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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
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ARTICLE

DEVELOPMENT, CHANGE, AND IMPLEMENTATION IN THE CURRICULUM OF CLINICAL PHARMACOLOGY INJAHROM UNIVERSITY OF MEDICAL SCIENCES: THE INTEGRATIVE WORKSHOP MODEL BY SIMULATION

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ABSTRACT

Introduction: *One of the most important and basic necessities in order to prevent and treat diseases is proper understanding of pharmacology. The method of teaching pharmacology needs radical changes. The purposes of this study were training-needs assessment, design and implementation of an integrated workshop program for emulation and models in teaching Clinical Pharmacology and evaluating it.* ***Method:*** *By changing the course curriculum, this workshop program was held based on the assessment of students and teachers needs. Then, by using the Pharmacy model, medicine dosage forms were shown to students. Furthermore, prescriptions that had errors were presented to students in the form of a case study and a realistic simulator. Finally, a group discussion was conducted regarding cases. The forum was used to practice working on the bugs in prescriptions. As well as dealing with bugs in prescriptions, students studied them in an interactive environment and responded to a quiz designed in this area.* ***Results:*** *The results showed that the program had a significant role in improving students' knowledge of pharmacology. As well as attracting students consent, this program was applicable and had a favorable impact on the integration of theoretical and practical content and can be used applicably in the clinical settiang (9.56 after intervention versus 6.45 before intervention).* ***Conclusion:*** *Teaching through workshops and the use of simulators and real models has a salutary impact on learning, helps students in real encounter with patients, making appropriate clinical decisions with high knowledge and skills while maintaining their confidence, in an environment free from anxiety.*

KEY WORDS
Pharmacology course, simulators, educational workshops, case study

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INTRODUCTION

Annually, billions of dollars are allocated to continue activities in medical education sector [1, 2]. In many countries it has been shown that continuous medical education is performed under the supervision of organizations such as: professional health, health system and some other stimuli), which greatly contribute to increase activity in this field [3]. There is an assumption that continuous medical education can improve patient treatment [4, 5]. The way that teachers train students irrevocably affects the improvement and quality of learning. So it is widely considered in the development programs of Schools [6-12]. According to new learning methods, procedures and processes common in educational institutions that have acted well before training may be considered as a request from trainees [13,14]. Students' learning begins with a real problem or a mystery through which the educator tries to find the problem and work out an appropriate solution for it [15]. This is one of the issues and innovative approaches in medical education [16]. There are several methods in medical education. The traditional method of teaching has several advantages such as investing less time for teachers and for students as well. On the other hand, new educational strategies and methods demand more time and practice [17]. Training sessions are one of the most common activities in continuous medical education (1,17). Training sessions include courses and workshops and are different in shape. The nature of training sessions are whole different due to several factors including: the content, number of participants, the degree and type of interaction and target groups [18]. Educational workshop is a teaching method which concentrates on the mutual relationship between student-teacher in real situations. In this teaching method, students have enough time to think, analyze and use their knowledge to deal with the existing educational problems [19,20]. Medical school graduates across the country have the skills necessary to prevent and diagnose diseases and treat patients with mental and physical diseases [21]. Since medical students are in direct contact with the health of patients, it is very important to investigate the factors affecting their learning [23]. Several factors such as personality traits, the number and culture of students may affect teaching strategies. However, teaching methods is a critical factor that may improve the learning process of the students [22] . Interactive workshops can make a very big difference in professional practice [23]. Teaching correct principles and methods consistent with the choice of proper medicines will not be formally implemented in teaching medical students. As a result, at the start of clinical course, medical students do not have a clear vision of the principles of drug prescription and logical medication. On the other hand, due to overcrowding and lack of space in educational hospitals' pharmacies, these deficiencies will be felt more tangible. Conducted education is a means that present students, who would-be future doctors, can take advantage of it in order to perform prescribing in a conscious and rational way based on scientific and practical principles. Prescribing proper medication (rational drug prescribing), such as accurate and timely diagnosis and appropriate drug selection earns a significant importance. Irrational prescribing can lead to increased mortality, social and economic problems for the patient and the community as well. Another problem with medical students is their lack of awareness and usage of

medicine forms. The purposes of the present study are developing familiarity with medicine forms, their usage, precautions, and drug interactions and reducing existing errors.

METARIALS AND METHOD

Given the importance of learning Pharmacology and the role of teaching this course appropriately in reasonable and appropriate prescribing of, a number of changes in presentation of Pharmacology course units were applied as follows:

1. Changing teaching pharmacology courses from compressed mode to continuous mode during the term in accordance with the blocks offered in each semester
2. Changing the course Pharmacology from four one-unit Pharmacology to two two-unit Pharmacology courses that each two units are presented during a semester.
3. Removing less important topics such as anti-malarial drugs and adding highly necessary and important topics such as anti-cancer drugs and drugs affecting bone.
4. Beginning early learning of how to prescribe logically.

In the next step

- Making a list of common drugs available and widely used in Jahrom health care centers and making them consistent with the course as much as possible.
- Providing simple, compact and thoroughly informative texts prepared for each drug regarding the resources referred to in the process.
- Ordering needed medicines and providing them from health care centers (preferably expired drugs).
- Evaluating purchased medicines and ensuring the accuracy of order and compliance with the scientific literature and fixing deficiencies.
- Classification of drugs with prepared text.
- PowerPoint presentations and training videos concerning medicine dosage forms during class.
- The presence of students in clinical skill centers and pharmacy model.
- Convening clinical pharmacology classes and workshops based on predicted objectives.
- Conducting pre-test and post-test from workshops regarding Pharmacology knowledge.
- Receiving their feedback and ideas to reform the proposed cases and modify the relevant defects.

In the next part

- Preparing raw prescription
- Collecting common prescriptions from medical centers or hospitals
- Collecting prescriptions erros which made by interns
- Preparing a bank of indigenous common clinical cases and logical prescription for treatment and conducting training sessions of clinical prescription for students.

1- Diversity in teaching the target (prescription) through prescription errors scenario in the forum (Problem Based Learning)

2- Forum is a cyber space in which we set a scenario of illness diagnosis and prescription errors to encourage students' higher involvement and increase more interactive learning. This makes group-reflection possible for students. The students' pre-test and post-test information was reviewed by a series of corresponding two-part questions and their satisfactions as well as the impact of workshops on their learning and performance were evaluated.

Pharmacology course is among courses which is presented through 4 units in the form of theory and 2 practical units (clinical pharmacology) to medical students in the third and fourth year of educational programs of GP and the second and fourth semester of nursing (2 of theory and one practical unit in the form of Clinical Pharmacology). The main objectives of this course are the familiarity of medical and nursing students with theoretical and practical knowledge for pharmaceutical dosage forms, cognitive traits, drug category, scientific and Persian names of drugs, their indication, adverse effects, rational drug prescription and the correct principles of prescription. Since the content of this course is such that it requires students to be connected continuously to these topics in order to learn and increase the level of their knowledge and skills to identify dosage forms and improve their vision in rational drug prescription, hence, the use of the new technologies that are helping educational methods, enhances their ability to enjoy more of the above areas. In this regard, providing a pharmacy model containing the most commonly prescribed medicines prepared by hospitals(Motahari and Peymanieh) and health centers along with a brief explanation including Persian name, accurate scientific name, dosages forms of drugs, clinical application, side effects, drug interaction and precautions were included in the agenda of an experimental pharmacology. The project, performed with the help of all students who have chosen this units in the semester, accounts for 30 per cent of the final score from the corresponding unit.

RESULTS

Pharmacy model provides educational materials to students and offers little information on each medicine using short texts. According to the results of this study, one can enumerates higher students' desire to study pharmacology, appropriate approach to logical prescription through facilitating learning and increasing their knowledge and skill level in the process of learning this lesson. The results of students' knowledge evaluation in pharmacology courses showed a significant difference before and after the intervention [Table 1].

Table 1: Results of clinical pharmacology courses before and after the intervention

N = 64

Test position	Number	Mean	Standard deviation	T	Significant values
Before	64	6.45	1.57	22/28	0.0001
After	53	9.56	0.72		

Other results showed that :

- Easy access to pharmacy model is effective in increasing willingness to learn. It can also be considered as a learning mode during education.
- Optimum satisfaction of student with supplementary pharmacology courses (90%)
- Significant improvement in students' knowledge score in Pharmacology
- Increasing the number of correct answers to checking prescriptions with errorsin the forum
- Students' appropriate answers to quizzes on manuscripts containing the scenario discussed in the forum
- Changing the system and reducing medical errors in prescription writing.
- Students' tendency to have more access to pharmacy model to complete Pharmacology information and knowledge [Fig.2].

Workshop assessment by students showed in[Table 2] .

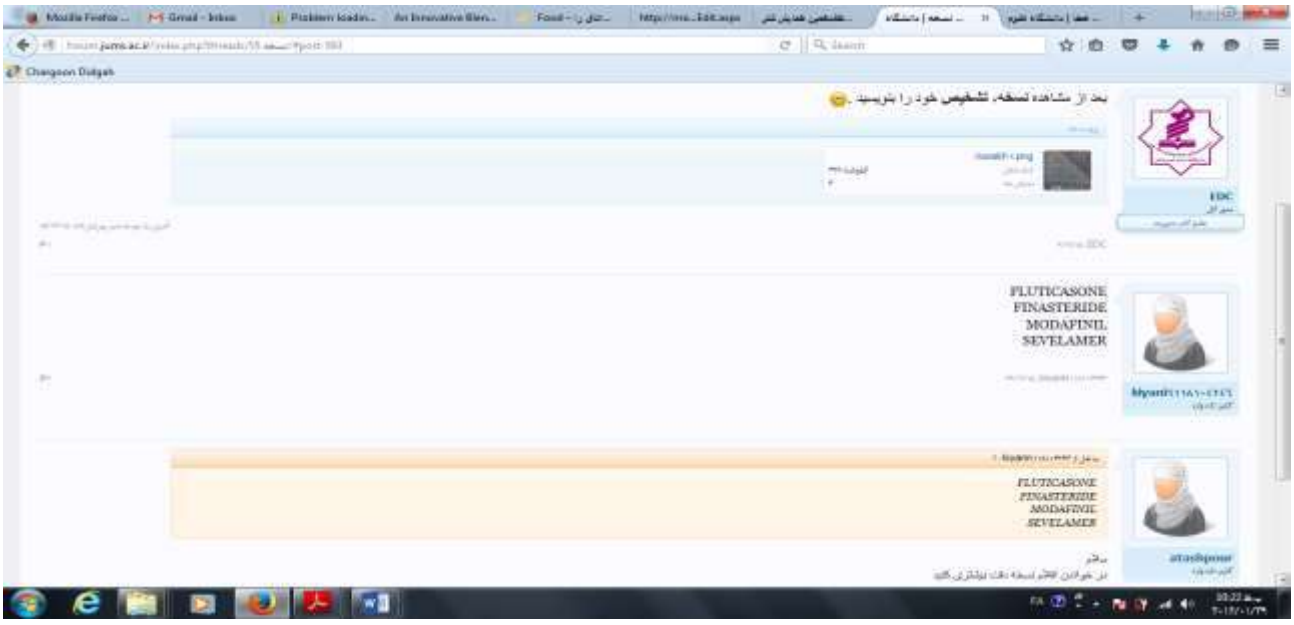

Fig.1 : Teaching prescription through prescription errors scenario in the forum.

Table 2: Descriptive indicators of assessment of workshop program impact on students

Title	Very weak	Weak	Average	Good	Very Good
Workshop content proportion with the students' required topics	------	------	------	3(9/1)	50(90/9)
Quantity and quality of the program in terms of presenting new scientific topics	------	------	5(9/4)	10(18/8)	38(71/8)
The quantity of material presented in the workshop	------	------	------	12(22/64)	41(77/36)
Eliminating defects in students' performance	------	------	------	15(28/3)	38(71/7)
The impact of program on the students' clinical decision-making	------	1(1/88)	3(5.6)	8(15/09)	41(77/35)
The impact of the program on the depth of learning and motivation	------	2(3/77)	5(9/43)	14(26/42)	32(60/38)
The impact of the program on promotion of scientific knowledge in pharmacology courses	------	2(3/77)	4(7/54)	15(28/30)	32(60/37)
The relationship between learn theoretical and practical material	------	2(3/77)	3(5/55)	48(90/56)	------
Using previous students' experiences and its relationship with new content	------	1(1/9)	1(1/9)	6(11/32)	46(86/8)
The impact of program on practical skills in the clinical setting	------		------	3(9/1)	30(90/9)
The rate of your overall satisfaction with the holding of the workshop program	------	1(1.7)	------	17(32)	35(66.3)
The teachers' mastery of the material and their ability to attract participants	------	------	------	53(100)	------

DISCUSSION

One of the most important and basic necessities in order to prevent and treat diseases is proper understanding of pharmacology. Bereft of any incentive to learn, most medical students learn this subject in a teacher-centered approach based on the theories of this science. The lack of an active role in this filed makes the whole learning process incomplete. That is why they face difficulty in logical prescribing of appropriate and effective medications for the patients in clinical encounter. Therefore, reconsidering teaching methods and other measures to promote teaching and learning and ultimately enhancing the level of knowledge and skills of medical students seem essential. Having evaluated the clinical applications of the students' pharmacological knowledge, Vasundhara and his colleagues stated that the teaching method of pharmacology requires fundamental changes [24]. Due to its unique features such as: encouraging students to learn, providing critical thinking and cooperating with the teachers in the learning

materials (teacher-based), environmental simulation similar to real environment and therefore improving students' confidence etc, teaching by workshops creates a more profound learning for students and strengthens their knowledge and skills as well. Findings from this study that were compared in the form of pre- and post-test revealed a significant increase in the level of students' knowledge and skill. The results of this study were consistent with those of other studies established on teaching workshop. For example, The Accreditation Council for Pharmacy Education (CAPE) has dealt with providing production standards of pharmacy model simulators in pharmacy Universities [25]. Some, also, emphasize knowledge transfer originating from these simulations on patient care to set the ground for making training pharmacology courses practical (25). Furthermore, some of the researches have pointed out the necessity of using simulations in the initial phase of learning pharmacology and then extending it to other functional skills[26]. According to a new model of teaching pharmacology developed by Kanchan Gupta et al. (2014), the students think with the help of a simulated clinical environment. The simulation was as follows: a patient consulted his physician, complained about process of his disease and explained the administered medicines as well as the way of managing medication. In addition, this caused students to make an active effort to prescribe the appropriate drug. Ultimately, the students stated that factors such as familiarizing and preparing them prior to their presence at the bedside (pre-clinical) and forcing them to learn the basics and applicable issues of this lesson led to increased learning [27]. Students' comments about the effectiveness of the workshops are very valuable. Most participants in this study declared their satisfaction with holding this workshop classes and its positive effect on their knowledge and skills. Similarly, a number of other studies have indicated that patient-centered learning (a workshop) increases students' satisfaction with their learning and asserted that this method was much superior to traditional methods of teaching [28]. The results of these studies support the students' attitude towards the workshops and the use of models and simulations.

Workshop training methods can also be useful in other courses and professions. In the study by PuncikovaRaicharoen et al (2015) that was performed on 189 medical students, the students were divided into two categories. The first group included 77 students who completed the course in emergency medicine with traditional methods and the second group includes the rest of the students who attended the course by attending workshops. Finally, three workshops attained the highest rating in terms of medical students' satisfaction who took part in workshops, among which one can mention: trauma workshops (internal bleeding), training in the patient's presence and the workshop of Emergency Medicine Services. Finally, they came to the conclusion that training emergency medicine to medical students through workshops would lead to higher satisfaction of students with emergency medicine training. According to the same study, trauma workshops can put students in real situations and causes that each student plays the role of a member of the group in support of patients during advanced cardiopulmonary resuscitation. In the end, these workshops teach real practical hints to the students. There are four very important components in these workshops which may help them to learn: Simulation or real action, team learning, immediate feedback and clinical thinking [29]. According to the results of studies done by Bazrafkan et al (2015), resident-teacher educational workshops can be effective in improving residents' teaching skills [30]. A study by Roudbari M (2006)[29], carried out on medical students to examine their views on the influence of research workshops on the development of their thesis, finally came to the conclusion that such workshop programs should be reviewed and that a part of these workshops' time should be replaced by better cases in teaching [31]. According to the study carried out by Jennifer Yost et al.[41], physicians who had attended workshops showed significant changes in their knowledge base, compared to other physicians who had not participated in these courses [33]. According to the study done by L Fritsche et al. (2005), after holding a three-day course in evidence-based medicine, significant progress has been observed in the physicians' knowledge and practice [8]. The expressed results, in line with current studies, are indicative of positive effects of workshop programs on students' knowledge and high satisfaction with these programs.

CONCLUSION

As a conclusion we can say that, as it is clear from the findings of studies, teaching through workshops andusing simulators and real models has a salutary impact on learning and helps students, in real encounter with patients, to make appropriate clinical decisionswith high knowledge and skills while maintaining their confidence, in an environment free from anxiety.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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AUTHOR CONTRIBUTION

ST was responsible for design and implementing of research, and final editing. LM was responsible for idea of title, writing of paper, editing the manuscript and data analysis.

ETHICAL ASPECTS

The study protocol was approved by the Ethics Committee of the Jahrom University of Medical Sciences, Jahrom, Iran

FINANCIAL DISCLOSURE

None

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ARTICLE

PHARMACOLOGICAL EFFECT OF SEVEN MEDICINAL PLANTS AS A TRADITIONAL PREPARATION

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ABSTRACT

Medicinal plants have been utilized as a part of essentially all societies as a wellspring of medicine. Affirmation of the security, quality, and adequacy of therapeutic plants and natural products has now turned into a key issue in industrialized and in developing nations. Medicinal plant species are known for producing beneficial active compounds or secondary metabolites with many therapeutic values, for centuries. Traditional medicines or folk medicines in worldwide are the synthesis of the therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Recently, Herbal products are increasingly used, mainly in human illnesses. This review investigates the available studies on the pharmacological effects of some medicinal plants (Silybum marianum, Taraxacum officinale, Fumaria officinalis, Cynara scolymus, Cichorium intybus, Echium amoenum and Viola odorata) on different human diseases. The present article incorporated a detailed interpretation of the seven medicinal plants, emphasizing its therapeutic uses, pharmacological properties such as anti-hyperlipidemic, anti-hepatitis, anti-obesity and antioxidant activities, and mechanism of action based on preclinical and clinical studies, safety issues along with the current research potential of the medicinal plants.

KEY WORDS
Medicinal plants; Anti-hyperlipidemic activity; Antioxidant activities; Anti-hepatitis Activity; Anti-cholesterol activity; Anti-obesity activity

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INTRODUCTION

Plants are among the main natural bio-factories on Earth, and are capable of producing many valuable and unique biochemical compounds. Medicinal plant species are known for producing beneficial active compounds or secondary metabolites with many therapeutic values, for centuries. Recently, the huge demand for the plant secondary metabolites to support various health and pharmaceutical purposes has put tremendous pressure on the planet's already depleting natural resources [1]. The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus there is a need to explore alternative therapies particularly from herbal sources as these are cost effective and possess minimal side effects. Plant secondary compounds are usually classified according to their biosynthetic pathways. Three large molecule families are generally considered: phenolics, terpenes and steroids, and alkaloids. A good example of a widespread metabolite family is given by phenolics: because these molecules are involved in lignin synthesis, they are common to all higher plants. For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy, and horticulture. In the area of phyto chemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds [2]. Traditional medicines or folk medicines in worldwide are the synthesis of the therapeutic experience of generations of practicing physicians of indigenous systems of medicine. In different traditional medicinal systems plants are the primary component to treat a diseases and subsequently so many plants, fruit, vegetable are used to keep the body healthy. The decision was based on two foundations; first, lack of access of a great number of people (up to 80% in some counties) to primary healthcare and second, dissatisfaction from the outcomes of treatments by modern medicine, especially in relation to chronic diseases and the side effects of chemical drugs [3]. Till date, numerous medicinal plants have been reported to be effective in different diseases, however plenty of research is still needed to be done. This article focuses on the various plants that could be effective in the treatment of illnesses.

Characteristics of seven medicinal plants

Silybum marianum, commonly known as 'milk thistle' (Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. The plant itself grows as a stout thistle in rocky soils with large purple flowering heads. The leaves are characterized by milky veins, from which the plant derives its name [4].

Taraxacum officinale, the common dandelion (Often simply called "dandelion"), is a flowering herbaceous perennial plant of the family Asteraceae (Compositae). With a long history of traditional use in the treatment of hepatobiliary problems, its root has been shown to have sesquiterpene lactones, triterpenes, carbohydrates, fatty acids (myristic), carotenoids (lutein), flavonoids (apigenin and luteolin), minerals, taraxalisin, coumarins, and cichoriin. Aesculin has been reported from the leaf [5].

Fumaria officinalis (common fumitory, drug fumitory or earth smoke) is a herbaceous annual flowering plant in the poppy family Papaveraceae It is the most common species of the genus *Fumaria* in Western and Central Europe [6].

Cynara scolymus (artichoke) from Apiaceae family, a species of perennial thistle and with a Mediterranean origin, is traditionally bused for the treatment of digestive disorders, moderate hyperlipidemia, and liver and bile diseases. The leaf extract of *C. scolymus* has been used for its hepatoprotective effects. Leaves contains several polyphenolic compounds flavonoids and sesquiterpenes (cynarin, luteolin, isochlorogenic acid, chlorogenic acid, caffeic acid and quinic acid [7].

Cichorium intybus is a perennial plant with blue or white flowers is easy to grow and can be used for many medicinal purposes. *Cichorium* means field and intybus are partly derived from the Greek "to cut," because of the leaves, and partly from the Latin tubus to indicate the hollow stem [8].

Echium amoenum (Borage) is a wild annual herb that belongs to Boraginaceae family which grows in large parts of Europe, Mediterranean region, and also in parts of Iran. The flowers and the leaves of borage are known as a traditional remedy and possesses antioxidant, analgesic, antibacterial, anxiolytic, antidepressant, and immunomodulatory properties. It is a rich source of anthocyanins including cyanidin and delphinidin [9].

Viola odorata commonly known as sweet violet in English, belongs to the family Violaceae. It is called Banafsha in Indo-Pakistan. The plant is native to Asia, North Africa and Europe. Its history as a medicinal herb dates back as far as 500 BC, where it was known to be used to relieve pain due to cancer. Viola odorata contains alkaloid, glycoside, saponins, methyl slicylate, mucilage and vitamin C [10].

Anti- hyperlipidemic activity

The extract from roasted chicory (*Cichorium intybus*) root (chicory root extract), which contains inulin-type fructans, has favorable effects including anti-hyperglycemic and anti-dyslipidemic effects and the improvement of bowel movement [11].

It has been reported that hydroalcoholic extract of *Echium amoenum* has hypoglycemic effect on diabetic rats and leads to valuable changes in blood lipid profiles as well as lipoprotein levels [12]. *Viola odorata* leaf extract caused a reduction in total cholesterol and triglyceride levels in tyloxapol-induced dyslipidemia model, the plant extract caused significant decrease in total cholesterol, LDL-C and atherogenic index and prevented the increase in average body weight [10].

Cynara scolymus extract can be conducive to the reduction in phosphatidate phosphor hydrolase activity and liver triglyceride. *C. scolymus* has benefits for controlling of hyperlipidemia, oxidative stress in hyper lipidemic regimes, and abnormalities in lipid profiles [13].

Abd El Azeem et al., (2016) suggested that leaf extract of *Cynara scolymus* could be helpful in decreasing the incidence of several fatty liver disease through a reduction in TC, LDL and TG and an increase in HDL level. In addition, it appears to exert these effects through suppressing lipogenesis in the liver and promoting lipolysis in white adipose tissue [14].

The water extract of *C. intybus* showed an antioxidant effect on low density lipoprotein (LDL) and inhibitory effects on the production of thiobarbituric acid reactive substance and the degradation of fatty acids in LDL. Chicory root aqueous extract decreased cholesterol absorption by 30% in the jejunum and by 41% in the perfused ileum [Table 1] [8].

Anti-hepatitis activity

The extracts of milk thistle became a favored medicine for hepato biliary diseases in 16th century and the drug was revived again in 1960 in central Europe [4, 15]. In Silymarin, amongst the flavonoids, which have proven anti oxidative, antiviral or anti carcinogenic properties like glycyrrhizin, phyllanthin, silybin, picroside and baicalein, can serve as primary compounds for further development as hepato protective drugs [4].

Even though silymarin does not affect viral replication it may have beneficial role in viral hepatitis by its inhibitory action on inflammatory and cytotoxic cascade of events induced by the viral infection. Also, it can improve the regeneration process and normalize the liver enzymes by its action on protein synthesis [16]. So it can use to treat alcoholic liver disease, acute and chronic viral hepatitis and toxin-induced liver diseases.

Among the many therapeutic properties that have been traditionally ascribed to the artichoke, the liver-protecting action is not certainly the least important, to the extent that its use was recommended to patients affected by hepatitis, jaundice, cirrhosis and liver steatosis. Cynarin and caffeoylquinic acids are thought to be the substances that are chiefly responsible for the protective action against such hepatotoxic agents as carbon tetrachloride [14].

The folkloric use of chicory as hepato protectant has been well documented. Ethanolic extract of chicory given orally at doses of 6, 18, and 54 mg/kg BW per day showed a significant hepato protective effect by reducing the liver enzymes (aspartate transaminase [AST] and alanine transaminase [ALT]). The results were highly significant at the dose of 54 mg/kg BW per day [Table 1] [8].

Anti-oxidant activity

Free radicals induced oxidative stress is now believed to be a fundamental mechanism underlying a number of human cardiovascular, neurologic and other disorders. Antioxidants are our crucial defense against free radical induced damage, and are critical for maintaining optimum health and wellbeing. Antioxidant properties of *Silybum marianum*, Free radicals, including the superoxide radical, hydroxyl radical (.OH), hydrogen peroxide (H2O2), and lipid peroxide radicals have been implicated in liver diseases. These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to xenobiotics [17]. The cyto protective effects of *silymarin* are mainly attributable to its antioxidant and free radical scavenging properties. *Silymarin* can also interact directly with cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity [18].

The pharmacological rationale for the use of *silymarin* relates to its antioxidant action, selective inhibition of leukotriene formation by Kupffer cells as well as its antiapoptotic action. *Silymarin* is found in the entire plant but it is concentrated in the fruit and seeds. Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation, has anti-fibrotic activity and may act as a toxin blockade agent by inhibiting binding of toxins to the hepatocyte cell membrane receptors [17].

The artichoke leaf extract induces the concentration- dependent inhibition of induced oxidative stress in human neutrophils. Cynarin, caffeic acid, chlorogenic acid and luteolin have been found to be the active ingredients that play the major role in the antioxidant protective activity. The antiradical properties of aqueous and alcoholic artichoke extracts, as well as their capability of inhibiting lipid peroxidation, were recently confirmed [8].

Chicory has promising potential to be considered as a natural substance for ameliorating oxidative stress and hepatic injury induced by nitrosamine (sodium nitrite, 0.05% in DW) compounds. Red chicory was also studied for its polyphenol content and the antioxidant activity by using the synthetic 2, 2-diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl radical scavengingm activity. Total phenolic content is correlated with antioxidant activity in both the synthetic radical scavenging activity and the enzyme-catalyzed reactions (xanthine oxidase, myeloperoxidase, and diaphorase). A high level of anthocyanins, present in the seeds of C. intybus, might exert a direct scavenging effect against reactive oxygen species (ROS) formation due to antioxidant activity [8]. The antioxidant activity of violet flavonoids results from the combination of their iron chelating activity and their ability to scavenge the aging-inducing free radicals. Flavonoids can inhibit oxidases such as lipooxygenase (LO), cyclooxygenase (CO), mieloperoxidase (MPO), NADPH oxidase and xanthine oxidase (XO), thus preventing the in vivo formation of reactive oxygen species and organic hydro peroxide. Additionally, it has been found that flavonoids inhibit enzymes indirectly involved in oxidative processes, such as the A2 phospholipase and stimulate other enzymes with well-known antioxidant properties, such as catalase and superoxide dismutase (SOD). These are the mechanisms through which flavonoids interfere with the propagation of free radicals and also their formation [19]. E. amoenum (Boraginaceae) is known as a traditional remedy for depression and possesses antioxidant activity because of the presence of anthocyaninv [Table 1] [9].

Anti-obesity activity

Obesity, a pathological condition characterized by excessive accumulation of body lipids, is by far the most prevalent metabolic disease affecting hundreds of millions of people worldwide. The leading cause of this excessive lipid accumulation is a chronic positive energy balance combined with energy partitioning toward lipids [20]. The number of people with obesity and obesity related diseases, such as diabetes mellitus, hypertension, coronary artery disease, and cancers, has increased at an alarming rate all over the world [21]. Consequently, the idea of developing anti-obesity drugs with no undesirable side effect has become a hot topic. Herbal medicine has been looked at as a complementary treatment [22].

Recent studies had shown that the *Cynara scolymus* L. leaf extract (CLE) is effective on visceral fat levels and hepatic lipid accumulation in mice with high fat diet-induced obesity. In addition [21, 22]. It has been found that the hydroalcoholic extract of Iranian *Echium amoenum* has hypoglycemic effects and can be used to prevent weight loss due to diabetes. These effects might be due to the presence of compounds such as flavonoids and saponins and some of the plant's antioxidant properties [Table 1] [12].

Other activities

It has been reported that chicory has anti-diabetic activity. The effect of methanolic extract of *C. intybus* (CME) on glucose transport and adipocyte differentiation in 3T3-L1 cells was studied by radiolabelled glucose uptake and lipid accumulation assays, respectively. CME exhibited a significant increase in glucose uptake with a dose-dependent response. It also inhibited the differentiation of preadipocytes [8].

Viola odorata leaves extract, which tested positive for alkaloids, saponins, tannins, phenolics, coumarins and flavonoids, caused a dose-dependent (0.1-1.0 mg/kg) decrease in mean arterial blood pressure in anaesthetized rats [10].

The anti-cancer activity of *Viola odorata* has been documented. Cycloviolacin O2 (CyO2), a cyclotide from *Viola odorata* (Violaceae) has antitumor effects and causes cell death by membrane permeabilization [23]. *Echium amoenum* induce antidepressant effective in part by increasing level CSF serotonin and dopamine [24]. Silymarin treatment produced significant reduction in daily and fasting blood glucose, daily glucosuria, glycosylated haemoglobin values, malondialdehyde values and a drop in insulin requirement and fasting insulinaemia [25].

Taraxacum officinale is a rich source of a variety of vitamins and minerals, including beta carotene, nonprovitamin A carotenoids, xanthophylls, chlorophyll, vitamins C and D, many of the B-complex vitamins, choline, iron, silicon, magnesium, sodium, potassium, zinc, manganese, copper, and phosphorous. *Taraxacum officinale* might possess blood sugar modulating activity [26]. *Taraxacum officinale* also can restore experimentally-induced suppressed immune function in animals by enhancing cell-mediated, humoral, and non-specific immunity [27]. Evidence also suggests *Taraxacum officinale* influences nitric oxide production [28].

Phytochemical effects of *Fumaria officinalis* related to several alkaloids such as adlumidiceine, copticine, fumariline, perfumine, protopine, fumaranine, fumaritine, paprafumicin and paprarine [29]. Extracts of *Fumaria officinalis* have been traditionally used for treatment of some skin diseases (rashes or conjunctivitis), rheumatism, stomach ache, abdominal cramps, fever, diarrhea, syphilis and leprosy. Fumaria extracts also possessed strong antihypertensive, diuretic, hepato protective and laxative effects, mainly because of the isoquinoline alkaloids [30]. Herbal medicinal study of Fumaria has shown that it used for the treatment of diabetes mellitus, hypertension diseases and cardiac disorders [31]. It also has anti-bactericidal activity against the Gram-positive organisms like Staphylococcus and Bacillus anthracis.

A few compounds found in the *Cynara scolymus* (artichoke) exhibited a significant hypo glycemizing activity. Chlorogenic acid was identified by Arion and collaborators as a powerful and specific glucose-6-phosphate-translocase inhibitor. This enzyme is essential for the formation of endogenous glucose during the gluconeogenesis process, as well as for the glycolytic process. Artichoke leaf extracts was proved to have anti carcinogenic, anti-oxidative, anti-inflammatory, antibacterial, anti HIV, bile expelling, and urinative activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation [Table 1] [32].

Future direction

Considering therapeutic potential of these seven medicinal plants in terms of their efficacy and adaptability is such that combination of them as one organic product can be noticed in future, since obesity, hyperlipidemia and fatty liver are becoming more epidemic around the world especially in developing countries as an organic product by using local knowledge can reduce many problems associated with the use of chemical drugs and their side effects to a large extent.

Table 1: Seven medicinal plants with evidence of their activities

Plant	Plant part used	Bioactive compounds	Screened activity
Silybum marianum	seeds	silymarin	Anti-hepatit, antioxidative, antiviral or anticarcinogenic [4]
Taraxacum officinale	Roots and leaves	sesquiterpene lactones, triterpenes, carbohydrates, fatty acids (myristic), carotenoids (lutein), flavonoids (apigenin and luteolin), minerals, taraxalisin, coumarins, and cichoriin	Anti-bacterial, anti-inflammatory, anti-hepatitis and appetizer [26, 28]
Fumaria officinalis	Leaves, flowers and stems	adlumidiceine, copticine, fumariline, perfumine, protopine, paprafumicin and paprarine	Amphicholeratic, appetizer, antihypertensive, diuretic, hepatoprotective and laxative effects and anti-diabetic [30]
Cynara scolymus	Leaves	cynarin, luteolin, cynaroside, scolmoside; phenolic acids such as caffeic, coumaric, hydroxycinnamic, ferulic, caffeoylquinic acid derivatives	Anti-hepatitis, anti-cholesterol, anti-carcinogenic, antioxidative, anti-inflammatory, antibacterial, anti HIV [32]
Cichorium intybus	Roots and leaves	thiobarbituric acid, inulin-type fructans, phenol, flavonoid, tocochromanol (tocopherol and tocotrienol)	Anti-hyperglycemic, anti-dyslipidemic, anti-oxidative, anti-cholesterol, and anti-diabetic [8]
Echium amoenum	Leaves and flowers	phenolic compounds like rosmarinic acid, cyanidin, and delphinidin	Anti-obesity, anti-hyperlipidemic, anti-cholesterol , antibacterial, anti-diabetic, anti-oxidative and anti-depressant [25]
Viola odorata	Leaves and flowers	alkaloid, glycoside, saponins, methyl slicylate, mucilage and vitamin C, Cycloviolacin O2 (CyO2)	Anti-hyperlipidemic, anti-cholesterol, anti-depressant, anti-bood pressure, anti-cancer and anti-tumor [23]

CONFLICT OF INTEREST

There is no conflict of interest.

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None

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ARTICLE

STUDY OF ALBUMIN USE PATTERN IN AYATOLLAH KASHANI TEACHING HOSPITAL OF SHAHREKORD IN 2015

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ABSTRACT

Human plasma albumin has many therapeutic indications and excessive and indiscriminate use of this product leads to high costs each year in many hospitals across the world. This study was conducted with the aim of studying albumin use pattern in Ayatollah Kashani Hospital, Shahrekord in 2015. This cross-sectional study was a conducted within a period of three months in 2014 in the 300-bed Kashani Hospital on 57 patients. Data were analyzed by descriptive and deductive statistical tests in SPSS 22. The period of receiving albumin by patients was 1-39 days with mean period of 8.8 days. Total number of vials received during hospitalization period was 1-68 with mean number of 14.01. In this study, the patients admitted to the ICU received the greatest amount of albumin (71.9%, n=41). The majority of patients (19.3%, n=11) who received albumin were accidental patients with multiple trauma and coronary artery disease (14%, n=8). Surgical and anesthesia services, with 63.2% and 21.1%, respectively haf the most requests for albumin from pharmacy. Clearly, modifying pattern of utilization and using medicines rationally according to appropriate strategies is essential and highly important.

KEY WORDS
Albumin, hospital, patient, rational consumption

INTRODUCTION

Drug utilization research (DUR) is one of the approaches to investigate use of drugs qualitatively and quantitatively. The ultimate aim of DUR is to investigate whether pharmacotherapy is rational or not. According to the World Health Organization (WHO), rational use of drugs refers to appropriate use of drugs according to clinical needs, in doses that meet individual requirements, for an adequate period of time, and with the lowest cost to the patient and his/her community [1-2]. Rational use of drugs is representative of an individual approach to treatment. The success of treatment depends mainly on physician's ability to diagnose disease, select appropriate drug, dosage form, and proper prescription, predict potential response(s), side effects, and drug interactions, an prevent unnecessary and life-threatening repetitions in treatment.

Moreover, rational use of drugs depends on the performance of pharmacists in preparing drugs and nursing teams in prescribing those [3].
Studies of DUR is an approach to investigate use of drugs qualitatively and quantitatively. These studies are considered to be the basis to revolutionize pharmacotherapy in addition to controlling medical expenses. Clearly, modification of pattern of utilization and establishment of a system of drugs rational use are conducted by detection and, if possible, suggestion of appropriate strategies and it is essential to conduct studies on this area [4]. The ultimate aim of DUR is to investigate whether pharmacotherapy is rational or not. A review of DUR has shown that successful research on this field requires multidisciplinary cooperation among physicians, clinical pharmacologists, pharmacists, and epidemiologists, and if these studies are not supported, they will not be fruitful [5].

Human serum albumin is the most important water-soluble protein in human plasma. Albumin has many benefits; however, preparation and production of albumin is greatly costly. Moreover, albumin increases risk for transmitting blood-borne infections. Several studies have reported excessive use of this product [6-9].

Inappropriate use of albumin imposes stupendous costs on hospitals each year across the world. Studies have reported inappropriate prescription of albumin to be 50%-70% with great costs [10-11]. A study reported that over 90% of albumin was not prescribed according to the available guidelines and was inappropriate [12]. However, according to these guidelines, less expensive alternatives to albumin are available for many diseases.

To develop a single standard protocol for rational prescription of albumin, first, pattern of its prescription for inpatients should be investigated by DUR. DUR are considered to be the basis for pharmacotherapy alongside contributing to control of treatment costs. The present study was conducted to investigate pattern of albumin use to achieve a standard guideline for prescription of albumin for patients in Kashani Hospital, Shahrekord.

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MATERIALS AND METHODS

This descriptive, cross-sectional study was conducted within three months in 2015 on the patients hospitalized in 300-bed Kashani Hospital, Shahrekord. During this period, the patients administered with human albumin hospitalized in each of the 17 wards of the hospital were studied. During this period, 57 patients were enrolled into the study. The study protocol consisted of four phases: Planning, data gathering and assessment, intervention, assessment programs.

The data were drawn from the software used for the inpatients' pharmacotherapy, the inpatients' medical file, and nursing report were used. To gather the data on the inpatients, a standard form of indications for treatment with albumin according to the available reliable guidelines was used. The demographic characteristics of the patients administered with albumin consisting of age, gender, weight, and the albumin-ordering service, clinical data on albumin prescription, reason for prescription, duration of administration, the number of used vials, and clinical and laboratory observations were recorded in a special form. If albumin was prescribed according to the standard guideline 2010, it was considered an appropriate prescription, and if not, it was considered an inappropriate prescription.
The data were analyzed by descriptive tests, ANOVA, independent t-test, Pearson's correlation coefficient, and chi-square in SPSS 22.

RESULTS

The mean age of the patients was 55.24 (range: 16-90) years. Most (n: 36 [63.2%]) patients were male and the rest (n: 21) female. The mean weight of the patients was 66.66 (range: 37-110) kg. The mean duration of the patients' stay in hospital was 26.42 (range: 3-87) days. The mean duration of albumin administration was 8.8 (range: 1-39) days. The total number of administered albumin vials during stay in hospital ranged from one to 68 vials with mean number of 14.01 vials. The mean albumin level of the patients, according to the laboratory findings, was 2.6 (range: 1.5-4.4) g/dL [Table 1].

Table 1: Comparison of total number of used albumin vials for age, gender, and the requesting service and physician

Variables	Range	Total number of used albumin vials (mean± SD)	ANOVA test
Age (year)	15-30	17/36±20/76	0/768
	30-45	11/42±9/25	
	45-60	9/55±9/21	
	60-75	16/58±20/53	
	Over 75	12/30±13	
Gender	Male	8/61±7/51	0/05
	Female	17/16±19/03	
Ward	ICU	15/07±17/89	0/241
	Burn	35±11/31	
	Surgery	6/66±5/68	
	Neurosurgery	7/80±6/41	
	Internal	8/66±5/71	
Ordering service	Surgery	15/91±19/14	0/78
	Internal	9/75±4/92	
	Anesthesia	12/41±10/94	
	Oncology	2±0	
	Neurology	9±5/47	
Ordering specialist	General surgeon	21/14±24/02	0/05
	Neurosurgeon	12/20±15/02	

	9/5±6/36	Internist	
	20/66±23/57	Neurologist	
	33/33±8/50	Plastic surgeon	
	10/44±8/61	Anesthetist	
	6/13±5/75	Cardiac surgeon	
	4±2/82	Sub oncologist	
0/94	19/60±25/22	Surgery resident	Ordering resident
	18/33±17/09	Anesthesia resident	

The highest dosage of albumin was administered to the patients in intensive care unit (ICU) (n: 41, [71.9%]). Moreover, most patients administered with albumin were patients with accident-induced injuries with multiple trauma (n: 11, [19.3%]) and coronary artery disease (n: 8, [14%]). Surgical and anesthesia services (63.1% and 21.1%, respectively) ordered albumin most frequently. Meanwhile, cardiologists (26.3%), anesthetists (15.8%), general surgeon (12.3%), and neurologists (10.5%) ordered albumin for patients most frequently.

Plasma freeze was the most common reason for ordering albumin followed by edema, and albumin ordering according to serum albumin level (7%, 7%, and 5.3%, respectively). Among 57 patients throughout the studied period, 15 (26.3%) deceased. Moreover, the number of used albumin vials was higher for the deceased patients than other ones (16.8 vs. 13) [Tables 2 and 3].

Table 2: Frequency and absolute frequency of reasons for albumin prescription and death among patients (n: 57)

Percentage	No.	Range	Variables
5/3	5	According to the albumin level in the test	**Reason for prescription**
0/7	4	Edema with Lasix	
1/8	1	Extensive burn	
1/8	1	Tonic	
3/5	2	malnutrition	
0/7	4	Plasma freeze	
1/8	1	Hypotension	
26/3	15	Yes	**Deceased**
37/7	42	No	

Table 3: Comparison of mean total amount of used albumin vials for prescription reasons and patients' decease

P-value of ANOVA test	Total number of used albumin vials (mean± SD)	Range	Variables
0/43	26±26/28	According to albumin level in the test	**Reason for prescription**
	5/75±1/05	Edema with Lasix	
	27±0	Extensive burn	
	7±0	Tonic	
	7/5±7/77	malnutrition	
	9±5/47	Plasma freeze	

	5±0	Hypotension	
0/43	13±14/93	Yes	**Deceased**
	16/86±19/76	No	

According to correlation coefficient test, the patients administered with greater number of albumin vials were hospitalized for a significantly longer period of time (*P*=0.004).

DISCUSSION

According to the findings in Kashani Hospital, Shahrekord, 4200 albumin vials, costing 2,400,000,000 Rials, were used within nine months (from March to February) in 2015. This figure is very great for this single drug in a teaching hospital compared to other commonly used drugs such as pantoperazole, ciprofloxacin, etc. Therefore, from economic and medical perspectives, it is necessary to ensure appropriate prescription of albumin. This study investigated the pattern of albumin use with reference to the available reliable guidelines.

According to the findings in this study, the highest dosage of albumin was administered to the patients in intensive care unit (ICU). Moreover, most patients administered with albumin were patients with accident-induced injuries with multiple trauma and coronary artery disease. Surgical and anesthesia services ordered albumin most frequently. Meanwhile, cardiologists, anesthetists, general surgeon, and neurologists ordered albumin for patients most frequently. In Jahangard et al. study, over 3/4 (over 870) vials of the used albumin was not prescribed according to the available guidelines referred to in the studied healthcare center. The most common inappropriate prescription of albumin was reported to be for cardiac surgery [13]. According to the majority of reliable guidelines, albumin is the last choice, following crystalloid and nonprotein colloid solutions, after open heart surgery.

Generally, nonprotein colloid solutions are not used in this hospital and albumin is used as the second-line treatment after crystalloid solutions. In Jahangard et al. study, a case of irrational prescription of albumin was for patients with malnutrition. For these patients, albumin was prescribed as a food supplement. The percentage of this inappropriate prescription was reported to be 46.6% [13]. In the present study, 15 of 57 patients deceased. Moreover, the mean number of used vials was greater for the deceased patients than other ones. Matos et al reported that among 3400 admitted patients, 40% were admitted to ICU and one third of all the admitted patients died. In this study, it was seen that use of over four albumin vials increased the likelihood of death up to 30% (even in cases that the prescription was conducted according to the severity and type of the services) [14].

It is argued that for patients with cardiac disease, albumin should not be prescribed even for short periods unless certain conditions such as hyperbilirubinemia, as an associated complication, occur. In Talasaz et al study, prescription of albumin was reported to be inappropriate for 36.2% of impatients. Hyperalbuminemia and food supplementation were the most common conditions for which albumin should not be prescribed. However, human albumin as protein source of energy should not be prescribed for the patients in need of receiving nutrients. Increased serum albumin concentration (for no specific reason) up to over 4 g/dL can cause increase in renal catabolism.

In this study, the number of used vials and the patients' mortality were significantly associated. Developing a pattern of albumin use in compliance with ASHP protocol not only determines the suitability of albumin prescription for the patients but also prevents associated problems, mortality, and additional treatment costs. Finally, it has been suggested that cooperation among physicians, pharmacists, and specialists in any hospitals to develop guidelines of albumin use helps optimize use of this important and valuable pharmaceutical product [15].

CONCLUSION

To develop a single standard protocol for rational prescription of albumin, first, pattern of its use in inpatients should be investigated by DUR. DUR is considered to be the basis for revolutionizing pharmacotherapy in addition to controlling treatment costs. Clearly, modification of utilization pattern and establishment of a system for drugs rational use is conducted through identification and, if possible, recommendation of appropriate strategies and it is necessary to do research in this field.

CONFLICT OF INTEREST
There is no conflict of interest.

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RTICLE

A CASE OF CARBAMAZEPINE INDUCED SYSTEMIC LUPUS ERYTHEMATOUS (SLE)

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ABSTRACT

Carbamazepine is an anticonvulsant drug that used to treat seizures and nerve pain and such as most drugs have side effect. Systemic lupus erythematous is one of connective tissue disorders that often caused by an auto immune mechanism of unknown, although some drugs cause SLE. This report aimed to demonstrate the carbamazepine induced SLE after its oral consumption for Convulsion. This is the report carbamazepine-induced systemic lupus in a 16- year-old patient who had been treated with carbamazepine for 2 years because of seizures. . After cessation of consumption and treatment, symptoms improved and antinuclear antibodies disappeared and indicated the patient's definite recovery. So far, few cases have been reported of carbamazepine-induced lupus after years the start of treatment. This is report about carbamazepine-induced systemic lupus erythematosus with serological confirmation after 2 years of treatment. Systemic lupus erythematosis is a syndrome of positive ANA associated with symptoms such as fever, malaise, arthritis or intense arthralgias/myalgias, serositis, and/or rash. The syndrome appears during therapy with certain medications and biologic agents, occurs predominantly in whites, has less female predilection than SLE, rarely involves kidneys or brain. Many drugs like carbamazepine. Hydralazine and phenytoin, cause SLE, but carbamazepine is a rare cause in literature. But according to physical exam and laboratory data this case occurred.

KEY WORDS
Carbamazepine, systemic lupus, erythematous (SLE), Hydralazine

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INTRODUCTION

Carbamazepine is an anticonvulsant that used to treat seizures and nerve pain. Although carbamazepine is usually well tolerated by most people, the potential side effects of therapy can vary from mild symptoms to severe, which may cause side effect such as slurred speech, vomiting, severe weakness, heart disease, dizziness, liver or kidney disease, glaucoma, drowsines, or dry mouth, swollen tongue, jaundice, headache, high blood pressure, high cholesterol or triglycerides, a thyroid disorder; lupus; porphyria [1].

Drug-Induced Lupus is a syndrome of positive ANA associated with symptoms such as fever, malaise, arthritis or intense arthralgias/myalgias, serositis, and/or rash. The syndrome appears during therapy with certain medications and biologic agents, occurs predominantly in whites, has less female predilection than SLE, and rarely involves kidneys or brain [2]. The list of substances that can induce lupus-like disease is long such as the anticonvulsants carbamazepine and phenytoin. ANA usually appears before symptoms [3].

MATERIALS AND METHODS

A 16-year-old man with a medical history of seizure disorder whose use anticonvulsant carbamazepine from 24 months prior to this admission, presented with the chief complaint of edema and dyspnea. His symptoms begun two weeks ago. His vital signs were BP of 100/60 mm Hg; heart rate, 114 beats/min; respiratory rate, 30 to breaths/min; and temperature, 38C. The patient had not a pulsus paradoxus. On physical examination, the patient's chest was decreased auscultation bilaterally, and cardiac auscultation heart sounds, with regular S1, S2.

laboratory data included a BUN, 11 mg/dL; creatinine, 0.7 mg/dL; total bilirubin, 3.7 mg/dL; aspartate amino transaminase (serum glutamic oxalacetic transaminase), 431 IU/L; alanine aminotransaminase (serum glutamic pyruvate transaminase), 183 IU/L; albumin, 3.7 g/dL; creatinine phosphokinase, 143 IU/L; WBC count, 10. × 103/μL with a normal differential; hemoglobin 13.2 g/dL; and hematocrit, 40.5%. The ECG demonstrated sinus tachycardia and low voltages without electrical alternant.

RESULTS

A chest radiograph revealed a "water bottle" cardiac silhouette [Fig. 1], bilateral pleural effusion without evidence of pulmonary infiltrate.

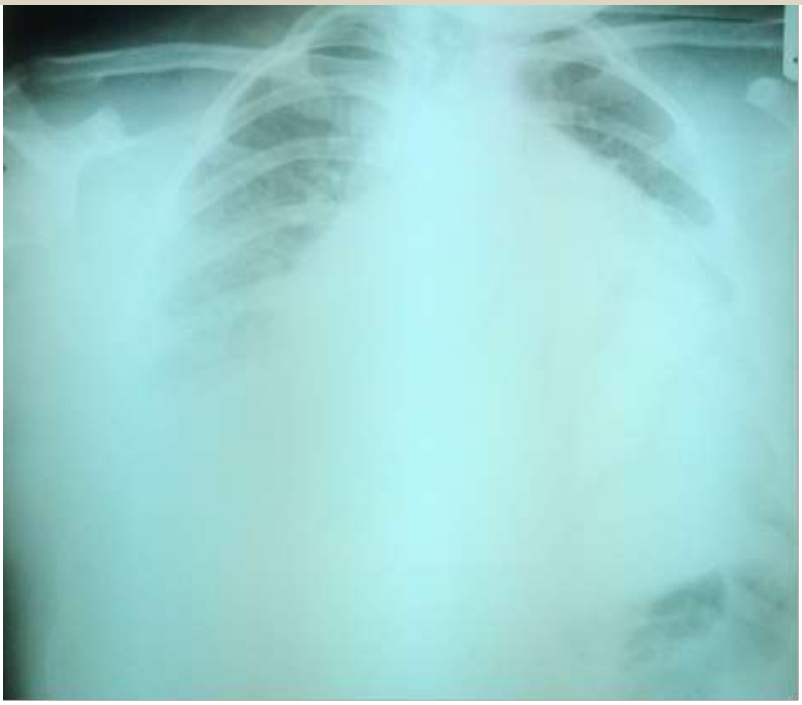

Fig. 1: "water bottle" cardiac silhouette, bilateral pleural effusion without evidence of pulmonary infiltrate.

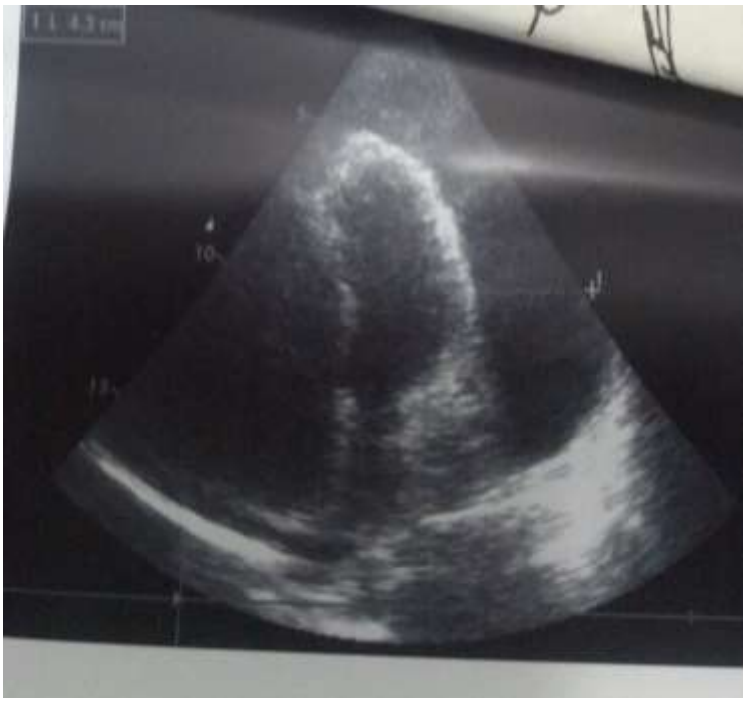

Fig. 2: A massive pericardial effusion in carbamazepine induced SLE.

An emergent echocardiogram showed a massive pericardial effusion, no tamponade, no RA and RV collapse.[Fig. 2]

Pericardiocentesis was performed with approximately 1000 mL of fluid removed. The patient began to improve breathing. Echocardiogram repeated and showed reduction in the pericardial effusion. pericardial fluid studies showed a; RBC count, 8500 cells/μL; WBC count, 235 cells/μL (neutrophils 65%, lymphocytes 35%); protein, 6 mg/dL; glucose, 20 mg/dL; and lactate dehydrogenase, 14 IU/L. Blood serologic studies showed antinuclear antibodies positive (1:320), anti-double-stranded-DNA (dsDNA) negative, antihistone antibodies positive. Serum studies for ADA and HIV were negative. Complement levels of C3, C4, and CH50 were normal limits. According neurology consult begun for antiseizure therapy and was discharged.

DISCUSSION AND CONCLUSION

Although some drugs cause pleural effusion and pericardial effusion and even ascites like concato disease without collagen vascular disorder, that reported by Hamid Rouhi and et al [4], but some drugs can cause systemic disease, that serositis is one of the presented symptoms.

Drug –induced SLE is generally equally common in males and females, and more common in older people and white populations. The risk for developing drug-induced lupus varies substantially between different medications, ranging from 15 to 20 percent of those taking procainamide, and 7 to 13 percent of those taking hydralazine, to as low as 2 per 1000 for those taking anti-tumor necrosis factor (TNF) agent, and 5 per 10,000 of those taking minocycline The diagnosis of SLE is based on characteristic clinical features and autoantibodies [5-6].

In many patients, criteria accrue over time. Antinuclear antibodies (ANA) are positive in >98% of patients during the course of disease. High-titer IgG antibodies to double-stranded DNA and antibodies to the Sm antigen are both specific for SLE and, therefore, favor the diagnosis in the presence of compatible clinical manifestations , but drug –induced SLE is rarely associated with anti-dsDNA, is commonly associated with antibodies to histones [2], and usually resolves over several weeks after discontinuation of the offending medication. In this case ANA and Anti histone anti body is positive and Anti ds DNA is negative, according manifestations and serologic studies drug-induced SLE considered for patient.

CONFLICT OF INTEREST
There is no conflict of interest.

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REVIEW

HYPOLIPIDEMIC HERBALS WITH DIURETIC EFFECTS: A SYSTEMATIC REVIEW

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ABSTRACT

Due to the high side effects of synthetic drugs, also versatile plant rather than poly-therapy by chemical drugs, the use of medicinal plants is common. Many medicinal plants in addition to effects on hyper lipidemia, are diuretic. The aim of this systematic review was introduced lipid-lowering plant with diuretic effects. The international research databases including MEDLINE; Google scholar, Web of Science SciVerse Scopus (SCOPUS); EBSCO Academic Search; Cochrane Central Register of Controlled Trials (CENTRAL); and a Chinese database (China Network Knowledge Infrastructure [CNKI]) were searched from their respective inceptions up to Mars 2015 with the search terms of "hyperlipidemia", "herbal medicine", "medicine traditional", "extract plant", "Traditional Medicine" and " Chinese Herbal Medicine" "Botany in hyperlipidemia"," Herbal in hyperlipidemia"," Herbal in hyper cholestrolemia", "Herbal therapy in hypertriglyceridemia", "Systematic Review of herbal in hyperlipidemia", "Natural remedies for hyperlipidemia", "Herbal medicine for cholestrolemia", "Herbal with antilipid effect", "Herbal therapy for Atherosclerosis"," Hyperlipidemia diet"and :" herbal as diuretics", "herbal therapy for edema", herbal medicine as diuretics", "herbal diuretic list", "Herbal diuretic review" without narrowing or limiting search elements. Finally, among of 2003 article, 20 plants that not only effected on hyperlipidemia but also was diuretic, selected and introduced.

KEY WORDS
Hypolipidemic, herbals, diuretic effects, cholestrolemia

INTRODUCTION

Herbal medicine, use of plants for medicinal purposes, has a long history in disease treatment and in health maintenance. Side effects of chemical drugs and lack of access to poly therapy chemical drugs have given more significance to the therapeutic importance of herbal medicine for the improvement of human health. So far, a wide variety of plants with therapeutic effect on serum lipid profile are explored and more than 200 plants with lipid lowering effect have been investigated. Nevertheless, there are great numbers of unknown plants with lipid lowering activity yet to be investigated. On the other hand, there are many hyperlipidemic patients who are diagnosed with hypertension or edema as well. Therefore, the importance of herbs which have diuretic properties in addition to their lipid lowering effect is more appreciated. Herbs with long term lipid lowering effect and diuretic side effects can replace the multi therapeutic medicines. The purpose of this systematic study was to explore lipid lowering plants with diuretic properties and their particular applications [1-24].

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MATERIALS AND METHODS

First, 2003 published papers including abstracts, full papers and 31 review papers were evaluated and were classified in two separate checklists. The first checklist was comprising information on plants with anti lipid effect along with their dosage and important consumption information. For this information, a comprehensive literature review on reliable databases such as PubMed, Google Scholar, Scopus, Medline and Web of Science was conducted using following main keywords: Botany in hyperlipidemia, Herbal in hyper cholestrolemia, Herbal therapy in hypertriglyceridemia, systematic Review of herbal in hyperlipidemia, natural remedies for hyperlipidemia, herbal medicine for cholestrolemia, herbal with anti lipid effect, herbal therapy for Atherosclerosis, Hyperlipidemia diet. In addition, for information on diuretic plants main keywords such as Herbal as diuretics, herbal therapy for edema, herbal medicine as diuretics, herbal diuretic list, herbal diuretic review, herbal tea as diuretic were used. At the end, using the tables, plants which had diuretic effect in addition to their lipid lowering properties were identified and were positioned into [Table 3].

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RESULTS

47 plants have high diuretic effects, including: *Aloysia citrodora, Ananas comosus, Anethum graveolens, Aparagus officinale, Artemisia vulgaris, Avena sativa, Braberis vulgaria, Boswellio carteri, Brassica rapa, Calendula officinalis, Chicorium intybus, Citrus limon, Cucumis sativus, Curcubita pepo, Cydonia oblonga,*

Daucus carota, Descurainia Sophia, Equiestum arvense, Ferula assa-foetida, Fisus carica, Hurdeum vulgare, Hypericum perforatum, Laurus nobilis, Medicago sativa, Mentha puregium, Morus alba, Morus nigra, Nasturtium officinale, Nepeta cataria, Nigella sativa, Petroselinum crispum, Phaseolus vulgaris, Pistacia vera, Pronus avium, Punica granatum, Purtolaca oleraceae, Satureja hortensis, Solanum lycopersicum, Solanum, melongena, Tea sinensis, Terminalia chebula, Tussilago farfara, Urtica dioica, Vicia faba and *Zea mays*. Other properties listed in [Table 1].

Achillea milefolium, Allium cepa, Allium sativum, Anethum graveolens, Apium graveolens, Arctium Loppa, Avena sativa, Braberis vulgaria,Brassica oleracea, Capsicum frutescens, Carthamus tinctorius, Chicorium intybus, Cicer arientinum, Citrus aurantifolia, Citrus aurantium, Commiphora mukul, Coriandrum sativam, Cornus mas, Cucumis melo L, Cucurbita pepo, Cynara cardunculus, Eleuthero coccus,Eugenia jambolana, Ferulago angulate, Ginko biloba, Glycine soja, Juglans regia, Malus orientalis, Miristica fragrans, Monascus purpureus, Musa paradisiaca, Oenothera biennis, Ocimum basilicom, Osmium sanctum, Orchis latifolia, Persea Americana, Plantago Lanceolata, Plantago ovate, Phaseolus unlgaris, Poryulaca oleracea, Prunus cerasus, Punica granatcm, Sipybum marinum, Solanum melongena, Solanum lycopersicum, Tamarindus indices, Thea sinesis, Terminalia chebula, Thymus volgaris, Trigonella foenum, Vaccinium myrtillus, Vitis vinifera, Zingiber officinalis and Zea mays had favorable effects on hyperlipidemia. Other plant characteristics are summarized in [Table 2].

20 plants containing both lipid-lowering effect and a diuretic, which includes: *Achillea milefolium, Allium cepa, Allium sativum, Anethum graveolens, Apium graveolens, Avena sativa, Braberis vulgaria, Chicorium intybus, Cornus mas, Ocimum basilicom, Plantago Lanceolata, Prunus cerasus, Punica granatcm, Solanum lycopersicum, Thea sinesis, Terminalia chebula, Thymus volgaris, Vaccinium myrtillus, Zingiber officinalis* and *Zea mays* [Table 3]. In addition to the scientific name of the plant, the common name, the use and safety of these plants in pregnancy is shown.

Table 1: Herbs with diuretic effects

Scientific name	Common name	Family	Part of used	Special Notes	Refrences
Achillea milefolium	Yarrow	Asteraceae	Leaf	Prohibited in pregnancy	1
Aloysia citrodora	Lemon verbena	Verbenaceae	Leaf	Do not use more than two grams.	2
Ananas comosus	Pineapple	Bromeliaceae	Fruit	The synergistic effect of anticoagulant drugs.	3
Anethum graveolens	Dill	Apiaceae	Leaf	Do not use herbal remedies contain this herb in pregnancy.	4
Aparagus officinale	Gorden Asparagus	Asparagaceae	Root	Prohibited in pregnancy and lactation	5
Artemisia vulgaris	Mugwort	Compositae	Leaf	Prohibited in pregnancy and lactation	6
Avena sativa	Oats	Germinaceae	Fruit	Don't use in celiac disease.	7
Braberis vulgaria	Barbery	Berberidaceae	Root and fruit		8
Boswellio carteri	Indian tree	Burceraceae	Resin	Maximum dose 2 g daily	9
Brassica rapa	Turip	Brassicaceae	Fruit , Seed	-	10
Calendula officinalis	Marigold	Compositae	Flower	Prohibited in pregnancy and lactation	11
Chicorium intybus	Chicory	Compositae	Root	May cause dermatitis	12
Citrus limon	Lemon	Rutaceae	Fruit	To be used for diluted	13
Cucumis sativus	Cucumber	Cucurbitaceae	Fruit	-	14
Curcubita pepo	Pumpkinseed	Curcubitaceae	Fruit	-	15
Cydonia oblonga	Quince	Rosaceae	Fruit	-	16
Daucus carota	Carrot	Umbelliferae	Fruit	Seed, don't use in hypertension and pregnancy.	17
Descurainia sophia	Flix weed	Brassiacaceae	Seed	-	18
Equiestum arvense	Horsetail	Equisetaceae		Do not use orally during pregnancy and latation	19
Ferula assa-foetida	Asafetida	Umbelliferae	Gum	Do not use orally during pregnancy and latation	20
Fisus carica	Fig	Moraceae	Leaf and fruit	Do not use if allergic to fig leaf acquired.	21
Hurdeum vulgare	Barely	Germinaceae	Seed	Don't use in celiac disease.	22
Hypericum perforatum	St.john's wort	Hypericaceae	Flowering branches	Do not use orally during pregnancy and lactation.	23
Laurus nobilis	Laurol	Lauraceae	Fruit	Do not use orally during pregnancy and lactation.	24
Medicago sativa	Alfalfa	Leguminaceae	Leaf	Dont use orally during pregnancy and lactation.	25
Mentha puregium	European pennyroyal	Labiateae	Leaf	The maximum dose of dried plant is 4 g/day. Do not use orally during pregnancy.	26
Morus alba	Block mulberry	Moraceae	Fruit, Leaf	-	27
Morus nigra	Block mulberry	Moraceae	Fruit, Leaf	-	28

Nasturtium officinale	Water cress	Brassicaceae	Leaf	Do not use orally during pregnancy and lactation.	29
Nepeta cataria	Catnip	Labiatae	Leaf	Do not use herbal remedies contain this herb in pregnancy.	30
Nigella sativa	Black cumin	Ranunculaceae	Seed	Black seed oil supplementation in pregnancy should be avoided.	31
Petroselinum crispum	Parsely	Umbelliferae	Leaf	Supplementary of this herb should be avoided in pregnancy.	32
Phaseolus vulgaris	Common bean	Leguminasae	Fruit	Contraindicated in gout.	33
Pistacia vera	Pistachio	Anacardiaceae	Fruit	-	34
Pronus avium	Cherry	Rosaceae	Fruit, Cherry tails	Cherry tails has potent diuretic effect.	35
Punica granatum	Pomegranate	Punicaceae	Fruit	-	36
Purtolaca oleraceae	Purstane	Purtulaceae	Leaf	-	37
Satureja hortensis	Savory	Labiatae	Leaf	-	38
Solanum lycopersicum	Tomato	Solanaceae	Fruit	Maximum 100 g	39
Solanum melongena	Brinjal	Solanaceae	Fruit	Contraindicated in acute asthmatic patient.	40
Tea sinensis	Tea	Theaceae	Leaf	-	41
Terminalia chebula	Haritali	Combretaceae	Fruit	supplementary in pregnancy should be avoided.	42
Tussilago farfara	Cast foot	Compositae	Umbelliferae	Do not use orally during pregnancy and lactation.	43
Urtica dioica	Nettle	Urticaceae	Root. Leaf	Do not use orally during pregnancy and lactation.	44
Vicia faba	Bell bean	Fabaceae	Fruit	Contraindicated in Favism and use caution with MAO Inhibitors.	45
Zea mays	Corn	Geramineae	Tasell	Potent diuretic	46

Table 2: Herbs with hypolipidemic effects

Scientific name	Common name	Family	Part of used	Special Notes	Refrences
Achillea milefolium	Yarrow	Asteraceae	Flower	Prohibited in pregnancy	47
Allium cepa	Onion	Liliaceae	Bulb	--	48
Allium sativum	Garlic	Liliaceae	Bulb	GI problems	49
Anethum graveolens	Dill	Apiaceae	Seed, Leaf	Prohibited in pregnancy	50
Apium graveolens	Celery	Umbellifera	Leaf	Asthma	51
Arctium Loppa	Burdock	Compositae	Root	Prohibited in pregnancy	52
Avena sativa	Oats	Germinaceae	Seed	Prohibited in pregnancy	53
Braberis vulgaria	Barbery	Berberidaceae	Fruit	Root and fruit	54
Brassica oleracea	Cabbage	Brassicaceae	Leaf	In hypertension and hypothyroidism	55
Capsicum frutescens	American peooer	Solanaceae	Fruit	Prohibited in pregnancy	56
Carthamus tinctorius	Saf flower	Compositae	Flower	Prohibited in pregnancy	57
Chicorium intybus	Chicory	Compositae	Root	May cause dermatitis	58
Cicer arientinum	Chickpea		Seed	--	59
Citrus aurantifolia	Bitter orange christm lime	Rutaceae	Fruit	Prohibited in pregnancy	60
Citrus aurantium	Orange	Rutaceae	Fruit	--	61
Commiphora mukul	Guggul	Burseraceae	Gum	Prohibited in pregnancy	62
Coriandrum	Coriander	Umbelliferae	Fruit	-	63

sativam					
Cornus mas	Cranberry	Cornaceae	Fruit	-	64
Cucumis melo L	Melon	Cucurbitaceae	Fruit	--	65
Cucurbita pepo	Pumpkin	Cucurbitaceae	Seed	Prohibited in pregnancy	66
Cynara cardunculus	Artichoke	Compositae	Leaf	Dermatitis susceptibility	67
Eleuthero coccus	Ginseng	Araliaceae	Rhizome	Prohibited in pregnancy	68
Eugenia jambolana	Eugenol	Myrtaceae	Leaf	Prohibited in pregnancy	69
Ferulago angulata	Schelecht	Umbelliferae	leaf	-	70
Ginko biloba	Ginko	Ginkgoaceae	Leaf	--	71
Glycine soja	Soy	Legomuminosae	Seed	Prohibited in pregnancy	72
Juglans regia	Walnut	Juglandaceae	Core	--	73
Malus orientalis	Apple	Rosaceae	Fruit	--	74
Miristica fragrans	Nutmeg	Myristicaceae	Seed	Prohibited in pregnancy	75
Monascus purpureus	Red yeast rice	Monascaceae	Seed	Liver disease	76
Musa paradisiaca	Miswak	Musaceae	Rhizome	--	77
Oenothera biennis	Evening primrose	Onagraceae	Seed	Prohibited in pregnancy	78
Ocimum basilicom	Basil	Labiatae	Leaf	--	79
Osmium sanctum	Bulacy	Labiatae	Leaf	--	80
Orchis latifolia	Orchis	**Orchidaceae**	Root	--	81
Persea americana	Avocado	Lauraceae	Seed , Fruit	--	82
Plantago Lanceolata	Plantain	Plantaginaceae	Leaf , Seed	Prohibited in pregnancy	83
Plantago ovate	Blond plotitago	Plantaginaceae	Seed	--	84
Phaseolus unlgaris	Commanbean	Fabaceae	Seed	Gout	85
Poryulaca oleracea	Purslane	Portulacaceae	Leaf	--	86
Prunus cerasus	Black cherry	Rosaceae	Fruit	--	87
Punica granatcm	Pomegranate	Punicaceae	Seed	--	88
Sipybum marinum	Milk thistle	Asteraceae	Fruit , Seed	--	89
Solanum melongena	Brinjal	Solanaceae	Fruit	Allergic effects	90
Solanum lycopersicum	Tomato	Solanaceae	Fruit	--	91
Tamarindus indices	Tamarind	Fabaceae	Seed	Prohibited in pregnancy	92
Thea sinesis	Thea	Theaceae	Leaf	Prohibited in pregnancy	93
Terminalia chebula	Common Thyme	Umbelliferae	Fruit	Prohibited in pregnancy	94
Thymus volgaris	Thyme	Labiateae	Leaf	Prohibited in pregnancy	95
Trigonella foenum	Fenugreek	Leguminosae	Seed	Prohibited in pregnancy	96
Vaccinium myrtillus	Bilberry	Ericaceae	Fruit	GI problems	97
Vitis vinifera	Blackcurran	Vitaceae	Fruit	--	98
Zingiber officinalis	Ginger	Zingiberaceae	Root	Cardiac arrhythmia	99
Zea mays	Corn	Gerameineae	Seed	--	100

Table 3: Hypolipidemic herbal with diuretic effects

Scientific name	Common name	Family	Part of used	Special Notes
Achillea milefolium	Yarrow	Asteraceae	Leaf	Prohibited in pregnancy
Allium cepa	Onion	Liliaceae	Bulb	--
Allium sativum	Garlic	Liliaceae	Bulb	GI problems
Anethum graveolens	Dill	Apiaceae	Seed, Leaf	Prohibited in pregnancy
Apium graveolens	Celery	Umbellifera	Leaf	Prohibited in acute asthma and other allergic reaction
Avena sativa	Oats	Germinaceae	Seed	Prohibited in pregnancy
Braberis vulgaria	Barbery	Berberidaceae	Root and fruit	Root and fruit
Chicorium intybus	Chicory	Compositae	Root	May cause dermatitis
Cornus mas	Cran berry	Cornaceae	Fruit	Safe in pregnancy
Ocimum basilicom	Basil	Labiatae	Leaf	--
Plantago Lanceolata	Plantain	Plantaginaceae	Leaf , Seed	Prohibited in pregnancy
Prunus cerasus	Black cherry	Rosaceae	Fruit	--
Punica granatcm	Pomegranate	Punicaceae	Seed	--
Solanum lycopersicum	Tomato	Solanaceae	Fruit	--
Thea sinesis	Thea	Theaceae	Leaf	Prohibited in pregnancy
Terminalia chebula	chebulic myrobalan	Umbelliferae	Fruit	Prohibited in pregnancy
Thymus volgaris	Thyme	Lmiaceae	Leaf	Prohibited in pregnancy
Vaccinium myrtillus	Bilberry	Ericaceae	Fruit	GI problems
Zingiber officinalis	Ginger	Zingiberaceae	Root	Cardiac arrhythmia
Zea mays	Corn	Gramineae	Seed	--

DISCUSSION

In this study it was discerned that great number of plants with evident impact on hyperlipidemia, have not demonstrated significant diuretic effects or it has not been reported yet. One of the possible reasons for lack of sufficient studies in this area is that studies on plants with therapeutic effects are newly emerged. Plants such as *Ferulago angulate, Moringa peregrina* and *Peucedanum membranacum* have significant effect on hyperlipedimia; however, studies on their diuretic effects need to be conducted. In addition, there are some plants which have demonstrated both diuretic properties and effects on hyperlipidemia, the most well-known of which are Onion, Garlic, Pomegranate, Dill, Chicory, Dandelion, Green Tea, Stinging Nettle, Yarrow, Parsley, Celery, Apple Cider Vinegar, Tomatoes, Cranberry. Among them, plants with permitted consumption in pregnancy such as cranberry and pomegranate have caught more attention. In our study, many clinical studies carried out that most of the plants: Garlic, Zinger, Chicory, Thyme, Pomegranate, Cranberry, Sumac and Lemon done that meet both the therapeutic effect.

CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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HERBAL MEDICINE AND PULMONARY DISORDERS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF UPDATED CLINICAL TRIALS

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ABSTRACT

Background: Recently, particular attentions have been focused the effective role of herbal drugs in chronic disease conditions, malignancies, and also allergic and inflammatory diseases. The current systematic review and meta-analysis attempted to summate recent evidences on the use of herbal drugs to treat various types of lung diseases in different nations. Methods: Studies were identified by searching electronic databases including Cochrane Library, Medline, Embase and Cinahl databases, and the Social Sciences Citation Index, scanning reference lists of included articles and consultations with experts in the field. Our sample is based on data published during recent five years from 2009 to 2014. Among 215 studies reviewed based on the included keywords, 48 med the study criteria and finally reviewed. Results: Overall, 15061 patients included into the analysis that among those, 11852 had COPD, 1324 were diagnosed to have non-small-cell lung cancer (NSCLC), 1012 suffered asthma, 492 had pneumonia, 172 had ARDS, 146 had radiation-induced pneumonitis, and 63 had lung contusion. The used herbal drugs could affect by different mechanisms including increase of pulmonary functional parameters including FEV1/FVC, PaO2/FiO2, and peak expiration flow rate as well as lowering inflammatory biomarkers such as cytokines of interleukin-6 (IL-6), interleukin-8, tumor necrosis factor-alpha, and transformation growth factor-beta1 leading improvement of clinical manifestations (indicated by lowering symptom score), increase of survival rate (in malignant states), reduce of ICU stay, reduce of ventilation time, improvement of quality of life, and lowering level of depression and anxiety. Conclusion: According to the pointed beneficial effects of herbal therapy, this option can be a good alternative for treatment with chemical drugs in various types of malignant, inflammatory, obstructive, and sensitivity-based pulmonary disorders.

KEY WORDS
Herbal medicine, pulmonary, meta-analysis, clinical trials

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INTRODUCTION

A long history of herbal medicine has been recorded to treat various types of diseases. By developing drugs derived from medicinal plants, the use of chemical drugs has been relatively declining [1]. In any individual culture, the materials used were those that were available within the geographical location and addressed local health concerns; however with exposing cultural traditions as well as overwhelming traditional medicine by modern scientific concepts, the use of herbal remedies is global regardless of geographic, ethnic, or cultural aspects [2,3]. The developments of chemically synthesized drugs have revolutionized healthcare services in whole of the word, but large sections of the population in developing countries still rely on traditional and herbal medicines for their primary care [4]. In Africa, up to 90% and in India 70% of the population tend to use traditional medicine to achieve healthcare needs. Besides, in China, herbal medicine accounts for about half of healthcare delivered and more than 90% of general hospitals in this country have especial units for herbal medicine [5]. Most importantly, using traditional medicine is not limited to developing countries so that during the recent decades, the interest in applying natural medicine has greatly increased in developed countries [6, 7]. This tendency has also expanded remarkably even in American and European countries so that about one-third of Americans [8, 9] and approximately 20% of Europeans [10, 11] tend to use herbal therapy. The most frequent reasons for applying herbal medicine include more affordability, more closely corresponding to the individual's ideology, allaying concerns about the adverse effects of chemical synthetic drugs, satisfying a desire for more personalized healthcare, and allowing greater public access to health information [12]. However, the use of herbal medicines is mostly considered unfortunately when conventional medicine is ineffective in the treatment of disease.

Recently, particular attentions have been focused the effective role of herbal drugs in chronic disease conditions, malignancies, and also allergic and inflammatory diseases. Recent investigations have been demonstrated beneficial effects of herbal medicine in many types of pulmonary diseases such as obstructive pulmonary lung disease (COPD), lung cancer, asthma, and even pneumonia in both children and adults. The current systematic review attempted to summate recent evidences on the use of herbal drugs to treat various types of lung diseases in different nations.

MATERIALS AND METHODS

Study population & data collection

Methods of the systematic review were specified in advance and documented in a published protocol in the International Prospective Register of Systematic Reviews (PROSPERO). Studies were identified by searching electronic databases including Cochrane Library, Medline, Embase and Cinahl databases, and the Social Sciences Citation Index, scanning reference lists of included articles and consultations with experts in the field. Our sample is based on data published during recent five years from 2009 to 2014 because of large volumes of studies on selected keywords and also knowledge of the latest results of studies on effects of herbal therapy in lung diseases. Only English language manuscripts and the manuscript with the available full texts were reviewed. Controlled vocabulary and keywords focused on "herbal medicine", "pulmonary disease", "lung", "herb" and "clinical trial". The clinical trial study design was only imposed. Studies were included regardless of study quality. The following data were extracted from each paper: country, description of the sample, type of pulmonary disease, type of herbal drugs, age range of patients, date of data collection, criteria measured, and standards used to judge quality, and the results. Two reviewers independently assessed studies identified by the search for eligibility based on the title and abstract. Selected full text papers were then assessed independently by the two reviewers. Among 205 studies reviewed based on the included keywords, 47 med the study criteria and finally reviewed [13-59].

Quality assessment of studies:

The selected studies were heterogeneous in terms of studied pulmonary disease, country, and scientific rigor. It was therefore inappropriate to aggregate or conduct a detailed quantitative analysis of the data. Instead, we assessed the studies using the following criteria: type of disease, patient's age range, sampling strategy, and sample size. These criteria were applied in a structured way to each of the selected studies. As for the data extraction, the quality assessment of the study methodology was performed. The quality criteria were found to be easy to apply to the papers and no differences were found between assessors. The main reviewed endpoint was the effects of different types of herbal drugs on pulmonary function status, symptoms score, inflammatory biomarkers, survival rate, as well as quality of life and depression-anxiety status.

Statistical analysis:

In meta-analysis phase, the k statistic was used to assess the agreement between two reviewers for study selection. The pooled relative risk (RR) was calculated for each outcome using the inverse-variance method for random effect, as well as for fixed effects [14]. The data heterogeneity was assessed using the Cochrane Q test via a $\chi 2$ test and quantified with the I2 test [15]. We used the log RR as the dependent variable. The log RR standard error was used to measure the within-study variability, and the residual maximum likelihood method was used to estimate the between-study variance. All analyses were performed using STATA version 11.0 (Stata Corp; College Station, TX) and SPSS version 21.0 for windows (SPSS Inc., Chicago, IL).

RESULTS

Among 205 records retrieved from the initial search, 47 studies were reviewed in full-text that all included in the meta-analysis. The inter-reviewer agreement for the study selection was high with k= 0.93. Overall, 15061 patients included into the analysis that among those, 11852 had COPD, 1324 were diagnosed to have non-small-cell lung cancer (NSCLC), 1012 suffered asthma, 492 had pneumonia, 172 had RDS, 146 had radiation-induced pneumonitis, and 63 had lung contusion. Two studies were published in 2014, 9 in 2013, 12 in 2012, 16 in 2011, and 7 in 2010. No obvious heterogeneity was also identified among the included studies ($x2 = 6.55$, p for $x2 = 0.428$; $I2 = 6.6\%$). In 11 studies, a combination of traditional Chinese herbal drugs was used as the intervention protocol. In studies which assessed the efficacy of herbal medicine on improvement of COPD, the use of this treatment protocol resulted in increase of FEV1/FVC ratio, reduce of inflammatory biomarkers such as interleukin-6 (IL-6), interleukin-8, tumor necrosis factor-alpha, and transformation growth factor-beta1, reduce of acute exacerbation frequency, reduce of lung symptoms score, increase of 6-min walking diameter, as well as improvement of health-related quality of live and reduce of depression-anxiety score. In patients who suffered NSCLC, using herbal drugs could considerably increase survival rate, improve quality of life, reduce inflammatory markers, reduce bone marrow suppression, and also reduce tumor markers. Those who were diagnosed to have asthma faced improvement of FEV1/FVC ratio, reduce of symptoms score, reduce of inflammatory biomarkers, increase of peak expiration flow rate, reduce of recurrence rate, increase of IgG immunoglobulin, and also increase the asthmatic drug effective rate. Herbal therapy could effectively result in increase of FEV1/FVC, reduce of inflammatory marker as well as reduce of ICU stay and ventilation time. In those patients with pneumonia, applying herbal therapy led to reduce symptoms score, reduce inflammatory biomarkers as well as improve level of quality of life. In the patients who experienced radiation-induced pneumonitis, herbal medicine could achieve increased Karnofsky Performance Status Scale (KPS) score and also reduced lung injury and dyspnea score. In a study that assessed the effects of

herbal medicine on acute respiratory distress syndrome (ARDS), PaO2/FiO2 index was successfully improved.

DISCUSSION

Many herbs and their,s compounds have been used for Asthma, Bronchitis and COPD [.60, 61].
Our review could show beneficial effects of different types of herbal drugs on a variety of inflammatory, sensitivity-based, obstructive, and even malignant pulmonary disorders in both children and adults. In this regard, reviewed clinical trials performed within recent six years showed high efficacy of herbal medicine in treatment of COPD, asthma, NSCLC, pneumonia, radiation-induced pneumonitis, and pulmonary contusion. In fact, the used herbal drugs could affect by different mechanisms including increase of pulmonary functional parameters including FEV1/FVC, PaO2/FiO2, and peak expiration flow rate as well as lowering inflammatory biomarkers such as cytokines of interleukin-6 (IL-6), interleukin-8, tumor necrosis factor-alpha, and transformation growth factor-beta1 leading improvement of clinical manifestations (indicated by lowering symptom score), increase of survival rate (in malignant states), reduce of ICU stay, reduce of ventilation time, improvement of quality of life, and lowering level of depression and anxiety. It seems that in some trials on disorders with immunosuppressive basis, the use of herbal drugs led to increase of serum immunoglobulin. In total, according to the pointed beneficial effects of herbal therapy, this option can be a good alternative for treatment with chemical drugs.

Besides the efficacy of herbal drugs on benign obstructive or inflammatory pulmonary disorders, the effects of herbs on cancer-related survival may be mediated by different mechanisms such as their anti-inflammatory effects (because inflammation is linked to increased risk of cancer), influence on carcinogen bioactive mediators such as cytochrome P450, alteration the proliferation of several cultured cancerous cells, as well as their antioxidant effects [62-67]. Of 177 drugs approved worldwide for treatment of cancer, more than 70% are based on natural products or mimetics, many of which are improved with combinatorial chemistry. Cancer therapeutics from plants include paclitaxel, isolated from the Pacific yew tree; camptothecin, derived from the Chinese “happy tree” Camptotheca acuminata and used to prepare irinotecan and topotecan; and combretastatin, derived from the South African bush willow [68]. More than 100 natural product–based drugs are in clinical studies [69], and of the total 252 drugs in the World Health Organization’s (WHO) essential medicine list, 11% are exclusively of plant origin [70]. However, herbal drugs against lung cancers are now under-assessed in world laboratories. Because of beneficial effects of herbal drugs, the side effects of these drugs should not be also ignored. Herbal extracts may be contaminated, adulterated, and may contain toxic compounds. The quality control of herbal medicines has a direct impact on their safety and efficacy. But, there is little data on the composition and quality of most herbal medicines not only due to lack of adequate policies or government requirements but also due to a lack of adequate or accepted research methodology for evaluating traditional medicines. In addition, there is very little research on whole herbal mixtures because the drug approval process does not accommodate undifferentiated mixtures of natural chemicals. These contents can be basis for further studies.

Table 1: literacher review

Author	Year	N. of patients	Diagnosis	Herb	Effect
Guo	2014	140	COPD	BuFei granule: Radix Codonopsis Radix spp Pericarpium Citri Reticulatae	↓ Inflammatory markers, QOL↑
Wang	2014	331	COPD	Herbal formulae	↑ FEV1/FVC, ↓nflammatory markers, 6MWD
Chen	2013	63	Contusion	Xuebijing: Cartami flos Angelica sinensis Saliva militoriza Radix spp	↑ FEV1/FVC, ↓nflammatory marker, ICU stay
Li	2013	75	Asthma	Herbal formulae: Sofora flavescnse Glycyrrhiza glabra Gonoderma lucidum	↓ Inflammatory marker, Survival rate
Li	2013	352	COPD	Bu-Fei Jiang-Pi: Astragalus propinquus Codonopsis pilosula Atractylodes macrocephala Poria cocos	↓ Symptom score, ↑OL, ↓ depression-anxiety

				Ging seng	
Tang	2013	151	Asthma	Yang Warming Aconite root Ging seng rubra Zingiber officinalis Glycyrrhiza glabra	↓ Symptom score
Li	2013	98	NSCLC	Herbal formula Tussilago farfara Rhus versini Morus alba Platycodon grandiflorum Perilla frutescens Prunus armenica	↑ Survival rate
Liu	2013	60	NSCLC	Scutellariae Barbatae Oregano spp	↓ Inflammatory marker
Tian	2013	100	Asthma	Ginger	↓ Symptom score
Cai	2013	100	COPD	Lung supportive Pulmonaria officinalis Oregano spp	↓ Symptom score
Li	2012	240	Pneumonia	TCM	↓ Symptom score, ↑ QOL
You	2012	91	NSCLC	TCM	↓ Bone marrow suppression, ↑ survival
Feng	2012	90	NSCLC	TCM	↓ Tumor marker, ↑ QOL
Xu	2012	180	NSCLC	TCM	↑ Survival, ↑ QOL
Wang	2012	504	COPD	TCM	↓ Symptom score, ↑ QOL, ↑ 6MWD
Liu	2012	172	ARDS	xuebijing	↑ PaO2/FiO2
Li	2012	120	Asthma	TCM	↓ Symptom score, ↓ inflammatory markers
Yao	2012	118	NSCLC	Lavandula officinalis	↓ depression-anxiety ↑ Expectorant
Tian	2012	60	Asthma	Glycyrrhiza glabra	Asthmatic drug effective rate
Park	2012	148	Asthma	Magnolia flos	↑ FEV1, ↑ peak expiration flow rate
Long	2011	133	NSCLC	Red Radix Ginseng Radix Aconitum Carmichaeli	↓ Symptom score, ↑ QOL
Li	2011	244	COPD	Zingiber officinalis Glycyrrhiza glabra	↓ Symptom score, ↑ QOL, ↑ 6MWD ↓
Han	2011	84	Asthma	Astragalus propinquus	Recurrence rate, ↑ IgG
Jiang	2011	50	NSCLC	TCM	↑ QOL
Guo	2011	136	NSCLC	Astragalus	↑ Survival, ↑ QOL
Li	2011	244	COPD	Bu-Fei Yishen Condopsis pilosopa Radix Astrix Pricarpium citri	↓ Symptom score, ↑ QOL, ↑ 6MWD
Shan	2011	91	NSCLC	TCM	↓ Symptom score, ↑ QOL
Xue	2011	168	COPD	panax ginseng	↑ FEV1/FVC, ↑ QOL
Kligler	2011	154	Asthma	TCM	↑ QOL
Yan	2011	74	NSCLC	Kangliu Zengziao	↓ Symptom score, ↓ inflammatory markers, QOL
Qi	2011	80	Pneumonia	Astragalus spp Cassia Twig	↓ Inflammatory markers
Wei	2011	60	Asthma	Chaipo granule Ganoderma lucidum Radix Sophora flavescens Radix Glycyrrhiza uralensis	↓ Symptom score, ↓ inflammatory markers

Xu	2011	121	NSCLC	TCM	↑Survival
Mukaida	2011	24	COPD	Desmodium triflorum	↓Symptom score
Jeong	2010	82	NSCLC	HangAm-Dan	↑Survival
Ye	2010	82	Pneumonia	Gingfeihuayu	↓Inflammatory marker
Xiao	2010	100	Pneumonitis	Liangxue	↑KPS score, ↓lung injury
Dou	2010	46	Pneumonitis	Dixiong	↑KPS score, ↓dyspnea score
Li	2010	90	COPD	Tanreqing	↓Symptom score, ↓Inflammatory markers, QOL
Liu	2010	90	Pneumonia	TCM	↓Symptom score, ↓Inflammatory markers
Rouhi	2009	76	Cough	Althea officinalis	↓Symptom score
Rouhi	2009	60	Asthma	Zingiber officinalis Althea officinalis	↓Inflammatory markers, ↑ QOL, Symptom score
Fazio	2009	9655 (5181 children)	COPD	Prospan® Ivy (Hedera helix)	↑ FEV1, ↑ peak expiration flow rate. Inflammatory markers

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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ARTICLE

A SURVEY ON THE CAUSES OF MEDICATION ERRORS FROM THE PERSPECTIVES OF PHYSICIAN, PHARMACISTS, NURSES, PARAMEDICS IN TEACHING HOSPITALS AND HEALTHCARE CENTERS OF CHAHARMAHAL VA BAKHTIARI AND PRACTICAL STRATEGIES TO REDUCE THEM

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ABSTRACT

Background and purpose: Medication errors represent a class of unforgivable human errors. Lack of national statistics and ethnographical studies of medication errors, and refusal to report these errors are some of challenges ahead. It is essential to detect these challenges to develop strategies to deal with committing or recommitting medication errors. This study was conducted to survey the perspectives of physicians and paramedics in Chaharmahal va Bakhtiari province about the causes of medication errors. Methods: First, 91 physicians and paramedics who attended a 2-day workshop in May, 2016 were randomly divided into eight groups of 10 people and a group of 11 people and asked to enlist medication errors. Then, the medication errors were recorded in a questionnaire consisting of items rated by 5-point Likert scale, with confirmed validity and reliability. Data were analyzed by descriptive statistics (frequency and percentage) and Fisher's exact test in SPSS 16. The level of significance was considered 0.05. Results: The errors related to physician bad handwriting (83.3%), physicians' moodiness (65.9%), and excessive fatigue (56.8%), new personnel's lack of familiarity with drug storage conditions (59.3%), pharmaceutical calculations (58.2%), drug prescription (55.6%), lack of compliance of pharmacological courses with pharmaceutical duties (58.1%), lack of pharmacists' mastery over scientific topics (58.0%), accessibility to pharmacist in three shift works (52.5%), and medication rounds (59.8%), drug shape confusion (56.8%), and caregiver's error (79.3%) were reported to be (very) highly and significantly important, and the frequency and causes of medication errors were different ($p<0.05$). Conclusion: Detecting the causes of medication errors in different wards and hospitals, and holding in-service training for nurses, physicians, hospital pharmacists, and patients caregivers can help prevent incidence of medication errors.

KEY WORDS
Medication errors, pharmacist, physician, nurse, patient caregiver

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INTRODUCTION

In all human communities, it has been acknowledged that human is likely to do wrong. Although medical errors are considered to be some kind of human errors, people do not tolerate any errors committed by medical community for several errors, which doubles the significance of this issue. Medication errors are a class of medical errors that all medical professionals have experienced at least once, and it is essential to pay attention to these errors [1]. A medication error refers to any preventable event that may cause or lead to inappropriate drug use or patient harm while this error can be related to the performance of health care professionals, pharmaceutical products, system, or processes including prescribing to using pharmaceutical product [2].

Each year, thousands of medication errors committed by medical and paramedical staff in the USA are reported. According to the latest figures, At least 100000 people die due to medication errors and side effects in the USA every year. The majority (49%-56%) of unintentional drug events have been reported to occur due to prescription errors committed by physicians [3]. Nurses and pharmacy personnel have been reported to commit 26%-34% of the medication errors [14]. In European countries, 19%-28% of inpatients are affected by medication errors. The primary and expectable outcome of such errors is the lengthened duration of hospitalization and increased expenses, and occasionally severe harm and even death [5].
According to evidence, one per five drug prescriptions in the USA is associated with medication error. Given the side effects of different drugs, the figures represent the depth of risk that threatens patients. A study demonstrated that only in two hospitals, 40 patients lost their lives due to medication errors and each patient during hospitalization was exposed to two medication errors by average [6].Therefore, collaboration among physicians, nurses, pharmacists, and other members of treatment team is essentially required. Undoubtedly, many of these errors remain unreported and real figures can be even more regrettable [7].Besides that, although incidence of these errors may be similar in most cases and highly consistent with the findings of studies conducted in other countries, it is not necessarily the same [8].
Therefore, it is essential to conduct ethnographical studies on medication errors in Iran. Moreover, many medication errors are left unreported in many cases for several reasons [9]. Therefore, indirect yet practical approaches can contribute significantly to preventing the repeated incidence of the errors. This study was conducted to ethnographically investigate incidence of medication errors in hospitals from the perspectives of physicians, nurses, pharmacists, and paramedics about the frequency of medication errors and strategies to prevent them.

MATERIALS AND METHODS

First, 91 general practitioners, teaching and non-teaching specialists working in hospitas, nurses, paramedics, and hospital pharmacists working in teaching health care centers, and university-affiliated and Social Security Organization-affiliated hospitals who attended a workshop on hospital safety principles were randomly assigned to 10 workgroups of 10 people each and one workgroup of 11 people and asked to write down the most important medication errors on a paper and deliver it to the workgroup leader. Then, with a statistician's assistance, the medication errors written down by the participants were listed, duplicate errors were removed, and a questionnaire consisting of items rated by 5-point (of very low importance, of low importance, of moderate importance, important, and highly important) Likert scale was developed to investigate the frequency of the medication errors. The validity and reliability of the items were investigated and confirmed. Then, data were encoded according to different hospital wards and analyzed by descriptive (frequency and percentage) and analytical (Fisher's exact test) statistics.

RESULTS

Of the 91 participants, 9 (10.8%) worked in ICUs and nursing office, 16 (19.3%) in orthopedics and surgical wards, 29 (34.9%) in neurology and internal wards, 23 (27.7%) in emergency and medical emergency wards, and 6 (7.3%) in ENT and ophthalmology wards. Eight people did not respond to the question "Which ward do you work in"?

Table1: Frequency and percentage of medication errors reported by the studied people

Row	Medication errors	Highly and very highly important	Moderately important	Lowly and very lowly important
1	Lack of physicians' mastery over drug interactions	35 (38.5)	47 (51.6)	9 (9.9)
2	Lack of physicians' full knowledge about food-drug interactions	38 (41.8)	44 (48.4)	9 (9.9)
3	Lack of physicians' full knowledge about drug incompatibilities (syringe-serum)	41 (45.6)	34 (37.8)	15 (16.7)
4	Physicians' bad handwriting	75 (83.3)	10 (11.1)	5 (5.6)
5	Moodiness and bad temper that cause stress to the nurse.	60 (65.9)	20 (22.0)	11 (12.1)
6	Lack of physicians' paying attention to drugs prescribed by advisory physicians	37 (41.6)	31 (34.8)	21 (23.6)
7	*lack of pharmacy's informing the staff about different drug shapes or changing them on time (e.g. **ENOXA 4000** instead of **ENOXA 8000**)*	48 (53.9)	33 (37.1)	8 (9.0)
8	Lack of nurses' mastery over different shapes of a drug (ophthalmic-cutaneous tetracycline, etc.)	36 (39.6)	31 (34.1)	24 (26.4)
9	Lack of physicians' correct diagnosis	42 (46.2)	35 (38.5)	14 (15.4)
10	Wrong dose	38 (41.8)	35 (37.4)	19 (20.9)
11	Lack of paying attention to patients' pharmaceutical history	42 (47.2)	34 (38.2)	13 (14.6)
12	Lack of paying attention to patients' physiological conditions (pregnancy, etc.)	34 (37.4)	31 (34.1)	26 (28.6)
13	Not labeling the drugs appropriately after dilution	39 (43.3)	27 (30.0)	24 (26.7)
14	Not implementing drug orders at appointed time	29 (31.9)	22 (24.4)	39 (43.3)
15	Injecting many drugs before conducting relevant tests	36 (39.6)	32 (35.2)	23 (25.3)
16	Lack of similarity between the same medical equipment (injection pump, insulin syringe, etc.) with different brands	27 (29.7)	39 (42.9)	25 (27.5)
17	Lack of familiarity with drug storage conditions	30 (33.0)	43 (47.3)	18 (19.8)
18	Lack of nurses' familiarity with pharmaceutical calculations	46 (50.5)	32 (35.2)	13 (14.3)
19	Lack of physicians' familiarity with pharmaceutical calculations	35 (38.5)	39 (42.9)	17 (18.7)
20	Lack of nurses' mastery over the methods of drug administration	37 (45.1)	24 (29.3)	21 (25.6)
21	Drug name confusion	42 (51.9)	21 (25.9)	18 (22.2)
22	Drug shape confusion	46 (56.8)	21 (25.9)	14 (17.3)

23	Handling beds by patients or caregivers	33 (41.8)	30 (38.0)	16 (20.3)
24	Patuents' or caregivers' prescribing drug by oneself or unknowledgeably	42 (51.9)	21 (25.9)	18 (22.2)
25	Nurses' fear of reporting medication errors	41 (50.6)	24 (29.6)	16 (19.8)
26	Lack of pharmacy managers' ward monitoring	47 (58.0)	26 (32.1)	8 (9.9)
27	Lack of technical officials' pharmaceutical round	46 (56.8)	30 (37.0)	5 (6.2)
28	Lack of technical officials' mastery over drug scientific materials	37 (46.2)	33 (41.2)	10 (12.5)
29	Lack of pharmacy staff's mastery over drug scientific materials	39 (48.8)	30 (37.5)	11 (13.8)
30	Medication errors due to wrong containers or labeling	35 (43.2)	25 (30.9)	21 (25.9)
31	Errors in transferring the physician's order from the medical file to the Kardex	32 (40.5)	28 (35.4)	19 (24.1)
32	Lack of injection of patient with sufficient dose of drug due to low quality injection products	32 (40.0)	25 (31.2)	23 (28.8)
33	Mistaking medical equipment in emergencies (blood collection set, serum collection set)	30 (37.5)	24 (30.0)	26 (32.5)
34	Lack of paying attention to expiry date and using it	32 (39.5)	25 (30.9)	24 (29.6)
35	Lack of physicians' mastery over drug gavage	30 (38.5)	20 (25.6)	28 (35.9)
36	Lack of nurses mastery over drug gavage	24 (30.0)	29 (36.2)	27 (33.8)
37	Lack of selecting appropriate solvent or diluter for venous infusion	28 (30.8)	21 (26.9)	29 (37.2)
38	Nurses' excessive fatigue	41 (51.2)	26 (32.5)	13 (16.2)
39	Physicians' excessive fatigue	46 (56.8)	21 (25.9)	14 (17.3)
40	Lack of faculty trainers' full knowledge about pharmacological materials	42 (52.5)	32 (40.0)	6 (7.5)
41	Lack of novice staff's familiarity with drug prescription	45 (55.6)	29 (35.8)	7 (8.6)
42	Lack of novice staff's familiarity with pharmaceutical calculations	46 (58.2)	24 (30.4)	9 (11.4)
43	Lack of novice staff's familiarity with drug storage conditions	48 (59.3)	25 (30.9)	8 (9.9)
44	Use of inappropriate abbreviations in physicians' prescribing drugs	35 (43.8)	30 (37.5)	15 (16.5)
45	Mismatch between the studied drug information and pharmacological materials during academic nursing studies and pharmaceutical duties in the ward after graduation	50 (58.1)	32 (37.2)	4 (4.7)
46	Physicians' incomplete order (e.g. prn or administration method)	46 (52.3)	37 (42.0)	5 (5.7)
47	Cordial belief in sensitivity of medication errors issue	45 (51.2)	34 (38.6)	9 (10.2)
48	Lack of paying attention to patients' physiological conditions (pregnancy, lactating, etc.)	41 (47.7)	34 (39.5)	11 (12.8)

From the perspectives of most participants, lack of implementing drug orders at the appointed time (43.3%) and lack of selecting appropriate solvent or diluter for venous infusion (37.2%) were of low or very low importance, and lack of physicians' mastery over drug interactions (51.6%), lack of physician's full knowledge about food-drug interactions (48.4%), lack of similarity between the same medical equipment (injection pump, insulin syringe, etc.) with different brands (42.9%), lack of nurses' familiarity with drug storage conditions (47.3%), lack of physicians' familiarity with pharmaceutical calculations (42.9%), and lack of nurses' mastery over medication gavage (36.2%) were moderately important.

Highly important and important causes of the medication errors, from the participants' perspectives, were physicians' bad handwriting (83.3%), physicians' moodiness and bad temper that cause stress to the nurse (65.9%), lack of novice staff's familiarity with drug storage conditions (59.3%), mismatch between the studied drug information and pharmacological materials during academic nursing studies and pharmaceutical duties in the ward after graduation (58.1%), lack of pharmacy managers' ward monitoring (58.0%), drug shape confusion (56.8%), lack of medication rounds by technical officials (56.8%), physicians' excessive fatigue (56.8%) (Figure 1), lack of novice staff's familiarity with drug prescription (55.6%), lack of pharmacy's informing the staff about different drug shapes or changing them on time (e.g.

ENOXA 4000 instead of ENOXA 8000) (53.9%), and lack of faculty trainers' full knowledge about pharmacological materials (53.2%).

Medication errors related to lack of physicians' familiarity with drug-food interactions, lack of physicians' familiarity with drug incompatibilities (e.g. syringe-serum), lack of pharmacy's informing the staff about different drug shapes or changing them (e.g. ENOXA 4000 instead of ENOXA 8000), lack of implementing drug order on the appointed time, drug shape confusion, and errors in transferring the physician's order from the medical file to the Kardex were significantly associated with the wards ($p<0.05$), and other errors were not associated with the wards ($p>0.05$).
Most participants working in the ICU and nursing office (66.7%), orthopedics and surgical wards (62.5%), and neurology and internal wards (58.6%) considered lack of physicians' full knowledge about drug-food interactions to be moderately important, most of them in emergency and medical emergency wards (60.9%) considered it to be highly important or very highly important, and most of them in ENT and ophthalmology wards (50%) considered it to be of low or very low importance.
Most participants working in the ICU and nursing office (66.7%), neurology and internal wards (62.1%), considered lack of physicians' full knowledge about drug incompatibilities to be moderately important, most of them in emergency and medical emergency wards (65.2%) and orthopedics and surgical wards (43.8%) considered it to be important or highly important, and most of them in ENT and ophthalmology wards (66.7%) considered it to be of low or very low importance.

Most participants working in the ICU and nursing office (55.6%), orthopedics and surgical wards (62.5%), and ENT and ophthalmology wards (66.7%) considered lack of pharmacy's informing the staff about different drug shapes or changing them on time (e.g. Enoxaparin 4000 instead of Enoxaparin 8000) to be moderately important, and most of them in neurology and internal wards (50%) and emergency and medical emergency wards (72.7%) considered it to be important or highly important.

Most participants working in the ICU and nursing office (62.5%), emergency and medical emergency wards (47.8%), and ENT and ophthalmology wards (83.3%) considered lack of implementing drug orders at the appointed time to be highly or very highly important, and most of them in neurology and internal wards considered it to be of low or very low importance (43.8%) and highly or very highly important (43.8%).
Most participants working in the ICU and nursing office (87.5%), neurology and internal wards (48%), and ENT and ophthalmology wards (100%), and emergency and medical emergency (52.4%) considered drug shape confusion to be highly or very highly important, and most of them in orthopedics and surgical wards (53.3%) considered it to be moderately important.

Most participants working in the ICU and nursing office (71.4%) and neurology and internal wards (56%) considered errors in transferring the physician's order from the medical file to the Kardex to be highly or very highly important, most of them in emergency and medical emergency (50%) considered it to be of low or very low importance, most of them in the ENT and ophthalmology wards (100%) considered it to be highly important, and 33.3% of them in orthopedics and surgical wards considered it to be of low or very low importance, 33.3% moderately important, and 33.3% highly or very highly important.

The errors related to physicians' bad handwriting, physicians' moodiness and bad temper, excessive fatigue, physicians' incomplete order, lack of observing prescription principles, and lack of taking into account previous conditions of the patient and advisory physician's order were the most frequent causes of medication errors committed by the physicians, and lack of novice staff's familiarity with pharmaceutical calculations and drug storage conditions, nurses' fear of reporting medication errors, drug shape and name confusion, lack of hospital pharmacist's monitoring and ward rounds, and lack of pharmacy's informing the staff about different drug shapes or changing them on time were the most frequent causes of medication errors committed by nurses and pharmacists [Table1].

DISCUSSION

The incidence of medication errors in hospitals is unavoidable, but frequency of these errors can be reduced by adoption of sensible and practical aporoaches and detecting the most common ones. Study of the type and causes of medication errors is the first step to prevent incidence of them.
Medication errors are a multidimensional issue and therefore, multidimensional approaches should be sought out to resolve them. In this study, lack of cordial belief in the sensitivity of medication errors issue and especially the nurses' fear of reporting medication errors were found to be highly frequent, which causes other medication errors to occur. Jolaei et al. study on medication errors committed by nurses and its association with working conditions in university-affiliated hospitals in Iran demonstrated the mean number of nurses' medication errors during three months 5.19 and the mean number of the reported errors 3.1 [10].

In the current study, physicians' fatigue and physicians' moodiness and bad temper were among the highly frequent causes of medication errors. Although problems in the incidence of medication errors in hospitals have already been reported, the frequency of such errors' causes, especially physicians' bad handwriting

has been reported to be much less frequent than that in our study. It seems that practical strategies including training prescription principles should be adopted to reduce medication errors.

In addition, lack of novice staff's familiarity with drug prescription, pharmaceutical calculations, and drug storage conditions after dilution were found to be among the most important causes of medication errors in this study. According to the findings of a study in Japan, the most important medication error committed by recently graduated nurses was wrong intravenous injection and the most important reason for this error low pharmacological knowledge [11]. leape et al. study demonstrated that 15% of medication errors committed by nurses are due to insufficient pharmacological knowledge [12].

This study also indicated that mismatch between the studied drug information and pharmacological materials during academic nursing studies and pharmaceutical duties in the ward after graduation in the ward had a highly important role in incidence of medication errors. Obviously, in the three-credit course of pharmacology in academic nursing studies, the main purpose is to achieve an introductory familiarity with drugs because of the density of the material and what is presented largely in the second and third terms, and fewer subjects on pharmaceutical calculations, drug storage and maintenance conditions, drug incompatibilities, and drug interactions are taught.

Besides that, in the current survey, lack of pharmacists' sufficient information and repeated ward rounds was reported to be an important cause of medication errors. Unfortunately, in pharmacology curriculum, full familiarity with hospital drugs or the minimum scientific skills of hospital pharmacy has not been taken into account. In addition, a study found that few refresher courses for hospital pharmacists has been considered by the relevant authorities and formal and scientific courses are deeply needed. Moreover, continuous training for physicians is needed to enhance hospital pharmaceutical care [13].

In the current study, drug name and shape confusion, e.g. Confusion of colors, shapes, names, and units of ceftriaxone and cefazolin is an example, was one of the highly frequent medication errors. Unfortunately, pharmaceutical companies have not yet take serious measures to assign different colors and packaging to different hospital intravenous drugs, but alas, this deficiency is on rise. Lack of injection of patient with sufficient dose of drug due to low quality injection products was one of the highly frequent medication errors. Furthermore, some products are usually reported when the product has been consumed, which causes a lot of problems [14].

Lack of nurses' mastery over pharmaceutical calculations was one of the frequently reported causes of medication errors. Studies have indicated that nurses need in-depth training about how to calculate the dose of injection soluble drugs, combination drugs, and additive drugs to serum. Kuzoowa et al. found that nurses faced certain problems due to not paying attention to the prescribed drugs doses, converting drug units erroneously, and not being able to apply their theoretical knowledge in clinical services [15].
Moccia et al. reported that 58% of nurses were unable to calculate pharmaceutical doses appropriately. Moreover, Bindler et al., consistent with Santanaria et al. study, reported that 81% of nurses were unable to calculate pharmaceutical doses appropriately [16-17].

Errors related to patients' care givers interference were reported to be highly frequent. Unfortunately, handling beds by patients or caregivers, prescribing drug by oneself or unknowledgeably, which was frequently reported, may cause incidence of bitter and occasionally fatal medication errors. These errors have been less frequently investigated to date, which doubles the significance of ethnographical study of medication errors. In addition, the causes of medication errors had simiar frequency in different wards.
According to this survey, first, although the hospital medication errors reported by the participants in this study were largely similar to those reported by previous studies, the causes of such errors in Iran are not necessarily similar to those in other countries and therefore it is necessary to conduct ethnographical studies on medication errors. Secondly, the incidence frequency of errors are not considered to have equal levels of importance in different wards, and the incidence frequency of each error should be closely determined in each ward and then basic strategies should be developed based on prioritization of preventing incidence of the errors.

Thirdly, the contents of nursing and pharmacy courses should be revised to make fundamental changes such that essential skills of hospital pharmacology are inserted in the curricula. Moreover, continuous and occasionally face-to-face training sessions should be held for pharmacists and nurses working in hospitals should be conducted. Moreover, short-term training at the onset of employment and introductory training on hospital medication errors errors should be conducted for novice staff and skills of hospital phamacy related to medication errors should be trained to pharmacists and physicians by officials in charge.
Conclusion: In the light of the findings of this survey, medical professionals in hospitals and teaching, treatment healthcare centers have low levels of information about hospital pharmaceutical calculations, serum-syringe drug incompatibilities, drug storage and maintenance conditions, and scientific principles of gavage therapy. Therefore, it is essential to train these subject matters to staff to reduce the incidence of medication errors.

CONFLICT OF INTEREST

There is no conflict of interest.

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None

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ARTICLE

DETERMINATION THE BACTERICIDAL AND BACTERIOSTATIC EFFECT OF HYDRO - ALCOHOLIC EXTRACTS OF THE AERIAL PARTS OF SATUREJA BACHTIARICA BUNGE ON STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS FAECALIS IN VITRO

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ABSTRACT

Introduction: As a result of excessive use of antimicrobial drugs in the treatment of infectious diseases, bacterial resistance against many antibiotics has increased also use of medicinal plants with antimicrobial effect has found its place in traditional medicine. According to the local sources of traditional medicine the medicinal plants offer a rich source of new antimicrobial agents. The effect of ethanol extracts of the aerial parts of (S. bachtiarica Bung) indigenous Weed of Chaharmahal and Bkhtiari on Staphylococcus aurous and Enterococcus faecalis bacteria has been studied. Method: In This laboratory research of Bakhtiari savory the aerial parts of ethanol extracts concentrations in the range 5 / 0-10 milligrams per ml was obtained by maceration method. Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) was performed using the binary dilution method. For statistical analysis SPSS version 18 was used, a significant level of P≤0.05 is intended and Vancomycin From antibiotics were used as a reference. Results: regards to the findings, over 2 and 4 mg/ml doses of MIC and 16 and 32 mg/ml doses of MBC were identified for Staphylococcus aurous and Enterococcus faecalis respectively. Conclusion: results of this study indicated that savory of Bakhtiari has remarkable antimicrobial effect on the bacteria Staphylococcus aurous and Enterococcus faecalis, which seems more studies in this field would achieve an alternative to synthetic antibiotics as antimicrobial resistance is increasing day by day. Further studies recommended to use a variety of clinical isolates for in vitro and in vivo assessment.

KEY WORDS
Enterococcus faecalis, Staphylococcus aurous, Savory of Bakhtiari, MIC, MBC

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INTRODUCTION

Confronting with agents of infectious diseases and poisoning is one of the most important health challenges because of the high outbreak and spread [1]. The indiscriminate use of antibiotics besides being expensive and not cost-effective to be produce, trigger larger problems such as antibiotic-resistant of any pathogens. On the other hand, long-term use of antibiotics and even cross-sectional, left behind side effects that are sometimes more dangerous than the disease itself. Hence, it seems essential to find new antibiotics with better performance and less side effects [2]. *Enterococcus faecalis* is facultative anaerobe, gram-positive, from streptococci and group D, which plays crucial role in nosocomial infections [3]. This bacterium is inherently resistant to many antibiotics. *Enterococcus faecalis* have been seen widely in root dental canal of teeth that have been under manipulation [4]. *Enterococcus faecalis*, would cause diseases such as endocarditic, bacteremia, urinary tract infections and meningitis in humans and has proved a variety of virulence factors [5]. Several virulence factors were describing in *Enterococcus faecalis* that includes Agg aggregation compounds, Enterococcus surface protein (Esp) and cytolysine (Cyl) that has bactericidal and hemolytic activity of gelatinize enzyme and assumed that these factors are the convergence by facilitating access to the quorum and setting active quorum-sensing and increasing the virulence. This leads to deeper tissue damage of bacterial invasion [6,7]. Recent studies have been shown that E. faecalis forms biofilm and quorum- sensing system controlling biofilm development in these bacteria [8]. Staphylococcus aurous is a gram-positive cocci that can be found in nosocomial infections as an important factor and in many diseases such as boils, toxic shock syndrome, endocarditis, osteomyelitis, etc. [9]. Due to indiscriminate use of antibiotics, pathogen resistance to available drugs is increasing [10]. In 1940 many strains of *Staphylococcus* were resistant to penicillin. After a decade the multiple resistant strains to tetracycline, chloramphenicol, and erythromycin have been reported. In 1960, Methicillin pickup an effective antibiotic for penicillin-resistant *Staphylococcus aurous* strains. But soon after, methicillin-resistant *Staphylococcus aurous* strains were observed [11].

The high prevalence of infectious pathogenic agents, antibiotic resistance and drug side effects caused today new approaches of using medicinal plants including their antimicrobial effects. As numerous studies on the antimicrobial effects of different plant extracts and essential oils are in progress [12].

Savory genus (*Satureja hortensis* L.) is one of the (Labiates) family. In Iran 14 species of annual and perennial herbaceous plant is available that grow in various areas of the country such as the provinces of Chahar Mahal and Bakhtiari, Lorestan, Khuzestan, Ilam, Kermanshah, Isfahan, northeast and some other parts. Iran endemic species are: *S. edmondi, S. sahandica, S. kallarica, S. Bachtiarica, S. Intermedia, S. Isophylla, S. Khuzestanica, S. Atropanata, S.rechingeri* and species of *S. mutica, S. macrantera, S.*

spicigera and *S.boissieri* have been seen also in Turkmenistan, Anatolia, the Caucasus, Transcaucasia and Iraq [13].
Species *S. bachtiarica* has a large dispersion in Iran and is collected from the western provinces, central and southwestern [14]. This kind of plant branched off to a height of 30-20 cm with a wooden base, short among nodes, early gray stems, downy fluff with a very short and soft, glandular spotted, flowering branches erect, thin, cylindrical, non-branched more or less branched, pale brown flowers are in clusters has several flowers [15].
This study aimed to determine the antimicrobial effects of hydro alcoholic extract of savory against standard strains of *Staphylococcus aurous* and *Enterococcus faecalis* Bakhtiar *in vitro* condition.

MATERIALS AND METHODS

Analyzing method

This experimental study was performed in 2015 at the University of Medical Sciences. Bakhtiari savory prepared from Shirmardi village of Lordegan was recorded by Professor of Pharmacognosy at the University of Medicinal Plants and identified with code 423-A.

Extraction

Dried hydro alcoholic (30:70) extract of savory Bakhtiari was prepared by maceration method and then condensed in Rotary and finally in the oven at 37 °C were dried.

Preparation of bacterial strains

Bacterial strains of microorganisms used in this study *S. aurous* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), was prepared in lyophilized form fungal and microbial Industry Research Center of Iran.

Determination of the lowest inhibition concentration (MIC) and minimum bactericidal concentration (MBC): The experiment of minimum inhibitory concentration was done in 96-cell sterile plate by broth dilution method (Micro broth dilution) so that 1,000 ml of the suspension was diluted 1 to 75 with a half-Mac into the pit 5/1 × 810cfu / ml Far land bacteria concentrations between 2 and 75 micrograms equivalent to 5,000 ml was added in Mueller Hinton broth. Controls were bacterial suspension in a row, and culture medium and concentrations of the extracts was poured in next row. Micro plates were incubated at 37 ° C for 24 hours. Subsequently, 10 ml tubes were cultured by Müller Hinton agar and incubated at 37° C for 24 hours to determine the MBC to MIC. Then, MBC, respectively after that results in the lowest concentration of the extract which turbidity observed resulting from bacterial growth in the target inhibitory concentration [16,17].

After collecting the data entered into SPSS software version 18 and P≤0. 05 was considered as significant level.

RESULTS

The results of the antimicrobial effect of hydro-alcoholic extract of Savory by extract distribution on the cell culture (all over) are shown in [Table 1]. These findings showed that this extract at a minimum concentration of 2 and 4 mg/ml were inhibited the growth of *Staphylococcus aurous* and *Enterococcus faecalis* respectively. It should be noted that the concentration of 2 mg/ml of the savory extract was chosen for onset of action based on the pervious researches have been done on this plant [18]. This concentration had inhibitory effect on the staph bacteria however 1 mg/ml concentration of the extract on has no considerable inhibitory effect on the growth of *Staphylococcus aurous* [Table 1].

Table 1: Minimum Inhibitory Concentration (MIC) of the extract from different concentrations of Savory Bakhtiari

Density Microorganism	1	2	4	8
Staphylococcus	-	+	+	+
Enterococcus	-	-	+	+

Sign (+) indicates lack of microorganism growth in cell culture and antimicrobial activity of ethanol extracts of savory. Sign (-) indicates bacterial growth in cell culture and absence of antimicrobial activity of savory extracts.
The findings from determination of minimal bactericidal concentration (MBC) of savory hydro alcoholic extracts on *Staphylococcus* and *Enterococcus* indicated that MBC for *Staphylococcus* and *Enterococcus* are respectively 16 and 32 milligrams per milliliter of savory hydro alcoholic extract [Table 2].

Table 2: Minimum bactericidal concentration (MBC) in different concentrations of Bakhtiari's savory extractin

Density Microorganism	2	4	8	16	32	64
Staphylococcus	-	-	-	+	+	+
Enterococcus	-	-	-	-	+	+

DISCUSSION

The aim of this study was to evaluate the antimicrobial effect hydroalcoholic extract of Bakhtiari's savory on standard strains of *Staphylococcus aurous* and *Enterococcus faecalis* in vitro. In this study, 2 mg/mL and 16 mg/ml doses were identified as MIC and MBC respectively for the *Staphylococcus aurous*. Also concentration over than 4mg/ml and 32mg/ml were presented for MIC and MBC of *Enterococcus faecalis* respectively. Regards to the results of the study, antibacterial effects of Savory extract on *Staphylococcus aurous* and *Enterococcus* was significant. In particular the most antibacterial effect was observed on *Enterococcus faecalis.*

According to study by Zarei et al. alcoholic extract of savory Bakhtiari indicated inhibitory effects on four pathogenic bacteria including: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aurous* and *Streptococcus agalactiae in vitro* [19].

In another study conducted by Habibian et al. alcoholic extract of savory Bakhtiari were showed antibacterial effect on pathogenic bacteria in red meat, involving Staphylococcus aurous and *E. coli* [20].
Also Savory Bakhtiari have indicated antimicrobial effect on the bacteria that cause cancer hair crown (Radio bacteria) in study was established by Ashrafi et al. [21].

According to Heydari et al. aqueous, ethanol and methanol extract of Savory Bakhtiari have antimicrobial effect on *Staphylococcus* epidermidis, *Streptococcus progenies* and *Pseudomonas aeruginosa* [22]. In another study, also conducted by Heydari et al. antibacterial effect of savory Bakhtiari's hydroalcoholic extract was proved on *Escherichia coli* and *Staphylococcus aurous* [23]. In our study, antibacterial effects Bakhtiari Mountain Savory against *Staphylococcus aurous* approved and for the first time, the effect of this plant against *Enterococcus* was confirmed.

In the survey conducted by Ansari et al. Savory essential oil of Khuzestan (*Satureja khuzestanica*) has showed significant antimicrobial effect on *Lactococcus garvieae* [24].

The Evaluation of antimicrobial effects of essential oils of two Savory species called *S. bachtiarica* and *Satureja khuzistanica* on a number of gram-positive and gram-negative was demonstrated positive antibacterial effects of the both. In addition this effect relates to the presence of phenol compounds such as caracole and thymol in the essential oil of these plants. Also in this study, which was conducted by Ahmad et al. in 2009 found that Savory essential oil of Khuzestan in have antimicrobial effects in both pre-flowering and flowering stage and Savory Bakhtiari essential oil is effective only before flowering. According to previous researches, total phenol compounds, thymol of Savory Bakhtiari before flowering (39%) was more than the flowering stage (31%), hence its antimicrobial effect is greater than the flowering stage [25,26].

According to studies have mentioned, extract of savory Bakhtiari seemed to have antimicrobial effects. Thus this study was investigated its effect on standard strains of *Staphylococcus aurous* and *enterococcus* which didn't carried out before.

Also, in the study of Pirbaloti et al. the antimicrobial impact of several plants on *Streptococcus* has been examined. SSEO is the mountain that one of the most valid plants having antibacterial effects with low MIC (39 micrograms per ml) was introduced. Their conclusion was also confirmed by this study [27].

the study of Mohammadpur et al. were compared three genus of Thymus and Ziziphora clinopodioides of Shiraz, savory essences of Bakhtiari was showed stronger antibacterial effect. In addition, comparing the diameter of the inhibition of this essential oil has been shown that it has significant inhibitory effect on *Candida albicans* [28]. In 2003 Shahin et al. were investigated anti-fungal properties of Savory's essential oil and were gain similar conclusion about the effectiveness of this plant on fungus [29].
Savory Bakhtiari's ethanol extract has antimicrobial effect against gram-negative bacteria such as salmonella typhoid and gram-positive bacteria such as *Enterococcus faecalis* demonstrated by Behbahani et al. 2014 [30].

In a study conducted by Bezic cuneifolia et al. essential oils with anti-bacterial and anti-fungal effects was studied against microorganisms including*, Bacillus subtilis, Enterococcus faecium, Staphylococcus aurous, Pseudomonas aeruginosa, Serratia, Candida albicans, Aspergillus fumigatus, Saccharomyces cerevisiae* [31].

In another study entitled, "Evaluation of the antimicrobial activity of ethanol extract of red peppers", amaranth and Savory against antibiotic-resistant *Staphylococcus aureus* were stated. In spite of

Staphylococcus aurous resistance to antibiotics, trimethoprim, ampicillin, erythromycin, penicillin, Cefixime and amikacin, herbs are effective against these bacteria; but compared with amaranth and Savory, red pepper extract showed higher antimicrobial activity [32].
Savory can be recommended for further studies and clinical trials as an anti-bacterial medications for control and treatment of infections.

Despite the fact that in many studies, the expression of antimicrobial effects of savory have been noted, in this study, we have investigated the effect of the plant on resistant bacteria, and the most common *Enterococcus faecalis* and among the study its effect on *S. aurous* was possibly less impressive.
Surely many plant compete with savory in the field of antimicrobial effects, but it doesn't reduce the importance of this plant (the native and the availability of savory and its success in multiple studies). According to the cited references it seems that savory could have been effective, not only in the field of bacterial infections but also fungal infections and could have been helpful in the treatment of some tumors. Antimicrobial Effects of various plant species is different thus, further investigation would appropriately identify effects of any ones.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

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ARTICLE

FORMULATION AND CLINICAL TRIAL STUDY OF AJMT CREAM IN TREATMENT OF ECZEMA

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ABSTRACT

Background and Purpose: Topical corticosteroids are first-line treatment for eczema, but the use of these drugs is followed by specific problems such as eczema, atrophy, depigmentation, and so on. In addition, continuous use of these medications leads to the reduced effectiveness of topical corticosteroids. These problems and a tendency to use herbal medicines inspired this study aimed at determining and comparing the effects of an herbal compound in the form of cream AJMT fenugreek (Trigonella foenum), chamomile (Matricaria chamomilla), walnuts (Juglans regia L), and marshmallow (Althae officinalis) with those of Fluocinolone acetonide on hand eczema.The aim of this study was Formulations andpreparation of herbal cream to treat eczema with minimal side effects. Methods: In this clinical trial, 64 patients with hand eczema that referred to the dermatology clinic of the University of Medical Sciences were randomly divided into two equal groups. One group was treated with AJMT cream, and the other received a two-week treatment with Fluocinolone acetonide. The results were analyzed using the McNemar and Chi square tests. Findings: The results indicated that the AJMT cream significantly improved the symptoms of burning, itching, redness, bumps, scaling and fissures, while Fluocinolone improved the symptoms of irritation and redness compared to before treatment (P<0.05). A comparison of the two groups indicated that after intervention, AJMT therapeutic effects on burning, itching, and redness were equal to those of Fluocinolone cream (P>0.05) and were significantly better on the symptoms of bumps, scaling, and cracke (P<0.05). Conclusion: Given the better effects of AJMT herbal cream than metal Fluocinolone acetonide on hand eczema symptoms and the long-term effects of topical steroids, use of the herbal cream AJMT is recommended.

KEY WORDS
Eczema, Chamomile, Marshallow, Fenugreek, Fluocinolone Acetonide,Walnut

INTRODUCTION

Eczema is the most common inflammatory disease of the skin [1], and hand eczema is considered the most abundant type of eczema (15%) [2-3]. About 2-10% of the world's population suffer from hand eczema. Because of contact with water and detergent, two times more women suffer from this disease than men. Eczema is also more prevalent among people under the age of 40 years [1]. It occurs because of discrete factors or a combination of factors, including internal ones, such as eczema naturally, or external factors, such as irritation or allergic eczema. Contact eczema, a simulative dermatitis, contracted through direct contact with foreign materials (such as water, soap, and detergent), leads to direct damage caused by the cell. In the allergic contact type of eczema, IV cell sensitivity is delayed in response to allergens (such as nickel, dyes, plastics, and perfumes) that are in direct contact with the skin [4-5].

Contact eczema comprises 90-95% of all occupational diseases; stimuli-caused eczema makes up 80% of cases, and 68% of skin problems cause career changes [6]. The most common types of hand eczema are by stimuli (35%), natural (22%), and allergies (19%) [1]. Moreover, for the past 50 years, topical corticosteroids have been used to treat skin diseases. Corticosteroid creams and ointments are often prescribed to relieve itching and inflammation caused by skin diseases like eczema. These materials prevent the release of the chemicals that cause inflammation [7-8]. However, long-term use of corticosteroids, especially high-power ones, could lead to systemic or local effects. Children are more prone to these complications. The local effects are greater than the systemic side-effects. The most common side-effects include skin atrophy, a temporary reduction in the use of pigments, and decreased immunity at the site of usage [8].

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Due to sensitiveness of a few patients to some of these drugs and many pathogens become resistant to them, scientists turn to natural and herbal remedies. Today, research in the field of herbal medicines, their prescription and use is expanding in countries throughout the world. The search for more effective drugs with less side effects is essential [9-10-11].

The aim of this study was Formulations and preparation of herbal cream to treat eczema with minimal side effects.

MATERIALS AND METHODS

This study is a double blind clinical trial including patients who were referred to the special clinic in Kashani Hospital. After receiving permission from the Ethics Committee and Deputy University of Medical Sciences and the consent of the participants, 64 patients with hand eczema were randomly divided into two groups of 32 patients each. After some patients withdrew from the study, 30 patients remained in each group. The first group was treated with the herbal cream AJMT, and the other group with Fluocinolone acetonide 2%. Patients were examined by a dermatologist before treatment and two weeks after treatment, and symptoms such as burning, itching, erythema level, papules and vesicles bumps, and fissures of the skin were evaluated and recorded in a questionnaire designed for this purpose. To avoid risk factors such as detergent, water, and soil, patients in both groups were provided with chemicals, the continued use of cotton gloves under plastic gloves, and other necessary training.

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The plan was to try to avoid any error, and the time of drug consumption was kept as near the same as possible in both groups. Patients in both groups were advised to apply a sufficient amount of cream twice a day to the affected area and that washing that area must be avoided.

Patients over ten years of age and having no systemic disease or other skin condition (such as infection or fungus) or a history of oral and topical medication use before and during treatment, and non-pregnant women were included in this study.

Extraction Methods and Herbal Cream Preparation: To prepare the cream AJMT, four medicinal herbs, fenugreek seeds, walnut leaves, chamomile and marshmallow root, produced by academic research in order to identify and then dry extract was prepared. Then based on final concentrations of the powder in the formulations, herbal extracts were prepared. Concentrations of these plants were as follows: 5% of fenugreek seeds, 5% of marshmallow, 5% of chamomile, and 5% of walnut leaves.

Cream formulation: An oil-in-water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol) were dissolved in the oil phase (Part A) and heated to 75°C. The preservatives and other water soluble components (methyl paraban, propyl paraban, triethanolamine, propylene glycol, ethanol extract of *Matricaria chamomilla*, *Trigonella foenum graceum, Juglans regia* L., and *Althaea officinalis*) were dissolved in the aqueous phase (Part B) and heated to 75°C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until the emulsifier was cooled [Table 1].

Determination of stability of formulation: Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The cream was poured into a bottle and kept in the humidity chamber, maintained at 32 ± 2°C/70 ± 5% RH, and 42 ± 2°C/80 ± 5% RH for two months. At the end of the experiments, samples were analyzed for their physical properties and viscosity and other physicochemical tests that are shown in [Table 2].

At baseline, this project is divided into two groups of healthy volunteers and, treatment was continued in the usual way. The information collected from the two groups of patients at different stages of treatment using indicators was subjected to McNamara and chi-square analyses.

Characteristics of plants used in this study

1. Marshmallow (*Althaea officinalis*) is an herbaceous plant that grows to a height of 2 m. Its roots, leaves, and flowers are used in herbal medicine. It affects smooth skin irritation, and the marshmallow root has anti-inflammatory properties [12-14]. Marshmallow is one of the most important medicinal plants. Althea mucilage compounds (plant mucilage) include sugars, starch, pectin, and other ingredients. This plant used to treat skin diseases such as eczema.

2. Walnut *(Juglans regia* L.) is a beautiful, base tree whose leaves, bark, and buds are used in herbal medicines. It's most important compounds include Tanen, Zhoglon, naphthoquinone, and vitamin C. Walnut can be used to treat eczema along with the secretion of anti-inflammatory and astringent properties [15]. Cream 2% is used for external treatment [16].

3. Common chamomile (*Matricaria Chamomilla*) is an annual herbaceous plant growing 20 to 80 cm in height. The flower color is greenish-yellow, and the flowers are used in making medicine. Chamomile and its extract are the most widely used plant in herbal treatment in the world [17]. This plant grows in some areas of Lorestan and Khuzestan, Iran. Its flowers have essential oils (Recubizul, Farnesol, and Chamazulene), Taten, glycosides, and flavonoids. Chamomile is used for the treatment of dermatitis, urine burns, pediatric rash, and cracking of the nipple (16). Its antibacterial and antifungal properties are used [18] for external treatment at concentrations of 3-10% [19].

4. Fenugreek (*Trigonella foenum graceum* L.) is an annual plant that grows up to 50 cm tall. Its grain is used and contains mucilage, sapogenin, aromatic materials, and large amounts of iron and phosphorus. It also contains an alkaloid called trigonellin at the index amount. Plant mucilage compounds have healing, softening, and anti-inflammatory properties and can be applied topically, particularly to treat eczema [20].

Given the high prevalence of hand eczema and its complications, common medications (topical steroids) have a relatively small effect; therefore, there is a growing tendency to use natural remedies. This study examined a total of four plants, each having anti-inflammatory, emollient, astringent, and anti-bacteria and fungi properties and being effective against eczema. This study aimed to determine the efficacy and effects of herbal creams AJMT and compare them with those of fluocinolone acetonide on hand eczema in patients referring to a dermatology clinic in Shahrekord.

RESULTS

In the group treated with herbal cream AJMT, 60% of participants were females (n=19) and 40% were male (13 persons). Thirteen patients (40%) were housewives, 6 (20%) were construction workers, 3 (10%) were farmers, and 10 (30%) had other jobs. The most frequent age was 30-40 years old. The group treated with Fluocinolone acetonide cream comprised 30 patients with eczema, 21 of whom (70%) were female and 9 (30%) were male; 15 (50%) were housewives, 6 (20%) were farmers, and 10 patients (30%) had other jobs. In terms of demographic characteristics, the two study groups were not significantly different (P> 0/05).

Table 1: Composition of cream

Material	% of material in formulation W/W
Dry extract of *T. Foenum*	5
Dry extract of *M. Camomilia*	5
Dry extract of *J. regia*	5
Dry extract of *A. officinalis*	5
Cetyl alcohol	3
Stearic acid	12
Glycerol	4
Methyl paraben	0.02
Tri ethanolamine	Qs
Water	Qs

Table 2: Physical properties of AJMT cream

pH of the Cream	Viscosity	Acid value	Saponification value	Homogeneity	After feel	Irritancy test	Appearance	Removal
6.35±2	28001±20	6.3±2	29.1±0.7	Good	Emolient	Not reaction	No change in color	Removed by washing with water

Table 3: Comparison of AJMT cream and fluocinolone cream in the treatment of eczema

	AJMT				Fluocinolone Acetonide			
Clinical signs	Before treatment		After treatment		Before treatment		After treatment	
	Percent	Number	Percent	Number	Percent	Number	Percent	Number
Burning and itching	80	25	10	3 *	90	29	0	0 *
Red	80	25	20	6 *	90	29	10	3 *
Ness **	40	13	10	3 *	80	25	60	19
Papules **	10	3	0	0	20	6	0	0
Scaling **	80	25	30	10.	100	32	80	25
Fissure **	60	19	10	3 *	70	22	60	19

*P>0.05 before treatment between the two groups in all variables
**P<0.05 between the two groups after treatment.
-AJMT Cream (combination of extracts of fenugreek, chamomile, walnuts, and marshmallow).
n = 30 in each group.

McNemar's test indicated that in the group treated with AJMT, all symptoms except papules, i.e. burning, itching, redness, bumps, scaling, and fissures, were significantly different before and after treatment (P<0.05), and recovery was achieved. Fluocinolone also resulted in statistically significant differences before and after the intervention for symptoms of skin irritation, itching, and redness (P<0/05).

A comparison of the two groups using the chi-square test indicated that after treatment, the therapeutic effects on burning or itching and redness AJMT and Fluocinolone had no statistically significant difference (P>0.05), while the therapeutic effects in the group treated with AJMT on symptoms of bumps, papules, scaling, and fissures were significantly better than those of the group treated with Fluocinolone (P<0.05) [Table 3].

DISCUSSION

The results indicated that in the group that used AJMT, except for papules signs, other symptoms such as burning, itching, redness, and so on, were significantly different after treatment. In the group using Fluocinolone, the symptoms of irritation and redness had statistically significant differences before and after intervention, but these differences were not significant for other symptoms.

A comparison of the two groups indicated that after intervention with AJMT, burning or itching and redness in the AJMT and Fluocinolone group had no statistically significant difference in symptom relief, while the

therapeutic effects of AJMT on scaling and fissures were significantly better than in the group treated with Fluocinolone.

Several studies have shown the twofold properties of medicinal plants in the treatment of eczema. For example, Paller compared plant Tacrolimus ointment and pimecrolimus cream and found the Tacrolimus ointment to be more effective in the treatment of eczema, but the effects of these two drugs are identical [22]. This study shows that this compound is effective on the treatment of eczema.
In the results of Tai in a study of herbal medicine, Sanfujiu indicated that, in the treatment of eczema, the plant was effective in 6.44% of patients, and 52.1% of them had no effects from treatment [23].
Based on the study findings, it seems that the herbs used in cream AJMT somehow have anti-inflammatory properties. Fenugreek has skin-softening properties. The plant is used as a poultice to treat inflammation of the skin [24]. Moreover, it is used as a skin cleanser and to treat wounds and abscesses [25].

Pereira et al. determined that the walnut has antifungal and antimicrobial effects, and walnut leaves are claimed to have antioxidant properties [26]. Walnut has Zhaglon (a naphthoquinone) that is effective in treating cutaneous and especially fungal diseases of the skin and dermatitis, such as rash and hives [14]. It seems that a walnut is likely to prevent secondary infection; it is effective in patients with eczema and prevents patients from becoming resistant to the usual treatment.

Marshmallow is also effective in relieving irritation and has a softening effect. The root has anti-inflammatory properties that can be used in the treatment of burns. [14]. In general, flowers and plant roots can be used as a skin moisturizer, anti inflammator and increase the water used on the skin [25].

The anti-itch effect of combining AJMT cream is probably from the chamomile compounds. chamazulene, The sesquiterpene compounds, bisabolol, and flavonoids in chamomile extract have anti-inflammatory and anti-allergy effects. Azole compounds in the essential oil of the plant inhibit the release of histamines and possibly play a major role in the treatment of dermatitis and itching. Skin cream Chamomile (MC) is now available and used as the treatment for skin inflammation, dryness, and cracked skin. [17]. This plant has antimicrobial, regenerative, and antioxidant properties that allow its topical use in the treatment of wet eczema, impetigo and open wounds [27-28-29].

One reason for the different therapeutic effects of herbal creams AJMT and topical steroids on the same characteristic may be the chamomile. Unlike corticosteroids, this plant has antibacterial and skin-healing properties while as mentioned earlier topical corticosteroid effects of this plant are the photos.

Gharavi et al. used a combination of cornflower extract, mallow, chamomile, and marigold in the treatment of disorders of skin dryness. They found that this combination was effective in treating skin roughness [29]. Kazemipour et al. combined garlic, chamomile, and marshmallow in healing surface wounds of the common carp. They came to the conclusion that this combination is effective in healing such ulcers [30]. These studies confirm that marshmallow and chamomile may also be effective in combination AJMT on both inflammation and skin dryness.

Evaluating the anti-inflammatory effects of chamomile cream (MC) and steroids and non- steroid drugs in the treatment of dermatitis, Aertgeerts came to the conclusion that chamomile cream (MC) of both non-vegetarian combination was more effective [31]. The findings of this study were met with recent research direction.

In the present study, the efficacy of chamomile, Marshmallow, walnut leaves, and fenugreek was studied in the treatment of dermatitis that does not exist and thus the effectiveness of this combination of properties inflammatory, antimicrobial, healing, antioxidant and emollient is concerned on these plants.

No overall features exist in a single plant and the cream Fluocinolone alone, and the results may be due to the properties of the composition of the herbal cream. Since chronic eczema requires the long-term use of topical medications such as cortisone and steroids and because all symptoms were resolved with no known long-term side effects from these drugs, such as discoloration of the skin, atrophy, striae and secondary infections, and so on, it can be claimed based on the findings that the herbal cream AJMT is one of the most convenient and effective treatments, and very few side effects in the treatment of hand eczema were seen. At the same time, the need for further investigation and follow-up studies regarding long-term use of the drug is acknowledged.

CONCLUSION

The results show that both Fluocinolone acetonide and cream AJMT can instigate the partial recovery of hand eczema lesions, but the long-term use of corticosteroid can cause skin complications. Because the combination of plants in AJMT improved some symptoms of hand eczema to a greater extent than topical Fluocinolone, it is advised that cream AJMT can be used for the treatment of hand eczema.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

AN INVESTIGATION OF TREES AND SHRUBS IN KERMANSHAH AND NORTHERN ZAGROS REGION

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ABSTRACT

Kermanshah is located at west of Iran which most part of it is covered by range of Zagros. According to the latest statistics, latitude of Kermanshah forests has been estimated to be about 500000 Hectares. If correct, 20 % of the provinces are covered by forest. Zagros forests are spread in 11 provinces of Iran with a latitude about 6 million Hectares which comprised 40 % of Iran's forests. Among these 11 provinces located in growth zone of Zagros, Fars, Lorestan and Khozestan are at first, second, and third place, respectively, in terms of growth latitude. Kermanshah has a cold and dry climate according to amperage to formula. It is mountain region with average annual precipitation of 414/72 mm and average high temperature of 37 ºc in July while average low temperature is – 20 ºc in December. Herbal cover was investigated by floristic method and then biological shapes and spread of each plant was identified. Among most dominant trees and shrubs in Zagros are: Daphne, Q. libani Quercus brantii Var persica, Q. infectoria. Amygdalus prientalis subsp. orientalis, Acer monspessulanum subsp. cinerascens mucronata, cerasus microcarpa subsp. Tortuosa. Species that are seen in the collection just once: Pyrus glabra, Ficus carica var. rupestris, Lonicera nummularifolia, Pistacia attlantica subsp kurdica, Rhamnus kurdica var. persica. The most known species under – investigation region are a part of Iranian – Toranian Ones. The number of each species in the region shows that all species are more than 1700 SPP.

KEY WORDS
Kermanshah, Zagros forest, geographical spread, biological shape, Flora

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INTRODUCTION

Forest is comprised of a group of herbs and trees which are in balance with their own environment. In silvers, forests are a society of trees as its dominant members. Alongside trees, there are shrubs, bush, small trees, and alive grass cover, useful and useless animals which are always under influence environment [1-5]. A person who studies forest should have practical experiences along ecological knowledge and he should be in the know about his job place that is base or habitat. Zagros forests as the widest are spread in 11 provinces with an area of 6 million Hectares and comprises 40 % of Iran's forests [6-8]. Among these 11 provinces located in growth region of Zagros, Fars, Lorestan and Khozestan have first, second and third place in terms of trees and forests habitats. Kermanshah, in western Iran, is a mountain region which is covered by a part of Zagros. According to the latest data, latitude of Kermanshah s forests has been estimated to be about 500000 Hectares. If correct, 20 % of the provinces are covered by forest. The forest in south part is spread over forests of Ilam, Lorestan and Bakhteyari. It connects northern forests of Iraq from west and western north combined with forests of Kurdistan. The Kermanshah province is comprised of 14 counties, 31 districts and 86 rural districts. Zagros forest is the widest ones in Iran are spread in 11 provinces with an area of 6 million hectares [7-10] which is 40 % of all forests in Iran. Forests of northern zagros are spread from the north part of Iranshahr in western Azarbijan to Shaho crest across Kermanshah and Kurdistan's boundaries. Northern Zagros is cold and snowy. The average of annual precipitation is 414/72 mm and the average of high temperature is 37 ºc in July the average low temperature is – 20 ºc in December. Fig. 1 shows rain curve and temperature of Kermanshah region.

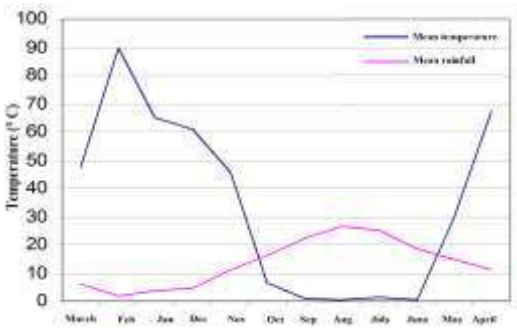

Fig.1: Rain and temperature of Kermanshah region.

MATERIALS AND METHODS

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To investigate the flora in the region, the plants were collected in different parts from early April 2013 to the end of January 2014. In this study, perfect and healthy samples were used. After herbarium preparation, all samples were investigated in herbarium of Payamenoor University. Then in agricultural faculty of Razi University in Kermanshah. The identification was done by various sources: Yazd flora [14] and Iranica flora [19], names of plants dictionary [14], Iraq flora [21], Turkey flora [22], growth elements [6], common families and genus of flora in Iran [14], and Oalaho flora in Kermanshah [13].

RESULTS AND DISCUSSION

Floristic investigation in Zagros region shows that subspecies of *Acer monspessulanum* L. grow in all parts of the region in forests of western Azarbijan, Kordestan, Kermanshah, Lorestan, Chaharmahal, Bakhtiari, Kohgiluyeh and boyer – ahmad, Isfahan, and Khozestan. Table 1 and -2 give the species found under this study.

This species regenerates in two generic or non-generic ways. It has a great capacity for propagation or regeneration of coppice scrub. It is light – orienting and resistant to lights of heights. Among land forms, valley has the best condition for this species. Also, it tends towards west more than other geographical directions. In terms of soil, the best conditions for this kind are habitats with a remarkable percentage of lime, carbonate and bicarbonate with magnesium, phosphorus and nitrogen in lower layers. Iranian oak is a common species in Kermanshah's forests which has changed from seed plants to coppice due to excessive destruction.

Table 1: Species and family name of plant in area

Family	Taxon	Karyotype
Aceraceae	*Acer monspessulanum L.*	IT-ES
Aceraceae	*Acer negundo L.*	IT-ES
Anacardiaceae	*Pistacia atlantica Desf.*	IT
Anacardiaceae	*Pistacia mutica Fisch.et mey.*	IT-SS
Caesalpinaceae	*Cercis siliguastrum L.*	IT-ES
Campanulaceae	*Campanula erinus L.*	IT-SS
Caprifoliaceae	*Loniceranumm ulariifolia jaub.et spach.*	IT-SS
Corylaceae	*Corylus avellana L.*	IT

Table 2: Species and family name of plant in area

Family	Taxon	Karyotype
Cupressaceae	*Platycladus orientalis (L.) Franco*	IT
Fagaceae	*Quercus brantii Lind L.*	IT
Fagaceae	*Quercus infectoria olive. roy, Emp*	IT
Fagaceae	*Quercus Libani*	IT
Juglandaceae	*Juglana regia L.*	IT-ES
Loranthaceae	*Loranthus europaeus Jacq Enum Stirp*	IT
Loranthaceae	*Loranthus grewinkii Boiss et Buhse*	IT
Moraceae	*Ficus carica*	IT-ES
Moraceae	*Morus nigra*	
Moraceae	*Morus alba L.*	IT-ES-SS
Oleaceae	*Fraxinus rotundifolia(Foangustifolia Vahi) L.*	IT-ES
Oleaceae	*Ligustrum Vulgare L.*	IT
Papilionaceae	*Spartium junceum L.*	IT-ES
Pinaceae	*Pinus eldarica Medw.*	ES
Plantaceae	*Platanus orientalis L.*	IT
Punicaceae	*Punica grantum L.*	IT-ES
Rosaceae	*Amygdalus haussknechtii (c.k Schneider.) Bornm.*	IT
Rosaceae	*Amygdalus lycioides Spach Var.*	IT
Rosaceae	*Amygdalus scoparia spach.*	IT
Rosaceae	*Cerasus Vulgaris Miller, Gard.*	IT-ES
Rosaceae	*Crataegus pontica C.koch*	IT
Rosaceae	*Cydonia oblong Miller, Gard.*	IT
Rosaceae	*Rosa sp.*	IT
Rosaceae	*Rosa sp.*	IT
Saliacaceae	*Populus caspica Bornm*	IT-ES
Saliacaceae	*Salix acmophylla Boiss.*	IT-ES
Saliacaceae	*Salix alba L.*	IT-ES
Saliacaceae	*Salix excels J.F. Gmel*	IT-ES
Tamaricaceae	*Tamarix sp*	IT-ES
Thymelaece	*Daphna mucronata Royle.*	IT-ES
Vitaceae	*Vitis sylvestris Gmelin*	IT

Ulmaceae	*Celtis caucasica Willd.*	IT
Ulmaceae	*Ulmus campetris L.*	IT-ES

Among common trees and shrubs species are: *Quercus brantii*, *Q. infectoria, Q. libani,* var *persica, cratagus pontica, Daphne mucronata, Cerasus microcarpa* subsp. *Tortuosa, Acer monspessulanum* subsp. *cinerascens, Amygdalus orientalis subsp orientalis.*

Species which have been in the collection once: *Ficus carica* var. *rupestris, Lonicera nummularifolia, Pistacia atlantica* subsp *kurdica, Pyrus glabra, Rhamnus kurdica* var *persica*

The Zagros's oaks is spread from western and eastern Azarbijan to Bakhteyari forests and western south of Iran. The western Azarbijan is full of *Quercus*. The species are *Q. brantii* in Sardasht and *Q. infectoria* and Q. libanii and profound in western Azarbijan.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

OUTCOME OF PRIMARY PERCUTANEOUS CORONARY INTERVENTION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASES IN CENTRAL IRAN, ISFAHAN

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ABSTRACT

It is still unclear whether chronic obstructive pulmonary disease (COPD) has an impact on the outcome of patients with ST-segment elevation myocardial infarction (STEMI) who underwent primary percutaneous coronary intervention (PCI). Accordingly, the present study aimed to evaluate the incidence of COPD in patients with ST-segment elevation myocardial infarction who underwent primary PCI and also its role to predict short-term and long-term clinical outcome in these patients following primary PCI. In this prospective study, 336 patients with acute STEMI undergoing primary PCI at our institute were consecutively included into the study. Of the 336 patients recruited in this study, 45 (13.4%) fitted the criteria of COPD. Both in-hospital and one-year cumulative mortality rates were higher in COPD than in non-COPD groups; however the length of stay in hospital did not differ between them. Among long-term cardiac events, the incidences of recurrent MI and readmission for heart failure were significantly higher in COPD than in non-COPD group. Univariate Cox regression analysis demonstrated that the baseline variables, including advanced age, female gender, and the presence of diabetes mellitus as well as COPD were strongly predictive one-year MACE. In multivariable Cox analysis, COPD showed a strong positive correlation with one-year MACE. In conclusion, COPD is an independent predictor of one-year MACE for patients with STEMI undergoing primary PCI.

KEY WORDS
pulmonary diseases, central Iran, Isfahan, chronic

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common finding in patients with ischemic cardiac events and also can be frequently occurred following cardiac revascularization [1-3]. This clinical condition can result in high mortality and morbidity for hospitalized patients in various disease settings and it has been demonstrated to be cause of surgery-related life-threatening evens in those with acute myocardial infarction (AMI). COPD is a chronic inflammatory phenomenon involving only the lung parenchyma and is also accompanied with increased level of circulating inflammatory biomarkers, including cytokines and C-reactive protein [4-6]. Because various types of disorders such as cardiovascular diseases have an inflammatory basis, the occurrence of COPD as an underlying event may affect the severity and extension of the diseases [7, 8]. Of particular importance is that ischemic heart disease has been reported to be a leading cause of death in patients with COPD [9, 10]. Indeed, the similarity between cardiovascular diseases and COPD are noteworthy [11, 12]. Recent clinical studies have revealed that cardiac ischemic event and COPD tend to coexist and share similar prevalence in the population [13-16]. Additionally, coronary artery disease (CAD) has been shown to be the main cause of death in COPD patients [17, 18]. COPD has been identified as an independent predictor of death after MI [7,19-22]; however, a number of patients suffered MI is scheduled for undergoing primary percutaneous coronary intervention (PCI) as a well-recognized therapeutic strategies for reducing long-term poor outcome in these patients. Hence, it is still unclear whether COPD has an impact on the outcome of patients with ST-segment elevation myocardial infarction (STEMI) who underwent primary PCI. Accordingly, the present study aimed to evaluate the incidence of COPD in patients with ST-segment elevation myocardial infarction who underwent primary PCI and also its role to predict short-term and long-term clinical outcome in these patients following primary PCI.

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MATERIALS AND METHODS

In this prospective study, 336 patients with acute STEMI undergoing primary PCI at our institute were consecutively included into the study. The main inclusion criteria were the experience of acute ST-segment elevation myocardial infarction that led to primary PCI within 12 hours. All patients gave written informed consent for primary PCI. Detailed in-hospital and follow- up data including age, gender, coronary risk factors, Killip score on admission, peak level of creatine phosphokinase (CPK), arrival time, duration from puncture to first balloon inflation, reperfusion time, duration of procedure, pre- and post-PCI TIMI flow grades, angiographic results, number of diseased vessels, in-hospital adverse events, and in-hospital mortality were collected from hospital recorded files and were prospectively entered into a digital database. In definition, STEMI was defined as typical chest pain lasting for more than 30 minutes plus ST-segment elevation > 1 mm in two consecutive precordial or inferior leads or typical chest pain lasting for

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more than 30 minutes with a new onset complete left bundle branch block. COPD was defined based on one of the following criteria: (1) the need for pharmacologic therapy using bronchodilator agent; (2) Past history of a 1-second forced expiratory volume < 70% of the predicted value (by pulmonary function test); (3) physical examination that showed expiratory wheezing and further confirmed by blood gas and chest radiograph; or (4) current use of bronchodilators prior to STEMI. In this context, of the 336 patients recruited in this study, 45 (13.4%) fitted the criteria of COPD.

Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using t test or Mann-Whitney U test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. Categorical variables were, on the other hand, compared using chi-square test. Univariate Cox regression analysis was utilized to determine correlations between COPD and one-year MACE defined as occurring at least one of these events: mortality, recurrent MI, or admission because of heart failure. Hazard ratio (HR) for this time was used using multiple Cox- regression analysis only parameters with a value of $p < 0.1$ in univariate analysis were evaluated. For the statistical analysis, the statistical software SPSS version 20.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

RESULTS

Comparing two groups with and without COPD showed that the patients in former group were older, but other baseline parameters including the gender, risk factors of CAD, and the incidences of previous ischemic events were similar in the two groups. Electrocardiographic assessment showed also no difference in infarction location between them. However, the peak levels of cardiac enzymes level were remarkably higher in COPD. In addition, LVEF was notably lower in group with COPD patients than those without COPD. The incidences of current medical use, including beta-blockers, calcium-channel blockers, angiotensin converting enzyme inhibitors/angiotensin II type I inhibitors and statins did not differ between the two groups. Regarding angiographic findings, it was demonstrated similar distribution of infarct-related artery between the two groups. But, those in COPD group had more severe obstruction of the affected artery prior to primary PCI.

Table 1: Baseline characteristics of study patients

Characteristics	COPD (+) (n = 45)	COPD (-) (n = 291)	P-value
Age	68.52 ± 4.45	59.25 ± 8.82	< 0.001
Male gender	43 (95.6)	266 (91.4)	0.847
Hypertension	22 (48.9)	142 (48.8)	0.995
Hyperlipidemia	10 (22.2)	75 (25.8)	0.691
Diabetes	12 (26.7)	72 (24.7)	0.831
Current smoking	23 (51.1)	132 (45.4)	0.666
Previous stroke	6 (13.3)	32 (11.0)	0.683
Previous MI	3 (6.7)	14 (4.8)	0.618
Anterior wall infarction	28 (62.2)	190 (65.3)	0.852
Peak CPK level (unit/L)	3191 ± 2997	2665 ± 2998	< 0.001
Peak CK-MB level (unit/L)	972 ± 2561	648 ± 2137	< 0.001
LVEF (%)	51.4 ± 3.2	56.5 ± 5.8	0.021
Advanced CHF	9 (20.0)	51 (18.2)	0.738
Multi-vessel disease	24 (53.3)	143 (49.1)	0.764
B-blocker utilization	32 (71.1)	198 (68.0)	0.859
Calcium channel blocker Utilization	6 (13.3)	32 (11.0)	0.683
Statin utilization	26 (57.7)	169 (58.1)	0.985
ACE-inhibitor	39 (86.7)	243 (83.5)	0.875

Table 2: Early and late outcome

Characteristics	COPD (+) (n = 45)	COPD (-) (n = 291)	P-value
Length of stay in hospital	11.2 ± 2.2	10.9 ± 3.1	0.224
In-hospital mortality	4 (8.9)	7 (2.4)	0.031
Recurrent MI	2 (4.4)	2 (0.6)	0.035
Re-admission for CHF	8 (17.8)	12 (4.1)	0.004
Cumulative mortality in one year	9 (20.0)	15 (5.2)	0.004
One-year MACE	18 (37.5)	31 (10.7)	< 0.001

Table 3: Multivariate analysis of predictors for MACE

Variable	Hazard Ratio	95% CI	P-value
Age	1.111	1.023 – 1.254	0.032
Male gender	2.214	1.456 – 3.245	0.001
Hypertension	1.147	1.112 – 5.214	0.024
Hyperlipidemia	3.314	1.247 – 6.478	< 0.001
Diabetes	2.789	1.147 – 4.457	< 0.001
Current smoking	1.799	1.144 – 2.247	0.040
Previous stroke	1.478	0.789 – 2.217	0.068

Previous MI	2.258	1.199 – 4.147	0.002
Anterior wall infarction	3.378	1.478 – 4.994	< 0.001
Peak CPK level (unit/L)	1.547	1.478 – 1.898	0.001
Peak CK-MB level (unit/L)	1.898	1.002 – 4.478	0.002
LVEF (%)	1.478	0.755 – 2.147	0.098
Advanced CHF	1.478	1.132 – 1.775	0.042
Multi-vessel disease	1.478	0.256 – 2.478	0.112
COPD	2.745	1.457 – 3.357	0.025

Regarding PCI outcome, both in-hospital and one-year cumulative mortality rates were higher in COPD than in non-COPD groups; however the length of stay in hospital did not differ between them. In addition, among long-term cardiac events, the incidences of recurrent MI and readmission for CHF were significantly higher in COPD than in non-COPD group. In Univariate Cox regression analysis demonstrated that the baseline variables, including advanced age, female gender, and the presence of diabetes mellitus as well as COPD were strongly predictive one-year MACE. In multivariable Cox analysis, COPD showed a strong positive correlation with one-year MACE.

DISCUSSION

This study investigated the incidence of COPD in patients with STEMI undergoing primary PCI and also to determine the impact of COPD on the prognostic outcome of the patients.

In the setting of STEMI, the incidence of COPD in our population was nearly similar to that in the Eastern and Western population [7, 19-22]. In our study COPD was an independent predictor of short-term mortality and also it was a significant predictor of one-year MACE after adjusting traditional risk factors. The results of previous studies [7, 19-22] could demonstrated that COPD was independently predictive of both short-term and long-term outcome. Our findings, therefore, were consistent with those of the previous studies. These consistent findings between the present studies may be explained by the following reasons. First, the exclusion criteria and the diagnosis of COPD may be identical between our investigation and studies by others. The diagnosis of COPD was obtained from hospital records and questioning the patient or only by 1-second forced expiratory volume < 70% of the predicted value. Second, primary PCI, which was a criterion for study enrollment in the majority of recent studies, was performed in all patients in the present study. Convincing data have established that primary PCI is one of the most effective life-saving procedures that also help in preserving heart function and reducing short-term and long-term mortality in patients after AMI [23-25].

The results of the current study imply that successful primary PCI may confirm the independent influence of COPD on the clinical outcome of patients after AMI. Campo et al. [26] showed that COPD was an independent predictor of mortality and also hospital readmissions for recurrent MI were significantly more frequent in patients with COPD as compared with those without. Also, hospital readmissions for COPD were more frequent in patients with a previous history of COPD as compared with those without. In total, in their study, patients with a hospital readmission for COPD showed a fourfold increased risk of death. In another study by Lazzeri et al. [27] Kaplan-Meier survival curve documented a significantly worse outcome in COPD patients. Also, at multivariate analysis, the following variables were independent predictors for death at follow up: age, GFR, COPD, and discharge left ventricular ejection fraction. In the current study, univariate analysis showed that COPD was strongly associated with MACE and multivariate Cox regression analysis also produces consistent result. These findings suggest that, besides traditional risk factors, COPD was a predictor of clinical outcome in STEMI patients undergoing primary PCI. COPD and CAD are frequently found to share common risk factors such as smoking and inflammation [7,13,14]. This could explain the prevalence of these two disease entities in patients with AMI [15,16]. In summary, COPD is an independent predictor of one-year MACE for patients with STEMI undergoing primary PCI (HR = 2.745, P = 0.025).

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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ARTICLE

EXTRACT OF *PISTACIA ATLANTICA* L. ON HYPERLIPIDEMIA AND BIOMARKERS OF OXIDATIVE STRESS IN RATS FED A HIGH-FAT DIET AND HYPOGLYCEMIC EFFECT IN DIABETIC RATS INDUCED WITH ALLOXAN

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ABSTRACT

Cardiovascular disorders are considered one of the greatest widespread diseases of human life. herbal cures were used traditionally to treat or prevent such of chronic disease and many of them have been indicated precise effect on hyperlipidemia. This study was performed to determined Pistacia atlantica leaf extract on lipid profile. 80 rates divided in 8 groups of 10 randomly, which feeds by normal diet, high fat diet (3 groups), high fat diet plus 200mg/kg and 400mg/kg of hydroalcoholic extract of pistachia atlantica, high fat diet plus 10 mg/kg atorvastatin respectively in prevention phase. After 90 days, low-density lipoproteins (LDL), High-density lipoproteins (HDL), Triglyceride, Fast blood sugar, C - Reactive Protein, malondialdehyde (MDA), Acyl-Coenzyme A, acetyltransferase (ACAT), Glutathione S-Transferase(GST), Catalase, superoxide dismutase (SOD), Total glutathione (GSH) and triglyceride and histopathologic test of liver and aortic arch were assessed. Then 3 groups of hyperlipidemic rats remains for 30 days feeding normal diet, normal diet plus 400mg/kg pistachia atlantica extract and normal diet plus 10mg/kg atorvastatin in treatment phase. All the tests were repeated in 120th day of experiment. After prevention phase, glucose, cholesterol, TG, LDL and HLDL in groups fed by herbal extract were decreased, also HDL increased significantly specialy in 400mg/kg dose (p=0.000). in addition, plant diet reducing MDA, OxLDL, plasma carbonyl, NO and increased catalase, SOD, thiol groups of plasma. Moreover, pathologic studies determined atherosclerotic plaques formed by 90 days high fat diet that prevented in groups fed by both doses of pistachia atlantica. In treatment phase, 400mg/kg of pistachia atlantica caused absolute decrease in GL, TC, LDL, VLDL, ALT, MDA and liver weight in hyperlipidemic group. Consequently, Pistachia atlantica leaf significantly decreased lipid profile and atherosclerotic biomarkers in 90-day prevention and 30-day treatment phases.

KEY WORDS
leaf extract, Pistacia atlantica L, hyperlipidemia, alloxan

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INTRODUCTION

Nowadays, cardiovascular disease is the most prevalent cause of mortality which is closely associated with formation of atherosclerotic plaques, which in turn is derived from certain stressors such as hyperlipidemia, hypertension, diabetes, and even fatty liver [1, 2]. Among these factors, hyperlipidemia, particularly high blood LDL level, contributes more fundamentally and markedly to pathogenesis of the disease [3, 4]. Severity and type of dyslipidemia alongside risk factors associated with bad habits such as over-eating, obesity, physical inactivity, and adverse high-fat diets are considered to be predisposing factors for formation of atheroma plaques in the vessel wall, particularly coronary arteries [5]. Many drugs are used to treat dyslipidemia. Despite high efficacy of these drugs, lack of appropriate overlapping effects on both cholesterol and triglyceride, relatively prevalent complications (increasing liver enzymes, rhabdomyolysis, allergic complications,etc.) as well as high interaction with other drugs and contraindications in many physiological states and diseases have encouraged the researchers to seek out better drugs [6,7].

Medicinal plants have long been investigated for treatment of dyslipidemias, and over 200 plants have been demonstrated to be effective in decreasing lipidemia (8). However, there are certain plants that are considered to be effective in decreasing lipidemia according to popular belief, are used as additives in foods and are recommended to study. Pistacia atlantica leaf is an Iranian traditional plant which has long been used a sweetener and antibacterial agent. P. atlantica tree can reach a height of 9 m and its tree is mainly used. Another product of this tree is gum which is green when it exudes from the trunk and is considered an oleoresin in terms of chemical compounds and physical characteristics. Unfortunately, most studies have been conducted on P. atlantica fruit and no study has yet investigated on P. atlantica leaf [9, 10]. The aim of this study was to investigate the effects of hydroalcoholic P. atlantica leaf extract on lipid profile, glycemia, and atherosclerosis-inducing biomarkers.

MATERIALS AND METHODS

Preparation, extraction, and selection of appropriate solvent

P. atlantica was identified and then collected from Shirmard village in Iran. After the pharmacognosy expert of the Isfahan University of Medical Sciences approved the primary identification and the code 461 of the Herbarium was specified to P. atlantica sample, it was shadow-dried. Then, botanolic, hexane, methanolic, and methanolic/aqueous extracts were obtained from the plant and the hydroalcoholic extract

(80:20) selected according to the assays of phenolic, flavonoid, and flavnolic groups and prepared by percolation. Finally, the extract was evaporated in an evaporator and dried by an oven at 37 °C [11].

Selection of dose

Using the method of acute toxicity study and Foreman (OECD guideline) and the Software AOT 425 (determining UP and DOWN method), and no mortality in 5000m/kg, LD50 of this plant, was considered to be higher than 5000 mg/kg. Then, the doses 25, 50, 100, 200, 400, and 800 mg/kg of the plant were examined for chronic toxicity within 28 days. Regarding the conducted experiments, we found no chronic toxicity. According to two doses of 200mg/kg and 400mg/kg, it was considered effective and safe dose [12].

Formulation of atherogenic diet

In the light of hyperlipidemic food ingredients for human, we attempted to prepare a diet with greatest similarity. In this formulation, a combination of cow fat, impure saturated fat palm oil, egg cholesterol, and sugar (20%) were used. An emulsion (1 cc) was prepared to gavage according to the suitable dose of cholesterol 25mg/kg [13].

Study design

In this study, the effects of P. atlantica were investigated in prevention and treatment phrases.

Prevention phase

Eighty male Wistar rats weighing approximately 200-250 G and aged approximately eight weeks were divided into eight groups of 10 each as follows:

Group 1: Fed with normal food;
Group 2: Fed with fat only;
Group 3: Fed with fat and 200mg/kg of P. atlantica;
Group 4: Fed with fat and 400mg/kg of P. atlantica;
Group 5: Fed with fat and atorvastatin (10mg/kg);
Group 6: Fed with fat alone;
Group 7: Fed with fat alone; and
Group 8: Fed with fat alone.

On day 90, the rats in the groups 1-5, fed with water only for 12 h, were anesthetized, their blood samples taken, and then their liver, kidney, and aortic arch isolated and kept in formalin 9%.

Biochemical tests were conducted on lipid profile, hepatic profile, renal profile, and inflammatory biomarkers. Furthermore, the weights of the rats were measured and recorded on days 1, 10, 20, 30, 40, 50, 60, 70, 80, and 90.

Treatment phase

In the remaining three groups (6-8) that were fed with fat alone until the day 90, after the blood samples were taken from the eyes, a veterinary participated in the second phase of the study as follows: (The duration of the study in the second phase was 30 days and biochemical tests were repeated).

Group 6: Previously hyperlipidemicized rats with normal diet;
Group 7: Previously hyperlipidemicized rats with normal diet and atorvastatin 10mg/kg; and
Group 8: Previously hyperlipidemicized rats with normal diet and 400mg/kg of P. atlantica (because this dose had better effect on lipid profile in phase 1).

On the day 120, the rats were fed with water only for 12 h and the whole procedure in the prevention phase was duplicated. The weights of the rats were recorded on the days 100, 110, and 120.

Study of hypoglycemic effects of P. atlantica

Regarding that P. atlantica was found to have reducing effects on fasting glycemia in the prevention and treatment phases, the groups below were studied to establish this effect using subcutaneous administration of alloxan120mg/kg:

10 control rats fed with normal diet;
10 control rats fed with normal diet and 1 cc normal saline subcutaneously administered;
10 rats diabetized with alloxan120mg/kg
10 rats diabetized with alloxan and 200mg/kg hydroalcoholic P. atlantica extract.
10 rats diabetized with alloxan and 400mg/kg hydroalcoholic P. atlantica extract.
10 rats diabetized with alloxan120mg/kg and metformin (100 mg/kg).

On the day 30, the rats were anesthetized after 12-h fasting, blood samples were taken, and the relevant tests conducted.

Measurement of antioxidant activity and total phenolic and flavonoid contents of the plant

To measure antioxidant activity, Khalighi et al's method was used [14]. According to the inhibition rate of free radical DPPH and the solution absorption, antioxidant activity was measured by UV spectrophotometer at 517-nm wavelength after 30-min presence in a dark environment, and RSA measured.

To measure total phenolic and flavonoid contents, Kim and Khalighi method was used [15]. Total flavonoid content was measured according to rutin equivalent amount (1 mg) and P. atlantica extract and dried powder.

Measurement of total plasma antioxidant capacity

To measure total plasma antioxidant capacity, FRAP (the ratio of changes to the measurement and inside measurement weights, 3.3% and 1.18% respectively) offered by Benzik et al was used [16].

Measurement of NO

To measure NO amount, Barkel et al's method according to Griess reaction was conducted using spectrophotometry.

Briefly, from 0.1 M sodium nitrite, 100 micromole of solution was prepared and serial triple concentrations (as standard concentrations to plot standard curve) were prepared from this concentration.

1000 microL of the serum sample was introduced, as paired, into 96-well plates. One hundred microL of sulfanilamide solution (1 g sulfanilamide in 100 cc of phosphoric acid 5%) was introduced into all wells containing the sample and standards. The plate was incubated at room temperature in the dark for 5-10 min. One hundred microL of N-(1-Naphthyl) ethylenediamine dihydrochloride (NED) was introduced and the plate was incubated at room temperature in the dark for 5-10 min again. Half an hour later, maximom optical absorbance was read using spectrophotometer at 530-nm wavelength and the amount of nitrite in the samples determined with reference to the standard curve [17].

Plasma carbonyl

Levin et al's method was used to measure plasma carbonyl groups. Reagent 2,4 dinitrophenyl hydrazine created Schiff base with carbonyl groups in the proteins and a yellow complex was formed with the color intensity measured spectrophotometrically at 380-nm wavelength [18].

Plasma thiol

Hu's method of colorimetry was used to measure plasma thiol [19]. The Ellman's reagent dTNB formed a yellow complex with thiol-containing groups with maximum absorbance at 412-nm wavelength.

Measurement of superoxide dismutase (SOD) enzymatic activity

SOD was measured according to Carrillo et al's method using xanthine oxidase as an oxygen delivery system for infants with nitro-blue tetrazolium (NBT) indicator. To identify other enzyme types, KCN was used [20].

Measurement of catalase enzymatic activity

Catalase was investigated according to Carrillo's method. Catalase enzymatic activity was measured at 37°C temperature and 240-nm wavelength and reported [21]

The data were analyzed by one-way ANOVA, paired t-test, and Tukey's post-hoc test in SPSS 21. $P < 0.05$ was considered to be the level of significance.

RESULTS

Mean weight of the animals in 10 time periods (first day, 10th day, 20th day, 30th day, 40th day, 50th day, 60th day, 70th day, 80th day and 90th day) of the studied groups were different in phase 1 of the study [Fig. 2].

Table 2-3: Comparison of variables in the studied group of phase I.

Groups	Normal	Hyper	Hyper+ atorvastatin	Hyper+ *P.a*.200mg/kg	Hyper+ *P.a* 400mg/kg	P-value
GL	103.79±11.53	142.08±25.44	9.27 109.43±	59.44±6.67	56.27±3.79	0.000
TC	59.89±12.27	134.30±26.48	80.00±7.11	82.03±4.93	75.83±7.16	0.000
TG	38.33±4.87	109.40±45.47	61.75±12.70	39.05±4.22	29.13±8.14	0.000
LDLC	18.78±5.19	48.92±5.75	27.51±1.66	39.36±1.63	33.13±11.75	0.000
VLDL	7.67±0.97	21.88±9.09	12.32±2.51	7.82±4.13	5.82±2.00	0.000
HDL	23.86±6.15	22.29±4.93	13.23±2.51	31.20±4.26	32.29±7.19	0.000
OXLDL	3806.89±526.79	4592.30±241.24	4103.12±229.60	3837.00±379.98	3714.83±321.81	0.000
ALT	369.11±51.00	451.80±129.63	315.42±80.27	334.26±13.81	307.20±20.12	0.000
UA	2.76±0.23	4.87±2.88	2.24±0.38	1.26±0.23	1.80±0.41	0.002
CR	0.77±0.09	0.97±0.13	0.69±0.08	0.63±0.09	0.60±0.05	0.000
GPT	49.44±5.98	51.00±13.47	41.25±11.97	46.29±2.12	43.20±4.28	0.000
UREA	47.33±11.80	45.10±18.65	35.62±8.25	13.20±3.17	11.02±8.43	0.000
CAT	8.42±0.31	5.02±0.71	5.74±0.40	6.35±0.47	6.71±0.77	0.000
SOD	42.36±5.57	35.61±3.29	36.34±4.34	41.20±5.17	42.10±2.70	0.000
THIOL	202.04±13.02	164.00±24.09	203.92±14.04	200.10±4.16	201.31±11.02	0.000

CARBO	0.77±0.09	0.87±0.04	0.62±0.07	0.51±0.07	0.49±0.08	0.000
MDA	2.33±0.48	6.31±0.76	3.03±0.43	2.71±0.37	239±0.60	0.000
DPPH	266.57±80.22	301.18±39.25	403.67±18.49	413.21±7.18	428.16±20.29	0.000
Liver Weight	4.40±0.34	7.21±0.90	4.95±0.18	4.94±0.31	4.49±0.30	0.000
NO	3.59±0.64	11.26±1.80	3.78±0.64	3.78±0.39	3.69±0.26	0.000

Table 5: Camparison of variables in 91th and 121th days of each group in phase II

variables	Days of sampling	Hyper+normal diet	Hyper+Atorvastatin	Hyper+P. a 400mg/kg
GL	91th day 121th day p- vaue	135.38±32.00 136.75±39.50 0.666	135.50±59.03 137.10±61.91 0.345	124.20±10.48 89.51±13.24 0.004
TC	91th day 121th day p- value	159.12±41.37 139.62±50.19 0.297	117.70±30.80 110.40±26.51 0.004	127.12±17.15 89.17±28.76 0.018
TG	91th day 121th day p- value	80.12±18.51 74.38±22.37 0.588	98.90±36.13 79.60±41.20 0.310	107.18±22.39 59.15±12.31 0.000
LDLC	91th day 121th day p- value	71.84±15.10 69.21±19.61 0.617	65.35±12.61 59.57±12.32 0.389	85.30±12.17 49.34±10.24 0.002
VLDL	91th day 121th day p- value	17.62±4.02 14.85±4.50 0.174	17.98±4.39 13.18±5.63 0.012	21.04±6.21 11.81±7.20 0.000
HDL	91th day 121th day p- value	17.06±7.70 18.90±7.50 0.116	11.69±3.75 13.28±3.54 0.328	11.98±5.17 10.30±2.84 0.853
OXLDL	91th day 121th day p- value	4090.62±872.60 3786.50±722.96 0.452	3811.00±451.62 3801.10±700.07 0.953	4051.73±156.80 3919.22±503 0.236
ALT	91th day 121th day p- value	386.88±44.61 384.12±33.85 0.746	453.90±136.71 528.60±228.93 0.252	583.31±200.38 529.13±101.69 0.795
UA	91th day 121th day p- value	2.30±0.59 2.18±0.50 0.083	4.15±0.29 4.22±0.37 0.566	4.20±0.52 4.13±0.45 0.767
CR	91th day 121th day p- value	0.74±0.12 0.65±0.05 0.087	0.91±0.20 0.90±0.16 0.910	1.10±0.12 0.90±0.18 0.168
GPT	91th day 121th day p- value	140.00±8.32 138.75±7.29 0.774	111.70±39.49 112.62±39.22 0.965	141.31±29.17 129.17±23.11 0.159
UREA	91th day 121th day p- value	33.50±3.63 33.62±2.77 0.946	58.60±8.98 58.10±6.56 0.875	64.10±13.42 57.10±4.75 0.164
GOT	91th day 121th day p- value	113.50±18.02 110.88±10.29 0.643	132.70±25.32 130.40±25.03 0.828	160.50±44.24 123.30±12.87 0.020
CAT	91th day 121th day p- value	4.57±0.95 4.82±0.74 0.351	4.73±0.89 5.08±0.59 0.424	4.40±0.21 5.12±0.01 0.004
SOD	91th day 121th day p- value	33.64±2.47 34.03±2.44 0.048	33.80±1.65 35.36±2.12 0.086	33.17±2.78 37.21±7.04 0.043
THIOL	91th day 121th day p- value	157.38±22.86 166.50±17.22 0.346	151.40±18.29 182.40±9.73 0.001	157.23±17.41 182.95±11.41 0.001
CAR	91th day 121th day p- value	0.88±0.05 0.82±0.08 0.045	0.91±0.06 0.76±0.09 0.002	0.98±0.04 0.73±0.06 0.000
MDA	91th day 121th day p- value	6.17±0.57 5.89±0.65 0.285	6.03±0.81 4.29±0.33 0.000	6.28±1.03 4.12±0.35 0.000
FRAP	91th day 121th day p- value	274.04±39.13 294.28±38.68 0.225	280.38±37.30 331.31±53.15 0.018	281.06±29.24 362.43±27.38 0.000
WLIV	91th day 121th day p- value	7.09±0.56 6.59±0.46 0.008	7.14±0.68 5.84±0.45 0.000	7.26±0.40 5.32±0.43 0.000

Table 6: Glycemic variations in the studied rats

Number	Day	Control	Treatment with *P.a*	Treatment with *P.a*	Diabetic	Diabetic and treated with metformin	Diabetic and treated with 200 mg/kg *P. M.*	Diabetic and treated with 400 mg/kg *P.M.*	One-way ANOVA
1	0	115.1±7/8	109.4±5.4	120.6±3.9	121.4±2.9	128.7±3.1	127/5±5/1	131/2±4/9	P<0.05
2	7	118/1±1/7	120/2±4/1	116.9±4.9	383/5±18.2	413.2±21.1	407/7±21/3	411/5±31/6	P<0.05
3	30	122.4±5.7	104.3±2.7	89.4±7.2	320.7±5.9	301±7.3	281.3±11.6	254.3±31.3	P<0.05

- The values were expressed as mean (±standard deviation) for each group.
 * Significant difference from healthy controls (P<0.05).

According to [Table 1], both the 200 mg/kg and 400 mg/kg of P. atlantica could remarkably reduce weights of rats feed by fat and plant diet. Meanwhile 400mg/kg indicated better effect in reducing weight than 200mg/kg (p< 0.05). In addition cross relation between treating group and weight in time was observed (F= 84.413, DF= 12.714, p=0.000).

Means of variants, glucose, cholesterol, TG, LDL and HLDL in groups fed fat and plant (both 200mg/kg and 400mg/kg) decreased in comparison with groups just getting fat diet, also HDL increased significantly. 400mg/kg dose of P. atlantica in reducing glucose, cholesterol and elevating HDL was more effective than 200mg/kg dose (p=0.000). Moreover, both doses of plant diet triggered reducing MDA, OxLDL, plasma carbonyl, NO and increased catalase, SOD, thiol groups of plasma; which significantly differed from control group.

Plasma antioxidant capacity was noticibly different in groups which received P. atlantica diet than groups feed fat diet; 400mg/kg dose showed better effects (p=0.000).
To be noticed the data of weight loss, lose abdomen fat, decreasing fat profile and increasing HDL, effect of 400 mg/kg dose on inflammation factors was determined in phase 2 of this study. Also pathologic studies indicated that high fat diet in phase 1 (90 day) caused formation of atherosclerotic plaques and 90 day treating by both 2 doses of plant inhibited formation of atheroma plaques.

Phase 2 (determination of therapeutic effects of plant in three studied group from 91th till 120th day)
In duration of 91th and 120th days, mean variation of gradual weight loss in hyperlipidemic rats with normal diet and 400mg/kg of plant comparing to hyperlipidemic rats with normal diet demonstrated significant difference (p=0.000). Also remarkable decreasing of GL, TC, LDL, VLDL, ALT, MDA and liver weight was observed in hyperlipidemic group feed with 400mg/kg of P. atlantica during phase 2 (91th till 121th day) and plasma antioxidant capacity, catalase, plasma thiol and SOD were increased (p=0.000). Furthermore decreased blood sugar was presented in Alloxan induced diabetic rats fed with plant diet while 400mg/kg dose of plant indicated more evident hypoglycemic effects (p<0.05).

DISCUSSION

This study demonstrated that P. atlantica leaf, in both 90-day prevention and 30-day treatment phases, could decrease lipid profile and exerted optimal effects on atherosclerotic biomarkers. Potent antioxidant compounds and properties of P. atlantica have caused decrease in oxidative stress. In previous studies, the cholesterol-lowering effect of P. atlantica essential oil was attributed to decreased PAP (22). The findings of this study indicated the presence of one or more active compounds in P. atlantica such that this plant caused increase in cholesterol in all lipoprotein fractions in the short term.

Besides that, this increasing effect was not observed on cholesterol in the long term. This finding can be due to the essential fatty acids abundantly found in P. atlantica, linoleic acid and linolenic acid that cause decrease in cholesterol in the long term. Because phosphatidate phsphohydrolase contributes importantly to development of fatty liver, and P. atlantica was found to cause decline in the activity and synthesis of this enzyme in the long term, researchers have recommended that P. atlantica be used to treat fatty liver.
In the present study, which was conducted on P. atlantica leaf, the cholesterol-lowering effects of this plant were obviously observed. Moreover, decreased ALT, AST, and liver weight as well as increased SOD, catalase, GSH, and GPX confirmed the protective effects of P. atlantica against fatty liver and increase in insulin sensitivity.

SOD is a metalloenzyme that helps regulate master eukoryotic free radicals. SOD causes free radicals to convert into OH. In addition, catalase decomposes hydrogen peroxide and therefore protects the tissues against highly active hydroxyl radicals. Catalase is an antioxidant enzyme which is extensively spread in animal tissues and is mostly active in liver and red blood cells. Glutathione is an important antioxidant which plays a fundamental role in removing the system of toxic peroxides and aldehydes, and indirectly causes increase in and survival of vitamins C (water-soluble antioxidant) and E (adipose-soluble

antioxidant). Conversion into water is the fundamental role of gluthatione and catalase. This conversion appears to occur in these steps [23].

NO levels are subjects with metabolic diseases and are negatively correlated with body mass index (BMI), blood pressure and triglyceridemia. Abnormal lipid metabolism, lipid peroxidation (oxidized low-density lipoprotein, ox-LDL) plays an important role in the formation and progression of arteriosclerosis. Its content responds to the speed and intensity of lipid peroxidation and indirectly responds to the damage severity of free radical. Malondialdehyde (MDA), the product of lipid peroxidation, has one of the greatest toxic actions [24, 25, 26].

In this study, decreased MDA and OxLDL, and modified NO caused prevention of lipid peroxidation and cell damage induced by the plant.
In addition, P. atlantica, in both study phases on lipid profile and study on rats with alloxan-induced diabetes, caused decrease in fasting blood sugar. In this study, this inconsistency in the findings was resolved.

In two phases, prevention and treatment, P. atlantica leaf was found to cause decrease in oxidative stress, and an independent study on rats with alloxan-induced diabetes confirmed the significantly reducing effects of this plant on fasting blood sugar ($P<0.05$).

Therefore, regarding to potent antioxidant activity of P. atlantica, its optimal effects on oxidative stress and in preventing atherosclerotic plaques as well as its low toxicity, clinical trials should be conducted on the effects of P. atlantica in decreasing cholesterolemia and blood sugar.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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ARTICLE

ANALYSIS ON RELATIONSHIP AMONG ANGER RUMINATION AND AGGRESSION WITH MEDIATING ROLE OF COGNITIVE EMOTION REGULATION IN ADOLESCENTS

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ABSTRACT

Background: *The current study was intended to analyze relationship among anger rumination with aggression and mediating role of cognitive emotion regulation.* ***Methods:*** *The statistical population of research includes all of high school students (female and male) in Qom city. 377 of them were chosen as sample of this study. They filled out Anger Rumination, Aggression, and Emotion Regulation questionnaires. The method of execution of this study was of descriptive survey and correlation type. Path analysis model was utilized with LISREL software for data analysis.* ***Results:*** *Results of path analysis indicated that the effect of post-thoughts of anger, retaliatory thoughts, anger related memories, and recognition of reasons might be positive and significant. Retaliatory thoughts, anger related memories, and recognition of reasons have positive and negative significant effect on emotion regulation. The post-thoughts of anger, retaliatory thoughts, and anger-related memory have positive and significant effect on aggression.* ***Conclusions:*** *However, recognition of reasons has not significant effect on aggression. Positive emotion regulation has negative and significant effect on aggression. Negative emotion regulation has positive and significant effect on aggression.*

KEY WORDS
Aggression, Anger rumination, Positive emotion regulation, Negative emotion regulation

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INTRODUCTION

The emotionally capable persons act more adaptively in treating with life events and challenges and for this reason they also enjoy further mental health [1]. These persons also recognize their emotions under various conditions and perceive implicit the related concepts and express efficiently their emotional states for the others. These individuals more succeed in coping with negative experiences and show more suitable adaptation in relation to environment and others [1]. Therefore, emotions are subjective, biologic, purposeful, and social phenomena. The natural phenomena that may emerge in different persons under the same conditions including emotions playing important and efficient role in life of all people are emotions of anger and aggression. As a fundamental emotion, anger is related to threat and negative assessment. It activates physiological responses and influences in behavioral dispositions [2]. Similarly, anger may be conceptually defined as strong sense of protest or sadness about a person or situation that can be synonymous to sadness, uneasiness, anger, spite, hostility or irritation [2].

In this sense, aggression usually denotes behaviors that are executed by different techniques to exert physical or spiritual damages to others. Sometimes, this injury includes destruction of personal and/ or public properties and assets. For instance, if in soccer field a football player may unintentionally reason legs of other player to be broken is not assumed as aggression but if he deliberatively hits legs of other player to exit him from game field this behavior is certainly considered as aggression [3]. In this regard, if anger is generally seen as an emotion, anger rumination may be defined as thinking about this emotion. Phenomenology of anger rumination includes repeated and automatic experience of angry moments and the fantasies relation to revenge [4].

Concerning diagnostics of anger rumination and aggression, Berkewitz [5] expressed this possibility that the failed experiences, aggression, and stimulation are led to aggression through creation of negative emotion. Similar to this possibility, relationship among anger rumination- aggression can be mentioned. Iterative and unavoidable (imposed) thoughts about past irrigational experiences improve possibility of aggression by activation of negative emotions. Fact-phobic thought i.e. dominance of ifs and conceptual consequences based on which an individual deals with assessment of past events is also another mechanism that relates anger rumination to aggression. Due to fact-phobic and reality- phobic nature, the fact- phobic thought is strengthened by repeated rumination of ifs and unrealistic thoughts [6] and increases possibility for aggression.

Concerning above variables, researchers of psychological pathology argues that failure in employing and adjustment of emotion regulation skills may predict mental impairments of a person in the in the future. For this reason, when someone is exposed to an emotional situation, good sense and optimism are not adequate to control emotion but s/he also needs to have the best cognitive function under such situations [7]. Cognitive emotion regulation is called to all cognitive styles any person uses them to increase or decrease and/ to or keep his/ her emotion where they are examined within two important frameworks as follows: 1) emotion regulation strategies are activated before occurrence of accident or at the beginning of occurrence; 2) emotion regulation strategies are activated after occurrence of accident and/ or after formation of emotion. Those emotion regulation strategies, which are activated before occurrence of accident may play essential role in control of negative emotions caused by accidents [1]. Since they cause the given event to be interpreted in such a way that to reduce negative emotional responses. Thus, no one

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can ignore role of cognitive emotion regulation in adaptation of individuals with life events [8]. Emotion regulation has been considered as a group of processes a person may employ them to call up a positive or negative emotion, keep that emotion, and control or change it. The present research is intended to examine relationship among anger rumination and reactive aggression by taking mediating role of self-regulation in adolescents.

MATERIALS AND METHODS

The current research has been executed by descriptive (non- trial) method and correlational research design is of path analysis type because the relations between variables are examined in this study within causal model framework. The techniques of data-collection can be generally divided into two groups of librarian and field study. The studied population of research includes all high school female and male students (adolescents at age group of 15-18 years) in Qom city where they studied in academic year (2015-6). With respect to extent of present population, multistage clustered sampling technique was utilized to select research sample.

The sample was calculated 377 by this formula n=(N×t2×p×q)÷(N×d2+ t2×p×q) [9]. Given the rate of loss in number of testees, 400 participants will be chosen as sample size.

$$n = \frac{N \times t^2 \times p \times q}{N \times d^2 + t^2 \times p \times q} = \frac{(3.8416)\,(.25)\;\;20000}{(0.0025)\,(3.8416)\,(.25)\;\;20000} = \frac{19208}{50.96} = 376.96 \approx 377$$

Determination validity and reliability of research measurement tools

Cronbach alpha coefficient method was adapted in order to determine reliability of test in this study. In order to calculate Cronbach alpha, initially variance of scores in any subgroup of questions in questionnaire and total variance should be computed. Then, we calculate alpha coefficient value using following formula.

$$r_\alpha = \frac{J}{J-1}\left(1 - \frac{\sum_{j=1}^{n} s_j^2}{S^2}\right)$$

Where, J is number of subgroups of questions of questionnaire or test; S_j^2 denotes subtest variance, S^2 is total variance of questionnaire or test [10]. In this study, high alpha coefficient (0.7) has been designated as suitable value for reliability of tools. Thus, reliability was measured by means of Cronbach alpha coefficient and SPSS software (v. 18). The results of Cronbach alpha coefficient have been reported in [Table 1]. Confirmatory factor analysis test was employed to examine validity of measurement tools rather than content validity.

Table 1: Cronbach alpha coefficients of research variables

Variables	Cronbach alpha
Post-thoughts of anger	0.78
Retaliatory thoughts	0.82
Anger- related memories	0.79
Recognition of reasons	0.77
Positive emotion regulation	0.80
Negative emotion regulation	0.81
Aggression	0.86

Introducing research tools

The measured variables included anger rumination, cognitive emotion regulation, and aggression in this study. Standardized questionnaires have utilized to measure each of these variables. Aggression questionnaire (Bass and Perry, 1992) was adapted to measure adolescent aggression. This inventory is a self-reporting tool comprises of 29 items and 4 subscales of physical aggression, verbal aggression, anger, and hostility. In order to measure anger rumination, Anger Rumination Scale ARS questionnaire was used that has been prepared by [4]. These inventory includes 19 questions with 4 subscales i.e. post-thoughts of anger, retaliatory thoughts, anger- related memories, and recognition of reasons. Cognitive emotion regulation questionnaire was used for measurement of cognitive emotion regulation that has been prepared by [8]. The main version of this questionnaire includes 9 components (self-blame, acceptance, rumination, positive refocusing, and planning, positive reappraisal, put into perspective, catastrophizing, and other-blame) with 36 articles.

Data analysis techniques

Descriptive and inferential tests were utilized for data analysis in this study. Factors of percentage, mean, standard deviation were used in descriptive part and Pearson's correlation tests and path analysis were utilized in inferential process. SPSS and LISREL software was employed for data analysis in this survey. Confirmatory factor analysis and path analysis techniques were employed for data analysis using LISREL software.

Fitness parameters in structured equations model

There are several parameters to evaluate model in which they have been introduced in three general classes of absolute, relative, and adjusted parameters. These parameters are listed in [Table 2].

Table 2: Fitness indices

Absolute indices	Relative indices	Adjusted indices
Chi-square	Normalized fitness index NFI	PGFI
X^2/df ratio	Non-normalized fitness index NNFI	Parameter of normalized fitness index PNFI
Root of mean residues RMR	Incremental fitness index IFI	
GFI	Comparative fitness index CFI	
AGFI	-	

RESULTS

Confirmatory factor analysis of data

Primarily, in order to enter into structured equations, research tools should be tested by confirmatory factor analysis to determine construct validity. Confirmatory factor analysis was adapted to confirm each of variables and also the relevant items for each of them. In fact, confirmatory factor analysis is used for determination of fitness of measurement model. Similarly, a reliable method is proposed to researcher to evaluate construct validity as well thereby researcher can saliently test hypotheses about factorial structure of data resulted from a predetermined model with certain number and composition of factors. Confirmatory method tests optimal agreement of the observed and theoretical factorial structures for group of data to determine fitness of predetermined factorial model.

Confirmatory factor analysis of anger rumination

The confirmatory factor analysis method was adapted to determine validity of anger rumination. T-coefficients are reported in [Fig. 1]. Numbers on paths include factorial loadings. With respect to LISREL output in [Table 3], value of X2/df was calculated 2.31; the existing X2/df is smaller than 3 indicates goodness of fit for the model. Similarly, Root Mean Square Error of Approximation (RMSEA) should be smaller than 0.08 where it is 0.06 in the given model. Values of indices (GFI, AGFI, CFI, and NFI) should be also greater than 0.9 so that these values are determined respectively greater than the given level in studied model. Therefore, data of this study are well fitted with factorial structure of this scale and this represents coordination of questions with variable of anger rumination.

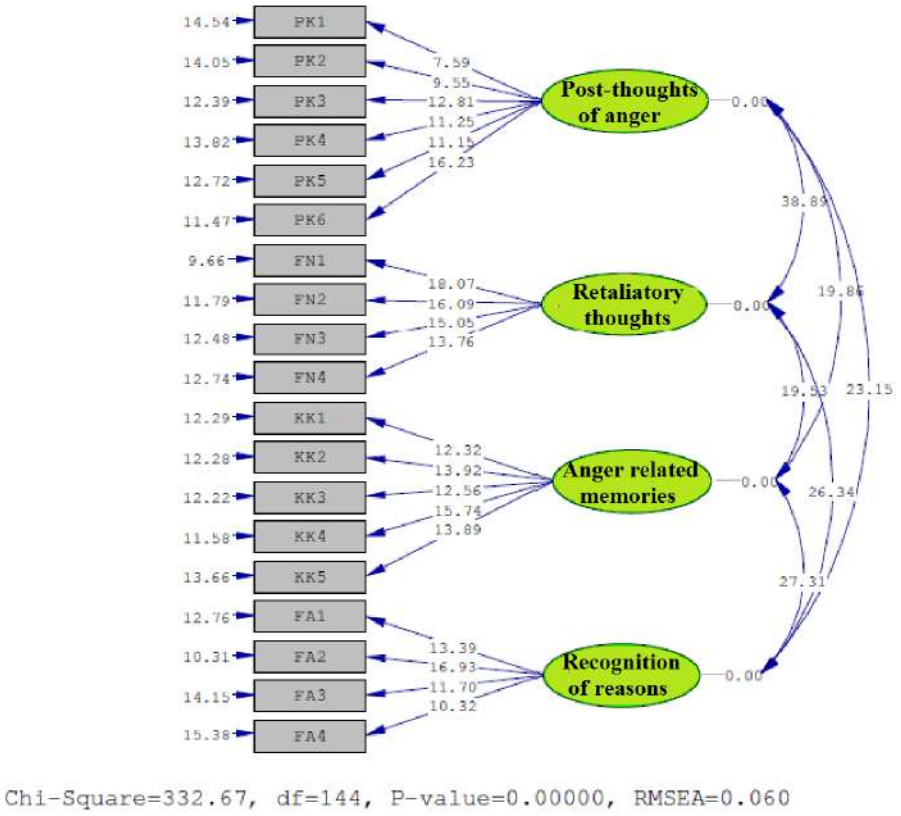

Fig.1: T- coefficients for variable of anger rumination .

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Table 3: Fitness indices for scale of anger rumination

Trait	Estimation	Criterion
Ratio of chi-square to degree of freedom (X^2/df)	2.31	X^2/df<3
Root mean square of error of approximation (RMSEA)	0.06	RMSEA<0.08
Goodness of fit index (GFI)	0.93	GFI>0.9
Adjusted Goodness of fit index (AGFI)	0.91	AGFI>0.9
Comparative fitness index (CFI)	0.98	CFI>0.9
Normalized fitness index (NFI)	0.95	NFI>0.9

The confirmatory factor analysis for cognitive emotion regulation

The confirmatory factor analysis was used in order to determine validity of cognitive emotion regulation. T-coefficients are reported in [Fig. 2]. With respect to LISREL output in [Table 4], value of X2/df was calculated 2.27; the existing X2/df value smaller than 3 indicates goodness of fit for the model. Likewise, Root Mean Square Error of Approximation (RMSEA) should be lesser than 0.08 where this value is 0.059 in the given model. The values of indices i.e. GFI, AGFI, CFI, and NFI should be also greater than 0.9 so that the given values are respectively higher than the determined level in the studied model. Therefore, data in this study are well- fitted to factorial structure of this scale and this expresses coordination of questions with variable of cognitive emotion regulation.

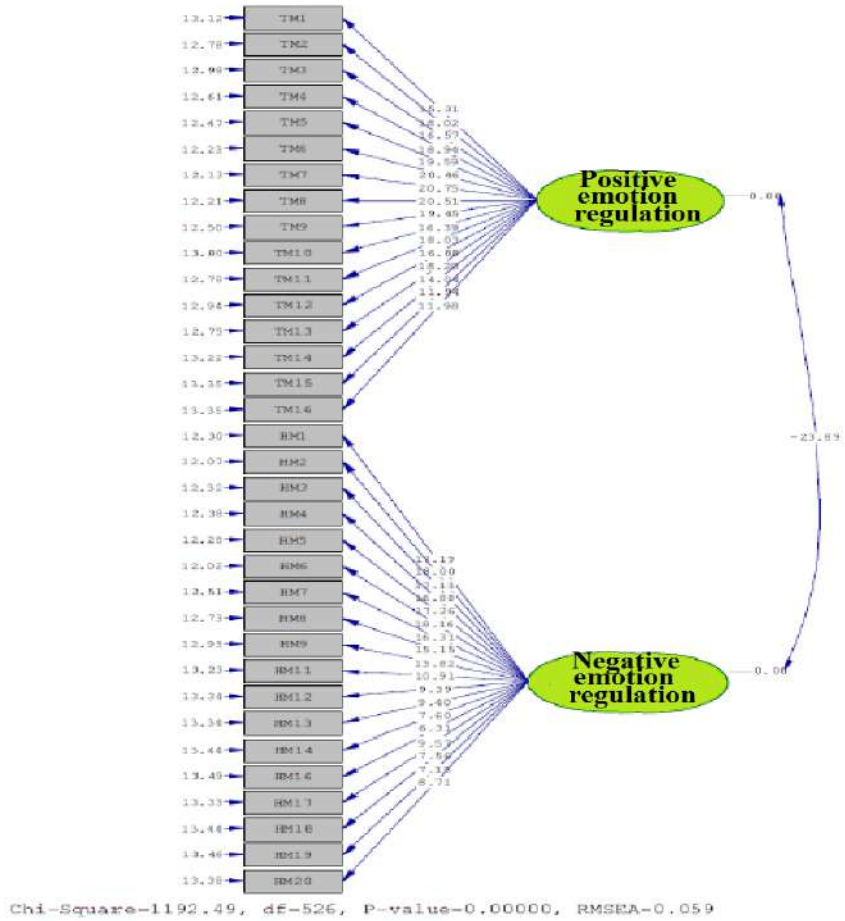

Fig. 2: T-coefficients for variable of cognitive emotion regulation .

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Table 4: Fitness indices for scale of cognitive emotion regulation

Trait	Estimation	Criterion
Ratio of chi-square to degree of freedom (X^2/df)	2.27	X^2/df<3
Root mean square of error of approximation (RMSEA)	0.059	RMSEA<0.08
Goodness of fit index (GFI)	0.93	GFI>0.9
Adjusted Goodness of fit index (AGFI)	0.90	AGFI>0.9
Comparative fitness index (CFI)	0.97	CFI>0.9
Normalized fitness index (NFI)	0.95	NFI>0.9

Confirmatory factor analysis of aggression

The confirmatory factor analysis was adapted to determine validity of variable of aggression. In [Fig. 3], factorial loadings are reported. The path locating on paths are factorial loadings in which all of factorial loadings are higher than 0.3. With respect to LISREL output in [Table 5], value of X2/df was calculated 2.48; the existing X2/df value smaller than 3 indicates goodness of fit in this model. Similarly, Root Mean Square Error of Approximation (RMSEA) should be smaller than 0.08 where in the given model this value is 0.063. Values of indices of GFI, AGFI, CFI, and NFI should be also greater than 0.9 where these values are respectively greater than the determined level in the studied model. Thus, data of this study are well-fitted with factorial structure of this scale and this denotes coordination of questions with variable of aggression.

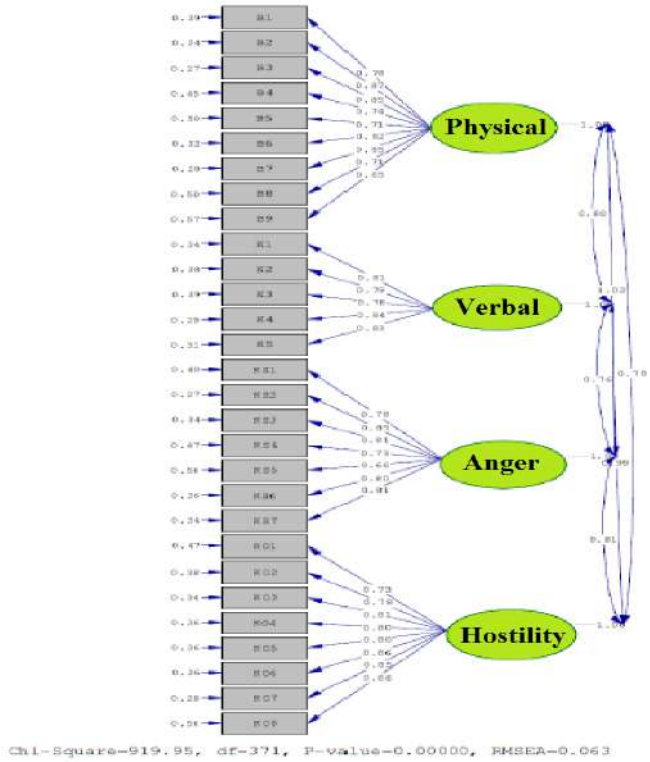

Fig. 3: LISREL output for variable of aggression.

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Table 5: Indices of fitness for scale of aggression

Trait	Estimation	Criterion
Ratio of chi-square to degree of freedom (X^2/df)	2.48	X^2/df<3
Root mean square of error of approximation (RMSEA)	0.063	RMSEA<0.08
Goodness of fit index (GFI)	0.94	GFI>0.9
Adjusted Goodness of fit index (AGFI)	0.92	AGFI>0.9
Comparative fitness index (CFI)	0.95	CFI>0.9
Normalized fitness index (NFI)	0.94	NFI>0.9

Correlation coefficient between variables

Pearson's correlation coefficient was used for recognition of relationship between current variables in this model. The findings derived from correlation coefficient among research variables were listed in [Table 6]. The findings indicate that coefficients of variables of post-thoughts of anger (r =-0.46), retaliatory thoughts (r = -0.50), anger related memories (r = -0.58), and recognition of reasons (r = -0.62) is correlated with positive emotion regulation negatively and significantly. The coefficient of variables of post-thoughts of anger (r = 0.52), retaliatory thoughts (r = 0.50), anger related memories (r = 0.49), and recognition of reasons (r = 0.50) is correlated with negative emotion regulation positively and significantly. The coefficient of positive emotion regulation is correlated with aggression (r = -0.58) positively and significantly. The coefficient of negative emotion regulation is correlated with aggression (r = 0.63) positively and significantly.

Table 6: Correlation matrix of research constructs

Variables	1	2	3	4	5	6	7
1. Post-thoughts of anger	1						
2. Retaliatory thoughts	0.70 **	1					
3. Anger related memories	0.55 **	0.57 **	1				
4. Recognition of reasons	0.61 **	0.64 **	0.64 **	1			

5.	Positive emotion regulation	-0.46 **	-0.50 **	-0.58 **	-0.62 **	1		
6.	Negative emotion regulation	0.52 **	0.50 **	0.49 **	0.50 **	-0.66 **	1	
7.	Aggression	0.43 **	0.44 **	0.34 **	0.42 **	-0.58 **	0.63 **	1

* p < 0.05 ** p < 0.01

The tested model plus standardized values are listed on each of paths in [Fig. 4]. The findings indicate that except the variables of post-thoughts of anger on positive emotion regulation and recognition of reasons do not significantly impact on aggression and the rest of coefficients are significant. T-coefficients of tested model are given to analyze significance of path coefficients in [Fig. 5]. Higher T- coefficients (±1.96 to ±2.58) are significant at level 0.05 while T-coefficients higher than ±2.58 are significant at level 0.01. In [Table 7], coefficients of direct and indirect and total effects and adjusted variance are given for research variables.

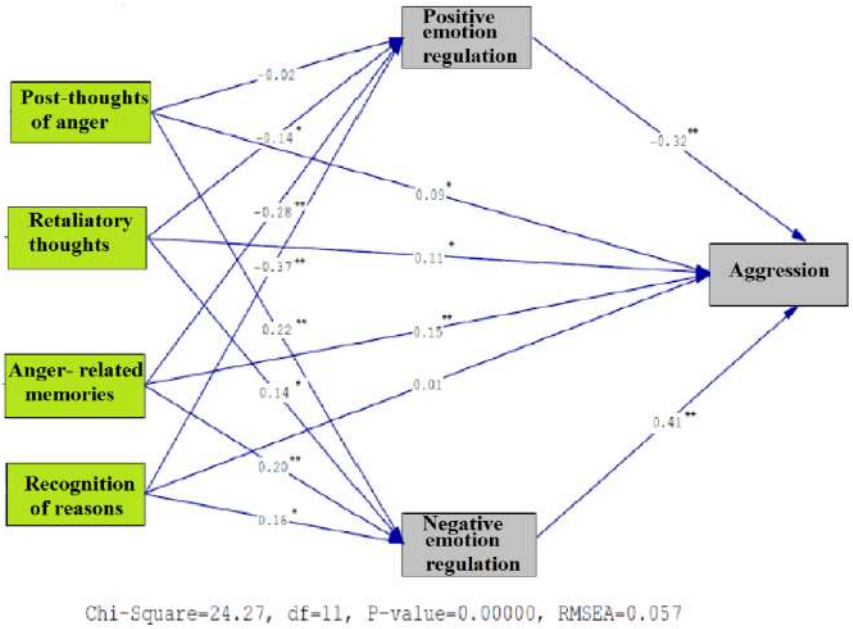

Fig 4: Standardized coefficients of tested model of research (* p< 0.05 ** p<0.01).

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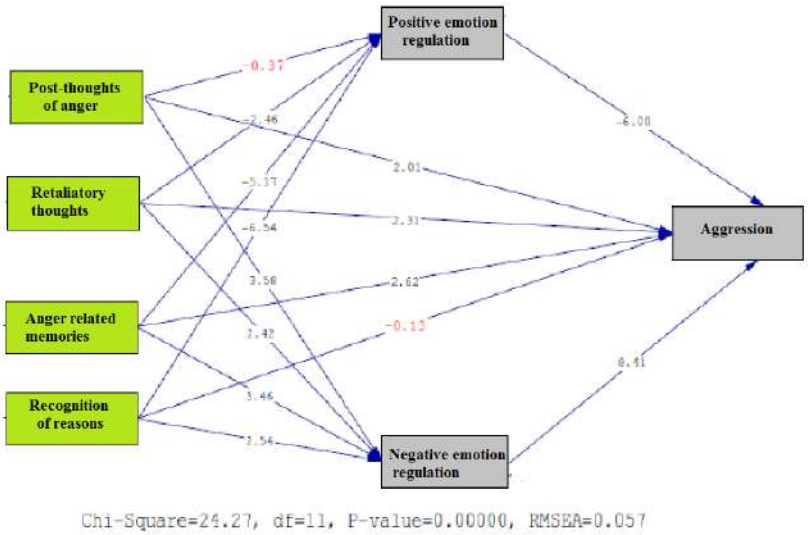

Fig. 5: T-coefficients of tested model of research (* p< 0.05 ** p<0.01).

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As it observed in [Table 7], the variables of post-thoughts of anger, retaliatory thoughts, anger related memories, and recognition of reasons impacts positively and significantly on negative emotion regulation. The retaliatory thoughts, anger related memories, and recognition of reasons have negative and significant effect on aggression but effect of recognition of reasons is not significant on aggression. Positive emotion regulation impacts negatively and significantly on aggression. Negative emotion regulation has positive and significant effect on aggression. Totally, 43% of variance of aggression, 44% of variance of positive emotion regulation, and 36% of variance of negative emotion regulation are interpreted by research model.

Table 7: Results of coefficients of direct, indirect, and total effects

Path	Direct effect	Indirect effect	Total effect	Interpreted variance
On aggression by				
Positive emotion regulation	-0.32 **	-	-0.32 **	
Negative emotion regulation	0.41 **	-	0.41 **	
Post-thoughts of anger	0.09 *	0.10 *	0.19 *	43%
Retaliatory thoughts	0.11 *	0.10 *	0.21 **	
Anger related memories	0.15 **	0.17 **	0.32 **	
Recognition of reasons	0.01	0.18 **	0.19 **	
On positive emotion regulation by				
Post-thoughts of anger	-0.02	-	-0.02	
Retaliatory thoughts	-0.14 *	-	-0.14 *	44%
Anger related memories	-0.28 **	-	0.28 **	
Recognition of reasons	-0.37 **	-	-0.37 **	
On negative emotion regulation by				
Post-thoughts of anger	0.22 **	-	0.22 **	
Retaliatory thoughts	0.14 *	-	0.14 *	36%
Anger related memories	0.20 **	-	0.20 **	
Recognition of reasons	0.16 *	-	0.16 *	

The fitness indices derived for tested model indicate in [Table 8] that RMSEA index (0.057) has reasonable level in the estimated model and other fitness indices (e.g. CFI, GFI, NFI, and AGFI) are totally at suitable level (0.96, 0.94, 0.94, and 0.93 respectively) and these traits of goodness of fit show the data in this study are well-fitted to factorial structure of this model.

Table 8: Fitness traits of the fitted model

Trait	Estimation	Criterion
Ratio of chi-square to degree of freedom (X^2/df)	2.21	X^2/df<3
Root mean square of error of approximation (RMSEA)	0.057	RMSEA<0.08
Goodness of fit index (GFI)	0.94	GFI>0.9
Adjusted Goodness of fit index (AGFI)	0.93	AGFI>0.9
Comparative fitness index (CFI)	0.96	CFI>0.9
Normalized fitness index (NFI)	0.94	NFI>0.9

Testing of research hypotheses

Hypothesis I: There is relationship among variables of post-thoughts of anger and aggression in adolescents.

The results of research indicated that the post-thoughts of anger had positive and significant effect on aggression. The results of present research are consistent with findings from [11], [12], [5]. Today, hostility and aggression are considered as a great problem of the world and an origin for many crimes, disorders, deviations, and even wars and also with its destructive psychological and physical effects at individual and social levels. Aggression is a disorder occurs among children and adolescents in different forms and a growing problem in adolescents. In this sense, anger rumination has been known as a factor that prepares

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ground for many psychological disorders especially emotional disorders. Anger rumination causes reduction of self-control and consequently it is led to rise of aggression. Rumination is a coping method with negative temperament that includes self- focusing attention. According to theory of response styles, this technique is characterized by self-thinking. In addition, it comprises of passive and iterative focus on negative emotions. The ruminative thoughts are often related to negative thoughts that cause sadness and depression and increase aggression [13] and [14] and they lead to reduction of well-being [4]. Post-thoughts of anger are one of subscales of anger rumination. Those persons, who report high levels of post-thoughts of anger, possess specific characteristics; for example, after a quarrel, these persons continually dispute with that individual in their minds. Similarly, these individuals review their diaries about anger in their mind before going to sleep. When they experience anger, they think about it for a period of time. Even partial diaries may cause neurosis in these persons. Moreover, if a subject makes them angry they review it in their mind for several times. Thus, sum of these traits causes to increase aggression levels in these persons.

Hypothesis II: There is relationship among variable of retaliatory thoughts of anger rumination and aggression of adolescents.

The results of study showed that retaliatory thoughts had positive and significant effect on aggression. The results of research are consistent with the findings of [11], [12], and [5]. Anger rumination is an unavoidable and iterative phenomenon that appears during anger experience and it may be accompanied to assessment of past events. In other words, anger rumination is assumed as one type of thinking rumination styles that take place as involuntary and iterative process after experiencing of anger period. The persons who exercise anger rumination, they experience anger as subjective image with all of aggressive and fantastic details relating to revenge. Thus that person naturally shows more aggression. The evidences indicate that anger rumination improves negative emotion and it is led to exacerbation and continuity of aggression. Rumination about anger-raising events impacts on performance [13]. The studies on process of rumination about reality have examined recall or anger imagination and shown that rumination intensified aggression [15]. It increases aggressive behavior and reduces forgiveness and delays improvement in blood pressure [15]. Inter alia, retaliatory thoughts are one of the subscales of anger rumination. The persons who enjoy retaliatory thoughts at high level possess characteristics that lead to rise of aggression in them. They imagine about revenge for long period after a serious conflict. Similarly, these individuals have difficulty in forgetting the persons who injured them. In addition, these individuals imagine and dream about nature of anger and when someone makes them angry they could not stop thinking about how to take revenge from them. Therefore, presence of such characteristics causes increasing aggression in these individuals.

Hypothesis III: There is relationship among variable of anger rumination related memories and aggression in adolescents.

The results of research showed that anger rumination related memories had positive and significant effect on aggression. The results of research are consistent with findings from [11], [12], and [5].
Rumination is a fact in life of humans and normal experience for many people. Rumination includes behaviors and thoughts that passively focus person's attention on symptoms of depression and reasons for these symptoms [16]. Rumination is defined as passive and iterative focus on distress symptoms and description of these symptoms which are related to negative thoughts, failure, and depression. Thus anger rumination is tendency to thinking about events that make them angry over the time. [15] showed that rumination about an angry-raising event might increase aggression while it reduced absent-mindedness. Similarly, anger rumination increases aggression more than the specified period. Empirical studies indicate that the rumination play causal role in intensification of negative emotion and negative cognition. Compared to absent-mindedness, it causes sadness to increase negative thoughts and defective problem-solving ([15]. In general rumination is considered as maladaptive and it is involved in intensification and continuity of types of destructive consequences for health such as aggression [13], depression and Post-Traumatic Stress Disorder PTSD [15]. Among them, anger rumination related memories are one of the other subscales of anger rumination. The persons who report anger rumination related memories at high levels possess the characteristics which lead to increase aggression level in them. These individual are thinking a lot about unfair activities done against them and constantly think about the events made them angry for long time. Similarly, these persons think about the accidents already occurred and ones that make still them angry. Moreover, these persons feel sense of anger about specific objects in life and presence of such traits in them is led to rise of aggression level.

Hypothesis IV: There is relationship among variable of recognition of reasons of anger rumination and aggression of adolescents.

The results of study showed that anger rumination reasons had no significant effect on aggression. The results of current research are consistent with findings of [12] and [17]. Rumination is a response that may take place because of different events that occur in life of person or different emotions a person may exercise such as anger, depression or anxiety [18]. Rumination comprises of a class of conscious thoughts that are focused on the same subject and they may recur even in the absence of immediate environmental requirements needed for such thoughts. Rumination is the result of difference between individual goals and one's real status. The upper and lower levels of these objectives are linked together and therefore deprivation from trivial objectives may also lead to rumination. Rumination is iterative, unwanted and often abhorrent and it may obstruct human from focus on more important subjects. Recognition of anger

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rumination reasons is one of the other subscales of anger rumination. Those persons who acquire high score in subscales of anger rumination have some characteristics; for example, they analyze the events that make them angry and there are some periods when they could not stop their mental preoccupation by specific conflict. Similarly, when someone makes these persons angry they surprise of this fact that this event constantly takes place for them. Nonetheless, despite of these features, results of present research indicated that anger rumination reasons had no significant effect on aggression and also anger rumination reasons was not led to rise of aggression.

Hypothesis V: There is relationship between variables of anger rumination and aggression with mediating role of cognitive emotion regulation (positive and negative) in adolescents.
The result of study indicated that post-thoughts of anger rumination had indirect positive and significant effect on aggression of adolescents. Thus, positive and negative cognitive emotion regulation plays mediating role in relationship among post-thoughts of anger rumination and aggression of adolescents. As a result it can be concluded that post-thoughts of anger rumination causes increase in aggression of adolescents through effect on cognitive emotion regulation among adolescents. In other words, post-thoughts of anger rumination may lead to reduction of positive cognitive emotion regulation and rise of negative cognitive emotion regulation and thus rise of aggression. Retaliatory thoughts have indirect positive and significant effect on aggression among adolescents. Therefore, positive and negative cognitive emotion regulation plays mediating role in relationship among retaliatory thoughts and aggression of adolescents. As a result, it can be mentioned that the retaliatory thoughts are led to rise of aggression in adolescents by effect on cognitive emotion regulation in adolescents. In other words, retaliatory thoughts are led to reduction of positive cognitive emotion regulation and rise of negative cognitive emotion regulation and consequently rise of aggression. Likewise, results of study showed that recognition of reasons had indirect positive and significant effect on aggression among adolescents. Therefore, positive and negative cognitive emotion regulation plays mediating role relationship among recognition of reasons and aggression in adolescents. As a result, it can be implied that recognition of reasons is led to rise of aggression of adolescents through effect on cognitive emotion regulation among adolescents. In other words, recognition of reasons is led to reduction of positive cognitive emotion regulation and increases of negative cognitive emotion regulation and thus raises aggression.
Results of testing research hypotheses are given in [Table 4].

Table 9: Results of testing research hypotheses

Hypotheses	-	Approval of hypothesis	Rejection of hypothesis
There is relationship among post-thoughts of anger rumination and aggression of adolescents.	-	Approved	-
There is relationship among retaliatory thoughts of anger rumination and aggression of adolescents.	-	Approved	-
There is relationship among anger rumination related memories and aggression of adolescents	-	Approved	-
There is relationship among recognition of reasons of anger rumination and aggression of adolescents.	-	-	Rejected
There is relationship among variables of anger rumination and with mediating role of cognitive emotion regulation (positive and negative) in adolescents.	Post-thoughts of anger rumination	Approved	-
	Retaliatory thoughts	Approved	-
	Anger-rumination related memories	Approved	-
	Recognition of reasons	Approved	-

CONCLUSION

Hypothesis I: There is relationship among post-thoughts of anger rumination and aggression in Adolescents. The results of study showed that post-thoughts of anger had positive and significant effect on aggression. **Hypothesis II**: There is relationship among retaliatory thoughts of anger rumination and aggression of adolescents. The results of study showed that retaliatory thoughts had positive and significant effect on aggression. **Hypothesis III**: There is relationship among anger rumination related memories and aggression in adolescents. The results of study indicated that anger rumination related memories had positive and significant effect on aggression. **Hypothesis IV:** There is relationship among recognition of reasons of anger rumination and aggression in adolescents. The results of study indicated that the reasons of anger rumination had no significant effect on aggression. **Hypothesis V**: There is relationship between variables of anger rumination and aggression by mediating role of cognitive emotion regulation (positive and negative) in adolescents. Thus, positive and negative cognitive emotion regulation plays mediating role in relationship among post-thoughts of anger rumination and aggression of adolescents. Similarly, results of study showed that recognition of reasons has indirect positive and negative effect on aggression in adolescents. Therefore, positive and negative cognitive emotion regulation plays mediating role in relationship among recognition of reasons and aggression of adolescents. As a result, it can be implied that recognition of reasons causes increase in aggression of adolescents by effect on cognitive emotion regulation of adolescents.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

FINANCIAL DISCLOSURE
None

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ARTICLE

PHYTO-PHARMACOLOGICAL EFFECT OF NINE MEDICINAL PLANTS AS A TRADITIONAL TREATMENT OF DEPRESSION

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ABSTRACT

Anxiety, stress and depression are characterized by widespread and highly comorbid psychiatric conditions in the world that are defined as a negative emotional experience and are associated with biochemical, cognitive, behavioral and psychological changes. Herbal medicine has been widely used among suffering and anxiety disorders since ancient times. The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus there is a need to explore alternative therapies particularly from herbal sources as these are cost effective and possess minimal side effects. This review investigates the available studies on the pharmacological effects of some medicinal plants on depression. The studied plants include: Melissa officinalis, Lavandula angustifolia, Cinnamomum zeylanicum, Viola Odorata, Echium amoneum, Valeriana officinalis, Aloysia triphylla, Citrus aurantium and Salix aegyptica. The present article is a comprehensive review of the pharmacological properties, especially anti-depressants, anti-anxiety of nine medicinal plants that could be useful for clinical studies to produce an herbal product which use treat depression.

KEY WORDS
Anxiety, Depression, Medicinal Plants, Traditional medicine

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INTRODUCTION

The interest in medicinal plant research and the aroma-therapeutic effects of essential oils in humans has increased in recent years, especially for the treatment of neuropathologies with profound social impact such as depression [1]. The medicinal use of essential oils has been known since the early times [2]. Popularly, they are used in the control of emotions and mood, for their sedative, anxiolytic [3], antidepressant effects [4], and anticonvulsant [5] among others [6, 7, 8]. Depression has become a common psychological illness in recent years. According to an investigation by the World Health Organization International Consortium of Psychiatric Epidemiology (WHO-ICPE), 6.3 – 15.7% of the world's population has been estimated to get depression once in their life [9]. It is estimated that by the year 2020, depression will result in the second greatest increase in morbidity after cardiovascular disease, presenting a significant socioeconomic burden. Although a wide variety of antidepressant drugs are available to treat depression, most of the synthetic drugs are not without side effects. Therefore, the search for regularly eaten foods with an antidepressant activity seems to be an essential approach to finding an effective antidepressant treatment without side effects. Modern research on herbal medicine in psychiatry although still in its infancy, has increased in recent years [10].

In Iranian and other traditional medicines, an antidepressant effect has been indicated for some medicinal plants. These include lemon balm (*Melissa officinalis*), lavender (*Lavandula angustifolia*), Cinnamon (*Cinnamomum zeylanicum*), Banafsha (*Viola Odorata*), Echium (*Echium amoneum*), valerian (*Valeriana officinalis*), Aloysia (*Aloysia triphylla*), Citrus (*Citrus aurantium*) and Salix (*Salix aegyptica*) [11, 12]. The association of various plants in a single medication is a relatively common practice in several countries. As examples, in France, Euphytose, an association of *Passiflora incarnata*, *Valeriana officinalis*, *Crataegus oxyacantha*, and three other plants, has been used for its anxiolytic properties [13]. In Chinese therapeutics, the practice of associating various plants with a view to producing one specific therapeutic effect is widely known [14]. This review focuses on the various plants that could be effective in the treatment of depression.

PHYTOPHARMACOLOGICAL EFFECTS OF NINE MEDICINAL PLANTS

Aloysia triphylla

Aloysia triphylla (Verbenaceae) is a perennial, bushy plant, originally from South America, and cultivated in various areas in the Middle East. *Aloysia triphylla* has long been used in traditional medicine. *Aloysia triphylla* has been reported to have a gentle sedative action and helps to counter depression [15]. The plant has tonic effect upon the nervous system and has reputation for soothing abdominal discomfort [16]. The plant has been found to possess antioxidant effect [17]. Phytochemical investigation of the aerial parts of *Aloysia triphylla* has led to the isolation of two compounds artemitin and hesperidin [18]. Artemitin has been claimed that it has an anti-inflammatory effect. Additionally, artemitin was found to induce relaxation in smooth muscle [19]. Hesperidin is a bioflavonoid, which has been reported to possess a wide range of pharmacological properties. It has been reported to have significant anti-inflammatory and analgesic effects [20]. Several mechanisms have been suggested to explain such activity including: inhibition of histamine release [21]; and inhibition of eicosanoid synthesis [22]. Additionally, hesperidin was found to have central nervous system depressant effects [23].

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Citrus aurantium

Citrus aurantium L. (Rutaceae), commonly known as sour orange (local name: laranja-amarga, laranja-azeda, laranjacavalo), is used in Brazilian folk medicine and other countries to treat anxiety, insomnia, and as an anticonvulsant, suggesting depressive action upon the central nervous system (CNS), among other properties. Essential oils, especially those of citrus fragrances, are popularly used as therapies for their effects on mood states and depression [24], and the orange essential oil is believed to induce an effect of mental relaxation [25]. A total of 22 phenolic compounds were identified in bitter orange seeds, including hydroxybenzoic acids, hydroxycinnamic acids, flavanones, flavanols, flavonols, flavones, simple phenol and coumarin [26]. Clinical studies suggest that the exposure to the inhalation of various kinds of essential oils is effective in reducing psychological stress, anxiety state, as well as the levels of cortisol in hypertensive patients [27]. The positive effects of essential oils on anxiety and depression symptoms have aroused interest, since they might be an alternative to synthetic substances which induce various side effects such as sedation, memory alterations and interaction with other drugs [28].

Echium amoneum

Echium (*Echium amoenum*) from Boraginaceae family, as an important Iranian traditional remedy, is widely used as a tonic, tranquillizer, diaphoretic, and as a remedy for cough, sore throat, and pneumonia [29]. It is believed that this plant possesses antibacterial, antioxidant, analgesic, anxiolytic, anti-depressant and immunomodulatory properties [30; 31]. Also it has been shown that E. amoenum aqueous extract was effective in the treatment of obsessive-compulsive disorder [32]. Dried violet-blue petals of E. *amoenum* have been recently recognized as an important source of phenolic compounds like rosmarinic acid, cyaniding, and delphinidin [33]. Cyanidin 3-glucoside, the most common anthocyanin, which is present in petals of E. *amoenum* attenuates PGE2 production and cyclooxygenase-2 expressions by inhibiting activation and translocation of c-Jun and NF-κB factors into nucleus [34]. Also the neuroprotective activity of cyanidin 3-glucoside has been investigated by Min et al. They suggested that the beneficial effect was related to attenuation of brain superoxide levels resulted from blocking apoptosis-inducing factor release in mitochondria [35].

Lavandula angustifolia

Lavender (*Lavandula angustifolia*) is a famous herb that has a long history in folk medicine and is still therapeutically used today. The essential oil obtained by steam distillation from the fresh flowering tops of this plant is often used in aromatherapy as a relaxant [36]. Inhalation of the vapor of the lavender essential oil and its main constituent, linalool, has shown sedative effects in both human and animal studies [37]. Other pharmacological effects of this oil, including anticonvulsive [38] anxiolytic [39], antidepressant [40], and anticonflict effects [41], have also been reported. On the other hand, lavender is also used as a tea infusion (i.e., aqueous extracts) to treat restlessness, insomnia, and nervous disorders of the stomach and intestines [42]. Furthermore, lavender contains aqueous phenolic constituents, such as hydroxycinnamic acids and flavone glycosides [43], which have been associated with the antioxidant activities of Lamiaceae plants including lavender [44].

Melissa officinalis

Melissa officinalis (Lamiaceae) or lemon balm is an herbal medicine native to the eastern Mediterranean region and western Asia. This plant is known as “Badranjboyeh” in Iran, and grows widely in provinces of Tehran, Golestan, Azarbayjan, Lorestan and Kermanshah [45]. Dried or fresh leaves and top aerial section of the plant are the parts which are used as medicine [45]. Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic, strengthening the memory, antidepressant and relief of stress-induced headache [46]. In Iranian traditional medicine, lemon balm has also been used in treatment of irritability and nervousness in young girls and women, lack of interest and energy, and depression [47]. Ibn Sina (Avicenna), the well-known Iranian scientist, recommended *Melissa officinalis* for above indications. The main components of the essential oil are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial. In addition, this oil contains such as threeterpinene, phenol carbon-acid (rosmarinic acid), and flavonglychoside acids in low ratio. Furthermore, it is stated that the essential oil of lemon balm which is, used in aromatherapy, may be beneficial for mild depression (3). The leaves of *Melissa officinalis* also known as lemon balm, are used in traditional medicine to prepare a tea for its nerve calming and spasmolytic effects [48] although there are a great variety of phytopharmaceutical preparations containing this plant or its extracts. Furthermore, this plant is used by food industry to flavor different products due to its particular taste. The number of people suffering from neurological disorders has lately increased worldwide, specially in the developed countries [49]. Between them, neurodegenerative diseases (Parkinson, Alzheimer) as well as psychiatric ones (anxiety and depression) are the most common [50].

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Salix aegyptiaca

Salix aegyptiaca commonly known as Musk Willow is a flowering plant and generally cultivated in some provinces of Iran for hedge and ornamental purposes [51]. Individual flowers are either male or female, but only one sex is to be found on one plant, so both male and female plants must be grown if seed is required and are pollinated by bees. The male inflorescences distillate of the plant has long been used in Iranian folklore medicine as cardiotonic, treatment of anemia, vertigo and depression, as well as a fragrance additive. Phenolic compounds from the extracts indicated the presence of gallic acid, caffeic acid, vanillin and p-coumaric acid, myricetin, catechin, epigallocatechin gallate, rutin, quercetin as well as salicin. The aqueous extract and essential oil of these inflorescences are also being used in confectionary, flavorful syrups and especially in the preparation of a local candy (Noghl-e Urmia) [52]. In addition, S. *aegyptiaca* is used as laxative, cardioprotective, nervous, sedative, hypnotic, somnolent, aphrodisiac, orexigenic, carminative and gastroprotection. The decoction of S. aegyptiaca leaves in honey still is used as a nervonic functional food [52].

Valeriana officinalis

Valeriana officinalis (Valerianaceae family) is a medicinal plant used in complementary and alternative medicine for its sedative and anxiolytic properties [53]. There are three main chemicals that are thought to be the active components of the plant. These are the essential oils, valerenic acid and valenol, valepotriates, and a few alkaloids. Valerian's effects on the central nervous system have been well documented and attributed to many of its active compounds: valepotriates, baldrinals, valerenic acid, valerenal and valeranone, and other constituents in the essential oils [53]. Consequently, the therapeutic properties of *Valeriana officinalis* have yet to be conclusively demonstrated [54]. The pharmacological effects attributed separately to each plant of a phytotherapeutic product – CPV (dry extract of *Crataegus oxyacantha* L., *Passiflora incarnata* L., and *Valeriana officinalis* L.) are well described in the literature. For instance, P. incarnate exerts anxiolytic, sedative and anticonvulsant actions [55], C. *oxyacantha* possesses cardiotonic, antiatherogenic, and antioxidant effects, which would enhance its action on atherosclerosis [56] and V. officinalis was utilized traditionally as a sedative for light insomnia, in addition to being indicated for its anxiolytic properties when administered in smaller doses [57]. Root extracts from *Valeriana officinalis* are popular herbal supplements and are widely used in the treatment of sleep disorders, anxiety, depression and epilepsy. Sleep disturbance can be associated with poor work performance, increased anxiety and depression, poor cognitive functioning, and impairment of overall QOL [58].

Viola odorata

Viola odorata is a species of the genus Viola native to Europe and Asia, but has also been introduced to North America and Australasia. It is commonly known as wood violet [59], sweet violet, English violet, common violet, or garden violet. The sweet scent of this flower has proved popular throughout the generations particularly in the late Victorian period, and has consequently been used in the production of many cosmetic fragrances and perfumes [59].
In the traditional system, it has been used in anxiety [60], insomnia and to lower blood pressure [61]. Violet is mainly used as an herbal remedy in cases of various respiratory ailments. It can be very beneficial in treatment of congestion, coughs and sore throat. Recent studies have shown the presence of glycoside of salicylic acid in Common Violet leaves, which explains its efficient use in cases of headaches and body pains. Syrup made from Common Violet's flower has anti-septic, anti-inflammatory, laxative and expectorant properties. It can be helpful in cases of various respiratory conditions, but also in treatment of headaches, insomnia, dizziness and exhaustion [62]. Viola contains alkaloid, glycoside, saponins, methyl slicylate, mucilage and vitamin C. The plant has been reported to possess antioxidant and diuretic activities along with other beneficial effects but no study has been found regarding its blood pressure lowering or lipid-lowering activity [61].

Cinnamomum zeylanicum

Cinnamon and its extract, irrespective of source, have been associated with a variety of health beneficial effects, including anti-microbial, anti-viral, antioxidant, and antidepressant activities. Many of the corresponding bioactivities are possibly attributed to cinnamaldehyde, a major constituent of the essential oil responsible for the flavor and aroma of whole cinnamon. In addition, a number of polymeric polyphenol molecules known as proanthocyanidins are present in the aqueous extract that are likely responsible for the majority of the antioxidant properties of cinnamon [63]. While the health-beneficial effects of bio-flavanoids in general are traditionally thought to be due to their antioxidant activity, proanthocyanidins exhibit other properties that may be important for their bioactivities [64]. Cinnamon bark contains procyanidins and catechins. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins extracted from cinnamon and berries also possess antioxidant activities [65]. Cinnamon can also improve the health of the colon, thereby reducing the risk of colon cancer. Cinnamon is a coagulant and prevents bleeding [66]. Cinnamon also increases the blood circulation in the uterus and advances tissue regeneration. This plant plays a vital role as a spice, but its essential oils and other constituents also have important activities, including antidepressant, antimicrobial, antifungal, antioxidant, anti-diabetic and anti-inflammatory [66].

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FUTURE DIRECTION

Considering therapeutic potential of these nine medicinal plants in terms of their efficacy and adaptability is such that combination of them as one organic product can be noticed in future, since depression is becoming more epidemic around the world especially in developing countries as an organic product by using local knowledge can reduce many problems associated with the use of chemical drugs and their side effects to a large extent.

Table 1: Nine medicinal plants with evidence of their activities

Plant	Plant part used	Bioactive compounds	Screened activity
Aloysia triphylla	Roots	Flavonoids (Artemitin and Hesperidin)	Antidepressant, anti-inflammatory [23]
Citrus aurantium	Flowers	phenolic compounds like flavanone glycosides, hydroxycinnamic acids	Antidepressant, anticonvulsant, antianxiety, antioxidant [24]
Echium amoneum	Leaves and flowers	phenolic compounds like rosmarinic acid, cyanidin, and delphinidin	Antidepressant, anti-hyperlipidemia, anti-cholesterol, antibacterial, anti-diabetic and antioxidant [30, 31]
Lavandula angustifolia	Flowers	phenolic compounds like hydroxycinnamic acids and flavone glycosides	Antidepressant, anticonvulsive, anxiolytic, antioxidant [40]
Melissa officinalis	Leaves and stems	Citronellal, citral (citronellol, linalool), geranial, threeterpinene, phenol carbon-acid (rosmarinic acid), and flavonglychoside acids	Antidepressant, antimicrobial, antispasmodic, antioxidant [47]
Salix aegyptiaca	Leaves and stem bark	phenolic compounds like gallic acid, caffeic acid, vanillin and *p*-coumaric acid, myricetin, catechin, epigallocatechin gallate, rutin, quercetin and salicin	Antidepressant, antioxidant, anti-vertigo, anti-anemia [52]
Viola odorata	Leaves and flowers	alkaloid, glycoside, saponins, methyl slicylate, mucilage and vitamin C, Cycloviolacin O2 (CyO2)	Antidepressant, anti-hyperlipidemia, anti-cholesterol, anti-blood pressure, anti-cancer and anti-tumor [60]
Valeriana officinalis	Leaves	alkaloid, glycoside, saponins, methyl salicylate and mucilage	Antidepressant, antioxidant, anti-inflammatory, laxative, anti-septic, anti-hyperlipidemia [57]
Cinnamomum zeylanicum	Stem bark and leaves	Eugenol. Cinnamaldehyde, camphor, procyanidins and catechins	Antidepressant, antimicrobial, antioxidant, anti-diabetic and anti-inflammatory [66]

CONFLICT OF INTEREST

There is no conflict of interest.

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None

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ARTICLE

EFFECT OF PLANTING BED CONDITIONS ON SOME GROWTH CHARACTERISTICS OF PLANE TREES IN NORTH EASTERN PART OF IRAN

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ABSTRACT

Root environment and soil composition play an important role on growth and development of the roots and, consequently, the trees. In this research, the effect of some physical and chemical properties of two soil types, including a typical agricultural soil and an urban soil with three soil depths (0-40cm, 40-80cm and 80-120cm), on the growth of plane trees in an urban environment were examined. Plan trees were selected as the typical tree types planted in Mashhad urban landscape. The agricultural soil type was supplied from local agricultural fields and the urban soils type was sourced from urban street landscapes in Mashhad. The results showed that there were significant differences in all measured traits including soil density, lime percentage, soil pH and EC, organic carbon percentage, nitrogen, phosphorus and potassium content. The results of leaf analysis showed that the trees cultivated in the urban soil had lower nitrogen and phosphorus absorption compared with trees planted in agricultural soil. In urban soils, soil density increased, which can be due to urban planning standards. In addition to soil compaction, nutrient shortages, inappropriate pH and high EC and other adverse soil conditions, tree growth will reduce and shortage of tree nutrients will occur. Due to the importance of trees in the city's life, a suitable planting bed for receiving water and nutrient for trees in urban soils should be provided.

KEY WORDS
Soil density; Plane tree ; early decay; Soil lime; urban soil.

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INTRODUCTION

Urban landscapes are major urban structures which provide oxygen for the cities and reduce air pollution. Also, urban landscapes can play a role in creating relaxation environments, controlling floods, creating visual aesthetics and reducing energy consumption. There are credible documents which show trees with shading in the summer and preventing winds in the winter will reduce energy consumption [1]. The ability of roots to explore the belowground environment in urban settings influences tree health, stability, and longevity. Quantitative studies on the response of urban trees to certain factors in bedding and underground environments are found [2].

Of all soil stresses in urban areas, the most common may be poor aeration due to soil compaction. Compaction destroys macro porosity; and because the pore space is reduced, soil resistance, hardness and bulk density are increased. In Washington, recently developed sites were found to have higher soil bulk densities than older sites, presumably due to more stringent engineering standards and more effective compaction equipment. Site development practices often entail removal of upper soil horizons (especially O and A) during grading, leaving denser subsoil at the surface, and the soil underlying pavement is typically compacted to provide structural support [2].

The plan tree (*Platanus orientalis*) is fast-growing, hydrophilic, with high optical requirements, deep-rooted, and dust-resistant roots that are recommended to be applied in temperate and humid areas to prevent soil erosion [3]. Plantain trees cannot grow well in small environments, such as sidewalks and streets [4]. In recent years, plantain trees in Iranian urban environments suffer from many problems, such as early grazing and signs of food shortages [5]. Water stress can limit plane tree life in Easter Europe[6]. Although much research has been done on the plan tree disorder in Iran, there has not been any evidence of the effect of beding effect on the plane tree.

MATERIALS AND METHODS

In this experiment plan tree *(Platanusorientalis*) was selected as the typical tree of landscaping of Mashhad. Mashhad is located in the northeast of Khorasan Razavi province. The city is located at 36.20° North latitude and 59.35° East longitude. By existence of over 60,000 plane trees of different ages in Mashhad urban landscape, this species was the most dominant tree species. Landscape of this city has severe management problems, especially demolition and early stage deterioration severe problems trees.

Two groups of trees were selected. The first group planted in an agricultural soil type and the second group was planted in urban soils with a minimum distance from the first group of planting. The diameter of the selected trees were15-20 cm (measure from 50 cm distance from the soil surface) and the soil samples were taken from 60 to 90 cm radius around the tree trunks. Six soil samples were taken from each of the nine experimental sites selected in different parts of the city which accounted 54 soil samples for whole experiment. In each sample point in the selected sites across the city three soil depths of A= 0- 40 cm, B= 40-80 cm, C= 80- 120 cm for taking the samples were selected. In addition to soil samplings and measurements in each sample point, vitality of the trees was assessed observing general morphological quality the trees at the end of the summer season. The quality measurements were based on the two qualitative factors of visual degree of crown defoliation and the intensity of leaf necrosis. Based on two

conditions for trees were classified including healthy trees (0–10% defoliation, 0% necrosis) and partly damaged (25–50% defoliation, 5–20% necrosis).

Soil density, soil lime, pH, EC, soil lime, organic carbon %,amount of nitrogen, potassium and phosphorus of the soil and also nitrogen, phosphorus and potassium content of the leaves were measured Then total organic carbon(using the Walkley and Black method) total nitrogen (using the Kjeldahl method), available phosphorus (using the Murphy-Riley method) and available potassium (Flame photometer) were measured.

This research was conducted as a completely design in 6 treatments and 9 replicates. The results were subject to analyses of variance as a factorial experiment based on a completely randomized block arrangement. Mean comparisons was performed based using LSD test 5% probability levels The statistical analyses were conducted using JMP 8 software package.

(A) **(B)**

Fig.1: Soil types used for this experiment. A) Urban compact soil. B) Agriculture soil.

Soil density, soil lime, pH, EC, organic carbon (%), soil nitrogen, potassium and phosphorus content and also nitrogen, phosphorus and potassium content of the leaves were measured. Soil density was determined by manual method and using a cylinder (5 cm height and 5 cm diameter) then dried and soil density was calculated. Soil lime amount was determined by the total neutralizing value (TNV%) method on the basis of calcium carbonate was measured using acid acetic volume consumed to neutralizing carbonates. Soil pH was measured in 1:1 [w/w] soil/water suspension; the electrical conductivity (EC) was measured from the saturation soil paste extract by an EC meter device. Then total organic carbon (Walkley and Black, 1934) total nitrogen determined by the Kjeldahl method (Hinds and Lowe, 1980), available phosphorus was measured by the Murphy-Riley method (1962) and available potassium was measured by Flame photometer device (Jenway-pfp7 model). This research was conducted as a completely design in 6 treatments and 9 replicates. The leafe nitrogen, phosphorus and potassium were measured by the Kjeldahl method (Hinds and Lowe 1980), available phosphorus was measured by the Murphy-Riley method (1962) and available potassium was measured by Flame photometer device (Jenway-pfp7 model). The results were subject to analyses of variance as a factorial experiment based on a completely randomized block arrangement. Mean comparisons was performed based using LSD test 5% probability levels The statistical analyses were conducted using JMP 8 software package

RESULTS

Soil pH

The results showed that there were significant differences between the soil types and depths at 5% probability level [Table 1]. Also, comparing the means [Table 2] showed that the highest soil pH was in0-40 cm depth in the urban soils. Lowest soil pH was in agriculture soil the 0-40 cm depth. Soil alkalinity is a common consequence of urbanization and therefore a more common impediment to tree health. This is mainly due to the use of concrete and other calcareous construction [2].

Previous research has confirmed the results of the present research in that urban soils tend to have higher soil pH than their natural counterparts. In Berlin, Germany, a pH of 8 was observed in street side, compared to a pH of less than 4 within a forest a short distance from the street. Over half of soils sampled in Hong Kong, China, were rated strongly alkaline (pH 8.5–9) to very strongly alkaline (pH 9–9.5), while surrounding soils were acidic at pH 4–5 [7].

Table 1: Analysis of Variance (mean squares) for soil characteristics plane trees in the studied experiment

	df	pH	EC	Density	Lime	O.C.	N	P	K
Soil type	5	1.15*	4.66**	0.51**	76.72**	0.26**	0.39**	209.27**	9183.48**
Error	49	0.39	0.19	0.01	4.73	0.007	0.006	23.34	919.29

**,* and ns: significant at 1 and 5% probability levels and non significant, respectively

Table 2: Mean comparison of the soil characteristics of plane trees in the studied sites

Treat	pH	EC d.s/m	Density gr/cm^3	Lime%	O.C.%	N%	P%	K%
Agricutral soil 0-40cm depth	7.14c	2.24d	1.36e	10.81cd	0.68a	0.74a	25.33a	220.7a
Agricutral soil 40-80cm depth	7.42bc	2.31d	1.47de	8.96d	0.46b	0.36b	19.22b	192.4ab
Agricutral soil 80-120cm depth	7.36bc	2.78cd	1.51d	11.55c	0.26d	0.21cd	12.22c	135.7d
Urban soil 0-40cm depth	8.15a	3.61b	1.97a	17.11a	0.34c	0.23cd	18.44b	206.2ab
Urban soil 40-80cm depth	7.74ab	4.05a	1.82b	14.75b	0.29cd	0.26c	15.22bc	179.1bc
Urban soil 80-120cm depth	7.69ab	3.09c	1.62c	12.22c	0.22e	0.18d	13.22c	154.4cd

In each column, means followed by the same letter are not significantly different at P≤0.05 according to LSD test.

Table 3: Analysis of variance (mean squares) for N, P, K in leaf contents of plane trees in the studied experiment

	df	N	P	K
Soil type	1	0.97**	0.13**	0.13ns
Error	17	0.11	0.009	0.04

**,* and ns: significant at 1 and 5% probability levels and non significant, respectively

Table 4: Mean comparisons of N, P, K leaf content of plane trees in the studied experiment

	N%	P%	K%
Trees planted in agricultural soil	2.45a	0.69a	1.66a
Trees planted in urban soil	1.99b	0.52b	1.49a

In each column, means followed by the same letter are not significantly different at P≤0.05 according to LSD test.

Soil EC

The results showed that there were significant differences among the soil types and depths in terms of soil electrical conductivity (EC) at 5% probability level [Table 1]. Comparison of the means showed that the highest soil EC was in urban soils at 40 to 80 cm depth, and the lowest in agricultural soils with depth of 0-40 cm [Table 2]. In Northeast parts of China, heavy snow in winter is usually accompanied with large amounts of snow-melting salt utilization. This salt utilization probably increases soil EC, and it is likely a pattern that urban central regions had more utilization of these salts [8]. The present findings suggest that, in addition to the above natural factors, human activities also affect soil electrical conductivity in the areas surrounded. Pervious study expressed in the low penetration depth; this reduced soil pore sizes, which furthered capillary rise and promoted high electrical conductivity even after a few days after the rain [9].

Soil density

The results showed that there were significant differences between the treatments at 5% probability level [Table 1]. Comparison of the means showed the lowest soil density was associated with 0-40 cm depth in agricultural soil and greatest in the 0-40 cm depth of urban soil [Table 2]. Perhaps the most important stress in urban soils is the reduction of soil porosity due to increased soil compaction. Soil compaction arising from urban land development and use is a more pervasive cause of root restriction for landscape trees. Compaction occurs as soil is compressed, which degrades structure, diminishes porosity, and increases strength the soil's physical resistance to penetration. Soil compaction in urban areas is widespread. In a study of 48 sites in Moscow, Idaho, and Pullman, Washington, recently developed sites were found to have higher soil densities than older sites, presumably due to more stringent engineering standards and more effective compaction equipment. Site development practices often entail removal of upper soil horizons (especially O and A) during grading, leaving denser subsoil at the surface, and the soil underlying pavement is typically compacted to provide structural support. Root penetration depth can be restricted by soil density [7]. The highest root activity including absorption of water and food and also aeration is carried out at soil depths of 0 to 30 cm. As we know root cells of the trees need to breathe. When breathing, if carbon dioxide produced from the surrounding environment cannot be removed and fresh oxygen is replaced by the atmosphere, the root is tense and cannot well absorb water and food [10]

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Soil Lime

The results showed that there is a significant difference between the samples at 5% probability level [Table 1]. Comparison of the means showed that the highest amount of soil lime was observed in urban soil and at 0-40 cm depth [Table 2].

In previous research on the urban soil showed increased concentrations of calcium and magnesium were also found, probably a result of contamination from building materials, such as bricks and concrete. Soil reaction is changed by higher concentrations of calcium and magnesium [11].

Total organic carbon

The results showed that there were significant differences between the soil types and depths at 5% probability level [Table 1]. Comparison of the means showed that the highest organic carbon content was observed in agricultural soil and disregarding the soil types total organic carbon decreases as soil depth increases [Table 2].

Due to the operation of submersion in urban soils, the soil layer is removed, which reduces the organic carbon of the soil, reducing the organic matter of the soil, which reduces the quality of root nutrition by the soil. By removing organic matter, the physical conditions of the soil make it harder for the food to be absorbed by the root. The removal of grass clippings, tree leaves, and other organic debris can further reduce inputs to the soil organic matter pool; while, organic additions such as top soil replacement, mulch, root turnover, microbes, earthworms, grass clippings, and leaf litter left on site help to build soil organic matter [9].

Soil nitrogen, phosphorus and potassium

The results showed that there is a significant difference in soil nitrogen, phosphorus and potassium levels between different soil types and depths with a 1% probability [Table 1]. Comparison of the means showed that in agricultural soils with a depth of 0-40 cm depth, the highest amount of root absorbable materials was found and this amount was reduced as the soil depth decreased. However in urban soils due to the structural changes and the removal of surface layer amount of these nutrients is reduced [Table 2]. Urban soils generally lack organic matter cycling and its nutrient contribution that typifies soil of the natural ecosystem. This is mainly because beneficial organic nutrient-containing materials (especially nitrogen, sulfur and phosphorus) such as leaves, litter, and animal remains are removed as wastes, or are produced in small quantities due to stressful conditions. Also, some urban soils do not rest on parent material or bedrock and do not receive the continuing benefit of nutrients released from inorganic mineral weathering [12].

In a study conducted in Beijing in 2014, it was determined that the total nitrogen and the total phosphorus in the soil in urban areas are lower than that in the surrounding area. In this study it was observed that the total P concentration in Beijing had a decreasing trend form the center of the city to its outskirts Compared to other cities around the world, the level of total N in Beijing was lower than that of Stuttgart, Germany (1400 to 7200 mg/kg) and Shanghai (370 to 2260 mg/kg, with an average of 1120 mg/kg) and especially this shortage increased in residential and commercial areas of the city [13].

Nitrogen, phosphorus and potassium of leaves content

The results showed that there was a significant difference ($p <= 0.05$) in nitrogen and phosphorus content of the leaves of the trees planted in agricultural soils and urban soils [Table 3]. Comparison of the means showed that leaves of the trees in agricultural soils had higher levels of adsorption nitrogen and phosphorus than the trees planted in urban soils. There were no significant differences in potassium content of the leaves planted of all both type of soil in this study [Table 4]. Nitrogen and phosphorus are macro nutrients in plant nutrition. Plants need these two elements during their entire growth period. Further nitrogen and phosphorus deficiency can reduce plants' growth and increase physiological disorders in plants and ultimately lead to an inability in the plants to absorb other nutrients.
In desert ecosystems such as that of (Mashhad climatic region), low soil moisture coupled with high soil alkalinity acts to decrease soil N and P availability. Infrequent and low precipitation limits soil weathering, organic matter production, and mineralization, leading to slow P release from primary material, low soil organic matter content, and N bound in organic matter. A study from 224 dry land sites indicated an increased decoupling of carbon (C), N and P with increased aridity resulting in greater P compared to N availability. Plant N fixation rates in arid regions have long been considered to be low because of low soil moisture and high temperatures. In contrast, ammonia volatilization of dry land soils can be high, as volatilization rates are positively related to soil pH, total salt content and $CaCO_3$, and negatively related to soil organic matter, cation-exchange capacity and clay content [14].

Availability of P is also reduced in alkaline soils. Elevated pH may also alter the composition and abundance of endomycorrhizal fungi that inhabit soil, which could influence root system colonization and therefore nutrient uptake capacity [3]

Although urban soils are heterogeneous and can defy generalization, it is common to find impenetrable horizons relatively near the surface; examples include buried asphalt, subsoil's compacted by construction activity, and poorly drained horizons. Analogous conditions in forest settings (e.g., bedrock, hardpans, shallow water tables) result in shallower root systems than occur for the same species on less restrictive

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sites. Soil compaction is very common in urban areas and can result in severe root restrictions. Plant species interaction with the environment plays a role here as well [3].

Root depth and extent can be severely limited and highly irregular in urban settings. When root restrictions are minimal, root spread shows a strong relationship with trunk diameter, which is a more reliable predictor than canopy diameter or tree height [3].Soil hardness and lack of ventilation reduce root spread. Also, nitrogen deficiency in cities is one of the most common deficiencies in urban trees. Nitrogen deficiency causes poor trees with a small crown and yellowish-green foliage, causing death in the acute conditions.

CONCLUSION

Urbanization has caused significant changes in the natural environments. Soil, which is the main bed for plant and root activities, has also been changed a lot. Increasing the soil density, reduction in soil aeration, reduction in soil nutrients, changes in pH, EC, have caused green plants especially trees to live in cities with many problems. This research identified some of these problems. Such findings can assist urban planners and managers towards more sustainable urban landscapes in urban environments.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY OF KEFIRAN EXTRACTED FROM *PEDIOCOCCUS PENTOSACEUS* STRAIN TNAR03

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ABSTRACT

Background: *Kefir is a microbial symbiont mixture that produces slimy grains with collection of bacteria and yeast. The polysaccharide extracts (kefiran) from kefir have antimicrobial, antioxidative and anticancer activity.* ***Methods:*** *Extraction of kefiran in laboratory environment was performed by batch culture of Pediococcus pentosaceus strain TNAR03. The supernatant was used to evaluate the antibacterial, anticancer and antioxidative activities of kefiran. Antimicrobial activity was estimated by Disc Plate Method, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration. Antioxidative activity, including radical-scavenging effects was analysed by DPPH method. Anticancer activity by MTT assay were investigated therein.* ***Results:*** *The intestinal pathogens from MTCC showed excellent decline in the growth when the kefiran extract were added to it. The antioxidant-scavenging properties by DPPH showed an enhanced antioxidant effect in the extract of kefiran. Vero and HepG2 cell were exposed to serial concentrations of kefiran to evaluate its cytotoxic activities. Results showed that kefiran significantly affected the viability of both tested cancer cell lines in a dose-dependent manner with IC_{50} values of 298.8 ± 1.71 and 371.2 ± 1.32 µg/ml for Vero and HepG2 cells, respectively.* ***Conclusion:*** *These findings have demonstrated that kefirs possess antioxidant activity, thereby suggesting that kefirs are potential applicants for the role of useful natural antioxidant enhancements for the human diet.*

KEY WORDS
Kefiran, Antimicrobial, Antioxidant, Anticancer activity, EPS

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INTRODUCTION

Probiotic bacteria are microorganism that can be used as a nutrition for the benefit for health. The kefir grains initiating the fermentation are a combination of lactic acid bacteria (LAB) and yeasts in a matrix of proteins, lipids, and sugars. This symbiotic culture of bacteria and yeast forms "grains"[1]. Kefir is a microbial symbiont mixture that produces jelly like grains as it grows, that contain both lactic acid bacteria (*Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, etc.) and yeasts (*Candida*, *Saccharomyces* sp. etc.). Both bacteria and yeasts are surrounded by a polysaccharide matrix, named kefiran, a water-soluble branched glucogalactan, which has been reported to have antibacterial and anticancer activity [2,3].

Kefir is needed to act against the pathogenic genera and to have anti-inflammatory activities. The main properties of kefir might be of use as an alternative medication for patients infected with a single or multi-resistant strains of bacteria [4].

The importance of probiotics in food industry is growing nowadays and further research between different microorganism and their interactions can result in curing and preventing human diseases (Irritable bowel syndrome, Baterial vafinosis, traveler's diarrhea, small interstinal bacterial overgrowth) and other disorders[5]. Antibiotic use became widespread that resulted in obstinately developed resistance in bacteria. Because of this, efforts have been made to develop and study new compounds outside conventional antibiotic therapy [6]

This work focuses on the production, characterization of kefiran and its antimicrobial, anticancer and antioxidative analysis.

MATERIALS AND METHODS

Microorganisms used

The microorganisms used were *Bacillus subtilis* MTCC 441, *Bacillus cereus* MTCC 1272, *Staphylococcus aureus* MTCC 1144, *Pseudomonas aeruginosa* MTCC 741, *Escherichia coli MTCC* 739, *Klebsiella aerogenes* MTCC 39 *Vibrio cholerae* MTCC 3906. All strains were purchased from MTCC and revived by using appropriate media following the standard instruction provided [7].

Kefir

Starter grain was purchased and maintained under appropriate condition. After 24 hours, the grains appeared to settle down at the bottom of the flask. The sour taste and the turbid appearance of the kefir drink indicated that it has been fermented. The medium was changed at 24h intervals and the grains washed with sterile water [7]. The organisms from kefir were isolated, identified by biochemical method and molecular 16S rRNA identification method and the sequence was submitted to NCBI using BANKIT Tool. Suspensions and kefir grains contained significant number of *Pediococcus* sp, and *Leuconostoc* sp.

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Extraction of kefiran

Kefir was extracted for kefiran production for use in the antibacterial experiments. The polysaccharide matrix (kefiran) also used was isolated from kefir grains using the method described by Micheli et al. [8]. The stirred grains were washed with boiled distilled water for 1 hour (1/100 v/v).The mixture was then cooled and centrifuged (Remi R-4C) at 10000 rpm for 15 min. The procedure was repeated with the sediment. The polysaccharide dissolved in the combined supernatants was precipitated by the addition of an equal volume of cold ethanol at 4°C overnight. The precipitate was re-dissolved in hot water (1:100) for 1 h at 70°C with stirring and the precipitation procedure was repeated twice. The precipitate was finally dissolved in 100mL distilled water, and subjected to dialysis in distilled water.

Susceptibility studies

Antibiotic susceptibility was studied by modified Kriby -Bauer method. The wells were made using sterile agar gel borer and loaded with different concentration of kefiran extract on pre-swabbed plate with organism in it [9].

Antimicrobial susceptibility was analyzed and interpreted using the guidelines for reference broth microdilution method as described by the Clinical & Laboratory Standards Institute (CLSI) [10].

The Minimum Inhibitory Concentration (MIC) was defined as the lowest antimicrobial concentration able to completely inhibit bacterial growth up to 24h. MIC parameters were determined in triplicates using 0.1mL of bacterial suspensions (5×10^8 CFU/mL) in tubes containing 10mL of minimal media and the same amounts of kefiran as described above. Tubes were mixed by vortexing and incubated at 37°C for 24 h. Minimal bactericidal values were obtained based on the results for MIC values. Plates containing 25mL of Brain Heart Infusion (BHI) agar medium were inoculated with 0.1mL of the tubes showing no growth and incubated for 24 and 48h at 37°C. Controls were analysed similarly using the antimicrobial agents listed above [11].

Scavenging effect upon DPPH radicals

The effect of kefiran upon DPPH radicals was measured according to the method [12]. Various concentrations of kefiran (0.8 ml, 0-4 mg/mL) were separately mixed with 0.2 mL of a methanolic solution containing DPPH radicals to give a final concentration of 0.2mM DPPH. The mixture was shaken vigorously and left to stand for 30min in the dark, and its absorbance was than measured at 517 nm. The capability to scavenge DPPH radicals was calculated as

DPPH radical-scavenging assay%
= 1- (absorbance of sample at 517 nm) / (absorbance of control at 517 nm) ×100

The percent DPPH decolonization of the sample was calculated. L-Ascorbic acid was used as a positive control [13].

Anticancer activity of kefiran

Vero and HepG2 cell lines were grown on RPMI-1640 medium supplemented with 10% inactivated foetal calf serum and 50µg/mL of antibiotic. The cells were preserved at 37°C in a humidified environment with 5% CO_2 and were subcultured two to three times a week. Potential cytotoxicity of the compounds was evaluated on tumour cells using the method of Bertram [14]. Working dilutions were freshly prepared on the day of testing. After 72h incubation, the cell growth rate was evaluated by performing the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which detects dehydrogenase activity in viable cells. Each test was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from concentration–response curves using linear regression analysis by fitting the test concentrations that give values above and below the reference value (i.e.,50%). If however, for a given cell line all of the tested concentrations exceeding the respective reference level of effect, then the highest tested concentration is assigned as the default value, which is preceded by a '>' sign. Each result is a mean value from three separate experiments.

$$1-\frac{OD_t}{OD_C}\times100$$

Where, OD_t is the mean optical density of wells treated with the tested sample and
OD_c is the mean optical density of untreated cells.

Statistical analysis

All values were expressed as mean ± S.D. Antimicrobial activity data from diffusion experiments were evaluated using the least squares method adjusted to the data and by one-way ANOVA using SPSS[15].

RESULTS

Isolation

The organism isolated from milk kefir was further screened and identified as *Pediococcus* sp. Confirmation of the organism was done using 16S rRNA method and the sequence was submitted in NCBI. The accession number was obtained as KY817786 [Fig. 1]. Further the organism was enhanced to produce kefiran using batch culture

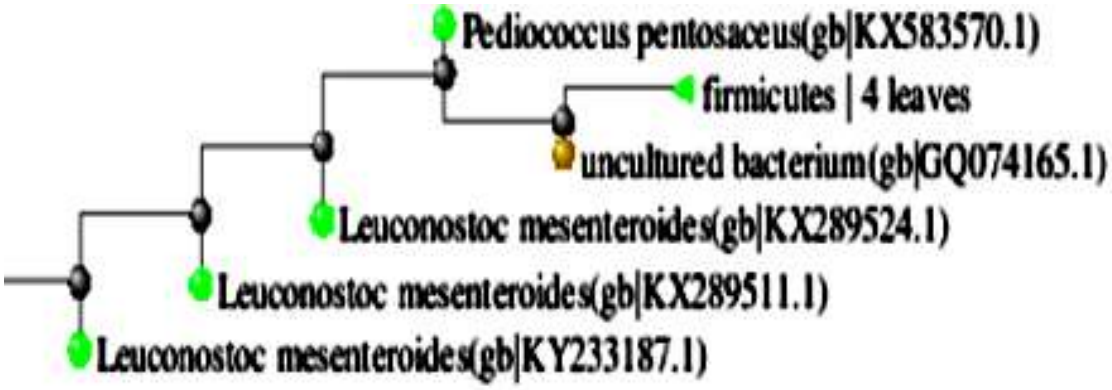

Fig. 1: Phylogenetic analysis of organism

Sequencing results of *Pediococcus pentosaceus*

GCTAGGGAACCGGTAGTTGGTTTCCCCTGGATTTTGATCGTGGTCAGGCATCGAACAGGTAACCGTAGAGGATAAAAAA
AGTTGGTAGGCGCATTGTACGAATAYTGTTACGACTTCCTGAGCCAGGGTCAAACTCTAGGTTCCTTGTTAGATCTTGTTA
CAACTTCCTGACTAGGGTCAAACTCTATAGGTTCCTTGTTACAACATCTTGATACACCTTCCTGATCTATGGTCACACTCTA
TGGATTCCCTGTTTCGACATCCTYCCCAACTTAAAAACCCATGATCAACCTTATCGGAACCTAGAACATTCCTGACAATAAG
GGGCATGATGATCTGACGTCGTCCCCGCCTTCCTCCGGTTTGTCCCGGCGTCTCGCTAGAGTGCCCATCTGAATGCTGG
CAACTAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACACCATGCACACC
TGTCACTTTGTCTCCGAAACACTTCTATCTCTAAAAGCTTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTC
GAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAG
GCGGAACACTTAATGCGTTAGCTTCGGCACTAAGAGGCGGAAACCTCCTAACACCTAGTGTTCATCGTTTACGGTGTGG
ACTACCAGGGTATCTAATCCTGTTTGCTACCCACACTTTCGAGCCTCAACGTCAGTTGCAGTCCAGTAAGCCGCCTTCGC
CACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGAGTTCCACTTACCTCTACTGCACTCAAGTTACCAGTT
CSATGCCATTCCGGAGTTGAGCT

Susceptibility tests

Inhibition ratios of kefiran against the pathogenic strains were determined from minimum least squares applied to different concentration at 5, 20 and 50. The results show *Pseudomonas aeruginosa* to be the most sensitive microorganism to kefiran, followed by *Bacillus subtilis* and *Bacillus cereus*. *Vibrio cholerae* and *Staphylococcus aureus* were less sensitive to kefiran [Table -1] and *Klebsiella aerogenes* and *E. coli* the least sensitive. Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for kefiran against all strains tested, ranged from 256 (MIC) to 128 mg/L (MBC) showing only a small increase in concentration to achieve a killing effect

Table 1: Antibiotic sensitivity pattern of various antibiotics on different organisms

S.No	Name of the Antibiotics	Zone of inhibition in mm						
		SA	BS	BC	PA	EC	KA	VC
1	Amoxicillin	16.5	21.2	27.2	24.5	22.1	11.9	23.3
2	Amikacin	17.6	29.4	12.3	34.6	11.2	12.5	25.5
3	Cefotaxime	25.1	28.1	23.4	35.2	28.5	22.1	28.1
4	Chloramphenicol	22.4	24.1	28.6	13.4	7.3	9.5	19.2
5	Erythromycin	15.5	11.5	46.7	23.6	12.7	16.4	18.4
6	Gentamycin	17.7	42.2	49.5	46.1	11.9	8.7	10.9
7	Streptomycin	19.8	28.9	24.4	43.3	8.4	12.9	7.7
8	Ciprofloxacin	18.1	32.8	46.6	19.6	19.6	12.6	16.2
9	Tetracycline	16.8	26.3	6.4	3.2	6.4	8.3	6.4
10	Kefiran	29.2	30.1	53.2	42.5	30.9	28.5	31.4

SA-*Staphylococcus aureus* MTCC 1144; BS - *Bacillus subtilis* MTCC 441; BC- *Bacillus cereus* MTCC 1272; PA - *Pseudomonas aeruginosa* MTCC 741; EC - *Escherichia coli* MTCC 739; KA- *Klebsiella aerogenes* MTCC 39;VC -

Vibrio cholerae MTCC 3906;

The results represent the mean zone diameters (in mm) using the agar diffusion method. MIC/MBC values of kefir and kefiran showed within a narrow range.

Scavenging effect upon DPPH radicals

DPPH is a compound that possesses free radical and used to determine its radical scavenging action. DPPH exhibits a characteristic absorption at 517 nm and its purple color fades when it encounters radical scavengers. At a dosage of 4.0 mg/ml, kefiran showed a significantly greater level of scavenging activity of DPPH radicals. The antioxidant activity for the milk based products were reported in different literature [5,8,10]. Hence the compound synthesized by the microorganism may have a similar property.

Evaluation of *in vitro* cytotoxicity of kefiran polysaccharides

The kefiran polysaccharides produced by *Pediococcus pentosaceus* strain TNAR03 were evaluated for its *in vitro* cytotoxic properties against Vero and HepG2 cells using standard MTT assay. [Fig. 2] shows the cytotoxic effect of kefiran, which was type-dependent. The IC_{50} values for Vero and HepG2 cells were 298.8 ± 1.71 and 371.2 ± 1.32 µg/mL, respectively. In increasing concentration of Kefiran extract, the cytotoxicity was prominent in the Vero cell line, compared with that of Vero.

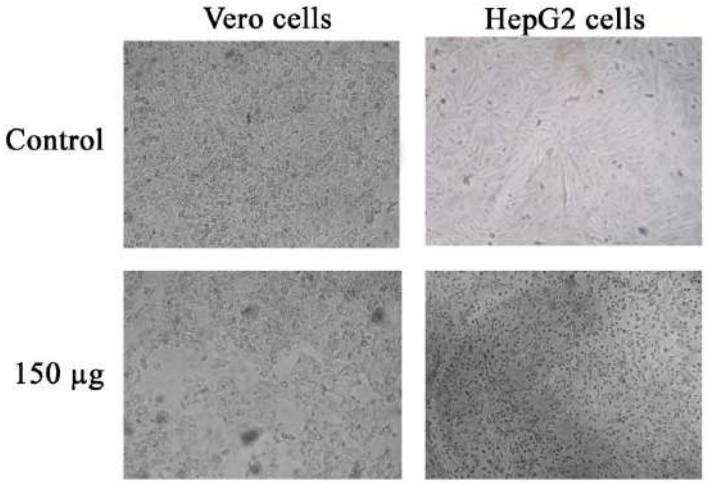

Fig. 2: Effect of kefiran on morphological characteristics of Vero and HepG2 cells after 24 h. Images were captured using inverted contrast microscope at 10x magnification.

CONCLUSION

Kefir is a symbiont organism mixture that produces slimy grains with collection of bacteria and yeast. The polysaccharide extracts (kefiran) from kefir have antimicrobial, antioxidative and anticancer activity. Therefore, kefirs are potential candidates for the role of useful and natural antioxidant supplements in the human diet.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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ARTICLE

ANTI-TUBERCULOSIS ASSAY OF NANOHERBAL AND ETHANOLIC EXTRACT OF *LANTANA CAMARA* LINN FLOS IN VITRO AND IN VIVO

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ABSTRACT

Background: *M. tuberculosis has been resistant to the synthetically anti-tuberculosis drugs. Traditionally Lantana camara* Linn-*flos (tembelekan in Indonesia) has been used to treat cough and bloody sputum. This study was conducted to investigate the potency of nanoherbal and extract of tembelekan flos as anti-tuberculosis in vitro and in vivo.* ***Method:*** *Anti-tuberculosis in vitro assay of nanoherbal and extract were performed at the concentration of 50 mg/ml and 25 mg/ml by Lowenstein-Jensen (LJ) method. Anti-tuberculosis in vivo assay was conducted in guinea pigs infected with M.* Tuberculosis *H37Rv into the bronchi using nebulizer. The nanoherbal and the extract were administered once a day at the dosage of 50 mg/kg BW and 25mg/kg BW. Anti-tuberculosis assessment was conducted by examining M.* Tuberculosis *on bronchial fluid specimens that were taken every week and assayed with LJ method.* ***Result:*** *In vitro assay showed that nanoherbal and extract could inhibit the growth of M.* Tuberculosis*: the 50 mg/ml of nanoherbal showed M.* Tuberculosis *negative for 5 weeks, while the 50 mg/ml extract showed positive +1 of M.* Tuberculosis *for 6 weeks. In vivo assay showed that both the nanoherbal and the extract performed anti-*tuberculosis *activity in guinea pigs infected with M.* Tuberculosis*: single daily dose of 50 mg/kg BW nano herbal for 4 weeks or extracts for 5 weeks decreased the bacterium from +3 to negative.* ***Conclusions:*** *In vitro nanoherbal and ethanol extracts of Lantana camara* Linn *flowers have strong activity to M. tuberculosis growth retardation. In vivo nanohebal and ethanol extracts of flowers tembelekan concentration of 50mg / Kg BW has a strong activity as anti-*Tuberculosis *in animal experiments.*

KEY WORDS
Lantana camara Linn, flos, nano herbal, ethanolic extract, anti tuberculosis acti

INTRODUCTION

Tuberculosis (TC) is one of the leading causes of death diseases, due to infection by *M. tuberculosis* that can infect latently or progressive, and the majority (80%) attacks respiratory tract and lungs. In general, in the world there are 2 billion people are infected and 2-3 million people die of Tuberculosis every year. Indonesia ranks third in the number of Tuberculosis infected people after India and China. TC is a disease threat in Indonesian, especially for the productive age between 15-55 years, and it is the third cause of death after heart disease and acute respiratory disease in all ages.

Increasing the number of TC cases is caused by a variety of factors, namely the lack of patient compliance rate for treatment, because treatment of this disease requires a long time; approximately 6 months, also the incidence of dual resistance, lack of endurance host against mycobacterium, and reduced power bactericidal drugs. People with TC can be treated and hospitals may provide optimal therapy with medicines such as rifampicin, ethambutol, isoniazid, pyrazinamide, and streptomycin or a combination of these drugs that are known as OAT KDT. The possibility of microbial resistance makes it is necessary to look for an alternative medicine; for example from natural ingredients that can help the treatment of patients of infected Tuberculosis.

To supply the medicines of TC, this paper discusses the experimental research result on *Lantana camara* Linn (in Indonesia is called as *tembelekan*). Indonesia is very rich with a diverse range of useful plants as medicine such as *Lantana camara* Linn., family of *Verbenaceae*. According to the *Ensiklopedia Tanaman Obat Tradisonal* [1, 2], *tembelekan* traditionally been used for various treatments, including for the stop of the bleeding, cough and bloody phlegm and asthma. The way of usage: taken 6 - 10g dried flowers boiled in 3 cups water until it is remaining 2 cups. Cold taxable income, filtered boiling water, drink 3 times a day.

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Several previous studies, including the methanol extract of leaves of *tembelekan* in vitro to inhibit the growth of *M. tuberculosis H37Rv* [3],the results of the test structure elucidation of leaves *tembelekan* obtained derived *flavonoids* can inhibit the growth of *M. tuberculosis* in vitro [4], *tembelekan* plants in vitro inhibit the growth of bacteria *Escherichia coli, Pseudomonas vulgaris, Pseudomonas aeroginosa, Streptococcus aureus*, the combination of garlic and leaf of *tembelekan* inhibits the growth of *M. tuberculosis* [5].

Referring to the efficacy of *tembelekan* that has been traditionally used to treat cough and bloody phlegm [6, 1], and the methanol extract of leaves *tembelekan* in vitro to inhibit the growth of *M. tuberculosis* [3], it is likely that flowers of *tembelekan* have been potential for the treatment of Tuberculosis. Due to large volume, it is difficult in the storage and transportation of *tembelekan* leaves, so it has to be developed in a more practical way; such as nanoherbal or extract form. Niño herbal has a very small particle size of the nanometer measurement. It is found that the effect of drug particle size on the rate of dissolution and bioavailability and comprehensively is demonstrated by drugs that absorbing the gastrointestinal tract. Particle size reduction of it may increase the rate of absorption and bioavailability, and one of particle size reduction efforts is to create the nano-scale shapes [7].

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In practical way, natural materials are made in the form of nano herbal likely to have advantages over the form of extracts for chemical compounds that might be still relatively intact, more soluble and easily

absorbed so that the possibility of onset of the drug will be faster and smaller doses [7]. On the other hand, the production of nano herbal requires less time and low costs because it does not use filters.

Based on these processes, the authors thus made the nanoherbal and ethanol extracts of *tembelekan*'s flowers and observed its potential in vitro of anti-tuberculosis with Lowenstein-Jensen method in LJ media, and in vivo with the infected Guinea pigs with *Mycobac-terium* Tuberculosis H37Rv. The results were expected to prove the efficacy of anti-tuberculosis of *tembelekan*'s flowers, and found the potential anti-tuberculosis difference between nano herbal and extracts of *tembelekan* flowers. So the flowers of *tembelekan* have a high possibility to be developed into anti-Tuberculosis medicines from natural materials with a rational way, cheap budget, and easily available.

Types of Anti-Tuberculosis Drugs

Tuberculosis drugs are commonly divided into two groups; namely, primary and secondary.

a. Primary drugs: *Isoniazid, rifampicin, pyrazinamide,* and *streptomycin Etam-butol* (*canamycin, amicacin*). These medications are most effective and have low toxicity, but causing any resistance quickly when used as a single agent. So, given the combination of 3-4 drugs, for sensitive TB germs are widely used in a combination of *isoniazid, rifampicin* and *pirazinamuida*.

b. Secondary drugs: *clofazimine, flourcinolon, cycloserine, rifabutin*, and *p-aminosalicylic* acid (PAS). These drugs have weaker activity and typically only used when there is resistance or intolerance facing primary drugs, also against infection with *M. avium intrasellulare* in HIV patients

Category of Tuberculosis

Tuberculosis has several categories namely:

The first category is that have the characteristics such as: a) new patients with positive pulmonary Tuberculosis acid-resistant bacteria (AFB). b) New patients with negative pulmonary Tuberculosis acid-resistant bacteria (AFB) and positive chest radiograph.c) Patients with extra pulmonary Tuberculosis

The second category has characteristics such as:

a. Relapse patients have been treated and recovered, but relapsed again

b. Failure patients, have been treated but less disciplined

c. Patient treatment is interrupted, has been treated but stops before recovering

Tuberculosis Treatment Measures

Tuberculosis therapy actions are performed by category of:

1. The first category treated through two phases is known with the code: 2 HRZE/4H3R3

a. Intensive phase; every day is given to the combination drug *isoniazid, pyrazinamide,* and *ethambutol* for two months.

b. Advanced phase: After the completion of the continuation phase is given by the combination of drugs *isoniazid* and *rifampicin* for four (4) months and 3 times a week

2. The second category treated through two phases namely known with the code: 2 HRZES/ HRZE

a. Intensive phase; every day is given to the combination of drug *isoniazid, rifampicin, pyrazinamide,* and *ethambutol,* and *streptomisin* for two months.

b. Advanced phase: Once completed given the drug combination of *isoniazid* and *ethambutol* for 5 months, with provision 3 times a week

If after two months of smear is still positive, plus 1 month intensive phase as inserts with HRZE

Lantana camara Linn (*Tembelekan*) Plants

Lantana camara Linn (*Tembelekan*) is an herbaceous plant, erect or slightly climbing, height 0.5 to 4 m, a characteristic odor. Woody stems, branched, twigs quadrangular, prickly, hairy, single leaf, face, round eggs, pointed tip, Compound interest grain shape, the inside of the crown-haired, white color, pink, orange, yellow, and so forth [6].

Determination of *Lantana camara* Linn (Tembelekan)

Division	: Spermatophyta
Sub Division	: Angiosperms
Class	: Dicotyledoneae
Sub Class	: Dialypetalae
Ordo	: Solanales
Family	: Verbenaceae
Genus	: Lantana
Species	: *Lantana camara* Linn

Synonym : *Lantana aculenta* Linn = *Lantana aculeata* = *Lantana antillana* Rafin = *Lantana mutabilis* Salisb = *Lantana polyacanthus* SCH. = *Lantana scabrida* Soland = *Lantana viburnoides* Blanco. The name of the region: Sumateran: Interest fence, Singapore wood, Tahi ayam (Malayan). Java: Kembang satek, saliyara, tai hayam, tai kotok, cente (Sundanese), *Kembang telek, oblo, punyengan, pucengan, tembelek, tembelekan, teterapan, wauna, wileran, kamanco, mainco, tamanjho (Madura*)

Fig. 1: *Lantana camara* Linn (*Tembelekan*) Plants

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Chemical contents of *Tembelekan* Flos

Based on the result of previous research, *tembelekan* contains the chemical compounds namely *alkaloid, cardiac glycosid, steroid, saponin, flavonoid, tannin,* essential oil, *triterpenoid*. Some chemical compounds namely *lantadene, lantadene* A, B, *lantanolic acid, humulene,* β- *Caryophyllene,* γ-*terpinene,* α-*pinene*, and p-cymene *triterpenoids, camarin, lantacin, camarinin, lantadienone, camaradienone, lantanoic acid, camaranoic acid, camarolic acid and lantrigloylic, lantadene, lantadene A, B, lantanolic acid, lantic acid, humulene* (4).

Some benefits

Traditionally, the root of *Lantana camara* Linn is as a reliever fever (*antipyretics*), detoxifying (*antibiotics*). Pain relievers (*analgesics*), rheumatism, stop bleeding (*hemostatic*), influenza, Tuberculosis glands, and vaginal discharge. The leaves are slightly poisonous (toxic), nutritious eliminate itching (*anti pruritic*), antitoxic, eliminate swelling, stimulating vomiting, skin diseases, bruises, sores, swelling, itching, fever, and arthritis. The flowers stop bleeding, cough and bloody phlegm, and asthma [6, 1, 2].

Several studies have been conducted to prove the efficacy of *tembelekan* plant based on the methanol extract of leaves in vitro to inhibit the growth of *M. tuberculosis* H37Rv [3]. The test results of the leaf structure elucidation *tembelekan* of obtained *flavonoid* derivatives inhibit the growth of *M. tuberculosis* in vitro [4]. *Tembelekan* plants in vitro retarded the growth of bacteria *E coli* ATTC 25 922, ATTC 13315 *Pseudomonas vulgaris, Pseudomonas aeroginosa* ATTC 15 442, 15 748 *V.chlareae* ATTC, *Streptococcus aureus* ATTC 12692. A combination of garlic and *tembelekan* obstructed *M. tuberculosis* [5]. *Tembelekan* leaf extract can heal the wounds of the artificial skin of mice [8]. *Tembelekan* leaf ethanol extract has antibacterial activity [9]. *Tembelekan* has anti-inflammatory effects [10]. The leaf of *tembelekan* has the effect of anti motility and indigestion in the intestines [11]. The leaf of *tembelekan* has the effect of anti-fever and potentially as an anti-malarial [12].

Nano Herbal

In this research, nano material has a particle size 10^{-9} meters. Various form materials are: nano materials, nano particles, nano composites, nano magnets, nano energy, nano medicine, and nano herbs. Some of the uniqueness of the nano particles such as:

a. The particle size is very small.

b. Contact surface area in nano material becomes greater cause physical, chemical, and biological changes such as its reaction kinetics, reaction rate, a bond is formed.

c. There were changes in the optical properties and magnetic properties. A change of this nature provides a variety of benefits including more soluble.

d. Easy to get into the cell organ, easily absorbed so that the possibility can increase the rate of absorption, onset of drug bioavailability will be faster the better, with smaller doses.

e. Particles that having a size smaller than 200 m are more easily sterilized by filtration with a sieve size of 0.22 µm.

Therefore, the particle size of the ingredients has given smaller size than the cells in the human body, then the ingredients are easy to enter cells and reaction bioavailability is better, the case against bacterial cells, with ingredients having a particle size smaller compared the bacterial cell will cause the ingredients easily penetrate bacterial cells, thereby the retardation the growth of bacteria is faster and stronger. Some particle size of some cells can be seen in [Table 1] below:

Tabel 1: Particle size of some cells

The Object	Size (nm)
Carbon Atoms	0.1
DNA double helix	3
The ribosome	10
The Virus	100
Bacteria	1000
Red blood cells	5000

The production of Nano Herbal

Top down approach:

The production size of nano from the great slab to the nano material got through in the process of grinding, cutting (crushing), grafting, until formed the size of nano. Such production of the flour; it is made from the mashed rice first, so also with the top down approach.

Bottom up approach:

This method is commonly used in the industry, because it can be constructed structure nano-sized objects from below. This method produces a uniform size so that no longer needs a separation process. Therefore, it can be designed conditionally for the properties that will be generated from these nanostructures

After completion of the production of nano particles, some observations on the particle size characteristics and a test were done by using SEM (Scanning Electron Microscopy), XRD (X-ray Diffraction), DTA (Differential Thermal Analyzer), and PSD (Particle Size Distribution).

MATERIALS AND METHODS

Plants Extraction

Tembelekan flos was dried and powdered, and then *phytochemistry* screening and quality assay of simplicy was done. Extraction by percolation then conducted using 80% ethanol.

Materials

Percolator, rotary evaporator, freeze dryer), a tool determination of water content, scales, HEM (high energy milling), SEM (Scanning Electron Microscopy), XRD (X-Ray Diffraction), DTA (Differential Thermal Analyzer), PSD (Particle Size Distribution), autoclave, oven, hot plate, incubator, refrigerator, inspisator, homogenizer glass, microscope, thermometer. The animal experiment that used were male guinea pigs weighing 300 to 400 grams

Anti-tuberculosis test in Vitro

Potential or effectiveness nano herbal and flower extracts tests of *tembelekan* were done with ability test and potential inhibition of the growth of bacteria *M. tuberculosis* H37Rv that has been included in the media LJ used in concentrations of each nano herbal and extracts around 25 mg / ml and 50 mg / ml, *rifampicin* comparison 40µg / ml, and *ethambutol* 10µg / ml, also conducted on blank media with the phases of work as follows:

The Production of bacterial suspension

One drop of sterile distilled water is dripped into a mixing glass; wire transferred one loopful of the colony from the culture medium into a mixing glass. Then it destroyed by rotating the tool until homogeneous and poured 7 ml sterile distilled water. Then take 0.1 ml diluted with 9.9 ml of sterile distilled water obtained bacterial suspension concentration of approximately 0.01 mg / ml [13].

The Inoculated bacteria suspension

It is inoculated 0.1 ml bacterial suspension concentration of 0.01 mg / ml into three tubes containing LJ medium containing each test material and the comparative material of various concentrations. Then, razed the entire surface of the medium and incubated at a temperature of 370C for 6 weeks was observed growth of bacteria in every week with readability criteria:

(-) : no growth

(+1) : seen there are little yellow colony 1-200 colonies

(+2) : ½ from media covered by the yellow color (200-500 colonies)

(+3) : ¾ from media covered by the yellow color (500-2000 colonies)

(+4) : media fully covered by the yellow colony (more than 2000 colonies)

Anti-tuberculosis in Vivo Test

Anti-tuberculosis activity of *ethanolic* extract of *mimba* cortex was determined using infected Guinea-Pigs. Guinea-Pigs were spread using 3 ml suspension of *M. tuberculosis H37RV* (from *CV. Varka Bayak Medan*) directly on bronchus by using nebulizer every 24 hours during 7 days respectively, continued with once every 2 days during 7 days and once every 3 days during 7 days. The spread of 20 ml *aquadest* was taken from esophagus. Identification and cultivation of *M. tuberculosis* was done on LJ medium. The positive Tuberculosis Guinea-Pigs then divided in 5 groups. 1st group: Extract 50 mg/kg BW, 2nd group: Extract 25 mg/kg BW, 3rd group: Nano herbal 50 mg/kg BW, 4th group: Nano herbal 25 mg/kg BB. 5th group: OAD KDT one times a day. After administrated with each treatment, specimen sampling was done each week for 4 – 5 samplings. Specimens were placed in assay tube then homogenized with phosphate buffer pH 7 and inoculated into two assay tubes contain with LJ medium. Inoculums were incubated at 37°C for 6 – 8 weeks and the growths were observed with criteria:

(-) : no growth

(+1) : medium covered with slight colony, 1 – 200 colonies

(+2) : ½ of medium surface covered with yellow colony, 200 – 500 colonies

(+3) : ¾ of medium surface covered with yellow colony, 500 – 2000 colonies (13).

RESULT AND DISCUSSION

Table 2. Results of in vitro test

Material Test	Concentration	Colony Growth on x week					
		1st	2nd	3rd	4th	5th	6th
Control	0 µg/ml	-	-	1+	2+	3+	4+
		-	-	1+	2+	3+	4+
Rifampicin	40 µg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
Ethambutol	10 µg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
Tembelekan flos Nano herbal	50 mg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
	25 mg/ml	-	-	-	-	1	1
				-	-	1	1
Tembelekan flos Extract	50 mg/ml	-	-	-	-		1+
				-	-		1+
	25 mg/ml	-	-	-	-	2+	2+
				-	-	2+	2+

Table 3. Result of In Vivo Test

Material Test	Concentration	Colony Growth on x week					
		1st	2nd	3rd	4th	5th	6th
OAT KDT	¼ of tablet	3+	2+	1+	-	-	-
		3+	2+	1+	-	-	-
Tembelekan flos Nanoherbal	50 µg/ml	3+	2+	1+	-	-	-
		3+	2+	1+	-	-	-
	25 µg/ml	3+	2+	2+	1+	1+	-
		3+	2+	2+	1+	1+	-
Tembelekan flos Extract	50 mg/ml	3+	-	-	1+	-	-
		3+	-	-	1+	-	-
	25 mg/ml	3+	3+	2+	2+	1+	1+
		3+	3+	2+	2+	1+	1+

The result of [Table 2] shows Colony growth on sample week

a. On the media that given by *Rifampicin* 40 µg/ml and *Ethambutol* 10 µg/ml, look no growth of Tuberculosis began the first week until the sixth week.

b. In nano herbal, the concentration of 50 mg / ml, look no growth of Tuberculosis began the first week to the sixth week, and a concentration of 25 mg / ml at week starts to look their V-harbor Tuberculosis per-tum category +1

c. In the extract with a concentration of 50 mg / ml, look no growth Tuberculosis began the first week until week-V, and a concentration of 25 mg / ml at week IV began their growth categories Tuberculosis +

The result of [Table 3] shows that:

a. All of test animals were infected with positive *M. tuberculosis* bacteria Tuberculosis infected with categories +3.

b. In granting OAT KDT, seen to have negative Tuberculosis at the third week.

c. In the production of nanoherbal dose of 25 mg / Kg BW seen to have negative Tuberculosis at week 5, and at doses of 50 mg / Kg BW, was negative at the third week.

d. At a dose of 25 mg extract / Kg BW seen to have negative Tuberculosis at sixth week, and at doses of 50 mg / Kg BW, was negative at the fourth week.

In vivo test results demonstrate the potential of anti-tuberculosis nanoherbal stronger than the extract, but still lower when compared with the OAT KDT so, nanoherbal *tembelekan* flowers can be used as a companion drug OAT KDT in the treatment of Tuberculosis, especially in coping resistance.

CONCLUSION

(i) *In vitro* nanoherbal and ethanol extracts of *Lantana camara* Linn have strong activity to *M. tuberculosis* growth retardation, with a concentration of 50 mg / ml; nanoherbal gave a negative result until week 5, and extracts 1+ at sixth week. (ii) *In vivo* nanohebal and ethanol extracts of flowers *tembelekan* concentration of 50mg / Kg BW has a strong activity as anti-Tuberculosis in animal experiments. (iii) Anti-tuberculosis nanoherbal strongly cures the Tuberculosis in animals of 3+ becomes negative for 4 weeks compared to extract for 5 weeks.

This research suggested to the medical staff providing the extracts of nanoherbal made of *tembelekan* as a companion drug OAT KDT dealing with the treatment of Tuberculosis patients to accelerate the destruction of *M. tuberculosis* . It is very vulnerable to resistance. For the pharmaceutical industry is expected to produce the nanoherbal *tembelekan* capsule; it is a companion to the anti-tuberculosis drugs.

CONFLICT OF INTEREST

There is no conflict of interest.

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