المنتجات وكيفية استخدامها. (32.9%) من المشاركين يقضون أكثر من 6 ساعات يوميا على الانترنت ووسائل التواصل، (94.2%) كانوا يرون اعلانات ممولة لمنتجات طبية خلال تصفحهم وسائل التواصل. (45.3%) من المشاركين جربوا هذه المنتجات بسبب الانترنت والصفحات على وسائل التواصل، (16.7%) من الاعلانات الممولة. المنتجات الأكثر استخاما كانت منتجات البشرة بنسبة (76.7%)، ثم منتجات الشعر بنسبة (72.2%)، ثم الفيتامينات بنسبة (58.8%). الشكل الصيدلاني الأكثر استخداما هو الكريمات والدهون بنسبة (71.3%)، (54.7%) من مستخدمي المنتجات الطبية أعادوا هذه التجربة مرة أخرى. (64.1%) من المشاركين وصفوا تجربتهم للمنتجات عبر الانترنت بأنها تجربة "سيئة" وغير صحية، (50.9%) من المستخدمين واجهوا اثار جانبية للمنتجات، (33.6%) من المشاركين توقفوا عن استخدام المنتجات التي اشتروها بسبب مواجهة الاثار الجانبية.

الخاتمة: استخدام المنتجات الطبية عبر الانترنت دون اللجوء الى استشارة طبية يعتبر شائع لفئة الشباب في فلسطين، وهذا يساهم في زيادة المشاكل الصحية، ولمعالجة وتخفيف ومحاولة منع هذه السلوكيات لتخفيف الاضرار نؤكد على دور وأهمية استشارة أهل الاختصاص وزيادة الوعي بين الناس عن أضرار هذه المنتجات.

الكلمات الدالة: المنتجات الطبية، الانترنت، طلاب الجامعات، السلوكيات الخاطئة، فلسطين .

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Uric Acid Relationship with Noninsulin-Based Insulin Resistance Indices in Selected Metabolic Disorders: A Systematic Critical Review

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ABSTRACT

OBJECTIVES: Uric acid (UA) has a role in pathogenesis of several metabolic abnormalities including insulin resistance (IR) and their related disorders. The aim of this report is to review the available evidences that reveal the association between UA, IR and related disorders via both noninsulin and insulin-based IR indices.

METHODS: The published literature was surveyed using Google Scholar and PubMed entering the terms Obesity, UA, IR, Metabolic Syndrome (MetS), Gestational Diabetes (GDM), Polycystic Ovarian Syndrome (PCOS).

RESULTS: UA had substantial positive relationships with IR, as well as obesity, MetS, DM, GDM, and PCOS. Evidently UA with a role in oxidative stress, endothelial dysfunction and induction of inflammation may cause IR in totality, the major factor in development of MetS and related diseases. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), insulin based IR index, correlated positively with UA. Moreover, specifically triglyceride to high density lipoprotein cholesterol ratio (TG/HDL-C), visceral adiposity index (VAI), lipid accumulation product (LAP), triglyceride to fasting glucose (TyG) index are noninsulin-based IR indices of positive correlations with UA. MetS score for IR (MetS-IR), a non-insulin based IR index, had significantly proportional correlations with MetS components as well as UA level. UA to HDL-cholesterol ratio (UHR) was a pronounced statistical predictor of MetS and diabetes control. UA positively associated with hyperinsulinemia and IR in prediabetes.

CONCLUSION: Succinctly UA can be an emerging biochemistry marker of predictive role in IR, MetS and related anomalies. More hyperuricemia related studies are warranted to be oriented from being correlational to mechanistic.

Keywords: Uric acid, insulin resistance, nascent Metabolic Syndrome, Gestational Diabetes, Polycystic Ovarian Syndrome (PCOS).

INTRODUCTION

Uric acid (UA) is an end product of metabolic catabolism of purine nucleotides (adenine and guanine) and the apprehensive causative agent of severe, acute and chronic inflammatory arthritis, gout. It has the role of antioxidant in liver, vascular endothelial cells and in the nasal sectretions[1]. UA has a relationship with several diseases and conditions such as diabetes mellitus (DM), nascent metabolic syndrome (MetS), polycystic ovarian syndrome (PCOS), and obesity through different pathological mechanisms. Many studies were conducted to verify these correlations.

Pathophysiological Mechanisms of UA

UA has different roles in inflammation and coronary endothelial dysfunction. Inflammatory markers such as high-sensitivity-C-reactive protein (hsCRP) and neutrophil count as well as increased homeostatic model

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assessment of insulin resistance (HOMA-IR) and so the risk of T2DM positively correlated with high serum UA level.[2a] Several pathological mechanisms were proposed for the role of UA in different diseases and conditions. Predominantly UA may induce inflammation through other several pathways including its pivotal role in Toll-like receptors (TLR)/NOD like receptor P3 (NLRP3) dependent activation and release of IL-1β and IL-18.[2b] First of all, UA irreversibly inhibits the production of nitric oxide (NO) leading to oxidative stress and endothelial dysfunction in different body tissues. This inhibition may lower insulin sensitivity and increase insulin level in skeletal muscles. It may induce proinflammatory markers in adipose tissues; this mechanism may increase the risk of hypertension, insulin resistance (IR) and MetS.[3] UA may change the lining of vascular system, exposing it to thrombosis and inflammation and so may increase the risk of cardiovascular diseases (CVD) such as DM and hypertension (HTN).[4] High level of UA may increase the production of reactive oxygen species (ROS) which may result in inflammation via augmenting the expression of inflammatory markers and in low insulin secretion and sensitivity.[5]. Another supposed mechanism of UA is increasing the activity of platelets and therefore thrombotic risk of many conditions such as arrhythmia, heart failure (HF), DM, MetS, and chronic kidney disease (CKD).[6] Markedly asymptomatic hyperuricemia was more likely a consequence of body mass index (BMI) in metabolically unhealthy obesity, unlike metabolically healthy obesity and metabolically unhealthy and healthy non-obesity.[7a] In displaying effectively the role of gut microbiota diversity and abundance in hyperuricemia; the antibiotic-fed high-purine-induced hyperuricemia rats had *significantly lower* UA content compared to the hyperuricemia-only rats, and gut microbiota (fecal microbiota transplant; FMT) from hyperuricemia rats increased UA content of recipient rats. The genera *Vallitalea, Christensenella* and *Insolitispirillum* may associate with hyperuricemia.[7b] Further Abnormal metabolism of gut microbiota reveals the possible molecular mechanism of nephropathy induced by hyperuricemia.[7c] In addition; as IR can be linkage between hyperuricemia of gouty arthritis and dyslipidemia.[8a] UA has a positive correlation with cholesterol production, and so on the risk of MetS; it modifies the homeostasis of cholesterol by increasing its endogenous synthesis. It is proportionally correlated with surrogate markers of cholesterol synthesis represented by ratio of lathosterol to total cholesterol and the ratio of lathosterol to compesterol index which represents the ratio of biosynthesis to intestinal absorption of cholesterol. In addition, it was found that UA has a positive correlation with triacylglycerol (TAG), low density lipoprotein-cholesterol (LDL-C), and negative correlation with high density lipoprotein-cholesterol (HDL-C).[8b]

UA correlations with Metabolic Abnormalities

UA and noninsulin based- as well as insulin-based Insulin Resistance (IR)

Insulin Resistance (IR) is a characteristic of several clinical conditions such as obesity, DM, and cardiovascular diseases. The major mechanism of IR is the low grade systemic inflammation. Inherent immunity and infection are supposed mechanisms of IR pathogenesis.[9] Several studies reported a correlation between UA and IR. A study which was conducted on non-diabetic patients showed that UA level was positively associated with IR using TyG index as a measure of IR.[10] High UA was correlated positively for HOMA-IR as a measure of IR in females but not in males.[11] IR is associated with autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). IR was measured in these patients by using TyG index, and it was found to be correlated positively with high UA level.[12] Another study conducted on patients with different levels of glucose tolerance to measure IR.

Four surrogate markers were used in the study and showed positive correlation with UA level. These markers include triglyceride to high density lipoprotein cholesterol

ratio (TG/HDL-C), visceral adiposity index (VAI), LAP, triglyceride to fasting glucose (TyG) index.[13] Opposite results were found in a cross sectional study which was conducted on non-diabetics with different levels of BMI. UA was found to be increased with increasing BMI. HOMA-IR as a marker of IR was found to be higher in overweight and obese, but no significant association was found between UA and IR. It is ambiguous weather high UA level is a result or a cause of IR, so UA cannot be used as alternative marker of IR.[14a] Conversely BMI, hyperinsulinemia, and insulin resistance (HOMA-IS) were independent risk factors of hyperuricemia in obese subjects. [14b]

UA and nascent Metabolic Syndrome (MetS)

MetS is a cluster of interrelated metabolic risk factors that increase the risk of cardiovascular morbidity and mortality.[15] It occurs simultaneously in the same individual, and results from systemic alterations in several organs and tissues; it includes abdominal obesity, dyslipidemia, hypertension, and hyperglycemia.[16-17] Many surrogate markers are used to predict MetS by measuring insulin resistance (IR). In a cross sectional study, triglyceride-waist circumference (TG-WC) and lipid accumulation product (LAP) were the most potent markers of IR that predict MetS.[18] Another cross sectional study reported that triglyceride-glucose index (TyG index) as a measure of IR is the most powerful marker for diagnosing of MetS compared to other IR indices such as fasting glucose, triglycerides, and HOMA-IR.[19] MetS score for IR (MetS-IR) is an indirect measure of non-insulin based IR which correlates fasting glucose with fasting triglycerides, BMI, and HDL-C. It has positive correlations with MetS components and it may be used to predict T2DM.[20] The same index was found in another study to have a positive relationship with hypertension as a component of MetS in patients who have normal BMI.[21] Most significantly the TG/HDLc, TyG, and METS-IR all had positive correlations with UA level in subjects without self- reported use of antihyperuricemic agents, hypoglycemic agents, or lipid-lowering drugs.[22] UA has correlations with MetS and its criteria. A cross sectional study which was the first study conducted on American children and adolescents to identify the prevalence rate of MetS at different concentrations of UA. The study showed increased prevalence of MetS with increased serum concentration of UA.[23] Higher UA level was reported in obese children compared to controls, the same study showed a correlation between UA concentration and MetS criteria.[24] In Liu et al. study (2010), the risk of MetS and its criteria increased with high serum concentration of UA in both men and women. The same study reported a significant positive correlation between central obesity and high UA in women but not statistically significant correlation in men.[25] In the study by Zhu et al. (2017), high serum level of UA associated with metabolic abnormalities such as high blood pressure, blood sugar, and lipid profile, as well as BMI.[26] Another cross sectional study was conducted on obese and non-obese diabetic inpatients. A positive association was found between high serum UA and obesity markers such as BMI and waist to hip ratio (WHR) in diabetic patients.[27] In a retrospective study that used for the first time UA to HDL-cholesterol ratio (UHR) as a predictor of MetS and diabetes control, UHR was found to be lower in well controlled diabetes compared to poorly controlled ones, as well as it was lower in diabetic patients without MetS compared to diabetic with MetS.[28] UA levels significantly correlated with 2-hour insulin levels and HOMA-IR scores in the prediabetes of impaired glucose regulation group.[29a] Obviously, that increasing the number of MetS components is associated with increasing level of UA. Pronouncedly favipiravir, as a proposed COVID19 treatment, can adversely increase serum UA in MetS patients. This can be exacerbated by combination therapy as MetS can be a risk factor of COVID19 progression and prognosis. [29b]

UA and Diabetes mellitus

DM has become a prevalent problem around the world. Many factors and mechanisms may involve in the development of DM. IR is a main pathological mechanism which contributes in the incidence of DM as well as other metabolic disorders.[30] The relation between UA and DM has become a hot topic of research, but controversy still exists. Some studies showed that high level of UA increase the risk

of development of DM. The study which was conducted on diabetic, non-diabetic, and prediabetic patients reported that patients who had impaired glucose tolerance (IGT) and DM had greater level of UA compared to normoglycemic ones.[31] In a cross sectional observational study conducted on diabetic and non-diabetic persons, higher serum UA concentration was noticed among diabetic and prediabetic compared to controls.[32] Another large cohort study was conducted on normoglycemic and prediabetic patients aged over 45 years. The study showed a positive association between UA level and the incidence of prediabetes (preDM) in normoglycemic ones, but no significant increase in incidence of DM among preDM patients. This may be due to the correlation of UA to the early phase mechanisms of DM.[33]

In a population based study, healthy persons were followed up for development of DM. The persons who had high serum UA were at more risk of development of DM.[34] several studies were conducted to correlate UA level with blood glucose level and HbA1c, as in Deb et al. study (2019) which was conducted on healthy persons and diabetic patients who had different level of blood glucose and HbA1c. The study demonstrated higher level of UA among diabetic participants compared to controls. In addition, the study reported that increasing level of blood glucose and HbA1c was associated with increasing level of UA.[35] Similar findings were found in a Saudi study, in which diabetic patients had higher level of UA compared to healthy ones, and HbA1c positively but not significantly correlated with UA level in diabetic patients.[36] Inverse findings were found in a cross sectional study conducted on US adults. A negative correlation between UA level and DM was reported. The proposed mechanism for this finding is the inhibition of UA reabsorption by high blood glucose level.[37] UA had been found to increase the risk of complications associated with DM such as albuminuria and renal damage.[38-39a] Interestingly intestinal microbiota of gout (mainly *Bacteroides caccae* and *Bacteroides xylanisolvens*) are enriched, thus are more similar to those of T2D than to liver cirrhosis. This may be related to disorders of purine degradation and butyric acid biosynthesis in gout patients. [39b] Substantially T2D patients infected with SARS-CoV-2 had decreased levels of BMI, lymphocytes, UA, and albumin, and increased CRP levels.[39] Evidently COVID-19 patients had UA and UA/Creatinine values lower than normal at admission. Male COVID-19 patients with low UA levels had a significantly higher risk of developing severe symptoms than those with high UA levels. During the aggravation course of the disease, the level of UA gradually decreased until discharge. At follow-up exam, the level of UA is similar to the levels at admission.[39c]

In terms of gestational diabetes mellitus (GDM), several studies demonstrated a positive correlation between high UA level in first trimester and the risk of developing GDM as in Laughon et al. study (2009).[40] Similar findings were found in El-Gharib et al. study (2013).[41] A case control study on pregnant women with and without GDM reported higher UA level among GDM women compared to controls.[42] In a retrospective study on registered births, the level of UA in the first period of pregnancy positively correlated with GDM.[43] In another study, 300 first trimester pregnant women were screened for serum UA, and followed till term. The study reported amazing correlation between UA level in the first trimester and the incidence of GDM.[44a] Substantially UA in patients of pregnancy hyperglycemia was much higher than those of healthy pregnant women. In the third trimester, COVID-19 infection in pregnant patients raised high risks of ketonuria, hypercoagulable state, and hyperfibrinolysis, which may lead to severe complications. COVID-19 increased the inflammatory responses of placenta, and fetuses and neonates had potential organ dysregulation and coagulation disorders. There was a potential intrauterine transmission while pregnant women had high titer of SARS-CoV-2, but it is necessary to detect SARS-CoV-2 in the blood cord, placenta, and amniotic fluid to further confirm intrauterine infection of fetuses.[44b]

UA and Obesity

Obesity is strongly correlated with IR; which may play a role in pathogenesis of obesity complications. Obesity may cause impairment and dysfunction of insulin sensitive tissues.[45] Obesity strongly associated with UA level. A cross sectional study was conducted on Thai adolescents' boys and girls showed high UA level in obese boys compared to those who had normal weight.[46] Similar findings were reported between UA level and obesity in Japanese children.[47] Another cross sectional study on obese and non-obese Bangladeshi adults showed significant positive correlation between UA level, obesity and its markers such as body mass index (BMI), waist circumference (WC), and hip circumference (HC). Higher UA level among overweight and obesity group compared to underweight group.[48] In a cohort study, it is found that UA had a correlation with all-cause mortality rate, but no significant correlation with cardiovascular mortality rate in obese adults.[49] On the contrary, the first study on obesity and its correlation with UA reported positive but weak relationship between high UA and obesity due to co presence of other factors affecting the obesity.[50a] Importantly in the general population, gout is associated with many risk factors for poor COVID-19 outcomes; it is more common in men than in women, it occurs more often in older individuals, and it is strongly associated with overweight and obesity, CVD, T2D, and chronic kidney disease. Thus even when gout patients are not prescribed immunosuppressants, their comorbidities mean that they have among the highest risk of poor COVID-19-related outcomes of all patients with rheumatic diseases.[50b]

UA and Poly Cystic Ovarian Syndrome (PCOS)

PCOS is an endocrinologic disease occurs in premenopausal women. Several studies have reported that patients with PCOS may suffer from deterioration of insulin secretion which may progress into other diseases such as DM.[51] Reproductive hormones play a role in hyperuricemia in females with PCOS. A retrospective cross sectional study was conducted on women with PCOS showed higher concentration of serum UA in those women compared to women without PCOS.[52] Another study showed a significant higher UA level in women with PCOS, this may be due to increased metabolism of UA by androgens which are present in high concentration in PCOS.[53] The same findings were found in Swetha et al. study (2013).[54] On the other hand, conflicting results were found in Leustean et al. study (2015). The study reported non-significant difference in serum UA concentration between PCOS and non PCOS groups; this may be due to small sample size. The same study demonstrated a positive relationship between sera UA, BMI, and WC as markers of obesity.[55a] Mechanistically the testosterone level was positively associated with the prevalence of hyperuricemia in females with PCOS. [55b] Visceral adiposity index (VAI) was independently associated with hyperuricemia in PCOS patients. [55c] Principally FMT were promising subordinate therapy option in altered microbiota-relevant hyperuricemia. [7b] Besides urease inhibitors are nominated treatment option in hyperuricemia induced nephropathy.[7c] Of importance, diversity and abundance of gout treatment options are widely elaborated and discussed in light of COVID19 infection. [50b].

Conclusion

UA has several correlations with different conditions and diseases. Positive association was found between UA and each of IR, DM, GDM, MetS, obesity, and PCOS. UA can be used to predict the incidence of these diseased conditions, but further causality longitudinal investigations are required to confirm that.

CONFLICT OF INTEREST the authors declare no conflict of interest.

CONTRIBUTIONS OF AUTHORS the authors equally contributed to this manuscript composition

Abbreviation Table

BMI	Body mass index
CKD	Chronic Kidney Disease
CRP	C-reactive protein
DM	Diabetes mellitus
GDM	Gestational diabetes mellitus
HC	Hip circumference
HDL-C	High density lipoprotein-cholesterol
HF	Heart failure
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
hs-CRP	High-sensitivity C-reactive protein
HTN	Hypertension
IGT	Impaired glucose tolerance
IR	Insulin resistance
LAP	lipid accumulation product
LDL-C	Low density lipoprotein-cholesterol
MetS	Metabolic syndrome
NO	Nitric Oxide
PCOS	Poly cystic ovarian syndrome
preDM	Prediabetes
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
TAG	Triacylglycerol
TG/HDL-C	Triglyceride to high density lipoprotein cholesterol ratio
TyG-WC	Triglyceride-glucose index*waist circumference
UA	Uric acid
UHR	Uric acid to HDL-cholesterol ratio
VAI	Visceral adiposity index
WC	Waist circumference
WHR	Waist to hip ratio

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علاقة حمض اليوريك مع مؤشرات مقاومة الأنسولين الغير المعتمدة على الانسولين في والاضطرابات ذات الصلة بالأيض : مراجعة نقدية منهجية

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ملخص

الأهداف: يمتاز حمض اليوريك بأن له دور في التسبب في العديد من الاضطرابات الأيضية بما في ذلك مقاومة الأنسولين والاضطرابات المرتبطة بها. ان الهدف من هذا التقرير هو مراجعة الأدلة المتاحة التي تكشف عن الارتباط بين حمض اليوريك ومقاومة الانسولين والاضطرابات ذات الصلة عبر مؤشرات مقاومة الانسولين المعتمدة وغير المعتمدة على الانسولين.

الطريقة: تم مسح الأدلة المنشورة باستخدام **Google Scholar** و **PubMed** بإدخال مصطلحات السمنة، حمض اليوريك، مقاومة الانسولين، متلازمة التمثيل الغذائي،سكري الحمل، ومتلازمة تكيس المبايض.

النتائج: أظهر حمض اليوريك علاقات إيجابية كبيرة مع مقاومة الانسولين ، بالإضافة إلى السمنة ،متلازمة الأيض الغذائي، مرض السكري،سكري الحمل، ومتلازمة تكيس المبايض. من الواضح أن حمض اليوريك له دور في الإجهاد التأكسدي ، والخلل البطاني وتحريض الالتهاب الذي قد يسبب مقاومة الانسولين والتي تعد العامل الرئيسي في تطور متلازمة الأيض الغذائي والأمراض ذات الصلة. نموذج تقييم مقاومة الأنسولين المتجانس والمعتمد على الانسولين أظهر ارتباطا إيجابيا مع حمض اليوريك.علاوة على ذلك و على وجه التحديد ، نسبة الدهون الثلاثية إلى نسبة كوليسترول البروتين الدهني عالي الكثافة ،مؤشر السمنة الحشوية ، منتج تراكم الدهون ، مؤشر الدهون الثلاثية إلى جلوكوز الصائم والتي هي مؤشرات مقاومة انسولين غير معتمدة عليه أظهرت ارتباطات إيجابية مع حمض اليوريك.كما أظهرت نسبة متلازمة الأيض الغذائي الى مقاومة الانسولين ، والتي هي مؤشر مقاومة انسولين غير معتمد على الانسولين ارتباطات متناسبة بشكل كبير مع مكونات متلازمة الأيض الغذائي وكذلك مستوى حمض اليوريك.وقد كانت نسبة حمض اليوريك إلى نسبة كوليسترول البروتين الدهني عالي الكثافة مؤشراً إحصائياً واضحاً للتنبؤ بمتلازمة الأيض الغذائي ومرض السكري.كما أظهرحمض اليوريك ارتباطا إيجابيا بفرط انسولين الدم ومقاومة الانسولين في مرض ماقبل السكري.

الخلاصة: يمكن أن يكون حمض اليوريك بإيجاز علامة كيميائية حيوية ناشئة للتنبؤ بحدوث مقاومة الانسولين ومتلازمة الأيض الغذائي والاضطرابات ذات الصلة. هناك ما يبرر توجيه المزيد من الدراسات المتعلقة بفرط حمض يوريك الدم من كونها مترابطة إلى آلية

الكلمات الدالة: حمض اليوريك ، مقاومة الأنسولين ، متلازمة التمثيل الغذائي ، سكري الحمل ، متلازمة تكيس المبايض.

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Prevalence of Hypogonadism in β-Thalassemia Major Patients from Gaza Strip

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ABSTRACT

This study was conducted to determine the prevalence of hypogonadism among β-Thalassemia Major (β-TM) adolescent patients, and the role of iron chelation therapy (ICT) in its prevention. Data was collected from 60 β-TM patients and 35 controls aged 13─ 30 years through a questionnaire and a review of medical history to collect sociodemographic characteristics, transfusion regimen, chelating history and pubertal status. Complete blood count, serum ferritin, and pituitary-gonadal function tests were analyzed 1–2 weeks after blood transfusion. Logistic regression, independent t-test, and chi-square test were used to analyze our results and the significance was tested at $p \leq 0.05$. The overall prevalence of hypogonadism among β-TM patients was 75%, the mean of serum ferritin was 6704.83± 2849 ng/ml and all patients had serum ferritin above 2000 ng/ml indicating a severe iron overload. Low hemoglobin level (p=0.006), high ferritin level (p=0.018), high blood transfusion per year (24-blood transfusions) (p=0.007), and poor compliance to iron chelation therapy programs (p=0.001), were detected had significant partial effect in hypogonadism. In conclusion, hypogonadism is highly prevalent among Gaza strip β-TM patients. Its presence being associated with lower hemoglobin values, iron overload, high blood transfusion and low compliance of iron chelation therapy.

Keywords: β-thalassemia major, Hypogonadism, Iron overload, Chelation therapy, Gaza strip.

1. INTRODUCTION

β-Thalassemia major (β-TM) is the term applied to patients who have no effective or severely limited production of β-globin chains. This type is the most severe form of thalassemia which is often associated with life-long blood transfusion dependent anemia[1]. More than five decades ago, β-TM was fatal in the first decade of life, this poor prognosis changed since the survival rates started to increase progressively; thanks to intensive blood transfusion programs combined with chelation therapy[2]. Humans have a very limited ability to excrete iron, so regular blood transfusion inevitably leads to iron overload. Evidence of marked iron deposition in the liver, heart, pancreas, pituitary, thyroid, parathyroid, adrenal, gonads, renal medulla, bone marrow, and spleen are commonly reported[3]. To date, there are 3 major classes of iron chelators. The first is Deferoxamine (DFO), which cannot be orally absorbed, so, it must be administered subcutaneously or intravenously. Having a short plasma half-life of 20–30 minutes, DFO should be administered by slow subcutaneous (SC) infusion over a span of 8-12 hours using an infusion pump for 6 nights per week[4]. The second iron chelator is Deferiprone (DFP), which can be administrated orally once daily[5]. Also, the third iron chelator Deferasirox (DFX) is recommended orally once or twice daily and is characterized by its high affinity for iron [6].

Abnormalities in pubertal onset and hypogonadism are the most frequently reported endocrine complications that

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affect 50–100% of β-TM patients [7], and is likely to result from impaired gonads (primary), pituitary gland (secondary), and hypothalamus (tertiary). Secondary hypogonadism, often referred to as hypogonadal hypogonadism, is the most common cause of hypogonadism in thalassemia major. Several worldwide studies examined the risk factors for these complications, and the results varied between them. Some of them showed that pituitary iron overload due to intensive blood transfusion and low efficient chelation is the main risk factor for hypogonadism[8,9] . These studies observed significant relationship between serum ferritin level and hypogonadism rate, however other studies did not observe significant relationship between serum ferritin and hypogonadism rate, these studies attributed hypogonadism for factors other than iron overload like multi-endocrine dysfunction, chronic anemia, infections, and malnutrition[10,11].

In Gaza Strip, β-thalassemia is a major public health problem where the screening studies estimated the prevalence of β-thalassemia gene carriers between 2.6% and 4.3%. The relatively high consanguinity rate, as well as the remarkable prevalence of the defects in HBB gene, resulted in more than 300 β-TM patients currently being managed through blood transfusions and iron chelation at the government hospitals[12,13].

The present study evaluated the prevalence of hypogonadism among β-thalassemia major patients from the Gaza Strip, and how it associates to the level of ferritin, the main indicator for body iron overload. The outcomes of our evaluation study could be used to improve or modify the management protocols, such as blood transfusions and iron chelation, for our patients in Gaza Strip.

2. SUBJECTS, AND METHODS

2.1. Study design

This study is a quantitative analytical retrospective case control study in which the prevalence of hypogonadism among β-TM patients from the Gaza Strip was evaluated, and correlated with ferritin level to detect the main risk factors for the resulted rate of hypogonadism, thus the main measures for its prevention could be determined.

2.2. Patients

2.2.1.Study Population and Sample Size

Sixty β-thalassemia major patients were selected randomly from two hospitals in the Gaza strip, where thalassemia patients are usually monitored and attend regularly for blood transfusion and iron chelation therapy administration, in Al Shifaa hospital, and European Gaza hospital.

Also, thirty-five healthy subjects with the same age and sex distribution as the experimental group served as the control group. Clinical and laboratory evaluation of patients and controls was performed in the same way after taking written consent to participate in this study after explaining its objectives to them.

2.2.2. Inclusion criteria

Patients were considered eligible in this study upon meeting the following criteria: (1) β-TM with 13-30 years old. (2) on a regular blood transfusion program. (3) subjected to suitable iron chelating program.

2.2.3. Exclusion criteria

Exclusion criteria were as follows: (1) non-transfusion-dependent thalassemia's. (2) mental illness. (3) bone marrow transplanted patients. (4) acute diseases as a sudden increase in serum ferritin may be caused by infections, or inflammatory conditions[14]. (5) treatment with hormones or anabolic steroids within the last 3 months.

2.3. Data collection.

Data was collected through questionnaire and a review of medical history records. The questionnaire included major sets of questions that include demographic characteristics, age at first transfusion and the interval between transfusions, age of starting iron chelation therapy (ICT), and compliance rate with ICT programs which was defined as percent of doses administered in the week out of those prescribed (The use of DFO regularly 5–7 days a week was considered as excellent, while the use of DFO

less than 5 days a week and less than two days a week was considered as good and poor compliance, respectively according to Haghpanah, et al.(2014)[15] and Trachtenberg, et al. (2011)[16].

2.4. Blood sampling and analytical procedures

All blood samples were collected in the morning (08.00 – 09.00 am) after an overnight fasting, and 1–2 weeks after blood transfusion. The investigations included complete blood count, serum ferritin analysis, liver function tests, pituitary-gonadal function analysis which included follicle-stimulating hormone (FSH) analysis, Luteinizing hormone (LH) analysis, estradiol analysis in females and testosterone analysis in males. **2.5. Assessment of hypogonadism and delayed puberty:**

2.5.1. Laboratory evidence for hypogonadism

Laboratory evidence for hypogonadism was based on finding of low FSH and LH with low estradiol or testosterone (hypogonadotrophic hypogonadism), or normal FSH and LH with low estradiol or testosterone (normogonadotrophic-hypogonadism) according to Noetzli et al.[17].

2.5.2. Pelvic ultrasound and careful physical examination

Hypogonadism and delayed puberty in males was defined by absence of testicular enlargement (>4ml) by the age of 18 years or the lack of complete pubertal development for more than 5 years after the start of puberty. While in females it is defined as: Absence of breast development by the age of 13 years, Primary amenorrhea or Secondary amenorrhea[18,19].

2.5.3.Tanner staging

According to Tanner staging which known as Sexual Maturity Rating (SMR), and defined as an objective classification system that providers use to document and track the development and sequence of secondary sex characteristics of children during puberty according to separate scales for the development of external genitalia: phallus, scrotum, and testes volume in males; breasts in females; and pubic hair in both males and females, hypogonadism is diagnosed by Tanner stage 2 or 3[20].

2.6. Data analysis

Statistical package for social science (SPSS)version 22 was used for analysis of data, and statistical relationship between hypogonadism and all variables were assessed using logistic regression chi-square and independent t-test and with (CI) of 95%.

3. RESULTS

3.1. Sociodemographic characteristics

As shown in table (1) out of 60 patients included in this study; 29(48.3%) patients were males, while 18(51.5%) of controls were males. The mean age of study patients was 21.08±3.8 years and study controls was21.11 ± 3.86 years. BMI in thalassemic patients was about 19.97 ± 2.74 that significantly lower than controls (P = 0.001) and high percentage of thalassemic patients about 28(46.67%) were underweight. Splenomegaly was reported to be a major complication among our β-TM patients that was mainly managed by splenectomy in 75% of them.

3.2. Hemoglobin level and iron status of the study sample

Reviewing the medical files of the studied patients revealed that all of them were diagnosed in the first year of life through hemoglobin electrophoresis and began blood transfusion. The mean age at the first transfusion was four months (range 1–10 months).

Table (2) shows that the mean current pre-transfusion hemoglobin (Hb) levels was about 7.64 ± 0.87 g/dl, and 7.48 ± 0.86 g/dl for males and females respectively, which was significantly lower than the mean Hb levels of study controls (P<0.005). The distribution of study sample according to three Hb level intervals showed about 56(93.33%) of thalassemic patients were below the expected pre-transfusion hemoglobin level (9-10) g/dl. To correct the anemic status among thalasssemic patients, all of them were given packed red blood cells. Data shown in table (2) represents the distribution of patients according to the number of blood transfusions per year which estimated from the week interval between transfusions

and range between 12-24 blood transfusions per year.

Frequent transfusions are associated with iron overload that usually is reflected by serum ferritin. The mean serum ferritin concentration for β-TM males was 6381 ± 2813 ng/ml significantly higher than the mean serum ferritin concentration of study control males 170 ± 25 ng/ml (P<0.005). Also, the mean serum ferritin concentration for thalassemia females was 7007 ± 2895 ng/ml was significantly higher than the mean serum ferritin concentration of study control females 106 ±29 ng/ml (P<0.005). This revealed that all thalassemic patients of the study sample had severe iron overload with ferritin level more than (2000 ng/ml) which serves as a risk factor for multiple endocrine dysfunctions.

3.3.Iron chelating therapy characteristics and compliance rate

All patients who participated in this study were using iron chelating agents, specifically Deferoxamine (Desferal)® due to unavailability of other chelators at the ministry of health for about one year. The mean dose of (Desferal)® for study patients was 49.27 (±SD 23.6) mg/kg/day (range1500-5000mg/day) administered by slow subcutaneous (SC) infusion over a span of 8-12 hours using an infusion pump for 5-7 nights per week. As shown in table (2) only about 40(66.67%) of patients used chelating agents at earlier dates before the age of 10 years, and the majority of patients 32(53.33%) had poor compliance to the therapy regimen, that resulted from practical issues such as difficulty with DFO infusions, or from psychological / psychosocial issues, socio-economic status, and lack of family support.

3.4.Characteristics of patient's liver function test levels

Table (3) shows significantly worsened liver function test levels results for β-thalassemia major patients compared to controlled persons (ALT 82.68 ±38.9 IU/l; AST 76.5 ± 41.4 IU/l; ALP 355.42±108.98U/l; and INR 1.25±0.12, P < 0.001).

3.5. Patient's Gonadal hormones level

In the gonadal profile, LH, FSH, total testosterone and estradiol were significantly different between cases and controls in males and females as shown in table (3).

3.6. Patients distribution according to gonadal function status

The prevalence of hypogonadism in β-TM was about 75%, while it was only 11.5 % in the control group. About 53.33% of β-TM patients had hypogonadal hypogonadism, while 21.67 of them had normo-gonadal hypogonadism.

3.7. Symptoms of hypogonadism

The main symptoms of hypogonadism in our β-TM females were menstrual irregularities as primary amenorrhea and secondary amenorrhea which applied to 29% and 42% of females respectively. In male's hypogonadism was in the form of sexual infantilism: this applied to 72% of them. Also, a grossly impaired pattern of sexual maturity rating (SMR) was found in our β-TM patients. About 2 (3.33%), 6 (10%), 19 (21.6%) of cases group had an SMR of T1, T2, and T3 respectively.

3.8. Risk factors for hypogonadism

In order to predict risk factors for hypogonadism, simple and multiple logistic regression analyses were employed after taking into consideration the absence of multicolinearity between the variables of interest.

The predictor variables in first multiple logistic regression analysis were age, gender, hemoglobin level, BMI, ferritin level, blood transfusion rate per year and age of using iron chelation therapy. A test of the full model versus a model with intercept only was statistically significant, $\chi 2 = 44.434$, $p < 0.001$. The model was correctly classified 88.3% of cases.

Table(4) shows the logistic regression coefficient, Wald test, and odds ratio for each of the predictors. Employing a 0.05 criterion of statistical significance, hemoglobin level (p=0.006), ferritin(p=0.018), and one blood transfusion program had significant partial effect.

The odds ratio for different variables indicates that 1% increment in hemoglobin level will decrease

hypogonadism risk about 98% when holding all other variables constant, while 1% increment in ferritin level will increase hypogonadism risk about 0.1% also when holding all other variables constant. Regarding to different programs of blood transfusion only there was significant difference between 12-blood transfusion program per year and 24- blood transfusion program per year(p=0.007) with odds ratio about (0.000) which indicates that patients who need blood transfusion once monthly (12-blood transfusion per year) were at very low risk to have hypogonadism compared to patients who need blood transfusion twice monthly (24-blood transfusion per year).

Second logistic regression was employed to predict the effect of compliance to ICT on hypogonadism. A test of the full model versus a model with intercept only was statistically significant, $\chi 2 = 23.88$, $p < 0.001$. The model was correctly classified 83.3% of cases. As shown in Table(4) significant difference was predicted between groups with poor and excellent compliance to ICT program (p=0.001), and patients with poor compliance to ICT program will have hypogonadism 28.6 times more than patients with excellent compliance to ICT program when holding ferritin constant. Finally , there was no significant di erence between hypogonadism and normal groups in percentage of splenectomy, and presence of other endocrine disease or levels of liver enzymes (Table5).

4. DISCUSSION

4.1. Prevalence of hypogonadism

This is, to the best of our knowledge, the first study that focused on endocrinopathies of β-TM in the Gaza strip. Our findings regarding the range of hypogonadism in patients with β-TM are consistent with findings in previous studies as in the West Bank and Saudia Arabia with rate about 46.7%, and 52.7% respectively[21,22]. while these findings are much higher than findings in Dubai and Taiwan with rate about 25%, and 23.1% respectively. This lower rate could be explained by the availability of treatment according to the latest guidelines to all patients including the latest medical treatments such as deferasirox (DFX) iron chelator which can be administrated orally once daily thus ensuring patient continuity with treatment [23,24].

4.2. Symptoms of hypogonadism and delayed puberty

Primary amenorrhea and secondary amenorrhea were the main symptoms of hypogonadism in our β-TM females, while sexual infantilism was the main symptom of hypogonadism in male participants with β-TM which was applied to 72% of them. This become in agreement with different studies carried out by Merchant & Shirodkar, 2011; Moayeri, H. & Oloomi, Z.,2006 in India and Iran respectively[25,26].

Also, a grossly impaired pattern of sexual maturity rating (SMR) which was found in our β-TM patients become in agreement with different studies carried out in India and Eygpt[25,27].

4.3. Relationship between hypogonadism and other variables

The main risk factors associated with hypogondism were anemic status and the overloaded iron due to chronic blood transfusion regimen associated with inadequate chelation therapy. This study revealed that most of the study patients about 93% had Hb level lower than (9g/dl) that is recommended by Thalassemia International Federation (TIF) and also lower than median baseline of 10.0g/dl reported by previous studies[14,28]. This reflects a severe anemic presentation of the study group. These results were consistent with the results of across sectional study in Tunis among twenty-eight β-TM patients and showed chronic hypoxia due to anemia could be a mechanism involved in pituitary-gonadal dysfunction[29].

The measurement of serum ferritin concentration used in this study to assess the iron status of patients as it is an easy, cost effective and noninvasive indicator of iron overload. The average of serum ferritin level for patients in the current study was 6704.83ng/ml (±SD 2847.8) and all patients had serum ferritin ≥2000ng/ml. Such increase in serum ferritin level is well known to put these patients at a great risk of developing

iron overload complications. According to TIF guidelines, serum ferritin cut off limit must be kept below 1000ng/ml[14]. This rise of serum ferritin in our participants resulted despite the fact that all patients were on standard regimen of iron chelation therapy but the main problems were that Deferoxamine (Desferal)® chelator which need parenteral administration was the only available drug and oral chelators were unavailable to replace it at the ministry of health for long period of time, which resulted in that majority of patients with poor adherence to the chelation regimen.

Other risk factor for hypogonadism was frequency of blood transfusion which indicates that patients who need blood transfusion twice monthly (24-blood transfusion per year) were at very high risk to have hypogonadism compared to patients who need blood transfusion once monthly (12-blood transfusion per year). The present results were consistent with the results of a retrospective cohort analysis of β-TM patients attending an ambulatory transfusion clinic in Australia which showed that the age of first transfusion and frequency of blood transfusion correlated with the number of endocrinopathies[30], while these results were inconsistent with other cross sectional study in India among 35 patients with β-TM which stated that there was no direct relationship between the amount of iron accumulated and organ dysfunction, and it is possible that endocrine glands are extremely sensitive to iron toxicity that even small amounts of iron accumulated in the early years of life can cause irreversible damage[25].

In our survey also, compliance to ICT program was detected as significant predictor for hypogonadism and majority of patients with poor compliance at high risk for hypogonadism. This result attributed mainly to the availably of only DFO as an iron chelators at the MOH, that is only given either subcutaneously through the pump or by intravenous infusion.

Both ways of therapy were not preferable by patients who found it painful. In addition, the pump was not always provided and sometimes broken down and need a long period of time to be repaired. All of the studied patients, said that they hope to use oral therapy to avoid the harmful pain. Despite that iron overload of tissues is the most important complication of β-thalassemia and is a major subject of management iron chelation therapy is still not efficient in Gaza Strip as indicated by the high ferritin level observed in our patients even after desferrioxamine chelation therapy. Thus, strategies to improve chelation regimens should be of the highest priority. These strategies must include continuance providing of oral iron chelators such as deferasirox (Exjade) and deferiprone to improve compliance**.**

5. Conclusion and Recommendations

Results of the current study revealed a high prevalence of hypogonadism as well as a gross impairment of pubertal development in β-TM patients in Gaza strip which was associated with iron overload and severe anemia. Higher blood transfusion and low compliance of iron chelation therapy was particularly associated with pubertal abnormalities. Such findings emphasize the need for a protocol for early detection and prevention of iron overload which leads to cell damage in various endocrine glands. Assessment of blood transfusion frequency, early (prepubertal), monotherapy, or combined chelation therapy, together with appropriate monitoring and continuous health education programs can protect or ameliorate such consequences.

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Limitations

This study was carried out, with all its blood tests at the student's personal expense, without any financial donation from anyone. Therefore, blood tests were limited and did not include any other endocrine disorder.

Ethical Approval

The study was approved by Helsinki Committee of ministry of health-Palestine at 4/12/2017.

Conflict of Interest

There is no conflicts of interest.

Table (1). Sociodemographic characteristics of patients and control groups

Variables		Cases (n=60)	Controls (n=35)	P-Value[a]
Gender	Male	29 (48.3)	18(51.5)	0.833 (X2)
No. (%)	Female	31 (51.7)	17(48.5)	
Age	Mean ± SD	21.08 ± 3.9	21.11± 3.9	0.970
Age groups (year)	13 – 21	36 (60)	22(63)	0.783 (X2)
No. (%)	22 – 30	24 (40)	13(37)	
Height (cm)	Male	152.76± 6.4	164.78± 2.9	0.001*
Mean ± SD	Female	149.71± 7.7	162.94± 2	0.001*
Weight (kg)	Male	47.52± 9.4	65.39± 10.01	0.001*
Mean ± SD	Female	46.19± 9	63.29± 9.3	0.001*
BMI	Mean ± SD	19.97± 2.7	23.91± 3.1	0.001*
BMI groups	<18.5	28 (46.67)	1(2.9)	0.001†*
No. (%)	18.5-24.99	32 (53.34)	22(62.9)	
	25-29.99	--	9 (25.7)	
	≥30	--	3 (8.5)	
Splenectomy	Yes	45 (75)	--	0.001(X2)*
No. (%)	No	15 (25)	35(100)	
Other endocrine disease				0.082 (X2)
No. (%)	Present	6 (10)	--	
	Absent	54 (90)	35(100)	

BMI; body mass index (calculated based on (NCD Risk Factor Collaboration, 2016, page 35).
(a)Statistical testing using chi-square test(X^2),Fisher's exact test (†) or independent sample t test.
(*) Difference is significant at the 0.05 level (2-tailed).

Table (2). Distribution of study sample according to hemoglobin level, transfusion regimen and iron status.

Variables	Cases (n=60)	Controls (n=35)	P-Value[a]
Blood hemoglobin (g/dl) [Mean ± SD]			
Males	7.64 ±0.87	14.14 ±0.48	0.001*
Females	7.48 ±0.86	12.18 ±0.47	0.001*
Blood hemoglobin categories			0.001 (X^2)*
6-7.49	29(48.33%)	--	
7.5-8.99	27(45%)	--	
9≥	4(6.67%)	35(100%)	
Blood transfusion regimen per year			
12 Blood transfusions	12(20%)	---	
16 Blood transfusion	20(33.33%)		
24 Blood transfusion	28(46.67%)		

Variables	Cases (n=60)	Controls (n=35)	P-Value[a]
Age of using ICT			
Before 10 years old	40(66.7%)		
After 10 years old	20(33.3%)		
Compliance to ICT regimen			
Excellent Compliance	13(21.7%)		
Good Compliance	15(25%)		
poor Compliance	32(53.3%)		

(a)Statistical testing using chi-square test(X^2), or independent sample t test.

(*)Difference is significant at the 0.05 level (2-tailed).

Table (3). Medical characteristics of patients and control groups

Variables	Cases (n=60)	Controls (n=35)	P-Value[a]
	Mean ± SD	Mean ± SD	
Ferritin (ng/ml)			
Females	7007± 92895	106 ± 29	0.001*
Males	6381± 2813	170 ± 25	0.001*
ALT(IU/l)	82.68 ± 38.9	24.71± 5.4	0.001*
AST(IU/l)	76.5 ± 41.4	24.63± 6.3	0.001*
ALP (U/l)	355.4 ±109	220.1± 209	0.001*
T. Protein(g/dl)	6.29±0.6	7.44±0.5	0.001*
Albumin (g/dl)	3.87 ± 0.5	4.11± 0.2	0.001*
D. bilirubin (mg/dl)	0.61± 0.3	0.1397± 0.04	0.001*
T. bilirubin (mg/dl)	2.13±1.4	0.72± 0.1	0.001*
INR	1.25±0.1	0.93± 0.1	0.001*
LH (mIU/ml) Female	2.70 ± 2.7	6.15 ±1.2	0.005*
Male	1.87 ± 1.8	6.27 ±0.8	0.001*
FSH (mIU/ml Female	3.46 ± 3	5.42±1.2	0.010*
Male	2.11 ± 1.9	5.62± 0.7	0.001*
Estrogen in females (Pg/ml)	28.07 ±21.9	67.22 ±27.5	0.001*
Total testosterone in males (ng/dl)	168.62 ± 174	486 ±118.1	0.001*

(a)Statistical testing using independent t-Test.

(*) Difference is significant at the 0.05 level (2-tailed).

Table (4). Detailed estimates from logistic regression models for determination risk factors for hypogonadism .

Predictor	Regression coefficient(B)	Wald	P-Value[a]	Odds ratio
Gender(1)	-3.501	3.175	0.075	0.030
Age	-0.171	0.933	0.334	0.843
BMI	-0.543	2.712	.1000	.5810
Hb	-3.912	7.524	.006*0	.0200
Ferritin	0.001	5.644	0.018*	1.001
Blood transfusion per year				
12- transfusions per year	-8.626	7.184	0.007*	0.000
16- transfusions per year	-4.485	3.517	0.061	0.011
Age of using ICT	-0.377	2.613	0.106	0.686
ICTcompliance				
ICTcompliance(1) (poor)	3.356	10.303	.001*0	28.678
ICTcompliance(2)(good)	0.855	.9170	0.338	2.352

(*)Statistical significant for individual predictor at the 0.05 level.

Table (5). Relationship between hypogonadism and medical characteristics.

Variable	Categories	Hypogonadism(n=45)	Non-Hypogonadism(n=15)	p-value [a]
Splenectomy	Yes	34 (75.56)	11 (24.44)	0.556†
	No	11 (73.33)	4 (26.67)	
Other endocrine disorder	Present	6 (100)	0 (0)	0.321†
	Absent	39 (72.22)	15 (27.78)	
ALT	Mean ± SD	87.2 ± 39.60	69 ± 34	0.11
AST	Mean ± SD	80.6 ± 42.80	64.1 ± 35.4	0.18
ALP	Mean ± SD	366.91 ±117.82	320.93±68.72	0.073

(a)Statistical testing using chi-square test(X^2), Fisher's exact test (†) or independent sample t test.
(*)Difference is significant at the 0.05 level (2-tailed).

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مدى انتشار قصور الغدد التناسلية في مرضى التلاسيميا العظمى (β) في قطاع غزة

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ملخص

أجريت هذه الدراسة لتحديد مدى انتشار قصور الغدد التناسلية بين المراهقين من مرضى التلاسيميا العظمى ((β، ودور أدوية إزالة الحديد في الوقاية منه. تم جمع البيانات من (60) من مرضي التلاسيميا العظمى((β و(35) شخصا سليما تتراوح أعمارهم بين 13 و 30 عامًا من خلال الاستبانة ومراجعه السجلات الطبية لجمع الخصائص الاجتماعية والديمغرافية ، نظام نقل الدم، تاريخ البدء باستخدام أدوية ازالة الحديد ومدى الانتظام بأخذها وحالة البلوغ. كذلك تم إجراء العديد من التحاليل التي شملت تحليل الدم الكامل، تركيز الفريتين في الدم واختبارات وظائف الغدة النخامية و الغدد التناسلية بحيث تمت جميعها في الصباح الساعة (08.00 –9.00 صباحا) بعد ليلة من الصيام ، و أسبوع الى أسبوعين من نقل الدم. تحليل البيانات تم باستخدام تحليل الانحدار اللوجستي ،اختبار (ت) للعينات المستقلة واختبار كاي المربع. أظهرت النتائج أن معدل انتشار قصور الغدد التناسلية لدى مرضى التلاسيميا العظمى((β في قطاع غزة يصل إلى 75 ٪ ، وقد كان تركيز الفريتين لدى المرضى حوالى 6704.83 ± 2849جم /مل وكان جميعهم لهم تركيز الفريتين أعلى من 2000 جم/مل مما يشير إلى زيادة شديدة في الحديد لديهم. ومن خلال تحليل الانحدار اللوجستي تبين أن انخفاض مستوى الهيموغلوبين ($p = 0.006$) ، وارتفاع مستوى الفريتين ($p = 0.018$) ، وارتفاع نقل الدم سنويًا (24 عمليات نقل دم) ($p = 0.007$) ، وسوء الالتزام بأدوية إزالة الحديد ($p = 0.001$) هي من أهم العوامل المسببة لقصور الغدد التناسلية وتخلص هذه الدراسة إلى أن قصور الغدد التناسلية منتشر بشكل كبير بين مرضى التلاسيميا العظمى((β في قطاع غزة، ويرتبط وجودها بانخفاض قيم الهيموجلوبين، زيادة تركيز الحديد في الدم، ارتفاع معدل نقل الدم وانخفاض الالتزام باستخدام أدوية إزالة الحديد.

الكلمات الدالة: التلاسيميا العظمى (β)، قصور الغدد التناسلية، الزيادة في مستويات الحديد، أدوية إزالة الحديد، قطاع غزة.

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Essential oil composition and antiproliferative activity of *Ecballium elaterium* (L). aerial parts:A Medicinal essential oil bearing plant from Jordan

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ABSTRACT

Background & aims: This study aims to provide GC-FID and GC-MS analyses of the essential oil of dried aerial parts of Ecballium elaterium L. grown in Jordan and examining its cytotoxicity capacity. **Methods**: Essential oil was obtained by hydrodistillation using Clevenger apparatus. MTT assay method was used to investigate the plant's in vitro antiproliferative activity against MCF-7, Caco-2 and Panc-1 cancer cell lines in addition to normal fibroblast cells. **Results**: E. elaterium hydrodistilled oil yielded thirty one components, accounting for 76.3% of the total oil content. High contents of nonterpenoidal compounds, sesquiterpenes, and monoterpene characterized the volatile fractions with hinesol (17.2%), the principal compound, benzaldehyde (12.3%) and E-β-ionone (7.8%) as the major constituents. E. elaterium ethanolic extract showed good activity against MCF-7 and Caco-2 cells (IC50 values=29.67 µg/mL and 17.64 µg/mL, respectively). Moreover, all extracts were safe on normal human cells. In conclusion: Evaluation of E. elaterium volatile oil has been conducted for the first time in Jordan; also various extracts were tested for the first time against Panc-1 cells. Furthermore, based on the obtained results, ethanol extract of E. elaterium may be advocated as candidate for breast and colorectal cancers management.
Keywords: *Ecballium elaterium* L., essential oil, Jordan, antiproliferative activity, MCF-7, Caco-2, Panc-1..

Abbreviations

EOs	Essential oils
RI	Retention index
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's modified Eagle's medium
GC-MS	Gas chromatography – mass spectroscopy
GC-FID	Gas chromatography – flame ionization detector

INTRODUCTION

Natural products still stand as a significant source of new drugs especially those used as anticancer and antihypertensive[1]. Drugs of plants origin accounts for 25% of the total drugs prescribed worldwide with 121 active compounds still used until now, and according to the World Health Organization (WHO) 11% of 252 basic and essential drugs come from plant origin[2]. Although Jordan is considered a small country, it is rich in plant species, as a result of the diversity of geography and climate circumstances[3]. About 2,543 plant species, of which about 485 species, belonging to 99 families, are recorded as medicinal plants [4].

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Ecballium elaterium (L.) A. Rich is called squirting cucumber in addition to many other common names such as spitting cucumber, bitter melon, and wild balsam-apple. Squirting cucumber of *Ecballium* is poisonous wild decumbent perennial herb, restricted to the Mediterranean Basin[5]. The plant leaves are fleshy, prostate, covered by bristly and coarse hairs, the flowers have a yellowish petal leaf, the fruits are green hairy, have plum-shape and explode when they are ripe to scatter stream of mucilaginous liquid containing their brown egg shaped seeds violently to a considerable distance in response to light pressure[6]. Squirting cucumber of *Ecballium* occupied an important position in traditional medicine prescriptions and had a long tradition of uses in the Mediterranean region, as a natural remedy for several indications, especially as an analgesic, antipyretic and antiphlogistic, also was used as a emetic, purgative, for the treatment of jaundice, oedema, otitis, malarial fever and hydrophobia[7].

The few comprehensive studies investigated thoroughly both the essential oil composition and the anticancer activities for *E. elaterium* grown in Jordan despite the well- known benefits of them encouraged us to evaluate (qualitatively and quantitatively) the complete chemical composition of the essential oil hydro-distilled from aerial parts of wild growing *E. elaterium* collected from Jordan by means of GC-FID and GC-MS systems and to investigate the *in vitro* anticancer activities of the aqueous and alcoholic extracts of the of aerial parts of the plant against selected breast cancer cell line (MCF-7), colon cancer cell line (Caco-2) and pancreatic cancer cell line (Panc-1) using MTT assay.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

Aerial parts (500 g roughly) of *E. elaterium* (L) were collected from north Amman (Jubaiha) in early summer time of 2016. All plants were air dried at room temperature and set aside in a cool place for further study. The collected plants were identified by Prof. Khaled Tawaha (Professor of Pharmacognosy and Phytochemistry, School of Pharmacy, The University of Jordan). Respective voucher samples were reserved in the Department of Pharmaceutical Science, School of Pharmacy, The University of Jordan, and given Voucher-Specimen (Reference) Numbers: (*Ecb*. Am. R. 24/7/16). At the beginning, visual inspection was made for the dried aerial parts for any contamination and then they were powdered to about 0.5 mm in order to enhance the efficiency of extraction.

2.2.Crude plant extraction

Ethanol extracts were prepared from *E. elaterium* by soaking 100 g of dried powdered aerial parts in 1L of ethanol (70%) and kept macerated for 48 h, then the extracts filtered and the solvent evaporated until dryness using rotary evaporator at 40°C. Also water extracts were prepared separately by boiling 250 g and 200 g of dried powdered aerial parts of *E. elaterium* with 2.5 L of tap water for 3 h, and then the boiled material was filtered and evaporated until dryness using rotary evaporator at 75°C. All evaporated extracts were kept in refrigerator for further study.

2.3.Volatile oil isolation

Using Clevenger type apparatus; Volatile oil extraction was conducted by hydrodistillation of 250 g of dried material of *E. elaterium* with 2.5 L of tap water for 3 h. The hydrodistillation was carried out twice for each plant material and the volatile oils obtained for each were pooled, dried over anhydrous sodium sulphate (anh. Na2SO4) and stored in closed in amber vials at 4°C until analysis.

2.4. GC-MS analysis

About 1µL aliquot of the oil sample obtained by hydrodistillation, appropriately diluted in GC grade n-hexane, was injected into Varian Chrompack CP-3800 GC- MS-200 equipped with DB-5 GC capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) consisting

of (5% diphenyl 95% dimethyl polysiloxane). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The GC was coupled to mass detector operating with electron ionization source of 180°C and 70 eV ionization voltage. At the beginning of GC analysis a steady temperature was held at 60°C for 1 min, then a linear temperature programming was applied from 60 °C to 246 °C at 3 °C/min ramping rate, then the temperature was held steady at 246 °C for 3 min. The oil of each individual plant material was analyzed twice. Using the same column, a hydrocarbon mixture of n-alkanes (C8-C20) was separately analyzed under the same chromatographic conditions.

2.5.GC–FID Analysis

Quantitative analysis (% area) was carried out using a Hewlett Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio, 1:50) and a FID detector. The column was an OPTIMA-5 (5% diphenyl 95% dimethyl polysiloxane) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 film thickness). The analysis of the oils was carried out under linear temperature programming applied at 10 °C/min from 60 °C to 250 °C, and then kept steady at 250 °C for 5 min (isothermal). Temperatures of the injector and detector (FID) were maintained at 250 °C and 300 °C, respectively. For each sample, the relative peak areas for individual constituents were averaged (as average % area of all replicates). Assuming a unity response by all oil components, concentrations were calculated as percentage contents (%contents) using their corresponding normalized relative areas obtained by FID. Each oil sample was analyzed twice[8].

2.6.Identification of oil components

The identification of oil components was performed by using their recorded mass spectra matched by library searching with data bank mass spectra (NIST, Wiley and Adams-2007 libraries) and by comparing their calculated retention indices (RI), with literature values measured on columns with identical polarities, mainly using the Adam's library[9]. The identities of α-pinene, β-pinene, limonene, *p*-cymene, γ- terpinene, 1,8-cineole, linalool, α- and β-thujones, camphor, thymol and carvacrol were further confirmed by chromatography of their authentic standards under the same GC-MS conditions[10].

2.7.Cell culture condition

Three solid human tumor cell lines; breast adenocarcinoma (MCF-7), Colorectal adenocarcinoma (Caco-2), pancreatic adenocarcinoma (Panc-1) and normal human skin fibroblast (as control) were purchased ATCC (USA). Malignant and non-malignant cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/mL streptomycin. Cells were maintained in vented flask at 37 °C in a humidified atmosphere (90%) containing 5% CO_2 and sub-cultured every 3–4 days. All procedures were carried out in a sterile condition using sterile tools, materials, and safety laminar cabinet.

2.8.Extracts preparation for antiproliferative assay

Appropriate weights of previously prepared ethanol extracts of the two plants were initially dissolved in filtered DMSO so that we get concentration of 10mg/ml, and passed through a 0.2 mm filter, then the extracts were diluted in medium to produce concentrations range (0.1-300 µg/mL) of each extract, while in case of previously prepared water extracts of the same two plants appropriate weights of them were dissolved initially in DMEM so that we get concentration of 10 mg/mL, and passed through a 0.2 mm filter, then the extracts were diluted in medium to produce concentrations range (1-1000 µg/mL) of each extract. Doxorubicin and cisplatin were used as positive controls at the concentrations range (0.05 - 50 µg/mL). The final dilution used for treating the cells contained not more than 2% of DMSO, which was shown to be non-cytotoxic

by previous researches, this concentration being used in the solvent control wells.

2.9. Antiproliferative assay

Based on optimization experiments, all procedures followed in screening antiproliferative activity were conducted according to standard protocols verified by the same laboratory research group[11]. The antiproliferative assay was performed using a modified 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay. For sake of optimization of outcome, a slight modification of incubation time was performed by increasing it from 48 h to 72 h which was shown to work better for natural and synthetic materials[12-13]. Absorbance measurements were made at 570 nm using a microplate reader (BioteK, USA). Viability was expressed as the fraction of optical density of treated cells that survived relative to the average of optical density of untreated cultures (negative control). The antiproliferative effect was calculated according to the following equation: Toxicity % = $100(A_t/A_C)*100$

Where:

A_t: is the absorbance of the test sample.

A_C: is the absorbance of the control sample.

The IC_{50} value was defined as the concentration at which 50 % of cell viability was inhibited. All experiments were performed in triplicate and repeated at least twice.

2.10.Determination of IC_{50} values

The toxicity % of each treated well was calculated and since the experiments were performed in triplicate the average of toxicity % was also calculated for every treatment concentration, and the standard deviation (SD) was calculated for reading that expresses the same concentration, then a curve of % cytotoxicity against logarithm of concentration was drawn and the IC_{50} value was calculated from the equation of logarithmic line. Optimally crude herbal extracts are considered effective antineoplastic if their IC_{50}s < 30 µg/mL[14-16].

3. RESULTS AND DISCUSSION

3.1.Essential Oil analysis

The quantitative and qualitative composition of essential oil hydrodistilled from aerial parts of *E. elaterium* is summarized in Table 1. All components with their percentage contents are listed in ascending order based on their linear retention indices (Kovats indices) calculated on DB-5 equivalent column as according to Van Den Dool and Kratz relationship in Table 1. In addition, representative total ion current (TIC) chromatogram of the analyzed oil is shown in Figure 1.

As shown in Table 1, the number of components identified in the oils was 31, corresponding to an average of 76.3% of the total oils hydrodistilled from the plant. The dominant fraction was the non-terpenoidal hydrocarbons and miscellaneous (30.1%), the sesquiterpene fraction was accounting for 26.7%, the monoterpene fraction was 17.0%, while, diterpenes accounted for not more than 2.5% of the total oil. Most notably, four compounds were major component of the oil: hinesol (17.2%), an unknown compound has the same content (17.2%) like hinesol but eluted after, followed by benzaldehyde (12.3%), and lastly E-β-ionone (7.8%).

Generally speaking, the diversity in squirting cucumber of *Ecballium* composition was obvious and some commonality exists. Of that, aliphatic esters were present like palmitic and phthalic acid esters; also aromatic aldehydes such as benzyldehyde, benzene acetyldehyde, *o*-tolualdehyde and safranal, characteristic to the oil in addition to aliphatic aldehydes such as decanal and β- cyclocitral. The majority of these aldehydes are flavoring compounds. Aliphatic acetones were also found to present in the oil such as rose ketones (e.g. β-damascone and β-ionone), neryl acetone, and vistitenone. According to our intensive literature survey and to the best of our knowledge; our study is unprecedented in evaluating the EOs of this plant in Jordan. In one similar study conducted in Iran, leaves and fruits were analyzed separately.

Unlike our tested plant oil which was dominated by terpenoidal compounds, the fruits of Iranian plants was dominated by aromatic compounds followed by aliphatic hydrocarbons, while, the aliphatic esters were dominating in leaves[18]. Moreover there were substantial variations in the principal and major constituents. Tables 2 and 3 summarize chemical class distribution of the EO composition and the main identified components in the oils obtained from Iranian *E. elaterium* compared to our tested Jordanian plant.

These differences may be attributed to: 1) the difference in parts that the oil was obtained from; our sample oil was obtained from the whole aerial parts, while in Iranian specimens oils were from leaves and fruits; 2) the difference in geographical locations (source), which stands as one of the main variability factors as shown above; 3) the difference in time of collection, the Iranian plant specimens were collected in September, while our samples in July; and 4) cultivation conditions and drying methods can also affect the chemical constituent of EOs.

3.2.Antiproliferative activity

Table 4 displays the IC_{50} values antiproliferative activity using MTT assay in a panel of cancer cell lines, namely breast adenocarcinoma (MCF-7), Colorectal adenocarcinoma (Caco-2), and pancreatic adenocarcinoma (Panc-1). Normal human skin fibroblast cell line was recruited to verify selective cytotoxicity. All IC_{50} values listed are mean ± SD (standard error of mean) (µg/mL). Antineoplastic agents' cisplatin and doxorubicin were the reference controls.

Importantly; significant antiproliferative effectiveness of ethanol extracts for *E. elaterium* (mainly displayed in both MCF-7 and Caco-2) was demonstrated with IC_{50}s < 30 µg/mL. Water extract was found to be non cytotoxic against any cancer cell line; furthermore all extracts examined in the study had a high safety profile exhibited in normal human fibroblasts. Figures 2-5 express the effect of each crude extracts on the four cell lines used in the study.

The evaluation of antiproliferative activity of aerial parts of *E. elaterium* using two solvents with different polarities (water and ethanol) against MCF-7, Caco-2 and Panc-1 cancer cell lines was conducted. Moreover the safety of the extracts of plants was determined by studying their activities against normal fibroblasts.

According to the American National Cancer Institute guidelines (NCI), crude extract is considered cytotoxic of promising anticancer product for future bio-guided studies only if it exerts an IC_{50} value < 30 µg/mL[19]. The ethanol extract from *E. elaterium* showed good cytotoxic activity on MCF-7 and Caco-2 cells with IC_{50} values of 29.67 µg/mL and 17.64 µg/mL respectively. Water extract was not found to be cytotoxic against any cell line; this lack of activity can be attributed to low water solubility for the organic compounds. Furthermore all extracts used in the study are considered safe against normal human cells because IC_{50} values determined for all extracts against normal fibroblast cell line was far away from that recorded against cancer cell lines under our study.

Generally, *E. elaterium* is known for its anticancer effect, thus the cytotoxic activity of the screened plant ethanol extracts on MCF-7 and Caco-2 was not surprising, for MCF-7 the result of this study is in accordance with previous finding for Egyptian crude cucurbitacins extract from the plant since IC_{50} value of 19.70 µg/ mL previously reported by Saker[20] was comparable to ours. Against Caco-2 cells, *E. elaterium* showed more potent cytotoxic effect. This cytotoxic effect could be due to the presence of active phyto-constituents in the extracts that have been reported previously with highly cytotoxic activities on other cell lines such as curcubitacins compounds which were isolated and characterized from *E. elaterium*. Appreciably the isolated cucurbitacins from other genus of the same family were specifically and strongly cytotoxic for Caco-2 cells[21]. Profoundly combination of

cucurbitacin B and gemcitabine synergistically potentiated the antiproliferative effects of gemcitabine on pancreatic cancer cells other than Panc-1 cells[22]. Notably studying cytotoxicity against Panc-1 cells was for the first time with this plant, to the best of our knowledge.

3.3. Conclusions

Squirting cucumber of *E. elaterium* oil composition was characterized by the presence of high contents of non-terpenoidal compounds (hydrocarbons, ketones, aldehydes, etc.), sesquiterpenes, and very lower contents of monoterpenes and diterpenes. Hinesol, benzaldehyde, and E-β-ionone were among the major components with the highest contents. With regard to cytotoxicity results, the ethanol extract from *E. elaterium* showed good cytotoxic activity on MCF-7 and Caco-2. Notably the tested extracts under the present study were found to be safe on normal human cells.

Acknowledgment

This work was funded by the Deanship of Scientific Research_ the University of Jordan. Authors are also grateful for Mr. Ismail Abaza technical assistance.

Table 1. Chemical composition of the essential oil hydrodistilled from aerial parts of *E. elaterium* from Jordan

No.	Rt	RI^{cal}	RI^{lit}	compound	%Content
1	**7.0**	**960**	**960**	**Benzaldehyde**	**12.3%**
2	7.7	1033	1035	Mixed peak, identified 2-isobutyl thiazole	1.6%
3	8.6	1042	1042	Benzeneacetyldehyde	0.9%
4	10.7	1068	1068	*o*-Tolualdehyde	0.8%
5	11.7	1093	1093	Ethyl sorbate	0.8%
6	12.2	1105	1106	α-Fenchocamphorone	2.0%
7	13.4	1134	–	Unknown	2.6%
8	15.4	1198	1197	Safranal	1.4%
9	16.1	1206	1202	n-Decanal	1.5%
10	17.0	1218	1217	β-Cyclocitral	0.8%
11	20.7	1303	NA	4,6-dimethyl-3,5,7-trioxatetracyclo[7.2.1.0(4,11).0(6,10)]Dodecane^{ms}	1.1%
12	20.9	1307	1309	p-vinyl-Guaiacol	2.1%
13	24.4	1389	1390	Isolongifolene (tobeshifted 6 m/zvalues)	1.9%
14	25.0	1405	1414	β-Damascone	1.1%
15	26.8	1446	1436	Neryl acetone	2.5%
16	27.2	1457	1447	Vestitenone	1.4%
17	**28.0**	**1476**	**1489**	**E-β-Ionone**	**7.8%**
18	28.1	1491	1490	cis-Eudesma, 6, 11 diene	0.9%
19	28.6	1510	1509	Germacrene A	0.7%
20	29.5	1513	–	Unknown	1.2%
21	31.4	1561	1563	E-Nerolidol	1.5%
22	33.6	1619	1624	10-epi-γ-Eudesmol	2.1%

No.	Rt	RI^{cal}	RI^{lit}	compound	%Content
23	**34.3**	**1639**	**1642**	**Hinesol**	**17.2%**
24	35.0	1656	1653	Eudesmol	1.0%
25	35.5	1670	1675	β-Bisabolol	0.7%
26	36.6	1713	1715	Longifolol	0.7%
27	41.3	1834	NA	2-methyl-7-octadecyne^{ms}	2.6%
28	**41.5**	**1839**	**–**	**Unknown**	**17.2%**
29	42.0	1853	NA	Phthalic acid, 6-ethyl-3-octyl isobutylester^{ms}	0.8%
30	42.2	1858	–	Unknown	0.8%
31	43.5	1876	1878	Cubitene	1.2%
32	43.7	1898	1900	n-Nonadecane	1.5%
33	43.9	1905	1902	epi-Laurenene	1.3%
34	44.3	1923	1921*	Hexadecanoicacid, methyl ester	2.8%
35	45.1	1947	NA	Butylcyclohexyl phthalate^{ms}	1.3%
36	46.3	1991	NA	Unknown	0.7%
Terpenoids:					**46.2**
Monoterpenes					**17.0**
HydrocarbonMonoterpenes					–
Oxygenated Monoterpenes - No.: 6,8,10,15,16 - No.: 14,17 (Rose ketones, carotenes degradation)					**17.0**
Sesquiterpenes					**26.7**
HydrocarbonSesquiterpenes - No.: 13,18,19					**3.5**
Oxygenated Sesquiterpenes - No.: 21-26					**23.2**
Diterpenes - No.: 31,33					**2.5**
Miscellaneous (all other compounds)					**30.1**
Total identified					**76.3**
Unidentified and Traces					**23.7**

*Rt: Retention time; RI^{cal}: linear (arithmetic) retention index calculated on a DB-5 equivalent column; RI^{lit}: retention index from literature[9];NA: data not available in literature; Compounds in bold are the major oil components (%content>5.0%);^{ms}:compound is identified according to MS only; *: RI^{lit}retention index is according to Santos et al.[17].*

Table 2. **Chemical class distribution of the essential oil components hydrodistilled from three samples of *E. elaterium***

Country Part used Collection time	Aliphatic hydrocarbons (%)	Aliphatic esters (%)	Aromatic compounds (%)	Alicyclic hydrocarbons (%)	Terpenoides (%)
Jordan Aerial parts July Our study sample	5.6%	0.8%	19.0%	4.7%	46.2%
[18]Iran Leaves					
Fruits	16.7%	30.8%	6.4%	20.4%	20.4%
September	33.0%	3.5%	45.3%	7.8%	7.8%

Table 3. Main, major and some components identified in the essential oils hydrodistilled from fruits, leaves and aerial parts of *E. elaterium* collected from Iran and Jordan.

Country Part used	**Main principal**	**Majors (<5% of the identified portion)**	**Components (≥ 2% of the identified portion)**
Jordan Aerial parts Our study sample	Hinesol (17.2%) Unknown (17.2%) Benzaldehyde (12.3%)	E-β-Ionone (7.8%)	p-vinyl–Guaiacol (2.1%) Neryl actone (2.5%) 10-epi-γ-Eudosmol (2.1%) 2-methyl- 7-octadecyne (2.6%) 14-methyl-petadecanoic acid (2.8%)
[18]Iran Fruit	E-anethol (31.6%)	3-(6,6-Dimethyl-5-oxohept- 2-enyl)- cyclohexanone (8.8%) Tetracosane (7.4%) Hexadecane (5.2%) Octadecane (5.7%)	β-Thujone (3.0%) Heptadecane (2.1%) Octyl octanoate (3.5%) Hexahydrofarnesyl acetone (2.1%) Dibuthyl phtalate (3.2%) Eicosane (2.7%) Henicosane (2.8%) Tricosane (4.9%)
[18]Iran Leaves	Octyl octanoate (30.0%) 3-(6, 6-Dimethyl-5- oxohept- 2-enyl)- cyclohexanone (20.4%) Hexahydrofarnesyl acetone (19.1%)		Hexadecane (2.5%) Octadecane (2.9%) Tricosane (2.0%) Tetracosane (3.7%)

Table 4. *In vitro* Antiproliferative activity of *E. elaterium* crude extracts on three cancer cell lines and normal human fibroblast cell line.

Treatment	Cytotoxicity (as of % control) (IC_{50} value: mean ± SD (µg/ ml)			
	MCF-7	**Caco-2**	**Panc-1**	**Fibroblasts**
E. elaterium AE	132.95 ± 5.9	972.63 ± 142.3	1141.39 ± 57.0	1236.45 ± 24.7
E. elaterium EE	**29.67 ± 2.9**	**17.64 ± 2.2**	107.77 ± 7.0	159.97 ± 1.3
Doxorubicin	0.35 ± 0.03	0.59 ± 0.1	1.38 ± 0.1	1.12 ± 0.1
Cisplatin	1.65 ± 0.2	1.44 ± 0.2	6.11 ± 0.1	9.91 ± 0.3

AE: aqueous extract. EE: ethanol extract. Results, representing IC_{50} (µg/ mL), are mean ± SD (n= 3 independent replicates). IC_{50}values (concentration at which 50% inhibition of cell proliferation took place in comparison to non- induced basal 72 h incubations) were calculated within treatment concentration range 0.05-1000 µg/ mL for all extracts and controls.

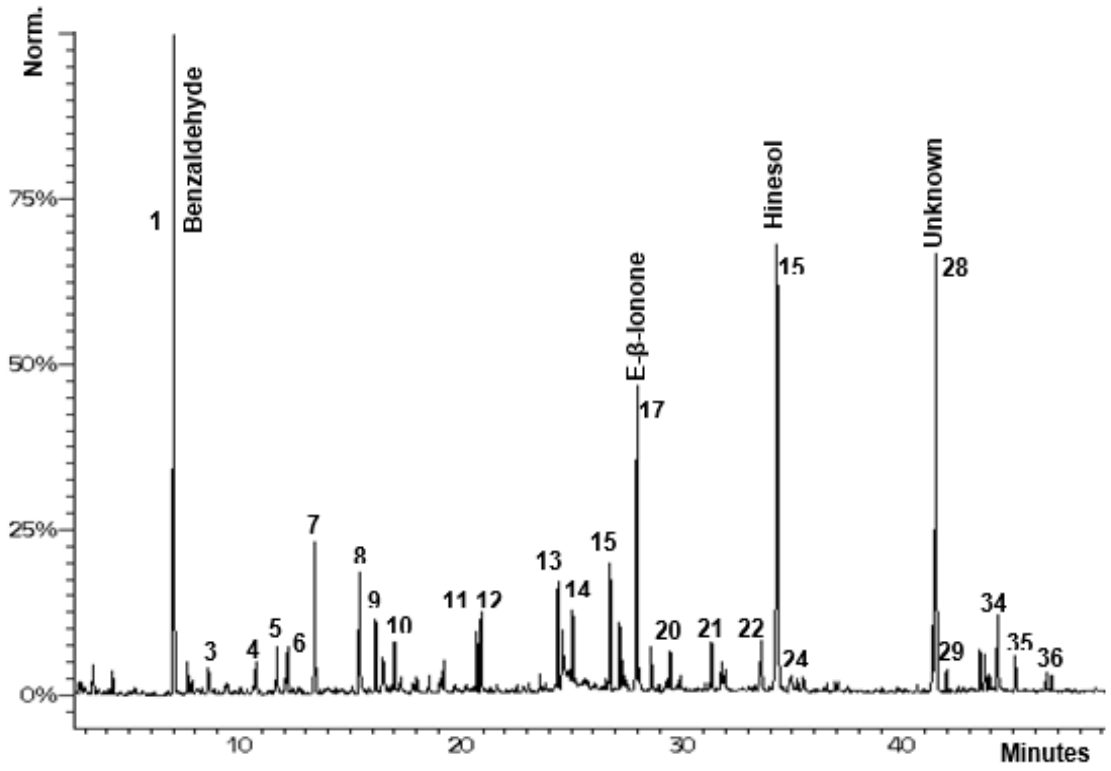

Figure 1. GC-MS TIC chromatogram of the essential oil hydrodistilled from aerial parts of *E. elaterium* grown in Jordan eluted on DB-5 column.

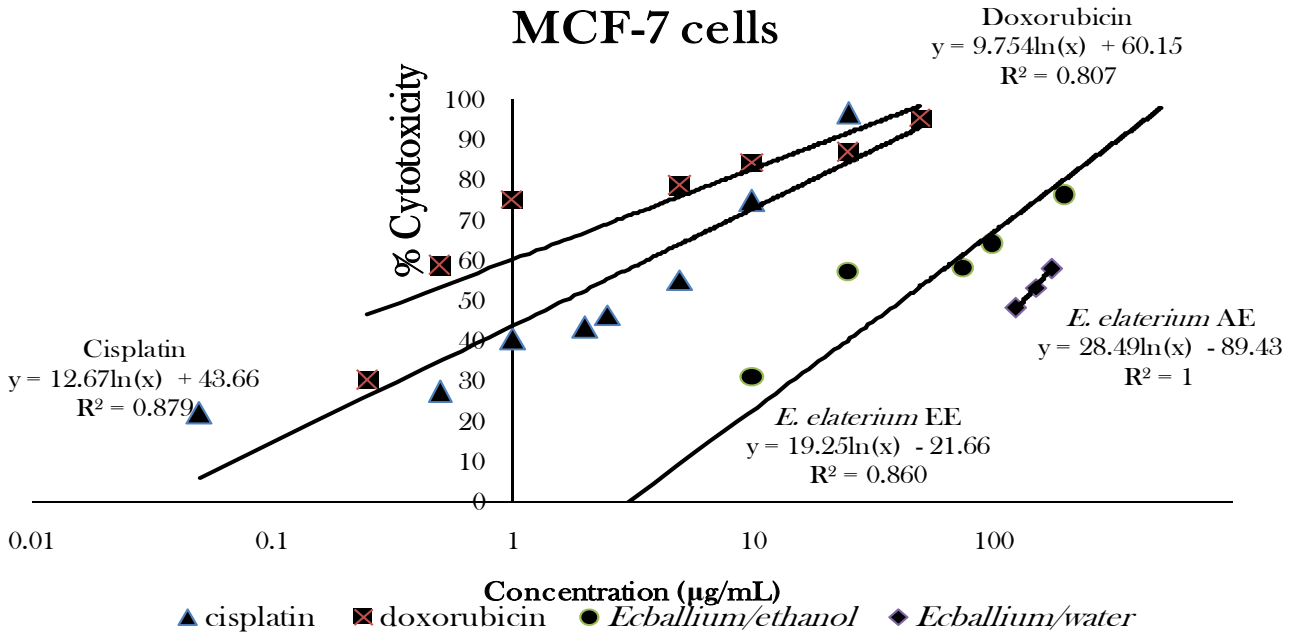

Figure 2. ***In vitro*** **antiproliferative activity of** ***E. elaterium*** **crude extracts, doxorubicin and cisplatin on MCF-7 breast cancer cells.**

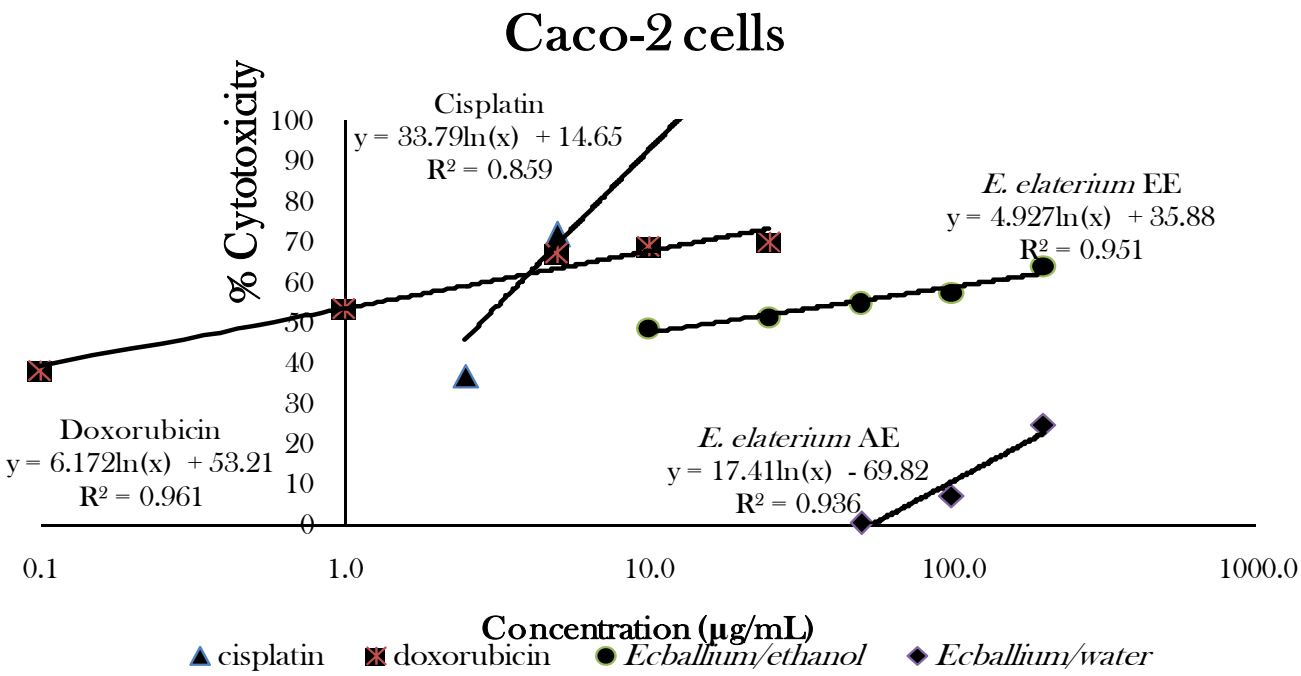

Figure 3. ***In vitro*** **antiproliferative activity of** ***E. elaterium*** **crude extracts, doxorubicin and cisplatin on Caco-2 colorectal cancer cells**

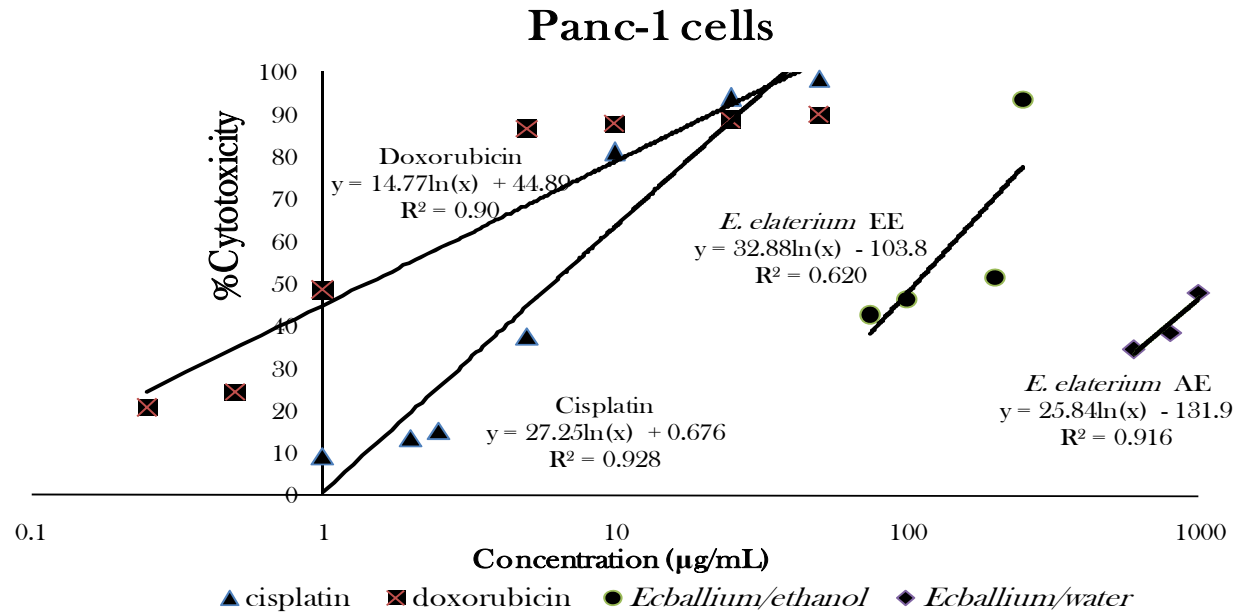

Figure 4. ***In vitro*** **antiproliferative activity of** ***E. elaterium*** **crude extracts, doxorubicin and cisplatin on Panc-1 pancreatic cancer cells**

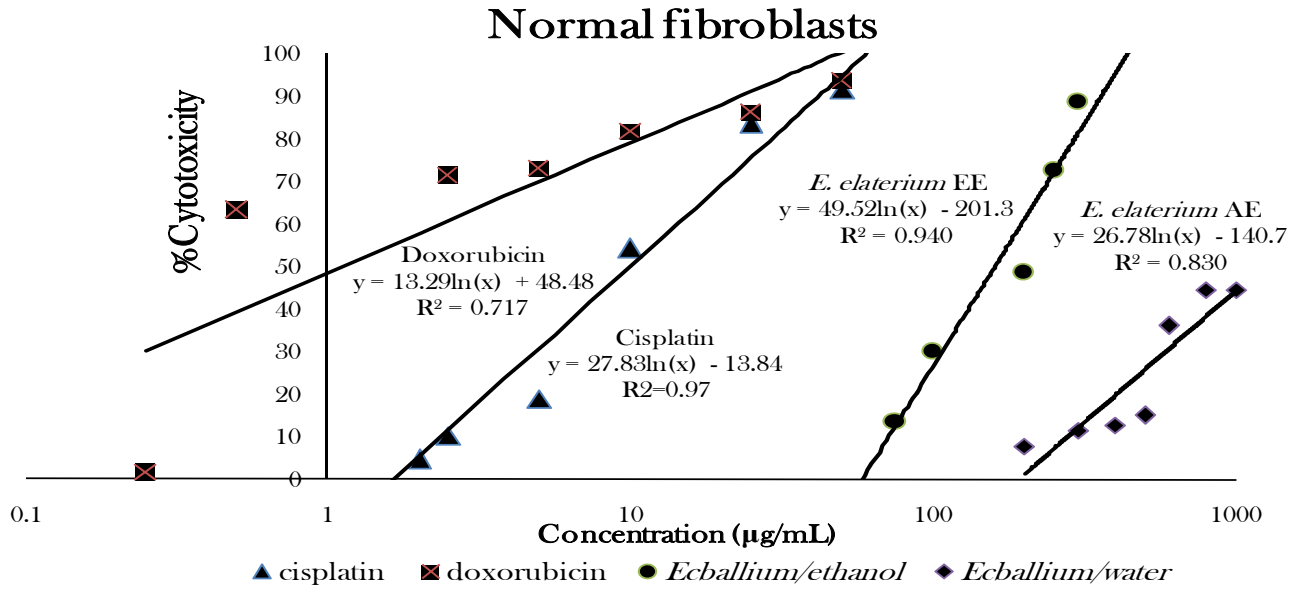

Figure 5. ***In vitro*** **antiproliferative activity of** ***E. elaterium*** **crude extracts, doxorubicin and cisplatin on normal fibroblast cells**

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دراسة مكونات الزيت العطري والنشاط المضاد لتكاثر الخلايا السرطانية للأجزاء الهوائية لنبات قثاء الحمار *Ecballium elaterium* (L) نبتة حاملة للزيوت العطرية الطبية من الأردن

ريم رايق جبارة[1] ، محمد م.د هديب [1،2] ، ياسر ك. بستنجي [1] ، نور ط الحوراني [1] ،ريم العباسي [1] وفيوليت كسابري

[1] كلية الصيدلة ، الجامعة الأردنية ، عمان ، الأردن

[2] كلية الصيدلة ، جامعة العين للعلوم والتكنولوجيا ، أبوظبي ، الإمارات العربية المتحدة .

ملخص

الخلفية والأهداف: هدفت هذه الدراسة إلى توفير تحليلات GC-FID و GC-MS للزيت العطري للأجزاء الهوائية المجففة من .Ecballium elaterium L في الأردن ودراسة قدرتها على السمية الخلوية .

الطريقة: تم الحصول على الزيت العطري بالتقطير المائي باستخدام جهاز .Clevenger تم استخدام طريقة MTT للتحقيق في النشاط المضاد لتكاثرالخلاياالسرطانية في المختبر للنبات ضد سلالات الخلايا السرطانية MCF-7 و Caco-2 و Panc-1 بالإضافة إلى خلايا الخلايا الليفية الطبيعية .

النتائج: أنتج زيت E. elaterium المقطر بالماء واحد وثلاثين مكوناً ، وهو ما يمثل 76.3٪ من إجمالي محتوى الزيت. محتويات عالية من nonterpenoidal لمركبات ، سيسكيتيربين ، ومونوتربين ميزت الكسور المتطايرة مع الهينيسول (17.2٪) ، المركب الرئيسي ، البنزالديهايد (12.3٪) و E-β-ionone (7.8٪) (كمكونات رئيسية. أظهر المستخلص الإيثانولي لنبتة E. elaterium نشاطًا جيدًا ضد خلايا MCF-7 و) Caco-2 قيم IC50 = 29.67 ميكروغرام / مل و 17.64 ميكروغرام / مل ، على التوالي). علاوة على ذلك ، كانت جميع المستخلصات آمنة على الخلايا البشرية الطبيعية. وبالمحصلة: تم إجراء تقييم لزيت E. elaterium المتطاير لأول مرة في الأردن. كما تم اختبار مستخلصات مختلفة لأول مرة ضد خلايا .Panc-1 علاوة على ذلك ، بناءً على النتائج التي تم الحصول عليها ، يمكن تقديمالمستخلص الإيثانولي من E. elaterium كمرشح لمعالجة سرطان الثدي والقولون والمستقيم.

الكلمات المفتاحية: Ecballium elaterium L. ، زيت عطري ، الأردن ، نشاط مضاد لتكاثرالخلايا السرطانية ، MCF-7، Caco-2، Panc-1.

الاختصارات

EOsالزيوت الأساسية

RIمؤشرالاحتفاظ

DMSO ثنائي ميثيل سلفوكسيد

DMEMوسيط Dulbecco’s modified Eagle’s

GC-MSكروماتوغرافيا الغاز – التحليل الطيفي الشامل

GC-FIDكروماتوغرافيا الغاز – كاشف التأين باللهب

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Development of a novel filtered-based pharmacophore for the identification of human equilibrative nucleoside transporter 1 inhibitors

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ABSTRACT

The human equilibrative nucleoside transporters (hENTs) are important proteins that allow nucleosides and nucleobases permeation into the cell. hENT 1 is a promising target against heart and Huntington's diseases as its inhibition mediates cardiac- and neural protection effects, respectively. However, the current hENT1 inhibitors have significant off-target effects and poor pharmacological profile, hence there is need for new novel inhibitors. Therefore, we developed a computational protocol that identified and selected inhibitors of hENT1 in an efficient and specific manner. First, several pharmacophores were created using a set of known inhibitors. Consequently, the best inhibitor pharmacophore exhibited as high selectivity and specificity rates as 92% and 88%, respectively. Furthermore, another pharmacophore was validated for the oppositely acting type of the hENT1 molecules (i.e. permeants) to act as an extra refinement step in our search for hENT1 inhibitors. Interestingly, employing the inhibitor pharmacophore as a filter-in along with the permeant pharmacophore as a filter-out resulted in up to two-fold enhancement of docking-based virtual screening results of hENT1 inhibitors. This in silico approach can prove very useful in the development of new cardio- and neuroprotective hENT1 inhibitors.

Keywords: Drug design, human equilibrative nucleoside transporter 1, inhibitor, permeant, pharmacophore.

INTRODUCTION

Equilibrative nucleoside transporters (ENTs) are transmembrane integral membrane proteins responsible for the transport of nucleosides and nucleobases across membranes of cells. Purine and pyrimidine nucleosides and nucleobases are crucial biological molecules for prokaryotic and eukaryotic cell physiology as they are the building blocks for DNA/RNA synthesis.[1,2] Certain cell types such as the brain and bone marrow cells are incapable of nucleotides *de novo* synthesis. Hence, they rely solely on the ENT salvage pathway for their nucleobase/nucleoside requirements. Moreover, ENTs regulate the flux of nucleosides such as adenosine across the cell membrane and therefore indirectly influence the purinergic cell singling pathway.[1-3] ENTs role is not limited to its physiological contribution but pharmacologically, ENTs are primary drug targets shown to modulate the efficacy of > 30 FDA/EMA approved drugs, including cardiovascular agents.[1] ENTs serve as a molecular target for drug binding, such as antihypertensive and antiarrhythmic agents,[1] that mediates their cardioprotective effects.

To date, four human ENTs isoforms (hENT1-4) have

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been identified with hENT1 being the most studied and best characterized.[1,4] hENT1 is ubiquitously distributed and primarily found in the plasma membranes of various tissues [1,2,5]. The structure of hENT1 consists of 11 TM helices with extracellular C-terminus and cytoplasmic N-terminus.[1,6] The structure was confirmed by glycosylation scanning mutagenesis[7] and more recently by the first crystal structure of this protein.[8] Crystallography structural studies of the inhibitors nitrobenzylthioinosine (NBTI) and dilazep-hENT1 complexes show that these two inhibitors shared a binding site termed "orthosteric site" within the hENT1 central cavity.[8] Potent hENT1 inhibitors include the adenosine analog, NBTI, and coronary vasodilators, dipyridamole and dilazep, with K_i values reported to be in the nanomolar range.[2] Furthermore, hENT1 is inhibited by a vast array of structurally diverse agents such as tyrosine- and serine/threonine kinase inhibitors, benzodiazepines, and calcium channel blockers but to a lesser extent than the aforementioned potent inhibitors.[1,2]

Therapeutically, hENT1 is a target for drugs that are inhibitors which blocks its transport activity. ENT1 inhibitor's therapeutic role in ischemic heart disease and cardioprotection has been well established. ENT1 genetic knockout or pharmacological inhibition via NBTI and dipyridamole resulted in attenuation of myocardial damage evident by the reduction in infarct size and ventricular dysfunction following ischemia/perfusion injury.[9-12] The nucleoside adenosine mediates ENT1 cardioprotective effect. ENT1 inhibition increase adenosine extracellular concentration, which potentiates adenosine receptors-purinergic signaling cardioprotective effects.[2,12,13]

Several lines of evidences have shown that ENT1 inhibition and enhanced adenosine mediated signaling has a neuroprotective effect. Prior research has shown that ENT1 was upregulated in Huntington's disease mouse models and patients at the early severity stage.[14,15] Moreover, extracellular striatal level of adenosine was significantly lowered in two Huntington's disease mouse models than controls,[14] potentially due to enhanced intracellular uptake of adenosine via the upregulated ENT1. It was established that ENT1 pharmacological inhibition via JMF1907 or ENT1 genetic removal increased striatum extracellular adenosine and enhanced survival of Huntington's diseased mice.[15] Furthermore, N6-(4-hydroxybenzyl) adenine riboside (designated T1-11), an adenosine receptor A2AR activator and ENT1 inhibitor, was shown to reduce deterioration of motor coordination and striatal Htt aggregates, a major pathological feature of Huntington's disease.[16] Hence, ENT1 inhibitors can serve as a promising therapeutic target for delaying the onset and treatment of Huntington's disease.[14,17]

Despite the therapeutic application of hENT1 inhibitors, limitations/unfavourable pharmacological properties associated with the present potent inhibitors has been reported. NBTI off-target effects have been reported in the cardiovascular system.[18] and the nitro group of NBTI has been suggested to cause potential liver toxicity.[18] Furthermore, it has been suggested that the polar nature of NBTI reduces its oral bioavailability and crossing through the blood-brain barrier.[19] Dipyridamole has a high binding affinity for α1-acid glycoprotein, which lowers its availability and efficacy as a nucleoside transporter inhibitor.[18] **Given the significant limitations of the current hENT1 inhibitors,** there is an imperative need to develop new hENT1 inhibitors. In this project, we aim to develop a computational approach that can efficiently identify the therapeutically valuable hENT1 inhibitors by investigating their physicochemical characteristics and establishing their pharmacophoric features. The data extracted from this step will be then employed in a novel structure-based drug discovery approach.

2. METHODS

2.1 Pharmacophore generation

As a starting point for developing the pharmacophore model, the inhibitor database underwent ligand preparation by Maestro software using LigPrep where accurate, energy minimized 3D structure for each ligand with all tautomeric and ionization states (at pH 7.4 ± 0.2, i.e., physiological pH) were generated. Additionally, ring conformations, and

stereoisomerism were taken into consideration at this stage to ensure the generation of each molecule's biologically active form in the inhibitors dataset.

For the pharmacophore generation, the pharmacophore features (queries) are constructed based on ligands superimposed conformations (aligned on top of each other) in MOE software to determine features that are common to the aligned ligands.[20] Initially, a "training set" was prepared for the inhibitor's library based on several selection criteria such as the number of rotatable bonds, molecular weight, various structural scaffolds, and IC_{50}/ K_i values.

2.2 Docking into the hENT1 transporter

For docking, co-crystallized structure for the hENT1 transporter with an inhibitor was obtained from the protein data bank (PDB, ID: 6OB7)[8]. The co-crystallized inhibitor and the water molecules were removed from the hENT1 structure. The removal of water was considered in this case as we do not have enough data about the presence of highly structured waters in the active site that are usually considered during docking.[21]

The protein structure preparation was then carried out by MOE using the protein preparation wizard that checks for missing residues, atoms, or loops and makes the corrections accordingly.[22] Later, H-bond optimization for the protein was performed by Protonate 3D method to maximize H-bond networks and reduce the overall self-energy. Further protein preparation was performed by the Maestro protein preparation module prior to Grid generation.[23] Using Receptor Grid Generation in Glide, a grid box was created to contain the docking site. The ligand-binding site was considered a centroid for grid box generation, consisting of a three-dimensional network of regularly spaced points. For all VS protocols, the ligands were docked into the hENT1 binding site using the Glide docking tool in Maestro using the GLIDE-XP method.[24]

3.3 Analysis of hENT1–Inhibitor complexes

The protein-ligand interaction fingerprint (PLIF) method has been previously described.[25] The generated PLIF graph showed the interaction occupancy of all residues present within the hENT1 binding site.

3. RESULTS AND DISCUSSUION

3.1 hENT1 inhibitors database building

The first step of the study was the development of the hENT1 inhibitors library to determine their general structural characteristics and pharmacophoric features. An extensive search of the literature was performed to compile a library of known hENT1 inhibitors along with their K_i/IC_{50} values. Using various search engines, a set of 56 known inhibitors were identified from the literature mining (Supporting Information Table S1, S2). For comparative analysis, we also prepared an additional library that constitutes of 18 known hENT1 permeants (Supporting Information Table S3, S4), which are substrates that are transported intracellularly via hENT1.[1] Then, a 3D structures of all ligands were built and prepared, via the MOE software[26] resulting in two databases, one that contained the inhibitors molecules, while the other one contained the permeants.

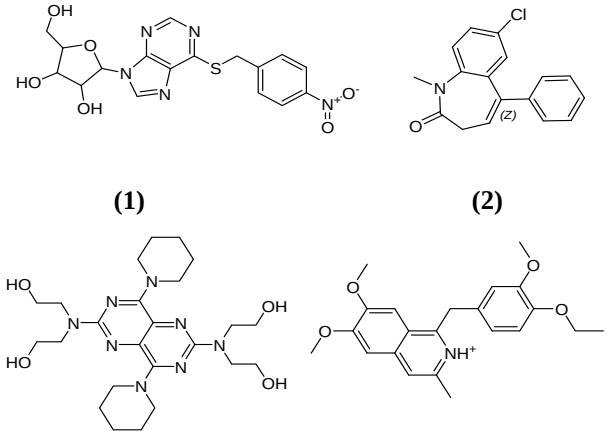
3.2 Physiochemical properties assessment

To determine the general structural characteristics of the inhibitors, several physicochemical properties were computationally calculated using the MOE descriptors calculator function[27] and compared to the permeant's physicochemical properties (Table 1). It was observed that the mean molecular weight (mean MWT) for inhibitors was 473.52 Da, which was 76% larger than permeants (269.7 Da). Also, inhibitors seemed to be much more flexible than permeants, as they had a greater number of rotatable bonds by more than four-fold, with a mean value of 10 in comparison to 2.22 in permeants. In terms of polarity, inhibitors showed higher hydrophobicity than permeants as they exhibited a higher value of partition coefficient and hydrophobic surface area (Log P 1.87, HSA 708.63 Å2) than that of permeants (Log P: -1.56, HSA: 423.79). Furthermore, hydrogen bonding capability was less in inhibitors than in permeants as the mean number of hydrogen bond acceptors for inhibitors and permeants was 4.82 and 6.05, respectively, while the mean

number of hydrogen bond donors was 2.0 and 3.72, respectively. Hence, in terms of binding, the inhibitors are more likely to bind through hydrophobic contact. To sum up, while permeants seem to occupy small hydrophilic pocket, the hENT1 inhibitors appear to sit in a larger pocket (that might be partially shared with permeants), with the ability to make a mixed type of polar and nonpolar contacts with the surrounding residues.

3.3 Pharmacophore hENT1 inhibitor model generation

Four molecules from different scaffolds, that were small in size, least flexible and had different levels of K_i/IC_{50} values, were chosen as the training set for the hENT1 inhibitor pharmacophore generation (i.e., NBTI, Dipyridamole, Diazepam, and Dimoxyline) (Figure 1, Supporting information Table S1)



(1) **(2)**

(3) **(4)**

Figure 1. Structures of inhibitor's training set. (1) NBTI, (2) Diazepam, (3) Dipyridamole, (4) Dimoxyline.

The hENT1 inhibitors training set was then used to generate the pharmacophore models using the MOE software pharmacophore elucidator. The objective of pharmacophore elucidation is to search for the pharmacophore queries (denoted as PH4) with the best overlay of the superimposed conformations of the training set.[26,28] Also, we specified seven pharmacophore features which were Hydrogen bond donor/acceptor (Don/Acc), Aromatic (Aro), Hydrophobic (Hyd), Hydrogen bond donor/acceptor projection (Don2/ Acc2), Pi ring (PiR), cationic (Cat) and anionic (Ani) to be taken into account when generating the pharmacophore queries (Supporting information Table S5). This step resulted in the generation of many queries, from which the top 50 with the highest overlaying score were selected. These pharmacophore queries were then validated using a "test set" of known hENT1 ligands, which were either inhibitors or permeants (Supporting information, Table S2, S4). These inhibitors and permeants test sets were used in assessing the selectivity (Se) and specificity (Sp) rates of the pharmacophore queries. Selectivity assesses the efficiency of the developed pharmacophores in selecting the desired ligand (true positives), while specificity measures the pharmacophore efficiency in eliminating those ligands that belong to the other class of hENT1 ligand (which were added as true negatives in this case). [29]

A four- and five- feature pharmacophore queries were generated from the training set's four inhibitors. Table 2 lists the top pharmacophoric queries with the best selectivity and specificity rates. Although PH4-40 had the highest specificity value of 100% among other pharmacophores, it couldn't pick up more than 90% of the

inhibitors compared to those that picked 92.3% and 94.23% of the screened inhibitors as seen in PH4-36 and PH4-4. The pharmacophore PH4-36 showed the best balance of selectivity and specificity, with Se and Sp values of 92.3% and 87.5%, respectively (Table 2). This means that PH4-36 was able to pick more than 90% of the screened inhibitors while filtering out more than 80% of the screened permeants. Figure 2a shows the most active hit inhibitor aligned to PH4-36, fulfilling all pharmacophore features. On the other hand, Figure 2b shows one of the filtered-out permeant along with PH4-36 that did not fulfil the pharmacophore where the two hydrophobic features were absent, as well as the absence of H-bond acceptor projection feature.

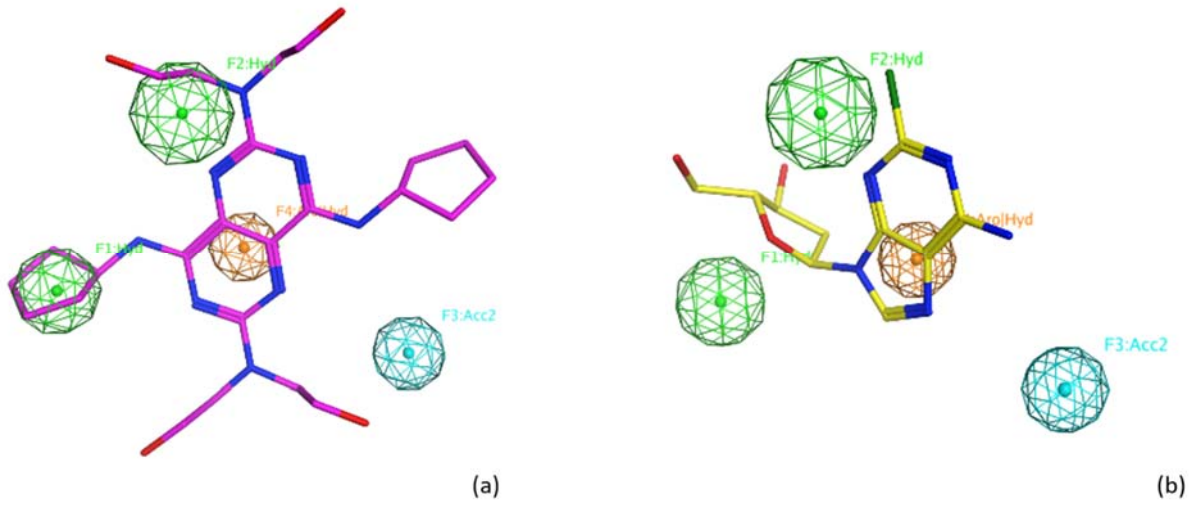


Figure 2. (a) hENT1 inhibitor aligned to PH4-36 fitting all pharmacophore features. (b) hENT1 permeants aligned to PH4-36 missing the two hydrophobic and acceptor projection features of the pharmacophore.

Figure 3a shows the overlay of the four training set inhibitors that resulted in generating query PH4-36 which exhibited the following features: (1) hydrophobic centroid feature with 1.2 in diameter; (2) hydrophobic centroid feature 1.4 in diameter; (3) Hydrogen bond acceptor projection and (4) aromatic or hydrophobic centroid feature (Figure 3b, 3c). Distance and angles between the features were shown in Figure 3d. For the other best performing inhibitor pharmacophore queries features listed in Table 2, see Supporting information Figure S1.

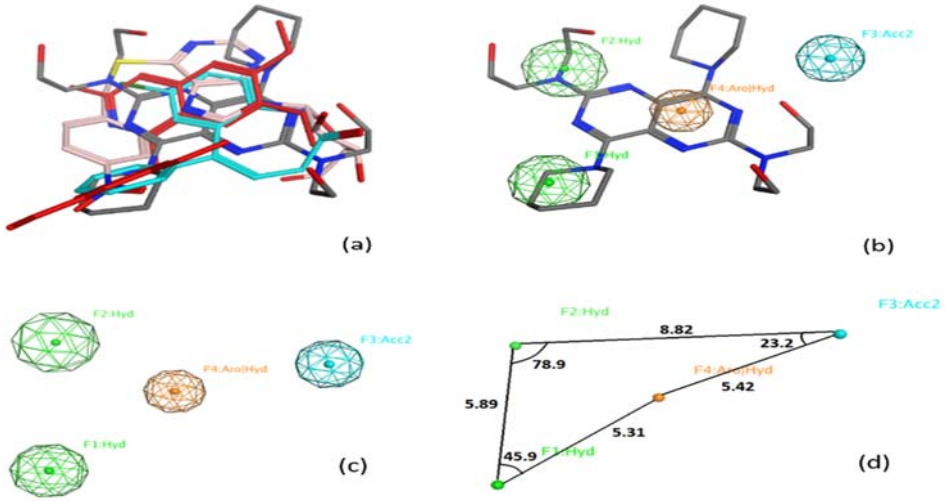


Figure 3. Pharmacophore query PH4-36 of the hENT1 inhibitors. (a) The inhibitors training set, consisting of 4 ligands, molecular superimposition. (b) An inhibitor molecule aligned with the pharmacophore query no. 36. (c) pharmacophoric features. (d) distances and angles between the pharmacophore features.

Similar to the PH4-36 features, prior research had shown that the best performing pharmacophoric features of ENT1 inhibitors were three hydrogen-bond acceptors, two aromatic rings, and a hydrophobic group generated by the PHASE modeling program using the potent ENT1 inhibitor NBTI and its two structurally related analogs.[30] Also, in partial agreement with our findings, Chen, et al. (2011) constructed a pharmacophore model that consisted of one aromatic ring and two hydrogen bond acceptors using the Catalyst program, based on one scaffold training set of structurally related 25 hENT1 inhibitors.[31]

Generally, some of the pharmacophoric features such as hydrogen bond acceptor and aromatic ring were common between our study and the studies mentioned above. However, the number of these pharmacophoric features differed among the studies. The variation in the number of features is due to the differences in the modeling program used and the different training compound sets utilized for pharmacophore model generation. It is also important to note that the previously mentioned studies had utilized a single scaffold in their relevant training sets.[30,31] On the other hand, we used four different scaffolds for pharmacophore generation, and hence PH4-36 shall be considered as a generalized pharmacophore that can help in discovering other new and novel scaffolds of hENT1 inhibitors.

For permeant pharmacophore generation, the training set consisted of two different scaffold molecules (Guanosine and Gemcitabine) (Supporting information Table S3, Figure S2) with low molecular weight, a low number of rotatable bonds and a high affinity to hENT1. The pharmacophore queries generated for hENT1 permeants consisted of four – five features (Table 3). A selectivity rate of 100% and a specificity rate of 84.62% was exhibited by PH4-23, which can be considered as the best performing and most discriminatory permeant pharmacophore (Table 3).

Figure 4a shows the superimposition of the two permeants Guanosine and Gemcitabine that resulted in generating PH4-23. Features of this pharmacophore were: one aromatic/planar system, two hydrogen bond donor projections and one hydrogen bond acceptor (Figure 4b, 4c). Distances and angles were calculated for PH4-23 (Figure 4d).

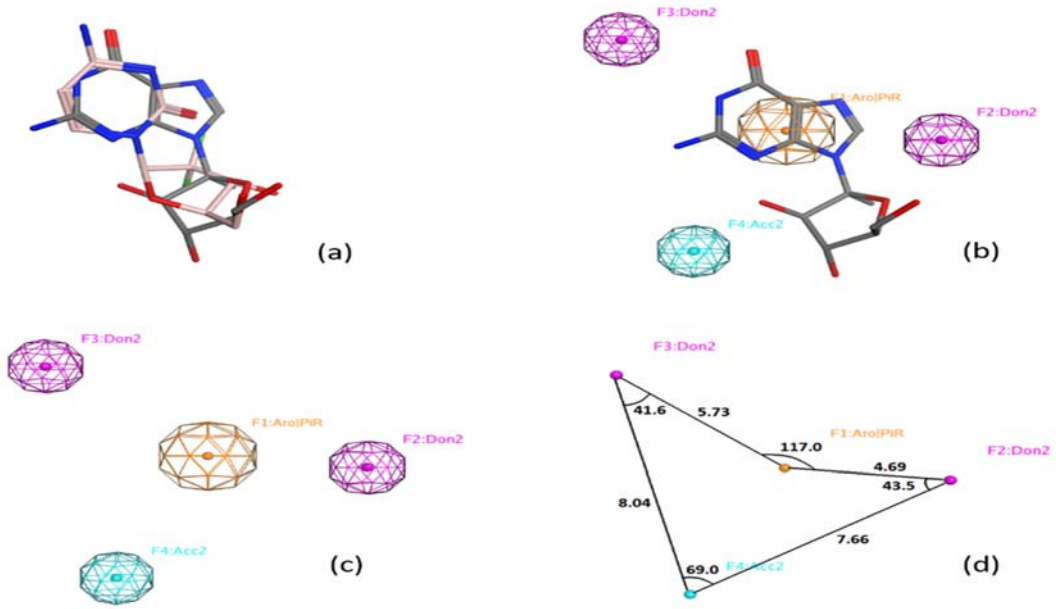


Figure 4. Pharmacophore query no. 23 of hENT1 permeants. (a) permeant's training set, consisting of 2 ligands Gemcitabine and Guanosine overlay. (b) A permeant molecule aligned with the pharmacophore query no. 23. (c) pharmacophoric features. (d) Distances and angles between pharmacophore features.

In terms of substrate structural requirement for ENT1 transport, substrate pentose sugars ribose/deoxyribose and arabinose moieties are primary determinates for transport.[1,6,32] Moreover, the substrate's pentose sugar C(3') hydroxyl group is essential for ENT1 binding.[1,32] Permeants such as nucleosides and nucleoside analog drugs are hydrophilic and, hence their permeation is presumed to be facilitated via pentose sugar C(3') hydroxyl group-hydrogen bonding interaction with ENT1. Similarly, the permeant pharmacophore PH4-23 did exhibit hydrogen bonding features.

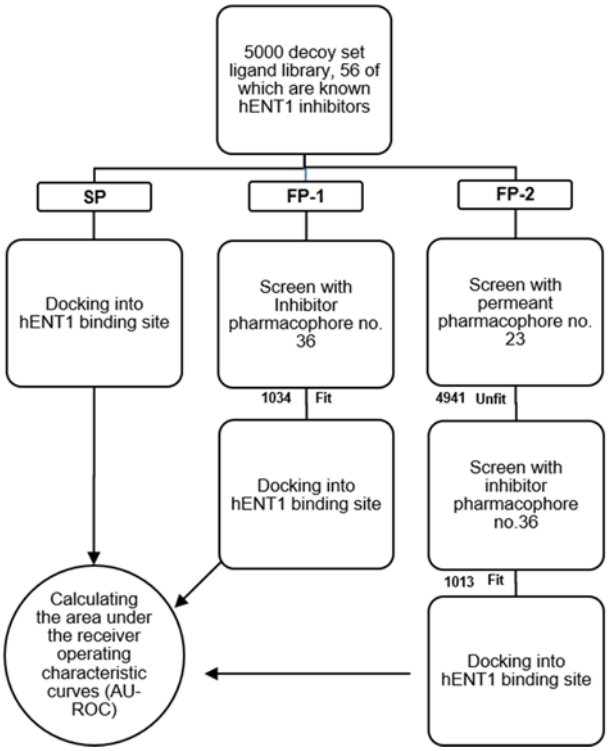
3.4 Establishment of virtual screening using hENT1 inhibitor's pharmacophore

To examine the efficacy of the PH4-36 pharmacophore and its application in the drug design of new hENT1 inhibitors, we used this pharmacophore as a filter prior to a small-scale virtual screening (VS). The drug-like ligand library consisted of 5000 compounds; 56 were hENT1 inhibitors while the remaining compounds were the decoy set, obtained from a commercial ligand library (i.e., TimTec), all were filtered according to Lipinski's rule of five.[33] Then, the ligand library was docked into the crystal structure of hENT1 where the co-crystallized ligand was used for binding site identification purposes.

Three protocols were applied to the drug-like ligand library to screen virtually for the 56 hENT1 inhibitors (Scheme 1). These protocols were: SP, a standard docking-based VS with no filters; FP-1, a VS protocol preceded by the use of hENT1 inhibitor pharmacophore PH4-36 as filter-in. For the third protocol, FP-2, a further optimization step was added to the VS protocol by including a hENT1 permeants pharmacophore that filter-out permeants. Hence the third VS protocol, FP-2, was preceded by the use of the hENT1 permeants pharmacophore PH4-23 as filter-out, combined with hENT1 inhibitor pharmacophore PH4-36 as filter-in. Furthermore, to assess the screening protocol's success, the area under receiver operating characteristic (AU-ROC) curves were calculated. ROC graph is a

graphing method used for visualizing, evaluating and comparing the performance of the three VS protocols.[34,35] Each ROC curve depicts the correlation between the number of retrieved ligands of interest (true positive) and the number of decoys (true negative) at various portions of top-ranked ligands obtained from VS. The higher the value of AU-ROC, the better the performance of that screening protocol.

The initial glance of implementing the FP-1 protocol is that it immediately resulted in drastic downsizing for the 5000 ligands library by around 79% (i.e., down to 1034 compounds) relative to the SP protocol (Scheme 1). As for FP-2, the prepared ligand library was first passed through the permeant pharmacophore. The ligands that fit the permeant pharmacophore were excluded, while the remaining ones that did not fit were then passed through the second filter of the inhibitor pharmacophore. Similarly to FP-1, FP-2 resulted in a significant reduction of the 5000 ligand library down to 1031 compounds. FP-2 slightly downsized the ligand library to be docked onto the binding site (1013 compounds) compared to FP-1 (1034 compounds) (Scheme 1).



Scheme 1. The three virtual screening protocols utilized in the decoy set validation test.

We then determined the rate of retrieved inhibitors of the standard, one and two filtered-based protocols and performed a ROC analysis. In the top 1% of the 5000-docked ligands, retrieved rates of 19.6% and 21.3% were seen with the single-filter and dual-filter protocol, respectively, in comparison to the SP approach which showed less %EF of 12.5% (Table 4). Hence, the filter-based protocols were able to pull up a higher number of inhibitors at the top 1 % of the docked ligands compared to the non-filtered protocol. Similarly, the rate in the top 3% of the docked ligands was higher in the filter-based protocols with values of 35.3% in FP-1 and 34% in FP-2

than the standard non-filter approach which showed only 17.8% (Table 4). Remarkably, in the top 20% of docked ligands, while FP-1 and FP-2 were capable of retrieving almost all docked inhibitors (with %EF values of 98% and 100%, respectively), the SP approach was only capable of retrieving 57% of the hENT1 inhibitors (Table 4). Based on the rate of retrieved inhibitors (true positive rate) and the rate of retrieved decoys (true negative rate) at various portions of top-ranked ligands obtained from the three VS protocols, a ROC curves were plotted for the three protocols where AUC value for each curve was then calculated. AU-ROC values of 0.802, 0.927 and 0.941 were seen for SP, FP-1 and FP-2, respectively. The higher the AU-ROC value the better the performance is. Overall, the one and two filtered approaches consistently performed better, evident by the significantly higher AU-ROC value, relative to the non-filtered SP approach (Figure 5).

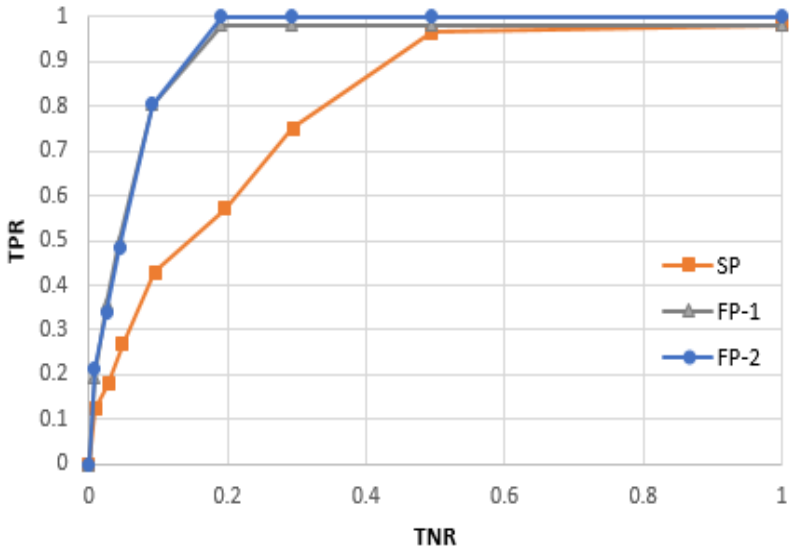


Figure 5. ROC analysis of the three assessed protocols: SP-1, non-filtered protocol; FP-1, one filter protocol; FP-2, two filters protocol. TPR: True Positive Rate; TNR: True Negative Rate.

Standard protocols for VS of a large-sized library have limitations; including the lengthy period of docking and scoring compounds, and the generation of many false-positive compounds that are ranked highly in docking scores ahead of the true positive compounds.[36] Similar to previous studies,[28,36,37] the VS results demonstrated the efficiency of the pharmacophore filter-based approach to pick up true hENT1 inhibitors over decoys, evident by the improved retrieval rates of the VS output compared with the nonfilter-based approach. Moreover, a significant reduction in the size of the ligand library was obtained by the filtered based approach to be docked on the hENT1 binding site, which resulted in the speed up of VS.

3.5 Analysis of inhibitors–hENT1 complexes

To identify the essential residues for inhibitors binding to hENT1, a PLIF was generated for the VS hENT1-docked inhibitors complexes using MOE software.[26] The PLIF-interaction occupancy of all interacting residues in the hENT1 binding site was then determined. The interaction occupancy is defined as the percentage of ligands interacting with the side or main chain of a given amino acid. A total of 20 residues were found to participate in the protein-ligand

interaction (Figure 6a). Trp29, Met33, Asn338, and Asp341 showed the highest binding interactions evident by their high % occupancies of ~ 45% in comparison to other residues (Figure 6b). Furthermore, hydrogen bonding and arene interaction with Met33; and hydrogen bonding with Trp29, Asn338, and Asp341 appeared to facilitate the inhibitors binding to hENT1 (Figure 6b).

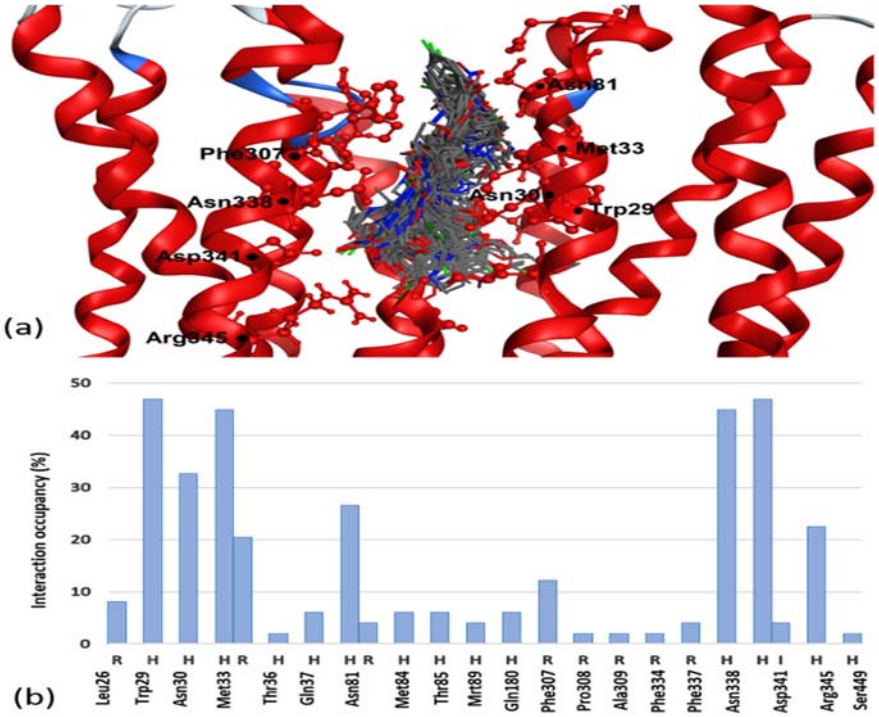


Figure 6. (a) The docked poses of inhibitors onto the crystal structure of the hENT1 α-helical TM domain. (b) Protein ligand interaction fingerprint of ENT1 inhibitors (H: H-bond attraction, I: ionic attraction, R: arene attraction).

Consistent with our structural findings, Trp29, Met33, and Asn338 have been previously shown to be necessary for the inhibitor Dilazep binding to hENT1.[8] Dilazep's trimethoxybenzoic acid group was shown to interact with Trp29, present within the orthosteric site of the hENT1 central cavity while the central diazepane ring of Dilazep interacted hydrophobically with Met33. In the hENT1 opportunistic site, located proximally to the orthosteric site, Dilazep's other trimethoxyphenyl ring formed hydrogen bonding with Asn338.[8] Similarly, we observed that arene interaction (possibly hydrophobic) with Met33 and hydrogen bonding with Asn338 facilitated the inhibitors interaction with hENT1 (Figure 6b). Asp341, another identified key residue that facilitated the inhibitors interaction with hENT1 in the current study, was reported to be essential for the binding of the inhibitor NBTI to hENT1. It was demonstrated that 3′-OH of the ribose moiety of NBTI interacted with Asp341 present within the hENT1 orthosteric site,[8] likely via hydrogen bonding. Overall, the results highlighted the key interaction residues for inhibitors binding to hENT1 and further confirms VS protocol's validity and applicability in selecting real hENT1 inhibitors given that the identified key interacting residues Trp29, Met33, Asn338, and Asp341, has been previously reported to be essential for inhibitors binding to hENT1.

4. CONCLUSION

In conclusion, we have reported the successful development of highly sensitive and selective pharmacophoric model for hENT1 inhibitors. Also, the present study demonstrated a new virtual screening protocol where the aforementioned inhibitor pharmacophore was used as filtration tools within the hENT1 inhibitors VS scheme. Furthermore, the proposed protocols were successfully able to reduce the size of the screened ligand library by almost 80% (prior to docking) and to accurately select the true hENT1 inhibitor among decoy ligands, which led to a more rapid and effective VS approach. The novel computer-aided protocol proposed in this work can help in the discovery of new hENT1 inhibitors as potentially acting cardio- and neuroprotective agents. Future studies will focus on utilizing the proposed virtual screening protocol to identify new prospective hENT1 inhibitors and testing their effect on hENT1 transport *in vitro* and *in vivo*.

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TABLES

Table 1. Mean values (±standard deviations) of calculated physicochemical parameters for inhibitors.

Physicochemical property	Inhibitors (± SD)	Permeants (± SD)
Mean MWT [a](Da)	473.52 ± 136.24	269.70 ± 34.36
Mean #HBA [b]	4.82 ± 2.35	6.05 ± 1.51
Mean #HBD [c]	2 ± 1.82	3.72 ± 1.13
Mean Log P	1.87 ± 2.08	-1.56 ± 0.65
Mean No. of rotatable bonds	10 ± 6.13	2.22 ± 0.65
Mean PSA [d] (Å2)	180.8 ± 88.7	37.01 ± 86.31
Mean HSA [e] (Å2)	708.63 ± 117.32	423.79 ± 89.54

[a] Molecular Weight; [b] Number of Hydrogen Bond Acceptor, [c] Number of Hydrogen Bond Donor, [d] Polar Surface Area, [e] Hydrophobic Surface Area.

Table 2. The selectivity and specificity of the best performing pharmacophore for inhibitors

PH4 no.	**Features**	**Selectivity 'Se' (%)**	**Specificity 'Sp' (%)**
1	1: Hyd 2: Acc2 3: Acc 4; Aro | Hyd	92.30	75
4	1:Hyd 2:Acc2 3:Aro|Hyd 4:Don|Acc	94.23	43.75
6	1:Hyd 2:Hyd 3:Acc2 4:Aro|Hyd	82.69	81.25
21	1:Hyd 2:Acc2 3:Aro|Hyd 4:Acc|Cat	86.53	75.00
30	1:Hyd 2:Hyd 3:Acc2 4:Acc	92.30	62.5
36	**1:Hyd 2:Hyd 3:Acc2 4:Aro|Hyd**	**92.30**	**87.5**
40	1:Hyd 2:Aro|Hyd 3:Aro|Hyd 4:Don|Acc	88.5	100

Aro: Aromatic center, Don: H-bond donor, Hyd: hydrophobic centroid, Acc: H-bond acceptor, Acc2: H-bond acceptor projection, Cat: cationic

Table 3. The selectivity and specificity of the best performing pharmacophore for hENT 1 permeants.

PH4 no.	Features	Selectivity Se (%)	Specificity Sp (%)
8	1:Don2 2:Acc2 3:Aro|Hyd 4:Don|Acc 5:Acc|Cat	93.75	69.23
9	1:Aro|PiR 2:Hyd 3:Don 4:Acc 5:Aro|Hyd	87.5	82.69
16	1:Aro|PiR 2:Hyd 3:Acc2 4:Don	100	75
17	1:Aro|PiR 2:Hyd 3:Acc2 4:Don|Ani	100	75
21	1:Aro|Hyd 2:Don|Acc 3:Don|Acc 4:Don|Acc 5:Acc|Cat	100	65.38
23	**1:Aro|PiR 2:Don2 3:Don2 4:Acc2**	**100**	**84.62**
28	1:Aro|PiR 2:Acc2 3:Acc 4:Aro|Hyd	93.75	61.54
49	1:Don2 2:Aro|Hyd 3:Don|Acc 4:Don|Acc 5:Acc|Cat	93.75	75

Aro: Aromatic center, Don: H-bond donor, Hyd: hydrophobic centroid, Acc: H-bond acceptor, Acc2: H-bond acceptor projection, Don2: H-bond donor projection, Cat: Cationic, PiR: Pi ring or Aromatic

Table 4. Retrieved rate of hENT1 inhibitors at various portions of the sorted docked ligands from filter-based and non-filtered-based VS.

Output percentage of the top-ranked docked 5000-ligand library	The rate of retrieved inhibitors		
	No-filter	One filter	Two filters
1	12.5	19.6	21.27
3	17.8	35.29	34.04
5	26.78	49.02	48.93
10	42.85	80.39	80.85
20	57.14	98.04	100

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تطوير فارماكوفور جديد لاستخدامه كمرشح نوعي خلال عملية اكتشاف مركبات مثبطة لناقلات النوكليوزيدات البشرية المتوازنة

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ملخص

تعتبر ناقلات النيوكليوسيدات البشرية المتوازنة **(hENTs)** بروتينات مهمة تسمح للنيوكليوسيدات والقواعد النيتروجينيه بالنفاذ إلى الخلية. يعتبر **HENT 1** هدفًا واعدًا ضد أمراض القلب وأمراض هنتنغتون حيث أن تثبيطه يترتب عليه تأثيرات علاجية محتملة مثل الحماية من بعض أمراض القلب والأعصاب. ومع ذلك، فإن مثبطات **hENT1** المتعارف عليها حاليا لها خصائص دوائية ضعيف، بالتالي بحاجة إلى تطوير مثبطات جديدة. لذلك، قمنا بتطوير بروتوكول حاسوبي له القدرة على تمييز وانتقاء مثبطات **hENT1** بطريقة فعالة ومحددة. أولاً، تم إنشاء عدد من فارماكوفور (حامل الخصائص الدوائية) باستخدام مجموعة من المثبطات المعروفة مسبقا. ومن المثير للاهتمام، أن أفضل فارماكوفور للمثبطات قد أظهر معدلات انتقائية وخصوصيه عالية بلغت **92**٪ و**88**٪ على التوالي. إضافة الى ذلك، أدى استخدام مثبط فارماكوفور كمرشح إلى تحسين نتائج الغربلة الافتراضي القائمة على تقنية **docking** الى ما يقارب الضعف. يمكن أن يكون هذا النهج في تطبيق المحاكاة الحاسوبية **(in silico)** مفيدًا جدًا في تطوير مثبطات **hENT1** جديدة.

الكلمات الدالة: تصميم الدوائي ، ناقلات النيوكليوسيدات البشرية المتوازنة 1، المثبط، حامل الخصائص الدوائية.

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Women's opinions, beliefs, and practices towards using different medicinal plants for postpartum health problems care

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ABSTRACT

Objectives: The aim of this study is to evaluate Jordanian Women's opinions, beliefs, and practices towards using different medicinal plants for postpartum health problems care.

Method: A cross-sectional observational study was conducted on 300 mothers aged 18 years and above. A structured valid and reliable questionnaire was used for collecting personal, medical and nutritional related data including: gestational weight gain characteristics, the effect of delivery and breast feeding on postpartum weight gain and herbal tea consumption for the management of different postpartum health problems such as postpartum colic, flatulence, spasm, maternal bleeding, lactation and weight gain. The above data where collected through a personal interview by the trained investigators.

Results: Around 45% of participants were overweight or obese with average post-pregnancy BMI of 25.1±4.94 kg/m2. Majority of participants (84%) used one or more medicinal plants after delivery to control their postpartum health problems. The participants may seek herbal help mainly for maternal purposes such as decreasing post-delivery colic, flatulence and spasm (52.9%), treating maternal postpartum bleeding (41.7%) and lactation enhancement (41.0%). Conversely, only 9.0% of participants used herbals for weight control. The most commonly used herbals were cinnamon (49.0%), sage (42.0%), and anise (38.0%).

Conclusions: The potential risk of medicinal plant self-medication is high for managing postpartum complications that need a professional evidence-based practice recommendation.

Keywords: Phytotherapy, complementary and alternative medicine, postpartum health problems, gestational weight gain.

INTRODUCTION

Women after delivery are at high risk for postpartum maternal comorbidities[1] such as fatigue (>50%),[2] depression (6.5% - 12.9%),[3] gestational weight gain (20% - 40%) over recommended[4] in addition to constipation, lactation problems, pain, and others which developed during first few weeks of delivery and may extend up to 1 year.[1-7] Many Studies have reported the effectiveness of using medicinal plants alone or beside steam baths, diet and prescriptions in the management of such postpartum health problems.[8-10] Particularly, postpartum overweight and obesity, which is strongly related to increased gestational weight above what is recommended during pregnancy.[11-14] The later factor is a critical contributor to higher rates of obesity in women of reproductive age even among those with normal weight before pregnancy.[15,16] Studies have indicated that, gestational weight gain associated with long-term with obesity and weight gain after delivery.[2,17,18]. Healthcare professionals encourage

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postpartum managements of gestational weight gain and obesity prevention even during pregnancy.[1,4,6] A recent review found that, diet, exercise or both interventions during pregnancy could reduce risk of postpartum obesity by 20%.[4] However, women are regularly seeking traditional methods to control postpartum obesity.

There are many conventional treatment options for obesity management including low-calorie diet consumption, exercise regimen, pharmacological and bariatric surgical treatments.[19-21] The non-surgical management is generally a multicomponent behavioral therapy includes dietary changes by decreasing energy intake, increasing physical activity, and using different pharmacotherapies such as medication and complementary and alternative medicine (CAM). Options for weight management include medicinal plants,[19,22,23] acupuncture,[24,25] homeopathy,[26] and sleep therapy[27] also play a crucial role in body weight control.

Women are the largest consumers of healthcare resources, and this extends to their big utilization and strong believes in the effectiveness CAM such as the use of medicinal plants.[28-30] With regard to socio-demographics, CAM users are more likely to be educated and middle-aged females, better as they are more committed to practices of health care processes than males.[31-33] Furthermore, they preferred CAM alone or combined with conventional medical treatments as a first line therapeutic option for themselves or their families in managing many acute and chronic conditions.[34-36] Musculoskeletal problems, different types of pain, severe headaches/migraines, depression, insomnia, and stomach or intestinal illnesses were the most common condition treated with CAM.[32,37] In addition, women widely use medicinal plants to manage gynecological and obstetric problems.[8,36,38-40] Women perhaps feel comfortable using medicinal plants because of believes in their perceived safety, accessibility, and the widespread availability of information about them using Internet, TV, magazines and books.[30] On the other hand and based on misconception that medicinal plants are natural then they are safe products, women still use these plants during the period of pregnancy and lactation regardless of possible high risk on mother and fetus health during these critical periods,[35,38,39] keeping in mind that the published data regarding safety during pregnancy are very limited.[30] A number of medicinal plants have lately been investigated for obesity management. Many studies approved that medicinal plants are effective in obesity management such as ginger (*Zingiber officinale*),[41] green tea (*Camellia sinensis*),[42] cumin (*Cuminum cyminum*),[43] and cardamom (*Elettaria cardamomum*).[44]

At Arab countries, few studies in gulf and Mediterranean regions investigated medicinal plant use for women health mainly during pregnancy in Oman,[45] Qatar,[46] Palestine,[47] Egypt,[48] but only one study from Saudi Arabia studied the use of medicinal plat during pregnancy and after delivery.[8] The studies revealed that between 22% - 82% of women have consumed medicinal plants during pregnancy and the most commonly used medicinal plants were peppermint, green tea, thyme chamomile, aniseeds, sage, fenugreek, ginger, and garlic.[39]

Here in the Hashemite Kingdom of Jordan (29°11 N 33°22 E) more than 49 plant families having more than 120 plant species are used in alternative medicine.[35] However, up to our knowledge, studies about medicinal plants usage for post-delivery health problems including obesity are also not available in Jordan as well as other Arab countries.

The connection between medicinal plant usage and maternal benefits that leads women to use these plants through generations based on their own believes and opinions is not well defined among Jordanian women. It is so important to know why women habitually do believe and use certain plants and what the benefits from their own point of view.

The aim of this study is to assess Jordanian women's opinions, beliefs, and practices towards using different medicinal plants for postpartum health problems care.

Methods

Study design

This cross-sectional observational study was conducted in Amman city; the capital of Jordan. Participants' recruitment was conducted during weekdays over a period of six months from March to August 2017. Participants' interviews were conducted in community nutrition clinics in Amman. The study protocol met the Declaration of Helsinki and Good Clinical Practices principles and was approved by the institutional review board at Balqa Applied University in Jordan.

Measures

A closed and open-ended questionnaire was used to explore Jordanian women's opinions, believes and practices towards using different medicinal plants phytotherapy in postpartum health complications. Data were collected through structured interviews conducted by three trained pharmacy students in their senior year.

Participants

A convenient non-random sampling approach was used to cover any women older than the age of 18 years who were able to speak Arabic and with full mental capacity and eligible for study entry. Participants were provided with information about the study and were invited to be interviewed while waiting for their clinic appointment. Women who agreed to participate had the research procedure, goals and methods explained to them and signed an informed consent. A signed written informed consent was obtained from each woman before participation in this study.

Procedure

This study adopted a survey methodology through structured interviews using validated anonymous questionnaire. For questionnaire development, a comprehensive literature review was conducted to ensure the content validity of the questions. Every effort was made during literature review to ensure content validity. Moreover, various drafts of the questionnaire were evaluated individually by two clinical pharmacists, two dietitians, one statistician and one sociologist in order to ensure face validity. This was followed by pilot testing and discussions to ensure clarity and overcome any ambiguities in the questions. The study was piloted on 30 educated and non-educated women before the official start of the definitive study where trained pharmacy students in their senior year were collect the data and help the participants.

Few questions were added or deleted accordingly and the data of the pilot part were excluded from the final analysis.

The questionnaire was in Arabic and consisted of several parts: the first one covered demographic data, obstetric characteristics data and some laboratory tests of the involved participants. These data were including age, body mass index (BMI), type of delivery method, random blood glucose (RBG) level, hemoglobin (Hb) level, vitamin B_{12} level and thyroid stimulating hormone (TSH) level. The next part of the questionnaire involved questions about the postpartum weight gain characteristics of recruited women; mainly the extent of weight gain and the methods used to retain to usual weight such as diet, physical activity or depending on lactation as a tool for weight reduction. Another part of the questionnaire assessed women's opinions and believes towards the effect of delivery and breast feeding on postpartum weight gain. The last part evaluated the habitual consumption of medicinal plants at least three times per week selected as herbal tea and they were also asked about the main indication of use for different postpartum health problems.

The medicinal plant use was evaluated by asking directly about the local name of the medicinal plant and a clear picture of each medicinal plant. The studied medicinal plants giving as common (*scientific*) names were; sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum verum*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), mint (*Mentha pipreta*), clove **(***Syzygium aromaticum***)**, common

silver nail root (*Paronychia argentea*), chamomile **(***Matricaria chamomilla***)**, anise **(***Pimpinella anisum***)**, cumin **(***Cuminum cyminum***),** cardamom **(***Elettaria cardamomum***)**, fenugreek **(***Trigonella foenumgraecum*), caravea **(***Carum carvi***)** and green tea (*Camellia sinensis*).

Statistical analysis

Data were coded and entered using Statistical Package for Social Sciences (SPSS©); Version 22.0) database for statistical analysis. Descriptive statistics with corresponding 95 % confidence intervals (CIs) were constructed. Differences between various groups were evaluated using the Pearson chi-Square correlation test and Fisher exact tests for categorical variables. *P* values less than 0.05 were considered statistically significant.

Results

Participants' characteristics

A total of 400 females were approached to participate in the study of which 300 were interviewed (response rate 75.0%). Table 1 summarizes demographic and obstetric characteristics of the study sample. The participants were females from 18 to 45 years with average postpartum BMI of 25.1 ± 4.94 kg/m^2 and normal average hemoglobin level of 12.6 ± 1.4 g/dL. Majority of participants (60%) had history of normal delivery method only and about 15% of participants had history of both normal delivery and caesarean section methods.

Table 1: Demographic and obstetric characteristics of the study sample (N=300)

Parameter	**N (%) or Mean (SD)**
Postpartum BMI (%)	
Under Weight	6 (2%)
Normal	154 (51.3%)
Over Weight	92 (30.7%)
Obese	48 (16.0%)
Type of delivery method	
Normal delivery	180 (60%)
Caesarean section	75 (25%)
Both	45 (15%)
Thyroid function test (TSH)	
Low	15 (5%)
Normal	267 (89%)
High	21 (7%)
B_{12} Level	
Deficient	24 (8%)
Normal	276 (92%)
Hemoglobin level (g/dL)	
Minimum	9.0

Parameter	N (%) or Mean (SD)
Average± SD	12.6 ± 1.4
Maximum	16.7
Random Blood Glucose (RBG)	
Minimum	58.0
Average ± SD	111.7 ± 42.4
Maximum	289.0

Most of participants (90%) suffered from postpartum weight gain where only half of them retained to their usual weight after one year of delivery. Around 62% of women gain around 1-10 Kgs after delivery which enforced them to one method or more such as special diet and/or physical activity to control her weight. Half of participants tried many methods to retain to their usual weight before pregnancy such as following self-planned diet (25.0%), increasing physical activity (9.0%) and seeking dietitian supervision (5.0%). Results are shown in table 2.

Table 2: Postpartum weight gain characteristics of the study sample (N=300)

	(%)
Suffering from postpartum weight gain	270 (90%)
Extent of weight gain	
1-5 kg	75 (25.0%)
6-10 kg	111 (37.0%)
11-15 kg	51 (17.0%)
16-20 kg	24 (8.0%)
>20 kg	9 (3.0%)
No weight gain	30 (10.0%)
Retain to usual weight after one year of delivery	150 (50%)
Methods used to retain to usual weight	
Self-planned diet	75 (25.0%)
Dietitian supervision	15 (5.0%)
Physical activity	27 (9.0%)
Lactation	33 (11.0%)

Participants' opinions and beliefs

A good percent of participants believes that both

frequent deliveries (62.0%) and breast feeding (53%) affect weight gain positively, but only 20% of the participants stopped breast feeding just in order to retain to their usual weight. On the other hand, around 11.0% of participants depend on lactation as a useful way in losing weight after delivery. Less than half of participants do believe that the gender of the baby has impact on the mother weight gain after delivery; in which they believe that male gender has higher impact on weight gain than female gender. Table 3 showed the opinions and believes of recruited women towards the effect of delivery and breast feeding on postpartum weight gain.

Table 3: Opinions and believes of recruited women towards the effect of delivery and breast feeding on postpartum weight gain (N=300)

	(%)
Baby Gender impact on weight gain	
Yes	120 (40%)
Male	66 (22%)
Female	54 (18%)
No impact of gender on weight gain	180 (60%)
Effect of frequent deliveries on usual weight	
Yes there is effect	186 (62%)
Increase usual weight	162 (54%)
Decrease usual weight	24 (8.0%)
No effect	114 (38%)
Effect of breast feeding on weight gain	
Yes there is effect	159 (53%)
No effect	141 (47%)
Would you stop breast feeding to prevent weigh gain	
Yes	60 (20%)
No	240 (80%)

Herbals use

The participants were asked about 14 herbals regarding their use of these herbals in general. Majority of participants (84%) used one or more of the mentioned medicinal plants after delivery. The most commonly used herbals were cinnamon (49.0%), sage (42.0%), and anise (38.0%). While the least used herbals were fenugreek (10.0%), clove (10.0%) and rosemary (9.0%). The participants may seek herbal help mainly for maternal purposes such as decreasing postpartum colic, flatulence and spasm (52.9%), treating maternal postpartum bleeding (41.7%) and lactation enhancement (41.0%). On the other hand, only 9.0% of participants use herbals for weight control where overweight and obese participants used medicinal plants to control their weights more than others (p <0.05). Table 4 summarizes the significance of each

medicinal plant use for the management of different postpartum health problems.

Table 4: Correlation between medicinal plant commonly used and different postpartum health problems (N=300)

	Herbal common name	Enhance lactation	Relieve delivery colic, flatulence and spasm	Treat maternal postpartum bleeding	Weight control	Improve sleeping, mood and cognition
1.	**Sage**	< 0.001	< 0.001	< 0.001	< 0.001	0.278
2.	**Rosemary**	0.004	< 0.001	0.19	0.07	0.004
3.	**Cinnamon**	< 0.001	< 0.001	< 0.001	< 0.001	0.087
4.	**Common silver nail root**	0.003	< 0.001	0.16	0.041	0.009
5.	**Chamomile**	< 0.001	< 0.001	0.007	0.012	< 0.001
6.	**Clove**	< 0.001	< 0.001	0.421	0.722	0.978
7.	**Anise**	< 0.001	< 0.001	0.07	0.005	0.015
8.	**Thyme**	< 0.001	< 0.001	0.005	0.669	0.845
9.	**Ginger**	0.001	< 0.001	0.06	0.002	0.669
10.	**Cumin**	< 0.001	0.002	0.162	0.017	0.871
11.	**Mint**	< 0.001	< 0.001	0.272	0.006	0.143
12.	**Cardamom**	< 0.001	< 0.001	0.037	0.783	0.084
13.	**Fenugreek**	0.91	0.037	0.001	0.382	0.03
14.	**Green Tea**	0.111	< 0.001	0.502	< 0.001	< 0.001

Discussion

Regardless of increasing medicinal plant consumption worldwide;[28,29,34,36] safety issues especially among mothers in postpartum period still warranted extra caution.[38] Little information is available about the use of medicinal plants in postpartum health care in neither Arab world in general nor Jordan in particular. In addition, no previous studies highlight the maternal opinions, beliefs and practices towards using different commonly known medicinal plants for these complications.

The present study noticed a high percent (84%) of medicinal plants consumption among participants for different postpartum health problems. When we compared the results in the current study, with that of a neighboring country such as Saudi Arabia[8] we found that although there are comparable results between Jordanian women (9.0%) and Saudi women (7.7%) regarding using medicinal plants for postpartum weight management, there is different prevalent regarding traditional use of medicinal plants for treating other postpartum health problems. In our study, medicinal plants were mainly used for decreasing postpartum colic, flatulence and spasm (52.9%), treating postpartum bleeding (41.7%) and for lactation enhancement (41.0%). However, and according to a study by Al-Ghamdi *et al*. (2017) a lower prevalence of such medicinal plants consumption for the mentioned postpartum health problems (2.8% to decrease bleeding, 6.5% to increase lactation and 25.1% to relieve pain) has been shown.[8] The reasons behind these differences may be due to different study population and sample size, the traditional and cultural believes among both population may also different in this regards between gulf (Saudi Arabia) and Mediterranean (Jordan) region.

In a previous Asian study by Manderson *et al*. (2003) ginger and cumin were used to control postpartum bleeding in a recipe of boiled rice and vegetables.[49] However, our results were inconsistent with that finding; as women consumption of ginger and cumin to treat postpartum bleeding were insignificant (p>0.05). The Asian life style and culture may contribute for these results. On the other hand, participants in our study strongly believed and consumed medicinal plants such as ginger, cumin and green tea (p = 0.002, 0.017 and <0.001 respectively) in order to manage postpartum obesity. This result is comparable to what is approved in the literature for the efficacy of ginger and cumin in obesity management.[41-43]

According to the current study, more than 40% of mothers used medicinal plants to enhance lactation. This is consistent with a previous study in Jordan concluded that, employed mothers tend to frequently used medicinal plants to treat minor health problem and seeking health benefit during lactation period not only for themselves but for their infants too as they strongly believed in medicinal plant efficacy and safety.[35] Other studies have also shown prevalent use of medicinal plant among lactating women.[38,39] In present study, the most commonly used medicinal plants were cinnamon (49.0%), sage (42.0%) and they were significantly (p<0.05) associated with all studied postpartum complication except for improve sleeping, mood and cognition (p>0.05). Studies were in disagree with our findings,[38,50,51] as these plants were shown to have adverse effects on breastfeeding such as cinnamon may cause cramps and diarrhea in infants, while sage may reduce the milk production.[50,51] Nevertheless, the safety of medicinal plants use by childbearing women and infants is questionable because the published data in this era is scarce, diverse and inconclusive.[30,52-54] Those plants, were used based on traditional thought, believes or previous experience of friends and relatives without seeking the health care professional advise because they think that medicinal plants are safe and effective.[28,38] This study showed that (53%) of participants believed that breastfeeding leads to weight gain, but even though only 20% of the participants stopped breast feeding just in order to retain to their usual weight. On the other hand, around 11.0% of participants depend on lactation as a useful way in losing weight after delivery. Although the benefit of breastfeeding on baby development, health and immunity well known in the literature,[55-57] its impact on maternal

postpartum weight is still inconclusive.[58-60] For instance, in a systematic review highlights the difficulties of evaluating the association between breastfeeding and weight change in an observational research, Neville *et al*. (2014) have found that many studies showed no or little association between breastfeeding and postpartum weight change.[60] The increase hunger in addition to sleep deprivation and decrease activity of the mothers while nursing are suggested reasons for this finding.[61,62] On other hand, Jarlenski *et al*. (2014) in this regard reported a positive association in a study found that mothers who exclusively breastfed for more than three months lose about 1.5 kg in the first and they have higher probability (6%) to return to their pre-pregnancy weight than non-exclusively breastfeeding mothers do.[59] The possible causes behind these results are the increased energy expenditures, hormonal changes, the duration and frequency of breastfeeding on maternal weight in postpartum period.

In this study, many participants (40%) do believe that the gender of the baby has impact on the ability of the mother to gain weight after delivery in which they believe that male gender (22.0%) has higher impact on weight gain than female gender (18.0%). This result is in agree with a recent study, which is conducted in the University of Georgia's College of Agricultural and Environmental Sciences in The United States of America looked at data from the Centers for Disease Control and Prevention and involved over 46 million births covering a 23-year period. The study found that a lower gestational weight gain is more associated with female gender baby.[63] According to the author, the possible justification for this result is the higher metabolic demands associated with male gestation.[63]

In present study, more than (60%) of participants believe that both frequent deliveries (multiparous) positively affect postpartum weight. This is in accordance with a study by Paulino *et al*. (2016) who reported high prevalence obese or overweight (62.5%) among multiparous women.[64] Regardless of the significant higher BMI among multiparas, the researchers also noticed an inverse correlation between parity and the total postpartum weight.[64] These findings support the important of obtaining a healthy weight before and during pregnancy through adapting a healthier lifestyle related to nutrition and physical activity.

Conclusions

The study highlights the use of medicinal plants among Jordanian females for managing postpartum gestational weight gain and other postpartum health complications based on traditional thought or previous experience of friends and relatives or even self-experience.

Many female connected postpartum obesity with infant gender, type and frequency of delivery and they tried many methods to retain for their normal weight this beliefs and behavioral practice were not relays on evidence-based practice.

Our results put spotlight on the urgent need for national strategies to educate and increase the awareness degree about medicinal plants efficacy and safety especially in childbearing mothers and infants. Managing postpartum complications based on evidence-based practice, through training programs and workshops by health care professionals, maternity care center and community workers focusing on the potential risk of self-medication.

Statement of Ethics

The study protocol met the Declaration of Helsinki and Good Clinical Practices principles.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The authors of the manuscript have equal contributions to the study design, work, analysis and interpretation of data, drafted the work and revised it, approved the final version to be published. The authors agreed to be accountable for the accuracy or integrity of any part of the work.

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آراء ومعتقدات وممارسات النساء تجاه استخدام النباتات الطبية المختلفة لرعاية المشاكل الصحية بعد الولادة

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ملخص

الأهداف: الهدف من هذه الدراسة هو تقييم آراء ومعتقدات وممارسات المرأة الأردنية تجاه استخدام النباتات الطبية المختلفة لرعاية المشاكل الصحية بعد الولادة .

الطريقة: أجريت دراسة رصدية مقطعية على 300 أم تتراوح أعمارهن بين 18 سنة وأكثر. تم استخدام استبيان منظم وصالح وموثوق لجمع المعلومات الشخصية والطبية والغذائية بما في ذلك خصائص زيادة الوزن أثناء الحمل ، وتأثير الولادة والرضاعة الطبيعية على زيادة الوزن بعد الولادة واستهلاك الشاي العشبي لإدارة المشاكل الصحية المختلفة بعد الولادة مثل مغص ما بعد الولادة ، انتفاخ البطن ، تشنج ، نزيف الأم ، الإرضاع وزيادة الوزن من خلال مقابلة شخصية من قبل المحققين المدربين.

النتائج: كان حوالي 45٪ من المشاركات يعانون من زيادة الوزن والسمنة بمتوسط مؤشر كتلة الجسم بعد الحمل 25.1 ± 4.94 كجم / م 2. استخدم غالبية المشاركين (84٪) واحدًا أو أكثر من النباتات الطبية بعد الولادة للسيطرة على مشاكلهم الصحية بعد الولادة. قد يطلب المشاركون المساعدة العشبية بشكل رئيسي لأغراض الأم مثل تقليل المغص وانتفاخ البطن والتشنج بعد الولادة (52.9٪) ، وعلاج نزيف ما بعد الولادة (41.7٪) وتعزيز الرضاعة (41.0٪). بالمقابل ، فقط 9.0٪ من المشاركين يستخدمون الأعشاب للتحكم في الوزن. أكثر الأعشاب استخداما هي القرفة (49.0٪) والمريمية (42.0٪) واليانسون**38.0**) ٪.(

الخلاصة: يوصى بشدة بإدارة رعاية المشكلات الصحية بعد الولادة بناءً على الممارسة القائمة على الأدلة ، مع التركيز على المخاطر المحتملة للتطبيب الذاتي.

الكلمات الدالة: العلاج بالنباتات ، الطب التكميلي والبديل ، المشاكل الصحية بعد الولادة، زيادة الوزن بعد الولادة.

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Use of medicinal plants in dermato-cosmetology: An ethnobotanical study among the population of Izarène

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ABSTRACT

An ethnobotanical study was carried out among the population riverine of the Izarène forest to enhance and safeguard ancestral knowledge of medicinal plants used in traditional pharmacopeia for the treatment of dermatological diseases. Using 480 questionnaire sheets, ethnobotanical field surveys were conducted during two campaigns (2013 to 2015). The determination of the different survey media was carried out using stratified probability sampling techniques. The ethnobotanical data were analyzed through the calculation of quantitative indices, such as Relative Frequency of Citation (RFC), Family Importance Value index (FIV), Fidelity Level (FL), Informant Consensus Factor (ICF), and the use-value of the Plant Part (PPV). The results show 62 useful plant species, belonging to 34 botanical families. The Lamiaceae family was the most represented (8 species, FIV= 0.037). The highest Relative Citation Frequency (RFC) (0.137) was recorded for Olea europaea L. Concerning the diseases treated, cosmetic use has the highest ICF (0.96), the leaf was considered to be the most used part of the plant (PPV=0.34) and the majority of the remedies were prepared under the form of a poultice. The results obtained could constitute a basis for further studies for the valorization of medicinal plants used against dermatological diseases through biological and phytochemical studies of the inventoried plants.

Keywords: Dermatological diseases, ethnobotanical surveys, Izarène forest, medicinal plants.

INTRODUCTION

The use of plants for medicinal purposes has led for several centuries to a multitude of herbal recipes to prevent, cure, relieve or improve human well-being [1]. Today, despite the development of chemical drugs to combat urinary tract diseases, there is often a return to plants as a source of active ingredients. Besides, an important part of the population, especially in rural areas, prefers medicinal plants, for economic reasons and sometimes because of difficulty in accessing medical care [2].

Thus, numerous studies in the field of ethnopharmacology, show that plants tested in traditional medicine are almost devoid of toxicities and are often effective in pharmacological models [3]. At present, natural substances present in plants are still the first reservoir of new drugs. They account for almost 60% of the drugs we have [4]. The remaining 39% of synthetic drugs are often the result of chemical modification of natural molecules or parts of natural molecules taken as heads of the series [5–7].

Since the beginning of time, the Moroccan people have used many medicinal plants as a popular medicine to cure

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many human and livestock health problems. In Morocco, given the diversity of medicinal plants with great therapeutic power, the Moroccan population adopts herbal medication for various diseases; and in this range, there is an important place occupied by dermatological affections. Dermatological diseases are numerous and widespread, they are considered as a set of pathologies whose most visible symptoms occur at the target organ level, including the skin, mucous membranes, and partners. In this context, we carried out a study based on ethnobotanical surveys and which aims to identify and inventory the medicinal plants used for the treatment of dermatological diseases with the population bordering the forest of Izarène known for its floristic, ecological diversity, which offers the local population a fairly rich knowledge of traditional herbal medicine.

Methods

Study area

According to the territorial division of 2015, the forest massif of Izarène is part of the Tangier-Tetouan-Al Hoceïma area. It is located in the North-Western part of the Kingdom, 12 Km North-East of the town of Ouezzane and covers an approximate forest surface of 14 600 ha between the parallels 34° 45' and 34° 58'N and the meridian lines 5° 25' and 5° 32'W. Limited to the southern part by marls of the Cretaceous pre-rifaine nappe, this forest massif is characterized by a rugged relief where altitudes vary approximately between 350 and 680 m [8].

The study area, the Circle of Mokrisset, is a part of the province of Ouezzane and includes 3 Caïdats (Zoumi, Mokrisset, and Brikcha), and 3 rural communes (Zoumi, Ain Baïda, and Brikcha) (Figure 1); it contains a population estimated at 25 000 inhabitants [9]. The geological formation of the forest massif is characterized by a tormented relief due to the presence of several hills forming the beginning of the Rifaine Mountains on the southern side. These hills are characterized by a deep clayey-schist or clayey-marly soil that can reach in some places more than 3 meters of depth. The climate of the area is subhumid with temperate winters, with an average annual rainfall of about 1 000 mm. These precipitations are distributed over approximately 70 days all the year (November to April). A mild winter with almost no frost and very rainy follows a dry and hot season lasting 3 to 4 months [10].

The vegetation cover is characterized by a rich and diversified forest formation, consisting mainly of vegetation that shows a degradation of the climatic formation of the cork oak [11]. The forest of Izarène has a vital economic, ecological, and social importance for the bordering population. It assures the needs of the population for the firewood and work and constitutes the principal source of fodder for the cattle [9]. Beyond its paramount functions, it plays a role in traditional medicine thanks to the use of medicinal plants as means of subsistence among the bordering population.

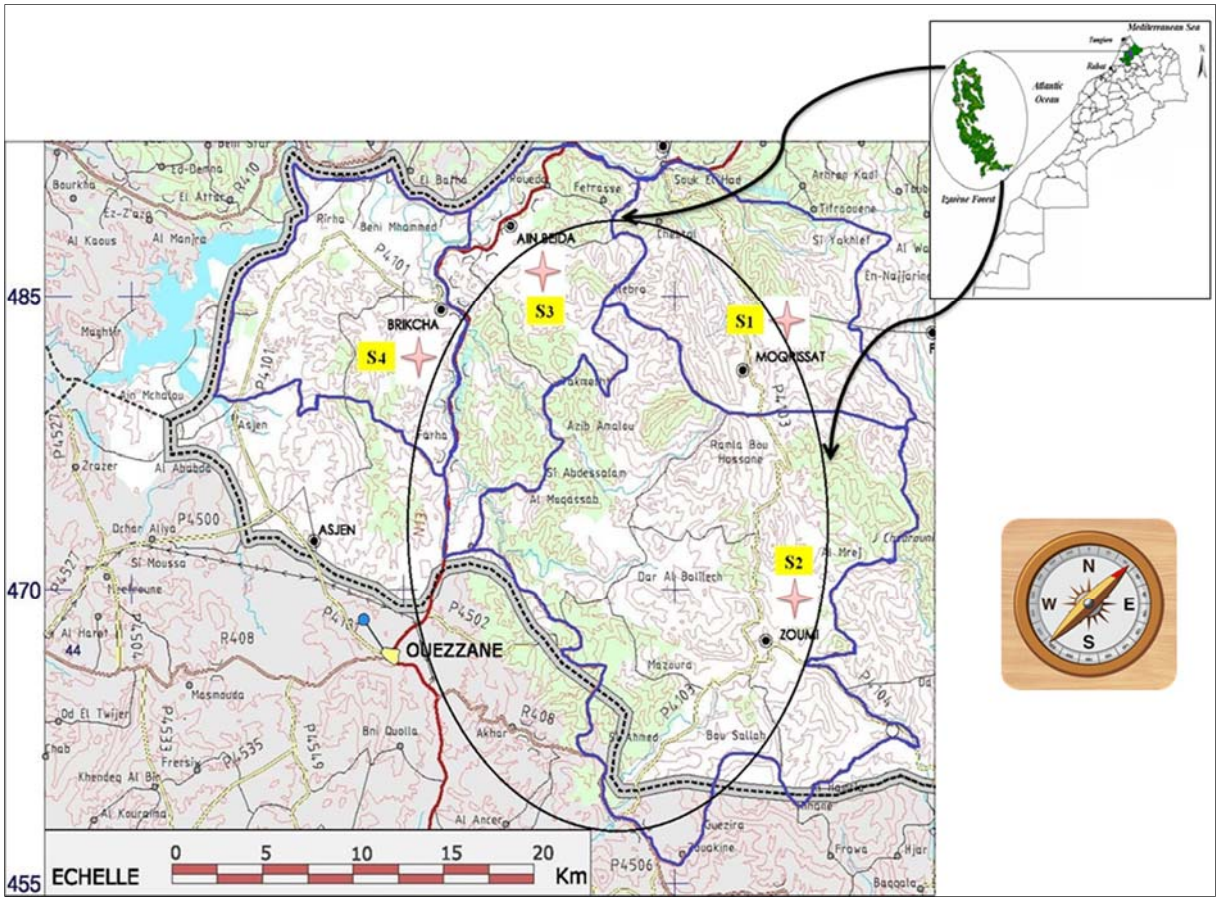

Figure 1. Location of the study area

Data collection

To gather as much information as possible about the use of medicinal plants in the treatment of dermatological diseases, ethnobotanical surveys were carried out in different localities, villages, and towns, bordering the forest of Izarène during a period from 2013 to 2015. The determination of the different survey media was carried out using stratified probability sampling techniques [12]. In this study, the sample is divided into 4 homogeneous strata (S1, S2, S3, and S4), 3 of which correspond to the numbers of rural communes in the Mokrisset Circle (Table 1). The investigation was carried out on 480 people inhabitants of villages and towns of the studied zone. By adopting a random stratified sampling, samples of 120 people are then formed for each of the 4 strata and they are put together to constitute the total sample (480 people). The field studies required us to deal with two groups: those who knew and/or used plants for medicinal purposes and those who used plants and plant products for commercial purposes (plant collectors, herbalists, traditional practitioners). The interviews with the informants were conducted in the Arabic dialect of the region. A questionnaire was prepared from the preliminary surveys in the study area and validated by a team from the Plant, Animal Productions and Agro-industry Laboratory of the Faculty of Sciences of Kenitra. The questionnaire used consists of two parts: the first part deals with the demographic characteristics of the informants and the second one focuses on the plants

used in the treatment of diseases (Appendix 1). The time devoted to each interview was approximately two hours, and the oral and written consent of the informants was collected for each interview. All persons who shared their traditional knowledge were informed about the principle of the study, its interest, and the procedure used while respecting the anonymity of the information collected.

Table 1. Distribution of the surveys by strata

Strata	Names of strata	Number of inquiries
Strata 1	Mokrisset	120
Strata 2	Zoumi	120
Strata 3	Ain Baïda	120
Strata 4	Brikcha	120
Total 480		

Identification and conservation of plant species

The plants indicated by the informants were systematically photographed and botanical samples were collected, classified, accumulated and placed in the herbarium or a plastic bag with a label indicating its vernacular name. The taxonomic validation of the species was carried out in the laboratory using herbaria and completed with the following documents: "Medicinal plants of Morocco" [13]; "Practical flora of Morocco" [14,15] and " Catalogs of vascular plants of northern Morocco, including identification keys volumes I and II" [16]. Fertile specimens for the present study were collected in the field (30 plant species), in herbal stores (20 plant species), and at the homes of traditional healers (12 plant species) in the Izarène. All voucher specimens have been preserved during documentation and deposited in the Ibn Tofail University, Morocco Herbarium for future reference.

Data Analysis

The data collected on the survey sheets were entered and transcribed into a database then processed by SPSS version 21 and the Excel spreadsheet (version 2010). Descriptive statistical methods were used to analyze the socio-demographic data of informants.

To determine the socio-demographic parameters influencing the orientation of the riverside population towards traditional medication, an analytical study using logistic regression was conducted. Analysis of ethnobotanical data was carried out using the Relative Frequency of Citation (RFC), the Family Importance Value (FIV), the Plant Part Value (PPV), Fidelity Level (FL), Informant Consensus Factor (ICF), and the Plant Part Value (PPV).

Relative frequency of citation (RFC)

RFC is calculated to appreciate the local importance of each species. The RFC is the result of the citation frequency (Fc), that is the number of informants who mentioned the use of the species, divided by the total number (N) of respondents[17], with ($0 < RFC < 1$). $RFC = Fc/N$

Family Importance Value (FIV)

The Family Importance Value (FIV) identifies the importance of medicinal plant families. It is calculated using the method: FIV = FC family/Ns. where $FC_{familly}$: RFC is the number of informants mentioning the family and Ns: total number of species in each family. Family use-value is a culturally significant index that can be applied to ethnobotany to calculate the biological value of the plant taxon [18].

Fidelity Level (FL)

The fidelity index is calculated to identify the species most commonly used in the treatment of a particular disease. It is calculated according to the method proposed [19]:

FL= (Fc/ Ft) x 100. Where Fc = frequency of citation of the species in the treatment of a particular disease and Ft = total number of citations of the species.

Informant Consensus Factor (ICF)

It is calculated for each category to assess the agreement between informants on the use of plants for specific use categories. It is obtained using the following formula [20]: ICF= (Nur - Nt) / (Nur – 1). Where Nur = number of use citations for a disease category and Nt = number of species used by

informants in a given use category. The value calculated from this consensus ranges from 0 to 1 and is close to 1 when the plant is used by a large number of the respondents for a particular disease and/or if the information is exchanged between informants on the use of the species for a particular disease, and close to 0 (low) when the plant is randomly selected or if there is no exchange of information about use among informants [21].

Plant Part Value (PPV)

The PPV is calculated to assess the importance of each part of the plant used by the respondents, it is obtained by the following formula [22]: PPV =$RU_{plant\ part}$/ RU. Where $RU_{plant\ part}$ = is the sum of reported uses per part of the plant and RU = the number of reported uses of all parts of the plant.

Results and Discussion

Sociodemographic profile of the respondents

The ethnobotanical surveys were carried out on a sample of 480 people, and the results obtained show that men and women are concerned with traditional medicine. However, women have slightly more knowledge about medicinal species compared to men, with a frequency of 75% compared to 25% for men (Table 2), which corresponds to a sex ratio of 3.

The predominance of women can be justified by the fact that women are always on the lookout for natural remedies based on medicinal plants, to improve their knowledge of these plants and to maintain their health and that of their families since they are closer to the plants either in their children's medication or in their use in cooking. These results confirm the results of other ethnobotanical work carried out at the national level [23–25].

The use of medicinal plants concerns all age groups at the scale of the riparian population of the forest massif of Izarène. The results obtained show a predominance of the elderly, so those with an age over 60 represent 36.5%. The age groups of [50-59], [40-49], [30-39], [18-29] have a percentage of 16.7%, 17.9%, 15% and 14%, respectively. Indeed, older persons are expected to provide more reliable information on the traditional use of medicinal plants, because they hold much of the ancestral knowledge that is transmitted orally. The transmission of this knowledge is currently in danger because it is not always ensured [26]. The relative transmission of traditional practices from one generation to the next may explain the mistrust of some people, particularly young people, who tend not to believe too much in this traditional medicine [27].

Concerning the level of education of the respondents in the study area, the results obtained show that the majority of users had been illiterate with a percentage of 48.8%. This relatively high percentage is in direct correlation with the level of education of the local population. Nevertheless, those with primary and secondary education had a significant percentage (27% and 17% respectively), while those with university education had a percentage of 8%. Other studies have shown that the knowledge of the population on the use of medicinal plants is held by illiterate people [28,29]. The majority of the residents surveyed in this region are married, with a percentage of 85% married versus 15% single. Concerning the socio-economic level, 45.2% of the people surveyed had a low socio-economic level, 42.5% are unemployed and 12.3% had a medium level.

Table 2. Socio-demographic characteristics of the participants in the study area.

	Distribution	**Number of informants**	**Percentages (%)**
Gender	Male	120	25.0
	Female	360	75.0
Age groups	[18-30[	67	14.0
	[30-40[	72	15.0
	[40-50[	86	17.9
	[50-60[	96	20.0

	Distribution	Number of informants	Percentages (%)
	> 60	159	33.1
Educational level	Illiterate	234	48.8
	Primary	128	26.7
	Secondary	81	16.9
	University	37	7.7
Family situation	Married	407	84.8
	Single	73	15.2
Monthly income	No income	204	42.5
	Low income	217	45.2
	Average income	59	12.3

According to the logistic regression model, it appears that the variables: gender, age, and least expensive use are predictors influencing the orientation of the riverside population towards traditional medication, pointing out that the least expensive use factor represents an influence of 12 times more than the other factors (Table 3). The high cost of modern medical treatments, their side effects, and the unfavorable socio-economic conditions of the local population, are the essential factors that push the local population to make extensive use of herbal medicine. These results confirm the results achieved by El Hassani in Middle Moulouya [30].

Table 3. Statistical analyses of the influence of socio-demographic parameters on confidence in-plant use the

		IC for 95%		
	Odds-Ratio	**Lower**	**Upper**	**p-value**
Gender	0.189	0.08	0.445	0.000
Age groups	0.294	0.208	0.415	0.000
Educational level	1.147	0.893	1.474	0.282
Family situation	1.203	0.340	4. 254	0.774
Monthly income	1.018	0.572	1.709	1.812
Least expensive use	12.403	5.915	26.009	0.000

Botanical families most represented in the study area

The study of medicinal plants led to the census and identification of 62 species belonging to 34 families and divided into 58 genera. These plants are presented in alphabetical order. For each plant listed, the scientific name, family, local name, the part used, and the method of preparation adopted by the local population are given, as well as the data of FL, FC, RFC, and FIV are shown in Table 5. The FIV index revealed 6 families with high importance values which are therefore preponderant in the treatment of dermatological conditions (Table 4). These are Lamiaceae (8 species with FIV = 0.037), Rosaceae (4 species with FIV = 0.033), Asteraceae (4 species with FIV = 0.032), Apiaceae (3 species with FIV = 0.032), Solanaceae (3 species with FIV = 0.03) and Fabaceae (3 species with FIV = 0.023). The remaining families had only one or two species. The dominance of Lamiaceae was also observed during similar ethnomedical surveys carried out in others regions of Morocco [2,25,31].

Table 4. Distribution of medicinal plants according to the family importance value (FIV) in the study area.

Familly	RFCs	NS	FIV	Familly	RFCs	NS	FIV
Amaranthaceae	0.025	1	0.025	Rubiaceae	0.049	2	0.024
Amaryllidaceae	0.071	2	0.035	Gentianaceae	0.062	1	0.062
Anacardiaceae	0.023	1	0.023	Iridaceae	0.025	1	0.025
Apiaceae	0.097	3	0.032	Lamiaceae	0.3	8	0.037
Apocyanaceae	0.056	1	0.056	Lythraceae	0.16	2	0.08
Arecaceae	0.046	1	0.046	Moraceae	0.02	1	0.02
Aristolochiaceae	0.033	1	0.033	Myrtaceae	0.193	2	0.096
Asteraceae	0.13	4	0.032	Nitrariaceae	0.044	1	0.044
Boraginaceae	0.045	2	0.022	Oleaceae	0.137	1	0.137
Brachytheciaceae	0.002	1	0.002	Ranunculaceae	0.095	2	0.047
Brassicaceae	0.018	2	0.009	Rhamnaceae	0.029	1	0.029
Cannabaceae	0.11	1	0.11	Rosaceae	0.132	4	0.033
Cistaceae	0.068	1	0.068	Sapotaceae	0.077	1	0.077
Cupressaceae	0.06	1	0.06	Solanaceaea	0.091	3	0.03
Euphorbiaceae	0.091	2	0.045	Tamaricaceae	0.039	1	0.039
Fabaceae	0.07	3	0.023	Thymelaeaceae	0.116	2	0.058
Fagaceae	0.039	1	0.039	Urticaceae	0.012	1	0.012

Relative Frequency of Citation (RFC) and Fidelity Level (FL)

Some species were more recommended by the local population than others; this is reflected by a high citation frequency (FC). The value of relative frequency of citation (RFC) as numerical representatives in the quantitative ethnobotanical survey highlights the importance of traditional knowledge. Species with a very significant relative citation frequency are those with a high level of use. Among these plants, 8 species belonging to 7 botanical families were frequently used by the bordering population in the treatment of dermatological conditions. These are: *Olea europaea* L. (RFC=0.137), *Lawsonia inermis* L. (RFC=0.125), *Cannabis sativa* L. (RFC=0.11), *Eugenia caryophyllata* Thunb. (RFC=0.104), *Daphne gnidium* L. (RFC=0.089), *Myrtus communis* L. (RFC=0.089), *Rosa damascena* Mill. (RFC=0.083) and *Salvia verbenaca* L. (RFC=0.083). Some of these plants have been reported by an ethnomedical survey in the treatment of dermatological conditions [25]. Among these plants are *Lawsonia inermis* L., *Eugenia caryophyllata* Thunb., *Olea europaea* L., *Daphne gnidium* L., *Myrtus communis* L., and *Salvia verbenaca* L.. Other recent studies had experimentally proven the anti-inflammatory activity of *Lawsonia inermis* L. and the antimicrobial activity of *Allium cepa* L. and *Coriandrum sativum* L., which partly explain their use in the treatment of infectious diseases by population bordering the Izarène forest. In addition, among the listed plants, some are recognized by their toxic power such as *Ricinus communis* L., *Peganum harmala* L., *Solanum nigrum* L. [32], and *Atractylis gummifera* L. [33]. Indeed, the majority of physiotherapists are unaware of the toxicity of the plants used as well as the modalities of their use, in particular the methods of preparation and the recommended doses. The use of medicinal plants must be rationalized and the benefit/risk determined. Studies on these objectives are therefore necessary.

The fidelity index value (FL) is an important means of determining, which disease a particular species is more effective, a high FL indicates a high use of plant species for a particular disease, while a low FL demonstrates a wide range of medicinal uses but with a low frequency for each disease

[21]. In the present study, the majority of plants had a high FL, and the highest 100% FL was recorded for 53 plant species (Table 5). Only 2 species have low fidelity values (FL <70). Among the species with the highest fidelity for the treatment of a wide range of dermatological conditions are *Lawsonia inermis* L. which has the highest FL for cosmetic use (FL = 85%), *Allium cepa* L. which is used most often to treat infectious diseases (FL = 71%), *Cistus monspeliensis* L. which is used to treat skin lesions (FL = 64%) and *Solanum nigrum* L. to treat allergic conditions (FL = 87%)

Table 5. List of medicinal plants used for the treatment of dermatological diseases in the study area.

Family and scientific name	Local name	Plant parts	Administration	Medicinal uses	FL %	FC	RFC
Amaranthaceae							
Chenopoium ambrosioides L.	Mkhinza	Leaves	Cataplasm	ID, SL	75	12	0.025
Amaryllidaceae							
Allium cepa L.	Lbesla	Other part	Cataplasm	ID, SL	71	14	0.029
Allium sativum L.	Toum	Other part	Friction	CU	100	20	0.042
Anacardiaceae							
Pistacia lentiscus L.	Drô	Leaves	Cataplasm	CU	100	11	0.023
Apiaceae							
Ammi majus L.	Trîlân	Seeds	Powder	AD	100	5	0.010
Coriandrum sativum L.	Qezbor	Seeds	Cataplasm	ID	100	30	0.062
Daucus carota L.	Khizzu	Roots	Cataplasm	AL	100	12	0.025
Apocyanaceae							
Nerium oleander L.	Deflaâ	Leaves	Other forms	ID	100	27	0.056
Arecaceae							
Chamaerops humilis L.	Jummar	Root	Power	CU	100	22	0.046
Aristolochiaceae							
Aristolochia baetica L.	Bereztam	Root	Powder	SL	100	16	0.033
Asteraceae							
Anacyclus pyrethrum (L.) Link.	Tigenticht	Root	Powder	AD	100	4	0.008
Atractylis gummifera L.	Addâd	Root	Powder	CU	100	10	0.021
Dittrichia viscosa (L.) Greuter	Terrahlâ	Leaves	Cataplasm	AL, ID	81	21	0.043
Matricaria chamomilla L.	Mansaniya	Flower	Cataplasm	CU	100	28	0.058
Boraginaceae							
Borago offinalis L.	Hurrîcha	Aerial parts	Cataplasm	SL	100	14	0.029
Cynoglossum officinale L.	Lossîq	Root	Cataplasm	SL	100	8	0.016
Brachytheciaceae							
Homalothecium aureum (Spruce) H.Rob.	Sunbul	Aerial parts	Cataplasm	CU	100	1	0.002
Brassicaceae							
Lepidium sativum L.	Habbrchad	Seeds	Powder,	ID	100	6	0.012
Sinapis alba L.	El khardel	Seeds	Powder, Cataplasm	CU	100	3	0.006
Cannabaceae							
Cannabis sativa L.	L'kif	Seeds	Powder, Cataplasm	CU	100	53	0.11
Cistaceae							

Family and scientific name	Local name	Plant parts	Administration	Medicinal uses	FL %	FC	RFC
Cistus monspeliensis L.	Chtâppa	Leaves	Cataplasm	ID, SL	64	33	0.068
Cupressaceae							
Tetraclinis articulata Benth.	Al'Araâr	Leaves	Cataplasm	AL, CU	69	29	0.06
Euphorbiaceae							
Mercurialis annua L.	L-hurriga L'melsâ	Roots	Cataplasm	CU	100	13	0.027
Ricinus communis L.	Lkharwa'	Seeds	Cataplasm	CU	100	31	0.064
Fabaceae							
Ononis natrix L.	Afezdade	Roots	Cataplasm	CU	100	2	0.004
Trigonella foenum-graecum L.	Al'houlba	Seeds	Macération, friction	CU	100	14	0.029
Vicia faba L.	Fûle	Seeds	Powder, Cataplasm	ID	100	18	0.037
Fagaceae							
Quercus suber L.	D'bbagh	Other parts	Powder	CU	100	19	0.039
Gentianaceae							
Centaurium Erythraea Rafn	Gossat Al'Hayya	Aerial parts	Powder	SL	100	30	0.062
Iridaceae							
Crocus sativus L.	Za'âfrane lhor	Flower	Powder	SL	100	12	0.025
Lamiaceae							
Lavandula stoechas L.	Al'Halhal	Leaves	Macération, friction	ID	100	15	0.031
Marrubium vulgare L.	Merriwta	Leaves	Cataplasm	ID	100	25	0.052
Mentha viridis L.	Naanaâ	Aerial parts	Cataplasm	SL	100	17	0.035
Ocimum basilicum L.	Lahbak	Leaves	Cataplasm	CU	100	23	0.048
Origanum compactum Bentham.	Zaâtar	Leaves	Powder, Cataplasm	SL, CU	89	9	0.018
*Rosmarinus officinalis*L.	Aazir	Leaves	Cataplasm	CU	100	4	0.008
Salvia Officinalis L.	Assalmiya	Leaves	Cataplasm	SL	100	12	0.025
Salvia verbenaca L.	Al'khiyata	Aerial part	Powder, Cataplasm	SL	100	40	0.083
Lythraceae							
Lawsonia inermis L.	L'hennâ	Leaves	Powder, Cataplasm	AL, ID, CU	85	60	0.125
Punica granatum L.	Rummân	Fruit	Powder	SL	100	17	0.035
Moraceae							
Fucus carica L.	L'kermôs	Leaves	Other forms	ID	100	10	0.02
Myrtaceae							
Eugenia caryophyllata Thunb.	Qronfel	Flower	Powder, maceration	CU	100	50	0.104
Myrtus communis L.	Arraihan	Leaves	Powder	CU	100	43	0.089
Nitrariaceae							
Peganum harmala L.	L'hermel	Seeds	Powder	CU	100	21	0.044
Oleaceae							

Family and scientific name	Local name	Plant parts	Administration	Medicinal uses	FL %	FC	RFC
Olea europaea L.	Zitoun, Zabbouj	Seeds	Other forms	CU	100	66	0.137
Ranunculaceae							
Delphinium staphisagria L.	Habbat râs	Seeds	Powder	CU	100	30	0.062
Nigella sativa L.	Assânûj	Seeds	Macération, Powder	CU	100	16	0.033
Rhamnaceae							
Ziziphus lotus L.	Nberg	Leaves	Cataplasm	ID	100	14	0.029
Rosaceae							
Prunus amygdalus stokes var. amara DC.	Lûz mûrr	Fruit	Powder	CU	100	7	0.014
Prunus amygdalus stokes var. dulcis DC.	Lûz Lahlou	Fruit	Friction	CU	100	10	0.021
Rosa damascena Mill.	L'werd L'beldi	Flower	Powder, Cataplasm	CU	100	40	0.083
Rubus ulmifolius Schott.	El-ûllîk	Leaves	Powder, Cataplasm	SL	100	7	0.014
Rubiaceae							
Coffea arabica L.	Al'qahwa	Fruit	Powder, Cataplasm	SL	100	9	0.018
Rubia tinctorum L.	Al'Fouwa	Roots	Powder, Cataplasm	ID	100	15	0.031
Sapotaceae							
Argania spinosa L. Skeels.	Argane	Fruit	Friction	CU	100	37	0.077
Solanaceae							
Hyscyamus albus L.	Gingît	Leaves	Cataplasm	AL, ID	80	10	0.021
Solanum lycopersicum L.	Maticha	Fruit	Cataplasm	CU	100	26	0.054
Solanum nigrum L.	Buqnîna	Fruit	Cataplasm	AL, SL	87	8	0.016
Tamaricaceae							
Tamarix aphylla (L.) Karst.	Takkaût	Other parts	Powder	CU	100	19	0.039
Thymelaeaceae							
Daphne gnidium L.	Lazaz	Leaves	Powder	CU	100	43	0.089
Thymelaea lythroides L.	L'metnâne	Leaves	Powder	ID	100	13	0.027
Urticaceae							
Urtica dioîca L.	Hourrîka	Aerial part	Cataplasm	CU	100	6	0.012

CU: Cosmetic Uses; **ID:** Infectious Diseases (Abscesses and furuncles); **SL:** Skin Lesion (Burns, wounds, and injuries); **AL:** Allergy (Eczema); **AD:** Autoimmune diseases (Vitiligo).

Frequency of use of the medicinal plants according to their origin

The ethnobotanical study allowed us to identify 62 medicinal plants used in the treatment of dermatological diseases. Among them, 24 species were imported from other regions of the country, while 15 species were cultivated and 23 species had collected from the forest of Izarène. This high use of local species can be explained by the high price of imported medicinal plants by the free and proximity of local medicinal plants. It should be noted that among the spontaneous medicinal species collected in the forest of Izarène, four medicinal plants: *Salvia verbenaca*

L., *Centaurium erythraea* Rafn, *Solanum nigrum* L., and *Origanum compactum* Bentham. are scarce in the region [27] and risk disappearing from the forest if no protective measures are taken by the services concerned, due to the intensive collection of these species.

Used parts

Several parts of the listed plants were used for the treatment of dermatological diseases. The calculation of the PPV usage index showed that leaves were the most used parts with an index PPV= 0.34, followed by the seeds with an index PPV= 0.24, then flowers (PPV= 0.11). The other parts were used to a lesser degree (Figure 2). The dominance of leaf use is confirmed by similar ethnobotanical studies carried out in other regions of Morocco [23,25,34–37]. Indeed, leaves were the most used because they are at the same time central to photochemical reactions and reservoirs of organic matter derived from them, therefore rich in active principles, and they are easy to harvest.

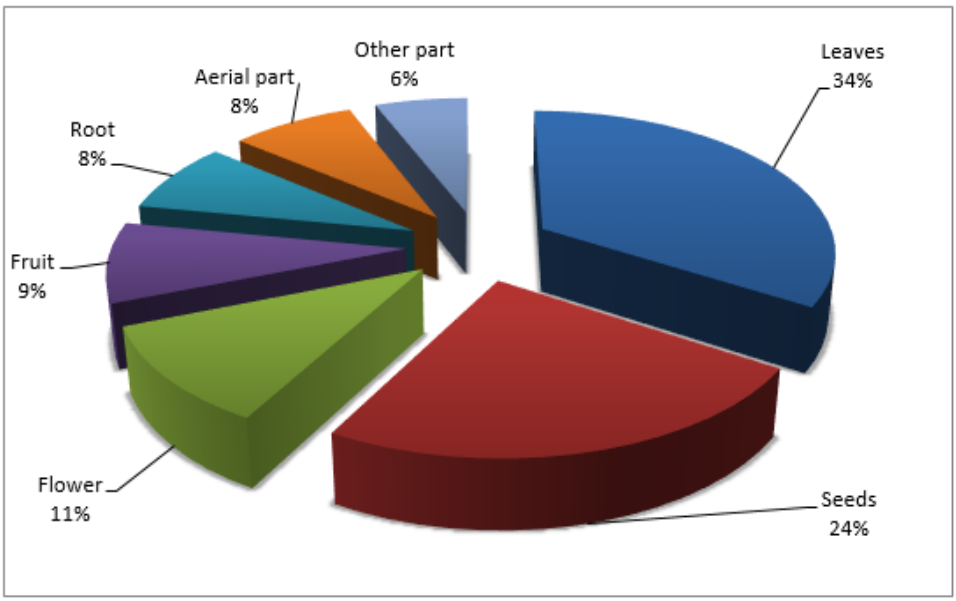

Figure 2. Different parts used for the treatment of dermatological diseases in the zone of study.

Method of preparation and administration used

Different therapeutic practices were used by the local population, for the treatment of dermatological affections (cataplasm, maceration, friction, and powder) to administer the active ingredients contained in the medicinal plants. Cataplasm was the most used mode of administration with a percentage of 46.7% (Figure 3), followed by powder preparation with a percentage of 37.7%, the other modes of preparation namely friction, maceration, and other forms had represented 15.6%. However, the majority of respondents ignore the weights, the dose, and the precise measures to be prescribed in the preparation and dosage of phytomedicines.

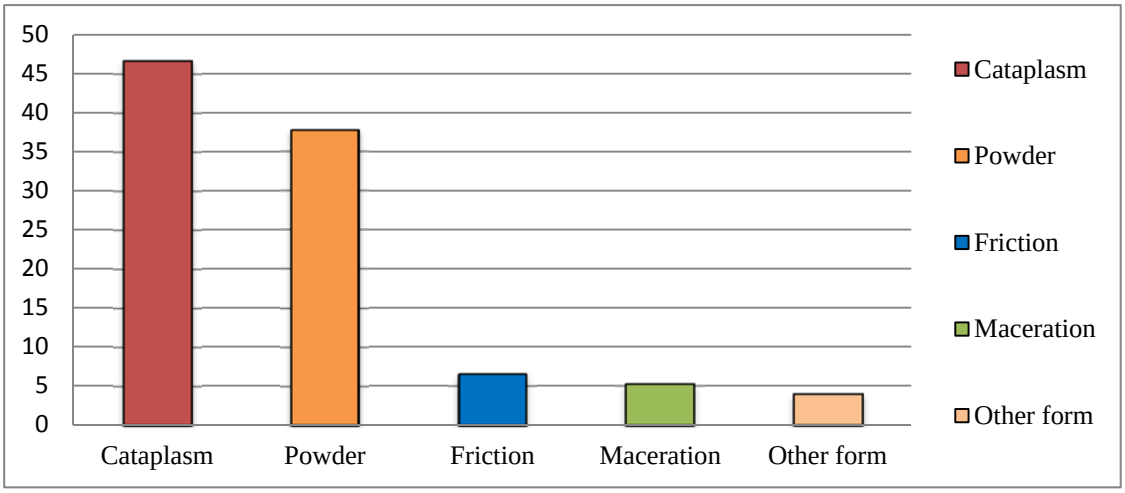

Figure 3. Percentages of different modes of remedies preparation used in the zone of study.

The Informant Consensus Factor (ICF)

The Informant Consensus Factor (ICF) reflects the homogeneity of information provided by different informants regarding the medicinal plants used to treat a category of diseases. Table 6 shows that the ICF values range from 0.87 to 0.96 per use category. A total of 62 species was identified to treat dermatological diseases. The categories with the highest ICF values were cosmetic use (0.96), followed by infectious diseases (0.93) and skin lesions (0.93). These high ICF values reflect the reasonable reliability of informants on the use of herbal species [38] and indicate that natural remedies are considered to be extremely effective. Consequently, species with a high ICF should be prioritized for further pharmacological and phytochemical studies to discover new active molecules.

The lowest agreement among informants was observed for medicinal plants used to treat allergies (Eczema) (0.91) and autoimmune diseases (Vitiligo) (0.87). These values highlight a marked non-homogeneity in the consensus for these categories and may indicate a lack of specific use for a set of species. In agreement with [39], a low ICF could also be due to the availability of readily available synthetic drugs that represent a better alternative to traditional drugs.

Table 6. ICF values by categories for the treatment of dermatological diseases.

Categories	**List of plant species used (number of citations)**	**Total number of**		**ICF**
		Species	**Use citations**	
Cosmetic Uses (CU)	*Allium sativum* L. (20), *Pistacia lentiscus* L. (11), *Chamaerops humilis* L. (22), *Atractylis gummifera* L. (10), *Matricaria chamomilla* L. (28), *Homalothecium aureum* (Spruce) H.Rob. (1), *Lepidium sativum* L. *Sinapis alba* L. (3), *Cannabis sativa* L. (53), *Tetraclinis articulata* Benth. (9), *Mercurialis annua* L. (13), *Ricinus communis* L. (31), *Ononis natrix* L. (2), *Trigonella foenum-graecum* L. (14), *Quercus*	33	753	0.96

Categories	List of plant species used (number of citations)	Total number of		ICF
		Species	Use citations	
	suber L. (19), *Ocimum basilicum* L. (23), *Origanum compactum* Bentham. (8), *Rosmarinus officinalis* L. (4), *Lawsonia inermis* L. (51), *Punica granatum* L. (17), *Eugenia caryophyllata* Thunb. (50), *Myrtuscommunis* L. (43), *Peganum harmala* L. (21), *Olea europaea* L. (66), *Delphinium staphisagria* L. (30), *Nigella sativa* L. (16), *Prunus amygdalus* stokes var. amara DC. (7), *Prunus amygdalus* stokes var. dulcis DC. (10), *Rosa damascena* Mill. (40), *Argania spinosa* L. Skeels. (37), *Solanum lycopersicum* L. (26), *Tamarix aphylla* (L.) Karst. (19), *Daphne gnidium* L. (43), *Urtica dioîca* L. (6).			
Infectious Diseases (ID): Abscesses and furuncles	*Allium cepa* L. (10), *Coriandrum sativum* L. (30), *Nerium oleander* L. (27), *Dittrichia viscosa* (L.) Greuter (17), *Lepidium sativum* L. (6), *Chenopoium ambrosioides* L. (9), *Cistus monspeliensis* L. (12), *Vicia faba* L. (18), *Lavandula stoechas* L. (15), *Marrubium vulgare* L. (25), *Lawsonia inermis* L. (6), *Fucus carica* L. (10), *Ziziphus lotus* L. (14), *Rubia tinctorum* L. (15), *Hyscyamus albus* L. (2), *Thymelaea lythroides* L. (13).	16	229	0.93
Skin Lesion (SL): Burns, wounds and injuries	*Allium cepa* L. (4), *Aristolochia baetica* L. (16), *Borago offinalis* L. (14), *Cynoglossum Officinale* L. (8), *Chenopoium ambrosioides* L. (3), *Cistus monspeliensis* L. (21), *Centaurium Erythraea* Rafn (30), *Crocus sativus*L. (12), *Mentha viridis* L. (17), *Origanum compactum* Bentham. (1), *Salvia Officinalis* L. (12), *Salvia verbenaca* L. (40), *Rubus ulmifolius* Schott. (7), *Coffea arabica* L. (9), *Solanum nigrum* L. (1).	15	195	0.93
Allergy (AL): Eczema	*Daucus carota* L. (12), *Dittrichia viscosa* (L.) Greuter (4), *Tetraclinis articulata* Benth. (20), *Lawsonia inermis* L. (3), *Hyscyamus albus* L. (8), *Solanum nigrum* L. (7).	6	54	0.91
Autoimmune diseases (AD): Vitiligo	*Ammi majus* L. (5), *Anacyclus pyrethrum* (L.) Link. (4),	2	9	0.87

Origin of information on medicinal plants

Regarding the therapeutic use of medicinal plants, the majority of respondents gathered information through the experience of people around them (62.8%), which reflects the image of the relative transmission of traditional practices from one generation to the next. Herbalists are ranked as the second source of information (20.6%) and a small minority (16.6%) through their own experience via television programs (Figure 4).

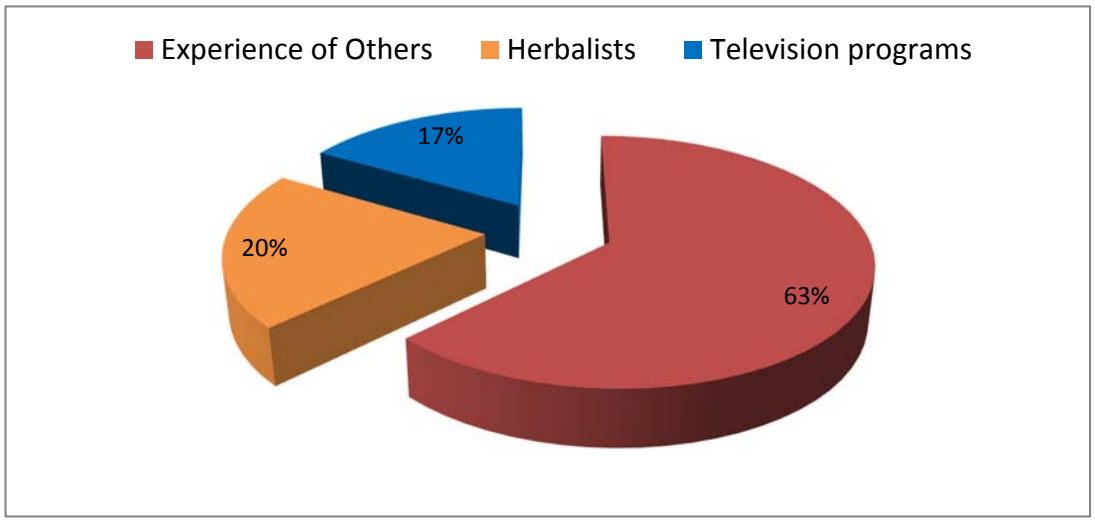

Figure 4. Modes of acquisition of traditional knowledge

Conclusion

The ethnobotanical surveys conducted as part of this study have highlighted the medicinal plant potential and the traditional knowledge related to the use of plants among the population of the Izarène forest. A total of 62 plants of therapeutic value were identified and used in the treatment of respiratory ailments. The qualitative analysis showed a strong association between the orientation of the local population towards traditional medication and certain socio-demographic characteristics low cost, gender, and age are the essential factors that push the local population towards this medication. Similarly, the list of species collected contains toxic plants. In the light of this study, we would like to make the local population aware of the risks of the anarchic use of plants for therapeutic purposes. This work constitutes a source of information which contributes to knowledge of the medicinal flora and the safeguarding of local popular know-how. It can also constitute a database for phytochemists and pharmacologists for the valorization of medicinal plants used in dermatological affections to discover new active ingredients that can be used in pharmacology.

Declarations

List of Abbreviations

SPSS: statistical package for social science
Fc: frequency of citation.
RFC: relative frequency of citation
FIV: family importance value
PPV: plant part value
ICF: informant consensus factor
CU: Cosmetic Uses
ID: infectious diseases (Abscesses and furuncles)
SL: skin lesion (Burns, wounds and injuries)
AL: allergy (Eczema)
AD: autoimmune diseases (Vitiligo).

Ethics approval and consent to participate

Before conducting interviews, all participants gave their prior consent knowing the reason for the study.

Author's contributions

HO: Compiled the literature sources, data analysis, and interpretation, NC: wrote the manuscript. EM and NF: Helped in data, LZ and AD: supervised the work and improved the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

Consent for publication

Not applicable

Availability of data and materials

The data was not deposited in public repositories.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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استخدام النباتات الطبية في مستحضرات التجميل الجلدية: دراسة استقصائية عرقية–عقاقيرية في منطقة إزارين

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ملخص

تم إجراء دراسة استقصائية عرقية–عقاقيرية بين سكان إزارين لتعزيز وحماية المعرفة السلفية للنباتات الطبية المستخدمة في الأدوية التقليدية لعلاج الأمراض الجلدية. باستخدام 480 ورقة استبيان ، تم إجراء مسوحات ميدانية عرقية نباتية خلال حملتين (من 2013 إلى 2015م). تم تحديد أوساط المسح المختلفة باستخدام تقنيات أخذ العينات الاحتمالية الطبقية. تم تحليل البيانات العرقية النباتية من خلال حساب المؤشرات الكمية، مثل التردد النسبي للاقتباس (RFC)، ومؤشر قيمة أهمية الأسرة (FIV) ، ومستوى الإخلاص (FL) ، وعامل توافق المخبر (ICF) ، وقيمة استخدام جزء النبات (PPV). أظهرت النتائج 62 نوعا نباتيا مفيدا تنتمي إلى 34 عائلة نباتية. كانت عائلة الشفهيات هي الأكثر تمثيلاً (8 أنواع0.037 = FIV). تم تسجيل أعلى تردد اقتباس نسبي (0.137) (RFC) لـ *Olea europaea* L.. فيما يتعلق بالأمراض المعالجة ، فإن استخدام مستحضرات التجميل يحتوي على أعلى معدل (ICF 0.96) ، واعتبرت الورقة هي الجزء الأكثر استخدامًا من النبات (PPV = 0.34) وتم تحضير غالبية العلاجات على شكل كمادة. يمكن أن تشكل النتائج التي تم الحصول عليها أساسًا لمزيد من الدراسات لتثمين النباتات الطبية المستخدمة ضد الأمراض الجلدية من خلال الدراسات البيولوجية والكيميائية النباتية للنباتات التي تم جردها في المنطقة.

الكلمات الدالة: الأمراض الجلدية، المسوح الإثنية النباتية، غابة إزارين، النباتات الطبية.

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Influence of Castor Oil on Glycated Hemoglobin (HbA1c) on Induced Type 2 Diabetes Mellitus in Rats

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ABSTRACT

Many T2D use CAO as a laxative. We did not find sufficient research to explain CAO's potential effect on the levels of HbA1c in T2D patients. This study will study this effect. Rats (n=80) were divided into eight groups (n=10). Five groups (n=50) were injected with streptozotocin intravenously to induce T2D. One group was given CAO with empagliflozin, and the second was assigned CAO only daily. The third was assigned CAO every two days, with empagliflozin, which was given daily. A fourth was assigned CAO alone daily. Also, the fifth was given empagliflozin alone. In the healthy groups, one group was given CAO, and the other was given empagliflozin. Also, the last healthy group was not assigned any drug. CAO's result on HbA1c in healthy rats was noted to decrease when delivered alone for eight weeks. HbA1c of the diabetic groups showed no significant difference (P-value<0.05) when comparing the rats given CAO with empagliflozin, and the rats were given CAO only. There was also no noticeable effect among the groups of rats given CAO daily and every two days. This study explains that CAO does not lead to a significant difference in HbA1c levels in diabetic rats, even it did for healthy rats, and if given alone, CAO could affect HbA1c levels if given over a long period. Also, CAO has a noticeable impact on experimental rats that co-administered Empagliflozin on HbA1c levels, and that Empagliflozin effect is not significantly affected if taken with CAO.

Keywords: Castor Oil, HbA1c, Type 2 Diabetes Mellitus, Rats, Empagliflozin, Blood Sugar.

INTRODUCTION

Diabetes mellitus is a metabolic disorder generally characterized by a rise in glucose levels that need regular monitoring and effective control. Each single monotherapy pharmacology agent, especially in comparison to lifestyle modifications, magnified the number of patients reaching target levels of HbA1c below 7 percent by 2 to 3 times[1]. Owing to inadequate regulation of diabetes, most patients will need multiple therapies to maintain reasonable long-term glycemic control[2]. Empagliflozin is a potent, highly selective, sodium-glucose cotransporter-2 (SGLT2) inhibitor, is an effective

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and generally well-tolerated antihyperglycemic agent approved for the treatment of adults with type 2 diabetes (T2D)[3]. SGLT2 is the primary carrier taking responsibility for glucose absorption back into circulation from the glomerular filtrate. Empagliflozin is an SGLT2 inhibitor. With the inhibition of SGL T2, empagliflozin decreases the reabsorption and renal level of filtered glucose, thus improving urinary glucose excretion. Empagliflozin/linagliptin (Glyxambi®) is a fixed-dose drug approved once daily in the U.S. in addition to the nutrition and adult exercise T2DM when empagliflozin and linagliptin are both treated appropriately. Using 5 mg linagliptin, Glyxambi® combines 10 mg or 25 mg empagliflozin using different complementary action pathways to improve glycemic control in T2D patients. Empagliflozin removes glucose from the urine by blocking blood glucose reabsorption into the kidney, with linagliptin lowering glucose activity by increasing the pancreas' hormones to create more insulin and decrease the blood glucagon the circulation.

Furthermore, this drug combination is moderately putting down weight and blood pressure without significant safety problems. Empagliflozin/linagliptin's fixed-dose combination generally enhances glycemic control over the individual components when used as an early treatment or as a metformin supplement[4]. Castor Oil (CAO) is known as a potent laxative property. HbA1c test has become the most famous indicator of chronic glycemia in epidemiological trials, clinical studies, and diabetes management[5]. Glycated hemoglobin (HbA1c) analysis in your blood provides evidence of a person's average blood sugar levels over the previous 2 to 3 months, which is predicted to represent the half-life of red blood cells (RBCs)[6]. The HbA1c level, formed via the non-enzymatic adhesion of glucose to hemoglobin, is commonly seen to represent the integrated mean blood sugar during the last 8–12 weeks, the 120-day lifetime of the erythrocyte being defined. HbA1c appears to be in a new stable state 3–4 months after changes in glucose[7]. All previous studies showed a link between the factors of HbA1c and average blood glucose used relatively rare daytime monitoring that is vulnerable to sampling errors and does not track glucose levels nighttime or true 24-hour Glycaemia[5]. ADA recently suggested HbA1c with a cutpoint < 6.5% for the diagnosis of diabetes as an option to plasma glucose fasting (FPG) based on 7.0 mmol / L requirements based[8]. This research's primary purpose is to investigate the effect of CAO on HbA1c levels in hyperglycemic rats treated with the SGLT2 inhibitor "empagliflozin" as an indicator in type 2 diabetes mellitus.

Results and Discussion

HbA1c (Glycated hemoglobin) is suggested for D.M.'s diagnosis as a possible replacement for fasting blood sugar. In this experiment, HbA1c was adopted and considered important as a long-term glycemic regulation predictor with the potential to represent the previous accumulated glycemic history. Accordingly, the HbA1c level of 6.5% or above indicates diabetes. Pre-diabetes is considered normal between 5.7% and 6.4%, and less than 5.7% is health[6].

In this experiment, rats were divided into eight groups, either healthy or diabetic, and treated with CAO for several periods. The results were compared with the corresponding positive and negative controls, as indicated in the experimental section. Comparison between the first group rats (healthy without any given drug) with seventh group rats (healthy rats which given the CAO daily) revealed a significant difference ($P<0.05$) between the two rats groups starting from the ninth week of the experiment until the last week, as can be seen in **Figure 1 and Table 1**. Besides, there is a decrease in values of HbA1c in the Healthy Rats Group, which was given the CAO daily. However, in the Healthy Rats Group, we did not provide any drug and increased the values of HbA1c.

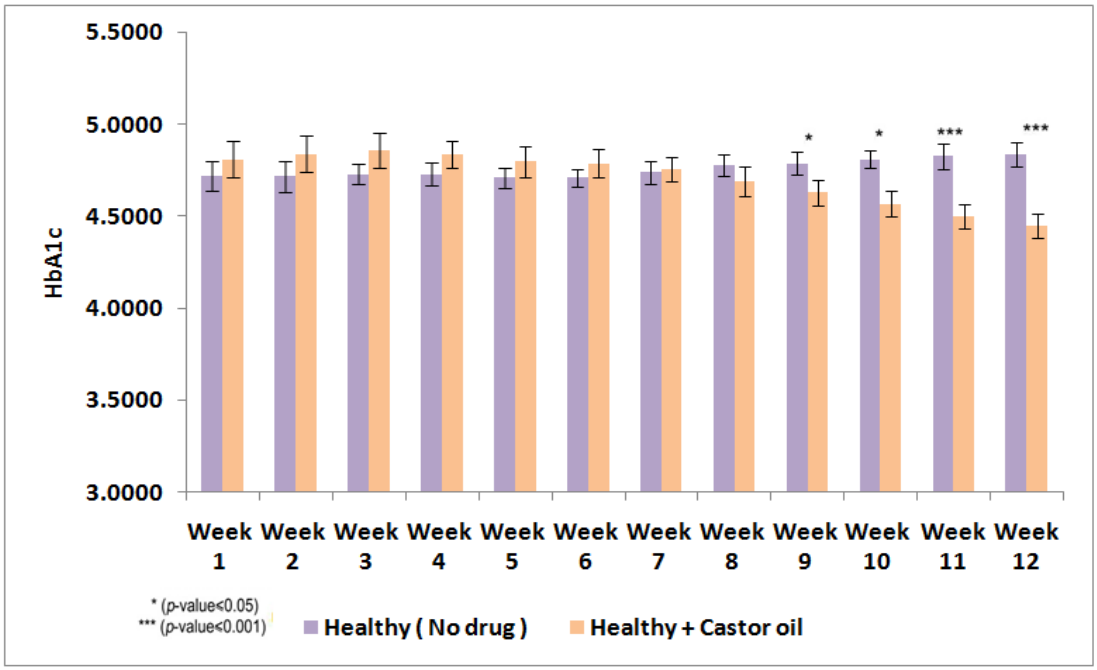

Figure 1: HbA1c % in first and seventh group rats.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	RAT_H_1 - RAT_H_c_1	-.09000	.29231	.09244	-.29911	.11911	-.974	9	.356
Pair 2	RAT_H_2 - RAT_H_c_2	-.12000	.31903	.10088	-.34822	.10822	-1.189	9	.265
Pair 3	RAT_H_3 - RAT_H_c_3	-.13000	.26687	.08439	-.32091	.06091	-1.540	9	.158
Pair 4	RAT_H_4 - RAT_H_c_4	-.11000	.22336	.07063	-.26978	.04978	-1.557	9	.154
Pair 5	RAT_H_5 - RAT_H_c_5	-.09000	.25144	.07951	-.26987	.08987	-1.132	9	.287
Pair 6	RAT_H_6 - RAT_H_c_6	-.08000	.20440	.06464	-.22622	.06622	-1.238	9	.247
Pair 7	RAT_H_7 - RAT_H_c_7	-.02000	.22509	.07118	-.18102	.14102	-.281	9	.785
Pair 8	RAT_H_8 - RAT_H_c_8	.09000	.25144	.07951	-.08987	.26987	1.132	9	.287
Pair 9	RAT_H_9 - RAT_H_c_9	.16000	.22211	.07024	.00111	.31889	2.278	9	.049
Pair 10	RAT_H_10 - RAT_H_c_10	.24000	.22706	.07180	.07757	.40243	3.343	9	.009
Pair 11	RAT_H_11 - RAT_H_c_11	.33000	.23118	.07311	.16462	.49538	4.514	9	.001
Pair 12	RAT_H_12 - RAT_H_c_12	.39000	.19692	.06227	.24913	.53087	6.263	9	.000

Table 1: Table showing results for healthy rats in Group 1 and compared with healthy Rats in Group 7

There are statistically significant decreases in the HbA1c starting from the ninth week in either healthy and diabetic rats treated with CAO compared to non-treated healthy and diabetic rats' groups, respectively, suggesting that treatment with CAO reduced blood sugar in both types as indicated with the decrease in HbA1c for an extended period, i.e., nine weeks.

These results are confirmed by the results showing no statistical difference between any of the remaining conjugation groups, as apparent in **Table 2**. Also, CAO could lower the HbA1c to a certain degree but does not have Empagliflozin's strength to reduce the HbA1c levels (**Figure 2**). The extent of the decrease in HbA1c in healthy rats treated with Empagliflozin is more significant than that of healthy rats treated with CAO alone, and the effect of treatment started earlier in the Empagliflozin treated rats, i.e., after the fourth week in Empagliflozin treated rats, **Figure 2** and **Table 2**. Another conclusion we can get from these results is that CAO could have the power to decrease HbA1c in healthy rats but to a much lower extent in diabetic rats.

Table 2: Comparisons of the conjugation of Groups of Rats (p-value= 0.05, NS: no statistical difference)

Groups	**Status**	**Comparison result**
Group 1 (healthy)	No drug	With group 4: there is a stat. differences
Group 2 (diabetic)	No drug	With group 8: NS
Group 3 (diabetic)	Empagliflozin only	With group 5: NS
Group 4 (healthy)	Empagliflozin only	
Group 5 (diabetic)	Empagliflozin + CAO	With group 6: NS
Group 6 (diabetic)	Empagliflozin + CAO (every two days)	With group 3: NS
Group 7 (healthy)	CAO only	With group 1: there is stat. differences
Group 8 (diabetic)	CAO only	With group 2: N.S.

However, comparing groups, 1 with 4 (health rats without any treatment with health treated with healthy rats treated with Empagliflozin) revealed the statistically difference ($P<0.05$) starting from week number 4, from which we conclude that the Empagliflozin has a noticeable effect in decreasing HbA1c in Fourth group Rats Compared to the first group rats ensuring that there are no any problems in the injections of the rats, and the study was going very-well **Figure 2**.

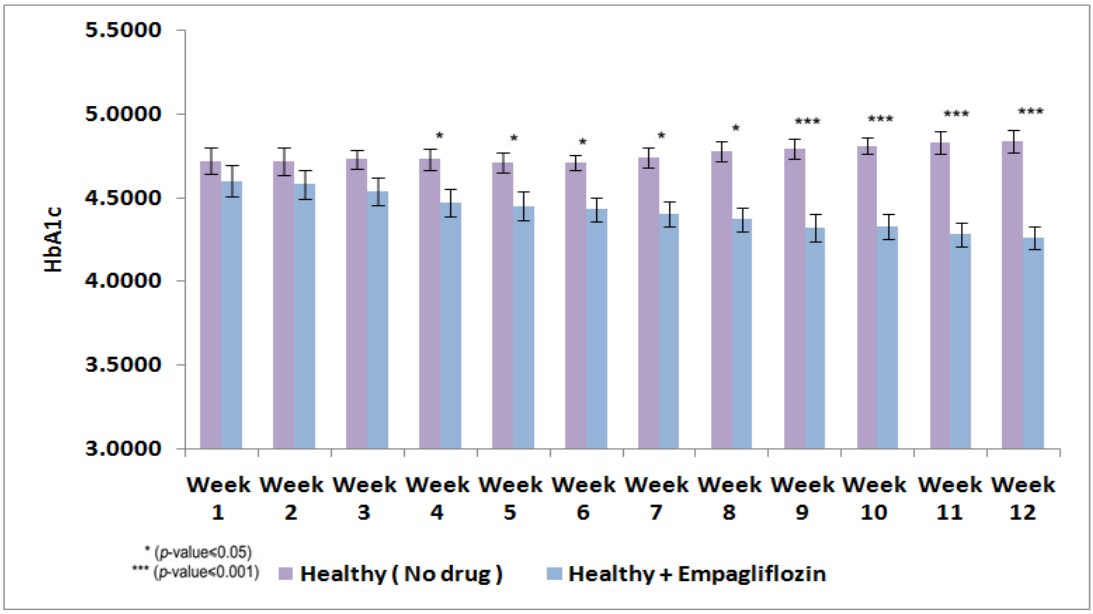

Figure 2: HbA1c % first group rats and fourth group rats

Experimental

Materials

Empagliflozin (Jardiance®) was obtained from the local market and manufactured by Boehringer Ingelheim Pharmaceuticals company (Germany) as a tablet form 10 mg/tablet and used for D.M. type-2 treatment. CAO was obtained from the local market, manufactured by Amman Pharmaceutical Industries (Jordan) as a soluble form of 0.1% as a stimulant laxative agent. Streptozocin 75% α-anomer basis powder, purchased from the local market and manufactured by Sigma Aldrich, known in Merck Germany (B.N: S6130SA), is used to induce type-2 D.M. for medical research.

Instruments

The iCHROMA™ reader is a handheld fluorescent scanner of the first generation that analyzes blood, urine, and other samples and shows the measurement results. More than 30 cartridges can be counted in immunoassay testing, and iCHROMA™ Reader HbA1c is a human blood hemoglobin A1c immunoassay form used for iCHROMA™ Reader. The procedure is used to track glycemic status in D.M. patients regularly. Incubadori Chamber is an incubation chamber for tests HbA1C. Cartridge (kits) for testing chemical composition and measuring test specimens' concentration like the liquid biological sample, use kit for rat hemoglobin A1c (HbA1c). Accu-Chek products were already created to aid you in diabetes management. Explore creative monitoring systems for blood glucose. EDTA tube stands for Ethylenediaminetetra-acetic acid. EDTA works through binding calcium throughout the blood and preventing blood from coagulation. Plastic Capillary Tube, 100% plastic capillary microhematocrit tubes Plain untreated Plastic microhematocrit tubular of Globe Scientific are fully unbreakable. They eliminate the dangers of damage and contamination due to breakage in glass tubes. In the laboratory, micropipettes are used to transfer small amounts of fluid, usually beneath 0.1 uL. The most common micropipettes in pharmaceutical and medicinal discovery labs are 1-10 uL and 20-200 uL. Pipette tips, often with pipette tips, are used for speed processing and cross-contamination with pipette tips and pipettes—those that are available in different styles and components.

Reagents

- Sodium Citrate buffer is used to dissolve Streptozocin.
- Hemolysis Buffer is individually pre-dispensed in a small tube, consisting of a cationic detergent, and stabilized for up to 20 months when positioned at 4-30 ° C.
- Detection Buffer contains HbA1c-peptide fluorescence marked, BSA stabilizer, and sodium azide as a preservative in PBS, is flexible up to 20 months if stored at a temperature of 2-8°C, enables detection buffer to achieve a room temperature (20-30 ° C) before running the test.

Preparation of Solution

Preparation of Streptozocin solution

Streptozocin at dose 45 mg, was prepared by dissolve 45 mg of Streptozocin in 12 mL of Sodium Citrate buffer, and mixing forced in a blender to get homogenous solution 3.75% (w/v), the concentration of Streptozocin, which give (3.75 mg/mL) concentration of solution injected I.P. for each rat[9,10].

Preparation of Empagliflozin solution

Empagliflozin at dose 10 mg (10 mg/70 kg),(0.142 mg/1 kg), (0.035 mg/0.25 kg) was prepared daily, prepared by dissolving 10 mg of Empagliflozin in 100 mL of distilling water and mixing forced in a blender to get a homogenous solution of 0.01%(w/v), the concentration of Empagliflozin, which give (0.035 mg/0.28 mL) concentration of solution orally for each rat[11].

Preparation of CAO solution

CAO at a 60 mL dose, which gives 2 mL of solution orally for each rat[12].

Preparation of sodium citrate buffer solution

Sodium citrate buffer solution prepare by adding distilled water 800 mL in a container, add 24.0g of sodium citrate dehydrate, add 3.5g of citric acid to the solution, adjust the solution to 4.5 pH, storage in 4-10°c.

Study Protocol and preclinical study

The Faculty of Pharmacy and Medical Sciences, University of Petra Appendix No. (3) accepted the study protocol's ethical work. The Wistar Albino rats were supplied and housed at the Faculty of Pharmacy – animal house at Applied Science Private University - Amman.

Animal Handling

Wistar Albino adult male and female laboratory rats were above eight weeks, weighing 250-300 g. The Applied Science Private University animal house was delivered, placed in the air-conditioned room at 20 ° C and subjected to a photographic cycle (12 hours of light / 12 hours of darkness) daily. All the rats weighed and randomized to eight groups for identification purposes were markers on their tails.

Group #1: Was the control - healthy group (No given any drug), which consists of 10 rats given a regular basal diet with water, and this group conducts this study for about 12 weeks under the same environmental conditions.

Group #2: Was the diabetic group (No given any drug), which consists of 10 rats that are not given any drugs, just only given a regular basal diet with water, and this group continued in this study for about 12 weeks under the same environmental conditions.

Group #3: Was the diabetic group (given only Empagliflozin), which consists of 10 rats given only Empagliflozin solution in 0.1mg/mL concentration daily for 12 weeks, delivered using suitable oral gavage and given a regular basal diet with water.

Group #4: Was the healthy group (given only Empagliflozin) which Consists of 10 rats which given only Empagliflozin solution in 0.1mg/mL concentration daily for 12 weeks, delivered by using suitable oral gavage, and given regular basal diet with water

Group #5: Was the diabetic group (given Empagliflozin and CAO, which consists of 10 rats which given Empagliflozin solution in 0.1mg/mL concentration daily for 12 weeks, and given CAO solution in 2 mL/rat concentration daily for 12 weeks, given by using suitable oral gavage, and given a regular basal diet with water.

Group #6: Was the diabetic group (given Empagliflozin and CAO) which consists of 10 rats, which

given Empagliflozin solution in 0.1mg/mL concentration daily for 12 weeks, and given CAO solution in 2 mL/rat concentration every two days for 12 weeks, given by using suitable oral gavage, and given a regular basal diet with water.

Group #7: Was the healthy group (given only CAO), consisting of 10 rats, which was given only CAO solution in 2 mL/rat concentration daily for 12 weeks, given by using suitable oral gavage given a regular basal diet with water.

Group #8: Was the diabetic group (given only CAO), consisting of 10 rats, which was given only CAO solution in 2 mL/rat concentration daily for 12 weeks, given using suitable oral gavage, and given a regular basal diet with water. As summarized in **Table 3**.

Table 3: Groups of the rats

Groups	Status
Group 1 (healthy)	No drug
Group 2 (diabetic)	No drug
Group 3 (diabetic)	Empagliflozin only
Group 4 (healthy)	Empagliflozin only
Group 5 (diabetic)	Empagliflozin + CAO
Group 6 (diabetic)	Empagliflozin + CAO (every two days)
Group 7 (healthy)	CAO only
Group 8 (diabetic)	CAO only

Sample Collection and Processing

A blood sample is drawn through the rat's optical vein by a capillary tube after 12 hours of fasting before sample collecting. The sample was collected at zero time to determine the baseline and then collected weekly until twelve weeks. When sample drawn from rats and putten in tube collector (EDTA, heparin, and NaF), take the sample and measure HbA1c by putting blood sample in Cartridge (kit-Special for rats), and put it in an incubator in the chamber for 12 minutes and finally put in the ichroma™ Reader to read the HbA1c range.

All the experiments were achieved by the University of Petra and Applied Science Private University institutional guideline on animal use, which adopts the Federation of European Laboratory Animal Science Association (FELASA) guideline.

Acknowledgment:

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تأثير زيت الخروع على سكر الدم التراكمي في الجرذان المستحدث فيها داء السكري من النوع الثاني

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ملخص

العديد من مرضى السكري من النوع الثاني يستخدمون زيت الخروع كمليّن للأمعاء. لم نجد أبحاثاً كافية تبحث في التأثير المحتمل لزيت الخروع على مستويات سكر الدم التراكمي في مرضى السكري من النوع الثاني. تهدف هذه الدراسة الى دراسة هذا التأثير. تم تقسيم الفئران (ن = 80) إلى ثماني مجموعات (ن = 10). تم حقن خمس مجموعات (ن = 50) بالستربتوزوتوسين عن طريق الوريد للحث على الإصابة بمرض السكري من النوع الثاني. أعطيت مجموعة واحدة زيت الخروع مع دواء إمباجليفلوزين ، والمجموعة الثانية تم اعطاؤها زيت الخروع فقط يوميًا. أما المجموعة الثالثة ، فتم اعطاؤها كل يومين زيت الخروع، مع دواء إمباجليفلوزين ، والذي كان يُعطى يوميًا. المجموعة الرابعة تم اعطاؤها زيت الخروع فقط وحده يوميا. أيضًا ، تم إعطاء المجموعة الخامسة دواء إمباغليفلوزين وحده. في المجموعات السليمة ، أعطيت إحدى المجموعتين زيت الخروع ، وأعطيت الأخرى دواء إمباغليفلوزين. أيضًا ، لم يتم اعطاء أي دواء للمجموعة الصحية الأخيرة. لوحظ أن نتيجة إعطاء زيت الخروع على مستويات سكر الدم التراكمي في الجرذان السليمة أدى الى انخفاض مستويات سكر الدم التراكمي عند تناوله بمفرده لمدة ثمانية أسابيع. أظهرت نتائج سكر الدم التراكمي لمجموعات الجرذان المصابة بمرض السكري من النوع الثاني عدم وجود فرق احصائي (P-value <0.05) عند مقارنتها مع الجرذان التي أعطيت زيت الخروع مع دواء إمباغليفلوزين او الجرذان التي أعطيت زيت الخروع فقط. لم يكن هناك أيضًا تأثير ملحوظ بين مجموعات الفئران التي أعطيت زيت الخروع يوميًا اوكل يومين. توضح هذه الدراسة أن زيت الخروع لا يؤدي إلى اختلاف كبير في مستويات سكر الدم التراكمي في الجرذان المصابة بداء السكري ، على الرغم من أنها كانت ذات تأثير بالنسبة للفئران السليمة ، وإذا تم إعطاؤه بمفرده ، وانما يمكن أن يؤثر زيت الخروع على مستويات سكر الدم التراكمي إذا تم إعطاؤه لفترة طويلة. أيضًا ، كان لـ زيت الخروع تأثيراً ملحوظاً على الفئران التجريبية التي اعطيت دواء إمباغليفلوزين على مستويات سكر الدم التراكمي وأن تأثير دواء الإمباغليفلوزين لا يتأثر بشكل كبير إذا تم تناوله مع زيت الخروع.

الكلمات الدالة: زيت الخروع، سكر الدم التراكمي ، داء مرض السكري من النوع الثاني، الجرذان، دواء الإمباغليفلوزين.

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Prevalence of Anxiety among Pregnant Women Attending Antenatal care in Jordan: A Single Center Study

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ABSTRACT

Background: Pregnancy is a stressful physiological period, where some women may suffer from some degree of anxiety. Our aim is to study the prevalence of anxiety among pregnant women attending routine antenatal care clinics.

Methodology: This was a cross sectional study for pregnant women coming for their routine antenatal care at Jordan University Hospital, during the period from October 1st to 27th, 2019.They were interviewed while waiting for their turn by a trained 6th year medical student using the Generalized Anxiety Disorder -7 (GAD-7) questionnaire.

Results: A total of 200 women were interviewed with a mean age of 30 ± 5.3years, ranges between (18 and 45years). Their gravidity mean was 3.3±2.9 (ranges from 0-22) and their mean parity was 1.6 ± 1.4. 59 women (29.5%) had previous history of miscarriage. Number of miscarriages ranged from 1 to 12 with a mean of 2.5 ± 3.3.In our study, 66 (33.0%) women had moderate and 42 (21.0%) had severe symptoms. For women with history of previous miscarriages, there was no significant correlation with anxiety, except for those with recurrent miscarriages (p-value= 0.019).

Conclusions: Recurrent miscarriage can affect women's psychological well-being; with an increase in the possibility of experiencing anxiety. Implementing mental health assessment in antenatal care has long-lasting benefits for both mother and infant.

Keywords: Pregnancy, Anxiety, miscarriage.

INTRODUCTION

Pregnancy and birth are regarded as joyful experience (1).About 10–15 % of all pregnant women experience some degree of anxiety during their pregnancy (2).A prior pregnancy loss is an established risk factor for developing anxiety during future pregnancies (3). The feeling of self-blame that women usually suffer after a miscarriage is associated with high levels of anxiety and depression as well as post-damaging disorder syndrome (4).These women have also higher level of pregnancy-related fear in the next pregnancy(5).Although controversial; the presence of anxiety in a new pregnancy constitutes a risk factor for perinatal complications (1).A woman's ability to adapt to the changes and challenges of pregnancy is unique and varies from one to another(6).The purpose of this

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study was to assess the impact of having previous misscarriege on the anxiety in pregnant women attending prenatal care clinics at our university hospital.

MATERIALS AND METHODS:

Study design

This study is a cross-sectional study performed at the University of Jordan Hospital, a university hospital based in Amman, the capital of Jordan. Patients were recruited from prenatal care clinics at Jordan University Hospital. The study was approved by the Ethics Committee for Medical Research at the Jordan University Hospital and the University of Jordan, and was conducted according the declaration of Helsinki.

Participants

We recruited 200 women during the period extending from October 1st to 27th, 2019.

These women attend the clinic for their routine antenatal care. They were interviewed at the clinic while waiting for their turn by a trained 6thyear medical student. We did not include women who were diagnosed with psychological diseases, or those who were on psychological medications.

Variables

Patients were informed about the study aim prior to initiation of the interview, verbal consent was then obtained. All women who declined to participate were excluded from the study. A structured clinical interview was conducted using the translated and validated version of Generalized Anxiety Disorder-7 (GAD-7), a short screening measures used in medical and community settings to assess depression and anxiety severity (7). They were asked to rate the frequency of anxiety symptoms on a Likert scale ranges from 0-3; 0 for not at all, 1 for several days, 2 for more than half the days and 3 for nearly every day. The total scores ranged from 0 (no anxiety symptoms) to 21 (all symptoms occurring daily). A total score of 0-4 represents minimal or no anxiety symptom from 5-9 is considered mild, 10-14 is moderate and 15-21is severe.

Statistical analysis

Data were analyzed using SPSS 23. Frequency and percentage were calculated for the categorical data. We used Pearson's chi-square test to compare anxiety disorder with each of the included variables, and we used independent sample t-test for continuous data. Fisher exact test was used when the cell is less than5. Significance level was set at $p < 0.05$.

Result:

A total of 200 women were interviewed. Their mean age was 30 ± 5.3years; ranges between18 and 45years. Their gravidity mean was 3.3±2.9; ranges from 0 to 22. 59 women (29.5%) had previous history of miscarriage. Their mean parity was1.6 ± 1.4; ranges from 0 to 6. Number of miscarriages ranged from 1-12with a mean of 2.5 ± 3.3(Table 1).

Regarding other medical illnesses during pregnancy, 5 women (0.25%) had hypertension, 8 women (0.04%) had diabetes mellitus, one woman had both diseases(0.05%) and another one (0.05%) had gestational diabetes mellitus. 14 women (7%) had neonates admitted previously to the neonatal intensive care unit (NICU), 37 (18.5%) had previous one cesarean section(CS), and 14 (7%) with more than one previous CS. Regarding level of education; 16 (8%) had high school degree,121(60.5%) with Bachelors degree,22(11%) had Diploma degree,4 women (2%) had master degree and 2 women (1%) with PHD degree(Table 1).

Regarding anxiety; 32 (16.0%) women had minimal symptoms,60 (30.0%)had mild symptoms, 66(33.0%)had moderate symptoms while42(21.0%)had severe symptoms (Figure 1).No significant correlation was found between degree of anxiety and previous history of miscarriage (Table 2).However, when we divided these women into those who had one previous miscarriage and those with

two or more miscarriages, we noticed that women with recurrent miscarriages had significantly severe anxiety level (p-value = 0.019)(Table 2).

Upon comparing anxiety levels between women who were pregnant for the first time to those who had delivered before (Parity more than one); we did not find any significant deference(Table 2).We did not find significant difference between degree of anxiety and each of mother's age (0.473), level of education (0.50), previous NICU admission (0.755), and previous CS's (0.388).

Discussion

In this study that included a coherent cohort of women from Jordan, with no previous psychological or mental issues, we found a significantly higher anxiety levels among women with prior history of multiple miscarriages. No significant anxiety impact was found forage, level of education, prior gynecological history.

Prenatal mental health concerns are particularly prevalent in Low-Middle Income Countries, where access to mental health services is scarce(8,9).Local studies had shown stigma toward mental illnesses(10-12).Arab cultural traditions, values and beliefs towards mental illnesses are different from those of Westerners(13); The percentage of Arab people who seek psychological help is much lower (5).This study shows our questionnaire's results for prenatal maternal anxiety levels among a sample of Jordanian women attending routine prenatal care clinics. We noticed that 66 women (33%) had moderate anxiety and 42 (21%) had sever anxiety levels in comparison to other studies which showed 15% (14) in one study, 25% and 26.8% (15) in another. A number of factors that may affect the development of pregnancy related anxiety and can subsequently influence maternal mental health, such as: frequent miscarriages, number of parity, socio-economic circumstances and many others (16).

There is an agreement in the literature that women who miscarry may suffer from some morbidity after pregnancy loss and in subsequent pregnancies (16-18). In addition to women having a live child, those who had previous miscarriage appeared to experience severer anxiety symptom (19). A previous study done on Jordanian women found several social factors may lead to anxiety and depression among women after pregnancy, however, the study did not assess those who experienced previous miscarriages (20)

We noticed that having a history of previous miscarriage per se does not significantly correlate with degree of anxiety, however frequent miscarriages(i.e., two or more)is significantly associated with severe symptoms (p value=0.019).Another more recent study also found several predictors for depression and anxiety among women after pregnancy, including family stressors, non-family stressors, education, and life-satisfaction, but they did not assess prior miscarriages history among the predictors (21).

Maternal age was shown to significantly correlate with prenatal anxiety in some countries such as Karachi, Pakistan and China (22), on the contrary this was not significant in our study.

It is noteworthy that we did not find significant relationship between prenatal anxiety and maternal level of education, previous NICU admissions, nor previous CS. Our study is one of few studies which assessed anxiety levels in the antenatal period using standardized scales where the diagnosis was performed following a structured clinical interview. However, the limitation of the study is the small sample size. Moreover, the prevalence of anxiety among pregnant women should be compared with the general population of women who are matched but not pregnant, which would provide important differences between the groups.

Conclusion:

History of multiple miscarriages may affect women's psychological well-being and can aggravate levels of anxiety in subsequent pregnancies. Implementing good mental health assessment in antenatal care clinics has long-lasting benefits for both mother and infant**.**

Table 1- Maternal characteristics

Measure	**Frequency**	**(%)**
Age (years)		
Mean age ± SD	30 ± 5.3	
Range	18-45	
Age group		
15 -24	29	14.5
25 -34	129	61.5
≥ 35	48	24
Level of education		
High school	16	8
Bachelors	121	60.5
Diploma	22	11
Masters	4	2
PHD	2	1
Medical Disease		
HTN	**5**	
DM	8	
DM+HTN	1	
GDM	1	
Gravida		
Mean Gravida± SD	3.3±2.9	
Range	0-22	
Parity		
Mean parity± SD	1.6±1.4	
Range	0-6	
Number of women with Miscarriages	59	29.5
No of miscarriages		
Mean ± SD	2.5±3.3	
Ranges	1-16	
NICU	14	7
CS	52	
No CS	148	74
One CS	37	18.5
≥Two CS	15	7.5

Table 2: Comparison in anxiety disorders for presence of miscarriages; number of miscarriages, and number of parity.

		Minimal or No	Mild	Moderate	Severe	P value
Miscarriage	Yes	5	21	16	17	0.079
	No	27	39	39	39	
Number of Miscarriage	0-1	31	52	59	32	**0.019**
	≥ 2	1	8	7	10	
Number of parity	0	6	19	22	9	0.286
	≥ 1	26	41	44	33	

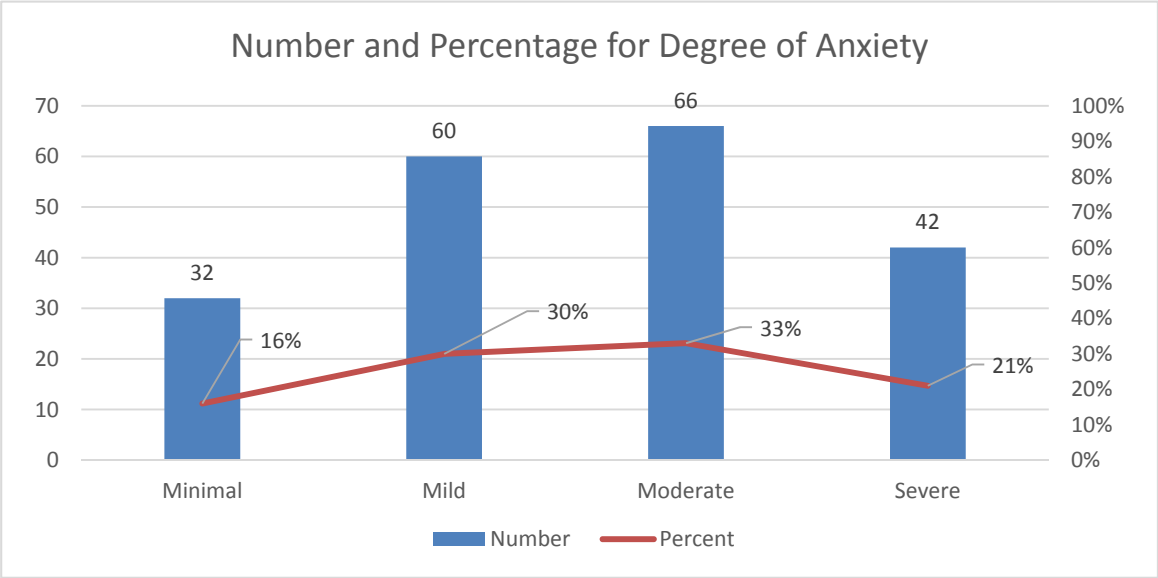

Figure1: Number of patients who suffered anxiety during pregnancy and percentagesof their degree of anxiety

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قلق بين النساءالحوامل اللاتي يتردد نعلى رعاية ما قبل الولادة في الأردن: دراسة مركزية واحدة

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ملخص

الخلفية: الحمل فترة فسيولوجية مرهقة. قد تعاني بعض النساء مندرجة معينة من القلق. . هدفنا دراسة انتشارالقلق بين النساء الحوام اللواتي يبترددن على عيادات الرعاية السابقة للولادة.

المنهجية: كانت هذه دراسة مقطعية للحوامل القادمات للرعاية الروتينية السابقة للولادة في مستشفى الجامعة الأردنية ، خلال الفترة من **1** إلى **27** أكتوبر **2019** ،حيث تمت مقابلتهن أثناء انتظار دورهن من قبل طلاب متدربين من طب سنة **6** باستخدام استبيان اضطراب القلق العام **7.**

النتائج: تمت مقابلة ما مجموعه **200** امرأة بمتوسط عمر **30** ± **5.3** سنوات، تتراوح بين (**18** و **45** سنة). كان متوسط عدد الأحمال **3.3** ± **2.9** (تتراوح من **0** إلى **22**) وكان متوسط عدد الولادات **1.6** ± **1.4**. كان هناك **59** امرأة (**29.5**٪) لديهن تاريخ سابق للإجهاض. عدد حالات الإجهاض تراوحت من **1** إلى **12** بمتوسط **2.5** ± **3.3**. في دراستنا كان لدى **66** (**33.0**٪) من النساء أعراض معتدلة و **42** (**21.0**٪) كانت لديهن أعراض شديدة القلق، بالنسبة للنساء اللواتي لديهن تاريخ إجهاضات سابقة، لم يكن هناك ارتباط كبيرمع القلق، باستثناء النساء اللواتي عانين من حالات الإجهاض المتكررة **.** قيمة **(p=0.019).**

الاستنتاجات: يمكن أن يؤثرالإجهاض على الصحة النفسية للمرأة؛ قد يزيد هذا أيضًا من احتمال التعرض للقلق من حالات الحمل اللاحقة. تنفيذ تقييم الصحة النفسية في رعاية ما قبل الولادة له فوائد طويلة الأمد لكل من الأم والرضيع.

الكلمات الدالة: الإجهاض، القلق، الاكتئاب.

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Identification of Biologically Important Compounds In Neem Leaves Through GC-MS Analysis

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ABSTRACT

Objective: Neem (Azadirachta indica A. Juss.) is an important tree species that is known to posses anthelmintic, contraceptive, antimicrobial, antidiabetic and sedative properties. Therefore, the present study aimed to identify various compounds present in the leaf extract of neem and to enlist various biological activities of these compounds through literature survey. Methodology: Extract of neem was obtained by soaking 100 g dry powdered leaves in 1000 mL methanol followed by filtration. This was successively fractioned using n-hexane and chloroform. **Results**: GC-MS analysis of the chloroform fraction was performed and 7 compounds namely 2-pentanol, acetate (9.72%); decane (8.96%); 11-oxa-dispiro[4.0.4.1]undecan-1-ol (6.56%); nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)- (7.13%); quinoline-4-carboxamide, 2-phenyl-N-n-octyl- (9.79%); nonacosane (44.27%) and tetratriacontane (13.43%) were identified. Conclusion: Literature survey showed that the identified compounds possessed antibacterial, antifungal, anticancer, cytotoxic, anti-inflammatory, antioxidant, and antiviral properties.

Keywords: *Azadirachta indica*, Bioactive components, GC-MS analysis, Neem leaves.

INTRODUCTION

As a rich source of ingredients, medicinal plants can be used in the synthesis and development of drugs.[1] In addition, these plants also play a crucial role in human cultures as these are recommended for various therapeutic properties.[2] In the recent decade, neem (*Azadirachta indicia*) has attracted importance worldwide due to its diverse medicinal properties.[3] It is an evergreen plant native to Pakistan, India, Bangladesh, Burma, Thailand, Indonesia, Malaysia and Sri Lanka.[4] The tree has the adaptability to survive under a wide range of edaphic, topographic and climatic factors.[5] It naturally grows in areas where the rainfall is in range of 400 to 1150 mm, soil pH lies between 4.5 to 10, and temperature range from 0 to 50 °C.[6] It has been used in folk medicines for more than 4000 years ago with proven antipyretic, antiviral, antiseptic, antifungal, antibacterial, anti-inflammatory, antigastric, antimalarial and antiulcer properties.[4,7] It possesses a vast array of pharmaceutical and biologically active compounds which are structurally complex and diverse in nature.[8,9] Over 140 compounds have been identified in different parts of this plant including terpenes, limonoids, meliacin, margocin, margocilin, isoazadirolide, azadirachtin, salanin, gedunin, valssin, nimbin, nibonolone, gallic acid and others.[4,10,11] In agriculture, neem-based products are highly effective, easily biodegradable, cheap, easy in preparation with less or no hazardous effects. The registered neem formulations such as Neemark, Nimbicidine, Bioneem, Achook, Neemix, Margosan, Nim-76, Nim-20 and Azatin are in practice for the management of plant diseases.[12,13] On the other hand,

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all parts of this plant are potentially beneficial for human health and are used traditionally for the treatment of jaundice, indolent ulcer, intestinal wounds, syphilitic sores, blood morbidity, itching, skin ulcer, rheumatism, leprosy, indigestion, pthysis, constipation, burning sensations, intestinal helminthiasis, pleuropneumonia, asthma, ringworm, urticaria, throat and respiratory disorders.[10,14,15] In West African counties, neem leaves and bark aqueous extracts are enriched with gedunin, an active anti-malarial component used particularly resistant to chloroquine strains.[16] Furthermore, neem bark possesses strong antiseptic properties that is used in the preparation of herbal tooth paste and dental gel to reduce the bacterial count and oral plaque index.[17] In a recent study, Khan et al.[18] reported promising antifungal activity of soil amendment with leaves of neem against *Sclerotium rolfsii*. Therefore, this study was carried out to analyze chloroform fraction of methanolic leaf extract through GC-MS for the identification of various antifungal and other biologically active compounds.

MATERIALS AND METHODS

Preparation of extract

Leaves of neem were collected from Lahore, Pakistan and dried under shade. The dried leaves were crushed thoroughly and 100 g material was dipped in 1000 mL methanol for 14 days and filtered. The solvent was evaporated on a rotary evaporator and the recovered material was mixed in 100 mL of distilled H_2O and mixed well. This mixture was partitioned with 100 mL of *n*-hexane using a separating funnel. After collecting the *n*-hexane soluble fraction, the process was repeated 5 times to separate all the *n*-hexane soluble compounds. Thereafter, the aqueous phase was partitioned with 100 mL of chloroform. The chloroform phase was collected, filtered through a Millipore filter paper and used for GC-MS analysis. In our previous studies, we found that generally chloroform fractions of methanolic extracts of different plant species were highly antimicrobial in nature and also contained other biologically important compounds.[19,20] Therefore, this fraction was selected for identification of biologically important compounds in the present study.

GC-MS analysis

This study was performed to evaluate the possible biologically active phytochemical constituents in chloroform fraction. For this, samples were run on Agilant Technologies machine (Model GC-7890A) attached with a capillary column (0.25 µm × 0.25 µm × 30 m) and a mass spectrometer (MS 5975C). There was an operating system that operated the electron impact mode having 70 eV ionization energy. A carrier gas helium was used at a flow rate of 1 mL min^{-1}. The injector temperature was 260 °C, ion-source temperature was maintained at 200 °C and the oven was adjusted from 50 °C with an increase of 10 °C min^{-1} ending with a 4 min isothermal at 310 °C.

Mass spectra were taken at source temperature of 250 °C, 70 eV with MS Quad temperature 150 °C. The total running time for GC-MS was 36 min with a solvent delay of 4 min. In order to identify the components of the extract, retention indices were used. In addition, these were also identified by comparing their mass spectral fragmentation patterns with those already reported and stored in the MS library (NIST database). The concentrations of these compounds were calculated from total area of GC peaks without applying any correction factor.

A thorough literature survey was carried out to collect the information regarding bioactivities of various identified compounds. Structures of the identified compounds were drawn using software ChemDraw.

RESULTS

The GC-MS investigation of chloroform fraction of methanolic leaf extract of neem indicated that there were eight phytochemicals as shown in Figure 1. The details of these constituents are shown in Table 1. Their bioactivities, as available in the literature, are given in Table 2 whereas their structures are illustrated in Figure 2. The identified compounds belonged to various classes of organic compounds. The most prevailing compound was

nonacosane with 44.27% peak area. The compounds namely, tetratriacontane; quinoline-4-carboxamide, 2-phenyl-N-n.-octyl-; 2-pentanol, acetate; decane; nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, (Z,Z,Z)- and 11-oxa-dispiro[4.0.4.1]undecan-1-ol were moderately abundant with peak areas ranging from 6.56 to 13.43%.

DISCUSSION

Among the identified phytoconstituents, nonacosane was found as the most abundant one. It has also been identified from ethanolic extracts of *Cayratia trifolia* and *Moringa oleifera* leaves with strong anti-inflammatory, antioxidant and cytotoxic properties. It showed the highest antimicrobial potential against a wide range of bacterial strains including *Staphylococcus aureus, Klebsella pnemoniaea, Escherichia coli, Enterobacter faecalis* and *Salmonella typhi.*[21,22] Similarly, this compound was also found in ethanolic extract of *Ipomoea mauritiana* with strong antidiabetic potential and it can also be utilize on commercial basis.[23] Tetratriacontane was the second most abundant compound in this study and was previously identified from the methanolic extract of a medicinal plant *Plantago lanceolata* with excellent pharmaceutical, medicinal, antioxidant, anticancer and antibacterial activities against *Escherichia coli* and *B. cereus.*[24] Likewise, decane was identified from methanolic extracts of *Camponotus fellah* and it is known for antifungal and antibacterial characteristics against a wide range of pathogens. Generally, it interacts with specific substances in the cell or either destabilizes the pathogenic cell wall or cell membrane permeability which eventually disrupts the signaling pathways leading to cell death.[25,26] Recently, nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)- was isolated from the methanolic leaf extracts of *Clinacanthus nutans* with potent antioxidant, cytotoxic, therapeutic and antiproliferative activities. It also exhibited excellent anticancer potential against aggressive HeLa cell line which is responsible for cervical cancer in humans.[27] Moreover, methanolic extracts of *Digitalis purpurea* revealed the presence of 11-oxa-dispiro[4.0.4.1]undecan-1-ol with promising antimicrobial potential.[28]

Conclusion

The present study concluded that neem leaf extract is a source of biologically important phytoconstituents such as nonacosane; tetratriacontane; decane and 11-oxa-dispiro[4.0.4.1]undecan-1-ol.

Author contributions: IH Khan conducted the study and wrote a part of paper. A. Javaid supervised the work and contributed in writing of manuscript.

Table 1. Compounds identified from chloroform leaf extract of *Azadirachta indica* through GC-MS analysis.

	Names of compounds	Formula	Weight (g mol^{-1})	Retention time (min)	Peak area (%)
1	2-Pentanol, acetate	$C_7H_{14}O_2$	130	4.542	9.72
2	Decane	$C_{10}H_{22}$	142	7.746	8.96
3	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	$C_{10}H_{16}O_2$	168	9.851	6.56
4	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	24.229	7.13
5	Quinoline-4-carboxamide, 2-phenyl-N-*n*.-octyl-	$C_{24}H_{28}N_2O$	360	26.754	9.79
6	Nonacosane	$C_{29}H_{60}$	408	27.236	44.27
7	Tetratriacontane	$C_{34}H_{70}$	478	29.504	13.43

Table 2. Nature and properties of compounds identified from leaf extract of neem through GC-MS analysis.

Comp. No.	Names of compounds	Nature	Property	Reference
1	2-Pentanol, acetate	Ester	-	-
2	Decane	Alkane	Antimicrobial, antifungal, antibacterial	26
3	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	Alcohol	Antimicrobial	28
4	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-	Ester	Antioxidant, antimicrobial, therapeutic agent, antiproliferative activity	27
5	Quinoline-4-carboxamide, 2-phenyl-N-*n*.-octyl-		-	-
6	Nonacosane	Aliphatic hydrocarbon	Antibacterial, antimicrobial, anti-inflammatory and antidiabetic	21,22
7	Tetratriacontane	Aliphatic hydrocarbon	Antimicrobial, antibacterial, anticancer, antioxidant, hypoglycaemic	24

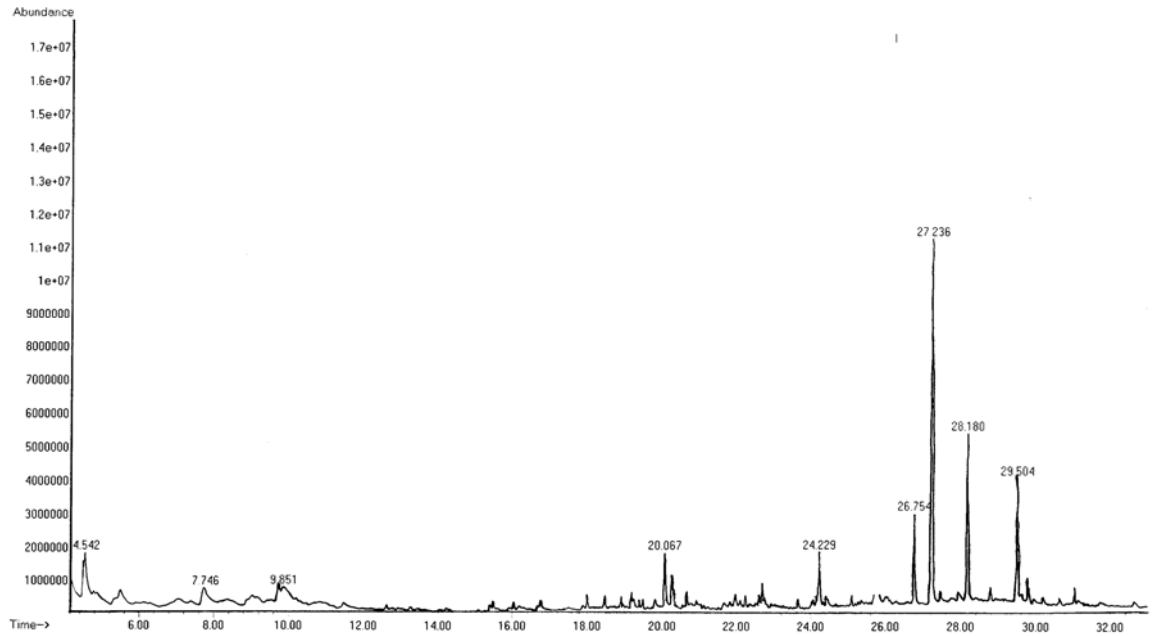
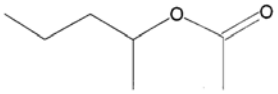
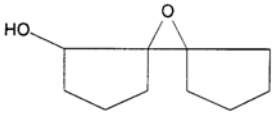


Fig. 1. GC-MS chromatogram of chloroform leaf extract of *Azadirachta indica.*

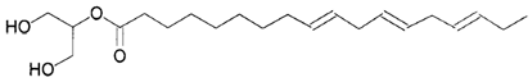


2-Pentanol, acetate

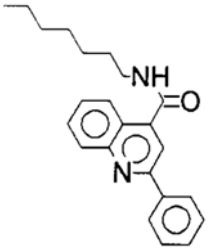
Decane



11-Oxa-dispiro[4.0.4.1]undecan-1-ol



Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-



Quinoline-4-carboxamide, 2-phenyl-N-*n*-octyl-

Nonacosane

Tetratriacontane

Fig. 2. Structures of compounds identified through GC-MS analysis of chloroform leaf extract of *Azadirachta indica.*

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تحديد المركبات المهمة بيولوجيًا في أوراق النيم من خلال تحليل GC-MS

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ملخص

خلفية: النيم (Azadirachta indica A. Juss.) هو أحد أنواع الأشجار الهامة المعروف بامتلاكه، خصائص طاردة للديدان، مانعة للحمل ، مضادة للميكروبات .مضادة لمرض السكر ومهدئة لذلك ، هدفت الدراسة الحالية إلى تحديد المركبات المختلفة الموجودة في مستخلص أوراق النيم وتسجيل الأنشطة البيولوجية المختلفة .لهذه المركبات من خلال مسح الأدبيات

الاساليب: تم الحصول على مستخلص النيم بنقع 100 .متبوعًا بالترشيح جم من أوراق البودرة الجافة في 1000 مل ميثانول. تم تجزئة هذا على التوالي باستخدام chloroform و n-hexane

النتائج: .الكلوروفورم وتم تحديد 7 مركبات تم إجراء تحليل GC-MS لكسر ديكان (8.96٪) ؛ بنتانول ، أسيتات (9.72٪) ؛ 2- وشملت هذه ؛ (6.56%−11) nonanoic acid, 9-(3- (oxa-dispiro[4.0.4.1]undecan-1-ol ; ٪hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)- (7.13); quinoline-4-carboxamide, 2-phenyl-N-n-octyl- (9.79٪)؛ (13.43٪) تتراتراكونتان و (٪نوناكوزان (44.27

الاستنتاجات: أظهر المسح الأدبي أن المركبات المحددة لها خصائص مضادة للجراثيم، ومضادة للفطريات ، ومضادة للسرطان، وسامة للخلايا ، ومضادة للالتهابات ، ومضادة للأكسدة. ومضادة للفيروسات

الكلمات الدالة: أوراق النيم ، GC-MS تحليل ، المكونات النشطة بيولوجيا ، Azadirachta indica .

تاريخ استلام البحث 2020/12/23 وتاريخ قبوله للنشر 2021/7/11.

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المجلة الأردنية في العلوم الصيدلانية

تعريف بالمجلة الأردنية في العلوم الصيدلانية

تأسست المجلة الأردنية في العلوم الصيدلانية بقرار لجنة البحث العلمي/ وزارة التعليم العالي والبحث العلمي رقم 367/2/10 تاريخ 2007/1/11 بشأن إصدار "**المجلة الأردنية في العلوم الصيدلانية**" ضمن إصدارات المجلات الأردنية الوطنية، وهي مجلة علمية عالمية متخصصة ومحكمة، وتصدر بدعم من صندوق دعم البحث العلمي والجامعة الأردنية تعنى بنشر البحوث العلمية الأصيلة المقدمة إليها للنشر في كافة مجالات العلوم الصيدلانية والعلوم الأخرى المرتبطة بها. وتصدر عن عمادة البحث العلمي وضمان الجودة في الجامعة الأردنية باسم الجامعات الأردنية كافة، خدمة للمتخصصين والباحثين والمهتمين في هذه المجالات من داخل الأردن وخارجه. وهي مجلة تصدر أربع مرات في العام أعتبارا من 2021، ومواعيد صدورها (آذار وحزيران وأيلول وكانون أول) من كل عام.

وباسمي وباسم أعضاء هيئة التحرير نود أن نشكر الزملاء الذين أسهموا بإرسال أبحاثهم إلى مجلتنا وتمكنا من إخراج العدد الأول. ونأمل من جميع الزملاء بإرسال ملاحظاتهم الإيجابية إلينا لنتمكن من النهوض بمجلتكم بالشكل الذي يليق بها.

وهذه دعوة إلى كافة الزملاء لإرسال اسهاماتهم العلمية من الأبحاث الأصيلة إلى عنوان المجلة.

والله ولي التوفيق

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