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Editorial

I feel privileged to be a part of the Central Council for Research in Unani Medicine (CCRUM), an apex organization functioning under the Ministry of AYUSH, Government of India. Unani System of Medicine is one of the traditional Indian systems of medicine. It is a comprehensive medical system and provides preventive, promotive, curative and rehabilitative healthcare. The fundamentals, diagnosis and treatment modalities of the system are based on scientific principles and holistic concept of health and healing. Further, its holistic approach considers individual in relation to his environment and stresses on the health of body, mind and soul. The available literature on Unani Medicine owes its immediate origin to ancient Greece (*Yunan*). Since the Greeks adopted Medicine (Tibb) from Egypt, the roots of this system go to Egypt and its sister civilization Mesopotamia. It was further adopted by the Romans and in the Middle Ages it travelled to the Arab world, Central Asian countries and parts of Europe where it developed to great heights. This system came to India from Arab and Iran and made a long journey to establish itself as one of the preferred medical systems in the country. Realizing its importance, the Government of India accorded great importance to it along with other Indian systems of medicine. As a result, Unani system of medicine today forms an integral part of the national healthcare delivery system and India is considered its world leader with the largest infrastructure and network of educational, research and healthcare institutions. In an effort to bring out the scientific evidences of various new drugs, the CCRUM has been publishing various publications and the notable being the Hippocratic Journal of Unani Medicine, a peer reviewed quarterly journal.

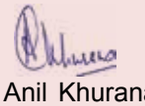
In Unani system of medicine diseases are treated by using different modes of treatment namely; regimenal therapy (Ilaj-bil-Tadbeer), dietotherapy (Ilaj-bil-Ghiza), drug therapy (Ilaj-bil-Dawa) and surgery (Ilaj-bil-Yad). Tadbeer means regimen and Ilaj-bil-tadbeer refers to treatments through various regimes. It includes various methods like dalak (massage), riyazat (exercise), hammam (Turkish bath), hijamah (cupping), fasd (venesection), taleeque (leeching), amal-e-kai (cauterization) etc. Many regimes like cupping therapy, exercise, leech therapy etc. are used in a number of diseases including musculoskeletal disorders like neck pain, back pain, osteoarthritis, rheumatoid arthritis etc. 'Clinical Study to Evaluate the Efficacy of *Ḥijāma bi'l Sharṭ* (Wet Cupping) in the Management of Musculoskeletal Pain A Case Series' included in this issue testifies the role of this regimen in alleviating the pains effectively.

Role of unani medicines in gynaecological conditions is explored in the 'Clinical Study on Unani Formulations *Qurs e Kushta Khabs al-Hadeed* and *Habb-e-Marwareed* in *Sayalan-al-Rahim* (Leucorrhea)'.

A Pilot Clinical Study- 'To Evaluate the Safety and Efficacy of a *Unani* Formulation in *Iktisabi Qillat-e-Ifraz-e-Darqia* (Autoimmune Hypothyroidism) opens up the avenues for undertaking larger study for addressing the diseases of auto immunity which are on the rise and none of the medical system has convincing answer to these maladies.'

In the areas of preclinical and standardization studies this issue includes 'Acute and Sub-acute Oral Toxicity Studies of Majoon IQ – A Unani Brain Tonic' 'HPTLC Fingerprint Studies and Evaluation of Pharmacopoeial Standards for the Drug Habb-e-Sadar - A Unani Formulation', 'Pharmacognostical Evaluation and HPTLC Fingerprinting Studies of *Millingtonia hortensis* L. f. Leaf' and 'Standardization and Phytochemical Screening of a Unani Compound Formulation UNIM 041 (*Mushil* Drug) along with Modern Analytical Technique'.

I sincerely believe the papers included in this issue would be of great value to the scientists and the readers. I take this opportunity to thank the authors for their contributions to this issue and encourage all the scientists and scholars to submit their research papers for publication in this journal.



New Delhi
March 27, 2018

Dr. Anil Khurana
Director General I/c

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The Concept of *Ṭabī'at* in Unani System of Medicine

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Abstract

The Unani Medicine is a comprehensive medicine and based on certain basic principles i.e. the concept of *ṭabī'at, mizāj* (temperament) *akhlāt* (theory of humours), *quwā* (faculties) and *rūḥ* (pneuma). The whole philosophy of Unani Tibb revolves around these concepts and by understanding these; the complete Unani Tibb can be comprehended. But the understanding of the concept of *ṭabī'at* among scholars varies from ancient time to present era. Regarding *ṭabī'at* controversies exist among scholars and people of the community. They criticise the concept due to the lack of understanding and clarity. The aim of this paper is to minimize the level of difference in understanding of this core concept so that it becomes more acceptable and generalised among scholars of Unani Tibb and other systems of medicine. *Ṭabī'at* is the administrative hidden power in the human body which is a matter of prime concern and apprehension for physicians of Unani Medicine.

Keywords: *Ṭabī'at*; Mizāj; Akhlāt; Quwā; Rūḥ; Tibb; Hidden power.

Introduction

In the perspective of Allopathic Medicine, diseases are supposed to be caused by micro-organisms present in the atmosphere (Nadvi, 1995).The human beings are surrounded by many factors and causes which disturb the equilibrium of the body. In spite of these factors which affect the healthy condition of the body, all the population is not equally affected. This implies that there is a hidden power inside every individual which protects him/ her when this power is strong and it varies from person to person. The name of this hidden power is known as "*ṭabī'at-i-insānīyya*".

Ṭabī'at is a broad and central concept of Unani Medicine. Some of the scholars compare it with immunity. The concept of immunity is not equivalent to *ṭabī'at* but it is a part of *ṭabī'at*. In relation to the concept of *ṭabī'at, Cameron Gruner* is the person who has strong faith in this concept. He delineates that in Unani Tibb the concept of *ṭabī'at* is more comprehensive than the germ and any other theories (Ahmad, 1983). He further says that he likes *Ibn Sina* simply due to his acceptance of this concept. *Dr. Weil Fraid Narth Field* and *Loyal Sherald* were also the strong supporter of the concept or theory of *ṭabī'at. Dr. Weil Fraid Narth* also says that he does not care whether anyone accepts this concept or not (Rahman, H.S.Z, YNM 2015). As these scholars were not the scholars of Unani Tibb but they had a strong faith in the concept of *ṭabī'at*. Therefore, one should not criticise this concept only due to the dominance of Allopathic Medicine. In Allopathic Medicine the concept of autoimmunity, auto-circulation, intrinsic factors and idiopathic factors are accepted as such by every person without any criticism and controversies. Here, when these concepts are accepted as such

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then there is no matter of criticising the concept of *ṭabīʿat* as we know both of the medical systems (Unani Medicine and Modern Medicine) have different basic principles and their limits. The basic principles of the related pathy are the pillars on which complete system depends. Hence, the concept of *ṭabīʿat* is a unique basic qualitative concept and accepted as such as said in Unani Tibb. *Ṭabīʿat* is the power which governs and administers the human body involuntarily and unconsciously (Jilani, 1998). It can be interpreted with other Systems of Medicine within the limits so that the concept of *ṭabīʿat* existence can be maintained because interpretations within the limits would maintain the dignity of Unani Tibb and its existence.

Explanation of *Ṭabīʿat*: Ancient scholars have explained *ṭabīʿat* in different manners but all explanations are interlinked. In the present era, the same trend is present and everyone is explaining and understanding it in own way. From ancient period to present era, the scholars defined *ṭabīʿat* in different ways and thus, controversies still exist among them. Some of the definitions specified by eminent Unani scholars are as follows:

Hippocrates says that the physician is the servant of *ṭabīʿat* and that if *ṭabīʿat* resists, all measures are ineffective (Anonymous, 1973).

Aflatoon: *Ṭabīʿat* is a power gifted by God which works for the welfare of the body through which all the functions of the body are accomplished (Jilani, 1998; Arzani, 2010).

Aristotle defined *ṭabīʿat* as the source of being in motion or at rest (Russell,1972) in that to which it belongs primarily by virtue of itself and not by chance (Aristotle's,1998).The word motion means change from pre-existing state of any thing, while rest is retention of pre-existing state. There are various kinds of motion such as growth, decay, genesis, destruction etc. (Jalinoos, YNM). The word motion is the initiation of existence of all the things and *intihā* (climax stage) reaches due to rest (Tabri, 2010). Therefore, we can say that *ṭabīʿat* is responsible for existence and bringing end of everything, including human beings.

Ali Ibn Abbas Majoosi delineates that sometimes our intention by the word *ṭabīʿat* is *quwwat-i-mudabbira badan* (supreme planner of the body), *mahiyat-i-badan* (essence of the body) and *mizāj* (temperament) (Majoosi, 2010).

Ali Ibn Rabban Tabri says that practically, *ṭabīʿat* may be defined as a *quwwat-i-mudabbira badan* (supreme planner) of the body (Tabri, 2010). Here, the word supreme planner stands for all planning adopted for maintaining the equilibrium of the body before the implementation of the functions. Therefore, we can say that *ṭabīʿat* actually knows what, when and how to plan to restore normalcy. Due to this reason, the physicians in Unani Tibb are called as subservient or assistant of *ṭabīʿat* and the role of drugs is to help or support *ṭabīʿat* (Rahman, H.S.Z., YNM 2015)

Abu Bakr Mohammad bin Zakariya Razi and Jalinoos (Galen) stated that *ṭabī'at* expels the fuzlat (waste materials) out of the body through different normal channels and sometimes the fuzlat (waste materials) are disposed off from one 'uḍw (organ) to other (Razi, 2000; Luqa, 2007). Therefore, retention or expulsion of any mādda (materials) in the body is decided by *ṭabī'at* of the individual. The one most important fact is that the *ṭabī'at* uses more processes of expulsion (*istifrāgh*) to maintain the internal harmony than retention.

Ibn Sina says that *ṭabī'at* develops, after the formation of *mizāj* (temperament) but it is not a *mizāj* (Ibn Sina, 2006).

Allama Qarshi calls *ṭabī'at* as a capacity which is responsible for preserving the *kamal'at* (extremes) of that body in which it is found (Arzani, 2010).Here, *kamal'at* of that body means *ṭabī'at* produces movement suitable to related body or compound.

Allama Ibn Qayim Jauzi is of the opinion that God the great has produced *ṭabī'at* in every individual as the armament of body and health. It administers the body up to the last movement of life (Jauzi, 1993).

From the above definitions, we can say that all the definitions are complementary to each other but scholars were not satisfied with any particular definition of *ṭabī'at*. This implies dissatisfaction with the definition and this tendency still persists and scholars keep on searching the exact definition and explanation of *ṭabī'at*.

Synonyms of Ṭabī'at: Phusis/Physis (Russell, 1972), *Quwwat-i-Mudabbira Badan, Quwwat-i-Jismani* (Jilani, 1998) and *Ḥarakat-i-Nafs* (Arzani, 2010) are the synonyms of *ṭabī'at*.

Literal Meaning of Ṭabī'at: *Ṭabī'at* is derived from the word *'taba'* which means *mizāj, fitri adat* or *fitri khaslat* (natural habit). *Gulam Jilani* says that the term *ṭabī'at* is applied for different technical meanings by the physicians which are as follows:

- *Mizāj-i-Insānīyaa*
- *Haiat-i -Tarkībyaa*
- *Quwwat*
- Habit
- Bowel habit (Jilani, 1998).

Functions of Ṭabī'at: *Ṭabī'at* performs different bodily functions and maintains internal harmony of the body so that the mizāji af'āl (temperamental functions) can be accomplished and healthy condition can be maintained. The two main functions of *ṭabī'at* are as follows:

- Maintains the normal physiological functions of the body and preserves the existing health. *Hkm. Syed Ishtiyaq Ahmad* clarifies the functions by saying

that the organs functions origin depend on *ṭabīʿat* and these functions are manipulated by *ṭabīʿat* according to the requirement of the body.

- Protects the body from various diseases and removes the disease when it occurs. These functions are also illustrated by Hkm. Syed Ishtiyaq Ahmad (Ahmad, 1983).

Causes of Weakness of Ṭabīʿat: Everything has certain *asbāb* (causes) either the stability or any changes that occur. Nothing will exist without a cause even health and disease. *Ṭabīʿat* simply becomes weak when the *asbāb* becomes *ghalib* (dominant) on *ṭabīʿat*. But after sometime it prepares itself and again combats against the cause and restores health. The physicians by keeping in view the importance of *ṭabīʿat* in combating the causative factors unsuitable for individuals, they pointed out the causes which may reduce the body combating power (*ṭabīʿat*) and provide favourable condition for the causes to complete casualty. These causes are as follows:

- Malnutrition results in weakness of body and finally *ṭabīʿat* becomes debilitated.
- Air pollution (Jilani, 1996)
- Increase or decrease in body functions effect *ḥarārat-i-gharīziyya* (innate heat) (Majoosi, 2010) and thus, result in debility of *ṭabīʿat* because *ḥarārat-i-gharīziyya* is an *Āla-i- ṭabīʿat* (tool of *ṭabīʿat*).
- Abnormal evacuation
- Toxic substances
- Age (Jilani, 1996)
- Emotions such as fear, sorrow etc. are responsible for maltemperament and weakness of *ḥarārat-i-gharīziyya* as well as *ṭabīʿat* (Majoosi, 2010).
- Miscellaneous diseases (Jilani, 1996).

All the above mentioned causes either directly or indirectly influence the *ṭabīʿat*.

Āla-i- Ṭabīʿat (Tools of Ṭabīʿat): Each organ of the body works in co-ordination with each other to achieve the specific functions of the body. This co-ordination between the organs is maintained by *ṭabīʿat* with the help of their different tools. Here, the *'Āla-i-ṭabīʿat'* implies the relations to channels without which the functions and process of *ṭabīʿat* cannot occur (Kabiruddin, YNM 1970). In relation to this statement *Abu Sahl Maseehi* in *Kitabul Miah* says that *ṭabīʿat* depends on support for performing their functions and processes of the body. According to *Abu Sahl Maseehi* the tool (*Āla*) of *ṭabīʿat* is "*Mizāj* (Maseehi, 2008) and *Ibn Sina* in *Kulliyat-i-Qanoon* says that, *ṭabīʿat* needs a tool i.e. "*ḥarārat-i-gharīziyya*" for its functions (Ibn Sina, 2006). Therefore, the tools of *ṭabīʿat* are *mizāj* and *ḥarārat-i-gharīziyya* and with help of these tools, *ṭabīʿat* maintains the equilibrium

of the body. So, the direct relationship betweeen *mizāj*, *ḥarārat-i-gharīziyya* and *ṭabīʻat*, exists.

Relation Between Mizāj and Ṭabīʻat: *Arkān* are *ajzā' awwaliyya* (primary constituent) of the human body and *mizāj* is formed by the interaction of opposite *kayfiyāt* (qualities) present in arkān (Ibn Sina, 2010). As soon as mizāj is formed, *ṭabīʻat* lodged in the respected compound mādda (matter). But *ṭabīʻat* is not a mizāj, neither it is precursor of mizāj; it is only a power which develops after mizāj and is associated with mādda (Ibn Sina, 2006). Once *ṭabīʻat* lodges in any compound, then it works for the preservation of the mizāj by using its different "*Āla*" (tools). So, the mizāji afʻāl can be performed.

Relation Between Ḥarārat-i-gharīziyya and Ṭabīʻat: *Ḥarārat-i-gharīziyya* (innate heat) is considered as a tool of *ṭabīʻat*. It helps *ṭabīʻat* in maintaining all bodily functions that are performed by various *quwā* (faculties). Each and every *quwwat* requires *ḥarārat-i-gharīziyya* for its functions. In case of any deviation in this *ḥ*arārat, *ṭabīʻat* tries to bring back the moderate *ḥarārat-i-gharīziyya*, so that all the *quwā* can perform their functions properly (Jurjani, 2010).

Quwā and Ṭabīʻat: The word *quwwat* stands for different technical meanings in literature e.g. potency, capacity and efficiency i.e. power of effecting others (Nafis, 1954). For all living beings certain functions are important to save the life. The continuation of these essential functions depends up on *quwwat*. The *quwwat* of the body is responsible for planning the following three different functions:

- *Quwwat-i-Ḥaywāniyya* (Vital Faculties)
- *Quwwat-i-Nafsāniyya* (Psychic Faculties)
- *Quwwat-i-Ṭabīʻiyya* (Vegetative Faculties) (Ibn Rushd, 1987).

With the help of these three faculties and other subordinate faculties working for *ṭabīʻat* such as quwwat-i-jādhiba (absorptive faculty), quwwat-i-māsika (retentive faculty), quwwat-i-mughayyira (transformative faculty) and quwwat-i-dāfiʻa (expulsive faculty) (Ahmad, 1980), it maintains the physiological functions of the body. *Ṭabīʻat* with the help of *quwwat-i-* dāfiʻa eliminates the waste products out of the body through normal channels (Tabri, 1995) and this eliminative function of *ṭabīʻat* is very important for health maintenance. It works more for elimination than retention. As per the need of the body *ṭabīʻat* manipulates all *quwā* and awakes the appropriate psychological instinct for restoration of health such as desire to sleep after fatigue, desire for cold beverages in sue mizaj har, etc.

Disease and Ṭabīʻat: As we know the change is universal phenomenon, the same sequence can be seen in health and disease condition. For the occurrence of disease, it is essential that *ṭabīʻat* be defeated because when *ṭabīʻat* is stronger than disease, it overcomes the *mādda-i-maraḍ* (causative materials) in

the preliminary phase by directing it out of the body (Razi, 2000). According to Unani Medicine, disease consists of four stages. Therefore, *ṭabīʿat* must play certain vital role in each stage for restoration of health. The four stages of *maraḍ* (disease) are as follows:

- *Zamāna-i- Ibtidā*' (Onset Phase): In this stage, *ṭabīʿat* does not do the process of *nuḍj* (concoction) in *bawl* (urine).
- *Zamāna-i-Tazayyud* (Increasing Phase): In this stage, *ṭabīʿat* starts the process of *nuḍj* in *bawl* and slight alteration in colour of urine occurs, it indicates that *ṭabīʿat* is giving the *nuḍj* in *mādda-i-maraḍ*.
- *Zamāna-i- Intihā*' (Climax Phase): In this stage, *ṭabīʿat* completes the process of *nuḍj* which is evident by the existence of rasūb (sediments) in bawl.
- *Zamāna-i-Inḥiṭāṭ* (Convalescence Phase): In this stage, *ṭabīʿat* becomes *ghalib* (dominant) over the disease (Ibn Rushd, 1987). Once *ṭabīʿat* becomes dominant, it will surely restore health by eliminating the causative matter through normal channels.

Treatment of Disease and Ṭabīʿat: *Ṭabīʿat* plays the prime role in the treatment of the diseases. Its importance can be understood by the statement of *Zakariya Razi*. He says that:

- *Ṭabīʿat* is the best physician.
- *Ṭabīʿat* fights against the disease (Razi, 2000).

In Unani therapeutics, the immense confidence is placed on *ṭabīʿat* and the chief aim of physicians is to support rather than produce hindrance in the action of *ṭabīʿat* (Anonymous, 1973).The general principles of treatment modalities which a physician can adopt for assisting *ṭabīʿat* are three:

- Regimen and diet.
- Use of drugs
- Manual operation (Ibn Sina, 2006).

Whatever the regimen, he chooses, in the treatment of any disease that must be in favour of *ṭabīʿat* action. Because, Raban Tabri says that if the physician (by his/her regimens), patient and his or her attainer develop a helpful environment, the disease can easily be controlled. But, if they go against the desire or urge of *ṭabīʿat* in disease eradication process, *ṭabīʿat* is defeated and disease will occur (Tabri, 2010).So, it is clear that the physician assisting *ṭabīʿat* from the initial phase of disease by using various treatment modalities is only to avoid the defeat of *ṭabīʿat* and makes it strong enough to combat the disease condition. Once *ṭabīʿat* becomes strong enough, it will surely maintain normalcy. Therefore, in Unani Medicine, the concept of *ṭabīʿat* is the matter of chief concern for all the practitioners.

Conclusion

Ṭabīʿat is a central and broad concept in Unani Medicine. It is an inherent power gifted by the creator (God) in every individual which can be sensed through its action but cannot be measured quantitatively. It is an administrator of the body which works involuntarily and unconsciously for the welfare of human beings throughout the life. *Ṭabīʿat* is also responsible for co-ordination of the different functions, maintenance of the internal harmony of the body and adjustment of the body according to the surrounding atmosphere.

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सारांश

यूनानी चिकित्सा पद्धति में तबियत की अवधारणा

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यूनानी चिकित्सा एक व्यापक चिकित्सा है और कुछ बुनियादी सिद्धांतों अर्थात् तबियत, मिज़ाज (स्वभाव), अख़्लात (स्वभाव पर सिद्धांत), कुवा (संकाय) और रूह (वायवीय) की अवधारणा पर आधारित है। यूनानी तिब्ब का समस्त तत्वज्ञान इन्हीं अवधारणाओं के चारों ओर घूमता है और इन्हें समझते हुए पूरे यूनानी तिब्ब को समझा जा सकता है। परन्तु तबियत की अवधारणा को समझते हुए विद्वानों के बीच में प्राचीन काल से वर्तमान युग तक भिन्नता पाई गई है। तबियत के संबंध में विद्वानों और समुदायों के लोगों के बीच विवाद अभी भी है। वह समझ और स्पष्टता की कमी के कारण अवधारणा की आलोचना करते हैं। इस लेख का उद्देश्य इस मूल अवधरणा को समझने में अंतर के स्तर को कम करना है ताकि यह यूनानी तिब्ब और अन्य चिकित्सा पद्धति के विद्वानों के बीच अधिक स्वीकार्य और सामान्यीकृत हो। तबियत मानव शरीर के भीतर छिपी एक प्रशासनिक शक्ति है जोकि यूनानी चिकित्सा के चिकित्सकों के लिए मुख्य चिंता और आशंका का केन्द्र है।

HPTLC Fingerprint Studies and Evaluation of Pharmacopoeial Standards for the Drug Habb-e-Sadar - A Unani Formulation

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Abstract

The use of natural products with therapeutic properties has been described by the practitioners of traditional medicines for several disorders. Habb-e-Sadar is a Unani formulation which is a combination of two single drugs namely, Momkham - bee wax and Mastagi - resin from *Pistacia lentiscus* L. It is the simplest formulation of one animal origin and one plant origin drug. As honey, the bee wax is also characterized by several therapeutic properties of great interest though not scientifically proven. Mastagi is also one of the widely used single drugs in Unani system of medicine without any proven scientific background. A very brief review of the literature on these two ingredients laid a way for standardisation of the drug Habb-e-Sadar, expecting that the synergistic effect of drug will result in exploiting many therapeutic properties for treating several ailments in future.

Keywords: Habb-e-Sadar Standardisation, Physicochemical analysis, TLC/ HPTLC analysis.

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization. Therapeutic efficacy of many traditional classical products prepared from minerals, plants and animal have been prescribed by the practitioners of traditional medicines for curing several disorders. Habb-e-Sadar is one such Unani formulation prepared in the form of pills with the combination of two ingredients namely; Mom Kham (Bee Wax) and Mastagi Roomi – Resin (*Pistacia lentiscus* L.). It is the simplest combination of one animal origin drug (bee wax) and one plant origin drug (resin) in equal proportion. The drug is prescribed for treating Waj-ul-Sadar (Chest pain) patients and in some cases it is to be used as Musakkin (sedative) (NFUM, 2011).

Mom kham (bee's wax) is obtained by squeezing or pressing the honeycomb mostly produced by *Apis mellifera* or *Apis cerana*, after extraction of honey. It is a yellowish solid mass, harder than butter, has honey like odour, soluble in petroleum ether, slightly soluble in cold alcohol (3%), chloroform (25%) and insoluble in water. It is a complex mixture of hydrocarbons, free fatty acids, esters of fatty acids, diesters, exogenous substances like residues of propolis, pollen, small pieces of floral components and pollution. Generally the composition of the bees wax may vary between and among different families and different breeds of bees. Many medicinal properties of the bee wax have been known from ancient times. The "father of medicine", Hippocrates, recommended the use of bees wax in case of purulent tonsillitis (Filippo Fratini *et al.*, 2016; Pawan Kumar Sagar *et al.*, 2015). In Ayurveda, the bee wax is used under the name "Madhuchishtha"

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in treating wounds, burns and in curing heel cracks (Bagade Sarojini, 2013). Apart from that, Unani literature survey also reveals that bee wax is used in the treatment of various ailments like Sual-e-Yabis (Dry cough), Ishal-e-Muzmin (Chronic diarrhoea), Bawaseer (Haemorrhoids), Diq (Tuberculosis) and Humma (Fever). It is also used as one of the ingredients in the unani formulation Zimad Niswan.

The ingredient Mastagi Roomi is the resin obtained from the plant trunk *Pistacia lentiscus* L. (Family - Anacardiaceae). It is light yellow in colour with slight agreeable taste and fragrance. It is available in market as pear, ovoid or globular shaped small tears of 4-8 mm in diameter. The main constituents of the resin are α and β masticonic acids, α-masticoresene, β-masticoresene, α and β masticinic acids, volatile oil, masticolic acid, α-pinene and β-myrcene (Evans 1996 ; Wallis, 1997; Boelens and Jimenez, 1991). In Unani system of medicine, physicians are using mastagi since centuries for the treatment of many ailments like gastrointestinal disturbances, hepatobiliary disorders, gynaecological diseases, fractures, wounds and ENT problems (Shaikh Imtiyaz *et al.*, 2013). It is also used as one of the ingredients in many Unani formulations like Jawarish-e- Jalinoos, Jawarish-e-Mastagi, Jawahar Mohra and Habb-e- Ambar Momyai (NFUM, 2006 and 2008). The drug Habb-e-Sadar which is the combination of Momkham and Mastagi may be a right choice for further investigation of pharmacological activities, as the synergistic effect of drug will result in exploiting many therapeutic properties for treating several ailments. With this view, the study aimed to investigate the TLC/HPTLC fingerprint pattern of the drug with analysis of its physicochemical and quality control parameters to prove the scientific validation and to lay down pharmacopoeial standards for the drug Habb-e- Sadar.

Material and Methods

Collection of the Raw Drug and Preparation of the Formulation

Authentic raw drug Momkham (bee wax DSM-A-05) and Mastagi (resin DSM-149) were procured from Chennai market (R N Rajan Stores). The formulation Habb-e-Sadar was prepared in laboratory scale in the Drug Standardisation Research Unit, Regional Research Institute of Unani Medicine, Chennai, with utmost care by adopting good manufacturing practices as prescribed by the European Commission Brussels guidelines (2008) and NFUM (2011).

To the finely powdered mastagi, equal quantity of sliced, melted momkham was added and mixed thoroughly to obtain the lubdi mass. Manually the lubdi mass was rolled in between the fingers into sticks of required size, thickness and were cut into pieces using a knife. The cut pieces were further rolled to get the round shaped pills and stored in air tight containers. The drug was prepared in

three batches, each batch comprising a minimum of 500 pills weighting about 300 – 400 mg per pill.

Physico-chemical Analysis

The physico-chemical analysis viz., moisture content, extractive values, ash values and pH value were analysed for the prepared drug Habb-e-Sadar as per the standard methods (WHO, 2011)

Quality Control Analysis

Quality control parameters like microbial load, heavy metals, aflatoxins and pesticidal residues for the samples of Habb-e- Sadar drug were undertaken and analyzed. The microbial load estimation was carried out as per the guidelines (WHO, 2007). Heavy metal analysis was done by Atomic Absorption Spectrophotometer (AOAC, 2005). Analysis for aflatoxins was performed by TLC method (WHO, 2007). Pesticide residues were analysed using GC MS Agilent instrument equipped with mass selective detector as per the methods AOAC (2005).

TLC/HPTLC Fingerprint Analysis

The three batches sample of Habb-e-Sadar (each 5 gm) were extracted with 20 ml each of petroleum ether and chloroform separately and reflux on water bath for 30 mins and made up to 10 ml in a standard volumetric flask. The extract (5µl each) was applied over aluminium plate pre coated with silica gel 60 F_{254} (5 x 10 cm, E.Merck) by employing CAMAG ATS4 sample applicator. The plates were developed up to the distance of 8 cm in the chamber (10 x 10), using 10 ml of the developing system Toluene: Ethyl acetate (6: 4) as mobile phase for petroleum ether extract and 12 ml of Toluene : Ethyl acetate: Petroleum ether (9: 1: 2) for chloroform extract, dried at room temperature, observed and scanned under UV 254nm and 366nm. Finally, the plates were dipped in vanillin sulphuric acid reagent (200 ml) for a minute and heated at 105º C till coloured spots appear (Wagner and Bladt, 1984 and Sethi P D, 1996).

Results and Discussion

Physico-chemical Analysis

The physico-chemical standards for the drug Habb-e-Sadar is given in Table 1.

The pH and moisture content of the drug were found to be 7.4 and 0.394% respectively. Quantitative standards reveal the presence of negligible amount of siliceous matter in the sample where the total ash content was found to be 3.68% and acid insoluble ash was found to be 1.11%. The extractive value shows that the solubility of phytoconstituents of the drug was more in petroleum

ether (88.83%). Only negligible amount of phytoconstituents is soluble in alcohol (2.42%) and water (0.13%) when compared to petroleum ether. It is inferred from the data that the drug contains more fat soluble phytoconstituents that are soluble in non polar solvents.

Quality Control Analysis

A. Microbial Load

The microbial content of the sample is given in Table 2.

The estimation of microbial load gives the tentative idea to assess the quality and safety of the drug prepared. The assessment done for estimating the total viable count of bacteria, total fungal count, count of bacteria belonging to the Enterobacteriaceae family, count of pathogens like Escherichia coli, Staphylococcus aureus and Salmonella spp. indicates that the microbial load to be within the permissible limits of WHO stating that the drug is safe for internal use for the treatment of prescribed ailments.

B. Heavy Metal Analysis

The amount of various heavy metals found in the sample is given in Table 2.

Heavy metals are hazardous to human and animal health, their content in any drug used for consumption or medicinal purposes must be limited. Heavy metal contained in Habb-e-Sadar was found to be within the permissible limits of Ayurvedic Pharmacopoeia of India (API) and Unani Pharmacopoeia of India (UPI) stating that the drug is safer from toxic substances point of view.

C. Detection of Aflatoxins

Results of aflatoxin content in the sample tested are given in Table 2.

Aflatoxins are toxic metabolites produced by a variety of molds such as Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. Reports of the present study do not show any evidence for the presence of any of the aflatoxin content (B_1, B_2, G_1, G_2) in the sample.

D. Detection of Pesticide Residues

The pesticide residue content of the sample is given in Table 3.

Production of herbal drugs according to good agricultural practices with no pesticide residues is highly uncontrollable due to several factors. Detection of pesticide in the samples also became a major task though several techniques have been developed. In the present study the pesticide residue was analysed using the GC-MS instrument which has the detection limit up to 0.01 ppm. The results suggested that the sample is free from pesticides.

TLC/ HPTLC Fingerprint Analysis

A. TLC/HPTLC of Chloroform Extract

The suitable mobile phase Toluene: Ethyl acetate: Petroleum ether (9: 1: 2) with appropriate proportion has been determined for chloroform extract of the drug Habb-e-Sadar. The TLC photographs of chloroform extract are shown in (Figure-1).

B. HPTLC Fingerprint of Chloroform Extract

HPTLC fingerprint profile of chloroform extract of Habb-e-Sadar showed 14 peaks (Figure-2). The densitometric chromatograms of three batch sample of the drug Habb-e-Sadar are recorded at 254 nm (Figure-3).

A. TLC/HPTLC of Petroleum ether Extract

The suitable mobile phase Toluene: Ethyl acetate (6: 4) with appropriate proportion has been determined for petroleum ether extract of the drug Habb-e-Sadar. The TLC photographs of petroleum ether extract are shown (Figure-4).

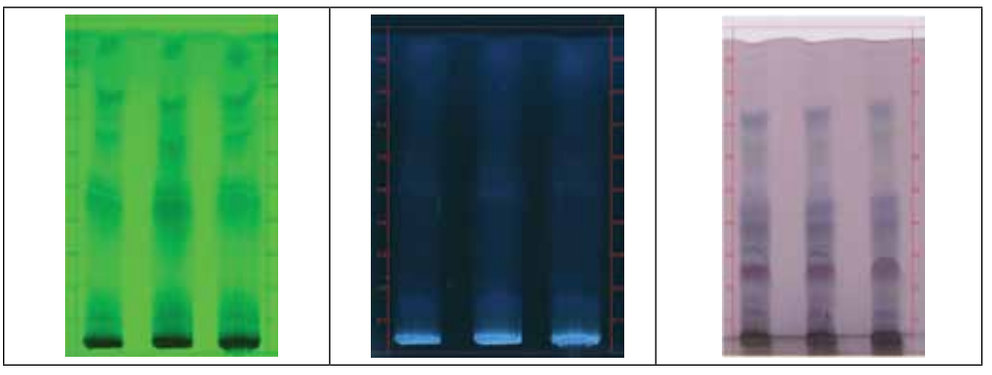
B. HPTLC Fingerprint of Petroleum ether Extract

HPTLC fingerprint profile of petroleum ether extract of Habb-e-Sadar showed 10 peaks (Figure-5). The densitometric chromatograms of three batch sample of the drug Habb-e-Sadar are recorded at 254 nm (Figure-6).

Conclusion

Up on investigation of the literature survey, the single drug mom kham and mastagi are found to exhibit numerous pharmacological activities as stated above though no work has proved scientifically. The drug Habb-e-Sadar, the combination of these two single drugs will be an appropriate choice to carry out many future pharmacological studies. The present approach for standardisation of the drug Habb-e-Sadar will serve as available scientific standards.

Chloroform extract



Solvent System: Toluene : Ethyl acetate : Petroleum ether (9:1:2)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

Rf values

UV 254nm	UV 366nm	VS Dipped
0.91 (Green)	0.90 (Blue)	0.76 (Blue)
0.78 (Green)	0.50 (Blue)	0.74 (Light blue)
0.71 (Green)	0.42 (Violet)	0.68 (Yellowish green)
0.68 (Green)	0.29 (Violet)	0.61 (Light violet)
0.51 (Green)	0.21 (Blue)	0.52 (Light violet)
0.41 (Green)	0.18 (Blue)	0.40 (Violet)
0.10 (Green)		0.32 (Blue)
		0.28 (Pink)
		0.20 (Light yellow)
		0.19 (Light blue)
		0.12 (Violet)

Fig. 1 : HPTLC photographs of Habb-e-Sadar

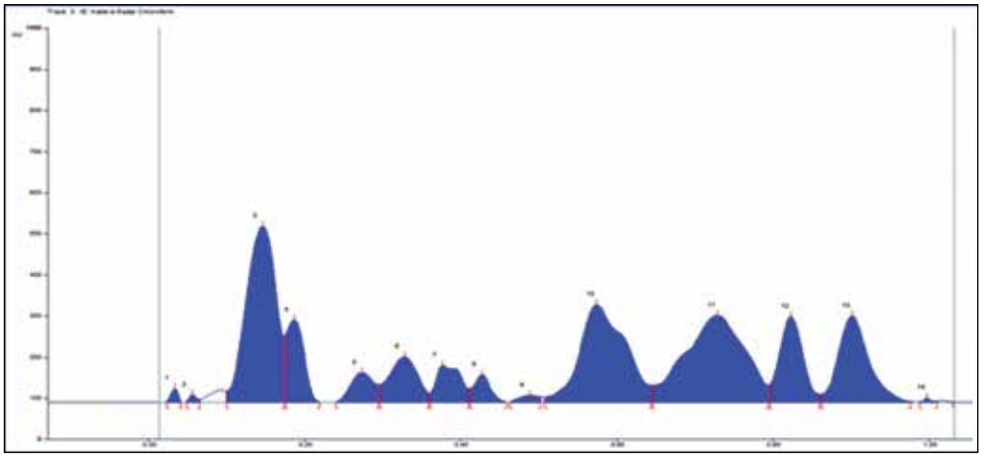


Fig. 2 : HPTLC fingerprint of Habb-e-Sadar chloroform extract at 254nm

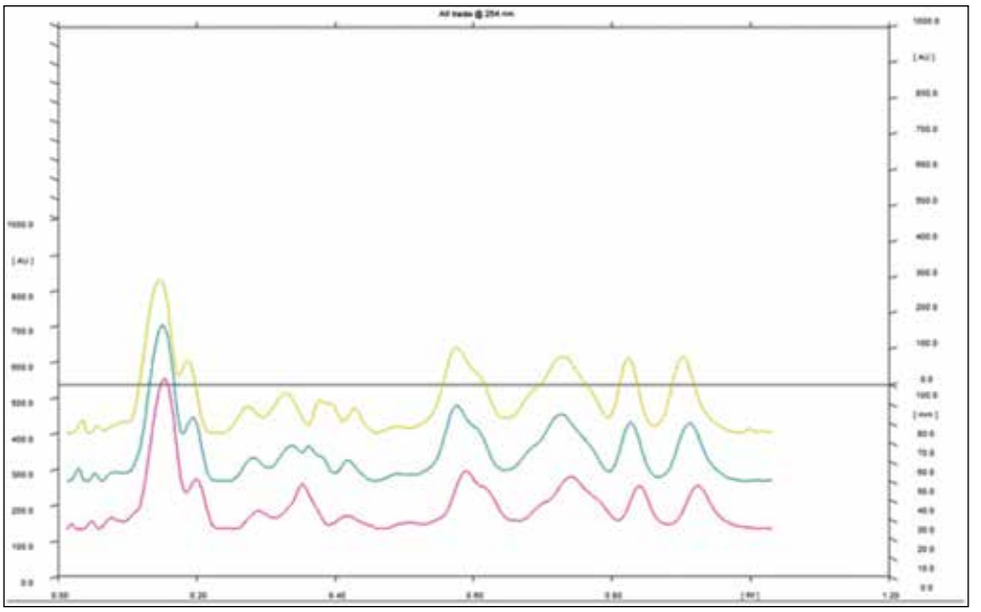
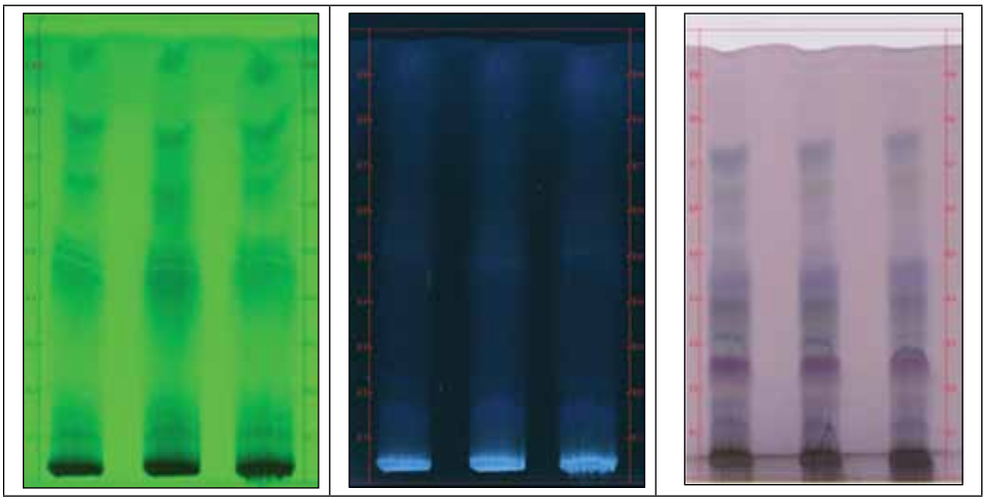


Fig. 3 : Densitometric chromatograms of Habb-e-Sadar chloroform extract at 254nm

Petroleum ether extract



Solvent System: Toluene : Ethyl acetate (6 : 4)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

Rf values

UV254nm	UV 366nm	VS Dipped
0.75 (Green)	0.91 (Violet)	0.90 (Violet)
0.70 (Green)	0.70 (Blue)	0.71 (Grey)
0.68 (Green)	0.59 (Blue)	0.65 (Yellowish green)
0.48 (Green)	0.55 (Blue)	0.59 (Light violet)

0.39 (Green)	0.51 (Blue)	0.50 (Light violet)
0.35 (Green)	0.48 (Blue)	0.46 (Violet)
0.34 (Green)	0.35 (Blue)	0.41 (Dark violet)
	0.31 (Blue)	0.38 (Yellowish green)
	0.18 (Blue)	0.32 (Violet)
	0.11 (Violet)	0.30 (Yellow)
		0.28 (Pink)
		0.17 (Light violet)
		0.10 (Violet)

Fig. 4 : HPTLC Photographs of Habb-e-Sadar

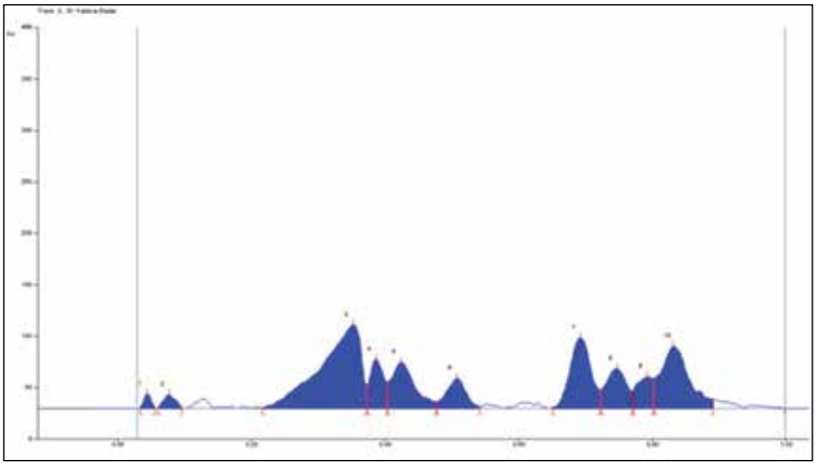


Fig. 5 : HPTLC fingerprint of Habb-e-Sadar Petroleum ether extract at 254nm

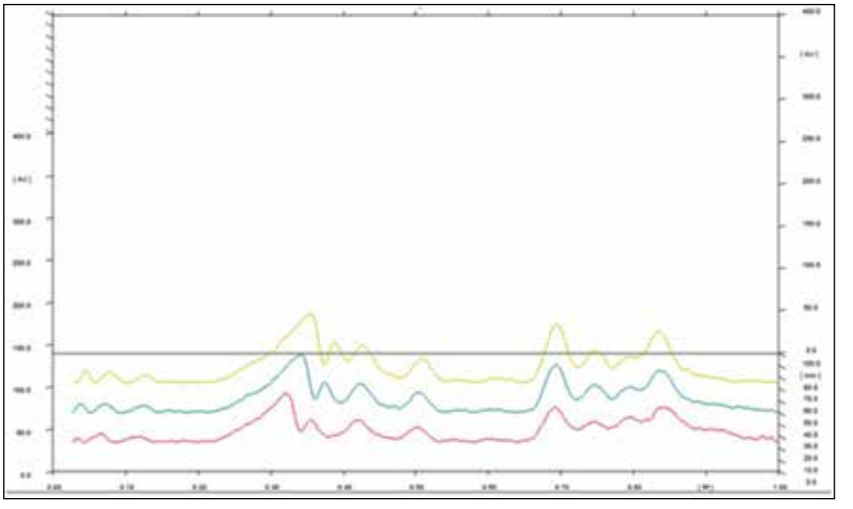


Fig. 6 : Densitometric Chromatograms of Habb-e-Sadar Petroleum ether extract at 254nm

Table 1: Physico-chemical Analysis of Habb-e-Sadar

Parameter Analysed	Batch Number		
	I	II	III
Extractive values			
Petroleum ether	88.86%	88.76%	88.87%
Alcohol	2.30%	2.46%	2.52%
Water	0.14%	0.13%	0.13%
Ash value			
Total Ash	3.69%	3.68%	3.69%
Acid insoluble ash	1.00%	1.13%	1.20%
Moisture	0.451%	0.375%	0.357%
pH Values			
1% solution	7.5	7.4	7.4
10% solution	5.2	5.2	5.1

Table 2 : Quality Control Analysis of Habb-e-Sadar

S. No.	Name of the Analysis	Parameter	Results	Permissible Limits
1.	Microbial Load	Total bacterial content	< 10 cfu/gram	$x10^5$ cfu/gm
		Total fungal content	<10 cfu /gram	$x10^3$ cfu/gm
		Enterobacteriaceae	Absent	Absent
		Escherichia coli	Absent	Absent
		Salmonella spp.	Absent	Absent
		Staphylococcus aureus	Absent	Absent
Permissible limits: World Health Organisation (WHO), 2007; Cfu/gm: Colony forming units per gram.				
2.	Heavy metals	**Name of the element**	**Results**	**Permissible Limits (ppm)**
		Lead	ND	10
		Cadmium	ND	0.3
		Mercury	ND	1
		Arsenic	ND	3

Permissible limits: The Ayurvedic Pharmacopoeia of India (API), 2008; The Unani Pharmacopoeia of India (UPI), 2016; ND: Not Detected; ppm: parts per million				
3.	**Aflatoxin**	**Parameters**	**Results**	**Inference**
		B1	ND	Absent
		B2	ND	Absent
		G1	ND	Absent
		G2	ND	Absent
ND: Not Detected				

Table 3: Pesticide Residue of Habb-e-Sadar

S. No.	Name of the pesticide compound	Results
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected
2	HCH (sum of all isomers)	Not detected
3	Endosulphan (all isomers)	Not detected
4	Azinphos-methyl	Not detected
5	Alachlor	Not detected
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected
7	Chlordane (cis & trans)	Not detected
8	Chlorfenvinphos	Not detected
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected
10	Endrin	Not detected
11	Ethion	Not detected
12	Chlorpyrifos	Not detected
13	Chlorpyrifos-methyl	Not detected
14	Parathion methyl	Not detected
15	Malathion	Not detected
16	Parathion	Not detected
17	Diazinon	Not detected
18	Dichlorvos	Not detected
19	Methamidophos	Not detected

20	Phosalone	Not detected
21	Fenvalerate	Not detected
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected
23	Fenitrothion	Not detected
24	Deltamethrin	Not detected
25	Permethrin (sum of isomers)	Not detected
26	Pirimiphos methyl	Not detected

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सारांश

हब्ब-ए-सदर-एक यूनानी मिश्रण का एच.पी.टी.एल.सी. अगुलांक अध्ययन एवं भेषजकोशीय मापदंड का मूल्यांकन

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चिकित्सीय गुणों से भरपूर प्राकृतिक उत्पादों का उपयोग पारंपरिक औषधियों के चिकित्सकों द्वारा बहुत सारी बिमारियों के ईलाज में किया जाता है। हब्ब–ए–सदर एक यूनानी मिश्रण है जिसे दो एकल औषधियों मोमखाम–मधुमक्खी के मोम और मस्तगी–पिश्तेसिया लेंटिस्कस एल की राल के मिश्रण से बनाया जाता है। यह एक पशु और पौधे के मूल से विकसित औषधि का सरलतम रूप है। शहद अर्थात् मधुमक्खी का मोम वैज्ञानिक तरीके से सिद्ध नहीं होने के बावजूद भी मधु के समान भरपूर चिकित्सीय गुणों से युक्त होता है। मस्तगी भी बिना किसी सिद्ध वैज्ञानिक सबूत के यूनानी चिकित्सा पद्धति में व्यापक रूप से इस्तेमाल की जाने वाली एकल औषधियों में से एक है। इन दो घटकों पर साहित्य की एक बहुत संक्षिप्त समीक्षा ने हब्ब–ए–सदर औषधि के मानकीकरण के लिए एक तरीका निकाला। दवा हब्ब–ए–सदर के सहक्रियात्मक प्रभाव से यह उम्मीद की जाती है कि भविष्य में यह औषधि कई बीमारियों के लिए कारगर सिद्ध होगी।

Pharmacognostical Evaluation and HPTLC Fingerprinting Studies of *Millingtonia Hortensis* L. f. Leaf

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Abstract

Millingtonia hortensis L.f. is commonly known as Cork tree belongs to the family Bignoniaceae. It is an important medicinal plant in Southern Asia such as India, Burma, Thailand and South China. The leaves of the plant were collected freshly and subjected to macro-microscopic, physico-chemical and quality control parameters to fix the authenticity and quality standards of the plant. Microscopical studies showed the presence of wavy epidermal cells with anomocytic stomata; palisade and spongy parenchyma cells; wide pitted vessels with tails at one or both the ends; fibres; glandular trichomes up to 50µ with 16 head cells and unicellular trichomes up to 200µ length. The physico-chemical data showed moisture content as 8.42%, total ash 8.93%, acid insoluble ash 0.098% and alcohol and water soluble extractive values as 5.92% and 24.44% respectively. TLC/HPTLC studies of chloroform and alcohol extracts showed various spots / peaks at 254nm, 366nm and in derivatized plates (Vanillin-sulphuric acid reagent). Quality control parameters such as microbial content and the heavy metals (As, Cd, Pb and Hg) were found to be within the permissible limit. The aflatoxins B_1, B_2, G_1 and G_2 were not detected. The study will be useful for the identification and authentication of the plant in dry form as well as in fresh form. The evaluated phytochemical data will serve as pharmacopoeial standards in the near future for any analytical and biological studies.

Keywords: *Millingtonia hortensis*, pharmacognostical characters, physico-chemical analysis, TLC/HPTLC studies, Quality control parameters.

Introduction

Millingtonia hortensis L.f. the sole species of the genus *Millingtonia* belongs to the family Bignoniaceae (Lindley and Moore, 1866) and originated from South-East Asia and South Asia (Gamble, 1921). It is also found in Central India, Myanmar (Burma) and Thailand. In India it is widely distributed and cultivated in many parts including the semi-arid regions of Rajasthan (Kaushik and Saini, 2008). The plant is commonly known as Indian Cork tree. Some of its other names are Akas Nim, Nim Chameli, Betati Nim, Mini Chameli, Maramall, Tree jasmine, Karkku, Kat Malli and Kavudi (Ramasubramaniaraja, 2010).

It is a very tall deciduous tree which grows up to 25m with straight trunk and a few branches. The leaves are pinnately compound and ornamental. The tree usually blooms from October to December, sheds leaves between January and March and renew during April and May. It flowers at night and shed early in the

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morning. The flowers are long tubular, silvery white with delightful fragrance. The arrangement of flowers is corymbose and bears capsule type of fruits (Mayuranathan, 1981).

It is an important medicinal plant in Southern Asia such as India, Burma, Thailand and Southern China where the leaves are used as tonic in folklore medicine and reported to have many medicinal values like antipyretic, sinusitis and cholagogue. The plants also have reported to possess antibacterial, antifungal, anticonvulsant and larvicidal activities (Ramasubramaniaraja, 2010). Flowers are reported to possess antioxidant, hepatoprotective, antiphlorgistic and antiasthmatic properties (Surendra Kumar *et al.*, 2014). Stem bark is used as antioxidant, antihelmintic drugs and also have other pharmacological activities like induction of apotosis on RKO colon cancer cell lines (Siwapong Tansuwanwong *et al.*, 2006).

Various phyto-constituents have been reported to be identified and isolated from various parts of the plants like lapachol, β-sitosterol and poulownin (roots), β-sitosterol (heart wood and bark), hispidulin, rutinoside, a flavonoid dinatin together with β-carotene (leaves), scutellarein, hispidulin and scutellarein-5-glucuronide (flowers) and acetyl oleanolic acid (fruit) (Aruna Kumari and Sharma, 2013) etc. Studies on the mutagenicity and antimutagenicity of hispidulin and hortensin (flavonoids) of the plant were also reported (Blatter and Millard, 1954).

The present investigation deals with the evaluation of morphological, anatomical, physico-chemical, TLC/HPTLC fingerprints and quality control parameters of leaves of the plant *M. hortensis*. The morphological and anatomical studies will provide the information for correct identification and authentication of the plant material whereas the other phytochemical studies will serve as pharmacopoeial standards for the plant in the near future for any phytochemical and biological related studies.

Material and Methods

Pharmacognostical Studies

The leaves of the plant *M. hortensis* were collected from the herbal garden of Regional Research Institute of Unani Medicine (RRIUM), Chennai, during the month of October 2016 and identified with the help of Flora of Presidency of Madras (Gamble, 1921.). The morphological authenticity of the plant was referred and compared with the herbarium specimen (*M. hortensis* - voucher specimen No. RRIUMCH12525), Department of Survey of Medicinal Plants Unit (SMPU), RRIUM, Chennai.

The fresh leaves were macroscopically examined for shape, size, surface characteristics, texture, color, odour and taste. The macroscopical, microscopical and powder microscopy were carried out using standard methods (Johansen, 1940). Free hand sections of the leaves were taken and its photo micrographs were recorded using MIPS camera attached with the microscope. Quantitative microscopical studies like vein islet number, veinlet termination number, palisade ratio, stomatal number and stomatal index were studied as per the standard procedures (Wallis, 1985; Kokate, 1994; UPI, 2009).

Physico-chemical Parameters

Physico-chemical parameters like foreign matter, total ash, acid insoluble ash and loss on drying at 105°C, alcohol and water soluble extractives were carried out as per the standard method (WHO, 2011).

TLC/ HPTLC Analysis

The TLC/HPTLC analysis was performed for chloroform and alcohol extract of the leaves of *M. hortensis*. The sample (5µl each extract) was applied on pre-coated silica gel 60 F_{254} TLC plate (E Merck) and developed using Toluene: ethyl acetate (1:1) solvent systems as mobile phase for both extracts. The developed plates were scanned densitometrically at 254nm, 366nm and derivatized using spray reagent Vanillin sulphuric acid. The Retention factor (Rf) values, peak area and peak height were determined (Wagner, 1984, Sethi, 1996).

Quality Control Parameters

Quality control parameters like microbial load and aflatoxin were carried out as per the WHO guidelines (WHO, 2007). Heavy metals analysis was done by atomic absorption spectrophotometer (AOAC, 2005). Pesticide residues were analyzed using GC-MS agilent instrument equipped with mass selective detector as per the methods of AOAC (AOAC, 2005).

Results

Macroscopic

Leaves large, imparipinnate and ornamental (Figure-1) Long leaf bears two or three widely spaced pinnae, each with 5-7 smooth leaflets; Leaflets oval, pointed, slightly toothed and 1-3 inches long. Sometimes the lower pinnae again divided and bear one pair of three leaved pinnae, 1-2 pairs of leaflets with one leaflet at the end. The leaves are slightly bitter in taste and odourless.

Microscopic

Petiole - The T. S. of petiole almost circular in outline (Figure-2); measures 3mm in median vertical plane and 5mm in horizontal plane; the upper part somewhat flat and the lower part semicircular; epidermis consists of single layer of thin walled parenchyma cells; cortex consists of 2 - 3 layers of collenchyma, chlorenchyma and parenchyma cells. Pericycle consists of group of sclerenchyma cells in bundles. Closed circular cylindrical vascular system (Figure-3) with xylem towards the centre and phloem outwards with wide pith in the centre. The petiole of the leaflets (Figure-2a, 2b, 2c and 2d) almost circular with two lateral wings on either side.

Leaf - The transverse section of the leaf shows prominent midrib and thick lamina; the lamina slightly raised above the level of the midrib forming shallow concavity on the upper side (Figure-4a).

Midrib - The T. S. of the midrib slight concave on the upper side and semi-circular on the lower side (Figure-4b), measures 500µm in vertical plane; cortex consists of 2 - 3 layers of collenchyma, chlorenchyma and parenchyma cells; the vascular bundle bowl shaped consists of 5 to 7 parallel rows of compactly arranged xylem elements with thin layer of phloem beneath the xylem strand.

Lamina - The T. S. of lamina shows 100µm thick; dorsiventral; upper epidermal cells rectangular (Figure-5a) with thick and smooth cuticle; the lower epidermal cells contain numerous stomata; the mesophyll region differentiated into upper two layer of compactly arranged palisade cells and lower four to five layers of loosely arranged spongy parenchyma cells.

Glandular Trichomes (Figure-5b) - Glandular trichome sessile on the epidermis of the lamina, it has two parts - the basal stalk cell and head. Head cell consists of 16 thin walled cells measuring up to 50µm wide; the glands are embedded on both the surfaces of the epidermal cells at position slightly lower the level of the epidermis.

Leaf Venation

The venation pattern clearly not visible. Veins fairly thick and vein lets form wide, angular islets. The vein-islets wide, distinct, rectangular, squarish or polygonal. The vein terminations arise from the vein islets and are long, slender, either branched or un-branched (Figure-6a).

Covering Trichomes (Figure-6b)

Covering trichomes seen on the epidermis; it is uniseriate and unicellular; the terminal cell of the trichome pointed and narrow whereas the basal cells broad and rectangular.

Epidermal Cells and Stomata

The epidermal cells thin, highly wavy (Figure-7a & 7b) with anomocytic stomata; stomata present only on the lower surface, the guard cells long and elliptical with narrow stomatal pore. The lower surface consists of 67.5 stomata per sq.mm with stomatal index of 24 per sq.mm; the vein islet number 9.25/sq.mm; veinlet termination number 19.25/sq.mm and palisade ratio 6.15 (Table 1).

Powder Microscopy (Figure-8)

Green color powder; evidenced for the presence of epidermal cells with anomocytic stomata (8a), numerous glandular trichomes (8a), covering trichomes (8b); glandular trichome with a single basal stalk cells and 16 head cells up to 50µm wide (8c); spiral vessels up to 30µ (8d); pitted vessels up to 25µ with tails at one or both the ends (8e & 8f) and thin walled fibres with broad lumen up to 30µ (8g).

Physico-chemical Studies

The physico-chemical parameters of the powdered drug were analyzed and the results are shown in Table 2. The loss on drying at 105°C was found to be 7.23% and the content of total ash and acid insoluble ash was found to be 8.92% and 0.099% respectively. The alcohol soluble extractive values 5.94% and water soluble extractive value 24.44 % show the extraction of polar constituents.

TLC/HPTLC Studies

TLC/HPTLC profile of chloroform and alcohol extract of the leaf was developed using Toluene: Ethyl acetate (1:1) as mobile phase. TLC/HPTLC profile at UV-254 nm, UV-366 nm and after derivatized with vanillin - sulphuric acid are shown in Figure-9 and 10. The Rf values of both the extracts are given in Table 3 and 4.

HPTLC Fingerprint Profile of Chloroform Extract

HPTLC fingerprint profile of chloroform extract, showed 8 peaks at 254nm and 6 peaks at 366 nm (Figure-11 and 12). Of which one major peak was seen at

254nm (Rf value of 0.37) and two major peaks were seen at 366nm (Rf values 0.73 and 0.79). The others were moderately smaller peaks.

HPTLC Fingerprint Profile of Alcohol Extract

HPTLC fingerprint profile of alcohol extract showed 6 peaks each at 254nm and 366nm (Figure-13 and 14). Of which 1 major peak was noted at both 254nm and 366nm (Rf value of 0.66). The others were moderately smaller peaks.

Quality Control Parameters

The microbial contents were found to be within the permissible limit (Table 5). The other parameters such as heavy metals, aflatoxin and pesticide residue were not detected from the samples (Table 6, 7 and 8) which indicate that the sample is free from toxic substances.

Discussion

The microscopical studies clearly reveal the wavy parenchyma cells of the epidermis in surface view with anomocytic type of stomata on the lower side and no stomata in the upper epidermis. Evidences are seen for the presence of glandular trichome with 16 head cells and a single basal cell immersed in the surface of the epidermis; numerous unicellular covering trichomes are also present on both the surfaces of leaf. The findings of the study are similar to the earlier reports of Metclafe and Chalk, 1957 where the reports stated the presence of external hairs of glandular and non-glandular forms, stomata confirmed to the lower surface surrounded by a fairly number of ordinary epidermal cells.

The usage of fingerprint chromatogram in the study helped in the identification of various phyto-constituents and quality evaluation of the study sample. The preliminary study of microscopy and phytochemistry carried on leaf part of the plant *M. hortensis* will act as a basic tool for correct identification of the plant and serve as phytochemical standards in the coming years, though a long-term study is required to evaluate the therapeutic efficacy and toxicity nature of the plant to establish the plant as a drug in the pharmaceutical industries.

Conclusion

The pharmacognostic studies, physico-chemical properties and TLC/HPTLC fingerprint analysis of the leaf of *M. hortensis* have been carried out for the first time which could serve in the identification and preparation of a monograph of the plant.

Millingtonia hortensis L.f.

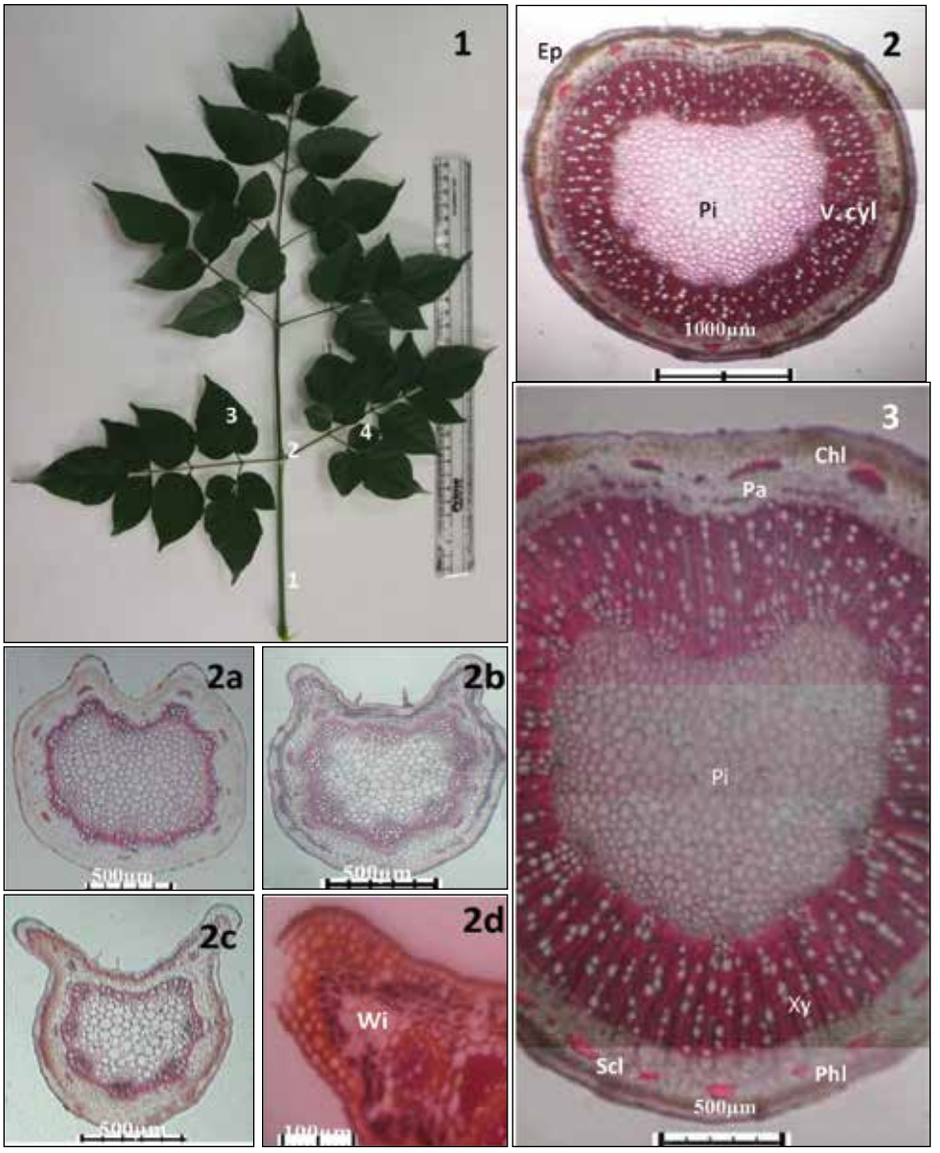


Fig. 1 : Leaf; **Fig. 2 :** T. S. of Petiole; **Fig. 3 :** T. S. of Petiole (Enlarged); **Fig. 2a, 2b, 2c** and **2d :** T. S. of Petiole of the leaflets with lateral wings; Ep – Epidermis, Chl – Chlorenchyma; Pa- Parenchyma, Scl – Sclerenchyma, Xy – Xylem, Ph – Phloem, Pi – Pith, Wi - Wings

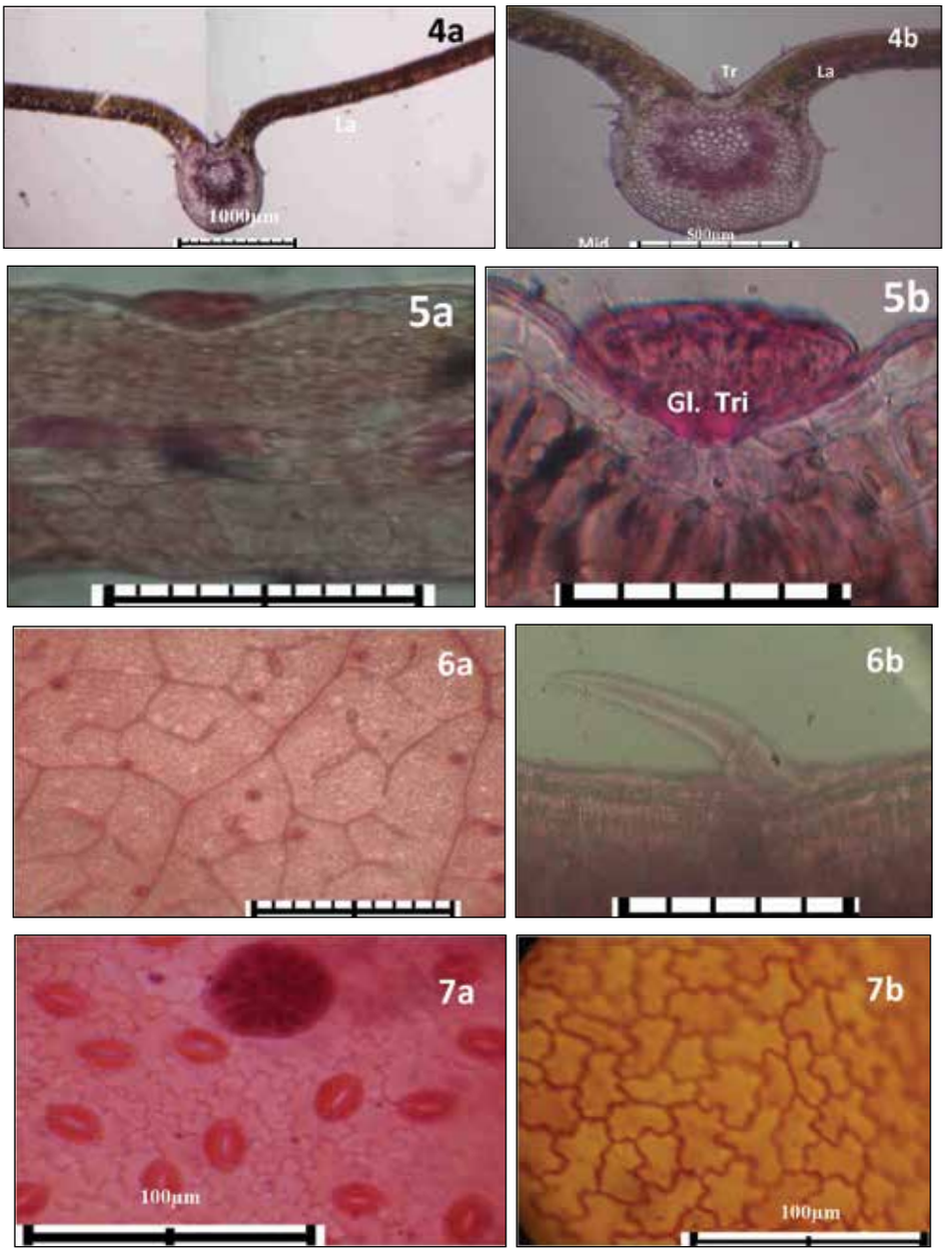
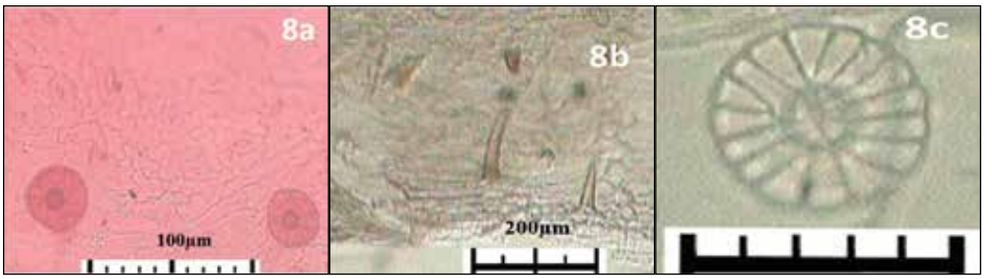


Fig. 4a : T. S. Leaf; **Fig. 4b :** T. S. of Leaf through midrib;
Fig. 5a : T. S. Leaf through lamina; **Fig. 5b :** Glandular trichomes on the lamina;
Fig. 6a : Vein islet and vein termination of the lamina;
Fig. 6b : Covering trichomes on the lamina;
Fig. 7a : Lower epidermal cells with stomata and glandular trichomes;
Fig. 7b : Upper epidermal cells without stomata



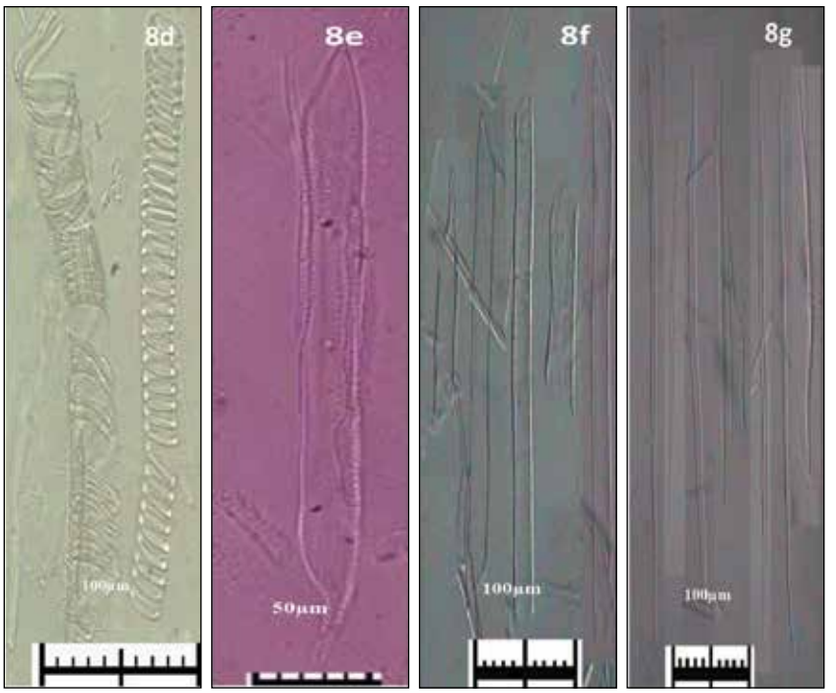


Fig. 8 : Powder Microscopical studies -

a. Epidermal cells with stomata and glandular trichomes;

b. Epidermal cells with covering trichomes;

c. Glandular trichomes

d. Spiral vessels

e. Pitted vessels

f. Pitted vessels

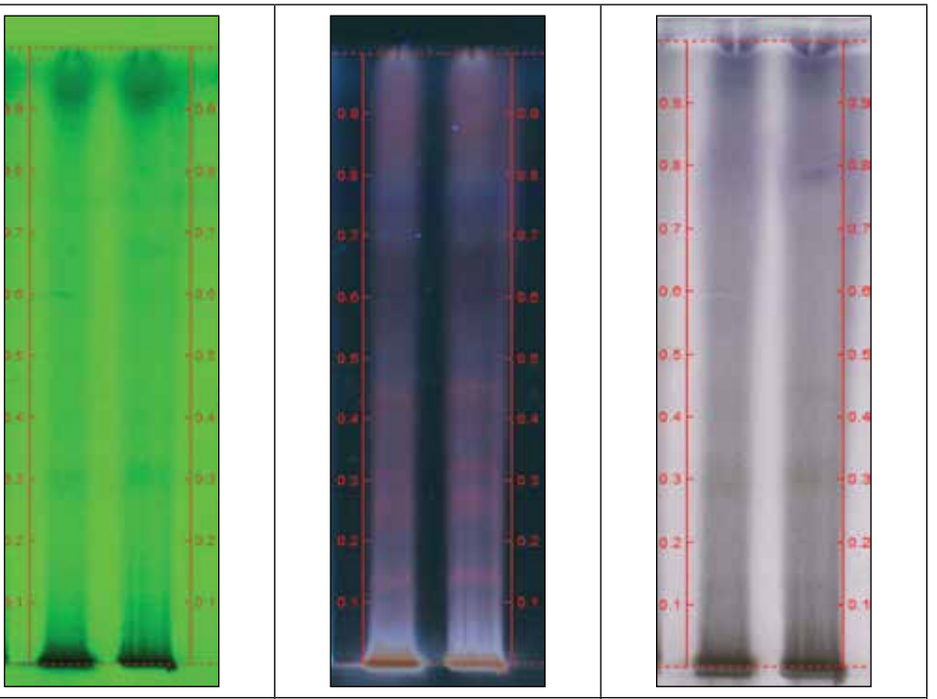
g. Fibres

Table 1 : Quantitative Microscopy

S. No.	Parameters Analysed	Observations	
		Range	Mean
1	Stomatal Number – lower epidermis	63 – 72 / sq. mm.	67.5
2	Stomatal Index – lower epidermis	24.28	24
3	Vein islet number	8.5 – 10.5 / sq. mm.	9.25
4	Veinlet termination number	15.5 – 22.0 / sq. mm.	19.25
5	Palisade ratio	5.5 – 6.8	6.15

Table 2 : Physico-chemical Parameters

S. No	Parameters	Results in % (w/w); n = 3
1.	Foreign matter	Nil
2.	Loss on drying at 105ºC	7.23
3.	Total ash	8.92
4.	Acid insoluble ash	0.099
5.	Alcohol soluble extractive values	5.94
6.	Water soluble extractive values	24.44

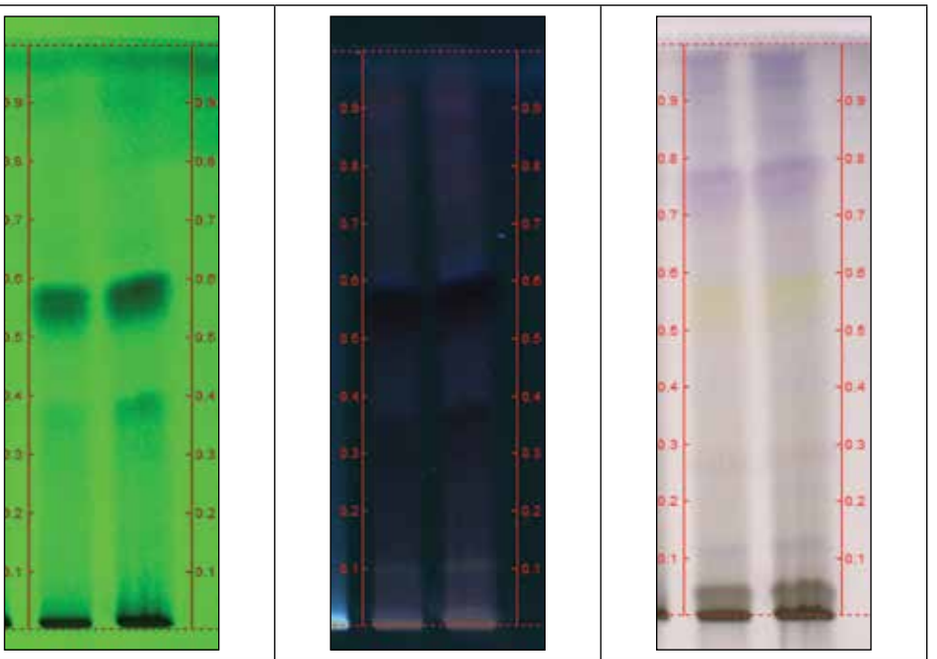


UV-254nm	UV-366nm	Visible light (After derivatized using Vanillin sulphuric acid reagent

Fig. 9 : TLC profile of Chloroform extract

Table 3 : Rf Values of Chloroform Extract (1:1)

Solvent system	Rf Values		
	UV 254nm (9 spots)	**UV 366nm (9 spots)**	**VS reagent (10 spots)**
Toluene : Ethyl acetate (1:1)	0.93 Dark green	0.89 Red	0.93 Dark green
	0.80 Green	0.78 Violet	0.80 Violet
	0.77 Green	0.53 Red	0.76 Light violet
	0.68 Dark green	0.46 Red	0.57 Light violet
	0.61 Green	0.42 Red	0.44 Light blue
	0.52 Green	0.39 Violet	0.32 Light blue
	0.40 Green	0.32 Red	0.29 Dark grey
	0.31 Dark green	0.28 Red	0.26 Light violet
	0.14 Green	0.15 Red	0.18 Light grey
			0.12 Dark grey



UV 254nm	UV 366nm	Visible light (After derivatized using Vanillin sulphuric acid reagent

Fig. 10 : TLC profile of Alcohol extract

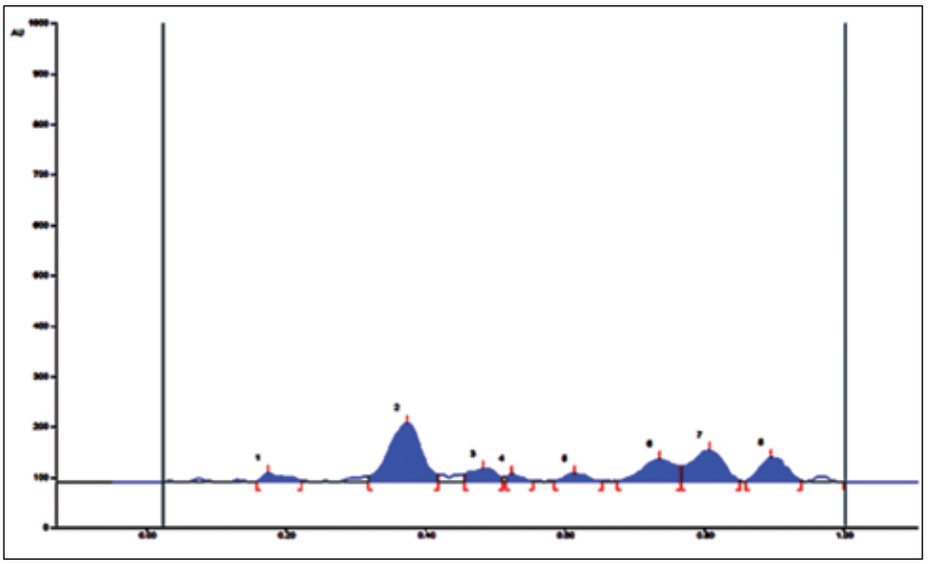


Fig. 11 : HPTLC fingerprint profile of Chloroform extract at 254nm

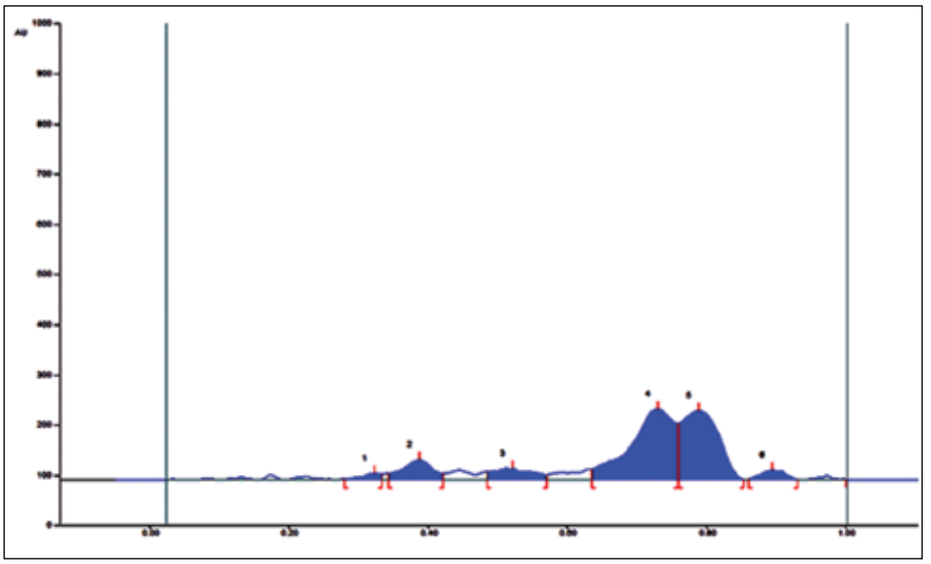


Fig. 12 : HPTLC fingerprint profile of Chloroform extract at 366nm

Table 4 : Rf Values of Alcohol Extract

Solvent system	Rf Values		
	UV 254nm (7 spots)	UV 366nm (7 spots)	VS reagent (10 spots)
Toluene : Ethyl acetate (1:1)	0.90 Green	0.91 Red	0.91 Violet
	0.80 Green	0.88 Light violet	0.83 Violet
	0.64 Green	0.85 Red	0.77 Dark violet
	0.57 Dark green	0.80 Light violet	0.57 Yellow
	0.38 Green	0.78 Red	0.39 Light violet
	0.29 Green	0.59 Violet	0.32 Yellow
	0.15 Green	0.57 Dark red	0.28 Light violet
		0.33 Dark red	0.20 Light violet
		0.30 Red	0.16 Yellow
		0.11 Light yellow	0.11 Blue

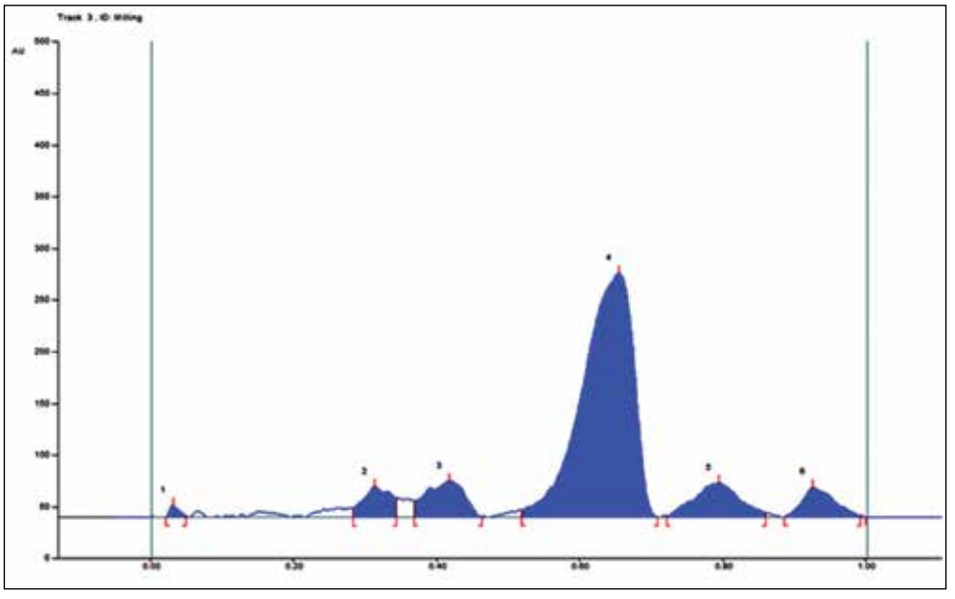


Fig. 13 : HPTLC fingerprint profile of Alcohol extract at 254nm

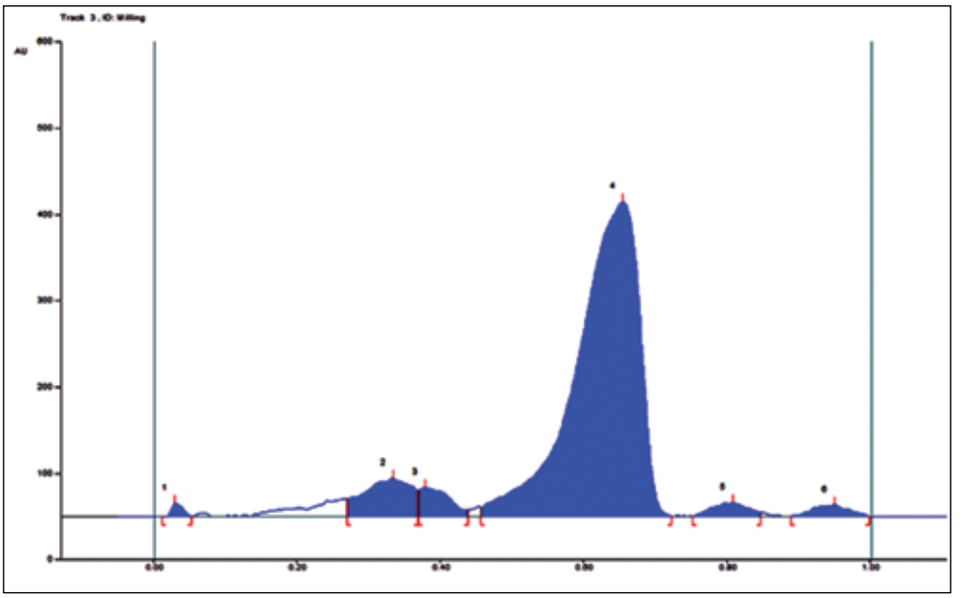


Fig. 14 : HPTLC fingerprint profile of Alcohol extract at 366nm

Table 5 : Microbial Load

S. No.	Parameter Analyzed	Results	Limits
1	Total Bacterial Count	2,600 CFU / gm	105 CFU / gm
2	Total Fungal Count	Absent	103 CFU / gm
3	Enterobacteriaceae	Absent	103 CFU / gm
4	*Salmonella* Spp.	Absent	Nil
5	*Staphylococcus aureus*	Absent	Nil

Table 6 : Heavy Metals

S. No.	Parameter Analyzed	Results	Limits
1	Lead	0.0142 ppm	10 ppm
2	Arsenic	Nil	3 ppm
3	Cadmium	Nil	0.3 ppm
4	Mercury	Nil	1 ppm

Table 7 : Estimation of Aflatoxins

S. No.	Aflatoxins	Results
1	B_1	Not detected
2	B_2	Not detected
3	G_1	Not detected
4	G_2	Not detected

Table 8 : Analysis of Pesticide Residues

S. No.	Pesticide Residues	Results
1	Organo Chlorine Group	ND
2	Organo Phosphorus Group	ND
3	Acephate	ND
4	Chlordane	ND
5	Dimethoate	ND
6	Endosulphan	ND
7	Endosulfan	ND
8	Endosulfon	ND
9	Ethion	ND
10	Endosufon sulphate	ND
11	Fenthion	ND
12	Lindane	ND
13	Methoxychlor	ND
14	Phorate sulfoxide	ND
15	Phorate sulfone	ND
	ND – Not detected	

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सारांश

मिलिंगटोनिया हार्टेंसिस एल.एफ लिफ का भेषजज्ञान मूल्यांकन एवं एच.पी.टी.एल.सी अगुलांक अध्ययन

[1]मागेश्वरी एस, [1]पवन कुमार सागर, [1]मीरा देवी श्री पी, [1]मुरूगेश्वरन आर., [2]रामप्रताप मीणा
[3]शमसूल आरीफीन और [1]आसिया खानम

मिलिंगटोनिया हॉर्टेंसिस एल.एफ. आमतौर पर कार्क पेड़ के रूप में जाना जाता है जोकि बिगनोनिएसी परिवार के अन्तर्गत आता है। यह दक्षिणी एशिया देशों जैसे भारत, बर्मा, थाईलैंड तथा दक्षिण चीन में पाया जाने वाला एक महत्वपूर्ण औषधीय पौधा है। पौधों की प्रमाणिकता और गुणवत्ता मानकों के लिए पौधे की ताजा पत्तियों को एकत्रित किया गया और मैक्रो–माइक्रोस्कोपिक, भौतिक रसायनिक और गुणवत्ता नियंत्रण मापदंडों का अध्ययन किया गया। माइक्रोस्कोपिक अध्ययन में पाया गया कि इसमें अंदर एनोमोसाइटिक स्टोमेटा के साथ वेवी एपिडर्मल सेल, पेलिसेड और स्पोंजी पैरेन्काइमा सेल्स, एक या दोनों किनारों पर टेल्स के साथ वाइडपिटेड वेसिल्स, फाइबर्स, 16 हेड सेल्स के साथ 50µ तक गलेनड्यूलर ट्राईकोम्स और 200µ लम्बाई के एक कोशिए ट्राईकोम्स विद्यमान है। भौतिक–रसायनिक आकड़ों से पता चलता है कि इसमें नमी 8.42%, कुल ऐश 8.93%, एसिड इन्सोल्यूबिल ऐश 0.098% और एल्कोहोल और वाटर सोल्यूबिल एक्स्ट्रैक्टिव मात्रा क्रमशः 5.92% और 24.44% थी। क्लोरोफॉर्म एवं एल्कोहोल एक्स्ट्रेक्ट के टी.एल.सी./एच.पी.टी.एल.सी. अध्ययन ने 254 एन.एम, 366 एन.एम और डेरीवेटाइज़्ड प्लेट्स (बेनीलिन–सल्फ्यूरिक एसिड रिएजेन्ट) के विभिन्न स्पॉट्स/पीक्स दर्शाए। औषधि की गुणवत्ता नियंत्रण मानदंड जैसे कि सूक्ष्म दर्शीय जीवाणु, भारी धातुऐं (जैसे, एएस, सीडी, पीबी, और एचजी) भी स्वीकार्य सीमा के भीतर पाए गए। एफलैटॉक्सिन जैसे, बी1, बी2, जी1, एवं जी2 भी अनुपस्थित पाए गए। यह अध्ययन दवा के सूखे एवं ताजा रूप में पहचान और प्रमाणीकरण करने के लिए बहुत ही उपयोगी साबित होगी। किसी भी विश्लेषणात्मक और जैविक अध्ययन के लिए मूल्यांकित किए गए फाइटोकेमिकल डेटा निकट भविष्य में भेषजकोशकीय मानकों के रूप में काम करेंगे।

Standardization and Phytochemical Screening of a Unani Compound Formulation UNIM 041 (Mushil Drug) along with Modern Analytical Technique

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Abstract

Herbal medicine has seen quite phenomenal growth in the recent years. India has a wealth of flora with hundreds of plants possessing medicinal or curative properties. Despite this wealth, India has a small share in medicinal plants trade in the world market. This dismal condition is attributable to several factors including non-identification of bio-active molecules, lack of uniformity in extraction and formulation processes, quality control, standardization of drugs etc. There is a great need for the standardization of drugs, development of standard operating procedures and scientifically validated analytical methods to provide evidence. The current formulation under study UNIM 041 is a Unani compound drug (Mushil) prescribed to the patients by the Unani Physicians in order to prepare the waste products for excretion which occurs by the expelling process and to bring the body in equilibrium. There are different types of Munzij and Mushil drugs which are administered to the patients depending on the humour involved in that particular disorder for the patient of the bars (vitiligo). Therefore, UNIM 041 has been taken up in this study to carry out standardization.

Key Words: UNIM 041, Standardization, Physico-chemical analysis, TLC.

Introduction

In Unani system of medicine the Munzij-e-Balgham (maturative for phlem) and Mushil (expulsion by purgation) therapy forms a unique and specific line of treatment. The Munzij and Mushil (MM) therapy is for humoral derangement of the body. This therapy is useful in chronic and established diseases. According to drug Soorate Naueya (structural property), the drugs pass so many substances through intestine. So some drugs help to excrete Phlegmatic matter, some bile matter and some Phlegmatic matter. Mus-hil (Purgative) increases frequency of stool. These act in several ways either by squeeze or by increasing peristaltic movement or the other way. These also pass balghem, sauda and safra and so they are also called as Mus-hil balghem and Mus-hil sauda, Mus-hil safra. The Mushil-e-balghem (Phlegmagogue and Phlegm purg) drugs due to their particular structural property excrete Phlegm through intestine (Hifzul Kabir, 2002).

Munzij brings about the correction of the abnormal humour at the level of Hazm-e-Uzvi (digestion at the tissue level), thus facilitating the nutrient to assemble and prepare the waste products for excretion which occurs by the expelling process of Mushil therapy. There are different types of Munzij and Mushil drugs which are administered to the patients depending on the humour involved in that particular disorder here for patient of the bars (vitiligo). It is given as per the mode of administration of Munzij-e-Balgham (maturative for phlem) and Mushil

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(purgative/expulsive) (Anonymous, 1986). *Bars* (Vitiligo) is a chronic disease and usually caused by excessive accumulation of *balgham-e-ghaleez* (thick phlegm). Therefore, all the *Unani* physicians are of the opinion that its treatment should be started with *Tanqiyah-e-Badan* (removal of morbid material from the body) through *Munzij* (concoctive) and *Mushil e-balgham* (laxative to phlegm) (Alam et al., 2014). Munzij-e-Balgham was given in cases of Bars as it was supposed that Balgham (Phlegm) plays an important role in the causation of the disease.

The current formulation under study UNIM 041 is a Unani compound drug (Mushil) prescribed to the patients according to the Unani classical method by the Unani Physicians in order to prepare waste products for excretion which occurs by the expelling process and to bring the body in equilibrium. Hence UNIM 041 has been taken up to carry out the UPLC analysis with fingerprinting profiling which may help in the quality control and authentication of the formulation and also in batch to batch analysis for consistency. This method is also extended to quantitative estimation and identification of compounds. The formulation under study is a Unani coded compound formulation i.e., UNIM 041. It is rough or coarse powder. The formulation consists of 1. Zanjabeel (*Zingiber officinale* Rosc) 2. Sana (*Cassia angustifolia* Linn.) and 3. Turbud (*Operculina turpethum* (L.) Silva Manso.). WHO has emphasized the need to ensure quality control of herbal drugs by using modern techniques (Imam, et al., 2009); (Rasheed, et al., 2010a; 2010b; 2010c;2010d; 2011; 2012,2013; 2014a; 2014b) and (Naikodi et al., 2011) and applying suitable parameters and standards. It was subjected to the analysis of physico-chemical parameters, microbial load, aflatoxin and thin layer chromatographic studies (Anonymous, 2009). The present paper describes the salient features of UNIM 041 in terms of phytochemical screening and drug standardization.

Material and Methods

Samples of UNIM 041 and its ingredients Zanjabeel, Sana and Turbud were procured from the pharmacy, Central Research Institute of Unani medicine, Hyderabad and reference standards viz., Sennoside A, Sennoside B and 6-gingerol were procured from Sigma Aldrich, Bangalore, India (Figure 1). Syringe filter PTFE membrane of 0.22 µm pore size, dia. 25mm (Axiva). All reagents used were of HPLC grade. HPLC-grade methanol, acetonitrile, water, formic acid, orthophosphoric acid (Fischer scientific, India) and triflouroacetic acid (Loba Chemie, India) were used.

Physico-Chemical Parameters

The Physico-Chemical parameters of compound formulation UNIM 041 was studied in terms of total ash, acid insoluble ash, alcohol soluble matter and water soluble matter, microbial load and aflatoxins as per the methods described in

Anonymous, 2009. Thin layer chromatography was carried out in the methanolic extract of UNIM 041 and its ingredients. Phyto-chemical screening was carried out in different solvents extracts such as methanol and aqueous as per the methods described by Evans, 2002. Microbial load, fungal count and Aflatoxins contamination were analyzed as per the methods described in WHO guidelines (Anonymous, 1998).

Thin Layer Chromatography Profile

Preparation of Extract of the Sample Drug

Five grams of fine powder of UNIM 041was dissolved in 100 ml of aqueous and methanol separately in a Stoppered conical flask and was kept for 2 hours and in the meantime shaking of the flask continued at regular intervals. Later the contents were filtered through whattmann No. 41 paper and evaporate the solution to 20 ml. Zanjabeel, Sana and Turbud powder was taken and prepared similar to UNIM 041. The resultant extracts were used to carry out TLC.

Development and Determination of the Solvent System

The samples were spotted as 5mm band on Precoated Aluminium Sheets of Silica Gel 60 F254 (Merck). After trying with various solvent system with variable volume ratios, the suitable solvent system as stated in Table 2 was selected in its proportional ratio and developed in the Twin through TLC chamber to the maximum height of the plate so that components are separated on the polar phase of silica gel and mobile phase of solvent system.

Detection System

After developing, the TLC plate was dried completely and detected under the UV and also exposed to iodine vapours for detection of spots and photographed as shown in Figure 2.

Preparation of Sample Solutions

Initially fine powder formulation UNIM 041 and its ingredients viz., Zanjabeel, Sana and Turbud was prepared. Accurately weighed 2gms each and transferred to a 100 ml beaker and extracted with 20ml methanol by Ultrasonic extraction method with the help of Ultrasonicator for 15 minutes. Sample extract was filtered through syringe filter PTFE membrane of 0.22 µm pore size and stored. Thus obtained solution was used for further analysis.

Accurately weighed 2gms each and transferred to a 100 ml beaker and extracted with 20ml each of water and 50% hydroalcoholic (50:50) for aqueous and hydroalcoholic (50:50) extracts by Ultrasonic extraction method with the help of

Ultrasonicator for 15 minutes. Sample extract was filtered through syringe filter PTFE membrane of 0.22 µm pore size and stored. Thus obtained solution was used for further analysis.

UPLC analysis

The study describes the UPLC analysis which was carried out on Waters Acquity UPLC H-Class Bio system with the BEH C18 reversed-phase analytical column (2.1 × 100 mm, 1.7 µm) at a flow rate of 0.2 mL/min. The detection wavelength was set at 280 nm. The injection volume was 2 µL, and the column temperature was maintained at 40°C. The mobile phase consisted of the solvent A (0.1%, v/v solution of formic acid in water) and solvent B (0.1%, v/v solution of formic acid in methanol) filtered through a 0.22 µm membrane filter using the gradient elution as 0 min 0% B, 0.20 min 15% B, 8 min 25% B, 24 min 30% B, 32 min 50% B, 50 min 90% B and 55 min 90% B, and 60 min 15% B. The data were collected and analyzed using Waters Acquity UPLC Empower 3 Software.

UPLC Analysis of Different Solvent Extracts of Aqueous, Hydroalcoholic (50:50) and Methanolic Extracts of UNIM 041

Aqueous, hydroalcoholic (50:50) and methanol extracts of UNIM 041 were subjected to reversed phase UPLC-PDA on Waters Acquity BEH C18 column under the optimized chromatographic conditions and the assay for the 6-gingerol, Sennoside A and Sennoside B were carried out.

Batch to Batch Analysis for UNIM 041 Formulations

The assay for the twelve batches of UNIM 041 formulation was subjected to methanolic extraction through ultrasonication method separately. The extract was filtered through 0.22µm PTFE syringe filter; the solution obtained was used for analysis.

Results and Discussion

Standardization and Quality Control Analysis

The Physico-chemical parameters were analyzed in the formulation as per the Unani pharmacopoeial methods as shown in Table 1. The parameters values for Zanjabeel as total ash was found to be 4.32-5.21 gm%, and acid insoluble ash 0.61-0.69 gm%, alcohol soluble extract 3.46-3.57 gm% and water soluble extract 14.79-15.48 gm% whereas for Sana as total ash was found to be 10.23-10.43 gm%, and acid insoluble ash 0.45-0.48 gm%, alcohol soluble extract 14.61-15.06 gm% and water soluble extract 40.38-41.26 gm% whereas for Turbud as total ash was found to be 4.60-4.68 gm%, and acid insoluble ash 1.00-1.20 gm%,

alcohol soluble extract 11.77-13.24 gm% and water soluble extract 9.50-10.10 gm% and in the UNIM 041 as total ash was found to be 7.52-8.90 gm%, and acid insoluble ash 4.39- 4.99 gm%, alcohol soluble extract 10.57-12.80 gm% and water soluble extract 25.96-28.45 gm%.

TLC Analysis

The methanolic extracts of formulation and its ingredients were subjected to TLC on Silica gel 'G' plate using Toluene: Ethyl acetate: methanol (7:2:1) and detected using the UV visible chamber which clearly showed various spots at UV 366nm and under iodine vapours. The formulation UNIM 041 under UV 366 nm detection shows nine spots at Rf values 0.18 (pink), 0.25 (blue), 0.32 (pink), 0.40 (blue), 0.54 (Fluorescent blue), 0.64 (light blue), 0.76 (yellow), 0.90 (red) and 0.94 (red). Up on exposure to Iodine vapour shows three spots at Rf values 0.61, 0.79 and 0.90 (All brown). Zanjabeel extract under UV 366 nm detection shows one spot at Rf value 0.78 (Green). Upon exposure to Iodine vapour shows one spot at Rf value 0.79 (brown).

Sana extract under UV 366 nm detection shows nine spots at Rf values 0.03 (blue), 0.06 (blue), 0.18 (red), 0.25 (red), 0.32 (red), 0.67 (red), 0.76 (red), 0.90 (red) and 0.94 (red). Upon exposure to Iodine vapour shows three spots at Rf values 0.06, 0.60 and 0.89 (all brown). Turbud extract under UV 366 nm detection shows two spots at Rf values 0.37 (light blue) and 0.88 (light blue). Upon exposure to Iodine vapour shows two spots at Rf values 0.61 and 0.79 (all brown). The data are presented in Table 1. Thus established a TLC profile which helps in the quality control analysis of formulation as a reference.

Phyto-chemical screening was carried out as qualitative test for the phytoconstituents presence. It was observed that alkaloids, carbohydrates, fixed oil, glycosides, phenols, proteins, steroids, saponins, tannins and flavonoids are shown to be positive and negative for starch as shown in Table 2. The results of total microbial load and total fungal count studies were found to be within the permissible limits and the other parameters were found to be absent in the formulation which are given in Table 3.

Ultra-Performance Liquid Chromatography-Photo Diode Array Detector Analysis

The UPLC analytical method was successfully used to simultaneously determine three components in UNIM 041 samples. Standard mixture of Sennoside B, Sennoside A and 6-gingerol were subjected to UPLC-PDA analysis. The corresponding chromatogram at 280nm is shown in Figure 3a. In the UPLC chromatogram of standard mixture, the reference compound peaks detected at retention times t_R 25.707 minutes for Sennoside B, 31.158 minutes for Sennoside A and t_R 40.078 minutes for 6- gingerol. Their corresponding UV absorbance

max at 267.5, 363.4nm for Sennoside B, 267.5, 340.4 nm for Sennoside A, and 281.0 nm for 6- gingerol. The methanolic extract of UNIM 041 formulation was subjected to UPLC-PDA analysis. The corresponding chromatogram at 280nm is shown in Figure 3b and the peak list with retention time is given in Table 4. In the chromatogram the peaks detected at retention times t_R 25.391 minutes for Sennoside B, 31. 014 minutes for Sennoside A and t_R 40. 025 minutes for 6- gingerol. Their corresponding UV absorbance max at 267.5, 363.4nm for Sennoside B, 267.5, 340.4 nm for Sennoside A, and 281.0 nm for 6- gingerol.

Different Solvent Extract Assay for the UNIM 041

Aqueous, hydroalcoholic (50:50) and methanol extracts of UNIM 041 were subjected to reversed phase UPLC-PDA on Waters Acquity BEH C18 column. The UPLC analysis under the optimized chromatographic conditions the peaks for the marker compounds of Sennoside B, Sennoside A and 6- gingerol were detected and identified with the UV data. The amount of Sennoside B, Sennoside A and 6- gingerol present in three different extracts in mg/g was estimated. In aqueous extract of UNIM 041, the amount of sennoside A and 6 Gingerol were found as 0.0486 and 0.0837 mg/g respectively; whereas in the hydroalcoholic extract, the amount of the Sennoside B, Sennoside A and 6-gingerol were found as 0.5333, 0.2598 and 0.3781 mg/g respectively and in the methanolic extract of UNIM 041 formulations, the amount of Sennoside B, Sennoside A and 6-gingerol were found as 0.7449, 0.4480 and 0.6322 mg/g respectively. It was found that the content of Sennoside B, Sennoside A and 6- gingerol is more in methanol extract as compared to that of aqueous and hydro alcoholic extract of UNIM 041.

Batch to Batch Analysis of UNIM 041

The assay for the twelve batches of UNIM 041 formulation was subjected to methanolic extraction through ultrasonication method separately. Under the optimized chromatographic conditions the results obtained are as shown in Figure 4. The corresponding peaks Sennoside B, Sennoside A and 6-gingerol in formulation were identified in the chromatogram by injecting the standard marker compounds. Identification was done with respect to retention times of sennoside B, Sennoside A and 6-gingerof and UV spectra were recorded by PDA detector. The assay of 12 different batches of UNIM 041 for the three components was identified by comparing retention times and UV spectra with authentic standards in the methanolic extract of UNIM 041

The assay for quantification of Sennoside B, Sennoside A and 6-gingerol in various batches for UNIM 041 formulations were found in the range 0.09% -0.12%, 0.046%-0.050% and 0.06%-0.07% respectively expressed in mg/g and the graphical representation is shown in Figure 4. It can be seen that the

measured amount agree with the actual values.

Conclusion

The Unani formulation UNIM 041 (Mushil drug) under study was subjected to Physico-chemical analysis which is helpful in establishing the standard along with the other parameters such as phyto-chemical screening and TLC analysis. The safety evaluation for aflatoxins contamination analysis was done and found absent; microbial load was found within the permissible limits of WHO guidelines. Modern analytical technique of UPLC analysis was employed with respect to separate compounds and generate fingerprint pattern for the formulation. The reverse phase UPLC-PDA method was successfully applied to determine the three active compounds in twelve different batches of UNIM 041 for the quantitative estimation of Sennoside B, Sennoside A and 6-gingerol. The assay for quantification of Sennoside B, Sennoside A and 6-gingerol in various batches for UNIM 041 formulations were found in the range of 0.09 %-0.12%, 0.046%-0.050% and 0.06%-0.07%, respectively expressed in mg/g. Thus the method could be used for batch to batch quality control analysis development as well as for quality assurance of UNIM 041. The different solvent extracts viz. UPLC analysis found that the amount of Sennoside B, Sennoside A and 6- gingerol is more in methanol extract as compared to that of aqueous hydro alcoholic extract of UNIM 041. Thus, the formulation UNIM 041 was successfully standardized along with physico-chemical parameters, TLC, UPLC-PDA analysis, batch to batch assay and different solvent extract assay.

Table 1 : Physico-Chemical Parameters of the Compound Formulation UNIM 041 and Ingredients.

S.No.	Name	Total Ash (gm%)	Acid insol. Ash (gm%)	Alc. Sol. Ext (gm%)	Wat. Sol. Ext (gm%)	TLC of methanolic extract on Silica gel 'G' plate using Toluene: Ethyl acetate: methanol (7:2:1)
1	**Zanjabeel**	4.32-5.21	0.61-0.69	3.46-3.57	14.79-15.48	Shows under UV (366 nm) one spot at R_f value 0.78 (Green). Upon exposure to Iodine vapour shows one spot at R_f. 0.79 (brown).

S.No.	Name	Total Ash (gm%)	Acid insol. Ash (gm%)	Alc. Sol. Ext (gm%)	Wat. Sol. Ext (gm%)	TLC of methanolic extract on Silica gel 'G' plate using Toluene: Ethyl acetate: methanol (7:2:1)
2	**Sana**	10.23-10.43	0.45-0.48	14.61-15.06	40.38-41.26	Shows under UV (366 nm) nine spots at R_f Values 0.03 (blue), 0.06 (blue), 0.18 (red), 0.25 (red), 0.32 (red), 0.67 (red), 0.76 (red), 0.90 (red) and 0.94 (red). Upon exposure to Iodine vapour shows three spots at R_f Values 0.06, 0.60, 0.89 (all brown).
3	**Turbud**	4.60-4.68	1.00-1.20	11.77-13.24	9.50-10.10	shows under UV (366 nm) two spots at R_f values 0.37 (light blue) and 0.88 (light blue). Upon exposure to Iodine vapour shows two spots at R_f values 0.61, 0.79 (all brown).
4	**UNIM 041**	7.52-8.90	4.39-4.99	10.57-12.80	25.96-28.45	shows under UV (366 nm) nine spots at R_f values 0.18 (pink), 0.25 (blue), 0.32 (pink), 0.40 (blue), 0.54 (Fluorescent blue), 0.64 (light blue), 0.76 (yellow), 0.90 (red) and 0.94 (red). On exposure to Iodine vapour shows three spots at R_f values 0.61, 0.79 and 0.90 (All brown).

Table 2 : Phytochemical Screening in Methanol and Aqueous Extracts of UNIM 041.

S. No.	Phytoconstituent	Methanol ext.	Aqueous ext.
1.	Alkaloid	+	+
2.	Carbohydrates	+	+
3.	Fixed oil /Resinified volatile oils	+	+
4.	Glycosides	+	+

S. No.	Phytoconstituent	Methanol ext.	Aqueous ext.
5.	Phenols	+	+
6.	Saponins	+	+
7.	Proteins	+	+
8.	Steroids	+	+
9.	Tannins	+	+
10.	Flavonoids	+	+
11.	Starch	-	-

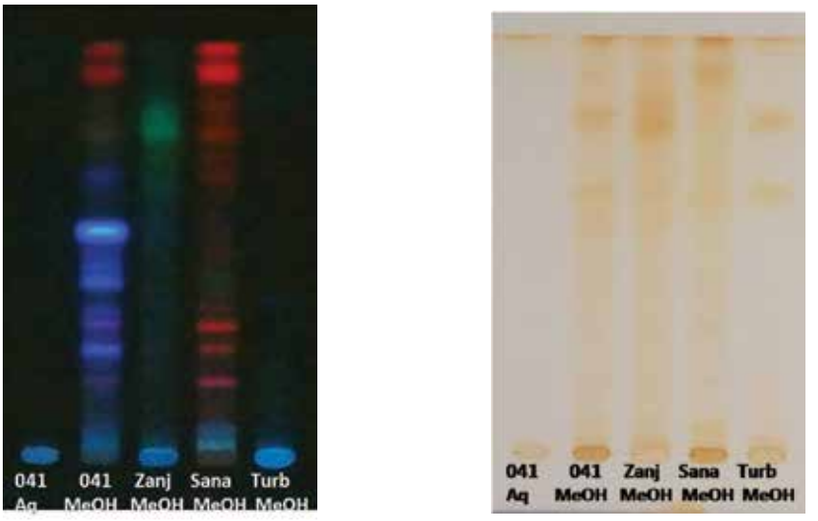
Table 3 : Aflatoxins, Microbial and Fungal Contamination in the UNIM 041.

Aflatoxin Contamination (Permissible limits as per WHO)					
S. No.	**Name**	**B1 (NMT 0.50 ppm)**	**B2 (NMT 0.10 ppm)**	**G1 (NMT 0.50 ppm)**	**G2 (NMT 0.10 ppm)**
1	Zanjabeel	Nil	Nil	Nil	Nil
2	Sana	Nil	Nil	Nil	Nil
3	Turbud	Nil	Nil	Nil	Nil
4	UNIM 041	Nil	Nil	Nil	Nil
Microbial and fungal Contamination (Permissible limits as per WHO)					
S. No.	**Name**	**Total Microbial Load (NMT 105/g)**	**Salmonella Spp (Nil)**	**Escherichia Coli (Nil)**	**Total Fungal count (NMT 103/g)**
1	Zanjabeel	27 x 102	Nil	Nil	15 x 102
2	Sana	12x103	Nil	Nil	1x10
3	Turbud	Nil	Nil	Nil	Nil
4	UNIM 041	81 x 103	Nil	Nil	21 x 102

Table 4 : Peak List and Retention Time of Unim 041 Under Optimized Chromatographic Condition.

S. No.	Name	Retention Time	Area	% Area	Height	% Height
1	Sennoside B	25.391	104198	12.24	9115	6.12
2	Sennoside A	31.014	329278	38.67	62168	41.75
3	6-gingerol	40.025	418089	49.10	77610	52.12

Fig. 1 Photograph of formulation UNIM 041 and its ingredients used



At UV 366nm Exposed to Iodine vapours

Fig. 2 TLC plate of UNIM 041 formulation 1.Aq:Aqueous extract 2.MeOH:Methanolic extract 3.Zanj:Zanjabeel 4.Sana 5.Turb:Turbud *(Mobile Phase: Toluene: Ethyl acetate: methanol (7:2:1))*

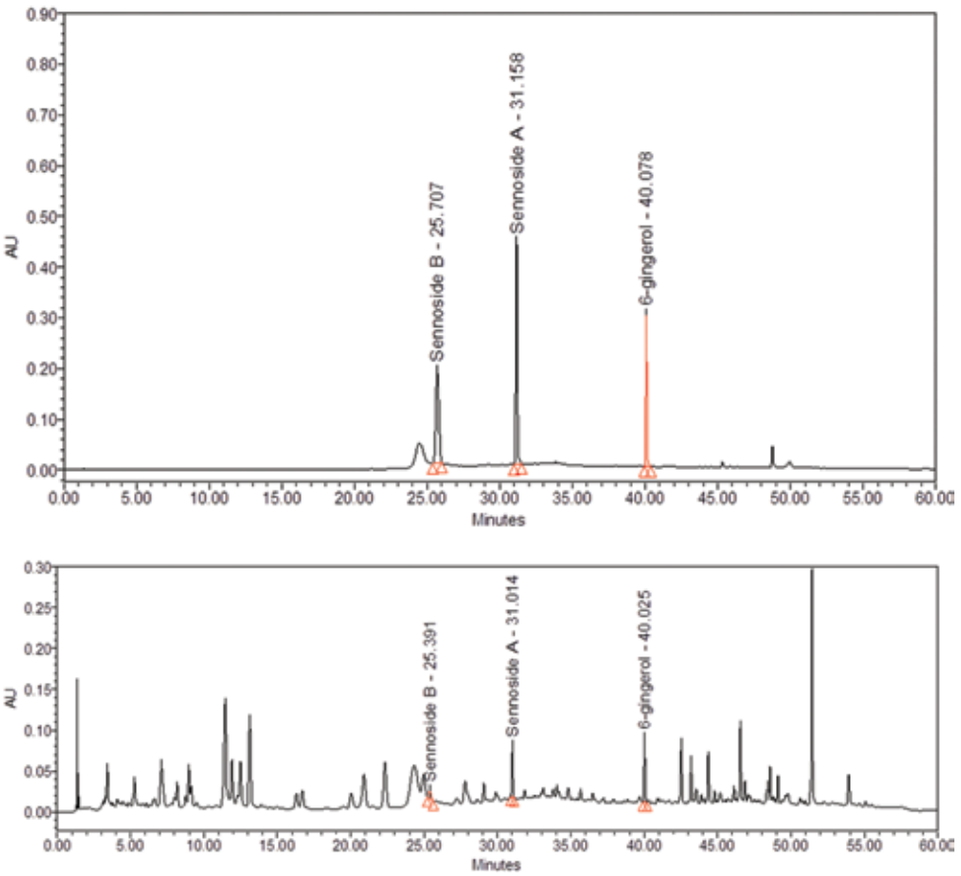
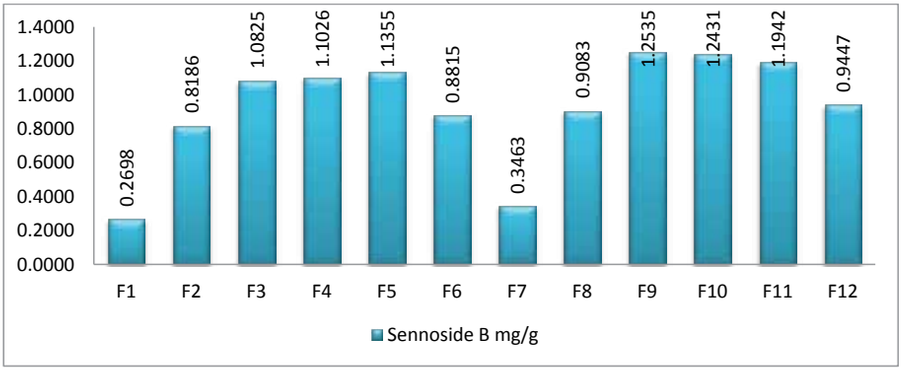
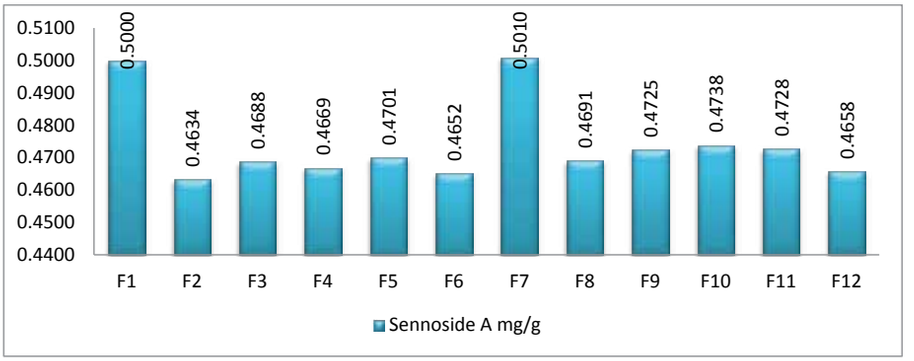


Fig. 3 UPLC chromatogram of a) standards of Sennoside B, Sennoside A, 6-gingerol. (b) UNIM 041 formulation.





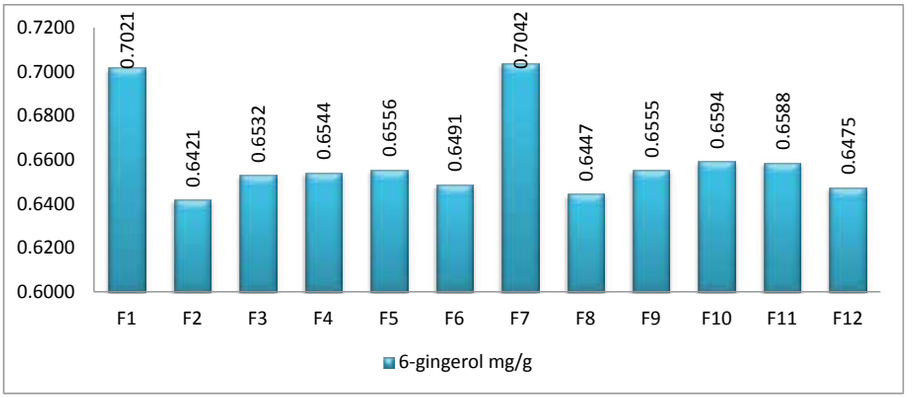


Fig. 4 Assay of 12 batches UNIM 041

Acknowledgement

The authors would like to record their gratitude to the Director General, CCRUM, New Delhi, for providing necessary facilities to carry out this study. The authors also thank the associated staff of Central Research Institute of Unani Medicine, Hyderabad, for their constant support and encouragement throughout the study.

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सारांश

यूनानी कंपाउंड फॉर्मूलेशन यूनिम-041 (मुसहिल औषधि) का आधुनिक विश्लेषणात्मक तकनीक द्वारा मानकीकरण एवं पादप-रसायनिक जाँच

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पिछले कुछ वर्षों से हर्बल औषधियों में काफी अभूतपूर्व वृद्धि देखी गई है। भारत एक वनस्पति औषधियों का भंडार है जिनमें सैकड़ों पौधों में औषधीय तथा रोग निवारक गुण पाए जाते हैं। इस विशाल भंडार के बावजूद, विश्व बाजार में औषधीय पादप व्यापार में भारत का छोटा सा स्थान है। यह निराशजनक स्थिति कई कारकों के कारण है जिनमें जैविक–सक्रिय अणुओं की पहचान न होना, निष्कर्षण और निर्माण प्रक्रियाओं में एकरूपता की कमी, गुणवत्ता नियंत्रण एवं औषधियों का मानकीकरण आदि शामिल है। आज के युग में औषधियों को गुणवत्ता प्रदान करने के लिए इनका मानकीकरण करना, मानक संचालन प्रक्रियाओं को विकसित करना एवं वैज्ञानिक तरीके से आधुनिक विधियों द्वारा विश्लेषण करना अति आवश्यक है। वर्तमान अध्ययन के अन्तर्गत यह देखा गया है कि औषधि यूनिम–041 एक यूनानी मिश्रित औषधि है जिसे यूनानी चिकित्सक रोगियों के उत्सर्जन एवं निष्कासन प्रक्रिया द्वारा शरीर को संतुलन में लाने के लिए उपयोग करते हैं। यूनानी पद्धति में अलग–अलग तरीके की मुंज़िज एवं मुस्हिल औषधियाँ पाई जाती हैं जिन्हें रोगियों की मनोवृत्ति के आधार पर एक विशेष रोग जैसे विटिलिगो आदि में दी जाती है। चूँकि औषधि यूनिम–041 विटिलिगो बीमारी में बहुत ही उपयोगी पाई गई है। अतः यूनिम–041 को मानकीकरण अध्ययन करने के लिए चुना गया है।

Acute and Sub-acute Oral Toxicity Studies of Majoon-IQ – A Unani Brain Tonic

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Abstract

Majoon-e-IQ is a Unani herbal formulation and used as a brain tonic. The objective of this study was to investigate the acute and sub-acute oral toxicity of Majoon-IQ in Wistar Albino rats of either sex. Acute oral toxicity study was conducted as per OECD-425 guideline in which Majoon-IQ was administered at a dose level of 5000mg/kg b.w. to both male and female rats and the animals were then observed individually for 30 minutes, 4 hour post dosing and at least twice daily for 14 days. Sub-acute oral toxicity study was conducted as per OECD-407 guidelines. In sub-acute oral toxicity study Majoon-IQ was administered at the dose level of 4800 mg/kg b.w. in a single bolus everyday for 28 days. The rats were observed daily during the period of study. After overnight fasting, rats were sacrificed on 15th day and 29th day. Observation parameters included a comparative evaluation of the general appearance/behaviour, morbidity/mortality, body weights, food/water consumption, haematology parameters, bio-chemistry parameters and histopathology of major organs of treated and control groups. No any adverse effect was observed in both the toxicity studies indicating that Majoon-IQ is free from any toxic effects under the conditions of these studies.

Keywords: Majoon-IQ; Brain tonic; OECD guidelines; Acute toxicity; Sub-acute toxicity.

Introduction

Unani system of Medicine originated from Greece. Hippocrates (460-377 BC) was the ancient Greek philosopher-physician who freed Medicine from the sphere of magic and superstition (Ahmad *et al.* 2010). The fundamentals of Unani medicine are based on his teachings. After Hippocrates, a number of other Greek scholars enriched the system significantly. Among them, Galen (131-210 AD) was the one who strengthened its foundation on which Arab physicians like Rhazes (850-1037 AD) and Avicenna constructed a huge and magnificent structure (Anonymous 2007; Chaudhary *et al.*, 2013). The Unani system of medicine in which plants (whole or parts) are used as herbal drugs to cure various ailments. Its use is quite prevalent and has potential for improving health and lowering the cost of treatment and thus makes health care affordable by all. A lot of herbs are used still for treating various diseases; the reason behind this is that most of the people believe that they have less toxic effects and more synergic effects (Azmat *et al.*, 2012). The literature on herbal medicine mentions several herbs exerting influence on brain function in general and memory in particular (Steven *et al.*,2002) Various Unani preparations are being used as a brain tonic. One such preparation is Majoon-IQ which is used as a brain tonic. Efficacy of Majoon-e-IQ is well defined but limited data are available pertaining to its toxicity profile as per the standard guidelines.

In this study, an effort was made to generate the toxicity profile of Majoon-IQ as per internationally accepted standards. Dosage of Majoon-IQ in Humans is 16

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grams per day. Majoon-IQ was administered to human subjects every day which amounts to 230mg/kg/day dose. The dosage in acute study was 5000mg /kg body weight which is the limit of dose while as the dosage for sub-acute study was 4800mg/kg body weight as it corresponds to the 3X of the rate extrapolated dose, while as X is the extrapolated dose. In order to assess the potential toxic effects of Majoon-IQ, it was administered to young, healthy rats at different dose levels in the two different acute and sub-acute safety studies.

Table 1: Constituents of Majoon-IQ

S. No.	Ingredients	Botanical/ English Name	Part Used	Quantity
1.	Brahmi	*Bacopa monnieri* L.	Whole plant	40 g
2.	Asgandh	*Withania somnifera* L.	Root	40 g
3.	Filfil Safaid	*Piper nigrum* L.	Fruit	40 g
4.	Badam	*Prunus amygdalus*	Seed	80 g
5.	Khajoor	*Phoenix dactylifera* L.	Fruit	80 g
6.	Asal	Honey	---	1.2 kg

Material and Methods

Test Item.

The work was carried on Majoon-IQ supplied by CRIUM, Hyderabad and the date of manufacture was August 2014. Majoon-IQ was available as a semisolid paste which was mixed with distilled water to make the suspension.

Animals and Exposure Conditions

The experimental animals (young, healthy Albino rats of Wistar Strain of either sex) were procured from Indian Institute of Integrative Medicine, Jammu. These rats were kept in the animal house as per the International standards (Animal Research Review Panel, 2002) and observed during the quarantine and acclimatization period (Capdevila *et al.*, 2007). A veterinary examination was done on the rats prior to and at the end of the acclimatization and quarantine period of 14 days. The rats were housed under standard environmental condition i.e. temperature of 22 ± 2^{o}C with 12:12 hour dark and light cycle. The rats were provided pelleted feed procured from Pranov Agro Industries, New Delhi and distilled water ad libitum. The experimental work was carried out as per the guidelines set by CPCSEA, India. The study was approved by the IAEC, Regional Research Institute of Unani Medicine, Srinagar which is registered with CPCSEA, India with registration No. 927/GO/C/06/CPCSEA.

Acute Oral Toxicity Study

The acute oral toxicity test was carried as per (OECD 425, 2008). Albino rats of either sex 100-150 grams body weight (8-12 weeks of age) were used. Five animals of each sex were used in each of the two treated and control groups (total 20 animals). All the rats were weighed to record their initial body weight at

intial stage and on the 8th and 15th day of the experiment. The test substance was administered orally by using feeding canula. Rats were fasted overnight but allowed water ad libitum prior to feeding of drug. The Group I and II were the male and female Controls and given RO water in comparable volumes to the treated animals in a single bolus (Vehicle only). Animals of Group III (males) and IV (females) were treated with the Majoon-IQ at the dose level of 5000 mg per kg body weight suspended in distilled water. All the rats were observed individually for any acute toxicity signs and behavioural changes at an interval of 30 minutes, 4 hour post dosing and once daily for 14 days. During the study period of 14 days, the feed and water consumption / animal / 24 hours were recorded at the initial stage, after 1 week of dosing and at the end of the study. On the 15th day all the animals were sacrificed by exsanguinations by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under ISOFLURANE anaesthesia. Two millilitre of blood was added to EDTA vacutainer for the study of Haematological parameters and 3 ml blood was added to Red tap vacutainer containing the clotting activators. The clotted blood was centrifuged and the serum was separated for the study of bio-chemistry parameters. The internal organs were examined macroscopically for the visualization of morphological changes, if any.

Sub-Acute Oral Toxicity Study

The Sub-acute Oral Toxicity was conducted in accordance with the OECD-407 Guideline. The rats were randomly divided into 4 groups and each group consisted of 5 rats. The Groups I and II were the male and female Controls and orally treated with distilled water (Vehicle) and Groups III and IV were the male and female experimental and orally treated with Majoon-IQ single dose of 4800mg/kg body weight for 28 days daily. All the animals were closely observed for the first 1 and 4 hours of dosing to examine any adverse toxic signs, behavioural changes etc. The body weight of the rats was evaluated weekly. Food and Water consumption / animal / 24 hours were recorded before dosing and then weekly up to 4 weeks. On the 29th day, after over-night fast, all the animals were sacrificed by exsanguinations by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under ISOFLURANE anaesthesia. Two millilitre of blood was added to EDTA vacutainer for the study of Haematological parameters and 3 ml blood was added to Red tap vacutainer containing the clotting activators. The clotted blood was centrifuged and the serum was separated for the study of bio-chemistry parameters. All the animals were dissected to check macroscopic morphology of the body organs. The organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/testes and heart were collected to determine the relative organ weight followed by grossing for the collection of tissues for Histopathological studies.

Assessment of Haematological Parameters

Haematological parameters were analyzed in freshly collected blood in blue top vacutainer containing EDTA anticoagulant. The blood was gently mixed with the

EDTA anticoagulant coated on the tube walls. Haematological parameters were determined on a fully automatic haematological analyzer (Sysmex XT2000iV Sysmex Corporation, Japan). Haematological parameters such as Haemoglobin conc., WBC count, RBC count, haematocrit value, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin, Platelet count, differential leukocyte count – Neutrophil %, Lymphocyte %, Monocyte %, Eosinophil % and basophil %, and Reticulocyte count were studied.

Assessment of Bio-Chemical Parameters

Bio-chemical parameters were studied in serum obtained after centrifugation of blood at 2000 RPM for 15 minutes on the day of the rat sacrifice. Bio-chemical parameters were determined on a fully automatic bio-chemistry analyzer (XL640 TRANSASIA) using ERBA kits. Liver function tests- aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphotase ALP, Total bilirubin, total protein and albumin, kidney function tests- blood urea, uric acid, creatinine and other bio-chemical substances such as glucose, Cholesterol, Triglycerides and HDLC were estimated.

Histopathology

Tissue samples were collected from the organs of control as well as from treated male/female rats of the Sub-acute study. The tissue collected from the organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/ testes and heart ware numbered for identification and then transferred to tissue cassettes (SS) to enable fixation in 10 % formalin for 36-48 hours followed by the tissue processing which was carried on Automatic tissue processor Model No1020 (LIECA make, Germany). The tissue processing included dehydration in graded isopropyl alcohol, clearing in xylene I and xylene II, impregnation in paraffin wax and finally tissue blocks was prepared on paraffin block maker Model No1150H+C (LIECA make Germany). Section cutting of tissue blocks was done using microtome (YARCO) to the thickness of 4 – 5 microns.

The tissue sections were fixed on the slide by heat technique followed by staining (Haematoxylin and Eosin stain). The staining was carried on Automatic slide stainer (THERMO MAKE, Germany) haematoxylin and eosin staining. After staining, the tissue section was mounted with DPX to prevent any damage to the stained tissue. The stained tissue sections were examined under microscope 40x and 10x objective to check the adverse effects of drug on cell morphology as well as on the cell organelles.

Statistical Analysis

All the results are expressed as mean ± SD. Comparison of all the results on body weight, food and water consumption, haematological value and bio-chemical values were performed by one way analysis of variance (ANOVA) method using

statistical software Graph Pad Prism version 6.05. Probability of 0.05 or less ($p \leq 0.05$) was used as the criterion of significance.

Results

Acute Oral Toxicity Test

Group Mean Body Weight

The rats treated with Majoon-IQ at the dose of 5000mg/kg of body weight were found to grow and gain body weight normal and no deleterious effect was found on their body weight. Table 2 shows the body weight of rats in acute toxicity study.

Table 2: Body Weight of Rats in Acute Toxicity Study

Group	**Days**		
	Day 0 Mean ± SD	**Day 7 Mean ± SD**	**Day 14 Mean ± SD**
Male Control	140.8 ± 5.5	187.4 ± 14.3	213.4 ± 19.4
Male Treated	128.4 ± 5.8	168 ± 13.9	191 ± 19.4
Female Control	120.4 ± 5.8	154.8 ± 6.0	166.4 ± 9.4
Female Treated	133 ± 18.2	154 ± 11.9	177.6 ± 9.0

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Group Mean Food and Water Consumption

No significant change was observed in the feed and water consumption in rats after treatment with Majoon-IQ as compared to the control group rats as shown in Table 3.

Table 3 : Average Feed and Water Consumption by Rats

Group Mean Food Consumption Per Rat/24 Hours. (Grams)				**Group Mean Water Consumption Per Rat/24 Hours.(Millilitre)**	
	Male (Mean±SD)	**Female (Mean±SD)**		**Male (Mean±SD)**	**Female (Mean±SD)**
Control	18.46 ± 1.5	18.22 ± 2.0		27.54 ± 1.1	26.33 ± 2.0
Treated	18.39 ± 1.1	17.24 ± 1.0		26.27 ± 2.2	27.32 ± 0.5

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Haematological Parameters

The results of haematological parameters of the treated male and female rats did not show any significant change in the values when compared to the respective controls as indicated in Table 4.

Table 4: Haematological Parameters of Rats in Acute Toxicity Study

Parameter	Male		Female	
	Control (Mean±SD)	Treated (Mean±SD)	Control (Mean±SD)	Treated (Mean±SD)
WBC (10^3/µl)	11.17 ± 3.26	10.16 ± 0.91	11.54 ± 0.41	10.31 ± 1.04
RBC (10^3/µl)	7.63 ± 0.32	8.38 ± 0.26	7.59 ± 0.24	7.49 ± 0.23
Hb (grams %)	15.10 ± 0.45	16.10 ± 0.50	15.46 ± 0.33	14.68 ± 0.47
HCT (%)	44.54 ± 1.87	45.27 ± 1.09	45.48 ± 1.00	42.20 ± 1.54
MCV (Femtolitre)	58.44 ± 1.43	59.97 ± 0.59	60.04 ± 1.93	56.03 ± 0.83
MCH (pico grams)	19.84 ± 0.47	20.40 ± 0.17	20.40 ± 0.44	19.62 ± 0.27
MCHC (grams %)	33.96 ± 0.41	34.00 ± 0.40	34.00 ± 0.51	34.82 ± 0.32
Reticulocytes (%)	3.15 ± 0.08	2.6 ± 0.09	3.17 ± 0.66	3.55 ± 0.73
Platelet count (10^3/µl)	1140 ± 64.54	1261 ± 113.3	1005 ± 77.93	1018 ± 62.90
Differential Leucocyte Count				
Neutrophils %	16.03 ± 0.77	17.17 ± 1.75	14.44 ± 1.78	13.08 ± 0.93
Lymphocytes %	74.86 ± 1.28	74.00 ± 1.03	80.50 ± 1.02	75.14 ± 1.61
Monocytes %	5.43 ± 0.65	4.50 ± 0.46	6.22 ± 1.44	6.18 ± 0.87
Eosinophils %	3.56 ± 0.16	4.23 ± 0.99	2.62 ± 0.62	2.46 ± 0.94
Basophils %	0.28 ± 0.09	0.16 ± 0.03	1.22 ± 0.60	0.14 ± 0.02

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Bio-chemistry Parameters

The results of bio-chemical parameters did not show any significant change in the group treated with Majoon-IQ when compared to the respective control groups (Table 5).

Table 5: Bio-chemical Parameters of Rats in Acute Oral Toxicity Study

Parameter	Male		Female	
	Control (Mean±SD)	Treated (Mean±SD)	Control (Mean±SD)	Treated (Mean±SD)
Liver Function Tests				
ALT (IU/l)	60.98 ± 5.18	69.63 ± 2.68	66.36 ± 2.42	67.64 ± 3.75
AST (IU/l)	121.1 ± 9.54	122.3 ± 4.60	117.7 ± 4.57	115.7 ± 8.20

ALP (IU/l)	174.2 ± 21.06	164.3 ± 14.38	169.4 ± 12.66	181.2 ± 22.01
Total bilirubin (mg/dl)	0.092 ± 0.01	0.097 ± 0.01	0.096 ± 0.005	0.082 ± 0.01
Total protein (g/dl)	7.39 ± 0.18	6.93 ± 0.13	7.93 ± 0.13	6.76 ± 0.13
Albumin (g/dl)	4.27 ± 0.04	4.37 ± 0.15	4.49 ± 0.10	4.01 ± 0.07
Kidney Function Tests				
Urea (mg/dl)	50.50 ± 1.41	45.83 ± 2.38	50.98 ± 1.48	45.38 ± 2.65
Uric Acid (mg/dl)	2.86 ± 0.33	2.82 ± 0.23	2.80 ± 0.13	2.03 ± 0.34
Creatinine	0.52 ± 0.02	0.44 ± 0.01	0.53 ± 0.01	0.45 ± 0.01
Metabolic Function Tests				
Glucose (mg/dl)	101.6 ± 16.01	104 ± 2.86	105.1 ± 13.76	105.8 ± 6.44
Cholestrol (mg/dl)	57.40 ± 2.70	56.00 ± 3.05	60.80 ± 2.28	65.40 ± 3.23
Triglyceride (mg/dl)	90.6 ± 4.26	86.33 ± 3.84	102.4 ± 13.25	96.80 ± 5.94
HDLC	29.68 ± 0.98	27.30 ± 0.10	37.66 ± 1.45	29.55± 1.60

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Sub-Acute Oral Toxicity Test

Group Mean Body Weight (At 4800 mg/kg)

The body weight of rats treated with Majoon-IQ at the dose of 4800mg/kg was found to increase normally as compared to the control rats as shown in Table 6.

Table 6 : Body Weight of Rats in Sub Acute Oral Toxicity Study

Group	Days				
	Day 0 Mean ± SD	**Day 7 Mean ± SD**	**Day 14 Mean ± SD**	**Day 21 Mean ± SD**	**Day 28 Mean ± SD**
Male Control	118.4 ± 7.4	160 ± 2.8	183 ± 4.6	185.8 ± 3.7	216.4 ± 5.6
Male Treated	128.4 ± 6.1	160.2 ± 11.9	180.8 ± 14.5	196.4 ± 19.5	205.6 ± 21.0
Female Control	119.2± 5.8	143.4 ± 8.9	161.8 ± 8.6	170.2 ± 9.6	183.6 ± 8.1
Female Treated	128.7 ± 1.8	155.5 ± 3.4	168.2 ± 5.1	182.7± 5.9	184 ± 8.5

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Average Food and Water Consumption (At 4800 mg/kg)

The average feed consumption of both treated groups was found to be unaffected by Majoon-IQ treatment compared to control group rats. No significant decrease in the average water consumption of treated rats was found when compared to the respective controls as shown in Table 7.

Table 7: Average Feed and Water Consumption by Rats

Group Mean Food consumption per rat/24 hours (Grams)				Group Mean Water Consumption per rat/24 hours (Millilitre)	
	Male (Mean±SD)	Female (Mean±SD)		Male (Mean±SD)	Female (Mean±SD)
Control	19.7 ± +1.5	18.3 ± 2.5		29.3 ± 3.05	29.3 ± 5.03
Treated	19.6 ± 1.5	17.5 ± 0.25		27.6 ± 1.4	27.3 ± 2.5

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Haematological Parameters (At 4800 mg/kg)

No significant change was observed in the haematological parameters of Majoon-IQ treated rats as compared to the control group rats as indicated in Table 8.

Table 8: Haematological Parameters of Rats in Sub-acute Oral Toxicity Study

Parameter	Male		Female	
	Control (Mean±SD)	Treated (Mean±SD)	Control (Mean±SD)	Treated (Mean±SD)
WBC (10^3/µl)	12.08 ± 5.03	10.50 ± 3.57	10.4 ± 2.07	11.15 ± 3.10
RBC (10^3/µl)	8.49 ± 0.50	8.92 ± 0.90	8.60 ± 0.20	7.98 ± 0.82
Hb (grams %)	16.34 ± 0.90	16.4 ± 1.2	16.7 ± 0.70	15.25 ± 1.64
HCT (%)	49.10 ± 2.9	49.1 ± 3.6	50.3 ± 1.7	45.9 ± 4.2
MCV (Femtolitre)	57.8 ± 3.3	55.2 ± 2.7	58.4 ± 1.6	57.6 ± 2.1
MCH (pico grams)	19.2 ± 0.90	18.4 ± 0.60	19.44 ± 0.30	19.10 ± 0.78
MCHC (grams%)	33.2 ± 0.40	33.4 ± 0.60	33.2 ± 0.60	33.1 ± 0.62
Reticulocytes (%)	4.38 ± 1.12	3.8 ± 0.50	4.1 ± 1.02	4.4 ± 1.55
Platelet count (10^3/µl)	1149 ± 187.3	1235 ± 138	1138 ± 144.8	1108.2± 106
Differential Leucocyte Count				
Neutrophils %	14.1 ± 1.9	15.9 ± 2.55	13.1 ± 4.90	13.3 ± 2.23
Lymphocytes %	79.8 ± 10.7	73.9 ± 8.4	78.3 ± 4.60	79.1 ± 2.40
Monocytes %	3.6 ± 1.01	6.7 ± 3.3	5.7 ± 1.4	5.4 ± 1.17
Eosinophils %	1.8 ± 0.90	1.16 ± 0.92	1.54 ± 0.9	1.6 ± 1.36
Basophils %	0.46 ± 0.23	0.16 ± 0.08	0.48 ± 0.22	0.50 ± 0.73

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Bio-chemistry Parameters (At 4800mg/kg)

The bio-chemical parameters of rats treated with 4800mg/kg b.w. of Majoon-IQ showed no significant change as compared to the bio-chemical parameters of control group rats as shown in Table 9.

Table 9: Bio-chemical Parameters of Rats in Sub-acute Oral Toxicity Study

Parameter	Male		Female	
	Control (Mean±SD)	Treated (Mean±SD)	Control (Mean±SD)	Treated (Mean±SD)
Liver Function Tests				
ALT (IU/l)	93.20 ± 13.2	91.80 ± 18.09	93.04 ± 14.6	96.2 ± 25.1
AST (IU/l)	102.3 ± 19.7	106.7 ± 15.7	109.4 ± 20.1	107.8 ± 13.04
ALP (IU/l)	183.8 ± 34.5	187 ± 78.30	179.20 ± 21	170.5 ± 15.43
Total bilirubin (mg/dl)	0.07 ± 0.014	0.08 ± 0.005	0.07 ± 0.01	0.09 ± 0.02
Total protein (g/dl)	8.2 ± 0.36	7.8 ± 0.5	8.05 ± 0.2	8.3 ± 0.34
Albumin (g/dl)	4.30 ± 0.15	4.50 ± 0.14	4.5 ± 0.10	4.30 ± 0.80
Kidney Function Tests				
Urea (mg/dl)	47.00 ± 3.7	50.6 ± 8.5	46.3 ± 6.01	51.7 ± 13.4
Uric Acid (mg/dl)	2.2 ± 0.61	2.2 ± 0.23	2.7 ± 0.70	2.1 ± 1.05
Creatinine	0.50 ± 0.01	0.50 ± 0.05	0.50 ± 0.01	0.50 ± 0.05
Metabolic Function Tests				
Glucose (mg/dl)	106.6 ± 13.2	99.8 ± 19.6	97.5 ± 21.4	107.0 ± 19.9
Cholestrol (mg/dl)	53.60 ± 6.5	48.90 ± 7.6	57.40 ± 3.36	56.00 ± 12.51
Triglyceride (mg/dl)	86.80 ± 12.2	82.40 ± 29.80	94.60 ± 31.0	94.25 ± 40.55
HDLC	31.34 ± 6.89	24.92 ± 2.03	36.16 ± 3.58	35.92 ± 11.51

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Relative Organ Weight of Treated and Controls

No significant change in the relative organ weights of Majoon-IQ treated rats was observed (Table 10)

Table 10 : Relative Organ Weight of Rats in Sub-acute Oral Toxicity Study

Organ	Male (control) (Mean ±SD)	Male (treated) (Mean ±SD)	Female (control) (Mean ±SD)	Female (treated) (Mean ±SD)
Brain	1.7±0.05	1.5±0.07	1.5±0.07	1.6±0.07
Spleen	0.6±0.007	0.5±0.06	0.7±0.02	0.7±0.15
Rt Adrenal	0.03±0.0	0.03±0.0	0.02±0.0	0.02±0.0
Lt Adrenal	0.03±0.0	0.03±0.0	0.02±0.001	0.03±0.001
Heart	0.81±0.18	0.71±0.06	0.68±0.07	0.66±0.02
Lung	1.51±0.2	1.42±0.13	1.31±0.05	1.42±0.05
Rt kidney	0.9±0.11	0.66±0.04	0.68±0.07	0.70±0.03

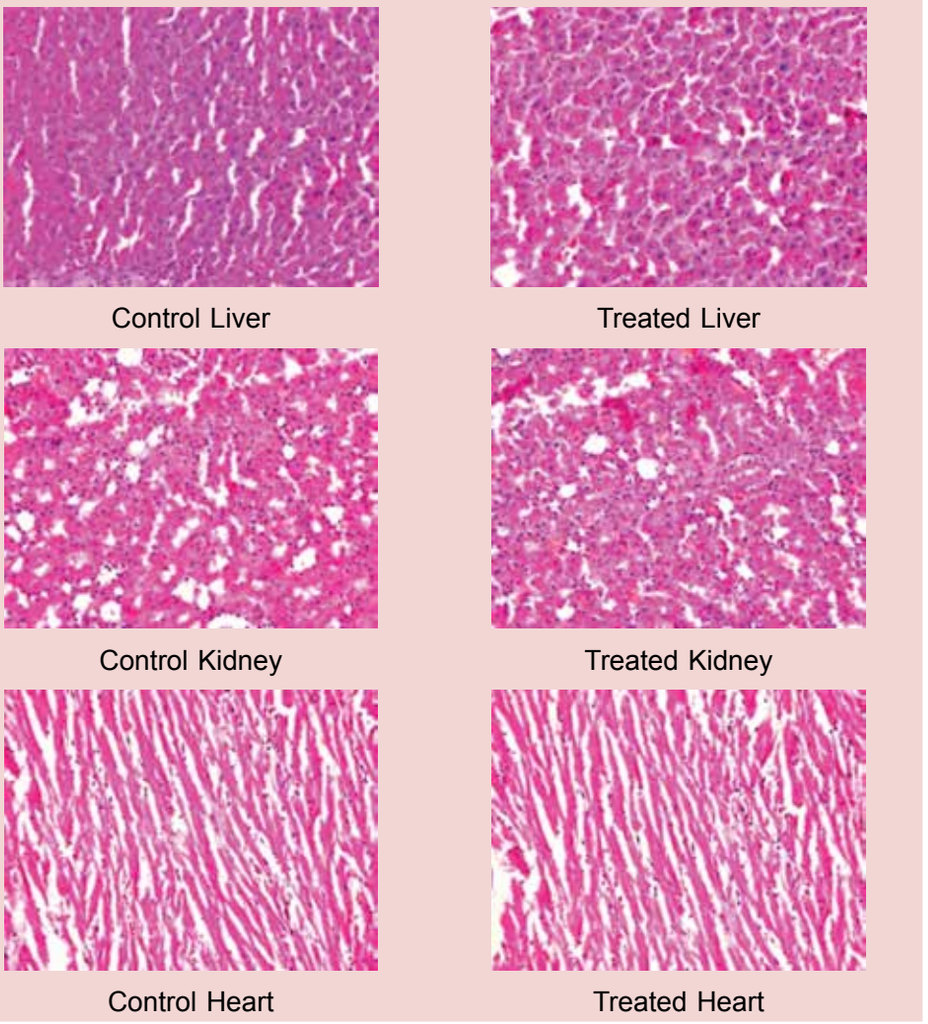
Lt kidney	0.9±0.12	0.66±0.03	0.66±0.04	0.68±0.05
Rt testis/Ovary	1.2±0.12	0.7±0.43	0.06±0.07	0.045±0.07
Lt testis/Ovary	1.2±0.09	0.7±0.31	0.06±0.04	0.05±0.14
Liver	7.12±2.3	6.74±0.007	6.25±0.42	6.85±0.2
Pancreas	0.52±0.12	0.47±0.16	0.44±0.08	0.42±0.11

*The values are expressed as mean ± SD n=5 in each group. *p<0.05 as compared to control at the same time (one-way ANOVA followed by Dunnett's multiple comparison test).*

Histopathological Parameters

The histopathological examination of the treated animals also indicated that there was no damage to the tissues/organs when compared to the control animals as shown below:

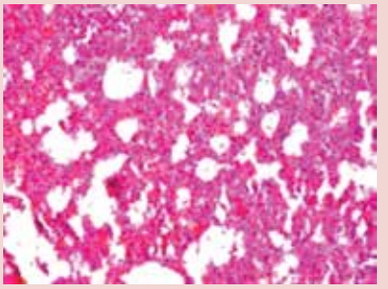
Histopathology of Control and Treated Rats



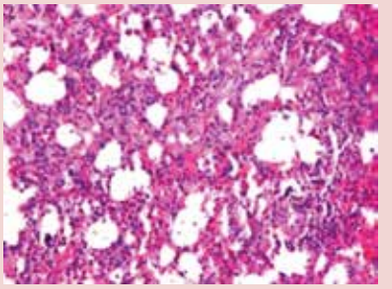
Control Liver | Treated Liver

Control Kidney | Treated Kidney

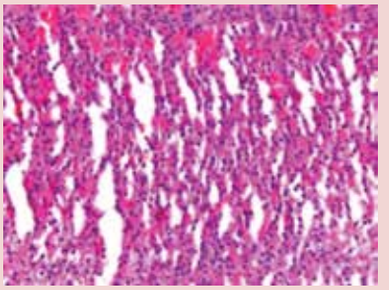
Control Heart | Treated Heart



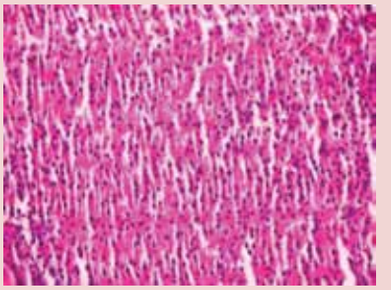
Control Lung



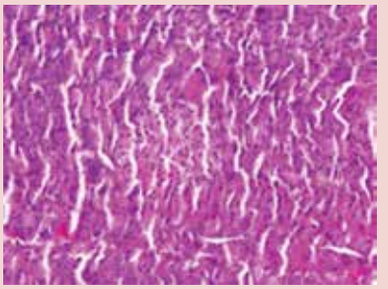
Treated Lung



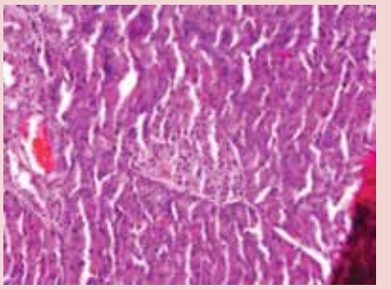
Control Adrenal



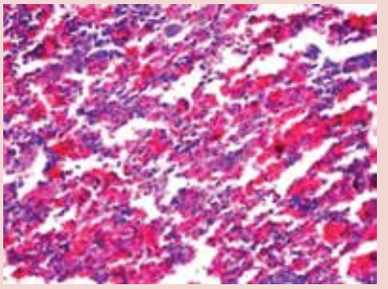
Treated Adrenal



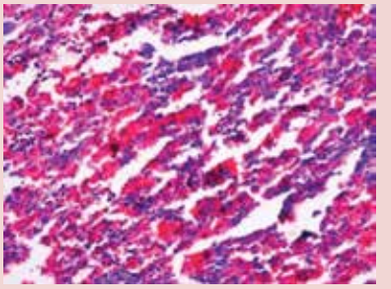
Control Pancreas



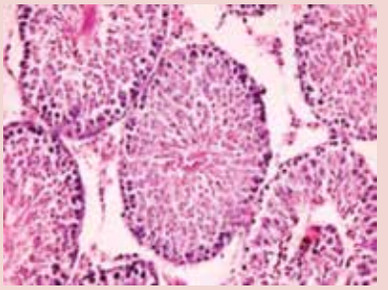
Treated Pancreas



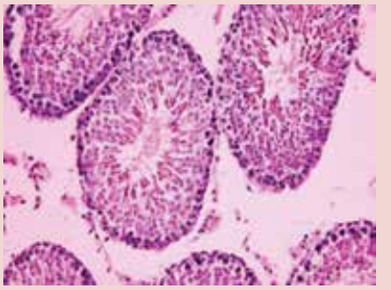
Control Spleen



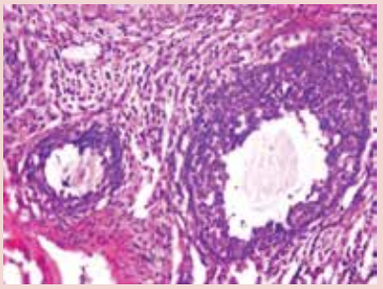
Treated Spleen



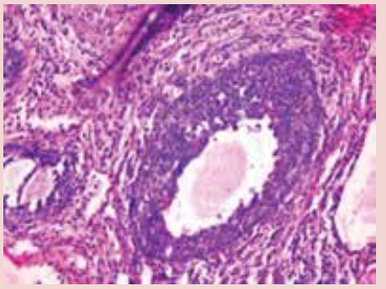
Control Testis



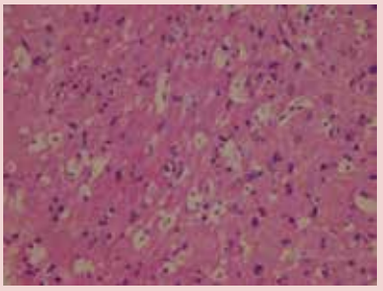
Treated Testis



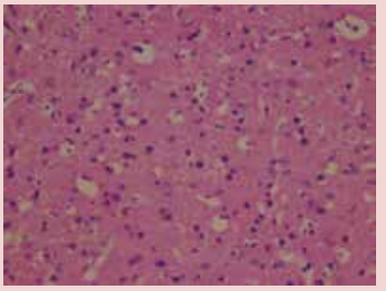
Control Ovary



Treated Ovary



Control Brain



Treated Brain

Discussion and Conclusion

With the enormous global consumption of herbal medicines, it is high time that they are included in pharmacovigilance systems. In terms of population exposure alone, it is essential to identify the risks associated with the use of herbal medicine and in this regard, the safety of these products has become an issue of great public health importance (WHO, 2004, 2005b). There is no doubt that the increasing cases of poisoning associated with use of herbal medicines in many parts of the world in recent times is necessitating the need to ensure thorough toxicity assessment alongside active pharmacovigilance on these products in order to promote their safe use and protect public health (Zhou *et al.,* 2013). In view of the above facts the acute and sub-acute oral toxicity study of Majoon-IQ was undertaken.

Acute Oral Toxicity Study

The drug Majoon-IQ was found to have no negative effect on the body weight gain of the treated male and female rats. The treated rats were found to grow normally. There was no significant change in the feed and water consumption of the treated male and female rats when compared to the respective controls. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Blood bio-chemical and haematological parameters were also normal. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated groups.

Sub-acute Oral Toxicity Study

The male and female treated rats were found to have a normal weight gain. There were no signs of abnormal behaviour in the treated rats. There was a slight

decrease (Statistically insignificant) in the average water consumption per day by the male and female rats treated with the drugs as compared to the respective controls. The drug was found to have no effect on the average feed consumption by the drug treated rats. Gross examination of the tissues revealed the normal appearance of the tissues/organs. The results of bio-chemical parameters did not show any significant change in the values when compared to the controls. The liver and kidney function tests were found to be normal in the drug treated groups. The lipid profile of the drug treated male and female rats was found to be unaffected when compared to the respective controls. The blood parameters were unaffected by the drug as the values of drug treated rats are within the range of control rats. No treatment related morphological changes were observed in the vital organs such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested. There was also no significant difference observed in the organ weights of male and female treated rats when compared to the respective controls.

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सारांश

माजून-ए-आईक्यू का तीव्र एवं उप-तीव्र मौखिक विषाक्तता अध्ययन - एक यूनानी दिमागी शक्ति वर्धक औषधि

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माजून–ए–आईक्यू एक यूनानी जड़ी–बूटी औषधि है जिसका प्रयोग दिमागी शक्ति वर्धक टॉनिक के रूप में किया जाता है। इस अनुसंधान का मुख्य उद्देश्य माजून–ए–आईक्यू की तीव्र और उप–तीव्र मौखिक विषाक्तता का विस्तार अलबिनो चूहों की दोनों जाति (नर व मादा) में अध्ययन करना था। तीव्र मौखिक विषाक्तता का अध्ययन आईसीडी–425 दिशा–निर्देशों के अनुसार किया गया। इस अध्ययन में माजून–ए–आईक्यू दवा को 5000 मिलीग्राम/किलोग्राम नर और मादा चूहों के शरीर के वजन के स्तर के अनुसार दी गई। इसके पश्चात् चूहों को 30 मिनट के लिए, खुराक के चार घंटे बाद और अगले 14 दिनों तक दिन में कम से कम दो बार व्यक्तिगत रूप से निरीक्षण किया गया। चूहों में उप तीव्र मौखिक विषाक्तता का अध्ययन आईसीडी–407 के दिशा–निर्देशों के अनुसार किया गया। इस अध्ययन में माजून–ए–आईक्यू औषधि की खुराक चूहों के शरीर के वजन के अनुसार 4800 मिलीग्राम/किलोग्राम की अस्तत मात्रा को 28 दिनों के लिए प्रतिदिन दिया गया एवं अनुसंधान अवधि के दौरान प्रतिदिन चूहों का निरीक्षण किया गया। एक रात उपवास के बाद चूहों को 15वें और 29वें दिन अनुसंधान करने के लिए मार दिया गया। निरीक्षण मानदंडों में सामान्य उपस्थिति/व्यवहार, रूग्णता(अस्वस्थता)/मृत्यु–दर, शरीर का वज़न, भोजन/पानी की खपत, रक्त–रोग मापदंड, जैव–रसायनिक मापदंड आदि का तुलनात्मक मूल्यांकन किया गया। चूहों में किसी प्रकार की मृत्यु–दर, रूग्णता एवं प्रायोगिक जाँच में दोनों तीव्र और उप–तीव्र मौखिक विषाक्तता नहीं पाई गई। इस अनुसंधान अध्ययन से यह पता चलता है कि माजून–ए–आईक्यू किसी भी प्रकार की विषाक्तता से मुक्त है।

Clinical Study to Evaluate the Efficacy of Ḥijāma bi'l Sharṭ (Wet Cupping) in the Management of Musculoskeletal Pain: A Case-Series

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Abstract

Chronic pain is a global public health problem, causing major consequences for the quality of life of the sufferer and a major burden on the healthcare system in the world. Chronic pain of moderate to severe intensity has been estimated to occur in 19% of adult Europeans, seriously affecting their daily activities, social and working lives. *Ḥijāma* (cupping) is one among various regimes of *Ilāj bi'l Tadbīr* (Regimen Therapy) used in Unani System of Medicine to alleviate pain since ages. This case-series study was designed to evaluate the effect of *Ḥijāma bi'l Sharṭ* (wet cupping) in musculoskeletal pain conditions. This study was conducted at the Central Research Institute of Unani Medicine, Hyderabad, during 2016-17. Seventy seven patients of either gender, aged 20-60 years, having moderate to severe musculoskeletal pain, including *Waja' al-Khāṣira* (low back pain), '*Waja' al-Rukba* (knee pain) and '*Waja' al-'Unuq* (neck pain) and *Waja' al-Katif* (shoulder pain) were included in this case series. Two cupping therapy sessions were performed one week apart. Post-treatment follow-up was conducted after two weeks of treatment. Response to therapy was evaluated by visual analogue scale (VAS) and patient global assessment of response to treatment (PGART). Student-'t' test was applied and P-value <0.05 was considered significant. There was a significant reduction in pain after two sessions of *Ḥijāma bi'l Sharṭ* as assessed by VAS (P<0.05). This case series suggests that *Ḥijāma bi'l Sharṭ* (wet cupping) is effective in the management of musculoskeletal pain; however, there is a need to conduct randomised controlled clinical trials with larger sample size and more cupping therapy sessions before arriving at any firm conclusion.

Keywords: *Ḥijāma bi'l Sharṭ,* Wet cupping, Musculoskeletal pain, Unani, Low back pain

Introduction

Chronic pain is a global public health problem, causing major consequences for the quality of life of the sufferer and a major burden on the healthcare system in the world. Chronic pain of moderate to severe intensity has been estimated to occur in 19% of adult Europeans, seriously affecting their daily activities, social and working lives. Chronic non-cancer pain substantially affects more than 60 million Americans and a significant population of India. (Leverence *et al.*, 2011) Indian epidemiological study conducted by Dureja et al (2014) revealed that patients of chronic pain were no longer to maintain an independent life and about 32% of the patients lost ≥4 hours of work due to pain.

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Although, there is no common definition of what constitutes chronic (persistent) non-cancer pain, the term is often used to describe continuous, long-term pain of more than 12 weeks duration or pain that persists beyond the expected period of healing after trauma or surgery. (Pergolizzi J) Physicians are facing a lot of troubles in dealing with the patients of chronic pain conditions. Though, a number of drug classes are presently available to combat pain but there is a need for some alternative therapies to deal with the chronic pain conditions. *Ḥijāma* (Cupping Therapy) may be a solution for suffering faced in many diseases manifested by pain. (El Sayed S *et al.*, 2013)

Ḥijāma bi'l Sharṭ, an Arabic term for wet cupping, is one of the ancient traditional therapies in which special cups are put on the patient's skin for a few minutes to create suction and blood is drawn by vacuum from small skin incisions for therapeutic purposes. Eber's papyrus, one of the oldest medical texts written in 1550 BC had also described cupping therapy. Ancient Egyptians, Chinese, Greek, and Arab Physicians had performed cupping therapy in different kinds of ailments. (El Sayed *et al.*, 2013; Al-Bedah *et al.*, 2016)

In Unani System of medicine, *Ḥijāma* (Cupping Therapy) is a well-known regime, which comes under '*Ilāj bi'l Tadbīr* (Regimen Therapy); an important part of treatment modality of the system. Scholars of Unani Medicine were very well acquainted with the procedure of *Ḥijāma* (Cupping) and its indications, contraindications and pre and post procedure precautions. (Abū al-Qāsim Zahrāwī, 2012; Ibn al-Quff). *Ḥijāma* is done by creating negative pressure inside the cups on pre-determined skin area, through suction or fire. The types of *Ḥijāma* include *Ḥijāma bi'l Sharṭ* (wet cupping or cupping with bloodletting) and *Ḥijāma bilā Sharṭ* (dry cupping or cupping without bloodletting). *Ḥijāma bi'l Nār* (Fire Cupping) is a kind of dry cupping in which vacuum is created by fire (*Nār*). It is not commonly practised nowadays. (Akhtar *et al.*, 2008) *Ḥijāma bi'l Sharṭ* (wet cupping) works under the principle of *Tanqiya Mawād* (evacuation of morbid humour), while *Ḥijāma bilā Sharṭ* (dry cupping) is done to divert the morbid humour from the diseased area. (Abū al-Qāsim Zahrāwī, 2012; Ibn al-Quff, Akhtar *et al.*, 2008)

Ḥijāma (Cupping) is indicated in various musculoskeletal disorders of back and extremities, e.g., '*Waja' al-Rukba* (knee pain), '*Irq al-Nasā* (sciatica), *Niqris* (gout), *Waja' al-Khāṣira* (low back pain), *Shaqīqa* (migraine); diseases of respiratory system, e.g., *Waram-i-Ḥalaq* (pharyngitis), *Iltihāb al-Anf* (rhinitis); gynaecological disorders, e.g., *Ihtibās al-Tams* (amenorrhoea), *Kasrat-i-Tams* (menorrhagia), *Iltihāb al-Raḥim* (metritis), pelvic pain; skin diseases, e.g., *Jarab* (scabies), *ikka* (pruritus), *Kalaf* (melasma), etc.

Currently, *Ḥijāma* (Cupping) is being practised in different parts of India by Unani scholars, however; there are limited scientific data on the efficacy of *Ḥijāma*. Keeping this in view, this case series study was conducted to evaluate the effect of *Ḥijāma bi'l Sharṭ* in the management of musculoskeletal pain.

Material and Methods

A clinical case-series study was conducted in patients having moderate to severe pain (VAS >3) for more than 12 weeks duration attending OPD of Central Research Institute of Unani Medicine (CRIUM), Hyderabad, during 2016-17. The procedure of therapy was explained to the participants along with the possible outcomes. Written informed consent was obtained from all the patients prior to initiation of the procedure. A detailed physical examination was done and *Mizāj* (temperament) and vitals were recorded. Each patient was asked to grade pain intensity on a 0-10 visual analogue scale (VAS) before the procedure.

Inclusion Criteria

Patients of either sex between the age of 20 and 60 years having the following musculoskeletal pain conditions:

- *Waja' al-Khāṣira* (low back pain)
- *'Waja' al-Rukba* (knee pain)
- *'Waja' al-'Unuq* (neck pain)
- *Waja' al-Katif* (shoulder pain)

Exclusion Criteria

- Use of NSAIDs since a week
- Cupping therapy in the last 3 months
- Any therapy for pain in the previous 2 weeks
- Known coagulopathy
- Severe anaemia
- Use of anticoagulant
- Other systemic diseases
- Pregnant and lactating women

Intervention

Ḥijāma bi'l Sharṭ (wet cupping) was performed twice at the baseline (day 0) and after one week (day 7) and post-treatment follow-up was done on 14th day of the therapy. Unani physicians have described cupping, puncturing and cupping (CPC) method in their legendary texts, (Abū al-Qāsim Zahrāwī, 2012; Ibn al-Quff) The detailed CPC method described by El Sayed *et al.*, consists of six steps viz. skin demarcation, sterilization, cupping, puncturing, cupping and sterilization.

1. **Skin Demarcation:** Firstly, skin demarcation was made by selecting the specific points on body (on back and other body parts). In case of *'Waja' al-Khāṣira* (low back pain), 2 medium size cups were applied between scapulae and 2 medium size cups on lower back. In *'Waja' al-Rukba* (knee pain), 2 medium size and 1-2 small cups were applied on affected joint, while in *'Waja' al-'Unuq* (neck pain) cases, 2 small size cups on posterior neck and 2 medium size cups on inter-scapular region were applied. In patients with *'Waja' al-Katif* (shoulder pain), 2 medium size cups were applied on inter-scapular region and 2-3 small size cups were applied on tender area of shoulder.
2. **Sterilization:** Selected area was sterilized by disinfectant (spirit) gently.
3. **Cupping:** Cups were placed on demarcated area and negative suction pressure was applied by manual suction (visco-elastic nature of skin helps it to be sucked to the inside of cups). The cups were clung to the skin and placed for 3-5 minutes or till the appearance of erythema and congestion on the surface.
4. **Puncturing or Scarification:** After removing the cups, immediate skin pricking (15-20 superficial incisions) for few millimeters depth was given by surgical blade no 11.
5. **Cupping:** Cups were again placed on the skin in the same manner as described above. Blood started oozing from injured capillaries towards the puncture site at the skin surface. Coagulation pathway stimulated and allowed clotting of blood. Cups were removed after 3-5 minutes or till blood was coagulated, whichever was earlier.
6. **Dressing:** In the last step, the area was cleaned by antiseptic solution and dressing was done to prevent any infection.

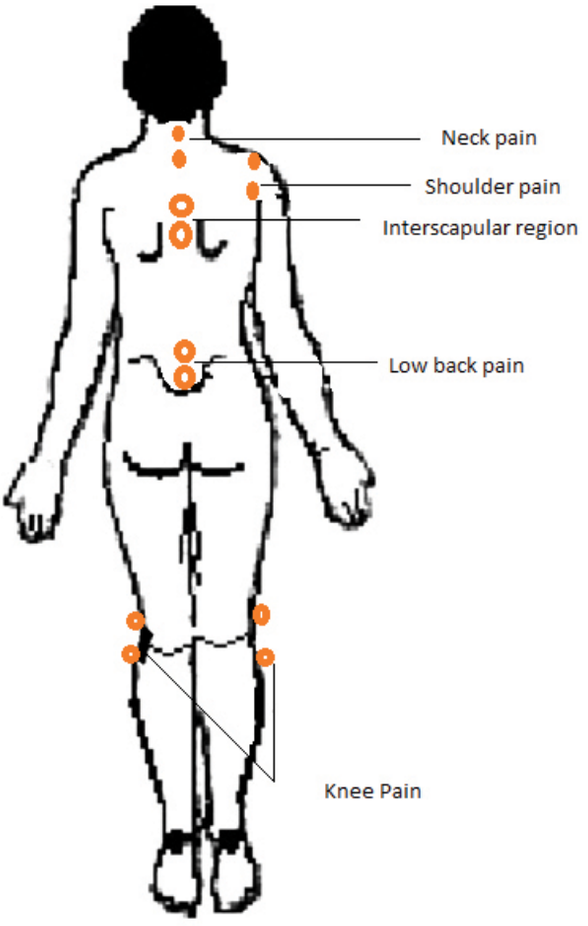

Fig. 1 : Anatomical Areas of Wet Cupping for Different Pain Conditions

Special Instructions

After the procedure, patients were kept under observation for at least one hour and liquid diet was allowed. Patients were advised to take rest for the next 24 hours. Patients were asked to note any skin changes at the site of puncturing.

Follow-Up

Two cupping therapy sessions were performed one week apart. Thus, follow-up was conducted after one week during the therapy. Post-treatment follow-up was done after two weeks of treatment.

Outcome

Assessment of response to therapy was made by VAS and PGART. Pain intensity was assessed qualitatively by a simple, reliable and commonly used validated scale, i.e., 0-10 cm visual analogue scale (VAS). Patient global assessment of response to therapy (PGART) consists of 4 categories, viz. poor, satisfactory, good and excellent.

Adverse Events

All the participants were asked to report any symptoms such as irritation, burning sensation, infection, increase of pain at the site of *Ḥijāma* (Cupping) and appearance of any new symptoms after the procedure.

Statistical Analysis

Descriptive and inferential statistical analysis were carried out in the present study. Student-'t' test was used to find out the significance of study parameters and P-value (<0.05) was considered significant.

Results

The demographic characteristics of each patient are presented in Table 1 and 2. A total of 77 cases were registered for *Ḥijāma bi'l Sharṭ* (wet cupping), out of which 22 were male and 55 female. The mean age of the participants was 44.7 ± 10.5 years. Most of the participants had 1 month to 2 years of chronicity and majority belonged to middle class. Of the 77 cases, 33 had *'Waja' al-Khāṣira* (low back pain), followed by 20 *'Waja' al-Rukba* (knee pain), 19 *'Waja' al-'Unuq* (neck pain), and 5 *'Waja' al-Katif* (shoulder pain) (Table 3).

Pain intensity score was assessed qualitatively by VAS. Table 4 presents response of *Ḥijāma bi'l Sharṭ* (wet cupping) assessed by VAS and reveals a significant reduction (p<0.05) in all kinds of pain conditions after intervention. Table 5 gives mean response of *Ḥijāma bi'l Sharṭ* (wet cupping) assessed by VAS and reveals that the response to the treatment in all pain conditions was >50%. Patient Global Assessment of Response to Therapy (PGART) was found good by 46 patients, satisfactory by 14 patients and excellent by 17 patients out of 77 registered patients. Nobody responded that the therapy was poor.Three patients informed to have burning at the site of *Ḥijāma* (Cupping), which was of mild degree and relieved itself in the next 24 hours. No other adverse events were reported by the patients.

Discussion

Ḥijāma (cupping) is one of the oldest medical techniques in the world. It is being practised in Unani system of medicine since antiquity. Abū al-Qāsim Zahrāwī (Abulcasis), known as father of surgery and Ibn al-Quff have given a detailed description of *Ḥijāma* (cupping) in their texts *Kitāb al-Taṣrīf* and *Kitāb al-'Umda fi'l Jarāḥa*. In the present study, *Ḥijāma bi'l Sharṭ* (wet cupping) was performed to relieve pain in patients with different kinds of musculoskeletal pain conditions. (Abū al-Qāsim Zahrāwī, 2012; Ibn al-Quff-1986)

In the present study, *Ḥijāma bi'l Sharṭ* (wet cupping) was performed twice at an interval of one week and post-treatment follow-up was done on 14th day of therapy. After two sessions of therapy, pain was significantly ($P<0.05$) reduced suggesting effectiveness of *Ḥijāma bi'l Sharṭ* (wet cupping). Hence, it may be suggested that wet cupping may be an alternative treatment in some musculoskeletal pain conditions. In this case series, low back pain was the most commonly reported condition followed by knee pain while in a study conducted by Dureja et al, knee pain was most prevalent. This is because, in this study, number of female patients was comparatively more and low back pain was reported more in case of in females by Indian study. (Ahdhi *et al.*, 2016)

According to Unani concept, pain originates due to accumulation of *Akhlāṭ Fāsida* (morbid humour) and *Balgham Ghayr Tabīʿī* in different parts of the body and line of management is the removal of *Akhlāṭ Fāsida* (morbid humour) through *Istifrāgh* (evacuation). *Ḥijāma bi'l Sharṭ* (wet cupping) works according to the principle of *Tanqiya Mawād* (evacuation of morbid humour). (Ibn Sina, 2007) Although, the physiological mechanism through which *Ḥijāma* works is not known but the evidence-based *Taibah* theory has already explained the therapeutic benefits of *Ḥijāma bi'l Sharṭ* (wet cupping) through clearing blood and interstitial spaces from causative pathological substances (CPS) which include pain causing and pain related substances. (El Sayed *et al.*, 2013)

Being a case series, the present study has several limitations, e.g., lack of control group (standard or placebo), small sample size and short duration of therapy. Thus, there is a need to conduct randomized controlled clinical trials with larger sample size to evaluate the significant effect of *Ḥijāma bi'l Sharṭ* (wet cupping). Further, long-term follow-up studies are required to establish the frequency of cupping therapy sessions.

Conclusion

In view of the above observations, it can be concluded that *Ḥijāma bi'l Sharṭ* (wet cupping) is effective in reducing pain of different musculoskeletal conditions. However, there is a need to conduct randomised controlled clinical trials with larger sample size and more cupping therapy sessions before arriving at any firm conclusion.

Table 1: Distribution of Patients According to Age, Sex and Chronicity

Age (Years)	Male (n=22)	Female (n=55)	Total (n=77) (%)
20 – 30	1	7	8 (10.4)
31 – 40	9	11	20(26.0)

41 – 50	5	21	26(33.7)
51 – 60	7	16	23(29.9)
Mean±SD			44.7 ± 10.5
Chronicity			
1 – 6 mo	3	20	23(29.9)
7 – 12 mo	10	8	18(23.4)
1 – 2 yrs	8	18	26(33.8)
3 – 5 yrs	1	8	9(11.7)
6 – 8 yrs	-	1	1(1.2)
Mean±SD			1.58 ± 1.3

Table 2: Distribution of Patients According to Temperament and Socio-economic Status

Mizāj (Temperament)	Male (n=22)	Female (n=55)	Total (n=77) (%)
Damawī	13	30	43(55.8)
Balghamī	8	25	33(42.9)
□*afrāwī*	-	-	-
Sawdāwī	1	-	1(1.3)
Socioeconomic Status			
High	-	1	1(1.3%)
Middle	20	44	64(83.1%)
Low	2	10	12(15.6%)

Data are presented as Mean±SD and percentage.

Table 3: Distribution of Patients According to Site of Pain

Musculoskeletal Pain	Male (n=22)	Female (n=55)	Total (n=77)	Percentage (%)
Low Back Pain	9	24	33	41.25
Knee Pain	8	12	20	25.0
Neck Pain	4	15	19	23.75
Shoulder Pain	1	4	5	6.25

Data are presented in percentage.

Table 4: Response of *Ḥijāma bi'l Sharṭ* (wet cupping) Assessed by VAS

Musculoskeletal Pain	n=77	BT	AT	Difference	P value
Low Back Pain	33	6.8±0.6	2.8±1.0	3.9±1.1	<0.05
Knee Pain	20	7.0±0.8	3.5±0.9	3.4±0.8	<0.05

Neck Pain	19	6.7±0.8	3.3±0.9	3.5±0.9	<0.05
Shoulder Pain	5	6.8±0.8	3.4±0.5	3.4±0.5	<0.05

Results are presented as mean±SD and analysed by Student t-test (dependent).

BT= Before Treatment; AT= After Treatment

Table 5: Mean Response of *Ḥijāma bi'l Sharṭ* (wet cupping) Assessed by VAS

Musculoskeletal Pain	No. of Cases	Mean Response (%) (Mean± S.D.)
Low Back Pain	33	58.2 ±15.3
Knee Pain	20	49.8 ±11.1
Neck Pain	19	51.6 ±12.4
Shoulder Pain	5	50.0 ±5.1
Total	77	53.8 ± 13.5

Results are presented as Mean±SD.

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सारांश

जोड़ों एवं मासपेशियों के दर्द के प्रबंधन में हिजामा-बिल-शर्त (वेट कपिंग) प्रभावकारिता के मूल्यांकन करने के लिए नैदानिक अध्ययन : एक प्रकरण श्रृंखला

*हिना रहमान, कमरूद्दीन, मुनव्वर हुसैन काज़मी और सय्यदा खदीरूननिसा

दीर्घकालीन दर्द एक वैश्विक सार्वजनिक स्वास्थ्य समस्या है, जोकि विश्व में पीड़ित व्यक्ति की जीवन की गुणवत्ता में और स्वास्थ्य देखभाल पद्धति पर एक बड़े बोझ और प्रमुख निष्कर्षों का कारण है। दीर्घकालीन दर्द की कम से अधिक तीव्रता का 19 प्रतिशत अनुमान व्यस्क यूरोपीय लोगों मे पाया गया है, जोकि गंभीरता पूर्वक उनकी दैनिक गतिविधियों, सामाजिक और कामकाजी जीवन में प्रभाव डाल रहा है। हिजामा(कपिंग) यूनानी चिकित्सा पद्धति के अन्तर्गत इलाज–बित्त–तदबीर की कई चिकित्साओं में से एक है जोकि प्राचीनकाल से दर्द को कम करने के लिए प्रचलित है। जोड़ों एवं मांसपेशियों के दर्द की स्थिति में हिजामा–बिल–शर्त (वेट कपिंग) की प्रभावकारिता का मूल्यांकन करने के लिए इससे संबंधित श्रृंखलाओं पर अध्ययन का रूपांकन किया गया। वर्ष 2016–17 के दौरान केन्द्रीय यूनानी चिकित्सा अनुसंधान संस्थान, हैदराबाद में यह अध्ययन चलाया गया। इस श्रृंखला में, 20–60 साल की आयु वाले दोनों लिंगों में से एक के 77 रोगी जिन्हें कम से अधिक जोड़ों एवं मांसपेशियों का दर्द था, जिसमें वजा–अल–कसीर (निचला पीठ दर्द), वजा–अल–रूकबा (घुटने का दर्द), वजा–उल–उनक(गर्दन का दर्द) और वजा–उल–कतिफ(कंधे का दर्द) शामिल थे, को सम्मिलित किया गया। एक हफ़्ते में दो कपिंग चिकित्सा सत्र प्रस्तुत किए गए। उपचार के दो सप्ताह के पश्चात पुनः जाँच के लिए बुलाया गया। विजुअल अनालोग स्केल(वी.ए.एस.) एवं पेशेन्ट ग्लोबल असिस्टमेंट ऑफ रेस्पान्स टू ट्रिटमेन्ट(पी.जी.ए.आर.टी) द्वारा चिकित्सा का मूल्यांकन किया गया। ''स्टूडेंट टी टेस्ट'' लगाया गया और पी वेल्यू <0.05 से कम को अर्थपूर्ण माना गया। वी.ए.एस. (पी<0.05) द्वारा मूल्यांकन करने पर हिजामा–बिल शर्त के दो सत्रों के पश्चात् दर्द में अर्थपूर्ण कमी हुई। इस अध्ययन श्रृंखला से पता चलता है कि हिजामा–बिल–शर्त(वेट कपिंग) जोड़ों–मांसपेशियों के दर्द के उपचार में प्रभावशाली है, यद्यपि किसी ठोस परिणाम पर पहुँचने से पहले बड़े सैम्पल साइज़ और अधिक चिकित्सा सत्रों पर नैदानिक यादृच्छिक नियंत्रित परीक्षण करने की आवश्यकता है।

Clinical Study on An Unani Formulation *'Qurs e Kushta Khabs al-Hadeed* and *Habb-e-Marwareed'* in *Sayalan-al- Rahim* (Leucorrhea)"

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Abstract

The objective of the study was to evaluate the safety and efficacy of Unani pharmacopoeial formulations viz. 'Qurs e Kushta Khabs Al-Hadded and Habb-e-Marwareed' in Sayalan-al-Rahim (Leucorrhea). One tablet of both drugs were administered orally to the patients, twice daily for 4 weeks. After the treatment, mean ± S.E.M. scores of clinical parameters of the disease including the amount of vaginal discharge, general weakness, backache, anaemia, excoriation and ulceration were subsided by 43.36% ($p<0.001$), 60.64% ($p<0.001$), 30.02% ($p<0.001$), 50.81% ($p<0.001$), 48.15% ($p<0.001$) and 44.26% ($p<0.001$) respectively as compared to the baseline. No adverse effect of the study drugs was found on the bio-chemical parameters of liver and Kidney function test. No adverse event was found either volunteered by the patients or elicited by the investigator by clinical as well as by laboratory investigations at the baseline and after the treatment. The Unani formulations Qurs e Kushta Khabs Al-Hadeed and Habb-e-Marwareed were found clinically very effective and safe in the treatment of Sayalan-al-Rahim (Leucorrhea).

Keywords: Qurse e Kushta Khabs Al-Hadeed and Habb-e-Marwareed, Unani formulation, Sayalan-al-Rahim.

Introduction

Sayalan-al-Rahim (Leucorrhea) is an excessive vaginal discharge which may be whitish, yellowish or greenish in colour. It is a frequent gynecological complaint of women and accounts for more than 1/4th gynaec patients who visit gynecologists (Dutta, 2007; Sabaratnum *et al.*, 1993). Peculiar vaginal discharge is generally associated with body ache and thirst (Yudin *et al.*, 2003). The most common cause of leucorrhea is physiological followed by vaginal infections due to bacteria, virus, fungi and parasites. Other causes include foreign bodies, cervicitis and atrophic vaginitis (Pravina, *et al.*, 1991). Sometimes, symptoms of disease are so severe that it over shadows the actual disease and women seek treatment only for symptoms (Sutton et al, 2007; Johnston *et al.*, 2008).

The Unani scholars have described Sayalan-al-Rahim (Leucorrhea) and its treatment in various Unani classical literature like Kamil al Sana'a, Al Hawi, Firdaus al Hikmat and Tibb-e-Akbar etc. According to them, disease is due to poor quwwat-e-ghadhiya (nutritive faculty) of the rahim (uterus) that causes accumulation of fuzlaat (waste materials) (Kabiruddin, 2003). According to

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humoural theory, Sayalan-al-Rahim is caused by the excess of humours and is of four types; sayalan-al-rahim damwi, sayalan-al rahim-safrawi, sayalan-al-rahim balghmi and sayalan-al-rahim sawdavi with discharge colours reddish, yellowish, whitish and blackish respectively (Khan, 2010). Associated symptoms of the disease are excessive vaginal discharge, weakness, backache, pain in the thighs and calf muscles, burning micturition etc. (Kabiruddin, 2003; Khan, 2002).

The drugs available in modern medicine produces more or less adverse effects in the human body and therefore natural, herbal or traditional medicines are now being seen by the people with an eye of great interest and hope. Unani medicine is one of them that not only provides the drugs information in abundance but also claims that the drugs have least or no adverse effect.

The Unani physicians have used Unani pharmacopoeial formulations viz. Qurs e Kushta Khabs Al-Hadeed and Habb-e-Marwareed in the treatment of Sayalan al Rahim (Leucorrhea) since ages and the Unani drug and its formulations have been mentioned in various Unani literature (Khan, 2002; Jeelani, 2005).The Unani formulations and their compositions have also been mentioned in National Formulary of Unani Medicine for treatment of Sayalan-al-Rahim (Leucorrhea) (Anonymous, 1993) but the clinical data to prove that Qurs e Kushta Khabs Al-Hadeed and Habb-e-Marwareed are safe and efficacious in treatment of the disease are not available. The international community will accept this Unani formulation only if it satisfies the safety and efficacy norms set by the International Regulatory Authority (Shetti *et al.*, 2011), therefore, the present clinical study was conducted with an aim to assess the clinical efficacy and safety of 'Qurs-e-Khusta Khabs-al-Hadeed and Habb-e-Marwareed' in the treatment of Sayalan al Rahim (Leucorrhea).

Material and Methods

Study Drugs

The study drugs were two Unani formulations viz. Qurs e Kushta Khabs Al-Hadded and Habb-e-Marwareed. The compositions are given in Table 1. The drugs manufactured by CRI, Hyderabad were supplied to the Regional Research Institute of Unani Medicine, Patna.

Study Design

The study was designed as open-label multi centric clinical study.

Patients Selection

Diagnosis of each case was made with the help of a detailed history in respect of the patients i.e. history and physical examination, allergic history and other systemic examinations as well as the laboratory investigations. The screened patients presenting one or more symptoms of Sayalan al Rahim (Leucorrhea), who met the inclusion criteria were selected for the study by Regional Research Institute of Unani Medicine, Patna, between August 2014 and March 2015.

Inclusion Criteria

- Female patients in the age group of 14-45 years.
- Patients having excessive white discharge with symptoms like general weakness, backache, anaemia, excoriation or ulceration etc.
- Patients willing to sign an informed consent form to participate in the study.
- Patients willing to comply with various demands of the study.

Exclusion Criteria

- Patients having acute/chronic PIDs.
- Patients on long-term medications.
- Patients on oral contraceptives / IUDs or taking hormonal therapy
- Pregnant and lactating women.
- Diabetes mellitus excluded by taking the history and blood sugar fasting examination.

Treatment of Patients

The screened patients selected for the present study were given tablets of *Qurs-e-Kushta Khabs -al-Hadeed* (each tablet 100 mg) and *Habb-e-Marwareed* (each tablet 250 mg) with water after the meals; one tablet each of both the drugs twice daily for a period of four weeks. No concomitant treatment was given.

Clinical Evaluation

The efficacy of Unani pharmacopoeial formulations viz. *Qurs e Kushta Khabs al-Hadeed* and *Habb e Marwareed* were assessed on clinical parameters of the Sayalan-al-Rahim (Leucorrhea) including the amount of vaginal discharge, general weakness, backache, anaemia, excoriation, ulceration etc. As, these clinical parameters differ in severity, such as absent, mild, moderate or severe

from patient to patient and therefore, severity of the clinical parameters including amount of vaginal discharge, anaemia, excoriation and ulceration were graded as absent=0, mild=1, moderate=2 and severe=3. General weakness and backache were assessed on a 10 point VAS for appropriate assessment and statistical evaluation of the efficacy of the study drugs. The patients were followed-up after 2 weeks and 4 weeks. And at every visit, they were clinically examined and asked about the improvement or worsening of their symptoms. Assessment of the temperaments of the patients was also done before and after the treatment.

Safety Assessment

The safety was assessed by monitoring adverse events reported by the patients or elicited by the investigator by clinical as well as by laboratory investigations at the baseline and after the treatment. The laboratory tests included Haematological Test (Hb.%, TLC, DLC, ESR), Liver Function Test (Serum bilirubin, SGOT, SGPT, Alkaline phosphatase) and Kidney Function Test (Blood urea, Serum creatinine).

Statistical Analysis

All the data were statistically analyzed by applying paired't' test to evaluate the efficacy and safety of the drugs. Probability level of less than 5% was considered as statistically significant.

Results

The distribution of characteristics / demographic data of patients in accordance with their ages and marital status; chronicities and status of the disease; dietaries habits and temperaments and socio economic status are summarized in Table 2, Table 3, Table 4 and Table 5 respectively.

The efficacy of the study drugs on the clinical parameters of Sayalan-al-Rahim (Leucorrhea) are depicted in Table 6. After the treatment, mean ± SEM scores of clinical parameters of the disease including the amount of vaginal discharge, general weakness, backache, anaemia, excoriation and ulceration were found decreased from 2.56 ± 0.06, 5.03 ± 0.13, 5.23 ± 0.12, 1.24 ± 0.08, 0.81 ± 0.11 and 0.61 ± 0.10 to 1.84 ± 0.05, 3.29 ± 0.14, 4.29 ± 0.15, 0.95 ± 0.08, 0.58 ± 0.09 and 0.44 ± 0.07 respectively. The reduction in Mean ± SEM scores of the amount of vaginal discharge, general weakness, backache, anaemia, excoriation and ulceration were 43.36% ($p<0.001$), 60.64% ($p<0.001$), 30.02% ($p<0.001$), 50.81% ($p<0.001$), 48.15% ($p<0.001$) and 44.26% ($p<0.001$) respectively as compared to the baseline. No adverse effect was detected by clinical examination

and/or laboratory investigations.

The effects of the trial compound drugs on haematological parameters (HB, TLC, DLC and ESR) and bio-chemical parameters (Liver Function Test parameters and Kidney Function Test parameters), as assessed by the laboratory investigations are depicted in Table 7 and Table 8 respectively.

After the treatment, heamoglobin in blood was found significantly increased by 1.94 % ($p<0.05$) as compared to the baseline. After the treatment, the variations in mean scores of other haematological parameters were found not significant as compared to the baseline (Table 7).

After the treatment, Mean ± SEM scores of the markers of Lever function test i.e. Serum bilirubin, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), and Alk.Phosphatase (ALP) were reduced from 0.79 ± 0.03, 17.46 ± 0.86, 22.9 ± 1.75 and 6.67 ± 0.31 to 0.76 ± 0.02, 16.24 ± 0.82, 18.9 ± 1 and 5.96 ± 0.31 respectively; the percentage reduction in these L.F.T. parameters were 3.87% , 7.0%, 17.7% ($p<0.05$) and 10.73% ($p<0.05$) respectively as compared to the baseline (Table 8).

After the treatment, mean ± SEM scores of renal markers i.e. S.creatinine, S.urea and S.uric acid were decreased from 0.84 ± 0.02, 24.12 ± 0.68 and 3.08 ± 0.11 to 0.77 ± 0.01, 21.04 ± 0.59 and 3.24 ± 0.12 respectively; the percentage reduction in these renal markers were 8.14% ($p<0.05$), 12.75% ($p<0.05$) and 4.75% respectively as compared to the base line (Table-8). During the course of the study, no adverse event was reported by the patients.

Discussion

Analysis of the results of 62 cases of Sayalan-al-Rahim (Leucorrhea) treated with 'Qurs e Kushta Khabs al-Hadeed and Habb e Marwareed' have revealed some interesting facts which have been discussed as below:

- The present study has revealed that the disease is prevalent among women in 14-54 years of their age and the highest incidences of disease 46.78% was found in the mid reproductive age group of 25-34 years followed by 30.64% in the age group of 14-24 years (Table 2).72.58% of the patients were married and the remaining 27.42% unmarried. The married women have impact on the occurrence of vaginal discharge with active sexual life, which was also shown in another study conducted by Rice and schachter (Rice *et al.*, 1991).
- The incidence of the disease was observed maximum (72.58%) in the middle

income group. It may be due to the fact that most of the women in the middle income group are either employed in Government job or private sectors job where they have to work under stress and tension. Maximum mental tension in women of middle income group may be a perceived cause of the disease and is in accordance with the finding of Dash 1974 who has described the mental tension as a cause of this disease.

- The prevalence of Sayalan al Rahim (Leucorrhea) was more common among the females who are non-vegetarian (75.8%) as compared to safravi (bilious) (24.2%) temperament. This fact needs to be further studied by taking a large sample size (Table 4).
- The study disclosed that 67.80% cases were known. Data in Table 3 present the patients who had taken some treatment earlier for the disease in other system of medicines before coming to Unani system of medicines for treatment. This finding suggests that patients who were not getting any relief by other system of medicines came for treatment by Unani system of medicines. The present study further disclosed that 51.61% patients had this problem for more than one year. It may be due to the fact that lack of awareness among most of the women about the disease ignored the problem for a long period till it becomes chronic (Table 3).
- The study shows that clinical symptoms of the disease were subsided after the treatment with Qurs e Kushta Khabs al-Hadeed and Habb e Marwareed. After the treatment, the clinical parameters of the disease including vaginal discharge, general weakness, backache, anaemia, excoriation and ulceration were found significantly reduced by 43.36% ($p<0.001$), 60.64% ($p<0.001$), 30.02% ($p<0.001$), 50.81% ($p<0.001$), 48.15% ($p<0.001$) and 44.26% ($p<0.001$) respectively (Table-6).The result shows that Unani formulations Qurs e Kushta Khabs al-Hadeed and Habb e Ma rwareed are very effective to subside the associated symptoms of the disease. The clinical data of the present study prove the scientific justification of the traditional use of these Unani formulations in relieving the symptoms of Sayalan-al -Rahim(Leucorrhea)
- The present study exhibited that after the treatment, the change in haematological parameters like TLC, DLC and ESR were found not significant but haemoglobin was found significant as compared to the baseline ($p<0.05$) (Table 7).
- The present study exhibited variations in S. Bilirubin and SGOT and found not

significant. Elevated level of SGPT and Alk. Phosphatise, which may cause malfunctioning of the livers in the patients were found reduced significantly by 17.7% and 10.73% respectively as compared to the baseline. It was also found that the mean scores of elevated level of renal markers viz. S. Creatine and S. Urea were reduced by 8.14% and 12.75% respectively. Elevated levels of S. Creatine and S. Urea in bloods which may cause malfunctioning of the Kidney were reduced after the treatment (Table 8).

Based on the findings it can be suggested that trial drugs were found very effective and safe in the treatment of Sayalan al Rahim (Leucorrhea).

Conclusion

On the basis of the above observations, it can be concluded that the Unani formulations viz. Qurs e Kushta Khabs al-Hadeed and Habb e Marwareed are clinically effective and safe in the treatment of Sayalan al Rahim (Leucorrhea) and hence these can be prescribed to the patients in treatment of Sayalan al Rahim (Leucorrhea). These Unani formulations are cheap, easily available and can be easily tolerated by the patients without any adverse effect on them.

Table 1: Composition of 'Qurs e Kushta Khabs al-Hadeed and Habb e Marwareed'

Unani Drugs	Constituents	Latin names	Quantity
Habb e Marwareed	Mastagi	Pistacia lentiscus Linn.	120 gm
	Tankar neem biriyan	Sodium borate decahydrate	60 gm
	Mazu muhraq	Quercus infectoria olivier	60 gm
	Azraqi Mudabbar	Strychnox nux vomica Linn	60 gm
	Marwareed	Mytilus margaritiferus	15 gm
	Ambar Ash-hab	Ambra grasea	15 gm
	Arq-e-Gulab	Rosa damascena Mill	Q.S.
Kusta e Khabs al-Hadeed	Constituents	Latin names	Quantity
	Khabs al Hadeed	Iron oxide ferric/ferous oxide	100 mg
	Sirka Naishakar	Malus domestica Syn. M.sylvestris	Q.S.
	Maghz e gheekawar	Aloe barbadensis Linn.	Q.S.
	Chaach	Whey	Q.S.

Table 2: Distribution of Patients According to Age and Marital Status

Age groups (in years)	Unmarried		Married		Total	
	No.	% age	No.	% age	No.	% age
14-24	15	24.19	04	6.45	19	30.64
25-34	02	3.23	27	43.55	29	46.78
35-44	00	00	12	19.35	12	19.35
45-54	00	00	02	3.23	02	3.23
Total	17	27.42	45	72.58	62	100

Table 3: Distribution According to Chronicity and Status of the Disease

Chronicity of disease	Status of disease				Total	
	New Status		Known status		No.	Percentage
	No.	Percentage	No.	Percentage		
Up to 1 Year	17	27.4	12	19.4	29	46.77
01-03 Years	03	4.8	22	35.5	25	40.32
03-05 Years	00	00.0	07	11.3	07	11.29
Above 5 Years	00	00.0	01	01.6	01	01.61
Total	20	32.2	42	67.8	62	100

Table 4: Distribution According to Dietary Habits and Temperament of the Patients

Temperament of patients	Dietary habits				Total	
	Veg.		Non-Veg.		No.	Percentage
	No.	Percentage	No.	Percentage		
Damvi (Sanguine)	03	4.8	08	12.9	11	17.7
Balghami (Phlegmatic)	04	6.5	15	24.1	19	30.6
Safravi (Bilious)	05	8.1	19	30.7	24	38.8
Saudavi (Melancholic)	03	4.8	05	8.1	08	12.9
Total	15	24.2	47	75.8	62	100

Table 5: Socio – Economic Status of the Patients

Socio Economic Status	No. of Patients	Percentage
Lower Income Group	11	17.74
Middle Income Group	45	72.58
Higher Income Group	06	09.68
Total	62	100

Table 6: Efficacy of Unani Formulations *'Qurs e Kushta Khabs al- Hadded* and *Habb e Marwareed'* on Clinical Symptoms of Sayalan al Rahim(Leucorrhea)

Clinical Symptoms		Mean ± SEM	Percentage Decrease (↓)		t-value	df	p-value
Amount of Vaginal Discharge	BT	2.56 ± 0.06	43.36	↓	13.91	61	<0.001
	AT	1.45 ± 0.07					
General Weakness	BT	5.03 ± 0.13	60.64	↓	18.67	61	<0.001
	AT	1.98 ± 0.13					
Backache	BT	5.23 ± 0.12	30.02	↓	12.29	61	<0.001
	AT	3.66 ± 0.16					
Anaemia	BT	1.24 ± 0.08	50.81	↓	7.82	61	<0.001
	AT	0.61 ± 0.08					
Excoriation	BT	0.81 ± 0.11	48.15	↓	5.50	61	<0.001
	AT	0.42 ± 0.07					
Ulceration	BT	0.61 ± 0.10	44.26	↓	4.80	61	<0.001
	AT	0.34 ± 0.06					

Paired 't' test, p<0.001 (Highly significant), p<0.05 (Significant), n=62

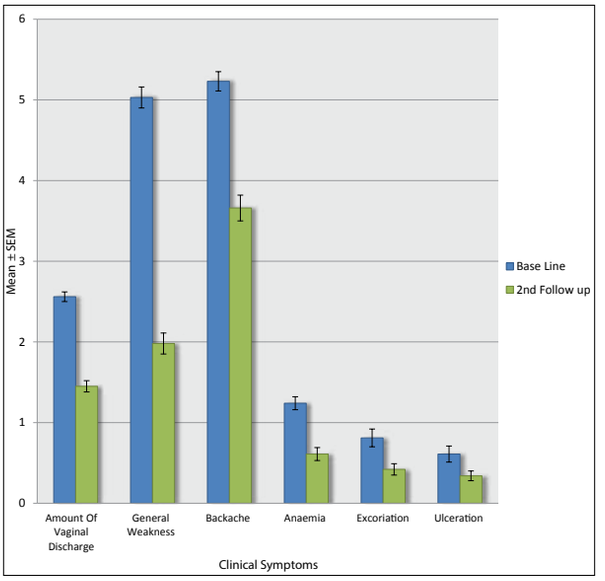

Fig. 1: Efficacy of Unani formulations '*Qurs e Kushta Khabs al- Hadded* and *Habb-e-Marwareed'* on clinical symptoms of Sayalan-al-Rahim (Leucorrhea).

Table 7: Effect of Unani Formulations on Hematological Parameters

Haematological parameters			Mean ± S.E.M		Percentage of Increase (↑) / Decrease (↓)		Paired 't' test	
			Base-line	After Treatment			t-value	p-value
C BC	Hb(gm/dL)		11.18 ± 0.13	11.4 ± 0.11	1.94	↑	2.15	0.035 (p<0.05)
	TLC(/mm)		6435.65 ± 164.93	6296.98 ± 172.55	2.15	↓	0.76	0.44
	D L C	N (%)	59.24 ± 0.95	58.6 ± 0.74	1.09	↓	0.57	0.54
		L (%)	35.5 ± 1.16	34.55 ± 0.77	2.68	↓	0.47	0.75
		E (%)	4.32 ± 0.33	3.92 ± 0.32	9.33	↓	0.36	0.096
		M (%)	2.03 ± 0.11	1.74 ± 0.09	14.29	↓	1.99	0.051
		B (%)	0 ± 0	0 ± 0	-	-	-	-
	ESR (mm)		17.9 ± 1.6	16.61 ± 1.38	7.21	↓	0.85	0.39

Statistical analysis- paired't' test; p>0.05 = Non Significant (N.S.); p<0.05 = Significant

Table 8: Effect of Unani Formulations on L.F.T and K.F.T. Parameters

Biochemical Parameters		Base-line	After Treatment	Percent decrease (↓) and increase (↑)		t value	p value
		Mean ± SEM	Mean ± SEM				
LFT	S.Bilirubin (mg/dl)	0.79 ± 0.03	0.76 ± 0.02	3.85	↓	1.13	0.25 p>0.05
	SGOT (IU/L)	17.46 ± 0.86	16.24 ± 0.82	7	↓	1.12	0.26 p>0.05
	SGPT (IU/L)	22.9 ± 1.75	18.9 ± 1	17.7	↓	2.4	0.019 p<0.05
	S.Alkaline Phosphatase (KA)	6.67 ± 0.31	5.96 ± 0.31	10.73	↓	2.13	0.036
KFT	S.Creatinine (mg/100 ml)	0.84 ± 0.02	0.77 ± 0.01	8.14	↓	2.88	0.005
	S.Urea (mg/dl)	24.12 ± 0.68	21.04 ± 0.59	12.75	↓	4.7	<0.001
	S.Uric Acid (mg/dL)	3.08 ± 0.11	3.24 ± 0.12	4.75	↑	1.07	0.28 p>0.05

Statistical analysis- paired't' test; p>0.05 = Non Significant (N.S.); p<0.05 = Significant

Acknowledgement

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सारांश

'सैयलन-अल रहीम (ल्यूकोरिया) में कुर्स-ए-कुश्ता खब्स-अल हदीद और हब्ब-ए मरवारीद' यूनानी औषधियों पर नैदानिक अध्ययन

[1]नजमुस सहर, [2]तसलीम अहमद, [3]मौ. वसीम अहमद, और [4]अर्निबेन गोस्वामी

इस अध्ययन का उद्देश्य 'सैयलन–अल रहीम'(ल्यूकोरिया) यूनानी भेषजकोशकीय मिश्रणों जैसे कुर्स–ए–कुश्ता खब्स अल–हदीद और हब्ब–ए मरवारीद' की सुरक्षा और प्रभावकारिता का मूल्यांकन करना है। दोनों औषधियों की एक गोली चार हफ्तों तक प्रतिदिन दो बार रोगियों को मौखिक रूप से दी गई। उपचार के पश्चात रोग के नैदानिक मापदंडों जैसे योनी स्राव की मात्रा, सामान्य कमज़ोरी, पीठ दर्द, खून की कमी, खरोंच और व्रणोत्पत्ति में क्रमशः 43.36% ($p<0.001$), 60.64% ($p<0.001$), 30.02% ($p<0.001$), 50.81% ($p<0.001$), 48.15% ($p<0.001$), और 44.26% ($p<0.001$), बेसलाइन की तुलना में कम कर दिया गया। अध्ययन औषधियों का कोई प्रतिकूल प्रभाव यकृत और गुर्दा क्रिया जाँच का जैव रासायनिक मापदंडों पर नहीं पाया गया। कोई प्रतिकूल प्रभाव न रोगियों द्वारा बताया गया और न ही बेसलाइन और उपचार के पश्चात् अन्वेषक द्वारा प्रयोगशाला के साथ–साथ नैदानिक जांचों में पाया गया। यूनानी मिश्रणों कुर्स–ए कुश्ता खब्स अल–हदीद और हब्ब–ए–मरवारीद को नैदानिक तौर पर सैयलन–अल–रहीम(ल्यूकोरिया) के उपचार के लिए बहुत ही प्रभावशाली एवं सुरक्षित बताया गया है।

To Evaluate the Safety and Efficacy of a *Unani* Formulation in *Iktisabi Qillat-E-Ifraz-E-Darqia* (Autoimmune Hypothyroidism) – A Pilot Clinical Study *(A Short Research Communication)*

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Abstract

The purpose of the study was to evaluate the safety and efficacy of new *Unani* formulation in *Iktisabi qillat-e-Ifraz-e-darqia* (autoimmune hypothyroidism) on five patients. For this purpose, decoction of semi-crushed powder made of six *Unani* herbal drugs was given twice daily in the morning and in the evening to the patients as per the method described in this paper for a duration of 90 days. The study was conducted in the year 2010-2011.The study period was one year. The patients were advised to take sour foods and fruits. After completion of the study, the therapy showed a significant improvement in the patients (*p=0.07*). The formulation had no any side effects and the patients felt overall sense of wellbeing and lighter. Details have been presented in this paper.

Keywords: Safety, Efficacy, Unani Formulation, Hypothyroidism

Hypothyroidism

This is known as *Qillat-e-Ifraz-e-Darqia* in *Unani* System of Medicine or hypothyroidism in Modern Medicine. It is a condition in which body lacks sufficient thyroid hormone (Norman; *et al.* 2011, Mathur, *et al. 2011*, Romshoo, et al 2009). Its other alternate names are autoimmune thyroiditis and Hashimoto's thyroiditis. The main purpose of thyroid hormone is to run the body's metabolism in order and the lack of sufficient thyroid hormone will affect metabolism (Norman et al; 2011). There are two common causes of hypothyroidism, the first which results due to inflammation of the thyroid gland, thus becoming incapable of producing sufficient hormone; and the second major cause is the broad category of medical treatments such as surgical removal of a portion or all of the thyroid gland. The thyroid gland uses iodine from foods such as seafood, bread and salt to produce thyroid hormones. The two most important thyroid hormones are thyroxine (T_4) and tri-iodothyronine (T_3), which account for 99% and 1% of thyroid hormones present in the blood respectively. If any disruption occurs at any of these levels, a defect in thyroid hormone production may result in a deficiency of thyroid hormone called as hypothyroidism (Mathur *et al 2011*).

The earliest reference in regard to Iodine use was found in Greek literature. The ancient Greeks, including *Galen*, used the marine sponge to treat swollen glands. The Swiss physician, Coindet, in 1813, hypothesized that the traditional treatment of goiter with seaweed was effective because of its iodine content and successfully treated goitrous patients with iodine. The French chemist Chatin in 1851 hypothesized that iodine deficiency was the cause of goiter (Zimmermann, et.al.; 2008). In Greek Medicine, both sea and rock salt were well known to the ancient Greeks and the healing methods of *Buqarat* (Hippocrates, 460 BC)

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especially made frequent use of salt. *Ibn Sina'*s (Avicenna 980-1037 A.D.) recipes also used salt. He emphasized the presence of Iodine and iron in coastal sea salt (Anonymous 2011). As such, there is neither the exact description of this disease nor any direct reference for the treatment is available in *Unani* System of Medicine but the descriptions with more or less similar signs and symptoms are found in the literature of Greek Physicians (Zimmermann, 2008; et al; Anonymous 2011) which nearly coincide with this disease.

A study was conducted on Hypothyroidism with intervention of a compound *Unani* formulation namely; *Akseer Darqeen* at Aligarh (Anees, 2002) but it was a preliminary one. An epidemiological study was also conducted in southern Kashmir in India on 886 patients from 2004-2009 including 724 (81.72%) females and 162 (18.28%) males in the age group of 12-75 years and it was found that the prevalence of primary hypothyroidism (especially subclinical hypothyroidism) is very high in Kashmiri population. It included 856 patients (96.61%) of primary hypothyroidism and 30 patients (3.86%) had subclinical hypothyroidism (TSH<10 µIU/ml). Primary hypothyroidism is the most common cause of elevated TSH (Romshoo, *et al.* 2009). The symptoms of hypothyroidism include fatigue, weakness, weight gain or increased difficulty in losing weight, coarse, dry hair, dry, rough pale skin, hair loss, cold intolerance, muscle cramps and frequent muscle aches, constipation, depression, irritability, memory loss, abnormal menstrual cycles and decreased libido. Hypothyroidism can often be diagnosed with a simple blood test such as Thyroid profile (Hasan *et al.*, 2006).

The patients of thyroid diseases, mainly hypothyroidism, have been attending to the OPDs of Regional Research Institute of Unani Medicine, Srinagar, for *Unani* treatment. The number is on an increasing trend and 98% is primary hypothyroidism which includes subclinical hypothyroidism up to 98% and rest 2% includes both mild hypothyroidism and overt hypothyroidism in which about 85 to 95% is female and rest male. On an average, 4-6 patients of primary hypothyroidism were reported at three OPDs of this institute which worked out to be about 2.5 to 3% patients of hypothyroidism. This observation coincides with a study conducted in southern Kashmir in 2004-2009. The management of hypothyroidism involves Thyroxin Replacement Therapy (TRT), starting form low dose of 50 micro grams daily for three weeks, then increase, thereafter to 100 micro grams daily for three weeks and finally, to a maintenance dose of 100-150 micro grams daily in single dose since its half-life is 07 days. Then repeat Thyroxin Function Test(TFT) was done after six weeks to maintain the dose usually in increments of 25 micro grams daily.

In view of increasing number of patients of hypothyroidism in Kashmir and failure of a number of the present conventional treatment, this study 'To Evaluate the Safety and Efficacy of a *Unani* Formulation in *Iktisabi Qillat-e-Ifraz-e-Darqia*

(Autoimmune Hypothyroidism) – A Pilot Clinical Study' was conducted on five patients during 2010-2011 at Regional Research Institute of Unani Medicine, Srinagar, Jammu and Kashmir, India.

Objective

To evaluate the safety of *unani* Formulation in *iktisabi qillat-e-ifraz-e-darqia* (autoimmune Hypothyroidism)

Material and Methods

Five female patients of diagnosed case of autoimmune (primary) hypothyroidism were included in the study, two belonged to the age range of 20-30 years, two 30-40 years and one 40-50 years. Their age ranged from 24 to 45 years with mean age of 33.6 years. Child-bearing women, pregnant and breast-feeding women were not included in the study. Though the patients were already screened and diagnosed by the Allopathic Doctors but Thyroid Profile was done before starting the treatment. After that, this formulation was given to the patients. The follow-ups were made on every 30th day. The last follow-ups were made on 90th day and post therapeutic Thyroid Profile in each patient was done on 91st day. The duration of therapy was 90 days and the duration of the study was one year. The patients were advised to take sour foods and fruits.

Two patients who had stopped Allopathic treatment for hypothyroidism two months ago and three patients who had detected it in the blood but had not taken any treatment were included in this study. Although, Thyroid Profile was done before and after the therapy for each patient, but for the sake of lucid presentation and explanation of response of therapy, only TSH level in each patient was taken into consideration.

The decoction of the following six *Unani* herbs in the form of semi-crushed powder of 30 gm, purchased from the local market, was given to the patients as per the method given below:

• *Aloo Bukhara* (*Prunus domestica*)	5 gm
• *Beikh Badyan* (root of *Foeniculum vulgare* Mill)	5 gm
• *Beikh Kasni* (root of *Cychorium intybus* Linn)	5 gm
• *Tamarhindi* (*Tamarindus indica*)	5 gm
• *Ustukhuddus* (*Levendula stoechs* Linn)	5 gm
• *Zeera Safaid* (*cuminum cyminum* Linn)	5 gm

Method of Preparation of Decoction and its Mode of Administration

The decoction made of semi-crushed powder of 6 simple Unani herbs(Unani Formulation), 05 gm each, total of 30 gm was prepared in 05 times of water,(i.e.,

150 ml of water), boiled for 05 minutes, filtered with ordinary filter paper and the decoction so prepared was given twice daily in the morning before breakfast. Decoction was again prepared (repeated) after boiling it for five minutes in the evening from the residue left in the morning in 150 ml of water and given after evening tea. The patients were asked to report for follow-up on every 30th day.

Results

After completion of the therapy, (90 days) it was found that the total TSH value of five patients was 227.39 µIU/mL on day 0 which came down to as low as 25.75 µIU/mL on 91st day. There was a reduction of 201.64 µIU/mL in five patients on 91st day. Similarly, the mean TSH value of the five patients on day 0 was 45.47 µIU/mL which came down to as low as 05.15 µIU/mL on 91st day. There was a mean reduction of TSH value of 40.32 µIU/mL in five patients after completion of the therapy (Table 1). A significant therapeutic response was seen Figure 1 (p=0.07).

Discussion

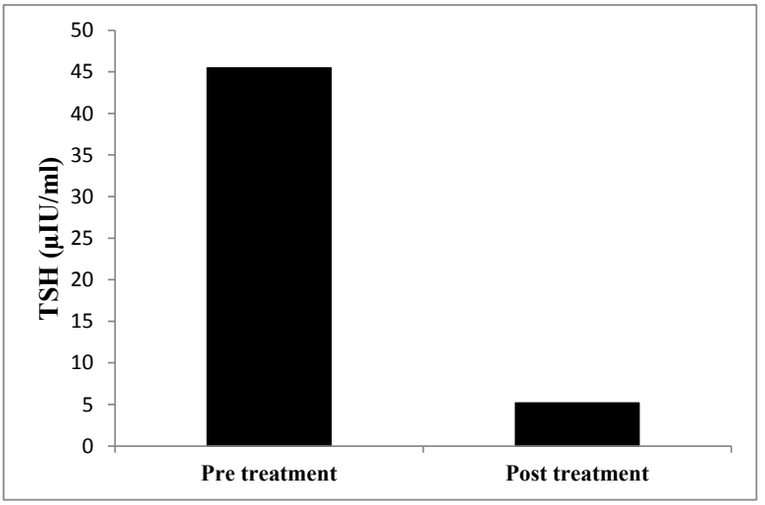
Since it was hypothesized that this disease is of yellow bilious-black bilious in origin, the first five Unani drugs, which are meant for the diseases due to yellow bilious-black bilious humors, were chosen in order to correct the imbalance of humors in the body which might have caused this disorder and the last(sixth) drug having diuretic action (Ali, 1979) was included in order to induce diuretsis to get body detoxified from ill efffect/s of the disease through complex phenomenon under the influence of decoction. It seems that, these drugs had tried to maintain a balance of humors thus enabling a significant reduction of TSH levels in all five patients. After the treatment, the patients felt overall a sense of wellbeing and lighter. The therapy had no any side effects.

Table1: Pre and Post Treatment TSH Values of Five Patients of Hypothyroid.

S. No (patients)	Value of TSH on day 0 (µIU/m L)	Value of TSH on day 91 (µIU/m L)	Reduction in TSH after day 91 (µIU/m L)
1.	65.92	10.75	55.17
2.	20.57	3.21	17.36
3.	112.34	0.01	112.33
4.	8.47	3.84	4.63
5.	20.09	7.94	12.15
Total	**227.39**	**25.75**	**201.64**
Mean	**45.47**	**05.15**	**40.32**

Conclusion

It is, therefore, concluded that this new Unani formulation may be tried in cases of autoimmune hypothyroidism as an alternate.



(p=0.07)

Fig. 1: Chart Presentation of Pre and Post Treatment Response of Unani Formulation in 05 patients of Autoimmune Hypothyroid:

Note:

1. The Chart presentation (*p=0.07*) also shows a significant therapeutic response
2. Data shown are the mean (n=5)

Acknowledgement

The authors are thankful to CCRUM, New Delhi, for giving permission to carry out this pilot study at Regional Research Institute of Unani Medicine, Srinagar, Jammu and Kashmir, India. Thanks are also due to the patients for their active participation and co-operation in completing this study.

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(Note: This research article was presented by the Corresponding Author at the 'National Seminar on the Role of Unani Medicine in Non-Communicable Diseases' held at CCRUM, New Delhi, during 14-15 January 2015.)

सारांश

इक्तिसाबी किल्लत-ए-इफराज-ए-दरक्विया (ऑटोइम्यून हाइपोथायराडिज़्म) में यूनानी औषधियों की प्रभावकारिता एवं सुरक्षा का मूल्यांकन करना - एक पायलट नैदानिक अध्ययन (एक संक्षिप्त अनुसंधानिक संचार)

[1]नक़िबुल इस्लाम [2]कौसर और [3]बशारत बुख़ारी

अध्ययन का मुख्य उद्देश्य पाँच रोगियों पर इक्तिसाबी किल्लत–ए–इफराज–ए–दरक्विया (ऑटोइम्यून हाइपोथायराडिज़्म) में यूनानी मिश्रणों की सुरक्षा और प्रभावकारिता का मूल्यांकन करना था। इस उद्देश्य के लिए, छः यूनानी हर्बल औषधियों से बना आधा पीसा हुआ जोशांदा (पेपर में वर्णित विधि के अनुसार) नब्बे दिनों की अवधि के लिए रोगियों को प्रतिदिन दो बार सुबह और शाम दिया जाता है। वर्ष 2010–2011 में यह अध्ययन किया गया। अध्ययन की समय अवधि एक वर्ष की थी। रोगियों को खट्टा भोजन एवं फल खाने की सलाह दी गई। अध्ययन के पूर्ण होने के उपरान्त, रोगोपचार से रोगियों (पी=0.07) में एक महत्वपूर्ण सुधार देखा गया। इन मिश्रणों का कोई भी दुष्प्रभाव नहीं था और रोगियों ने हल्का और सुखी महसूस किया। विवरण पेपर में प्रस्तुत किया है।

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