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Editorial Office

CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
Ministry of Ayush, Government of India
61-65, Institutional Area, Janakpuri, New Delhi - 110 058
Telephone: +91-11-28521981, 28525982
Email: unanimedicine@gmail.com
Website: www.ccrum.res.in

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Editorial

The Central Council for Research in Unani Medicine (CCRUM) is an autonomous organization under the Ministry of Ayush, Government of India entrusted with the mandate of research and development in Unani Medicine. Since it came into being in 1978, the CCRUM has been making concerted efforts for creating scientific evidences for this age-old system which has been treating and caring the mankind in a larger part of the world including India. Through its endeavors in the area of clinical research, preclinical research, survey and cultivation of medicinal plants, drug standardization and literary research, the CCRUM has been truly successful in increasing the system's acceptability among the modern and scientific society of the world, promoting its global visibility and developing viable solutions for health problems of the people.

The CCRUM publishes various periodical and non-periodical publications to propagate its research outcomes. The Hippocratic Journal of Unani Medicine (HJUM) is one such publication. It is a peer-reviewed scientific quarterly journal which covers papers on clinical research on single and compound Unani drugs, validation of regimen therapy, experimental pharmacological studies, standardization of single and compound drugs, development of standard operating procedures, ethnobotanical studies and development of agro-techniques thereof and literary research on classics of Unani Medicine. The journal is also open for studies on safety evaluation of Unani and other herbo-mineral drugs, nutraceuticals, cosmotherapeutics, aromatics, oral health, lifestyle disorders, sports medicine, etc. and such other newer areas which are the outcome of modern day living.

This issue of the HJUM comprises seven papers. The first paper entitled 'Dynamics of *Arkān Arbaʿa* for understanding of life in animate things' attempts to elucidate the substantial and basic precursors of life as stated in the literature of Unani Medicine and tries to explore it in the light of facts based on basic sciences. In the second paper, the author has presented a literature review on *Inkibāb* of herbal medicines as a unique mode of treatment of *Nazla* in Unani Medicine. The third paper 'Investigating the relationship between somatotype and *Mizāj* of young non-athletes' establishes a significant relationship between people's *Mizāj* and physical structure and physiological and psychological performance of individuals through a field study conducted on 200 individuals having different *Mizāj*.

In the fourth paper entitled 'UV-visible spectroscopic study on interactions of selected Unani herbs with calf thymus DNA *in-vitro*', outcomes of a study on six extracts of *Solanum nigrum* (*Mako*) and *Sesame indicum* (*Til*) having anticancer and antioxidant properties have been discussed. The fifth paper presents physico-chemical and pharmacognostical evaluation of anti-inflammatory poly-herbal formulation *Ḥabb Hindī Muḥallil* conducted at the Drug Standardisation Research Unit of Central Council for Research in Unani Medicine. The sixth paper entitled 'Therapeutic intervention of Unani pharmacopoeial formulations in the management of *Surʿat-i-Inzāl* (premature ejaculation)' is based on a study conducted to assess the safety and efficacy of Unani pharmacopoeial formulations *Safūf Mughalliẓ-ī-Manī, Maʿjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā*' in the management of *Surʿat-i-Inzāl* (premature ejaculation). The last paper of the issue elucidates outcomes of a preliminary study on efficacy of Unani formulation *Maʿjūn ʿAqrab* in the management of *Haṣāt al-Kuliya wa'l-Ḥālib* (kidney and ureter stone).

We have been constantly striving to reach to higher standards and make HJUM a leading journal of Unani Medicine and related sciences. In this context, we thank the authors for their contributions and our learned reviewers for their invaluable inputs in improving the manuscripts. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions.

Prof. Asim Ali Khan
Editor-in-Chief

Contents

Dynamics of *Arkān Arba'a* for Understanding of Life in Animate Things

[1]A. Rahman,
[2]Wasim Ahmad*,
[3]G. Sofi,
[2]Mohd. Zulkifle

[1]Department of Mahiyatul Amraz, National Institute of Unani Medicine, Bangalore

[2]Department of Kulliyat, National Institute of Unani Medicine, Bangalore

[3]Depart of Ilmul Advia, National Institute of Unani Medicine, Bangalore

Abstract

Unani Medicine has travelled a long way and flourished across different geographies. It is based on comprehensive concepts which govern its unique *Uṣūl-i-Tashkhīṣ* (principles of diagnosis) and *Uṣūl-i-'Ilāj* (principles of treatment). The system derives its principles from observation, logic, and philosophy and therefore the perception, understanding and discernment of this system without proper understanding of logic and observations is not possible. In the contemporary scientific era, fundamentals of Unani Medicine need to be understood from the prospects of modern sciences as well. This paper attempts to elucidate the substantial and basic precursors of life as stated in the literature of Unani Medicine and tries to explore it in the light of facts based on basic sciences.

Keywords: *Arkān*, Universe, Life, Transformation, Unani Medicine

Introduction

Unani Medicine is a medical system based on a conceptual framework organized around *al-Umūr al-Ṭabi'iyya* (Natural Fundamental Concepts). The basic concept of *Arkān* serves the material base for it. *Arkān* are the building blocks of all living and non-living things of the universe. The concept evolved has Greek origin. From a single basic block, scholars Argued for two, three, four and infinite basic building blocks. Unani scholars have consensus on *Arkān Arba'a* (four primordial substances) as the basic blocks. This consensus was arrived at through logical explanation of the necessary factors of the existence of a thing. They argued that these are sufficient and necessary for a thing to be formed and sustain. Thus, the concept of *Arkān Arba'a* was taken as one of the essential bases for the conceptual framework of Unani Medicine. This paper attempts to elucidate the substantial and basic precursors of life as stated in the literature of Unani Medicine and tries to explore it in the light of facts based on basic sciences.

Methodology

The concept of *Arkān Arba'a* was surveyed from the literature of Unani medicine. Various characteristics and dynamic manifestations were noted. The same was explained in the light of various facts of science that were gathered from books and web-based platforms.

*Corresponding author; Email: drwasimkulliyat@gmail.com

Observations and Discussion

Unani concept of *Arkān Arbaʿa*

According to Unani Medicine, *Arkān Arbaʿa* (four primordial substances) are responsible for the origin of *Mawalīd Thalātha* (three primary matters: solid, liquid, gases). These *Arkān* are *Nār* (fire), *Hawā*' (air), *Mā*' (water), *Arḍ* (earth). The *Arkān* are the basic building blocks of substances in the universe. The three important aspects of primordial matter, *Ṣūrat* (form) and *Ṣūrat Nawʿiyya* (specific configuration) are necessary for a substance to exist independently. Since any matter manifests itself through its *Kayfiyāt* (qualities) which are associated with the form, each of the *Arkān* has specific quality. There are two pair of basic qualities: One pair is considered active which includes 'hot' and 'cold', and the other pair is passive which includes 'moistness' and 'dryness'. These qualities manifest as the natural effects or properties of a substance.

Renowned Unani scholar *Jurjani* proposed that *Arkān* are the basic constituents for the origin, evolution, and sustenance of life (Jurjani, 2010). This concept is corroborated by the contemporary theory that the involvement of water and organic molecules is essential for the origin of life. Theories of evolution of solar system state that before the appearance of animate things on the earth, it was a sphere of gases and heat. On close reflection, it is clear that *Rukn Hawā*' (prebiotic atmosphere) comprised of gases, vapor, heat and solid organic and inorganic particles. By cooling, these things settled down and concentrated according to their density. Some of them became compact and heavy, occupied the central position in the hot and revolving gaseous mass. On further cooling, water vapor condensed around, and some gases could not condense and hence, they occupied the place above water which is called *Hawā*'*-i-Muḥīṭ* (atmospheric air), and surrounding this gaseous sphere is a sphere of heat (Zulkifle, 2013).

Similar analogy is seen when Unani literature describes the nature of four *Arkān*. Regarding physical properties of these *Arkān Arbaʿa*, Unani philosophers argue that the *Rukn* earth is a simple body, the natural position of which is below the other *Arkān* due to its heaviness. In nature, earth serves the purpose of making the objects firm and stable, and maintains their forms and figures (Anonymous, 1993; Antaki, 2008). *Rukn* water is a simple body, and its nature is *Bārid Raṭb* (cold and moist). Its position is below air. It provides easy acceptance of any shape (Jurjani, 2010; Sina, 2012; Arzani, 2010). Moisture in water means ability of gathering of dispersive substances again and molding into any kind of shape but is incapable of retaining it (Anonymous, 1993). *Rukn* fire is a simple body that is elusive and light in nature (Baghdadi, 2004) and it is most elevated among all other *Arkān* due to its absolute lightness. *Rukn* air is less subtle than fire and provides lightness, porosity, thinness, and expansion to any object and has the ability to raise things upward (Jurjani, 2010; Ibn Sina, 2012; Arzani, 2010).

The explanation of the four *Arkān* is also illustrated by Unani scholars by analyzing the phases of a substance. The physical state of a matter, and its physical condition, is determined by its physical properties. Two samples of a matter that have similar physical properties are in the same state (Atkins and Paula, 2010).

Ibn Sina (980-1037AD) mentions that the movement of the *Rukn* fire is upward, and *Rukn* earth is downward, hence, it is the *Ḥayyiz* (ordered position) that is sought by a particular *Rukn*. Once there is the mixture what is referred as *Murakkab* (compound), the simple body does have enough *Quwā* (power) permeating the whole that occurs after the mixture (Gutas, 2012; Ginnis, 2009; Magner, 2005). James T. Robinson mentions about mixing of these four basic substances to explain basic three origins of substances by the change of position of spheres of *Arkān*. By these spherical mixing of basic substances there is formation of three natural kingdoms in universe i.e., animal, plant, and mineral (Robinson, YNM).

Arkān in modern perspective

Various forms of *Arkān* are responsible for different types of characteristics originally like functions, activities, and their existence. Further, due to proper admixing of *Arkān* with their described characteristics change i.e., hot and cold are responsible for active characters, whereas moistness and dryness cause passive characters (Adamson and Taylor, 2005).

For the genesis of specific *Mizāj* of species, appropriate contribution from *Arkān* is necessary. Yet no animate has been observed there without the four *Arkān* and their *Kayfiyāt* (qualities). We can recognize the dominancy of *Arkān* in every animate with the help of their specific structures / morphology, habitat, and dietary habits. For instance, if *Rukn Mā'* is dominant in organisms then their skeleton will be soft, elastic and habitat will be aquatic. Likewise, the flying property of organisms is attributed to the dominance of *Rukn Hawā'*. *Rukn Nār* is responsible for the existence of thermophiles, and terrestrial life indicates the dominance of *Rukn Arḍ*. If the habitat of these organisms is shifted, they cannot survive. Here again it is compelling to observe the effect of predominance of *Arkān* linked to biodiversity.

By conceiving the dietary habits of animal kingdom, one can deduce the presence and dominance of a particular *Rukn*. A lion, because of dominance of *Rukn Nār*, takes *Ghidhā' Ḥārrah* (hot diets) like meat and blood. Unani philosophers stated that the *Mizāj* of human being is *Mu'tadil* (moderate) or *Qarīb Mu'tadil* (near to moderate) because of proportionate presence of *Arkān Arba'a*, therefore a human being takes vegetarian, non-vegetarian or both types of diet (Jalinus, 2008).

Two of the *Arkān* are light, namely *Rukn* Nār and *Rukn Hawā'*, while the other

two are heavy, namely *Rukn Arḍ* and *Rukn Mā'* (Saffari and Pakpour, 2012; Gruner, 1973; Shah, 2007). Another analogy for understanding *Arkān Arba'a* is related to their densities. Assuming that the earth's outermost layer is about 4 or 5 times denser than water and that water is one thousand times heavier than air, if all the planetary materials were expanded to the density of air, it would take up a space nearly 1,400,000 times larger than the earth's sphere (Kant, 2008).

Amalgamation of *Rukn Mā'* and *Rukn Arḍ* from below and heat (sunlight) and air (carbon dioxide) from above, plants have a relation with both *Rukn Arḍ* and the sky (Capra, 1996). Thus, the process of photosynthesis also indicates the role of *Arkān Arba'a in* the origin and existence of life.

One is inclined to consider that plants grow out of the *Rukn Arḍ*, but actually most of their substances come from the *Rukn Hawā'*. The cellulose and other organic compounds formed by photosynthesis consist of carbon (C) and oxygen (O) atoms, which plants obtain from air in the form of carbon dioxide directly. Therefore, the weight of a wooden piece comes almost completely from the *Rukn Hawā'* (air) as a piece of wood releases CO_2 and heat on burning (Capra, 1996).

Although all life is based on cellular structure, genetic information, and its duplication and development over time; these alone do not make life possible. The structures and functions form a viable unit only in environment which sustains them. Energy is essential for all processes of life. The only ultimate energy source of life on the earth is the sunlight even for an animal that uses a plant which takes the sunlight to grow by photosynthesis (Teerikorpi *et al.*, 2009). This transformational process of energy helps to understand the concept of *Istiḥāla* (transformation) through *Rukn Nār*.

Life also wants a solvent to liquefy and transfer the entire *Rukn Arḍ*. *Rukn Mā'* is the solvent for the existence of life because it appears to be the appropriate solvent for all biochemical reactions. Thus, *Rukn Mā'* is also a basic component for living beings.

Mā is origin for all forms of life, since animals originated in *Mā*, and the procreation substance of all animals is liquid. Augustine holds *Mā'* to mean formless matter. Water may be understood here in the sense of radical moisture (Paracelsus), which is absolutely essential to life, H_2O being thus as it was an instrument or substrate. The plant cannot shootout leaves, flowers, and fruit without it. Humans cannot thrive without this radical moisture, or innate moisture. This concept brings us to the domain of chemistry (Gruner, 1973).

The characteristic of water strongly affects the chemical properties of water. The electric polarity of water molecules gets frail electric interactions, or hydrogen bonds, to form among different molecules, exhibits the integrated behavior of water, as a weak bond network (Teerikorpi *et al.*, 2009). This validates the

concept of Unani scholars that water can accept any form and shape easily.

Rukn Mā stays in fluid form at a broad range of temperature. The molecular pulling of *Rukn Mā*' towards each other also resists the rising of temperature, and therefore more heat/energy is required to move up the temperature of *Rukn Mā*'. Similarly, more heat/energy is generated when water cools; this characteristic makes it a great temperature thermostat, both inside the cells and in a large environment. *Rukn Mā*' dissolves other charged molecules readily; this makes it a very efficient solvent for all ionic compounds. Water also liquefies the polar compounds where the negative and positive charges are yet together on a molecule but separated. On the other hand, water does not tend to dissolve non-polar molecules, such as hydrocarbon chains. So, it is very important biological feature, because these are hydrophobic molecules and in water solution tend to combine with each other rather than with water (Teerikorpi *et al.*, 2009). The hydrophobic and hydrophilic interactions also strongly influence the three-dimensional folding of molecules, including proteins, and assist them to provide stability. Because of hydrogen bonding, evaporation and surface tension, behavior of water is very perfect in the environment.

Air is a simple body, the natural position of which is above water and below fire. This is the explanation for its relative lightness. Its nature is hot and moist. It furnishes subtleness to the things and makes creations easier, frailer, and hollow. An object becomes equally squeezable and may adopt and release any shape easily due to its moistness (Anonymous, 1993; Nafees, 2010; Anonymous, 2012; Anonymous, 1973). Thus, it is confirmed that the dominance of *Rukn Hawā*' is observed in flying organisms.

However, by counterargument the natural scientist must find the natural causes of the role of *Arkān*, with no need to assume any special arrangements for the phenomenon. Immanuel Kant observes correctly that sea winds must go through periodic motions, even if no human beings would have lived on the island, it is no property other than the flexibility of *Hawā*', and it is also necessary for the growth of *Nabātāt*. Over the land the sun's heat upsets the equilibrium of *Rukn Hawā*' by rarefying it out, thus allowing the cooler sea air to go up from its position and take its place. It provides a number of benefits to living beings on the Earth. In Kant's words, these benefits are generally advantageous to our planet and life, and no other arrangements is essential to make them except the same general properties of *Rukn Hawā*' and heat (Kant, 2008).

Rukn fire is a simple body, the natural position of which is above all the elements. Fire is hot and dry in nature. The purpose of its existence is to produce maturation, firmness, lightness and intermingling. It penetrates the aerial substance and breaks the sheer coldness of the two heavy cold elements *Rukn Arḍ* and *Rukn Mā*' (Gruner, 1973; Anonymous, 2012; Anonymous, 1973).

Everything can grow, attenuate, rectify, and blend with other things easily due to absolute weightlessness and penetrative power of *Rukn Nār* and make the things flexible. Exactly the same is seen as stated by Charles Law where the expansion is the fundamental thing due to heat/energy. According to Unani classical texts, heat energy comes under the *Rukn Nār* (Clarke, 2017; Fullick, YNM). Evidence of dryness of fire is argue from the fact that dry wood burns easily than wet because transition of any *Rukn* to other state with similar quality is easier than to the state with opposing one (Nafees, 2010).

Of course, heat alone may not provide energy accessible to organisms, but heat can promote a physical setting that facilitates the derivation of metabolic energy from chemical or solar sources (Pudritz *et al.*, 2007).

A hot inanimate object, a rock, a block of iron, or even a cup of tea, cools according to Newton's law of cooling that the rate of cooling is proportional to the temperature difference between the object and its surroundings. Hence, an inanimate object cools fast at first and then progressively slower as its temperature approaches that of its surroundings.

The exact rate of cooling for any object depends on its composition and size; technically, it depends on a property known as its 'heat capacity', with objects of high heat capacity, effectively being hungry for heat, cool down slowly. Water has a high heat capacity, which is one reason why ice forms slowly on lakes in winter and why the oceans are a kind of thermal ballast and help to stabilize temperature of the planet. In so far as a human body is mostly water, it cools quite slowly to the temperature of its surroundings with the precise rate depending on the extent of thermal contact with them (Atkins, 2011).

Along with these qualities of *Rukn Nār*, heat energy is used to achieve the work in inanimate but animates are isothermal and utilize the chemical energy for growth. In such cases, energy might be transferred from one place to another or transformed into different forms of energies governed by law of conservation of energy (Deb, 2002).

Many physical and chemical properties depend on the energy associated with each of these modes of movement. For instance, a chemical bond may break down if a lot of energy becomes concentrated in it (Atkins and Paula, 2010).

Transition of matter (chemical composition and physical state), the spontaneous conversion of one stage into another stage, happens at a characteristic of *Ḥarārat* for given pressure. All the life activities depend on the coupling of the exothermic and endothermic reactions, for the oxidation of food drives other reactions forward. In biological cells, the energy released by the oxidation of foods is stored in Adenosine Tri Phosphate (ATP, 1). The essence of the action of ATP is its ability to lose its terminal phosphate group by hydrolysis and

to form Adenosine Di Phosphate (ADP) (Atkins and Paula, 2010). From the discussion above regarding energy, Unani Medicine generalize that *Ḥarārat* plays a key role in the process of life.

In case of solid matter, the lattice energy (form of energy) is the difference in potential energy of the ions packed together in a solid and widely separated as a gas. The lattice energy is always positive; high lattice energy indicates that the ions interact strongly with one another to give a tightly bonded solid matter (Atkins and Paula, 2010). This proves the quality of *Rukn Nār* which provides firmness and steadiness to the body by its *Kayfiyyāt* i.e. *Ḥarārat* and *Yabūsat*.

It is observed at cellular level that chemical reactions in the cell rely on extracted energy gained from nutrition. This requirement of energy should be provided continuously and constantly to maintain the energy transformation. In humans, alterations in *Ḥarārat* take place to achieve physiological function; for instances, haemoglobin saturation depends upon this energy differences. At 25°C, saturation of haemoglobin is 88% while it is 56% at 37°C (Campbel and Farrel, 2007). *Ḥarārat Gharīzī* (innate energy) accomplishes functions that are needed by animals (Harwi, 2007).

Peter Atkins observes that the life is the outcome of an alliance of molecules. To achieve the dissipation of energy, an aggregation of molecules needs to form, one molecule perhaps to harvest energy from the sun or from a local hotspot; another molecule to accept that captured energy and respond; another molecule perhaps to be welded to the second in what at first was achieved by simple incorporation. The simple incorporation of one molecule causes such aggregate an edge on incorporating others (Atkins, 2011). This explanation supports the view of ancient Greek philosophers on origin of life.

Green plants play an important role in the transformation of energy through almost all ecological cycles. Their roots use *Rukn Mā'* and *Rukn Arḍ*, and consequently juices go up to the leaves, where they combine with carbon dioxide (CO_2) from the air to form sugars and other *Nāmiyātī Murakkab* (organic compounds). In this marvelous process, known as photosynthesis, *Shamsī Tawānā'ī* (solar energy) is transformed into *Kīmīyā'ī Tawānā'ī* (chemical energy) to form *Nāmiyātī Ajzā'* (organic substances), while oxygen (O_2) is expelled into the air to be taken up again by other *Nabātāt* (plants), and by *Ḥaywānāt* (animals), through the respiration process (Capra, 1996; Ross, 1947). Here the concept of transformation of *Arkān Arba'a* into other form can be accounted. In the above mentioned process *Rukn Nār* is transformed into *Rukn Hawā'* and *Rukn Arḍ*.

Dynamic interaction of *Arkān* in present perspective

In the organization of *Arkān,* there is either absolute excess or deficiency of these

qualities. Heat and cold are two opposite directions of energy in space while dryness and moisture two contrary reactions of the mass in time. As Einstein has demonstrated in the world of phenomenon there is neither pure energy nor pure mass in any form except as a relative proportion of both in their various manifestations. Everything is made of mass and energy simultaneously; they are separated from each other in thought. Science also recognizes no form of mass or energy could have quality to the absolute degree, the generalization of the Unani Medicine that all objects including what we may term as the atoms, molecules, elements, compounds, genes, or chromosomes, have their own relative proportion of all the four qualities - two of mass and two of energy. The *Ḥarārat* is present in *Rukn Nār* and *Rukn Hawā'*, coldness in *Rukn Arḍ* and *Rukn Mā*, dryness in *Rukn Nār* and *Rukn Arḍ* and moisture in *Rukn Mā* and *Rukn Hawā'*. Thus, the building blockers come to differ in one aspect, and also resemble one another in other aspects. Each building blocker in this way has a resemblance and relationship through one of its qualities with building blocker below or higher to it and by its other qualities is of an opposite and different character. It is on account of their mutual similarities and differences that they tend to act and react upon each other. By differing from each other they can retain their identity and by resembling one another they tend to combine with each other.

The law of conservation of matter and energy is accounted by Unani scholars by generalizing that building blockers as the ultimate units of matter and energy are incapable of disintegrative alterations and that from one compound to other the change is merely a less or more of their quantity and quality (Anonymous, 1973).

The method of determining the molecular weight of various substances from the rising of boiling point and the lowering of freezing point of their solution is accounted by the Unani concept that greater the earth in a solution greater the resistance to change from liquid to gaseous or solid state and hence greater is the weight (Atkins and Paula, 2010; Anonymous, 1973).

Ultimately life manifests the characteristics found in the body as they represent in their building blockers. The *Rukn* of any sign shows the specific type of consciousness and method of most immediate perception to which the individual is attuned. Air signs have correlates of the mind's sensation, perception, and expression, specially related to geometric thought forms. Fire signs to express the warming, radiating, energizing life principle which can manifest as enthusiasm and love or as ego. Water signs are the cooling, healing, soothing, principle of sensitivity and feeling response. Earth signs reveal attunement with the world of physical forms and a practical ability to utilize the material world (Arroyo, 1975). These all signs are according to properties of *Arkān* which are mentioned by Unani philosophers.

Conclusion

The present facts that have been established after rigorous efforts of previous three centuries evidently furnish a lot of evidence to support the comprehensive framework of four *Arkān* as stated in the literature of Unani Medicine in context of creation and sustenance of life. This is the preliminary work done in this direction and further study is required for elaboration and better systematization of Unani concepts in the light of modern sciences.

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सारांश

सजीव वस्तुओं में जीवन को समझने के लिए *अर्कान अरबा* की महत्ता

ए. रहमान, वसीम अहमद*, जी. सोफ़ी, मो. ज़ुलकिफ्ल

सारांश

यूनानी चिकित्सा ने एक लम्बा सफ़र तय किया है और विभिन्न भौगोलिक क्षेत्रों में फली–फूली है। यह विस्तृत अवधारणाओं पर आधारित है जो इसके अनूठे *उसूल–ए–तशख़ीस* (रोगनिदान का सिद्धांत) और *उसूल–ए–इलाज* (उपचार का सिद्धांत) को संचालित करतीं है। इस पद्धति के सिद्धांत पर्यवेक्षकण, न्यायशास्त्र और तत्वज्ञान से उत्पन्न हुए हैं और इसीलिए इनकी उचित समझ के बिना इस पद्धति की अवधारणा को समझना संभव नहीं है। वर्तमान वैज्ञानिक युग में यूनानी चिकित्सा के मूल सिद्धांतों को आधुनिक विज्ञान की दृष्टि से भी समझने की आवश्यकता है। इस शोध पत्र में यूनानी चिकित्सा के साहित्य में बताए गए जीवन के आधाभूत तथ्यों और सिद्धांतों को स्पष्ट करने का प्रयास किया गया है तथा मूल विज्ञानों पर आधारित तथ्यों के प्रकाश में इनका पता लगाने की कोशिश की गई है।

शब्दकुंजी: *अर्कान,* संसार, जीवन, परिवर्तन, यूनानी चिकित्सा

Inkibāb of Herbal Medicines: A Unique Mode of Treatment of *Nazla* in Unani Medicine – A Literature Review

Zehra Zaidi

Department of Ain, Uzn, Anaf, Halaq wa Asnan, School of Unani Medical Education & Research Jamia Hamdard, New Delhi

Abstract

Introduction: In this study, an attempt has been made to highlight the relevance of *Inkibāb* (inhalation) for treating *Nazla* in the present scenario from Unani Medicine sources.

Method: Literature concerning *Inkibāb* was explored from the classical manuscripts of Unani Medicine and assessed. Recommended drugs for *Inkibāb* in the treatment of respiratory and other diseases were extracted from original sources. Electronic databases were searched for investigating the pharmacological efficacy of the inhalation of herbs.

Results: More than thirty plants recommended for *Inkibāb* in *Nazla* by Unani scholars have been identified. Various research studies have proved that steam inhalation acts as expectorant and relieves cough, have amelioration of symptoms of allergic rhinitis, helps in alleviation of cold symptoms and increased nasal patency. The essential oils of many herbs including cinnamon have shown antibacterial effects. *Inhalation of* camphor, menthol, and Eucalyptus oil provides relief in upper respiratory tract (URT) infection in children. Inhalation of *Nigella sativa* seeds has broncho-relaxant effect in asthma and also provides statistically highly significant symptomatic relief in cytokine release syndrome (CRS). These studies have proven the claims of Unani physicians regarding effectiveness of *Inkibāb* in nasal diseases.

Conclusion: *Inkibāb* (inhalation therapy) of medicinal plants can be taken for future research to provide economic, effective and safe remedy for allergic rhinitis, CRS, etc.

Keywords: *Inkibāb*, Unani Medicine, Nazla, Nasal diseases

Introduction

Inkibāb is one of the important modes of treatment which is in clinical practice by Unani physicians since ancient times to treat various diseases especially the respiratory disorders. *Inkibāb* means taking the vapours of *Joshānda* (decoction) of herbs on whole body or a on a particular part of the body by covering the whole body or the particular part by a blanket (Arzani, 1880; Ibn Nafees, 1984; Hamdani, 1980). In this study, an attempt has been made to highlight the relevance of *Inkibāb* (medicated steam inhalation) for treating *Nazla* in the present scenario from Unani Medicine sources.

*Corresponding author; Email: zehra.zaidi@jamaihamdard.ac.in

Method

Literature concerning *Inkibāb* was explored from the classical manuscripts of Unani Medicine and assessed. Recommended drugs for *Inkibāb* in the treatment of respiratory and other diseases were extracted from original sources. Electronic databases were searched for investigating the pharmacological efficacy of the inhalation of herbs.

Results

History of *Inkibāb*

Inkibāb was known to early men with the advent of early medicine. Thousands years ago early men got knowledge of inhalation therapy through hit and trial method. The earliest available record of nasal inhalation is 1,554 BC old; Egyptian Ebers Papyrus - which has the description of inhalation of black henbane (*Tukhm-i-Bhāng*) with many prescriptions of various subtypes of nasal inhalation, i.e. snuffs, vapours and smoke (Sanders, 2007). The most eminent Persian physician Jamshed described the inhalation of volatile vapours for the treatment of respiratory troubles. Ayurvedic physicians were the pioneer users of burnt black henbane (*Cannabis indica*) for refreshment and sedation. The great Unani physician Jalinoos (130-201 AD) described some powdered drugs for inhalation for the relief of nasal and brain diseases.

The Romans established general public baths, in their large cities with available warm water facility. In bathrooms, the warm steam was applied to the whole body for its beneficial effects in many body troubles.

In the eighth century, Unani scholars contributed a lot to the progress of inhalation therapy. In this direction, *Ḥammām* (Turkish baths) was modified as the medical center for physical therapy, treatment of many body troubles and also used as public baths. In early period of Unani Medicine, the drugs were heated on stove or burning wood, hot stone, hot iron plates and patients were directed to take the evolved vapors by covering head with towel. Wood snuff boxes were used for powdered drugs inhalation. Later on, Unani scholars invented clay jars or metallic pots and large bath tubs as tools for better application of *Lakhlakha*, *Inkibāb/ Bakhūr* and *Ḥammām* respectively. They invented many dosage forms for nasal drug delivery like sniffing powders, volatile vapors for inhalation, burnt smoke for breathing.

Unani scholars introduced many new drugs (of plant/animal origin) for *Inkibāb* like Eucalyptus, *Na'nā'/ Pudīna*, *Dārchīnī* (*Cinnamomum zylanicum*), *Hulba* (*Trigonella foenum- graecum*), *Yāsmīn* (*Jasminum grandiflorum*), *Za'frān* (*Crocus sativus*), *Usṭukhūddūs* (*Levandula stoechus*), *Kāfūr, Kundus* (*Helleborus niger*), *Bādiyān* (Foeniculum vulgare), Mustard (*Brassica campestris*), *Qaranful*

(*Syzygium aromaticum*), *Banafsha* and *Mushk* (*Moschus moschiferous*). They also invented *Satt-i-Lobān* (liquid of benzoin/storax), *Satt-i-Ajwāyin* (thymol) and *Satt-i-Pudīna* (peppermint) after invention of *'Amal-i-Taṣ'īd* (sublimation) for the treatment of nasal, throat and respiratory diseases (Shehatta, 2013).

Definition

Inkibāb means taking the vapours of *Joshānda* (decoction) of herbs on whole body or a on a particular part of the body by covering the whole body or the particular part by a blanket (Arzani, 1880; Ibn Nafees, 1984; Hamdani, 1980)

Inkibāb has been recommended as a part of the treatment of *Nazla Bārid/Muzmin*. Other nasal dosage forms like *Bakhūr, Qaṭūr, Naṭūl* and *Fatīla* have also been recommended for the treatment of *Nazla Bārid/Muzmin*.

Unani physicians formulated many types of nasal dosage forms like powders, solution and gaseous preparations of herbal, animal and mineral origin drugs. The target site was either in the nasal area or even in the upper regions like CNS. Besides these, they formulated nasal dosage forms for eye, ear, throat, gastric and CNS disorders.

Nasal dosage forms in Unani Medicine

Various nasal dosage forms for treatment of different diseases prescribed by Unani physicians are given below:

A. *Safūf* (powdered form): This is subdivided into the following:

   1. *Nafūkh* (nasal insuffation): Sprinkling of dry powder of herbal drugs in nose through a tube with forceful exhalation of air by mouth.

   2. *'Aṭūs* (errhine or sternutator medicine): Intake of dry powder of herbal drugs through deep nasal inhalation to induce *'Uṭās* (sneezing) and nasal secretions.

B. *Sayyāl* (liquid): This is subdivided into:

   1. *Sa'ūṭ/Qaṭūr* (nasal snuffng drop/nasal drop): Put drops of liquid of herbs in nose.

   2. *Nashūq* (liquid snuff formulation): Intake of liquid herbal drugs through nose with the help of forceful intake of air.

C. *Bukhārī* (gaseous): It is subdivided into:

   1. *Bakhūr* (fumigation): To take the smoke of burning herbs.

   2. *Inkibāb* (medicinal vapour bathes or boiled aqueous extracts): Taking the vapours of *Joshānda* (decoction) of herbs on whole body or a

particular part by covering the whole body or particular part by a blanket.

3. *Lakhlakha* (inhalation form): Inhalation of aromatic liquid herbal drugs from a glass container after forceful shaking.

4. *Ghāliyah/Shamūm* (aroma agents): Inhalation of aroma directly (Shehata, 2013; Arzani 1880; Ibn Nafees, 1984).

Inkibāb is recommended for the treatment of *Nazla-o-Zukām* and other nasal diseases like *Sudda-i-Bīnī* (nasal obstruction), *Khasham* (olfactory abnormalities), *Naksīr* (epistaxis), *Bawāsīr-i-Anf* (nasal polyps), *Bakhr al-Anf* (ozaena), *Qurūh al-Anf* (nasal ulcers), etc.

Unani scholars have also prescribed *Inkibāb* in the following diseases:

Amrāḍ-i-Ra's (head diseases): *Ṣudā'*, *Ṣudā' Ḥārr*, *Sarsām*, *Sahar*, *Shaqīqa*, *Laqwa*, *Fālij*, *Tashannuj*, *Mirgī*, *Sarsām Balghamī*, *Ghashī*, *Lītharghus*

Amrāḍ-i-Udhun (ear diseases): *Waja' al-Udun*, *Thiql-i-Simā't*, *Kān kī phunsī* (boil/furuncle), *Ṭanīn-o-Dawī*

Amrāḍ-i-'Ayn (eye diseases): *Ṭarfa*, *Rataundhī*

Amrāḍ-i-Ḥalaq (throat diseases): *Khunāq*

Amrāḍ-i-Baṭn (abdominal diseases): *Istisqā' Laḥmī*, *Istisqā' Ṭablī*, *Bawāsīr*

Other Diseases: *Waja' al-Mafāṣil*, *Kalaf, Khafaqān*

Inkibāb in *Nazla Bārid*

1. Inhale roasted *Kalonjī* tied in a cloth. It is stated that *Inkibāb* of *Kalonjī* is better than its use as *Bakhūr*.

2. Inhale vapors of *Bābūna, Marzanjosh, Nākhūna* and *Nammām* boiled in water.

3. Inhale vapours of water decoction of *Bābūna, Nākhūna, Darmina Turkī*, *Qayṣūm* 25 g each, *Barg-i-Ṣanobar*, *Sabūs Gandum*, *Aṣl al-Sūs Kofta*, *Jaw Kofta* 50 g each. (Tabari, 2009)

4. Inhale vapours of *Shonīz/Kalonjī Biryān*, *Anīsūn* after tying in a cloth.

5. Inhale vapours of water decoction of *Bābūna*, *Parsiāoshān*, *Zūfā Khushk*, *Marzanjosh*, *Nammām* and *Qayṣūm* (Khan, 2003).

6. Inhale vapors of water decoction of *Nākhūna*, *Bābūna* and *Marzanjosh* (Arzani, 1880).

7. Inhale vapours of *'Araq 'Ajīb* 2 drops in hot water (Anonymous, 2015)

Inkibāb in *Nazla Ḥārr*

1. Inhale vapors of *Sirka* sprinkled on hot stone for congestion of nose (Ibn Nafees, 1984)
2. Dip *Kalonjī* in *Sirka* for 24 hours, then roast and pound it and inhale its vapours after tying it in a cloth.
3. Inhale vapours of water decoction of *Bābūna*, *Banafsha* and *Jhā'o*.
4. Inhale vapours of water decoction of *Sausan* after boiling it in *Sirka* (Jurjani, 1903)
4. Take *Sabūs Gandum*, *Bāqlā, Ṣandal Safaid*, *Gul-i-Jhā'o*, *Gul-i-Surkh* 3.5 g each, *Kāfūr* 480 mg; make pills and put them in water and inhale (Arzani, 1988)

Inkibāb in *Nazla*

1. *Inkibāb* of water decoction of *Bābūna*, *Marzanjosh, Nākhūna* and *Nammām* (Ibn Sina, 2010)
2. Tie equal parts of *Qusṭ*, *Kalonjī* in a cloth, put it on hot iron pan and inhale regularly.
3. Inhale vapours of water decoction of *Jhā'o*.
4. Inhale vapours of water decoction of *Sundarūs*, *Kundur*.
5. Inhale vapours of water decoction of Shibt, *Bābūna*, *Iklīl al-Malik* and *Ṣa'tar* (Ghani, 1927)

Inkibāb in *Nazla Shadīd*

Inhale vapours of alcohol after dipping hot stone in it (Tabari, 2009).

Inkibāb in *Sudda-i-Bīnī* (nasal obstruction):

1. Inhale vapours of water decoction of *Sabūs Gandum, Sirka.*
2. Inhale vapours of water decoction of *Bābūna, Rā'ī*, *Zīra*, *Shīḥ*, *Nammām*, *Pudīna*, *Ṣa'tar*.
3. Inhale vapors of water decoction of *Lādan*, *Chob Gāz*, and *Shonīz* (Khan, 2009).

Inkibāb in *Khasham* (Anosmia)

1. Inhale vapours of *Pudīna*, *Ṣa'tar*, *Sudāb* after boiling in *Sirka* (Ghani, 1927)
2. Put boiled *Sirka* in a bottle and inhale.

3. Inhale vapours of water decoction of *Kalonjī, Khardal, Shaḥm-i-Ḥanẓal* and *Pudīna* (Khan, 2009).

4. Inhale vapours of *Sirka* after poring it on hot stone regularly (Kabiruddin, 1927).

Single drugs for *Inkibāb* (vaporisation, steam inhalation)

Aṣl al-Sūs (*Glycirrhiza glabra*), *Bābūna* (*Maticaria chamomilla*), *Banafsha* (*Viola odorata*), *Bāqla* (*Vicia faba*), *Barg-i-Ṣanobar*, *Chob Gāz*, *Darmina Turkī* (*Artemesia meritima*), *Gul-i- Surkh* (*Rosa damascena*), *Gul-i-Jhā'o*, *Iklīl al-Malik* (*Astragalus hamosus*), *Jaw* (*Hordeum vulgare*), *Jhā'o* (*Tamarix gallica*), *Kāfūr*, *Kalonjī/Shonīz* (*Nigella sativa*), *Kundur* (*Boswellia serrata*), *Lādan* (*Citrus ladanum*), *Marzanjosh* (*Origanum vulgare*), *Nākhūna* (*Melilotus indicus*), *Nammām* (*Ocimum canum*), *Parsiāoshān* (*Adiantum capillus-veneris*), *Pudīna* (*Mentha arvensis*), *Qusṭ* (*Saussurea lappa*), *Qayṣūm*, *Ṣa'tar* (*Zataria multiflora*), *Sabūs Gandum* (*Triticum aestivum*), *Ṣandal Safaid* (*Sanatalum album*), *Sundarūs* (*Trachylobium hornemannianum*), *Sausan* (*Iris ensata*), *Shakar*, *Shīḥ*, *Shibitt* (*Anethum sowa*), *Sirka* (vinegar), *Tukhm-i-Khaṭmī* (*Althaea officinalis*), *Tukhm-i-Khubbāzī* (*Malva sylvestris*), *Khardal* (*Brassica nigra*), *Zīra Siyāh* (*Carum carvi*), *Zūfā Khushk* (*Hyssopus officinalis*) (Kabiruddin, 2007).

Modern concept of inhalation

Due to the rich vascularisation, the olfactory and in particular the respiratory area of nasal cavity may serve as an efficient absorption surface for topically applied drugs. The limited area (150 cm^2) in the nasal cavity that offers an optimal absorption environment is perpetually cleansed by mucociliary clearance (MCC) removing all particles including vehicle. The olfactory region with its vicinity to the cerebrospinal fluid and direct nervous interface to the brain has attracted research interest for possible nose to brain delivery (Bitter *et al*., 2011).

Some researchers have advocated for intranasal (IN) medications and describe their advantages; the nasal cavity provides a direct route into blood stream for medications that easily cross mucous membrane. This route avoids gastrointestinal destruction and hepatic first pass metabolism, allowing more drugs to be cost effectively, rapidly and predictably bio-available than it were administered orally. For many IN medications, the rates of absorption and plasma concentrations are comparable to intravenous administration and are typically better than subcutaneous or intramuscular routes. IN drug administration is essentially a painless and does not require sterile technique, intravenous catheter or other invasive devices and it is immediately and readily available for all patients (Bitter *et al*., 2011, Szczeklik & Stevenson, 1999).

Methods of drug inhalation

1. Inhalation therapy: The use of therapeutic material whether powders, liquids, vapours or gases through the inspired air has revolutionized into a medical sub-specialty known as inhalation therapy (Ogbonna *et al.,* 2012).

2. Aromatherapy: Aromatherapy is the treatment or prevention of disease by use of essential oils. Aromatherapy refers to the inhalation and topical application of true, authentic essential oils from aromatic plants (Keville & Green, 1995).

Some of the commonly used essential oils in modern medicine are eucalyptus oil, menthol which are part of British Pharmacopoeia 1980 and are indicated for inhalation to relieve symptoms of sinusitis (Ernest, 1955).

Karvol Plus inhalant capsule of Indico Remedies (Menthol BP-35, 55.0 mg, Chlorbutol BP-2, 25.0 mg, Thymol BP-3, 15.0 mg, Total Pine Oil-112, 05.0 mg, Terpineol BP-66, 60.0 mg, Cinnamon Oil-12, 15.0 mg.) is prescribed as decongestant in cold.

Drug dosage forms: Different drug dosage forms are now available for inhalation therapy.

1. Snuff: Snuff powder that can be inhaled to the nose or mouth by a snuff box or a rubber pump.

2. Liquids: Liquids, used as drops, sprays or atomization to the nose, pharynx, larynx or chest. Very fine forms of atomizers are now available for nebulization.

3. Vapors: These can be inhaled by inhalers, vaporizers or humidifiers.

4. Water steam: It can be inhaled directly from water boilers or special devices.

5. Smokes: Smokes of the burnt reeds, plants or minerals, evolved from a stove or a heater. The habit of tobacco smoking in spite of its variable side effects is still prevailing in use for smoke inhalation.

6. Gases: This group includes many therapeutic gases such as oxygen, carbon dioxide, helium and nitrous oxide that are commonly inhaled for resuscitation, general anesthesia and medical therapy.

Methods

1. Simple method: Pour water in a bowl and add 3 drops of essential oil in it. Place your head about 12 inches (30 cm) above the bowl and cover your head with a towel in such a way that sides are totally covered and you in fact form a tent over the bowl. Keep your eyes shut and breathe deeply through your nose.

2. Through devices like Inhaler, Nebulizer, Neeti Pots

   (i) Inhaler/puff/pump: It is a medical device used for delivering medicines into the lungs through the work of a person's breathing. This allows medicines to be delivered to and absorbed in the lungs, which provides the ability for targeted medical treatment to this specific region of the body, as well as a reduction in the side effects of oral medications.

   (ii) Nebulizer: It is designed to deliver medications over an extended period of time over multiple breaths through a mouthpiece or face mask. They generate a continuous mist with aerosolized medication, allowing a patient to breathe normally and receive medications. They are commonly used in infants and toddlers requiring inhaled medications or in patients in the hospital who require inhaled medications.

Research studies

1. Steam inhalation is not advised for children because of the risk of scalding. Instead, it might help a child if they sit in a hot, steamy bathroom.

2. In a clinical study, it was found that steam inhalation at 42-44 degree C for 20 minutes resulted in alleviation of cold symptoms and increased nasal patency in a significantly higher percentage of patients in the actively treated group than in the placebo treated group (Ophir & Elad, 1987).

3. In a clinical study, it was found that inhalation of humidified warm air at 42-44^{0}C for 30 minutes resulted in amelioration of allergic rhinitis symptoms and in increased nasal patency in a high proportion of patients. There was no adverse side effect (Ophir *et al.,* 2000).

4. A clinical study found that after inhalation of hot steam at 42 degrees C there was an increased microcirculatory perfusion of nasal mucosa. Up to inhaling of 100 times, it leads to effective increase in microcirculation of nasal mucosa. (Kim *et al.*, 2000)

5. In a study it was concluded that Vaporub (VR) (camphor, menthol, eucalyptus oil) helps to fill the therapeutic void. Despite mild irritant adverse effects, VR provided symptomatic relief for children with URIs and allowed them and their parents to have a more restful night (Paul *et al.*, 2010).

6. The antibacterial activity of 14 essential oils and their major constituents in the gaseous state was evaluated and it was found that *H. influenzae* was most susceptible, followed by *S.pneumoniae* and *S. pyogenes*, and then *S. aureus*. A 128-fold difference in minimal inhibitory dose (MIDs) was observed

between the highly active cinnamon bark oil and the weakest citron oil. The results indicate that the antibacterial action of essential oils was most effective when at high vapour concentration for a short time (Inouye *et al.*, 2001).

7. In a comparative clinical study, *Nigella sativa* steam inhalation was found more effective than chamomile in relieving symptoms of chronic bronchial asthma (Al-Jawad *et al.,* 2012).

8. In a randomised, standard controlled clinical trial on 40 patients, it has been found that inhalation of *Kalonjī* with oral Unani formulation has statistically highly significant effect on CT total sinus score (Lund Mackay staging system) of CRS patient in comparison to statistically significant effect in standard control group (20 patients) *(Zaidi et al., 2019)*.

9. In a single blind, randomised, control trial of a Unani compound formulation on 40 patients of CRS patients, it was revealed that the oral Unani formulation with inhalation of *Kalonjī* (*Nigella sativa*) has statistically highly significant effect on major and minor symptoms of chronic rhinosinusitis in comparison to significant effect of standard medication (20 patients) *(Zaidi et al., 2021)*

10. A randomized, double-blinded, controlled, clinical trial was conducted on 93 patients with CRS and randomly allocated to the test drug and placebo group. The test drug group (31 patients) received 2 puffs /day of *N. sativa* nasal spray (2 puffs contained 1 g of *N. sativa*), and the placebo group (34 patients) had 2 puffs/day of sodium chloride nasal spray 0.65%. After 8 weeks of treatment, it was concluded that that *N. sativa* is effective at reducing symptom scores assessed by SNOT-22 and objective findings as determined by Lund-McKay and Modified Lund Kennedy scoring in patients suffering from CRS-related symptoms. (Rezaeian & Amoushahi Khouzani, 2018).

Discussion

Inkibāb is one of the important modes of treatment which has been used in Unani Medicine and is part of medical practice of Unani physicians from centuries. This mode of treatment has been adopted by modern medicine and named as inhalation therapy and aromatherapy. There are many researches on inhalation therapy and aromatherapy on herbal drugs used in the same way as Unani physicians with some modifications. But the researches on inhalation therapy of upper airway diseases has been significantly neglected in modern medicine and Unani Medicine also as there are very few studies conducted so far on this topic.

Inkibāb as a mode of nasal drug delivery of herbal drugs has gained more

attention nowadays as scientists are trying to find out effective chemical herbal natural phytoconstituents or phytoalexins (plant antibiotics) for better treatment of pulmonary diseases, i.e. pulmonary hypertension, asthma, chronic obstructive pulmonary disease, (COPD), occupational lung diseases.

Chemical herbal natural phytoconstituents or phytoalexins like Curcumin from *Curcuma longa*, Resveratrol from *Veratrum grandiflorum*, Quercetin flavonoid from fruits and vegetables, Apigenin from *Apium spss* like parsley, celery and carrot, Baicalein from *Scutellaria baicalensis* Georgi of Lamiaceae, Fisetin from vegetables and fruits, Naringenin from *Citrus aurantium* L., *Citrus medica* L., *Drynaria fortunei* (Kunze ex Mett.) J.Sm., Lignan-Honokiol from genus *Mangolia* (officinalis, obovata, grandiflora), Diaterpinides-Andrographolide from *Andrographis paniculata* of family Acanthaceae, Atropine from *Atropa belladonna*, *Datura spss-inoxia*, *Stramonium metel* and Cannabinoid-Dronabinol from *Cannabis sativa* have been found to be effective in various lung diseases (Mehta *et al.,* 2018).

Natural phytoconstituents or phytoalexins have showed a unique treatment array with minimum side effects and great capability to treat intrapulmonary and extra pulmonary diseases compared to synthetic drugs. With the progress in the field of nanotechnology, material science and particle engineering these particles can be further improved in the treatment capability and efficiency of clinically proved phytoconstituents. In 2009, a lab was established at Kansas USA with the name of Aerosol Research and Engineering Labs Inc. (ARE Labs) which is actively engaged in development of phytoconstituents based Dry Powder Inhalers (DPI) for treatment of various pulmonary and extra pulmonary disease (Mehta *et al.,* 2018).

So far some clinical researches have been conducted on the Unani drugs i.e. *Kāfūr, Satt-i- Pudīna, Satt-i-Ajwāyin, Kalonjī*, *Eucalyptus*, and *Rowghan-i-Dārchīnī,* etc. The efficacy of these drugs during studies verify the claims made by Unani scholars centuries ago about the effectiveness of inhalation of steam and aroma of aromatic drugs in common cold, nasal obstruction, allergic rhinitis, cough, chronic rhinosinusitis, etc.

Conclusion

Through the above discussion it can be concluded that *Inkibāb* is very potent mode of treatment of Unani Medicine but lacking clinical data in the era of evidence-based medicine. Researchers of Unani Medicine should prepare comprehensive research programme on *Inkibāb* to combat various challenges in the field of medicine especially respiratory diseases and provide economic, effective and safe medical solutions to the society.

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सारांश

हर्बल औषधियों का *इन्किबाब* (अंतःश्वसन): यूनानी चिकित्सा में *नज़ला* के उपचार की एक आनोखी विधि – एक साहित्यिक समीक्षा

ज़ेहरा ज़ैदी*

सारांश

परिचयः इस अध्ययन में यूनानी चिकित्सा स्रोतों से वर्तमान परिदृश्य में नज़ला के उपचार के लिए *इन्किबाब* (अन्तःश्वसन) की प्रासंगिकता को उजागर करने का प्रयास किया गया है।

विधिः यूनानी चिकित्सा की क्लासिकल पांडुलिपियों से *इन्किबाब* से सम्बन्धित साहित्य की खोज की गई और इसका मूल्यांकन किया गया। श्वसन संबंधी और अन्य रोगों के उपचार में *इन्किबाब* के लिए बताई गई औषधियां मूल स्रोतों से ली गईं। श्वसन औषधियों की भेषजकोशीय प्रभावकारिता की जांच करने के लिए इलेक्ट्रॉनिक डाटाबेस को खंगाला गया।

परिणामः नज़ला में *इन्किबाब* के लिए यूनानी विद्वानों द्वारा बताए गए 30 से अधिक पौधों का पता लगाया गया। विभिन्न शोध अध्ययनों से प्रमाणित हुआ है कि भाप द्वारा अन्तःश्वसन बलग़म निकालता है और खांसी में राहत प्रदान करता है, एलर्जिक राइनाइटिस के लक्षणों में सुधार करता है, सर्दी के लक्षणों को कम करने और नाक की सहनशीलता को बढ़ाने में मदद करता है। दालचीनी सहित कई जड़ी–बूटियों के आवश्यक तेलों ने जीवाणुरोधी प्रभाव दिखाए हैं। कपूर, मेन्थॉल और यूकलिप्ट्स के तेल द्वारा अन्तःश्वसन से बच्चों की ऊपरी श्वास नलिका के संक्रमण में राहत मिलती है। कलौंजी के बीज के अन्तःश्वसन से अस्थमा में ब्रोंकों–रिलैक्सेंट प्रभाव पड़ता है और साइटोकाइन रिलीज़ सिंड्रोम (सीआरएस) में सांख्यिकीय रूप से अत्यधिक महत्वपूर्ण लक्षणात्मक राहत प्रदान करता है। इस अध्ययन ने नाक संबंधी रोगों में इन्किबाब की प्रभावशीलता के संबंध में यूनानी चिकित्सकों के दावों को सिद्ध किया है।

निष्कर्षः चिकित्सीय पादपों के इन्किबाब को एलर्जिक राइनाटिस, सीआरएस इत्यादि में किफायती प्रभावी रूप से सुरक्षित उपचार प्रदान करने हेतु भविष्य में शोध के विषय के तौर पर लिया जा सकता है।

शब्दकुंजीः *इन्किबाब,* यूनानी चिकित्सा, नज़ला, नाक संबंधी रोग

Investigating the Relationship between Somatotype and *Mizāj* of Young non-athletes

[1]Saeed Vahedi,
[1]Milad Rahati,
[2*]Saba Zaidi,
[1]Amin Zanjani
and
[3]Ali Rasti

[1]Department of Sport Sciences, Faculty of Sport Sciences, Ferdowsi University of Mashhad, Iran

[2]Department of *Munafeul Aza*, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh

[3]Department of Sport Sciences, Faculty of Sport Sciences, Islamic Azad University of Mashhad, Iran

Abstract

Introduction: Evaluation of body indicators is an important part of the talent search process. Anthropometry is one of the effective factors in predicting the success of athletes in various sports. Somatotype can be a determining factor in individual differences. In Unani Medicine / Traditional Iranian Medicine, the relationship between people's *Mizāj* and physical structure and physiological and psychological performance of individuals has been discussed. Therefore, the aim of this study was to investigate the relationship between somatotype and *Mizāj* of young non-athletes.

Materials and Methods: The type of study was comparative, and conducted by field method with a comparative-correlational design. The number of samples in the study was 200 people who were divided into four groups of 50 people: hot and humid, hot and dry, cold and wet and cold and dry *Mizāj*. The relationship between *Mizāj* index and somatotype was investigated using the ETA correlation coefficient method. Data analysis and hypothesis testing were conducted on SPSS software version 21 with a significance level of $P \geq 0.05$.

Results: Statistical results showed that the ETA correlation coefficient between endomorphic type and *Mizāj* was 0.89 and the results of one-way analysis of variance showed that cold and wet temperament had a significantly higher endomorphic score than other *Mizāj* (4.91 ± 0.58). Also, the correlation coefficient of eta between mesomorph type and *Mizāj* was 0.924, and significantly the highest mesomorph score was assigned to warm and wet temperament (5.12 ± 0.49). The correlation coefficient of eta between ectomorph type and *Mizāj* was 0.946 and cold and dry temperament had the highest ectomorph score significantly compared to other *Mizāj* (4.83±0.45).

Conclusion: The results of this study showed that there is a significant relationship between *Mizāj* and the body type of young men. Therefore, since the structure of a person's body type can be a predictor of success in any of the various sports, *Mizāj* can be used as a factor in determining a person's athletic talent.

Keywords: Somatotype, *Mizāj*, Physical talent

Introduction

Evaluation of physical indicators is an important part of the talent identification process (Ackland *et al.*, 2009). Due to the diversity of these indicators, several

*Corresponding author; Email: sabazaidi437@gmail.com

studies have focused on body type, and the results of various studies indicate the relationship between this index and athletic performance, similarity of body type of young and adult players in a field and physical type stability throughout life (Bayios *et al.,* 2006). Given that physical indicators, especially body type, are influenced by genetic factors and have little effect on the environment from exercise and nutrition, these indicators can be useful in identifying talented people (Eston & Reilly, 2009).

Body type is a definition of the shape and form of the human body, which is the structure of a person according to the constituent elements of the body. Each sport requires its own body type, and for success and championship in that field, athletes have a body type specific to that sport. Body type consists of three numerical scales. Each part is defined as an index of a particular physical property, such as 1-5.5-1, which is expressed on a continuous numerical scale from zero to seven.

Body type components are endomorphic or obese, mesomorphic or muscular, and ectomorphic or lean. Body type and physical characteristics of individuals is one of the effective factors in predicting the success of athletes in various sports. Because, special training can be effective only up to 30% in a person's performance and a major part of athletes' ability is due to genetic capacity, which can be effective in reaching the peak of championship performance and greater stability at the peak of athletes (Santos *et al.,* 2014). Studies in this field indicate that every sport needs athlete with specific body dimensions and sizes, and body shape plays an important role in selecting people to participate in sports activities (Singh *et al.,* 2010).

In this regard, Carter and Heath also believe that body type provides important information about chances of success in a particular sport. Comparing Olympic athletes, they concluded that athletes in different sports are different in terms of anthropometry, body composition and body type and the champions of each sport show a similar pattern of body size and body type at the highest level of skill performance (Zuniga *et al.,* 2011). Rich and Fulton also believed that body structure was one of the best signs of a person's biological diagnosis (Rich & Fulton, 1992). Singer reports that individuals are born with certain innate characteristics and can function by determining these characteristics (Singer, 1972). According to the theories of Unani Medicine and Persian Medicine, the factor that can affect the characteristics and physical capacities is the inherited part of human existence, namely *Mizāj*. *Mizāj* literally means to merge, and in Persian medicine it means the same and new quality that results from the merging of the elements with each other and their interaction and interaction in a composite body (Naseri *et al.,* 2008). Each person's physical and mental functions are adjusted to his or her life, and he or she has the neurohormonal structure and system and the benefits he or she inherits genetically. It regulates

his reaction against external and internal environmental factors and is always a function of physiological, anthropometric and psychological characteristics (Molakazemi, 2013; Avicenna, 1386 H; Shahabi *et al.,* 2007). In Persian Medicine, 9 types of *Mizāj* are considered, which are cold, warm, wet and dry for single *Mizāj*, cold and wet, cold and dry, warm and wet, warm and dry for mixed temperament, and moderate *Mizāj* (Jorjani, 2005; Shirazi, 2001; Chaghmini, 1996)

According to the theories of complementary and traditional Iranian medicine or Unani Medicine, *Mizāj* affects the psychological and physical characteristics and physiological function of the body. According to this view, each person has a unique personality and a unique *Mizāj*, which are classified through morphological, physiological and psychological manifestations (Molakazemi, 2013; Avicenna, 1386 H) and researchers in this field believe that the shape and size of the body are determined by nature (*Mizāj*) and nutrition (Setooni *et al.,* 2016). One of the few studies that have been done in this field can be the research of Alam *et al.* in 2019, which examined the relationship between body mass index (BMI) and *Mizāj* in 100 men and women and concluded that people with warm and wet temperament had significantly higher body mass index than people with cold and dry temperament (Alam *et al.,* 2019). In addition, Rahati *et al.* in a study conducted in 2018 on physical fitness factors on 40 people with different *Mizāj* showed that warm-tempered people have significantly better anaerobic fitness capabilities than cold-tempered people enjoyed (Rahati *et al.,* 2018).

Also, one of the important researches in this field is the research of Vahedi *et al.* in 2018, which was performed on 140 men with different *Mizāj* and the results showed significant differences in most physiological indicators in different *Mizāj* (Vahedi *et al.*, 2018). According to the above studies and evidence of modern medicine on the relationship between genetics and physical activity and the important role of physical conditions in it (Maia *et al.,* 2002) and the emphasis of traditional medicine scholars on the relationship between *Mizāj* and physical activity and on the other hand because researchers have considered the structure of a person's body type as a predictor of success in each of the various sports, so the present study aims to examine the relationship between body type and *Mizāj* in young men.

Materials and methods

The present study is a cross-sectional comparative study and was conducted by field method with four groups of warm and wet, warm and dry, cold and wet and cold and dry temperament with an intergroup comparison plan. The study population consisted of single non-athlete men who were not professional athletes and did not have regular training for at least three months. The subjects

were students in Mashhad with an average age of 20 to 24 years, who did not have any drug addiction. This community did not have physical disabilities or diseases affecting their physical and physiological characteristics and did not take any medication. Sampling in this study was done by simple random method and the number of samples in the present study was determined by G-power software version 3.12 and by regression method and using the formula n* = n / 1-x, the ratio of people who were expected to leave the study was determined and with the departure of 15 subjects, the sample size was determined to be 200, which was determined using a standard *Mizāj* questionnaire (Mojahedi *et al.*, 2014) and with the guidance of a Persian medical expert *Mizāj* were determined and four groups of 50 people formed warm and wet, warm and dry, cold and wet and cold and dry. In order to gain the informed consent of individuals to participate in the study, after the introductory session and receiving personal information, general health and medical history were assessed to enter the study. Sampling was performed every day for two weeks in the evening in one of the sports halls of Mashhad.

Inclusion criteria for the study

1. Single men aged 20 to 24 years
2. No smoking and alcohol
3. Do not take special medicine
4. No history of surgery
5. Not be a professional athlete and do not have regular training

Determining body type

All body measurement characteristics were measured according to the standards of the International Association for the Advancement of Sports Body Image (ISAK). In order to measure body type, standing and sitting height of participants was measured with a wall gauge (Seca mark with an accuracy of 1 mm) and their weight with a scale (Seca mark with an accuracy of 0.1 kg). Measurement around the limbs (relaxed arm, bent arm, waist, pelvis and leg) was taken with a tape measure (Lufkin brand with an accuracy of one millimeter), thickness of subcutaneous fat in six points (back of the arm, under the scapula, supraspinatus, abdomen, quadriceps and legs) with a caliper (Slime guide brand with an accuracy of 0.5 cm), arm and thigh bone widths were measured with a caliper (Mitutoyo mark with an accuracy of 0.1mm). Using Carter's six-part equation, information on body fat percentage was calculated.

Men's fat percentage = (0.1051) Total thickness of skin folds six points + (2.585)

To measure body type components (endomorphic, mesomorphic, ectomorphic)

and determining the body type of the measured data was entered into somatotype software version 1.25, the validity and reliability of this software has been measured and confirmed by Mahmoudkhani and Barati (Mahmoudkhani & Barati, 2012).

Statistical analysis

After collecting and entering data in SPSS software version 21, descriptive statistics were used to calculate the central tendency and scatter indices and plot the variables. After checking the normality of data distribution by Shapiro ilk test, correlation and data correlation were performed using ETA correlation coefficient method and significant one-way analysis was performed. Hypotheses were tested with a significance level of $p \geq 0.05$.

Results

Information about the personality traits and anthropometrics of the participants is presented in Table 1.

Statistical results showed that the correlation coefficient of eta between endomorphic type and *Mizāj* was 0.89 (p=0.001) and the highest mean endomorphic score was significantly observed in cold and wet temperament (4.91±0.58). On the other hand, the correlation coefficient of Eta between mesomorphic type and *Mizāj* is 0.924 (p=0.001). The highest mean endomorphic score was significantly observed in warm and wet temperament (5.12±0.49). Finally, the correlation coefficient of eta between ectomorphic type and *Mizāj* was 0.946 (p=0.001). The highest mean ectomorphic score was observed in cold and dry temperament (4.83±0.45).

Statistical analysis of one-way analysis of variance also showed that warm and wet individuals had the highest number of mesomorphic types, cold and wet endomorphic types, and cold and dry ectomorphic types and this difference was significant at the level of 0.05 (p=0.0). Somatotype chart that is entered by Somatotype Calculation and Analysis software by entering test data version 2.1 shows that the highest density of cold and dry temperament is in the ectomorphic region, warm and dry temperament between ectomorph and mesomorphic, warm and wet temperament in the mesomorphic region and cold and wet temperament has the most endomorphic region (Chart 1).

Discussion and Conclusion

This study aimed to investigate the relationship between *Mizāj* and three types of body types: mesomorphic, endomorphic, and ectomorphic. The results of this study showed that there was a high correlation coefficient between endomorphic

Table 1: Data related to the descriptive statistics of the subjects

Variables	Groups	N	Deviation standard ± Mean	Degrees of free	F	P-value
Age (years)	Warm and wet	50	23.05 ±1.25	3	2.5	%23
	Cold and wet	50	23.05 ±1.25			
	Warm and dry	50	23.50 ±1.23			
	Cold and dry	50	22.90 ± 1.53			
Height (cm)	Warm and wet	50	177.6 ±3.8	3	2.2	%18
	Cold and wet	50	173.1 ±4.3			
	Warm and dry	50	178.4 ±5.6			
	Cold and dry	50	178.2 ±5.3			
Weight (kg)	Warm and wet	50	68.67 ±8.74	3	1.6	%10
	Cold and wet	50	70.38 ±7.02			
	Warm and dry	50	64 ± 6.2			
	Cold and dry	50	63.4 ±7.4			
BMI (kg/m)	Warm and wet	50	20.44 ±2.5	3	1.9	%15
	Cold and wet	50	22.17± 3.19			
	Warm and dry	50	19.33 ±1.9			
	Cold and dry	50	19.03 ±1.61			
Endomorphic score (Eta= 0.89)	Warm and wet	50	2.91± 0.51	3	188.34	%0
	Cold and wet	50	4.91 ±0.58			
	Warm and dry	50	1.79 ±0.51			
	Cold and dry	50	2.03 ± 0.53			
Mesomorphic score (Eta =0.924)	Warm and wet	50	5.12 ±0.49	3	121.94	%0
	Cold and wet	50	3.25 ±0.73			
	Warm and dry	50	2.23 ± 0.43			
	Cold and dry	50	3.91 ±0.47			
Ectomorphic score (Eta=0.946)	Warm and wet	50	1.93 ±0.37	3	270.44	%0
	Cold and wet	50	1.91 ±0.35			
	Warm and dry	50	4.03 ±0.59			
	Cold and dry	50	4.83 ± 0.45			

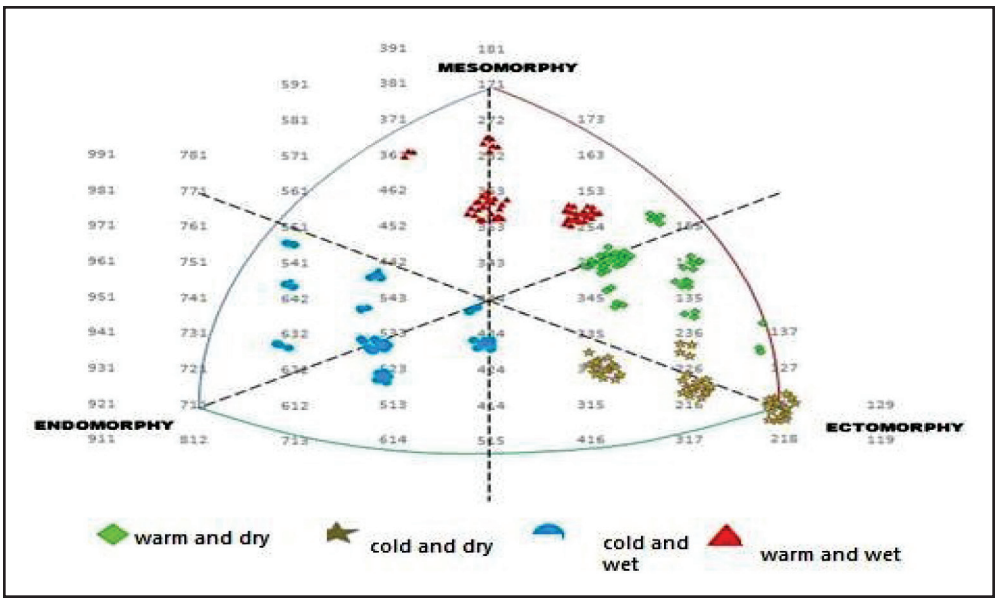

Chart 1: Dispersion of *Mizāj* types in the somatochart

score and *Mizāj* (89%), while the subjects with cold and wet temperament had the highest endomorphic score (4.91±0.58). According to Iranian traditional medicine scientists, cold-tempered people have high visceral fat and also have porous bones with low density compared to other *Mizāj* (Avicenna, 1386 H; Jorjani, 2005; Shirazi, 2001; Ahvazi, 2008; Rhazes, 1987; AghiliAlavi, 2006). According to Mehdizadeh *et al.* in terms of desire for physical activity and sports, cold and wet temperament have the least desire for physical activity and it is probably their physical characteristics that affect this behavioural characteristic (Mehdizadeh *et al.,* 2013).

Also, cold and wet people who have been introduced to people with fat body in the basics of ancient Iranian medicine had the highest score of endomorphic type and this is in line with the results of Vahedi, *et al.* which showed the highest relative fat content of cold-tempered people compared to other *Mizāj* (Avicenna, Year 1386 H; Vahedi *et al.,* 2018). A high correlation coefficient was observed between *Mizāj* and mesomorphic score (924%) among *Mizāj* groups, warm and wet temperament had the highest mesomorphic score (5.12±0.49) among other *Mizāj*. Due to the fact that the mesomorphic type has been introduced as a muscle type (Carter & Heath, 1990) and the dominant muscle volume has always been composed of protein and water (Vuori *et al.*, 1999), people with warm and wet temperament have more relative muscle and whole-body water than other *Mizāj*. In their research in this field, Vahedi *et al.* have pointed out that the highest percentage of muscle mass and higher protein and water content of the whole body is warm and wet people (Vahedi *et al.,* 2018), whose achievements are in line with the results of this study.

The correlation coefficient in relation to *Mizāj* and ectomorphic score (946%) also indicated a high correlation between these factors and the highest ectomorphic score significantly belonged to cold and dry temperament (4.83±0.45). Cold and dry people are mentioned in Persian medicine with a thin and elongated body (Avicenna, 1386 H; Jorjani, 2005; Shirazi, 2001). Researchers have studied bone mass (minerals) and muscle protein volume, which were the lowest in cold and dry people, and these physiological factors seem to be directly related to body shape and structure. Body size and obesity and thinness is one of the factors in determining *Mizāj* in Persian medicine sources, which is considered as one of the indicators of somatotype. According to Ibn Sina, the dimensions of the body are the size of the limbs, joints, and chest. The largeness of these organs is a sign of warmth and their smallness is a sign of cold temper. Warm temperament causes enlargement of the chest and limbs, width and protrusion of superficial veins, size of muscles and their proximity to the joints, and vice versa in cold temperament, all of the above indicators are below average. (Avicenna, 1386 H)

While the Heath and Carter method is used to measure the joints and muscles (Carter & Heath, 1990), Jorjani mentioned a wide chest and large veins as a sign of warmth and the opposite of these indicators as a sign of cold temper (Jorjani, 2005). Ahwazi mentions the strength of the limbs as a sign of a warm temper and the size of the chest, especially along with the lack of head size, a sign of warmth of the heart, and the opposite signs as a sign of a cold temper of the heart (Ahvazi, 2008). Razi mentions this index as one of the main indicators of determining *Mizāj* and the size of the nostrils and ducts of the limbs in warm temperament; and their tightness in the cold temperament. He mentioned the appearance of joints in the symptoms related to wet body, lack of joint protrusion, and in the symptoms of dry temperament, and the symptoms of hot and dry temperament, the appearance of tendons, bones, and joints. He considers the prominent larynx, long neck and elongated nose as a sign of dryness and largeness and protrusion of the eyes, wide nose, a lot of cheek flesh, a little facial hair, soft and thin nails as a sign of wet temperament. He also considers the shortness and thickness of the fingers as a sign of cold temperament and wet temperament, respectively (Rhazes, 1987; Aghili Alavi, 2006).

Increased fat or muscle tissue compared to the average person in that community is a sign of a wet temperament, a little bit of fat and muscle tissue or being thinner than the average person in the same community is a sign of a dry temper. Obese body with fat predominance is a sign of cold and wet, which is introduced as endomorph in somatotype, and muscular body is a sign of warmth and wet, which can be referred to as mesomorphic type and slimming is usually a sign of a dry temperament and is not an argument for a warm temperament or a cold temperament alone. Of course, cold and dry people are mostly thinner than warm and dry people; because hot and dry people,

although thin, have acceptable muscle tissue, but cold and dry people do not have much muscle tissue that belongs to the ectomorphic type (Mojahedi *et al.*, 2012; Mirtaheri *et al.,* 2015; Saifi *et al.,* 2017). According to the four *Mizāj* types mentioned in Persian medicine sources, the three *Mizāj* of warm and wet, cold and wet and cold and dry in somatotype had a high correlation with mesomorphic, endomorphic, and ectomorphic scores, respectively and also, hot and dry temperament with mean (4.0.03±0.59) were close to the cold and dry temperament group in ectomorph score and close to warm and wet *Mizāj* group in mesomorphic score with mean (3.91±0.47). Based on this, it can be considered among these body types. Putting this type of *Mizāj* in somatotype classifications can make it more complete.

The results of this study showed that there is a significant relationship between *Mizāj* and the body type of young men. Therefore, since the structure of a person's body type can be a predictor of success in any of the various sports, *Mizāj* can be used as a factor in determining a person's athletic talent.

Conflict of interest

This research has been conducted without any financial support from any particular institution or organization and none of the authors claim conflict of interest.

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सारांश

नॉन–एथलीट युवाओं के कद–काठी और *मिज़ाज* (स्वभाव) के बीच संबंधों की जांच

सईद वहेदी, मिलाद राहती, सबा ज़ैदी*, आमीन ज़नजानी और अली रास्ती

सारांश

परिचयः शारीरिक संकेतकों का मुल्यांकन प्रतिभा खोज प्रक्रिया का एक महत्वपूर्ण भाग है। एंथोपोमेट्री विभिन्न खेलों में एथलीटों की सफलता का पूर्वानुमान करने के लिए मानवनिर्मित प्रभावी कारकों में से एक है। कद–काठी (सोमाटोटाइप) व्यक्तिगत विभिन्नताओं के लिए निर्धारण कारक हो सकती है। यूनानी चिकित्सा/पारंपरिक ईरानी चिकित्सा में लोगों के *मिजाज़* (स्वभाव) एवं शारीरिक संरचना और शारीरिक तथा मनोवैज्ञानिक कार्य प्रदर्शन के बीच संबंध पर चर्चा की गई है। अतः इस अध्ययन का उद्देश्य नॉन–एथलीट युवाओं के कद–काठी और *मिजाज़* के बीच संबंधों की जांच करना था।

सामग्री और कार्यविधिः यह अध्ययन तुलनात्मक प्रकार था और तुलनात्मक सहसंबंधात्मक डिज़ाइन के साथ क्षेत्र विधि द्वारा किया गया। अध्ययन में 200 लोगों को नमूनों के रूप में लिया गया जिन्हें 50 लोगों के चार समूहों अर्थात् गर्म और आर्द्र, गर्म और शुष्क, ठण्डा और तरल तथा ठण्डा और शुष्क *मिजाज़* में विभाजित किया गया। ईटा सहसंबंध गुणांक विधि का उपयोग करके *मिजाज़* सूचकांक और कद–काठी के बीच संबंधों की जांच की गई। डाटा विश्लेषण और परिकल्पना परीक्षण एसपीएसएस सॉफ्टवेयर संस्करण 21 पर पी ≥ 0.05 के महत्व स्तर के साथ किया गया।

परिणामः सांख्यिकीय परिणामों से पता चला कि एंडोमोर्फिक प्रकार और मिज़ाज के बीच ईटा सहसंबंध 0.89 था और एकतरफा प्रसरण विश्लेषण के परिणामों से पता चला कि ठंडे और तरल *मिजाज़* में अन्य मिजाज़ (4.91±0.58) की तुलना में काफी अधिक एंडोमोर्फिक स्कोर था। इसके अलावा मेसोमोर्फ प्रकार और *मिजाज़* के बीच ईटा का सहसंबंध गुणांक 0.924 था और महत्वपूर्ण रूप से उच्चतम मेसोमोर्फ स्कोर गर्म और तरल स्वभाव (5.12±0.49) को सौंपा गया। एक्टोमोर्फ प्रकार और *मिजाज़* के बीच ईटा का संहसंबंध गुणांक 0.946 था और ठंडे और शुष्क स्वभाव में अन्य स्वभाव (4.83±0.45) की तुलना में उच्चतम एक्टोमोर्फ स्कोर था।

निष्कर्षः इस अध्ययन के परिणामों से पता चला कि *मिजाज़* और युवा पुरुषों के शरीर के प्रकार के बीच एक महत्वपूर्ण संबंध है। चूंकि किसी व्यक्ति के शरीर के प्रकार की संरचना विभिन्न खेलों में सफलता पूर्वसूचक हो सकती है, अतः *मिजाज़* को किसी व्यक्ति की एथलेटिक प्रतिभा को निर्धारित करने में एक कारक के रूप में उपयोग किया जा सकता है।

शब्दकुंजीः शब्दकुंजीः कद–काठी, *मिजाज़,* शारीरिक प्रतिभा

UV-visible Spectroscopic Study on Interactions of Selected Unani Herbs with Calf Thymus DNA *in-vitro*

[1*]Mohd. Farooq Naqshbandi,
[2]Imran Ali,
[1]Mohd. Husain
and
[3]Munawwar Hussain Kazmi

[1]Department of Biotechnolgy,
Jamia Millia Islamia,
New Delhi – 110 025

[2]Department of Chemistry,
Jamia Millia Islamia,
New Delhi – 110 025

[3]National Research Institute of
Unani Medicine for Skin Disorders,
Hyderabad – 500 038

Abstract

DNA has been recognized to be the cellular target for numerous cytotoxic anticancer drugs for a very long period of time. The understanding of the process of mechanism of interaction between DNA and phytochemical ligand has become an active research area at the interface between different branches of biology and medicine. When the interactions of drugs with DNA are explored, it helps in designing more efficient and specifically targeted drugs with lesser side effects for diseases like cancer. Due to the high toxicity associated with present chemotherapeutic drugs to the normal cells of the human body, researchers all over the world are attracted towards the plants for discovery of new cytotoxic anticancer drugs. Among many Unani medicinal plants, *Solanum nigrum* (Mako) and *Sesame indicum* (*Til*) are found to have anticancer and antioxidant properties. The berries of *Solanum nigrum* and white seeds of *Sesame indicum* were successively extracted with six solvents. After addition of different concentrations of Ct-DNA to fixed concentration of plant extract, UV-visible absorption spectra were generated and recorded. The DNA binding constants for six extracts of *Solanum nigrum* and *Sesame indicum* ranged from 2.3 x 10^4 to 75 x 10^4, 3.6 x 10^4 to 34 x 10^4 respectively, showing good interactions with DNA. The DNA binding interactions were due to intercalative and ϖ-ϖ electron forces which provide the evidence that these extracts contain phytochemicals which strongly interact with DNA, hence they can be used for development of anticancer drug targeting DNA.

Keywords: DNA binding, *Solanum nigrum*, *Sesame indicum*, Extraction, Spectrometry

1. Introduction

According to data available with the World Health Organization (WHO), more than 80% of the world's community depends on traditional therapy for their primary healthcare needs and essentially 20,000 medicinal plants are found in 91 countries including 12 mega biodiversity countries. India is bestowed with huge botanical diversity and many traditional systems of medicine known as Ayurveda, Unani and Siddha system of medicine are practiced in India which use different herbs and medicinal plants to cure many diseases since centuries. Since ancient times, the local communities have built a valuable knowledge about the use of the rich bio-resources of herbal remedies. The practitioners of Unani Medicine have been employing the locally available medicinal plants for the treatment of different diseases of human beings. For past thousands of years, traditional medicines are used in the form of drugs which have been

*Corresponding author; Email: drfarooqnaqshbandi@gmail.com

empirically used in the treatment of various human disorders. The different systems of traditional medicine like Ayurveda, Siddha and Unani use several plant species to treat different human as well as animal ailments. The herbs used in traditional systems of medicines like Ayurveda and Unani contain phytochemicals which are used as medicine in different countries and are considered rich source of many potent and pharmacologically active powerful drugs (Fabricant & Farnsworth, 2001; Mahesh *et al.*, 2008). Phytochemicals can be defined as the constituent found in traditional medicinal plants that exhibited a potential action for modulating human metabolism for the prevention of chronic and degenerative diseases (Newman *et al.*, 200). Serious attempts have been made by scientists all over the world to make use of the biologically active phytochemicals from plant resources used in traditional systems of medicines in India which include pharmacological screening, isolation and characterization of the bioactive compound, clinical evaluation and toxicological analysis (Srivastava *et al.*, 1996). Nowadays scientists use natural medicinal plants as lead products for the development and production of curative drugs. It has been observed by present scientific community that relatively small number of plant species have been studied for possible medical applications in spite of the fact that plant based herbal medicines are being used for treatment of different ailments over many centuries. Data regarding the safety and efficacy of medicinal plants for use in different diseases is available for a very smaller number of plants (Jazari *et al.*, 2011 Geyid *et al.*, 2005).

As mentioned above, whole plant, plant parts and their products have been employed for treatment of various ailments for possessing distinctive medicinal properties since earliest times and this practice is still continuing. Scientists all over the world have studied many plant-based natural compounds which have exhibited their strong efficacy as anti-microbial, anti-cancer, antiviral as well as anti-inflammatory agents. Moreover, many potent cancer drugs used in modern medicine have been derived from plants e.g. morphine is derived from the fruit of *Papaver somniferum*, Taxol from *Taxus baccata* and Vincristine, Vinblastine from *Catharathus rosea,* etc. (Martins *et al.,* 2014). The National Cancer Institute have tested near about 114,000 extracts of plants for control of the disease of cancer after collection of about 35,000 plant samples from 20 different countries. It is assessed that about 30-40% of the drugs used worldwide for the treatment of cancer are obtained from plant sources (Newman *et al.,* 2003). It is believed that the use of medicinal plants in future continues to hold important potential for the prevention and cure of the cancer (Huang *et al.,* 2010). The treatment of the disease of cancer through Unani medicines may have some apparent benefits due to low side effects associated with these drugs over the treatment by synthetic drugs (Vickers *et al.*, 2002). The anti-tumor and antioxidant properties of the extracts of various herbs and medicinal plants have been proved beyond doubt by laboratory experiments and clinical

experiences (Bonham *et al.*, 2002). It is now proved experimentally that huge diverse of naturally occurring products from plants sources provide defense from carcinogenic exposure. There has been a growing awareness that many plant species are possessing antioncogenic properties. It has been researched that herbs used in Unani Medicine contain phytochemicals which are targeting the DNA of the cancer cells. It is very important to show the presence of phytochemicals in any herb which can interact with DNA *in-vitro*. A good amount of interactions between any herb and DNA gives a clue about the presence of phytochemicals which can react with DNA of cell very strongly and can be pharmacologically very active. This kind of herb can be used in future to target DNA of cancer cells during chemotherapy. In this background, we selected two classical Unani herbs for their interactions with Ct DNA by UV-visible Spectrometer, which include *Til* (*Seasame indicum*) and *Mako* (*Solinum nigrum*). *S. nigrum* commonly known as *Mako* or black nightshade, usually grows as a weed in moist habitats in different kinds of soils. *S. nigrum* is an important herb in Unani Medicine and is called *Mako*. Infusions are used in dysentery, stomach complaints, and fever. The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a tonic, laxative, appetite stimulant, and for treating asthma and excessive thirst (Jain *et al.*, 1968). Traditionally, the plant was used to treat tuberculosis (Kaushik *et al.*, 2009). The boiled extracts of leaves and berries are also used to alleviate liver-related ailments, including jaundice. *S. nigrum* is a widely used plant in modern medicine where it is considered antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic (Jain *et al.*, 2011). *S. nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation and fever (Acharya *et al.*, 2006).

Sesame (*Sesamum indicum*) is a flowering plant in the genus *Sesamum*. It is called *Til* in Unani Medicine. Sesame is a common cultivated herb. The sesame oil is mildly laxative, emollient and demulcent. Sesame seeds and fresh leaves are also used as a poultice .Oil has wide medical and pharmaceutical application. The phytochemical Sesamin found in Sesame has been found to protect the liver from oxidative damage. Sesame oil has been used for healing wounds for thousands of years. It is naturally anti-bacterial for common skin pathogens such as staphylococcus and streptococci as well as common skin fungi such as athlete's foot. It is anti-viral and anti-inflammatory. The oil has been used in the treatment of several chronic diseases including hepatitis, diabetes and migraines. Other uses of sesame include the treatment of blurred vision, dizziness, and headaches. Indians have used sesame oil as an antibacterial mouthwash, to relieve anxiety and insomnia. The leaves are rich in a gummy matter and when mixed with water form rich bland mucilage that is used in the treatment of infant cholera, diarrhea, dysentery, cataract, boils, carbuncle, menstrual irregularities, poly-urea, stomach- trouble, serious burns, skin diseases, alopecia and used also as a tonic (Trivedi *et al.*, 1965).

It is well-known now that many anticancer drugs used during chemotherapy are targeting the DNA of the cancer cells. For the sake of design of new drugs, the processes of study of interaction of DNA with drug are very stimulating and important (Hajian *et al.*, 2009). The precise mode of exchanges between DNA and drug molecules is quiet not completely clear. It is, therefore, important to present extra simple approaches for studying the mechanism of interaction between DNA and drug molecule. The understanding of the process of mechanism of interaction will be highly helpful for planning of new DNA specific targeted drugs and *in-vitro* investigation of these drugs (Mattes *et al.*, 1986).

It is vital, therefore, to study the interactions of newly discovered compounds with DNA. This kind of study will be useful to give idea about their anticancer activities and likely mode of action. The covalent interactions can lead into the permanent adduct formations with DNA while as non-covalent interactions are temporary and reversible interactions with DNA. When a labile drug is substituted and gets bounded to an atom of DNA base, such as N^7 of guanine, it is called covalent binding and when there are interactions like intercalative, electrostatic and groove binding it is called as non-covalent binding. Basically, in covalent interactions, covalent bond formation takes place between the nitrogenous bases of DNA and drugs or ligands (Hurley *et al.*, 1989, Neidle *et al.*, 1986, Wang *et al.*, 1992). The covalent bond formations are not common and only few examples of this kind of bonding can be found. An interaction which involves a sandwich-type of configuration between the base pairs of DNA and the ligand is known as non-covalent binding. Generally, covalent intercalation takes place because of ϖ–ϖ interaction between ligand molecule and the aromatic bases of DNA. This kind of interaction of ligand and DNA results into the increase of distance between the base pairs of DNA. When there are interactions between cationic ligands and the negatively charged phosphate backbone of DNA, they result into electrostatic interactions between DNA and ligand, which are another non-covalent mode of binding of ligands with DNA. When the molecules approach to the grooves (major or minor) of the DNA, it results into the groove binding with DNA non-intercalative mode (Wang *et al.*, 1984, Kelly *et al.*, 1958). The groove binding types of interactions are mostly stabilized by van der Waal's forces, hydrophobic interactions, and hydrogen bonding. (Indumathy *et al.*, 2008; Rajski *et al.,* 1998).

Another oldest class of anticancer drugs which interact with DNA and are still in use is called alkylating drugs. They cause significant DNA damage to destroy malignant cells cell (Liu *et al.*, 2001). They are used in the management of many kinds of malignances. The maximum alkylating drugs are mono-functional methylating agents, bifunctional alkylating agents or chloroethylating agents (Kelly *et al.,* 1985). Alkylating agents can interact with DNA through three

mechanisms. First mechanism involves an attachment of an alkyl groups to DNA bases by alkylating agent, resulting in the DNA fragmentation by repairing enzymes in their efforts to substitute the alkylated bases. The second mechanism involves, networking composed of two bases of DNA by an alkylating agent that has two DNA binding sites. The cross networking of the two strands of DNA; created by the bifunctional alkylating agents; leads the prevention of replication and transcription because this template of linked DNA cannot be used for further DNA and RNA synthesis, which in turn leads to inhibition of replication and transcription; leading to cell death. A huge number of chemical compounds are alkylating agents under normal physiological conditions, and a wide range of these compounds have displayed antitumor activities. The third mechanism of action of alkylating agents involves the mis-pairing of the nucleotides of the DNA. The mis-pairing of nucleotides results into mutations. The formation of covalent links between alkylating agents and DNA result in mis-pairing, substitutions, or excision in DNA. These are called "mistakes" in the DNA. The cellular response of cancer cells to these changes or mistakes may lead to the prevention of DNA synthesis and proliferation or they may lead to apoptosis of cancer cells (Mei *et al.*, 1986). One more type of binding, which is electrostatic in nature, is called external binding. In this kind of bonding, ligands and drugs have the property of forming non-specific, outside edge piling interactions with the DNA phosphate backbone. This type of binding commonly takes place where the anticancer drug self-associates to produce higher-order aggregates, which may pile on the anionic DNA backbone in order to decrease charge–charge repulsion between drug molecules.

There are several spectroscopic, biochemical, and physical techniques which can be used for monitoring the above mentioned different interactions between a ligand and DNA. The monitoring techniques include circular and linear dichroism, voltammetric, agaraose gel electrophoresis, NMR, IR, Raman, viscometry, genosensors, fluorescence and absorption methods. But, absorption spectroscopic method (UV-vis. spectroscopy) is commonly used technique for monitoring the interactions of DNA with the ligand. This technique is based on the principal that the configuration of DNA changes when added ligand interacts with it. Absorption spectroscopic method (UV-vis. spectroscopy) provides a clue about the conformational changes of DNA structure upon addition of ligand or drug. UV-Vis. spectroscopy changes into DNA on addition of ligand include hypochromic and hyperchromic shifts in absorption, appearance of isobestic points, red-shift or blue shift in the absorption maxima and increase in luminescence. These changes make the characteristics of DNA interactions with the ligand observed during UV-vis. spectroscopy study. It is evident that a change in absorbance of a drug or ligand in UV-vis. spectroscopy on addition of DNA is an indication of the binding of ligands with DNA. Generally, a covalent binding between a ligand and a drug with DNA results in hyperchromism (increase in

absorbance) and red shift (wavelength shifts to higher value) (Chauhan *et al.*, 1992). The processes of red shift happening during spectroscopic study of ligand with DNA is a suggestion of the organization of a compound with DNA through N^7 position of guanine (Liu *et al.*, 2005). On the other hand, the hypochromism (decrease in absorbance) and bathochromism (red shift) (due to decrease of the $\pi \rightarrow \pi^*$ transition energy as π^* orbital of the intercalated ligand couples with the orbital of the base pairs) shifts take place when a ligand interacts and bounds to DNA through intercalation. There is strong stacking interaction between aromatic chromophore and the base pairs of DNA during the intercalative mode of binding (Shahabadi *et al.*, 2009). The extent of hypochromism is directly proportional to the strength of intercalation (Liu *et al.*, 2001; Pyle *et al.*, 1989). Commonly, when there is electrostatic interaction of a compound with DNA, it can result into hypochromicity with no bathochromic shift (Pasternack *et al.*, 1983). No or slight alteration in UV-vis. spectra, sometimes with minor hyperchromicity, may characterize and indicate outside binding between the DNA and ligand. Contrarily, when there are characteristics of the intercalative binding approach present but to a slight grade, it indicates outside binding with self-stacking (Zhao *et al.*, 2008; Zhao *et al.*, 2014; Guo *et al.,* 2011). The formation of adducts (more than one type of interaction) between DNA and ligand results into the hyper- and hypochromicities with adequate blue shift of the absorption band (Sirajuddin *et al.*, Zhao *et al.*, 2014.)

2. Experimental

2.1. Chemicals and reagents

All the solvents, reagents and chemicals used were of analytical grade and were put to use without more purification in original form. The chemicals used were hexane ethanol, methanol ethyl acetate and chloroform. These were purchased from Merck, India. Deoxyribonucleic acid (DNA) from calf thymus was purchased from Sigma Chem. Co., and used as received.

2.2. Instruments used

Millipore water was collected from Millipore-Q, Bedford, MA, USA system. The DNA interaction studies with plant extracts were carried out using UV-visible spectrophotometer (model T 80) from PG Instruments Ltd., UK. Other equipment used were sonicator, digital pH meter, centrifuge machine and weighing balance, water bath.

2.3. Plant collection

The white seeds of *S. indicum* and berries of *S. nigrum* were purchased from Khairebaile Market, New Delhi.

2.4. Plant identification

The purchased plant samples were authenticated by Department of Illmul Advia (pharmacology), School of Unani Medicine, Faculty of Medicine, Jamia Hamdard, New Delhi. The standard procedure was followed for the identification of the plant materials.

2.5. Preparation of *Solinum nigrum* and *Sesamum indicum* powder

After procurement, the plant materials were cleaned manually and dried carefully under shed for ten days. After drying, these berries and seeds were crushed to make into fine small particles by means of a blender. The fine small particles or powder thus produced was stored in a desiccator at ambient temperature for the extraction purpose.

2.6. Extraction of berries of *Solinum nigrum* (*Mako*) and seeds of *Sesamum indicum* (*Til*)

In order to make extracts in the different successive solvents of different polarity, 50 g *Solinum nigrum* and *Sesamum indicum* fine particles were saturated separately in 200 mL of double distilled water, methanol, acetonitrile, ethyl acetate, chloroform and acetone in separate round bottom flasks and extracted in Soxhlet apparatus. This method of extraction was continued and repeated for many cycles. During each new cycle, fresh clove powder and fresh solvents were used. All the extracts were allowed to stand for 24 hours before filtration with Whatman no.1 filter paper. The filtrate was then allowed to evaporate with the aid of a rotary vacuum evaporator. The solid residues were used to make final solution before being transferred to a water bath for further drying. The stock solutions of 100 mg/mL of each extract of the two plants were prepared for all 6 extracts and evaluated for DNA binding.

2.7. DNA binding studies

The stock solution of disodium salt of Ct-DNA [$(1.0 \times 10^{-5}$ to 30.0×10^{-4} M)] was made in *tris*-HCl buffer at pH 7.2-7.4 and refrigerated at 4 °C temperature. After making the stock solution of DNA, it was used within 4 days. Concentration of the solution was calculated with the help of spectrometer. In order to ascertain that Ct-DNA was amply free of protein, its absorbance at wavelength of 260 and 280 ($\geq$ 1.8) was determined and ratio was calculated (**Fig.1**). The extinction coefficient at 260 nm (6600 $M^{-1}cm^{-1}$) was used to measure the concentration of Ct-DNA.

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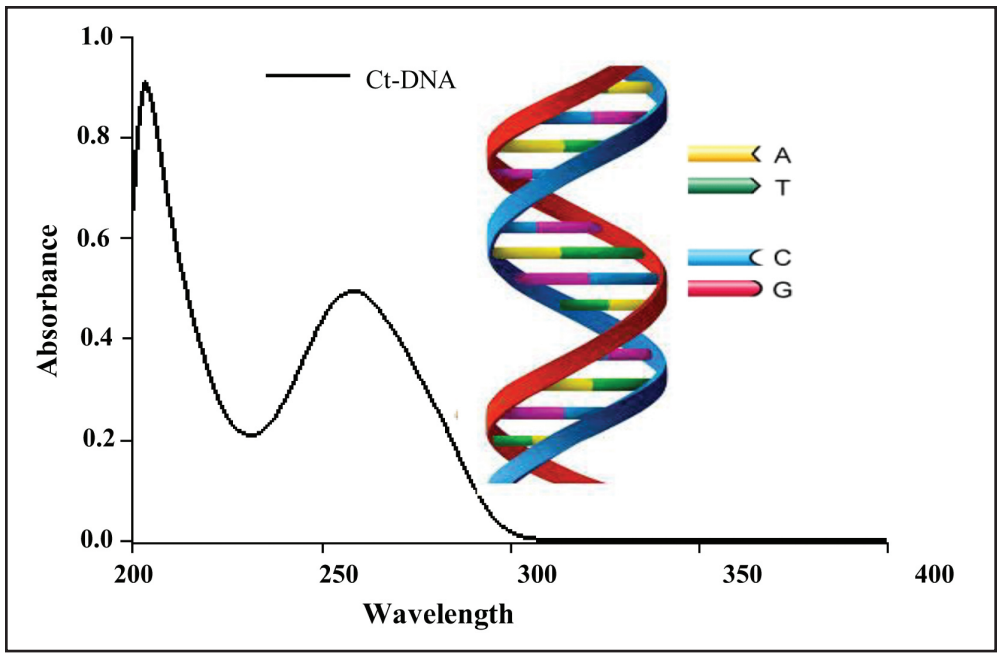


Fig. 1

The dilution of Ct-DNA stock solution was done by using *tris*-HCl buffer. The dilution of the solution was done for the purpose of titration. The plant fractions were dissolved in small quantity of MeOH/DMSO and maintained with buffer (~ 10^{-4} M). The solution was filtered to remove any precipitate present in solution. After addition of different concentrations of Ct-DNA to fixed concentration of plant fraction, UV-vis. absorption spectra were generated and recorded. By adding varying concentrations (~ 10^{-4}) of Ct-DNA, absorption titration was achieved. The study of interactions of the plant extracts fractions with Ct-DNA was carried out at pH 7.4 in a solution of tris-(hydroxymethyl)-amino methane (Tris, 10^{-2} M) in double distilled water. The absorption spectra were generated at room temperature against the blank solution of Tris-HCl buffer at pH 7.4. In the beginning of the experiment, UV-vis spectrophotometry is used to calculate the concentration of newly prepared Ct-DNA at a wavelength of 260 nm (ε = 6600 M^{-1} cm^{-1}) (Reichmann *et al.*, 1954).

In order to study the binding interactions, the absorption spectra of freshly prepared plant extracts at fixed concentration of (0.01 mg/mL) were mixed with the different concentrations of DNA (1.0×10^{-5} to 30.0×10^{-4} M) in increasing order i.e. at first lower concentration of DNA was used then gradually increased. The absorbance of each concentration was noted. At beginning, λ_{max} and absorbance of pure DNA and plant extract fractions were recorded separately in tris-buffer solutions. Then λ_{max} and absorbance of mixture of 2.0 mL of each solution containing DNA and plant extract were recorded. To study the changes in the absorption spectra, readings were recorded after addition of each new increased concentration of the of DNA solution (2.0 mL) to the solution of the

plant extract fractions. To avoid bias, the titration experiments were repeated five times. Benssi-Hilderbrand equation modified by Wolfe *et al.* (1987) was applied to calculate the intrinsic binding constants (K_b). Due to the variance in absorbance, the intrinsic binding/association constants (Ks) of the plant extracts with the DNA were calculated by the following equation.

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_a-\varepsilon_f) + 1/K(\varepsilon_b-\varepsilon_f) \text{ ----------} 1^{st}$$

Where, absorption coefficients, ε_a, ε_f, and ε_b correspond to A_{obs}/[plant extract], extinction coefficient for the plant fraction in free form and the extinction coefficient for the plant fraction in the fully bound form, respectively. The slopes and the intercepts of the plots of [DNA]/ (ε_a - ε_f) vs [DNA] were used to calculate binding constants of different fractions. The purity of stock solution of Ct-DNA was ascertained by considering ratio of absorbance ratios (A260/A280 and A260/A230). It was found well within the range of 1.8-1.9 and indicated the sufficiently protein free nature of DNA (Pratviel *et al.*, 1998). The concentration of stock solution of DNA was experimentally determined by utilizing ε = 6600 M^{-1} cm^{-1} at 260 nm. The additional DNA solutions were made from a stock solution (30×10^{-4}M) with a fixed concentrated solution of plant extracts (0.01 mg/mL).

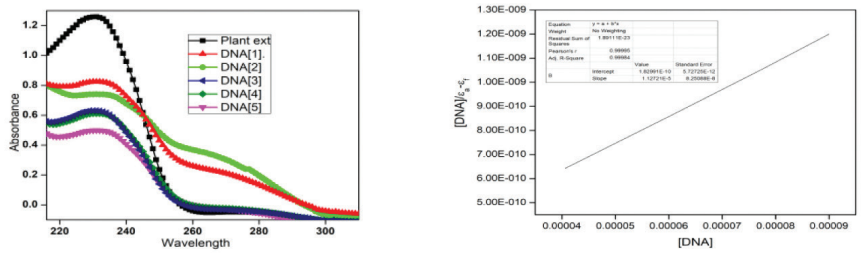
3. Results and Discussion

3.1. Extraction of the plant materials

During the extraction procedure, the colourless extracting solvents became coloured in Soxhlet apparatus indicating that the plant material constituents have dissolved into the extracting solvents. The extraction was carried out till the extracting solvents became colourless in the Soxhlet apparatus arm. It was observed that the colours of the extracting solvents were different with respect to each other after the extraction was complete. This observation clearly reveals that each extracting solvents contain plant constituents who are different from other extracting solvents. It was because of the varying polarities of the solvents used for extraction. Furthermore, the colour of the extracting solvents becomes dark on reducing the volumes of the extracting solvents by rotary evaporator apparatus under vacuum.

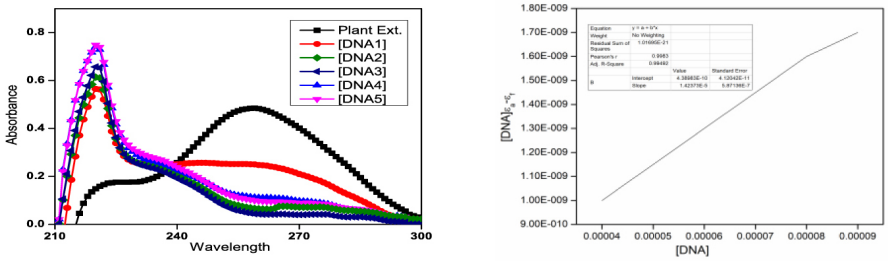
3.2. DNA binding study

The DNA binding studies of *Solinum nigrum* and *Sesamum indicum* extracts were experimented out as described above. The results are given in Fig. 2-13. Figure 2 to 7 depicts DNA binding spectra of *Solinum nigrum* and Fig. 8 to 13 depict DNA binding spectra of *Sesamum indicum*. The different calculated parameters of interactions of plant extracts with DNA are depicted in Tables 1 and 2. The



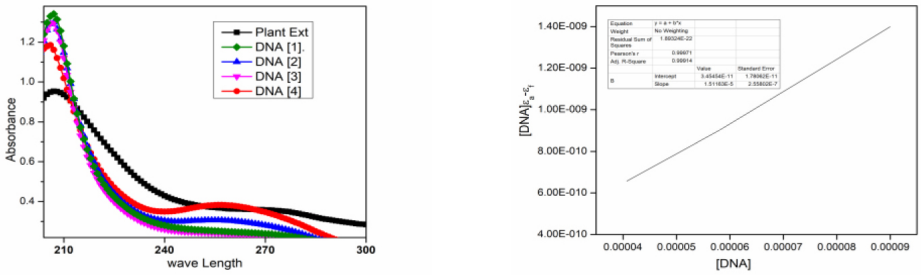
DNA Binding Spectra and DNA Binding Constant of Acetone Extract of Solinum Nigrum

Fig. 2



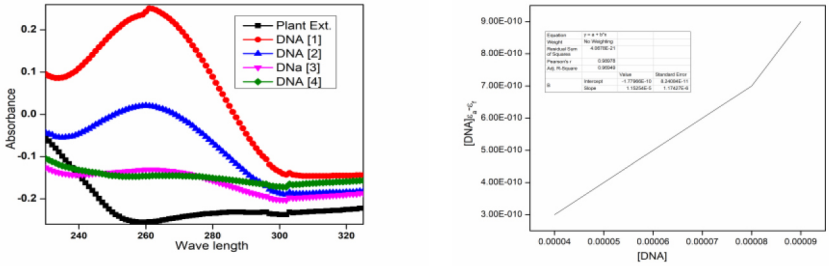
DNA Binding Spectra and DNA Binding Constant of Ethyl Acetate Extract of Solinum Nigrum

Fig. 3



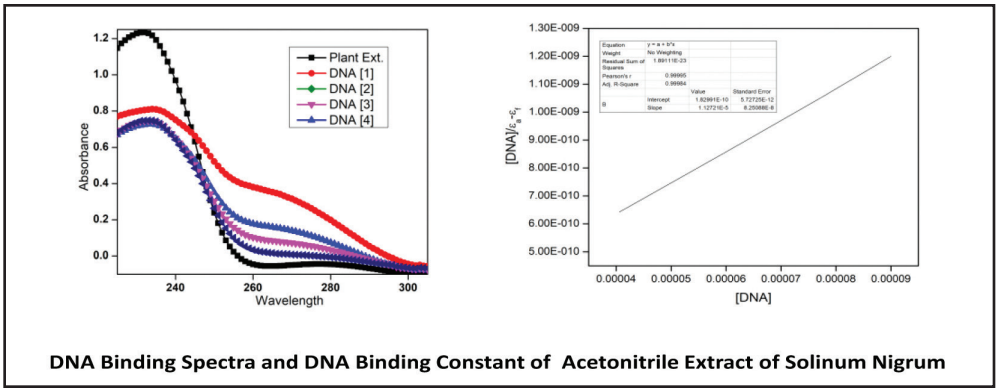
DNA Binding Spectra and DNA Binding Constant of Water Extract of Solinum Nigrum

Fig. 4



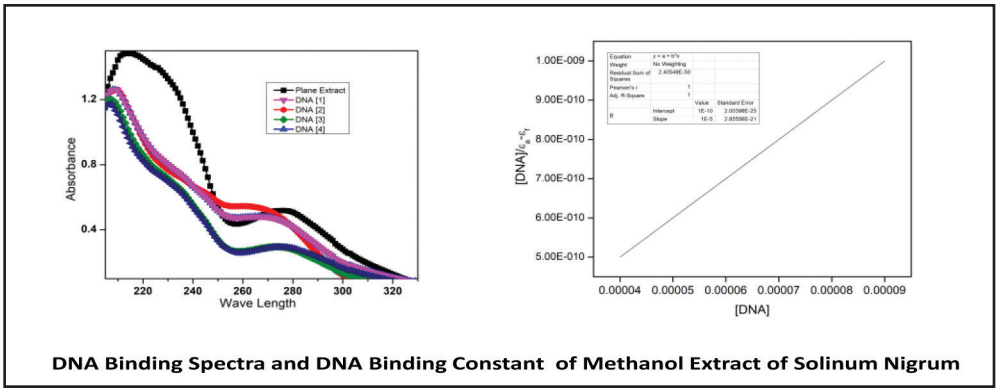
DNA Binding Spectra and Binding Constant of Chloroform Extract of Solinum Nigrum

Fig. 5



DNA Binding Spectra and DNA Binding Constant of Acetonitrile Extract of Solinum Nigrum

Fig. 6



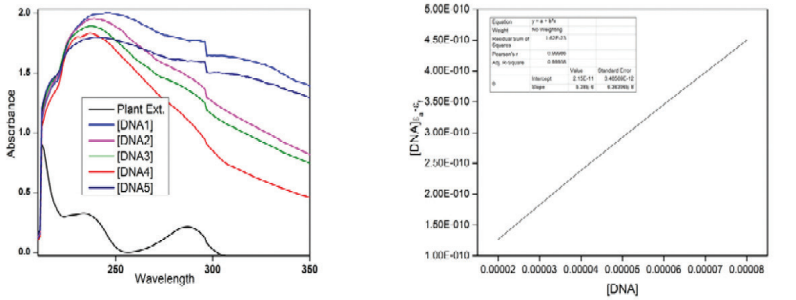
DNA Binding Spectra and DNA Binding Constant of Methanol Extract of Solinum Nigrum

Fig. 7

Table 1: Different parameters used for DNA binding of extracts of *Solinun nigrum*

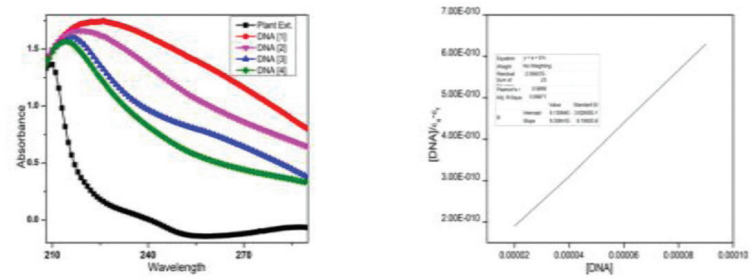
Plant Extracts	λ_f (nm)	λ_b (nm)	$\Delta\lambda_{max}$ (nm)	A_f	A_b	ΔA	(H%)	K_b (M^{-1})
Acetone	234	204	20	1.25	1.14	0.70	9%	2.3 x 10^4
Acetonitrile	232	207	25	1.23	1.01	0.22	18%	9.8× 10^4
MeOH	202	207	0.5	0.95	1,40	0.11	92.3%	50× 10^4
Water	206	207	1.0	0.11	0.29	0.18	16.3%	75× 10^4
Ethyl acetate	260	230	30	.45	.75	,30	66.6%	32× 10
Chloroform	258	259	1.00	1.48	1.15	.33	33%	4.3× 10^4

% Hyperchromism (H%) = [Change in absorbance /A_f]×100, where A_f and A_b represent the absorbance of free and bound plant extracts; $\lambda_f = \lambda_{max}$(free extracts), $\lambda_b = \lambda_{max}$(extracts bound to DNA) and K_b = Binding constants.



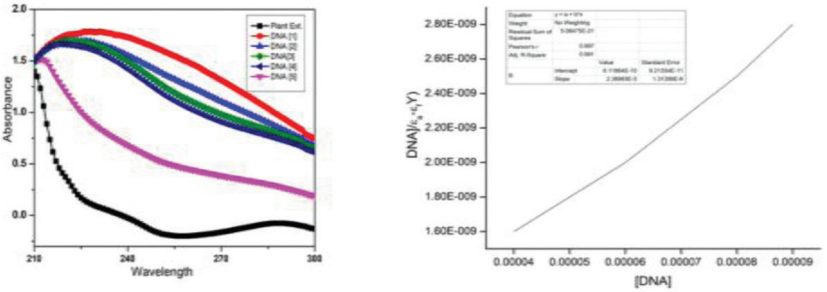
DNA Binding Spectra and DNA binding Constant of Acetone Extract of Sesame Indicum.

Fig. 8



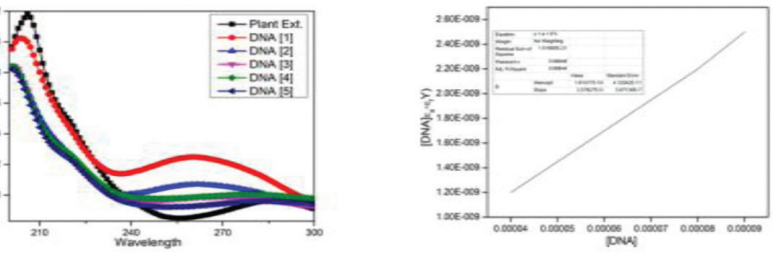
DNA Binding Spectra and DNA Binding constant of Actonitrile Extract of Sesame Indicum

Fig. 9



DNA Binding Spectra and DNA Binding Constant of Chloroform Extract of Sesame Indicum

Fig. 10



DNA Binding Spectra and DNA Binding Constant of Water Extract of Sesame Indicum

Fig. 11

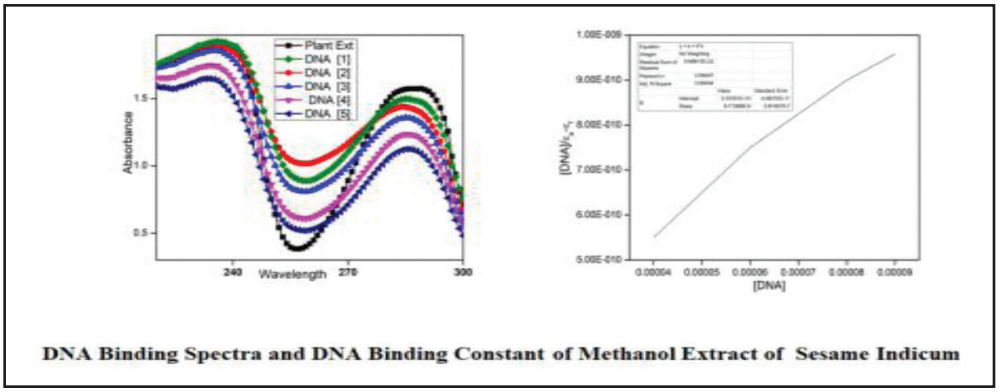


Fig. 12

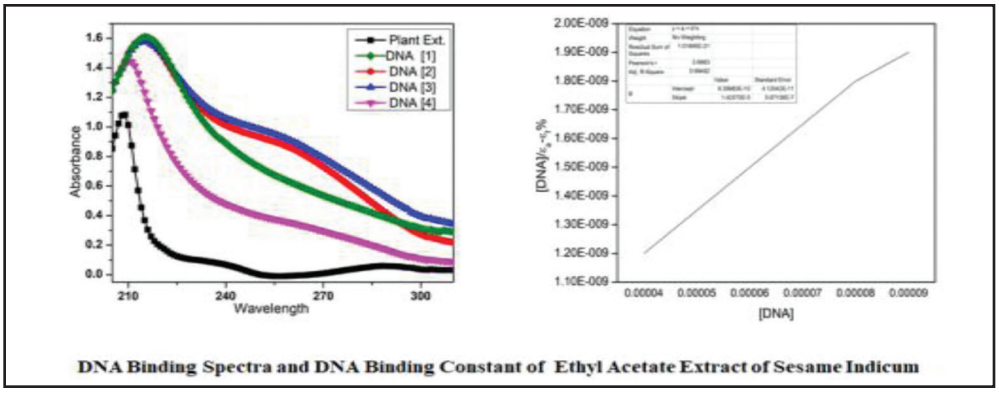


Fig. 13

Table 2: Different parameters used for DNA binding of *Sesamum indicum* extracts

Plant Extracts	λ_f (nm)	λ_b (nm)	$\Delta\lambda_{max}$ (nm)	A_f	A_b	ΔA	(H%)	K_b (M^{-1})
Acetone	211	235	24	1.34	1.82	0..48	48%	3.6x 10^4
Acetonitrile	226	210	26	1.36	1.74	0.38	38%	11.9x 10^4
MeOH	237	285	48	1.91	1.42	.81	81%%	34× 10^4
Water	206	203	3.0	1.18	0.86	0.32	32%	25× 10^4
Ethyl acetate	206	213	7	1.01	.1.61	,15	15%	22× 10^4
Chloroform	210	231	21.00	1.39	1.78	.39	39%	3.7× 10^4

% Hyperchromism (H%) = [Change in absorbance /A_f]×100, where A_f and A_b represent the absorbance of free and bound plant extracts; $\lambda_f = \lambda_{max}$ (free extracts), $\lambda_b = \lambda_{max}$(extracts bound to DNA) and K_b = Binding constants.

parameters include λ_{max} (nm), free plant extracts (λ_f), plant extract bonded to DNA (λ_b), change in wavelengths after binding with DNA ($\Delta\lambda_{max}$), absorbance of free plant extract (A_f), absorbance of plant extract bonded to DNA (A_b), and change in absorbance after binding with DNA (ΔA). Fig. 2 to 13 show the interactions of the six extracts of the two plants with DNA. The insert graphs are representation of the plot of [DNA] / (ε_a - ε_f) vs [*DNA*] calculated to determine the intrinsic binding constant (K_b) (Kalu *et al.*, 2010). The marked changes were observed in the absorption bands by the addition of increasing concentrations of DNA to the plant extracts. These figures also confirm bathochromism (red shift) and hyporchromism. The intercalative modes resulted in stacking interactions between the base pairs of DNA and aromatic chromophores. The degree of the hypochromism was usually steady with the level strength of intercalative interactions, which decided the extent of the hypochromism observed in the graph (Liu *et al.*, 2002). These observations can be explained by the process of π electrons combination of the fractions with ϖ electrons of DNA bases. The level of energy of the π-π electron transitions decreased and, consequently, this decrease in electron transitions led to the red shifts and caused the hypochromic effects (Sirajuddin *et al.*, 2012; Falcioni *et al.*, 2008). The order of strength of the binding constants of *Solinum nigrun* extracts was acetone >chloroform >acetonitrile >ethyl acetate >methanol >water and for *Sesamum indicum* order was acetone >chloroform >acetonitrile >ethyl acetate >water >methanol. At the same time, the plant extract may be having charged moieties. These positive charged moieties are anticipated to openly and directly affect DNA (Liu *et al.*, 2002) binding in the form of classical electrostatic interactions. Besides, there are negatively charged oxygen of the phosphate group of DNA, which may also have electrostatic interactions and finally lead to further spectral changes. The more interactions such as hydrogen bondings between base pair in DNA and the plant fractions may also be present (Tysoe *et al.*, 1993; Zhang, 2004). All these interactions discussed can counter the predominant hypochromic effect of the plant fractions and can have declining effect on it.

4. Conclusion

The DNA binding constants of two Unani herbs *Solinum nigrum* and *Sesamum indicum* ranged from 2.3 x 10^4 to 75 x 10^4 and 3.6 x 10^4 to 34 x 10^4 respectively, showing good interactions with DNA. The DNA binding interactions were due to intercalative and π-π electron forces. Finally, water extract of *Solinum nigrum* and methanol extract of *Sesamum indicum* were found to be the most active. Briefly, it was concluded that these two extracts i.e. water extract of *Solinum nigrum* and methanol extract of *Sesamum indicum* contain phytochemicals which are binding with DNA significantly and may be used to discover a drug which works at DNA level to treat cancer effectively.

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सारांश

काफ थाइमस डीएनए के साथ चयनित यूनानी जड़ी–बूटियों के अंतःक्रियाओं पर इन–विट्रो यूवी–विज़िबल स्पेक्ट्रोकोपिक अध्ययन

मो. फ़ारूक़ नक़्शबन्दी*, इमरान अली, मो. हुसैन, और मुनव्वर हुसैन क़ाज़मी

सारांश

डीएनए को लंबे समय से कई साइटोटोक्सिक कैंसररोधी औषधियों के लिए कोशिका लक्ष्य के रूप में मान्यता प्राप्त है। डीएनए और फाइटोकेमिकल लिगैंड के मध्य अंतःक्रिया तंत्र की प्रक्रिया को समझना जीव विज्ञान और चिकित्सा की विभिन्न शाखाओं के बीच इंटरफेस में एक सक्रिय अनुसंधान क्षेत्र बन गया है। जब डीएनए के साथ औषधियों की अंतःक्रियाओं का पता लगता है तो इससे कैंसर जैसी बीमारियों के लिए कम दुष्प्रभाव वाली कुशल और विशेष रूप से लक्षित औषधियों को डिज़ाइन करने में मदद मिलती है। मानव शरीर की सामान्य कोशिकाओं में वर्तमान कीमोथेराप्यूटिक औषधियों से जुड़ी उच्च विषाक्तता के कारण दुनिया भर के अनुसंधानकर्ता नई साइटोटोक्सिक कैंसररोधी औषधियों की खोज करने के लिए पौधों की ओर आकर्षित हुए हैं। अनेक यूनानी औषधीय पौधों में *सोलेनम नाइग्रम* (मको) और *सेसम इंडिकम* (तिल) में कैंसररोधी और ऑक्सीकरणरोधी गुण पाए जाते हैं। *सोलेनम नाइग्रम* के बेज और *सेसम इंडिकम* के सफ़ेद बीज क्रमिक रूप से छः द्रावकों के साथ निकाले गए। पौधों के सत्त की निश्चित सांद्रता में सीटी–डीएनए की विभिन्न सांद्रता मिलाने के बाद यूवी–विज़िबल अवशोषण स्पेक्ट्रा उत्पन्न तथा रिकॉर्ड किए गए। *सोलेनम नाइग्रम* और *सेसम इंडिकम* के छः सत्तों के लिए डीएनए बाध्यकारी स्थिरांक क्रमशः 2.3×10^4 से 75×10^4, 3.6×10^4 से 34×10^4 था जिससे डीएनए के साथ अच्छी अंतःक्रिया का पता चलता है। डीएनए अंतःक्रियाएं इंटरकलेटिव और π-π इलेक्ट्रॉन बलों के कारण थीं जो इस बात को प्रमाणित करते हैं कि इन सत्तों में फाइटोकेमिकल्स होते हैं, जो डीएनए के साथ दृढ़ता से अंतःक्रिया करते हैं इसलिए इनका उपयोग डीएनए–लक्षित कैंसररोधी औषधि के विकास के लिए किया जा सकता है।

शब्दकुंजी: डीएनए, *सोलेनम नाइग्रम, सेसम इंडिकम,* निष्कर्षण, स्पेक्ट्रोमेट्री

Physico-chemical and Pharma-cognostical Evaluation of Anti-inflammatory Poly-herbal Formulation *Ḥabb Hindī Muḥallil*

[1*]Akhlaq Mustafa,
[1]Farah Ahmed,
[1]Kiran Negi,
[1]Asma Sattar Khan,
[1]Anas Iqbal Alvi,
[1]Mahesh Chandra,
[1]Mustehasan,
[2]Zaki Ahmed Siddiqui,
and
[2]R. P. Meena

[1]Drug Standardisation Research Unit, Central Council for Research in Unani Medicine, New Delhi – 110 058

[2]Central Council for Research in Unani Medicine, New Delhi – 110 058

Abstract

Standardization of drugs is of a paramount importance today in order to put drug industry on a sound footing. Unani Medicine offers poly pharmaceutical formulations in a variety of forms like *Safūf*, *Ḥubūb*, *Ma'jūnāt, Kushtajāt, Murabbajāt* and *Marham*. *Ḥabb Hindī Muḥallil* is an anti-inflammatory formulation used mainly for the treatment of cervical adenitis which is one of the global public health concerns nowadays. The formulation is mainly prepared by mixing thirteen ingredients belonging to the drugs of plant origin. For the assurance of safety, efficacy and reproducibility, it is mandatory that every herbal product pass the tests of identification, authentication and chemical standardization.

The present investigation deals with the physico-chemical and pharmacognostical standardization of three different batches of the drug based on the various pharmacopoeial parameters, e.g. salient organoleptic and pharmacognostic characters, alcohol and water soluble extractives, pH values, ash values, successive extractives and detection of qualitative and quantitative organic constituents, thin layer chromatographic studies of successive extracts, etc. Besides toxicological scanning including microbial load, pesticide residues, aflatoxin, heavy metal estimations and HPTLC were also carried out which may serve as a prelude to fix the pharmacopoeial standards or prove helpful in laying down the SOP's for this preparation.

Keywords: *Ḥabb Hindī Muḥallil*, Herbal drug, Physico-chemical standards, Poly-herbal formulation, SOP, Soxhlation

Introduction

Herbal medicines play a significant role in the management of both major and minor medical illness (Bahuguna *et al.*, 2014) and there is an increasing awareness and general acceptability of indigenous drugs in today's medical practice. Over 80% of world's population depends on the herbal drugs and herbal products for healthy living. Unfortunately, a number of these drugs in the market are adulterated with cheap, less or spurious plant materials posing danger to the public health. Studies on the identity, purity and quality of genuine drug will enhance the information in checking the adulteration.

Ḥabb Hindī Muḥallil is a poly-herbal formulation prepared on the basis of the formula mentioned in the National Formulary of Unani Medicine, Part-I comprising thirteen ingredients belonging to the drugs of plant origin which is widely used for the treatment of cervical adenitis and inflammation (MHFW, 2006).

*Corresponding author; Email: amh786786@gmail.com

In the present study, an attempt has been made to find out the analytical characteristics e.g. organoleptic characters, microscopical, physico-chemical, thin layer chromatography along with aflatoxin, microbial load, heavy metals and pesticide residues analysis of the drug *Ḥabb Hindī Muḥallil*.

Material and Methods

The crude drug was procured from a local market of Delhi and authenticated by Pharmacognosy Section of Drug Standardization Research Unit, New Delhi. Three batches of the poly-herbal formulation *Ḥabb Hindī Muḥallil* were prepared as per the formula mentioned in National Formulary of Unani Medicine, Part-I, 2006 by following the method as given in *Qarābādīn-i-Aʿẓam* (Khan, 1320H). For preparation of the drug, take all the ingredients of pharmacopoeial quality. Clean all the ingredients 1–12 and make fine powder separately. Mix the powdered mixture of 1–7 and keep aside (Part A). Soak ingredients 8–12 in water and leave overnight. Now boil till the content remains one third. Mesh the boiled mixture and strain. Now this material so obtained is mixed with ingredient No. 13 and boil the decoction till it becomes thick (Part B). Now mix Part A and B to make *Lubdī* and prepare pills. Store these pills in a sealed container protected from light and moisture. The composition of the drug is as given in Table 1.

Table 1: Composition of Unani formulation *Ḥabb Hindī Muḥallil*

S. No.	Ingredient	Botanical/Scientific Name	Part used	Quantity
1	*Hīl Khurd*	*Elettaria cardamomum* (L.) Maton	Fruit	5 pcs
2	*Zanjabīl*	*Zingiber officinale* Rosc.	Rhizome	4.75 g
3	*Filfil Siyāh*	*Piper nigrum* L.	Fruit	4.75 g
4	*Filfil Darāz*	*Piper longum* L.	Fruit	4.75 g
5	*Halela Zard*	*Terminalia chebula* Retz.	Fruit	4.75 g
6	*Balela*	*Terminalia bellirica* (Gaertn.) Roxb.	Fruit	4.75 g
7	*Āmla*	*Phyllanthus emblica* L.	Fruit	4.75 g
8	*Halela Zard*	*Terminalia chebula* Retz.	Fruit	262.50 g
9	*Balela*	*Terminalia bellirica* (Gaertn.) Roxb.	Fruit	262.50 g
10	*Āmla*	*Phyllanthus emblica* L.	Fruit	262.50 g
11	*Turbud*	*Operculina turpethum* (L.) Silva Manso	Root	262.50 g
12	*Maghz-i-Amaltās*	*Cassia fistula* L.	Pulp	262.50 g
13	*Muqil*	*Commiphora wightii* (A.) Bhandari	Exudate	525 g

Microscopic Studies

Crush 5 pills and mix it with 50 ml of water in a beaker. Stir gently and discard the supernatant. Take the residue without loss of the material and repeat the process until clear of gummy material. Finally, wash the residue with distilled water and reject the supernatant. Clear the residue with the solution of chloral hydrate and after staining with different reagents like safranin, iodine soiution, ferric chloride, etc. mount it with glycerine (Johansen, 1940; Trease & Evans, 1983; Wallis *et al.*, 1969). The representative photographs were taken from the computer with microscopic attachment.

Observation and Results

Microscopic observations

Upon examination under the microscope, the following cells/ tissues/cell contents were observed:

Hīl Khurd **(Fig. 1, 2)**: Fragment of arillus in surface view consisting of thin walled, elongated, irregular fusiform cells and fragment of testa in surface view consisting of thick walled Sclerenchymatous cells.

Zanjabīl **(Fig. 3, 4)**: Fragment of thin walled parenchyma cells filled with single, flattened oblong oval starch grains with terminal beak like projections measuring 9-45µ in length and 5-25µ in width, pieces of unlignified fibres which are pitted, thin walled, septate having width 31.5-36µ.

Filfil Siyāh **(Fig. 5, 6)**: Group of beaker shaped stone cells of the endocarp and groups of parenchyma cells filled with compact mass of round angular starch grains measuring 1-5 µ in diameter.

Filfil Darāz **(Fig. 7)**: Group of oval elongated stone cells.

Halela Zard **(Fig. 8, 9)**: Pieces of fibres present mostly in groups, thick walled, lignified, with or without pits, group of sclereids that are oval – isodiametric in shape, narrow lumen and with pits.

Balela **(Fig. 10, 11)**: Elongated sclerotic cells with pointed and flattened ends striated walls, pitted and highly lignified, fragment of epidermal cells with uniseriate, elongated trichomes.

Āmla **(Fig. 12)**: Group of sclereids with very broad lumen and pitted walls.

Properties

Action : *Muḥallil-i-Waram* (anti-inflammatory)

Therapeutic use : *Khanāzīr* (Cervical adenitis)

Fig. 1: x 40 fragment of arillus of *Hīl Khurd*

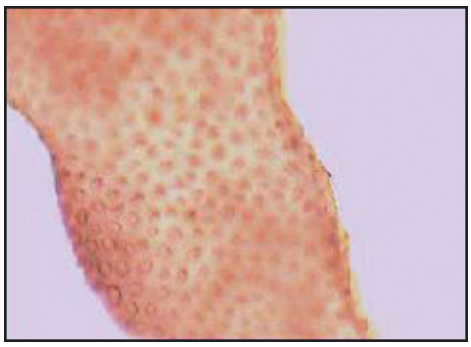


Fig. 2: x 40 fragment of testa of *Hīl Khurd*

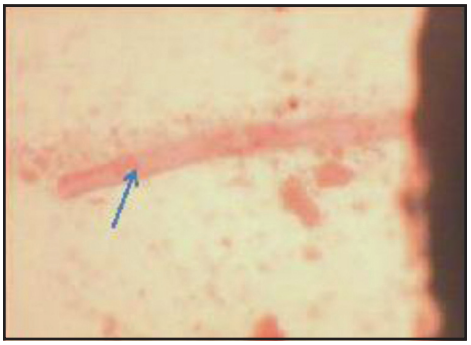


Fig. 3: x 40 piece of fibre of *Zanjabīl*

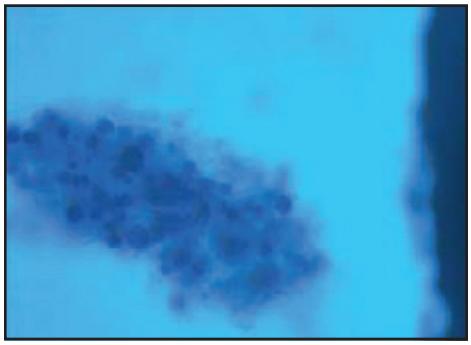


Fig. 4: x 40 parenchyma cells filled with starch grains of *Zanjabīl*

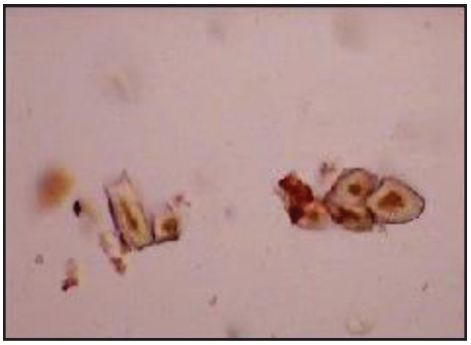


Fig. 5: x 40 stone cells of *Filfil Siyāh*

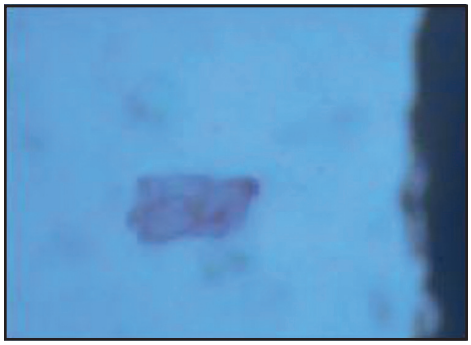


Fig. 6: x 40 parenchyma cells filled with starch grains of *Filfil Siyāh*

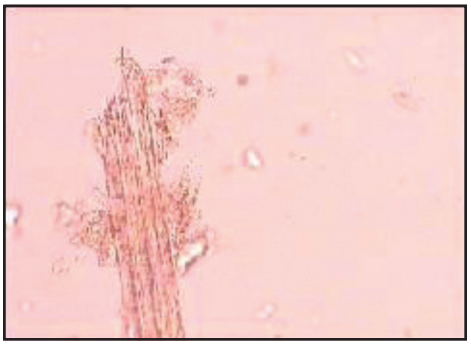


Fig. 7: x 40 group of fibres of *Halela Zard*

Fig. 8: x 40 group of sclereids of *Halela Zard*

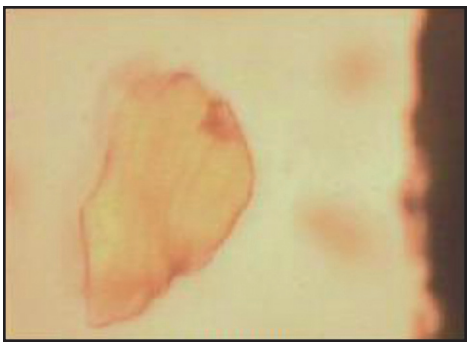


Fig. 9: x 40 sclerotic cells of *Balela*

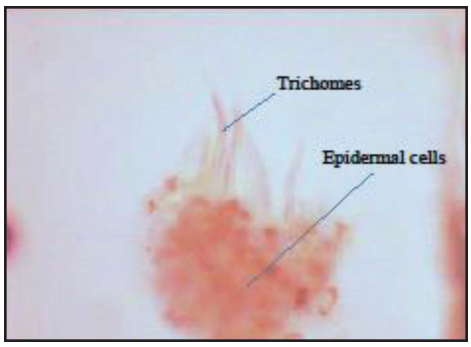


Fig. 10: x 40 fragments of epidermis with trichomes in *Balela*

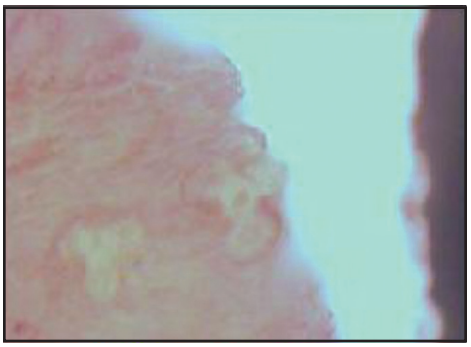


Fig. 11: x 40 group of Stone cells of *Filfil Darāz*

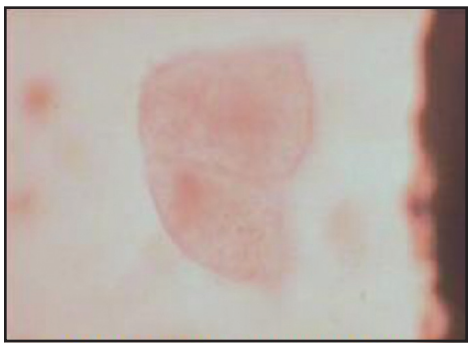


Fig. 12: x 40 group of pitted sclereids of *Āmla*

Pharmacopoeial Standards

Pharmacopoeial parameters, e.g. total ash, acid insoluble ash, alcohol and water soluble extractives, pH values (1 and 10%), loss in weight on drying at 105^0C along with the detection of aflatoxin, microbial load, pesticide residue and heavy metals were carried out as per the method described in WHO guidelines (Anonymous, 1998). The study of organic constituents present in the drug was conducted as per the methods mentioned by Trease and Evans (1983). For the chromatographic estimations, data of Rf values in HPTLC profile is reported for separately prepared chloroform and ethanol extracts whereas successive extracts of pet ether, chloroform and ethanol were prepared by using Soxh-let apparatus and subjected to find out their characteristic pattern of chromatograph in different solvent systems following the Stahl (1969) and Harborne (1973) method on percolated Silica gel 60 F264 TLC plates as given in Table 6 (Wagner & Bladt, 1984).

Table 2: Organoleptic characters

Appearance	Pill
Colour	Black
Smell	Characteristic odour
Taste	Bitter

Table 3: Qualitative analysis of phyto-constituents

Chemical Constituents	Test / Reagent	Result
Glycoside	Liebermann test	+ve
Sterol	Salkowski reaction	
Liebermann-Burchard's test	+ve	
Phenolics	Phenol	+ve
Tannins	Ferric chloride test	+ve
Resins	Acetic anhydride test	+ve
Amino acids	Ninhydrin solution	+ve
Saponin	Sodium bi-carbonate solution	+ve
Protein	Xanthoproteic test	
Biuret's test	+ve	
Flavonoids	Magnesium chips test	+ve

(-ve) represents absence and (+ve) presence of constituent

Table 4: Physico-chemical constants

S. No.	Parameters	Results (%)			
		Sample I	Sample II	Sample III	Mean + SD
1	Alcohol soluble extractive	6.82 - 6.99	6.88 - 6.93	6.56 - 6.89	6.83 + 0.07
2	Water soluble extractive	49.29 - 49.65	49.35 - 50.09	50.03-50.61	50.15 + 0.15
3	Hexane soluble extractive	2.00 - 2.15	1.80 – 1.90	1.70 – 1.80	1.89 + 0.16
4	Total ash (%)	5.55 - 5.60	5.65 - 5.70	5.65 - 5.75	5.67 + 0.08
5	Acid insoluble ash (%)	1.85 - 1.94	1.85 - 1.90	1.80 - 1.90	1.88 +0.25
6	Water soluble ash (%)	1.38 - 1.40	1.50 – 1.52	1.40 – 1.42	1.43 +0.01
7	Loss in wt. on drying at 105^0C	7.90 - 8.25	7.75 – 8.00	7.40 – 7.90	7.80 +0.58
8	Bulk density (gm/ml)	1.289	1.287	1.291	1.256 + 0.06
9	pH of 1% aqueous soln.	5.10-5.20	5.27 - 5.35	5.30 - 5.40	5.32 + 0.11
10	pH of 10% aqueous soln.	4.48 - 4.50	4.47 - 4.52	4.45 - 4.50	4.52 + 0.03
11	Successive extractives a. Pet ether $(60\text{-}80)^O$ b. Chloroform c. Ethanol	 3.50 7.50 10.50	 3.25 7.00 10.00	 4.00 7.25 10.80	 3.58+0.34 7.03 + 0.30 10.43 + 0.34

Values are expressed as mean % + SD except for pH values which are expressed as mean + SD

Table 5: Estimation of phyto-constituents

S. No.	Phyto-constituents	Results (%) (Mean Values)		
		Sample I	Sample II	Sample III
1.	Total phenolics	4.41	3.41	3.36
2.	Tannins	0.33	0.48	0.44
3.	Resins	5.46	6.10	5.63
4.	Proteins	7.14	6.04	6.53

Table 6: Chromatographic evaluation

S. No.	Extract	Solvent System	Spray/Treatment	No of Spots	Rf Values
1	Pet ether	Benzene: Chloroform (8 : 2)	2% Ethanolic sulphuric acid and heated at 105^0C	3	0.21,0.45 (brown), 0.52 (violet)
2.	Chloroform	Pure chloroform	2% Ethanolic sulphuric acid and heated at 105^0C	3	0.45 (purple), 0.55, 0.65 (brown)
3.	Ethanol	Benzene : ethanol : acetic acid (3 :1: 0.5)	5% Ethanolic sulphuric acid and heated at 105^0C	4	0.38 (light green), 0.50 (Purple) 0.69, 0.92 (violet)

Quality control parameters

In quality control analysis, various parameters including heavy metals, pesticide residue, aflatoxin and microbial load were conducted. Heavy metals analysis and pesticide residue were carried out by following the method of AOAC (2005) while the microbial load and aflatoxin were performed as per the WHO guidelines (WHO, 2007). Results are given in Table 7, 8, 9 and 10.

Table 7: Quantitative estimations of heavy metals

S. No.	Name of element analysed	WHO & FDA limits of detection	Results
1.	Mercury	1 ppm	Below detection value
2.	Lead	10 ppm	Below detection value
3.	Cadmium	0.3 ppm	Below detection value
4.	Arsenic	3 ppm	Below detection value

Table 8: Analysis of microbial load

S. No.	Parameter analysed	Result	WHO limit
1.	Total bacterial count	Less than 1CFU/g	10^5 CFU/g
2.	Total fungal count	Less than 1CFU/g	10^3 CFU/g
3.	Enterobacteriaceae	Absent	10^3 CFU/g
4.	Salmonella	Absent	Nil
5.	Staphylococcus aureus	Absent	Nil
6.	Escherichia coli	Absent	Nil

Table 9: Estimations of aflatoxin

S. No.	Aflatoxin	Result	WHO limit (ppm)
1.	B1	Not detected	0.5
2.	B2	Not detected	0.1
3.	G1	Not detected	0.5
4.	G2	Not detected	0.1

Table 10: Analysis of pesticide residues

S. No.	Pesticide Residues	Result	Limit
1	Alachlor	Not detected	LOQ : 0.01
2	Aldrin	Not detected	LOQ : 0.01
3	Azinphos–methyl	Not detected	LOQ : 0.01
4	Chlordane (cis & trans)	Not detected	LOQ : 0.01
5	Chlorfenvinphos	Not detected	LOQ : 0.01
6	Chlorpyrifos	Not detected	0.071
7	Chlorpyrifos-methyl	Not detected	LOQ : 0.01
8	Cypermethrin	Not detected	LOQ : 0.01
9	DDT	Not detected	LOQ : 0.01
10	Deltamethrin	Not detected	LOQ : 0.01
11	Diazinon	Not detected	LOQ : 0.01
12	Dichlorvos	Not detected	LOQ : 0.01
13	Dimethoate	Not detected	LOQ : 0.01
14	Dieldrin	Not detected	LOQ : 0.01
15	Endosulphan	Not detected	LOQ : 0.01

16	Endrin	Not detected	LOQ : 0.01
17	Ethion	Not detected	LOQ : 0.01
18	Fenitrothion	Not detected	LOQ : 0.01
19	Fenvalerate	Not detected	LOQ : 0.01
20	Heptachlor	Not detected	LOQ : 0.01
21	Hexacholobenzene	Not detected	LOQ : 0.01
22	Lindane (gamma HCH)	Not detected	LOQ : 0.01
23	Malathion	Not detected	LOQ : 0.01
24	Parathion	Not detected	LOQ : 0.01
25	Parathion-methyl	Not detected	LOQ : 0.01
26	Permethrin	Not detected	LOQ : 0.01
27	Phosalone	Not detected	LOQ : 0.01
28	Primiphos methyl	Not detected	LOQ : 0.01

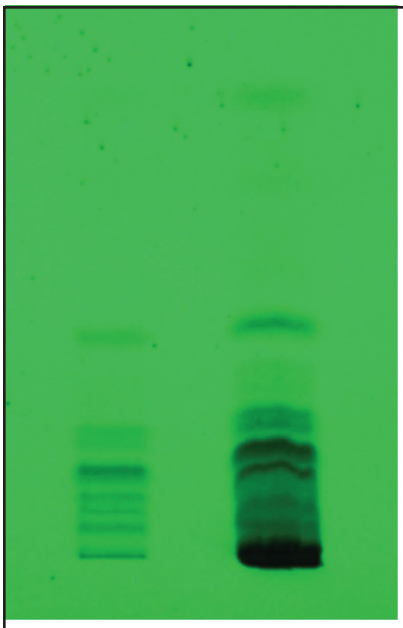
LOQ = Limit of quantification

HPTLC profile

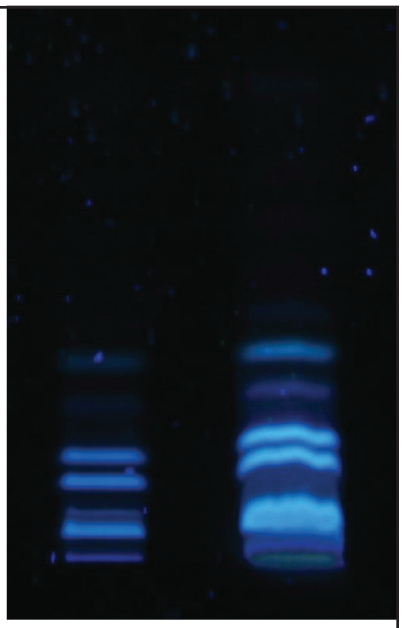
The drug in the quantity of 2 g was extracted separately with 50 ml of chloroform and ethanol for 30 minutes and filtered. The filtrates were concentrated and made up to 10 ml in a volumetric flask separately. These solutions were used for HPTLC finger print analysis by Desaga HPTLC apparatus using pre-coated silica gel aluminium plate. The chromatograms were developed using solvent system toluene: ethyl acetate (9:1). The plate was dried at room temperature and examined under UV 254 nm and under UV 366 nm. Further, the plate was dipped in 1% vanillin-sulphuric acid and heated at 105^{O}C for 10 minutes till colour of the spots appeared without charring (Fig. 13 (A–C)).

The chromatogram of the chloroform extract of the drug shows 6 spots under UV 254nm at R_f0.15 (dark green), 0.05, 0.08, 0.11, 0.29, 0.40 (all green), 4 spots under UV 366nm at R_f 0.06, 0.14, 0.19 (all fluorescent blue) and 0.35 (blue) and 9 spots after derivatization at R_f 0.10 (brownish black), 0.14, 0.19 (light pink), 0.25 (dark pink), 0.39 (yellowish pink), 0.49 (light purplish pink), 0.60 (brownish pink), 0.75 (very light purple), 0.88 (pinkish purple).

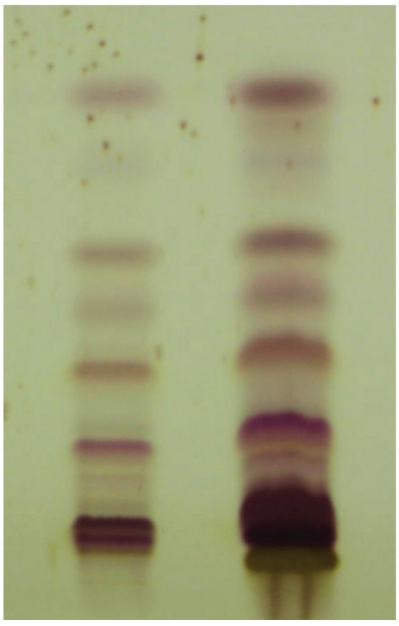
The chromatogram of the ethanol extract of the drug shows 6 spots under UV 254nm at R_f0.06, 0.10, 0.26, 0.43 (all green), 0.16 and 0.20 (both dark green), 4 spots under UV 366nm at R_f 0.30 (blue), 0.08, 0.19, 0.23, 0.30 (all fluorescent blue) and 7 spots after derivatization at R_f 0.19, 0.51 (pinkish brown), 0.26 (pinkish dark purple), 0.40 (purplish brown), 0.59 (brownish purple), 0.73 (light purple), 0.86 (pinkish purple).



Chloroform ext. Ethanol ext.
UV 254nm



Chloroform ext. Ethanol ext.
UV 366nm



Chloroform ext. Ethanol ext.
After Derivatization

Fig. 13 (A–C)

Discussion

Phytochemical screening of the chemical constituents present in the drug revealed that it contains glycosides, steroids, phenolic, tannins, resins, proteins, saponins, flavonoids and amino acids (Overtone, 1963).

These chemical compounds may be responsible for the therapeutic efficacy of that drug (Table 3). In quantitative estimation for physico-chemical parameters, three experiments were conducted for each sample and their % range & mean values were reported (Table 4). These physico-chemical parameters are considered as tools of checking the quality, identity, purity and strength of Unani drugs. The solubility and extractive values sometimes are used as an index of purity for formulations. Therefore, for establishing the standards of any drug, these extractive values and solubility play an important role as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jerkins *et al*., 1967). The loss of weight on drying on heating tells us about the moisture contents of the test drug, which determines the release of active ingredients as well as their chemical, physical, microbial, shelf-life properties and adulterations. Organoleptic characteristics of the drug indicate that it is black in color with characteristic odor, sticky in nature and bitter in taste (Table 2). The pH values of all three samples were found to be acidic. Estimation of ash values is an important criterion for judging the impurities along with identity and quality of drugs because adulteration of herbal drugs with unwanted materials like earthy matters results in higher ash percentage. Bulk density is also one of the important parameters which can be used as a quality control measure to check the uniformity of bulk chemicals and in selecting the paper size of container and packing materials (Subrahmannyam, 2009). The study was extended for quantitative estimation of phyto-constituents, e.g. phenolics, tannins, resins and proteins as presented % mean values of all the three samples in Table 5.

In chromatographic studies, HPTLC of separately prepared chloroform and ethanolic extracts was conducted while successive extracts of petroleum ether, chloroform and ethanol were utilized to carry out thin layer chromatography by using different solvent systems and their Rf values were reported (Table 6). In quality control parameters, the bacterial load and total fungal count of microbial studies, aflatoxin, heavy metals and pesticide residues were either within the permissible limits or found to be absent as shown in Table 7, 8, 9 & 10 respectively. At the same time, bacteria Enterobacteriaceae, Salmonella spp, Escherichia coli, Staphylococcus aureus were found to be absent which shows that the drug is not the carrier of these organisms.

Conclusion

In this study, an attempt has been made to establish the scientific basis of the formulation. The result of phyto-chemical screening, physico-chemical, microscopic studies and other parameters in the present investigation such as aflatoxins, heavy metal analysis, microbial load and pesticide residues contamination will serve not only to assess the quality of the drug but will reflect

the purity of ingredients used in the drug as well. Thin layer chromatography profile is another significant tool to identify and assess the presence of an active and other phyto-constituents in the drug while HPTLC fingerprint profile of chloroform and ethanol extracts provide a suitable method for monitoring the identity and purity of the drug. In other words, it can be concluded that the standardisation of the drug is essential for their proper utilisation due to the fact that any adulterant in these drugs will not only reduce the efficacy of the drugs but sometimes may even lead to adverse effects.

Acknowledgement

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सारांश

एंटी–इन्फ्लामेटरी पॉली–हर्बल मिश्रण हब्ब–ए–हिन्दी मोहल्लिल का भौतिक–रासायनिक और फार्माकोग्नॉस्टिक मूल्यांकन

अख़लाक मुस्तफ़ा*, फ़राह अहमद, किरन नेगी, असमा सत्तार ख़ान, अनस इक़बाल अल्वी, महेश चन्द्र, मुस्तेहसन, ज़की अहमद सिद्दीक़ी और आर. पी. मीना

सारांश

औषध उद्योग को सुदृढ़ स्थिति में लाने के लिए आज औषधियों का मानकीकरण बहुत महत्वपूर्ण है। यूनानी चिकित्सा में *सफ़ूफ़, हुबूब, माजून, कुश्ता, मुरबबा* और *मरहम* आदि विभिन्न प्रकार के बहुऔषधीय मिश्रण होते हैं। हब्ब–ए–हिन्दी मोहल्लिल एक एंटी–इन्फ्लामेटरी मिश्रण है जिसका उपयोग मुख्य रूप से सर्वाइकल एडेनाइटिस का उपचार करने के लिए किया जाता है जो आजकल वैश्विक स्तर पर एक सार्वजनिक स्वास्थ्य समस्या है। यह मिश्रण मुख्य रूप से औषधीय पौधों के 13 अवयवों को मिलाकर तैयार किया जाता है। सुरक्षा, प्रभावकारिता और पुनरुत्पादनीयता का आश्वासन सुनिश्चित करने के लिए प्रत्येक हर्बल उत्पाद की पहचान, प्रमाणीकरण और रासायनिक मानकीकरण अनिवार्य है। वर्तमान अध्ययन विभिन्न भेषजकोशीय मापदंडों जैसे सैलियन्ट ऑर्गेलेप्टिक और भेषजकोशीय वर्ण, एल्कोहल और पानी में घुलनशील सत्त, पीएच मात्रा, राख मात्रा, गुणात्मक और मात्रात्मक कार्बनिक घटकों की पहचान, क्रमिक सत्त का थिन लेयर क्रोमैटोग्राफिक अध्ययन, इत्यादि के आधार पर औषधि के तीन अलग–अलग बैंचों के भौतिक–रासायनिक और औषधीय मानकीकरण से संबंधित है। माइक्रोबियल लोड, कीटनाशक अवशेष, एफ्लाटॉक्सिन, भारी धातु आकलन और एचपीटीएलसी सहित विषाक्त स्कैनिंग भी की गई जोकि फार्माकोपियल मानक तय करने औषधि की तैयारी के एसओपी निर्धारित करने में मददगार साबित हो सकती है।

शब्दकुंजी: हर्बल औषधि, भौतिक–रासायनिक मानक, पॉली–हर्बल मिश्रण, एसओपी, सोक्स्लेशन

Therapeutic Intervention of Unani Pharmacopoeial Formulations in the Management of *Surʿat-i-Inzāl* (premature ejaculation)

[1]Mohd Tariq Khan,
[1]Mohd Naseem,
[2]Nighat Anjum,
[2]Naheed Parveen,
and
[2]Asim Ali Khan

[1]Clinical Research Unit – Meerut, Central Council for Research in Unani Medicine, New Delhi

[2]Central Council for Research in Unani Medicine, New Delhi

Abstract

Introduction: *Surʿat-i-Inzāl* (premature ejaculation) is the most common type of sexual dysfunction in men younger than 40 years of age wherein a man ejaculates sooner during sexual intercourse than he or his partner would like.

Objective: To assess the safety and efficacy of Unani pharmacopoeial formulations *Safūf Mughalliẓ-ī-Manī, Maʿjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā'* in the management of *Surʿat-i-Inzāl* (premature ejaculation).

Design: An open-label clinical study was carried out on 155 patients for 2 weeks.

Result: The mean PEDT score decreased as compared to the baseline findings. After treatment, variation in all haematological and biochemical parameters was found within the normal range. No adverse effects were reported in the patients. The test drugs were found to be effective and safe and demonstrated a good response in premature ejaculation.

Conclusion: *Safūf Mughalliẓ-ī-Manī, Maʿjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā'* are very effective and safe in the management of *Surʿat-i-Inzāl* (premature ejaculation).

Keywords: *Mughalliẓ-ī-Manī*, premature ejaculation, Unani, *Surʿat-i-Inzāl*

Introduction

Surʿat-i-Inzāl (premature ejaculation/rapid ejaculation) is the most common type of sexual dysfunction in men younger than 40 years of age. Most professionals treating premature ejaculation define this condition as the occurrence of ejaculation prior to the wishes of both sexual partners (Laumann *et al.*, 2005). According to Waldinger *et al.*, men with an IELT (Intra vaginal ejaculatory latency time) of less than 1 minute (belonging to 0.5 percentile) have 'definite' premature ejaculation, while men with IELTs between 1 and 1.5 minutes (between 0.5 and 2.5 percentile) have 'probable' premature ejaculation (Waldinger *et al.*, 2005). In addition, another grading of severity of premature ejaculation should be defined in terms of associated psychological problems. Thus, both definite and probable premature ejaculations needs further psychological sub-classification in non-symptomatic, mild, moderate, and severe premature ejaculation.

*Corresponding author; .....

According to classical literature of Unani Medicine, reproductive functions related to the generation of *Manī* or semen (sperm and ovum) depend on *Quwwat Tanāsuliyya* (reproductive faculty). However, *Quwwat-i-Bāh* (faculty of sexual potency and libido) governs sexual functions and carries the meaning of virility, lust, venereal passion and generative poser (Kantoori, 1930). This faculty namely *Quwwat-i-Bāh* depends upon the overall health and normal functioning of four vital organs, viz. brain, heart, liver and testes. Any pathological condition affecting any of these organs adversely results in *Surʻat-i-Inzāl* (premature ejaculation) (Kantoori, 1879; Kabiruddin, 1926). According to renowned Unani scholar Hakim Ajmal Khan, *Surʻat-i-Inzāl* (premature ejaculation) when accompanied by *Ḍuʻf-i-Bāh* (sexual debility) causes embarrassment and distress in the relationship (Ishtiyaque, 1980; Hameed, 1932).

Surʻat-i-Inzāl (premature ejaculation) may be primary or secondary. Primary premature ejaculation applies to individuals who have had the condition since they became capable of functioning sexually (i.e. post puberty). Secondary premature ejaculation means the condition in which an individual previously experienced an acceptable level of ejaculatory control, and then for unknown reasons, he experienced premature ejaculation. Secondary premature ejaculation does not relate to a general medical disorder and is usually not related to substance inducement, although, rarely, hyper excitability might relate to a psychotropic drug and resolves when the drug is withdrawn (Godpodinoff, 1989; Cihan *et al.*, 2009; Vignozzi *et al.,* 2005).

Testosterone is thought to play a role in the ejaculatory reflex. Higher testosterone (free and total) levels have been demonstrated in men with premature ejaculation than in men without premature ejaculation (Vignozzi *et al.*, 2005). Semen from men with premature ejaculation contains significantly less acid phosphates and alpha-glycosidase than the semen of controls. A research concluded that these biochemical parameters may reflect dysfunction of the prostate and epididymis, possibly contributing to premature ejaculation. In other biochemical parameters like prolactin hormone, it was found to be decreased in quantity in many patients with premature ejaculation (Waldinger, 2007). Biochemical markers such as prolactin may contribute to premature ejaculation, organic and psychological associations (i.e. anxiety) suggest that biochemical parameters play only a partial role in premature ejaculation. There are some other factors that have been found to contribute greatly to premature ejaculation beyond merely the time to ejaculation. While patients with premature ejaculation show significantly lower intra-vaginal ejaculatory latency time (IELT), the IELT in those who fit the criteria for premature ejaculation overlaps with the IELT in patients who do not fit the criteria. However, while a shorter IELT has been the measure of premature ejaculation in many studies, the perception of ejaculation control has been shown to mediate patient and/or partner satisfaction with sexual intercourse

and ejaculation-related distress. While premature ejaculation is most likely not a purely psychological disorder, such associations demonstrate a significant psychological role in the disorder (Peeters & Giuliano, 2008).

In recent years, great emphasis has been laid on the utilization of natural agents in the management of various diseases due to the harmful effects posed by synthetic drugs. There are several drugs in allopathic medicine to treat premature ejaculation but various side effects consistent with these drugs often render treatment difficult; so the demand of new types of medicine emerged. Ancient Unani physicians devised a complete management plan and successfully treated *Surʻat-i-Inzāl* or premature ejaculation using natural agents. This centuries-old natural way of treatment must be explored further to provide an alternative and better treatment for premature ejaculation (Khan, 1883; Khan, 1895).

Therefore, the present study was designed to evaluate the efficacy of Unani pharmacopoeial formulations *Safūf Mughalliẓ-ī-Manī, Maʻjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā'* and to validate its use in the management of premature ejaculation.

Material and Methods

The present open-label clinical study was carried out at the Clinical Research Unit (Unani), Meerut, Uttar Pradesh after obtaining the approval from the Institutional Ethical Committee of Clinical Research Unit (Unani), Meerut. The study drugs were prepared at Central Research Institute of Unani Medicine, Hyderabad as per the formulation of the drugs mentioned in the National Formulary of Unani Medicine (NFUM). *Safūf Mughalliẓ-ī-Manī* (7 g twice daily), *Maʻjūn Ārad-i-Khurmā* (10 g twice daily) and *Ḥabb Iksīr-i-Shifā'* (1 tablet once daily) were given to the subjects for 2 weeks.

The study was carried out on affected men between 18 and 55 years of age with an IELT (intravaginal ejaculatory latency time) of less the 1 minute and persistent or recurrent ejaculation with minimal sexual stimulation, before, on, or shortly after penetration. Patients with erectile dysfunction and diseases requiring long-term treatment were excluded from the study. The patients were assessed on baseline, day 7 and day 14 and the efficacy of the test drugs was evaluated on the basis of improvement and worsening according to the premature ejaculation diagnostic tool (PEDT) score.

The safety of the drugs was monitored on the basis of the laboratory investigations like CBC (Hb%, TLC, DLC, ESR), LFT (S. Bilirubin, SGOT, SGPT, S. Alkaline Phosphates), KFT (Blood Urea, S. Creatinine, Uric Acid) and urine for routine and microscopic examination done at the baseline and at the end of the study. The safety of the drug was also assessed clinically on the basis of adverse events as reported by the patients or observed clinically during follow-up.

Clinical subjective parameters, haematological and biochemical parameters were statistically analyzed by using the student paired t-test. The results were expressed as the Mean ± SEM. P<0.05 has been considered as statistically significant and P<0.001 has been considered statistically highly significant.

Results and Discussion

The present open-label clinical study was carried out at the Clinical Research Unit (Unani), Meerut. The clinical trial was conducted to evaluate the efficacy of Unani formulations *Safūf Mughalliẓ-ī-Manī, Maʻjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā'* in patients of *Surʻat-i-Inzāl* attending the general outpatient department (GOPD). Out of 155 registered cases, 24 cases did not complete the study (drop out) while the remaining 131 selected cases of *Surʻat-i-Inzāl* were treated with the selected Unani pharmacopoeial formulations.

Demographic interpretation

Age group

The patients were divided into four age groups - 18–25, 26–35, 36-45 and 46–55 years and the highest incidence of *Surʻat-i-Inzāl* i.e. 40.65% was observed in the age group of 36–45 years followed by 37.42% in 26–35, 12.25% in 46–55 years and the least 9.68% in 18–25 years of age as depicted in Table 1 and Figure 1. As per Unani literature the problem is greater in *Sinn-i-Shabāb* and present study report is going hand in hand with the literature.

Table 1: *Surʻat-i-Inzāl* cases according to age groups

Sex & Age	18–25 years (A)	26–35 years (B)	36–45 years (C)	46–55 years (D)	Total
Male	15 (9.68%)	58 (37.42%)	63 (40.65%)	19 (12.25%)	155 (100%)

Dietary habits

90% of the patients enrolled in the study were non-vegetarian and this is due to the catchment area of the hospital (Table 2 and Figure 2)

Table 2: *Surʻat-i-Inzāl* cases according to dietary habits

Dietary habits	Male
Vegetarian	15 (10%)
Non-vegetarian	140 (90%)
Total	**155** (100%)

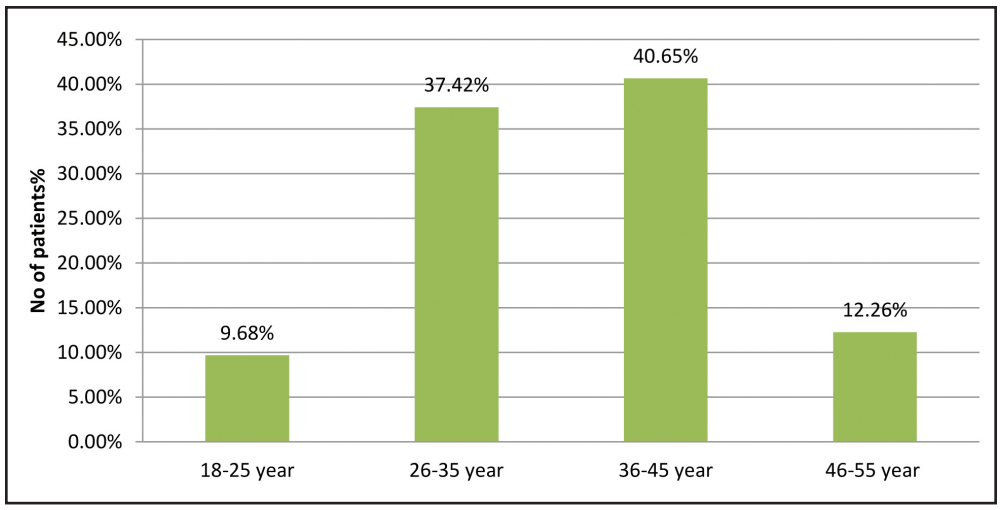

Figure 1: *Surʻat-i-Inzāl* cases according to age groups

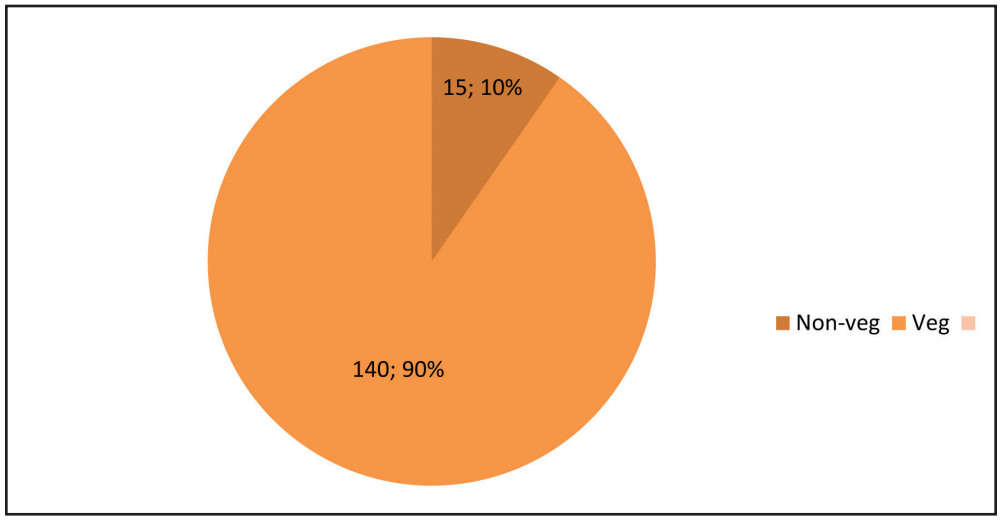

Figure 2: *Surʻat-i-Inzāl* cases according to dietary habits

Socio-economic status

As per the Kuppuswamy scale, maximum number of patients (51.61%) in the study belonged to middle income group, while 47.74% patients were from low income group as shown in Table 3 and Figure 3.

Table 3: *Surʻat-i-Inzāl* cases according to social status

Social status	No. of registered cases	Percentage
Low income group	74	47.74%
Middle income group	80	51.61%
High income group	01	0.64%
Total	**155**	**100%**

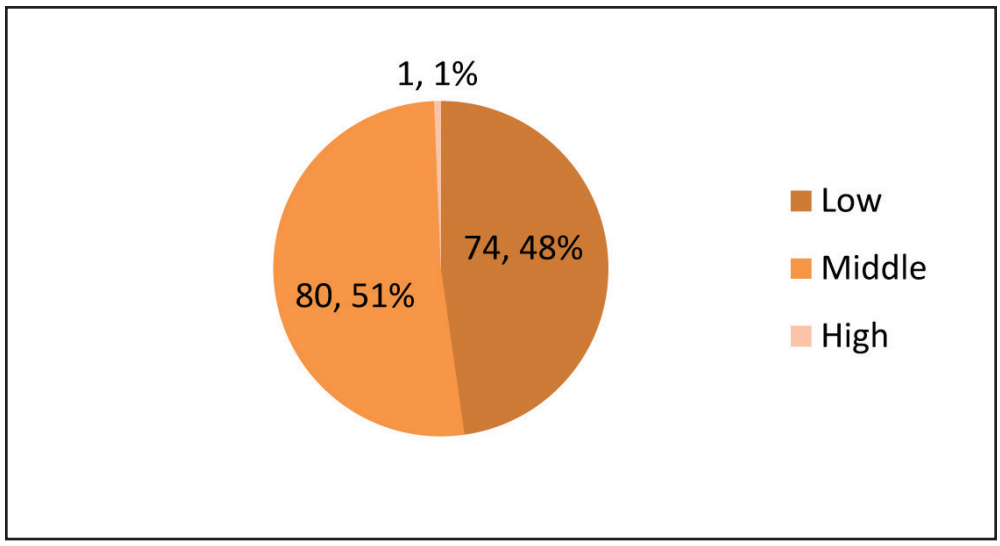

Figure 3: *Surʿat-i-Inzāl* cases according to social status

Mizāj of patients

Out of 155 patients, 75 (48.38%) had *Balghamī* (phlegmatic) *Mizāj*, 69 (44.51%) had *Ṣafrāwī* (bilious) *Mizāj*, 08 (5.16%) had *Damwī* (melancholic) and 03 (1.93%) had *Sawdāwī* (sanguine) *Mizāj* as shown in Table 4 and Figure 4.

Table 4: *Surʿat-i-Inzāl* cases according to temperament

Temperament observed	Number of cases	Percentage %
Balghamī (Phlegmatic)	75	48.38%
Ṣafrāwī (Bilious)	69	44.51%
Sawdāwī (Melancholic)	03	1.93%
Damwī (Sanguine)	08	5.16%
Total	**155**	**100%**

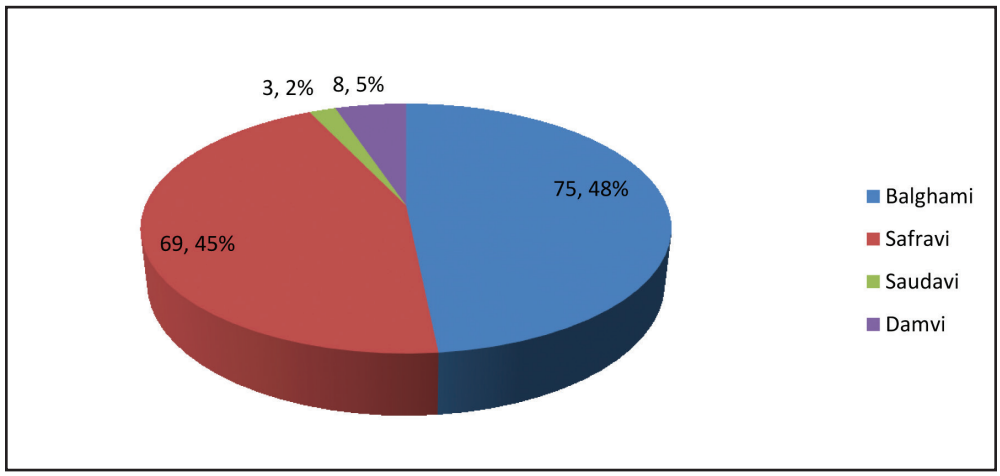

Figure 4: *Surʿat-i-Inzāl* cases according to temperament

Safety evaluation

For evaluation of the safety of the test drugs, all the patients were assessed on haematological and biochemical parameters on baseline and day 14.

Haematological and biochemical parameters

All haematological and biochemical parameters remained normal throughout the treatment and it was concluded that the test drugs are safe and don't cause any alteration in the safety parameters. While evaluating statistically, p-value was not significant in the parameters evaluated under Haemogram (Hemoglobin, TLC, DLC and ESR) and kidney as shown in (Table 5–6 and Figure 5–6)

Table 5: Variation in haematological parameters of *Surʿat-i-Inzāl* patients

Laboratory tests		Mean ±S.E.M.	Percentage(%) increase or decrease		t- value	p-value
Hemoglobin (gm/dl)	BT	11.053±0.101	2.83	increase	1.954	p >0.05
	AT	10.740±0.099				
ESR (mm/hr)	BT	28.446±0.490	2.053	increase	0.2007	p >0.05
	AT	27.862±0.385				
Total leukocyte count (1000/cu.mm)	BT	8012.7±77.159	6.68	increase	1.157	p >0.05
	AT	7477.3±71.159				
Polymorphs (%)	BT	61.546±0.488	-0.788	decrease	0.4945	p >0.05
	AT	62.031±0.370				
Lymphocytes (%)	BT	32.792±0.462	-0.23	decrease	0.8314	p >0.05
	AT	32.869±0.407				
Monocytes (%)	BT	1.685±0.126	4.569	increase	0.6991	p >0.05
	AT	1.608±0.123				
Eosinophils (%)	BT	3.538±0.208	0.847	increase	0.146	p >0.05
	AT	3.508±0.176				

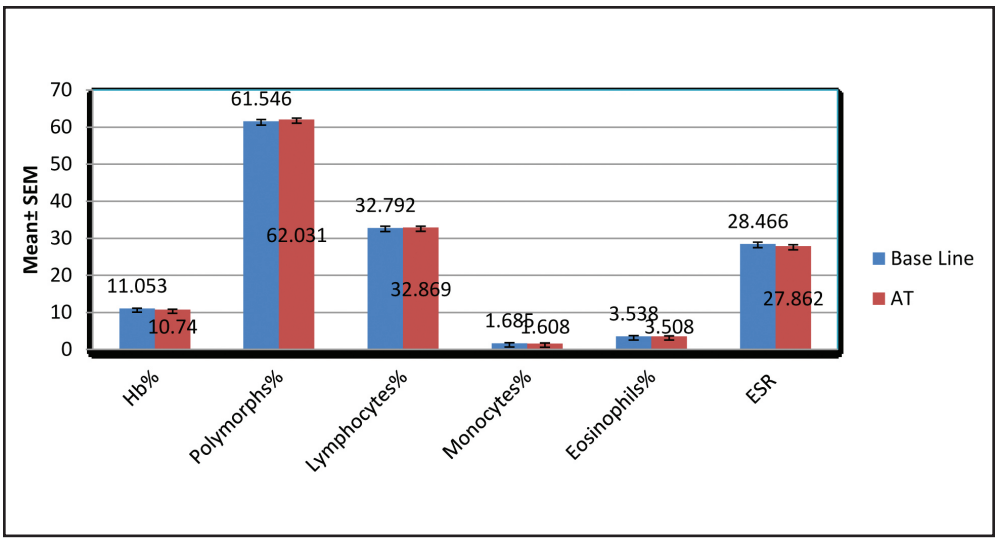

Figure 5: Variation in haematological parameters of *Surʿat-i-Inzāl* patients

Table 6: Variation in biochemical parameters of *Surʿat-i-Inzāl* patients

Laboratory tests		Mean ± S.E.M.	Percentage(%) increase or decrease		t-value	p-value
Serum bilirubin (mg/dl)	BT	0.8477±0.033	-4.171	decrease	2.106	p <0.05
	AT	0.8846±0.036				
SGOT (U/L)	BT	24.357±0.779	3.28	Increase	0.8185	p >0.05
	AT	23.577±0.797				
SGPT (U/L)	BT	32.765±1.56	14.597	Increase	1.965	p >0.05
	AT	27.862±0.99				
Alkaline phosphatase (U/L)	BT	105.39±1.368	-2.235	decrease	0.9315	p >0.05
	AT	107.80±1.587				
Serum creatinine (mg/dl)	BT	0.877±0.014	8.209	increase	0.2146	p >0.05
	AT	0.805±0.015				
Blood urea (mg/dl)	BT	26.191±0.706	-2.7833	decrease	0.9629	p >0.05
	AT	26.920±0.964				

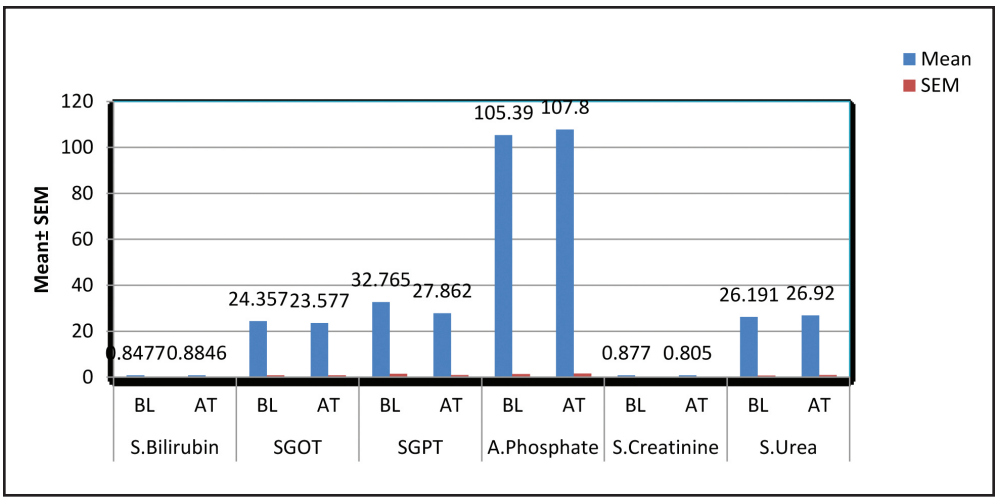

Figure 6: Variation in biochemical parameters of *Surʿat-i-Inzāl* patients

Efficacy evaluation

Clinical parameter - Premature ejaculation diagnostic tool (PEDT)

The PEDT is composed of five items (questions), grouped in a single domain:

1. How difficult is it for you to delay ejaculation?

Out of 131 patients, 26 (19.85%) had experienced extremely difficult to delay ejaculation, 101 (77.10%) experienced very difficult to delay ejaculation while 4 (3.05%) moderately difficult to delay ejaculation before starting treatment and after the treatment 45 (34.35%) patients found delaying ejaculation somewhat difficult, 51 (38.93%) felt moderately difficult to delay ejaculation and 35 (26.72%) very difficult to delay ejaculation as shown in Table 7 an Figure 7.

Table 7: PEDT question 1 evaluation

Severity	Before treatment		After treatment	
	Patient	(%)	Patient	(%)
Not difficult at all	0	0	0	0
Somewhat difficult	0	0	45	34.35%
Moderately difficult	4	3.05%	51	38.93%
Very difficult	101	77.10%	35	26.72%
Extremely difficult	26	19.85%	0	0
Total patients	131	100%	131	100%

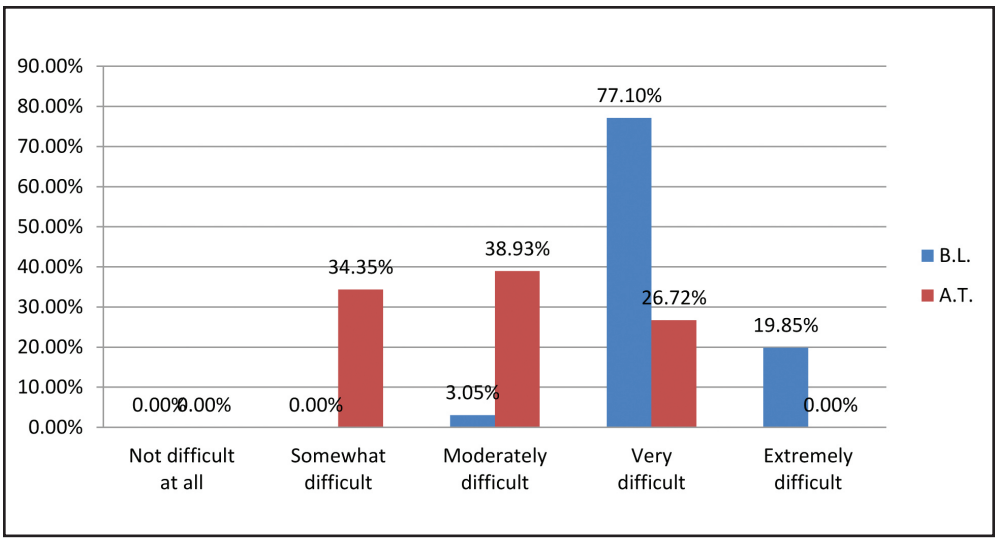

Figure 7: PEDT question 1 evaluation

2. Do you ejaculate before you wish?

Before starting treatment, out of 131 patients, 14 (10.69%%) had complaint about ejaculating 50% times before wish, 101 (77.10%) were ejaculating 75% times before wish and 16 (12.21%) were ejaculating 100% times before wish and after the treatment 4 (3.05%) patients were ejaculating as per their wish, 41 (31.03%) were ejaculating 25% times before wish, 66 (50.38%) were ejaculating 50% times before wish and 20 (15.27%) were ejaculating 75% times before wish as shown in Table 8 and Figure 8.

Table 8: PEDT question 2 evaluation

Severity	Before treatment		After treatment	
Never	0	0	4	3.05%
25% times	0	0	41	31.30%
50% times	14	10.69%	66	50.38%
75% times	101	77.10%	20	15.27%
100% times	16	12.21%	0	0
Total patients	131	100%	131	100%

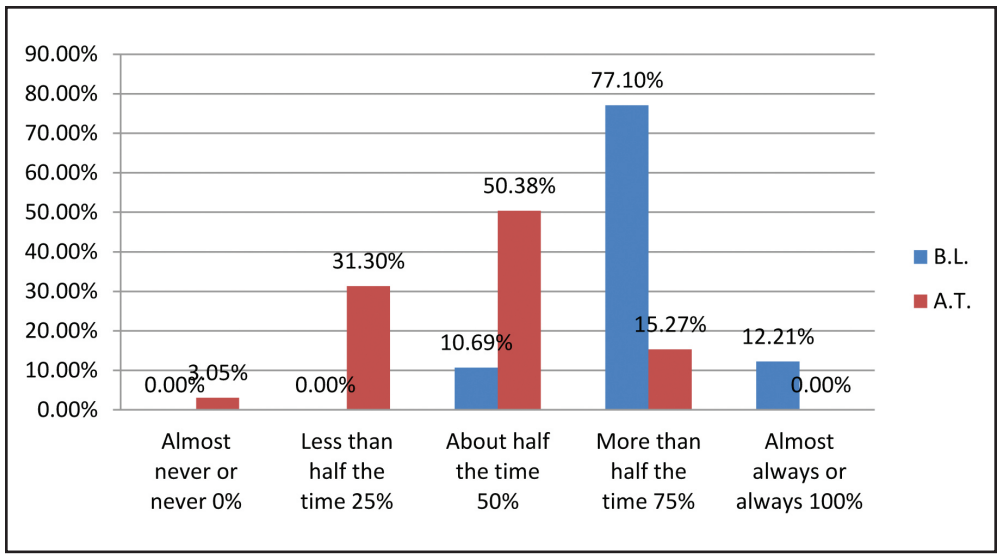

Figure 8: PEDT question 2 evaluation

3. Do you ejaculate with very little stimulation?

Before starting treatment, out of 131 patients, 04 (3.05%) patients had complaint about ejaculating with little stimulation 25% times, 31 (23.66%) were ejaculating with little stimulation 50% times, 89 (67.94%) were ejaculating with little stimulation 75% times and 7 (5.34%) were ejaculating with little stimulation 100%. After the treatment, 06 (4.58%) patients were ejaculating with high stimulation, 42 (32.06%) were ejaculating with little stimulation 25% times, 78 (59.54%) were ejaculating with little stimulation 50% times and 5 (3.82%) were ejaculating with little stimulation 75% time as shown in Table 9 and Figure 9.

Table 9: PEDT question 3 evaluation

Severity	Before treatment		After treatment	
Never	0	0	06	4.58%
25% times	04	3.05%	42	32.06%
50% times	31	23.66%	78	59.54%
75% times	89	67.94%	05	3.82%
100% times	07	5.34%	0	0
Total patients	131	100%	131	100%

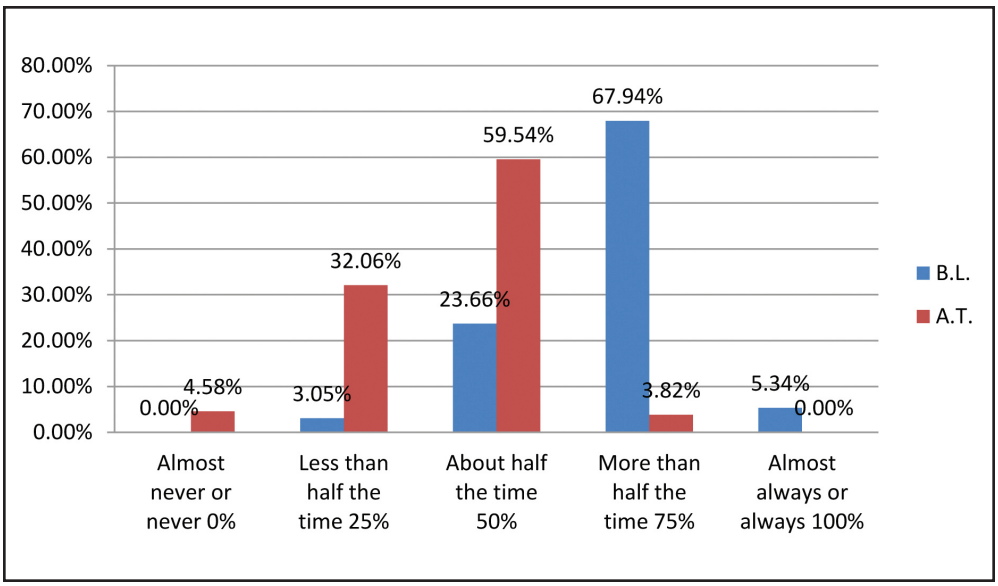

Figure 9: PEDT question 3 evaluation

4. Do you feel frustrated because of ejaculating before you want to?

Out of 131 patients, 85 (64.89%) patients felt moderately frustrated, 44 (33.59%) patients were mildly frustrated because of early ejaculation and after the treatment, 19 (14.50%) patients had no frustration, 37 (28.24%) had slight frustration, 73 (55.73%) patients had mild frustration because of early ejaculation shown in Table 10 and Figure 10.

Table 10: PEDT question 4 evaluation

Severity	Before treatment		After treatment	
Not at all	00	00	19	14.50%
Slightly	02	1.53%	37	28.24%
Mildly	44	33.59%	73	55.73%
Moderately	83	63.36%	02	1.53%
Severely	02	1.53%	00	0
Total	131	100%	131	100%

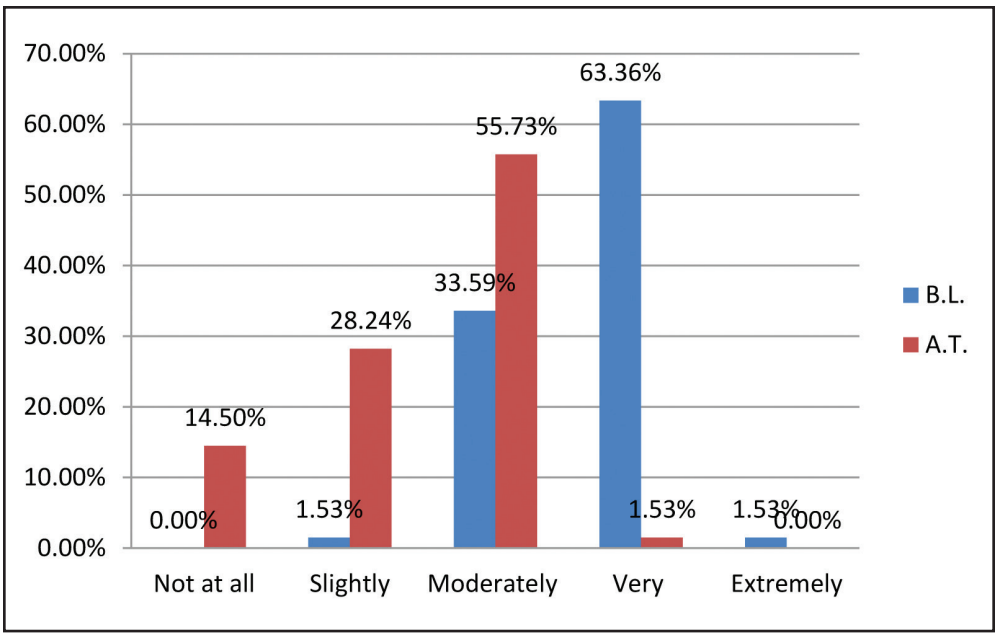

Figure 10: PEDT question 4 evaluation

5. How concerned are you that your time to ejaculation leaves your partner sexually unfulfilled?

Out of 131 patients, 82 (62.5%) patients had complaint about partner dissatisfaction while after the treatment 16 (12.21%) revealed that their partners had appreciated, 52 (39.69%) patients' partners were unsatisfied, 61(46.56%) patients' partners were mildly unsatisfied as shown in Table 11 and Figure 11.

Table 11: PEDT question 5 evaluation

Severity	Before treatment		After treatment	
Not at all	00	00	16	12.21%
Slightly	04	3.05%	52	39.69%
Mildly	45	34.35%	61	46.56%
Moderately	80	61.07%	02	1.53%
Severely	02	1.53%	0	00
Total	131	100%	131	100%

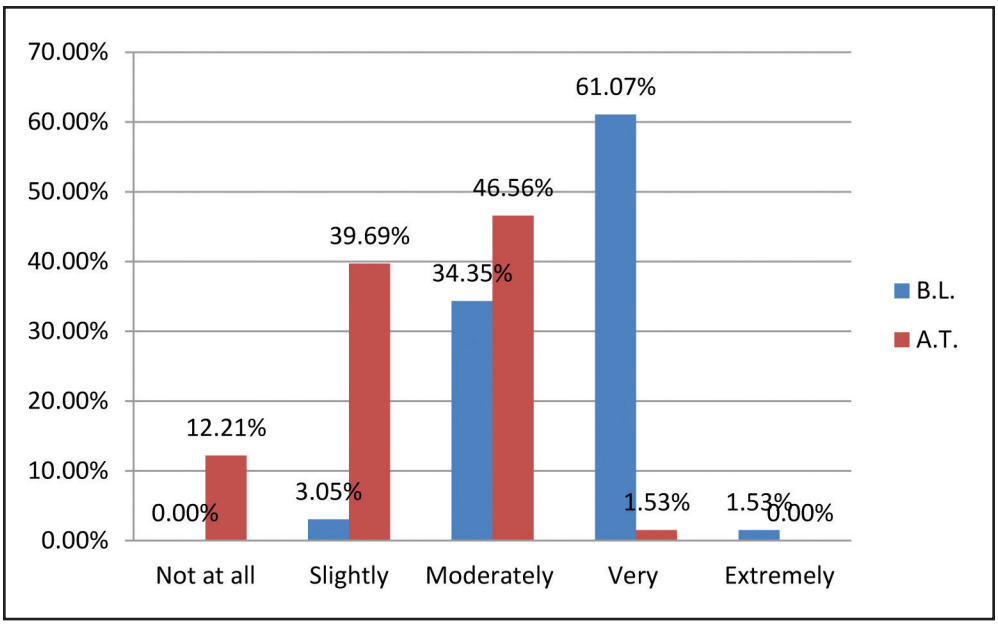

Figure 11: PEDT question 5 evaluation

In the present study, the efficacy of Unani pharmacopoeial formulations was evaluated over a period of 6 weeks on the basis of improvement in sign and symptoms. There was significant improvement reported in difficulty to delay in ejaculation (37.12%), ejaculation time before his sexual partner does (40.50%), ejaculation with very little stimulation (40.71%), feeling of frustration due to incomplete or unsatisfactory ejaculation (45.42%) and feeling of concerned when sexual partner unfulfilled due to short leaving time of ejaculation (47.38%) as shown in Table 12 an Figure 12.

Table 12: PEDT improvement

Presenting symptoms		Mean ± S.E.M	Decrease in percent (%)	t-value	p-value
How difficult is it for you to delay ejaculation?	BT	3.168±0.039	37.12↓	14.413	<0.001
	AT	1.992±0.071			
Do you ejaculate before time you wish?	BT	3.015±0.042	40.50↓	15.828	<0.001
	AT	1.794±0.065			
Do you ejaculate with very little stimulation?	BT	2.756±0.052	40.71↓	14.600	<0.001
	AT	1.634±0.057			
Do you feel frustrated because of ejaculating before you want to	BT	2.649±0.047	45.42↓	14.631	<0.001
	AT	1.450±0.067			
How are you concerned that your time to ejaculation leaves your partner sexually unfulfilled	BT	2.611±0.050	47.38↓	15.400	<0.001
	AT	1.374±0.063			

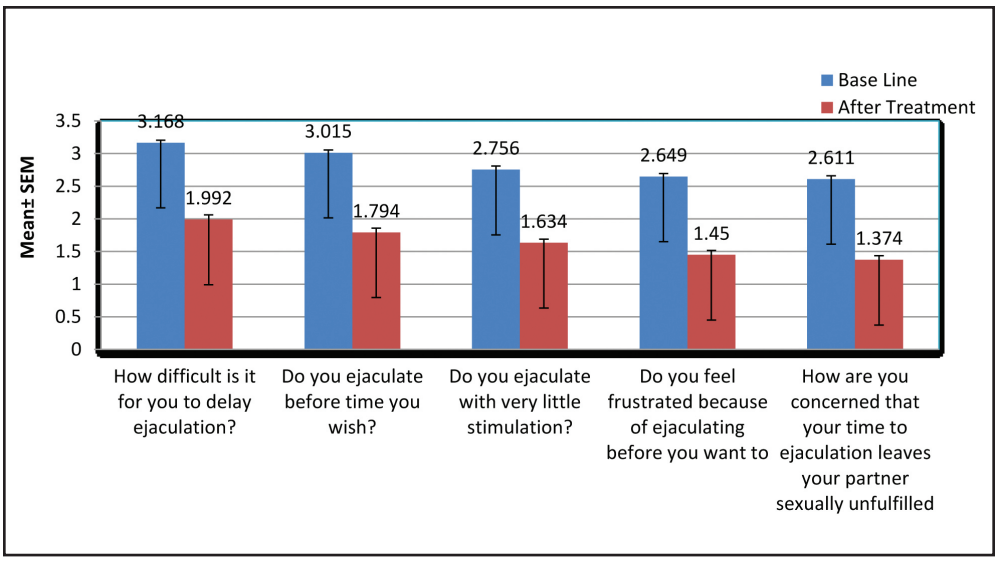

Figure 12: PEDT improvement

Assessment of PDET score

Out of 131 patients, 03 (2.29%) patients got cured, 28 (21.37%) patients got relieved, 54 (41.22%) got partially relieved and 46 (35.11%) did not get any relief as shown in Table 13 and Figure 13.

Table 13: Response of drugs in *Surʿat-i-Inzāl* according to PDET score

Response	Male	Percentage
Cured (90-100%)	3	2.29%
Relieved (60-89%)	28	21.37%
Partially relieved (30-59%)	54	41.22%
Not relieved (<30%)	46	35.11%
Total	131	100%

Discussion and Conclusion

An open-label clinical study was carried out at Clinical Research Unit (Unani), Cantonment General Hospital, Meerut, with the aim to validate the safety and efficacy of selected Unani pharmacopoeial formulations in the management of

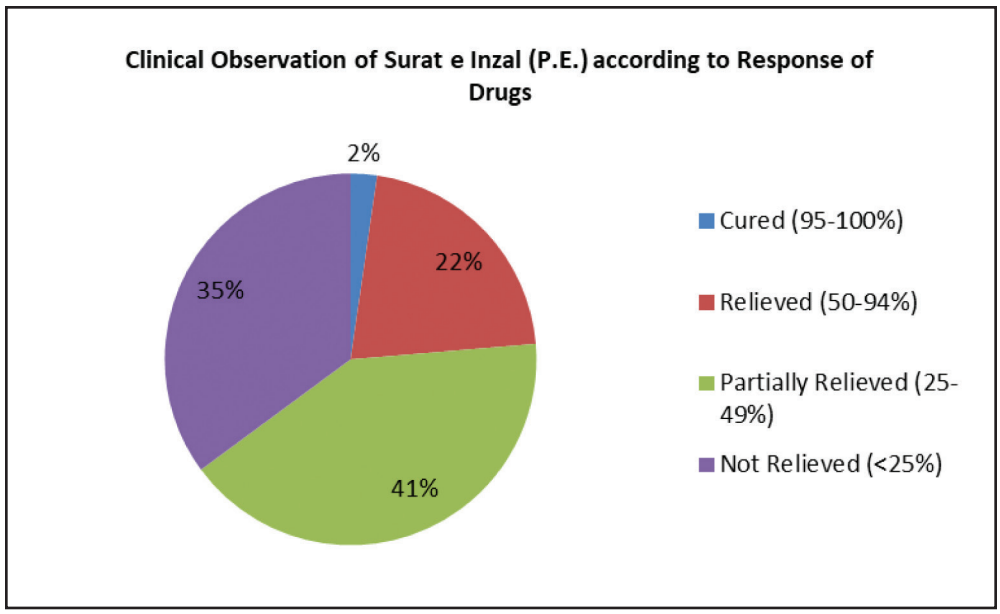

Figure 13: Response of drugs in *Surʻat-i-Inzāl* according to PDET score

Surʻat-i-Inzāl (premature ejaculation). *Safūf Mughalliẓ-ī-Manī* (7 g twice daily), *Maʻjūn Ārad-i-Khurmā* (10 g twice daily) and *Ḥabb Iksīr-i-Shifā'* (1 tablet once daily) were given to the subjects for 2 weeks. Efficacy of Unani pharmacopoeial formulations was evaluated clinically on the basis of PDET questionnaire. For the safety of the test drug, all the patients were assessed on haematological and biochemical parameters on the basalline and on day 14. The data was analyzed by using IBM® SPSS Statistics 2.0 using statistical tools that include student t-test.

The *premature ejaculation* diagnostic tool (SNAQ) used to assess the clinical efficacy of the Unani pharmacopoeial formulations showed that 23.66% got completely relieved while 41.22% had improved a lot. *Safūf Mughalliẓ-ī-Manī, Maʻjūn Ārad-i-Khurmā* and *Ḥabb Iksīr-i-Shifā'* possess pharmacological activities which are effective directly or indirectly in controlling premature ejaculation. These formulations are considered to have semen inspissant as well as *Mumsik-i-Manī* (semen retentive) property and could be the reason for its usage in *Surʻat-i-Inzāl* by prominent Unani physician since ages. Thus, the findings of the study are in conformity with the claims of ancient Unani physicians who used these drugs to treat *Surʻat-i-Inzāl* with favorable therapeutic effects.

Throughout the study, no adverse effect was reported and haematological and biochemical parameters were done at the baseline and at the end of the study remained normal which signifies that Unani pharmacopoeial formulations *Safūf Mughalliẓ-ī-Manī, Maʻjūn Ārad-i-Khurmā* and *Ḥabb Ikīsr-i-Shifā'* are safe at the given dosage schedule.

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सारांश

सुरअत–ए–इंज़ाल (शीघ्रपतन) के उपचार में यूनानी भेषजकोशीय मिश्रणों का चिकित्सीय प्रभाव

मो. तारिक़ ख़ान, मो. नसीम, निग़हत अन्जुम, नाहीद परवीन और आसिम अली ख़ान

सारांश

परिचयः *सुरअत–ए–इंज़ाल* (शीघ्रपतन) 40 साल से कम उम्र के पुरूषों में सबसे सामान्य प्रकार का यौन रोग है जिसमें पुरूष संभोग के दौरान अपनी या अपने पार्टनर की इच्छा से पूर्व स्खलित हो जाते हैं।

उद्देश्यः *सुरअत–ए–इंज़ाल* (शीघ्रपतन) के उपचार में यूनानी भेषजकोशीय मिश्रणों *सफूफ़ मुग़ल्लिज़–ए–मनी, माजून आरद–ए–खुर्मा* और *हब्ब इक्सीर–ए–शिफ़ा* की सुरक्षा और प्रभावकारिता का आकलन करना।

डिज़ाइनः 155 रोगियों पर दो सप्ताह तक ओपन लेबल पर नैदानिक अध्ययन किया गया।

परिणामः आधाररेखा निष्कर्षों की तुलना में औसत पीईडीटी स्कोर में कमी आई। उपचार के बाद सभी रुधिर विज्ञान और जैव रासायनिक मापदंडों में भिन्नता सामान्य सीमा के भीतर पाई गई। रोगियों में किसी प्रकार का प्रतिकूल प्रभाव नहीं देखा गया। परीक्षण औषधियों को प्रभावशाली और सुरक्षित पाया गया तथा शीघ्रपतन में इनकी अच्छी प्रतिक्रिया रही।

निष्कर्षः *सुरअत–ए–इंज़ाल* (शीघ्रपतन) के उपचार में *सफूफ़ मुग़ल्लिज़–ए–मनी, माजून आरद–ए–खुर्मा* और *हब्ब इक्सीर–ए–शिफ़ा* बहुत ही प्रभावशाली और सुरक्षित हैं।

शब्दकुंजीः *मुग़ल्लिज़–ए–मनी,* शीघ्रपतन, यूनानी, *सुरअत–ए–इंज़ाल*

Efficacy of Unani Formulation *Ma'jūn 'Aqrab* in the Management of *Haṣāt al-Kuliya wa'l-Ḥālib* (kidney and ureter stone) - A Preliminary Study

[1]Zaid Ahmed,
[2*]M Shakeel Ansari,
[3]Ansari Mushir
and
[4]Hamiduddin

[1]Department of *Tashreeh ul Badan*, Dr M.I.J.T.U.M College, Mumbai

[2]Department of *Ilmul Jarahat*, National Institute of Unani Medicine, Bengalore

[3]Department of Ilmul Advia, Dr M.I.J.T.U.M College, Mumbai

[4]Department of *Ilmul Saidla*, National Institute of Unani Medicine, Bangalore

Abstract

Introduction: Unani Medicine is used to cure renal stones since antiquity and many Unani drugs have lithotriptic property that causes disintegration and excretion of stone through urine. The aim of the following study was to evaluate the efficacy of Unani formulation *Ma'jūn 'Aqrab* in renal stone.

Methodology: It was an open-label preliminary clinical study with a sample size of 30 patients. The patients were treated with *Ma'jūn 'Aqrab* 5 g twice a day for 60 days. This study was conducted at OPD / IPD of National Institute of Unani Medicine (NIUM), Bangalore. A comprehensive protocol was framed and approval was obtained from the Ethical Committee of the NIUM. The assessment of subjective parameters was done every 15th day of follow-up. Out of 30 patients, 28 completed the study with protocol. The assessment of objective parameters was carried out before and after the treatment.

Result: The study outcomes showed that *Ma'jūn 'Aqrab* is effective significantly in both subjective and objective parameters. There was 96.4% ($p<0.001$) relief in pain in abdomen, 78.6% ($p<0.001$) in burning micturation, 35.7% ($p=0.061+$) in haematuria and 39.3% ($p=0.0137$) in nausea. Objective parameters showed improvement in USG upto 28.6% ($p<0.001$). Further, no side effects were observed during and after the study.

Keywords: *Haṣāt al-Kuliya*, *Ma'jūn 'Aqrab*, Renal stone, Unani Medicine

Introduction

Renal stone is one of the most painful and common urological diseases globally. The evidence of urinary calculi has been found in 7000 year old Egyptian mummy and incidence has increased considerably during the 20th century. India is included in the stone belt region as high incidence of renal stone has been reported consistently. It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract stone disease. The commonest type of stone contains calcium in combination with either oxalate or phosphate which makes at least 80% of all kidney stones (Jan, 2008; Malhotra, 2008)

According to Unani scholar *'Alī Ibn 'Abbās Majūsī*, renal stone is formed due to increased *Ḥarārat Gharīziyya* (innimate heat) of kidney and viscous matter produced from viscous humour. Due to excessive *Ḥarārat* (heat), *Ruṭūbat* (moisture) of this viscous matter gets dried which after a period of time leads

*Corresponding author; Email: msnium@rediffmail.com

to stone formation (*Majūsī*, 2010; *Qumrī*, 2008). According to *Ḥasan bin Nūḥ Qumrī,* one of the causes of kidney and bladder stone is constriction of neck of these organs. In the beginning, small particles are collected and if persist for a long period, lead to stone. (*Qumrī*, 2008; *Ibn Zuhr*, 1986). According to Avicenna, bladder and kidneys are favorable sites for the formation of calculi. He further states that prolonged stay of morbid matter in the urinary tract is instrumental to form a stone (*Kabīruddīn*, 2003). Two things are responsible for the development of kidney stone, one is viscous matter (*Ghalīẓ Khilṭ*) and other is power (*Quwwat)* which converts the matter (*Mādda)* into stone. The *Mādda* is *Ruṭūbat* (liquid) which is viscous and originates either from *Balgham* or from *Dam* humours. Sometimes, the origin of stone formation is injury, the injured area can cause the collection of morbid matter at that site of the kidney (Ibn Sina, 2007). According to Galen, stone formation in kidney is due to injury and as the pus is not excreted and collect in the kidney helps in forming stone (*Majūsī*, 2010; *Arzānī*, YNM; *Ibn Sīnā*, 2007; *Kabīruddīn*, 2003). *Rāzī* (2002) in western known as Rhazes in his book described the symptoms of renal stone as kidney pain, incontinence of urine and difficulty in micturation (*Rāzī*, 2002; *Rāzī*, 1991; *Arzānī*, YNM; *Ibn Zuhr*, 1986).

Most of the renowned Unani physicians agreed that *Shadīd Ḥarārat* (excessive heat) and *Khilṭ Ghalīẓ* (viscous humour) are two important factors which promote urolithiasis. According to Avicenna, stone formation needs two things, *Ghalīẓ Mādda* and stasis of these *Ghalīẓ Mādda*. (*Majūsī*, 2010; *Arzānī*, YNM; *Ibn Sīnā*, 2007; *Kabīruddīn*, 2003).

Most of the stones should be treated by minimal access and minimally invasive techniques. Open operations are still needed when appropriate expertise is not available or newer techniques fails. The minimal access techniques are Percutaneous nephrolithotomy (PCNL), Extracorporeal Shock Wave Lithotripsy (ESWL). Minimal invasive surgery has its own complication like it needs expertise and is costly. Traditional medicine particularly Unani Medicine have very wide therapeutics options to deal with renal calculi.

Several studies have validated its effect in kidney stone. A study by Aziz *et al.* (2011) proved that *Sharbat Buzūrī Mu'tadil* is antiurolithiatic and it can prevent the recurrence of stone formation by forming soluble calcium compound with citric acid and it possesses lithotriptic, diuretic and alkalinizing effect)*Azīz*, 2011). Another study showed that Unani formulation *Safūf Ḥajr al-Yahūd* is effective in the treatment of kidney stone (Rajesh, 2016). Ara *et al.* (2015) concluded that *Ma'jūn 'Aqrab* and *Sharbat Ālū Bālū* in combination are effective in kidney stone particularly in symptoms such as flank pain, dysuria and hematuria, reduces the size of stones and are also effective in eliminating the stones (Ara *et al.,* 2015). Herbal remedies for prevention and cure of this ailments can be more effective than other available treatments, with lesser side effects and is also

economical (Sangeetha *et al.,* 2015). Unani formulation *Ma'jūn 'Aqrab* has both antiurolithiatic and diuretic property as per Unani text. The objective of the study was to assess the efficacy and safety of Unani formulation *Ma'jūn 'Aqrab* in the management of *Haṣāt al-Kuliya wa'l-Ḥālib* (kidney and ureter stone).

Methodology

It was an open-label preliminary clinical study with a sample size of 30 patients. The patients were treated with *Ma'jūn 'Aqrab* 5 g twice a day for 60 days. The study was conducted at OPD / IPD of National Institute of Unani Medicine (NIUM), Bangalore after ethical clearance. Clinical study was started by enrolling the patients as per eligible criteria. Criteria for selection of cases were based on inclusion and exclusion criteria, subjective and objective parameters. This study spanned for a period of 10 month.

Inclusion criteria: Diagnosed case of *Haṣāt al-Kuliya wa'l-Ḥālib* (radiological evidence of stone up to 15 mm in kidney and 8 mm in ureter). Patient in the age group of 15-60 years of either sex. Patients agree to sign the informed consent form and follow the protocol.

Exclusion criteria: Stone size above 15 mm in kidney and above 8 mm in ureter. Patients aged below 15 years or above 60 years. Patient with uncontrolled diabetic, hypertensive, pregnant and lactating women, patients with gross hydronephrosis, pyelonephrosis, impacted stone, impaired renal function, bladder outlet obstruction. Patients undergoing treatment for other serious illness.

Subjective parameters selected were pain in abdomen in renal area, burning micturation, haematuria, nausea and vomiting. Objective parameters included urine routine and microscopic, USG abdomen with pelvis including post void residual urine before and after treatment. X-ray (KUB), blood urea and serum creatinine were done as safety parameters.

Study design: The study was designed as open-label, observational, randomized, preliminary clinical trial with a sample size of 30 patients. The patients were randomly allocated into the study. The treatment period was ascertained as 60 days.

Selection of drug: For the rational and effective conservative treatment of renal stone a potent Unani formulation is required, which could break and remove the stone via urinary system, revert the pathological condition to normalcy. Therefore, the test formulation *Ma'jūn 'Aqrab* was selected for the work which is used as *Mufattit-i-Ḥaāāt* and *Mudirr-i-Bawl* (Anonymous, 1993). The formulation used in the study was purchased from market, manufactured by Dehlwi Ambar Pvt. Ltd. as per *Bayāḍ-i-Kabīr*, vol. II with following ingredients without '*Aqrab Muḥarraq* (*Hottentotta pachyuruf*). The ingredients of each 5 g formulation are

Kāknaj (*Physalis Alkekanj*) 292 mg, *Jund Bedastar* (*Castoreum*) 242 mg, *Juntiyānā* (*Guntiyana lutea*) 242 mg, *Filfil Safaid* (*Piper nigrum* without pericarp) 146 mg, *Filfil Siyāh* (*Piper nigrum*) 146 mg, *Zanjabīl* (*Zingiber officinale*) 49 mg, *Qand Safed* 3.873 g.

Dosage and mode of drug administration: 5 g with plain water 2 times a day orally.

Efficacy assessment: The assessment of efficacy in the test group was based on subjective and objective parameters. Subjective parameters include symptoms like pain in abdomen, burning micturation, haematuria, nausea and vomiting. As these subjective parameters differ in severity from patient to patient, an arbitrary grading scale was used for assessment of all these subjective parameters. Subjective parameters were assessed on day 0, 15, 30, 45, 60. Objective parameters were USG, X-ray KUB and urine examination.

Withdrawal criteria were (a) Failure to follow the protocol, (b) Any adverse reaction or adverse event, and (c) Drop out. Adverse effect documentation was done and no adverse event or reaction was noted during the test drug administration. The case record form and consent form properly documented throughout the study were submitted to the Department of Ilmul Jarahat at NIUM, Bangalore after completion of the study.

Statistical analysis: The assessment of subjective parameters were recorded and assessed objectively by using arbitrary scale. The assessment of objective parameters was carried out before and after the treatment using paired proportion test (Bernard, 2000; Riffenburg, 2005; Sunder, 2006; Suresh, 2012).

Result

Out of 30 patients, 28 completed the study with protocol and 60-day therapy, while 2 patients dropped out. The patients were treated with Unani formulation *Ma'jūn 'Aqrab* 5 g twice a day for 60 days. The assessments of subjective parameters were done on every 15th day of follow-up. The study outcomes from baseline to different follow-ups in the form of subjective symptoms were recorded and assessed objectively by using arbitrary scale. The assessment of objective parameters was carried out before and after the treatment. The study outcomes and their findings with statistical values are put here with demographic, efficacy and safety data.

Demographic data

Age: 2 (7.1%) patients were in age group 15-20 years, 7 (25%) patients in 21-30 years, 7 (25%) patients in 41-50 years, 6 (21.4%) patients in 31-40 years and 6 (21.4%) patients in age group between 51-60 years of age. This observation

coincides with Bailey and Loves description as approximately 50% of patients present between the age of 30-50 years (William, 2008) and with API description as predominantly in young individual with peak in late 20s and early 30s (Shah, 2008).

Sex: 19 (67.9%) patients were male and only 9 (32.1%) patients were female. This finding coincides with description of API and Das textbook of surgery, males are more affected than females and also coincides with the description of men affected two to three times more frequently than women because of enhancing capacity of testosterone and inhibiting capacity of estrogen in stone formation (Shah, 2008; Das, 2014).

Socio-economic status: Out of 1 (3.6%) patient was found to be of upper class (I), 16 (57.1%) patients were of upper middle class (II), 9 (32.1%) patients were of lower middle class (III) and 2 (7.1%) in upper lower class (IV). No patient in lower class (V) was found. Middle class (upper middle and lower middle) was affected mostly.

Assessment at different study points

Pain in abdomen: The baseline score for pain in abdomen was noted before starting treatment. 1 (3.6%) patient had mild pain, 25 (89.3%) patients had moderate pain, and 2 (7.1%) patients had severe pain in abdomen. After completion of treatment, out of 28 patients, 21 (75%) patients had no pain and the change was 75%. Detail of pain in abdomen parameter done at different interval is depicted in Table 1. Assessment of pain in abdomen by Arbitrary scale in study before and after treatment is statistically significant ($p<0.001$) using paired proportion test. Improvement of 96.4% was observed in mild pain to no pain (0-1 score). **(Table 1)**

Table 1: Pain in abdomen at renal angle: Assessment at different study points

Pain in Abdomen	D - 0	D – 15	D - 30	D – 45	D - 60	% change
0	0(0%)	1(3.6%)	0(0%)	9(32.1%)	21(75%)	75.0%
1	1(3.6%)	8(28.6%)	21(75%)	18(64.3%)	7(25%)	21.4%
2	25(89.3%)	19(67.9%)	6(21.4%)	1(3.6%)	0(0%)	-89.3%
3	2(7.1%)	0(0%)	1(3.6%)	0(0%)	0(0%)	-7.1%
Total	28(100%)	28(100%)	28(100%)	28(100%)	28(100%)	-

P<0.001**, Significant, paired proportion test: Improvement of 96.4% (0-1 score)

Burning micturation: Before starting the treatment, 2 (7.1%) patients had no burning micturition, 13(46.4%) patients had mild burning micturition, 13 (46.4%) patients had moderate degree and there was no patient of severe degree of burning micturition. After 2-month treatment, 24 (85.7%) patients had no burning micturition, a change of 78.6%, while 4 (14.3%) patients had mild degree of burning micturition, a change of -32.1%, and also there was no patients of moderate (change -46.4%) and severe degree of burning micturition.

Assessment of burning micturition in patients of study before and after treatment is statistically significant ($p<0.001$) using paired proportion test. The improvement is 78.6% (in 0 score) i.e. No burning micturition. The Mean ± SD before treatment was 1.39±0.63 and after treatment was 0.14±0.36. The drug is effective in reducing burning micturition **(Table 2).**

Table 2: Burning micturition: Assessment at different study points

Burning Micturition	D - 0	D – 15	D - 30	D - 45	D - 60	% change
0	2(7.1%)	6(21.4%)	14(50%)	17(60.7%)	24(85.7%)	78.6%
1	13(46.4%)	17(60.7%)	14(50%)	11(39.3%)	4(14.3%)	-32.1%
2	13(46.4%)	5(17.9%)	0(0%)	0(0%)	0(0%)	-46.4%
3	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0.0%
Total	28(100%)	28(100%)	28(100%)	28(100%)	28(100%)	-
Mean ± SD	1.39±0.63	0.96±0.64	0.50±0.51	0.39±0.50	0.14±0.36	-

P<0.001**, Significant, paired proportion test: Improvement of 78.6%(0 score)

Hematuria: Out of 28 patients, 18 (64.3%) patients were with no hematuria, 3 (10.7%) patients had mild degree of hematuria and 7 (25%) patients had moderate hematuria. After taking medicine for 2 months, all 28 (100%) patients displayed no hematuria. Detail is depicted in Table 3. Assessment of hematuria in patients of study before and after treatment is statistically significant p = 0.061+ using paired proportion test. The improvement is of 35.7% (in 0 score) i.e. no hematuria **(Table 3).**

Table 3: Hematuria assessment at different study points

Hematuria	D-0	D-15	D-30	D-45	D-60	% change
0	18(64.3%)	19(67.9%)	24(85.7%)	25(89.3%)	28(100%)	+35.7%
1	3(10.7%)	9(32.1%)	4(14.3%)	3(10.7%)	0(0%)	-10.7%
2	7(25%)	0(0%)	0(0%)	0(0%)	0(0%)	-25.0%
3	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0.0%
Total	28(100%)	28(100%)	28(100%)	28(100%)	28(100%)	-

P=0.061+, Significant, paired proportion test: Improvement of 35.7% (0 score)

Nausea: 14 (50%) patients presented with no nausea, 10 (35.7%) patients were with mild nausea and 4 (14.3%) patients presented with moderate degree of nausea and there were no patient of severe nausea at baseline score. After taking medicine for 2 months, 25 (89.3%) patients were having no complaints of nausea, a change of 39.3%, 3 (10.7%) patients were having mild degree of nausea with a change of -25%, and there was no patient of moderate nausea (change -14.3%) and severe degree of nausea. Result is statistically significant with p = 0.0137 using paired proportion test and improvement of 39.3% was seen. Results indicate that the test drug is also effective in associated nausea symptoms.

Vomiting: Prior to treatment, 20 (71.4%) patients had no vomiting, 6 (21.4%) patients presented with mild vomiting and 2 (7.1%) patients presented with moderate degree of vomiting. After treatment, all 28 (100%) patients had no complaints of vomiting. The change occurred after treatment is 28.6%, -21.4%, - 7.1% for no vomiting, mild & moderate degrees of vomiting categories respectively.

Assessment of vomiting in the study before and after treatment was statistically not significant, p = 0.115, using paired proportion test, improvement of 28.6% was noted.

USG (Ultrasonography) and X-ray KUB (kidney, ureter, and bladder): USG and X-ray KUB are the two standard objective parameters which were taken to assess the efficacy of the test formulation. USG and X-ray KUB were done in all the patients before and after the treatment, but the calculus was not depicted by X-ray in most of the patients, but presence of stone was confirmed by USG.

Before treatment, USG of abdomen and pelvis of all 28 patients was done. Of them, 13 (46.42%) patients were having B/L renal stone, 9 (32.14%) patients left

renal stone, 5 (17.9%) patients right renal stone and 1 (3.6%) patient were having right ureteric calculus. After the treatment, USG of 7 (25%) patients displayed normal study, 9 (35.7%) patients with B/L renal stone, 7 (25%) patients with left side stone, 4 (14.3%) with right side and 1 (3.6%) patient with stone at left vesicoureteric junction. The change was 25% in normal study category, before and after treatment, -10.72%, -7.14%, -3.6%, -3.6%, in case of B/L renal calculi, Lt renal calculi, Rt renal calculi and Rt ureteric calculi, respectively. While in case of Lt ureteric calculi the change was 3.6% after treatment. Significant result with p=0.002 using paired proportion test was observed (**Table 4**).

The evaluation of the formulation after the treatment displays statistically significant result with p=0.002 using paired proportion test in USG test. In X-ray KUB the assessment before and after treatment are statistically not significant p=0.315 using paired proportion test. Improvement of 10.7% in normal study was noted.

Table 4: USG abdomen & pelvis: Assessment at different study points

USG Abdomen & Pelvis	BT	AT	% change
NS	0(0%)	7(25%)	25.00%
B/L renal cal	13(46.42%)	9(35.7%)	-10.72%
Lt ren cal	9(32.14%)	7(25%)	-7.14%
Rt ren cal	5(17.9%)	4(14.3%)	-3.60%
Rt ureteric cal	1(3.6%)	0(0%)	-3.60%
Lt ureteric cal	0	1(3.6%)	3.60%
Total	100	100	-

P=0.002**, Significant, paired proportion test: Improvement of 25%, Cal: Calculi

Other urine examination: On evaluation of urine analysis, out of 28 patients, 21 patients presented with crystals in urine before treatment. After the treatment, only 9 patients had crystals in urine, while remaining of 12 patients displayed no crystals in urine. The p value was P < 0.001 highly significant using paired proportion test, improvement of 42.9%.

Treatment with the test drug *Maʿjūn ʿAqrab* displays significant effect on various parameters of patients with *Haṣāt al-Kuliya* validating *Mufattit-i-Ḥaṣāt* and *Mudirr-i-Bawl* actions of the test drug.

Discussion

The study was conducted at NIUM, Bangalore about 57 % patients in the study belongs to Upper class (I). Outcomes showed that *Ma'jūn 'Aqrab* is effective significantly in both subjective and objective parameters. Significant improvement noted in subjective parameters was up to 96.4% reduction in pain in abdomen, 78.6% ($p<0.001$) in burning micturition, 35.7% in hematuria and 39.3% in nausea. The test drug displays significant property of decreasing the symptoms of pain in renal stone disease.

Objective parameters of USG displayed 28.6% significant improvement at probability level of ($p<0.001$). Further, no significant side effects were observed during and after the study. The study was designed as an open-label, observational clinical study for preliminary evaluation of *Ma'jūn 'Aqrab* in subjective and objective parameters displaying significant results, with subjective parameters displaying more result in comparison to objective. In urine analysis, the significant result was in crystal in urine and other parameters of urine was not significant. Further clinical study with sophisticated methodology and parameters/test is required for further detail comment on the efficacy of this famous classical Unani formulation. In this study, modified market formulation was used which does not contain its prime ingredient i.e. *'Aqrab*. This study also supports the effect of the drug without this. This study also suggests that in case of regulatory restriction and unavailability of some drugs in classical formulation, other ingredients of the formulation can also be utilized for clinical use. The study validates other combination of drugs in the formulation. The study indicates that the test formulation is effective to expel out stone through urine but detail analysis is needed in this regard for further exploration of mechanism of lithotriptic activity of the formulation.

Conclusion

The Unani formulation *Ma'jūn 'Aqrab* was effective in both subjective and objective parameters of the study. It has the effect of reducing the symptoms in patients with renal stone and is also effective in reducing the size of stone.

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सारांश

हसात अल–कुलिया व *अल–ह़ालिब* (गुर्दे और मूत्र वाहनी की पथरी) के उपचार में *माजून अक़रब* की प्रभावकारिता – एक प्रारंभिक अध्ययन

ज़ैद अहमद, एम. शकील अंसारी*, अंसारी मुशीर और हामिदुद्दीन

सारांश

परिचयः गुर्दे की पथरी के उपचार के लिए यूनानी चिकित्सा का उपयोग प्राचीन काल से होता रहा है। कई यूनानी औषधियों में लिथोट्रिप्टिक गुण होते हैं जो मूत्र के माध्यम से पथरी के विघटन और उत्सर्जन का कारण बनते हैं। निम्नलिखित अध्ययन का उद्देश्य गुर्दे की पथरी में यूनानी मिश्रण *माजून अक़रब* की प्रभावकारिता का मूल्यांकन करना था।

शोधविधिः 30 रोगियों के नमूने पर आधारित यह एक ओपन लेबल का प्रारंभिक नैदानिक अध्ययन था। 60 दिनों तक *माजून अक़रब* दिन में दो बार 5 ग्राम की मात्रा में देकर रोगियों का उपचार किया गया। यह अध्ययन राष्ट्रीय यूनानी चिकित्सा संस्थान (रा.यू.चि.सं.), बैंगलोर की ओपीडी/आईपीडी में किया गया। एक व्यापक प्रोटोकॉल तैयार किया गया और रा.यू.चि.सं. की नैतिक समिति से उसकी स्वीकृति ली गई। जांच के प्रत्येक 15वें दिन व्यक्तिपरक मापदंडों का मूल्यांकन किया गया। 30 रोगियों में से 28 रोगियों ने प्रोटोकॉल के साथ अध्ययन पूरा किया। उपचार से पहले और बाद में वस्तुनिष्ठ मापदंडों का मूल्यांकन किया गया।

परिणामः अध्ययन के परिणामों से पता चला कि माजून अक़रब व्यक्तिपरक और वस्तुनिष्ठ दोनों मापदंडों में महत्वपूर्ण रूप से प्रभावशाली है। 96.4% ($p<0.001$) को पेट में दर्द, 78.6% ($p<0.001$) को मूत्र में जलन, 35.7% ($p=0.061+$) को हेमट्यूरिया में और 39.3% ($p=0.0137$) को मतली में राहत मिली। वस्तुनिष्ठ मापदंडों ने यूएसजी में 28.6% ($p<0.001$) तक सुधार दिखाया। इसके अतिरिक्त अध्ययन के दौरान और बाद में कोई दुष्प्रभाव नहीं देखा गया।

शब्दकुंजीः *हसात अल–कुलिया, माजून अक़रब,* गुर्दे की पथरी, यूनानी चिकित्सा

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Ministry of Ayush, Government of India
61 - 65, Institutional Area, Janakpuri, New Delhi - 110 058
Telephone: +91-11-28521981, 28525982
Email: unanimedicine@gmail.com
Website: http://ccrum.res.in