Editorial

Unani journals should play leading role in Unani Medicine’s development

Tajuddin

The task of bringing out a journal of international standards is tremendously arduous as it entails a lot of complexities. The problem compounds when one has to envisage the publication of a journal of Unani Medicine, because of the paucity of a representative journal that may help to provide a lead and insight for proceeding to start a new journal and also because of the shortage of personnel well-equipped with the art of scientific writing and its presentation. Carving out a design from the whole ethos of Unani Medicine and with limited but committed human resources remains the only option in such a situation. Thus, from preparing the blueprint to setting the standard for a journal devoted to the cause of Unani Medicine, everything has to be done to launch a journal of Unani Medicine.

The information generated out of the new data or collected from existing one fails to transform into a body of knowledge, if not shared, reviewed and subjected to the scrutiny of peers and finally published. Publication makes the findings accessible to all, and invites comments, reactions, suggestions etc and provides a forum for debate that helps ultimately in rectifying the shortcomings and leads to further improvement of the clarity of the concepts and paradigms, and to the final goal of finding the truth.

It is being realized that there is little space available for the publication of works related to Unani Medicine in main stream journals of medical sciences because of their peculiar outlook and inflexible policies not to accommodate the works that are not in full commensuration with their paradigm and scheme of things. Unani scholars, therefore, frequently compromise and content themselves with the minimum description of Unani concepts, terminologies, methodologies and the underlying theories and philosophies of a concept, in order to publish their work. They communicate their research findings mostly in respect of the efficacy of Unani drugs without discussing the Unani theories and the rationale behind them. In the process they sometime misinterpret the Unani view points. Such a work never finds a platform for further debate and thus fails to add an iota of information to the existing stock of knowledge. Since, the outlook of Unani Medicine towards health and diseases is different from other medical systems specially the modern medicine, therefore, it requires relatively different research methodology to design a study, verify a hypothesis, analyze the data and arrive at a conclusion. It is obvious, therefore, that works designed according to Unani principles, will not find proper space for publication in the journals of modern medicine. Further the topics related to the fundamentals of Unani Medicine such as Mizaj, Arkan, Arwah etc., and Usule Ilaj, Ilaj Bittadbeer, Zulkhassah, Surate Nauyeeya and many more, will hardly find a place for publication. It means that the debate on such important topics cannot be started.

Unani scholars have been engaged in research activities with modern overtones for nearly a century on various aspects of Unani Medicine. A number of scientific studies have been undertaken during this period but unfortunately very few of them have been published and that too in the journals of relatively low impact factor. Thus, the entire exercise of conducting research is not yielding the desired results and not succeeding in improving the theoretical knowledge and its practical application. Therefore, the publication of a journal of international standard devoted to the cause of Unani Medicine becomes inevitable.

The Faculty of Unani Medicine, Aligarh Muslim University at Aligarh is happy to take the initiative to start such a journal, namely, Unani Medicus. This journal will provide sufficient space for the publication of the works conducted on a topic related in any way to Unani Medicine. It will also provide an international forum for debate on Unani topics and also for collaboration with other sciences and bodies of intellectuals, scientists and researchers of other traditional medicines as well as the modern medicine and allied sciences. It will accommodate the works of the scholars of Unani and other traditional medicines and give equal importance to the works of modern scientists if their work helps improve the understanding of Unani Medicine and its various components and products. Therefore, the work related to Pharmaceutics, Medicinal Chemistry, Pharmacognosy, Phytochemistry, Pharmacology, Pharmacy, Clinical trials, Hospital management, Physiotherapy, Pharmacogenomics, Biotechnology, Pharmacovigilance, Biostatistics etc. may be included in this journal. In light of the importance of what can be called literary research, such historical, philosophical and other type of theoretical works as well as reviews of poorly known aspects of classical Unani information and recent advances are also welcome.

I hope the publication of this journal will begin a new chapter in the history of Unani Medicine and go a long way to bring back the glory of its golden past.
Unani medicine should occupy centre stage by rediscovery and research

Kunwar Mohammad Yusuf Amin

Unani Medicine has been finally noticed by the contemporary world. Now, it should move to centre stage. The time has come for Traditional Medicines (TMs) to move from the Complementary slot to the centre. Unani Medicine, the only transnational TM, should obviously form the nucleus of Traditional Medicines. It has to be at the centre for other reasons too: more balanced Philosophical & Scientific dimensions; greater but not over riding place for Chemistry; more systematic theory; clear principles for correlation with Western Medicine; out puts to and in puts from nearly every Traditional Medicine etc.

It is in the World’s interest to make pervasive use of Unani Medicine. The WHO recommends Unani Medicine chiefly because of its accessibility to the masses. But scholars like Gruner strove to preserve it as a perfect medicine offering things which Western Medicine can never provide. So, Unani Medicine should be used in a big way and for big reasons. Though, already fit for that role, it has to be made fitter still. This will be done chiefly by Unani physicians and scientists.

The advancement of Unani Medicine requires educating the World to appreciate its existing superiority and abilities and to use them in a big way. It also requires research – both traditional and modern – to improve it. But, more than that it needs to be rediscovered by most of its practitioners themselves. Although, the contemporary Unani educational system has on the whole succeeded in capturing and transferring the traditional character of Unani Medicine but certain misinterpretations and misunderstandings, as well as, lacunae, do remain in the teaching of Unani Medicine according to its true and complete traditional character.

The World, particularly its policy makers, need to be educated that Unani Medicine, indeed all Traditional Medicines, are much wider than Western Medicine. They use both the philosophical as well as the scientific method in studying and describing Man, his Environment and Therapy, whereas, Western Medicine uses only the scientific method. By the twin methods of Philosophy and Science, Unani Medicine discovers and manipulates both the physical and the supra-physical level of Man and Drug. Since, the supra-physical level is simpler it can be grasped, at least in its outlines, in the whole by - what can be called for simplicity’s sake as – the philosophical method. Since, the physical correlates or indices of the supra-physical entities have also been envisioned and described by ancient sages, therefore, the physician can grasp these supra-physical entities by observing their physical correlates. Secondly, the physical level is described within the framework of the supra-physical descriptions. So, the latter too is known as a whole. Thus, Man, Disease and Drug are known as a whole, twice over: by knowing both the levels of these entities and by knowing each level as a whole. This allows total contrast matching of disease and drug. For instance, a cold and wet disease is treated by a hot and dry drug.

Every one hails Traditional Medicines for their Holism and the resultant safety of treatment and radical cure of disease. But few appreciate that this Holism is dependent upon TM’s ability to describe both the physical and supra-physical levels, each as a whole. If they would understand that the much prized Holism of Unani Medicine depends upon its supra-physical descriptions, they would lay greater stress on them. They would also not demand ‘scientific’ proof of these entities, such as, the Arwah (Pneuma) and even of Akhlat (Humours). It is the clinical implications of these entities that are to be tested empirically (scientifically). But they as such have been discovered by Intuition and developed by the philosophical method, so it is illogical to demand their scientific proof. Thus, it can be appreciated how crucial it is to educate people regarding the traditional character of Unani Medicine so that the society and governments may make demands and apply policies that would help Unani Medicine to advance with its practically valuable traditional character rather than make it a caricature of Western Medicine and in the process unwittingly strike a deadly blow to the unique and valuable healthcare contributions of this system of medicine.

Research is undoubtedly important for Unani Medicine’s advancement but its profound rediscovery is more important. For the simple reason that its extremely valuable past and present performance depends upon its traditional character. So, while research may add new dimensions to it, a completely satisfactory knowledge of its traditional character will stabilize and maximize its existing core contributions whose immense worth and value is known and sought after by every one.
This requires many things: proper and effective teaching of Falsafa (Traditional Islamic Philosophy), crucial to the understanding of classical Unani Patho-physiology, Pharmacology etc.; improvement of Urdu, Arabic and Persian proficiency of students to ensure full access to classical texts; publication of rare classical books and manuscripts; their translation; greater enrolment in Unani Colleges of the products of Oriental Colleges who are proficient in classical languages and philosophy; compilation of the Mujarrabat genre of traditional Unani texts that include secondary pharmacological actions and therapeutic effects discovered by Unani physicians down the ages including the Nineteenth and Twentieth Century; preparation of better text-books etc.

Research, though important for Unani Medicine, will play a positive role only if it is tailored to the unique and very different character of this system of medicine. Otherwise, a blind imposition of Western Medicine’s research methods will play havoc with it. This fear is not hypothetical. Indiscriminate research has already done damage to Unani Medicine. For instance, the extreme quantitative nature of modern research has put focus on individual natural drugs and even on their compounds. This has led to the abandonment of traditional therapeutic ‘packages’ eg Munzij-Mushil (Cocction-Purgation) pretreatment before administering specific anti-arhritic drugs. Thus, research is necessary but its appropriateness to Unani Medicine is more important. Given the fundamental differences between Unani and Western Medicine, as well as, significant differences in practical circumstances, very critical and careful thinking is needed to pick appropriate elements from modern research methodology, adapt them and to amalgamate them with traditional Unani research methodology. It should not be forgotten that Unani Medicine has very elaborate and sophisticated traditional research methodology.

Having emphasized the need for carefully picking and choosing from modern research methodology, its importance and value for Unani Medicine cannot be denied. In addition to rather ‘technical’ applications e.g. efficient drug identification by pharmacognosy, physico-chemical standardization and quality control of drugs, pharmaceutical improvement of traditional drug forms, modern instrumentation in traditional diagnostics and pharmaceutical manufacture, checking for microbiological load and heavy metal contamination of crude drugs, minimal animal toxicity testing etc modern research methodology can also be used for some ‘rational’ sort of purposes.

Although, diseases have to be conceived according to Unani patho-physiology and therapeutic decisions should be primarily made on the basis of Unani pharmacological principles such as the Mizaj (Temperament) of the Drug, but later, fine tuning can be done on the basis of molecular actions of these drugs. For instance, for the treatment of Balghami (Phlegmatic) type of Arthritis, Hot and Dry Drugs should be used. However, out of the list of say 5 such drugs the final selection may be made on the basis of appropriate molecular actions. This calls for studying important Unani drugs for biochemical and molecular actions.

A very important application of modern research seems to be revalidation and sub-typing of Unani Drugs by Clinical Trials and Observational Studies. Although, Unani Drugs have been traditionally validated by clinical study but Clinical Trials seem to be an improvement upon traditional methods. So, revalidation by these more rigorous and precise methods, not to talk of the discovery of new details of their therapeutic behaviour, has considerable value.

The above task i.e. Clinical Trial would also create a place for experimental pharmacology which would be needed for validating and ranking the Unani drugs, and working out their pharmacological and safety profile, in order to select agents with very high chances of being successful in this long and expensive research exercise. Secondly, the relatively easier Clinical Observational Studies of Unani treatments will also help in revalidation and new information generation.

Unani Medicus aims to disseminate works of both research and rediscovery. It hopes to strengthen processes that will give central position to Unani Medicine. By these means, it wishes to make its contribution to the reform and revitalization of Healthcare in the present age. Lastly, we acknowledge the contribution of Prof. IH Zaidi who paved the way for the emergence of this Journal. I also affectionately acknowledge the hard work of my students, Saba Viqar, Azizur Rahman and Sumbul Rehman in launching the first issue of Unani Medicus.
Relationship between Huihui Yaofang and Unani Medicine: correlation of Hui Hui Prescriptions and certain ancient Islamic medical works

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Abstract

Many prescriptions in the the 14th century, ancient Chinese medical book Huihui Yaofang are same as the ones from The Canon of Medicine (“Al-Qanun fi al-Tibb”) by Avicenna (Ibn Sīnā) and that in the medical books written by Rhazes (Muhammad ibn Zakariyā Rāzī). In Huihui Yaofang, the entire description of fractures is exactly the same as in “On Fractures” by Hippocrates, and the management is the same as that of Aristotle to his student, Alexander the Great, and that of many Greek and Roman Doctors. The Unani Medicine has, therefore contributed to the development of Traditional Chinese Medicine very significantly and has directly lead to the development of Uygur (Xinjiang) Medicine. The related study need the enhanced medical communication between China and India, which will be helpful to the development of health science of human being and the development of both Unani and Chinese medicines.

Key Words: Chinese Medicine, Uighur Medicine, Ibn Sina, Huihui Yaofang, Razi
are over a thousand terms originated from Persian or Arabic that were transliterated into Chinese, which are not comprehensible for ordinary readers, nor can they be used by the medical doctors.

From March 1988, I began research on them by using my knowledge in Arabic, Persian and other relevant languages. My basic method is to make a detailed comparison between the book and Canon of Medicine by Ibn Sina, Al-Jaami Li-Mufradat al-Adwiya by al-Adhiya by Ibn Baitar and Burhan Qati. I found out that out of a total of 656 prescriptions contained in the Hui-Hui Prescriptions over 100 prescriptions are quite the same in contents and dosage as those recorded in the Canon of Medicine. For example, it includes the following prescriptions of The Canon of Medicine: Anush Daru, Majun al Yaguti, Majun Qubadh al Malik, Sanat al Fulunita al-Farsi, al-Juwarish al-Khusaw al-Maruf bi-Juwarish an al ambar, etc. I also found that the descriptions about fracture and Joints as contained in volume 34 of that Huhi Yaofang book are also very similar to that in the Canon of Medicine though the latter gives more detailed descriptions. All this indicates that the Canon of Medicine is the source of the Hui-Hui Prescription.

As for rest the over 500 prescriptions, they are basically the same as those in the Canon or other Islamic medical texts with slight difference of one or two medicines. Besides, many names recorded in the Canon of Medicine reappear in the Hui-Hui Prescriptions, such as Bughrat (Hippocrates), Aristatalis (Aristotle), Iskandar (Alexander), Jalinus (Calien or Galen, 131-201 CE), Arkashanis, Andarumakhis, Lughadhiya, Rufus, Baulis, Sabur, [Ibn] Sah, Saharbakht (full name Isa ibn Saharbakht). Islamic and Unani-related names in Huhi Prescriptions that do not appear in the Canon include: Ahmad Faruq, Hunayn ibn Ishaq, Marwazzi, etc. These indicate the Hui-Hui Prescriptions have recorded contents of other Islamic books also.

Based on the description in the Hui-Hui Prescriptions, we believe that the following medical works were introduced into the Yuan China:

1. Canon of Medicine, by Avicenna, born in Afshanah Village of Central Asia, of Persian nationality.
2. Medical work by al-Razi (865-925 CE) whose original name (Muhammad ibn Zakariya) were recorded in two places in the Hui-Hui Prescription but without mentioning the title of his book. Therefore we can not affirm the above-mentioned book is actually his Continens (al-Hawi) at the moment.
4. Medical work by Hunayn ibn Ishaq (809 – 873 CE), a Nestorian doctor. His name and one of his prescriptions are mentioned in the Hui – Hui Prescriptions.
5. A medical work called “Hia la Ba Din” (Persian for Qarabadin) is mentioned in Volume 30 of the Hui – Hui prescriptions.
6. A medical work by Sabur and Saharbakht was mentioned in Volume 30 of the Hui-Hui Prescriptions. Both of the two authors were medical doctors of ancient Persia City called Jundishapour. Later they were doctors practising in Baghdad. Their co-written work has never been mentioned in historical medical literature in Arabic, therefore we are not quite sure about its existence.

Apart form the above six works, works by Marwazzi and Ahmad Faruq might also have been possibly introduced into China. There are records about prescriptions related to them. According to Tarikh al-Hukama by Qifti (1248 CE), a ‘History of Philosophers’, Marwazzi was a medical doctor of Baghdad, whose home town was Marw (Marw shahjan), an ancient town of Persia. He was also a Nestorian doctor. Ahmad Faruq is unknown to us at the moment.

According to the Hui-Hui Prescriptions, we may conclude that works by the greatest medical writers in the Islamic history of medical science were introduced in the 13th century into China. Another important fact is that since the books preserved in the imperial library were exclusively used by emperor and his court, we have sufficient reasons to believe that those 13 medical works were considered at that time as classics. Thus these 13 classics, closely related with their original versions, that is to say, medical works by Rhazes, Avicenna, etc. had already been widely circulated for a considerable time by the 13th century in China. They have played an important role in the emergence and development of Chinese Medicine. The Hui-Hui Prescriptions is an edited and translated edition of these great works of medical science.

The emergence of such Islamic medical works as the Hui-Hui Prescriptions have also played a
significant role for the development and change of traditional Chinese Medicine. This is shown by the fact that a great deal of the Islamic prescriptions have been absorbed into Chinese Medical works. *Pu Ji Fang* (*Universal Healing Prescriptions* appeared in the 15th century), one of China’s biggest book of prescription as well as *Ben Cao Gang Mu* (*Compendium of Materia Medica*) by Li Shi Zhen all have included into their contents Islamic prescriptions for healing eye diseases and some of their prescriptions were translated into Chinese with Arabic or Persian pronunciations, such as *Tootiya, Anzarut, Afyun, Kateera* and *Zafaran*. These traces speak strongly for the fusion of Islamic medical science and traditional Chinese medical science after the entry of the former into Chinese land. This suggests the close relations in scientific and cultural exchanges between ancient oriental peoples.

At the close of my paper, I would like to declare that my research of *the Hui-Hui Prescriptions* and of the Islamic history of medical science in China is still at the initial stage and I would like to learn from experts of various countries. I am convinced that my research will yield, under the involvement of such experts, satisfactory results. This is also the hope of Chinese academic world.
‘Tibb al aemmah’ at a glance: an introduction of a book on medical teachings of the Imams of Shiites

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Abstract

The Holy Prophet Muhammad (Peace be upon him) has provided guidance on medical philosophy and ethics, taught prayers for healing and advised many medical interventions. In the same manner Shiite scholars have compiled the medical teachings of their Imams. Tib al Aemmah is one such well known book. The Paper presents a brief biography of the author, and describes the outline of the contents of the book. The scientific names of the herbal treatments have also been given. The recommendations described in the book can not only serve as interesting and valuable topics of research and exploratory clinical application but are also an impressive example of the broad and holistic approach towards health and treatment, with a wide notion of health including the mind, soul and spirit along with body, as well as interventions at all these levels by all the means related to each level as well as the impact of spiritual and psychic interventions on body and mind and the influence of physical interventions on not only the body but on the soul and the spirit. Further, the relation and integration of Health with nearly all other dimensions of life, in the broadest sense, including spiritual life, is also revealed. Thus, the medical teachings of the Holy Prophet (PBUH) and of the Imams (peace be upon them) should be utilized at global level not only as practices and treatments but also for reviving the true, wide nature of Health and Medicine.

Key Words: Tib al Aemmah, Tib al Nabawi, Prophetic Medicine, Unani Medicine

The traditions of Holy Prophet Mohammed (Peace be upon him) and his immaculate household (Peace be upon them) about hygiene and treatment of diseases have always been interesting for Muslims and among writings of Sunnites and Shiites chapters can be found on medicinal and curative foods and beverages. Additionally some books have been composed specifically on this issue, including the following: al Tib al Nabavi by Abdolmalek ibn Habib al-Andlusi (238 A.H.), al Shifâ fi al Tib al Mosnad an al Seyyed al Mostafâ by Ahmad ibn Yusef al Tifâshi (651 A.H.), al Tib al Nabavi by Ibn Qayyem al Juziyah (751 A.H.), al Tib al Nabavi by Mohammed ibn Ahmad ibn Othman al Zahabi (748 A.H.), al Manhaj al Savî and also al Rahmah min al Tib va al Hekmah, both by Jalal al Din al Soyuti (911 A.H.). All of the books mentioned above have been written by men of tradition.

Among twelve Imâm Shiites also, books in this field have been written, a few of which are: Tib al Aemmah by Hossein and Abdallah, sons of Bastâm Neyshaburi, and also Tib al Aemmah by Seyyed Abdallah Shobbar. The latter book that is discussed in this article. The first part of this article is a review of the life of Seyyed Shobbar and in the second part the contents of his book are discussed.

Biography

Seyyed Abdallah ibn Mohammedreza ibn Mohammed ibn Mohsen Hosseini Shobbar, was a
great jurisprudent, legal theoretician, traditio
and commentator who was born in 1188 A.H. in
Najaf. He migrated to Kâzemayn with his father
during his childhood and learnt the introductions to
Islamic sciences in this city. He then returned to
Najaf to continue his studies and he became
proficient in most of the Islamic sciences. He
dedicated his life to teaching, writing, and helping
people, and during his relatively short 54 year life
he trained many students and also managed to
write 70 scientific books. The large number of
books he wrote are the reason he is called
"Majlessi, the Second". This great scholar died in
Rajab, 1242 A.H. and was buried in Kazemayn
holy shrine.

His Teachers
1- Seyyed Mohammadreza Hosseini Shobbar, 2-
Seyyed MohsenToAddollah ibn Ismail Kâzemi
Shushtari, 3- Sheikh Jafar Kâshef al Ghetâ, 5- Mir Seyyed Ali
Tabâtabâi, 6- Sheikh Ahmad Ahsâi, 7- Mirza
Mohammed Mahdi Shahrestâni, 8- Mirza
Abolghâsem Ghomi.

His Students
He trained many illustrious students e.g. Seyyed
Hasan Hosseini Shobbar, Seyyed Ali Amin Âmeli,
Seyyed Mohammadali ibn Kâzem Aaraji etc.

His Books
He wrote important books in many genres of
Islamic Learning e.g., Safvat al Tafsir on Tafsir;
Jâme al Mâref va al Ahkâm (20 vols.) on Hadith; al
Balâgh al Mobin fi Usule al Din in Theology;
Risâlah fi al Kalâm in Kalam etc.

Tib Al Aemmah
This book contains 131 chapters and 1623
traditions. The first chapter has 7 traditions about
the fact that medicines and treatments are God's
gifts and that the main source of correct medicine
are in the hands of Prophets and Imâms. It is in this
chapter that the Treatise of Ahliljah that is about
the disputation between Imâm Sâdegh (on whom
be peace) and an Indian doctor is written.

The second chapter contains 24 traditions and
Treatise of the Divine Unity Theory of Mofazzal and
the discussion between Imâm Sâdegh (on whom
be peace) and Abuhanifah about comparison are
included in this chapter. In the third chapter which
includes 23 traditions, points on the permit to treat
and to consult a physician, and also on treatment
with different drugs except lethal poisons and
substances that are forbidden in Islam are covered.
This point is also mentioned that in case of need,
treatment is mandatory and in other cases, the
disease can be left to ameliorate on its own accord.
In the fourth chapter that contains 3 traditions, facts
are mentioned about abstinence and diets. In the
fifth chapter, which has 22 traditions, prohibition of
treatment with intoxicants and other forbidden
substances has been discussed. The sixth chapter
that includes 24 traditions is about preference of
tolerance during disease and not losing hope. In
the seventh and eighth chapters, that include 2 and
13 traditions relatively, points on preference of
tolerance during the illness of offspring or turning
blind and also hiding disease and not complaining
are mentioned. The ninth chapter has 3 traditions
and is about indecency of exaggeration about
disease and the next chapter, which includes 4
traditions, it has been advised to complain of illness
only in the presence of a religious and responsible
person, as this might have advantages like his help
or useful advice or blessings for the wellbeing of
the ill person. Chapter eleven contains 1 tradition
only that is about the patients should avoid walking
and the next chapter, that includes 3 traditions, is
about the preference of allowing Muslim brothers to
visit the ill.

The thirteenth chapter, that has 15 traditions, is
about approval of visiting patients especially in the
morning and afternoons. Chapter fourteen includes
2 traditions about the rules to visit a patient with
eye pain or a patient with chronic disease. In the
fifteenth chapter 5 traditions about the preference
of asking for a patients blessing and avoiding
enraging him are mentioned. The sixteenth chapter
that includes 2 traditions, paying patients short
visits is encouraged and it has been mentioned that
visits should be made longer only on the patients'
request. In the next chapter that has 2 traditions,
taking fruit, fragrances, and other things of this kind
as gifts for patients has been encouraged.

In the eighteenth chapter that includes 7 traditions,
not liking death and also getting away from areas
contaminated with cholera and plague have been
accepted. The nineteenth chapter that has 9
traditions discusses about repelling misfortunes
and diseases by praying. In the twentieth, twenty
first and twenty second chapters that include 6, 12
and 3 traditions, respectively, treating diseases
with alms, Torbat [soil of the holy shrine of Imâm
Hossein (on whom be peace)], and Armenian bole
has been mentioned.
The twenty third chapter that has 10 traditions, mentions treating diseases with a variety of useful methods such as cupping, bathing and so on. The twenty fourth chapter that has 14 traditions, is dedicated to nutrition therapy. The next chapter has 6 traditions, and is dedicated to washing hands before and after a meal. The twenty sixth chapter has 2 traditions and points out the advantages of massaging the face after its washing after a meal. In the twenty seventh chapter that includes 8 traditions, ways to treat fermentation and discomforts resulting from gluttony have been discussed. Chapters twenty eight and twenty nine that include 7 and 15 traditions, mention the curing capabilities of the leftovers of a meal and also the medicinal properties of table salt and preference of beginning and ending a meal with it.

The thirtieth (3 traditions), thirty first (8 traditions), thirty second (2 traditions), and thirty third (20 traditions) chapters are dedicated to the medicinal properties of Indian cypress (Soad=Cypress rotundus DC.), the types and benefits of toothpicks, rice cookies, and types of fried and pounded wheat or barley (sawigh) relatively. In the thirty fourth chapter that has 23 traditions, the healing properties of various meats and fats have been discussed.

Chapters thirty five, thirty six, thirty seven, and thirty eight, including 5, 3, 6, and 2 traditions respectively, point to the treatment qualities of cooked meat in milk, kebab, harisah (a soft dish consisting of ground wheat and meat), and hasw (a kind of soup). The thirty ninth chapter is composed of 18 traditions and in it the curing capabilities of honey have been discussed. In the fourtieth chapter, that contains 23 traditions, the healing properties of various meats and fats have been discussed.

Chapter 41 includes 18 traditions and is specifically about the medical qualities of milk. Chapters forty two (with 7 traditions), forty three (containing 10 traditions), and forty four (including 5 traditions) point to the treatment qualities and also disadvantages of cheese, the curing capabilities of vinegar, and the medicinal properties of olives and olive oil. In chapters forty five (2 traditions), forty six (10 traditions), forty seven (7 traditions), forty eight (5 traditions), and forty nine (6 traditions), the treatment qualities of barley, rice, lentil, Lens culinaris Medikus and pea Cicer arietinum L., broad bean Vicia faba L., vetch Vigna radiate (L.) Wikzek; Phaseolus radiatus L., French bean Phaseolus vulgaris L., and panicum Panicum miliaceum L., have been discussed relatively. Chapter fifty is dedicated to the medical qualities of fruits (in general and without referring to any specific fruit) and includes 7 traditions.

Chapters fifty one (20 traditions), fifty two (3 traditions), fifty three (9 traditions), fifty four (16 traditions), fifty five (17 traditions), fifty six (21 traditions), fifty seven (4 traditions), fifty eight (9 traditions), fifty nine (7 traditions), sixty (5 traditions), and sixty one (2 traditions) mention the treatment qualities of some fruits including dates, grapes, raisins (zabib), pomegranate, apples, quince Cydonia oblonga Mill., pears, fig, citron Citrus medica L., plums, and Russian olive Elaegnus angustifolia L.

The sixty second chapter (containing 2 traditions) is about the medical qualities of sugar cane. Chapters sixty three (6 traditions), sixty four (24 traditions), sixty five (6 traditions), sixty six (12 traditions), sixty seven (4 traditions), sixty eight (3 traditions), sixty nine (3 traditions), seventy (3 traditions), seventy one (5 traditions), seventy two (6 traditions), seventy three (4 traditions), seventy four (2 traditions), seventy five (9 traditions), seventy six (3 traditions), seventy seven (3 traditions), seventy eight (4 traditions), seventy nine (14 traditions), eighty (2 traditions), eighty one (15 traditions), eighty two (2 traditions) and eighty three (5 traditions) point to the treatment qualities of several vegetables and summer crops including the following: melon Cucumis melo L., chicory Cichorium intybus L., sweet basil Ocimum basilicum L., French leek Allium porrum L., celery Apium graveolens L., green purslane Portulaca oleracea L., garden lettuce, rue, garden cress, sugar beet and cabbage, truffle, dill, gourd, radish, carrot, turnip, eggplant, cucumber, onion and garlic, coriander, Ajwain caraway, and thyme.

In the eighty four chapter (with 16 traditions) the advantages and disadvantages of drinking water and also the way to drink water are discussed. The next chapter, with 51 traditions, is about the curing quality of Zemzem, Forât, Nile, Jaxartes, Oxus, and other waters. The eighty sixth chapter, that is considered the first chapter of the second volume of the book, includes 73 traditions, and contains practical points about hygiene and health during traveling or at home. The next chapter includes 24 traditions and discusses phenomena that increase and decrease lifetime and phenomena that cause affliction of misfortunes. In chapter eighty eight (containing 16 traditions) harms caused by an evil eye and their associated prayers are discussed.
Chapter eighty nine has 34 traditions and is about treatment of various fevers. Chapters ninety (28 traditions) and ninety one (23 traditions) discuss the medicines and prayers related to headaches and migraine and also other diseases associated with the head such as various pains, itches, pruritus, dandruff, hair fall and so on. Chapter ninety two (with 59 traditions) is about treating different types of eye diseases, while the next chapter (containing 28 traditions) is about tooth diseases and useful and harmful substances for teeth. In the ninety forth chapter (with 13 traditions) types of lips, tongue, oral, gum, uvula, and pharyngeal diseases also cough and sneeze and their treatment are discussed. Chapter ninety five (with 22 traditions) is dedicated to discussing the factors that are associated with facial diseases like freckles, lentigo and dryness of facial skin. The next chapter (with 19 traditions) mentions points on treatment of facial paralysis, palsy, jaundice, numbness, colic etc. Chapter ninety seven (with 15 traditions) mentions points on treatment of phlegm and excessive body moisture, tremors of the face and other parts of body, excessive thirst, and dryness of the mouth.

The next chapter, that includes 10 traditions, discusses memory and factors that increase it.

Chapter ninety nine comprises of 120 traditions and is about treating unpleasant diseases like rodent ulcer, leprosy, leukoderma, vitiligo, mania, epilepsy, mental retardation, amentia, types of swelling, frivolity, fear, temptation, apoplexy, tremor etc. The hundredth chapter of the book (with 44 traditions) is dedicated to the hygiene of mothers and newborns. In the next chapter (containing 11 traditions), points about the difficulty of giving birth and ways to confront it are mentioned. In chapter 102, that doesn’t have any traditions, points about abolition of spells are mentioned. In the next chapter, that includes 13 traditions, sexual hygiene and ways to increase chastity of family members are discussed. Chapter 104 (with 8 traditions) mentions medicines that increase sexual strength. Chapter 105 (including 19 traditions) is about drugs related to the male genitourinary system.

The next chapter (with 6 traditions) is related to diseases of anus and their treatment. Chapter 107 that includes 19 traditions is specifically about treating hemorrhoids. Chapter 108 (with 34 traditions) mentions the treatment of abdominal pain, gastralgia, borborygmus, taenia, helminth, cramps, leanness, dysentery, colic, gripes, hilum pains and so on. Chapter 109 (with 7 traditions) mentions the treatment of modalities of pelvic. In the next chapter, that has 6 traditions, muscular pains are discussed.

Chapter 111 (with 5 traditions) mentions the causes of tenderness and cruelty. Chapter 112 of the book (with 19 traditions) discusses the treatment of various types of heart diseases. In chapter 113 (including 11 traditions) points on prevention of fermentation and dyspepsia. The next chapter (with 10 traditions) is about various skin diseases such as boils, ulcers, wounds, swellings, scabies, papules, itch and their treatment. Chapter 115 includes 7 traditions and mentions the treatment of coryza and catarrh. The next chapter has 7 traditions and contains issues on ascite, diseases of spleen and liver, thirst, diarrhea and constipation. The hundred and seventeenth chapter has 8 traditions and contains prayers to rebel plague and cholera. The next chapter with 11 traditions has prayers to rebel fear, sadness and worrying. In chapter 119 (with 8 traditions) points on prevention and treatment of sciatica, gout and arthralgia are mentioned. The next chapter (with 20 traditions) discusses the treatment of poisonings and insect stings.

Chapters 121 (7 traditions), 122 (2 traditions), 123 (2 traditions), 124 (1 tradition), 125 (without any traditions), and 126 (with 2 traditions) mention points on the treatment of pains associated with foot and leg, curing cysts, wart, pneumonia, ringworm, and scrofula. In chapter 127, that contains 8 traditions, a few prayers related to rebel grasshoppers and other pests are written. In the next chapter (with 39 traditions), prayers that are read during travelling and in the time of fear of natural things are mentioned. Chapters 129 (5 traditions) and 130 (8 traditions) point to prayers related to divine aid for Pilgrimage and repaying of loans. The last chapter of the book includes 74 traditions and mentions different points associated with prayers and medicines.

The main references of this book include: 1- al Risâlah al Zahbîah attributed to Imâm Rezâ (on whom be peace), 2- al Kâfi by Sheikh Kolayni, 3- al Mahäsen by Sheikh Barghi, 4-Ghbor al Asnâd by Hemiari, 5-ûnâa al Akhûbû, 6-Eghâba al Aâmâl, 7- Elal al Shsharâe, 8-Thawâba al Aâmâl, 9-al Khesâl, 10-Maânia al Akhbâr, 11- Man Lâ Yahzaroho al Faghih, 12-al Amâl (all of them by Sheikh Sadough), 13- alMasâel by Ali ibn Jaafar, 14-al Tafsîr by Aïâshi, 15- Tîbb al Aemmah by Hossein and Abdallah, sons of Bastâm Neyshaburi, 16-Daâem al Islâm by Ghazi Nemân, 17-Fîggha al Rezâ (on whom be peace) by Shalmaghâni, 18-

The copy of “Tibb al Aemmah” used for this review was published by Dar al Alfain Publishers of Kuwait and reprinted in the year 1415 A.H. by the “Dar al Etesâm” of Qum, Iran.

References
Ritu related dysfunctions of Mahabhutarupa in the context of Thai Traditional Medicine

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Abstract

Thai Traditional Medicine was originated from Indian Ayurveda. Later, it has developed its own theory on health and diseases, having over 2500 years of development. It has interesting affinities with Unani Medicine too as it is based on Four Unani rather than the Five Ayurvedic Elements. Ritu or seasons, are recognised as one of the causes of human ailments, which are divided into 3 seasons in Thailand i.e. summer between March and July, rainy season between July and November and winter between November and March. This seasonal effects can be considered as the external cause which governs Dhatu dysfunction in human beings i.e. in summer when the weather is hot and damp; the fire element (Pitta) is the basis of dysfunction, in rainy season when the weather is wet and damp; the wind element (Vata) is the basis of dysfunction and in winter when the weather is cool and dry; the water element (Semha) is the basis of dysfunction. As a response of the human body in the effort of homeostasis i.e. trying to adapt the body function to the changing environment, results in Mahabhutarupa dysfunctions accordingly. April, August and December are months of dysfunctional period of Patta-Pitta, Apatta-Pitta and Gumdao (body thermal control), respectively. May, September and January are the dysfunctional periods of earth element (Pathavi Dhatu) i.e. Hatayan, Utraiyan and Grisan, respectively. July, November and March are months of dysfunctional Semha i.e. water element in the region of throat, chest and colon, respectively. October, February and June are months of Vata dysfunction i.e. Sattagavata, Sumanavata and Hatayavata, respectively. There are herbal formula for each dysfunction and combinations are unavoidable due to the effects of previous and past environment. Basic official herbal formulae will be described as examples.

Key Words: Thai Traditional Medicine, Mahabhutarupa, Ritu, Seasonal effects

Introduction

Thai Traditional Medicine (TTM) has been developed in the land now called Thailand (formerly Siam) since Buddha’s time i.e. 543 years B.C. or 2553 years ago. Some medicinal plants recorded in a book on Buddhist teachings (Mahavejsandorn Chadok) are still being used. These are Cinnamomum camphora, Cassia fistula, Holarrhena dysenterica, Oroxylum indicum, Piper retrofractrum, Carthamus tinctorius, Acacis catechu, Morinda citrifolia, Commiphora mukul, Phyllanthus emblica, Aegle marmelos, Ferula asafoetida, Cyperus rotundus, Terminalis alata, Asparagus racemosus etc [Luanratana 2001]. At the beginning TTM received influences from Indian Ayurvedic medicine through Buddhism and cultural exchanges. In 17th B.E. King Chaivoraman 7th built 17 Arokayasala (hospitals) on the route from Vimanaya city (location at present time: Phimai district,
Nakornrachasima, Thailand) to Pra Nakorn (location at present time: a city in West Cambodia). There were list of essential medicinal plants in each Arokayasala and assignment of staffs equivalent to doctors, pharmacists and nurses and others [Fine Arts Department 2005].

The present TTM medical textbooks and pharmacopoeia (Patayasart Songkrok or Vejasart Songkrok) also have Bali words (in Thai characters) at the beginning of a paragraph demonstrating the link between TTM and Buddhism. In the past, TTM was the only healthcare for Thai people until the introduction of western medicine ca. 100 years ago under the reign of King Rama V. Attempt had been made to teach both types of medicine in Siriraj hospital, Mahidol University at that time but was not successful and caused the disappearance of TTM from Thai education system until 2009. Originally TTM theory and principles were developed from Ayurveda but ancient Thai doctors had also synthesized theory and herbal formulas for specific treatments and availability of the medicinal flora in Thailand. Seasons are considered as external pathogens which affect normal function of human body. TTM has theory on how to formulate herbal formulas to help human body response to external stimuli and maintain good health or homeostasis and there are basic herbal formulas for each changing environment.

**Thai Traditional Medicine Theory**

**[Vejasartsongkrok 1923]**

TTM has recognized the existence of the physical and the spiritual components of human being like other ancient traditional civilizations (Greek, Chinese, Indian etc). It also accepts that both components must be maintained in conformity to live a long life, therefore, the basis of treatment is

1. **Dhatu Samuthan**
2. **Aryu Samuthan**
3. **Ritu Samuthan**
4. **Kala Samuthan**

### 1. Dhatu Samuthan

TTM classified body composition into four elements i.e. the pathavi dhatu (earth element), the arpo dhatu (water element), the vayo dhatu (wind element), and the techo dhatu (fire element). Each element has sub-components as follow.

The **pathavi dhatu** includes 20 components i.e. hair, body hair, nail, teeth, skin, tendon, muscle, bone, bone tissue, liver, lung, kidney, fascia, heart, spleen, big intestine (stomach and large intestine), small intestine, utariyan (the new food or the incompletely digested food in the stomach and duodenum), grisan (the old food or the completely digested food in the large intestine) and brain.

The **aroo dhatu** comprises 12 components i.e. blood, lymph, solid fat, liquid fat, joint lubricative liquid, bile (within gall bladder, outside gall bladder, saliva, sweat, nasal liquid, phlegm, tear, and urine.

The **vayo dhatu** includes 6 types i.e. athokamavata which moves up from toes to head, uthankamavata which moves down from head to toes, gothasayavata which is in the stomach, small intestine and large intestine, gushisayavata which is outside the gastro-intestinal tract, unkamank-anusarivata, which is in the heart and circulatory system, assasapassasavata which is the inhal and exhale air.

The **techo dhatu** comprises 4 components i.e. Santappachi the fire which keeps our body warm, Parutaiachi which governs human response in relation to the temperature, Shiranachi which causes aging, and parinamachi which digests the food.

### 2. Aryu Samuthan

TTM divides human life into 3 stages i.e. the first stage is from 0-15 years old the ailments are under semha basis, therefore the herbs for their ailments are sweet and bitter-sour in nature. The examples of sweet herbs are Glycyrrhiza glabra, Asparagus racemosus root, Abrus precatorius leaf, Saccharum officinarum, honey etc. The examples of bitter-sour herbs are Terminalia bellerica fruit, Terminalia chebula fruit, Phyllanthus emblica fruit, Mangifera indica leaf, Acalipa indica leaf etc. The second stage is from 16-30 years old, the ailments of people in this stage are under pitta basis, therefore the herbs for their ailments are puckery-sour and salty-sour in nature. Examples of puckery-sour herbs are Tribulus terrestris, Phyllanthus acidus leaf, Spondias pinnata leaf, Ziziphus mauritiana bark, Anacardium occidentale leaf, Bauhinia variegata bark, Embelia ribes leaf. Examples of salty-sour herbs are Citrus hystrix leaf, Tamarindus indicus seed, Solanum stramonifolium fruit, Phyllanthus acidus root, Psidium guajava root, Citrus hystrix leaf, Spondias pinnata root, Bouea macrophylla root, Atalantia monophylla root etc.
The third stage is from over 30 years old onwards the ailments are under vata basis, the herbs for their ailments are fatty-bitter and puckery-salty. Examples of fatty-bitter herbs are *Zingiber cassumunar* rhizome, *Plumbago indica* root, *Cinnamomum porrectum* heartwood, *Curcuma aromatic* rhizome, *Bridelia ovata* leaf etc. Examples of puckery-salty herbs are *Acorus calamus*, *Crateva religiosa* bark, *Echinochloa stagnina*, *Leersia hexandra*, *Cynodon dactylon*, *Digitaria violascens*, *Hygrophilla erecta* seed, *Connarus chochinchinesis* vine etc.

3. **Ritu Samuthan**

Thailand has 3 Ritus or seasons i.e. summer, rainy season, and winter. The numbering of months in a year is different from western countries i.e. December is considered as the first month and November is considered as the last month of the year. The beginning of seasons also depend on the moon position.

*Kimhantaritu or Summer* begins from the first waning moon of the fourth month (March) until 15\textsuperscript{th} waxing moon of the eighth month (July). During this period the weather is hot and damp, people will be suffered from techo dhatu in Mahabhutarupa.

*Wasantaritu or Rainny season* from the first waning moon of the eight month (July) until 15\textsuperscript{th} waxing moon of the twelfth month (November). During this period the weather is cool and damp, people will be suffered from vayu dhatu in Mahabhutarupa.

*Hemantaritu or Winter* from the first waning moon of the twelfth month (November), until 15\textsuperscript{th} waxing moon of the fourth month (March). During this period the weather is cold and dry, people will be suffered from arpo dhatu in Mahabhutarupa.

4. **Kalasamuthan**

The 24 hours of a day are divided into 12 hours by day and 12 hours by night. During the day and night there are 4 hours per period, a total of 3 periods. This principle is used with patients in critical conditions and are related to the tridosha principle (vata, pitta, semha or capha). The first period of the day (6-10 a.m.) and night (18-22 p.m.) needs attention on semha (capha) system, the second period of day (10 a.m.-14 p.m.) and night (23 p.m.- 2 a.m.) needs attention on pitta system, the third period of day (14 -18 p.m.) and night (2 - 6 a.m.) needs attention on vata system.

**The principles of disease development [Sirithamawanich 2006]**

There are five principles of disease development.

1. Mahabhutarupa
2. The 5 ailments ( bile, blood, semha, gumdao and vayu)
3. The tridosha (vata, pitta, semha or capha).
4. Pratedsamuthan (Geography)
5. Pranachakra

1. **Mahabhutarupa**

In this principle each of the four dhatus is organized into 3 sub-groups which are related to tridosha and the 5 ailments as follow:

Pathavi Dhatu consists of hatayan (heart), utariyan (new food or digestion system) and grisan (old food or the balance within the colon).

Arpo Dhatu consists of sorsemha (the mucous from the nose, throat through the end of esophagus), urasemha (the mucous within trachea, lung, the chest), kootasemha (the mucous within the intestines and anus).

Vayo Dhatu consists of Hataivata ( the vayu within the heart and circulatory system) , Satagavata ( the vayu within the internal organs i.e. heart, lung, liver, spleen, kidney) , Sumanavata ( the common vayu within pranachakra energy lines i.e. Ita, Pingkala, Sumana and Suchumunai).

Techo Dhatu consists of Pattapitta (the bile within the gall bladder) it is necessary for the digestion, Apattapitta (the bile outside the gall bladder i.e. in the liver and in the blood vessels) it is necessary for the immune system, Gumdao (the maintainance of normal body temperature).

2. The 5 ailments ( bile, blood, semha, vayu and gumdao)

TTM has identified 5 simple ailments due to the dysfunction of bile, blood, semha, vayu and gumdao systems. Bile ailment is involved with metabolism of the body which are related to the function of stomach, liver, blood and intestine. Blood ailment is involved with the whole system of blood i.e. plasma, platelet, lymphocyte etc in the blood. Semha ailment is involved with all secretions covering the cells and tissues of various organs. Vayu ailment is involved with the dysfunction of vata system in mahabhutarupa (hataivata, Satagavata and Sumanavata) which cause tension in the nervous system, blood vessels and tissues, results in the imbalance of the four dhatus in Mahabhutarupa. Gumdao ailment is related to the warmth of body which is the result of balanced bile, semha and vayu.

3. The tridosha (vata, pitta, semha or capha)

This principle is related to the energy system within human body which could be related to Kreb’s cycle.
in scientific knowledge. The vata system in tridosha refers to the Ita line starts from the left toe up to the knee down to the dorsal at the knee, goes up to the left hip then cross to the right of the spine into the brain to the right nostril. The pitta system in tridosha refers to the pingkala line begins at the right toe up to the knee and down to the dorsal at the knee, goes up to the right hip then cross to the left of spine into the brain and down to the left nostril. The capha system in tridosha refers to suchumunai line which originates in the coccyx goes up the spine and into brain and combines with Ita and Pingkala in the nostrils. This line is equivalent to dumai in TCM. These 3 lines are involved with the gurunadi or pulse reading.

4. Pratedsamuthan (Geography)

TTM has identified geography as a form of pathogen which causes certain ailments for people who live in such areas. These are the 5 simple ailments i.e. bile, blood, semha, vayu and gumdao. Gantaprated has a lot of fresh water i.e. rivers and canals, soil of clay types, a lot of sea water. People live in this area will suffer from semha and vayu more than the rest. Sakornprated has a lot of stones, sand and gravel, scant water and plants. People in this area will suffer from blood and gumdao more than the rest. Sataranaprated has plenty of fresh water, sea water, soil, stones, sand and gravel. People in this area will suffer from all 5 ailments.

5. Pranachakra Energy Lines [Sirithamawanich 2007]

This concept resulted from the observation by traditional doctors dated back thousands of years which cannot be explained by the available scientific knowledge at present. The ancient doctors accept the presence of these lines of energy and used them for massage and herbal formulation. There are 14 lines and 6 olarn chakras involve in the treatment with medicine. The top chakra (the seventh) is involved with spiritual aspect of the body. These olarn (big) chakras are called sahasara (crown), ajna (third eye), visuddha (throat), anahata (heart), manipura (navel), svadisthana (hip, genital), muladharra (root). The 14 energy lines are called ita*, sumana*, dhavari, ulanga, tischa, ongkamavata*, pingkala*, kalatari,
sahasarangsi, lawusang, nanthakrawat, sahasamonkol, suchumuna*, jantapusam* (the
star * indicates connection with the olarn chakras).

Basic herbal formulations for ritu related
dysfunction of Mahabhutarupa
As stated earlier that the numbering of months in a
year for TTM is different from the western calendar
i.e. December is considered as the first month and
November is considered as the last month of the
year. The beginning of seasons also depend on the
moon position i.e. the wax and the waning moon. In
general the summer months cause aggravation of
body functions, the rainy months cause reduced
activity of the body functions and the winter months
cause malfunction. The months which involve
techo dhatu are April, August and December, those
involve vayo dhatu are June, October and
February, arpo months are July, November and March and pathavi motns are May, September and
January (Figure 3). The basic herbal formulas for
each month are listed in Table 1-4.

Table 1
The basic herbal formulas for dysfunctional Mahabhutarupa (techo dhatu)

<table>
<thead>
<tr>
<th>Techo Dhatu</th>
<th>Month</th>
<th>Piper interruptum</th>
<th>Piper chaba</th>
<th>Piper sarmentosum</th>
<th>Zingiber officinalis</th>
<th>Plumbago indica</th>
<th>Terminalia bellirica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattapitta</td>
<td>April</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Apatapitta</td>
<td>August</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Gumdao</td>
<td>December</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>16</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: add some dried Z. officinale in every formula

Table 2
The basic herbal formulas for dysfunctional Mahabhutarupa (vayo dhatu)

<table>
<thead>
<tr>
<th>Vayo Dhatu</th>
<th>Month</th>
<th>Piper interruptum</th>
<th>Piper chaba</th>
<th>Piper sarmentosum</th>
<th>Piper nigrum</th>
<th>Plumbago indica</th>
<th>Terminalia chebula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hataivata</td>
<td>June</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Sattagavata</td>
<td>October</td>
<td>16</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Sumanavata</td>
<td>February</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: add some P. nigrum in every formula

Table 3
The basic herbal formulas for dysfunctional Mahabhutarupa (arpo dhatu)

<table>
<thead>
<tr>
<th>Arpo Dhatu</th>
<th>Month</th>
<th>Piper interruptum</th>
<th>Piper chaba</th>
<th>Piper sarmentosum</th>
<th>Zingiber officinalis</th>
<th>Plumbago indica</th>
<th>Phyllanthus emblica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorsemha</td>
<td>July</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Urasemha</td>
<td>November</td>
<td>1</td>
<td>8</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Kootasemha</td>
<td>March</td>
<td>3</td>
<td>16</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: add some Tripla in every formula (Tripla = Terminalia chebula, T. bellerica, Phyllanthus emblica)

Table 4
The basic herbal formulas for dysfunctional Mahabhutarupa (pathavi dhatu)

<table>
<thead>
<tr>
<th>Pathavi Dhatu</th>
<th>Month</th>
<th>Piper interruptum</th>
<th>Piper chaba</th>
<th>Piper sarmentosum</th>
<th>Piper nigrum</th>
<th>Zingiber officinalis</th>
<th>Plumbago indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatayan</td>
<td>May</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Uttariyan</td>
<td>September</td>
<td>8</td>
<td>16</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Grisan</td>
<td>January</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: add some Tripla in every formula (Tripla = Terminalia chebula, T. bellerica, Phyllanthus emblica)

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A review of *wajaulmafasil* (osteoarthritis) with special reference to *wajaurrakbā* (knee osteoarthritis)

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Abstract

*Wajaurrakbā* (Knee Osteoarthritis) is one of the most common diseases of human being and an important cause of disability. In the classical literature of Unānī Medicine, the illness of joint pain is mentioned under the caption of *Wajaulmafasil* which includes almost all forms of arthritis along with other joint diseases. *Wajaurrakbā* (Knee Osteoarthritis) is described in the context of *Wajaulmafasil* and is defined as the pain in the knee joint which causes immobilization of the joint and cessation of the functions. In *Wajaulmafasil* the *Akhlāt* (humours) accumulate within the joints thereby leading to pain swelling and articular tissue damage etc. Scholars of Unānī medicine have described that *Asbābe Fa’ila* (causative factors) for the *Wajaulmafasil* are *Sue Mizāj* (Inequitable Temperament) and *Mawāde Fāsida* (Morbid Humours) and on the basis of this concept they have classified the *Wajaulmafasil* into *Damvi* (Sanguine), *Balghami* (Phlegmatic), *Safrawi* (Choleric) and *Saudawi* (Melancholic) groups. The features and characteristics of these groups differ from each other. *Wajaurrakbā* has been discussed in Unani literature more extensively as compared to the other forms of arthritis simply because of its high prevalence. Even in common connotation, *Wajaulmafasil* is used frequently for *Wajaurrakbā*.

Key Words: *Wajaurrakbā*, *Wajaulmafasil*, Humours, Arthritis

Introduction

*Wajaurrakbā* is an Arabic word which means pain in the knee joints (*Wajā* = Pain and *Rakbā* = Knee Joint). In the classical literature of Unānī system of Medicine, *Wajaurrakbā* has been discussed under the caption of *Wajaulmafasil*. *Wajaulmafasil* is also an Arabic term used to describe the pain in joints (as *Wajā* stands for pain and *Mafāsīl* for joints).

According to Zakariyā Rāzi, *Wajaul Mafāsīl* is a wide term which is used to bdescribe the painful joints including *Niqras* (Gout) and *Irqun Nisā* (Sciatica) etc. It may have specific names according to the joints involved e.g. when the pain starts from hip and spreads down the length of leg it is called as *Irqun Nisā*, and it is named as *Niqras* when the pain is in toes (Razi, Pub.2005).

Ismāil Jurjānī defines *Wajaulmafasil* as the pain and inflammation due to the accumulation of secreted materials with in the joints. But if pain is in ankle joints and in metatarsophalangeal joints especially in great toe it is known as *Niqras* (Gout). If the pain occurs in hip joint it is called as *Wajaul Wark* and if the pain arises from hip joint and runs downwards, is called as *Irqun Nisa* (Sciatica). While the painful condition of knee joint and joints of upper limb, is known as *Wajaulmafasil* (Jurjani, 1878).

According to Akbar Arzānī, *Wajaulmafasil* is joint pain. It may or may not be associated with inflammation depending upon the causative factors. In case of *Sue Mizāj Sāda* there will be no inflammation while in case of *Akhlāte Fāsida* (Morbid Humours) inflammation will be the
important feature. Further he defined that the pain and inflammation, occurs in the joints of hands and legs, is called as Wajaulmafasil (Arzani, Pub.1890).

Ghulam Jeelani says, it is a disease in which ligaments of the joints become hard and prevent the movements of the joints (Jeelani, 1996). Kabiruddin, Ajmal Khan and Rizwan Ahmad have described it as a variety of pain with swelling which occurs in the joints of the body (Khan, 1983; Kabeeruddin, 1916 & Ahmad). According to Noor Kareem, Wajaulmafasil is a type of pain which occurs in joints. He also gave the same concept about Wajaul Wark, Irqun Nisā and Niqras as mentioned above (Kareem).

Classification of Wajaulmafasil

Wajaulmafasil can be classified based on the various factors, as follows:

1. Presence or absence of Akhlāte Fāsida (Morbid Humours)
2. Mizāj (Temperament)
3. The type of Māddā (Matter) involved
4. The number of Khilt (Humour) involved
5. The severity and duration of the disease
6. The joints involved

1. Presence or absence of Akhlāte Fāsida (Morbid Humours)
   I. Wajaulmafasil Sāda (where there is no morbid matter)
   II. Wajaulmafasil Māddi (where there is morbid matter)

2. Mizāj (Temperament)

Temperament is expressed by four qualities, of which two are active i.e. hararat (Hotness) & burudat (Coldness) and two are passive i.e. Rutubat (moistness) & Yabsurat (dryness).

(When one quality is involved)
   I. Ḥār (Hot)
   II. Bārid (Cold)
   III. Ratab (Wet)
   IV. Yābis (Dry)

(When two qualities are involved)
   I. Ḥār Ratab (Hot and Wet)
   II. Ḥār Yābis (Hot and Dry)
   III. Bārid Ratab (Cold and Wet)
   IV. Bārid Yābis (Cold and Dry)

According to Ibn Rushd, the above described qualities are some times Māddi (with matter) and sometimes Ghair Māddi (without matter), but he has clearly mentioned in that Marze Māddi can't persist with single quality, but it exists with two qualities. He further mentioned that Ḥār Ratab Ghair Māddi (Hot and Wet without matter) and Bārid Ratab Ghair Māddi (Cold and Wet without matter) diseases are not possible, but Ghair Māddi diseases can exist with Ḥār, Bārid, Ratab and Yābis kaiiyaāt, separately (Ibn Rushd, Pub.1987).

3. The type of Madda (Matter) involved
   I. Damvi (Sanguineous)
   II. Safrāwi (Bilious)
   III. Balghami (Phlegmatic)
   IV. Saudāwi (Melancholic)
   V. Reehi (Pneumatic)
   VI. Ufooni (Infectious)

4. The number of Khilt (Humour) involved
   I. Mufrad (due to single Khilt)
   II. Murakkab (due to more than one Khilt)

5. The severity and duration of the disease

6. The joint involved
   I. Wajauz Zehar is used for the pain in muscles and tendons around the vertebral column.
   II. Wajaul Wark is the pain in hip joint which does not radiate down wards.
   III. Irqun Nisā is the pain which starts from the hip joint and radiates downward to the thigh, even some times up to the knees and ankles.
   IV. Wajaurrakbā is a type of pain specifically in the larger joints like knee and hip joints.
   V. Niqras is the pain with swelling in ankle joint and other joints of the foot.
   VI. Wajaul Khasirā is the pain in the hip joint (Kulhe ka Dard).

Asbāb wa Mahiyate Wajaurrakbā (Etiopathogenesis of knee OA)

Ibn sinā explained that the cause of the joint pain is dilatation of Majarije Tabiya, due to sue Mizaj Mustahkam particularly the Mizaje Bārid (Ibn Sina, 1930). He has divided the causes into two types (Ibn Sina, Pub.1930).
• Al Asbābul Fā’ila
• Al Asbābul Munfa’ila

**Al Asbābul fā’ila** (Causative factors):
These factors are:
• Sue Mizāj (Inequitable Temperament)
• Mawāde Fāsidah (Morbid Materials)

The alteration in the Mizāj may be general (of entire body) or local (in a particular region of the body). The altered Mizāj may act as Multahib (Inflammatory), Mubarrid (Refrigerant), Mujammīd (Consolidant), Mujaffif (Desiccant) or Munqabiz (Astringent). These alterations get aggravated when any Rutūbate Gharibah (Abnormal Fluid) is also involved.

The morbid matters may be Dame Khālis (Simple Sanguine), Dame Balghami (Phlegmatic Sanguine), Suddaa Balgham Khām (Obstruction of Raw Phlegm), Mirrae Khālis (Simple Bile), Safrae Balghami (Phlegmatic Bile), Middah (Pus) and Rēhe Motashabbikah (Pent-up Air). The most common causative substances for Wajaulmāfāsil are as follows:

1. Balghame Middi
2. Balghame Khām
3. Dam
4. Safra
5. Saudā may also cause the disease but rarely.

**Al-Asbābul Munfa’ila**: These causes are related to the structure of the organ which is susceptible for the disease e.g.

2. Acquired dilatation of the natural passage.
3. Onset of a new unnatural passage due to movement, Tahallul and Tahkalkhul which may be acquired or congenital such as in Aziātul Ghudad (glandular tissues).

The diseased organ becomes the cause of occurrence of these diseases due to following factors:-

1. Its weakness due to Sue Mizāj Mustahkam (Stable Alteration of the Temperament)
2. Its congenital weakness which is not related to the temperament.
3. Intensive generation of the heat especially when it is supported by movement, pain and other external factors.
4. Being the normal position of the organ below as it lies in relation to other organs where the matters move naturally, that is the cause of its higher incidence in the legs and the hip.

All the above types of Asbābe Munfa’ila are induced by some previous factors, which may be called Asbābe Mu’iddah or predisposing factors. These are as follows:

1. The treatment of intestinal colic in the way that the intestine is strengthened and routine excretory products are diverted to it, but the intestine does not accept them, so they ultimately move to the extremities and joints.
2. Certain diets produce a kind of substance e.g. Luhoome Ghaleezā (Fatty meat), Samake Māleh (Salty Fishes), Chugandar (Beet Root) etc., which causes Wajaulmāfāsil.
4. Sedentary lifestyle (Tabri).
5. Sudden avoidance of exercise.
6. Excessive coitus.
7. Excessive use of Nishashta (Carbohydrates).
8. Ehtabāse Ghair Tabā’i (Abnormal Retention) e.g. amenorrhea and constipation, or to give up Fasād (blood letting) and Is’hāl (purgation) in habitual persons.
9. Exercise or coitus on full stomach (Tabri).
10. Taking bath immediately after meal.

According to Ismāil Jurjāni (Jurjāni, Pub.1873), there are two causes for joint disease. They are:

1. Asbābe Asli
2. Asbābe Aarzi

**Asbābe Asli**

There are three Asbābe Asli:

• Shortage of Rutubat
• Harkaāt
• Quwate Ḫāzima

**Shortage of Rutubat**: Excessive movement of joints utilizes the Rutubat present in the joints. Due to friction, joints become hot and dry, which in turn absorbs more of rutubat, thus causing more loss of Rutubat from the joints.

**Harkaāt**: Due to Harkat (movement), Harārat (heat) is produced, which absorbs the rutubat, and the movements in the joints causes the mawād to be absorbed in the joints, as seen in chirāgh ki batti (domestic lamp).

**Quwate Ḫāzima**: There is no quwate hazimā in the joints, due to which the excess zmount of
khilt that enters into the joint spaces persist and is not digested, thus the wastes can't be disposed off from the joints. Weakness of quwwate hazimā is due to structural peculiarity of joints which is made up of ghuzroof (cartilage), vatr (tendon) and rebāt (ligament). All these are cold and dry, while hazm (digestion) is facilitated by heat and moisture.

Asbabe Aārzi
1. Diminished Mobility.
2. Zoafe Meda (Defective Digestion)
3. Sue Tarteeb (Food intake without schedule).
4. Mastie Mutwātir (Excessive Indulgence).
5. Intake of alcohol in breakfast.
6. Intercourse and Riyāzat after meals.
7. Descend of Nazla and Zukām (Catarrhal fluid) from brain to joints.

Some times avoidance in the habitual evacuation may cause the Wajaulmafāsil.

Persons with cold temperament are susceptibility to develop Wajaulmafāsil. Similarly people with hot temperament may also develop this disease because domination of hot humour causes burning of good humour, and then chances to assume saudāwi temperament increases which is more susceptible to diseases than any other temperament (Jurjani, Pub.1878).

Clinical Manifestations (Ibn Hūbal, Pub.1346 AH)

Wajaulmafāsil Hār
- The affected joint is hot on touch.
- The symptoms are relieved by cold.

Wajaulmafāsil Damvi
- Onset is comparatively sudden and the signs and symptoms are severe.
- Generalized and localized symptoms of Ghalbae Dam (Dominance of Blood) are present.
- Affected part is edematous, tender and reddish.
- Symptoms are aggravated on heat exposure.
- Symptoms are relieved on Venesection.

Wajaulmafāsil Safrāwī
- Onset is again acute and sudden.
- Generalized and localized symptoms of Ghalbae Safrā (Dominance of Bile) are present as yellow colouration. There may also be a red tinge to yellow colouration.
- Joint swelling is lesser than Wajaulmafāsil Damvi.
- Throbbing pain is relatively more than Wajaulmafāsil Damvi.
- Symptoms are aggravated on heat exposure.
- Symptoms are relieved on cold exposure.

Wajaulmafāsil Bārid
- The affected part is cold to touch.
- The symptoms are relieved by heat exposure.

Wajaulmafāsil Balghamī
- It is most common variety of Wajaulmafāsil, the onset of symptoms and signs are gradual.
- Generalized and localized symptoms of Ghalbae Balgham (Dominance of Phlegm) are present.
- Affected joint is swollen, soft, whitish and cold on touch. Pain and throbbing is nominal.
- Symptoms are aggravated on cold exposure.
- Symptoms are relieved on exposure to heat.

Wajaulmafāsil Saudāwī
- It is an uncommon variety of Wajaulmafāsil; some times it occurs at the late and terminal stage of other types.
- Generalized and localized symptoms of Ghalbae Sauda (Dominance of Black Bile) are present.
- Affected joint is dusky, hard and cold on touch.
- Symptoms are aggravated on cold exposure.
- Symptoms may be relieved on warm and wet exposure.

Wajaulmafāsil Reehi
- Joint swelling is very nominal.
- Affected part is felt light.
- Pain is fleeting and radiating in nature.

Wajaulmafāsil Middī
- Affected part is extremely hot and itching with tickling sensation.
- Symptoms may worsen on hot exposure.
- Symptoms are relieved on cold exposure.

Wajaulmafāsil Mufrād
- When, single khilt is involved in the causation of disease.

Wajaulmafāsil Murakkab
- Combined symptoms of more than one involved Akhlāt are present.

Nowadays, the word ‘Wajaulmafāsil’ is used by the Unani physicians as a synonymous of arthritis. Arthritis is a broad term used in medical science for several types of painful conditions of
joints such as rheumatic arthritis, rheumatoid arthritis, gouty arthritis, psoriatic arthritis, gonococcal, typhic, juvenile arthritis, osteoarthritis etc. Whereas in Unani System of Medicine, various forms of arthritis are dealt under the single name i.e. \textit{Wajaulmafasil}.

The descriptions of this crippling disaster mentioned in the literature of Unani System of Medicine, are of paramount importance in the field of Rheumatology. The baseline of the treatment (\textit{Usool-e-Illaj}) should be made according to the classification mentioned above, for the better outcome in the management of this catastrophe of mankind.

References
Effects of herbal formulation diabateen in comparison with glibenclamide on Type 2 diabetics in Pakistani population

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Abstract
The Pakistani population faces a high risk for Type II Diabetes Mellitus. The objective of this study was to evaluate the effects of Diabateen (a herbal formulation) and Glibenclamide (an allopathic medicine) on glycemic control and control of type 2 diabetics. A total of 50 type 2 diabetics, 16 females and 34 males, aged between 27-67 years, were enrolled in the study. After applying the test of significance (Chi-square & Fisher Exact Test) there was no difference between these two drugs as p value was calculated to be 0.145 and both groups exhibited significant reductions in blood glucose levels. Thus, the study shows that Diabateen is as effective as the Glibenclamide in the treatment of Type II Diabetes Mellitus.

Key Words: Diabateen, Glibenclamide, Type II Diabetes Mellitus, Eugenia jambolana, Trigonella foenum-graecum

Introduction
Pakistan is a South-Asian country with a population of approximately 150 million. Diabetes prevalence in Pakistan is high: 12% of people above 25 years of age suffer from the condition (Sher et al., 2002). WHO ranks Pakistan 7th on diabetes prevalence list. In Pakistan, 6.9 million people are affected by diabetes with the International Diabetes Federation estimating that this number will grow to 11.5 million by 2025 unless measures are taken to control the disease.

In 2007, 246 million people world-wide suffered from diabetes making the disease one of the most common non-communicable global diseases and the fourth leading cause of death in the world according to IDF estimates (Anonymous, 2008).

Diabateen

Glibenclamide
As of 2007[update], it is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines (the other being Metformin) (Anonymous, 2007). As of 2003, in the United States, it was the most popular sulfonylurea (Anonymous, 2007).

Materials and Methods
Study Design: The study was Single blind, prospective, multicentre, randomized clinical trial. The main purpose of this study was to carry out clinical trials of herbal formulations among well established cases of Type II Diabetes Mellitus as diagnosed by Clinical Assessment and related Laboratory data. The study was conducted on patients seeking treatment and consultation at Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, Qasmi Clinic Gulshan-e-Iqbal and Diabetes Care Clinic at Sir Syed Town North Karachi, Karachi from November 2006 to February 2009.

Study Population
All the patients who fulfilled the inclusion criteria were selected. After final diagnosis and considering inclusion and exclusion criteria, fifty patients were enrolled in this study. Informed and written consent was taken from all patients. Approval from institutional ethical committee was also obtained.

Subjects
A total of 50 subjects (16 females and 34 males) were recruited. All subjects gave their written informed consent to participate in the study. The study was given approval by Medical Ethics Committee of the University and was conducted during February 2007 to February, 2009. Inclusion criteria were as follows: established Type II Diabetes Mellitus (> 6 months duration), not taking insulin, males or females aged between 27 to 67 years. Subjects were assigned to two groups.

Parameters
Fasting blood glucose (FBG) and Random blood glucose (FBG) were measured at initial level (before study) and follow-ups were taken after every 2 weeks. Total 6 follow-ups were taken.

- Fasting blood glucose (FBG) was determined by glucometer.
- Random blood glucose (RBG) was determined by glucometer.

Dosage and Administration
Glibenclamide tablet was prescribed to the patients with the dosage of one tablet (5mg) thrice daily. Whereas capsule Diabateen 500mg twice daily: a combination of four plants in equal parts named as; Lupinus Albus, Swertia chirata, Eugenia jambolana and Trigonella foenum-graecum.

Statistical Analysis: Initial baseline measurements were compared with final follow ups. The changes were compared between groups by Chi square and Exact Fisher test. Statistical tests were performed using SPSS Software (SPSS 12.0). Statistical significance of the change in results, from pre-study to the post-study is indicated.

| Table – 01 |
| Mean Number of Patients |
| Treatment Group | Mean | Number (n) | Std. Deviation |
| Test Drug (Diabateen) | | | |
| Male | 40.42 | 19 | 10.389 |
| Female | 43.33 | 6 | 13.367 |
| Total | 41.12 | 25 | 10.944 |
| Control Drug (Glibenclamide) | | | |
| Male | 43.93 | 15 | 12.601 |
| Female | 42.30 | 10 | 11.833 |
| Total | 43.28 | 25 | 12.074 |
| Total | | | |
| Male | 41.97 | 34 | 11.374 |
| Female | 42.69 | 16 | 11.993 |
| Total | 42.20 | 50 | 11.457 |
### Table – 02
**Distribution of Age Group in Total Patients**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Treatment Group</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (n)</td>
<td>Control (n)</td>
</tr>
<tr>
<td>27 – 35 Years</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>35 – 42 Years</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>42 – 49 Years</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>49 – 56 Years</td>
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<td>0</td>
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<td>56 – 63 Years</td>
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<td>4</td>
</tr>
<tr>
<td>63 – 70 Years</td>
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<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>

### Table – 03
**Effect of Test and Control Treatment on Fasting Blood Sugar**

<table>
<thead>
<tr>
<th>Fasting Blood Sugar</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Glibenclamide)</td>
</tr>
<tr>
<td>Before Tt</td>
<td>After Tt</td>
</tr>
<tr>
<td>Diabetic</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
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</tr>
</tbody>
</table>

### Table – 04
**Effect of Test and Control Treatment on Random Blood Sugar**

<table>
<thead>
<tr>
<th>Random Blood Sugar</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Glibenclamide)</td>
</tr>
<tr>
<td>Before Tt</td>
<td>After Tt</td>
</tr>
<tr>
<td>Diabetic</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
<td>00</td>
</tr>
</tbody>
</table>

### Results and Discussion

We analyzed the effects of Diabateen and Glibenclamide on Fasting and Random Blood Glucose levels in a Pakistani population with type 2 diabetes. The principle findings conclude that both medicines elicited significant improvements in blood glucose levels.

The data was collected in the years from February 2007 – February 2009, which completed the clinical trial protocol at baseline. The collected data includes 50 patients, among which male patients were 34 (percentage of male 68%), while 16 were female patients (percentage of female 32%). These 50 patients were selected out of 70 patients according to exclusion and inclusion criteria. In the mean of age of over all patients, which are assigned for Diabateen as well as allopathic Glibenclamide, there is no significant difference between them as shown in Table 1.

After exclusion of drop outs (changes in according to exclusion/inclusion criteria), the sample population with Type II Diabetes Mellitus comprised of 50 patients who had fulfilled the criteria at baseline or at follow up. The patient’s gender, age, and baseline clinical features at the time of enrolment were recorded in both treatment arms. So overall, 50 patients were selected and 25 patients assigned to (50%) herbal coded formulation (Diabateen) and 25 patients (50%) to allopathic Glibenclamide. The age distribution of patients for Diabateen and Glibenclamide are shown in Table 2.

All the patients were clearly categorized as having Type II Diabetes Mellitus. The demographic and baseline characteristics of the patients included in the gps. for evaluation was found to be similar for the two treatment groups and were comparable to those of the intent-to-treat population as p > 0.05. All of the patients recruited in this study were categorized in different class interval ranging from 27 years of age to 70 years of age. All patients had one or more pretreatment symptoms of Type II Diabetes Mellitus, which were almost same in both treatment groups as p > 0.05 in tables as mentioned below.

**Fasting Blood Sugar**

Clinically high fasting blood sugar at baseline in both the gps. was found to be similar in both gps. all patients enrolled with diabetes type II. After applying Chi-square and Fisher’s Exact Test p value was calculated to be 1.00, which is greater than 0.05 as shown in Table 3. After having complete follow-ups in test group, the 24 patients out of 25 showed controlled fasting blood sugar and only one patient displayed no improvement. Whereas, in control group out of 25 patients, 21 patients were recorded as having controlled fasting blood sugar and 4 patients showed un-controlled fasting sugar. After applying the test of significance (Chi-square & Fisher Exact Test) there was no difference between these two drugs as p value was calculated to be 0.145 as shown in Table 3.

**Random Blood Sugar**

Clinically relevant random blood sugar at baseline in both the gps. was found to be similar in all patients enrolled with Type II Diabetes Mellitus. After applying Chi-square and Fisher’s Exact Test p value was calculated to be 1.00, which is greater than 0.05 as shown in Table 4.

After having complete follow-ups in test gp., out of 25 patients 24 patients had controlled random...
blood sugar and only one patient showed no improvement. Whereas, in control gp., out of 25 patients, 21 were recorded as having controlled random blood sugar and 4 patients showed uncontrolled random blood sugar. After applying the test of significances (Chi-square & Fisher Exact Test) there was no difference between these two drugs with p value was calculated as 0.145 as shown in Table 4.

Acknowledgement
We thank Dr. Naseem A. Khan Vice Chancellor Hamdard University and all the patients who have volunteered in this study.

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Preventive practices against intestinal worm infestation and the role of qurs-e-deedan in it

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Abstract

Intestinal worm infestations are one of the commonest infectious diseases in the world. It causes long term as well as short term morbidity in children. To study the prevalence and associated factors of worm infestations among children and to test the efficacy of Qurs-e-Deedan in worm infestation. A community based cross sectional study was carried out in Grandpass (n=100) and Wellampitiya (n=100) areas of Colombo district. Sample was selected using three stage convenience sampling method. Efficacy of Qurs-e-Deedan was also tested in a sub sample of the main study population (intervention group n=60; control group n=60). Socio-demographic characteristics, knowledge on intestinal worm, personal hygiene practices and attitude to treatment of participants were recorded using an interviewer administered questionnaire. Efficacy was tested using Kato-katz method and scotch tape test. Indiscriminate defecation prevalence in urban children and rural children were 20% and 9% respectively. More than 50% of households used chlorinated water on both sector while 18% and 40% use boiled water for drinking in urban and rural area respectively. Personal hygiene was poor in both study populations (83% in urban and 63% in rural). Overall knowledge about intestinal worms was poor in both areas (90% in urban and 82% in rural). Western treatment was obtained mostly in both area (61% in urban and 51% in rural). But in rural sector 44% used home remedy only. There were associations between level of education and personal hygiene, and knowledge on intestinal worm. And between knowledge about intestinal worms and personal hygiene. Both Qurs-e-Deedan and Mebendazole produced highly significant decrease in worm egg count and symptoms produced by the test and standard drug were not significant statistically. These findings suggest that intestinal worms need to be still considered an important public health problem in the respective area. Qurs-e-Deedan was found to be efficacious in reducing worm egg counts and improving symptoms to the same extent as Mebendazole.

Key Words: Intestinal worms, Unani Medicine, Qurs-e-Deedan

Introduction

Intestinal worms / Deedan-e-Ama’a have been prevailing from the ancient times. The causes, types of worms and herbal treatment have been studied in detail by Ibn-e-Sina and by Al Razi (864-925 CE). The incidence of round worm infection is about one billion; whip worm infection is about 500 million; and hook worm infection is about 900 million worldwide. Worm infestation is a public health problem in most developing countries (Narhayti 2003).

Several researches have been carried out in Sri Lanka in different parts of the country. Research findings in Mahayyawa slums revealed that the
overall prevalence of intestinal parasites was 26.4%, to which Ascaris contributed by 23.8%, and Trichuria by 7.2% (Dee Silva et al, 1996.) Research conducted in two estate areas namely Meliboda and Ayr estate, should that the prevalence of Ascaris was 53.4% and 51%, respectively (Gunavardane et al, 2004).

A complexity of belief, attitude and practices concerning worm treatment and personal hygiene are relevant to worm infestation prevention. Therefore, health education and programs that promote personal hygiene could only be successful if they are based on the current level of knowledge, attitudes and practices of caregivers of children with respect to worm infestation and hygiene (Malik et al, 1992).

Therefore, the present study looked at the knowledge, attitudes and the level of hygienic practices associated with worm infestations among a vulnerable group of people. These findings will help to implement a comprehensive intervention program in future in these high risk populations.

Qurs-e-Deedan is a compound Unani medicine used since ancient times in the treatment of worm infestations (Anonymous,1983). The herbs in the Qurs-e-Deedan are freely available throughout Sri Lanka. The ingredients in Qurs-e-Deedan are cheap and it is easy to prepare the pills. Above factors prompted to test the efficacy of Qurs-e-Deedan against the standard treatment viz, Mebendazole for worm infestation in this study.

**General objective:**
To study the prevalence, preventive practices and associated factors of worm infestations and to assess the efficacy of Qurs-e-Deedan in the district of Colombo.

**Materials and Methods**

**Component I- Cross Sectional Study**
A community based cross sectional study was carried out from August to October in 2008 in Grandpass (urban) and Wellampitya (rural) areas of the district Colombo.

**Study setting**
Both components of the study were carried out in the district Colombo. All mothers or care-givers (in the absence of mothers at home) of children aged 2-16 years, at least one child of either sex living in a household located in the selected study setting were included in the study. Exclusion criteria were those residing in the area for a period of less than 1 year and health care workers.

**Sample size**
Sample size was decided to include a minimum of 200 households of which 100 households were located within each urban and rural setting.

**Sampling method**
These two settings were selected using a convenience sampling method. A three stage sampling method was used to obtain this sample size of 200 households.

**Study instrument**
Information was obtained from respondents by using a pre tested interviewer-administered questionnaire. This questionnaire included data on socio demographic characteristics, practices related to personal hygiene, knowledge on worm infestations and attitudes on worm treatment and preventive practice related to worm infestation.

Personal hygiene of respondents and children were classified according to a scoring system. Each correct practice was given one mark and decided to have a total score of 8.5 or above to be considered as “good” personal hygiene whereas a score below 8.5 was considered as “poor” personal hygiene. Overall knowledge on intestinal worms of the respondents also was categorized into ‘good’ and ‘poor’ knowledge according to another scoring system. Each correct answer was given one mark and a total score of 19 or above to be considered as ‘good’ knowledge, whereas a score below 19 was taken as ‘poor’ knowledge.

**Method of data collection**
Data were collected by the principal investigator and trained data collectors by visiting households. Information was obtained after getting the written informed consent from eligible respondents. While ensuring privacy and confidentiality of the collected data.

**Statistical analysis**
Data were analyzed using SPSS 14.0 statistical package. Knowledge, attitudes and practices of mothers / care givers of children were assessed using descriptive statistics. Associations of these variables were assessed for their significance using significance tests (chi square test).

**Component II- Randomized Clinical Trial**
A randomized, single blinded clinical trial was carried out in the same study setting from
February to July in 2009 with the objective of assessing the efficacy of Qurs-e-Deedan.

Study population

Inclusion criteria for the interventional study were symptomatic children with positive stool and scotch tape test for ova, who were aged 2-16 years and of either sex. Exclusion criteria were, children who were currently on medical treatment for worm infestation and children who were treated for worm infestations during the last 2 weeks.

Sampling method and sample size

From the 200 households visited during the first component, respondents who gave consent and also fulfilled the eligibility criteria were selected for screening to be included in the trial. Thereafter, these children were allocated to 2 groups of 60 by using a simple random method. Once the 2 groups were identified, Qurs-e-Deedan (intervention group) and standard drug treatment viz, Mebendazole (control group) were randomly allocated.

Hold Outcome Measures

Reduction in worm egg count was assessed by laboratory investigations.

Improvement of symptoms was assessed by clinical examination and questionnaire

Information on previous treatment, personal hygiene and detailed history was obtained using a pre tested proforma. A separate proforma was used to assess the temperament of individual subjects (Appendix-4). Investigations carried out were Kato Katz techniques (Anonymous, 1991), Scotch tape test and blood tests (Hb % and WBC-DC).

Method of data collection:

Informed written consent was obtained from parent or guardian of all the positive cases. All children included in the study were subjected to relevant clinical examination along with presence or absence of specific symptoms associated with worm infestation. The same examination and symptom inquiry were repeated at the same time after 2 weeks and laboratory investigations were performed.

Administration of the intervention

a) Preparing the Qurs-e-Deedan Composition of Qurs-e-Deedan.

1. Palaspapda / Butea monosperma (lam) 1 part
2. Maghz e tukhm e Karanj / Pogamia 1 part
3. Nankhua / Carum capticum 1 part
4. Qinbeel / Melatus philippinensis 1 part
5. Baubadang / Embelia ribes 1 part
6. Turbud / pomea tarpetham 1 part
7. Qandsiya / sugar 1 part

Qurs-e-Deedan pills were rolled weighing 250mg each. Rolled pills were dried in Drier and packed in an air tight polythene pack having six pills each. Mebendazole (100mg) was packed in sealed air tight polythene packs having 6 tablets each.

b) Baseline assessment of outcome measures

Prior to allocation of treatment, the following investigations were carried out on each participant to score for the Outcome Measures (Appendix-5).

The Mizaj of the Subjects was determined by the method of Alavi et al. (2005) (Appendix-4)

Stool examinations- Kato Katz techniques (Anonymous, 1991)
Scotch tape test
Blood examinations- Hb %, WBC-DC

c) Allocation of treatment

Each group was then randomly allocated to one type of treatment. Qurs-e-Deedan 1(250mg) pill twice a day for 3 days was administered in intervention group and Mebendazole 1(100mg) pill twice a day for 3 days for standard treatment group.

d) Follow up

Follow up was carried out on day 15 with repeated stool examinations / tape tests in both intervention and control groups. A detailed clinical examination and an evaluation of relief of symptoms were also carried out on the same day.

e) Outcome Measures

Statistical analysis

Efficacy of Qurs-e-Deedan tablets was assessed by the difference in the reduction of egg count and relief of symptoms before and after the period of treatment compared to that of Mebendazole. Paired ‘t’ test and independent ‘t’ test were used for this purpose.

Results

1. Component I (Cross sectional study)

Mean age of the children in Grandpas area was 6 years (SD = 4.5). Mean age of the children in
Wellampitiya area was 7 years (SD = 3.8). Most of the respondents in both areas were mothers who were housewives (>70%). In both areas, most of the children below 10 year old used toilets, however 20% of urban children and 9% of rural children also used open space for defecation. Majority of them in both areas used chlorinated water for dirking. 18% of urban households used boiled water in contrast to 40% in rural area using boiled water for drinking.

Most of the children in both areas washed hands after using toilet and before meals using soap and water. However majority of the children did not wash hands after playing. Almost all respondents in both areas washed hands after using toilet with soap and water. Most of them washed hands before meals. Prevalence of poor personal hygiene was 83% and 63% in urban and rural sectors, respectively. Overall prevalence of worm infestation was 40%.

Most of the respondents in both areas agreed that sweet is a causative factor for worm. They were not aware of other causative factors. Most of the respondents in both areas were not certain about the consequences of worm infestation.

Majority of them agreed that taking regular treatment and proper hand washing are the preventive measures but were not certain about other preventive aspects. Majority of the respondents were also not sure about the mode of transmission of worms. 90% and 82% of respondents had poor knowledge about worm infestation in urban and rural area, respectively.

Most of the time only the affected children were treated for worm infestation in both areas. In both areas mostly, initial treatment was given after completion of first birth day of children. 34% of children in urban area were treated every 3 months while similar percentages of children in rural area were treated every 6 months. Majority of them took western treatment in both areas while 44% of the rural population took home remedy only. Majority of them in both areas did not treat their children while having fever, Cold /cough, during rainy days and after immunization. They also avoid sour food and oily food during treatment.

Poor hygiene was higher among those with poor knowledge about worm infestation (89%) compared to those with good knowledge. There was significant association between poor knowledge on worms and poor personal hygiene (p<0.05).

Component II (Clinical Trial)

A total of 293 subjects were screened to select the 120 participants. 150 scotch tape test and 143 stool tests were done during the screening. Out of 150 scotch tape tests 103 were positive and out of 143 stool tests 17 were positive. Positive case with 52 pin worms and 8 whip worms were enrolled for intervention group while positive cases with 51 pin worms, 8 whip worms and 1 round worm were enrolled for control group (Table 1). Total withdrawal cases in both groups were 4 (3%).

Mean age of intervention group was 6.6 years (SD=2.9) and in control group was 6.7 (SD=3.1). The mean Hb percentage of intervention group was 12.78 g % (N=59; SD=1.46). Mean Hb percentage of standard treatment group was 12.8 g % (N=57, SD=1.55).

32 male and 28 female children were enrolled in intervention group. 25 male and 35 female children were enrolled for control group. The difference between intervention and control group in gender (p>0.17), hygiene (p>0.78), and taking last treatment (p>0.59) were statistically not significant. Most of the children’s Mizaj was Balghami (Phlegmatic) in both groups.

### Table 1

<table>
<thead>
<tr>
<th>Temperament (Mizaj) type of intervention and control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperament (Mizaj)</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
</tr>
<tr>
<td>Type</td>
</tr>
<tr>
<td>Balgham (Phlelematic)</td>
</tr>
<tr>
<td>Dammavi (Sangunic)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
a) Baseline assessment of outcome measures

Table 2
Baseline characteristics related to type of worm infestation of intervention and control group

<table>
<thead>
<tr>
<th>Worm infestation</th>
<th>Intervention Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thread</td>
<td>52</td>
<td>87</td>
</tr>
<tr>
<td>Whip</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Round</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3
Mean egg count and symptom difference in intervention and control group

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Intervention Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Mean (SD)</td>
<td>After Mean (SD)</td>
</tr>
<tr>
<td>Easter count</td>
<td>87.2 (77.6)</td>
<td>5.6 (8.8)</td>
</tr>
<tr>
<td>74.4 (77.2)</td>
<td>5.3 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Symptom score</td>
<td>0.18 (0.38)</td>
<td>0.04 (0.18)</td>
</tr>
<tr>
<td>0.19 (0.39)</td>
<td>0.02 (0.14)</td>
<td></td>
</tr>
</tbody>
</table>

Efficacy of Qurs-e-Deedan and Mebendazole in reducing worm egg count

Efficacy of Qurs-e-Deedan was assessed in relation to the mean egg count before and after the period of intervention. For this purpose, paired t t test was used. The mean egg count reduction in Qurs-e-Deedan was statistically significant (p<0.01). Similarly, when the efficacy of Mebendazole was assessed, it also showed a statistically significant mean score for improvement of symptoms (p<0.01).

Table 4
Efficacy of the individual drugs in improving overall symptoms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Symptom score (SD)</th>
<th>Difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td></td>
</tr>
<tr>
<td>Qurs-e-Deedan</td>
<td>0.18 (0.38)</td>
<td>0.04 (0.18)</td>
<td>0.14 (0.4)</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>0.19 (0.39)</td>
<td>0.02 (0.14)</td>
<td>0.17 (0.4)</td>
</tr>
</tbody>
</table>

Comparison of efficacy between two drugs in reducing worm egg count

Efficacy of Qurs-e-Deedan compared to Mebendazole was assessed in relation to the reduction of mean egg counts. For this purpose, independent t test was used. The difference in the mean egg count between Qurs-e-Deedan and Mebendazole was not statistically significant (p>0.05).

Comparison of efficacy between two drugs in improving symptoms

Efficacy of Qurs-e-Deedan compared to Mebendazole was assessed in relation to improvement of overall symptoms. The difference in the mean symptoms score between Qurs-e-Deedan and Mebendazole was not statistically significant (p>0.05).

Discussion

Mean age of children in this study was 6 years and 7 years in urban and rural households respectively. Previous research in Sri Lanka in the tea plantation sector revealed the highest prevalence of worm infestations among children aged 6-12 years (Gunawardene et al, 2004). Therefore, the children of the present study population were within the most vulnerable age group for worm infestations.

In 1996, another study reported an overall prevalence of intestinal worms of 26.4% in an urban slum area in the district of Kandy (D Silva et al, 1996). Overall prevalence of intestinal worm in this study was 40%. The knowledge of respondents on intestinal worms and personal hygienic practices of households was poor in both areas, which also could have contributed to the above high prevalence.
Another salient feature in relation to the prevalence of worm infestation was its variation between urban and rural settings. It was 46.9%, and 31.9%, in urban and rural population, respectively. Significant variation in urban and rural prevalence has been observed in previous research in Colombo district (Pulani et al, 1999).

Previous findings showed variation in hygienic standards between households and is known to be related to socio economic status and level education of families (Malik et al, 1992). In this study too such variation was seen in hygienic standards related to level education of families in rural sector. But no significant association was found between hygienic standards and socio economic status in both sectors.

A study in Nepal reported that there was a highly significant relationship between education and the knowledge of mothers regarding helminthic infestations and method of prevention (Shah & Baig, 2001). Similarly, a significant association was found in this study among rural sector respondents in level of education and knowledge on intestinal worm infestations.

91 % of urban respondents and 82 % of rural respondents opined that sweets cause worm infestation. Unani medical view also considers taking excessive sweets as a causative factor for worm infestation (Ibn Sina). In contrast, according to modern scientific point of view this is not true.

Majority of them treated their children in spite of poor knowledge about worm infestation in the present study population. A similar result was found in previous survey where 90% of primary school children were treated regularly (Parthemesuaran, 2001).

The major limitation encountered in cross sectional studies was that determinants of knowledge attitude and practices were assessed at a single point in time. It was not possible to account for the temporality of the relationships, which may have modified over time.

**Randomized clinical trial**

Mebendazole is known to be highly effective against round worm with a cure rate of 90% to 100% (De Silva, 1997). Present trial found there was no difference in the efficacy between Qurs-e-Deedan and Mebendazole. Since the conclusion is Qurs-e-Deedan is equally effective as Mebendazole, it is recommended for in treating intestinal worms particularly thread worm and whip worm infestation since it is cheaper, culturally congenial and more safe.
Appendix-1

Questionnaire of Cross Sectional Study

Preventive Practices of Intestinal Worms and the Role of Qurs e Deedan in the Prevention of Worm infestation

Serial No: 

G N Division

Household number Address / Road

Details of Household members:

1. Ethnicity: 1-Sinhalese, 2- Tamil, 3- Muslim, 4-Burgher, 5- Other (specify) 
2. Religion: 1-Buddhism, 2- Hindu, 3- Islam, 4- Catholic, 5- Other (specify) 
3. Number of household members:

<table>
<thead>
<tr>
<th>HHM No.</th>
<th>Age</th>
<th>Sex (m/f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Monthly household income

<table>
<thead>
<tr>
<th>&lt;10,000</th>
<th>10,000- 20,000</th>
<th>&gt; 20,000</th>
</tr>
</thead>
</table>

Description of the respondent:

5. Respondent: 1-mother, 2-father, 3-guardian (grandparent, relation, neighbour, servant in the absence of mother / father)

6. Current occupation:

<table>
<thead>
<tr>
<th>House wife</th>
<th>Currently employed</th>
<th>Previously employed</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

7. Level of education

<table>
<thead>
<tr>
<th>No schooling</th>
<th>G 1-6</th>
<th>G 7-10</th>
<th>O/L Passed</th>
<th>G 11-12</th>
<th>A/L passed</th>
<th>Diploma Vocational</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>
Sanitary facilities:

8. Toilets

8.1 Toilet facility: 1-public (shared by more than 1 family), 2-private

8.2 Location of toilet: 1-inside house and next to kitchen, 2-inside house but not next to kitchen, 3-outside the house

8.3 Type of toilet: 1-pit, 2-water seal: squatting/commode, 3-other

8.4 Frequency of cleaning toilet: 1-daily, 2-(3-4) days a week, 3-once a week, 4-once a month

8.5 Indiscriminate defecation of children < 10 years old

<table>
<thead>
<tr>
<th>HHM No.</th>
<th>1-Potty</th>
<th>2-Commode</th>
<th>3-Nappies</th>
<th>4-Open space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.6 Method of disposal of faeces of children < 10 years old

<table>
<thead>
<tr>
<th>HHM No.</th>
<th>1-Drainage</th>
<th>2-Toilet</th>
<th>3-Garbage</th>
<th>4-Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Water facilities:

9.1 Water facility: 1-public (shared by more than 1 family), 2-private

9.2 Type of water facility:
1-pipe borne, 2-well, 3-other

9.3 Drinking water:
1-boiled and cooled, 2-not boiled but chlorinated, 3-not boiled and not chlorinated

9.4 Storage for drinking water:
1-filter, 2-closed container, 3-clay pot, 4-no proper utensil, 5-other
10. **Food preparation and feeding practices**

10.1 Person preparing food at home

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Domestic aid</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>For adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For children &lt; 10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10.2 Sources used for hand washing by person who prepares food at home

<table>
<thead>
<tr>
<th></th>
<th>For adults</th>
<th>For children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soap and water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10.3 Washing of vegetables for cooking:

1. Only water,
2. Water with salt,
3. Not done

10.4 Fruits for children:

1. Washed and given,
2. Without washing,
3. Not applicable

10.5 Feeding children:

1. Using fingers,
2. Using spoon,
3. Bottle feeding,
4. Not applicable

10.6 Utensil for preparation of food and milk for children: Separate utensil

1. Yes, 2. No, 3. Not applicable

11. **Personal hygiene of respondent**

11.1 Hand washing of the respondent using soap and water

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a After using toilet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Before meals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Before preparing food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Before breast feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e After cleaning children nappy/ feces</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C

11.2 Hand washing of children < 10 years

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a After using toilet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Before meals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c After playing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11.3 What is used for hand washing in children < 10 years?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>1</td>
</tr>
<tr>
<td>Soap and water</td>
<td>2</td>
</tr>
<tr>
<td>Water only</td>
<td>3</td>
</tr>
</tbody>
</table>

11.4 Trimming the nails of children < 10 years and Respondent

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Respondent</th>
<th>Children</th>
<th>&lt; 2</th>
<th>2-5</th>
<th>&gt; 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Weekly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Monthly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 &gt; monthly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Not applicable(nail biting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11.5 Nail biting in children < 10 yr: 1-yes, 2-no, 3-not applicable

11.6 Bathing

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Respondent</th>
<th>Children</th>
<th>&lt; 2</th>
<th>2-5</th>
<th>&gt; 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 EOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Weekly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D

11.7 Wearing foot wear: going to toilet / playing / school

<table>
<thead>
<tr>
<th></th>
<th>Wearing foot wear</th>
<th>Respondent</th>
<th>Children</th>
<th>&lt; 2</th>
<th>2-5</th>
<th>&gt; 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Playing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b School</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Going to toilet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12. **Knowledge on worm infestations:**

12.1 Are you aware of the following types of worms?

<table>
<thead>
<tr>
<th>Round</th>
<th>Thread</th>
<th>Tape</th>
<th>Whip</th>
<th>Hook</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12.2 What foods produce worms in the body?

<table>
<thead>
<tr>
<th>Source</th>
<th>Agree</th>
<th>Not agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Sweets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Under cooked meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Green vegetables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Unclean water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12.3 Consequences of worm infestations

<table>
<thead>
<tr>
<th>Consequences</th>
<th>Agree</th>
<th>Not agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Growth impairment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Sleep disturbance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Learning problems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Vitamin deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12.4 Mode of transmission:

<table>
<thead>
<tr>
<th>Mode of transmission</th>
<th>Agree</th>
<th>Not Agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Person to person</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Contaminated food &amp; drink</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Self</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12.5 How can we prevent the spread of worms?

<table>
<thead>
<tr>
<th></th>
<th>Agree</th>
<th>Not agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Taking treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Proper hand washing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Proper washing vegetables / fruits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Bathing</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12.6 How do you recognize that your children have got worm problems?

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Seeing adult worm in stool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Nocturnal itching of anus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Teeth biting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Rash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e Abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f Vomiting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g LOA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>j Salivation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13. Worm treatment seeking behaviors:

13.1 Who are the household members taking worm treatment?

1 All members
2 Only children
3 Only the affected child

13.2 At what age do you start giving treatment?

<table>
<thead>
<tr>
<th>Age</th>
<th>1 yr</th>
<th>2 yr</th>
<th>6/12</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13.3 How often does your family take treatment? Every

<table>
<thead>
<tr>
<th>Frequency</th>
<th>3/12</th>
<th>4/12</th>
<th>6/12</th>
<th>Annually</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13.4 Type of treatment is taken in your family

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Herbal</th>
<th>Western</th>
<th>Home remedy</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13.5 If herbs, what kind

<table>
<thead>
<tr>
<th>Kind</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13.6 How do you obtain treatment for your family? (1 answer)

1 After consulting a Doctor
2 Self medication
3 Recommended by pharmacist
4 Home made
13.7 During pregnancy, do you take treatment? 1-yes, 2-no, 3-not applicable

13.8 If not, why?........................................................................................................

13.9 Is there any instance where you should not give worm treatment?

<table>
<thead>
<tr>
<th>Agree</th>
<th>Not agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Have fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Cold and cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c During rainy days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e After immunization</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13.10 Is there any precaution to be taken during treatment?

<table>
<thead>
<tr>
<th>Agree</th>
<th>Not agree</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Avoiding sour foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Avoiding oily foods</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G

Appendix-2

List of Personal Hygiene Scoring Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Minimum score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand washing in respondent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After using toilet</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Before meals</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Before preparing food</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Before breast feeding</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>After cleaning children nappy/ feces</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hand washing in children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After using toilet</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Before meals</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>After playing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Use of soap and water for hand washing</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Frequency Trimming nail -weekly</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bathing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EOD</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Wearing of foot wear while using toilet</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>12.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>
## List of Overall Knowledge Scoring Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Minimum score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Types of worm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Round</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Thread</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tape</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Whip</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hook</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>What produces worm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweets</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Under cooked meat</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Green vegetables</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Unclean water</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Consequences of worm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth impairment</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Learning problems</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin deficiency</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Mode of transmission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person to person</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Contaminated food &amp; drink</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Self</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Methods of prevention of worm infestation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking treatment</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Proper hand washing</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Proper washing vegetables / fruits</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bathing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms of worm infestation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeing adult worm in stool</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nocturnal itching of anus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Teeth biting</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LOA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Salivation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>19</td>
</tr>
</tbody>
</table>
Appendix-4

Proforma for assessing Temperament

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Name of the patient</th>
<th>Age</th>
<th>sex</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Dammavi / Sanguinous</td>
<td>Balgamy / Phlegmatic</td>
<td>Safravi / Cholaratic</td>
<td>Saudavi / Melancholic</td>
</tr>
<tr>
<td>Complexion</td>
<td>Reddish / wheat brown</td>
<td>1</td>
<td>Chalky / whitish</td>
<td>0.75</td>
</tr>
<tr>
<td>Body built</td>
<td>Muscular and broad</td>
<td>1</td>
<td>Fatty and broad</td>
<td>0.75</td>
</tr>
<tr>
<td>Touch</td>
<td>Hot and soft</td>
<td>1</td>
<td>Cold and soft</td>
<td>0.75</td>
</tr>
<tr>
<td>Hair</td>
<td>Bleakish</td>
<td>1</td>
<td>Brownish</td>
<td>0.75</td>
</tr>
<tr>
<td>Movement</td>
<td>Active</td>
<td>1</td>
<td>Dull</td>
<td>0.75</td>
</tr>
<tr>
<td>Diet Most liked</td>
<td>Cold and dry</td>
<td>1</td>
<td>Hot and dry</td>
<td>0.75</td>
</tr>
<tr>
<td>Weather most suitable</td>
<td>Spring</td>
<td>1</td>
<td>Summer</td>
<td>0.75</td>
</tr>
<tr>
<td>Sleep</td>
<td>Normal</td>
<td>1</td>
<td>In excess</td>
<td>0.75</td>
</tr>
<tr>
<td>Pulse</td>
<td>Normal rate 70-80/min</td>
<td>1</td>
<td>Slow 60-70/min normal volume</td>
<td>0.75</td>
</tr>
<tr>
<td>Emotions</td>
<td>Normal</td>
<td>1</td>
<td>Calm and quiet</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Total points

Range of temperament in numbers: Sanguine = 7.51 to 10, Phlegmatic = 5.10 to 7.50, Choleric = 2.51 to 5.00 and Melancholic = 0.00 to 2.50

Appendix-5

List of Symptoms included as Outcome Measure and the scores allotted to them

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Seeing adult worm in stool</td>
<td>1</td>
</tr>
<tr>
<td>2 Nocturnal itching of anus</td>
<td>1</td>
</tr>
<tr>
<td>3 Teeth biting</td>
<td>1</td>
</tr>
<tr>
<td>4 Rash</td>
<td>1</td>
</tr>
<tr>
<td>5 Abdominal pain</td>
<td>1</td>
</tr>
<tr>
<td>6 Vomiting</td>
<td>1</td>
</tr>
<tr>
<td>7 LOA</td>
<td>1</td>
</tr>
<tr>
<td>8 Diarrhea</td>
<td>1</td>
</tr>
<tr>
<td>9 Dry Cough</td>
<td>1</td>
</tr>
</tbody>
</table>
References

A study of Unani formulation Majoon-e-baladur for effect on drug induced catatonia in rats

NA Khan, MN Nasiruddin, MM Muzaffar and IA Qasmi

1Department of Ilmul Advia, A. K. Tibbiya College, Aligarh Muslim University, Aligarh, India.
2Department of Pharmacology, J. N. Medical College, Aligarh Muslim University, Aligarh, India.

Abstract

D2 antagonists are associated with extra pyramidal symptoms which are very distressing. The drugs available in modern medicine for therapy of Parkinsonism and drug induced extrapyramidal symptoms are also not free from adverse effects. The Unani formulation (Majoon-e-Baladur) is one of the known formulations which contains 21 compounds and used in Unani system of medicine as nervine tonic, in tremors for years. But there is no scientific basis for its use. The study was planned to evaluate its effect in drug (haloperidol) induced catatonia. The effect was compared with standard drug i.e. benzhexol. This study was conducted on adult albino rats weighing 150 – 200 gm of either sex. They were divided into four groups of six animals each. The animals were provided standard diet and water ad libitum. The animals in group I were administered distilled water. Groups II, III and IV were administered benzhexol majoon and alcoholic extract of Unani formulation in the dose of 0.15mg, 140mg and 10mg/100gm body weight, respectively. The treatment was given p.o. once daily for 30 days. On 30th day 45 minutes after treatment all animals in different groups were administered haloperidol in the dose of 0.30mg/100gm body weight intraperitoneally. All the animals were tested for the intensity of catatonia at every twenty minutes for a period of three hours. The percent inhibition of catatonia in test and standard drug was calculated and compared with control. It was found that there was significant decrease in duration of catatonia in test and standard drug treated groups as compared with control at different interval of time. It can be concluded that the Unani formulation in extract form was more effective than majoon in drug induced catatonia.

Key Words: Parkinsonism, Semecarpus anacardium

Introduction

Neuroleptics and some prokinetics are often associated with distressing and severe extra pyramidal side effects (Casey DE, 2000 & Kulkarni SK, Naidu PS, 2001). The phenomenon to induce catatonia in rodents by typical neuroleptics (Phenothiazines, haloperidol) is robust behavioral model to study nigrostriatal function (Turner, 1965, Albina A, 2007). Werth et al (1958) divided catatonia into 4 stages depending upon increasing intensity. He observed that the drugs able to antagonize catatonia of stage III and IV possessed good antiparkinsons activity.

Neuroleptics are most commonly used drugs in schizophrenia and other affective disorders (Alfred Goodman Gilman, 2006). Prokinetic drugs (metoclopramide) also induce catalepsy in mice (Ahtee L, 1974). The drugs available in modern medicine for the therapy of Parkinsonism and drug induced extrapyramidal symptoms are also having limitations to be used due to their adverse drug reactions.

The Unani Formulation (Majoon-e-Baladur) is one of the known formulations which contains 21 compounds and used in Unani system of medicine as nervine tonic, in tremors for years. But there is no scientific basis for its use. The
study was planned to evaluate its effect in drug (haloperidol) induced catatonia. The effect was compared with standard drug i.e, benzhexol.

Material and Methods

The study was conducted in the Department of Ilmul Advia, A. K. Tibbiya College, AMU, Aligarh in collaboration with the Department of Pharmacology, J. N. Medical College, AMU, Aligarh, after obtaining permission from Institutional Animal Ethical Committee.

Animals – Albino rats of either sex (Wistar Strain) Weighing 150 – 200 gm. were divided into four groups of 6 animals each. The animals were provided standard diet (Lipton India) and water and libitum. The temperature at 26 ± 2 °C and 12 hrs dark and light cycles were maintained.

Drugs: The ingredients as described in National Formulary of Unani Medicine, Part I (Anonymous 1981) (table – 1)were procured from Dawakhana Tibbiya College AMU, Aligarh and were identified by comparison for its macroscopic and microscopic characters with authentic specimens of the ingredients at the Botanical survey of India, Dehradun and Forest Research Institute Dehradun, India. The ingredients were administered in two forms (a) Majoon and (b)

50% alcoholic extract. The amount of ingredients as mentioned in National Formulary of Unani Medicine, Part I were mixed in Majoon and for extract.

Benzhexol was obtained from Sigma pharmaceuticals.

Haloperidol was obtained from Torrent pharmaceuticals.

Preparation of Majoon

All the ingredients except Crocus sativus, Linn (Zafran) and Pistacia lentiscus, Linn (mastagi) were powdered in an electric grinder and sieved 80 No mesh. P.lentiscus was powdered separately in porcelain mortar by slow and light movement and C. sativus was ground in 50 ml rose water separately in a china clay mortar. Qiwm (sugary base) was prepared from sugar and honey both, sugar (350gm) was dissolved in 200 ml of water at boiling stage, thereafter honey(1 kg) was added in the sugar solution and the mixture was heated. Powdered ingredients were added in the qiwm (sugary base) and mixed well. After cooling P. lentiscus powder and ground C. sativus in rose water were added to the above syrup containing all other ingredients and made homogenous by stirring.

### Ingredient of Majoon-e-Baladur

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Content</th>
<th>Botanical Name</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Kunjad</td>
<td>Sasamum Indicum Linn.</td>
<td>30 gm</td>
</tr>
<tr>
<td>2.</td>
<td>Maghz-e-Tukhum-e-Baladur</td>
<td>Semecarpus anacardium Linn.</td>
<td>30 gm</td>
</tr>
<tr>
<td>3.</td>
<td>Maghz-e-Badam</td>
<td>Prunus amygdalus, Bail</td>
<td>30 gm</td>
</tr>
<tr>
<td>4.</td>
<td>Maghz-e-Chilghoza</td>
<td>Pinus gerardiana, Wall</td>
<td>30 gm</td>
</tr>
<tr>
<td>5.</td>
<td>Asgand</td>
<td>Withania somnifera, Dunal</td>
<td>30 gm</td>
</tr>
<tr>
<td>6.</td>
<td>Aqarqarha</td>
<td>Anacyclus Pyrethrum DC</td>
<td>30 gm</td>
</tr>
<tr>
<td>7.</td>
<td>Khulanjan</td>
<td>Alpinia galanga, wild</td>
<td>30 gm</td>
</tr>
<tr>
<td>8.</td>
<td>Jauzabuwa</td>
<td>Myristica fragrans, Houtt.</td>
<td>30 gm</td>
</tr>
<tr>
<td>9.</td>
<td>Bisbasa</td>
<td>Myristica fragrans, Houtt.</td>
<td>30 gm</td>
</tr>
<tr>
<td>10.</td>
<td>Zanjabeel</td>
<td>Zingiber officinal, Roscae</td>
<td>20 gm</td>
</tr>
<tr>
<td>11.</td>
<td>Salab Misi</td>
<td>Orchis latifolia, Linn.</td>
<td>20 gm</td>
</tr>
<tr>
<td>12.</td>
<td>Filfil Daraz</td>
<td>Piper longum Linn.</td>
<td>15 gm</td>
</tr>
<tr>
<td>13.</td>
<td>Mastagi</td>
<td>Pistacia lentiscus Linn.</td>
<td>15 gm</td>
</tr>
<tr>
<td>14.</td>
<td>Tukhm-e-haliyun</td>
<td>Asparagus officinalis Linn.</td>
<td>15 gm</td>
</tr>
<tr>
<td>15.</td>
<td>Tukhm-e-Gazar</td>
<td>Daucus carota Linn.</td>
<td>10 gm</td>
</tr>
<tr>
<td>16.</td>
<td>Tukhm-e-Anjra</td>
<td>Blepharis edulis Pers.</td>
<td>10 gm</td>
</tr>
<tr>
<td>17.</td>
<td>Tukhm-e-Konch</td>
<td>Mucuna Pruriens Bak.</td>
<td>10 gm</td>
</tr>
<tr>
<td>18.</td>
<td>Zafran</td>
<td>Crocus sativus Linn.</td>
<td>10 gm</td>
</tr>
<tr>
<td>19.</td>
<td>Samundar Sokh</td>
<td>Argyreia speciosa, sweet</td>
<td>5 gm</td>
</tr>
<tr>
<td>20.</td>
<td>Qand Safaid</td>
<td>Saccharum officinarum, Linn.</td>
<td>375 gm</td>
</tr>
<tr>
<td>21.</td>
<td>Asal</td>
<td>Apis mellifera, Linn</td>
<td>1 Kg</td>
</tr>
</tbody>
</table>
Preparation of Extract

All the ingredients except honey, sugar, P. lentiscus and C. sativus were powdered in an electric grinder. P. lentiscus was powdered separately in a mortar. The powdered ingredients and C sativus were extracted in Soxhlet's apparatus for 6 hrs in 50% alcohol. The extract was filtered and dried on a water bath. The yield percentage of extract was calculated with reference to crude drug and was found to be 8.56%.

The animal dose of Majoon and extract were calculated in the usual manner by dividing the Unani Clinical dose by 50 then multiplying by 7 for albino rats (Dhawan, 1982).

Doses of the Unani formulation and drugs in rats

Majoon – 140 mg / 100gm body weight p o
50% alcohol extract 10mg / 100gm body weight po
Benzhexol 0.15 mg / 100 gm p o
Haloperidol – 0.30mg / 100gm body weight i.p.

Methods – Test was carried out by the method of Morpugro (1962). The animals were divided into four groups of 6 animals each. The animals in group I were administered distilled water and served as control. The animals in group II were treated with benzhexol 0.15 mg / 100gm once daily. The dose of benzhexol in human is 6-12 mg/day (Alfred Goodman Gilman, 2006). The animals in groups III and IV were administered majoon and alcoholic extract in the dose of 140 mg and 10mg / 100gm body weight once daily p.o. The animals of all groups were treated for 30 days. On the 30th day 45 minutes after administration of distilled water, standard drug & test drug, all animals in different groups were administered haloperidol in the dose of 0.30 mg / 100 gm body weight intraperitoneally. The maximum dose of haloperidol in human is 20 mg /day (Alfred Goodman Gilman, 2006). The dose of benzhexol and haloperidol in rats was calculated by formula given by Dhawan, 1982. All the animals were tested for the intensity of anticitatonia at every 20 minutes for a period of 3 hrs. The right and left fore limbs of each animal was successively placed on 3 cm high object. If the animal retained the position for 10 seconds a score of 0.5 was given (for each fore limb). Later the limbs were placed on 6 cm high object. If the animal retained the posture for 10 seconds a score of 1 was given (for each limb). Thus maximum score for a rat was 3.

The percent inhibition of catatonia in test drug and standard drug was calculated in comparison with control group by the formula.

\[
100 \times \left(1 - \frac{a}{b}\right)
\]

Where ‘a’ is the mean anti-catatonic score of the test / standard animals and ‘b’ is the mean anti-catatonic score of the control animals. The mean score of various group of animals were statistically compared for determine significance of difference by using one-way ANOVA followed by Dunnett’s test was perform by using Graph Pad software, version 3.00 ,San Diego California, USA.

Result: It was observed that percentage of inhibition of catatonia after 20 min. of haloperidol administration in group II (benzhexol treated group), III & IV were 69, 54 & 62 respectively. There was highly significant increase in duration of catatonia in group II, III & IV as compared with control group (p<0.01) depicted in table 2 and graph 1, 2.

After 40 min. of haloperidol administration the percentage inhibition in catatonia in group II, III & IV were 74, 59 & 67 respectively as compared with control. The increase in duration of catatonia in all three groups were highly significant (p<0.01). The observation was recorded for 3 hours at the interval of 20 min and it was observed that the increase in duration of catatonia in groups II, III & IV were highly significant statistically.

Discussion

The test formulation (Majoon e baladur) is a potent Unani formulation and mainly used in CNS disorders but it was not used in Parkinsons disease or drug induced extrapyramidal symptoms. The test formulation has significant catatonia antagonizing activity produced by haloperidol. The antagonism of catatonia produced by phenothiazines is a good index of antiparkinsons activity (Turner, 1965).

In the present study it was observed that all the test formulations (Majoon and alcoholic extract) & standard drug (benzhexol) in the dose of 140 mg, 10 mg and 0.15 mg/100gm body weight respectively produced significant inhibition of catatonia throughout the testing period (p<0.01). The unani formulation in Majoon form produced peak and significant inhibition (75%) at 60 min (p<0.01) but least inhibition at 20 min after
haloperidol administration (p<0.01). 50% alcoholic extract produced peak and highly significant (84%) inhibition in catatonia at 60 min of haloperidol administration (p<0.01). The standard drug (benzhaxol) produced peak and highly significant (88%) at 60 min of haloperidol administration while produced highly significant inhibition throughout the testing period. 50% alcoholic extract treated group showed almost similar response to benzhexol treated group. It can be concluded that the Unani formulation in extract form was as effective as benzhexol in drug induced catatonia. Its mechanism of action and safety profile can be explored in further studies.

Table 1: The Anti-Parkinsonism Effet of Majoon Bala dur & its Extract

<table>
<thead>
<tr>
<th>Group</th>
<th>Interval of Testing (Minutes)</th>
<th>Mean Score ± S.E.</th>
<th>% of Inhibition</th>
<th>p. value</th>
<th>Mean Score ± S.E.</th>
<th>% of Inhibition</th>
<th>p. value</th>
<th>Mean Score ± S.E.</th>
<th>% of Inhibition</th>
<th>p. value</th>
<th>Mean Score ± S.E.</th>
<th>% of Inhibition</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td></td>
<td></td>
<td></td>
<td>60 min</td>
<td></td>
<td></td>
<td>100 min</td>
<td></td>
<td></td>
<td>140 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group - I</td>
<td></td>
<td>2.17±0.21</td>
<td>-</td>
<td>-</td>
<td>2.67±0.10</td>
<td>-</td>
<td>-</td>
<td>2.58±0.08</td>
<td>-</td>
<td>-</td>
<td>2.42±0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group - II</td>
<td></td>
<td>0.67±0.10</td>
<td>69 p&lt;0.01</td>
<td>0.33±0.10</td>
<td>88 p&lt;0.01</td>
<td>0.50±0.00</td>
<td>81 p&lt;0.01</td>
<td>0.58±0.23</td>
<td>76 p&lt;0.01</td>
<td>0.58±0.11</td>
<td>74 p&lt;0.01</td>
<td>8.3±0.16</td>
<td>63 p&lt;0.01</td>
</tr>
<tr>
<td>Group - III</td>
<td></td>
<td>1±0.22</td>
<td>54 p&lt;0.01</td>
<td>0.67±0.10</td>
<td>75 p&lt;0.01</td>
<td>0.75±0.17</td>
<td>71 p&lt;0.01</td>
<td>0.92±0.15</td>
<td>62 p&lt;0.01</td>
<td>0.83±0.16</td>
<td>63 p&lt;0.01</td>
<td>0.75±0.11</td>
<td>69 p&lt;0.01</td>
</tr>
<tr>
<td>Group - IV</td>
<td></td>
<td>0.83±0.10</td>
<td>62 p&lt;0.01</td>
<td>0.42±0.08</td>
<td>84 p&lt;0.01</td>
<td>0.58±0.08</td>
<td>78 p&lt;0.01</td>
<td>0.75±0.11</td>
<td>69 p&lt;0.01</td>
<td>0.67±0.10</td>
<td>70 p&lt;0.01</td>
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<td>69 p&lt;0.01</td>
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Microbiological study, of a Unani preparation, Safoof e ithrifal (Thripaladi Choorna) in Sri Lanka with a view to defining an acceptable microbial quality standard

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Abstract

All herbal materials contain a natural inherent microbial flora and also may be contaminated during processing. World Health Assembly has emphasized the need of ensuring the microbial quality standards of medicinal plant products. Safoof e Ithrifal is a Unani preparation also used as Thripaladi Choorna in Ayurveda which is widely used in Sri Lanka by all the Indigenous medical practitioners for many health problems. The basic ingredients are Terminalia chebula (Haleela), Terminalia belarica (Baleela), and Phyllanthus emblica (Amla). The main indications are Cough, Asthma, Fever and as Purgative. Recent studies proved it is an effective remedy for Diabetes mellitus, Hyperlipidemia and Obesity. The main objective of this study was to study microbial load and identify microorganisms in the market samples of Safoof Ithrifal and to define a suitable microbial quality standard of these products. Thirty three market samples of different manufactures were tested for microbial load and for microorganisms present. Microbial load was noted as Colony Forming Units (CFU). Detection of Coliforms and Salmonella were tested according to the international standards ISO 9308-2-1990 (E). Identification of microorganisms was done through standard biochemical tests. The statistical range of the microbial load for bacteria and fungi was found to be 2.0x10⁵ to 1.2x10⁷ and 0 to 1.5x10⁴ respectively.

Through the biochemical tests the Bacillus present in the preparation was found to be Bacillus Furmis. However, none of the drug sample was positive for Coliforms or Salmonella. Hence these results scientifically evaluate that these tested samples were microbiologically safe and up to the microbial quality standard.

Key Words: Microbial Load, Thripaladi Choorna, Phyllanthus emblica

Introduction

Herbal medicine, a form of complimentary and alternative medicine, is becoming increasingly popular in both developing and developed countries. A World Health Organization (WHO) survey indicates that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare. WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicine (Anonymous 2007).

Further medicinal plants are important sources for pharmaceutical industry. Hence medicinal plants and herbal medicines account for a significant percentage of the pharmaceutical market (Anonymous 1988). The basic ingredients of most of the traditional medicinal products are plant...
materials, minerals and animal origin ingredients. All these materials contain a natural inherent microbial flora and also may be contaminated during harvesting, processing, preparation and storage. Considering these facts the World Health Assembly in its resolutions WHA 31:33, WHA 40:33, WHA 42:43 has emphasized the need to ensure the microbial quality standards of medicinal plant products by using modern techniques and application of suitable standards (Anonymous 1988). The main objective of this study was to enumerate the total viable count of bacteria and fungi, identify the organisms presents, identify the other specific microorganisms such as Coliforms and Salmonella in the market samples and to define a suitable microbial quality standard of this product.

**Materials and Methods**

Drug samples were taken from open market with sealed packs. Fifteen Market samples of different manufacturers were tested for microbial load and for specific microorganisms. Microbial load of the drug samples were studied through pour plate technique and spread plate technique by using Nutrient agar and Potato dextrose agar for bacteria and fungi, respectively. Microbial load was noted as Colony Forming Units (CFU). It was assumed that one bacterium produced one colony. Detection of Coliforms and Salmonella were tested according to the international standards ISO 9308-2-1990 (E) (Anonymous 1990). Coliforms were tested by using most probable number technique and Salmonella was tested following the pre-enrichment in non selective liquid medium, enrichment in selective medium and plating out in solid media. Routine sterilization techniques were applied in all steps.

All the biochemical tests were performed within 24 hours of culturing. The following bio chemical tests were done on these cultures. The methods adapted are routine methods mentioned in laboratory manuals. Grams stain, Spore stain, Motility test, Catalase activity, Oxidase activity, Glucose acid test, Oxidative and fermentative activity, tests for Carbohydrate utilization (tests for Arabinose, Mannitol, Xylose, Glucose, Sucrose and Fructose were done) Methyl red test, Voges – proskauer reaction, Indole test, Starch hydrolysis, growth in 7% Sodium chloride, growth in 65°C, growth in 45°C, Gelatin hydrolysis, Casein hydrolysis. All these test were repeated whenever necessary (Cowan and Steel 1974). Coliform test was performed by using 9 ml. Single strength MacConkey broth. 1.0 ml of each drug sample was transferred into these tubes which contain a Durham tube and mixed gently and kept in the Incubator at 37°C for 24 hours. Test tubes which showed acid and gas productions were considered as positive for Coliforms. (Cowan and Steel 1974). Salmonella test was performed after the enrichment process in Buffer peptone. 1.0 ml of the drug was transferred into 20ml of sterile buffer peptone and kept in a shaker at gently shaking position for 24 hours. Within 24 hours 1.0ml of this buffer peptone was transferred in to separate 10ml of sterile Tetrathionate broth and Selenite broth tubes. These tubes were kept in incubator at 37°C for 36 hours. Within 36 hours 1 loop of this broth was separately streaked on sterile Bismuthsulphite agar (B.S) plates and Brilliant Green Bile agar (BGB) plates. Black colonies on B.S. agar and pink colonies on BGB agar were considered as positive for Salmonella. (Cowan and Steel 1974) (Anonymous1998). Isolated black and pink colonies were again streaked on Nutrient agar plates and tested for confirmation of Salmonella. Indole test and Urease test were done as negative confirmation test. Kliglers Iron agar test was done as positive confirmation test (Cowan and Steel 1974). All the above test procedures were repeated three times on each sample for confirmation of the test results.

**Results**

According to the limits adapted from the provisional guidelines established by World Health Assembly, the microbial colony counts observed in this study were within the limits acceptable by WHA. The Colony count for Bacteria was between 10x68 and 10x10. Fungi Colony count was 1x10 to 36x 10. These results were statistically analyzed by using Student’s ‘t’ Test. Mean of the colony count is not significant at p < 0.05. There is no difference between the standard mean of the colony count and sample colony count. The Bacteria present in this preparation was belonged to Bacillus group. Through the biochemical tests it was identified as Bacillus firmus. However, none of the drug sample was positive for Coliforms or Salmonella. Hence these results scientifically show that the tested samples are microbiologically safe and up to the microbial quality standard. The microbial load of bacteria and fungi can be summarized as follows:
Discussion
According to WHA limitations for internally used plant based medicinal preparations the aerobic bacterial count should be less than $10^5$ per gram. Yeast and moulds should be less than $10^3$ per gram. *E.-Coli* should not exceed 10 per gram. Other Enterobacterial count should not exceed $10^3$ per gram and *Salmonella* should be totally absent (Anonymous1988).

Microorganisms in these preparations can enter into the body through digestive system. The effects of the organisms depend on the number and the immune power of the individuals. Therefore herbal medicines however are not necessarily always safe simply because they are natural (Anonymous1988). The microorganisms these preparations or the biological by products of these organisms may cause serious adverse reactions and can produce long terms side effects. These medicines will only be beneficial to the human health if they are manufactured and used properly (Anonymous1988). Therefore, good quality control and microbial quality standardization are essential for any medicinal product. Especially for herbal medicinal products as they may contain inherent and contaminated microorganisms. The colony count observed in this study was within the limits acceptable by WHA.

These results scientifically validate that the tested preparations are microbiologically safe and up to microbial quality standard. Introducing the proper Good Manufacturing Practice (GMP) according to the WHO- GMP manual can leads to a microbiologically sterile herbal product. (Anonymous2005)

Acknowledgement
The National Science Foundation of Sri Lanka is gratefully acknowledged for financial support.

References
3. Anonymous, 1998; Ayurveda Pharmacopoeia Vol. 1, Published by the Ministry of Health & Indigenous Medicine, Department of Ayurveda, Sri Lanka.
4. Anonymous, 1998; WHO Guidelines for the appropriate use of Herbal medicine; Region Office for the Western Pacific Manila.

<table>
<thead>
<tr>
<th>Name of the Manufacturer</th>
<th>Number of sample tested</th>
<th>CFU positive samples</th>
<th>CFU/g in N.A</th>
<th>CFU/g in PDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ay/Dr/Corp</td>
<td>03</td>
<td>03 02</td>
<td>10 x 24</td>
<td>10 x 02</td>
</tr>
<tr>
<td>Link</td>
<td>03</td>
<td>No No</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Mansuwa</td>
<td>03</td>
<td>No 01</td>
<td>--</td>
<td>3.6 x 10^2</td>
</tr>
<tr>
<td>Gampaha</td>
<td>03</td>
<td>01 03</td>
<td>10 x 10</td>
<td>1x10</td>
</tr>
<tr>
<td>Nu-osu</td>
<td>03</td>
<td>03 03</td>
<td>2.8x10^3</td>
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</tr>
<tr>
<td>Bimal</td>
<td>03</td>
<td>02 No</td>
<td>6.8 x 10^2</td>
<td>--</td>
</tr>
<tr>
<td>Pasyale</td>
<td>03</td>
<td>02 No</td>
<td>4.8 x 10^2</td>
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</table>
Wound healing activity of Berge lajwanti (*Mimosa pudica* Linn) in animal model

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Abstract

Research on wound healing drugs is a developing area in modern biochemical sciences. Unani Medicine is a treasure trove of a number of wound healing drugs. Therefore, an experimental study was designed to investigate the comparative wound healing activity of aqueous and methanol extracts of Berg-e-Lajwanti (*Mimosa pudica* Linn) in rat models. Excision, incision and dead space wound models were used to evaluate the wound healing activity in rats, of either sex. In excision wound model the test extracts were applied topically, till the complete healing of the wound, and the healing was assessed by the percentage of wound contraction and reduced period of epithelization while in incision wound models the treatment was continued for 10 days only and the healing of the wound was assessed by tissue breaking strength, where as in the dead space wound model test drug was administered orally for 10 days and the healing effect was determined on the basis of breaking strength of granulation tissue, dry granulation tissue weight, hydroxyproline content and histopathology of the granulation tissue. The test extracts promoted the wound healing activity significantly in all the wound models. High rate of wound contraction, decrease in period of epithelization, high breaking strength and granulation strength, increase in dry granulation tissue weight, elevated hydroxyproline contents and increased collagenation in histopathological sections were observed in animals treated with test drug extracts when compared to the control group of animals. Thus, the study showed the Methanol and Aqueous leaf extracts of *Berge Lajwanti* to possess striking wound healing activity. However, methanol extract showed greater wound healing activity.

Key Words: Berge Lajwanti, *Mimosa pudica*, Wound contraction, Epithelization, Breaking strength, Hydroxyproline

Introduction

*Berge lajwanti* (*Mimosa pudica* Linn,) is known in Urdu and Persian as *Lajalu* (Ibn-e-Sina 1981, Azam 1867, Sheerazi, Allama 1951, Chughtayee 2004, Nadkarni 1982,). It is a wild plant which grows in hot and humid regions in India (Ghani 1971). In Unani literature it has been described to posses haemostatic, resolvent, astringent, blood purifier, sedative, antiseptic, antipyretic etc properties (Azam 1867, Sheerazi, Allama 1951, Chughtayee 2004, Ghani 1971, Hakeem 2002, Anonymous 1987) and to be useful in the management of various ailments like piles, fistula, scrofula, skin eruptions, chronic ulcers, hemorrhoids, ascitis and also reported to possess nerve regenerative property, (Prasad et al. 1975) hyperglycemic and (Amalraj and Ignacimuthu 2002) moderate diuretic activity (Pillai et al. 1978), nematicidal activity, and to inhibit the hyaluronidase and protease activity of Indian snakes venom (Girish et al. 2004).
It is reported to contain a number of phytochemicals, some of the important constituents viz. flavonoids, saponin, tannins, glycosides, quercetin, phyto hormone, tubulin, β-sitosterol, D-pinitol, norepinephrine, mimosine crocetin dimethyl ester, trace elements, mimosine and turgorine (Chaterjee 1992, Nadkarni 2004, Anonymous 2004, Ghosh 1998, Pankaj 2006, Yuan et al. 2006) have been shown to possess important pharmacological activities, constituents such as flavonoids and quercetin etc are reported to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity; tannins, triterpenoids and sesquiterpenes are reported to possess anti inflammatory and analgesic effects; they also have astringent and antimicrobial properties and are used topically to treat burns and wounds (Manjunatha et al. 2005).

On the basis of above-mentioned references, the present study was carried out to determine the wound healing effect of methanolic and aqueous extract of Berge lajwanti by excision, incision and dead space wound healing models in rats.

Material and Methods

Animals: Healthy inbred Wistar rats of 4-6 month, weighing 150-200gms of either sex were used in the study and were procured from animal house of the National Institute of Unani Medicine (NIUM), Bangalore. The study was approved by the Institutional Ethical Committee (IEC) NIUM, Bangalore, India, under Reg. No. 953/C/106/CPCSEA. The animals were maintained in standard conditions of temperature and humidity and were fed with a commercial diet and water ad libitum during experiment.

Plant material: Fresh Berge Lajwanti (Mimosa pudica Linn leaves) was collected from NIUM herbal garden and was authenticated by the pharmacognost of the Institute, and a voucher specimen deposited in the laboratory for further reference.

Preparation of extracts

1. Methanolic extract: Berge Lajwanti was dried under shade and powdered mechanically; 250gm of powder was subjected to extraction by Soxhlet apparatus with 70% methanol for 8hrs. The extract was filtered and concentrated on water bath at 80° C. The yield was 18% w/w.

2. Aqueous extract: For aqueous extract, 250gms of powdered leaves were macerated with 1000ml of distilled water for 3days with intermittent stirring, then the extract was filtered and concentrated on water bath at 80° C. The yield was 13%w/w (Gamble1936).

Preparation of formulation

Two types of drug formulations (topical and oral) were prepared from each of the extracts. For topical administration, 5% w/w ointment was prepared in 2% sodium alginate. For oral administration, 200mg and 400mg/ml of aqueous and methanolic suspension of leaf extracts were prepared (Manjunatha et al 2005).

Acute toxicity studies

The acute toxicity study was carried out for both the extracts starting at 50mg/kg orally by stair case method (Ghosh 1984). No mortality or any other behavioral change was observed up to the administration of 4000mg/kg body wt. in the rats. However, 1/20th (200mg/kg) and 1/10th (400mg/kg) of the maximum administered dose were used for further studies.

Experimental procedures

Excision wound model: The animals were divided into 6 groups of 6 rats in each, as described below and kept in separate cages.

Group I - animals were left untreated and served as control.
Group II - served as standard, treated with 1% w/w Framycetin Sulphate cream.
Group III - Served as test treated with 50mg ointment prepared from 200mg of aqueous extract of test drug.
Group IV - Treated with 50mg ointment prepared from 400mg of aqueous extract of test drug.
Group V –Treated with 50mg ointment prepared from 200mg of ethanol extract of test drug.
Group VI – Treated with 50mg ointment prepared from 400 mg of methanol extract of test drug.

In this model the rats were fasted overnight and were inflicted with excision wound of about 4.88cm² (500mm²) as described by Morton and Malone (Morton 1972).

The percentage wound closure and epithelization time was calculated (Table 2). The ointment was applied topically once a day, starting from the day of operation till complete epithelization time. The
wounds were traced on mm$^2$ graph paper on day 3, 6, 9, 12, 15 and 18th and thereafter on alternate days until healing was completed; the percentage of wound closure was calculated as percentage change in initial wound size i.e.,

$$WC(\%) = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

The period of Epithelization was monitored by noting the number of days required for eschar to fall away, leaving no raw wound area behind (Kamath2006).

**Incision wound model:** Animals were divided same as in the excision wound model. Two para vertebral straight incisions of 6 cm length each were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel (Ehrlich and Hunt et al. 1968). After complete homeostasis the wound were closed by interrupted sutures placed at equidistance points about 1 cm apart; immediately after operation the rats were placed in the collars to prevent damage to the wound and the ointment was topically applied once in a day. On the 8th day, sutures were removed and on the 10th post wounding day, tensile strength was measured by tensiometer as described in the method of Lee et al. (Lee et al. 1968).

**Dead space wound:** The animals were divided into 5 groups of 6 rats in each and kept in separate cages (Table 4). Under the light ether anesthesia dead space wound was created by subcutaneous implantation of sterilized cylindrical grass piths 2.5×0.3cm, one on either side of the dorsal para vertebral surface of the rat (Turner1965, Patil and Kurkarni 1984).

Group I – served as control (treated with 1ml of distilled water once orally)

Group II – Treated with oral suspension of aqueous test extract 200mg/kg.

Group III – Treated with oral suspension of methanol test extract 400mg/kg.

Group IV – Treated with oral suspension of methanol test extract of 200mg/kg.

Group V – Treated with oral suspension of methanol test extract of Berg e lajwanti 400mg/kg.

Animals received test extract from 0 day to 9th post wounding day. On 10th post wounding day, the granulation tissue harvested on each implanted grass pith was carefully dissected out along with the grass pith and employed for determination of breaking strength (Lee et al.1968) by fixing a piece of granulation tissue between two Babcock forceps and it was measured by a constant and continuous water flow technique. The granulation tissue so harvested was subjected to hydroxyproline estimation following the method of Neuman and Logan (Neuman and Logan 1949). The granulation tissue was collected and dried at 60 °C for 24 h to get constant weight for the determination of dry granulation weight (Kaushal et al. 2007).

**Histopathological Study**

On 10th post wounding day, a small piece of the intact healed wound with edges was excised from an animal of each group and fixed in 10% neutral buffered formalin. Histopathological evaluation was carried out using hemotoxylin and eosin (H&E) stained 5-6 μm thin paraffin longitudinal section, to evaluate the effect of the extracts on collagen formation.

**Statistical analysis**

The results were analyzed statistically by using ANOVA- non repeated measure test and tests after ANOVA for pair comparison at significant levels of p<0.05 & p< 0.01

**Results**

**Acute toxicity studies**

The extract of Berge lajwanti was found to be safe upto 4000mg/kg body weight by oral route. After 24hrs, the doses were found to be well tolerated. There was no mortality and no signs of toxicity.

**Excision wound model**

The healing started steadily in groups treated with test drug; healing on 3rd day was found to be 24% (P<0.01), while it was only 11% in control group and 22% (P<0.01) in standard group.

Complete wound healing (100%) occurred by 15th day and 18th day in group V and VI respectively as compared to 97% healing in control group on day18th. Epithelization time was reduced from 22 days in control to 18.33, 17.16, 17.0 and 16.5 days in III, IV, V and VI groups, respectively. The group VI was almost equal to group II, 15.83 day.

Intergroup comparison of group II, III, IV, V & VI revealed no significant reduction in wound area in groups III & IV as compared to group II where as groups V & VI showed significant reduction with p<0.05 & p<0.01, respectively (Table 1 & 2).
Incision wound model
The breaking strength was observed as 575.83±15.63 (P<0.001) in standard treatment group, followed by 566.66±28.62 (P<0.01) in group VI and 514.58±1.51 (P<0.01) in group V (Table 3).
The breaking strength in group III & IV was 442.5±23.22 and 451.25±21.34, respectively. However, these were not significant statistically.

Dead space wound model:
Weight of dry granulation tissue (mg) increased significantly in groups IV & V with 156±0.01 (P<0.001) followed by group III with 120±0.02 (P<0.05) in comparison with the control. However, in group II it was 109±0.003 (P<0.05).
The breaking strength was found 368.66±2.66 & 383.66±2.59 (P<0.01) in group IV & V followed by 336.33±2.73 & 352.83±3.96 (P<0.001) in group III & IV, respectively.

There was a significant increase in the hydroxyproline content in the test groups as compared to the control, 2016.66±44.09 (P<0.01), 2133.33±91.89 (P<0.001) in groups IV & V followed by 1533.0±44.09 (P<0.05), 1733.0±67.90 (P<0.001) in group II in comparison with the control, respectively (Table 4).

Histopathological studies of the granulation tissue of control group in dead space wound model showed more aggravation of macrophages with few collagen fibers (Fig 1), and in case of Gp II and Gp II and III, moderate collagen deposition, macrophages, and fibroblast were noticed (Fig 2 & 3). Whereas the Gps IV and V evidenced significant increase in collagen deposition showing lesser macrophages and fibroblast (Fig 4 & 5), when compared with the control group.

Table – 1
Effect of topical application of aqueous and methanol extract of Berg-e-Lajwanti on excision wound model

<table>
<thead>
<tr>
<th>Group</th>
<th>Post wounding reduction in wound area in cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.886 ± 0.009</td>
</tr>
<tr>
<td>Standard</td>
<td>4.882 ± 0.009</td>
</tr>
<tr>
<td>Aqueous -A-1</td>
<td>4.882 ± 0.009</td>
</tr>
<tr>
<td>Aqueous A-2</td>
<td>4.887 ± 0.006</td>
</tr>
<tr>
<td>Methanol B-1</td>
<td>4.885 ± 0.008</td>
</tr>
<tr>
<td>Methanol B-2</td>
<td>4.887 ± 0.008</td>
</tr>
<tr>
<td>F</td>
<td>0.071 &gt;0.05</td>
</tr>
</tbody>
</table>

n = 6 *p<0.05, **P<0.01 and ***p<0.001 when compared to control
### Table – 2
Effect of aqueous and methanol extract of *Berg-e-Lajwanti* on Wound Contraction and Period of Epithelization

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent (%) wound contraction in days</th>
<th>Period of Epithelization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;0&quot; Day</td>
<td>3rd Day</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>11.27</td>
</tr>
<tr>
<td>Standard</td>
<td>0.00</td>
<td>22.73*</td>
</tr>
<tr>
<td>Aqueous-A1</td>
<td>0.00</td>
<td>18.12</td>
</tr>
<tr>
<td>Aqueous-A2</td>
<td>0.00</td>
<td>16.38</td>
</tr>
<tr>
<td>Methanol-B1</td>
<td>0.00</td>
<td>22.76</td>
</tr>
<tr>
<td>Methanol-B2</td>
<td>0.00</td>
<td>24.59**</td>
</tr>
</tbody>
</table>

\[n = 6 \quad *p<0.05, \quad **P<0.01 and \quad ***p<0.001 \text{ when compared to control}\]

### Table – 3
Effect of *Berg-e-Lajwanti* on Breaking Strength in Incision Wound Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Breaking strength(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>397.083 ± 14.640</td>
</tr>
<tr>
<td>Standard</td>
<td>575.833 ± 15.635***</td>
</tr>
<tr>
<td>Aqueous A-1</td>
<td>442.5 ± 23.229</td>
</tr>
<tr>
<td>Aqueous A-2</td>
<td>451.25 ± 21.348</td>
</tr>
<tr>
<td>Methanol B-1</td>
<td>514.58 ± 1.510**</td>
</tr>
<tr>
<td>Methanol B-2</td>
<td>566.66 ± 28.626***</td>
</tr>
</tbody>
</table>

\[n = 6 \quad *p<0.05, \quad **P<0.01 and \quad ***p<0.001 \text{ when compared to control}\]

### Table – 4
Effect of aqueous and methanol extracts of *Berg-e-Lajwanti* on healing of Dead Space Wound Model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Granulation tissue dry weight (mg/100g)</th>
<th>Breaking strength(g)</th>
<th>Hydroxyproline (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>83.5 ± 0.003</td>
<td>234.5 ± 2.930</td>
<td>733.33 ± 33.33</td>
</tr>
<tr>
<td>II. Aqueous A-1</td>
<td>109.0 ± 0.003</td>
<td>336.332.728***</td>
<td>1533.00 ± 44.906*</td>
</tr>
<tr>
<td>III. Aqueous A-2</td>
<td>120.3 ± 0.002*</td>
<td>352.83 ± 3.962***</td>
<td>1733.00 ± 67.905*</td>
</tr>
<tr>
<td>IV. Methanol B-1</td>
<td>156.7 ± 0.015***</td>
<td>368.66 ± 2.667***</td>
<td>2016.66 ± 44.096**</td>
</tr>
<tr>
<td>V. Methanol B-2</td>
<td>193.3 ± 0.008***</td>
<td>383.66 ± 2.591***</td>
<td>2133.33±91.894***</td>
</tr>
</tbody>
</table>

\[n = 6 \quad *p<0.05, \quad **P<0.01 and \quad ***p<0.001 \text{ when compared to control}\]
Fig – 1
Histological section of granulation tissue of control animal shows non specific granulation tissue.

Fig – 2
Histological section of granulation tissue of test group animal treated with lower dose of Berg-e Lajwanti aqueous extract shows healing ulcer with abundant collagen.

Fig – 3
Higher dose Berg-e-Lajwanti aqueous extract shows healing ulcer with formation of stratified epithelium and dense collagen.

Fig – 4
Lower dose of Berge Lajwanti extract shows the granulation tissue is covered with a thin layer of stratified squamous epithelium and shows abundant collagen fibres. Few thin walled blood vessels are also seen.

Fig – 5
Higher dose of Berge methanol Lajwanti methanol extract shows granulation tissue is covered with a thin layer of stratified squamous epithelium and shows abundant collagen fibres. Few thin walled blood vessels are also seen.

Discussion
In the present study excision, insicion and dead space wound models were used to investigate the extracts of Berge Lajwanti for wound healing activity.

The findings of the study show that the test extracts in varying concentration (200 and 400mg/kg) were capable of producing significant wound healing activity. It was found to have complete healing (100%) in methanol extract treated group, followed by aqueous treated group, when compared with the control group.
There was a significant difference in the period of epithelisation in test groups and control group, but almost equal to standard group, therefore, it is evident that the test drug is able to promote the natural healing process, reducing the time period of onset of healing and the completion of healing process until the epithelization took place. This may be because of the constituents of Berge lajwanti, like tannins, triterpenoids and sesquiterpenes which are reported to possess anti-inflammatory, analgesic, astringent and antimicrobial properties and hence seem to be responsible for wound contraction and increased rate of epithelization (Manjunatha et al., 2005).

The tensile strength of incision wound produced by aqueous treated group was comparatively lesser than the standard and methanol treated group but significantly better than the control group.

Breaking strength of granulation tissue was increased in methanol and aqueous extract treated groups indicating enhanced collagen maturation by increased cross linking and hydroxyproline content of granulation tissue when compared to control group; this may be because of flavonoids and quercetin etc, the constituents present in test drug which are reported to reduce lipid peroxidation by preventing cell necrosis, improve vascularity, and increase strength of collagen fibers to ultimately enhanced viability of collagen fibrils.

Increased granulation tissue weight and breaking strength in dead space wound attributed to prohealing activity of *Mimosa pudica* may be due to its influence on growth hormone, as growth hormone stimulate granulation tissue formation (William & Frohman, 1986).

The prohealing activity of *Mimosa pudica* is further substantiated by increase in hydroxyproline content in methanol followed by aqueous and control groups.

Both the extracts of test drug in two different doses produced significant effect in dose dependent fashion, as it is evident from the increased values of hydroxyproline, breaking strength and granulation tissue weight which are makers of prohealing activity.

In the light of above discussion, it can be concluded that the Unani test drug Berge Lajwanti (*Mimosa pudica* Linn) possesses significant healing effect in different types of wounds, being equieffective to framycetin in excision wound model. At the molecular and biochemical level, the healing effects may be attributed mainly to the presence of its phytochemicals, which are responsible for antioxidant, astringent and aseptic properties. Therefore, the present study supports Unani claims regarding the wound healing activity of the plant. However, further studies are needed to ascertain the exact mechanism of the molecular and biochemical actions.

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Antibacterial activity of Shahtra (Fumaria officinales Linn.) extracts against MRSA (Methicillin Resistant Staphylococcus aureus)

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Abstract

The present study has been done to evaluate the efficacy of Shahtra (Fumaria officinales Linn.) extract against Methicillin Resistant Staphylococcus aureus (MRSA). Agar well method was used according to CLSI Guidelines by W.H.O. The antibacterial activity was evaluated by measuring the Zone of Inhibition - ZOI (in mm.) of drug extract. The efficacy of the drug was evaluated by comparing it with the Standard drug- Methicillin. MIC and MBC were also determined and all the experiments were conducted in triplicates and in sterilized conditions. The results were analyzed statistically by using ANOVA. It was found that MRSA strain was sensitive to F. officinales Linn. showing significantly greater ZOI as compared to the standard drug. The study concludes that F. officinales Linn. is effective against MRSA. Results of the present investigation indicate that F. officinales possesses antimicrobial properties and hence can be developed as a natural plant based antimicrobial agent against the infectious diseases caused by the resistant strains of Staphylococcus aureus safely and effectively.

Key Words: Fumaria officinales Linn., MRSA, Methicillin, Shahtara

Introduction

Infectious diseases are the leading cause of death worldwide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Chahal et al., 2010). A wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively on the basis of empirical evidence (Cos et al., 2006). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Bandow et al., 2003).

Fumaria officinals Linn. (Fumariaceae) known as Common Fumitory (Evans and Trease, 2009), Shahtarah in Persian (Chopra, 1958) or Pitpapra in Hindi (Ainslie, 1826) is a perennial herb which has been used in the Unani Medicine for a long time in various health ailments (Ghani, 1921). It is extremely useful in syphilis, scrofula, leprotic affections (Ainslie, 1826), is used as diuretic, tonic, diaphoretic, alterative, blood purifier (Ainslie, 1826; Chopra, 1958; Hakeem, 1343H, Nadkarni, 2000). Traditionally, whole herb of F. officinales, which also features in a number of commercial Indian preparations, is used for liver
disorders (Evans and Trease, 2009; Hakeem, 1343H) for digestive problems, certain metabolic diseases, to purify blood (Hakeem, 1343H).

Phytochemical investigation revealed the presence of several alkaloids such as adlumidiceine, copticine, fumariline, perfumine, protopine fumaric acid (considered at one time as a treatment of psoriasis), fumaranine, fumaritine, paprafumicin and paprarine (Erdogan, 2009) Biologically active compounds like isoquinolone alkaloids including fumaricine, sanguinarine have been found in this (Evans and Trease, 2009).

A review of literature reveals that *F.officinales* has been in use in treating various health ailments including “colicky pain affecting the gallbladder and biliary system, together with the gastrointestinal tract” (Heidari, 2004) various pharmacological studies have been done like its use in Irritable Bowel Syndrome (Brinkhaus, 2005), Brine shrimp Lethality Bio assay was also done (Krishnaraju, 2005).

Despite the medicinal importance of this plant species, reports on its antibacterial and antifungal activities are self limited. During our search of literature via Google and Pubmed we found studies on other *Fumaria* spp. Namely *F. parvivora* (Bhattacharya, 1970), *F. indica* (Parekh and Chanda, 2008; Gilani, 2004). Antibacterial studies are reported on *F.parvivora*, *F. vaillantii* (Sener, 1994). Antibacterial activity of *F.officinales* (Dulger, 2004) has also been done, but there is no study reported on its activity towards MRSA. In fact there are very few researches available regarding the use of herbs against MRSA. And it is a need of the hour today to explore the natural flora, so that it can be used safely and effectively against the pathogenic organisms which have become resistant to the synthetically derived antibiotics. So, the present study was designed to find out the antibacterial efficacy of *F.officinales* against MRSA, so that it can be used as an anti-infective potential source to combat such noscomial pathogenic bacteria.

1.1 Methicillin Resistant *Staphylococcus Aureus* (MRSA)

Over the last three decades the bacterium is responsible for causing several difficult-to-treat infections in humans. Also known as Multidrug resistant *S.aureus* or Oxacillin resistant *S.aureus* (ORSA) emerged as a noscomial pathogen in early 1960s (Tyagi,2008) it is resistant to a large group of antibiotics called beta-lactams, which include the penicillin and the cephalosporins. A publication of the Centers for Disease Control and Prevention (CDC) estimated the number of MRSA infections in hospitals doubled nationwide. Another study led by the CDC and published in the October 17, 2007 issue of the Journal of the American Medical Asociation estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,650 hospital stay-related deaths in the United States in 2005. These figures suggest that MRSA infections are responsible for more deaths in the U.S. each year than AIDS. In 2007, the CDC reported that MRSA causes 19,000 deaths every year in the US, which is more than HIV/AIDS cases (en.wikipedia.org).

**Material and Methods**

The herb was procured from the local market Baradari of Aligarh city and was properly identified by the Botanical literature available and then confirmed by Prof. S. H. Afaq from the Pharmacognosy section, Department of Ilmul Advia, Aligarh Muslim University, Aligarh. Voucher specimens (V.No-SC0118/09-F) were preserved in the herbarium of Medicinal Plant Lab in the Department of Ilmul Advia, F/O Unani Medicine, Aligarh Muslim University, Aligarh for future reference.

**Preparation of plant extract**

Two different extracts were prepared for analysis in the present study viz. aqueous extract and ethanolic extract.

**For aqueous extract**: 10 gm of the powdered drug and 150 ml of the Double Distilled Water (DDW) were put into a soxhlet apparatus. The solvent was boiled at 40°C and refluxed for a period of 150 min (eleven extraction cycles).The extract was filtered and evaporated to dryness under reduced pressure in the Lypholizer (Macro Scientific works, Delhi). It was redissolved in DMSO to the desired concentration (20 mg/ml) for the study.

**For ethanolic extract**: 10 gm of the powdered drug and 150 ml of the ethanol (Solvent) were put into a soxhlet apparatus and the same procedure was repeated as stated above.

**Microorganisms Used**

Clinical strains of MRSA isolated from various sources viz. pus (Pus culture: PC), urine (Urine Culture: UC) and control strains N315, Mu50,
ORSA (Oxacillin resistant *Staphylococcus aureus*) of the tested microorganisms were obtained from Department of Microbiology, AMU Unit, Gandhi Eye Institute, Aligarh. The bacterial cultures were grown in Nutrient Broth (M002 Himedia Labs, Mumbai, India) and incubated at 37°C for 24 hours, followed by frequent sub culturing to fresh media and were used as test bacteria. The bacterial cultures were checked to confirm the presence of sufficient number of bacterial cells on nutrient broth and maintained on nutrient agar slant.

**Antimicrobial activity**

Antimicrobial assay of the crude extracts was performed against pathogenic strains by Agar well method (Ananthanarayan and Panikker, 2009). The nutrient agar plates were swabbed with a suspension (10^6 cfu/ml) of the bacterial strains. The wells of the equivalent size were prepared with the help of a cork borer and than the drug (40µl) was poured in the respective well with the help of a micropipette. Finally, the antibiotic –Methicillin disks (SD137, Himedia Labs, Mumbai, India) were placed on the prepared plates with sterile forceps and pressed properly to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre diffusion time) and then incubated at 37°C for 24 hours.

The antibiotic disk (6 mm) was used as Positive Control while the solvent used for diluting the test drug was used as the Negative Control. The diameter of the inhibition zone – Zone of Inhibition (ZOI) in mm was measured and is given in Table-1 and Graph. The experiment was done in triplicate and the mean values were calculated.

**Determination of minimum inhibitory concentration (MIC)**

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Jennifer, 2001). 96-well sterile microtitre plates were used and 50 µl of standardized suspension of a strain (10^6 cfu/ml) was added to each tube containing extracts at various concentrations, the plate also included a well with inoculation and uninoculated wells of test drug–free broth (the first controls the adequacy of broth to support the growth of the organism, the second was done for a check of sterility). Finally the plates were sealed with a sterile sealing tape and then incubated at 37°C for 24h. MIC was observed as the lowest concentration of the test drug that showed no visible growth from the wells in microtitre plates (Table-2). Minimum Bactericidal Concentration (MBC) was further determined from the same isolates MBC is the minimal concentration of drug needed to kill most (99.9%) of the viable organisms after incubation for 24 hours.

**Statistical analysis of data**

All the values have been expressed as Mean ± SEM (Standard error of mean). Statistical significance was determined by one way ANOVA using g-paid software for calculation.

**Results**

Both the aqueous and ethanolic extracts of Shahtara (*F.officinalis*) exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table-1). The MIC of crude extracts of the drug was determined at the concentrations ranging from 19.53 to 5000 µgm/ml (Table-2).

The extracts showed greater activity than the agent used for identifying MRSA ie Methicillin. However, the alcoholic extract was more effective than the aqueous extract: For N315 - ZOI by test drug was 30.0 ± 0.71(p-value<0.0001) and MIC (0.078 mg/ml) and MBC (0.312 mg/ml), whereas by Standard drug ZOI was just 7.0±0.32. Against PC strain the test drug exhibited ZOI, MIC and MBC as 27.4 ± 0.67 (p-value<0.001), 0.156mg/ml and 0.312mg/ml, respectively while ZOI by Standard drug was 7.4±0.24. For Mu50 ZOI was 25±0.83 (p-value<0.001), MIC (0.312mg/ml) and MBC (0.625 mg/ml) and ZOI by Standard drug was 7.4±0.24. For ORSA ZOI by test drug was 23.8±0.8 (p-value<0.001) while by Standard drug it was 7.2±0.20, MIC (0.312 mg/ml); MBC (1.25 mg/ml) and for UC the inhibition zone was 23.4±0.2 by the test drug used and by Standard drug it was 7.0±0.31, MIC (0.312 mg/ml) and MBC (1.25mg/ml). ZOI by standard drug shows a highly significant effect in most of the cases. Minimum activity was shown by aqueous extracts of the test drug, where the inhibition zones (IZ) were in the range of 8.8 to 12.0; MIC ranges from 0.625 to 1.25 mg/ml and MBC from 1.25 to 2.50 mg/ml.
Table – 1
Zone of Inhibition (in mm) of *Fumaria officinalis* Linn. Extracts against MRSA strains.

<table>
<thead>
<tr>
<th>MRSA Strains</th>
<th>Zone of Inhibition (ZOI) in mm (Mean ± S.E)</th>
<th>Extract of <em>F. officinalis</em> Linn.</th>
<th>Methicillin (30 µg)</th>
<th>DMSO (50µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanolic</td>
<td></td>
</tr>
<tr>
<td>N315</td>
<td>12 ±0.32</td>
<td>30±0.71**</td>
<td>7.0±0.32</td>
<td>6.6±0.32</td>
</tr>
<tr>
<td>Mu50</td>
<td>10±0.07</td>
<td>25±0.83*</td>
<td>7.4±0.24</td>
<td>6.6±0.32</td>
</tr>
<tr>
<td>ORSA</td>
<td>8.8±0.58</td>
<td>23.8±0.8**</td>
<td>7.4±0.24</td>
<td>6.4±0.24</td>
</tr>
<tr>
<td>UC</td>
<td>9.0±0.44</td>
<td>23.4±0.2*</td>
<td>7.2±0.20</td>
<td>6.6±0.32</td>
</tr>
<tr>
<td>PC</td>
<td>12±0.20</td>
<td>27±0.67*</td>
<td>7.0±0.31</td>
<td>6.6±0.24</td>
</tr>
</tbody>
</table>

PC: Strain isolated from a pus culture  
UC: Strain isolated from urine culture  
ORSAs: Oxacillin Resistant Staphylococcus aureus  
DMSO: Dimethyl Sulphoxaside  
* <0.001, ** <0.0001

Table – 2
MIC and MBC of the Aqueous and Ethanolic extract of *F. officinalis* Linn. Extract against MRSA strains

<table>
<thead>
<tr>
<th>MRSA Strains</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/ml)</td>
<td>MBC (µg/ml)</td>
</tr>
<tr>
<td>N315</td>
<td>625</td>
<td>1250</td>
</tr>
<tr>
<td>Mu50</td>
<td>1250</td>
<td>2500</td>
</tr>
<tr>
<td>ORSA</td>
<td>1250</td>
<td>2500</td>
</tr>
<tr>
<td>UC</td>
<td>625</td>
<td>2500</td>
</tr>
<tr>
<td>PC</td>
<td>625</td>
<td>1250</td>
</tr>
</tbody>
</table>

MIC: Minimum Inhibitory Concentration  
MBC: Minimum Bactericidal Concentration

Discussion

Medicinal plants are naturally gifted with invaluable medicinal effects which form the backbone of traditional medicines. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind (Chahal et al., 2010). In the present investigation, *in vitro* antimicrobial efficacy of the crude extracts of *F. officinalis* was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The study shows that the crude ethanolic extract of *F. officinalis* showed more pronounced antimicrobial activity as compared to aqueous extracts, and when efficacy of either extract was compared to Resistance identifying agent ‘Methicillin’, both were found to be much more significantly efficacious in terms of the ZOI.

The results of the present investigation suggest that *F. officinalis* has a striking antibacterial efficacy against Methicillin resistant strains of *S. aureus* which have become resistant to most chemotherapeutic agents of Western Medicine. Since, herbal drugs used in Traditional Medicines are generally safe, this finding is of even greater therapeutic significance. Shahtara, or its optimized forms, within the range allowed by Unani Medicine, eg aqueous extracts, are suggested by this study to be very valuable as they are likely to be effective against very virulent bacteria despite being safe, natural products.
At the molecular level, isoquinolone alkaloids including fumaricine, sanguinarine (Evans and Trease, 2009), fumariline, perfume, protopine, fumaric acid (Erdogan, 2009) etc could be possessing antibacterial activity. However, more investigations are needed in this direction before the use of its appropriate form in clinics, as the present study is just an in-vitro proof about its antibacterial efficacy. Many in vivo and clinical studies regarding its clinical use are still needed to be done.

The study suggests that *F. officinale* exhibit antimicrobial properties against MRSA which is an emerging cause of a number of infectious diseases and has developed resistance to the synthetic antibiotics. The potential antimicrobial activity of *F. officinale* towards the infectious micro-organism explains the basis for its use in future in combating the disease caused by such dreadful bacteria.

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Anti-ulcer effect of Tukhme kishneez (Coriandrum sativum Linn.) in stress induced gastric ulceration in albino rats

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Abstract

Tukhm Kishneez (fruits of Coriandrum sativum Linn.), in Unani literature is mentioned for its anti-gastritis and anti-ulcer activity and also for its sedative, hypnotic, anti-anxiety, and anti-stress effect. It has been studied scientifically for anti-ulcer activity on certain parameters but no study has been conducted so far, for its role in stress induced gastric ulcer. So, the present study was undertaken to study the hydroalcoholic extract of Tukhm Kishneez, both before and after induction, for effect in water immersion stress induced gastric ulcer in rats, with Ranitidine as the standard drug. The median ‘ulcer score’ and ulcer index were found to decrease significantly in pre treated and post treated groups as compared to Negative control group. The effect was found to be dose dependant and the efficacy of test drug was more marked in curative group. Histological findings were in agreement with the ulcer index and ulcer score in all the groups and demonstrated definite signs of improvement in the mucosal texture. The study demonstrated that Tukhm Kishneez possesses anti-ulcer effect against stress induced gastric ulcer.

Key Words: Tukhm Kishneez, anti-ulcer, stress ulcer, Unani medicine, Coriandrum sativum

Introduction

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorders, which causes a high rate of morbidity (Andreoli et al, 2001). It comprises both gastric and duodenal ulcers. These are benign defects in the gastrointestinal mucosa that extends beyond the muscularis mucosa, and are perpetuated by acid peptic activity. It has been generally accepted that gastric ulceration results from an imbalance between aggressive factors (gastric HCl, pepsin secretion, H. pylori infection, alcohol, NSAIDs etc.) and defensive factors (break down of gastric mucosal barrier, endogenous prostaglandins, secretin, somatostatin, epidermal growth factors, blood flow etc.) and the most common causes of peptic ulcer include H. pylori infection, excessive use of non-steroidal anti-inflammatory agents (like aspirin, ibuprofen, indomethacin, diclofenac sodium etc.) and stress (Piper et al, 1981). The management of peptic ulcer involves decreasing the secretion of acid with H2-receptor antagonist or proton pump inhibitor, neutralizing secreted acid with antacids and enhancing the mucosal protection mechanism by cytoprotective agents. The later one is being appreciated as equally important measure to that of anti-secretory agents in the management of peptic ulcer (Horn, 2000). Although these drugs have brought about remarkable changes in ulcer therapy but their efficacy and safety are still debated. Reports on
clinical evaluation of these drugs show that there are incidences of relapses, adverse effects and danger of drug interaction during ulcer therapy (Dharman and Palt, 2006). In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. Most of the herbal drugs used in the management of peptic ulcer have been reported to reduce the offensive factors. They have been proved to be safe and effective and showed better patient tolerance. Hence use of natural product alone or in combination with other drugs should be seriously considered in the management of PUD (Goel and Sairam, 2002).

Herbs including spices have been used in traditional medicines to treat a wide range of ailments, including gastrointestinal disorders such as dyspepsia, gastritis and peptic ulcer disease (Solanki et al, 2002). A large number of spices such as large cardamom, caraway, coriander, clove, ginger, saffron, turmeric etc have been shown to possess significant gastro protective activities. Other properties attributed to spices such as anti-oxidant, anti-spasmodic, carminative, anti-inflammatory etc. further advocate their use in the management of PUD (Al-Mofleh et al, 2007).

Coriandrum sativum Linn. is considered both a herb and a spice and both its leaves and seeds are used as a seasoning condiment (Saeed and Tariq, 2007). It is used as carminative, diuretic, tonic, stimulant, stomachic, refrigerant, aphrodisiac and analgesic (Chaudhry and Tariq, 2000). It has also been attributed to be anti-diabetic (Gray and Flatt, 1999), anti-inflammatory and antioxidant and reported recently for its cholesterol lowering effect (Chithra and Leelamma, 1999). Its protective effect against ethanol induced gastric lesion has been reported recently by Al-Mofleh et al (2007). Coriander and its constituents have also been shown to produce other related effects such as cytoprotective (Ammar et al., 1997), anxiolytic (Emamghoreishi et al., 2005) and anti oxidant (Chithra and Leelamma, 1999). Unani literature reveals that it possesses musakkin (sedative), mubarrid (refrigerant), qabiz (astringent), munawwim (hypnotic) and mujaffif (siccative) properties (Ibn Hubal, 2004; Khan, 1313 H) useful in dyspepsia, vomiting, biliousness, bleeding ulcer (Qurhe meda), inflammatory conditions etc. It is also used in anxiety, insomnia, hypertension and stressful conditions (Kabeeruddin, 2007; Ghani, ynm; Ibn Baitar, 2003). An association between qurhe meda (peptic ulcer) and brain/nervous system, behaviour and psychology of human being has been frequently discussed in Unani literature (Khan, 2003; Ibn Hubal, 2004). Kishneez and its preparations are therefore recommended in Amraze Hazm (gastric diseases), Amraze aasab (Nervous diseases) and many disorders where both the systems are involved.

Therefore, in view of its described effects in Unani literature and age-old practice of Unani physicians to use Kishneez and its preparations in the management of stress and associated disorders, and some recently published reports demonstrating anti ulcer, anxiolytic and cytoprotective effect etc, Coriandrum sativum Linn. was hypothesised to be effective in stress ulcer and the study was designed to evaluate its anti-ulcer activity in stress model of water immersion-induced restraint gastric ulcer.

Materials and Methods

Plant Material

The test drug Tukhm Kishneez (fruits of Coriandrum sativum Linn.) was purchased from Sunkadkatte, one of the local markets of Bangalore, Karnataka. The drug sample was authenticated by an authorized committee of National Institute of Unani Medicine (NIUM), Bangalore, comprising of a pharmacognosist, Unani experts and a medicinal chemist. The drug was dried in a hot chamber (40 – 45 0C) and powdered coarsely in an electric grinder. The powder was extracted in hydroalcoholic solution (50% each) in a ratio of 1: 5, (100 gm of powdered drug was taken into 500 ml of hydroalcoholic solution) with the help of a Soxhlet apparatus for 8 hrs. Thereafter, the extract was filtered and concentrated on water bath. The concentrated extract was weighed and the yield percentage was calculated with reference to the weight of crude drug. The yield was found to be 19.48% w/w.

Dosage of the test drug

The therapeutic dose of Tukhm Kishneez described in Unani literature is 5-7 gm (Ghani, ynm). The dose for albino rats was calculated by multiplying the maximum dose i.e. 7 gm by the conversion factor of 7 (Freirich et al, 1966) and found to be 820 mg/kg body weight for rats. To study the dose dependent effect of the test drug, a second dose was calculated by the method of Miller and Tainter (1944) and was found to be 820 mg/kg body weight for rats.
1400 mg/kg body weight. The dose of extract corresponding to the dose of crude drug i.e. 160 mg/kg and 273 mg/kg respectively was used in the study. Ranitidine (50 mg/kg) was used as the reference drug. Drugs were administered by oral route with the help of a gastric cannula. The dosage of the test/reference drug was prepared freshly every day before the administration, by suspending them in 1ml of 0.4% carboxymethyl cellulose (CMC).

Experimental animals
The study was carried out in healthy adult Wistar rats of either sex, weighing 150-200 gm. They were procured from central animal house facility, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. The rats were housed in polypropylene cages, under controlled conditions of light (12/24 hour) and temperature (23±2 0C) and fed with standard commercial food pellets (Hindustan Lever Ltd.) and tap water ad libitum, under strict hygienic conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), National Institute of Unani Medicine, Bangalore, Karnataka, India vide Reg. No. 953/C/06/CPCSEA, dated 28th August, 2008.

Water immersion-induced restraint ulcer
This test was carried out by the method of Hayaso and Takeuchi (1986) with little modification in the treatment schedule. Animals were divided into 8 groups of 8 animals each. The animals in Group I and Group II were treated with 1 ml of 0.4% CMC and served as Plain control and Negative control respectively, while the animals in Group III and IV were treated with hydroalcoholic extract of the test drug in the dose of 160 mg/kg and 273 mg/kg and served as Pre-treated test group A and B, respectively. The animals in Group V were given ranitidine in the dose of 50 mg/kg and served as Pre-treated standard group. The treatment continued for 5 days. On 5th day 12 hours fasted animals were treated routinely and after one hour after the treatment they were sacrificed under thiopentone anaesthesia.

The abdomen of all the anaesthetized animals was opened by the midline incision; stomach was dissected out carefully and opened along with the greater curvature. The mucosa of stomach was washed with tap water and spread over a cardboard with the mucus surface upwards. The mucosal surface was examined for ulceration with the help of magnifying lens (10 fold magnification). The stomach of 2 rats from each group was preserved immediately in 10% formalin and sent for histopathological examination.

Determination of the degree of ulceration
The degree of ulceration was determined by the method of Adami et al. (1964).

- 0.0-- Absence of any detectable lesion
- 0.5-- Small haemorrhagic effusion
- 1.0-- Haemorrhagic effusion
- 1.5-- Mucosal ulceration of limited diffusion involving not more than 1/3rd of whole surface of stomach
- 2.0-- Mucosal ulceration of limited diffusion involving not more than 2/3rd of whole surface of stomach
- 2.5-- Mucosal ulceration of generalized diffusion
- 3.0-- Deep ulceration of limited diffusion
- 3.5-- Deep ulcerations of generalized diffusion
- 4.0-- Perforated ulcer

The average degree of single ulceration (ADU) for each group was determined by adding together the degree of single ulceration (DSU) and dividing it by the number of animals. On the basis of the percentage of rats with ulceration (% RU), the ulcer index was calculated by the following formula of Srimal (1984):
Ulcer index = \( \frac{(ADU)\times RU}{100} \)

ADU-- Average degree of single ulceration
% RU-- Percentage of rats with ulceration

**Statistical analysis**

The observations in various groups were expressed as median with range. The ulcer scores of various groups were compared with Negative control group. The group comparison was analyzed using statistical test of Kruskal-Wallis with post hoc Dunn’s multiple pair comparison test. The difference of median was considered significant at p<0.05.

**Result**

Effect of hydroalcoholic extract of *Tukhm Kishneez* on Water immersion-induced restraint ulcer.

The median ‘ulcer score’ in Negative control group where the ulcer was induced by Water immersion and restraint method, was found to be 2.25 (2.0, 3.5), while in Pre-treated test group A & B it was found to be 0.5 (0.5, 1.5) and 0.75 (0.5, 1.5), respectively and showed significant decrease (P<0.05) as compared to Negative control. Similarly, in Pre-treated standard group where Wistar rats were first treated with ranitidine in the dose of 50 mg/kg orally for five days and on 5th day ulcer was induced, the median ulcer for this group was 0.5 (0.0, 1.5) and showed significant result at p<0.01. In Post-treated test group A & B, the median ulcer score for Post-treated group A was 0.75 (0.5, 1.0) which was found to be significant at p<0.05, and for Post-treated B it was 0.5 (0.0, 1.0) and was found to be highly significant (p<0.001) with respect to Negative control. The median ulcer score for Post-treated standard group was equal to that of Post treated group B.

The ‘ulcer index’ in Negative control group was found to be 2.5, while it decreased to 0.75 and 0.71 in Pre-treated test group A & B, respectively. In Pre-treated standard group the ulcer index was found to be 0.68, and in Post-treated test group A it increased slightly to 0.75, but in Post-treated test group B and standard group it significantly reduced to 0.38 & 0.44 (Table 1,2).

**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Median with range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Plain control</td>
<td>1 ml of 0.4% Carboxymethyl cellulose</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative control</td>
<td>Ulcer induction</td>
</tr>
<tr>
<td>Group III</td>
<td>Pre-treated test group A</td>
<td>Tukhm Kishneez (160 mg/kg) + Ulcer induction</td>
</tr>
<tr>
<td>Group IV</td>
<td>Pre-treated test group B</td>
<td>Tukhm Kishneez (273 mg/kg) + Ulcer induction</td>
</tr>
<tr>
<td>Group V</td>
<td>Pre-treated standard group</td>
<td>Ranitidine (50 mg/kg) + Ulcer induction</td>
</tr>
<tr>
<td>Group VI</td>
<td>Post-treated test group A</td>
<td>Ulcer induction + Tukhm Kishneez (160 mg/kg)</td>
</tr>
<tr>
<td>Group VII</td>
<td>Post-treated test group B</td>
<td>Ulcer induction + Tukhm Kishneez (273 mg/kg)</td>
</tr>
<tr>
<td>Group VIII</td>
<td>Post-treated standard group</td>
<td>Ulcer induction + Ranitidine (50 mg/kg)</td>
</tr>
</tbody>
</table>

n = 8  *p<0.05, **p<0.01, ***p<0.001 with respect to Negative control
Table – 2
Effect of hydroalcoholic extract of Tukhm Kishneez (Coriandrum sativum Linn.) on Water immersion-induced restraint ulcer

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer incidence</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>% age</td>
</tr>
<tr>
<td>Group I Plain control</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>Group II Negative control</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>Group III Pre-treated test group A</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>Group IV Pre-treated test group B</td>
<td>7/8</td>
<td>87.5</td>
</tr>
<tr>
<td>Group V Pre-treated standard group</td>
<td>6/8</td>
<td>75</td>
</tr>
<tr>
<td>Group VI Post-treated test group A</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>Group VII Post-treated test group B</td>
<td>6/8</td>
<td>75</td>
</tr>
<tr>
<td>Group VIII Post-treated standard group</td>
<td>8/8</td>
<td>100</td>
</tr>
</tbody>
</table>

n = 8

Histopathological findings
On microscopic examination, sections of the stomach revealed different pathomorphological changes in different groups of animals. The Negative control showed haemorrhages and engorged blood vessels. The mixed inflammatory cells were present at places. Erosions and ulcerations were also found. Pre-treated test group A, showed mild lymphocytic infiltrate in the lamina propria towards antrum. The congestion, oedema and inflammatory changes were also observed. In Pre-treated test group B, biopsy revealed congestion, otherwise no significant pathology was observed. While Pre-treated standard group, showed increased number of paneth cells. Post-treated test group A, (Group VI) revealed features of gastritis while Post-treated test group B, (Group VII) showed congestion otherwise no significant pathology was observed. In Post-treated standard group, slides revealed mild congestion otherwise no pathology was observed (Fig1-5; Table 3).

Table – 3
Summary of Histopathological Findings

<table>
<thead>
<tr>
<th>Groups</th>
<th>Congestion</th>
<th>Haemorrhage</th>
<th>Oedema</th>
<th>Necrosis</th>
<th>Inflamm changes</th>
<th>Erosion</th>
<th>Ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group III</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group IV</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group V</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group VI</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group VII</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group VIII</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Normal: (-), Moderate: (+), Severe: (++), Intensely severe: (+++)
Discussion

The study demonstrated that Kishneez produces significant anti ulcer effect against stress induced gastric ulcer in dose dependant manner. It possesses both protective effect (when given before stress) and curative effect (when given after stress), but the curative effect is more pronounced and the higher dose is equally effective as the standard drug ranitidine.

Water immersion-induced restraint ulcer is one of the best models of stress in rats to induce ulceration. This model provides both emotional stress as well as psychological/physiological stress to the animals. It was found that there was significant decrease in ulcer score and ulcer index.
of all Pre-treated groups. The Pre-treated test group A and B showed a significant decrease at p<0.05, while in Pre-treated standard group more significant reduction (p<0.01) was observed. The Post-treated groups also exhibited significant decrease in ulcer index. The Post-treated test group A rats, showed significant decrease in ulcer score at p<0.05, while the groups treated with higher dose and the standard drug showed almost equal effect and were found to be highly significant at p<0.001 (Table 1-3 & Fig 1-5).

In Histopathological slides inflammatory and degenerative changes, hemorrhages, engorged blood vessels, inflammatory cells, erosions and ulcerations were seen all around, in negative control group. The higher doses of both the test groups showed only congestion and no significant pathology was observed, indicating that the higher dose of the test drug is quite effective as a protective agent, as it did not allow the ulcerogenic dose of the test drug to act upon the gastric mucosa. Further, it also acted as a therapeutic agent and showed improvement when gastric lesions were induced prior to the treatment. The lower dose was also found to repair the histological changes and induced significant improvement but the effect of the higher dose was remarkable and equal to that of the standard drug.

It is well known that central nervous system is intimately concerned in the genesis of gastric ulceration. Stress is believed to be important in the causation of hyperacidity and ulceration (Levenstein et al., 1999). The techniques of restraint in albino rats provide a model for the study of stress induced gastric ulceration (Malairajan, 2008). Gastrointestinal erosion is one of the consistent findings in man and experimental animals subjected to different types of stress. Since the development of gastric lesions during stress enhances significantly by exposure to water immersion, the rise in acid secretion may be instrumental in aggravating the gastric lesions during water immersion (Parmar and Desai, 1993). The stress was induced with two stressing factors with an aim to develop the ulcer at the earliest and with clear features. Restrain itself is a good inducer of ulcer but when it is combined with cold or water immersion it produces more acute ulcers (Brodie, 1968). The major factor in the causation of ulcer has been reported to be the mucosal defense system and mucosal ischemia. Stress is associated with strong gastric contraction that can generate areas of focally reduced blood flow. Stress releases thyrotropin-releasing hormone (TRH) in the brain which is associated with reduction of overall gastric blood flow (Bhargava et al., 1980). Further, acid secretion is increased with stress which is considered essential for the development of ulcer. Stress also increases histamine release with enhanced acid secretion, which causes ulcers and reduces mucus production (Norton, 2001). The test drug by ameliorating the ulcers in experimental model induced by stress, demonstrated significant anti-ulcer activity. This anti-ulcer activity of the test drug may be attributed to its stress improving effect as well as to its ability to maintain a balance between aggressive and defensive factors by inhibiting the acid secretion and protecting the mucosa. It is also possible that the corrosive action of secreted gastric juice is neutralized by the extract either by decreasing the histamine secretion or by increasing the production of mucus and bicarbonate and also by improving the gastric blood supply. The healing property (Kabeeruddin, 2007; Anonymous, 2004) of the test drug may also be considered contributory in improving the ulcerogenic condition at least in curative regimen.

Kishneez in Unani literature has been described to possess sedative, hypnotic, anti-anxiety and anti-stress effects for which it is used frequently in the management of stress related diseases (Hakeem, 2002; Masihi, 1968) which has been proved by the findings of the present study too. It has also been reported to possess anxiolytic, sedative and muscle relaxant effects (Emamghoreishi et al, 2005). Thus we can say that the test drug is endowed with anti-stress effect which is one of the factors consistent with the improvement in stress induced gastric ulcer. The overall effect of the test drug is in consonance with another report that demonstrated that the oral administration of the coriander powder produced dose dependant effect against the ulcers induced by the ethanol and pylorus ligation (Al- Mofleh, et al, 2006).

According to Unani physicians the causes of gastric ulcer are Khilte Haad (hot and irritant humour), fuzlat (waste products), intake of hot and spicy foods, excessive use of alcohol, prolonged stress and strain, chronic gastritis and indigestion (Tabri, ynm). The temperament of Tukhme Kishneez has been mentioned to be cold and dry in second degree (Hakeem, 2002; Masihi, 1986), so it is quite possible that Kishneez is able to neutralize the Khilte Haad and because of its cold temperament it may
arrest the secretion of corrosive fuzlat. It has also been described to be qabiz (astringent), musakkin (sedative), munawwim (hypnotic), muhalil (resolvent) and munaqqi (cathartic) (Khan, 1313H, Masahi, 1986). These effects may have a role in improving the gastric lesions either through nervous system or by producing local effect such as neutralization, healing and cytoprotection. Unani scholars have also mentioned it to be useful in various gastric disorders like sue mizaj meda (altered temperament of stomach), wajae meda (gastralgia), zofe hazam (oligopepsia), sue hazam (indigestion), tukhma (food poisoning) and qurooh wa busoor meda (gastric ulcers) and all these pathological conditions are associated with making the mucosal defense system weak (Khan, 2003; Tabri, ynm). Therefore, it may be assumed that Kishneez plays an important role in improving the mucosal defense system. When taken orally Kishneez not only resolves haar madda (hot matter) but also strengthens the defensive system (Ibn Baitar, 2003).

Tukhm Kishneez contains several fatty acids, oleic, linolenic and palmitic acids etc (Rastogi and Mahrotra, 2002). The fatty acids are well known as a source of prostaglandins (Wichtl, 1994). A number of reports show that PGs have cytoprotective effects (Robert, 1977) and that they are reduced in the gastric mucosa of patients with gastric ulcer (Wright, 1982). Studies suggested that cytoprotective activity of Kishneez is, at the molecular level, at least partially, due to the presence of unsaturated fatty acids and quercetin which may together produce a synergistic effect (Ammar, 1997). The cytoprotective and antiulcer activity of many flavonoids including quercetin has already been confirmed (Borrelli and Izzo, 2000). Thus the findings of the present study and the reports available on Kishneez and its various constituents suggest that it produces the anti ulcer effect probably by evolving diverse mechanisms such as by reducing the stress, cytoprotective effect, minimising or neutralising the acid secretion, healing property and anti-oxidant activity but the anti stress effect appears to be the core effect as the other effects are anyhow routed through the stress mechanism.

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Gastro protective effect of the Unani drug Habbul aas (*Myrtus communis* Linn. berries) in experimental ulcer models in rats

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Abstract

The effects of aqueous and methanolic extract of dried berries of *Myrtus communis* Linn. (Myrtaceae) were studied for anti-ulcer activity by employing different experimental models in rats including indomethacin and pyloric ligation induced gastric ulcers and compared with the standard drug, omeprazole. Two doses of aqueous extract of *M. communis*, viz. 105 (AE1) and 175 (AE2) mg/kg, and of methanolic extract viz. 93 (ME1) and 154 (ME2) mg/kg were administered orally to animals prior to the exposure of ulcerogens. Oral treatment of aqueous and methanolic extract of *M. communis* significantly reduced ulcer index, gastric juice volume, total acidity and significantly enhanced the gastric pH and gastric wall mucus content. Omeprazole also exhibited significant ulcer protective results. The results suggest that Habbul Aas (berries of *M. communis*) has protective role against gastric ulcers.

Key Words: *Myrtus communis*, Anti-ulcerogenic activity, Indomethacin, Habbul Aas

Introduction

For over a century, peptic ulcer disease (PUD) has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *H. pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) (Hoogerworf and Pasricha, 2001). An estimated 15,000 deaths occur each year as a consequence of PUD (Valle, 2005). The prevalence of duodenal ulcers is dominant in Western populations and gastric ulcers are more frequent in Asia (Yuan et al., 2006; Falcao et al., 2008). A number of drugs including proton pump inhibitors, prostaglandin analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage (Ariyphisi et al., 1986). Many of these drugs do not fulfill all the therapeutic requirements and have side effects (Anoop and Jegadeeshan, 2003; Dharmani et al., 2005). The clinical evaluation of these drugs showed development of tolerance and incidence of relapse and side effects that make their efficacy arguable. This has been the rationale for the development of new antiulcer drugs, which includes herbal drugs. Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Herbal medicines are considered safer because of the natural ingredients with no side effects (Clouatre and Rosenbaum, 1994). The herbal drugs of Traditional medicines like Unani medicine are likely to be all the more safe and more effective too because they have been developed on
holistic, humoural basis. Unani Medicine possesses many safe and effective drugs for PUD. One such drug is Habbul Aas. *Myrtus communis* Linn (Myrtaceae) is an evergreen shrub, native to Southern Europe and North Africa and widespread in Mediterranean area. It is traditionally used as an antiseptic, disinfectant drug and hypoglycaemic agent (Elfellah, 1984). Its berries are frequently prescribed in the treatment of various gastro-intestinal disorders including gastric ulcer due to its stomachic, astringent, dessicant, haemostatic, analgesic, coolant and anti-inflammatory activities (Elfellah, 1984). Its berries are frequently prescribed in the treatment of various gastro-intestinal disorders including gastric ulcer due to its stomachic, astringent, dessicant, haemostatic, analgesic, coolant and anti-inflammatory activities (Ghani, 1917). Antimicrobial (Dominguez and Ortega, 1983), anti-inflammatory (Ahmad et al., 1981) and anti-oxidant properties (Montoro, 2006) of berries of this plant have been reported. There was no modern scientific report available on the traditional claim of the effects of *M. communis* in gastric ulcer. Therefore, the present study was undertaken to investigate the effects of the aqueous and methanolic extracts of the berries of *M. communis* in gastric ulcer against indomethacin and pyloric ligation induced ulcers in rats.

**Materials and Methods**

**Plant material**

The dried berries of *M. communis* were procured from the Khari Bawli, Delhi. The identity of the berries was established by NISCAIR (National Institute of Science Communication and Information Resources), Pusa Gate, New Delhi. The Voucher specimen (NISCAIR/ RHM/ CONSULT/ - 2007-08/914/98, dated 13/11/2007) of the test drug was retained and deposited for future reference in the department of Ilmul Advia (Pharmacology), Faculty of Unani Medicine, Hamdard University, New Delhi.

**Preparation of the extract**

The dried plant material (100gm) was coarsely powdered and exhaustively extracted with methanol by using soxhlet apparatus for 8 hours and the filtered extract was evaporated on a water bath to get a viscous aqueous extract. The extractive value (% w/w) of the aqueous dry extract was 24.57% and methanolic dry extract was 21.15%. These extracts were used for different ulcer models in rats. Both doses of the extracts were administered corresponding to 3 gm and 5 gm of crude drug, respectively (according to therapeutic doses mentioned in Unani literature) (Baitar, 1999) and converting to rat dose and extract yields.

**Experimental animals**

All the experiments were carried out in albino rats of wistar strain weighing 150-200gm supplied by Central Animal House, Hamdard University, New Delhi. The animals were kept at standard laboratory conditions and maintained on a standard pellet diet (Amrut Laboratory Animal-feed, Pune) and tap water *ad libitum*. Prior to experiments, rats were fasted for 24 hrs but were allowed free access to water. These experiments were conducted after getting approval from the Institutional Animals Ethical committee (IAEC) duly constituted according to CPSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines of Government of India.

**Indomethacin induced gastric ulceration**

In this model rats were randomly divided into different gps. having 6 animals each. Test samples in the form of aqueous extract at the dose of 105 and 175 mg/kg and methanolic extract at the dose of 93 and 154 mg/kg dissolved in distilled water and Omeprazole (standard drug) at the dose of 20 mg/kg were administered orally prior to the exposure to ulcerogens. After 1 hour of drug administration ulceration was induced by Indomethacin 20 mg/kg except in the control gp. The animals were sacrificed 5 hours later by an overdose of ether vapours and the stomach was removed and opened along the greater curvature and evaluated for ulcer index (Djahanguiri, 1969;Main and Wittle, 1975).

**Pyloric ligation induced gastric ulceration**

Rats were randomly divided into different gps. having six animals each in this model. Aqueous extract (105 and 175 mg/kg) as well as methanolic extract (93 and 154 mg/kg) of the test drug, Omeprazole (standard anti-ulcer drug) in the dose of 20 mg/kg (all dissolved in distilled water, 10 ml/kg) and control (distilled water) were administered orally prior to pyloric ligation. The pylorus was ligated after 30 minutes of drug administration as described by Shay et al. (1945). Animals were sacrificed 4 hours later and stomach was removed and opened along the greater curvature and examined for various parameters.

**Estimation of ulcer index, volume and pH of gastric juice**

The gastric juice was evacuated into a centrifugation tube. After centrifugation (3000 rpm, 10 min) the volume of the supernatant (ml/100 gm) and the pH values were measured (Shay, 1945). The sum of length (mm) of all
lesions for each stomach was used as ulcer index (UI). The severity of erosions in the glandular mucosa was assessed on a scale of 0 to 3. The scoring technique for ulcer index was assessed with the following criteria:

**EROSIONS SCORE**

<table>
<thead>
<tr>
<th>Normal stomach</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mm or less</td>
<td>1</td>
</tr>
<tr>
<td>1 mm to 2 mm</td>
<td>2</td>
</tr>
<tr>
<td>More than 2 mm</td>
<td>3</td>
</tr>
</tbody>
</table>

The inhibition percentage of the ulcer index was calculated by the following formula:

\[
\% = \left[ \frac{(UI_{\text{CONTROL}} - UI_{\text{TREATED}})}{UI_{\text{CONTROL}}} \right] \times 100
\]

**Total acidity**

For the determination of total acidity of the samples of gastric juice, a known volume of the gastric juice was titrated with 0.01N sodium hydroxide to pH 8.5 using phenolphthalein as an indicator (Parmair, 1984). The values of the total acidity were expressed as milliequivalents per litre per 100 gm (Mequiv./L/100 gm). The values of the total acidity of the gastric juice samples contaminated with blood were not included in the data.

**Gastric wall mucus (barrier mucus) determination**

In this method alcian blue a histological dye is used, which stains only the barrier mucus and does not penetrate the mucosal tissue. The dye complexed with barrier mucus can be recovered by immersion in a standard solution of MgCl₂. The amount of alcian blue recovered per gram of net glandular tissue was calculated as µg/g wt. of glandular tissue (Corne, 1974).

**Statistical analysis of data**

All the values have been expressed as Mean ± SEM (Standard error of mean). Statistical significance was determined by C.R.D. (completely randomized design) also known as one way ANOVA and Student-Newman-Keuls Multiple comparison test.

**Results**

Effect of aqueous and methanolic extract of *M. communis* on ulcer index against indomethacin induced gastric ulcers

Pretreatment of animals with AE₁ of Habbul Aas inhibited the ulcer index significantly by 3.67 ± 0.44 (p<0.001) as compared to 11 ± 0.81 in toxic control. The inhibition was in the order of 66.64 %. With AE₂ the ulcer index was 3.33 ± 1.08 (p<0.001) with a significant inhibition of 69.72%.

In these results high dose of aqueous extract showed better result than low dose (Table - 1).

ME₁ showed ulcer index of 0.16 ± 0.16 (p<0.001) as compared to 11 ± 0.81 in toxic control. The inhibition was in the order of 98.48%. With ME₂ a significant reduction in the ulcer index was seen viz. 3.83 ± 1.22 (p<0.001) with the inhibition by 65.18%. Omeprazole reduced the ulcer index to 0.5 ± 0.34 (p<0.001) with the inhibition by 95.45%. The low dose of ME₁ showed highly significant effect greater in comparison to Omeprazole (Table - 1).

Effect of aqueous and methanolic extract of *M. communis* on ulcer index, gastric juice volume, total acidity, pH and gastric wall mucus content against pyloric ligation induced gastric ulcers

AE₁ showed ulcer index of 5.67 ± 1.05 (p<0.001) as compared to 20.16 ± 3.24 in control. With AE₂ the ulcer index was 7.33 ± 2.21 (p<0.001). In this model, low dose of aqueous extract showed better results than high dose of aqueous extract (Table - 2).

ME₁ showed ulcer index of 5.83 ± 0.98 (p<0.001) as compared to 20.16 ± 3.24 in control. With ME₂ showed a significant reduction in the ulcer index by 6.67 ± 1.90 (p<0.001). Low dose of methanolic extract exhibited better results than high dose (Table-2).

Pretreatment of animals with AE₁ decreased the gastric juice volume to 3.65 ± 0.21 (p<0.001) as compared to 6.58 ± 0.35 in control gp. I. AE₂ showed significant decrease in gastric juice volume to 3.98 ± 0.50 (p<0.001). Omeprazole treatment also showed significant reduction of gastric juice volume to 1.70 ± 0.17 (p<0.001) (Table - 2).

ME₁ decreased the gastric juice volume to 4.15 ± 0.41 (p<0.001) as compared to 6.58 ± 0.35 in control gp. I. ME₂ significantly reduced gastric juice volume to 4.16 ± 0.40 (p<0.001) (Table - 2).

Pretreatment of animals with AE₁ decreased the total acidity to 114.33 ± 2.71 (p<0.001) as compared to 152.5 ± 1.70 in control gp. I. AE₂ produced significant decrease in total acidity to 128.83 ± 2.97 (p<0.001). Omeprazole treatment also significantly reduced total acidity to 110.33 ± 2.37 (p<0.001) in comparison to 152.5 ± 1.70 in control (Table - 2).

Pretreatment of animals with ME₁ decreased the total acidity to 115.5 ± 1.89 (p<0.001) as compared to 152.5 ± 1.70 in control group I. ME₂
significantly reduced total acidity to 115.0 ± 2.35 (p<0.001) (Table-2).

Pretreatment of animals with AE1 extract significantly enhanced the gastric pH to 3.20 ± 0.07 (p<0.001) as compared to 2.48 ± 0.12 in gp. I. AE2 significantly increased gastric pH to 3.09 ± 0.06 (p<0.001) (Table-3).

ME1 raised the gastric pH to 3.16 ± 0.04 (p<0.01) as compared to 2.48 ± 0.12 in gp. I. ME2 significantly increased pH to 3.14 ± 0.08 (p<0.001). Omeprazole significantly raised gastric pH to 3.24 ± 0.08 (p<0.001) in comparison to 2.48 ± 0.12 in control gp. (Table-3).

The gastric wall mucus in animals pretreated with AE1 was 15.05 ± 1.34 as compared to 12.11 ± 0.54 in control gp. I, which showed no significant effect on gastric wall mucus. The animals treated with AE2 showed increase in mucus content by 17.19 ± 1.43 (p<0.05) in comparison to 12.11 ± 0.54 in control gp. I. Omeprazole treatment showed non significant increment in mucus content to 13.31 ± 0.79 (Table-3).

The gastric wall mucus in animals pretreated with ME1 increased the mucus content by 17.89 ± 1.45 (p<0.05) as compared to 12.11 ± 0.54 in control gp. I. The animals treated with dose ME2 significantly raised the mucus content by 17.51 ± 1.63 (p<0.05) (Table-3).

### Table - 1
Effect of aqueous and methanolic extract of *M. communis* on ulcer index against indomethacin induced gastric ulcers in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose per os</th>
<th>Ulcer Index (mm) (Mean ± S.E.M.)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>11.0 ± 0.81</td>
<td>_</td>
</tr>
<tr>
<td>Toxic Control</td>
<td>11.0 ± 0.81</td>
<td>0.5 ± 0.34</td>
<td>95.45</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>20 mg/kg</td>
<td>3.67 ± 0.49</td>
<td>66.64</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>105 mg/kg</td>
<td>3.33 ± 1.08</td>
<td>69.72</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>175 mg/kg</td>
<td>0.16 ± 0.16</td>
<td>98.54</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>93 mg/kg</td>
<td>3.83 ± 1.22</td>
<td>65.18</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>154 mg/kg</td>
<td>3.83 ± 1.22</td>
<td>65.18</td>
</tr>
</tbody>
</table>

p < 0.001 statistically significant, when compared with toxic control gp.

### Table - 2
Effect of aqueous and methanolic extract of *M. communis* on ulcer index, gastric juice volume and total acidity against pyloric ligation induced gastric ulcers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose per os</th>
<th>Ulcer Index (mm)</th>
<th>Gastric Juice Volume (ml/100gm)</th>
<th>Total Acidity (MEq/L/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.16 ± 3.24</td>
<td>6.58 ± 0.35</td>
<td>152.50 ± 1.70</td>
<td>115 ± 2.35***</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>3.16 ± 0.74***</td>
<td>1.7 ± 0.17</td>
<td>110.33 ±2.37***</td>
<td>115 ± 2.35***</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>105 mg/kg</td>
<td>5.67 ± 1.05***</td>
<td>3.65 ± 0.21</td>
<td>114.33 ±2.71***</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>175 mg/kg</td>
<td>7.33 ± 2.21***</td>
<td>3.98 ± 0.50</td>
<td>128.83 ±2.97***</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>93 mg/kg</td>
<td>5.83 ± 0.98***</td>
<td>4.15 ± 0.41</td>
<td>115.50 ±1.89***</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>154 mg/kg</td>
<td>6.66 ± 1.90***</td>
<td>4.16 ± 0.40</td>
<td>115 ± 2.35***</td>
</tr>
</tbody>
</table>

*p = 0.001, when compared with control gp.*
Table – 3
Effect of aqueous and methanolic extract of *M. communis* on pH and gastric wall mucus content against pyloric ligation induced gastric ulcers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose per os</th>
<th>pH</th>
<th>Gastric wall mucus (µgm alcian blue/100 gm glandular tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.48 ± 0.12</td>
<td>12.12 ± 0.54</td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td>3.24 ± 0.08</td>
<td>13.31 ± 0.79**</td>
</tr>
<tr>
<td>Aqueous extract 105 mg/kg</td>
<td></td>
<td>3.20 ± 0.07</td>
<td>15.05 ± 1.34**</td>
</tr>
<tr>
<td>Aqueous extract 175 mg/kg</td>
<td></td>
<td>3.09 ± 0.06</td>
<td>17.19 ± 1.43*</td>
</tr>
<tr>
<td>Methanolic extract 93 mg/kg</td>
<td></td>
<td>3.16 ± 0.04</td>
<td>17.89 ± 1.4 5*</td>
</tr>
<tr>
<td>Methanolic extract 154 mg/kg</td>
<td></td>
<td>3.14 ± 0.08***</td>
<td>17.51 ± 1.63*</td>
</tr>
</tbody>
</table>

n = 6; **p < 0.001; * = p < 0.05; ns = non significant; when compared with control gp.

Discussion
The results of the study revealed that aqueous and methanolic extract of the berries of *M. communis* possess significant ulcer protective activity. NSAIDS like Indomethacin are known to induce gastric ulceration principally due to the inhibition of biosynthesis of cytoprotective prostaglandins which are cytoprotective to gastric mucosa e.g. PGEs and PGI$_2$ resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford, 1987). Pyloric ligation induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. Histamines play a mediating role in the gastric secretion stimulated by gastrin, vagal excitation and cholinergic agents (Glick et al., 1966). Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach by the accumulating acid (Shay et al., 1945). In pyloric ligation induced model, the drug showed significant reduction in gastric juice volume and increase in pH of gastric juice at both the doses. Decrease in mucosal secretion is considered important in gastric ulceration (Goel and Bhattacharya, 1991). The results of the study indicate that both the extracts of test drug increased the mucus. Thus the increased gastric wall mucus may be responsible to some extent in reducing the gastric lesions. It is believed that Omeprazole produces its antiulcer activity through acid suppression, scavenging of OH$^-$ radical and blocking apoptotic cell death (Biswas et al., 2003). The berries of *M. communis* possess significant antioxidant property (Montoro et al., 2006). There are extensive experimental evidences that indicate that free radical scavengers protect the gastric mucosa (Goel et al., 1985). Therefore the anti-ulcer action of the drug may be due to scavenging of the free radicals generated in the injured mucosa. Thus, anti-ulcerogenic activity of the extracts could possibly be due to various possible mechanisms including its prostaglandin synthesis increasing, lipoxygenase inhibitory, leukotrienes antagonistic, cytoprotection or anti-oxidant activity. Yet further studies are required to evaluate the exact mode of action of this drug in gastric ulcer.

The preliminary phytochemical studies revealed the presence of flavonoids, tannins, alkaloids and protein in the drug. Various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection (Alarcon, 1994). Glycosides have been shown to inhibit the gastric acid secretion and enhancement in gastric mucus content against several experimental ulcer models (Murakami, 1990). Tannins and volatile oil of some plants are also known to possess antiulcer activity (Al-Rehaily, 2002). So the anti-ulcer action of *M. communis* may be due to biological active compounds viz. flavonoids,
thus these observations indicated that Habbul Aas (M. communis) can be effectively used for the treatment of gastric ulcer. Although the mechanism involved was not determined in the present study, yet more detailed phytochemical studies are required to investigate the active principles and exact mechanism of the action of M. communis. The results support the traditional claim for the use of this plant as an effective anti-ulcer drug.

References


Hepatoprotective effect of Majeeth (Rubia cordifolia Linn.) against chemically induced hepatic damage

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Abstract

The objective of the present study was to evaluate the hepatoprotective activity of hydroalcoholic extract of root of Rubia cordifolia Linn. (Majeeth) using CCl₄ induced hepatic damage in Wistar albino rats. The hydroalcoholic extract of root was administered orally for 7 days in two doses viz. 350 mg/kg and 450 mg/kg. Hepatoprotective activity was studied in CCl₄ induced hepatic damage model using biochemical parameters, wet liver weight, and liver histology as the parameters and Silymarin as the standard drug. The effect on lipid peroxidation was also studied. There was significant increase in serum levels of bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, urea, cholesterol and wet liver weight with a decrease in total protein in CCl₄ treated animals, reflecting liver injury. In test drug treated group there was a decrease in serum levels of markers of liver damage and significant increase in total protein, indicating the recovery of hepatic cells. The lipid peroxidation indicated by MDA was also increased in CCl₄ treated group. The standard and test drug reduced MDA. Histopathology findings also showed protection and regeneration of liver. Thus, the hydroalcoholic extract of root of Rubia cordifolia Linn. Was seen to possess significant protection against CCl₄ induced hepatocellular injury, at least partially, produced by Anti-oxidant effect.

Key Words: Rubia cordifolia Linn., Majeeth, Rubiadin, Hepatoprotection, Silymarin

Introduction

The study of liver ailments is a priority area because liver is the vital organ of the body and is responsible for maintaining the body’s metabolic homeostasis (Kumar et al, 2003). Presently, the risk of liver intoxication has greatly increased due to higher exposure to environmental toxins, pesticides and by frequent use of chemotherapeutics. In spite of tremendous scientific advancement in the field of hepatology, in the recent years, liver problems are on rise, jaundice and hepatitis are the two major hepatic disorders that accounts for a high death rate (Gujrati et al, 2007). No effective measures are available for the treatment of liver ailments in modern medicine. In such a situation, man always looks towards nature as a source of alternative medicine. So, alternative medicines employing herbal drugs particularly Unani Medicine is approached. Root of Rubia cordifolia Linn., commonly known as Majeeth is frequently prescribed in treatment of Yarqaan (Jaundice), Tasuddud-e-Aaza (Visceral Obstruction), Izm-e-Tehal (Splenomagaly), Faalij (Paralysis) (Antaki, 1343H: Ibn Baitar, 1291H : Ghani, 1921,) and used as Mufatteh-e-Suddad (Deobstruent) (Antaki, 1343: Attar, 1888: Ibn Baitar, 1291H) Muhallil (Ghani, 1921), Mudir-e-Baul-e-Ghaleez (Strong Diuretics) (Razi, 1321H: Antaki,1343: Attar,1888: Ibn Baitar, 1291H) and were reported to contain phenolic constituents predominantly Hydroxyanthr-
aquinoes (Cai et al, 2004), Anthracene derivatives, Rubiasin A-C (Chang et al, 2007) and essential oil (Miyazawa & Kawata, 2006). Rubiadin, a glycoside is the major constituent isolated from Rubia cordifolia Linn. showed significant hepatoprotective effect against CCl₄ induced damage (Rao et al, 2005). Alcoholic extract of Rubia cordifolia Linn. possesses Immunomodulatory activity (Joharapurkar et al, 2003) and Anti-oxidant activity (Joharapurkar et al, 2003). Rubiadin also prevents elevation of hepatic MDA formation i.e. lipid peroxidation induced by CCl₄. Aqueous extract showed significant anti-inflammatory activity, comparable to that of phenylbutazone (Antarkar et al, 1983).

Majeeth, root of Rubia cordifolia Linn. is a drug which is used in liver disorders since long but no scientific data is available on the traditional claims of the roots of this plant in liver disorders. Therefore, of hydro-alcoholic extract of Rubia cordifolia Linn. was investigated in CCl₄ hepatic damage induced model to provide scientific support and validation to Unani claim regarding the clinical utility of Majeeth for hepato-protective activity in hepatic disorders.

Materials and Methods

Plant Material

The dried roots of Rubia cordifolia Linn. were procured from Khari Baoli Market in Old Delhi and authenticated by Department of Raw Materials Herbarium and Museum, NISCAIR, Dr. K.S. Krishnan Marg, New Delhi. A voucher specimen was also preserved with ref no:NISCAIR/ RHM/F-2005/cons/628/108 for future reference.

Experimental Animals

Albino rats of either sex of Wistar strain weighing 150-200 gms, used in the study, were obtained from animal House, Department of Ilmul Advia, Ajmal Khan Tibbiya College (AKTC). They were kept under standard laboratory conditions, commercial diet pellets and water was allowed ad libitum. Experiments were conducted after getting approval from the departmental ethical committee for animal care and use.

Extracts

The dried roots of Rubia cordifolia Linn. was crushed and powdered coarsely and then hydroalcoholic extract (50:50) was prepared using soxhlet’s apparatus. The solvent was removed under reduced pressure and the extract yield was 56% (w/w) in terms of starting material.

Liver Function Tests

The protective effect of the test and control drug was studied by estimating the concentration of biochemical and metabolic markers of liver function in blood viz Serum Bilirubin (Malley et al, 1937) Serum Alanine Transaminase (S ALT/S GPT) and Serum Aspartate Transaminase (S AST/S GOT) (Reitman et al, 1957) Serum Alkaline Phosphatase (S ALP) (Kind et al, 1954), Serum Total Protein (Biurat & Dumas, 1971), Serum Total Cholesterol (Wybeng et al, 1970), Serum Urea (Fearon, 1939: Martinek, 1969: Wybenga, 1971), and Wet Liver Weight (Kuttan, 2000).

CCl₄ induced hepatic damage

The animals were randomly distributed into 5 groups of 6 animals each. The animals of control group received vehicle (10 ml/kg) for 7 consecutive days. CCl₄ group animals were also received vehicle for 7 days. The standard group received silymarin (100 mg/ kg, orally) for 7 days. The Majeeth extract at a dose of 350 and 450 mg/kg was also administered in two groups of animals for seven days. Hepatic damage was induced on the fifth day by intraperitoneal route by CCl₄ in a dose of 2ml/kg to all the animals except the animals of plain control group. Then, the animals were sacrificed after 48 hrs of CCl₄ administration by cervical dislocation. After measuring the body weight, blood was collected for estimating the concentration of biochemical and metabolic markers of liver function. Then, the abdomen was opened and the liver dissected out and weighed after cleaning extraneous tissue.

Estimation of Lipid Peroxidation (TBARS Test)

The test was carried out according to the method (Okawa et al, 1979). In this procedure 0.2 ml of sub cellular fraction of liver (as 10 % w/v homogenate of chilled 0.15M KCl) was mixed with 1.0 ml of 20 % acetic acid. Subsequently 0.2 ml of 8.0 % aqueous sodium dodecyl sulphate was mixed. After this 1.5 ml of 0.8 % Thiobarbituric acid (pH 7.0) and 1.1 ml of double distilled water was added. The reaction mixture was incubated in a boiling water bath for an hour. After cooling to room temperature, 3.0 ml of n-butanol was mixed in each test tube. The reaction mixture was then centrifuged at 10,000 Xg for 15 minutes. A clear butanol supernatant was used for measuring the O.D. at 532 nm against the blank. The protein was also determined simultaneously by the method (Lowry et al, 1951).
Liver Histological Studies

For histological study, the sections of liver were removed and fixed in 10% formalin. The fixation was done immediately to check the autolysis and to preserve as nearly as possible the natural state of tissue cells. Care was taken to keep the volume of the fixative (Mukherjee & K L 1988). The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and studied for histopathological change by light microscopy under various magnifications.

Statistical analysis

All the values were expressed as mean ± S.E. Statistical significance of the differences was determined by using ANOVA Test followed by Pair-wise comparison by Student’s ‘t’ Test.

Results

Effect of Majeeth on liver function

In the animals in Group I, used as plain control, S ALT, S AST, S.Bilirubin,S.Alkaline Phosphatase, S.Total protein, S.Urea, S.Cholesterol and Wet liver weight were seen to be 34.70 ± 4.49 units/ml, 14 ± 4.23 units/ml, 1.33 ± 0.24 mg/dl, 5.40 ± 0.15 KAU/dl, 6.72 ± 0.16 gm/100ml, 47.72 ± 1.22 mg/dl, 101.75 ± 5.96 mg/dl, and 2.83 ± .28 gm/100gm, respectively.

In CCl₄ treated animals (Group II), S ALT, S AST, S. Bilirubin, S. Alkaline Phosphatase, S.Urea, S.Cholesterol were significantly increased, while S.Total Protein was significantly decreased as compared to Group I. The increase in liver weight was not significant statistically. In the animals treated with silymarin and CCl₄ (Gp III) showed significant decrease in S ALT, S AST, S. Bilirubin, S. Alkaline Phosphatase, S. Urea and Cholesterol while S.Total Protein was significantly increase as compared to CCl₄ treated Gp. The decrease in wet liver weight was not significant statistically. In the Group IV animals treated with lower dose of Majeeth (350 mg/kg) and CCl₄, S ALT, S AST, S.Bilirubin, S. Alkaline Phosphatases was significantly decreased but the decrease produced in S.Urea, S.Cholesterol and Wet liver weight was not significant statistically. Similarly, S.Total Protein also did not increase significantly. Group V, treated with higher dose of Majeeth (450 mg/kg) and CCl₄, S ALT, S AST, S.Bilirubin, S. Alkaline Phosphatase, S. Urea, were decreased significantly while the decrease produced in S.Cholesterol and Wet liver weight was not significant statistically. Similarly, S.Total Protein also did not show significant changes. In inter-treatment similarly comparison of lower and higher dose of Majeeth, it was observed that decrease produced with higher dose (450 mg/kg) in biochemical and metabolic parameters was slightly greater than with the lower dose (350 mg/kg), but the difference was statistically not significant (Table-1 & 2).

Table 1: Effect of Test drug on biochemical parameters of liver functions in CCl₄ induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S.ALT (Units/ml) (Mean ± SE)</th>
<th>S.AST (Units/ml) (Mean ± SE)</th>
<th>Serum Bilirubin (mg/dl) (Mean ± SE)</th>
<th>S.Alk.Phosphatase (KAU/dl) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Control</td>
<td>34.70 ± 4.49</td>
<td>14 ± 4.23</td>
<td>1.33 ± 0.24</td>
<td>5.40 ± 0.15</td>
</tr>
<tr>
<td>CCl₄ Control</td>
<td>87.30 ± 13.27</td>
<td>134.30 ± 15.86 c d y z</td>
<td>3.76 ± 0.33</td>
<td>50.87 ± 2.42</td>
</tr>
<tr>
<td>Standard (Silymarin)</td>
<td>46.70 ± 1.43 x</td>
<td>62 ± 6.24 x y c d</td>
<td>1.94 ± 0.24 x d</td>
<td>23.76 ± 3.33 x y c</td>
</tr>
<tr>
<td>Majeeth (350 mg/kg)</td>
<td>57.70 ± 3.32 x y z</td>
<td>96.00 ± 5.8 y x z</td>
<td>2.41 ± 0.44 y x z</td>
<td>33.60 ± 3.09 y x z</td>
</tr>
<tr>
<td>Majeeth (450 mg/kg)</td>
<td>53.20 ± 1.91 y x z</td>
<td>86.30 ± 6.18 y x z</td>
<td>2.10 ± 0.26 x z</td>
<td>27.98 ± 0.66 y x z</td>
</tr>
</tbody>
</table>

n = 6  x = Against CCl₄,  y = Against Plain Control,  1 = P< 0.05,  2 = P< 0.01,  z = Against Standard  c = Against Majeeth (350 mg/kg)  3 = NS (Not Significant)  d = Against Majeeth (450 mg/kg)
**Table – 2**

Effect of Test drug on metabolic parameters of liver functions in CCl₄ induced hepatic damage in rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Protein (gm/100 ml) (Mean ± SE)</th>
<th>Serum Urea (mg/dl) (Mean ± SE)</th>
<th>Serum Cholesterol (mg/dl) (Mean ± SE)</th>
<th>Wet liver wt (Liver wt / 100 g body wt) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Control (10 mg / kg)</td>
<td>6.72 ± 0.16</td>
<td>47.72 ± 1.22</td>
<td>101.75 ± 5.96</td>
<td>2.83 ± 0.28</td>
</tr>
<tr>
<td>CCl₄ Control (2 ml/kg of CCl₄)</td>
<td>5.16 ± 0.24</td>
<td>70.55 ± 3.99</td>
<td>149.55 ± 8.92</td>
<td>3.83 ± 0.31</td>
</tr>
<tr>
<td>Standard (Silymarin) (100 mg / kg)</td>
<td>6.18 ± 0.26</td>
<td>54.50 ± 3.05</td>
<td>104.70 ± 5.23</td>
<td>3.14 ± 0.06 (NS)</td>
</tr>
<tr>
<td>Majeeth (350 mg / kg)</td>
<td>5.24 ± 0.27</td>
<td>65.71 ± 2.71</td>
<td>141.82 ± 4.93</td>
<td>3.82 ± 0.34</td>
</tr>
<tr>
<td>Majeeth (450 mg / kg)</td>
<td>5.61 ± 0.27</td>
<td>59.44 ± 3.69</td>
<td>133.23 ± 8.11</td>
<td>3.70 ± 0.16 (NS)</td>
</tr>
</tbody>
</table>

n=6  x = Against CCl₄  y = Against Plain Control  1 = P < 0.05  
z = Against Standard  c = Against Majeeth (350 mg / kg)  2 = P < 0.01  
d = Against Majeeth (450 mg / kg)  3 = NS (Not Significant)

**Effect of Majeeth on Lipid Peroxidation**

The Malondialdehyde (MDA) concentration was found to be 3.21 ± 0.25, in the Plain control Gp (I), while it was significantly elevated to 10.44 ± 1.66 in Gp (II) nmole / mg of protein. The standard Gp (III) and Gp (V) showed significant decrease while the decrease in Gp (IV) was not significant statistically (Table-3).

**Table – 3**

Effect of Test drug in Lipid Peroxidation in CCl₄ induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid Peroxidation (n mole of MDA / mg of protein) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Control (10 ml /kg)</td>
<td>3.215 ± 0.25</td>
</tr>
<tr>
<td>CCl₄ Control (2 ml / kg of CCl₄)</td>
<td>10.44 ± 1.66 y³</td>
</tr>
<tr>
<td>Standard (Silymarin) (100 mg / kg)</td>
<td>4.53 ± 2.18 x²</td>
</tr>
<tr>
<td>Majeeth (350 mg / kg)</td>
<td>7.15 ± 0.57 NS</td>
</tr>
<tr>
<td>Majeeth (450 mg / kg)</td>
<td>5.94 ± 1.00 x¹</td>
</tr>
</tbody>
</table>

n = 6  x = Against CCl₄  y = Against Plain Control  1 = P < 0.05  
z = Against Standard  c = Against Majeeth (350 mg / kg)  2 = P < 0.01  
d = Against Majeeth (450 mg / kg)  3 = NS (Not Significant)

**Histological Studies**

In the histological study the Plain Control Gp showed well maintained liver architecture with no evidence of fatty change, necrosis or inflammation. The animals treated with CCl₄, showed massive disruption of hepatic architecture, fatty degeneration (Zone - 3 and Zone- 2), ballooning degeneration, extensive centrilobular, acidophilic necrosis (Zone - 3,2 and 1) extending to capsular area with area of confluent submassive necrosis, mild to moderate portal inflammation, markedly dilated portal vessels, areas of focal haemorrhage, heavy inflammatory cell infiltrate in necrotic zone and no evidence of hepatocyte regeneration.

The animals treated with silymarin, showed almost normal histology with well maintained liver architecture, no fatty degeneration, mild focal haemorrhagic necrosis around central vein, (Zone-3), mild inflammation (Zone - 3), and portal inflammation and evidence of regeneration (indicated by the presence of bi-nucleated cells).
The animals treated with lower dose of *Majeeth* (350 mg/kg), showed well preserved liver architecture with fatty change in Zone-3 and Zone-1, centrilobular acidophilic necrosis in Zone-3 (around central vein), mild portal inflammation, marked dilation of portal vessels with evidence of hepatocytic regeneration. The animals treated with higher dose of *Majeeth* (450 mg/kg) showed well maintained liver architecture with fatty change in Zone-3, mild centrilobular acidophilic necrosis, with evidence of regenerating hepatocytes (Figures 1–5).

**Fig. 1** Photomicrograph of Plain Control group

Section of liver showing well maintained liver architecture with no evidence of necrosis or inflammation (H & E stain, low power)

**Fig. 2** Photomicrographs of CCl₄ treated group

Section of liver showing fatty degeneration in zone-3 and zone 2, extensive centrilobular acidophilic necrosis, marked dilatation congestion of portal vessels (H & E stain, low power)

**Fig. 3** Photomicrographs of Silymarin treated group in CCl₄ induced damage

Section of liver showing normal liver architecture with central vein and mild portal inflammation (H & E stain, high power)

**Fig. 4** Photomicrograph of Majith (350 mg/kg) treated group in CCl₄ induced damage

Section of liver showing centrilobular acidophilic necrosis in zone-3 with fatty change in zone-3 and 1, mild portal inflammation and evidence of hepatocytic regeneration (H & E stain, low power)

**Fig. 5** Photomicrographs of Majith (450 mg/kg) treated group in CCl₄ induced damage

Section of liver showing mild centrilobular acidophilic necrosis with early fatty change in zone-3 and evidence of regeneration (H & E stain, high power)
Discussion
The present study showed that the hydroalcoholic extract of the Unani liver tonic drug Majeeth (R. cardifolia) on the whole produces significant improvement in the biochemical markers of liver function as well as causing significant decrease in lipid peroxidation. It also produces striking improvement in histological studies. Thus, it is scientifically shown to possess Hepato-protective and Anti-oxidant Activity. The study shows that on the whole there is no significant difference in the effects of the test drug and the standard agent Silymarin. Thus, Majeeth is shown to possess good efficacy as hepatoprotective agent equivalent to the efficacy of the standard hepatoprotective agent Silymarin.

The mechanism of producing hepatic damage by CCl₄ depends on reductive dehalogenation of CCl₄ catalyzed by cytochrome P₄₅₀ in the liver cell endoplasmic reticulum leading to generation of unstable complex of CCl₄ radical. This trichloromethyl radical reacts rapidly with O₂ to yield trichloromethyl peroxy radical (Paker et al., 1978). These free radicals attack microsomal lipids leading to its peroxidation (Rao et al., 2003). Protective agent exert their action against CCl₄ induced damage by impairment of CCl₄ mediated Lipid Peroxidation either through decreased production of free radical derivatives or due to the Anti-oxidant activity of protective agent itself (Ahmed et al., 1998). Majeeth exerted its liver protective effect probably by inhibiting Lipid Peroxidation mediated by CCl₄ due to its Anti-oxidant activity. This is in consonance with an earlier report of Rao et al., (2005) who had reported the significant hepatoprotective effect of Rubiadin (a glycoside isolated from <i>Rubia cordifolia</i> Linn.) against CCl₄ induced hepatic damage in rats. He also reported the significant prevention of the elevation of hepatic MDA formation in CCl₄ intoxicated rats. Thus, our findings of crude drug extract also cohere with the finding as reported earlier on the isolated active principle. Test drug also showed hepatocytic regeneration. So, they may be effective in the disorders where liver degeneration is extensive such as chronic hepatocytes and cirrhosis. The hepatoprotective effect of Majeeth is suggested to be, at least partially, due to their anti-oxidant activity.

The present study provides scientific support and validation to the Unani claim regarding the hepatoprotective effect of Majeeth.

Acknowledgement
The authors are thankful to the department of Ilmul Advia of Aligarh Muslim University, Aligarh, for providing all the facilities to carry out the study.

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The study of Kabab chini (Piper cubeba) for nephroprotective effect in cisplatin induced nephrotoxicity

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Abstract

In the present study Kabab chini (Piper cubeba) was studied for nephroprotective effect against Cisplatin induced nephrotoxicity in Wistar rats. The effect of the test drug was studied in Cisplatin (5 mg /kg / i.p.) induced nephrotoxicity and both protective as well as curative effects were evaluated in groups of albino rats. The powder of the test drug was administered in the dose of 820 mg/kg and 1230 mg/kg orally in the pre and post treated models. The nephroprotective effect was assessed on the basis of biochemical estimation of serum urea and creatinine levels and histopathological examination of the kidney. The test drug produced significant decrease in serum urea and creatinine and protected the histological structure of the kidney. The study demonstrated that Kabab chini (Piper cubeba) possesses significant protective and curative effect against Cisplatin induced nephrotoxicity.

Key Words: Cisplatin, Nephroprotection, Kabab chini, Piper cubeba, Unani Medicine

Introduction

It is being appreciated that traditional systems of medicine can offer some effective drugs in diverse pathological conditions of kidney and thus can be used to protect the renal function and prevent/slow the progression of renal diseases to Chronic Kidney Disease (CKD) or End Stage Renal Disease (ESRD) (Venkatesan, et al., 2000). A number of drugs from herbal sources have been shown to possess promising nephroprotective and related effects in certain recent studies and researchers are making it a point to concentrate seriously on the development of nephroprotective agents from traditional sources (Priya et al., 1999; Yokozawa, 1995; Panda et al., 1997; Zhen et al., 1992; Lu Wei et al., 1994).

Unani System of Medicine claims to possess a number of drugs that can be used successfully in the treatment of renal diseases. Some of these drugs have been shown to produce interesting pharmacological effects such as diuretic, anti-inflammatory, antioxidant and nephroprotective against known toxicants. These reports although of preliminary nature yet show great potential of Unani Medicine to deliver promising agents that can be used to treat kidney diseases or at least, preserve its function and slow the progression of renal disease.

The renoprotection / nephroprotection by view point of Unani Medicine comprises of the protection of the various faculties (quwa) the kidneys are imbibed with, to maintain their functioning. In case of mild degree of kidney disorder the drugs categorized to be kidney tonics are sufficient enough to deal with the situation to bring normalcy (Ibn Hubal, 2007; Azam Khan, 1987). However, when gross impairment in kidney function or it matrix takes place anyhow,
because of the high toxic effect of a substance or because one of the natural faculties is undermined owing to some local or systemic disease of the body, then the drugs having other pharmacological actions along with the tonic one, are used. Drugs ascribed to possess diuretic, anti-inflammatory, antioxidant, etc. activities along with tonic effect are frequently used with an aim to treat the pathology and invigorate the kidney to bounce back to its normal state to perform its assigned work. Further, in case of progression of kidney diseases some other drugs are included in the regimen along with the drugs mentioned above, to directly ameliorate the compromised condition by promoting the healing of injured tissue, removal of toxins and reducing the pressure of work on kidney by diverting the wastes to some other system or organs of the body from where they are excreted out easily (Ibn Rushd, 1987; Ibn Hubal, 2007; Khan, 1987; Arzani, 2006).

In the present study therefore, an important drug of Unani medicine widely acclaimed to be effective in various urogenital disorders, viz. Kabab chini (KC) was selected to study its nephroprotective effect in view of the various pharmacological actions that it has been described to possess by Unani physician such as Mudirre baul (diuretic), Mufattit wa Mukhrije Hasat (lithotriptic) Dafeye taffun (antiseptic), Muqawwiy e Kulyah (Kidney tonic), Mohafize Kulyah (nephroprotective) etc. (Razi, 1967; Ibn-e-Baitar, 2003; Ghani 1921; Dymock, 2005). It is reported to possess diuretic, anti-inflammatory, tonic, antileishmanial, antimicrobial, antioxidant, antileukemic and antimicrobial activities (Sumathykutty et al., 1999; Choi et al., 2003; Yam J et al., 2008; Hardik et al., 2007; Silva et al., 2007; Aqil et al., 2006; Karthikeyan et al., 2003; Taneja et al., 1991).

Therefore, KC was hypothesized to produce nephroprotective effect in acute renal impairment and studied for nephroprotective effect against chemically-induced nephrotoxicity by Cisplatin.

Materials and Methods

Test Drug

The dried berries of KC (Piper cubeba) were provided by the pharmacy of National Institute of Unani Medicine, Bangalore. Dr. Siddamallayya N, of Regional Research Institute (Ay.) Bangalore, authenticated the test sample vide Ref. No. RRI / BNG / SMP / Drug Authentication / 2008-09/356. The berries were powdered finely with the help of an electric grinder. The dose of the test drug for the rats was calculated by multiplying the human therapeutic dose of 7 gm as described in Unani literature, by the conversion factor of 7 (Freirich et al., 1968). The dose thus calculated for experimental study was found to be 820 mg. However, to study the dose dependent effect of the test drug another dose was also employed by increasing the calculated dose by 50 % which was found to be 1230 mg. 5 gm of gum acacia was taken into 100 ml of distilled water; it was shaken vigorously for quite some times to make a homogenous suspension. The suspension was divided into two equal parts. One part was mixed with 8.2 gm of KC powder while the other with 12.3 gm of powder. Both the samples were mixed and shaken well to get a suspension having uniformly distributed drug particles. The test samples were prepared a fresh every time, before the administration to the animals.

Experimental animals

Healthy adult albino rats of Wistar strain weighing 150 - 200 gm were used in the study. Animals were maintained on standard diet and water ad libitum and housed in clean polypropylene cages at room temperature (25 ± 2°C) with a 12h light: 12h dark cycle.

Cisplatin induced nephrotoxicity test

This test was carried out by the method of Annie et al., (2004). Male Wistar rats weighing 150-200 gm were divided into 6 groups consisting of 10 animals each and treated as follows:

Plain control: Normal saline in the dose of 3 ml orally for 14 days.

Negative control: Cisplatin 5 mg/kg (Babu, et al., 1995) intraperitoneally on 1st, 7th and 14th day.

Pre-treated Test group - A: Test drug in the dose of 820 mg/kg orally once daily for 14 days. On 8th day 1st dose of Cisplatin (5 mg/kg) was given intraperitoneally, while 2nd and 3rd dose of Cisplatin was administered on 15th and 22nd day of the treatment.

Post-treated Test group - A: Cisplatin injection 5 mg/kg intraperitoneally on day 1, 7, and 14, and from 8th day onwards the test drug was also given in the dose of 820 mg/kg once daily, orally for next 14 days.

Pre-treated Test group - B: Test drug in a higher dose of 1230 mg/kg orally, once daily for 14 days,
while the 1st dose of Cisplatin injection was given on 8th day in the dose of 5 mg/kg body weight. The 2nd and 3rd dose of cisplatin was given on 15th and 22nd day, respectively.

**Post-treated Test group - B:** Cisplatin injection in the dose of 5 mg/kg intraperitoneally on 1st, 7th and 14th day to induce toxicity, and from 8th day onwards KC was given to all the animals orally daily in the dose of 1230 mg/kg for next 14 days (Table 1).

At the end of the experiment the rats were sacrificed by being sequentially anesthetized with inhaled diethyl ether for about 30 – 40 seconds. 4 - 5 ml of whole blood was collected by cardiac puncture. The blood samples were allowed for complete clotting for about 3 – 5 hours before they were centrifuged at 2000 revolution per minute for 15 minutes. This was aimed at separating the sera from clotted blood cells. The sera were carefully separated into new, well labeled bottles at room temperature (23 - 26 ºC). The sera were assayed for serum urea, and creatinine, using Diagnostic kits.

**Histopathological study**

After sacrificing the animals their postmortem examination was performed. The kidneys were carefully dissected out en bloc for histopathological examination. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formo-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 µm thick sections stained with hematoxylin-eosin and observed under a photomicroscope.

**Statistical analysis**

The results were analysed by ANOVA one way with Dunnett’s multiple pair comparison test. The difference of mean was considered significant at p value of 0.05 or less.

**Result**

**Serum urea**

Serum urea in plain control group was found to be 31.2 ± 2.311 mg/dl. It increased to 122 ± 26.973 mg/dl in negative control (Cisplatin) group. The pre-treated group A, in which animals were treated with KC along with Cisplatin (5 mg/kg intraperitoneally), serum urea was found to be 46 ± 4.775 mg/dl showing a significant decrease as compared to the negative control group (p< 0.01). Similarly, the post-treated group A, in which animals were treated with Cisplatin (5 mg/kg) along with KC in the dose of 820 mg/kg serum urea was found to be decreased significantly to 57 ± 6.026 mg/dl (p< 0.01) when compared with negative control group. In the Pre-treated group B where the animals were treated with KC in the dose of 1230 mg/kg before Cisplatin administration, significant decrease in urea level was found, as it lowered down to 43 ± 5.586 mg/dl (p<0.01). The Post-treated group B which was given Cisplatin before the administration of the test drug, the level of mean serum urea decreased significantly to 51 ±10.657 mg/dl (p<0.01). Data is summarized in Table 1.

**Serum creatinine**

Serum creatinine in plain control group was estimated to be 0.6481 ± 0.07 mg/dl. It increased to 3.921 ± 0.3884 mg/dl in negative control group. In pre-treated group A, serum creatinine level was found to be 1.21 ± 0.1540 mg/dl (p< 0.01), while in post-treated group A it decreased significantly to 1.306 ± 0.1826 mg/dl (p< 0.01). In the Pre-treated group B and Post-treated group B the level of serum creatinine was found to decrease to 1.18 ± 0.1155 mg/dl and 1.042±0.1906 mg/dl, respectively (p<0.01). Data is summarized in Table 1.

**Histopathological examination**

In Negative control group, kidney structure was distorted by severe necrosis of tubules. The blood vessels were engorged and the stroma was edematous with separation of tubules. The tissue was moderately infiltrated by inflammatory cells composed of small lymphocytes. Many of the glomeruli showed diffuse eosinophilic sclerosis. Features suggested severe tubular necrosis (Slide No. 1).
In Pre-treated group A, histopathology revealed that the stroma had mild degree of edema. There was mild degree of glomerular congestion. The tissue was sparsely infiltrated by inflammatory cells. Features suggested moderate tubular damage (Slide No. 2).

Slide No. 2 (Pre-treated A) showing mild tubular damage

In Post-treated group A, histopathology of the kidney showed that the blood vessels were engorged and the stroma showed moderate degree of edema. The tissue was moderately infiltrated by inflammatory cells composed of small lymphocytes. Some of the glomeruli showed moderate degree of congestion. Histological features suggested moderate tubular necrosis (Slide No. 3).

Slide No. 3 (Post-treated A) showing moderate tubular necrosis

In Pre-treated group A, histopathology revealed that there was a mild interstitial edema. No peritubular congestion was seen. The tissue was free from inflammatory cells. The glomeruli showed no significant pathology. Features suggested minimal tubular changes (Slide No. 4).

Slide No. 4 (Pre-treated B) showing minimal tubular changes

In Post-treated group B, histopathology section showed mild interstitial edema, mild degree of glomerular congestion. Few congested blood vessels were seen. The tissue was sparsely infiltrated by chronic inflammatory cells. Features suggested mild tubular damage. Cut sections of the kidney are shown in the form of various slide plates (Slide No. 5).

Slide No. 5 (Post-treated) showing mild to moderate tubular necrosis Histopathological features as seen in the kidney of Cisplatin treated animals

Discussion

The test drug produced a significant effect against the cisplatin induced nephrotoxicity as was evidenced by the fact that the biochemical markers and histological features of kidney structure that were found deviated under the influence of cisplatin, showed significant movement towards normalcy after the administration of KC. It was found effective in both pre and post treated groups suggesting its protective and curative potential.
Cisplatin was observed to induce acute renal injury on expected lines as the serum urea and serum creatinine levels were found elevated and histopathological features were deranged suggesting massive functional and morphological impairment. The two markers in plain control group treated with normal saline were not found altered and histopathological study also showed maintained structural integrity of kidney tissue with no histological abnormality.

Treatment with KC prior to cisplatin administration brought the two serum markers to a significantly low level (p< 0.01) as compared to negative control group. In this group the histopathological features too showed marked improvement in kidney matrix as only mild degree of edema and peritubular and glomerular congestion were observed which suggested mild tubular damage. Similarly, the post-treated group A in which Cisplatin was administered along with the test drug, demonstrated a decrease in serum urea and creatinine level from 122 ± 26.973mg/dl to 57 ± 6.026 mg/dl and from 3.921 ± 0.3884 to 1.306 ± 0.1826 mg/dl, respectively showing a significant decrease (p< 0.01). The histopathological examination showed tremendous recovery as the features suggested only moderate degree of tubular necrosis.

Animals in pre-treated group B given KC prior to Cisplatin administration demonstrated good recovery as the urea and creatinine levels were found to be decreased significantly (p<0.01) and histological studies demonstrated mild tubular changes. While in Post-treated group B where the animals were treated with Cisplatin before the administration of test drug, the level of urea and creatinine decreased, significantly, (p<0.01) and the section of kidney studied, showed only mild degree of tubular damage. Thus, the test drug was found to exhibit nephroprotective activity in both pre and post-treatment groups but the pre-treatment device was more effective.

Cisplatin is known to produce toxic effect through oxidative mechanism (Arany, 2003) hence a drug that ameliorates the damage induced by reactive oxygen species will be considered to possess antioxidant property. It can be inferred therefore that the test drug produced nephroprotective effect most probably by scavenging the free radicals. This finding is in conformity with the other reports demonstrating anti oxidant property possessed by the test drug (Aqil, et al., 2006; Karthikeyan, et al., 2003). However it has also been reported to disturb the DNA synthesis therefore the toxicity may be assumed to develop through other mechanisms also (Montine and Borch, 1990). Cisplatin produces toxicity mainly in medullary region which is unarguably one of the difficult regions to be repaired (Pabla, 2008) but the test drug produced remarkable protective and curative effect which is the testimony of its striking nephroprotective effect. Further, in-group comparison between pre treated and post treated findings; it was observed that the effect of the test drug was more marked in pre treated groups as compared to the post treated groups. It clearly demonstrated that the protective effect of the test drug is more pronounced than its curative effect.

The protective effect demonstrated by the test drug fortified the Unani concept of tonic drugs that help to maintain the faculties (quwa) to perform the normal function of the organ even in deviated condition of structure and the function by strengthening the affected organ to fight against the toxicant (Ibn Hubal, 2007; Khan, 1987). Since the test drug possesses different pharmacological effect that addressed almost all aspect of toxic effect on kidney tissue to bring the chemically challenged kidney to a state of functional and structural normalcy therefore it can be said that the collective response actually translated into nephroprotection. Some of the compounds contained in Cubeb such as cubebin, hinokinin, yatein, dihydrocubebin etc have been shown to possess anti inflammatory, analgesic, antioxidant and anti cancer activities etc (Matsushima et al., 1998; Da Silva et al., 2005; Tepy et al., 2005; Desouza et al., 2005; Sumathykutty et al., 1999; Susan, 1989). This further corroborates the proposition that the test drug produced effect through diverse mechanisms complementing each other and the entirety of the effect is actually responsible for nephroprotection. It has been already discussed that for nephroprotection, drugs possessing effect beyond the tonic one are used to treat kidney diseases when massive functional and structural derangement takes place (Ibn Hubal, 2007; Khan, 1987).

In view of the findings of the study and discussion it can be concluded that the test drug possesses significant nephroprotective effect against Cisplatin induced nephrotoxicity. Thus, the study validated the claim of Unani System of Medicine that KC is a drug that can be used as nephroprotective agent in renal diseases.
Table 1
Effect of Kabab chini (Piper cubeba) on serum urea and creatinine in cisplatin induced kidney damage

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Urea (mg/dl) (Mean ± SEM)</th>
<th>Serum Creatinine (mg/dl) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Plain Control</td>
<td>31.2 ± 2.311*</td>
<td>0.6481 ± 0.07*</td>
</tr>
<tr>
<td>Group II Negative Control</td>
<td>122 ± 26.973*</td>
<td>3.921 ± 0.3884*</td>
</tr>
<tr>
<td>(Cisplatin 5mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III Pre-treated A</td>
<td>46 ± 4.775*</td>
<td>1.21 ± 0.1540*</td>
</tr>
<tr>
<td>(Test drug 810 mg /kg + Cisplatin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV Post-treated A</td>
<td>57 ± 6.026*</td>
<td>1.306 ± 0.1826*</td>
</tr>
<tr>
<td>(Cisplatin+Test drug 810 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V Pre-treated B</td>
<td>43 ± 5.86*</td>
<td>1.18 ± 0.1155*</td>
</tr>
<tr>
<td>(Test drug 1220 mg /kg + Cisplatin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group VI Post-treated B</td>
<td>51 ± 10.65*</td>
<td>1.042 ± 0.1906 *</td>
</tr>
<tr>
<td>(Cisplatin+Test drug 1220 mg/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.01

Table 2
Effect of Kabab chini (Piper cubeba) in Cisplatin induced kidney damage on kidney histology

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

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

References
A physicochemical study of Qurs-e-rewand

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Abstract
A number of drugs of Unani medicine particularly compound formulations have still not been scientifically evaluated for their described effects as well as for physicochemical standards. Qurs-e-Rewand is one such compound preparation described to be effective in liver diseases, but it has not been standardized so far on physico-chemical parameters. Therefore, in the present study it has been standardized according to certain physico-chemical and analytical parameters i.e. extractive values in different solvents (both successive and non-successive), alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, pH values, qualitative analysis for various chemical constituents, thin layer chromatography (TLC), weight and diameter of the tablets, disintegration time, friability and bulk density. The standardization thus carried out provides the analytical characteristics which may prove to be useful in fixing the physicochemical standard for this and other Unani tablets.

Key Words: Qurs-e-Rewand, Physicochemical standardization, Rheum emodi

Introduction
With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the safety and the quality of medicinal plant materials and finished products have become a major concern for health authorities, pharmaceutical industries and the public.

The efficacy of a drug mainly depends upon its physical and chemical properties therefore, the determination of physicochemical characters for the authenticity of a drug is necessary. Physicochemical study is also important, because it helps in characterization of constituents or groups of constituents that frequently lead to establish the structure-activity relationship and the likely mechanism of action of the drug. Phytochemical constituents present in the drug vary, not only from plant to plant but also among different samples of the same species, depending upon various atmospheric factors and storage and drying conditions. A little deviation from the normal in terms of quality or quantity of the constituents may alter the effect of the drug. Apart from the degradation in the quality of the drugs that occurs due to above conditions, adulteration also contributes to variability. Thus, keeping in view the above considerations, physicochemical studies on Qurs-Rewand were carried out.

The physicochemical studies included the determination of extractive values of Qurs-e-Rewand in different solvents (both successive and non-successive), alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, pH values, qualitative analysis for various chemical constituents, thin layer chromatography (TLC), colour of the tablet, appearance, texture, taste, weight and diameter of the tablets, disintegration time, friability and bulk density.

Material and Method
Ingredients of Qurs-e-Rewand

Rheum emodi Wall. (Revand chini) 17.5 gm
Rubi cordifolia Linn. (Majith) 10.5 gm
Collection of raw materials and preparation of Qurs

The crude drugs were procured from the local market (Bara Dwari, Aligarh). After the confirmation of purity and identity of the ingredients by the pharmacognosy section of the Department of Ilmul Advia, A.K. Tibbiya College, AMU, Aligarh, all the ingredients of Qurs-e-Rewand were powdered in an electric grinder and tablets were prepared according to the method described in the Pharmacopoeia of India (Anonymous, 1970) by compressing machine in Dawakhana Tibbiya College, AMU, Aligarh.

All the ingredients were prepared in a dry granular form passing through the compressing machine. The drug or a mixture of drugs in powder form was mixed and a suitable inert substance viz. Acacia arabica (Gum acacia) was used as adhesive or binding agent. The material in the requisite degree of fineness, mixed and damped with a moistening agent (Distilled Water). The moistened material was made into granules by compressing machine. The granules were dried in a current of air, at a suitable temp. not exceeding 60°C and again passed through a sieve, at last tablets of 500 mg was made by dye machine.(Anonymous, 1968; Anonymous, 1970).

The quality of the tablet was determined according to following parameters:

(I) Weight variation of Qurs-e-Rewand

Ten tablets were taken randomly and weighed individually to confirm the weight variation and average weight of tablet was expressed in mg weight ± 7.5% variation was considered acceptable in case of pills/tablets (Dandagi et al., 2006).

(II) Diameter of Qurs-e-Rewand

Three tablets were selected randomly and measured individually by using Vernier Calliper to make sure, the uniformity of diameter and denoted in mm (Dandagi et al., 2006).

(III) Determination of disintegration time

The rate of disintegration was taken by using a Disintegration Testing Machine. The two disintegration media were selected for determination of disintegration. Simulated gastric fluid VSP, without enzymes and pure water, simulated gastric fluid (pH about 1.2) was prepared by dissolving 1 gm of NaCl in 500 mL of deionised water, adding 7 mL of concentrated HCl and adjusting the volume to 1000 ml with water. A USP disintegration apparatus and 900 ml of either stimulated gastric fluid or water at 37°C was used in this investigation (Anonymous, 1989).

(IV) Friability test

Friability test of the tablets was carried out by Friability Tests Apparatus (Friabilator) of Macro Scientific Works, Delhi. This device subjects the tablets to the combined effect of abrasions and shock in a plastic chamber revolving at 25 rpm and dropping the tablets at a height of 6 inches in each revolution. Pre-weighed sample of tablets was placed in the 100 revolutions. Tablets were de-dusted using a soft muslin cloth and re-weighed the friability (f) was calculated by the following formula:

\[ f = \left(1 - \frac{W}{W_o}\right) \times 100 \]

Where, W is the weight of the tablets before the test and \( W_o \) is the weight of the tablet after the test (Vijay & Mishra, 2006).

(V) Determination of extractive values

The extractive values of ingredients of Qurs in different organic solvents viz. petroleum ether, diethyl ether, chloroform, benzene, alcohol and water were carried out by percolation in Soxhlet apparatus. The heat was applied for 6 hours on a water bath for each solvent except water, which was heated directly on a heating mantle. Powdered drug (of ingredients not tablet) (10 gm) was taken and subjected to successive and non-successive extraction with each solvent (200 ml). The extracts were filtered and after evaporation of the solvents; the extractive values were determined with reference to the weight of drug. The procedures was repeated 3 times and the mean value for each extract was calculated (Anonymous, 1968; Anonymous, 1987).

(VI) Determination of water and alcohol soluble contents

Five gm of drug was taken into 100 ml of distilled water in a glass stoppered conical flask. The mixture was carefully shaken for 6 hours frequently, and then allowed standing for 18 hours. Shaken well and filtered rapidly through
dry filter to make sure that no soluble matter left behind. The filtrate was transferred to a previously weighed and tarred flat-bottomed dish and water was evaporated to dryness on a water bath. The residue was dried at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay. The percentage of water soluble matter was calculated with reference to the amount of drug taken. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 1968).

(VII) Determination of moisture contents
The toluene distillation method was used for the determination of moisture contents. 10 gm of powdered drug was taken in the flask of the apparatus and 75 ml of distilled toluene was added to it. Distillation was carried out for 6 hours and the process was repeated for five times. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture calculated with reference to the weight of the air dried drug taken (Jenkins et al., 1967).

(VIII) Determination of ash values: Total ash
2 gm of drug was incinerated in a silica crucible of a constant weight at a temperature not exceeding than 450°C in a muffle furnace until free from carbon, cooled and weighed and the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible + ash. The percentage of total ash was calculated with reference to the weight of drug taken (Anonymous, 1968).

Water Soluble ash
The obtained ash was boiled with 25ml of distilled water for 5mins. The insoluble matter was collected in an ashless filter paper, (Whatman No. 42) washed with hot water and ignited in crucible, at a temp not more than 450°C, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug taken. (Anonymous, 1968).

Acid insoluble ash
The total ash was boiled with 25 mL of 10% hydrochloric acid for 5 mins. The insoluble matter was collected on ashless filter paper (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not exceeding more than 450°C and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken (Anonymous, 1968).

(ix) Loss of weight on drying
10 gm of drug was taken, spread uniformly and thin layered in a shallow Petri dish. It was heated at a regulated temperature of 105°C, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were constant. The percent loss in weight was calculated with respect to initial weight (Anonymous, 1987).

(x) Determination of bulk density
A clean, dry and previously weighed bottle (25 mL) was taken, it was filled with known quantity of distilled water and weighed, marked the water level and got the bottle emptied, rinsed with acetone and dried. The bottle was filled with the drug, allowed it to settle overnight and again adjusted the level up to the mark and weighed. The bulk density was determined from the weight of water and drug (Anonymous, 1991).

(XI) Determination of pH
Determination of pH was carried out by a synchronic digital pH meter 335 equipped with a combined electrode.

The pH value of 1% solution: 1gm of accurately weighed drug was dissolved in accurately measured 100 ml of distilled water, filtered and pH was measured with a pH meter.

The pH value of 10% solution 10gm of accurately weighed drug was dissolved in accurately measured 100ml of distilled water, filtered and pH measured with a pH meter (Anonymous, 1987).

(XII) Chromatographic Studies
Thin layer chromatography (TLC)
Thin layer chromatography was carried out on T.L.C. aluminium plates, pre-coated with silica gel 6 of 254 (layer thickness 0.25mm) for different extracts of Q.R. viz. pet. ether, diethyl ether, chloroform, benzene, alcohol etc, in various mobile phases. Later, sprayed by different spraying reagents. The \( R_f \) values of the spots were calculated by the following formula:

\[
R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}
\]

(Anonymous, 1968)

Qualitative analysis of chemical constituents
The qualitative analysis of different chemical constituents, present in the Qurs-e-Rewand was
carried out according to the scheme proposal by Bhattacharjee and Das (1969).

The powder of *Qurs-e-Rewand* was extracted with petroleum ether and the petroleum ether extract was tested for:

- Free phenols, alkaloids and sterols/terpenes.
- Fatty acids and again for alkaloids, phenols and sterols/terpenes for confirmation. The defatted marc was divided into two portions. One portion was extracted with hot water and other with ethanol (70%). The aqueous and ethanolic extracts were tested for alkaloids, flavonoids, saponins, sugars and tannins.

Aqueous extract was extracted further with ether and ether soluble portion and again it was tested for alkaloids, sterols where as, water soluble portion was tested for glycosides. The water soluble portion was again hydrolyzed with 05% hydrochloric acid and extracted with chloroform. The aglycone portion was tested for insoluble hydrochloride of alkaloid. Chloroform soluble portion was tested for alkaloids and sterols/terpenes whereas; water soluble fraction was tested for alkaloids. One part of this water soluble portion was basified with any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part was again tested for alkaloids.

**Tests for alkaloids**

(a) A drop of Dragendorff’s Reagent in the extract was added. The brown precipitate showed the presence of alkaloids.

(b) In a test solution, a drop of Mayer’s Reagent was added. The white precipitation was observed, which indicated the presence of alkaloids (Afaq et al., 1994)

**Tests for amino acids**

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes it gives a blue to red-violet colour indicated the presence of amino acids (Brewster and Mc Ewen, 1971).

**Tests for flavonoids**

(a) In the ethanolic extract, the concentrated hydrochloric acid was added. Red colour showed the presence of flavonoids.

(b) Magnesium ribbon was added to the ethanolic extract of the material follow drop wise addition of concentrated HCl. Colour changed from orange to red is a confirmatory test for flavonoids (Fransworth, 1966).

**Tests for glycosides**

The drug was first extracted with petroleum ether for removal of waxy substances followed by extraction to obtain glycosides. The extract was filtered and sugar was removed by precipitation with magnesium oxide or barium oxide. To confirm the complete removal of sugars the test for sugars was performed when the sugars were found absent, the ethanolic extract was supposed to contain the glycosides which were detected by the following methods:

The hydrolysis of the solution was done with concentrated sulphuric acid and after the hydrolysis, the presence of the sugar was determined with the help of Fehling’s solution.

The Molisch’s test was carried out for sugars using α-naphthol and concentrated sulphuric acid (Paech and Tracey, 1955).

**Tests for phenols**

The ferric chloride was mixed in the petroleum extract. A purple or red colour indicated the presence of phenol (Brewster and Mc Even, 1971).

**Tests for proteins**

(1) Biurette’s reaction

In the hot test solution 0.1 mL concentrated sodium hydroxide solution is to be added, followed by one drop of copper sulphate solution. A violet or red colour indicates the presence of proteins.

(2) Millon’s reaction

For the test solution, Millon’s reagent was mixed and white colour precipitate showed the presence of proteins.

(3) Xanthoproteinc reaction

Concentrated nitric acid was added in the solution. A yellow precipitate appears which dissolved in the strong ammonia solution and gave yellow colour that means proteins are present in the drug (Afaq et al., 1994).

**Test for resins**

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that was rapidly changed to violet, it indicated the presence of the resins (Afaq et al., 1994).
Test for sterols/terpenes

(i) Hosse’s reaction:
In the test solution of chloroform 2ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicated the presence of the sterols/terpenes.

(ii) Liebermann’s Burchard reaction
0.2 mL of acetic anhydride solution was mixed in the test solution (0.1 mL), followed by 0.2 mL concentrated sulphuric acid was added. A change in colour from red to blue showed the presence of sterols/terpenes.

(iii) Moleschott’s reaction
0.1 gm of the drug was mixed with 0.5 mL of distilled was, filtered and 0.5 mL concentrated sulphuric acid was poured from the side of the test tube and the colours was noted. A change in colour from red to violet showed the presence of sterols/terpenes (Afaq et al., 1994).

Tests for sugars

(a) Reducing sugars
Fehling’s solution test
In the aqueous extract, a mixture of equal parts of Fehling’s solution A and B previously mixed was added and heated. A brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

Molisch’s tests
In a aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A purple colour ring at the junction of the two solutions indicated the presence of the reducing sugar.

Non reducing sugars
After removing the free sugars by fermenting the test solution by baker’s yeast, the solution was hydrolysed with hydrochloric acid and the presence of non-reducing sugars (now reduced) was tested either by Fehling’s solution or α-napthol (Afaq et al., 1994).

Tests of tannins
Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, showed the presence of tannins (Afaq et al., 1994).

Observations and Result
The Physicochemical and phytochemical studies were carried out based on different pharmacopoeial parameters. The data is based on multiple observations. The results are depicted in Table 1 to 14 and Fig. 1.

| Table – 1 Organoleptic Description of Qurs-e-Reaward |
| Color | Reddish brown |
| Appearance | Tablet |
| Texture | Hard |
| Taste | Bitter |

<p>| Table – 2 Weight Variation of Qurs-e-Reaward |</p>
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>567</td>
</tr>
<tr>
<td>2</td>
<td>560</td>
</tr>
<tr>
<td>3</td>
<td>568</td>
</tr>
<tr>
<td>4</td>
<td>565</td>
</tr>
<tr>
<td>5</td>
<td>570</td>
</tr>
<tr>
<td>6</td>
<td>562</td>
</tr>
<tr>
<td>7</td>
<td>568</td>
</tr>
<tr>
<td>8</td>
<td>558</td>
</tr>
<tr>
<td>9</td>
<td>569</td>
</tr>
<tr>
<td>10</td>
<td>561</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>564.8 ± 1.34</td>
</tr>
</tbody>
</table>

<p>| Table – 3 Thickness and Diameter of Qurs-e-Reaward |</p>
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.40</td>
<td>13.25</td>
</tr>
<tr>
<td>2</td>
<td>5.45</td>
<td>13.35</td>
</tr>
<tr>
<td>3</td>
<td>5.50</td>
<td>13.30</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>5.45 ± 0.03</td>
<td>13.30 ± 0.03</td>
</tr>
</tbody>
</table>
### Table – 4
Disintegration time of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Disintegration time in the water (minutes)</th>
<th>Disintegration time in the simulated gastric fluid (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.37</td>
<td>2.46</td>
</tr>
<tr>
<td>2</td>
<td>3.52</td>
<td>2.57</td>
</tr>
<tr>
<td>3</td>
<td>3.41</td>
<td>2.51</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3.43 ± 0.04</td>
<td>2.51 ± 0.03</td>
</tr>
</tbody>
</table>

### Table – 5
Friability Test of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1.86</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.98 ± 0.07</td>
</tr>
</tbody>
</table>

### Table – 6
Alcohol and water soluble content of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Alcohol Soluble Content (%)</th>
<th>Water Soluble Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>26.90</td>
<td>33.60</td>
</tr>
<tr>
<td>2.</td>
<td>27.80</td>
<td>32.40</td>
</tr>
<tr>
<td>3.</td>
<td>27.40</td>
<td>33.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>27.36 ± 0.26</td>
<td>33.03 ± 0.35</td>
</tr>
</tbody>
</table>

### Table – 7
pH values of 1% and 10% solution of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>1% solution</th>
<th>10% solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.10</td>
<td>5.30</td>
</tr>
<tr>
<td>2.</td>
<td>5.40</td>
<td>5.40</td>
</tr>
<tr>
<td>3.</td>
<td>4.90</td>
<td>5.10</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>5.13 ± 0.14</td>
<td>5.26 ± 0.08</td>
</tr>
</tbody>
</table>

### Table – 8
Moisture content of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.8</td>
</tr>
<tr>
<td>2.</td>
<td>7.7</td>
</tr>
<tr>
<td>3.</td>
<td>7.5</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>7.66 ± 0.08</td>
</tr>
</tbody>
</table>

### Table – 9
Ash values of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Total ash %</th>
<th>Acid insoluble ash %</th>
<th>Water soluble ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.5</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>2.</td>
<td>5.3</td>
<td>0.9</td>
<td>1.9</td>
</tr>
<tr>
<td>3.</td>
<td>5.7</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>5.5 ± 0.11</td>
<td>1.06 ± 0.08</td>
<td>1.9 ± 0.11</td>
</tr>
</tbody>
</table>
Table – 10
Loss of weight on drying and Bulk Density of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Loss on drying %</th>
<th>Bulk Density (gm/ml) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>9.38</td>
<td>0.6209</td>
</tr>
<tr>
<td>2.</td>
<td>9.21</td>
<td>0.6178</td>
</tr>
<tr>
<td>3.</td>
<td>9.27</td>
<td>0.6215</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>9.28 ± 0.05</td>
<td>0.619 ± 0.01</td>
</tr>
</tbody>
</table>

Table – 11
Successive Extractive Values of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Petroleum ether %</th>
<th>Di-ethyl ether %</th>
<th>Chloroform %</th>
<th>Benzene %</th>
<th>Alcohol %</th>
<th>Water %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.78</td>
<td>3.66</td>
<td>10.24</td>
<td>0.32</td>
<td>15.06</td>
<td>7.92</td>
</tr>
<tr>
<td>2</td>
<td>1.76</td>
<td>3.69</td>
<td>10.27</td>
<td>0.29</td>
<td>15.13</td>
<td>7.81</td>
</tr>
<tr>
<td>3</td>
<td>1.72</td>
<td>3.60</td>
<td>10.18</td>
<td>0.27</td>
<td>15.17</td>
<td>7.4</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.75 ± 0.01</td>
<td>3.65 ± 0.02</td>
<td>10.23 ±0.02</td>
<td>0.29±0.01</td>
<td>15.12±0.03</td>
<td>7.71±0.15</td>
</tr>
</tbody>
</table>

Table – 12
Non-Successive Extractive Values of *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Petroleum ether %</th>
<th>Di-ethyl ether %</th>
<th>Chloroform %</th>
<th>Benzene %</th>
<th>Alcohol %</th>
<th>Water %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.80</td>
<td>4.63</td>
<td>17.39</td>
<td>2.63</td>
<td>35.7</td>
<td>41.4</td>
</tr>
<tr>
<td>2</td>
<td>1.77</td>
<td>4.57</td>
<td>17.42</td>
<td>2.68</td>
<td>36.2</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>1.81</td>
<td>4.65</td>
<td>17.49</td>
<td>3.1</td>
<td>34.8</td>
<td>42.6</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.79±0.01</td>
<td>4.61±0.02</td>
<td>17.43±0.02</td>
<td>2.80±0.14</td>
<td>35.56±0.40</td>
<td>42.1±0.36</td>
</tr>
</tbody>
</table>

Table – 13
Qualitative tests for various chemical constituents in *Qurs-e-Rewand*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Amino acid</td>
<td>+ve</td>
</tr>
<tr>
<td>3.</td>
<td>Protein</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Glycoside</td>
<td>+ve</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoid</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Phenol</td>
<td>+ve</td>
</tr>
<tr>
<td>7.</td>
<td>Resin</td>
<td>+ve</td>
</tr>
<tr>
<td>8.</td>
<td>Sterol/ Terpene</td>
<td>+ve</td>
</tr>
</tbody>
</table>
Table – 14
TLC Profile of *Qurs-e-Rewand* extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent System</th>
<th>Detection / Observations</th>
<th>Number of the spots</th>
<th>( R_f ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ether</td>
<td>Toluene: Ethyl acetate (4:1)</td>
<td>Yellow</td>
<td>4</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orange</td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple</td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light Yellow</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Chloroform : CCl4 (1:1)</td>
<td>Yellow (big spot)</td>
<td>5</td>
<td>0.84</td>
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<td></td>
<td></td>
<td>Violet</td>
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<tr>
<td></td>
<td></td>
<td>Violet</td>
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<td>0.30</td>
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<td></td>
<td></td>
<td>Orange</td>
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<tr>
<td>Benzene</td>
<td>Toluene: Benzene (1:1)</td>
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<td>4</td>
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<td></td>
<td>Red</td>
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<tr>
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<td>Toluene: Ethyl acetate (8:2)</td>
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<td></td>
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**Extract**

- Diethyl ether
- Chloroform
- Benzene
- Alcohol

**Movement of the solvent**

- Diethyl ether: 5.6
- Chloroform: 5.6
- Benzene: 5.2
- Alcohol: 5.4

**Fig 1**

**Fig 2**

**TLC Profiles of *Qurs-e-Revand* in different Extracts**

**Discussion**

A rapid increase, in the global use of herbal medicines over the last few years has led to concerns over the safety and quality of herbal materials and herbal products and physicochemical standardization has been
considered as a pre-requisite in quality control of Unani drugs, both single as well as compound formulations. Since Qurs-e-Reward is an important Unani Compound formulation, it was subjected to physicochemical standardization. For establishing the physicochemical standards of tablets weight variation test was conducted because a good quality tablet should be accurate and uniform in weight. The mean value of weight of Qurs-e-Reward was found to be 564.8 ± 1.34 mg. The diameter of a tablet can vary without any change in its weight. The mean of the diameter and thickness were found to be 13.30 ± 0.03 mm and 5.45 ± 0.03 mm, respectively. After administration, the tablet should disintegrate readily; therefore, the tablets were also subjected for the evaluation of disintegration time. The mean values of disintegration time in the water and in simulated gastric fluid were found to be 3.43 ± 0.04 minutes and 2.51 ± 0.03 minutes, respectively. It is mentioned that plain tablets / pills pass the test if each of the six plain uncoated tablets disintegrates in not more than 45 minutes (Anonymous, 1989). It was also noticed that as the disintegrate was changed from water to simulated gastric fluid the time taken for disintegration was reduced.

Friability test is done to evaluate the ability of tablets to withstand abrasions. For Friability, a loss of less than 1% is considered acceptable by industrial standard. The mean percentage of friability of Qurs-e-Reward was found to be 1.98 ± 0.07.

The extractive value is a parameter for detecting the adulteration in any drug. The amount of the extract that the drug yields in a solvent is often an approximate measure of the amount of certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jenkins et al., 1967). The percentage of successive extractive values of ingredients of Qurs-e-Reward in different organic solvents was found as 1.75 ± 0.01, 3.65 ± 0.02, 10.23 ± 0.02, 0.29 ± 0.01, 15.12 ± 0.03 and 7.71 ± 0.15 with petroleum ether, diethyl ether, chloroform, benzene, alcohol and water respectively. The non-successive extractive values were found as 35.56 ± 0.40, 42.1 ± 0.36, 17.43 ± 0.02, 4.61 ± 0.02, 2.80 ± 0.14 and 1.79 ± 0.01 with alcohol, water, chloroform, diethyl ether, benzene and petroleum ether, respectively. The mean percentage of alcohol and water soluble contents were found to be 27.36 ± 0.26 and 33.03 ± 0.35, respectively. The mean percentage of the moisture content was found to be 7.66 ± 0.08. Ash value is the residue that remains after complete incineration of the drug. Ash value plays an important role in ascertaining the standard of a drug, because the dust, earthy and un-required matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information related to its adulteration with inorganic matter (Jenkins et al., 1967). The mean percentage of the Total ash, Acid-insoluble ash and water soluble ash was found as 5.5 ± 0.11, 1.06 ± 0.08 and 1.9 ± 0.11, respectively.

Percentage of loss in weight on drying at 105°C indicates towards the loss of volatile substances along with water, which is determined by subtracting the moisture content of the drug from the loss in weight on drying. Mean percentage of loss of weight on drying was found to be 9.28 ± 0.05. Mean percentage of bulk density was found to be 0.620 ± 0.001 gm/ml. pH value of the drug is also an important parameter. The drugs in the opposite pH are unionized and absorbed rapidly from stomach. On account of having high acidic pH, the drugs get ionized in stomach because pH of stomach is reported to be about 3.5 (Gilman et al., 2001). The mean of the pH value of 1% and 10% solution was found to be 5.13 ± 0.14 and 5.26 ± 0.08, respectively.

Qualitative phytochemical analysis of the tablet was also carried out for the determination of the presence of alkaloids, amino acids, flavonoids, phenols, proteins, resins, sterols/terpenes, sugars, glycosides and tannins. As the therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs and the lower percentage of chemical constituents may cause lesser therapeutic values of the drugs and therefore, they are considered as low standard drugs. Thin layer chromatography is one of the important parameters used for detecting the adulteration for judging the quality of the drugs. The resolution of different kinds of chemical components are separated by using TLC and
calculating the \( R_f \) values after detecting the spots in order to standardize the drug for its identity, purity and strength. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the number of spots. On the other hand the exhausted or deteriorated drugs may lose the components and the number of spots appeared might be less. Keeping this in mind TLC studies of different extracts obtained in different organic solvents of the test drug (Q.R) have been conducted, and \( R_f \) values of various spots appeared in different solvents system have been noted(Fig.1). Their findings will be helpful in setting the physico chemical standards of Qurs-e-Reward, and predicting the biological activity of the drug. Further, the method used may be utilized for developing the SOP for standardizing of Unani tablets.

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