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Editorial Note
Editor will be glad to give due consideration to any authenticated material/illustrations etc. submitted for publication in this Journal, and he does not assume any responsibility for their safe custody or return, nor does he necessarily share the views expressed by the author.
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDITORIAL</td>
<td>Linking of Research to Unani Healthcare</td>
<td>1 – 2</td>
</tr>
<tr>
<td></td>
<td><em>Kunwar Mohammad Yusuf Amin, Editor</em></td>
<td></td>
</tr>
<tr>
<td>REVIEW ARTICLE</td>
<td>ADRs and ADR monitoring in Unani Medicine: A shifting paradigm</td>
<td>3 – 8</td>
</tr>
<tr>
<td></td>
<td><em>Farkhunda Jabin</em></td>
<td></td>
</tr>
<tr>
<td>EXPERIMENTAL PAPERS</td>
<td>Pharmacological effect of <em>Crocus sativus</em> (Saffron) and its constituent <em>safranal</em> on respiratory system</td>
<td>9 – 14</td>
</tr>
<tr>
<td></td>
<td><em>Mohammad Hossein Boskabady</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical study of Unani Drug Bishkapra (<em>Boerhaavia repens</em>) with raloxifene as control treatment</td>
<td>15 – 20</td>
</tr>
<tr>
<td></td>
<td><em>Khan Usmanghani, Amna Khalil, Shahabuddin, H. M. Asif and Muhammad Akram</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The anti-inflammatory activity of <em>Artemisia afra</em> and <em>Sutherlandia frutescens</em></td>
<td>21 – 27</td>
</tr>
<tr>
<td></td>
<td><em>Najwa Kisten</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of Taleeq (Leech therapy) on Dawali (Varicose Veins)</td>
<td>28 - 35</td>
</tr>
<tr>
<td></td>
<td><em>Mohammad Anwar Alam and Zarnigar</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preparation of Kushta Sammulfar (<em>calx of Arsenic</em>) by muffle furnace using the temperature pattern extrapolated from the classical method of its preparation as practiced in Unani medicine</td>
<td>36 – 39</td>
</tr>
<tr>
<td></td>
<td><em>Shamim Irshad, Abdul Wadud, Najeeb Jahan, Ghulamuddin Sofi and Ghufran Ahmad</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An experimental study of Maa-uz-zahab (Gold Preparation) for nootropic activity</td>
<td>40 – 42</td>
</tr>
<tr>
<td></td>
<td><em>Ashfaq Ahmad, Kunwar Mohammad Yusuf Amin, Iqbal Ahmad Qasmi and Abdul Lateef</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Factors favouring and disfavouring the popularity of Unani Medicine among patients and practitioners – a survey</td>
<td>43 – 51</td>
</tr>
<tr>
<td></td>
<td><em>Abdullah Bin Junaid, Reshma Nasreen, N. Ravichandran, Mohammed Junaid Siddiqui and Paras Wani</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physico-chemical and phytochemical study of <em>Ruta graveolens</em> Linn. seeds</td>
<td>52 – 57</td>
</tr>
<tr>
<td></td>
<td><em>Shabir Ahmad Parray, Najeeb Jahan and Ghufran Ahmad</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Identification and standardization of a pharmacopeal Unani formulation: estimation of marker compounds</td>
<td>58 – 62</td>
</tr>
<tr>
<td></td>
<td><em>Aziz ur Rahman, Tajuddin and Kunwar Mohammad Yusuf Amin</em></td>
<td></td>
</tr>
</tbody>
</table>
A pharmacokinetic approach to standardize Tukhm-e-Katan (*Linum usitatissimum*) seeds as a bioavailable source of β-sitosterol using High Performance Thin Layer Chromatography (HPTLC) 63 – 70
Sunita Shailajan, Sasikumar Menon, Manasi Yeragi and Harshada Hande

Effect of *Nigella sativa* on blood glucose in alloxan induced diabetic rabbits 71 – 75
Umme Aiman, Mohammad Nasiruddin, Rahat Ali Khan and Ahmad Najmi

Study of *Carthamus tinctorius* Linn. for diuretic and nephroprotective effect in albino rats 76 – 82
Wasim Ahmad, N.A. Khan, Ghufran Ahmad and Shamshad Ahmad

Pharmacological evaluation of a Unani formulation and estimation of its alkaloidal constituents 83 – 89
Shariq Shamsi, Tajuddin and S.H. Afaq

A comparative clinical study of Kabdeen and Lamivudine in Warm-e-kabid haad vairoosi (Acute Viral Hepatitis B) 90 – 96
Rafiullah, M.M.H. Siddiqui and M.H. Hakim

Efficacy of Irsa (*Iris ensata*) in the management of Iltehabe unqr rehm (Cervicitis): a clinical trial with standard chemotherapeutic regimen as control treatment 97 – 104
Salma Mirza, Wajeeha Begum and Umraz Mubeen

GUIDELINES FOR AUTHORS
CONTRIBUTORS FORM
SUBSCRIPTION INFORMATION
SUBSCRIPTION FORM
Tribute to the founding Editor-in-Chief

The Editorial Board of ‘Unani Medicus- An International Journal’ wishes to place on record the extra-ordinary contributions of Professor Tajuddin, the founding Editor-in-Chief, in conceiving the Journal and tirelessly working to translate it into reality. Although, the position of Editor-in-Chief, being an annexation to the rotatory Deanship of Faculty of Unani Medicine, Aligarh Muslim University, Aligarh, India, was relinquished by him a few months back, but Prof. Tajuddin’s founding imprint will ever remain on the Journal.

Prof. Tajuddin, who has always been a dedicated, dynamic and affable leader, employed his full energy and innovative potentials with ever-present geniality to bring out such a high standard Journal whose very first issue outshone most Journals on Traditional Medicine and drew appreciative acclaim from scientists and scholars of cognate fields such as Western Medicine, Chemistry, Biochemistry etc. He persuaded the University authorities to provide resounding support to the Journal and a respectable recurring annual grant to put the Journal under perpetual gratitude to him. He picked up his editorial team from amongst the dedicated faculty members and involved distinguished scholars and scientists known for their expertise in Traditional Medicine and allied sciences as members of the advisory board. He used his personal connections and established rapport with scientists, scholars and researchers the world over to invite them to contribute to this new but refreshing journal. He also helped in putting together a formidable team of referees and reviewers to ensure an effective peer review of the submissions. When the journals devoted to Unani Medicine are few and faltering, it is indeed laudable that he succeeded in launching a journal at such a high note. The Faculty of Unani Medicine will always remain indebted to him for this remarkable contribution. We take this opportunity to express deep appreciation of Prof. Tajuddin for launching a standard journal for consolidating research in Unani Medicine that was overdue.

Editors
Editorial

Linking of Research to Unani Healthcare

Kunwar Mohammad Yusuf Amin

Since, research in a discipline is carried out for its advancement, it is important to ask what is the contribution of a particular study to the advancement of Unani Medicine? This will also help in identifying the type of Research which is useful, useless or even harmful for Unani Medicine. So, this exercise will help in answering the question being asked by everyone, namely, ‘what is the Research appropriate for Unani Medicine?’

So, we will try to see the contribution of the studies included in this Issue of Unani Medicus. Three Studies, viz. Aziz ur Rahman et al., Shailajan et al. and Parrey et al. can be related to Identification and Standardization. These three studies provide only two new possibly significant contributions i.e. the HPLC Spectrum of Suddab (*Ruta graveolens*) and the method of estimation of plasma levels of β-Sitosterol after the administration of Tukhme katan (*Linum usititassimum*). The former can be used as one of a multi-parameter battery for identifying and possibly standardizing Suddab. But its role can only be partial and minor in this regard. It is difficult to understand the role of plasma estimation of β-Sitosterol as an efficient means of Standardization. However, it may obviously be used for delineating a partial kinetics of Tukhme katan.

Standardization is being done by certain physico-chemical parameters that relate to the whole Plant. They are acceptable. But the use of single Marker Compounds, particularly their quantification, is more questionable, as Unani Pharmacological Actions relate to the whole, integrated plant, rather than to the individual compounds. So, the use of Marker Compounds for Standardization should be critically examined for relevance, as a whole, as well as, from Plant to Plant. Biological Standardization seems to be more appropriate, a particular Biological Activity may be due to the overall effect of multiple rather than single compounds, eg the Cardiotonic Activity of Digitalis is due to the net effect of all its Cardiac Glycosides. Thus, Biological Activity relating to the whole Plant, like the HPLC Peaks, is a holistic standardization parameter and more relevant to Unani Medicine, as Unani Pharmacological Actions (Af’al) relate to the plant as a whole.

Similarly, it is not justified to consider classical forms and methods of Formulation to be unimportant and freely replaceable by new forms and methods of preparation. Rather, changes should be made after due theoretical and experimental checking to ensure that there is no ‘substantial’ deviation from Traditional Forms. Only those changes in drug form or instrumentation should be allowed which do not violate classical principles and prescriptions, and the resultant formulations should be stringently evaluated for safety and efficacy equivalence with the traditional formulation. If found equivalent, the modified formulations should be accepted only as ‘alternatives’ which can be used alongside traditional counterparts and not as ‘replacements’ putting an end to the traditional ones. The Study of Irshad et al. is a good example of this proper approach. They have mapped out the temperature variations of a traditional heating method and reproduced them while replacing it with a Muffle Furnace.

A very basic issue about formulation is: whether to use the whole crude drug or its fractions and compounds. There is great pressure to replace the crude drug by its compounds. But the Unani principles demand the usage of crude drug. Fortunately, experimental pharmacology too has demonstrated the superiority or at least the parity of crude drug. This Issue of Unani Medicus too contains two studies showing the parity or even superiority of whole Kalonji (Umme Aiman et al.) and Zafraan (Boskabady) with comparison to their individual compounds.

Usmanghani et al., Alam & Zarnigar, Rafiullah et al. and Mirza et al. have conducted Clinical Studies in comparing Unani treatments of various diseases with Standard Agents of Western Medicine and shown the superiority or parity of the Unani treatments. It is indeed valuable to revalidate Unani claims by modern clinical research methods but they have little to contribute to the advancement of Unani Medicine. Whereas
the same studies could have provided new information about the clinical behavior of the test treatments, if they had been designed within a wider perspective. For instance, they could have been easily used for providing additional information on their Safety if Parameters of expected Adverse Effects would have been included. Only Alam & Zarnigar have provided suggestions for the improvement of Unani Healthcare by showing that the use of Compression Bandages and Limb Elevation as Adjunct to Unani Therapy increases the level of improvement.

Boskabady, Ahmad, M. et al., Umme Aiman et al., Ahmad, W. et al. and Shamsi et al. have conducted Experimental Studies to examine Unani Drugs for The Actions reported in Unani Texts and shown them to possess these actions. All these studies used In vivo Tests and Patho-physiological Parameters, rather than Molecular Parameters to check for the Actions. This is appropriate as Patho-physiological Parameters eg Anti-inflammatory, Bronchodilator etc are more ‘Holistic’ than Molecular Parameters e.g. Corticosteroidal, β2-Agonist etc, so, the former are more similar to Unani Actions or Af’al. Secondly, all these studies have involved some interpretation or ‘translation’ of Unani Af’al into Western Medicine Pharmacological Actions eg Muhallil is interpreted as Anti-inflammatory, although, the meaning of Muhallil has many additional elements. However, most of these interpretations seem to be on the whole, at least broadly, acceptable. Some studies have gone beyond validation, which is obviously valuable if self, to provide new information. For instance, Umme Aiman et al. have shown Kalonji to be Anti-Hyperglycemic but not Hypoglycemic. Boskabady has shown some molecular mechanisms of Zafran’s Bronchodilator Action. By showing both β2-Agonistic and Anti-Muscarinic Activity in Zafran, it is indicated to be equally effective in Bronchial Asthma and COPD (Chronic Obstructive Pulmonary Disease). This suggestion could be tested clinically. Thus, Molecular Effect discovery could help in adding new details (not replacements) of Moalajat etc within the classical framework.

Two of the studies are especially interesting as they examine unexplored areas, viz. the study of Gold Preparation by Ahmad et al. and of Taliq (Leeching) by Alam & Zarnigar. Thus, studies, whether clinical or experimental, that focus on neglected areas are more valuable and should be taken up with greater frequency.

Another innovative study (Abdullah Bin Junaid et al.) is an epidemiological exercise for determining the factors that attract patients and physicians towards Unani Medicine or put them off. This can be used to maximize the plus points and minimize the minus points to increase the popularity of Unani Medicine.

Review Papers on Unani Medicine are generally disliked because they have nothing new to offer. But Jabin’s Review on ADRs & ADR Monitoring is interesting because it tries to identify a newly emerging phenomenon i.e. new types of ADRs. It also tries to propose strategies for dealing with them. This shows that Review Papers which have something new to offer, even unknown classical information or new analyses of classical information, not to talk of new problems as in Jabin’s paper, will be found interesting and useful.

Finally, Kisten’s study is an example of the evaluation of Folk Drugs, particularly those related to Classical Unani Drugs, in order to add new drugs to Unani Pharmacopoea.

It is hoped that this very modest attempt to relate Research to Unani Healthcare and Theory will encourage systematic reviews of research reports to derive actual benefit for Unani Medicine and also remind researchers to select problems and adopt methods for the sake of solving practical and theoretical problems and questions of Unani Medicine rather than conduct aimless studies.
ADRs and ADR monitoring in Unani Medicine: A shifting paradigm

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Abstract

It was not until the disaster caused by thalidomide in 1961 that the need for systematic international efforts were felt. With an objective of safe and rational use of drugs, WHO introduced pharmacovigilance and launched its international Adverse Drug Reaction monitoring programme. Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problem. Recently, its concerns have been widened to include herbal and traditional and complementary medicines (Craven, 1997). Pharmacovigilance is perhaps a new term in Unani Medicine as yet, but concept of adverse drug reaction and safety concern regarding drug prescriptions are not new. Unani physicians since, the inception of Unani Medicine have advocated safe and rational use of their drugs which is evident from literature review of ancient books of Unani Medicine. In present scenario renewed interest in traditional system of medicine and re-adaptation and re-application of Unani Medicine in modern clinical settings as well as various other factors have enhanced the possibility of appearance of ADR’s. Therefore, Unani drugs should also be subjected to rules of ADR monitoring and it should be applied to national drug policy, regulation of Unani drugs and in clinical practice; and other related steps should also be taken in order to ensure drug safety of Unani drugs.

Key words: Adverse Drug Reactions, Unani Medicine, Pharmacovigilance

Introduction

Harmful, unintended reactions to medicines that occur at doses normally used for treatment are called adverse drug reactions (ADRs). They are among the leading causes of death in many countries. WHO promotes global drug safety through its International Drug Monitoring Programme, which began in the 1960. Through the cooperative effort, Member States and WHO work together to identify possible relationships between the use of a drug and adverse effects. Nearly 100 countries now have national systems in place to report ADRs to the database managed by the WHO Collaborating Centre, the (UMC) Uppsala Monitoring Centre (Anonymous, 2008). Term side effects are often used synonymously with ADR, but both are different as side effects can sometimes be beneficial also. The study of ADR is the concern of the field called Pharmacovigilance (Nebeker, 2004). Pharmacovigilance activities are done to monitor, detection, assessment, understanding and prevention of any obnoxious adverse reactions to drugs at therapeutic concentration that is used or is intended to be used to modify or explore physiological system or pathological states for the benefit of recipient. These drugs may be any substance or product including herbs, minerals, etc. for animals and human beings and can even be that prescribed by practitioners of Unani or Ayurvedic system of medicine (Kumanan et al., 2010). In humans since, prehistoric times traditional herbal medicines are being used for treatment. Presently, 65-80% of the world’s population relies on herbal medicine for their primary health care. The use of such medicinal plant extracts for the treatment of various disorders is largely based on historical and anecdotal evidence (Rahman, 1998). In India Unani Medicine is playing a essential role in national health care delivery system, as one of the Indian Systems of Medicine (ISM), it’s drug
constitute a valuable share of herbal remedies used throughout the world. Along with allopathic medicines herbal remedies are also prescribed by Ayurveda, Unani and Siddha systems of medicine. As on January 1999, the estimated number of Registered Unani practitioners in India was 40748 and total domestic market of Unani was about 100 crore i.e. 2.3% of total ISM drugs (Anonymous, 2010). One of the blunt truth is no drug therapy or medical intervention is 100% safe for all people in all circumstances and while information about adverse reactions to allopathic (modern) drugs has been recorded for over three decades, it has become increasingly clear that the enterprise must be extended to herbal remedies not least because reports in which they are implicated in ADRs continue to be submitted (UMC-2004). Several studies showed that many adverse reactions to herbal remedies remain unnoticed since, personal experience is not a reliable basis for the exclusion of uncommon reactions. While ingestion of herbal drugs even in overdose generally produces minimal toxicity, life threatening events from severe intoxication may also occur as with other poisonings, an understanding of herbal therapeutic agents, mechanism of toxicity is key in planning specific management strategies. Post marketing as well as pre marketing surveillance of drugs of Indian System of Medicine is thus imperative to detect infrequent but significant adverse events (Rahman & Singhal, 2002).

Up to the end of June 2004, the Uppsala Monitoring Centre had received more than 11,500 reports of adverse reactions from top twelve countries like Germany, France, USA, UK, Spain, Australia, Sweden, Japan, Canada etc (Anonymous, 2004). As shown in figure-1. Henna (Lawsonia innermis linn) is used as decorating skin paint, hair conditioner and for nail colouring. Additives like paraphenylene diamine in henna can cause dermatitis. Further in G6PD deficient patient it can result in haemolysis.

Use of Garlic use has been shown to cause hypoglycaemia in diabetic patients when used in combination with anti diabetic drugs.

Ginkgo biloba, the most prescribed drug in the world, has been shown to cause serious adverse effects like bleeding disorder, rise in blood pressure. When used along with thiazide diuretics and coma in combination with trazodone (Gupta et al., 2006).

Adverse Hepatic reactions in association with herbal remedies have been widely reported in the literature over the recent years and implicated products are Senna, Germander, and Valerian root. Belgium reported the occurrence of renal failure who adopted slimming regimen, which included the herbs Stephana tetrandra and Mangnolia officinalis (Rahman, 2006).

In a recent clinical trial of Unani herbal compound formulation in the patients of Bronchial asthma, 20% patients reported Adverse effect of different types ranging from mild to moderate and severe. It included arrhythmia, sweating, insomnia, palpitation, blisters, redness, tremors, rhinitis, nausea, vomiting, headache, pruritis, myalgia, diarrhoea etc (Jabin et al., 2005). These reported events highlight the extreme importance of effective drug monitoring system for Unani drugs also.

Fig. 1

Concept of ADR in Unani Medicine

Tibb is defined as the branch of knowledge which deals with the states of health and disease in human body for the purpose of adopting suitable measures for preserving or restoring health. By aiming at the preservation of health Tibb promises to be well advance of time. To achieve this goal, it not merely depends on ordinary methods of hygiene but encompasses constitutional make up of body, climate, habit and other social and allied factors in order to maintain health (Shah, 1973). Literally, the word Pharmacovigilance is not present but the concept of ADR monitoring, safe and rational drug therapy are vibrant throughout the literature and practice of Unani Medicine. Unani physicians were vigilant enough, what to talk of drugs they even have illustrated the side effects of food. Al Qanoon fit Tibb (a classical text book of Unani Medicine) gives rather detailed pharmacological and pharmacotherapeutic characteristics of 811 drugs, among which those of vegetable kingdom
constitute 594 (73.7%), of animal kingdom 118 (14.5%), and of mineral origin 99 (12%) (Denisenko, 1999. Drugs have been classified into four categories according to their effects on the body i.e. I, II, III, and IV degree. First degree drugs are the safest drugs they do not produce any perceptible effect on body and can be used without any slightest fear of any toxic effect. Second degree drugs produce and easily perceptible effect but do not cause any harm to the functions of the body. Third degree drug produces effects which have harmful effects on the body and this type of drug is always used with corrective drug or Musleh. Fourth degree drug is so strong that physiological functions of the body are completely disturbed. Drugs of this group are avoided by Unani physicians and are not administered unless they are purified by specific methods then they are labelled as advia muddabarah, e.g. seeds of Strychnos nux-vomica are soaked in water for 5 days then 2 days in milk followed by boiling in milk, which reduces its toxic effect (strychnine content) to minimum (Masood et al., 2010). Further drugs are classified according to Temperament (constitutional makeup) and drug prescribed should always be opposite the temperament of diseased person i.e. hot against cold and cold against hot (Azmi, 1995). So prescribing drugs is not as simple as one formula fitting all, but drugs are totally individualised according to the patient and stage of disease. Ancient physicians used to prepare drugs by themselves and age, sex, temperament, stage of the disease climate, form and route of drug administration were always evaluated then drug was formulated according to individual needs. They were well aware of the associated ADR that’s why they were so cautious and in ancient textbooks of Unani Medicine the harmful effects of drugs are described in detail along with their Musleh or corrective drugs. Drug therapy is the second choice after the regimental therapy fails to give relief. Single ingredient drug is preferred over multiple ingredient formulation. Writing a drug prescription meeting the needs of the patient without eliciting any undesirable effects is the test of intelligence and ability of the Unani physician and is considered as an art. But, at present Unani Medicine is known for its herbal part only and its basic fundamentals, condition of application of Drug therapy are totally ignored. There are numerous challenges ahead in order to tap full potential of Unani Drugs with minimum side effects.

Possibilities of Emergence of New ADRs of Unani Drugs

In present scenario Unani drugs are mostly used outside their original, clinical, cultural or pharmaceutical context, or in combination with modern medicine, making them vulnerable to the emergence of ADR. Drugs prescribed are mostly manufactured by profit oriented pharmaceutical companies at large scale and fixed dosage (same formula for all), little attention is paid to the individualization of medicine. Moreover, they claim 100% safety of their drugs. Drug regulations governing the manufacture and sale are not well defined at both national and international level. In most of the situations a mixture of many plants is incorporated to make a drug resulting in far and wide number of organic and inorganic components. Sometimes different plants are known by the same name and same plant is known by different local names, a plant may be known by different botanical name, some plants have striking similarity leading to improper identification. All these factors sometimes result in choosing wrong drug e.g. in a multiple ingredient formulation, Ruta graveolens linn (suddab) was accidentally replaced by Euphorbia drancunculoidis, a toxic herb (Latif and Rahman, 2005). There are lots of shortcomings at the level of identification, collection, storage, and manufacturing leading to low quality and sometimes adulterated drug in the market. Single and multiple ingredient formulations are numerous; registration trials are inadequate to detect ADRs. Perhaps because of the firm belief among doctors and prescribers alike, that Unani drugs are safe, the detection of adverse reactions to these medicines (signal detection) is a major challenge and lack of reporting aggravates it further. Same drug can be prescribed for number of diseases and different drugs can be used for same disease creating confusion. Contamination of plant material with bacteria, fungi, yeast, pesticides or other substances add additional pharmacological actions which may be harmful. Unani drugs are sometimes given along with the drugs of other systems of medicine or drugs of low therapeutic margin resulting in chances of drug to drug interaction. Over the counter preparations for various indications are available, hence self medication with these drugs are very common. Soil type, soil contamination, season, climate in which herb is grown alters the medicinal value or influence toxicity. There are chances of deliberate or unintentional drug adulteration. Poor quality
control systems loose distribution channels (internet, mail orders) and casual approach in distribution have further aggravated the problem. Consumers and providers both are unaware about the emergence and monitoring of ADR. No data of safety is available at the time of launch of a product or medicine. Manufacturing units are under no obligation to inform about the contraindications or ingredients of their product creating an atmosphere of secrecy. Adverse drug reaction analysis for safety point of view thus becomes a challenge and poses even more difficulties in monitoring it.

Therefore, to ensure safety of Unani drugs concrete steps should be taken and ADR monitoring should be employed in:

a) regulation of medicines
b) In clinical practice

c) In national drug policy.

Suggestions for Controlling ADRs in Unani Treatment

1. Prescription of drugs should be according to the fundamental and pharmaceutical approach of Unani Medicine i.e. concept of individualization should always be considered.

2. Consumers and providers should be sensitized to the need and concept of ADR monitoring.

3. Unbiased drug information about Unani drugs including both classical and proprietary formulations should be made easily available.

4. Pharmacovigilance concepts should be introduced into the curriculum of Unani at the under-graduate and post-graduate level.

5. Uniformity and quality should be maintained by applying good manufacturing techniques. To ensure it, following methods can be employed: macroscopic appearance, microscopic examination (type of stoma, stomata number, trichomes etc.) volatile matter, ash value, extractive value, chromatographic profile and marker component, determination of heavy metals, pesticide residue etc. (Singhal, 2003)

6. The prescribers and consumers should be sensitized regarding the system of ADR monitoring. It is equally important to dispel the notion that natural means safe so that they are willing to participate in Pharmacovigilance programme.

7. Pharmaceutical companies should also maintain transparency regarding information about the ingredients and composition of their product. Unani medicinal products should include the registration of quantitative list of ingredients. It includes the plant names and plant parts used (i.e. Latin name), full product formula for imported herbal-based medicinal products (in the language of the importing and exporting countries), a set containing labels, pamphlet, carton and specimen sales pack, particulars of manufacturer and assembler; manufacturer’s license or certificate from the drug regulatory authority. Pre-export notification and certificate of free sale of the herbal-based medicinal product should be obtained from the concerned authority. Brand name of product, dosage form, indications, dosage, mode of administration, duration of use, adverse effects if any, contraindications, warnings, precautions and major drug interactions, date of manufacturing, expiry date of product, lot/batch number, storage condition should be mentioned.

ADR monitoring employed in clinical practice

Studies on drug safety should be encouraged. Need for toxicity testing is strongly related to what is known of their clinical properties. A long tradition in certain product in a country could itself be a better evidence of safety than any animal experiment, on the other hand distant experiences and introduction of newer compounds call for deeper considerations. Therefore for practical purposes, Unani drugs can be classified into three categories.

**Category 1:** Classical drugs and their formulations: They are based on classical original text and considered safe owing to the long history of use. They need no testing for safety.

**Category 2:** Proprietary medicine: They are also based on classical original text, except that they have been modified in some way either shape or form including dose, dosage form, mode of administration, herbal medicinal ingredients, methods of preparation and medical indications. They have to meet the national regulatory requirements of safety and efficacy of herbal medicines.

**Category 3:** Proprietary medicine (based on new herbs): The ingredients used in these types of formulations are not mentioned in the classical texts; hence these drugs are of uncertain safety therefore they should be evaluated for safety identical to that of any new drug.
ADR Monitoring employed in National Drug Policy

1. The prescribers and consumers should be sensitized regarding the system of ADR monitoring. It is equally important to dispel the notion that natural means safe so that they are willing to participate in pharmacovigilance programme.

2. A data base for safety monitoring of Unani drugs should be created and the practitioners should know how to analyze reports (signal detection) how, whom and what to report. Everyone involved in the manufacture, sale and consumption should be included in the reporting network.

3. Strict drug regulations regarding the proper launch, manufacture and sale of category 2 and 3 Unani drugs are needed so that drug is not launched into the market without any preliminary research about its safety and efficacy.

4. Terminologies used are different in Unani medicine, e.g. Dafe humma for antipyretic, Dafe Taffun for antiseptic etc., therefore, classification and coding of Unani drugs, with special consideration to terms used for ADR and treatment concepts e.g. imbalance in temperament should be made available in an internationally accepted format. WHO’s ATC classification is a step in this direction.

ADR Monitoring (Pharmacovigilance) as a way to ensure safety of Unani drugs

The provision of good quality, safe and effective medicines and their appropriate use is the responsibility of national governments. The establishment of a national medicine regulatory agency and a designated centre for the study of adverse reactions (ADR monitoring) are central to safe and rational use of Unani drugs. ADR monitoring ensures safety of the drugs in the following ways:

1. It provides information about potential hazards of the drug after collecting, monitoring, research, assessing and evaluating information from healthcare providers and patients on adverse effects, especially those which are serious or unexpected.

2. It serves public health and fosters a sense of trust among patients in the medicines they use by ensuring that medicines are of good quality, safe and effective and also by taking into account the concerns of the patient when therapeutic decisions have to be made.

3. It communicates adverse effects and toxicity—especially when previously unknown to an audience that has knowledge to interpret information.

4. The degree to which clinicians are informed about the principles of pharmacovigilance, and practice according to them, has a large impact on the quality of health care.

5. It ensures that risks in drug use are anticipated and managed.

6. It provides regulators with the necessary information to amend the recommendations on the use of the medicines.

7. It improves communication between the health professionals and the public.

8. It educates health professionals to understand the effectiveness/risk of medicines that they prescribe.

9. Education and training of health professionals in medicine safety, exchange of information between national pharmacovigilance centres, the coordination of such exchange, and the linking of clinical experience of medicine safety with research and health policy, all serve to enhance effective patient care.

10. A regular flow and exchange of information in this way means that national pharmacovigilance programmes are ideally placed to identify gaps in our understanding of medicine-induced diseases.

Conclusion

A drug is defined as being safe if it causes no known or potential harm to users. Nobody can claim 100% safety of drugs in all circumstances. Ancient Unani physicians were very well aware of this fact. They left no stone unturned to ensure safe and rational use of their drugs. Present era of globalization has put forward many challenges and has increased the likelihood of ADRs with Unani Drugs. Therefore these drugs should also be subjected to the rules of pharmacovigilance at the level of regulation of medicine, clinical practice and national drug policy. As this improves patient care and safety in relation to the use of medicine and contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use.
Other steps should also be taken to minimize the newly possible ADRs.

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Pharmacological effect of *Crocus sativus* (Saffron) and its constituent *safranal* on respiratory system

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Abstract

The extract of the stigma of saffron flower and an active principle of saffron viz, safranal were studied for bronchodilator effect and its possible mechanism of action with theophylline as the standard bronchodilator for comparison. The results showed a potent relaxant effect of different concentrations of the extract comparable to theophylline which was greater than the effect of safranal. The results also showed leftward shifts in isoprenaline curves obtained in the presence of the extract and safranal which indicated a relatively potent stimulatory effect of *Crocus sativus* on β₂-adrenoceptors which was partially due to its constituent, safranal. The results of inhibitory effect on histamine H₁ receptors also showed parallel right ward shift in concentration response curves of histamine in the presence of the extract and safranal compared to those of saline, indicating an inhibitory effect of *Crocus sativus* at histamine H₁ receptors which was also greater than safranal. In addition, the results showed a non parallel right ward shift in concentration response curves of methacoline compared to that of saline which indicated a functional antagonistic effect of the plant and safranal on muscarinic receptors. These results showed a potent relaxant effect of the extract from *Crocus sativus* with the suggested mechanism of stimulatory effect on β₂-adrenoceptors and to lesser extent histamine H₁ inhibitory effect and anti-muscarinic effect. The results also suggested that the effect of the plant is partially due to its constituent safranal. Thus, the study shows that the Unani drug Zafran is a very effective Bronchodilator as active as the synthetic agent Theophylline while being likely to be much safer. The study also provides support to the Unani view that Natural Products are likely to be more effective than their Active Principles.

Key words: Zafran, *Crocus sativus*, Safranal, β₂-adrenoceptors, Theophylline, Bronchodilator

Introduction

*Crocus sativus* L, (saffron), is a small perennial plant (Iridaceae family) which is cultivated in Morocco and Greece but particularly in Iran and India. It is known to posses crocin, *safranal*, picrocrocin, ketoisophorone, isophorone and glycosidic terpenoids (Tarantilis *et al.*, 1995).

Saffron (its stigma) is used in traditional medicine for treatment of various disorders (Rios *et al.*,1996; Abdullaev *et al.*, 2004).

Different pharmacological effects of this plant have been shown in previous studies including: anticonvulsant (Hossienzadeh *et al.*, 2002; Hossienzadeh *et al.*, 2005), antidepressant (Akhondzadeh *et al.*, 2007), anti-inflammatory (Hossienzadeh and Younesi, 2002), radical scavenger and anti-oxidant properties (Kanakis *et al.*, 2007) and antitumour effects (Rios *et al.*, 1996; Abdullaev *et al.*, 2004) The plant has also learning and memory improving properties (Pitsikas and Sakellaridis, 2006). Saffron extract also has chemopreventive and genoprotective effects and protects from genotoxins-induced oxidative stress in mice (Premkumar *et al.*, 2006). A blood pressure lowering effect (Rios *et al.*, 2002).
and relaxant effect of saffron on vascular smooth muscles and antitussive activity of stigma and petal extracts and its components, safranal and crocin, has been shown (Hossienzadeh and Gheenati, 2006).

In a series of studies the relaxant effect (Boskabady and Aslani, 2006), stimulatory effect on β-adrenergic receptors (Nemati et al., 2008) and inhibitory effects on histamine (H1) (Boskabady and Ghasemzadeh) and muscarinic receptors of aqueous-ethanolic extracts of *Crocus sativus* and safranal (Neamati and Boskabady) on tracheal chains of guinea pigs were examined.

In the present study, the extract of saffron flower stigma and one of its active principles, namely, safranal were studied for bronchodilator effect and its possible mechanism of effect, with theophylline as the standard agent for comparison.

**Materials and Methods**

**Plant and extracts**

*Crocus sativus* was collected from Torbat Heydarieh (Eastern Iran) (Herbarium No: 143-0319-1). The aqueous-ethanolic extract was prepared as follows: 10 grams of chopped, dried stigma was extracted with 25 ml distilled water and 25 ml ethanol by soxhlet apparatus. The solvent was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final extract was 10 g%.

**Tissue preparations**

Tracheal chain was prepared and suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseleit solution. The Krebs solution was maintained at 37°C and gassed with 95% O2 and 5% CO2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 hr while it was washed with Krebs solution every 15 min.

**Protocols**

**Relaxant effect:** The relaxant effects of four cumulative concentrations of aqueous-ethanolic extract (0.15, 0.30, 0.45, and 0.60 mg/mL), safranal (Fluka chemical AG, Switzerland) (0.03, 0.06, 0.09, and 0.12 mg/mL), four cumulative concentrations of theophylline anhydrous (Sigma Chemical Ltd UK) (0.15, 0.30, 0.45, and 0.60 mM) as positive control, and saline as negative control (0.6 ml) were examined on precontracted tracheal smooth muscle in three different experimental designs (n=6 for each group) as follows:

1. On tracheal chains contracted by 10 µM methacholine hydrochloride (Sigma Chemical Ltd UK) (group 1).
2. On non-incubated tracheal chains contracted by 60 mM KCl, (group 2).
3. On incubated tracheal chains with 1 µM propranolol hydrochloride (Sigma Chemical Ltd UK), 1 µM chlorpheniramine maleate (Sigma Chemical Ltd UK) and 1 µM atropine sulphate (Sigma Chemical Ltd UK) 30 min prior to beginning and during the testing relaxation of different solutions. In this series of experiments, tracheal chains were also contracted by 60 mM KCl (group 3).

**Beta adrenergic stimulatory effect:** The stimulatory effect of different solutions was examined on β2-adrenoceptors by producing cumulative log concentration-response curve of isoprenaline sulphate (Sigma Chemical Ltd UK) induced relaxation of pre-contracted tracheal chains by 10 µM methacholine hydrochloride (Sigma Chemical Ltd UK) 10 min after exposing tissue to the tested solutions. Different tested solutions were included: 10 nM propranolol (Sigma Chemical Ltd UK), two concentrations of aqueous-ethanolic extract from *Crocus sativus* (0.1 and 0.2 g% equivalent to 0.48 and 0.96 mL of 10 g% extract), safranal (1.25 and 2.5 µg equivalent to 1 and 2 mL of 10 g% extract), or 0.2 mL saline. This effect was tested on two different experimental conditions as follows:

a) Non incubated tracheal chains (group 1, n=9).

b) Incubated tracheal chains 30 min prior to the beginning and while obtaining the isoprenaline curve with 1 µM chlorpheniramine maleate (Sigma Chemical Ltd UK), (group 2, n=6).

**Histamine receptors inhibitory effect:** The inhibitory effect of *Crocus sativus* on histamine H1 receptors was examined by producing cumulative log concentration-response curve of histamine acid phosphate (BDH Chemical Co, Ltd UK). Test solution were 10 nM chlorpheniramine maleate (Sigma Chemical Ltd UK), two concentrations of aqueous-ethanolic extract from *Crocus sativus* (0.025, 0.05 and 0.1 g %), safranal (0.63, 1.25 and 2.5 µg), or 0.2 mL saline.

This effect was tested on incubated tracheal chains:
1. 1.4 µM indomethacin (Sigma Chemical Ltd UK), (group 1 experiments), (n=8).
2. 1.4 µM indomethacin, 1 µM propranolol hydrochloride (Sigma Chemical Ltd UK), and 10 nM atropine sulphate (Sigma Chemical Ltd UK), (group 2 experiments), (n=7).
3. 1.4 µM indomethacin and 1 µM propranolol hydrochloride (group 3 experiments), (n=6).

**Muscarnic receptors inhibitory effect:** The inhibitory effect of *C. sativus* on muscarinic receptors was examined similar to the effect on histamine receptors but on the cumulative log concentration-response curve of methacholine hydrochloride (Sigma Chemical Ltd, U.K.)

**Measurements**

For evaluating the beta adrenergic stimulatory, histamine and muscarinic receptors inhibitory effects the following measurements were performed:

1. The effective concentration of corresponding agonist causing 50% of maximum response (EC$_{50}$) in each experiment was measured using the log concentration-response curve of corresponding experiment.
2. Maximum responses to corresponding agonist obtained in the presence of extracts and corresponding antagonist in all sets of experiments were compared with that of saline.
3. Slope of the corresponding agonist-response curve of each experiment was measured and were compared with that of saline.
4. In experiments with parallel shift in histamine-response curve, the concentration-ratio minus one (CR-1) as competitive antagonism effect was calculated by the following equation: (EC$_{50}$ obtained in the presence of effective solutions/EC$_{50}$ obtained in the presence of saline - 1).

**Statistical analysis**

All data were expressed as mean ± SE. Data of the extract and safranal were compared with the results of negative and positive control and between three groups using ANOVA. The correlations between the concentrations and effect were tested using least square regression. The values of (CR-1) obtained in the presence of extract and safranal, were also compared with those obtained in the presence of corresponding antagonist and those of two different concentrations of extract and safranal using ANOVA with Tukey-Kramer multiple paired test. Significance was accepted at p<0.05.

**Observation and Results**

**Relaxant (Bronchodilator) effect**

In groups 1 and 2, all concentrations of theophylline, extract and safranal showed significant relaxant effects compared to those of saline (p<0.05 to p<0.001). However, In group 3, the effect of only the last concentration of the extract was significantly higher than the effect of saline (p<0.05, Table 1). The effects of the two higher concentrations of safranal were significantly lower than that of extract in groups 1 and 2 (p<0.05 to p<0.01), (Table 1).

The relaxant effect of most concentrations of extract in groups 1 and 2 were significantly higher than those of group 3 and the effects of most concentrations of safranal in group 2 were lower than those of group 1 (p<0.05 to p<0.001), (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Concentrations</th>
<th>saline</th>
<th>Saffron Extract</th>
<th>Saffranal</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>14.5±1.51*</td>
<td>16.4±3.51</td>
<td>26.5±6.35**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>52.3±6.16</td>
<td>37.4±4.33</td>
<td>57.5±8.55***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>82.7±5.50</td>
<td>48.6±3.50</td>
<td>73.5±7.85***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.45±0.51</td>
<td>98.6±4.23***</td>
<td>87.4±6.22***</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>1</td>
<td>14.8±5.89</td>
<td>4.4±1.75</td>
<td>18.9±4.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44.9±8.78</td>
<td>11.0±2.51</td>
<td>57.3±2.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>67.7±10.75</td>
<td>25.6±4.74</td>
<td>70.6±0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.02±0.52</td>
<td>98.6±4.84***</td>
<td>83.7±1.09</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>1</td>
<td>1.84±1.29</td>
<td>6.4±2.35</td>
<td>12.8±0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.7±3.60</td>
<td>17.8±5.52</td>
<td>26.8±3.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>17.8±5.52</td>
<td>26.8±3.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.23±0.82</td>
<td>26.8±3.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison with Plain Control: * = p<0.5, ** = p<0.1, *** = p<0.001.
Comparison of saffron vs safranal: + = P<0.05, ++ = P<0.01, +++ = P<0.001.
Comparison of Theophylline vs saffron and safranal solutions: ϣ = P<0.5, ϣϣ = P<0.01, ϣϣϣ = P<0.001.
Comparison of Group 3 vs Groups 1 and 2: ★★ = P<0.01, ★★★ = P<0.001.
Beta adrenergic stimulatory effect
Cumulative log concentration-response curves of isoprenaline with only higher concentration of the extract in group 1 and its both concentrations and higher concentrations of safranal in group 2 showed leftward shift compared to the curve in the presence of saline. The EC_{50} of isoprenaline of both concentrations of the extract and safranal in group 1 and only two concentrations of the extract in group 2 were significantly lower than that of saline (p<0.05 to p<0.001) (Table 2). The EC_{50} of isoprenaline with both concentrations of the extract, safranal and propranolol in group 2 were significantly higher than those of group 1 (p<0.01 to p<0.001) (Table 2).

Table 2  
Effect of Zafran extract, safranal, propranolol and saline (Plain Control) on EC_{50} (µM), maximum response, slope and (CR-1) of Isoprenaline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Solutions</th>
<th>EC_{50} (µM)</th>
<th>Maximum response</th>
<th>Slope</th>
<th>(CR-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.1 mg/mL</td>
<td>0.17±0.06</td>
<td>2.72±0.12</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.2 mg/mL</td>
<td>0.07±0.06</td>
<td>1.36±0.06</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 1.25 µg/mL</td>
<td>2.20±0.32</td>
<td>6.16±0.66</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 2.5 µg/mL</td>
<td>2.10±0.34</td>
<td>6.66±0.69</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Propranol 0.5 mg/mL</td>
<td>4.01±0.18</td>
<td>1.40±0.10</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.1 mg/mL</td>
<td>0.07±0.10</td>
<td>0.22±0.10</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.2 mg/mL</td>
<td>0.07±0.10</td>
<td>0.22±0.10</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 1.25 µg/mL</td>
<td>2.20±0.35</td>
<td>0.01±0.04</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 2.5 µg/mL</td>
<td>2.10±0.34</td>
<td>0.01±0.04</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Propranol 0.5 mg/mL</td>
<td>4.01±0.18</td>
<td>0.22±0.10</td>
<td>**</td>
<td>-</td>
</tr>
</tbody>
</table>

Comparison of the difference between the data of negative (saline) and positive (propranolol) controls vs other solutions: * = p<0.5, ** = p<0.1, *** = p<0.001.
Zafran vs safranal: + = P<0.05, ++ = P<0.01. Group 1 vs Group 2: ★ = P<0.05, ★★ = P<0.01, ★★★ = P<0.001.

All values of (CR-1) of higher concentration of the extract in group 1, it’s both concentrations and higher concentration of safranal in group 2 were negative and was significantly different with that of propranolol (p<0.01 to p<0.001) (Table 2).

Histamine receptor inhibitory effect
Cumulative log concentration-response curves of histamine obtained with all concentrations of the extract, safranal and chlorpheniramine showed clear rightward shift compared to the curves of saline in all three groups of experiments. The EC_{50} of chlorpheniramine in all three groups, all concentrations of the extract and safranal in group 1, low and high concentrations of the extract and all concentrations of safranal in group 2 and in medium and high concentrations of the extract and all concentrations of safranal were significantly higher than that of saline (p<0.05 to p<0.01) (Table 3).

Table 3  
Effect of Zafran extract, safranal, chlorpheniramine maleate and saline (Plain Control) on EC_{50} (µM), maximum response, slope and (CR-1) of Histamine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Solutions</th>
<th>EC_{50} (µM)</th>
<th>Maximum response</th>
<th>Slope</th>
<th>(CR-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>0.50±0.24</td>
<td>100.00±0.00</td>
<td>1.90±0.14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.025 mg/mL</td>
<td>0.05±0.05</td>
<td>0.40±0.14</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.05 mg/mL</td>
<td>0.10±0.08</td>
<td>1.50±0.20</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zafran 0.1 mg/mL</td>
<td>0.15±0.10</td>
<td>2.00±0.30</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Safranal 0.025 µg/mL</td>
<td>0.08±0.05</td>
<td>0.30±0.10</td>
<td>**</td>
<td>-</td>
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<tr>
<td></td>
<td>Safranal 0.05 µg/mL</td>
<td>0.10±0.10</td>
<td>0.50±0.20</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Safranal 0.1 µg/mL</td>
<td>0.12±0.10</td>
<td>0.70±0.30</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Safranal 0.2 µg/mL</td>
<td>0.14±0.10</td>
<td>0.90±0.40</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Safranal 0.63 µg/mL</td>
<td>2.00±0.40</td>
<td>5.00±0.60</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine 0.025 µg/mL</td>
<td>0.05±0.05</td>
<td>1.00±0.05</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine 0.05 µg/mL</td>
<td>0.08±0.08</td>
<td>1.50±0.20</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine 0.1 µg/mL</td>
<td>0.12±0.10</td>
<td>2.00±0.30</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine 0.2 µg/mL</td>
<td>0.14±0.10</td>
<td>2.50±0.40</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine 0.63 µg/mL</td>
<td>2.00±0.40</td>
<td>5.00±0.60</td>
<td>**</td>
<td>-</td>
</tr>
</tbody>
</table>

Comparison of the difference between the data of negative (saline) and positive (chlorpheniramine) controls vs other solutions: * = p<0.5, ** = p<0.1, *** = p<0.001. Zafran vs safranal: + = P<0.05, ++ = P<0.01. Group 1 vs Group 2: ★ = P<0.05, ★★ = P<0.01, ★★★ = P<0.001. Group 2 vs Groups 3: ¶ = p<0.05, ¶¶ = p<0.01.
The values of (CR-1) obtained in the presence of high concentrations of the extract and safranal in group 1 and low concentration of safranal in group 3 were significantly greater than those of chlorpheniramine (p<0.05 to p<0.001). The differences in EC₅₀ values between the extract and safranal was only significant for the low concentration in group 3 (p<0.01). In group 1, the slopes of medium and high concentrations of the extract were significantly lower than those of safranal (p<0.05 for both cases).

**Muscarnic receptor inhibitory effect**

Cumulative log concentration-response curves of methacholine with all concentrations of the extract, safranal and atropine showed rightward shift compared to methacholine curves produced in the presence of saline. The EC₅₀ methacholine obtained in the presence of atropine and all concentrations of safranal and the extract except low concentrations of safranal (0.63 µg/ml) were significantly higher than that of saline (p<0.05 to p<0.001) (Table 4). But the maximum responses to the extract and safranal showed that both concentrations of the extract and higher concentrations of safranal caused parallel leftward shift in isoprenaline concentration-response curves indicating stimulatory effect of the extract and safranal on β₂- adrenoceptors (Arunlakshana and Schild, 1959).

The smaller values of EC₅₀ in the presence of the extract and safranal in group 2 and negative values of (CR-1) confirm their stimulatory effect on β₂- adrenoceptors.

The results suggest that the β₂- adrenoceptor stimulatory effect of the extract from Crocus sativus is partially due to safranal.

The results of the inhibitory effect on histamine (H₁) receptors showed parallel rightward shift in histamine-response curves obtained in the presence of the extract and safranal to that of saline and significant improvement of maximum responses to histamine and significant increase in EC₅₀ in group 2 (incubated tracheal preparation with indomethacin, propanolol, and atropine), relative to those of group 1 indicating possible competitive antagonistic effects of the extract of Crocus sativus and safranal on histamine H₁ receptors. The differences between the effects of the extract and safranal suggests that the pharmacological properties of the extract is not solely due to its constituent, safranal.

In muscarinic inhibitory study, the results showed a non-parallel rightward shift in methacholine log concentration-response curves in the presence of the aqueous-ethanolic extract and safranal and lower maximum contraction effect to methacholine compared to those of saline but greater values of EC₅₀ in the presence of all concentrations of the extract and two higher concentrations of safranal compared to saline indicating a functional antagonistic effect of saffron and a possible competitive antagonistic effect of safranal at high concentrations.

**Table 4**

<table>
<thead>
<tr>
<th>Solutions</th>
<th>EC₅₀ (µM)</th>
<th>Maximum response</th>
<th>Slope</th>
<th>(CR-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saffron</td>
<td>0.025 mg/ml</td>
<td>8.94±1.99</td>
<td>0.99±0.004</td>
<td>1.29±0.44</td>
</tr>
<tr>
<td></td>
<td>0.05 mg/ml</td>
<td>8.7±12.9</td>
<td>0.97±0.005</td>
<td>3.7±152</td>
</tr>
<tr>
<td></td>
<td>0.1 mg/ml</td>
<td>4.9±2.48</td>
<td>0.88±0.009</td>
<td>3.65±0.84</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/ml</td>
<td>4.9±0.03</td>
<td>0.99±0.008</td>
<td>1.46±0.37</td>
</tr>
<tr>
<td></td>
<td>1.25 mg/ml</td>
<td>8.9±6.25</td>
<td>0.99±0.008</td>
<td>1.46±0.37</td>
</tr>
<tr>
<td></td>
<td>2.5 mg/ml</td>
<td>7.6±6.35</td>
<td>0.99±0.008</td>
<td>1.46±0.37</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.025 mg/ml</td>
<td>0.1±0.27</td>
<td>0.96±0.007</td>
<td>4.25±0.21</td>
</tr>
</tbody>
</table>

**Discussion**

In groups 1 and 2 all concentrations of theephyloline, saffron extract and safranal showed potent relaxant effect on tracheal smooth muscle but in-group 3, a weak relaxant effect was observed for the extract and safranal.

The weak relaxant effect in group 3 for the extract of the plant suggest that probably β-adrenergic stimulatory, muscarinic and/or histamine H₁ blocking properties of the plant extract may contribute to its relaxant effect on tracheal chains of the guinea pig.

The significant lower relaxant effect of safranal compared to that of extract in both groups of experiments suggests that other constituents of the plant may also contribute to the relaxant effect of the plant.

The results of the stimulatory effect of the aqueous-ethanolic extracts of Crocus sativus and safranal in group 2 (incubated tracheal preparation with chlorpheniramine to block histamine H₁) showed that both concentrations of the extract and higher concentrations of safranal caused parallel leftward shift in isoprenaline concentration-response curves indicating stimulatory effect of the extract and safranal on β₂- adrenoceptors (Arunlakshana and Schild, 1959).
muscarinic receptors of guinea pig trachea (Arunlakshana and Schild, 1959).

Thus, the study shows that the Unani drug Zafran is a very effective Bronchodilator as active as the synthetic agent Theophylline while being likely to be much safer. The study also provides support to the Unani view that Natural Products are likely to be more effective than their Active Principles.

Acknowledgment

This study was financially supported by Research Department of Mashhad University of Medical Sciences.

References

Clinical study of Unani Drug Biskhapra (Boerhaavia repens) with raloxifene as control treatment

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Email: ugk2005@yahoo.com

Abstract

To conduct a comparative study of Boerhaavia repens with raloxifene for the management of osteoporosis in different hospitals. This study enlisted 86 patients at random, of whom 48 were diagnosed as osteoporotic from different age group. Applying the designed format data was collected. The 48 patients so registered, the number of female and male were 46 and 2, respectively. The associated diseases data on hypertensive, diabetes, arthritis and thyrotoxicoses of these osteoporotic patients were recorded. The clinical study on raloxifene was conducted for a period of three years in which the number of responding patients continued with the therapy the tally remained at 14. Similarly in others designed group, which were treated with Boerhaavia repens 18 patients registered but after a period of three years only 8 patients continued with the treatment. Both types of the clinical evaluation were conducted for bone mineral density, bone alkaline phosphatase and serum calcium level. The exclusion criteria observed for the osteoporotic patients include the fracture of the long and hip bone. The range of alkaline phosphatase in postmenopausal women without any pathology should be 22-30 mg/dl. But in case of osteoporosis this range exceeded up to 80-100 mg/dl. After treatment of the selected group of post menopausal osteoporotic women by raloxifene the alkaline phosphatase was found to be in the normal limits up to 35 mg/dl. When the other group of postmenopausal women treated by Boerhaavia repens the level decreased to the range of 32 mg/dl. In case of serum calcium level decreases up to 6-8 mg/dl from the normal range of 8-10mg/dl. With raloxifene, the additional supplements of calcium were administered at the recommended dose of 800-1000 mg/day. The T – score indicates how many Standard Deviation (SD) a patient’s BMD is from the young adult BMD i.e. T – score expresses the percentage (%) Young Adult (YA) value in a different way. Patients with T – score above –1 considered as normal with the BMD up to 1.42 - 1.54 g/cm², while in case of osteoporosis the value of T – score suggestive of osteoporosis up to –2.5 to –5 with decreased value of BMD i.e. 0.82 – 0.58 g/cm² with increased risk of fracture. The group of patients treated with raloxifene shows the improvement in T – Score up to –2 to –1.5 with BMD up to 1.1 g/cm². The other group of patients treated with Boerhaavia repens shows significant improvement in the T – Score up to the range of –1.5 to –1 with BMD level 1.18 g/cm². Boerhaavia repens produced marked improvement in the level of bone alkaline phosphatase and bone mineral density whereas, calcium level was found static. Thus, the study provided scientific support to Unani usage of the test drug.

Key words: Osteoporosis, Biskhapra, Boerhaavia repens, raloxifene.

Introduction

Medicinal plants and herbs are utilized in alternate and complimentary therapy besides conventional therapy which has recently started inducting medicinal plants to cure diseases. In alternate and complementary therapy the system of treatment in Pakistan are using medicinal herbs and plants as Unani, Ayurvedic, and Homeopathic medicine. The discipline has undergone many changes over the years and recently medicinal plants basically used for the search of new drugs.
will be a 40% chance of developing osteoporotic fracture during a person's remaining lifetime (Hulth, 1989). A specific cause of osteoporosis in most women is a rapid increase in bone loss after menopause, primarily due to loss of estrogen. However, whenever estrogen is taken, the patients should be cautioned about the development of breast cancer. Another fact is that not all postmenopausal women who have lost estrogen develop postmenopausal osteoporosis. A weaker association was found between calcium intake during adulthood and bone mass (Kanis et al., 1994; Jianxin et al., 1996).

Some women with very low calcium intakes do not develop osteoporosis. Treatment with Vitamin-D and calcium supplements will prevent some degree of loss of skeleton and decrease the likelihood of fractures. Calcium and calcitonin supplements act by decreasing bone resorption. Calcium acts mainly by decreasing activation of new bone remodeling units (not by decreasing action of existing osteoclasts). Postmenopausal women who are not treated with estrogen require about 1,500 mg daily for calcium balance. High dietary calcium suppresses age-related bone loss and reduces fracture rate in patients with osteoporosis. Calcitonin has recently shown to be an effective agent in management of patients with osteoporosis, but the drug is expensive and difficult to administer.

Here the study so thoughtfully conceived that is better designated to be a conjoint approach and cure many bone metabolic disorders by mixing the basic with the applied sciences. Herein, after a case of research is presented which specify the results so obtained on basic parameters on plant Boerhaavia repens and the bioactive marker for osteoporotic activity. The plant extract was then tested from clinical perspective and the paradigm so pursued exhibited succeed of thesis and genesis. Boerhaavia repens contain natural forms of calcium, which prevent development of fractures in osteoporosis, which has been extensively used in the treatment of many osteodystrophic conditions, helps in relieving pain associated with osteodystrophic conditions and is also useful in people with general debility, nervous problems and muscular pain, increases the mineralization of the bones and is useful for relief of bony pains of osteoporosis. This is a comparative study of Boerhaavia repens with raloxifene was conducted in different hospitals. The protocol, which included the research methodology, investigators brochures and the
patients, consent forms, was approved by the ethical committee of the Faculty of Eastern Medicine, Hamdard University, Karachi, Pakistan.

Materials and Methods

This study enlisted 86 patients at random, out of whom 48 were diagnosed as osteoporotic aged between 45–60 group. The patients were registered from the general O.P.D. and clinical Research ward of the Hospital. All the patients selected for the study, were thoroughly examined and clinical history was recorded in the prescribed proforma of case sheet. This proforma designed on Microsoft Access 2000 database program. The 48 patients so registered, the number of female and male were 46 and 2 respectively. The associated diseases data on hypertensive, diabetes, arthritis and thyrotoxicoses of these osteoporotic patients were recorded.

The clinical study on raloxifene was conducted for a period of three years in which the number of responding patients continued with the therapy the tally remained at14 (Kanis et al., 1994). Similarly in others designed group, which were treated with Boerhaavia repens 18 patients registered but after a period of three years only 8 patients continued with the treatment. Both types of the clinical evaluation were conducted for bone mineral density, bone alkaline phosphatase and serum calcium level.

The exclusion criteria observed for the osteoporotic patients include the fracture of the long and hip bone. The range of alkaline phosphatase in postmenopausal women without any pathology should be 22-30 mg/dl. But in case of osteoporosis this range exceeded up to 80-100 mg/dl. After treatment of the selected group of post menopausal osteoporotic women by raloxifene the alkaline phosphatase found to be in the normal limits up to 35 mg/dl. When the other group of postmenopausal women treated by Boerhaavia repens the level decreased to the range of 32 mg/dl. In case of serum calcium, the calcium level decreases up to 6-8 mg/dl from the normal range of 8-10 mg/dl. With raloxifene, the additional supplements of calcium were administered at the recommended dose of 800-1000 mg/day. The T – score indicates how many Standard Deviation (SD) a patients BMD is from the young adult BMD i.e. T – score expresses the percentage (%)Young Adult (YA) value in a different way. Patients with T – score above –1 considered as normal with the BMD up to 1.42 - 1.54 g/cm², while incase of osteoporosis the value of T – score suggestive of osteoporosis up to –2.5 to –5 with decreased value of BMD i.e. 0.82 – 0.58 g/cm² with increased risk of fracture.

The group of patients treated with raloxifene shows the improvement in T – Score up to –2 to –1.5 with BMD up to 1.1g/cm². The other group of patients treated with Boerhaavia repens shows significant improvement in the T – Score up to the range of –1.5 to –1 with BMD level 1.18 g/cm². The Boerhaavia repens showed marked improvement in the level of bone alkaline phosphatase and bone mineral density whereas, calcium level was found static (Goldstein et al., ; Sato et al., 1994).

Results and Discussion

The some main parameters taken in signs and symptoms for osteoporosis were arthralgia, joint stiffness, cracking sound, bone pain, arthritis as presented in Table 1, and Fig 1. The data showed in Table 1 regarding osteoporosis signs and symptoms as 40 patients presented with arthralgia, 40 patients were recorded in joint stiffness, 10 patients were presented with cracking sound, 40 patients were enrolled with bone pain and 40 patients having arthritis.

The clinical study on raloxifene was conducted for a period of three years in which the number of responding patients continued with the therapy the tally remained at 14. Similarly in others designed group, which were treated with Boerhaavia repens 18 patients registered but after a period of three years only 8 patients continued with the treatment.

Improvement of Arthralgia

By the comparison of the data of the Boerhaavia repens and raloxifene, their effects observed on the patients of arthralgia. Presenting with complaint of arthralgia were specified as below studying the effect of Boerhaavia repens 60% of patients recorded marked improvement, 20% of patients observed moderate improvement, 20% of patients recoded mild improvement of the their complaint and there was no patient in failure rate. The effects of raloxifene, 42.85% of the patients observed marked improvement, 14.28% patients observed moderate improvement, 14.28% of patients recoded mild improvement of the their complaint and the failure rate was 28.57% as shown in Table 2 and graph.

Boerhaavia repens shows 18% more improvement than raloxifene.
Table 1
Distribution of Signs and Symptoms of Osteoporosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
<td>40</td>
</tr>
<tr>
<td>Joint Stiffness</td>
<td>40</td>
</tr>
<tr>
<td>Cracking Sound</td>
<td>10</td>
</tr>
<tr>
<td>Bone Pain</td>
<td>40</td>
</tr>
</tbody>
</table>

Fig. 1
Distribution of signs and symptoms of osteoporosis

Table 2
Comparison of Boerhaavia repens with raloxifene with regards to improvement of arthralgia

<table>
<thead>
<tr>
<th>Level of Improvement</th>
<th>Boerhaavia repens</th>
<th>Raloxifene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked Improvement</td>
<td>60%</td>
<td>42.85%</td>
</tr>
<tr>
<td>Moderate Improvement</td>
<td>20%</td>
<td>14.28%</td>
</tr>
<tr>
<td>Mild Improvement</td>
<td>20%</td>
<td>14.28%</td>
</tr>
<tr>
<td>No Improvement</td>
<td>0%</td>
<td>28.57%</td>
</tr>
</tbody>
</table>

Fig. 2
Comparison of Boerhaavia repens with raloxifene with regards to improvement of arthralgia

Improvement of Joint Stiffness
Presented with the complaint of Joint Stiffness showed that Boerhaavia repens marked improvement in 50% of the patients, moderate improvement was recorded in 16.66% of the patients, mild improvement showed to be 41.65% and no improvement showed in 8.33% as shown in Table 3 and graph.

Boerhaavia repens gives 9.65% more good result for joint stiffness.

Table 3
Comparison of Boerhaavia repens with raloxifene with regards to improvement of Joint Stiffness

<table>
<thead>
<tr>
<th>Level of Improvement</th>
<th>Boerhaavia repens</th>
<th>Raloxifene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked Improvement</td>
<td>50%</td>
<td>41.65%</td>
</tr>
<tr>
<td>Moderate Improvement</td>
<td>16.66%</td>
<td>8.33%</td>
</tr>
<tr>
<td>Mild Improvement</td>
<td>16.66%</td>
<td>41.65%</td>
</tr>
<tr>
<td>No Improvement</td>
<td>16.66%</td>
<td>8.33%</td>
</tr>
</tbody>
</table>

Fig. 3
Comparison of Boerhaavia repens with raloxifene with regards to improvement of Joint Stiffness

Table 4
Comparison of Boerhaavia repens with raloxifene with regards to improvement of Cracking Sound

<table>
<thead>
<tr>
<th>Level of Improvement</th>
<th>Boerhaavia repens</th>
<th>Raloxifene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked Improvement</td>
<td>33.33%</td>
<td>50%</td>
</tr>
<tr>
<td>Moderate Improvement</td>
<td>66.66%</td>
<td>0%</td>
</tr>
<tr>
<td>Mild Improvement</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>No Improvement</td>
<td>0%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Fig. 4
Comparison of Boerhaavia repens with raloxifene with regards to improvement of Cracking Sound
Improvement of Cracking Sound

By the comparison of the data of the *Boerhaavia repens* and raloxifene, their effects observed on the patients of Cracking Sound. Presenting with complaint of Cracking Sound were specified as below studying the effect of *Boerhaavia repens* 33.33% of patients recorded marked improvement, 66.66% of patients observed moderate improvement, while the rate of mild improvement and failure was nil. The effects of raloxifene, 30% of the patients observed marked improvement, 50% patients observed moderate improvement, the failure rate was 20% of patients and the mild improvement rate was nil as shown in Table 4 and graph.

*Boerhaavia repens* shows 3.33% more improvement than Raloxifene

Improvement of Bone Pain

Presented with the complaint of Bone Pain showed that *Boerhaavia repens* marked improvement in 14.28% of the patient, 42.85% showed moderate improvement, 28.57% showed mild improvement and no improvement rate was also 14.28. Whereas, raloxifene was found marked improvement in 50% of the patients, moderate improvement was recorded in 14.28% of the patients, mild improvement showed to be 21.42% and no improvement showed in 14.28% as shown in Table 5 and graph.

Bone Pain improvement with Raloxifene is along with the use of NSAID.

Table 5

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Boerhaavia repens</th>
<th>Raloxifene</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
<td>95%</td>
<td>76%</td>
<td>0.00</td>
</tr>
<tr>
<td>Not Improved</td>
<td>5%</td>
<td>24%</td>
<td></td>
</tr>
</tbody>
</table>

*Boerhaavia repens* addresses two factors associated with healthy bone architecture: adequate calcium supplementation and appropriate hormonal balance. Earlier studies with *Boerhaavia repens* demonstrate a dose-dependent increase in the bone mineral content and density. *Boerhaavia repens* treatment shows desired effect on inhibitors on bone reabsorption and stimulators of bone formation in experimental studies, thereby indicating a potential therapeutic usefulness as an anti-osteoporotic agent 15.

Table 6

| Comparison of *Boerhaavia repens* with raloxifene with regards to improvement |
|-------------------------------|-----------------|-----|
|                               | Boerhaavia repens | Raloxifene |
| Improved                      | 95%              | 76%  |
| Not Improved                  | 5%               | 24%  |

95% patients who were treated with *Boerhaavia repens* showed improvement and 5% showed no improvement. Whereas, those patients who were treated by raloxifene 76% showed improvements and 24% did not show any improvement.

After applying the test of significance there was significant difference between these two drugs with Fisher Exact Test was applied and p-value was calculated as 0.00 as shown in Table 7.

References


The anti-inflammatory activity of *Artemisia afra* and *Sutherlandia frutescens*

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**Abstract**

*Artemisia afra* and *Sutherlandia frutescens* are commonly used in South Africa for their wide range of medicinal properties. Their anecdotal uses range from Asthma, colds, influenza to the management of cancers and wasting due to chronic illnesses. It is useful in the management of inflammation at wound sites, oesophagitis, gastritis, dysentery, inflammatory skin conditions, rheumatoid arthritis, osteoarthritis, etc many of which are due to inflammation. This study investigated the cytotoxic and anti-inflammatory effect of these two indigenous South African herbs. Whole blood cells were used in this study and IL-6 was used as biomarker for inflammation. The whole blood cells with various concentrations of *Artemesia afra* or *Sutherlandia frutescens* were stimulated *in vitro* with endotoxin and incubated. Results show that *Artemesia afra* is only cytotoxic at high concentrations (5000 μg/ml). *Sutherlandia frutescens* was not toxic at any of the concentrations tested. The addition of either *Artemesia afra* or *Sutherlandia frutescens* to the stimulated whole blood cells resulted in a significant decrease in the IL-6 released (P<0.001 and P<0.05, respectively). Thus, the findings of this study validate the anecdotal use of *Artemesia afra* and *Sutherlandia frutescens* in the management of inflammatory conditions.

**Key words:** Interleukin 6, *Artemisia afra*, *Sutherlandia frutescens*

**Introduction**

*Artemisia afra* (Asteraceae) is one of the oldest and best-known of South Africa’s indigenous medicines. The plant is also found in parts of Eastern Africa and is used as a medicine in Ethiopia (Van Wyk and Gericke, 2005). Wormwood, its common name, is derived from the Old English word ‘wermode’, meaning “mind preserver”.

African tribes like the Zulu, Xhosa, and Sotho all have different traditional names for *Artemisia afra*. One of these popular names include ‘wilde als’ or ‘als’ in Afrikaans, translating as ‘all’ or ‘everything’ as the herb is a common folk remedy for nearly every ailment (Mukinda, 2007). Therapeutically *Artemisia afra* is used as an Analgesic and Antihelminthic. Because of the great diversity of ailments treated with *Artemisia afra*, it is widely considered a “cure all” remedy in South Africa and its neighbouring countries (Mukinda, 2007; Van Wyk and Gericke, 2005; Van Wyk and Wink, 2004). A related species, namely, *Artemisia absinthium* is an important drug of Unani Medicine, where it is known as Afsanteen and is used in inflammatory disorders of Liver, Spleen, Uterus and Stomach, Worm Infestation and in various types of Fevers (Husain, y.n.m; Ghani, y.n.m.). Another famous related species is *Artemisia annua*, used in Chinese Medicine. *Sutherlandia frutescens* is a perennial, flowering shrub of the pea family (*Fabaceae/Leguminosa*) (Sia, 2004). It is also a commonly used, versatile medicinal herb that is indigenous to Africa (Ojewole, 2008). Widely known as cancer bush, *Sutherlandia frutescens* has been used in the traditional medicine of different cultural groups in Africa such as Zulu, Xhosa, Sotho, Khoi-San and Cape Dutch.

These plant are used by many cultural groups for fever, poor appetite, indigestion, gastritis, oesophagitis, peptic ulcers, dysentery, cancer tonic (prevention and treatment), diabetes, cold,
influenza, cough, asthma, chronic bronchitis, kidney and liver conditions, rheumatism, heart failure, urinary tract infections and stress and anxiety (Van Wyk and Albrecht, 2008). According to *Medicinal Plants of the World* by Van Wyk and Wink (2004) it is also used as a bitter tonic, an adaptogen, appetite enhancer, tuberculosis remedy, immune stimulating properties, treatment for wasting in cancer and AIDS patients and topically used to treat burns, wounds and inflammatory skin conditions.

Emphasis will be placed on the effects these two indigenous South African herbs have on inflammation. Inflammation is an immune reaction which describes the body's immediate response to infection or damage (Edgar, 2006; Griffin et al., 2003; Janeway and Travis, 1994). It was originally defined by four Latin words dolor, rubor, calor and tumescence translated as pain, redness, heat and swelling respectively (Janeway and Travis, 1994). It is triggered by a range of stimuli including chemical or thermal damage and infection. Preformed mediators are released in response to a breach in the first line of defense. This results in immediate aggregation of platelets which is associated with the release of serotonin. This promotes vasoconstriction, further platelet aggregation and the formation of a platelet plug. Other preformed mediators released include histamine, heparin, lysosomal enzymes and proteases, neutrophil chemotactic factor and eosinophil chemotactic factor. These factors are responsible for vasodilation, i.e. increase in blood flow to the site of injury and the recruitment of specific inflammatory cells to the area (Edgar, 2006). This increase in vascular permeability gives rise to the four clinical signs of inflammation, i.e. pain, redness, heat and swelling which is crucial to the early inflammatory response and is initiated as a result of the activation of complement (Edgar, 2006; Meyer, 2009).

Signaling pathways within the immune system is initiated via cytokines. Cytokines are proteins that functions as intracellular mediators. They are produced by leukocytes and act on target tissues resulting in multiple biological actions (Kapsimalis et al., 2008). Interleukin (IL) 6 is a multi-potential cytokine. It is known to play a role in inflammation through the activation of T cells and differentiation of B cells. It is therefore commonly used as a biomarker for inflammation (Heyen et al., 2000). In this study, the inflammatory action of *Artemisia afra* and *Sutherlandia frutescens* are evaluated.

**Materials and Methods**

**Preparation of the herbs**

A 20% (w/v) extract of *Artemisia afra* and *Sutherlandia frutescens* were prepared in 94.4% ethanol separately by Parceval (Pty) Ltd pharmaceuticals (South Africa). The leaves of the plants were crushed (sieve size~ 2-3 mm) after which it was mixed with 94.4% ethanol at 20 g/100 ml ethanol. The mixture was incubated overnight then pressed to separate the leaves from the tincture. The tincture was then filtered to remove excess debris and stored at -4°C. The samples for the immune assays were prepared by air drying 5 ml of the ethanol extracts. The dried extracts were then reconstituted with 2 ml DMSO to give a final concentration of 5 g leave extract/ml DMSO.

**Blood collection**

Blood was obtained from healthy male volunteers, not on any medication, by the doctor or nurse at the campus clinic. Blood samples were collected by venipuncture directly into heparinised vacuum tubes. The blood was stored at room temperature and used within 4 hours of collection. Preparation of the whole blood cell cultures were done under sterile conditions in a laminar flow cabinet. Approval for the study was received from the University of the Western Cape's ethics committee and an informed consent was obtained from all participants. Whole blood cells were stimulated with Lipoplysalchiride (LPS). Stimulated whole blood cultures contained 1 volume of 10 μg/ml LPS in dimethylsulfoxide (DMSO), 10 volumes of blood and 89 volumes of RPMI-1640 medium. Unstimulated whole blood contained 10 volumes of blood and 89 volumes of RPMI-1640 and 1 volume DMSO. The (stimulated and unstimulated) blood (200 μl/ well) was incubated at 37°C for 24 hours. Culture supernatants were then collected and assayed for LDH and IL-6.

**Cell culture**

*Artemisia afra* and *Sutherlandia frutescens* were diluted with DMSO individually. The diluted *Artemisia afra* and *Sutherlandia frutescens* (2 μl/well) was added to separate wells of a 96 well plate. Stimulated or unstimulated whole blood (200 μl/ well) was added to wells containing *Artemisia afra* and *Sutherlandia frutescens* extract. Control cultures contain 2μl DMSO instead of *Artemisia afra* and *Sutherlandia frutescens* extracts. The 96 well plate was sealed.
with Platemax cyclerseal sealing film and thereafter incubated at 37°C. Plates containing LPS stimulated blood were incubated for 24 hours.

Cytotoxicity

Culture supernatants were collected after the incubation with and without immune stimulation. LDH was measured using a Cytotoxicity Detection kit (Biovision, USA). The kit includes all the components required for the assay. Total cellular LDH were obtained by lysing diluted whole blood with the detergent Triton X100. The lysed cells were used to determine total cellular LDH. Cell culture supernatants and the lysed cells were assayed on a 96 well plate (Nunc-Immuno plate, MaxiSorp). 100µl of the kit reaction mixture was added to respected wells and incubated for approximately 15 minutes. The absorbance of the reaction mixtures were measured at 492 nm using a plate spectrophotometer at various time intervals. Cytotoxicity was expressed as the amount of LDH in supernatant as % of total cellular LDH.

Cytokine analysis

Double antigen sandwich enzyme linked immune sorbent assay (DAS ELISAs) (e-Bioscience, Germany) was used to measure cytokine release from the supernatants of the whole blood cultures. Nunc maxisorp (NuncTM, Denmark) plates were used for the assays. This kit contained all the reagents, buffers and diluents needed for performing quantitative ELISAs. The ELISAs were carried out according to the manufacturer’s instructions. In summary: 96 well plates were coated with primary antibody against the respective cytokine and incubated overnight at 4°C. After incubation, the plates were washed with autoclaved phosphate buffered saline containing 0.05% Tween-20. Nonspecific binding sites were then blocked with assay diluent for 1 hour at ambient temperature after which the wells received either recombinant human cytokine standards or sample. The plate was sealed and incubated for 2 hours at ambient temperature on a shaker. After incubation the wells were washed. The wells then received Biotin-conjugated antibody against the respective cytokine. The plate was incubated for 1 hour at ambient temperature on a shaker followed by washing as before. The wells then received Avidin-HRP conjugate. The plate was then incubated for 30 minutes at ambient temperature on a shaker. After the last wash, the bound peroxidase was monitored by addition of Tetrathionatebenzidine solution (substrate solution) to each well, after which the plate was incubated for approximately 15 minutes. The reaction was stopped by adding 2M H2SO4 to each well. The absorbance was read at 450nm on an ELISA plate reader. Excel was used to generate a standard curve for each ELISA plate which was used to determine the cytokine concentrations of the culture supernatants.

Statistical analysis

Experiments were performed three times in duplicate. All data is presented as a mean ± Standard deviation (SD). Data was statistically analyzed via one-way ANOVA (P<0.05) and regression analysis.

Results

Cytotoxicity

Total LDH in the blood culture was obtained by lysing diluted whole blood with Triton X100% detergent. The total LDH from the blood cultures was considered to be 100% toxicity (after being released into the medium). A standard curve was constructed from dilutions of the 100 % toxicity sample.

Cytotoxicity for Artemisia afra

The curve in Figure 1 indicates good correlation between the absorbance values and the % toxicity (R2=0.983).

Fig. 1: Standard curve of toxicity of Artemisia afra. A graphical representation of the absorbance of the reaction mixtures measured at 492nm at various total cell lysate concentrations after 10 minutes of incubation.

Based on the results of previous studies (Mukinda, 2007), no toxicity of Artemisia afra was expected. Figure 2 is a diagrammatical representation of the data obtained for theLDH assay. Data shows that Artemisia afra was toxic
at 5,000 µg/ml, the highest concentration tested. At this concentration, toxicity reached a maximum of 39.9% which was much higher than that demonstrated by the control (DMSO). At lower concentrations, however, Artemisia afra is not cytotoxic.

**Fig. 2:** Cytotoxicity of Artemisia afra. Each point represents the mean and standard deviation of triplicate assays. Data is expressed as a % of the total cellular LDH.

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Cytotoxicity for *Sutherlandia frutescens*.

The curve in Figure 2 indicates good correlation between the absorbance values and the % toxicity ($R^2=0.998$).

Results obtained conclude that *Sutherlandia frutescens* does not cause LDH leakage at any of the concentration. This indicates that *Sutherlandia frutescens* is not toxic to the cells at any of the concentrations used for this study.

**Inflammatory activity**

IL-6 forms a vital mediator in inflammation which is the acute phase response in immune activation. It is mainly secreted by macrophages in response to microbial pathogens. Culture supernatants from LPS stimulated cultures were analyzed for inflammatory activity using IL-6 as a biomarker.

**Inflammatory activity for Artemisia afra**

The IL-6 released by stimulated whole blood cultures was $3,418.3\pm4588.1 \text{ pg/ml}$ while the reading obtained from unstimulated blood was $31.9\pm35.9 \text{ pg/ml} (n=9)$. The data shows that there is statistically significant difference between IL-6 secretions of the stimulated and unstimulated whole blood cell cultures ($P<0.042$) showing that this biomarker assay can be used to monitor inflammatory activity.

Discussion

Inflammation is an intricate biological response of the host’s immune system to harmful stimuli (Medeiros et al., 2009). IL-6 is a pleiotropic cytokine which has a proinflammatory action (Ahmed and Ivashkiv, 2000). Research has indicated that IL-6 levels are increased in
rheumatoid arthritis and mechanisms of IL-6-target blockade are currently being researched as a potential treatment (Nishimoto and Norihiro, 2006). Increased IL-6 levels have been associated with an increased risk of intervertebral disc disease (IDD) commonly characterized by sciatica (Noponen Hietala et al; 2005). Increased IL-6 levels have also associated with obesity, non-insulin dependent diabetes mellitus (Herder et al; 2007) and the metastasis of cancer (Smith, 2001). In 2004 Tackey et al. described elevated IL-6 levels as being a key factor in the immunopathology of systemic lupus erythematosus (SLE) and may be directly responsible for tissue damage. Enhanced inflammation has been linked to the development and progression of atherosclerosis (Gokkusu et al; 2010).

*Artemisia afra* is traditionally used as a topical application for back pain and neuralgia (Kramer, 2006). The above results indicate that *Artemisia afra* may have potential in thetreatment of these conditions. Traditional uses of *Artemisia afra* include colds, influenza, mumps and wounds (Kramer, 2000; van Wyk and Wink, 2004). The decrease in IL-6production induced by *Artemisia afra* would be beneficial in these conditions as it could bring about symptomatic relief from fever caused by infection and also reduce theamont of inflammation at wound sites. Other traditional uses include joint pain and rheumatism (van Wyk and Wink, 2004). As *Artemisia afra* is considered a traditional “cure-all”, it is used for a wide variety of complaints including diabetes (Mukinda, 2007).

*Sutherlandia frutescens* at high concentrations inhibits IL-6 indicating that the extract also has antinflammatory properties. The anti inflammatory properties of *Sutherlandia frutescens* validate its uses as an anti-inflammatory agent. It is reported to have a possible application in the treatment of septic shock and chronic inflammation (Albrecht, 2008). The anti inflammatory properties have been linked to flavonoids sutherlandin A-D, L17 canavanine and pinitol (Anfossi et al., 1999; Ojewole, 2004; Avula et al., 2010).

Flavonoids are known for its anti inflammatory effect (Evans, 2002). L-canavanine prevents the arginine derived synthesis of nitric oxide (NO). L-canavanine is an arginineantagonist that selectively inhibits NO synthase causing vasoconstriction hence contributes to *Sutherlandia frutescens* anti inflammatory action (Anfossi et al; 1999; Abramson, 2005). Pinititol decreases the production of pro-inflammatory cytokines (Ojewole, 2004). The proven anti inflammatory action of *Sutherlandia frutescens* validates its anecdotal use in the treatment of oesophagitis, gastritis, dysentery, inflammatory skin conditions, rheumatoid arthritis, osteoarthritis, asthma, bronchitis and cystitis (Albrecht, 2008; Gericke et al., 2001; van Wyk and Albrecht 2008; van Wyk and Wink, 2004).

**Conclusion**

Increasing emphasis is being placed on biomarkers as indicators for certain inflammatory and auto-immune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus. Expression of cell surface markers and cytokines produced by T and B lymphocytes can lead to a more detailed description of disease activity in patients and serve as indicators of the patient’s response to treatment (O’Hara et al; 2006). It is for this reason that cytokines are commonly used as diagnostic biomarker to monitor the effects of a pharmaceutical on immune pathways (Fiala and Veerhuis, 2010). The results obtained from this study are cohesive with traditional uses of the herbs investigated regarding inflammatory pathways. Data shows that both *Artemisia afra* and *Sutherlandia frutescens* acts as an inflammation inhibitor. This demonstrates their potential ability to reduce the effects of auto-immune conditions such as RA and SLE. Conditions such as arteriosclerosis, rheumatic pains, sciatica, neoplastic metastasis and non-insulin dependant diabetes mellitus have all been associated with abnormally high levels of IL-6. *Artemisia afra* or *Sutherlandia frutescens* could play a promising role in the treatment and prevention of these diseases. Fever is associated with numerous bacterial, viral, and parasitic infections, such as influenza, colds, mumps and malaria. In these conditions, symptomatic relief could be brought about by using either *Artemisia afra* or *Sutherlandia frutescens* to decrease the fever.

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Effect of Taleeq (Leech therapy) on Dawali (Varicose Veins)

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Abstract

The objective of the study is to evaluate the efficacy of Taleeq (Leach therapy) in Dawali (varicose veins) and to provide a safe and cost effective alternative treatment. Randomized controlled clinical open trial was conducted in Regimenal Therapy Unit of NIUM Hospital. Fifty patients were divided into 2 groups, 30 in test and 20 in control group. Test group was treated with Taleeq on alternate day and control group was treated with grade 2 compression stockings and limb elevation for 2 months. Response was measured by assessment of pain / leg discomfort, limb girth at calf, ankle, and feet, pigmentation area and colour on every 15th day. Hb% was assessed on every 15th day to check anaemia. Effect on anatomy of vein was assessed by colour flow Doppler USG before and after treatment. Test group showed significant reduction in pain, limb girth, pigmentation, number of perforators. Control group showed significant reduction in pain and limb girth, but there was no improvement on pigmentation. Both groups did not show significant improvement on SFJ and SPJ incompetency. Taleeq produced significant improvement in Dawali (Varicose Veins) in comparison with existing standard treatment. However, the study indicates that combination with compression stockings and other effective treatment modalities like weight normalization for obese patients, physical therapy, dietary modification etc produces optimal results.

Keywords: Leeching, Varicose Veins, Dawali, Hirudin

Introduction

The word varicose vein was probably first used as a medical description by Hippocrates in 460 BC. Varicose veins were first described in Ebers papyrus over 3500 years ago as “Serpentine windings”, (John, 2005). Dawali (varicose veins) is a disease in which veins of lower limbs become dilated, tortuous, prominent and greenish in colour (Jurjani, 2010; Arzani, ynm). The aetiology of varicose vein is still incompletely understood despite the fact that it is a very common disease affecting all ages from teenagers to elderly people. Greco-Arab physicians postulated that it is caused by accumulation of non-purulent balghami, saudavi or damvi matter in leg veins or due to weakness (Jurjani, 2010; Arzani, ynm; Ibn Sina, 2007; Quamri 2008). Today it is assumed that the etiology of varicose veins is multifactorial. Patients may report aching especially on standing, itching, restlessness in legs and ankle swelling. Complications of varicose veins may develop like venous eczema, venous pigmentation, lipodermatosclerosis, superficial thrombo-phlebitis, and venous ulceration which are most troublesome, distressing and painful condition for patient (Russel et al., 2004, Berkow et al., 1997). Health related quality of life is significantly impaired in individuals with vascular diseases. Approximately 15% adults have varicosity of veins (Shah et al., 2003). The prevalence of varicose veins being highest in the western world; mostly from 10-30% in men and 25-55% in women has been reported in population based studies (Robertson et al., 2008). A study showed over all prevalence was significantly higher among south Indian workers than North Indians (6.8%) (Malhotra,1972). Surgical treatment of varicose veins is widely used. The main principles of surgical treatment are to ligate the source of the venous reflux and to remove the incompetent saphenous trunks and the associated varices. Sapheno-femoral ligation is associated with a high rate of recurrence of
varices. Removal of the saphenous veins has the disadvantage that vein is accompanied by a nerve that may be damaged in the vein stripping operation (Canonico et al., 1998). Due to high rate of recurrence and disadvantages of surgical treatment the need of hour is to find efficient and low cost alternative management. All the sign and symptoms or complications of varicose veins develop due to plethora (venous congestion). In order to save the limb and relieve the sign and symptoms, the venous blood must be removed and pressure must be reduced. Greco-Arab physicians have mentioned bloodletting in cases of plethora. Razi 2004, Ibn Sina 2007, Jurjani 2010 etc. have recommended fasd in Dawali, but Khan (2003) and Arzani mentioned Taleeq (Leech Therapy) in Dawali (varicose veins) for bloodletting. Taleeq seems to be effective for the management of varicose veins and their complications. Therapeutic effect of Taleeq to control the complications of varicose veins may be attributed to the salivary secretions of leech which contains certain bio-chemicals with vasodilating, anticoagulant, anesthetic, thrombolytic, analgesic, antibiotic and anti-inflammatory properties (Kaestner, 1967; Anonymous, 1995). Indian leeches H. granulosa also has medicinal properties (Verma, 2006). Venous disease is typically progressive; no treatment can prevent the appearance of new varicose veins in future. Modes of treatment that offers efficacy (long term control of symptoms or complications) without medication or surgery are given most priority. The study aimed at evaluating the effect of taleeq in the rehabilitation of dawali (varicose vein) patients as it is acclaimed for the beneficial effects in the management of this disease by Unani physicians. It further evaluated the treatment procedure with the conventional measures of tight stocking and foot elevation. The study tried to validate the Unani claims. If these claims are found true, it will help in the better management of varicose vein patients.

**Materials and Methods**

The present study is a randomized controlled open study conducted to know the effect of Taleeq in dawali. This study was carried out over a period of 12 months from April 2009 to March 2010 in National Institute of Unani Medicine, Bangalore. Fifty patients were assigned randomly to 2 groups, 30 in test group and 20 in control group by using random allocation software. Test group was treated with Alaq (leeches) alternate day for two months and control group was given compression stockings grade 2 for wearing and also advised limb elevation. Diagnosis was made on the basis of history, physical examination and colour flow Doppler ultra sound while taking history Emphasis was given on the past history of hypertension, hyperlipidemia, diabetes mellitus, and myocardial infarction, claudication, varicose vein, and DVT. After history general examination was done with special emphasis on pulse, (rate, rhythm, character and volume), B.P, respiratory rate, respiratory distress, anaemia, oedema, and lymphadenopathy, BMI etc. CT, BT, blood sugar fasting, and P.P, HBsAg, Elisa test for HIV were carried out for not to perform leech therapy. Hb% assessment was done on every 15th day to check anaemia. Color flow Doppler was done before and after treatment to observe the effect of treatment on vein anatomy. Leeches were identified as Hirudinaria granulosa by Dr. P. Mahboob Basha department of Zoology, Bangalore University. Fresh unused, well cleaned leeches gathered 24 hours before starting a leeching session. Small sealable containers partly filled with water labelled with patient’s name for used leeches, water proof padding and towels, bandages or highly absorbent material, adhesive tape, water, scissor, disposable razor, surgical gloves, were required and gathered before starting a leeching session. Patients were advised not to use perfumes, chemicals to the skin at the intended application site for at least 2 days before treatment. Skin of the target area was thoroughly cleaned with soap and water. Application site was shaved and dry rubbed until the skin become rosy or red, it helps to get the animals to bite quickly. A dampen square gauze with 1 cm square hole in the middle was placed in close contact with the area to be treated to protect the leech from wandering. After wearing surgical gloves, active and healthy leeches were selected and the head of the leech was put in the hole of the gauze, attachment generally occurs quickly. If the leech was reluctant to bite, a small needle prick was made on the skin to produce a tiny droplet of blood, which results in enthusiastic attachment. The gauze square can be removed without disturbing the animal. The target area was kept warm and dark by covering it with a towel or other material. Leeches usually stay attached for 30-60 minutes and fell down itself. When the leeches drop off they were placed in a jar labeled with patients name to avoid confusion between used and unused animals and to
prevent use on another patient. The tripartite jaw of the leech makes a three pronged Y shaped bite wound. After the leech has dropped off it usually takes 3-48 hours for the wound to stop bleeding. The slow drainage of blood is an important part of treatment. The drainage of blood reduces venous congestion. When there was a good outflow of blood after leech feeding, the wound was loosely covered and checked the extent of bleeding 15-30 minutes later, if satisfactory, a loose dressing was applied. Patient was advised to avoid strenuous physical activity until the bleeding stop naturally. Primary dressing was consisting of a wide and thick sterile pad to absorb all the blood oozing from the wound. The layers of padding were loosely secured with a gauze bandage that is not so tight that it obstructs the blood flow. Area around leech bite was routinely observed for local infection. Patients in control group were advised to put the stockings on as soon as get out of bed, before gravity gets a chance to cause pooling of blood in varicose vein. They were advised to keep the stockings on all day and take them off when lying down, with legs rose above the level of the heart. For efficacy assessment the Baseline observations were recorded on zero day thereafter at an interval of 15 days till 2 months. At every visit the patients were asked about the improvement and worsening in their symptoms and subjected to examination to assess clinical findings. Concomitant treatment was not allowed during the protocol period. The patients who were taking any medicine were advised to observe abstinence from that drug for one week. An arbitrary grading of various parameters was improvised for appropriate assessment of the efficacy of leech therapy. Intensity of leg pain / leg discomfort during walking was assessed on 4 point scale ranging from 0-3 (0 for no pain, 1 for mild pain i.e. Irritating and uncomfortable, 2 for moderate i.e. Dreadful and Horrible and 3 for severe pain i.e. unbearable or Agonizing). Pigmentation was assessed by colour and area of pigmentation. Colour of pigmentation was scored as 0 for none, 1 for reddish to light brown, 2 for light brown to dark brown, 3 for dark brown to blackish. Area of pigmentation was obtained by multiplying the greatest length (head to toe) and greatest width (side to side) of pigmented area i.e. length × width. Both dimensions were measured by centimetre ruler. Oedema was measured by taking difference between the values of limb girth, before and after the treatment. Limb girth was measured at 3 points i.e. calf, ankle and foot. Colour flow Doppler ultra sound was carried out to exclude arterial disease and to determine the patency of veins; a bidirectional flow probe was used to detect venous reflux. This investigation was carried out with the patient standing. The assessment of safety of the treatment was done by TLC, DLC, ESR, CT, and BT (before and after treatment). The data was analyzed by computerized statistical package Graph pad (Instat version).

Result

Our study population predominantly comprised of males accounting 68% (34) while remaining 32% (16) were females. In present study population 30% (15) were house wives, 10% (5) Businessmen, 8% (4) Clerks, 4% (2) Drivers, 2% (1) Tailors, 2% (1) Editor, they constituted 56% (28) of study population. All were involved in prolong standing at work place or low level of physical activities. Study population also included laboures 16% (8), Shopkeepers 8% (4), Nurses 4% (2), Farmers 4% (2), Printers 4% (2), they constituted 36% (18).All were involved in prolong standing at work place and others 8% (4) (including Doctors 2% (1), Retired 4% (2), Engineers 2% (1).

In test group the median pain score on 0 day and 15th day was 2, on 30th and 45th day it was 1 and on 60th day was 0. Test group showed significant reduction (p<0.01) in pain from 30th day onwards. In control group the median pain score on 0 day, 15th day and 30th day was 2 and 45th day and 60th day was 1. Control group showed significant reduction (p<0.01) in pain from 45th day onwards.

On inter group comparison using Kruskall Wallis test with Dunn’s pair comparison test, it was found that the median pain score in test group at 60th day was significantly reduced (p<0.01) in comparison to median pain score of control group at 60th day (Table 1).

Table 1
Effect of leeching on pain in varicose vein patients
(Median rating with range)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>2 (1, 3)</td>
<td>2 (1, 2)</td>
<td>1 (0, 1)</td>
<td>1 (0, 1)</td>
<td>0 (0, 1)</td>
</tr>
<tr>
<td>Control</td>
<td>2 (2, 3)</td>
<td>2 (1, 2)</td>
<td>2 (1, 2)</td>
<td>1 (1, 2)</td>
<td>1 (1, 2)</td>
</tr>
</tbody>
</table>

*p < 0.01 w. r. t. control 0 day, ++ p < 0.01 w. r. t. test 0 day, **p < 0.01 w. r. t. test 60 day.
In test group limb girth at calf on 0 day was 37.17 ± 0.83 cm, on 15th day 36.03±0.83 cm, on 30th day 33.93 ± 0.78 cm, on 45th day 32.53±0.83 cm and on 60th day 32.67±0.78 cm. Test group showed significant reduction (p<0.01) in limb girth at calf from on 15th day onwards. In control group limb girth at calf on 0 day was 37.45±1.08 cm on 15th day, 36.75±1.07 cm on 30th day, 36.20±1.04 cm on 45th day, 33.90±1.08 cm, on 60th day was 33.7±1.01 cm. Control group showed significant reduction (p<0.01) in limb girth at calf from 30th day onwards when compared with median limb girth at calf on 0 day control. On inter group comparison using Kruskall Wallis test with Dunn’s pair comparison test it was found that test group showed significant reduction in mean limb girth at calf on 60th day in comparison to 60th day control (p<0.01).

In test group mean limb girth at ankle on 0 day was 26.93±0.54 cm, on 15th day was 26.06±0.53 cm, on 30th day was 22.30 ± 0.50 cm, on 45th day was 22.50 ± 0.68 cm and on 60th day was 22.50 ± 0.68 cm. Test group showed significant reduction (p<0.01) in mean limb girth at ankle from 15th day onwards in comparison to 0 day test. In control group mean limb girth at ankle on 0 day was 26.00 ± 0.70 cm, on 15th day was 25.40 ± 0.75 cm, on 30th day was 25.00 ± 0.70 cm, on 45th day was 22.55 ± 0.71 cm and on 60th day was 22.50 ± 0.68 cm. Control group showed significant reduction (p<0.01) in mean limb girth at ankle on 60th day when compared with mean limb girth at 0 day control. On inter group comparison using Kruskall Wallis test with Dunn’s pair comparison test it was found that the test group showed significant reduction in mean limb girth at ankle at 60th day with respect to 60th day control (p<0.01).

In test group median limb girth at foot on 0 day was 25 cm, on 15th day 24 cm, on 30th day 23 cm, on 45th day 23 cm and on 60th day 22 cm. In control group mean limb girth at foot on 0 day was 23 cm, on 15th day was 23 cm, on 30th day was 23 cm, on 45th day was 22.5 cm and on 60th day was 22 cm. On intra group comparison both groups showed reduction in median limb girth at foot on 60th day with respect to 0 day but statistically not significant. On inter group comparison the difference was not statistically significant (p>0.05) (Table 2).

In test group median score of pigmentation on 0 day was 3, on 15th day was 2, on 30th day was 2, on 45th day was 1 and on 60th day was 1. Test group showed significant reduction in pigmentation from 15th day on wards with respect to test 0 day. In control group median score of pigmentation colour on 0 day was 2, on 15th day was 2, on 30th day was 2, on 45th day was 2 and on 60th day was 2. Control group showed no significant reduction (p>0.05) in pigmentation when various assessment days were compared with 0 day control. When median rating for pigmentation were compared among two groups using Kruskall Wallis test with Dunn’s pair test it was found that median rating for pigmentation colour at 60th day test was significantly reduced (p<0.01) when compared with 60th day control (Table 3).

### Table 2

**Effect of leeching on Limb girth (cm) in varicose vein patient (Mean + SEM)**

<table>
<thead>
<tr>
<th>Assessment day</th>
<th>Test Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calf</td>
<td>Ankle</td>
</tr>
<tr>
<td>0 Day</td>
<td>37.17 ± 0.83</td>
<td>37.45 ± 1.08</td>
</tr>
<tr>
<td>15th Day</td>
<td>36.03 ± 0.83</td>
<td>36.75 ± 1.07</td>
</tr>
<tr>
<td>30th Day</td>
<td>33.90 ± 1.08</td>
<td>36.03 ± 1.08</td>
</tr>
<tr>
<td>45th Day</td>
<td>33.90 ± 1.08</td>
<td>36.03 ± 1.08</td>
</tr>
<tr>
<td>60th Day</td>
<td>22.90 ± 0.48</td>
<td>22.55 ± 0.48</td>
</tr>
</tbody>
</table>

N=3 * p<0.01 w. r. t. control 0 day, ** p<0.01 w. r. t. test 0 day, + p<0.01 w. r. t. test 60 day.

### Table 3

**Effect of leeching on pigmentation in varicose vein patients (Scores: Median with range)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Day</th>
<th>15th Day</th>
<th>30th Day</th>
<th>45th Day</th>
<th>60th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>3 (0, 3)</td>
<td>2 (0, 3)</td>
<td>2 (0, 3)</td>
<td>1 (0, 2)</td>
<td>1 (0, 2)</td>
</tr>
<tr>
<td>Control</td>
<td>2 (0, 3)</td>
<td>2 (0, 3)</td>
<td>2 (0, 3)</td>
<td>2 (0, 3)</td>
<td>2 (0, 3)</td>
</tr>
</tbody>
</table>

*- p < 0.01 w. r. t. control 0 day, + p < 0.01 w. r. t. test 0 day, ++ p < 0.01 w. r. t. test 60 day.
In test group the median area of pigmentation on 0 day was 210 sq cm, on 15th day 140 sq cm, on 30th day 120 sq cm, on 45th day 115 sq cm and on 60th day 90 sq cm. In test group median area of pigmentation was reduced from 30th day onwards with respect to test 0 day. In control group the median area of pigmentation on 0 day was 210 sq cm, on 15th day 225 sq cm, on 30th day 220 sq cm, on 45th day 230 sq cm and on 60th day 230 sq cm. control group showed reduction in area of pigmentation on 60th day compared with 0 day but it was not statistically significant (p>0.01). On inter group comparison using Kruskall Wallis test with Dunn's pair comparison test, test group showed significant reduction in area of pigmentation on 60th day with respect to control 60th day (Table 4).

Table 4
Effect of leeching on pigmentation area in varicose vein patients (Area: Median with range)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Day</th>
<th>15th Day</th>
<th>30th Day</th>
<th>45th Day</th>
<th>60th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>210 (72.754)</td>
<td>225 (70.350)</td>
<td>220 (70.350)</td>
<td>230 (62.350)</td>
<td>230 (62.350)</td>
</tr>
<tr>
<td>Test</td>
<td>210 (56.810)</td>
<td>140 (45.870)</td>
<td>120 (24.850)</td>
<td>115 (24.500)</td>
<td>90 (4.380)</td>
</tr>
</tbody>
</table>

*p < 0.01 w. r. t. control 0 day, + p < 0.01 w. r. t. test 0 day, ++ p < 0.05 w. r. t. test 60 day.

Before treatment in test group the median number of perforators was 11 and after treatment number of perforators were 4. Intra test group comparison was assessed by applying Friedman test. It showed significant reduction in number of perforators after completion of treatment (p<0.001). Before treatment the median number of perforators in control group was 11 and after treatment the median number of perforators was 7. Intra control group comparison was assessed by applying Friedman test, it showed significant reduction in number of perforators (P<0.05). inter group comparison by using Kruskall Wallis test with Dunn’s pair comparison test, test group showed significant reduction (p<0.01) in median number of perforators with respect to control group (Table 5).

Table 5
Effect of leeching on Colour Flow Doppler USG, Incompetent perforators in varicose vein patients (Median with range)

<table>
<thead>
<tr>
<th>Groups</th>
<th>BT</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>11 (10, 13)</td>
<td>4 (3, 6)</td>
</tr>
<tr>
<td>Control</td>
<td>11 (9, 13)</td>
<td>7 (4, 8)</td>
</tr>
</tbody>
</table>

*p < 0.001 w. r. t. control BT, + p < 0.001 w. r. t. test BT, ++ p < 0.05 w. r. t. test AT,

In test group before treatment total number of SFJ incompetence was 20 and after treatment total number of SFJ incompetence was 19. In control group before treatment total number of SFJ incompetence was 14 and after treatment total number of SFJ incompetence was 12. In test group before treatment total number of SPJ incompetence was 12 and after treatment total number of SPJ incompetence was 10. In control group before treatment total number of SPJ incompetence was 7 and after treatment total number of SPJ incompetence was 6. Inter and intra group comparison showed the difference is not significant (p>0.05) (Table 6)

Table 6
Number of incompetent valves before and after treatment

<table>
<thead>
<tr>
<th></th>
<th>Test group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>AT</td>
</tr>
<tr>
<td>SFJ</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>SPJ</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

(* = p< 0.05)

Discussion
Our study population predominantly comprised of males patients (68%) as compared to female (32%). Most studies showed higher incidence of varicose veins in females than in males (Laurkka et al., 1993; Canonico et al., 1998). The result of study published by Evans (1999) in which on the contrary, a higher occurrence was found in men (Evans et al., 1999). A Polish study showed that advanced form of venous pathology was more prevalent in male as compared to female (Jawien et al., 2003). This finding is comparable to our study.

In present study 56% (28) of study population, was involved in prolonged sitting at work place or low level of physical activities. Our results are in
In accordance of Framingham study that showed subjects with varicose veins had lower level of physical activities (Brand et al., 1988). In our study 36% (28) subjects were involved in prolong standing at work place. Prolong standing is associated with development of varicose veins. Our results are in accordance with a Danish study reported that working in standing position was even associated with subsequent hospitalization due to varicose veins for both men and women (Tuchsen et al., 2000).

In the present study both groups showed significant reduction in median pain score after treatment as compared to before treatment (p<0.01). Inter group comparison using Kruskall Wallis test with Dunn’s pair comparison test showed significant reduction in median pain score at 60th day test with respect to 60th day control (p<0.01) (Table 1). The result indicated that both regimens (Taleeq and compression stockings with leg elevation) were effective in reducing pain but Taleeq was found more effective in comparison to compression stockings and leg elevation.

In case of venous stasis the pathogenesis of the pain not only involves the concept of pain receptors but also the appearance of algogenic metabolites at the site of the microcirculatory units to which endothelial cells are particularly sensitive (Cofet et al., 1992). When leech bites the skin it sucks the stagnated blood thereby reduces the mechanical pressure. It also injects secretions containing anticoagulant, antithrombotic, vasodilating and anesthetic agents from the salivary ductules by pumping action (Whitaker et al., 2005; Elder et al., 1996). Hirudin, Calin and Factor Xa inhibitor present in leech saliva are anticoagulant. Hirudin binds to and inhibits only the activity of thrombin with a specific activity on fibrinogen (Rydell et al., 1991). Thus Hirudin prevents the formation of clots and thrombi and dissolves them. Calin present in leech saliva inhibit the Von Willebrand factor to bind itself to collagen, and it also inhibits the platelet aggregation caused by collagen. Factor Xa inhibitor present in leech saliva block the action of the coagulation factor Xa. Enzyme Destabilase has thrombolytic effect; it breaks up any fibrin that has formed. Leech saliva has 3 compounds that act as a vasodilator agent; they are the histamine like substance, the acetylcholine and the carboxypeptidase A inhibitor, these widen the vessels and increases the flow of blood to the bite site (Kamenew, 2009). Anticoagulant, thrombolytic and vasodilating substances present in leech saliva prolong bleeding and causes hypo-volumic haemo-dilution which reduces pressure of blood and also remove the metabolites at the site of the microcirculatory units, in combination of these, anesthetic substances present in leech saliva deaden pain on the site (Kamenew, 2009).

Limb girth was measured at 3 points i.e. at calf, ankle and foot. It is the measure of oedema. Reduction in limb girth showed reduction in oedema. Test group and control group showed significant reduction in mean limb girth at calf and ankle after treatment as compared to before treatment (Table 2) and the median girth at foot also reduces after treatment in both groups but was found statistically not significant (p>0.01). The result indicates that both regimens are effective in reducing limb girth (oedema) but Taleeq is more efficacious in reducing oedema than compression and leg elevation. In case of varicose veins the movement of blood toward heart is decreased due to incompetent valves and patient may develop stasis (pooling) of blood which contributes to oedema (Ogilive et al., 1997).

Biochemicals present in leech saliva due to their anticoagulant, thrombolytic and vasodilating effects that cause hypovolumic haemodilution thus reduce stasis or blood pooling (Kamenew, 2009).

The effect of leech therapy on pigmentation was assessed by change in colour and area of pigmentation. In test group median score of colour of pigmentation was significantly reduced after Taleeq as compared to before Taleeq (p<0.05) but control group did not show significant reduction in pigmentation colour at 60th day with respect to 0 day control (p>0.05).

On inter group comparison test group showed significant reduction in median rating for pigmentation colour (p<0.01) (Table 3). These results indicate taleeq reduces pigmentation colour. Test group showed significant reduction in median area of pigmentation after taleeq as compared to before taleeq (p<0.05). Control group showed no significant reduction in median area of pigmentation after treatment as compared to before treatment (p>0.05). On inter group comparison, test group showed significant reduction in area of pigmentation. (p<0.01) (Table 4). Pigmented lesions in stasis dermatitis are caused by deposition of haemosiderin in the dermis.
Haemosiderin is formed from the decomposition of haemoglobin within the cytoplasm of phagocytic cells in association with post inflammatory pigmentation that induces pigment in continence. Dermal haemosiderin deposition has a stimulatory effect on melanogenesis (Rosarco et al., 2003). Macrophages of reticulo-endothelial system play the major role in relieving iron, from catabolism of erythrocyte haemoglobin to plasma for reuse in haem synthesis, part of this iron is rapidly returned to plasma and part is exchanged with shortage iron in macrophages and is reutilized slowly. Hypovolumic haemodilution caused by Biochemicals present in leech saliva improve circulation of skin on the affected site, thus the haemosiderin deposited in skin is reutilized as a source of Iron (Normen, 1994).

Colour flow Doppler USG was done in all patients of both groups before and after treatment to detect SFJ and SPJ incompetency. Both groups showed insignificant reduction in number of patients with SFJ and SPJ incompetency (p>0.05) (Table 5). This may be attributed to degeneration of valve cusps (Russel et al., 2004). On inter group comparison test group showed significant reduction in number of perforators (Table 6).

Conclusion

Taleeq has significantly positive effect on the course of superficial phlebitis; patients perceive a noticeable improvement of symptoms right after treatment due to potent anti-inflammatory, blood thinning and lymph flow accelerating effect of leech secretion. Compression stockings and leg elevation also showed significant improvement but less than Taleeq. Thus from the above result we conclude that Taleeq was safe and well tolerated and has encouraging potential in prevention of complications of varicose veins. We must stress that leech therapy should be administered in combination with compression stockings and other effective treatment modalities like weight normalization for obese patients, physical therapy, dietary modification, etc for optimal results.

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Preparation of Kushta Sammulfar (*calx of Arsenic*) by muffle furnace using the temperature pattern extrapolated from the classical method of its preparation as practiced in Unani Medicine

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Abstract

Kushta is an important dosage form prepared by incinerating some of the drugs of mineral and animal origin at a high temperature so as to get the calx (*calcined material*). It is prepared by the classical methods as described in Unani Medicine. The methods are time-tested, proven and are in extensive use of Unani physicians and pharmacists. But since the materials used to produce the heat and the procedure adopted for incineration have been alleged not to invariably ensure the required degree of temperature, therefore, it was thought reasonable to use muffle furnace to prepare Kushta by maintaining the temperature pattern recorded during the preparation of Kushta by classical method. It was prepared first by the classical method (KSCM), and a thermogram was developed using a thermocouple (an electronic instrument assembled for recording of core temperature), which depicted the pattern of temperature variation during the process. Similar pattern of temperature was followed when Kushta Sammul Far was prepared by muffle furnace (KSMF). Further, Atomic Absorption Spectroscopy Analysis was done on the two samples to determine the concentration of elemental arsenic which determines the safety/toxicity profile of the drug. Concentration of elemental arsenic as shown by Atomic Absorption Spectrometer was found to be 6.388 ± 0.711 (ppm) and 3.62 ± 1.327 (ppm) in KSCM and KSMF, respectively (Tab.2). When KSCM and KSMF were weighed, loss of weight in classical method was found to be 90% and that in muffle furnace to be 93% with respect to total weight of crude drug. Thus, the study showed that traditional method for calcinations of arsenic is dependable. It also provided the method of optimizing modern method of calcination by muffle furnace to the traditional Unani method viz. by applying the thermogram generated from the traditional method.

Key words: Arsenic, kushta, calcined arsenic, muffle furnace

Introduction

Most of the metals and other drugs of mineral origin and some of the drugs of plant and animal origin that are known for their toxic effect in Unani Medicine are not used in the natural form for therapeutic purposes because of their ability to induce toxicity even at a low dose levels. Rather they are subjected to various physical and chemical processes in order to eliminate or minimize their toxicity. One such important and commonly used method in case of many mineral and a few animal origin drugs is the burning of the drugs at a high temperature so as to get a calcined product which is considered more effective and safe. The plausibility of their safety lies in the fact that they are converted into oxide form, become highly subtle and thereby highly soluble. Their elemental status which is considered injurious is changed into oxide form and the dose becomes very low which further minimizes the chance of toxicity. The calcined material is known as Kushta (calx), a Persian word that means killed (Aziz *et al.*, 2002). It has a high dissolution rate and ability to get absorbed in the body quickly, therefore, a low dose of Kushta
induces quick onset of action and high magnitude of effect (Hafeez, 1931).

Kushta is prepared with a special technique as described in Unani texts. The drug intended to be calcined is first treated with the decoction, distillate, extract etc of another drug or mixed with the powder of some other drug of plant or mineral origin. The new preparation is now put into Boota (Crucible) covered with another Boota of same diameter and sealed with a specially made mud plaster known as Gile Hikmat. The sealed boota after drying in sun is kept in a pit of specific dimension; the pit is then filled with cow dung cake and burnt. The quantum of heat is all important in the preparation of Kushtha because different degree of heat is required (usually from 300˚C–1000˚C) for different drugs; accordingly the size of pit as well as the number/quantity of cow dung cakes vary with each preparation. In this method the temperature increases gradually until it reaches a plateau and comes down slowly to atmospheric temperature. It has been reported that changes in crude drugs take place and certain intermediaries are produced at specific temperature before the Kushtha is prepared (Bhagwat, 2004). Though the method is simple and is in use since ancient times and a number of drugs are being prepared by this conventional method, still it is argued frequently that the process is less objective, as the degree of heat cannot be guaranteed every time. The consistency of cow dung and their heat producing ability varies. The atmospheric condition (temperature, relative humidity, current of air etc.) where the pit is dug also determines the maximum temperature as well as it’s the rate of ascent. Thus, the uniformity of characteristics in different samples of Kushtha cannot be ensured in the absence of a standardized method of heat application. It was thought therefore useful to standardize the temperature pattern during the course of preparation of a Kushtha by classical method and to develop a thermogram of the same so as to use the same pattern of temperature while preparing the Kushtha by a muffle furnace.

In the present study, Kushtha Sammul Far (Calx of Arsenic trioxide) was prepared by classical method and also in a muffle furnace. The temperature recorded in the classical method at different time intervals was utilized to develop a thermogram which was used to prepare the Kushtha by the muffle furnace. The two samples were subjected to Atomic Absorption Spectroscopy Analysis to determine the concentration of elemental Arsenic which was taken as the marker of the quality and safety of Kushtha.

**Materials and Methods**

**Materials**

- Arsenic: Arsenic Trioxide (As$_2$O$_3$) was procured from Nice Pvt. Ltd., Kerala, India.
- Alum: Alum was procured from local market of Bangalore.

**Thermocouple**: An electric instrument with a long metallic rod having a free end and a device of recording and digital display of temperature at the other end. The free end is exposed to heat to record the temperature.

**Boota and Gile Hikmat**

Boota is an earthen pot of bowl shaped, prepared with special technique and specific materials so as to make it heat resistant. The drug is placed in it and another Boota of similar dimension is used to cover it. While Gile Hikmat is the application of a specific semi solid material prepared in the mud base, all around the bowl and specially the junction point of the brim of two bowls to make it air tight (Anonymous, 2006).

**Preparation of Kushtha by Classical Method (KSCM)**

The Kushtha was prepared by the method as described in National Formulary of Unani Medicine (Anonymous, 2006). Arsenic and alum were taken in the ratio of 1:2; mixed well and placed in a Boota which was sealed by Gile Hikmat as described above and dried in sun. A 2×2×2 Cubic feet pit was dug and the Boota was placed within a pile of 2.5 kg of cow dung cakes set in the pit. Another 2.5 kg of cow dung cakes were placed over the Boota so that it would remain in the middle. The heat-exposed part of thermocouple was inserted into the pit placing the tip close to the Boota so as to measure the temperature. The cakes were ignited from the lower end and the temperature variation was recorded from this point of time till the whole of the cow dung cakes burnt to ashes and temperature receded back to the point where from further decrease was not recorded for an hour (Table - 1)
Development of Thermogram

A Thermocouple (single channel battery operated hand held type K (Thomas, 1988) was used to record temperature at every 5 minutes till the Kushta was formed. Three samples were taken and correspondingly three sets of temperature recordings were made. The temperatures recorded were observed for peak temperature (P. Temp.), mean rising temperature (MRT1), mean receding temperature (MRT2) and over all mean temperature (OAMT) in all the three samples.

Preparation of Kushta by Muffle Furnace (KSMF)

The temperature recorded during the preparation of KSCM was used to develop a thermogram to have an accurate record of gradual increase and decrease of temperature. The temperature pattern of the Thermogram was followed and similar temperature was maintained in muffle furnace when the Kushta was prepared in it (Christian and Feldman, 1970). As in KSCM three samples of Kushta were prepared in muffle furnace. The temperature was noted and compared statistically for homogeneity of variation in temperature. The mean of homogeneity calculated for the values of varied samples served as template for temperature variation in muffle furnace.

Atomic Absorption Spectrometry

The two samples of Kushta prepared by classical and Muffle Furnace methods were also subjected to Atomic Absorption Spectrometry (Christian and Feldman, 1970) by using AAS Sensa, GBC, Australia, to find the concentration of elemental arsenic. The samples were weighed to know the loss in weight, if any.

Results and Discussion

The three sets of temperature as recorded in KSCM were compared statistically. Sample I and Sample III were found homogenously varied and there was no significant difference between the two. Whereas Sample II was significantly different in respect of mean rising and mean receding temperatures. So, the sample II was discarded and the mean of the two samples was taken for setting up the temperature pattern for muffle furnace. The quality of cow dung cake (dryness, consistency etc) may be considered responsible for not showing the same temperature pattern in sample II as that in sample I and III. The peak temperature of 820˚C was set with gradual increase from atmospheric temperature (20˚C approximately) onward and then it was again set to the same level of atmospheric temperature to allow it to cool (Table 1; Fig.1, 2).

The concentration of elemental Arsenic as shown by Atomic Absorption Spectrometry was found to be 6.388 ± 0.711 (ppm) and 3.623 ± 1.327 (ppm) in KSCM and KSMF, respectively (Tab.2). When KSCM and KSMF were weighed, loss of weight in classical method was found to be 90% and that in muffle furnace to be 93% with respect to total weight of crude drug showing non-significant difference in the two values. In another study conducted for sub-acute toxicity the two samples have been shown to be comparably safe with regard to Body Weight, DLC, SGOT, SGPT, S Urea and S Creatinine (Shamim, 2011). However, the significant difference in elemental arsenic level

### Table 1

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Sample (I)</th>
<th>Sample (II)</th>
<th>Sample (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over all Mean</td>
<td>173.835 ± 23.302</td>
<td>143.942 ± 24.435</td>
<td>198.090 ± 26.332</td>
</tr>
<tr>
<td>Peak Temperature</td>
<td>813</td>
<td>1047</td>
<td>894</td>
</tr>
<tr>
<td>Mean of rising Temperature</td>
<td>290.476 ± 38.250</td>
<td>267.727 ± 52.403</td>
<td>327.048 ± 44.570*</td>
</tr>
<tr>
<td>Mean receding Temperature</td>
<td>190.298 ± 25.973</td>
<td>137.328 ± 23.458</td>
<td>201.716 ± 26.881*</td>
</tr>
</tbody>
</table>

Test used ANOVA one way, *p>0.05, *p<0.05 comparison with reference to S1 and S2.

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Arsenic (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSCM</td>
<td>6.388 ± 0.711</td>
</tr>
<tr>
<td>KSMF</td>
<td>3.623 ± 1.327</td>
</tr>
</tbody>
</table>
Development of Thermogram using classical method of preparation of Kushta

Comparative temperature parameters of samples subjected to classical procedure

of classical method was not followed while preparing the Kushta in muffle furnace. As discussed earlier that gradual increase or decrease of temperature is important in converting the crude form of drug into intermediates and finally the actual Kushta form. Therefore, use of same pattern of temperature is a must and is in consonance with Unani practices.

Conclusion

It can be concluded that the classical method of preparation of Kushta practiced by Unani physicians is a useful method by which an effective and safe dosage form can be prepared. A Kushta can also be prepared with the help of muffle furnace applying the temperature pattern recorded from classical method. The thermogram prepared by us can be used for the preparation of Kushta Sammull Far by muffle furnace and may also serve as a model for using thermogram of traditional calcination methods of other formulations to be applied to prepare their Kushta in muffle furnace.

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An experimental study of *Maa-uz-Zahab* (Gold Preparation) for nootropic activity

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Abstract

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system. Traditional Systems of Medicine possess a large number of nootropic agents (Moharrik-Hararat-e-Gharizia and Muqawwi-Aaza-e-Raisa) which have been in use for thousands of years. Despite many such drugs mentioned in classics of traditional medicine, until recently no Unani and only a few Ayurvedic drugs have been studied for nootropic activity. So, in the present study ‘Maa-uz-Zahab’ (gold solution) was subjected to scientific study for nootropic activities. Tests for Memory: Elevated Plus–Maze Test. The elevated plus-maze test study shows that the test drug improves ‘retrieval’ of memory. The study shows that the test drug possesses appreciable nootropic activity. The study scientifically substantiates the Unani usage of the test drug in disorders related to stress and memory-impairment.

Key words: Moharrik-Hararat-e-Gharizia, Nootropic, Muqawwi-Aaza-e-Raisa, Maa-uz-Zahab, Gold

Introduction

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capacity and memory (Jaisawal and Bhattacharya, 1992). Various neurodegenerative disorders including Alzheimer’s, Parkinson’s, and Huntington’s diseases are reported to be associated with dementia.

Despite the fact that the study of novel drugs is accepted as a high priority area of research on traditional drugs, until recently no Unani and only a few Ayurvedic drugs have been studied for nootropic activity.

A pioneering work was carried out in the Department of Ilmul-Advia, Ajmal Khan Tibbia College, Aligarh Muslim University, Aligarh in which a very well known Unani tonic preparation *Maa-uz-Zahab* (Gold solution) was selected for the present study (Ahmad, 1998). It is one of the many mineral origin drugs that is considered to possess nootropic activity (Azam Khan, 1902; Ibn Baitar, 1248; Ibn Sina, 1906; Nandkarni, 1976; Razi, 1968; Saeed, 1997; Shirazi, 1790). Earlier in the same institution some Unani simple and compound drugs were subjected to study only adaptogenic activity and shown to possess the effect with striking degree (Amin et al., 1995). The drugs of mineral origin have been generally neglected by modern scientific researchers, while the fact is that some of the most exciting therapeutic successes of Unani Medicine are achieved by such drugs.

Since the test drug is considered in Unani Medicine to improve memory (Azam Khan, 1902; Shirazi, 1790), it was studied for Nootropic activity by Elevated plus-maze test.

Materials and Methods

Preparation of Test Drug

*Maa-uz-Zahab* (MZ) was prepared according to standard pharmacopoeia viz. ‘Bayaz-e-Kabir’ (c,
Gold foil (\textit{Waraq-e-Tila}) 1 gm
Nitric acid (\textit{Tezab-e-Shora}) 2 ml
Hydrochloric acid (\textit{Tezab-e-Namak}) 6 ml
Distilled water 30 ml

\textit{Waraq-e-Tila} (Gold foil) was procured from Dawakhana Tibbiya College, Muslim University Aligarh and its identity was confirmed by chemical parameters. The two acids were mixed together to form aqua regia and kept in a clean bottle, then gold foil was added to it. When the latter was dissolved, a sufficient quantity of distilled water was added so that no harmful effect is produced. The solution was then filtered and kept in a bottle. The required quantity of the solution was taken out and suspended in distilled water just before the administration. This process yields gold chloride (Selwood, 1972).

The test drug 'Maa-uz-Zahab' was administered by the oral route as practiced by Unani physicians, in the dose corresponding to their Unani clinical dose by multiplying the latter with appropriate conversion factor. The test drug was accordingly studied in the dose of 5 mg/kg body weight.

Test for memory

Elevated plus-maze test: The test was carried out by the method of (Sharma and Kulkarni, 1992). Albino rats of either sex, weighing 150-200 gm were selected for the study and divided into 3 groups of 6 animals each. The animals in Group I and II were treated orally with the test drug in the dose of 5 mg/kg, daily for 7 days. The animals in Group III were administered distilled water in the same volume and served as control.

The plus-maze consisted of two opposite open arms 50x10 cm crossed with two closed arms of the same dimension with walls 40 cm high. The arms were connected with a central square 10x10 cm to give the apparatus a plus sign appearance. The maze was elevated 70 cm above the floor in dimly lit room.

The elevated plus-maze was employed for the measurement of Transfer Latency (TL) on the 7th day 30 min. after the drug/vehicle administration, the rats were placed individually at the end of one open arm facing away from the central platform and the time took to move from the open arm to either of the enclosed arms, was recorded.

TL was the time elapsed between the times animal was placed on the open arm, and the time when it fully entered (all four paws) in the enclosed arm.

On the first day rats were allowed to explore the plus-maze for 30 seconds after the measurement of TL. The rats were returned to their home cages after the first trial. Twenty four hours later, the animals were again placed individually on the elevated plus-maze after 45 min. of drug or distilled water administration and the TL was measured. The TL measured on first and second day served as parameter for acquisition and retrieval of memory, respectively. The result was analyzed statistically by Student’s ‘t’ test.

**Observation and Results**

In the control group, the TL1 and TL2 were found to be 86.5±7.29 and 72.16±5.88 sec. respectively. The TL2 was considerably lesser than TL1 in the same group, but the decrease was not significant statistically. In group 2 (animals treated with M.Z.) the TL1 and TL2 were found to be 76.16±9.13 and 60.00±5.65 sec. respectively. The TL2 was significantly lesser than TL1 in this group. The TL1 of group 2 was marginally lesser than the TL1 in the control group. The TL2 of group 2 was considerably lesser than the TL2 in the control group, but this decrease was not significant statistically (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>TL 1 in Sec (\text{Mean} \pm \text{S.E.})</th>
<th>TL 2 in Sec (\text{Mean} \pm \text{S.E.})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86.5±7.29</td>
<td>72.16±5.88</td>
</tr>
<tr>
<td>M.Z.</td>
<td>76.16±9.13*</td>
<td>60.00±5.65</td>
</tr>
</tbody>
</table>

\(n=6\) \(*=P<0.02\) (Between TL.1 and TL.2 in the same group)

**Discussion**

In the Elevated plus-maze test for nootropic activity, the TL2 of M.Z. treated animals was significantly lesser than TL1 in the same group while the TL2 of control animals was not significantly lesser than the control TL1, therefore, the study shows that the test drug improves ‘retrieval of memory’ (indicated by TL2).

However, since the test TL2 is not significantly lesser vis-a-vis control TL2, the test drugs cannot
be said to possess a very striking nootropic activity. Thus, the present study reveals that the test drug (M. Z.) possesses appreciable nootropic activity. The Study scientifically substantiates the Unani usage of the test drug in disorders related to stress and memory impairment.

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Factors favouring and disfavouring the popularity of Unani Medicine among patients and practitioners – a survey

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Abstract

Unani system is one of the oldest systems of medicine, with its genesis in the present day Greece. It has been well known and popular system of medicine with no reported side effects on the human body. It is also well known for the permanent cure of a number of diseases. But there are also certain drawbacks such as form of the medicine and method of preparation etc. This study plans to find out the grass route/core factors affecting the popularity of this system and also the factors impeding its growth. The study will help in identifying gaps existing in the creation of awareness of this system, and other factors as well as identifying probable solutions/suggestions from established existing practitioners. The proposed sample size is 250 (200 patients and 50 doctors). A questionnaire has been prepared separately for patients and doctors. The area chosen for research is Delhi. The doctors and patients will be selected randomly from hospitals, nursing homes, private clinics and government dispensaries where Unani practice is being carried out. The primary data is being collected through questionnaire and interviews with doctors and will be statistically analyzed and presented.

Key words: Unani Medicine, Popularity Survey, Relapse

Evolution of Unani Medicine in India

Pre independence

Although Unani Medicine originated in the present day Greece, it travelled through the Arab province before entering India. It is assumed that Arab traders who came to India introduced this form of medication. This system of medicine was patronised by the Delhi sultans like Khiljis, The Tughlaks and the Mughal emperors. The golden era of Unani Medicine in India was the 13th century and the 17th century. It gained popularity amongst the masses during their reign. The system soon spread all over the country. It was favoured by the masses and continued to hold an unchallenged sway for a long period. There was a setback to Unani Medicine during the British period due to the withdrawal of its patron government but because the system had good faiths among the masses it continued to flourish.

1351 AD was the era in which Unani Medicine was introduced in India by Arabs. Hakim Zia Mosood Rasheed Zangi was the first know hakim. The non toxicity and efficacy of unani drugs is the main reason because of which it got acceptance by masses. There are Eminent Scholars of that period who worked for Unani system of medicine and these are Akbar Arzani (d.1721 AD), Hakim M Shareef Khan (1725-1807), Hakim Ajmal Khan (1864-1927) and Hakim Kabeeruddin (April 1894-9th January 1976).

The first person who perceived and gave the research concept in Unani Medicine was Maseehul Mulk Hakeem Ajmal Khan in 1920. He belonged to
Sharifi family and founded the Ayurvedic and Unani Tibbiya College in Delhi in 1916.

The compound drugs which are used in the treatment of the disease are basically of two types. One is classical compound drugs and the second is the proprietary compound drugs. The classical compound drugs are used for the hundred and thousand years. On the other hand the proprietary are formulated by the individuals or institutions depending on their experience and research. Although Unani Medicine is one of the oldest system of medicine it is still practiced in India and is very popular in the other parts of the world.

However, very few objective studies have been conducted for assessing its popularity. So, the preset study was conducted to assess the popularity of Unani Medicine among patients and practitioners and the factors affecting it.

Research methodology

Objective of the study

a). To find out the problems associated with Unani system of medicine.
b). To know the views of Unani physicians and patients on Unani treatment.
c). To find out the ways by which Unani system of medicine can be improved.

Population defined

(i) Unani physicians as they have the best knowledge about the Unani drugs and had a good experience in this field.

(ii) Patients on Unani treatment because they are the real one who actually taking the Unani medicine and experiencing its effects. They are the best on for sharing their knowledge about the Unani medicine.

Area of research

Delhi and NCR

Sample size

Doctors = 57
Patients = 108

Research tools

Questionnaire, personal interviews and observations.

Data analysis

Doctors’ responses

Place where the doctor is carrying out the practice

How many patients are referred to you by other doctors

Total No. of Doctors Covered

No. of Doctors

Qualification of Doctors

Place of practice

No. of Doctors

No. of Patients

No. of Patients
What is the no. of patients who directly comes to you without taking any other alternative medicine in a week

For how long you are carrying out the practice of Unani Medicine

What is the average number of patients you diagnose in each of your OPD
What is the number of patients who come to you after taking alternative system of medicine in a week

In what stage of disease patients come to you for treatment

What are the adverse effects of Unani Medicine over the alternative system of medicine

What is the average cost of treatment by Unani Medicine in a week (in Rupees)

What advantages does the patient get in the Unani Medicine over other alternative system

How much relief does the patient get after taking Unani Medicine (In % age)
Are there any incidence in which patient leave the Unani Medicine in between the treatment

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>25</td>
<td>26</td>
</tr>
</tbody>
</table>

How you take any other alternative medicine before taking Unani Medicine

<table>
<thead>
<tr>
<th>Response</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>58</td>
<td>48</td>
</tr>
</tbody>
</table>

Patients Response

**Patients suffering from different disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal Disorder</td>
<td>25</td>
</tr>
<tr>
<td>Respiratory Disorder</td>
<td>18</td>
</tr>
<tr>
<td>Cardiovascular Disorder</td>
<td>14</td>
</tr>
<tr>
<td>Psychological Disorder</td>
<td>11</td>
</tr>
<tr>
<td>Gynaecological Disorder</td>
<td>4</td>
</tr>
<tr>
<td>Paediatric Disorder</td>
<td>3</td>
</tr>
<tr>
<td>Obstetric Disorder</td>
<td>1</td>
</tr>
<tr>
<td>Any other</td>
<td>1</td>
</tr>
</tbody>
</table>

How long you are suffering from this disease

<table>
<thead>
<tr>
<th>Time duration</th>
<th>1 week</th>
<th>2 week</th>
<th>3 week</th>
<th>4 week</th>
<th>above 4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Who told you about Unani Medicine

<table>
<thead>
<tr>
<th>Sources</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Patients</td>
<td>9</td>
</tr>
<tr>
<td>Other Doctors</td>
<td>13</td>
</tr>
<tr>
<td>Relatives</td>
<td>64</td>
</tr>
<tr>
<td>Media</td>
<td>0</td>
</tr>
<tr>
<td>Friends</td>
<td>12</td>
</tr>
<tr>
<td>Any other Source</td>
<td>10</td>
</tr>
</tbody>
</table>

How long have you been taking Unani Medicine

<table>
<thead>
<tr>
<th>Duration of Time</th>
<th>1 mth</th>
<th>2 mth</th>
<th>3 mth</th>
<th>4 mth</th>
<th>5 mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>75</td>
<td>60</td>
<td>45</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>
What are the adverse effects from which you suffer while taking Unani Medicine

<table>
<thead>
<tr>
<th>Adverse effects</th>
<th>No of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>96</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
</tr>
<tr>
<td>Rashes</td>
<td>0</td>
</tr>
<tr>
<td>Itching</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
</tr>
<tr>
<td>Any other</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

What is the cost of Unani Medicine as compared to other alternative systems

<table>
<thead>
<tr>
<th>Cost</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less</td>
<td>70</td>
</tr>
<tr>
<td>More</td>
<td>30</td>
</tr>
</tbody>
</table>

What advantages you get in Unani Medicine over other alternative system of medicine

<table>
<thead>
<tr>
<th>Advantage</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged relief</td>
<td>40</td>
</tr>
<tr>
<td>Low cost</td>
<td>35</td>
</tr>
<tr>
<td>Least side effects</td>
<td>20</td>
</tr>
<tr>
<td>Easy to administer</td>
<td>15</td>
</tr>
<tr>
<td>Good availability</td>
<td>10</td>
</tr>
<tr>
<td>Complete relief</td>
<td>1</td>
</tr>
</tbody>
</table>

Would you like to recommend Unani Medicine to your nearer and dearer for the treatment

<table>
<thead>
<tr>
<th>Response</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>20</td>
</tr>
<tr>
<td>Yes</td>
<td>100</td>
</tr>
</tbody>
</table>

Are Unani Drugs easily available

<table>
<thead>
<tr>
<th>Type of response</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>30</td>
</tr>
<tr>
<td>Yes</td>
<td>80</td>
</tr>
</tbody>
</table>

Findings

1. Most of the practitioners are BUMS and BUMS + MD.
2. Out of 57 practitioners 30 have an experience of less than 10 years.
3. The major advantage of unani system over other system is prolonged relief as both the doctors and patients gave similar response.
4. Unani drugs are not available in various dosage forms, which are required presently.
5. Most of the unani drugs contain sugar which cannot be prescribed to diabetic patients.
6. There is very less advertisement of Unani Medicines.
7. Emergency drugs are not available in Unani Medicine.
8. There are very few unani hospitals available.
9. There are no side effects but in very few cases GI toxicity was found.
10. GI and Sexual disorder patients are in majority.
11. Mostly the patients first go towards allopathic system of medicine and then come to Unani Medicine.
12. The result of unani treatment was found out to be very good.
13. The unani drugs are only available in the pharmacy of the hospitals where unani practice is carried out.

Recommendations

a) To increase the market of Unani Medicine, Sugar free Unani Drugs should be made available for diabetic patients as these patients might be added to this system.

b) For easy administration unani drugs should be made more in tablet and capsule forms. This will also help in better distribution and storage of the medicines, resulting in more reach. At present Unani Medicines are available at pharmacies associated with the hospitals with very few pharmacies elsewhere.

c) Emergency drugs should be made available in Unani System of Medicine, because there is a general perception amongst the patients that such treatment is not available. Therefore for emergencies patients take different system of medication and come to unani system for prolonged relief.

d) Advertisement of Unani System of Medicine should be done as most of the patients come through referrals (either of doctors or of patients). Those people who have not had prior exposure to this system might become aware of the usefulness and efficacy of Unani Medicines.

e) Research in Unani medicine should also be carried out. Most of the practitioners felt that the traditional system is prevalent and contemporary medicines to current diseases should be developed.

Results

The Unani Medicine was found out to be a good system of medicine. From the patients point of view Unani Medicine is better than other alternative system of medicine because it has no or very less side effects. Treatment with Unani Medicine will lead to almost complete relief to the patient. The one major problem in Unani Medicine found out is that the duration of treatment is very long because of which patient leave the treatment in between. And from doctors point of view Unani Medicine is done through natural ingredients because of which it is effective and has very few or no side effects. One of the major problems that were pointed out by unani physicians is that these unani drug preparations contains sugar and because of which these cannot be prescribed to patients who are suffering from diabetes also. So they said that the research should be carried out to find an alternative solution for this problem. They said that more of the unani preparation should be available in sugar free form for diabetic patients. The unani practitioner also said that most of the Unani Medicines are available in semisolid preparation so work should be carried out to make the unani drugs available in tabled and capsule form as well. The overall response from the doctors as well as from the patients under unani treatment is good for unani drugs. But in some areas this need to be improved to make it better and effective. The advertisement is also very less for Unani Medicine. The advertisement plays a very important role in making people aware about anything. There is general perception that this system of medicine is associated with a particular community. This myth should be dispelled through general promotions and organization of camps pan India. So advertisement should also be carried out for unani drugs and unani treatment. Finally we can say that the treatment through Unani Medicine is better than the alternative system of medicine on the basis of this research. But improvement is required in few areas so as to make it more popular and acceptable by the patients.

Conclusion

A market survey of unani physicians and patients was carried out to know the current scenario of Unani system of medicine. The basic aim behind this study was to know where Unani Medicine stands among the other alternative system of medicine and also to find out what are the problems associated with Unani Medicine and how these problems can be overcome. In this market research 57 unani physicians and 108 patients were covered who were on unani treatment. The research was carried out in
various unani hospitals, private clinics and dispensaries of Delhi. Doctors which are covered under the study are BUMS (Bachelor of Unani Medicine), BIMS (Bachelor of Medicine), BUMS+MD and BUMS+MS. Among these maximum physicians were carrying out there practice from the last 10 years. In each of their OPD they see around 20 patients but some physicians also see more than 40 patients in each of their OPD. Maximum numbers of patients that come for treatment are suffering from sexual disorders and gastrointestinal disorders and are in chronic condition. Most of the patients first go for alternative treatment and when they didn’t get the relief then they come for unani treatment. Most of the patients who come for unani treatment were suffering from last 4 week with the disease. The reason behind this is that they prefer other system of medicine. It was also found that there are no side effects in Unani Medicine but in some cases nausea and vomiting was there because of high doses of the drug. The average cost of treatment by Unani Medicine comes out to be around rupees 200 to 300 per week which is very less as compared to alternative system of medicine. Patients get 51% to 75% relief after taking Unani Medicine. Some patients also leave the treatment in between because of long treatment in case of unani system. Maximum number of patients gets to know about Unani Medicine from their relatives. Prolonged relief is the main advantage that patient gets in unani system of treatment. Patients who are hospitalized in government hospitals get the unani drugs easily from the hospital unani pharmacy but those who are treated in private clinics and private hospitals faces difficulty to find out the unani drugs. Out of 108 patients 104 patients said that yes they will recommend Unani Medicine to others.

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Physico-chemical and phytochemical study of *Ruta graveolens* Linn. seeds

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Email: ghufran97@yahoo.co.in

Abstract

*Ruta graveolens* Linn. belongs to the family Rutaceae and commonly known as garden rue. In India, it is commonly called as sudab or sadab. The seeds of the *Ruta graveolens* were subjected to the physicochemical, phytochemical and HPLC studies for purity. The result showed that seeds have highest extractive value in hydro-alcoholic extract (3.40±0.61) followed by aqueous (2.37±0.33) and alcoholic extract (2.25±0.53). The moisture content estimated by dry oven method and Toluene distillation method was found as 7.87% and 11%, respectively. The pH was determined in 1% and 10% aqueous solution at 35°C of temperature and found to be 5.60 and 5.40, respectively. The fixed oil was present and the volatile content was <1%. Phytochemical screening revealed the presence of alkaloids, saponins, resins, sugars and flavonoids. HPLC study shows the presence of 3 and 7 major chemical constituents in isocratic as well as gradient elution series, respectively, of the seed extract.

Key words: Sudab, HPLC, Physico-chemical parameters, Phytochemistry, *Ruta graveolens*

Introduction

The family Rutaceae consists of extremely wide variety of aromatic plants, mainly in tropical regions. Among them the genus Ruta is very rich (Fredj *et al.*, 2007). Now the plants are cultivated in many parts of the world. It is considered as indigenous in South Europe and North Africa and grows on waste stony ground (Bently and Trimen, 2004; Dymock *et al.*, 2005; Anonymous, 2004).

The plant is commonly cultivated in India and called as sudab or sadab (Bently and Trimen, 2004; Anonymous, 2004; Kirtikar and Basu, 2003). Two species of Ruta (genus) are reported to grow in India, among them *Ruta graveolens* (garden rue) is well known for its aromatic and medicinal uses (Anonymous, 2004). In traditional systems of medicine its leaves and stems are commonly used as stimulant, emmenagogue, diuretic, abortifacient, resolvent etc. in a number of diseases (Anonymous, 2004; Kirtikar and Basu, 2003; Ghani, y.n.m; Ibn Baitar, 1999; Nadkarni, 2005; Kabiruddin, 2007; Chopra *et al.*, 2002). However its seeds in Unani literature, have been described to be Mulattif (Demulcent), Mufatteh (Deobstruent) and Muhallil (Resolvent), useful in the management of hyperlipidemia, obesity and to resolve the atheroma plaque, making the therapeutic potential of the plant wide and diverse (Razi d. 860 CE). The seeds of *R. graveolens* Linn. are ovoid, rounded on the back, flattish in front, angular, testa blackish, rough; embryo slightly curved from the base to the apex and is surrounded by scanty fleshy endosperm (Kirtikar and Basu, 2003; Nadkarni, 2005). Almost in all traditional medicine, the medicinal plants play an important role and constitute the backbone for the same. In order to make sure the safe use of these drug plants, an important step is to establish their standards of identity, quality, safety and efficacy to check adulteration. The standardization of stem and leaf of *R. graveolens* has been done earlier (Nazish *et al.*, 2009) but the authors have left the
standardization of the seed. Therefore, the seeds of *R. graveolens* have been taken into consideration to establish its physicochemical and phytochemical standards and HPLC finger printing.

**Materials and Methods**

**Drug identification**

The seeds of *Ruta graveolens* were procured from Gandhi Krishi Vigayan Kendrya (G.K.V.K, Bangalore) - a Govt. of India Institution known to grow and supply authentic drug samples (under Receipt No. 13134). The seeds were further identified by Dr. Shiddamallayya N. (Botanist at Regional Research Institute (RRI) of Ayurveda, Bangalore). The identification certificate of the seeds was issued by the said Botanist, under Reference No.-RRI/BNG/SMP/Drug authentication/2009-10/337(a). The specimen of the plant material was retained in the RRI for reference purpose. Same specimen of the plant material has been submitted in the NIUM herbarium library for record and future reference.

**Preparation of extract**

The seeds of *Ruta graveolens* were shade-dried and coarsely powdered by using electrical grinder. The coarse powder was subjected to the successive extraction in different solvents using soxhlet apparatus in the series of petroleum ether, diethyl ether, chloroform, benzene, methanol and distilled water. Another extract of hydro-alcohol was made in the same manner. The extraction was carried out for 6 hours in each solvent on a water bath, but the extraction of alcohol and distilled water was done by direct heating. After cooling, the extract was filtered by Whatmann paper no 41 and the filtrates were dried by evaporating the solvent in previously weighed petridishes.

**Preliminary phytochemical studies**

The different extracts were subjected to different phytochemical screening for detection of various chemical constituents viz. Phenols, tannins, sterols/terpenes, alkaloids, flavonoids, sugar, saponins and resins by the methods as mentioned by Evans et al., (2002) and Kokate (2007).

**Physicochemical parameters**

The physicochemical parameters of the powder of the seeds of *Ruta graveolens* viz. Total ash, water soluble ash, acid insoluble ash, pH, volatile oil content, moisture content, presence of fixed oil, and specific gravity were determined (Anonymous, 1968; Jenkins et al., 1967; Peach et al., 1955; Bhattacharjee et al., 1969; Khandelwal, 2008).

**HPLC Studies**

The hydro-alcoholic extract of the seeds of *Ruta graveolens* was prepared and subjected to the HPLC finger printing in both types of series i.e. isocratic elution and gradient elution of HPLC finger printing.

**Results and Discussion**

**Physicochemical studies**

The successive extractive values, total ash, acid insoluble ash and water soluble ash are given in the Table 1. The results showed that the highest extractive value was in hydro-alcoholic solvent (3.40±0.61) followed by aqueous (2.37±0.33) and alcoholic solvents (2.25±0.53). The moisture content estimated by dry oven and Toluene distillation method showed 7.87% and 11%, respectively. The volatile content was found to be <1%, while the presence of fixed oil was observed. The pH was determined in 1% and 10% aqueous solution at 35°C of temperature and found to be 5.60 and 5.40, respectively. The values are given in Table 2.

**Table 1**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Organic solvent</th>
<th>Mean %age of extracts</th>
<th>Colour of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>0.93±0.35</td>
<td>Reddish Brown</td>
</tr>
<tr>
<td>2</td>
<td>Ether</td>
<td>1.3±0.020</td>
<td>Reddish Brown</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>0.98±0.007</td>
<td>Reddish Brown</td>
</tr>
<tr>
<td>4</td>
<td>Benzene</td>
<td>0.21±0.01</td>
<td>Reddish Brown</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol (methanol)</td>
<td>2.25±0.53</td>
<td>Reddish Brown</td>
</tr>
<tr>
<td>6</td>
<td>Aquous</td>
<td>2.37±0.33</td>
<td>Black</td>
</tr>
<tr>
<td>7</td>
<td>Hydro alcoholic extract</td>
<td>3.40± 0.51</td>
<td>Reddish brown</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Ash values</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>8.75%</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.50%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.53%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>11%</td>
</tr>
<tr>
<td>Volatile content</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.015</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Present</td>
</tr>
<tr>
<td>pH Values</td>
<td></td>
</tr>
<tr>
<td>1% solution</td>
<td>5.60</td>
</tr>
<tr>
<td>10% solution</td>
<td>5.40</td>
</tr>
<tr>
<td>Loss on drying at 105°C</td>
<td>7.87%</td>
</tr>
</tbody>
</table>
Table 3
Phytochemical screening of different extracts of seeds of *Ruta graveolens*

<table>
<thead>
<tr>
<th>Chemical Test</th>
<th>Aqueous extract</th>
<th>Benzen extract</th>
<th>Petroleum ether extract</th>
<th>Ether extract</th>
<th>Methanol extract</th>
<th>Chloroform extract</th>
<th>Hydro Alcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragendorff’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Mayer’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hager’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Wagner’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2. Glycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fehling test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molish’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molish’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
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<tr>
<td>Fehling test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Reduced sugar test</td>
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<td>4. Steroids/Terpenes</td>
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<td>Lieberman burchard</td>
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<td>Molechott’s reaction</td>
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<td>5. Phenolic compound and Tannins</td>
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<td>Shinoda test</td>
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Phytochemical screening

Petroleum ether extract was tested for the presence of free phenols, alkaloids, sterols/terpenes by dilute HNO₃ and lead acetate test. There was no change in the colour, suggesting the absence of phenols and tannins. The presence of phytosterols and terpenes were detected by Leiberman Burchad reaction, Moleschott’s reaction test and Hosse’s reaction test. All three tests gave negative results indicating the absence of phytosterols and terpenes. For the presence of alkaloids, the Dragendorff’s test, Mayer’s test, Wagner’s and Hager’s test were done. In case of Mayer’s reagent test, white precipitate was observed while in case of Dragendorff’s reagent test, appearance of brown precipitate confirmed the presence of alkaloids; in Hager’s test and Wagner’s test, yellow precipitate and reddish brown precipitate were observed. The aqueous extract and methanolic extract were tested for alkaloids, flavonoids, saponins, carbohydrates, proteins, amino acids and tannins. Flavonoids were detected by lead acetate test. Appearance of yellow colour indicated the presence of flavonoids. It was again confirmed by Magnesium hydrochloric acid test. The presence of saponin was confirmed by Honey comb frothing test and Haemolysis test. There was froth of about 1 cm, indicating the presence of saponins. The presence of haemolysis also confirms the saponins. The presence of sugar was detected by the Molish’s, Fehling’s and Benedict's tests; which indicated the presence of carbohydrate in the extract. Ether soluble portion was tested for alkaloids and sterols / terpenes. Ethanolic extract was tested for glycosides. First, the extract was fermented with baker’s yeast and after hydrolysis, Fehling solution test and Molish’s test were performed, the presence of glycoside was indicated in the extract. The chloroform soluble portion was tested for alkaloids and sterols / terpenes. Benzene soluble portion was tested for fixed oil. Hydro alcoholic extract was tested for alkaloids, flavonoids, saponins, carbohydrate, protein, amino acids and tannins. The phytochemical screening is shown in Table 3.

HPLC

Hydro-alcoholic extract of test drug was subjected to HPLC for the isolation, calculation and identification of various substances present in the material. Both types of methods viz. isocratic elution and gradient elution were done to measure the various substances which may be used as markers for the quality evaluation and standardization of the test drug. The results obtained were found suggestive of the presence of 39 peaks (chemical constituents) in isocratic elution and 70 peaks (chemical constituents) in gradient elution of test drug. However, there were 3 and 7 large peaks in isocratic and gradient elution respectively, suggesting the major constituents of the test drug.

The HPLC finger print data of hydro alcoholic extract is shown in Fig. 1, 2 and 3.

Fig. 1 HPLC Isocratic Elusion
Conclusion

In the light of the above results, it can be concluded that the findings of physicochemical and phytochemical studies, especially that of HPLC finger printing may be helpful in the identification and purity determination of *R. graveolens* and the consequent comparability of samples will help in increased reproducibility of the biological results.

References


Identification and standardization of a pharmacopoeal Unani formulation: estimation of marker compounds

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Abstract

Unani classical literature mostly contains more or less fractional description of drugs based on organoleptic characters only. Now Unani Medicine is adopting comprehensive scientific methods for identification and standardization of single drugs as well as polypharmaceutical preparations. In this regard estimation of marker compounds in a Unani pharmacopoeal compound formulation containing *Zingiber officinale* (Ginger), *Colchicum luteum* (Colchicum), and *Aloe vera* (Aloe) was done. This study includes total alkaloidal estimation, colchicine estimation, identification of colchicine by HPLC method and volatile oil estimation. The mean concentration level of total alkaloids, colchicine and volatile oil was found to be 0.45±0.09%, 0.38±0.04% and 0.62±0.07%, respectively. The last two values are within the range mentioned for *C. luteum* and *Z. officinale*, respectively. The retention time of colchicines was found at 2.8 min. The present study added a new precise parameter for identification and standardization of the test pharmacopoeal Unani preparation viz. HPLC peak of colchicine.

Key words: *Zingiber officinale*, *Colchicum luteum*, *Aloe vera*, Colchicine

Introduction

The drugs of Unani Medicine are derived from natural sources, where plants form the dominant component over other natural resources. Now Unani Medicine is adopting comprehensive scientific methods for identification and standardization of single drugs as well as polypharmaceutical preparations. Therefore, in the present study estimation of marker compounds of an Anti-arthritic Unani formulation mentioned in Unani Pharmacopoeia, ‘Ilaj ul Amraz’ (Sharif Khan, 1870), was conducted. This formulation is in powder form and contains (a) Ginger (*Zingiber officinale* Linn.—Dried Rhizome- 3.5 g) (b) Colchicum (*Colchicum luteum* Baker—Dried Corm- 3.5 g) (c) Aloe (*Aloe vera* Linn.—Dried Exudate- 7.0 g). It is modified for use in the form of Tablet (Qurs) and additionally Gum Acacia (S.d. Fine Chemical Ltd.) was used as excipient.

*Z. officinale* (Zanjabeel) is an underground stem or rhizome of the plant, belonging to family Zingiberaceae (Chopra et al., 1958). The chemical composition of dried ginger is: starch 40-60%, proteins 10%, fats 10%, fibres 5%, inorganic material 6%, residual moisture 10% and essential oil (oleoresin) 1-4 per cent. The essential oil of ginger contains various terpins and sesquiterpenes. The predominant sesquiterpene hydrocarbon is zingiberene. The characteristic pungent odour is due to its oleoresin content which is an oily liquid containing oxymethyl phenol like shogaol, zingerone and gingerol etc. In all more than 200 different volatile substances have been characterised in the essential oil fraction (Verma and Bordia, 2001).

*C. luteum* (Suranjan Talkh) also known as Indian Colchicum belongs to the family Liliaceae. It is an annual herb, dark brown in colour on drying (Chopra et al., 1958). It is a root (corm) of small size plant (herb). The whole corms are 2.5-5 cm long and 1.5-2.5 cm broad. They are translucent or opaque and gibbously ovoid with tapering apex and prominent longitudinal groove on one side.
Corms are odorless and have a bitter and acrid taste (Anonymous, 1987). Chemical analysis shows that *C. luteum* contains a large amount of starch, a small quantity of oily resinous matter and a bitter alkaloid. Following the assay methods the percentage of the alkaloid in the *C. luteum* (rhizome) was found to be from 0.21 to 0.25 and in the seeds from 0.41 to 0.43 % (Nadkarni, 2000). From Colchicum, thirty one different alkaloids have been isolated. Colchicine is the main alkaloid isolated from all species of the genera, Colchicum. The major phenolic compounds obtained from the genus, Colchicum, are 4-hydroxy-3-methoxybenzaldehyde (vanillin) 4-hydroxybenzoic acid (vanillic acid), 3-(3-hydroxyphenyl)-2-propanoic acid (coumaric acid), 3-(3,4-dihydroxyphenyl)-2-propanoic acid (caffeic acid), and 3, 4, 5, 7-tetrahydroxylflavone (luteolin) (Ahmad, 2010).

*A. vera* (Sibr or Elwa) is one of the earliest known purgative used in Unani system of medicine belongs to the Family Liliaceae or Asphodelaceae. Botanically it is the dried leaf juice of the plant. The plant is a coarse looking perennial with short, thick, somewhat divided stem, 30-60cm high. The leaves are glucose green, sessile crowded, lanceolate, erect, spreading, rather concave, spiny toothed at the margin and full of juice (Anonymous, 1968). *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. Recently, a glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from *Aloe vera* gel. It provides 12 anthraquinones, which are phenolic compounds traditionally considered as laxatives. Aloin and emodin act as analgesics, antibacterials and antivirals. It provides 20 of the 22 human required amino acids and 7 of the 8 essential amino acids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin (Surjushe et al., 2008).

**Materials and Methods**

**Collection of plant material**

The raw materials were purchased from the local market of Aligarh and the sample were authenticated in Pharmacognosy section of the Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh and Elwa and Zanjabeel Khushk were found within range of standards as mentioned in the Unani Pharmacopoeia of India (Anonymous, 2007) and Surjanan was found as mentioned in Standardization of single drugs of unani medicine (Anonymous, 1987).

**Preparation of formulation**

*Z. officinale* and *C. luteum* were powdered in an electric grinder and *A. vera* was made Mushawwa (broiled) by keeping in an Apple, which was covered by the process of Kaprauti, and heated in an oven, till it was brown. Then Elwa (A. vera) was taken out of apple and dried and then used. All the three ingredients were mixed together (before mixing, *A. vera* was dissolved in distilled water as required) in order to make Lubdi (dough). The mixture was dried in shade and then powdered in a mortar. This powder was mixed with a suitable inert substance viz. powder of Gum Acacia (Sd. Fine Chemical Ltd.) as excipient. The material in the requisite degree of fineness was mixed and moistened with a moistening agent (distilled water). The moistened material was made into granules by passing through a sieve. The granules were dried in shade and again passed through a sieve. Tablets of 500 mg were made by Automatic tablet making machine in Dawakhana Tibbiya College, AMU, Aligarh (Anonymous, 1968).

**Total alkaloidal estimation**

10 gm of powdered drug was extracted in a Soxhlet’s apparatus with chloroform and little ammonia. After extraction the solvents were evaporated and in the extract 100 ml of distilled water was added. Later on, the extract was acidified (02 pH) with dilute hydrochloric acid for the conversion of alkaloid in to salt. The chloroform soluble portion was separated with the help of separating funnel. The water portion was neutralized with ammonium hydroxide to release the alkaloid and this fraction was again extracted with chloroform to obtain the free alkaloids. The chloroform was evaporated and the content was weighed, the alkaloid percentage was calculated with reference to the drug taken (Paech and Tracey, 1955).

**Colchicine estimation**

3 gm of powdered drug was extracted twice with 150 ml of petroleum ether with frequent shaking for 1 h, followed each time by filtration. The solid residues were air dried and then extracted with 60 ml of dichloromethane at room temperature for 30 min with frequent shaking. Then 10% solution of ammonia (3 ml) was added to the mixture with...
vigorously shaking for 10 min; the mixture was left undisturbed for 30 min and then filtered. The residue was washed twice with 60 ml of dichloromethane and then combined with the filtrate. The organic phase was evaporated to dryness and then dissolved in 6 ml of 70% ethanol to yield the test sample, which was ensured in samples by comparison with the standard containing 10 mg/ml colchicine as control (Bharathi et al., 2006).

High Performance Liquid Chromatography (HPLC)

Identification of colchicine was done by comparing the retention time of the sample with that of the standard obtained from Otto, Mumbai. A Cyber Lab’s HPLC system equipped with a single pump and porous silica with 5 µm diameter C18 4.6 × 250 mm column was used for separation. The mobile phase consisted of acetonitrile: 3% acetic acid (60:40), at a flow rate of 1 ml/min. The peaks eluted were detected at 245 nm and identified with authentic standards. The HPLC method was used to estimate the colchicine content in the Unani Formulation (Bharathi et al., 2006).

Volatile oil estimation

The Clavenger’s Apparatus is most common for determination of Volatile oil (V.oil) percentage. In this equipment, the quantity of oil obtained, can be determined directly in the receiver of apparatus (Afaq et al., 1994). A suitable quantity of the coarsely powdered drug together with 75ml of glycerin and 175ml of water in 1 litre distilling flask, and a few pieces of porous earthen ware also put in the distilling flask and then the condenser is attached. The content of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate which keeps the lower end of condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides. At the end of specified time (3-4 hrs) heating is discontinued, the apparatus is allowed to cool for 10 min. and the tap is opened and the tube lowered slowly; as soon as the layer of the oil completely enters in to the graduated part of the receiver, the tap is closed and the volume is read. The tube is then raised till the level of water in it is above the level of joint when the tap is slowly opened to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. The measured yield of volatile oil is taken to be the content of V. oil in the drug (Lohar et al., 2008).

Results

Alkaloid estimation

The mean concentration level of total Alkaloids was determined in the formulation and it was found to be 0.45±0.09%, the results are presented in Table 1.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Alkaloid (%)</th>
<th>Colchicine (%)</th>
<th>Volatile oil (%)</th>
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<td>1.</td>
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<td>0.75</td>
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<tr>
<td>2.</td>
<td>0.31</td>
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<td>0.80</td>
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<td>3.</td>
<td>0.44</td>
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<tr>
<td>Mean±SE</td>
<td>0.45±0.09</td>
<td>0.38±0.04</td>
<td>0.62±0.07</td>
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Colchicine estimation

The mean concentration level of Colchicine in the formulation was found as 0.38±0.04%, the results are shown in Table 1.

High Performance Liquid Chromatography (HPLC)

Colchicine was identified in the Unani Formulation and compared with the standard. Colchicine was eluted at 2.8 min. The chromatogram is depicted in Fig 1 (A, B and C).

Volatile oil estimation

The mean percentage of Volatile Oil in the formulation was found to be 0.62±0.07%, the results are given in Table 1.

Discussion

An impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Doughari, et al., 2008). Traditional medical traditions can offer a more holistic approach to drug design and myriad possible targets for scientific analysis. For traditional medicines, newer guidelines of identification, standardization, manufacture and quality control will be required (Patwardhan, 2009).

For the purpose of standardization and efficacy of a Unani pharmacopoeal compound formulation having Z. officinale, C. luteum and A. vera, the estimation of marker compounds viz. Isolation and estimation of Total Alkaloids, Colchicine and Volatile Oil were done. All the
three contents of this formulation, Ginger, Colchicum and Aloe contain Alkaloids. For instance *C. luteum* has an alkaloid named as Colchicine which is a vital compound of *C. luteum*. *Z. officinale* also has alkaloids but in traces and *A. vera* also has a glycosidal alkaloid named as Aloin. Consequently, estimation of total alkaloid in a formulation which is constituted of such single drugs which contain alkaloid in them is very basic for standardization of that formulation and it was found to be $0.45 \pm 0.09\%$.

The test formulation contains *C. luteum* as an
important ingredient which contains the alkaloid Colchicine. It is a marker and important compound of C. luteum. Colchicine is useful in the treatment of gout and acts against the inflammatory response (Rang et al., 2008). It inhibits the deposition of uric acid (urate) crystals and decreases serum uric acid (Anonymous, 2001). It produces a significant inhibition of joint swelling in both, formaldehyde and Freund's adjuvant induced arthritis. Serum TNF-α level is also reduced significantly by it (Nair et al., 2011). These properties make colchicine a very significant compound. So, its identification, isolation and estimation are necessary for assessing the efficacy and authenticity of this Unani formulation. Thus, the mean concentration level of Colchicine in the formulation was estimated and found as 0.38±0.04%. This value was found within prescribed range (Rastogi and Mahrotra, 1999).

Since, colchicine is of prime importance, therefore, it was considered useful to confirm the identity of colchicine extracted from the test formulation by HPLC by comparison with standard agent. The study showed that the peak obtained was identical with the peak obtained with standard colchicine (Fig. 1-A, B, C). The peaks of colchicine in both the samples were eluted at 2.8 min. indicating authenticity of Unani formulation.

Volatile oil estimation was also done in the Unani formulation for standardization, because, of the presence of Z. officinale. It is the only constituent of the formulation which contain V. oil. Estimation of V. oil is also an important parameter for standardization of test drug and it was found to be 0.62±0.07%. This value was found within range as described by Verma and Bordia, (2001). The parameters applied for standardization of lab samples of the Unani Formulation may be taken as standard parameters for future reference. The Formulation may be studied for possible synergistic interactions and / or chemical changes occurring due to ingredient interaction and the compounding process.

References
A Pharmacokinetic approach to standardize Tukhm-e-Katan (Linum usitatissimum) seeds as a bioavailable source of β-sitosterol using High Performance Thin Layer Chromatography (HPTLC)

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Abstract

β-sitosterol is one of the key phytoconstituent of seeds of Tukhm-e-katan (Linum usitatissimum Linn.) a Unani drug which has therapeutic action in female reproductive disorders. β-sitosterol in pure form has low bioavailability. Therefore, bioavailability and pharmacokinetics of β-sitosterol from seeds of Tukhm-e-katan was evaluated using High Performance Thin Layer Chromatography (HPTLC). The study was conducted on Male albino rabbits of New Zealand strain. Blood samples were collected at different time intervals following a single oral administration of refluxed residue of Tukhm-e-katan in olive oil (1g Kg⁻¹). Absorption and elimination of β-sitosterol after administration of Tukhm-e-katan refluxed residue was monitored using change in the concentration of the marker band in the HPTLC profile. A marker from Tukhm-e-katan at Rf = 0.48 was detected in rabbit plasma after half an hour of ingestion of the plant refluxed extract. The marker reached maximum concentration at 6 hrs post dose and was not detectable in plasma after 8hrs post dose. The results of this study can form baseline for making polyherbal formulation containing Tukhm-e-katan with defined bioavailability of β-sitosterol leading to a possible extrapolation to humans.

Key words: β-sitosterol, Linum usitatissimum, Bioavailability, Tukhm-e-katan

Introduction

The drugs of the Unani system of medicine are derived from the plant, animal and mineral sources and these drugs are administered to the patients in various forms. In order to prevent further deterioration of the authenticity and quality of Unani drugs, there is need for standardisation of the Unani drugs. Linseed (Linum usitatissimum L.) or Tukham-e-katan is an annual crop growing worldwide either for the oil extracted from the seed or fiber from the stem (Fig. 1 and 3). In Unani medicine Tukham-e-katan is reported to be useful for various human ailments (Anonymous, 1983; Afaq, 1984). The meal that remains after oil is extracted from the seed is fed to animal as a protein supplement. Tukham-e-katan meal is 35-40% protein and together with cottonseed and sunflower supplies about 23% of the world’s oilcake/meal. Though, it is a small plant of 60-80cm but all of its parts are being used for various human diseases. It is the phytoconstituent present in the Tukham-e-katan which imparts bioactivity and is responsible for bio-potency of it. Tukham-e-katan contain β-sitosterol, ferulic acid, p-coumaric acid (Strandas, 2008). Phytoesters are present as important class of chemical compound in Tukham-e-katan (Wahid, 2009). Phytoesters are known to have a wide range of biological activities and physical properties. Therefore, the development of food technology has created some foods enriched with phytoesters. For evaluation of their natural levels, reliable data on plant sterol concentrations in various plant seeds is needed. The most common phytoester present in katan in large concentration is β-sitosterol (Fig. 2).

β-sitosterol have pharmacological activities which are potentially useful to man (Borgstrom, 1968; Gould, 1955). β-sitosterol is pharmacologically
active marker which have proven to be potent against hyperlipidemia, breast cancer, and gynecological disorders. Numerous international journals have published scientific studies that prove \( \beta \)-sitosterol is an extremely effective, natural treatment for an enlarged prostate.

**Owing to the significant nutritional, medicinal and commercial value of the Tukham-e-katan and considering the importance of \( \beta \)-sitosterol, the objective of the present work is to develop a simple, rapid and effective quantitative method for the analysis of \( \beta \)-sitosterol in Tukham-e-katan using High Performance Thin Layer Chromatography. In humans, the only source of \( \beta \)-sitosterol is the diet. It is very necessary to check absorption-elimination pattern of \( \beta \)-sitosterol from Tukham-e-katan. Thus in the present work pharmacokinetics of Tukham-e-katan with reference to \( \beta \)-sitosterol was evaluated.**

### Materials and Methods

#### Plant material

Seeds of *Linum usitatissimum* (Tukham-e-katan) were procured from Mumbai, Maharashtra, India. The samples were authenticated by National Institute of Science Communication and Information Resources (Auth No. NISCAIR/seeds 1133/165) and voucher specimens were deposited in our Herbal Research Laboratory. The samples were stored at 25°C in air-tight containers and powdered to 85 mesh when required.

#### Chemicals

All the chemicals used in the experiments were of analytical grade. Standard \( \beta \)-sitosterol (95% purity) was procured from Sigma Aldrich Chemie (Steinheim, Germany).

#### Chromatographic system

Camag HPTLC system with Cats 3 Version Software was used for the analysis. Camag Linomat IV was used as spotter and Camag Scanner II with mercury lamp was used for scanning. Chromatography was performed on aluminium backed silica gel 60 F\textsubscript{254} HPTLC pre-coated plates. Before use, the plates were pre washed with methanol and dried at 110°C for 10min. Samples (10 µL) were spotted using Camag Linomat IV sample applicator and the plates were developed to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated with the mobile phase; toluene-ethyl acetate-methanol-glacial acetic acid, 8+1+0.5+0.3 (v/v/v/v). The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After derivitization in 10% methanolic sulphuric acid reagents, chromatograms were evaluated densitometrically at \( \lambda = 366 \)nm by means of a Camag Scanner II in fluorescence/reflectance mode using mercury lamp in conjunction with Cats 3 Version Software.
Preparation of Tukham-e-katan refluxed residue (KRR)

55g of dried powder of Tukham-e-katan was extracted with 550 ml methanol by refluxing for 3 h at 55°C, and filtered through Whatmann filter paper No. 41 (E. Merck, India) and the filtrate was evaporated to dryness on a rota evaporator at 65°C. The procedure was subjected to two cycles to obtain 11.27g of residue after evaporation. It was filtered through Whatman filter paper No. 1.

Preparation of Standards and quality control samples

A stock solution of β-sitosterol (1000 ppm) was prepared in methanol. Eight calibrators of β-sitosterol were prepared by dilutions of stock solutions followed by spiking drug-free plasma, three replicates prepared to calibrators of β-sitosterol for each concentration. The calibration range was 10 -100 µg /ml of β-sitosterol. Quality control samples were prepared at low (15 µg /ml), medium (30 µg /ml) and high (90 µg /ml) concentrations for β-sitosterol. The calibration range was 0.035 -0.35 µg /ml of plasma. Quality control samples were prepared at low (0.050 µg /ml), medium (0.15 µg /ml) and high (0.30 µg /ml) concentrations of plasma.

Safety Evaluation

The study is carried out to assess the acute toxicity of KRR using Albino Swiss mice as the experimental model. The study is conducted as per the methodology laid down in the OECD guideline 425. The protocol and dosage chart is given in Table 1 and 2.

Pharmacokinetic study

Male albino rabbits of New Zealand strain were starved for 18hr before administration of KRR. A blank sample (0h) of blood (2ml) was collected from the marginal ear vein before dosing and then the rabbit was fed orally with 1 g/Kg body weight of KRR suspended in 10ml Olive oil using a number 10 gavage needle. After administration of the suspension, blood samples at 0.5h, 1h, 2h, 4h, 6h, 8h, 10h, 12h and 24h were collected in heparinized eppendorf tubes.

Plasma sample preparation

0.5ml of blank rabbit plasma was transferred to clean and dry stopper test tubes. Each tube was spiked with the above concentrations of β-sitosterol. 5ml of Chloroform was added to each test tube and the tubes were shaken well for 10min and then kept undisturbed for 30min. The organic layer was isolated carefully and transferred to evaporating tubes. The tubes were placed in water bath at 50°C and the residue obtained after evaporation was reconstituted in 200µl of chloroform. Each of these solutions (10µl) was applied to the plate, the plate was
developed, and the detector response for the different concentrations was measured.

**Results and Discussion**

**Toxicity study of KRR**

KRR was administered orally as per the dosage chart (Table 1). The animals were observed for 14 days. Daily body weight, food and water intake were recorded. A cage side observation of the study is mentioned in the Table 3. The animal treated with the KRR did not show any major change in the body weight as compared with the control i.e. those treated with olive oil. Animal treated with KRR and olive oil in the respective groups had normal food and water consumption throughout the study period. No mortality was recorded in animals treated with KRR and olive oil. It was seen that there was no significant change in body weight (Table 4), food intake (Table 5), and water intake (Table 6) of the animal when compared with the animal of control groups. No mortality was recorded too. Thus, KRR is not toxic at 2g/kg dose. Data obtained from toxicity study formed the basis for pharmacokinetic study.

### Table 3

Cage side observations for acute toxicity study

<table>
<thead>
<tr>
<th>Parameters Observed</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition of the fur</td>
<td>Normal</td>
</tr>
<tr>
<td>Skin</td>
<td>Normal</td>
</tr>
<tr>
<td>Subcutaneous slug</td>
<td>Nil</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>Nil</td>
</tr>
<tr>
<td>Dullness of eyes</td>
<td>Nil</td>
</tr>
<tr>
<td>Opacity of the eyes</td>
<td>Nil</td>
</tr>
<tr>
<td>Discharge from the eyes</td>
<td>Nil</td>
</tr>
<tr>
<td>Ptosis of the eyes</td>
<td>Nil</td>
</tr>
<tr>
<td>Pupil diameter</td>
<td>Normal</td>
</tr>
<tr>
<td>Colour and consistency of faeces</td>
<td>Normal</td>
</tr>
<tr>
<td>Condition of teeth</td>
<td>Normal</td>
</tr>
<tr>
<td>Breathing abnormalities</td>
<td>Nil</td>
</tr>
<tr>
<td>Gait</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Specificity**

The purity of band due to β-sitosterol in the KRR extract as well as plasma samples was confirmed by overlaying the absorption spectra with standard β-sitosterol are shown in Fig. 6 at visible region.

### Table 4

Daily body weight record of Group I (Control) in comparison with Group II

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I (Control)</th>
<th>Group II KRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>36.5</td>
</tr>
<tr>
<td>1</td>
<td>48.5</td>
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</tr>
<tr>
<td>2</td>
<td>51.0</td>
<td>39.5</td>
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<tr>
<td>3</td>
<td>47.0</td>
<td>38.5</td>
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<td>4</td>
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<td>6</td>
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<td>39.0</td>
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<tr>
<td>7</td>
<td>47.0</td>
<td>39.0</td>
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<tr>
<td>8</td>
<td>47.0</td>
<td>38.0</td>
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<tr>
<td>9</td>
<td>47.0</td>
<td>37.0</td>
</tr>
<tr>
<td>10</td>
<td>47.0</td>
<td>37.5</td>
</tr>
<tr>
<td>11</td>
<td>47.0</td>
<td>36.5</td>
</tr>
<tr>
<td>12</td>
<td>47.0</td>
<td>37.0</td>
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<tr>
<td>13</td>
<td>47.0</td>
<td>37.0</td>
</tr>
<tr>
<td>14</td>
<td>46.5</td>
<td>36.0</td>
</tr>
</tbody>
</table>

### Table 5

Daily food intake record of Group I (Control) in comparison with Group II

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I (Control)</th>
<th>Group II KRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>5.5</td>
<td>7.5</td>
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<td>6</td>
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<td>8.5</td>
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<td>7</td>
<td>6.5</td>
<td>8</td>
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<td>8</td>
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<td>7</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
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</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>8.6</td>
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<td>11</td>
<td>5.5</td>
<td>8.2</td>
</tr>
<tr>
<td>12</td>
<td>5.5</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>6.5</td>
<td>8</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>
Method development

The method development and selection of a suitable mobile phase involved several trials because of the complexity of the chemical composition of the herbals and the affinities of the components towards various solvents. Precoated silica gel HPTLC plates were used and toluene-ethyl acetate-methanol-glacial acetic acid, \( 8+1+0.5+0.3 \) (v/v/v/v) as mobile phase resulted in good separation of \( \beta \)-sitosterol band from matrix components. Fingerprint of spiked plasma with KRR along with \( \beta \)-sitosterol and KRR methanolic extract is shown in Fig 4 and 5.

Calibration and validation

Linearity of detector response for \( \beta \)-sitosterol standard

The method was validated for precision, repeatability and accuracy.

A good linearity was achieved in the concentration range of 10-100 \( \mu \)g ml\(^{-1} \) for \( \beta \)-sitosterol. The regression equations and correlation coefficient for the reference were:

Calibration Graph:

- **Fig. 4** Chromatographic plate of fingerprint of KRR

\[ \text{Rf} = 0.48 \]

Fig. 4 Overlay of visible spectra of 0h plasma, \( \beta \)-sitosterol, sampling point plasma and KRR.
Fig. 5 Chromatographic plate of fingerprint of spike rabbit plasma with KRR along with \( \beta \)-sitosterol

\[ \beta \text{-sitosterol } \left[ y = 29.02x + 89.96, R^2 = 0.996 \right]. \]

Table 7

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>10 to 100 ( \mu \text{g mL}^{-1} )</td>
</tr>
<tr>
<td>LOD</td>
<td>1 ( \mu \text{g mL}^{-1} )</td>
</tr>
<tr>
<td>LOQ</td>
<td>5 ( \mu \text{g mL}^{-1} )</td>
</tr>
<tr>
<td>System Suitability (n=5 % CV)</td>
<td>0.09</td>
</tr>
<tr>
<td>Instrument Precision (n=6 % CV)</td>
<td>0.11</td>
</tr>
<tr>
<td>Intraday (precision) (n=3 % CV)</td>
<td>0.06</td>
</tr>
<tr>
<td>Interday (precision) (n=3 % CV)</td>
<td>0.12</td>
</tr>
<tr>
<td>Rf</td>
<td>0.48</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>29.02</td>
</tr>
<tr>
<td>Intercept</td>
<td>89.96</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.996</td>
</tr>
</tbody>
</table>

Instrumental precision and intra-day and inter-day precision

Instrumental precision was checked by repeated scanning of the same spot of \( \beta \)-sitosterol five times each. Standards of \( \beta \)-sitosterol (15, 30, 90 \( \mu \text{g mL}^{-1} \)) was spotted both at intra-day (spotting each concentration thrice within 24 hour) and inter-day (spotting each concentration three times at 3 days interval) interval to check the precision. The results were expressed as %RSD and were found to less than 2%.

Recovery

The recovery was used to evaluate the accuracy of the method. The present recovery as well as average percent recovery was calculated. Recovery studies at three different levels were carried out using sample 2 by accurately spiking various concentrations of standard just prior to the extraction.

Limit of Detection and Limit of Quantitation

The limit of detection and limit of quantification were obtained with signal-to-noise ratios of 3 and 10 respectively. The LOD and LOQ were found to be 1 and 5 \( \mu \text{g mL}^{-1} \) respectively for \( \beta \)-sitosterol.

Linearity of detector response for plasma spiked with \( \beta \)-sitosterol standard

\( \beta \)-sitosterol at eight different concentrations ranging from 35ng–350ng were prepared in methanol and shown in Table 8. A graph was plotted of the concentration of \( \beta \)-sitosterol in spiked plasma against peak area. This experiment was performed in triplicate and the mean value was used for calculations. The plot was linear in the range from 35 to 350ng with a correlation coefficient of 0.986. The line of regression had a slope of 5.48 and intercept of 97.96.

Table 8

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>35 to 350 ng mL(^{-1} )</td>
</tr>
<tr>
<td>LOD</td>
<td>5 ng mL(^{-1} )</td>
</tr>
<tr>
<td>LOQ</td>
<td>10 ng mL(^{-1} )</td>
</tr>
<tr>
<td>System Suitability (n=5 % CV)</td>
<td>0.09</td>
</tr>
<tr>
<td>Instrument Precision (n=6 % CV)</td>
<td>0.11</td>
</tr>
<tr>
<td>Intraday (precision) (n=3 % CV)</td>
<td>0.06</td>
</tr>
<tr>
<td>Interday (precision) (n=3 % CV)</td>
<td>0.12</td>
</tr>
<tr>
<td>Rf</td>
<td>0.48</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>5.48</td>
</tr>
<tr>
<td>Intercept</td>
<td>97.96</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.986</td>
</tr>
</tbody>
</table>

Pharmacokinetic applicability

This validated method was applied to monitor the plasma concentrations of \( \beta \)-sitosterol in rabbits after oral administration of Tukham-e-katan Refluxed Residue (KRR) at a dose of 1g (containing 0.3978mg of \( \beta \)-sitosterol)/ Kg body weight. The pharmacokinetic parameters were estimated using WinNonlin version 3.0 software. The pharmacokinetic parameters are presented in Table 9. A graph showing the absorption-elimination pattern of \( \beta \)-sitosterol from KRR in

Unani Medicus 2011 1(2)
rabbit plasma is given in Fig 7 as a plot of peak area against time.

<table>
<thead>
<tr>
<th>Table 9 Pharmacokinetic parameters of β-sitosterol in rabbit after administration of KRR (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
</tr>
<tr>
<td>Tmax</td>
</tr>
<tr>
<td>AUC 0- t</td>
</tr>
<tr>
<td>AUC 0- ∞</td>
</tr>
<tr>
<td>t1/2</td>
</tr>
<tr>
<td>Kel</td>
</tr>
</tbody>
</table>

A marker from KRR at Rf = 0.48 was detected in rabbit plasma after 0.5hr of ingestion of the refluxed residue. The marker reached maximum concentration at 6hrs post dose and was not detectable in plasma after 12hr post dose. Absorption and elimination of the marker after ingestion of KRR is followed using changes in the concentration of the marker in the HPTLC profile. The marker could be identified as β-sitosterol using the corresponding bands obtained in the plant extract.

The bioavailability of β-sitosterol in humans is generally reported to be low (Gould, 1955; Borgstrom, 1968; Gould et al., 1969). Recent data suggest that extrapolation of in vivo preclinical pharmacokinetic data tends to be the most accurate method for predicting human pharmacokinetic parameters (Jolivette, 2005). Therefore, it will be of great therapeutic interest to evaluate the relative bioavailability of β-sitosterol from Tukham-e-katan powder with that from a formulation of β-sitosterol.

Bioavailability studies of β-sitosterol from Tukham-e-katan have not been reported earlier. Also preclinical pharmacokinetics data and related findings for such a kind of study has not been documented.

Also since, Tukham-e-katanmeal is reported to have a high nutritional potential, not only based on its high protein content (Oomah and Mazza, 1993a), but also because of its water-soluble fiber fraction (Warrand et al., 2005) and lignan content (Hyvärinen et al., 2006a), therefore, Tukham-e-katan as source of β-sitosterol has great potentials as a dietary supplement. The pharmacokinetic parameters of β-sitosterol suggest that it may be used as a marker compound to characterize some profiles of the herbal extract.

![Fig. 7 Plot of the mean concentration of β-sitosterol in the plasma of rabbit vs time after oral administration of KRR](image)

**Conclusion**

This paper describes a simple HPTLC method for quantitation of β-sitosterol in rabbit plasma. Also safety evaluation of Tukham-e-katan refluxed residue was monitored by conducting acute toxicity study and it was found that it was not toxic at 2g / kg dose. It was applied to the pharmacokinetic study of β-sitosterol from linseed. The study can form baseline for making polyherbal preparation of defined bioavailability of β-sitosterol leading to a possible extrapolation to humans. Further a better understanding of the pharmacokinetics and bioavailability of phytoconstituent can also help in designing rational dosage regimens.

**Acknowledgement**

This work was supported by University Grants Commission, Ref No. 34-175/2008 (SR), New Delhi, India for financial assistance to carry out this project.

**References**


Effect of *Nigella sativa* on blood glucose in alloxan induced diabetic rabbits

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Abstract

To study the effect of *Nigella sativa* (NS) on blood glucose in alloxan induced diabetic rabbits, diabetes was induced by a single i.v. injection of alloxan monohydrate in a dose of 150 mg/kg. Aqueous extract (20mg/kg/day), alcoholic extract (45 mg/kg/day), whole seed (200 mg/kg/day) and seed oil (0.5 ml/kg/day) of NS were administered to normal as well as diabetic rabbits for 4 weeks. Glibenclamide (0.5 mg/kg/day) was used as the standard drug. Blood samples were collected at day 0, 7, 14, 21 and 28 days for estimation of blood glucose. There was no significant reduction in blood glucose of normal rabbits following treatment with any component of NS. NS seed, aqueous extract, alcoholic extract and oil reduced blood glucose of diabetic rabbits which was comparable to glibenclamide. *Nigella sativa* has antidiabetic property in alloxan induced diabetic rabbits but does not have hypoglycemic activity.

Key words: Kalonji, *Nigella sativa*, Alloxan, Diabetes mellitus, Hypoglycemia

Introduction

Diabetes mellitus has emerged as a disorder of global importance. It is a heterogeneous metabolic disorder characterised by phenotype of hyperglycemia. This disorder can result either from impaired insulin secretion or reduced sensitivity of tissues to insulin or from both. Long standing diabetes is associated with various complications which lead to morbidity and mortality. Available drugs for diabetes have adverse effects and they start failing after variable time. Therefore it is necessary to search for more effective and safer drugs.

*Nigella sativa* L. (Black cumin or kalonji) (NS) is a herbaceous plant that has been used for centuries for treatment of various ailments, including diabetes mellitus. As an oriental spice, NS has long been used as a natural medicine for the treatment of many acute as well as chronic conditions. It has been reported to have analgesic (Abdel-Fattah AM et al., 2000), anti-inflammatory (Marsik P et al., 2005), antimicrobial (Hannan A, et al., 2008), antineoplastic (El-Najjar N et al., 2010), nephroprotective, (Yildiz F et al., 2010), bronchodilator (Boskabady MH et al., 2007), antioxidant (Soleimani H et al., 2008) and neuroprotective (Radad K et al., 2009) properties. Although antidiabetic property of NS has been reported in the past (Khanam M et al., 2008, Kanter M et al., 2010), it has not attained a well defined place in the therapy of diabetes. Therefore, this study was conducted to evaluate its antidiabetic property in detail and to compare its seed, its extracts and oil for their antidiabetic effect in both normal (euglycemic) and diabetic animals.

Materials and Methods

This work was conducted in the department of pharmacology, Jawaharlal Nehru Medical College, Aligarh from March 2009 to December 2010. Ethical clearance was taken from Institutional Animal Ethical Committee of J N Medical College.

Experimental Animals

The study was conducted on adult healthy albino rabbits of either sex weighing 1.5-2.5 kg. They were kept in the Central Animal House of the J N Medical College, for two weeks before the
commencement of experiments, under standard laboratory conditions. All the animals were fed standard animal diet (Hindustan Lever Ltd. Mumbai, India) and water *ad libitum*.

**Plant material, Drugs and Chemicals**

Seeds of NS were procured from a local dealer at Aligarh. They were authenticated by a pharmacognocist, Deptt. of Ilmul Advia, A. K. Tibbiya College, AMU, Aligarh, by its macroscopic and microscopic properties. The whole lot of seeds used in the study was purchased in one batch and kept in refrigerator at 8°C.

NS oil (Kalonji oil, Mohammedia products, Karimnagar – 505001, A.P., India) was procured from local market at Aligarh. As per manufacturer’s information, it was prepared by steam distillation.

To induce diabetes in experimental animals, Alloxan monohydrate was used. This drug was obtained from Sigma Aldrich (USA).

**Preparation of extracts**

The aqueous and alcoholic extracts of NS seeds were prepared by cold maceration of 150 gm of seed powder in 500 ml of respective solvents (drinking water and ethyl alcohol respectively) for 7 days. The extract was filtered, concentrated, dried *in vacuo* and the residue stored in a refrigerator at 2-8°C for further use (Shirwaikar A et al., 2004).

**Induction of diabetes**

Diabetes was induced in rabbits by a single injection of alloxan monohydrate in a dose of 150 mg/kg into the marginal vein of the ear (Akhtar MS et al., 1981). Diabetes was confirmed 72 hrs after alloxan injection and animals showing blood glucose ≥ 200mg/dl were selected for the study.

**Collection of blood and determination of blood glucose**

Blood was collected from marginal ear vein and glucose levels were estimated using a glucose oxidase reactive strips and a glucometer (Gluco Chek® Blood Glucose Monitoring System, Major Biosystem Corp. Taiwan) (Freshwater JD et al., 2002).

**Experimental design**

Rabbits were divided into 11 groups as follows:

- **Group I**: normal control rabbits administered normal saline
- **Group II**: normal rabbits + NS seed (200 mg/kg/day)
- **Group III**: normal rabbits + aqueous extract of NS seed (20 mg/kg/day)
- **Group IV**: normal rabbits + alcoholic extract (45 mg/g/day)
- **Group V**: normal rabbits + NS seed oil (0.5 ml/kg/day)
- **Group VI**: diabetic control rabbits
- **Group VII**: diabetic rabbits + NS seed (200 mg/kg/day)
- **Group VIII**: diabetic rabbits + aqueous extract of NS seed (20 mg/kg/day)
- **Group IX**: diabetic rabbits + alcoholic extract (45 mg/g/day)
- **Group X**: diabetic rabbits + NS seed oil (0.5 ml/kg/day)
- **Group XI**: diabetic rabbits + glibenclamide (0.5 mg/kg/day) (Ahmed S et al. 2005)

All the treatments were given orally for 28 days.

**Statistical analysis**

Data were statistically analysed using one-way ANOVA, followed by Tukey post-hoc test using SPSS 16 software. P value <0.05 was considered as significant.

**Results**

**Aqueous extract**

It was a yellow coloured powder, with slight pungent odour. Extractive value was 8.8%.

**Alcoholic extract**

It was a brownish yellow powder. It had a strong pungent odour. Extractive value was 21.7%.

There was no significant change in blood glucose of group I to group V during the period of study (Table 1). The blood glucose of diabetic control rabbits gradually increased over a period of 28 days. All the components of NS reduced blood glucose of diabetic rabbits. NS whole seed and aqueous extract caused a significant reduction at day 14, 21 and 28. With NS alcoholic extract reduction was significant at day 21 and 28. NS seed oil caused significant reduction at day 28. Glibenclamide also reduced blood glucose of diabetic rabbits which was significant at day 14, 21 and 28.
Table 1
Effect of *Nigella sativa* on blood glucose in normal rabbits

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Mean blood glucose (mg/dl)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal control</td>
<td></td>
<td>103.1± 10.21</td>
<td>100.4 ± 25.07</td>
<td>113.6± 7.11</td>
<td>98.4± 21.03</td>
<td>113.4± 12.21</td>
</tr>
<tr>
<td>Group II</td>
<td>Seed (200 mg/kg/d)</td>
<td></td>
<td>110.3± 12.08</td>
<td>96.5± 7.32</td>
<td>102.8± 20.68</td>
<td>120.2± 10.39</td>
<td>117.1± 13.16</td>
</tr>
<tr>
<td>Group III</td>
<td>Aqueous extract (20 mg/kg/d)</td>
<td></td>
<td>120.0± 8.32</td>
<td>117.2± 21.48</td>
<td>110.1± 11.02</td>
<td>115.0± 25.47</td>
<td>121.5± 6.21</td>
</tr>
<tr>
<td>Group IV</td>
<td>Alcoholic extract (45 mg/kg/d)</td>
<td></td>
<td>102.6± 42.12</td>
<td>100.8± 21.06</td>
<td>107.1± 8.02</td>
<td>102.0± 9.29</td>
<td>97.3± 13.33</td>
</tr>
<tr>
<td>Group V</td>
<td>Oil  (0.5 ml/kg/d)</td>
<td></td>
<td>96.6± 12.35</td>
<td>113.4± 9.16</td>
<td>120.1± 20.03</td>
<td>111.4± 32.02</td>
<td>104.6± 12.03</td>
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</tbody>
</table>

Values are mean ± SD, n=6, *p<0.05 as compared with diabetic control

Table 2
Effect of *N*. *sativa* on blood glucose in diabetic rabbits

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Mean blood glucose (mg/dl)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Percentage reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VI</td>
<td>Diabetic control</td>
<td></td>
<td>251.8± 10.59</td>
<td>254.3± 20.03</td>
<td>273.1± 15.63</td>
<td>295.6± 20.97</td>
<td>315.1± 16.01</td>
<td>—</td>
</tr>
<tr>
<td>Group VII</td>
<td>Seed (200 mg/kg/d)</td>
<td></td>
<td>284.5± 22.81</td>
<td>247.8± 15.77</td>
<td>217.5± 13.35*</td>
<td>217.5± 9.87*</td>
<td>216.3± 12.37*</td>
<td>18.1%</td>
</tr>
<tr>
<td>Group VIII</td>
<td>Aqueous extract (20 mg/kg/d)</td>
<td></td>
<td>259.1± 18.38</td>
<td>249.6± 9.77</td>
<td>236.5± 13.86*</td>
<td>219.3± 10.13*</td>
<td>220.5± 15.21*</td>
<td>15.4%</td>
</tr>
<tr>
<td>Group IX</td>
<td>Alcoholic extract (45 mg/kg/d)</td>
<td></td>
<td>288.6± 30.63</td>
<td>274.6± 14.38</td>
<td>251.3± 29.54</td>
<td>238.0± 27.81*</td>
<td>219.1± 14.35*</td>
<td>23.9%</td>
</tr>
<tr>
<td>Group X</td>
<td>Oil  (0.5 ml/kg/d)</td>
<td></td>
<td>296.1± 29.83</td>
<td>266.1± 26.84</td>
<td>254.6± 25.75</td>
<td>242.1± 29.99</td>
<td>223.8± 14.74*</td>
<td>20.6%</td>
</tr>
<tr>
<td>Group XI</td>
<td>Glibenclamide (0.5 mg/kg/d)</td>
<td></td>
<td>277.5± 28.03</td>
<td>246± 18.65</td>
<td>232± 20.76*</td>
<td>228± 12.16*</td>
<td>217± 7.48*</td>
<td>21%</td>
</tr>
</tbody>
</table>

Blood glucose values of group VII (seed), VIII (aqueous extract), IX (alcoholic extract), X (oil) and XI (glibenclamide) were also statistically compared with each other. No significant difference was found between these groups.

Percentage reduction in blood glucose of diabetic animals was 18.1%, 15.4%, 23.9%, 20.6% and 21% with NS seed, its aqueous extract, alcoholic extract, its oil and glibenclamide respectively (at day 28 as compared to their pretreatment values).

**Discussion**

Current medications for the treatment of diabetes mellitus are limited, majority of the drugs act by one of the two mechanisms of action, either by increasing insulin secretion or by decreasing insulin resistance. Agents increasing insulin secretion suffer from the adverse effect of hypoglycaemia and weight gain. Hypoglycaemia is frequently problematic and sometimes fatal. Agents decreasing insulin resistance are not very potent and have to be used along with other drugs. Diabetic patients continue to develop insulin resistance and drugs start failing after variable period of time. No one medication can enhance insulin secretion and sensitivity simultaneously; there is an essential need for new and more effective therapeutic agents.

In the present study, the NS seed, its aqueous extract, alcoholic extract and oil were given to
normal as well as alloxan-diabetic rabbits for 28 days. As described above in the results, there was no significant fall in blood glucose of normal rabbits. Similar results were shown by Le PM et al., (2004). They reported that fasting plasma glucose remained stable during 4 week treatment of normal rats with petroleum ether extract of NS seed. Absence of hypoglycaemic effect in the presence of normal blood glucose levels is a desirable feature of any potential antidiabetic drug. Agents showing this type of action are called as antihyperglycemic rather than hypoglycaemic. This property of the drug can save the patient from episodes of hypoglycaemia which is common in elderly patients or those taking insulin or drugs for concomitant diseases.

*N. sativa* seed, its aqueous extract, alcoholic extract, its oil and glibenclamide caused a reduction in blood glucose of 18.1%, 15.4%, 23.9%, 20.6% and 21% respectively, in diabetic rabbits after 4 weeks of treatment as compared to their control (pretreatment) value. Effect of NS seed and aqueous extract started earlier (day 14) as compared to alcoholic extract (day 21) and oil (day 28). The mechanism of antidiabetic effect of NS may be increase in insulin sensitivity, decrease in hepatic gluconeogenesis, regeneration of pancreatic \( \beta \) cells or its free radical scavenging activity or a combination of all these.

Lack of hypoglycaemia in normal animals with NS might also be due to the fact that antioxidant potential of NS is one of the proposed mechanism of its antidiabetic activity and antioxidant effects are better exerted in the presence of oxidative damage (by alloxan in case of experimental diabetes) (Begum NA et al. 2006).

From these results, we conclude that the antidiabetic effect of NS seed (200 mg/kg/d), aqueous extract (20 mg/kg/d), alcoholic extract (45 mg/kg/d), and its oil (0.5 ml/kg/d) are comparable to that of glibenclamide (0.5 mg/kg/d). Further it can be concluded that antidiabetic effect of NS is exerted by both its water soluble and lipid soluble components.

The seeds produced nearly the same level of reduction as Glibenclamide while Aqueous Extract produced somewhat lesser reduction. So, the study on the one hand points out that the use of the drug as whole crude drug as in Unani Medicine is very effective, and on the other hand indicates that the use as Decoction in Unani Medicine may be less effective. So, it suggests that the use of whole seeds is probably the best way of using it in Unani Medicine.

**Conclusion**

*Nigella sativa* seed, its aqueous and alcoholic extracts and oil have antidiabetic property comparable to that of glibenclamide in alloxan induced diabetic rabbits. Further studies are warranted to assess the efficacy and safety of *Nigella sativa* in diabetic patients.

**References**


Study of *Carthamus tinctorius* Linn for diuretic and nephroprotective effect in albino rats

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Abstract

70 % Ethanol extract of the seeds of *Carthamus tinctorius* Linn. of family Compositae, was investigated for its protective and curative effects against gentamicin (40 mg/kg) induced acute renal injury and also for the diuretic effect, in albino rats. Elevation of blood urea and serum creatinine level, and appearance of histopathological features of acute tubular injury were taken as the indicators of nephrotoxicity, while an improvement in these indicators following the test drug administration was taken as nephroprotective effect. An increase in urine output and sodium and potassium concentration was taken as the indices of diuretic activity. In the preventive regimen, the extract showed reduction in the elevated blood urea and serum creatinine level and almost normalized the structural disintegration of kidney tissue caused by gentamicin. In curative group too both biochemical markers of kidney function decreased significantly and definite signs of regenerative process were also seen in histological examination. Similarly, the test drug increased the total volume of urine and the concentration of sodium and potassium in it, significantly and exhibited diuretic effect which was almost equal to the effect produced by furosemide. The findings suggest that the extract of seeds of *Carthamus tinctorius* possesses marked nephroprotective and diuretic activity.

Key words: Nephroprotective Effect, Nephrotoxins, Diuretics, *Carthamus tinctorius*, Furosemide

Introduction

Kidney diseases are emerging as a global threat to human health. Although, renal replacement therapy has got tremendous popularity and recognition however it is not possible for every patient to receive the treatment because of the high cost of dialysis and the acute shortage of kidneys for transplantation. Unfortunately the new patients of renal replacement therapy are growing very rapidly at a rate of 7% per year and annual expenditure of 1 trillion dollar is incurred globally on the therapy. In United States alone 6% of total Medicare budget ($ 12.7 million which is expected to exceed $ 28.3 million by 2010) was incurred on transplantation and dialysis in 1999, and served only 0.7% of Medicare population (Norberto *et al*., 2005). Thus, it is virtually impossible to synchronise the available budget and the growing number of patients of kidney diseases. In poor countries where enough facilities are not available for all in need, a number of patients die because of uremia. Researchers, scientists and clinicians are, therefore, looking for ways to prevent the need of dialysis in as many patients as possible. A simple and inexpensive treatment in majority of the cases is plausible and possibly effective in treating the diseases and preventing the progression of initially a simple disease, into chronic and complicated one. It has been appreciated that traditional systems of medicine can offer some promising drugs (Afzal *et al*., 2004) that can be used in the prevention and management of renal diseases. Such agents can
either protect the kidney from various diseases or rob of the toxic effects on kidney or at least, slow the progression of disease and thus minimize the chances of renal replacement therapy.

Qurtum (Carthamus tinctorius, Linn) of the family Compositae is an important drug of Unani Medicine commonly used in renal diseases. It has been described in classical Unani literature, to be diuretic (Aawan, 1993), lithotryptic, tonic to kidney, nephroprotective and anti-inflammatory (Ghani, 1920). It has been also described in detail, in ethnobotanical literature to be anti-inflammatory, diuretic, nephroprotective, tonic to kidney and useful in chronic nephritis (Anonymous, 1950; Kirtikar and Basu, 1987; Chopra et al., 1956; Nadkarni, 2000; Anonymous, 1996; Khory and Katrak, 1985).

In view of the above, therefore, an attempt has been made to evaluate the ethanol extract of seeds of Carthamus tinctorius for its diuretic and nephroprotective effects in albino rats.

Materials and Methods
Preparation of ethanol extract
The seeds of Carthamus tinctorius were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, India. Dr. M. Inamuddin and Professor S. H. Afaq (Pharmacognosists), Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh confirmed the identity of the drug. A voucher specimen (No. WA/2005/1) has been deposited in the museum of the department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, India.

The seeds were dried at room temperature and reduced to coarse powder by grinding. Powdered drug was immersed in 70% ethanol and left for 12 h at room temperature and then extracted for 6 h in soxhlet apparatus. 100 gm of powdered drug was extracted in 500 ml of ethanol and filtered. The filtrate was concentrated using a water bath. The yield of the extract was found to be 7% of crude drug (w/w).

Experimental Animals
Wistar Albino rats of either sex weighing 100-150 g, divided into different groups of six animals each were used. They had free access to standard diet and water ad libitum unless stated otherwise. They were housed in clean polypropylene cages at room temperature (25±2°C) with a 12 h light and 12 h dark cycle. The Institutional Animal Ethics Committee approved the experimental protocol.

Treatment Schedule
Dose of the drugs for albino rats was calculated by multiplying the human therapeutic dose, described and practiced in Unani Medicine (Aawan, 1993; Ghani, 1920) by the conversion factor of seven (Freidrich et al., 1966) and the animals were treated as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Treatment</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (plain control)</td>
<td>Normal Saline</td>
<td>30 ml/kg</td>
<td>1st-12th days</td>
</tr>
<tr>
<td>Group II (negative control)</td>
<td>Gentamicin</td>
<td>40 mg/kg</td>
<td>1st-12th days</td>
</tr>
<tr>
<td>Group III (preventive group)</td>
<td>Gentamicin + ethanol extract</td>
<td>40 mg/kg + 100 mg/kg</td>
<td>1st-12th days</td>
</tr>
<tr>
<td>Group IV (curative group)</td>
<td>Gentamicin + ethanol extract</td>
<td>40 mg/kg + 100 mg/kg</td>
<td>1st-5th days</td>
</tr>
</tbody>
</table>

Nephroprotective effect
The albino rats were divided into four groups. The animals in Group I received 30 ml/kg body weight of 0.9% normal saline, intragastrically by a gastric canula, twice a day for 12 days. The animals in Group II were treated with gentamicin (IM) in the dose of 40 mg/kg body weight twice a day for 12 days. The animals in Group III serving as preventive group were treated with ethanol extract in a dose of 100 mg/kg body weight, by oral route, along with gentamicin in the dose of 40 mg/kg body weight intramuscularly, twice a day for 12 days. The animals in Group IV were treated with a daily dose of gentamicin 40 mg/kg body weight intramuscularly, twice a day for the first 5 days followed by oral administration of ethanol extract at a dose of 100 mg/kg body weight, from the 6th day onwards for next 7 days (i.e. until the 12th day) and served as curative group. The concentrated extract of seeds was reconstituted afresh in normal saline at the time of administration.

On the 13th day 12 h after the vehicle/drug administration all the animals were sacrificed under deep anaesthesia and blood was collected by cervical decapitation. Serum was separated from the blood and level of urea and creatinine was estimated. Kidneys were isolated after blood withdrawal and immersed in 10% formalin for histopathological studies. Tissue samples were embedded in paraffin; sectioned and stained with haematoxylin and eosin. Elevation of urea and creatinine level in the serum and presence of...
features of tubular injury and necrosis in the histopathological sections of the kidneys were taken as the indices of nephrotoxicity (Ali et al., 2001).

Diuretic activity
The animals were divided into three groups. Group I served as plain control and received the vehicle (normal saline). Group II was treated with furosemide in the dose of 4 mg/kg body weight, dissolved in normal saline and administered intragastrically by a gastric canula. While the animals in group III were given a single dose of the extract (100 mg/kg). Food and water were withdrawn 18 h before the administration of drug (Anwar et al., 1999).

Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by them over a period of 6 h was collected in a calibrated jar. Total urine output was recorded and concentration of sodium and potassium was determined with the help of a ‘flame photometer’.

Statistical Analysis
The results are given as mean ± S.E.M. Level of significance was determined by using the Student’s t test. P-value equal to or less than 0.05 showed significance.

Results
Nephroprotective effect
Rats treated with gentamicin only (Group II) showed definite signs of nephrotoxicity as compared to the control group (Group I). This was evidenced by the increase in two serum markers of the kidney function viz. blood urea and serum creatinine (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood urea (mg/dl) (Mean±S.E.M.)</th>
<th>Serum creatinine (mg/dl) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (plain control)</td>
<td>36.06±0.558</td>
<td>0.742±0.435</td>
</tr>
<tr>
<td>Group II (negative control)</td>
<td>66.05±0.606</td>
<td>2.287±0.302</td>
</tr>
<tr>
<td>Group III (preventive)</td>
<td>43.33±0.558</td>
<td>1.425±0.926</td>
</tr>
<tr>
<td>Group IV (curative)</td>
<td>45.45±0.663</td>
<td>1.607±1.775</td>
</tr>
</tbody>
</table>

n = 6  *P < 0.005

Peritubular and glomerular congestion, tubular casts, epithelial degeneration, interstitial oedema, blood vessels congestion and infiltration by inflammatory cells, which are the features of acute tubular necrosis, were also observed in the histopathological sections of the kidneys in this group, indicating the extent of damage caused at tissue level (Figure 2); such changes were not observed in the control group (Figure 1).

While in Group III and Group IV, the test drug produced a significant (P<0.005) decrease in the level of serum markers of the kidney function when compared with Group II, demonstrating significant nephroprotective effect. In histopathological sections of the kidneys of Group III and IV glomerular congestion, tubular cast and epithelial desquamation were not found. However, it continued to show slight peritubular congestion, blood vessel congestion, interstitial oedema and inflammatory cell but the scoring was very low (Table-2, Figure 3 and 4).

Diuretic effect
Total urine output
Ethanol extract of seeds of Carthamus tinctorius at the dose of 100 mg/kg body weight induced significant (P<0.005) increase in urine volume, as compared to plain control group. The diuresis produced by the test drug was almost equal to that of furosemide (Table 3).

Electrolytes
Ethanol extract of test drug was found to produce significantly (P<0.005) increased natriuresis and kaliuresis as compared to plain control group. Furosemide treated animals also produced similar effect (Table 3).
Table 2

<table>
<thead>
<tr>
<th>Histopathological features</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular congestion</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubular casts</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peritubular congestion</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epithelial desquamation</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood vessel congestion</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Interstitial oedema</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(-): Normal; (+): Little effect; (++): Appreciable effect; (+++): Severe effect

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urine volume (ml) (Mean±S.E.M.)</th>
<th>Sodium excretion(ppm) (Mean±S.E.M.)</th>
<th>Potassium excretion(ppm) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (CONT)</td>
<td>0.76±0.333</td>
<td>1888.66±1763</td>
<td>462.50±0.619</td>
</tr>
<tr>
<td>Group II (STD)</td>
<td>3.43±0.102</td>
<td>2227.50±0.885</td>
<td>670.33±0.557</td>
</tr>
<tr>
<td>Group III (TEST)</td>
<td>2.68±0.414 **</td>
<td>2177.50±0.428 *</td>
<td>656.50±0.562 *</td>
</tr>
</tbody>
</table>

n = 6 *P < 0.01 *P < 0.005
Discussion

The study demonstrated that *Carthamus tinctorius* possesses nephroprotective effect against gentamicin induced acute nephrotoxicity. It also possesses diuretic effect which is almost equal to the effect produced by furosemide. Thus the study validated the claim of Unani Medicine that *Carthamus tinctorius* is a nephroprotective and diuretic agent and useful in various renal diseases.

It was observed that gentamicin induced appreciable degree of renal toxicity / injury, which was evidenced by the elevated blood urea and serum creatinine levels (Table 1) and also by the histopathological features of acute tubular necrosis (Table 2, Figure 2), in group of animals treated with gentamicin only. Elevation of blood urea and serum creatinine is considered as one of the most important manifestations of tubular necrosis of kidney (Anwar et al., 1999; Shirwaikar et al., 2005) and impaired GFR, which ultimately leads to renal failure (Moran and Myers, 1985).

Gentamicin has been reported to produce nephrotoxicity even at normal therapeutic dose level (Smith et al., 1980) because it accumulates within the renal cortex owing to high energy demand of proximal tubules (Locatelli and Del, 2000) and binding ability of amino group with tubular cells (Namita et al., 2005). This accumulation causes local injury and necrosis and thus impairs the function as well as the structural integrity of kidney (Lietman and Smith, 1983) and aggravates the toxicity and the degree of damage further at high dose level (Arnot et al., 1983). It although, affects glomerulus and distal tubules also but the main sites of nephrotoxicity are S1 and S2 segments of proximal tubules. The morphological changes of gentamicin nephrotoxicity include increase in the number and size of the secondary lysosomes which in turn cause decrease in the density and height of the brush border microvilli, dilation of the cisternae of rough endoplasmic reticulum and cytoplasmic vacuolization of the tubular epithelium. As injury progresses, mitochondrial swelling, tubular necrosis and desquamation occur (Goldstein, 1993). Tubular damage caused by gentamicin leads to loss of urinary concentration power, low glomerular filtration rate (GFR) and albuminuria etc. It has also been suggested that gentamicin and other aminoglycosides interfere with the production and metabolism of prostaglandin in the kidney and that is actually related to reduced-GFR (Humes et al., 1984). GFR is therefore considered as a sensitive index of functional nephron mass (Newman and Price, 1999) which may have compromised in the negative control group treated with gentamicin, as both the functional indicators of kidney and the structural integrity as shown in histopathological examination, were grossly altered. However, administration of a high dose of gentamicin along with the ethanol extract of seeds of *Carthamus tinctorius* at the dose level of 100 mg/kg in preventive and curative groups did not produce nephrotoxicity at all, as the blood urea and serum creatinine levels were found within the normal limits and the integrity of kidney matrix was seen to be maintained in microscopic examination of protective group. The findings in curative group were suggestive of definite improvement in functional and structural disintegration caused by gentamicin.

Since the test drug did not allow the two important serum markers of kidney function to elevate significantly above the normal level, protected the kidney from the toxic effect of a known toxicant and helped recover the structurally impaired kidney therefore, it may be inferred that the test drug has strong protective and curative effect against the toxic effect of gentamicin.

This study also showed that seeds of *Carthamus tinctorius* produced striking increase in total urine output over a period of 6 h. It also increased the excretion of sodium and potassium significantly. These findings thus suggested that the test drug possesses diuretic, natriuretic and kaliuretic effects which may be associated with one of the bases of its therapeutic uses in various urinary ailments, such as nephritis, burning micturation, etc., and different oedematous diseases, as described in Unani literature. Since, the test drug increases structural disintegration caused by gentamicin. This study also provides some indication about the molecular mechanism of action of the test drug. Its diuretic effect has been shown to be more or less equal to that produced by furosemide. Diuretics in general have been reported to augment the toxic potential of gentamicin when administered concomitantly with it and consequently cause severe tubular necrosis (Mitchell et al., 1977). *Carthamus tinctorius* in spite of demonstrating significant diuretic
activity not only did not increase the degree of toxicity but rather protected the kidney even from the exaggerated toxic effect that was likely to arise after the administration of gentamicin along with the test drug. This further indicated its nephroprotective effect. The diuretic potential may complement the nephroprotective effect by helping the removal of various toxic substances. If these two potentials of the test drug could offer even small improvement in slowing the renal disease progression it can provide large benefit by preventing or at least delaying the dialysis and transplantation.

**Conclusion**

In conclusion, it can be said that the seeds of *Carthamus tinctorius* possess marked nephroprotective and diuretic effects and thus have promising role in the treatment of acute renal injury induced by nephrotoxins, especially gentamicin. It may also be used to slow the progression of kidney diseases and thereby can prevent or delay to a great extent, the need of dialysis and transplantation.

**References**


Pharmacological evaluation of a Unani formulation and estimation of its alkaloidal constituents

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Abstract

Unani Medicine claims to possess a number of effective and safe drugs useful in the treatment of nervine disorders. Habb-e-Hudar (HH) is one such pharmacopoeial Unani compound preparation is described to possess neuropharmacological activity and anti-inflammatory activity and is useful in nervine disorders and in arthritis. Therefore, in the present study Habb-e-Hudar was tested for neuropharmacological and anti-inflammatory activity. Mice were treated orally by HH at dosage level 50 and 75 mg/kg to evaluate its central effects by Pentobarbitone sleeping time test and Spontaneous motor activity test. Acute toxicity of HH was tested in mice. It was administered orally to rats (30 and 45 mg/kg) to study its anti-inflammatory activity in Carrageenan oedema test and diclofenac was used as reference drug. HH showed a potent anti-inflammatory activity as indicated by suppression carrageen induced rat paw oedema and has central nervous system activity as indicated by reducing the sleep time and increasing the spontaneous motor activity in mice. These pharmacological effects were dose dependent. Acute toxicity test showed that HH is relatively safe to use. The study revealed the anti-inflammatory and central stimulant activity of HH, to which the presence of alkaloids, strychnine and brucine may be contributing.

Key words: Habb-e-Hudar, Strychnos nux vomica, Anti-inflammatory, Central Stimulant

Introduction

Tibb-e-Unani claims to possess a number of effective and safe drugs useful in the treatment of nervine disorders. The physicians of Unani Medicine are using such drugs effectively since centuries for the treatment of nervine disorders with a good recovery rate and despite the fact that nervine disorders requires a long term treatment, these drugs have not been found to cause any major side effects. Researchers of Unani Medicine have always given priority to the study of those drugs which are lacking in western medicine. Many single and compound drugs of Unani Medicine treatise when subjected to experimental and clinical studies demonstrated very promising results, for example Haldi (Curcuma longa), Tulsi (Ocimum sanctum) and Asgandh (Withania somnifera) were demonstrated to possess antidepressant activity (Zhang, 2004). However, a large number of drugs which have been described in Unani Materia Medica, and are widely used by the physicians of Unani Medicine in the treatment of nervine disorders, have still not been studied scientifically for their reported effect as Evidence based medicine.

Habb-e-Hudar (HH) is a pharmacopoeial Unani formulation (Anonymous, 2001), containing only two ingredients (Table 1). Major portion of the formulation contains Strychnos nux-vomica and alkaloids are the main bioactive chemicals in the nux vomica (Bisset and Phillipson, 1971), which are responsible for the pharmacological and toxic effects exerted by nux vomica to a great extent.
A study showed that crude nux vomica contains 16 alkaloids, among which strychnine and brucine take up 80% (Cai et al., 1994). Many scientific studies reported that Strychnine and Brucine are the stimulus for the central nervous system (Agarwal, 1995; Anonymous, 1995) and also possesses Anti-inflammatory effect (Yin et al., 2003). In Unani classical literature HH is reported to be Muqawwi-e-Asab (Nervine tonic) and Mohallil-e-Warm (Anti-inflammatory) (Anonymous, 2001; Kabiruddin, 1967; Lubhaya, 1979; Nigrami, 1995). As far as the individual ingredients are concerned, *Strychnos nux-vomica* is reported to have Anti-inflammatory activity (Yin et al., 2003) and the other ingredient *Zingiber officinale* is reported to have Anti-inflammatory activity (Jana et al., 1999; Levy et al., 2006; Tripathi et al., 2008; Young et al., 2005), Anxiolytic activity (Vishwakarma et al., 2002; Lakshmi and Sudhakar, 2010) and Anti-convulsant activity in experimental studies (Vishwakarma et al., 2002), but the ingredients in formulation in the form of pill which is practically in use in the system of Unani Medicine, had not been evaluated for neuropharmacological and anti-inflammatory activity.

Therefore, in the present study the formulation was experimentally evaluated for neuropharmacological activity by Sodium pentobarbitone sleeping time test and spontaneous motor activity test. HH is also used in arthritic conditions, therefore, it was also experimentally studied for its Anti inflammatory activity by carrageenan-induced rat paw oedema test. Since, Azaraqi (*Strychnos nux-vomica* Linn.) is toxic drug even in detoxified form and is chief ingredient in the HH, the LD-50 was also determined. Though HH, contains two ingredients but the major portion of the formulation contains *Strychnos nux-vomica* hence, it was necessary to estimate the total alkaloid and marker compounds such as Strychnine and Brucine because proper identification is vital for the proper and rationale use of plant medicines.

### Materials and Methods

#### Preparation of Pill

Azaraqi (*Strychnos nux-vomica*) was procured from the local market and fresh Ginger was obtained from the market and the juice was taken with the help of Juicer and stored in a fridge for further use. The ingredients were identified by Prof S.H Afaq (Pharmacognosist), Department of Ilmul Advia, Faculty of Unani Medicine A.M.U, Aligarh. The voucher specimens (SC-0105/09-Z and SC-0106/09-L) have been kept in our museum for future references.

Azaraqi was detoxified by the method given in National Formulary of Unani Medicine [Anonymous, 1983], dried under shade and then powdered. The powder of Azaraqi was made in the form of a paste by adding sufficient quantity of Aab-e-zanjabeel; with the help of a cutter this lubdi was cut into small pieces equivalent to the prescribed dose of the pill, the pieces so obtained were put in to a rolling machine which rotates at a fixed speed to give the round shape to the pills and then dried in an oven at 60°C ± 5°C.

The required quantity of the finely powdered pills was taken out and suspended in the gum acacia suspension just before the administration. The drug was administered to the animals orally by a feeding canula after shaking the suspension well.

The doses used in the study were selected according to Freidrich (Freidrich et al., 1966), multiplying the Unani clinical doses reported in standard Unani text (Anonymous, 2001) by the conversion factor of 12 and 7 for mice and rat respectively.

The Departmental ethical committee for animal care and use approved the experimental design.

#### Experimental Animals

Swiss Albino mice (20-25 gm) and adult albino rats (150-200 gm) of either sex were used for this experiment. The animals were given standard pellet diet and given tap water ad libitum. The
experiments were performed in a quiet room with an ambient temperature of 22 ± 2°C.

**Reagents and Standards**

Chloroform and ethanol were purchased from Qualigens fine chemicals, Mumbai. Ammonia was from Merck, India. Solvent ether, sulphuric acid and nitric acid from Thomas Baker chemicals Pvt. Limited, Mumbai. Sodium nitrite from Glaxo Lab., Mumbai. Sodium hydroxide from Ranbaxy Laboratories Ltd chemical division S.A.S Nagar. Methyl orange from SD fine Limited, Mumbai. Carrageenin and Pentobarbitone sodium were purchased from Sigma Chemical Co., St. Louis, MO, USA.

**Extraction and Estimation of Total Alkaloids, Strychnine and Brucine**

The estimation of total Alkaloid from Habb-e-Hudar was done as per the procedure described in Indian Pharmacopoeia (Anonymous, 1970).

**Estimation of Strychnine**

Strychnine was estimated as per the procedure described in Indian Pharmacopoeia (Anonymous, 1970).

**Estimation of Total Alkaloids**

Total Alkaloid was estimated by using the method for the estimation of strychnine but without adding a mixture of 3% w/v Sulphuric acid, nitric acid (15:2) and a few crystals of sodium nitrite (Agrawal and Joshi, 1977).

**Estimation of Brucine**

The percentage of brucine calculated in terms of strychnine in each case was calculated on the basis of the difference in the weight of the total alkaloid and strychnine (Agrawal and Joshi, 1977).

**Determination of LD-50**

The mice of either sex were divided into 6 groups of 10 animals each. The animals were kept on fasting for 10 hours after that the powdered material of the test drug in the form of suspension in the gum acacia was administered in graded quantities (mg/kg B. wt) singly by oral route respectively to the animals of each group. Following the administration of the test drugs all the animals were kept in a cage singly and observed continuously for two hours and then at 6 and 24 hours for mortality (Turner, 1965). The number of animals dying within 24 hours in each group was recorded. LD-50 was calculated by Arithmetical Method of Reed and Muench (1938) (Turner, 1965).

**Neuropharmacological Activity**

**Sodium Pentobarbitone Sleeping Time**

Adult Swiss albino mice of either sex were divided randomly into three groups of six animals each. Habb-e-Hudar at doses 50 and 75 mg/kg b.wt and normal saline per orally were administered orally to separate groups. Thirty minutes after the administration of drug / Normal Saline, each animal was injected with Sodium Pentobarbitone (40 mg/kg, I.P.). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex (Dandiy and Columbine, 1959).

**Spontaneous Motor Activity Test**

Male Swiss albino mice were divided randomly into three groups of six animals each. SMA of the mice was recorded using a motor activity cage (photoactometer), which automatically counted the animal movements across the cage floor. HH at doses 50 and 75 mg/kg b.wt and normal saline were administered orally to separate groups. The animals were placed singly in the photoactometer and the number of movements was recorded for 1 hour beginning 45 minutes after p.o. administration of each test drug (Cutting et al., 1959). The number displayed at the conclusion of the experiment denotes the number of spontaneous movements made by the animal.

**Anti-inflammatory Activity**

**Carrageenin-induced Rat Hind Paw Oedema Test**

Albino rats of either sex were divided into 4 groups of 6 animals each the volume of right hind paw was measured plethysmographically. The first group served as the control was administered with distilled water. Second group of animals was administered with standard drug diclofenac sodium (5 mg/kg orally). Third group was treated with HH suspension (30 mg/kg) and Fourth group was treated with HH suspension (45 mg/kg). One hour after the drug / vehicle treatment 0.1 ml of 1% aqueous solution of carrageenin (lambda type) was injected under the plantar Aponeurosis of the right hind paw. The volume of the paw was again measured at 1,2,3,4 and 5 hours after carrageenin injection the percentage inhibition of oedema was calculated by the formula described by Newbould, 1963. The formula used was $i = 100 \left[1 - \frac{a - x}{b - y}\right]$ Where $i =$Percentage of inhibition, $a =$mean right hind Paw volume of Test/standard animals after carrageenin
injection, \( b \) = mean right hind Paw volume of control animals after carrageenin injection, \( x \) = mean right hind Paw volume of Test/standard animals before carrageenin injection, \( y \) = mean right hind Paw volume of control animals before carrageenin injection.

**Statistical Analysis**

All results from different pharmacological studies are presented as mean ± S.E.M. Data were using Student’s ‘t’ Test analysis and/or by Analysis of variance (ANOVA) followed by Tukey Kramer Multiple comparisons Test wherever necessary. Results were considered significant at \( P < 0.05 \).

**Results**

**Estimation of Total Alkaloid and Marker Compounds (Strychnine and Brucine)**

The percentage of total Alkaloid, strychnine and brucine in HH was found to be \( 0.84 \pm 0.02; 0.55 \pm 0.01 \) and \( 0.294 \pm 0.02 \) respectively.

**Toxicity Study**

The LD-50 (maximal tolerance dose) of HH was found to be 571 mg/kg b.wt.

**Sodium Pentobarbitone Sleeping Time**

In the drug treated groups, the sleeping time was found significantly reduced when compared with control. The higher dose of HH (75 mg/kg b.wt) showed highly significant reduction (\( P < 0.001 \)) while its lower dose (50 mg / kg b.wt) showed significant reduction in the sleeping time (\( P < 0.01 \)). In Inter-treatment comparison, the significant reduction in the sleeping time was found to be higher with 75 mg / kg of HH, which was higher than 50 mg / kg of HH (\( P < 0.001 \)). The results are presented in Table 2.

**Spontaneous Motor Activity Test**

The test drug HH produced significant increase in the number of movements as compared to the control group. Higher dose showed greater increment (\( P < 0.001 \)), while its lower dose showed lesser increment in the number of movements (\( P <0.01 \)). In Inter-treatment comparison, higher dose of HH produced a significantly higher increment in the number of spontaneous movements than its lower dose (\( P < 0.001 \)). The results are presented in Table 3.

**Carrageenin-induced Rat Hind Paw Oedema Method**

Results of Anti-inflammatory study are summarized in Table 4. The maximum increase in paw volume was found at 3rd hour. At 3rd hour both the doses of test drug (\( P < 0.001 \)) significantly reduced the paw volume, which is comparable to the control group. The percentage inhibition of oedema was found to be 68.75% in standard group. 68.75% with 30 mg/kg of HH and 77.08% (\( P<0.05 \)) with 45 mg/kg of HH. In Inter-treatment comparison, the significant reduction in oedema was found to be higher with 45 mg / kg of HH than that of 30 mg / kg of HH (\( P < 0.05 \)).

**Discussion**

The Unani formulation, Habb-e-Hudar which is described to be Muqawwi-e-Asab (Nervine tonic) and Mohallil-e-Warm (Anti-inflammatory) (Anonymous, 2001; Kabiruddin, 1967; Lubhaya, 1979; Nigrami, 1995), was subjected to testing for neuropharmacological and anti-inflammatory activity. The LD50 was found to be 571 mg / kg B. wt and the percentage of total Alkaloid, strychnine and brucine in H. Hudar was found to be \( 0.84 \pm 0.02; 0.55 \pm 0.01 \) and \( 0.294 \pm 0.02 \) respectively.

Neuropharmacological activity showed that both the doses of H.H significantly reduced the sleeping time (\( P < 0.01 \) and \( P < 0.001 \)). Therefore H.H was shown to possess central stimulant activity. In order to confirm this effect, its effect on Spontaneous Motor was studied and was found to be increased significantly (\( P < 0.01 \)) with lower dose and further increased (\( P < 0.001 \)) with the higher dose. These findings corroborate the results of Pentobarbitone sleeping time. The test drug also demonstrated the dose dependent effect. The mean number of movements within the period of 1 hour was 607.8l, which clearly indicated that H.H possesses significant central Nervous Stimulant activity.
### Table 3

Effect of test drug on spontaneous motor activity (photoactometer test)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of movements in one Hour (mean ± SEM)</th>
<th>P value (Inter group comparison)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal Saline)</td>
<td>537.5 ±11.68</td>
<td>-</td>
</tr>
<tr>
<td>Habb-e-Hudar (50mg/kg b.wt.)</td>
<td>607.8 ± 16.31</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Habb-e-Hudar (75mg/kg b.wt.)</td>
<td>904.3 ± 4.54</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a = Against Control, w = Against H.H 50 mg / kg. 2 = P < 0.01, 3 = P < 0.001

### Table 4

Effect of habb-e-hudar in carrageenin-induced rat paw oedema test

<table>
<thead>
<tr>
<th>Group</th>
<th>1 hour after carrageenin injection</th>
<th>2 hours after carrageenin injection</th>
<th>3 hours after carrageenin injection</th>
<th>4 hours after carrageenin injection</th>
<th>5 hours after carrageenin injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase in Paw volume in ml (Mean ± SE)</td>
<td>Percentage of inhibition</td>
<td>P value</td>
<td>Increase in Paw volume in ml (Mean ± SE)</td>
<td>Percentage of inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>0.32 ±0.02</td>
<td>:</td>
<td>:</td>
<td>0.41 ±0.02</td>
<td>:</td>
</tr>
<tr>
<td>Standard drug Dfc (5mg/kg)</td>
<td>0.15±0.009</td>
<td>a&lt;sup&gt;3&lt;/sup&gt;</td>
<td>53.12 a&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.15±0.009</td>
<td>a&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>H. Hudar (30mg/kg)</td>
<td>0.24±0.02</td>
<td>a&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25 a=</td>
<td>0.23±0.01</td>
<td>a&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>H. Hudar (45mg/kg)</td>
<td>0.18±0.01</td>
<td>a=</td>
<td>43.75 a=</td>
<td>0.17±0.01</td>
<td>a=</td>
</tr>
</tbody>
</table>

a = Against Control, b = Against Standard (Diclofenac Sodium), w = Against H. H (30mg / kg), x = Against H. H (45 mg / kg), 1 = P < 0.05, 2 = P < 0.01, 3 = P < 0.001
In case of anti-inflammatory study, the results also showed that HH exhibited anti-inflammatory activity against carrageenin-induced rat hind paw oedema. H.H significantly prevented carrageenan-induced swelling in a dose dependent manner after oral administration. The maximal inhibition (77.08 %) was exhibited by H.H at 5 mg/kg. While the reference drug diclofenac, at 5 mg/kg, afforded approximately 69 % significant protection against acute inflammation. This showed potent Anti-inflammatory effect of the formulation. This is expected to be due to synergism between the two ingredients. This Synergistic activity might be due to the fact that both ingredients of the formulation are reported to have Anti-inflammatory activity in different experimental studies (Yin et al., 2003; Jana et al., 1999; Levy et al., 2006; Tripathi et al., 2008; Young et al., 2005).

HH is attributed mainly for two medicinal virtues i.e. Muqawwi-e-Zanjabeel Tar (Nervine tonic) and Muhallil-e-Warm (Anti-inflammatory) in Unani system of Medicine and contains two ingredients; Azaragi Mudabbar (Strychnos nux-vomica-detoxified) and Aab-e-Zanjabeel Tar (Zingiber officinale -juice). The main ingredient Strychnos nux-vomica is reported to have anti inflammatory and analgesic effect (Antaki, 1343H; Halim, 1948) whereas other Zingiber officinale is an important and multipurpose ingredient as it is reported to have anti-inflammatory and Anodyne effect (Antaki, 1343H; Halim, 1948; Hakim, 1343H), apart from possessing anti-inflammatory and anodyne effect, it also has antiulcerogenic effect and antigastric effects, thus minimizing the chances of ulcer formation after the administration of test drug, which is a common side effect of anti-inflammatory agents. Thus, the combination appears to be rational as it can produce significant central stimulant and anti-inflammatory effect and can be used as a nervine tonic and antiarthritic agent.

The findings of the present study corroborate the description of Unani literature as H.H has been described to possess Neuropharmacological activity and Anti-inflammatory activity (Anonymous, 2001; Kabiruddin, 1967; Lubhaya, 1979).

References


A comparative clinical study of Kabdeen and Lamivudine in Warm-e-kabid haad vairoosi (Acute Viral Hepatitis B)

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Abstract

Warm-e-Kabid Haad Vairoosi (Acute Viral Hepatitis) is usually caused by one or more of the five viral agents. Among all, hepatitis viruses Hepatitis-B virus is one of the most grievous viral infection which may culminate in liver cirrhosis, carcinoma of the liver or fulminant hepatitis leading to death. In Unani Medicine, plants as a whole or their parts are extensively used for the cure of liver derangements. They are likely to be effective and much safer. In the present study a comparative therapeutic evaluation of Kabdeen and Lamivudine in 20 patients of Warm-e-Kabid Vairoosi (viral hepatitis B) was done. The study shows that the Unani Test Formulation Kabdeen produces significant improvement in cases of Hepatitis B. Comparison of Kabdeen with the standard agent Lamivudine shows that in most parameters the latter is more effective while in some parameters Kabdeen is more effective. Regarding the key improvement criterion i.e. Australia Antigen, Kabdeen is shown to clear it in 40% patients. (Lamivudine clears it in 60 % patients).

Key words: Acute Viral Hepatitis B, Kabdeen, Lamivudine, Hepatoprotective Activity, Antiviral Activity

Introduction

Kabdeen, a well-known Unani formulation used in liver disorders was studied for effect on Hepatitis B. Lamivudine was used as the Control Treatment.

In the present study 20 patients were selected randomly suffering from viral hepatitis due to hepatitis B virus infection confirmed by serological test. The patients were divided into two groups of 10 each. Tablet Lamivudine was chosen as a standard drug while Unani formulation Kabdeen as test drug. These drugs were given in both the groups for 60 days and the statistical analysis was carried out at appropriate intervals.

Here it is worth mentioning that our core consideration was to see the clearance of the hepatitis B surface antigen (HBsAg) from the blood in both the groups. Besides this, of course the amelioration in clinical features and biochemical abnormalities were also our focus of study. The diagnosis was made on the basis of clinical features and serological examination.

Materials and Methods

Ethical clearance and consent: The trial was carried out after the approval of departmental ethics committee and informed written consent.

Study location: Study was conducted at outdoor and indoor sections of department of Moalijat, Ajmal Khan Tibbiya College Hospital, Aligarh Muslim University, Aligarh.

Study size: The study included 20 cases of Warm-e-kabid Haad Vairoosi (Acute Viral hepatitis)

Inclusion/Exclusion Criteria: The patients in whom the presenting feature was Jaundice due to acute viral hepatitis B were included in the study. The patients suffering from surgical jaundice, hypothyroidism, hyperthyroidism, liver cirrhosis, and diabetes mellitus, chronic renal failure, nephritic syndrome, using estrogen containing oral contraceptives, chronic alcoholics, and having primary gout, were excluded from the study. Similarly smokers and those taking
hypolipidemic drugs like nicotinic acid statin and cortisone were also excluded from this study.

**Duration of study:** Total duration of study was 60 days.

**Treatment, route and dose:** The clinical trial was concerned with comparison between Unani phytoformulation ‘Kabdeen’ and Allopathic drug Lamivudine in the treatment of Warm-e-kabid Haad Vairoosi. The patients were divided into two groups A (Test) and B (Control) comprising of 10 each. In test group Syrup ‘Kabdeen’ 2 Tea spoon full (10ml) 8 hourly and in control group Tab Lamivudine 100 mg once a day was administered orally.

**Parameters studied:** In the present study clinical/biochemical/serological parameters studied were Jaundice, Anorexia, Nausea and vomiting, Arthralgia and Myalgia, Headache, Fever, Itching, Pain in right quadrant of abdomen, Tender hepatomegally, Dark urine, Clay color stool; Serum Bilirubin, Transaminases, Alkaline phosphatase, Prothrombin time and HBsAg (Australian antigen).

**Statistical Analysis:** The values of the different clinical and biochemical parameters were compared with each other and also with control group. All the results were statistically evaluated by applying paired ‘t’ test for the observation recorded before and after treatment.

### Ingredients of Kabdeen in each 5 ml of syrup

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achillea millefolium Linn (Leaves)</td>
<td>Baranjasaf 160 mg</td>
</tr>
<tr>
<td>Agrimonia eupatoria Linn (Flower)</td>
<td>Gula-e-ghafis 160 mg</td>
</tr>
<tr>
<td>Alpinia galanga Wild (Root)</td>
<td>Khulalanjan 80 mg</td>
</tr>
<tr>
<td>Aquilaria agallocha Roxb (Stem)</td>
<td>Uood-e-Hindi 80 mg</td>
</tr>
<tr>
<td>Butea frondosa Koen.ex Roxb (Flower)</td>
<td>Gula-e-tessoo 160 mg</td>
</tr>
<tr>
<td>Cassia occidentalis Linn (Leaves)</td>
<td>Kasandi 80 mg</td>
</tr>
<tr>
<td>Chamapodium album Linn (Seeds)</td>
<td>Tukhm-e-bathooy 80 mg</td>
</tr>
<tr>
<td>Dichorium intybus Linn (Root)</td>
<td>Bekh-kasni 80 mg</td>
</tr>
<tr>
<td>Dichorium intybus Linn (Seeds)</td>
<td>Tukhm-e-kasni 160 mg</td>
</tr>
<tr>
<td>Lycium sativus (Seeds)</td>
<td>Tukhm-e-khyaren 80 mg</td>
</tr>
<tr>
<td>Cuscuta reflexa Roxb (Seeds)</td>
<td>Tukhm-e-koasoo 80 mg</td>
</tr>
<tr>
<td>Fumaria officinalis Linn (Leaves)</td>
<td>Shahtara 80 mg</td>
</tr>
<tr>
<td>Mesua ferrea Linn (Flower)</td>
<td>Nar mushk 80 mg</td>
</tr>
<tr>
<td>Nymphaea alba Linn (Flower)</td>
<td>Gula-e-teefar 80 mg</td>
</tr>
<tr>
<td>Rheum emodi Wall (Rhizome)</td>
<td>Rewand chini 40 mg</td>
</tr>
<tr>
<td>Rosa damascena Mill (Flower)</td>
<td>Gula-e-surk 40 mg</td>
</tr>
<tr>
<td>Similax aspera Linn (Root)</td>
<td>Ushba maghribi 80 mg</td>
</tr>
<tr>
<td>Solanum nigrum Linn (Leaves)</td>
<td>Mako khusk 160 mg</td>
</tr>
<tr>
<td>Swertia chirata Buch-Ham (Whole plant)</td>
<td>Chiraita shirin 40 mg</td>
</tr>
<tr>
<td>Valeriana jatamansi DC (Root)</td>
<td>Balchar 80 mg</td>
</tr>
<tr>
<td>Jataria multiflora Boiss (Stem)</td>
<td>Satar farsi 80 mg</td>
</tr>
<tr>
<td>Cane sugar (Crystals)</td>
<td>Qand safaid 4.5 G</td>
</tr>
<tr>
<td>Lamivudine (Tablets)</td>
<td>100 mg per tablet (copia)</td>
</tr>
</tbody>
</table>

### Discussion

**Age**

All the patients in this study were between 10-60 years of age. However the maximum incidence was found to be between 20-30 years of age followed by 40-50 years of age. The male gender predominated, the reason may be due to the shaving habits in barber’s shop and untold extramarital relationship (Table 1).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Age group (in years)</th>
<th>Number of Patients</th>
<th>Total No of patients</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11-20</td>
<td>2</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>21-30</td>
<td>5</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>31-40</td>
<td>2</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>41-50</td>
<td>1</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>51-60</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>9</td>
<td>20</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Marital status**

The incidence was definitely more in married that is 12 (60%) as compared to unmarried person that is 8 (40%) the most obvious reason seems to be through sexual transmission in married couples (Table 2).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Marital Status</th>
<th>No. of Patients</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Married</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Unmarried</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

**Jaundice**

In all the groups clinical jaundice was present in all the patients which disappeared completely in both groups after completion of therapy that is 60 days. In the test group the improvement may be due to the presence of Kasni, Mako, Gul-e-Tesu, Revand Chini, Sumbul-uteeb, Tukhm-e-Bathooua and Ood-e-Hindi which are useful in clearing the excess bile pigments from blood (Chopra et al., 1968; Ghazrooni, 1311H; Ibn Baitar, 1291H; Nadkarni, 1986; Dhar et al., 1973; Dhar et al., 1968). In control group Lamivudine, by virtue of its inhibiting HBV
replication and reverse transcriptase activity brought earlier recovery (Wilson et al., 2008; Goodman and Gilman 2001) (Table 3).

### Table 3
**Effect of Drugs on Jaundice**

<table>
<thead>
<tr>
<th>Duration (in days)</th>
<th>Before Treatment (0 Days)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Jaundice</td>
<td></td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>% Impr.</td>
<td></td>
<td>0</td>
<td>20</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Lamivudine n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Jaundice</td>
<td></td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>% Impr.</td>
<td></td>
<td>40</td>
<td>80</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### Anorexia

As regards anorexia, the improvement was seen in 75% of cases in test group and 63.63% in control group. Apparently Lamivudine being a synthetic drug probably the persistence of anorexia is its side effect as compared to Kabdeen which has the digestive and carminative effect of Branasaf, Kasni, Mako, Gul-e-Tesu, Sumbulut Teeb and Satar Farsi (Chopra et al., 1968; Ghani, 1921; Baitar, 1291 H). Therefore, it can be concluded that Kabdeen has maximum beneficial effect in abolishing the anorexia which is a distressing symptoms in patients suffering from jaundice (Table 4).

### Table 4
**Effect of Drugs on Anorexia**

<table>
<thead>
<tr>
<th>Duration (in days)</th>
<th>Before Treatment (0 Days)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen n = 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Anorexia</td>
<td></td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>% Impr.</td>
<td></td>
<td>0.0</td>
<td>12.5</td>
<td>37.5</td>
<td>75.0</td>
</tr>
<tr>
<td>Lamivudine n = 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Anorexia</td>
<td></td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>% Impr.</td>
<td></td>
<td>0.0</td>
<td>18.18</td>
<td>54.54</td>
<td>63.63</td>
</tr>
</tbody>
</table>

### Nausea and vomiting

As far as nausea and vomiting is concerned, 83.33% of cases in test group showed improvement as compare to control group in whom there was recovery in all patients. The improvement in test group showed almost similar pattern which may be due to the carminative and digestive effect of Mako, Kasni, Gul-e-Surkh, Neelofar, Ood-e-Hindi, Mayen kalan and Kibar which are present in the test formulation. (Chopra et al., 1968; Ghani 1921; Baitar, 1291 H; Dhar et al., 1968). As far as the Lamivudine group is concerned there was a definite improvement which is due to its known effect of inhibiting HBV replication and thereby restoring the hepatic function at early stage (Wilson et al., 2008; Goodman and Gilman, 2001) (Table 5).

### Table 5
**Effect of Drugs on Nausea and Vomiting**

<table>
<thead>
<tr>
<th>Duration (in days)</th>
<th>Before Treatment (0 Days)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen n = 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Nausea and Vomiting</td>
<td></td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>% Improvement</td>
<td></td>
<td>0.0</td>
<td>16.66</td>
<td>50</td>
<td>83.33</td>
</tr>
<tr>
<td>Lamivudine n = 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with Nausea and Vomiting</td>
<td></td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Patients Improved</td>
<td></td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>% Improvement</td>
<td></td>
<td>33.33</td>
<td>77.77</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### Arthralgia and Myalgia

Arthralgia and Myalgia are clinically important features of Hepatitis B viral infection as compared to other type of Viral Hepatitis. Those who received Lamivudine had maximum improvement. While in Kabdeen half of the patients showed improvement. This can be explained on the basis that analgesic activity of Mako, Gul-e-Tesu, Revand cheeni, Gul-e-Neelofar, Ood-e-Hindi and Khulanjan present in Kabdeen. The anti-viral effect of Lamivudine seems to be the main cause of amelioration of pain associated with arthralgia and myalgia (Wilson et al., 2008; Goodman and Gilman, 2001) (Table 6).
### Table 6
**Effect of Drugs on Arthralgia and Myalgia**

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kabdeen</strong> n = 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>0.0</td>
<td>25</td>
<td>25</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td><strong>Lamivudine</strong> n = 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>55.55</td>
<td>55.55</td>
<td>66.66</td>
<td>88.88</td>
<td></td>
</tr>
</tbody>
</table>

Impr. = Improvement

### Table 7
**Effect of Drugs on Fever and Headache**

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kabdeen</strong> n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>20</td>
<td>70</td>
<td>70</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Lamivudine</strong> n = 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>36.36</td>
<td>72.72</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

### Fever and headache

The mechanism involved in ameliorating the fever and headache is probably the same as that in arthralgia and myalgia as agents that have analgesic property also have the antipyretic property (Table 7).

### Table 8
**Effect of Drugs on Itching**

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kabdeen</strong> n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Lamivudine</strong> n = 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

### Itching

In both groups there was a complete disappearance of itching. In test group this improvement may be due to Mufatteh Sudud, (Vasodilator), Choleretic and anti-inflammatory action on Kupffer cells thereby facilitating flow of bile due to the presence of Gul-e-Ghafis, Branjasaf, Mako, Kasni, Shahatra, Tukhm-e-Kasooos, Tukhm-e-Bathua, Khuanjan, Gul-e-Surkh (Ghani, 1921; Ibn Baitar, 1291 H) (Table 8).

### Table 9
**Effect of Drugs on Pain in Right Quadrant of Abdomen**

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kabdeen</strong> n = 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>0.0</td>
<td>0.0</td>
<td>16.66</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><strong>Lamivudine</strong> n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>0.0</td>
<td>0.0</td>
<td>20</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>
Tender Hepatomegaly

The mean enlargement below the right subcostal margin was 7 cm each in test as well as in control group which decreases by 71.42% and 85.71% in test and control group, respectively. The basic mechanism that is anti-inflammatory effect on hepatocyte there by decreasing the liver size and release of the tension of hepatic capsules may be the causative factor. The mechanism has already been discussed under the pain in abdomen (Table 10).

Table 10
Effect of Drugs on Tender Hepatomegaly in (cm)
Decrease Liver Size in Percentage

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td>n = 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Decrease liver size</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>0.0</td>
<td>0.0</td>
<td>28.57</td>
<td>71.42</td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>n = 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpable liver (in cm)</td>
<td></td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Decrease liver size (in cm)</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>14.28</td>
<td>28.57</td>
<td>42.85</td>
<td>85.71</td>
<td></td>
</tr>
</tbody>
</table>

Clay Colour Stool

The acute viral hepatitis passes through different stages one of which is the obstructive phase in the initial part of pre-clinical or clinical illness. This stage is due to the edema and obstruction in the flow of bile into the G.I.T which gives stool its characteristic clay color. The regaining of normal stool color is most probably due to the natural and uncomplicated course of illness or due to the anti-inflammatory action of Mako, Kasni, Branjasaf and Ood-e-Hindi which reduce the edema of the intra hepatic bile canaliculi. In control group the similar pattern is observed so, it can be deduced indirectly that the Unani formulation has antiviral activity (Ghani, 1921; Ibn Baitar, 1291 H) (Table 12).

Table 12
Effect of Drugs on Clay Color Stool Total

<table>
<thead>
<tr>
<th>Duration in days</th>
<th>Before Treatment 0 Days</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>25.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient Impr.</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Impr. (in % age)</td>
<td>75.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

L.F.T

Mean Serum Bilirubin

Before starting the treatment the mean serum bilirubin was 5.8 mg % in test group and 9.6 mg %
in control group, respectively, which after completion of the therapy i.e. 60 days, showed 79.32%, and 91.45% improvement, respectively. Greater improvement in control group is obviously because Lamivudine inhibits reverse transcriptase activity of HBV. However, the improvement in the test groups may be due to the anti-inflammatory, cholagogue, cholekinetic, choleric and diuretic action of the drugs like Mako, Kasni, Shahatra, Ood-e-hindi, Kibar and, Myen kalan. There is a definite superiority of Lamivudine in restoring the normal liver function in earlier stage (Table 13).

### Table 13
**Effect of Drugs on Serum Bilirubin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment (0 days)</th>
<th>After treatment (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean serum bilirubin (mg/dl) + SD</td>
<td>5.8 ± 1.69</td>
<td>1.2 ± 1.65</td>
</tr>
<tr>
<td>N = 10, t = 3.0, p &lt; 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean serum bilirubin (mg/dl) + SD</td>
<td>9.6 ± 1.59</td>
<td>0.82 ± 1.83</td>
</tr>
<tr>
<td>N = 10, t = 2.26, p &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Transaminases
The transaminases activity reached within normal limits after 45 days in test groups as well in control group. However, greater reduction was observed in those who received Lamivudine. Improvement was also observed in test group but at a slower pace which may be due to Mako and Kasni which have hepato-tonic effects (Table 14).

### Table 14
**Effect of Drugs on Transaminases**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment (0 days)</th>
<th>After treatment (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ALT (U/l) + SD</td>
<td>93 ± 1.47</td>
<td>24 ± 1.58</td>
</tr>
<tr>
<td>N = 10, t = 2.6, p &lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean AST (U/l) + SD</td>
<td>84.75 ± 1.57</td>
<td>21.44 ± 1.49</td>
</tr>
<tr>
<td>N = 10, t = 2.8, p &lt; 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean AST (U/l) + SD</td>
<td>98.35 ± 1.63</td>
<td>19.73 ± 1.88</td>
</tr>
<tr>
<td>N = 10, t = 3.0, p &lt; 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ALT (U/l) + SD</td>
<td>78.55 ± 1.39</td>
<td>18.23 ± 1.57</td>
</tr>
<tr>
<td>N = 10, t = 3.19, p &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Alkaline Phosphatase
It is evident from the table-15 that maximum effect was seen in control group which clears viral load from blood due to its antiviral and reverse transcriptase activity. In test group the improvement may be attributed to the presence of Mako, Kasni, Branjasaf and Kasundhi which have diuretic anti-inflammatory and hepatotonic actions.

### Table 15
**Effect of Drugs on Alkaline Phosphatase**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment (0 days)</th>
<th>After treatment (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Serum alkaline phosphatase (K.Au/ml) + SD</td>
<td>19.88 ± 1.44</td>
<td>7.2 ± 1.79</td>
</tr>
<tr>
<td>N = 10, t = 4.09, p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Serum alkaline phosphatase (K.Au/ml) + SD</td>
<td>19.45 ± 1.56</td>
<td>6.21 ± 1.74</td>
</tr>
<tr>
<td>N = 10, t = 2.97, p &lt; 0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Prothrombin Time
The synthesis of coagulation factors is one of the most important function of liver which may be deranged partially or near completely in any form of viral hepatitis especially hepatitis B viral infection. It is very sensitive indicator of liver function. Its degree of prolongation is directly proportional to the extent of liver damage. While going through the above tables it will be observed that mean Prothrombin time reached within the normal limits only in the control group. However there is also an improvement in test group. These results indicate that there is every likelihood that if the duration of the study would have been longer Prothrombin time would also have reached within normal limits i.e. around 14 seconds in test group too. However, the progression of PT towards normal limits in test group may be due to the presence of Mako, Kasni, Branjasaf, Gul-e-Tesu and Chiraita. Obviously Lamivudine induces rapid recovery thereby bringing the prolonged Prothrombin time within normal limits in a comparatively shorter duration (Table 16).
Table 16
Effect of Drugs on Prothrombin Time

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment (0 days)</th>
<th>After treatment (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean prothrombin time in sec. (PT) + SD</td>
<td>20.11 + 1.88</td>
<td>15.66 + 1.75</td>
</tr>
<tr>
<td>N = 10, t = 2.84, p &lt; 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean LDH (I.U) + SD</td>
<td>20.66 + 1.79</td>
<td>14.01 + 1.54</td>
</tr>
<tr>
<td>N = 10, t = 3.13, p &lt; 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HBsAg (Australia Antigen)

As already mentioned only HBsAg (Australia Antigen) positive cases were included in the study. Besides noting overall improvement in signs, symptoms and liver function, our main concern was to know whether any of these drugs convert HBsAg +ve patients to HBsAg –ve status. From observations it may be noted that none of the cases became HBsAg –ve during 60 days treatment. Moreover, some encouraging results are definitely observed that is in the kabdeen group 6 patients remain positive while in lamivudine group 20% patients remained positive respectively. It cannot be clearly deduced that whether this was a natural course of disease or it was because of drugs.

Statistically lamivudine group produced the maximum negativity in HBsAg positive subjects. This may be because of its known action of reversing transcriptase activity of HBV. Kabdeen may be clearing the Antigen due to Anti-Viral Effect or more likely by Immunomodulating Effect (Table 17).

Table 17
Effect of Drugs on HBsAg (Australia Antigen)

<table>
<thead>
<tr>
<th>Duration in Days</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabdeen n = 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>10</td>
<td>6 +ve</td>
</tr>
<tr>
<td>No. of Patients</td>
<td>10</td>
<td>4 +ve</td>
</tr>
<tr>
<td>No. of Patients Improved</td>
<td>--</td>
<td>05</td>
</tr>
<tr>
<td>% age of improvement</td>
<td>--</td>
<td>60</td>
</tr>
<tr>
<td>No. of Patients Improved</td>
<td>--</td>
<td>04</td>
</tr>
<tr>
<td>% age of improvement</td>
<td>--</td>
<td>40</td>
</tr>
</tbody>
</table>

References

6. Ghazrooni S, Al-Sadeedi, Part III, Munshi Naval Kishore, Lucknow, India, 1311H.
Efficacy of Irsa (*Iris ensata*) in the management of Iltehabe unqur rehm (Cervicitis): a clinical trial with standard chemotherapeutic regimen as control treatment

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Abstract

To study the efficacy of Irsa (*Iris ensata*) in the management of Iltehabe Unqur Rehm (cervicitis). This study was a randomized single blind standard controlled trial. All the patients were randomly allocated to test and control group (30 patients in test group and 15 patients in control group). Irsa was given in the form of majoon, 10 gm in two divided doses after menses for 15 days for three cycles. Extract of Irsa (10ml) was used locally in the form of humool (pessary) OD after menses for 15 days for three cycles. In the control group tablet doxycycline 100 mg BD was given orally after menses for 7 days for three cycles. Vaginal pessary of a combination of clindamycin, clotrimazole, and metronidazole OD was given locally after menses for 7 days for three cycles. All the patients were assessed by subjective parameters and per speculum examination once in fifteen days for three cycles. The results were analyzed statistically using Chi Square and Paired Student’s t test. Patients treated with the test drug showed a cured and relieved rate of 76.7%, partially cured and relieved rate was 16.7 % and not cured and relieved rate was 6.7% in comparison to control group where the results were found to be 33.3%, 26.7 % and 40 %, respectively (p<0.01). The study revealed that the test drug is effective in management of Iltehabe Unqur Rehm (Cervicitis).

Key words: Iltehabe Unqur Rehm, Cervicitis, Irsa, *Iris ensata*, Doxycycline

Introduction

Iltehabe unqur rehm (cervicitis) is very common, affecting more than half of all women at some point during their adult lives. Intercourse at an early age, high risk sexual behaviour, multiple sexual partners, and a history of sexually transmitted diseases increases the risk of cervicitis. Cervicitis may be acute or chronic and each of this acute and chronic cervicitis may be from non infective and infective causes (Esan OGO *et al*., 2006; Christine Navarro *et al*., 2002; Smith RP, 2002).

According to unani system of medicine Iltehabe unqur rehm may be Iltehabe har or Iltehabe barid. Iltehabe har is due to the domination of hot humours mainly safra and dam and Iltehabe barid is due to the domination of balgham. It may also arise following trauma, after abortion, difficult labour, delivery conducted in unhygienic conditions and excessive intercourse (Kabiruddin, 2003; Razi, 2001; Majoosi, 1889). Iltehabe unqur rehm can be caused by “Sue mizaj”. When Sue mizaj inflicts any organ, it results in certain changes in the functions of that organ and these aberrant changes leads to derangement in the normal functioning of intrinsic faculties which manifest in the emergence of diseases (Ibn Rushd, 1987; Hamdani, 2006; Ibn Sina, 2007; Razi, 2000; Kabir uddin, 1916).

In Iltehabe unqur rehm the usual manifestations which occur singly or in combination are vaginal discharge, backache, lower abdominal pain, dysuria, dyspareunia etc. On examination, cervix
is congested, hypertrophied with velvety appearance. Ectropions are present which may be inflamed and bleeds on touch; nabothian follicles are present on the cervix, and tender to touch with exudation of mucopurulent, opaque or clear discharges from the cervical os.

Prevalence rate is 10 to 40%. Initial infection of PID begins with cervicitis predominantly between the ages of 15 and 44 years. Gonococcal infection remains a major health problem, as more than sixty million cases are reported annually worldwide. Genital infection due to Chlamydia trachomatis is one of the most prevalent STD worldwide (Smith RP, 2002; Nichols DH and Clarke PDL, 2000). This disease is differentiated from vaginitis, cervical neoplasia, cervical metaplasia, cervical erosion, syphilitic ulcer and tuberculosis of cervix.

There are certain complications associated with this disease such as untreated chlamydial infection can lead to pelvic inflammatory diseases in affected women. PID can result in infertility, ectopic pregnancy, chronic pelvic pain, cervical atypia and neoplasia, salpingitis, leucorrhoea, cervical lacerations, chronic infection, cervical haemorrhages and cervical stenosis. It can complicate the pregnancy by premature rupture of membranes, premature labour.

Group of drugs are ascribed to be Mohallil (anti inflammatory), Musakkin (analgesic), Mufatteh (deobstruent), Mulattif (demulcent), Musaffi (blood purifier) Munaqqi (expectorant) in unani medicine which are frequently used in the management of Iltehabe Unqur Rehm, Irsa is one such drug. By careful forage into unani literature, Irsa was selected for the trial. Irsa is one such drug which has been extensively described in unani literature to possess Mohallil warm (anti-inflammatory), Mulattif (demulcent), Mufatteh (deobstruent), Munzij (concoctive), Musaffi (purifying), Jali (detergent) and Mushil safra wa balgham (bilious and phlegmatic purgatives) properties (Anonymous, 1987; Kabiruddin, ynm; Ghani, ynm). The present study was designed therefore to investigate the clinical efficacy of Irsa in the patients of Iltehabe Unqur Rehm.

Materials and Methods

Before embarking upon the project, a comprehensive protocol was checked out and put forth for clearance from the institutional scientific ethical committee of NIUM, Bangalore. After ethical clearance, clinical study was started.

Standard controlled randomized single blind study had been undertaken in the department of Ilmul Qabalat wa Amraze Niswan NIUM, Bangalore. The study group included 45 patients (30 in test group and 15 in control group) having the complaint of vaginal discharge, lower abdominal pain, low back ache, dysuria, dyspareunia etc.

Criteria for selection of the patients

Diagnosis of cervicitis was based on the following criteria:

- Exudation of mucopurulent, mucoid, cloudy, white or curdy discharges from the cervical canal.
- Hypertrophied and congested cervix.
- Presence of nabothian follicles.
- Velvety appearance of the cervix.
- Tenderness of the cervix on touch.

Following investigations were carried out in each patient.

Specific Investigations:

- USG-Pelvic: To exclude the pelvic pathology.
- Pap smear: It is a medical procedure in which a sample of tissue from cervix is collected and spread on a slide. The cells are examined under a microscope for pathologic changes.
- Cervical swab culture:- The ectocervix wiped clean with a large swab and samples of endocervical secretions obtained using the microloop technique than smeared directly onto slides for screening of infectious organism. It is repeated after treatment in those patients who have positive before.

Diseases such as VDRL, HIV I and II etc was excluded by using specific tests on the patients.

Safety Profile:

The ESR, LFT and RFT were used as safety parameter once at baseline and once after completion of trial to ensure the safety of the test drug.

Routine Investigations:

Hb%, TLC, DLC, ESR, RBS and CUE were done once at baseline and repeated after completion of trial.

Criteria for selection of the drug

The test drug Irsa (Iris ensata) was provided by the pharmacy of National Institute of Unani Medicine. Before preparing the formulation, drug was properly identified from Regional Research Institute (Ay.) Bangalore, (RRCBI/Mus. 5-39).
Dosage of the test drug

Irsa was given in the form of majoon, 10 gm in two divided doses after menses for 15 days for three cycles. Extract of Irsa (10ml) used locally in the form of humool OD after menses for 15 days for three cycles.

Preparation of Majoon

Qiwm (sugary base) was prepared from sugar. The drug was powdered separately and added in the qiwm and mixed well.

Preparation of extract

The powder was extracted in Distilled Water in a ratio of 1:5 (100 gm of powdered drug was taken into 500 ml of Distilled Water) with the help of soxhlet apparatus. Thereafter the extract was filtered and concentrated on water bath.

Standard Drug:

Tablet Doxycycline 100mg BD was given orally after menses for 7 days for three cycles. Vaginal pessary of clindamycin, clotrimazole, and metronidazole OD was given locally after menses for 7 days for three cycles.

Criteria for assessment of response of treatment:

On the basis of clinical relief and cervical swab culture findings before and after the treatment. The response was graded as follows:

- Therapeutic outcome.
- Symptomatic relief.

Therapeutic outcome: The therapeutic outcome was assessed by objective parameters i.e. Per speculum and cervical swab culture findings after the treatment.

On per speculum examination hypertrophy, congestion or redness, naboths etc should disappear considerably. The discharge should also diminish noticeably. The culture report which was positive in some patients should become negative such an improvement is regarded as cured.

Discharge was graded as: (Willmott FE, 1988)

1. No Discharge: ( - )
3. Moderate (++): Opaque (cloudy or curdy).

Congestion was graded as: ( Jian Xin Zhang et al., 2006 )

- Absent( - ): No congestion
- Mild (+): Congestion either around the os or upper lip or lower lip.
- Moderate (++): Congestion of whole cervix.
- Severe (+++): Congestion of whole cervix which bleeds on touch.

Hypertrophy was graded as: (Field ML and Coats AJS, 1999)

- Not Present: (-)
- Mild (+): Hypertrophy of cervix with patulous os.
- Moderate (++): Hypertrophy of cervix with slightly patulous os.

Scoring System for Overall Evaluation of Each Patient: The Pathological Status of each Patient was evaluated before administering the Treatment (Baseline) and after the Treatment (Post-Treatment), and expressed by a Scoring System. Scores of 3, 2, 1 and 0 were given for Parameters graded as ++++, ==, + and - . The Scores for all Parameters observed in each Patient were then added up. The % decrease in scores was determined by comparing the Baseline and Post-Treatment Scores.

Improvement Criteria:

The Improvement of Patients after the Test and Control Treatment was judged as Relief on the basis of Subjective Parameters and as Cure on the basis of Objective Parameters.

The Patients were considered Relieved and Cured as follows:

Relief: The Response was characterized as: (Trabuco EC et al., 2007)

- ≥83 % relief in all the parameters present at first visit is considered cured.
- 18-82 % relief in all the parameters present at first visit is considered partially cured.
- ≤ 17 %relief in all the parameters present at first visit is considered not cured.

Symptomatic relief

The discharge is classified as mild, moderate and severe. (Padubidri VG and Daftary SN, 2008)

- Mild (+): The normal moistness of vagina without staining or moistening the underclothes.
- Moderate (++): The underclothes are undeniably soiled require changing and washing frequently.
Severe (+++): Requires the wearing of some extra absorbent pads.

Pruritis vulva is classified as: (Akhyani M et al., 2005)
- none (-), mild (+), moderate (++) or severe (+++)

Dysuria is classified as: (Marickar YMF and Salim A, 2009)
- none (-), mild (+), moderate (++) or severe (+++)

Dyspareunia, backache, lower abdominal pain, was assessed by Visual Analog Scale as: (Wei Che Lin et al., 2005)
- none (-), mild (+), moderate (++) or severe (+++)

Assessment of Socioeconomic status: Socioeconomic status was assessed by modified Kuppuswamy’s classification scale (Kumar N et al., 2007).

At last visit, the patients were asked about the progression and regression of symptoms and Improvement Criteria:

Cure: The response was characterized as: (Trabuco EC et al., 2007)
- ≥77.7% relief in all the symptoms present at first visit is considered as relief.
- 18-76% relief in all the symptoms present at first visit is considered as partially relieved.
- ≤17% relief in all the symptoms present at first visit is considered not relieved.

Follow up:
- The patients were examined every fifteen days for three cycles, during which the clinical evaluation of the disease and related signs and symptoms and information about concomitant medication was also obtained.
- Initial symptoms and specific findings were recorded in case proforma at first visit. At every visit, the patients were asked about the progression and regression in their symptoms.
- At last visit, clinical examination and specific investigation were performed. Pre and post treatment values of symptoms and signs were analyzed and were subjected to comparison statistically to evaluate the response or effect of the treatment.

After completion of the trial patients was advised for follow up. At the follow up visit, complaint of vaginal discharge and other symptoms were enquired and the speculum examination was repeated.

Criteria for withdrawal of patients:
- Failure to follow protocol.
- The case in which adverse drug reaction is notice.

Results
1. Seventy nine patients were enrolled in the study. Seven were excluded, twenty seven denied and forty five were completed the trial (30 patients in test group and 15 patients in control group)

2. Our study revealed that Iltehabe Unqur Rehm more prevalent in the age group of 26-30 years (Table 1).

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Test group</th>
<th>Control group</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20</td>
<td>1</td>
<td>1</td>
<td>2 (4.40)</td>
</tr>
<tr>
<td>21-25</td>
<td>9</td>
<td>1</td>
<td>10 (22.20)</td>
</tr>
<tr>
<td>26-30</td>
<td>12</td>
<td>5</td>
<td>17 (37.80)</td>
</tr>
<tr>
<td>31-35</td>
<td>6</td>
<td>6</td>
<td>12 (26.70)</td>
</tr>
<tr>
<td>36-40</td>
<td>2</td>
<td>2</td>
<td>4 (8.90)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>15</td>
<td>45 (100)</td>
</tr>
</tbody>
</table>

3. The maximum number of patients with cervicitis belonged to upper lower and lower middle socio-economic groups (Table 2).

<table>
<thead>
<tr>
<th>Socio-economic status</th>
<th>No. of patients</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper (I)</td>
<td>2</td>
<td>3 (6.70)</td>
</tr>
<tr>
<td>Upper Middle (II)</td>
<td>2</td>
<td>5 (11.10)</td>
</tr>
<tr>
<td>Lower Middle (III)</td>
<td>13</td>
<td>18 (40)</td>
</tr>
<tr>
<td>Upper Lower (IV)</td>
<td>13</td>
<td>19 (42.20)</td>
</tr>
<tr>
<td>Lower (V)</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>

4. The maximum numbers of patients were of damvi temperament. (Table 3).
Table 3
Distribution of patients according to Mizaj

<table>
<thead>
<tr>
<th>Mizaj</th>
<th>No. of patients</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test group</td>
<td>Control group</td>
</tr>
<tr>
<td>Damvi</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Balghami</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Safarvi</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Saudavi</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

5. The maximum numbers of patients with cervicitis have ≥3 children. (Table 4).

Table 4
Distribution of patients according to Parity

<table>
<thead>
<tr>
<th>Parity</th>
<th>No. of patients</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test group</td>
<td>Control group</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>≥3</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

1. The patients responded immediately after giving the treatment, effect is long lasting (Table 5 and 6).

2. Statistically the test group shows 23 (76.7 %) patients were relieved, 5 (16.7%) partially relieved and 2 (6.7 %) not relieved. In the control group 5 (33.3%) patients were relieved, 4 (26.7%) partially relieved and 6 (40%) not relieved (Table 7).

3. Out of 45 patients were relieved, 4 (26.7%) partially relieved and 6 (40%) not relieved (Table 7).

4. Out of 45 patients 23 (76.7 %) were cured, 5 (16.7 %) partially cured and 2 (6.7 %) not cured. In the control group 5 (33.3%) patients were cured, 4 (26.7%) partially cured and 6 (40%) not cured (Table 8).

5. Student t test (paired) has been used to find the homogeneity of parameters. Here P.val > 0.05, it is considered non-significant so the drug has not any toxic effect on safety parameters.

6. No adverse effects of the drugs were noticed or reported by the patients after use.

7. There was no recurrence of similar complaint after the withdrawal of treatment.

8. It can be inferred that the research drug has affected on the clinical parameters through its effect on cervicitis.

Table 5
Effect of Test drug on Subjective Parameters

<table>
<thead>
<tr>
<th>Symptoms (Subjective Parameters)</th>
<th>No. of cases</th>
<th>%age</th>
<th>No. of cases</th>
<th>%age</th>
<th>No. of cases improved</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge P/V</td>
<td>30</td>
<td>100</td>
<td>17</td>
<td>56.7</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td>Abnormal vaginal Odour</td>
<td>9</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>Low Backache</td>
<td>30</td>
<td>100</td>
<td>14</td>
<td>46.7</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>Lower Abdominal Pain</td>
<td>29</td>
<td>96.7</td>
<td>8</td>
<td>27.6</td>
<td>21</td>
<td>72.4</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>14</td>
<td>46.7</td>
<td>2</td>
<td>14.3</td>
<td>12</td>
<td>85.7</td>
</tr>
<tr>
<td>Dysuria</td>
<td>22</td>
<td>73.3</td>
<td>3</td>
<td>13.6</td>
<td>19</td>
<td>86.4</td>
</tr>
<tr>
<td>Post Coital Bleeding</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Pruritis vulvae</td>
<td>25</td>
<td>83.3</td>
<td>5</td>
<td>20</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>
Table 6
Effect of Test drug on Objective Parameters

<table>
<thead>
<tr>
<th>Objective Parameters</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>No. of cases improved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>%age</td>
<td>No. of cases</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>30</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Congestion</td>
<td>30</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>Discharge</td>
<td>30</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Nabothian Cysts</td>
<td>14</td>
<td>46.7</td>
<td>1</td>
</tr>
<tr>
<td>Velvety Appearance</td>
<td>9</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Cervical Swab.Culture</td>
<td>5</td>
<td>16.7</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 7
Symptomatic relief

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relieved</th>
<th>Partially relieved</th>
<th>Not relieved</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Group (N=30)</td>
<td>23</td>
<td>5</td>
<td>2</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>Control Group (N=15)</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Table 8
Therapeutic relief

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relieved</th>
<th>Partially relieved</th>
<th>Not relieved</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Group (N=30)</td>
<td>23</td>
<td>5</td>
<td>2</td>
<td>&lt; 0.02**</td>
</tr>
<tr>
<td>Control Group (N=15)</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Present standard controlled randomized single blind study was conducted during 2009-2010 in the National Institute of Unani Medicine to evaluate the efficacy of unani formulation in the management of Iltehabe unqur rehm. The study aims to analyze the improvement in cervicitis in terms of objective cure and symptomatic relief.

The results of the study revealed that Irsa is effective in relieving the symptoms and signs of cervicitis. Both the subjective and objective parameters were found to be significantly...
improved suggesting that the local and systemic administration of Irsa is effective in the management of Iltehabe unqur rehm.

On the other hand Patients in the Control Group given the usual Chemotherapeutic Treatment (Oral Doxycycline, Topical Clindamycin + Clotrimazole + Metronidazole) were improved to a significantly lesser extent.

Relief in abnormal vaginal odour and purities vulvae is due to antibacterial and antifungal activity of test drug (Scalbert A, 1991). It may also be due to Musaffi and Jali property of Irsa as malodour is produced due to ufoonat (Kabiruddin, ynm; Maltaani HC, ynm). Mudirre bole and Mohallil warm properties of the test drug possibly played a role in improving dysuria (Anonymous, 1987; Kabiruddin, ynm). Irsa has been reported to possess tannins and flavonoids which inhibit the growth of many microorganisms like bacteria, fungi, yeast etc and relieve inflammation (Scalbert A, 1991; Nair Muraleedharan G et al., 2004) and also helps in inhibition of biochemical pathways related to pain or inflammation transmission. It has also anti-inflammatory and antibacterial properties which inhibit the growth of microorganisms thus preventing the source of discharge (Anonymous, 1987; Scalbert A, 1991).

As described earlier that apart from other factors domination of akhlat (humours) particularly safra and balgham is the major cause of Iltehabe unqur rehm. Since Irsa possesses Mushil safra wa balgham properties therefore it is more likely that it induced tanqya (removal) of dominant causative khilt and also relieved the pressure, hypertrophy and congestion. Due to Mufatteh-e-sudad (deobstruent) property of the test drug nabothian cysts were relieved (Anonymous, 1987; Kabiruddin, ynm). Our findings that majority of the patients included in the study belonged to upper lower (IV) class is in consonance with the Unani description that that malnutrition and poor sanitation which is common in low socio-economic group are the predisposing factors of Iltehabe unqur rehm (Kabiruddin, 2003; Razi, 2001; Majoosi, 1889). Low income, minor communities have higher risk than higher income community (Christine Navarro et al., 2002).

Relevance of Iltehabe unqur rehm and mizaj in the study can be inferred by the fact that maximum number of patients belonged to the reproductive age group. In these age groups (sine namoo and sine waqoof wa sine shabab) mizaj is usually har, it can be sometimes har ratab or har yabis. Khilt dam has har ratab mizaj and khilt safra has har yabis mizaj (Hamdani, 2006).

Maximum number of patients included in the study had ≥3 children. According to unani literature ibtedae jimah (early age of coitus) is included in the aetiology of Iltehabe unqur rehm and it is also associated with multiparty (Kabiruddin, 2003; Razi, 2001; Majoosi, 1889). It is also given that nulliparity was protective against infection and early age of sexual debut is also one of the risk factor (Christine Navarro et al., 2002).

Symptomatic relief in every patient was assessed and categorized as ≥ 77.7% relief in all the symptoms present at first visit is considered as relieved. 18-76% relief in all the symptoms present at first visit is considered as partially relieved and ≤ 17 % relief is considered not relieved and P.value < 0.01 for both the observations which is statistically significant. (Chi Square test)

Diagnosis and assessment of cure was done on the basis of speculum examination and cervical swab culture. It was assessed and categorized as ≥ 83 % relief in all the parameters present at first visit is considered cured, 18-82 % relief in all the parameters present at first visit is considered partially cured and ≤ 17 %relief in all the parameters present at first visit is considered not cured and P.value <0.01 for both the observations which is statistically significant. (Chi Square test)

On the basis of above observations it can be concluded that this drug is very effective in relieving the symptoms and signs of cervicitis. It is much more effective than the existing standard chemotherapeutic treatment of Western Medicine. The test drug is cheaper, easily available and well tolerated by the patients without having any side effects.

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