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Editorial

The traditional and herbal systems of medicine have been growing at a tremendous pace and are expected to witness even faster growth and popularity in the near future. According to a report, the global market size of herbal medicine is expected to grow at a CAGR 18.9% reaching to US$ 550 billion by 2030 from an estimated size of US$ 83 billion in 2019. This growth is driven by various factors including rise in the prevalence of liver and heart diseases, increased research and development activities in herbal and traditional systems of medicine, and growing awareness and consciousness among people about the side effects of chemical-based medicines and benefits of natural remedies. The increasing geriatric population, introduction of Current Good Manufacturing Practices (CGMP) by the USFDA for dietary supplements and cost-effectiveness of herbal drugs in comparison to their alternatives are some other factors fueling the growth and expansion of herbal medicine. The COVID-19 pandemic further boosted the popularity of traditional medicine due to the natural measures available for enhancing immunity against respiratory illnesses.

This positive change in the favour of traditional medicine comes with the challenge pertaining to issues of quality, safety and efficacy. The key objectives of WHO Traditional Medicine (TM) Strategy 2014–2023 focus to address this challenge. The goals of the WHO strategy are: '(1) harnessing the potential contribution of TM to health, wellness and people-centred health care; and (2) promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems, where appropriate'.

India has already aligned its policy related to traditional medicine with the WHO strategy and set up the National Ayush Mission to support Ayush medical system through cost-effective services, strengthening the educational system, enforcement of quality control of Ayurveda, Siddha, Unani and Homoeopathy drugs and ensuring sustainable availability of raw materials.

On its part, the Central Council for Research in Unani Medicine (CCRUM), through its research programmes, especially clinical research, drug research, literary research, and survey & cultivation of medicinal plants has been contributing significantly in the area of research and development in Unani Medicine and generating scientific data on quality control, safety and efficacy of Unani drugs.

To propagate data of research in Unani Medicine amongst academicians and researchers engaged in the scientific validation of traditional drugs, the CCRUM has been publishing *Hippocratic Journal of Unani Medicine* (HJUM), a peer-reviewed quarterly journal for over 15 years.

This issue of HJUM is comprised of eight papers. In the first paper entitled 'Relationship between *'Afiṣ* (acrid) taste, phytochemistry and pharmacological actions of drugs of Unani Medicine', the authors have explored the drugs having *'Afiṣ* (acrid) taste in terms of their *Afʿāl* (pharmacological actions) mentioned in Unani literature and the relationship of this particular taste with the reported pharmacological activities and chemical constituents of the drugs. The second paper based on a survey of 100 Unani physicians presents current perception and practice about the use of *Ḥabb Muṣaffī-i-Khūn* in cancer management. The third paper presents HPTLC characterization and quality standards of *Qurṣ Mafāsil Jadīd,* a multi-ingredient Unani formulation, effectively used for the management of joint pains of various etiologies. The fourth paper presents outcomes of a study conducted to evaluate the effect of detoxification (*'Amal-i-Tadbīr*) on the toxicity of *Semecarpus anacardium* (*Balādur*) by spectrophotometric estimation of total phenolic content. In the fifth paper, the authors have studied the demographic, epidemiological and clinical characteristics of *Kalaf* (melasma) and its impact on the quality of life of the participants. The sixth paper evaluates the immuno-modulatory action of Unani treatment against HBV induced compensated cirrhosis of liver through a case series on seven patients. The seventh paper is a case study on the effect of *Ḥabb Muṣaffī-i-Khūn, Iṭrīfal Shāhitara* and *Eczenil* ointment in a case of *Qūbā al-Badan* (tinea corporis). The last paper is based on a clinical study conducted to evaluate therapeutic efficacy of *Marham Dākhliyūn* in the management of vaginal candidiasis through a standard controlled single blind clinical trial.

We hope that the contents of this issue would be of great use for the researchers of Unani Medicine and other traditional medical systems. We are thankful to our contributors and learned reviewers for their valuable contributions, time and efforts.

Prof. Asim Ali Khan
Editor-in-Chief

Contents

Relationship between *'Afiṣ* (Acrid) Taste, Phytochemistry and Pharmacological Actions of Drugs of Unani Medicine

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Abstract

The drugs of Unani Medicine have been established through various means, of which determination of their *Mizāj* (temperament) has remained central one. *Mizāj* of drugs is determined through *Qiyās* (analogy) which assesses the potential *Mizāj* and then *Tajraba* (experimentation) that confirms the *Mizāj* of the drug. Taste is vital determinant for *Mizāj* assessment and thereby the drug action in humans. The present study explores the *'Afiṣ* (acrid) tasting drugs in terms of their *Af'āl* (pharmacological actions) mentioned in Unani literature and also the relationship of this particular taste with the reported pharmacological activities and chemical constituents of the drugs. *Af'āl* and *Mizāj* of thirty *'Afiṣ* tasting drugs were recorded from Unani literature and the reported pharmacological activities and chemical constituents were noted from indexed journals and other related available literature. Possible relationship between taste, *Af'āl*, reported activities and chemical constituents was explored. A fair degree of correlation was observed between taste, *Mizāj, Af'āl*, reported activities and chemical constituents of the drugs. The most common *Af'āl* observed were *Qābiḍ* (astringent), *Muḥallil* (resolvent) and *Muqawwī* (tonic), whereas the most common reported action was antioxidant activity. Anti-inflammatory and hepatoprotective activities were also reported in some of the drugs under consideration. The *'Afiṣ* taste drug samples chosen for the study showed a positive correlation between *Mizāj, Af'āl*, chemical composition and reported pharmacological activities of these drugs.

Keywords: *Af'āl, 'Afiṣ, Mizāj, Qiyās, Tajraba,* Phytochemistry, Unani

Introduction

The sense of taste is the ability of organisms to detect nutritionally important and beneficial compounds, including sugar and salt as well as potentially harmful substances, such as alkaloids and acids which are essential for survival (Kinnamon, 2000). Taste is elicited by water soluble molecules that interact with receptors on the tongue and in the oral cavity (Shallenberger, 1993) in the same way as drug molecule interacts with taste receptors on the tongue, to give bitter, sweet or salty taste sensation when dissolved in saliva. The sensation of the taste is the result of signal transduction from the taste buds (receptor organ) (Deepak *et al.*, 2012). In Ayurveda, taste is described under the heading of '*Rasa*'. *Rasa* is related to the total subjective experience, arising after putting the substances in the mouth, including not only the six primary tastes recognized by Ayurveda (sweet, sour, salty, bitter, pungent and astringent), but also the 'flavors' experienced by means of retro nasal olfaction (nasal smell receptor stimulation by food warmed in the mouth) (Joshi *et al.*, 2006).

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The number of basic tastes recognized as primary tastes has varied over the years. Applying the 'Doctrine of Opposites', Aristotle considered sweet and bitter tastes to be the example of the doctrine and believed that all other tastes lay between the two extremes. Linnaeus expanded the number of tastes like sweet, sour, sharp, salty, bitter, astringent, viscous, aqueous, and nauseous. Wundt, the founder of experimental psychology, first reduced the number to six (sweet, salt, bitter, sour, metallic and alkaline), then four (sweet, salt, sour and bitter) (Danish, 2016).

Pharmacological basis of drug action revolves around the universal pharmacological principle that similar structures have similar pharmacological activity. If the structure of a substance is known, then its pharmacological behavior can be inferred. Conventional pharmacology uses chemical structure as the basis for pharmacological basis of drug action. According to Charaka, *rasa* can be different in fresh condition and in dry state of the same substance, and have different pharmacological actions in those conditions, e.g. *Piper longum* L. is *Madhura* (sweet) in fresh condition whereas in dry form it is *Katu* (pungent) and accordingly fresh *P. longum* is heavier to digest (*guru*) than dry *P. longum* which is easy to digest (*laghu*) (Rath *et al*., 2014). Beauchamp *et al*. correlates the pharmacological action of *Ibuprofen* and *Oleocanthal* on the basis of their similarities in taste. They point out that both Oleocanthal from olive oil and solution of *Ibuprofen,* a non-steroidal anti-inflammatory drug, induce similar strong stinging sensations in the throat. Despite not being entirely similar structure, both molecules are anti-inflammatory and have similar profiles, being COX-1 and COX-2 inhibitors (Joshi *et al*., 2006). Therefore, it is obvious that since *rasa* indicates the pharmacological behavior of the substance as and when the substance is presented before the user, *rasa* can be used as a tool to test the substance in use. Modern pharmacology revolves around a central concept that the activity of a chemical is reflected in its chemical structure. Both qualitative and quantitative structure-activity relationship (QSAR) determines the biological activity of the substance and defines those alterations in structure that can change the overall properties of a compound (Rath *et al*., 2014).

The enormous experiences of Unani physicians are worthy when we look at the literature explaining minute details regarding these determinants of *Mizāj*. From the present scenario, their experiences will be more useful when they are interpreted by objective measurements (Parveen, 2015). Colour, smell and taste were attempted for objective measurement and it was found that variation in these determinants needs to be further evaluated (Parveen, 2015). The eight tastes defined by Unani Medicine have got unique importance as it reveals distinct *Tāthīr* in the body. So attempt was needed to study them separately (Danish, 2016). The present study was carried out in the light of the above discussion and it was decided to explore the relation between taste, *Mizāj, Afʿāl*

mentioned by Unani physicians, reported pharmacological activities and the chemical constituents of the drug.

Methodology

The concept of analogy for the determination of the effect of *'Afiṣ* taste drug was thoroughly reviewed and the pharmacological action of *'Afiṣ* taste drugs on the basis of their phytoconstituents was also explored. Thirty *Mufrad* (single) drugs of *'Afiṣ* taste were selected from different Unani classical books (Khan, 2012; Ibn Sina, 1998; Mohammad, 2002; Kabiruddin, 2007; Ghani, YNM). Phytoconstituents and reputed pharmacological actions of all drugs were searched from authentic sources like ethno-botanical literature, web search engines and indexed journals. For all pharmacological actions mentioned in Unani literature and reported in journals, the chemical constituent of drugs was compared to see the similarity between taste, chemical constituents, and action of the drugs.

Observation (Table 1)

Table 1: *Mizāj, Af'āl*, reported activities and chemical constituents of acrid taste drugs

Drug name	*Mizāj*	*Af'āl* (Actions)	Reported activities	Chemical constituents
***Āmla* fruit** (*Emblica officinalis* L.)	B1,[2,3] Y1,[1,2] Y2, Y3[3]	*Qābiḍ, Muqawwī-i-Mi'da,*[1,2,3] *Muqawwī-i-Bāh,*[1,3] *Mushil-i-Balgham wa Sawdā',*[1,2] *Mushtahī* [1]	Antioxidant[4] Hepatoprotective[5] Anti-inflammatory[6]	Flavonoid, phenols, proanthocynidin, emblicanin A and B, gallic acid, ellagic acids[6]
***Abhal* berry** (*Juniperus communis* L.)	H1,[1,2] H2,[3] Y2,[1] Y3[1,3]	*Muḥallil,*[1,2,3] *Muqawwī,*[2] *Mujaffif, Mulaṭṭif,*[1,2,3] *Qābiḍ Muqawwī-i-Mi'da, Mushil-i-Balgham wa Sawdā'*[3]	Hepatoprotective[7] Anti-inflammatory[8] Antioxidant[9]	Phenols, flavonoids,[7] essential oil[8]
***Arjun* bark** (*Terminalia arjuna* L.)	H1,[1,2,3] Y2 [1,2]	*Qābiḍ,*[2] *Muqawwī-i Qalb,*[1,2,3] *Musakkin-i-Alam,*[2] *Muqawwī-i-Bāh,*[3] *Dāfi'-i-Jarayān, Mushil-i-Balgham wa Ṣafrā'*[1]	Hepatoprotective[10] Antioxidant [11] Anti-inflammatory[12]	Flavonoids,[10,11] phenol[11]
***Atīs* root** (*Aconitum heterophyllum* Wall. ex Royle.)	H1,Y2,[2] H2, Y1[1,3]	*Qābiḍ,*[2] *Muqawwī-i-Bāh,*[1] *Muḥallil,*[1,3] *Mushtahī,*[1] *Qātil-i-Kirm-i-Shikam, Mushil-i-Balgham*[3]	Antioxidant[13] Hepatoprotective[14] Anti-inflammatory[15]	Alkaloids, glycosides, flavonoid, phenols,[13,15] sterols,[15] tannin, saponin[13]

Drug name	*Mizāj*	*Afʿāl* (Actions)	Reported activities	Chemical constituents
***Ḥabb al-Ās* leaf** (*Myrtus communis* L.)	B1,[2,3] Y1,Y2[3]	*Muqawwī-i-Miʿda,*[2,3] *Muḥallil-i-Riyāḥ, Qābiḍ,*[1,3]*Muḥalllil-i-Waram,*[3] *Muqawwī-i-Dimāgh,*[1,3] *wa Aḥshā', Mudirr-i-Bawl*[1]	Anti-inflammatory[16] Antioxidant[17] Hepatoprotective[17]	Tannin, polyphenolics, flavonoids, terpenoids, steroids[17]
***Zaytūn* fruit** (*Olea europaea* L.)	Riped-H1[1] Unripe-B1, Y1[1,3]	*Muqawwī-i-Bāh,*[1] *Muqawwī-i-Miʿda, Mushtahī,*[1,3] *Qābiḍ*[3]	Antioxidant[18] Anti-inflammatory[19] Hepatoprotective[20]	Flavonoid, phenol,[18] oleuropein, glucoside[19]
***Palās Pāprā* leaf** (*Butea monosperma* (Lam.) Taub.)	B1,Y1,[2] H1,R1[1]	*Muḥammir, Kāsir-i-Riyāḥ, Qābiḍ,*[2] *Mushil-i-Balgham wa Sawdā', Qātil-i-kirm shikam* [2,3]	Hepatoprotective[21] Antioxidant[22] Anti-inflammatory[23]	Flavonoids, polyphenol[21,23]
***Balela* fruit** (*Terminalia bellirica* (Gaertn.) Roxb.)	B1, Y2[1,2,3]	*Muqawwī,*[2] *Qābiḍ, Mushil-i-Sawdā' wa Ṣafrā', Muqawwī-i-Miʿda,*[1,2,,3] *Muḥallil-i-Waram,*[3] *Mulaṭṭif, Mushtahī*[1,3]	Antioxidan[24] Hepatoprotective[25]	Phenols, flavonoids[24]
***Banafsha* flower** (*Viola odorata* L.)	B1, R1[1,2,3]	*Muḥallil-i-Waram,*[1,3] *Mughadhdhī, Mulaṭṭif, Jādhib, Muzalliq, Qābiḍ,*[3] *Mulayyin,*[2,3] *Muʿaddil-i-Ṣafrā'*[1,2]	Antioxidant[26] Hepatoprotective[27] Anti-inflammatory[28]	Phenols, flavonoid,[26,28] salicylate, saponins, alkaloids, volatile oils[28]
***Bisbāsa* aril** (*Myristica fragrans* Houtt.)	H1, Y2[1,2,3]	*Muḥallil*[1,3] *Mujaffif,*[1,2,3] *Qābiḍ,*[2] *Muqawwī-i-Miʿda wa Kabid,*[1,3] *Mulaṭṭif*[3]	Antioxidant[29] Anti-inflammatory[30]	Terpenes, phenols, tannins, flavonoids,[29] myristicin[30]
***Chalia* fruit** (*Areca catechu* L.)	B2, Y2 [1,2,3]	*Qābiḍ,*[1,2,3] *Muḥallil,*[2] *Muqawwī wa Mubarrid-i-Miʿda,*[1,3] *Mushtahī, Mushil-i-Balgham wa Ṣafrā' wa Sawdā', Mujaffif, Muqawwī-i-litha wa Dandān wa Bāh*[3]	Anti-inflammatory[31] Antioxidant[32] Hepatoprotective[33]	Alkaloids, flavonoids, saponins, sterols, tannins, terpenes[31]
***Gā'ozabān* leaf** (*Borago officinalis* L.)	Fresh-H1,R1[3] Dry-H1[1,3] Y1[3]	*Muqawwī-i-Aʿḍā' Raʾīsa, Mulayyin-i-Ṭabaʿ, Mushil-i-Ṣafrā', Mushil-i-Sawdā'*[1,3]	Antioxidant[34] Hepatoprotective[35] Anti-inflammatory[36]	Carbohydrates, glycosides, sterols, saponins, tannins, alkaloids,[35] phenols, flavonoid[36]

Drug name	*Mizāj*	*Af'āl* (Actions)	Reported activities	Chemical constituents
Gul-i-Surkh (*Rosa damascena* Mill.)	Fresh- H1, R1[2] Dried- H1,[1,2,3] Y1[1,3]	*Mushil, Qābiḍ, Mulaṭṭif, Muḥallil-i-Waram, Muqawwī-i-Mi'da wa Bāh*[1,3] *Muqawwī-i-Litha*[,] *Mujaffif*[1]	Anti-inflammatory[37] Antioxidant[38] Hepatoprotective[38]	Phenol, alkaloid, flavoniod, terpenoid, saponin, tannin, glycoside, carbohydrates[38]
***Halela* fruit** (*Terminalia chebula* Retz.)	B1,Y2[1,2,3]	*Muqawwī-i-Mi'da, Mushil-i-Ṣafrā' wa Sawdā',*[1,2,3] *Muqawwī-i-Dandān wa Litha, Mujaffif, Mulayyin*[1,3] *Qābiḍ* [2,3]	Antioxidant[39] Hepatoprotective[40] Anti-inflammatory[39]	Flavonoids, phenols, tannins, triterpenoids[39]
***Jāmun* leaf** (*Eugenia jambolana* Lam.)	B2, Y3,[1,3]	*Muqawwī-i-Mi'da,* [1,2,3] *Qābiḍ*[1,3] *Hāḍim, Muqawwī-i-Litha,*[1] *Muqawwī-i-Bāh*[1,3] *Muḥarrik-i-Ishtihā'* [1,2,3]	Anti-inflammatory[41] Antioxidant[42] Hepatoprotective[43]	Flavonoids, saponins, phenols, steroid, tannin, coumarins[42,43]
***Khār-i-Khasak* fruit** (*Tribulus terrestris* L.)	B1,[3] Y1, H1[2,3]	*Mushtahī, Muḥallil-i-Waram, Muqawwī-i-Bāh,*[1,2,3] *Munḍij, Mulayyin,*[1,3] *Mudirr-i-Bawl wa Ḥayḍ* [1,2,3]	Antioxidant[44] Hepatoprotective[45] Anti-inflammatory[46]	Flavonoids, phenols[44]
***Majīth* root** (*Rubia cordifolia* L.)	H1,[2] Y2[2,3]	*Mufattiḥ-i-Sudad, Mudirr-i-Bawl, Muqawwī-i-Mi'da,*[2,3] *Muḥallil, Musaffi-i-Khūn*[3]	Hepatoprotective[47] Antioxidant[48] Anti-inflammatory[48]	Rubiadin,[47] terpenoids[48]
***Ma'īn* galls** (*Tamarix gallica* L.)	B1,[1,2,3] B2,Y2 [1,3]	*Qābiḍ,*[1,2,3] *Muqawwī-i-Litha,*[3] *Muḥallil-i-Waram-i-Ṭiḥāl,*[1] *Muqawwī-i-Mi'da,*[2,3] *Mujaffif* [1,2]	Hepatoprotective[49] Antioxidant[49] Anti-inflammatory[50]	Tannins, flavonoids, alkaloid, saponin, Phenols[50]
***Mazu* galls** (*Quercus infectoria* Oliv.)	B1,Y2, Y3 [1,2,3]	*Qābiḍ, Mujaffif,*[2,3] *Ḥābis,*[2] *Muqawwī-i-Litha, Muḥallil-i-Waram,*[1] *Dāfi'-i-Ta'affun*[2]	Hepatoprotective[51] Antioxidant[52] Anti-inflammatory[53]	Carbohydrate, alkaloid, sterol,[51] tannins, flavonoids, terpenoids [51,52]
Post-i-Anār (*Punica granatum* L.)	BY [1,2,3]	*Mujaffif,*[2] *Qābiḍ,* [1,2] *Muḥallil, Ḥabis*[2]	Antioxidant[54] Hepatoprotective[55] Anti-inflammatory[56]	Polyphenols, gallic acid, catechin, quercetin, rutin, flavonols, Anthocyanidins[55]
***Pudīna* leaf** (*Mentha arvensis* L.)	H1,[2] Y2 [2,3]	*Muḥallil, Mulaṭṭif,* [1,2,3] *Muqawwī-i-Mi'da,*[2] *Muqawwī-i-Litha,*[1] *Qābiḍ*[3]	Hepatoprotective[57] Antioxidant[58] Anti-inflammatory[59]	Alkaloids, flavonoids, polyphenols, tannins, cardiac glycosides, Triterpenoids[58]

Drug name	*Mizāj*	*Af'āl* (Actions)	Reported activities	Chemical constituents
***Pīpal* leaf** (*Ficus religiosa* L.)	HY,[1,2] BY [3]	*Qābiḍ*,[3] *Mujaffif*,[2] *Muqawwī-i-Bāh*, *Muḥallil-i-Waram*[1,2,3]	Antioxidant[60] Hepatoprotective[61] Anti-inflammatory[62]	Tannins, glycosides, saponins, flavonoids, carbohydrates[61]
***Kā'iphal* bark** (*Myrica nagi* L.)	H2, Y2 [1,2,3]	*Muḥallil, Qābiḍ, Muḥallil-i-Waram,* [1,2,3] *Mushil-i-Sawdā'*,[3] *Muqawwī-i-Mi'da wa Bāh*,[1,3] *Mujaffif* [2]	Antioxidant[63] Anti-inflammatory[64]	Flavonoids, steroids,[64] phenols[63]
Hadjor (*Cissus quadrangularis* L.)	H,Y [1]	*Muqawwī, Musakkin-i-Alam, Mushtahī* [3]	Antioxidant[65] Hepatoprotective[66] Anti-inflammatory[67]	Ascorbic acid, carotene, calcium[65]
Siras (*Albizia lebbeck* (L.) Benth.)	H2,[2] Y2 [2,3]	*Muqawwī-i-Litha wa Dandān*,[1,3] *Muḥallil, Mujffif, Muṣaffi-i-Khūn, Muqawwī* [2]	Antioxidant[68] Anti-inflammatory[69]	Tannins, phenols, alkaloids, steroids, triterpenoids, glycosides, saponins, anthroquinones [68,69]
Nār Mushk (*Mesua ferrea* L.)	H2, H3, Y3 [1,3]	*Mujaffif*,[2,3] *Muqawwī-i-Mi'da, Mulaṭṭif*,[1,2,3] *Qābiḍ*,[3] *Muḥallil-i-Riyāḥ*[1,3]	Hepatoprotective[70] Antioxidant[70] Anti-inflammatory[71]	Phenol[70]
Baheman (*Centaurea behen* L.)	H1,[2,3] Y1[2]	*Muḥallil-i-Riyāḥ*[1,3] *Muqawwī-i-Bāh*,[1,2,3] *Qābiḍ, Mulaṭṭif* [3]	Antioxidant[72] Hepatoprotective[73]	Phenols, flavonoid[72]
Gulnār (*Punica granatum* L.)	B1,[1,2,3] Y2 [1,3]	*Qābiḍ, Mujaffif, Ḥābis*,[1,3] *Mundamil-i-Qurūḥ*,[3] *Muqawwī-i-A'ḍā', Litha wa Dandān* [1,3]	Antioxidant[74] Hepatoprotective[75] Anti-inflammatory[76]	Alkaloids, saponins, tannins, coumarins, terpenoids, steroids, protein, carbohydrates,[75] phenols, flavonoids[75,76]
***Babūl* bark** (*Acacia nilotica* L.)	B1,Y1,[2] Y2,[3] B2[1]	*Muqawwī*,[1,3] *Qābiḍ*,[2] *Mujaffif*,[1,2] *Muḥallil-i-Riyāḥ*,[3] *Muqawwī-i-Litha wa Dandān*[1,3]	Antioxidant[77] Hepatoprotective[78] Anti-inflammatory[79]	Flavonoid[78,79] phenols, amino acids,[78] alkaloids, glycoside, saponins, tannins steroids[79]
Kāth (*Acacia catechu* Willd.)	B Y2 [2,3]	*Qābiḍ, Muṣaffī-i-Khūn, Mujaffif, Qātil-i-Kirm Shikam*[2,3]	Antioxidant[80] Anti-inflammatory[80] Hepatoprotective[81]	Catechins, epicatechins, flavonoids[80]

Abbreviation: B=*Bārid* (cold), H=*Ḥārr* (hot), R=*Raṭb* (moist), Y=*Yābis* (dry); 1, 2, 3=first, second, third degree of *Mizāj*

Sources: 1(Mohammad, 2002), 2(Kabiruddin, 2007), 3(Ghani, YNM), 4(Liu *et al*., 2008), 5(Bhuvaneswari *et al*., 2014), 6(Golechha *et al*., 2014), 7(Singh *et al*., 2016), 8(Han *et al*., 2017), 9(Elmastaș *et al*., 2006), 10(Chaudhari *et al*., 2016), 11(Shahriar *et al*., 2012), 12(Halder *et al*., 2009), 13(Prasad *et al*., 2012), 14(Konda *et al*., 2013), 15(Verma *et al*., 2010), 16(Rossi *et al*., 2009), 17(Kumar *et al*., 2011), 18(Faiza *et al*., 2011), 19(Sahranavard *et al*., 2014), 20(Kang *et al*., 2014), 21(Chavan *et al*., 2010), 22(Darshan *et al*., 2012), 23(Gupta *et al*., 2016), 24(Guleria *et al*., 2010), 25(Pingale, 2011), 26(Peshin *et al*., 2017), 27(Elhassaneen *et al*., 2013), 28(Koochek *et al*., 2003), 29(Sivaraj *et al*., 2017), 30(Ozaki *et al*., 1989), 31(Khan *et al*., 2011), 32(Phaechamud *et al*., 2009), 33(Pithayanukul *et al*., 2009), 34(Segovia *et al*., 2014), 35(Hamed *et al*., 2015), 36(Conforti *et al*., 2008), 37(Valiollah *et al*,. 2010), 38(Achuthan *et al*., 2003), 39(Rani *et al*., 2016), 40(Balakrishna *et al*., 2017), 41(Kota *et al*., 2010), 42(Shankar *et al*., 2012), 43(Kumar *et al*., 2012), 44(Durgawale *et al*., 2017), 45(Sugunavarman *et al*., 2013), 46(Sudheendran *et al*., 2017), 47(Rao *et al*., 2006), 48(Charde *et al*., 2010), 49(Sehrawat *et al*., 2006), 50(Chaturvedi *et al*., 2012), 51(Lodhi *et al*., 2012), 52(Rao *et al*., 2013), 53(Kaur *et al*., 2004), 54(Salwe *et al*., 2015), 55(Khan *et al*., 2017, 56(Labib *et al*., 2015), 57(Patil *et al*., 2012), 58(Ameen *et al*., 2017), 59(Verma *et al*., 2003), 60(Al-Ezzy *et al*., 2017), 61(Selvan *et al*., 2017), 62(Charde *et al*., 2010), 63(Rana *et al*., 2014), 64(Patel *et al*., 2011), 65(Prabhavathi *et al*., 2016), 66(Swamy *et al*., 2010), 67(Panthong *et al*., 2007), 68(Ariharasiva kumar *et al*., 2014), 69(Babu *et al*., 2009), 70(Rajopadhye *et al*., 2012), 71(Tiwari *et al*., 2012),72(Chougule *et al*., 2012),73(Pushplata *et al*., 2014), 74(Nalini *et al*., 2015), 75(Kau *et al*., 2006), 76(Xua *et al*., 2017), 77(Hegazy *et al*., 2013), 78(Verma *et al*., 2014), 79(Safari *et al*., 2016), 80(Stohs, 2015), 81(Pingale, 2010).

Results and Discussion (Table 2 & 3)

Table 2: Variation in *Afʿāl* of *ʿAfiṣ* taste drugs

S. No.	*Afʿāl* of *ʿAfiṣ* taste Unani drugs	Drugs having common *Afʿāl*	% age
1.	*Qābiḍ*	25	83
2.	*Muḥallil* (*Waram and Riyāḥ*)	20	66
3.	*Muqawwī-i-Miʿda*	16	53
4.	*Mujaffif*	13	43
5.	*Muqawwī-i-Bāh*	10	33
6.	*Muqawwī-i-Litha*	9	30

Table 3: Variation in reported activities of drugs of *ʿAfiṣ* taste drugs

S. No.	Common reported activities of acrid Unani drugs	No. of drugs having common reported activities	% age
	Antioxidant	30	100
	Anti-inflammatory	23	93
	Hepatoprotective	19	90

In Unani Medicine, taste is described under the heading of *Ṭaʿm* which is an Arabic word. Taste is defined as a sensation which can be immediately felt

and described in actual terms or feelings (Khan, 2012). Taste is classified into nine types – *Ḥulw* (*Shīrīn*/sweet), *Ḥirrīf* (*Charparā*/pungent), *Ḥāmiḍ* (*Khattā*/sour), *Dasm* (*Rowghani*/fatty), *Murr* (*Kadwā*/bitter), *'Afiṣ* (*Kasīla*/acrid), *Māliḥ* (*Namkīn*/salty), *Qābiḍ* (astringent) and *Tafih* (*Phīkā*/tasteless). These nine types are classified by ancient Unani physicians, while modern Unani physicians classify them into eight types (Danish, 2016; Khan, 2012). They also stated that those substances which bear a certain taste would be dense and earthy, tenuous or moderate in these attributes (*Jawhar*). In potency (*Quwā*), it would be hot, cold, or moderate. Now if the dense and earthy substance is hot, it would be bitter; if it is cold, it would be acrid; and if it is moderate, it would be sweet. In case of a substance being tenuous, if it is hot, it would be pungent; if it is cold, it would be sour and if it is moderate, it would be greasy, if the substance being hot, is of moderate density and tenuity it would be salty and if it is cold it would be astringent, if the substance is moderate in coldness and in hotness, according to physicians it would possibly be insipid (Danish, 2016, Khan, 2012).

The interrelated concepts are well-expressed in terms of *Mizāj* of a drug which is stated in reference to its (*Tāthīr*) in the body. To ascertain *Mizāj* of drugs, Unani Medicine follows the procedure of *Qiyās* and *Tajraba*. *Qiyās* predicts the action of a drug through the probable *Mizāj* whereas *Tajraba* confirms the same. *Qiyās* remains important step in assessing the drug action. The determinants of *Mizāj* through *Qiyās* are organoleptic characters and physical properties of the drug. Among organoleptic characters, taste is considered vital determinant followed by smells and then colours (Khan, 2012; Ibn Sina, 1998). The reason is that it is felt just when it meets the faculty of taste. In case of odour some vapors emanating from the rarefied parts of the drug are felt whereas no vapors arise from the condensed part of that drug. Similarly, a colour which is perceptible may be the colour of the external surface and not of the hidden part of the drug. Sometimes odours indicate taste, such as sweat odour, sour odour, pungent or bitter odour. This shows that the taste is the most precise in giving out the nature of a drug, and then comes odour and colour (Danish, 2016; Khan, 2012).

The observation of the study was utilized to see the correlation of *Afʿāl,* reported activities and the chemical composition of acrid taste drugs along with their *Mizāj*. Survey showed that out of 30 acrid drugs, 25 (83%) were ascribed with *Qābiḍ* (astringent) action. In this respect, it was noted that most of the drugs with *Muqawwī* (tonic) action were specific as *Muqawwī-i-Miʿda* (stomach tonic) (53%), *Muqawwī-i-Bāh* (aphrodisiac) (33%) and *Muqawwī-i-litha* (30%). It was also noted that 20 drugs (66%) had *Muḥallil* (resolvent) action, while 43% of the drugs were specific as *Mujaffif* (desiccant) action. The same drugs which have been subjected to screening of the pharmacological activities, the common reported activities that was observed as antioxidant (100%) and 93% were having anti-inflammatory activity while 90% have hepatoprotective action.

Table 4: Technical terms and their equivalents

Technical Unani Terms	English Equivalents
Af'āl	Pharmacological actions
Mulaṭṭif	Attenuant
'Afiṣ	Acrid
Mulayyin	Laxative
Bārid	Cold
Burūdat	Coldness
Dasm	Fatty/oily
Dāfi'-i-Jarayān	Antihemorrhagic
Dāfi'-i-Ta'affun	Antiseptic
Ḥābis	Styptic
Hāḍim	Digestive
Ḥāmiḍ	Khatta/sour
Ḥārr	Hot
Ḥarārat	Heat
Ḥirrīf	Charpara/pungent
Ḥulw	Shireen/sweet
Jādhib	Desiccant
Jawhar	Attributes
Kāsir-i-Riyāḥ	Carminative
Māliḥ	Namkeen/salty
Mizāj	Temperament
Mizāj Adwiya	Temperament of drug
Mu'addil-i-Ṣafrā'	Neutralize bile
Mubarrid	Refrigerant
Mudirr-i-Bawl	Diuretic
Mudirr-i-Ḥayḍ	Emmenagogue
Mufattiḥ-i-Sudad	Deobstruent
Mufrad	Single
Mughadhdhī	Nutritious
Muḥallil	Resolvent
Muḥallil-i-Riyāḥ	Antiflatulent
Muḥallil-i-Waram	Anti-inflammatory

Technical Unani Terms	English Equivalents
Muḥallil-i-Waram-i-Ṭiḥāl	Spleen anti-inflammatory
Muḥarrik-i-Ishtihā'	Appetite stimulant
Mujaffif	Desiccant
Mulayyin-i-Ṭaba'	Demulscent
Mundamil-i-Qurūḥ	Wound healing agent
Munḍij	Concoctive
Muqawwī	Tonic
Muqawwī-i-A'ḍā' Ra'īsa	Visceral tonic
Muqawwī-i-Bāh	Aphrodisiac
Muqawwī-i-Dimāgh wa Aḥshā'	Brain and visce tonic
Muqawwī-i-Kabid	Liver tonic
Muqawwī-i-Mi'da	Stomach tonic
Muqawwī-i-litha wa Dandāṉ	Teeth & gum tonic
Muqawwī-i Qalb	Cardiac tonic
Murr	Kadwa/bitter
Murakkab al-Quwā	Multiple actions
Muṣaffi-i-Khūn	Blood purifier
Musakkin-i-Alam	Analgesic
Mushil-i-Balgham	Phlegm purgative
Mushil-i-Ṣafrā'	Bile purgative
Mushil-i-Sawdā'	Melancholic purgative
Mushtahī	Appetizer
Muzalliq	Demulscent
Qābiḍ	Astringent
Qātil-i-Kirm-i-Shikam	Vermicides
Qiyās	Analogy
Quwā	Potency
Raṭab Mizāj	Moist temperament
Tajraba	Experiment
Ṭa'm	Taste
Tāthīr	Effect of the drug
Tafih	Pheeka/tasteless
'Unṣur-i-Arḍ	Earthy matter
Yābis Mizāj	Dry temperament

In spite of chemical composition of these drugs observed to be of highly varied nature, most of the drugs have tannin, flavonoid and phenolic compounds in their chemical composition besides alkaloids and glycosides. Saponins, carbohydrates, triterpenoids and sterols, etc. are other chemical constituents present in these drugs. All the drugs of acrid taste have *Yābis Mizāj* with *Ḥarārat* and *Burūdat* except *Gul-i-Banafsha* which has *Raṭb Mizāj* with *Ḥarārat* and *Burūdat*. In Unani literature, it is mentioned that *Jawhar* of acrid taste substance is dense and earthy, and it is also mentioned that due to the inclination of acrid taste drugs towards *'Unṣur-i-Arḍ* because of these substances acrid taste drugs may have *Yābis Mizāj*.

The most common pharmacological action observed was *Qābiḍ* (astringent) action. Astringent drugs create density in the state of parts of an organ and obstruct the channels due to its excessive movements. From the observation table, it was found that most of the drugs have tannin as chemical composition, and tannins are responsible for astringent action because either they bind and precipitate or shrink proteins. Tannin containing drugs have been used traditionally as styptic and internally for the protection of inflamed surfaces of mouth and throat. Actions related to reducing tissue stress load like antioxidant action may be associated with *Muqawwī* actions as described in Unani Medicine.

The most common reported activity of *'Afiṣ* drugs was antioxidant (100%). Out of 30 drugs, 24 drugs have phenols, 26 drugs have flavonoid and 15 drugs have tannins, which are responsible for antioxidant activity (Table 1).

Conclusion

Much information is available regarding reported pharmacological activities of many drugs in reputed journals on internet and many standard books reporting the pharmacological studies done on herbal or traditional drugs and after study it was observed that most of the actions claimed in classical texts were also reported for similar pharmacological activity.

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सारांश

यूनानी चिकित्सा की औषधियों के *अफ़िस* (ऐक्रिड) स्वाद, फाइटोकेमिस्ट्री और फार्माकोलॉजिकल क्रियाओं के मध्य संबंध

***ख़दीजा अब्दुल हफ़ीज, प्रो. ग़ुलामुद्दीन सोफ़ी, ग़ौसिया इस्लाम**

सारांश

यूनानी चिकित्सा की औषधियां विभिन्न माध्यमों से स्थापित की गईं हैं जिनमें इनके *मिज़ाज* (स्वभाव) का निर्धारण महत्वपूर्ण है। औषधियों का *मिज़ाज क़ियास* (समानता) के माध्यम से निर्धारित किया जाता है जो संभावित *मिज़ाज* का आकलन करता है और फिर *तजर्बा* (प्रयोग) औषधि के *मिज़ाज* की पुष्टि करता है। स्वाद *मिज़ाज* के मूल्यांकन तथा मनुष्य में औषधि क्रिया के लिए महत्वपूर्ण निर्धारक है। वर्तमान अध्ययन यूनानी साहित्य में वर्णित *अफ़आल* (फार्माकोलॉजिकल क्रियाओं) के अनुसार *अफ़िस* (ऐक्रिड) स्वाद वाली औषधियों की खोज करता है और रिपोर्ट की गई औषधीय गतिविधियों और औषधियों के रासायनिक घटकों के साथ इस विशेष स्वाद के संबंध की भी खोज करता है। यूनानी साहित्य से तीस *अफ़िस* (ऐक्रिड) स्वाद वाली औषधियों के *अफ़आल* और *मिज़ाज* रिकॉर्ड किए गए और रिपोर्ट की गई औषधीय गतिविधियों और औषधियों के रासायनिक घटकों को अनुक्रमित पत्रिकाओं और अन्य संबंधित उपलब्ध साहित्य नोट किया गया। स्वाद, *अफ़आल*, रिपोर्ट की गई गतिविधियों और रासायनिक घटकों के बीच संभावित संबंध का पता लगाया गया। औषधियों के स्वाद, *मिज़ाज*, *अफ़आल*, रिपोर्ट की गई गतिविधियों और रासायनिक घटकों के बीच काफी हद तक सहसंबंध देखा गया। सबसे साधारण पाए गए *अफ़आल क़ाबिज़* (एस्ट्रिन्जेन्ट), *मुहल्लिल* (रिज़ोलवेन्ट), *मुक़व्वी* (टॉनिक) थे जबकि सबसे साधारण रिपोर्ट की गई क्रिया एंटीऑक्सीडेंट गतिविधि थी। विचाराधीन कुछ औषधियों में एंटी–इन्फ्लामेटरी और हेपेटोप्रोटेक्टिव गतिविधियां भी पाई गईं। अध्ययन के लिए चुने गए *अफ़िस* स्वाद औषधि नमूनों से इन के स्वाद, *मिज़ाज*, *अफ़आल*, रिपोर्ट की गई गतिविधियों और रासायनिक घटकों के बीच एक सकारात्मक संबंध का पता चला।

शब्दकुंजी: *अफ़आल*, *अफ़िस*, *मिज़ाज*, *क़ियास*, *तजर्बा*, फाइटोकेमिस्ट्री, यूनानी

Current Perception and Practice of Using *Ḥabb Muṣaffī-i-Khūn* in Cancer Management

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Abstract

Background: *Ḥabb Muṣaffī-i-Khūn* (HMK) is a Unani formulation used traditionally as a blood purifier. The individual ingredients of HMK have reported anti-cancer activity, however, its use in the management of cancer has not been yet reported. Thus, a survey was carried out to find out the current perception and practice of Unani doctors towards using HMK in cancer management.

Methodology: After taking approval from Institutional Ethics Committee, a questionnaire was prepared and sent to 100 Unani physicians (Hakims) and their opinion regarding the usage, dosage, safety and tolerability of HMK was documented.

Results: HMK was found to be used in the treatment of blood related disorders by ~66% of Hakims. It has been usually prescribed alone or in combination with other drugs to enhance its effects or to increase its absorption. The average efficacy rate for HMK usage in blood disorders was found to be 8 (scale of 1 to 10). About 39% of Hakims perceived that HMK could be used in the management of various cancers such as breast, prostate, lung, leukemia, oral, renal, bladder and skin.

Conclusion: HMK is largely being used in the treatment of blood related disorders by Hakims in their clinical practice and is perceived to play a significant role in the management of cancers.

Keywords: *Ḥabb Muṣaffī-i-Khūn*, Survey, Hakims, Perception, Anticancer drug, Practice

Introduction

Cancer is regarded as one of the important causes of mortality and morbidity worldwide (Rafiemanesh *et al.*, 2016). The currently available cancer therapies have serious side effects and thus for more than a decade now, cancer research has shifted its focus towards natural products as well as traditionally used herbal remedies for possible use as adjunct therapies (Savjiyani *et al.*, 2012). The most recent systematic review of cancer patients surveyed globally has shown an increase in the use of Complementary and Alternative Medicine (CAM) (Tangkiatkumjai *et al.*, 2020).

Unani Medicine, one of the branches of Complementary and Alternative Medicine (CAM), is based upon Hippocratic theory of four humors, viz., blood, phlegm,

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yellow bile and black bile (Hongal *et al.*, 2014). Any change in the proportion of these humors, in terms of quality and quantity, modulates the viscosity of the blood. Unani blood purifying drugs with their heat, cold, dry and wet properties maintain the viscosity of the blood. Moreover, these drugs have anti-cancer, anti-oxidant, anti-inflammatory and immunomodulatory activities (Mushir *et al.*, 2019). In Unani text, cancer (*Saraṭān*) has been described as a malignant and melanotic swelling, which spreads rapidly in the body (Naz and Ahmad, 2017). According to Ibn Sina, cancer is a disease caused by accumulation of black bile produced from combustion of yellow bile (*Mirra-i-Sawdā'*) (Fahad and Shameem, 2018). Various *in-vitro* and *in-vivo* studies have reported anticancer activity of Unani medicinal plant extracts such as *Nigella sativa*, *Cuscuta reflexa* Roxb. in different human cancer cell lines and animal models (Al-Sheddi *et al.*, 2014; Ali *et al.*, 2020).

Ḥabb Muṣaffī-i-Khūn (HMK), a Unani compound formulation, is used as a blood purifier and for the treatment of boils, scabies, acne, pimples and problems associated with *Fasād i-Dam* (imbalance of humours). It is a unique combination of 16 medicinal plants that include *Berberis aristata, Cassia absus, Terminalia chebula, Trichoderma glaberrima, Cuminum cyminum, Tephrosia purpurea, Pterocarpus santalinus, Fumaria parviflora, Lawsonia inermis, Piper nigrum, Melia azadirachta, Melia azedarach, Coriandrum sativum, Bauhinia recemosa, Rosa damascene* and *Genitalia kurroo* Royale (Unani Pharmacopoeia of India). In Unani texts, most of the ingredients of HMK have been mentioned to be used in the management of *Sawdā'* and anti-cancer activity of most of the ingredients have also been proven scientifically (Kim *et al.*, 2008; Prasanna *et al.*, 2009; Pai *et al.*, 2012; Kapadia *et al.*, 2013; Rajkapoor *et al.*, 2013; Nithya and Sumalatha Jan, 2014; Serasanambati *et al.*, 2015; Santha and Dwidedi, 2015; Nahata *et al.*, 2017; Padmapriya *et al.*, 2017)

Based on the above properties of HMK, a survey was conducted wherein the opinion of Unani physicians (Hakims) regarding the proposed use of HMK as an anti-cancer drug as well as its current practice, safety and tolerability was recorded.

Materials and Methods

Study Design

A mixed-methods study, comprising survey and interview of Hakims from different cities of India, was conducted after taking approval from Institutional Ethics Committee. The survey was conducted at the Unani CME Conference held at Azam Campus, Pune.

Development of Survey Questionnaire

Literature search using the available databases (Ayush Research Portal, PubMed, Google Scholar) was carried out to collect information and define the theme for developing the questionnaire. A characteristic questionnaire was designed in order to collect the information related to the clinical use of HMK. The questionnaire was directed to 100 Unani physicians (Hakims) through personal communications as well as through emails and phone calls. All the Unani physicians filled it completely to the best of their knowledge and clinical expertise.

Questionnaire Structure

The survey was carried out with a set of questions that included experience in practicing Unani Medicine (in years); frequency and usage of HMK; opinion about the medicine as a blood purifier; opinion about the medicine with anticancer activity and if they had ever prescribed it to cancer patients; whether HMK was prescribed individually or in combination with other Unani formulations; number of tablets and duration prescribed; opinion on mechanism of action on humor and its type; efficacy and safety of the drug; name of the brand commonly used (supplementary information). All the questions were open ended. Questionnaire was given to the Hakims after explaining the study objectives and they were asked to revert with duly filled document.

Statistical Analysis

The survey results and the quantitative data have been reported descriptively. For qualitative variables, frequency with percentage was used to describe the data. Chi-square tests and binary logistic regression were used to compute bivariate odds ratio (OR) to evaluate the preference for using HMK as a blood purifier or as an anticancer drug.

Results

The survey was conducted with a sample size of 100 Unani physicians. The experience of Hakims practicing Unani Medicine varied: 37% with 2-3 years, 20% with 3-5 years, 20% with 5-10 years and 23% with more than 10 years. Survey revealed that 66% Hakims used HMK for the treatment of blood related disorders, specifically skin disorders and remaining 34% used the formulation along with other blood purifiers. The frequency of using HMK in the clinical practice varied among the Hakims: 41% used it frequently, 58% used it sometimes, and only 1% never used the drug. Interestingly, 98% Hakims ($p<0.0001$, OR-4.737; 95% CI: 2.607 to 8.609) recognized HMK to be an effective blood purifier. The odds ratio higher than one indicates Hakims preferably consider HMK as an effective blood purifier.

In the present survey, the physicians had different opinions regarding the mechanism of action of HMK: 45% assumed that HMK cures blood disorders by acting on blood (*Dam*), 20% assumed that it acts on yellow bile (*Ṣafrā'*), and 35% assumed its action on black bile (*Sawdā'*) (Fig. 1). This data suggested that since cancer was caused due to combustion of yellow bile and accumulation of black bile, HMK could help in controlling the progression of the disease. Thus, the opinion of Hakims was taken for the use of HMK as an anti-cancer drug. Only 39% Hakims suggested that HMK could be used in the treatment of different types of cancer such as leukemia, skin, renal and bladder.

Ḥabb Muṣaffī-i-Khūn (*Ḥabb* means pill), commonly given in the form of pills, was prescribed by Hakims along with other Unani formulations (mostly blood purifiers) that include *Rakt Ṣafā*, *Qurṣ al-Kalī*, *Sharbat-i-'Unnāb, Ma'jūn 'Ushba*, *Ma'jūn Muṣaffī-i-Khūn, 'Araq Muṣaffī-i-Khūn*, *Iṭrīfal Shāhitara*, *'Araq-i-Mundī*, *Mā' al-'Asal*. About 28% Hakims prescribed HMK to be given alone for blood purification, 50% prescribed it along with other Unani formulations and 22% believed that it could be given both either alone or in combination with other Unani formulations as per the patients' requirements.

The dose of HMK suggested by the doctors varied in different patients: 90% prescribed 2 pills (about 0.5 g) BD, and 10% prescribed one pill TDS (Fig. 2). The duration of the dosage prescribed also varied from patient to patient: 33% prescribed for one month, 20% for 2 months and 47% for 3 months (Fig. 2). The Hakims (99%) shared their experience about the beneficial effects of HMK in terms of excellent tolerability in the patients without any side effects. The prominent beneficial effect of the drug was found to be improvement in the skin related problems, with an efficacy of 8 (on the scale of 1 to 10). Only 1% doctors observed that the patients with hyper acidity experienced burning sensation in the chest after consumption of HMK.

Different Hakims prescribed different manufacturing brands of the drug. For example, 33% Hakims prescribed HMK from Hamdard; 8% made the pills

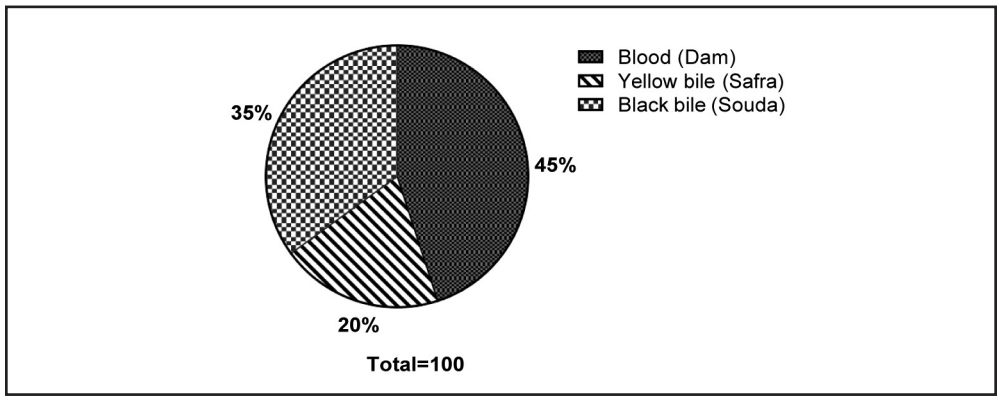

Figure 1: Pie chart representing mechanism of action of HMK as per Unani concept of cancer

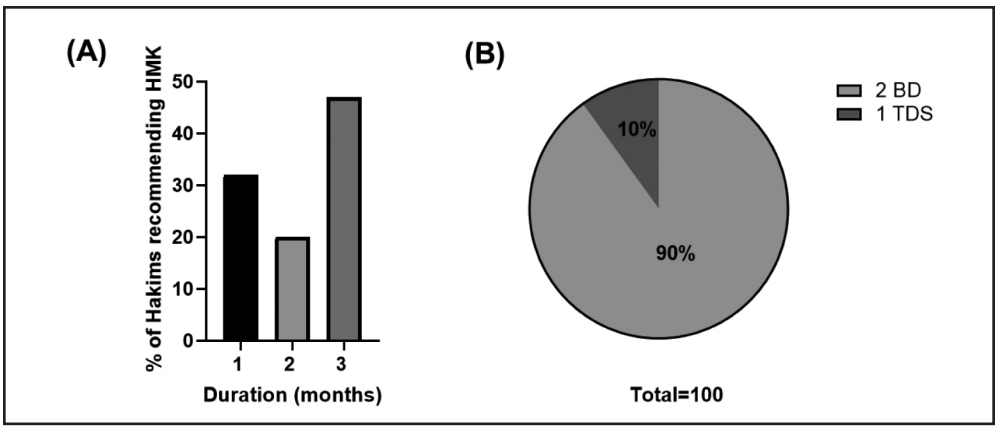

Figure 2: Recommendation of HMK by Hakims - (A) Number of HMK pills prescribed (B) Duration (months) of HMK to be taken

themselves as per the formulary of Unani Medicine; 7% prescribed it from Shama Herbals, 4% from Puritex and 7% from Tayyebi Dawakhana Unani (Indore) Pvt. Ltd., 4% recommended from Hermas Biotech Pvt. Ltd., 1% from Rex Remedies and 1% from Uniherb. Remaining 35% Hakims didn't comment upon any specific manufacturing company.

Discussion

As per the Unani concept, blood is considered to have different humours (*Akhlāṭ*) such as *Dam* (sanguine), *Balgham* (phlegm), *Ṣafrā'* (yellow bile) and *Sawdā'* (black bile) (Mushir *et al.*, 2019). Each person has a unique and balanced combination of different humours, which may get disturbed under the diseased conditions. According to the Unani principles, cancer is commonly produced from burning of black bile (*Iḥtirāq-i-Sawdā'*) and sometimes from burning of altered yellow bile and impure phlegm (*Iḥtirāq-i-Ṣafrā'-o-Balgham*). The causative material of cancer possesses heat, which helps cancer to progress rapidly. Galen had hypothesized in his book (Methods of Treatment) that cancer was a disease associated with black bile and had proposed its removal from the body with administration of appropriate purgative, which can prevent generation and accumulation of black bile in the blood vessels (Emami *et al.,* 2012). Galen had mentioned in his book entitled "Thick Substances with Abnormally High Concentrations" that humour was warm and could lead to ulcerative cancer. The vasculature of cancerous tissue being hyperemic and the blood contents being darker than the other swellings (Emami *et al.,* 2012), cancer could be cured by a drug that would prevent the combustion of yellow bile and phlegm, and thus would purify the blood.

Unani medicines, with blood purifying properties, could expel the excessive humours (either yellow or black bile) and maintain their normal balance

within the body. In Unani Medicine, cancer can be treated with the medicines having resolvent and repellent properties (Ibn Sina, 2010). Resolvent medicines help to subside the swelling whereas repellant medicines repel the excessive humour (e.g., yellow bile in case of *Saraṭān* in blood) that is balanced by equipoise medicine. HMK is a Unani formulation, used as a blood purifier and for the treatment of boils, scabies, acne, pimples and problems associated with *Fasād al-Dam* (imbalance of humours). Components of *Ḥabb Muṣaffī-i-Khūn* are known to have resolvent, repellant and equipoise properties e.g., *Berberis aristata*, *Coriandrum sativum*, *Santalum album* and *Pterocarpus Santalinus* have repellent properties. *Tephrosia purpurea, Sphaeranthus indicus*, *Tricholepis angustifolia*, *Chrozophora plicata*, *Fumaria officinalis*, *Lawsonia inermis*, *Melia azadirachta, Melia azedarach*, *Hedysarum pseudalhagi* have equipoise property, which counterbalances the yellow bile in the blood. On the other hand, *Cassia absus* has resolvent property (Kabeeruddin *et al.,* 2007).

A survey was conducted to know about the perception and practice of using HMK in cancer management. The survey revealed that being an excellent blood purifier, HMK was frequently recommended for blood related disorders, while some of the Hakims perceived its use for the management of cancer. Most of the ingredients of HMK formulation have been reported to exhibit anti-cancer activity. For instance, *B. aristata* has been reported to exhibit anticancer activity against human breast cancer cells (MCF-7) (Serasanambati *et al.,* 2015), and Ehrlich ascites mouse model carcinoma (Pai *et al.,* 2012). *C. absus* has been shown to induce cell cycle arrest and apoptosis in human breast (MCF-7) and larynx cancer (Hep-2) cells (Prasanna *et al.,* 2009). *S. indicus* has been reported to induce apoptosis in leukemia (HL-60) cells (Nahata *et al.,* 2017) while *T. purpurea* exhibited anti-cancer activity against hepatocellular carcinoma cells (HCC) (Padmapriya *et al.,* 2017). *S. album* showed anticancer activity against melanoma, breast and prostate cancer cells and in vivo skin cancer mouse models (Santha & Dwidedi, 2015). *P. Santalinus* has been shown to inhibit the proliferation of cervical (HeLa) (Kim *et al.,* 2008). *L. inermis* exhibited anti-cancer activity against skin cancer mouse model (Kapadia *et al.,* 2013). Cytotoxic activity of crude extract of *M. azedarach* was reported against HT-29, A-549, MCF-7, HepG-2 and MDBK cell lines (Kapadia *et al.,* 2013). *C. sativum* has been shown to inhibit the growth of HT- 29 cells (Nithya & Jain, 2014). The antitumor activity of ethanol extract of *Bauhinia variegata* (EBV) was reported against Ehrlich ascites carcinoma (EAC) in Swiss albino mice (Rajkapoor *et al.,* 2013).

The study also revealed that HMK was prescribed either individually or along with other Unani medications. These included *Rakt Ṣafā*, *Qurṣ al-Kalī*, *Sharbat-i-'Unnāb, Ma'jūn 'Ushba*, *Ma'jūn Muṣaffī-i-Khūn, 'Araq Muṣaffī-i-Khūn*, *Iṭrīfal Shāhitara*, *'Araq-i-Mundī*, *Mā' al-'Asal* having blood purifying and anti-

inflammatory properties that could enhance curative effect of HMK in blood disorders. The variable dosage and duration pattern of HMK in the patients highlighted the personalized treatment approach followed by the Unani practitioners. Traditional drugs have been generally considered to be safe for human consumption (Mukherjee, 2001), and this was confirmed from our study, wherein HMK was demonstrated to be safe in the patients.

The overall data suggested that HMK, being an excellent blood purifier, was perceived to be used in the management of cancer based upon its property of counterbalancing *Sawdā'* and *Ṣafrā'* found in the cancer.

Conclusion

The survey revealed that HMK was currently being used in the treatment of blood disorders and could balance the body humours and their production, which is important in cancer management. As suggested by the Hakims, HMK cures blood impurities by acting on black or yellow bile, thereby helping in controlling the cancer progression. Thus, future studies are warranted to explore the potential of HMK as an anticancer drug.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary Information

Questionnaire used in the survey to understand current perception and practice of using *Ḥabb Muṣaffī-i-Khūn* in cancer management

Questionnaire

1. **Since how many years are you practicing Unani medicines?**
   a) 2 to 3 years
   b) 3 to 5 years
   c) 5 to 10 years
   d) More than 10 years
2. **What do you prescribe for blood purification?**
3. **How frequently do you prescribe Habbe Musaffi Khoon for blood purification?**
   a) Very frequently
   b) Some times
   c) Never
4. **What do you think, is Habbe Musaffi khoon a good medicine (blood purifier)?**
   a) Yes
   b) No
5. **Have you ever prescribed Habbe Musaffi khoon for cancer patient?**
   a) Yes
   b) No
6. **What is your opinion, which cancer it can be prescribed for?**
7. **Do you prescribe Habbe Musaffi Khoon individually or in combination?**
8. **How many tablets and for how much duration?**
9. **What is the mechanism, on which humour does it affect?**
   a) Blood (Dam)
   b) Phlegm (Balgham)
   c) Yellow Bile (safra)
   d) Black Bile (souda)

सारांश

कैंसर के उपचार में *हब्बे मुसफ्फी–ए–खून* के उपयोग की वर्तमान धारणा और अभ्यास

आलिया ख़ान, निधि शर्मा, ग़ज़ाला मुल्ला, *रुचिका कौल–घनेकर

सारांश

पृष्ठभूमिः *हब्बे मुसफ्फी–ए–खून* (एचएमके) एक यूनानी मिश्रण है जिसे पारंपरिक रूप से रक्त शोधक के रूप में उपयोग किया जाता है। एचएमके के विशेष घटकों में कैंसररोधी गतिविधियां पाई गईं हैं, हालांकि कैंसर के उपचार में इसके उपयोग की अभी तक पुष्टि नहीं हुई है। इस प्रकार कैंसर के उपचार में एचएमके का उपयोग करने हेतु यूनानी डॉक्टरों की वर्तमान धारणा और अभ्यास का पता लगाने के लिए एक सर्वेक्षण किया गया।

विधिः संस्थागत नीति समिति से स्वीकृति लेने के बाद एक प्रश्नावली तैयार कर 100 यूनानी चिकित्सकों (हकीमों) को भेजी गई और एचएमके के उपयोग, खुराक, सुरक्षा और सहनशीलता से संबंधित उनकी धारणा का दस्तावेजीकरण किया गया।

परिणामः रक्त संबंधी विकारों के उपचार में एचएमके का उपयोग ~66% हकीमों द्वारा पाया गया। यह आमतौर पर अकेले या अन्य औषधियों के साथ मिलाकर इसके प्रभाव को बढ़ाने या इसके अवशोषण में वृद्धि के लिए निर्धारित किया गया। रक्त विकारों में एचएमके के उपयोग की औसत प्रभावकारिता दर 8 (1 से 10 के पैमाना पर) पाई गई। लगभग 39% हकीमों ने माना कि एचएमके का उपयोग स्तन, प्रोस्टेट, फेफड़े, ल्यूकेमिया, मौखिक, गुर्दे, मूत्राशय और त्वचा जैसे विभिन्न कैंसर उपचार में किया जा सकता है।

निष्कर्षः एचएमके का उपयोग हकीमों द्वारा उनके नैदानिक अभ्यास में रक्त संबंधी विकारों के उपचार के लिए किया जाता है और माना जाता है कि यह कैंसर के उपचार में महत्वपूर्ण भूमिका निभा सकता है।

शब्दकुंजीः *हब्बे मुसफ्फी–ए–खून*, सर्वेक्षण, हकीम, धारणा, कैंसररोधी औषधि, अभ्यास

HPTLC Characterization and Quality Standards of a Herbal Unani Formulation - *Qurṣ Mafāṣil Jadīd*

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Abstract

Qurṣ Mafāṣil Jadīd (QMJ) is a multi-ingredient Unani formulation, effectively used for the management of joint pains of various etiologies. It is in tablet form and has three herbal ingredients – *Sūranjān Talkh*, *Zard Chob* and *Gond Kikar*. QMJ is a pharmacopoeial formulation mentioned in *Qarābādīn Majīdī*, a standard pharmacopoeia of Unani Medicine. Quality control of herbal formulation is utmost important to get the consistent and optimal result. Though the standardization of multi-ingredient formulation is not an easy task, simple and sophisticated techniques like TLC, HPTLC can serve the basic aim of quality control. For the purpose of getting authentic and reliable results, QMJ was subjected to HPTLC fingerprinting profile, heavy metal analysis, pesticide residue, microbial load and aflatoxin. HPTLC fingerprinting profile of QMJ was developed and detected by UV λ 366 nm, UV λ 254 nm and visible light (UV λ 580 nm). The heavy metal analysis, pesticide residue, microbial load and aflatoxin were done as per WHO guidelines. The present study laid down the standards for purity and quality of QMJ and can be used as a reference in future to maintain quality and therapeutic authenticity of the formulation. The results obtained in this study may be used as a fingerprint for future reference.

Keywords: Aflatoxin, Heavy metal, HPTLC, Microbial load, Pesticide residue, *Qurṣ Mafāṣil Jadīd*, Unani formulation, Quality control

Introduction

Unani Medicine has a long and glorious history of promotion of health as well as prevention and management of diseases through its holistic approach. It has time-tested drugs and therapies for the management of various acute and chronic ailments. It offers a large variety of pharmacological drugs and therapies for the management of various types of joint pains such as osteoarthritis. There is a long list of single and compound formulations meant for the management of joint pains. *Qurṣ Mafāṣil Jadīd* (QMJ) is a very well-known anti-arthritic Unani formulation (Anonymous, 1986). It is mentioned in *Qarābādīn Majīdī*, a standard pharmacopoeia of Unani formulations. It is made up of three ingredients, viz., *Sūranjān Talkh* (*Colchicum luteum* Baker.), *Zard Chob* (*Curcuma longa* L.) and *Gond Kikar* (*Acacia nilotica* (L.) Willd.) (Table 1, Fig. 1, 2, 3).

It is usually used in the form of tablet; the weight of tablet is 500 mg. The dose of 500 mg tablet is two tablets once a day in the morning. It is recommended for every type of joint pain.

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Table 1: Composition of *Qurṣ Mafāṣil Jadīd* (QMJ)

S. No.	Unani name	Botanical name	Part used	Quantity
1.	*Sūranjān Talkh*	*Colchicum luteum* Baker.	Corm	5 part
2.	*Zard Chob*	*Curcuma longa* L.	Rhizome	5 part
3.	*Gond Kikar*	*Acacia nilotica* (L.) Willd.	Gum	1 part

Fig. 1: *Sūranjān Talkh* (*Colchicum luteum* Baker.)

Fig. 2: *Zard Chob* (*Curcuma longa* L.)

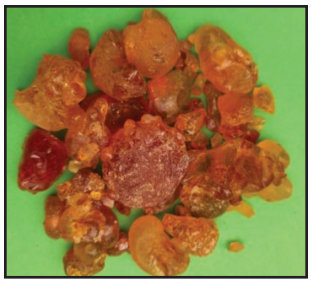

Fig. 3: *Gond Kikar* (*Acacia nilotica* (L.) Willd.)

The maintenance of quality of the herbal drugs is of prime importance as the demand of the herbal product is rising exponentially. The WHO has released guidelines for the quality control of herbal drugs and various methods have been described in various guidelines released by it (WHO, 1998a). The pharmacological effect of the drug is based on the secondary metabolites present in the plant parts in the form of alkaloids, glycosides, flavonoids, etc. The estimation of secondary metabolites in multicomponent herbal drug is not an easy task and needs sophisticated test. The adulteration and low quality crude drugs used in the manufacturing of the formulation would leads to substandard drug which might not produce desired therapeutic effects. The other aspect of the quality and safety of herbal drug is the contamination by microbes, fungus, aflatoxins, heavy metals and pesticides. The formulation having any of the contaminants above the permissible limit would be dangerous to the health of the individual (WHO, 1996).

Many herbal medicines can cause a health risk due to the presence of toxic ingredients like heavy metals. Heavy metals are dangerous in the form of their cations and are highly toxic when bonded to the short chains of carbon atoms (Kirmani *et al.*, 2011). Herbs may absorb heavy metals from soil, water or air. Medicinal herbs may be easily contaminated during growing and processing (Khan, 2008; WHO, 1998b). Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point sources such as mining, smelting and different industrial activities (Bin, 2001; Mosihuzzanman, 2008). Some other sources of soil contamination involve use of fertilizers, pesticides, sewage sludge and organic manures (Kirmani *et al.*, 2011). Furthermore, many

dangerous and lethal side effects have been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with drugs and other herbs (Mosihuzzanman, 2008).

The common effects of trace metal toxicity to living organisms include cancer, brain disorder, and gross deformities in development, carcinogenic effects and disruption of biological processes (Zevenhoven, 2001). Mercury, lead and arsenic are carcinogenic and affect the central nervous system while lead and cadmium affect the liver and kidneys (Zevenhoven, 2001; Mustafa, *et al.*, 2004; Khan *et al.,* 2008; Balammal *et al.*, 2012). Atomic absorption spectrophotometry (AAS) is the main method commonly employed to analyze the metals in trace quantities and quantitatively (Sarmani, 1999; WHO, 1998a).

Plant materials may be associated with microbial contamination by bacteria, fungi and viruses. The contamination of the drug by the microbes has adverse effect on the quality of the herbal product. Herbal drugs normally carry bacteria and molds from the soil and unscientific and unhygienic practice of harvesting, collection, drying, storage and transport may add contamination, especially E. coli or Salmonella spp. Therefore, estimation of microbial load becomes an important subject in the quality control of herbal drugs. Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopoeias, as well as in the WHO guidelines (WHO, 1998b; WHO 1998a). The fungal contamination should be monitored more carefully because some fungal species produce harmful toxin, especially aflatoxins. Aflatoxins in herbal formulations can be more harmful to health even if they are absorbed in minute amounts. Aflatoxin may be produced from the herbs during storage of the drugs (WHO, 1998a; WHO 1998b). The presence of harmful chemical pesticides should be within permissible limit to avoid harmful effects. The estimation of individual pesticide can be done by GC, MS, or GC/MS (WHO, 1998b; WHO, 2002).

Standardization of herbal drugs is an important aspect for maintaining and assessing the quality and safety of polyherbal formulations as these are combinations of more than one herb to attain the desire therapeutic effect (Sharma *et al.,* 2009). Standardization minimizes batch to batch variation; assures safety, efficacy, quality and acceptability of these herbal medicines (Ahmad *et al.,* 2006).

HPTLC is a sophisticated technique used in pharmaceutical industry for the identification and detection of adulterants, and for quality control of herbs and formulations (Soni, *et al.*, 2010). The benefit of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Accordingly, HPTLC can be used for simultaneous assay of several components in a multicomponent formulation (Thoppil *et al.,* 2001). HPTLC data can be

used as fingerprinting for the quality assessment of formulation in commercial settings. Phyto-constituents in crude drugs or formulation can be checked by standardized HPTLC methods (Dhalwal *et al.*, 2008).

Keeping in the mind the magnitude of the problem, the test drug QMJ was subjected to HPTLC fingerprinting and estimation of aflatoxin, pesticide residues, microbial load and heavy metals.

Material and Methods

Preparation of *Qurṣ Mafāṣil Jadīd*: The ingredients of QMJ were procured from the local market of Hyderabad and properly identified on the basis of Unani literature and authenticated by Pharmacy Incharge and confirmed by Incharge, SMP Unit of National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD), Erragadda, Hyderabad. The voucher specimens were preserved under SMPU/CRI-Hyd-14166, SMPU/CRI-Hyd-14167 and SMPU/CRI-Hyd-14168 for *Sūranjān Talkh* (*Colchicum luteum* Baker.), *Zard Chob* (*Curcuma longa* L.) and *Gond Kikar* (*Acacia nilotica* (L.) Willd.), respectively in SMP Unit of NRIUMSD for the purpose of record and future reference. Tablets were prepared as per the instruction given in *Qarābādīn Majīdī* and stored in air-tight container for further use. All the ingredients were grounded to fine powder, mixed thoroughly and sieved with a sieve number 80. After that, granules were prepared by mixing of sugar syrup in this powder. Finally, 500 mg tablets were prepared by these granules with the help of automatic tablet making machine (Anonymous, 1986).

HPTLC Analysis of *Qurṣ Mafāṣil Jadīd* (QMJ)

DESAGA Sarstedt Gruppe System (Germany) was used for analysis, automatic TLC applicator, UV visible cabinet (λ 254 nm, λ 366nm and λ 580 nm) as imaging system and Proquant 1.6 version software for analysis. Precoated TLC plates 60 F_{254} was used as stationary phase and development chamber was 20×10 cm. The test sample volume applied was 5 µl, distance of sample spotting from starting was 20 mm, distance of sample from bottom was kept as 10 mm, band length kept as 10 mm, distance between tracks was kept as 20 mm and development distance of TLC plate was 70 mm (Rasheed *et al.*, 2012).

Sample Preparation for PE, CHF and ME Extract

Five grams of powdered sample was mixed with 100 ml of petroleum ether (40-60 ^{0}C), 100 ml of chloroform and 100 ml of methanol respectively in separate flasks. The flasks were kept on a laboratory shaker for 6 hours. It was filtered and concentrated to 5 ml and used for TLC. The spot was applied on TLC plate and Chloroform:Methanol:Formic acid (9.6:0.4:0.1, v/v/v) was used as mobile phase for each extract.

A linear ascending development phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapours for 20 min at room temperature (25±2 °C). After the development, plates were air-dried, scanned by using densitometer of DESAGA Sarstedt Gruppe (Germany) at 254 nm, 366 nm and 580 nm wavelength separately and operated by Pro Quant 1.06 version software for analysis (Rasheed *et al.*, 2012).

Test for Heavy Metals

QMJ was analyzed for the detection of the presence of heavy metals like arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb), etc. by atomic absorption spectrophotometer method at DSRI, Ghaziabad as per WHO guidelines (WHO 1998b; Bin, 2001).

Test for Pesticidal Residue

The test drug QMJ was assessed for specific pesticide residues like organochlorine compounds, organophosphorus compounds and pyrethroids compounds using GCMS-MS at DSRI, Ghaziabad as per WHO guidelines (WHO 1998b).

Microbial Load Determination

The microbial load was assessed by disc diffusion assay mentioned in WHO guidelines (WHO 1998b).

Test for Aflatoxins

Different type of aflatoxins (e.g. aflatoxins B1, aflatoxins G1, aflatoxin B2 and aflatoxin G2) were determined by using LCMS-MS (WHO 1998b).

Results

Three different extracts of *Qurṣ Mafāṣil Jadīd* were subjected to HPTLC analysis, i.e. petroleum ether (PE), chloroform (CHF) and methanol (ME) extracts. The solvent system used was Chloroform: Methanol: Formic acid (9.6:0.4:0.1, v/v/v) and very good separation spots appeared by all three (PE, CHF and ME) extracts (Fig. 4,5,6). TLC plates were scanned under UV λ 254nm, UV λ 366nm and visible light λ 580nm; densitogram was obtained through scanning by HPTLC densitometer (Tables 1,2,3). The corresponding 3D densitogram was obtained for each extract and data is given in Fig. 7,8,9. The corresponding overlay densitogram (Fig. 10,11,12) and vertical representations of densitogram (Fig. 13,14,15) were also obtained for each extracts, i.e., PE, CHF and ME.

The peak obtained for spots appeared in TLC by scanning under densitogram is shown in Table 2,3,4 for each extract (PE, CHF & ME). Maximum number of

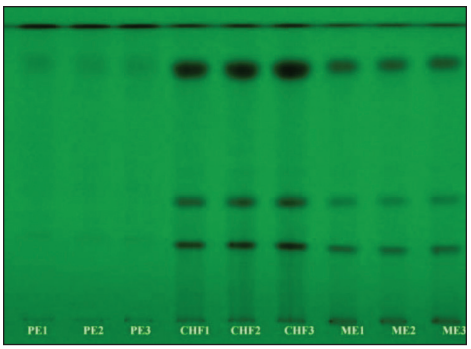

Fig. 4: HPTLC of PE, CHF and ME of *Qurṣ Mafāṣil Jadīd* at UV λ 254nm

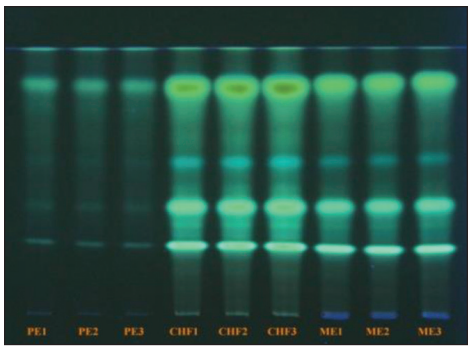

Fig. 5: HPTLC of PE, CHF and ME of *Qurṣ Mafāṣil Jadīd* at UV λ 366 nm

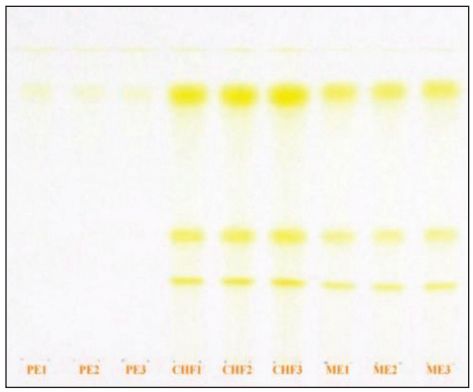

Fig. 6: HPTLC of PE, CHF and ME of *Qurṣ Mafāṣil Jadīd* at UV λ 580 nm (visible light)

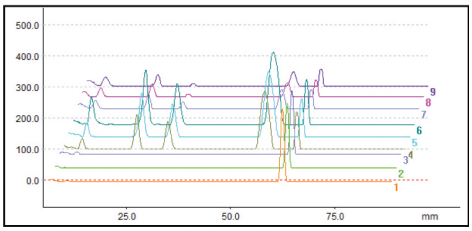

Fig. 7: 3D Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 254nm

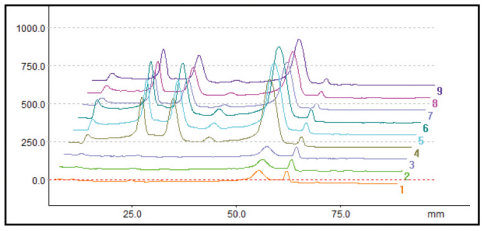

Fig. 8: 3D Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 366 nm

spots appeared in chloroform (CHF) extract as compared to PE and ME extracts (Table 5). Chloroform extracts showed five spots under UV 254 nm wavelength detection with Rf values as 0.02, 0.26, 0.40, 0.83, 0.97 with all black in colour. Similarly under UV 366 nm showed six spots with Rf values as 0.02, 0.27, 0.41, 0.57, 0.84, 0.98, all yellow colour spots appeared and detection under visible region at 580 nm showed three spots at Rf values 0.26, 0.41, 0.84 as brown colour spots. Petroleum extracts did not show any spot under UV 254 nm wavelength and detection under visible region but under UV 366 nm showed

Table 2: Peak list of PE, CHF and ME extract of *Qurṣ Mafāṣil Jadīd* at UV λ 254 nm

Sample extract	Peak no	Y-Pos	Area	Area %	Height	Rf value
PE1	1	68.0	604.67	100.00	537.75	0.96
PE2	1	68.1	534.38	100.00	480.86	0.97
PE3	1	10.0	23.10	4.23	21.39	0.02
	2	68.1	523.45	95.77	473.03	0.97
CHF1	1	10.2	84.79	3.82	74.52	0.02
	2	24.9	372.18	16.77	254.29	0.26
	3	33.4	342.11	15.41	203.96	0.40
	4	59.5	1118.49	50.39	424.05	0.83
	5	68.3	302.06	13.61	274.60	0.97
CHF2	1	10.2	123.39	4.52	104.71	0.02
	2	25.0	525.26	19.23	326.67	0.26
	3	33.4	433.15	15.86	241.89	0.40
	4	59.4	1340.74	49.08	482.07	0.83
	5	68.3	309.35	11.32	281.23	0.97
CHF3	1	10.2	281.35	8.15	199.18	0.02
	2	24.8	674.72	19.54	401.55	0.26
	3	33.4	566.88	16.41	302.00	0.40
	4	59.4	1563.57	45.27	537.46	0.83
	5	68.4	367.36	10.64	333.96	0.97
ME1	1	10.1	67.08	10.01	53.77	0.02
	2	24.3	131.75	19.66	106.55	0.25
	3	33.8	65.22	9.73	49.45	0.41
	4	60.4	251.86	37.59	143.22	0.84
	5	68.3	154.16	23.01	140.15	0.97
ME2	1	10.3	80.97	15.20	65.33	0.02
	2	24.1	119.92	22.52	92.61	0.25
	3	33.9	20.37	3.82	18.28	0.41
	4	60.8	177.70	33.37	102.60	0.85
	5	68.3	133.61	25.09	121.47	0.97
ME3	1	10.3	100.21	18.58	61.85	0.02
	2	24.4	105.01	19.47	84.03	0.25
	3	33.9	17.81	3.30	16.19	0.41
	4	61.2	183.09	33.95	103.08	0.86
	5	68.4	133.16	24.69	121.05	0.97

Table 3: Peak list of PE, CHF and ME extract of *Qurṣ Mafāṣil Jadīd* at UV λ 366 nm

Sample extract	Peak no	Y-Pos	Area	Area %	Height	Rf value
PE1	1	60.4	367.17	67.70	127.33	0.86
	2	68.0	175.15	32.30	146.92	0.98
PE2	1	60.2	296.45	62.52	108.66	0.85
	2	68.0	177.74	37.48	143.77	0.98
PE3	1	10.0	7.61	1.38	11.01	0.02
	2	60.1	364.42	66.09	123.95	0.85
	3	68.1	179.39	32.53	145.91	0.98
CHF1	1	10.4	210.51	2.81	127.12	0.02
	2	25.0	532.43	20.43	674.52	0.27
	3	33.5	767.73	23.57	643.67	0.41
	4	43.2	136.28	1.82	64.84	0.57
	5	59.6	688.11	49.17	935.22	0.84
	6	68.1	165.16	2.20	125.52	0.98
CHF2	1	10.4	279.62	3.29	166.71	0.02
	2	25.0	717.11	20.18	743.67	0.27
	3	33.5	996.91	23.47	706.24	0.41
	4	43.3	157.49	1.85	75.84	0.57
	5	59.5	162.72	48.92	991.24	0.84
	6	68.1	195.39	2.30	144.04	0.98
CHF3	1	10.4	279.62	3.29	166.71	0.02
	2	25.0	717.11	20.18	743.67	0.27
	3	33.5	996.91	23.47	706.24	0.41
	4	43.3	157.49	1.85	75.84	0.57
	5	59.5	162.72	48.92	991.24	0.84
	6	68.1	195.39	2.30	144.04	0.98
ME1	1	10.3	234.41	5.30	106.25	0.02
	2	24.5	834.14	18.86	466.77	0.26
	3	33.7	926.18	20.94	388.50	0.41
	4	43.6	29.05	0.66	21.79	0.58
	5	60.4	2312.41	52.28	657.69	0.86
	6	68.4	86.74	1.96	74.16	0.99
ME2	1	10.4	211.90	5.13	104.52	0.02
	2	24.3	824.26	19.96	447.05	0.26
	3	33.8	859.38	20.81	364.83	0.41
	4	44.1	36.32	0.88	23.22	0.58
	5	60.8	106.79	51.02	625.81	0.86
	6	68.4	90.97	2.20	74.96	0.99

Sample extract	Peak no	Y-Pos	Area	Area %	Height	Rf value
ME3	1	10.6	227.27	5.54	107.12	0.03
	2	24.6	819.82	19.97	443.68	0.26
	3	34.2	851.39	20.74	358.79	0.42
	4	44.5	27.13	0.66	17.52	0.59
	5	61.2	086.24	50.83	624.65	0.87
	6	68.6	92.49	2.25	74.88	0.99

Table 4: Peak list of PE, CHF and ME extract of *Qurṣ Mafāṣil Jadīd* at UV λ 580 nm (visible light)

Sample extract	Peak no	Y-Pos	Area	Area %	Height	Rf value
PE1	-	-	-	-	-	-
PE2	-	-	-	-	-	-
PE3	-	-	-	-	-	-
CHF1	1	24.9	195.92	19.08	130.34	0.27
	2	3.3	194.27	18.92	104.18	0.41
	3	59.4	636.42	61.99	213.04	0.84
CHF2	1	4.9	237.80	19.24	58.66	0.27
	2	33.3	71.25	21.95	34.22	0.41
	3	59.4	26.86	58.81	30.57	0.84
CHF3	1	24.8	285.92	19.80	82.57	0.26
	2	33.3	322.51	22.34	55.52	0.41
	3	59.3	835.41	57.86	40.50	0.84
ME1	1	24.4	98.36	24.30	61.20	0.26
	2	33.5	3.43	13.20	27.67	0.41
	3	60.3	253.00	62.50	106.45	0.85
ME2	1	4.2	94.21	26.31	51.69	0.25
	2	33.7	43.91	12.26	22.83	0.41
	3	60.6	19.95	61.43	94.18	0.86
ME3	1	24.5	92.88	25.78	50.12	0.26
	2	34.2	39.09	10.85	20.55	0.42
	3	61.0	28.31	63.37	93.99	0.87

only one spot with Rf value as 0.85 in yellow colour. Methanol extracts showed five spots under UV 254 nm wavelength detection with Rf values as 0.02, 0.25, 0.41, 0.86, 0.97 with all black in colour. Similarly under UV 366 nm showed six spots with Rf values as 0.03, 0.26, 0.42, 0.59, 0.87, 0.99 with all yellow in colour and detection under visible light region at 580 nm showed three spots at Rf values 0.26, 0.42, 0.87 as represented in Table 5.

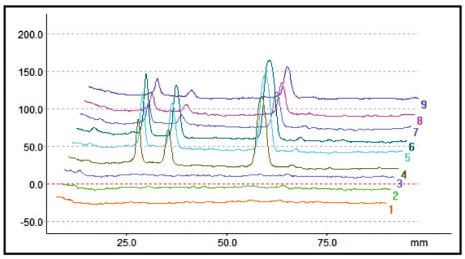

Fig. 9: 3D Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 580 nm (visible light)

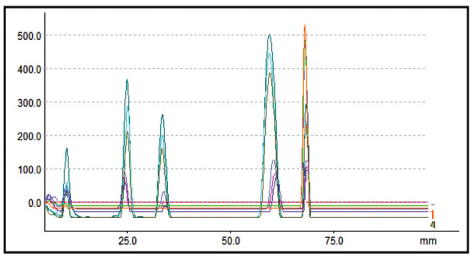

Fig. 10: Overlay Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 254nm

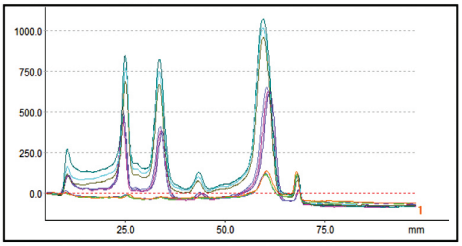

Fig. 11: Overlay Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 366 nm

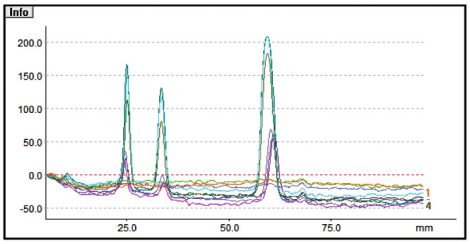

Fig. 12: Overlay Densitogram of QMJ, PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract at UV λ 580 nm (visible light)

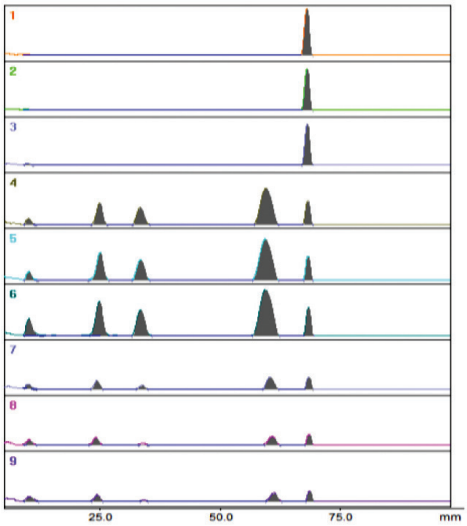

Fig. 13: Vertical Densitogram of PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract of QMJ at UV λ 254nm

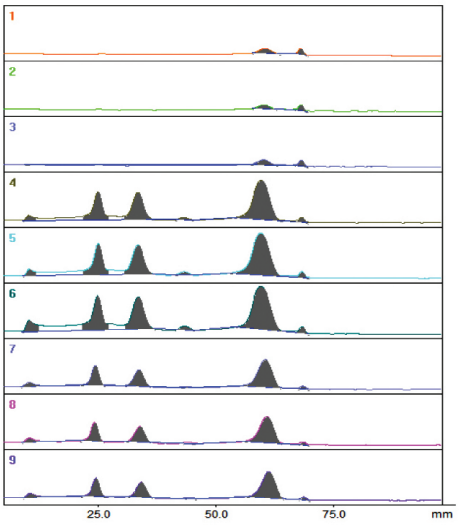

Fig. 14: Vertical Densitogram of PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract of QMJ at UV λ 366 nm

The heavy metal analysis revealed no traces of cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) in QMJ, results are recorded in Table 6. The total bacterial count (cfu/gm) of QMJ was found 1300 (1.3 x 10^3) cfu/gm, which is much lower than the permissible limit (1x 10^5) *cfu/gm*. The value of total yeast and mould count was found 190 (1.9 x 10^2) cfu/gm, which is much lower

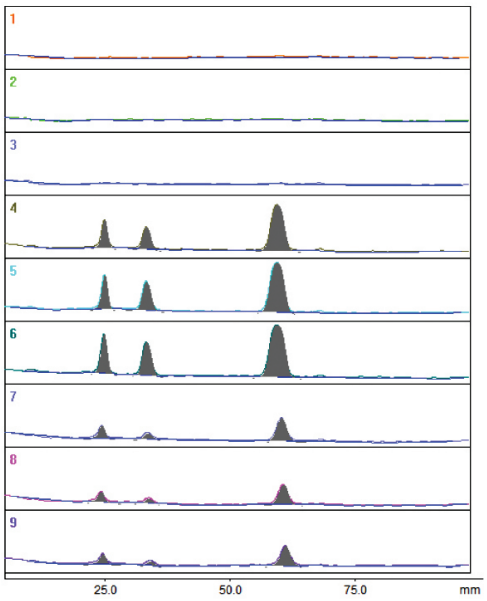

Fig. 15: Vertical Densitogram of PE (1,2,3), CHF (4,5,6) and ME (7,8,9) extract of QMJ at UV λ 580 nm (visible light)

Table 5: Comparison of PE, CHF and ME extract of *Qurṣ Mafāṣil Jadīd* (QMJ)

Solvent System	Chloroform: Methanol: Formic acid (9.6:0.4:0.1, v/v/v)								
Extracts	Petroleum ether extract			Chloroform extract			Methanol extract		
Detection system	UV 254 nm	UV 366 nm	under visible light	UV 254 nm	UV 366 nm	under visible light	UV 254 nm	UV 366 nm	under visible light
No. of Spots	1	2	0	5	6	3	5	6	3
Peak 1	-	-	-	0.02	0.02	-	0.02	0.03	-
Peak 2	-	-	-	0.26	0.27	0.26	0.25	0.26	0.26
Peak 3	-	-	-	0.40	0.41	0.41	0.41	0.42	0.42
Peak 4	-	-	-	-	0.57	-	-	0.59	-
Peak 5	-	0.85	-	0.83	0.84	0.84	0.86	0.87	0.87
Peak 6	0.97	0.98	-	0.97	0.98	-	0.97	0.99	-

than the permissible limit (1x 10^3) *cfu/gm. The* Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa were absent in the test drug QMJ (Table 7). Aflatoxin estimation was done by LCMCMS method for aflatoxins B1, G1, B2 and G2 and result showed no detection of aflatoxins in QMJ (Table 8). The pesticide residue analysis was done for 33 different pesticides

by GCMSMS and one by UV-Vis spectrophotometer. The result confirms the absence of all pesticide in QMJ (Table 9).

Table 6: Results of heavy metals content in *Qurṣ Mafāṣil Jadīd* (QMJ)

Heavy Metals	Method	Heavy Metals in QMJ	Limit of Quantification	Permissible Limit as per API
Cadmium (Cd) (mg/Kg)	AAS	ND	2.5	Not more than 0.3
Arsenic (As) (mg/Kg)	AAS	ND	0.5	Not more than3
Lead (Pb) (mg/Kg)	AAS	ND	1.25	Not more than 10
Mercury (Hg) (mg/Kg)	AAS	ND	0.25	Not more than 1

Not Detected = ND

Table 7: Results of microbial load in *Qurṣ Mafāṣil Jadīd* (QMJ)

Colony forming units on nutrient agar medium	Result	Permissible Limit as per API
Total bacterial count (cfu/gm)	1300	NMT 1x 10^5 *cfu/gm*
Total yeast and mould (cfu/gm)	190	NMT 1x 10^3 *cfu/gm*
Escherichia coli (cfu/gm)	Absent	Absent
Salmonella (cfu/gm)	Absent	Absent
Staphylococcus aureus (cfu/gm)	Absent	Absent
Pseudomonas aeruginosa (cfu/gm)	Absent	Absent

Not more than = NMT; Absent = -Ve

Table 8: Results of aflatoxin in *Qurṣ Mafāṣil Jadīd* (QMJ)

Aflatoxin	Method	Result	Limit of Quantification	Permissible Limit as per API
Aflatoxin B_1 (mg/Kg)	LCMCMS	ND	0.001	NMT 0.5
Aflatoxin G_1 (mg/Kg)	LCMCMS	ND	0.001	NMT 0.5
Aflatoxin B_2 (mg/Kg)	LCMCMS	ND	0.001	NMT 0.1
Aflatoxin G_2 (mg/Kg)	LCMCMS	ND	0.001	NMT 0.1

Not Detected = ND; Not more than = NMT

Table 9: Results of pesticide residue in *Qurṣ Mafāṣil Jadīd* (QMJ)

Pesticides	Methods	Results	Limit of Quantification	Permissible Limit as per API
Alachlor (mg/Kg)	GCMSMS	ND	0.02	0.02
Aldrin & Dieldrin (sum of) (mg/Kg)	GCMSMS	ND	0.04	0.05
Azinophos Methyl (mg/Kg)	GCMSMS	ND	0.04	1.00
Bromopropylate (mg/Kg)	GCMSMS	ND	0.08	3.00
Chlordane (sum of cis, trans, and oxychlordane) (mg/Kg)	GCMSMS	ND	0.04	0.05
chlorfenvinphos (mg/Kg)	GCMSMS	ND	0.04	0.5
Chlorpyrifos (mg/Kg)	GCMSMS	ND	0.04	0.2
Chlorpyrifos-methyl (mg/Kg)	GCMSMS	ND	0.04	0.1
Cypermethrin (and Isomers) (mg/Kg)	GCMSMS	ND	0.10	0.1
DDT (sum of P,P-DDE and P,P-TDE) (mg/Kg)	GCMSMS	ND	0.04	1.0
Deltamethrin(mg/Kg)	GCMSMS	ND	0.10	0.5
Diazinon (mg/Kg)	GCMSMS	ND	0.04	0.5
Dichlorvos (mg/Kg)	GCMSMS	ND	0.04	1.0
Dithiocarbamates (as CS2) (mg/Kg)	UV-VIS,			
Spectrophotometry	ND	0.01	2.0	
Endosulfan (Sum of Isomer of Endosulfan Sulphate) (mg/Kg)	GCMSMS	ND	0.04	3.0
Endrin (mg/Kg)	GCMSMS	ND	0.04	0.05
Ethion (mg/Kg)	GCMSMS	ND	0.04	2.0
Fenitrothion (mg/Kg)	GCMSMS	ND	0.04	0.5
Fenvalerate (mg/Kg)	GCMSMS	ND	0.10	1.5
Fonofos (mg/Kg)	GCMSMS	ND	0.04	0.05
Heptachlor (sum of heptachlor and heptachlor epoxide) (mg/Kg)	GCMSMS	ND	0.04	0.05
Hexachlorobenzene (mg/Kg)	GCMSMS	ND	0.04	0.1
Hexachlorobenzene isomer other than λ(mg/Kg)	GCMSMS	ND	0.04	0.3

Pesticides	Methods	Results	Limit of Quantification	Permissible Limit as per API
Lindane (λ-Hexachlorocylohexane)				
(mg/Kg)	GCMSMS	ND	0.04	0.06
Malathion (mg/Kg)	GCMSMS	ND	0.04	1.0
Methidathion (mg/Kg)	GCMSMS	ND	0.04	0.2
Parathion (mg/Kg)	GCMSMS	ND	0.04	0.5
Parathion methyl (mg/Kg)	GCMSMS	ND	0.04	0.2
Permethrin (mg/Kg)	GCMSMS	ND	0.04	1.0
Phosalone (mg/Kg)	LCMSMS	ND	0.04	0.1
Piperonyl butoxide (mg/Kg)	LCMSMS	ND	0.04	3.0
Primiphos methyl (mg/Kg)	LCMSMS	ND	0.04	4.0
Pyrethrins (sum of Isomers)				
(mg/Kg)	GCMSMS	ND	0.10	3.0
Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide) (mg/Kg)	LCMSMS	ND	0.10	1.0

Not Detected = ND

Conclusion

In Unani Medicine, more than one herb is used in many formulations. Standardization of polyherbal formulations is an important task and a central aspect for maintaining and evaluating the quality and safety as per WHO guidelines. It is required to minimize batch to batch variation, assurance of safety, efficacy, quality and acceptability (Ahmad *et al.*, 2006; Sharma *et al.*, 2009; Patil and Shettigar, 2010).

HPTLC is a sophisticated technique for the identification of chemical constituents in single as well as compound formulations. It is reliable and can be used for the quality control of herbal products (Siddiqui, 2020). Safety studies of plant materials intended to be used as drugs have become mandatory as per WHO guidelines. Therefore, heavy metals, pesticide residue, microbial load and aflatoxin estimation of herbal drugs are necessary to establish the quality standard of any drug (WHO, 1998b; WHO, 2002).

Microbial contamination of raw material of herbal drugs is a major concern and poses a health risk. Microbial contamination should be prevented at every stage

of the drug development, i.e. procurement, transport, storage, preparation and finished product storage. Therefore bacterial and fungal contamination of drugs, especially raw materials, should be prevented (WHO 1998b). Aflatoxins are mycotoxin produced by many species of a fungus Aspergillus, most commonly by *Aspergillus flavus* and *Aspergillus parasiticus*. The mycotoxins are considered carcinogenic substance, the adverse effects of aflatoxins in humans ranged from acute hepatic toxicity to chronic disease such as liver cancer (Agag, 2004). Aflatoxins are classified as the most potent natural carcinogens and generally affect the liver. The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 in the group 1 as a human carcinogen and aflatoxins G1, B2 and G2 in the group 2B as possible carcinogens to humans (Meritxell ventura *et al*., 2004).

Unscientific and haphazard use of synthetic pesticides is increasing day by day and posing health hazards to human beings. Pesticide residues are the remains of pesticides on drug material when it is collected for medicinal use. Pesticide poisoning is taking about 3 lakh deaths worldwide every year. The most lethal pesticides are organophosphates and organochlorines compounds (Goel and Aggarwal, 2007).

The data obtained through HPTLC of *Qurṣ Mafāṣil Jadīd* clearly depicted the importance of developing a HPTLC fingerprint pattern for record and future reference. It was found that chloroform extract has shown maximum numbers of spots followed by methanol extract. The sophisticated analytical method of HPTLC is very much valid and has more significance than other conventional techniques. Hence the present study serves as a reference standard for the future studies and also for comparison for quality control study in different batches of the formation. The findings of the present study demonstrated that the values recorded in respect of heavy metals and microbial loads were within the permissible limits while aflatoxin, pesticides and other pathogenic organisms were not found at all. It suggests that the crude drugs used in the preparation of QMJ were genuine and not afflicted with poor soil and environmental conditions and thus qualified the criteria for being used for studies and even for therapeutic use.

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सारांश

हर्बल यूनानी मिश्रण *कुर्स मफ़ासिल जदीद* के एचपीटीएलसी अभिलक्षण एवं गुणवत्ता मानक

ज़की अहमद सिद्दीक़ी, *ज़ुबैदा ए. अन्सारी, अर्ज़ीना जबीन, मो. अनवर, मोहम्मद ज़ाकिर, मोहम्मद अब्दुल राशीद नईकोडी, निशात खुर्शीद और मुनव्वर हुसैन काज़मी

सारांश

कुर्स मफ़ासिल जदीद (क्यूएमजे) एक बहु–घटक यूनानी मिश्रण है जो विभिन्न एटियोलॉजी के जोड़ो के दर्द के उपचार के लिए प्रभावी रूप से उपयोग किया जाता है। यह गोली के रूप में होता है और इसमें तीन हर्बल घटक – *सूरंजान तल्ख़, ज़र्द चोब और गोंद कीकर* होते हैं। क्यूएमजे एक भेषजकोशीय मिश्रण है जिसका उल्लेख *क़राबादीन–ए–मजीदी,* यूनानी चिकित्सा की एक मानक भेषजकोश, में किया गया है। रोग की स्थिति में नियमित और सर्वोत्तम परिणाम प्राप्त करने के लिए हर्बल मिश्रण की गुणवत्ता नियंत्रण अत्यंत महत्वपूर्ण है। हालांकि बहु–घटक मिश्रण का मानकीकरण आसान काम नहीं है, टीएलसी, एचपीटीएलसी जैसी सरल परिष्कृत तकनीकें गुणवत्ता नियंत्रण के मूल उद्देश्य की पूर्ति कर सकती हैं। प्रामाणिक और विश्वसनीय परिण ाम प्राप्त करने के उद्देश्य से क्यूएमजे का एचपीटीएलसी फिंगरप्रिंटिंग प्रोफ़ाइल, भारी धातु विश्लेषण, कीटनाशक अवशेष, माइक्रोबियल लोड और एफ्लाटॉक्सिन परीक्षण किया गया। क्यूएमजे की एचपीटीएलसी फिंगरप्रिंटिंग प्रोफ़ाइल को यूवी λ 366 एनएम, यूवी λ 254 एनएम और दृश्य प्रकाश (यूवी λ 580 एनएम) द्वारा विकास और पता लगाया गया। भारी धातु विश्लेषण, कीटनाशक अवशेष, माइक्रोबियल लोड और एफ्लाटॉक्सिन का विश्लेषण डब्ल्यूएचओ दिशानिर्देशों के अनुसार किया गया। वर्तमान अध्ययन ने क्यूएमजे की शुद्धता एवं गुणवत्ता को निर्धारित किया और भविष्य में मिश्रण की गुणवत्ता और चिकित्सीय प्रमाणिकता को बनाए रखने के लिए एक संदर्भ के रूप में उपयोग किया जा सकता है। इस अध्ययन में प्राप्त परिणामों को भविष्य में संदर्भ के लिए फिंगरप्रिंट के रूप में उपयोग किया जा सकता है।

शब्दकुंजी: एफ्लाटॉक्सिन, भारी धातु, एचपीटीएलसी, माइक्रोबियल लोड, कीटनाशक अवशेष, *कुर्स मफ़ासिल जदीद,* यूनानी मिश्रण, गुणवत्ता नियंत्रण

Spectrophotometric Estimation of Total Phenolic Content in Undetoxified and Detoxified *Balādur* (*Semecarpus anacardium*)

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Abstract

Objective: The objective of the study was to evaluate the effect of detoxification (*'Amal-i-Tadbīr*) on *Semecarpus anacardium* (*Balādur*) toxicity by spectrophotometric estimation of total phenolic content (TPC).

Methods: Qualitative analysis for the presence of phenols was performed. Quantitatively, TPC was determined using the Folin & Ciocalteu reagent, with gallic acid as the standard by the method described by Singleton and Rossi, 1965.

Results: The TPC in aqueous and ethanolic extracts of un-detoxified *Balādur* was found to be 186 mg/g and 210 mg/g gallic acid equivalent (GAE), respectively. The TPC in aqueous and ethanolic extracts of *Balādur* after applying hot compression (detoxified *Balādur*) was found to be 139 mg/g GAE and 183 mg/g GAE. However, application of *Patal Jantar* revealed the amount of TPC to be 74 mg/g (aqueous extract) and 167 mg/g GAE (ethanolic extract). The TPC in *Balādur* oil obtained by hot compression method was 172 mg/g GAE.

Conclusion: The study showed reduction in total phenolic content after detoxification which suggested the possible cause of decreased toxicity of *Balādur*. The TPC of *Patal Jantar* detoxified *Balādur* was lesser than detoxified by hot compression. The TPC can be used as an indicator for the standardization of *Balādur*.

Keywords: *Balādur*, *Semecarpus anacardium*, Spectrophotometer, Total phenolic content

Introduction

Balādur is *Semecarpus anacardium* which belongs to the family *Anacardiaceae* (Tondon & Sharma, 2011). The trees of *Balādur* are deciduous and found in the outer Himalayas from Sutlej to Sikkim, common throughout the hotter parts of India as far east as Assam (Anonymous, 1972). Its fruit is mainly used for medicinal purpose which shows resemblance with the heart of birds (Ibn Baitar, 1999). The fruit oil is also used for therapeutic activity. According to Ibn Baitar, '*Balādur* in the Indian language is Anacardia in Greek which means heart shaped' (Dymock *et al.*, 1972). *Belādin*, *Ḥabb al-Fahm*, *Ḥabb al-Qalb*, *Bhelā*, Marking nut, *Bhilāwāṉ*, *Agnīkā* and *Bhilānvanā* are some of its vernacular names (Chopra *et al.*, 2006). The fruits of *Balādur* are about 2.5 cm long oblong shape, smooth and shining, black when ripe seated on a fleshy hypocarp of about 2 cm long, smooth and yellow in colour when ripe (Kirtikar & Basu, 1996). It has characteristic odour and taste is highly acrid and irritating (Tondon & Sharma, 2011). With the consensus of various Unani authors,

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the temperament of *Balādur* oil or juice is hot and dry in the fourth degree while the temperament without oil is hot and dry in the third degree (Khan, 2012; Ghani, 2010; Kareem, 1875). It is therapeutically used in the treatment of nervous disorders like paralysis, facial palsy, tremors, numbness, chorea, memory loss, dribbling of urine and nervous debility (Khan, 2012; Kareem, 1875). *Anqaroyā Kabīr*, *Anqaroyā Saghīr* and *Ma'jūn-i-Balādur* are its important Unani formulations (Anonymous, 2007). The most significant constituents of *Balādur* oil are phenolic compounds. Vesicant reactions are possibly attributed to phenolic compounds (Raut *et al.,* 2017). Pillay and Siddiqui in 1931 isolated Bhilawanol (46% of the extract) as chief constituent from the juice of the pericarp (Chopra *et al.,* 2006). Its juice on contact with skin causes intense pain and swelling, produces deep bluish coloured vesicles and irritable sore. The mark does not disappear for many months or even for life (Khory & Katrak, 1985). Despite the presence of Bhilawanols as the toxic components, it is still used in the Indian system of medicine (Kumar *et al.,* 2017). In Unani Medicine, it is used after detoxification. There are two methods of its detoxification mentioned in classical Unani literature – Hot compression and *Patal Jantar* (Baitar, 1999; Khan, 2012). Its use as a therapeutic agent even in the indigenous system of medicine has dwindled to a great extent owing to the fact that the irritation produced by its application cannot be properly controlled (Chopra *et al.,* 2006).

Since the toxicity of *Balādur* is mainly due to the presence of phenolic compounds, the present study was designed to evaluate the effect of the two methods of detoxification mentioned in Unani literatures, i.e. hot compression and *Patal Jantar,* on *Balādur* by spectrophotometric estimation of phenolic contents.

Material and Methods

Procurement and Authentication of Drug

The fruits of *Semecarpus anacardium* (*Balādur*) were procured from the Khari Baoli Market, Delhi. The drug was identified by Pharmacognosy Section, Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh. Further, it was authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi with Ref. No. NISCAIR/RHMD/ Consult/2018//3294-95.

Detoxification of *Semecarpus anacardium* (*Balādur*)

But before detoxification the cap of *Balādur* was removed. Fig. A.1 and A.2 show the images of *Balādur* with cap and after removal of cap, i.e. uncapped *Balādur*, respectively.

Fig. A. 1: *Balādur* with cap

Fig. A. 2: Uncapped *Balādur*

In classical Unani literature, there are two methods of its detoxification:

1. Hot compression: After removing the cap, oil of the fruit is released by squeezing it in between two strongly heated rods (Khan, 2012; Ghani, 2010) (Fig. A. 3)

2. Patal Jantar: In this method, earthen pot was divided into two halves. A hole was bored at the central part of the lower half of the pot. The uncapped *Balādur* was put into the long neck round bottom flask with head covered by steel net. Then the flask was covered with *Gil-i-Ḥikmat* except at the open head and placed upside down fitting into the hole of the earthen pot. The temperature to the flask was provided by burning cow dung cake. A beaker was placed below the head of flask for receiving oil coming out of *Balādur* (Rafiquddin, 2001) (Fig. A. 4)

Fig. A. 3: Hot compression

Fig. A. 4: *Patal Jantar*

Preparation of Extract

The aqueous and ethanolic extracts of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* were prepared by refluxing 10 g powdered drug with 150 ml distilled water and 70% ethanol separately for six hours and then filtered and freeze dried in the lypholizer. Stock solutions were prepared at a concentration of 1 g/100 ml and subjected to spectrophotometric determination of total phenolic content.

Qualitative Analysis for Phenol

Qualitative analysis for the presence of phenols in detoxified and undetoxified *Balādur* was performed. The analysis for the presence of phenols in *Balādur* oil obtained by hot compression method was also performed. In the extract of petroleum ether, ferric chloride was mixed. A purple or red colour indicated the presence of phenols (Afaq *et al.*, 1994).

Estimation of Total Phenolic Content

Total phenolic content in aqueous and ethanolic extract of three different forms of *Balādur* and *Balādur* oil obtained by hot compression method were estimated by the method described by Singleton and Rossi, 1965 in terms of gallic acid equivalent in mg/g of the extract (Singleton & Rossi, 1965). The standard calibration curve of aqueous samples of gallic acid (Graph 1) was plotted by mixing 1 ml aliquots of 10, 50, 100, 150 and 200µg/ml of gallic acid solutions with 5.0 ml of Folin & Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (7.5g/l). The standard calibration curve of ethanolic samples of gallic acid (Graph 2) was plotted by mixing 1 ml aliquots of 10, 50, 100, 150 and 200µg/ml of gallic acid solutions with the same amount and concentration of reagents mentioned above. The absorbance was measured after 30 minutes at 765nm. 0.1ml of aqueous and ethanolic extract (1 g/100 ml) was mixed separately with the same reagents as performed in construction of calibration curve, and after 1 hour the absorbance was measured for the determination of total phenolic content. TPC of samples were calculated by using standard curve equation obtained from the graph, e.g. if SCE is y = 0.006x + 0.150; where, y = Absorbance of drug extract sample; x = Total phenolic content in that drug extract sample.

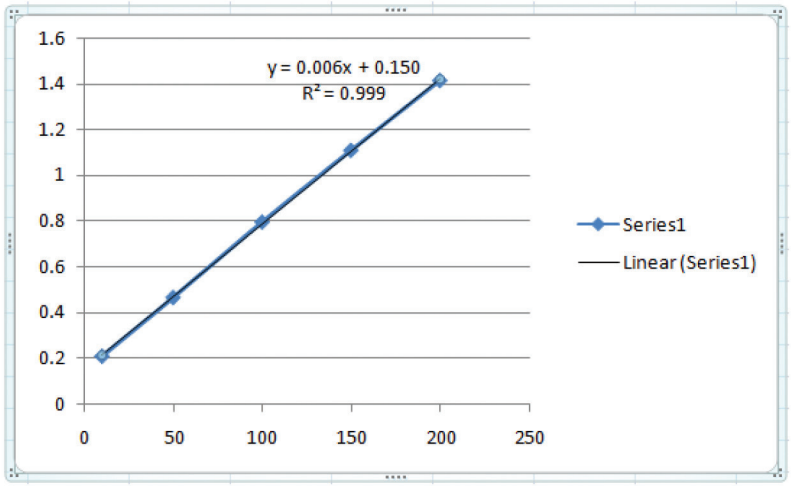
Results

Estimation of Total Phenolic Content

Qualitative analysis of aqueous and ethanolic extracts of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* showed the presence

of phenols. The analysis also confirmed the presence of phenols in *Balādur* oil obtained by hot compression method. So quantitatively the amount of total phenolics was determined by Singleton and Rossi method in terms of gallic acid equivalent in mg/g of the extract.

The total phenolic content of aqueous extract of all the three samples of *Balādur* was estimated with the help of standard curve equation obtained from Graph 1. The standard curve equation obtained from the graph was 0.006x + 0.150. The amount of total phenolic content in aqueous extract of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* calculated was 186 mg/g, 139mg/g and 74 mg/g of gallic acid equivalents, respectively. The *Balādur* oil is insoluble in water hence total phenolic content estimation in aqueous extract of oil not seems possible.

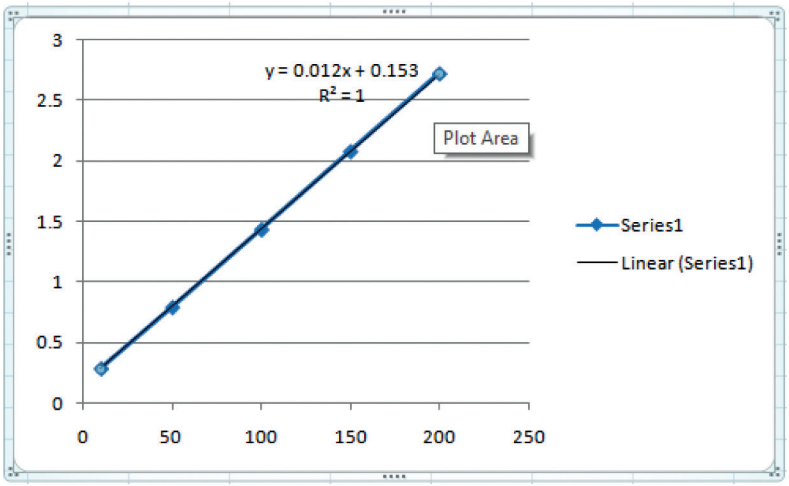


Graph 1: Standard curve of aqueous samples of gallic acid

The phenolic content of ethanolic extract of all the samples of *Balādur* was also determined with the help of standard curve equation obtained from Graph 2. The standard curve equation obtained from the graph was 0.012x + 0.153. The amount of total phenolic content in ethanolic extract of undetoxified *Balādur* and *Balādur* detoxified by hot compression and *Patal Jantar* calculated was 210 mg/g, 183 mg/g and 167 mg/g gallic acid equivalents, respectively. The TPC in ethanolic extract of oil calculated was 172mg/g GAE.

Discussion

It has been mentioned in the classical Unani literatures that *Balādur* (*Semecarpus anacardium*) possesses potential therapeutic activity. However, its toxicity restricts the use of *Balādur* as single or compound formulation. Unani Medicine directs its use only after detoxification. Two methods for detoxification of *Balādur* have



Graph 2: Standard curve of alcoholic samples of gallic acid

been mentioned in Unani literature which are hot compression and *Patal Jantar*. The toxicity of *Balādur* is mainly due to the presence of phenolic compounds (Kumar *et al.,* 2017). Therefore, it was imperative to evaluate the effect of detoxification on total phenolic content of *Balādur* or its toxicity.

Qualitative analysis of aqueous and ethanolic extracts of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* showed the presence of phenolic content. The qualitative analysis of *Balādur* oil obtained by hot compression method also confirmed the presence of phenolic content.

Quantitatively, total phenolic content was determined by Singleton and Rossi method in terms of gallic acid equivalent in mg/g of the extract. The amount of total phenolic content in aqueous extract of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* calculated was 186 mg/g, 139 mg/g and 74 mg/g gallic acid equivalents, respectively. Being insoluble nature of oil in water, total phenolic content estimation in aqueous extract of oil was impossible. The amount of total phenolic content in ethanolic extract of undetoxified *Balādur* and detoxified *Balādur* by hot compression and *Patal Jantar* calculated was 210 mg/g, 183 mg/g and 167 mg/g gallic acid equivalents, respectively. The TPC in ethanolic extract of oil calculated was 172 mg/g GAE.

Conclusion

The qualitative analysis confirmed the presence of phenols in undetoxified, detoxified *Balādur* and the oil obtained by detoxification method. Quantitatively, total phenolic content after detoxification of *Balādur* was decreased which justified the cause of its decreased toxicity. The TPC in *Patal Jantar* detoxified *Balādur* was lesser than *Balādur* detoxified by hot compression. But the charring of *Balādur*, increased ash value beyond the permissible limit and lack of standardized procedure are the limitations of detoxification by *Patal Jantar*.

The toxicity of *Balādur* is due to phenols and anacardic acids present in its oil (Ilanchezhian *et al.,* 2012). The sum of total phenolic content in oil and in detoxified *Balādur* is greater than undetoxified *Balādur* which suggests that the toxicity of *Balādur* in not only due to phenols, but other constituents also contribute to its toxicity like anacardic acids. There is also a possibility that on detoxification, due to chemical reactions, some constituents may convert into other constituents. The total phenolic estimation will be helpful in the development of new drug and standardization of the drug. This study was an attempt to evaluate the effect of detoxification on *Balādur*, however, it is essential to explore this study on more objective parameters and advanced scientific basis.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

KMYA contributed to conception and design of the study. He critically revised the manuscript and gave final approval. MZS is accountable for all aspects of work ensuring integrity and accuracy. MZS involved in conducting the whole experimental part, data acquisition, data analysis and prepared the manuscript for publication. The experimental part was performed with full assistance under the guidance of NS. She provided major contribution in statistical analysis and data interpretation. She also supported the preparation and finalization of manuscript. SA contributed technically ensuring the availability of accurate reagents and chemicals. She provided support in running spectrophotometer for estimation. MS supported in designing the research protocol. She critically looked into the statistical analysis. She revised the manuscript and approved the final draft. MU provided support in concept and design of the research proposal. He critically revised the manuscript as per journal guidelines and finalized the draft for submission.

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सारांश

अनडिटॉक्सिफाइड और डिटॉक्सिफाइड *बलादुर (सेमीकार्पस एनाकार्डियम)* में कुल फेनोलिक सामग्री का स्पेक्ट्रोफोटोमेट्रिक आकलन

***मो. ज़ाकिर सिद्दीक़ी, मरयम सरदार, कुंवर मोहम्मद युसुफ़ अमीन, मो. उरूज, नाज़िश सिद्दीक़ी, सदा अख़्तर**

सारांश

उद्देश्यः अध्ययन का उद्देश्य कुल फेनोलिक सामग्री (टीपीसी) के स्पेक्ट्रोफोटोमेट्रिक आकलन द्वारा सेमीकार्पस एनाकार्डियम (*बलादुर*) विषाक्तता पर विषहरण (*अमल–ए–तदबीर*) के प्रभाव का मूल्यांकन करना था।

विधिः फेनोल की उपस्थिति का पता लगाने के लिए गुणात्मक विश्लेषण किया गया। मात्रात्मक रूप से सिंगलटन और रॉसी, 1965 द्वारा बताई गई विधि द्वारा मानक के रूप में गैलिक एसिड के साथ टीपीसी को फालिन एवं सियोकाल्टू अभिकर्मक का उपयोग करके निर्धारित किया गया।

परिणामः अनडिटॉक्सिफाइड *बलादुर* के जलीय और एथेनॉलिक सत्त में टीपीसी क्रमशः 186 मि.ग्रा./ग्रा. और 210 मि.ग्रा./ग्रा. गैलिक एसिड इक्युवेलेन्ट (जीएई) पाया गया। गर्म संपीड़न लगाने के बाद डिटॉक्सिफाइड *बलादुर* के जलीय और एथेनॉलिक सत्त में टीपीसी 139 मि.ग्रा./ग्रा. जीएई और 183 मि.ग्रा./ग्रा. जीएई पाया गया। हालांकि, *पाताल जंतर* के प्रयोग से पता चला कि टीपीसी की मात्रा 74 मि.ग्रा./ग्रा. (जलीय सत्त) और 167 मि.ग्रा./ग्रा. जीएई (एथेनॉलिक सत्त) है। गर्म संपीड़न विधि द्वारा प्राप्त *बलादुर* तेल में टीपीसी 172 मि.ग्रा./ग्रा. जीएई थी।

निष्कर्षः अध्ययन में विषहरण के बाद कुल फेनोलिक सामग्री में कमी देखी गई जिसने *बलादुर* की विषाक्तता में कमी के संभावित कारण का सुझाव दिया। *पतल जंतर* द्वारा डिटॉक्सिफाइड *बलादुर* की टीपीसी गर्म संपीड़न द्वारा *डिटॉक्सिफाइड* से कम थी। टीपीसी को *बलादुर* के मानकीरण के लिए एक संकेतक के रूप में उपयोग किया जा सकता है।

शब्दकुंजीः *बलादुर*, सेमीकार्पस एनाकार्डियम, स्पेक्ट्रोफोटोमीटर, कुल फेनोलिक सामग्री

A Clinico-epidemiological Study of *Kalaf* (Melasma) and its Impact on Quality of Life

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Abstract

Introduction: *Kalaf* (melasma) is an asymptomatic cosmetic disorder having direct negative impact on quality of life (QoL) of the affected individuals. Lack of definitive therapies and higher rate of recurrence are the prominent challenges in the management of melasma.

Objective: To study the demographic, epidemiological and clinical characteristics of *Kalaf* (melasma) and its impact on QoL of the participants was the main objective of this study.

Materials and Methods: We conducted a cross-sectional study on the participants (n=88) of melasma of any sex aged between 18 and 55 years visiting outpatient department of NRIUMSD, Hyderabad. The data were collected on predesigned format in face-to-face interview and clinical examination, and analyzed retrospectively.

Observations and Results: We observed the average age (39 ± 8.1 years), average age of onset (34 ± 8.1 years), female to male ratio (4:1) and average chronicity of illness (5.2 ± 4.2 years) of the participants with melasma. We found mean MASI (17.84), mean DLQI (20.3) and mean MQOL (51.4) in the participants.

Discussion: This study showed that melasma was common in female in the age group of 30-40 years. The commonest clinical pattern was centro-facial. The probable contributing factors were sun exposure, pregnancy, OCPs and prolonged amenorrhea. The higher mean DLQI and mean MQOL demonstrated the negative impact of melasma on the QoL of the participants independent of severity of the disease.

Conclusion: In conclusion, this study provides the current understanding of the clinico-epidemiological characteristics of melasma. It impacts negatively on QoL of the participants.

Keywords: Dermatology, Hyperpigmentation, MASI, Melasma, Pregnancy, Unani

Introduction

Kalaf (melasma) is an asymptomatic cosmetic disorder commonly encountered in outpatient department of dermatology clinics (Charupalli *et al.,* 2018). It is defined as the hyperpigmentation of sun-exposed areas of the face characterized by symmetrical irregular grey to brown patches (Achar & Rathi, 2011). Its prevalence varies from 0.25% to 4% in South East Asia (Charupalli *et al.,* 2018; Pawar *et al.,* 2015). It is a female predominant disease accounting for 90% of the cases (Achar & Rathi, 2011; Charupalli *et al.*, 2018). It is most commonly

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observed in Fitzpatrick skin types IV to VI (Pawaskar *et al.,* 2007; Sarkar *et al.*, 2016; Sardesai *et al,* 2013). This clinical condition develops slowly and may last for many years.

The exact cause of melasma is still to be determined (Guarneri, 2016; Sonthalia & Sarkar, 2015). But various common contributing and risk factors have been identified such as sun exposure (exposure to UV light) (Jiang *et al.,* 2018), use of oral contraceptive pills (Arora *et al.*, 2017; Pawar *et al.,* 2015), pregnancy (Jiang et al., 2018), stress (Pawar *et al.,* 2015) and exogenous hormones (Pawar *et al.,* 2015). Cosmetics (Handel *et al.*, 2014), genetic predisposition (Arora *et al.,* 2017; Jiang *et al.,* 2018) and phototoxic drugs (Ali *et al.,* 2013) are reported to be linked with the causation of melasma. Unani literature says that dominance of melancholic humour (*Khilṭ Sawdā'*), prolonged amenorrhea and liver disorders may cause melasma (Khan, 1906).

A few studies have revealed that melasma has a direct negative impact on quality of life (QoL) and self-esteem of the patients (Jiang *et al.*, 2018; Yalamanchili *et al.*, 2015). The patient may suffer from psychosocial and emotional stress independent of severity of melasma or MASI score (Arora *et al.*, 2017). It directly affects the physical appearance of the patient leading to avoidance of public appearance and social gatherings.

Long duration therapy, lack of definitive therapy (Ravi & Pandya, 2013) and tendency of recurrence (Grimes *et al.,* 2010) are the challenges in the treatment of melasma. Excessive use of chemicals in cosmetics and lifestyle modification may have a link in the development of melasma. Now it becomes essential to have a current and comprehensive understanding and information of the demographic, epidemiological and clinical characteristics and risk factors of melasma. The present study was planned in order to determine the recent trends of clinico-epidemiological characteristics of melasma and its impact on QoL of the patients. The data in respect of clinical patterns, demographic characteristics and contributory factors was generated in this study. The outcome of this study would help in the understanding of the impact of melasma on the QoL of the patients. These data could be utilised to develop strategy for prevention and better management of melasma.

Materials and Methods

A cross-sectional study was conducted on the participants diagnosed with melasma at outdoor patient department (OPD) in National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD), Hyderabad. Melasma was diagnosed clinically on the basis of clinical features such as brown and grey symmetrical macules present on face with serrated or irregular border. Eighty-eight consecutive participants were enrolled into the study during August

2019 and July 2020. The data were collected in a case record form (CRF) specially designed for this study. Institutional ethics committee approved the protocol, informed consent form (ICF) and case record form of this study. The demographic data such as age, gender, chronicity of illness, age of onset, marital status, dietary habits and socio-economic condition were recorded in a face to face interview. Medical, socio-economic, treatment and gynecological (in female) histories were recorded to explore predisposing factors, natural course of the disease and relapses. The investigator assessed the temperament of the participants, melasma area severity index (MASI), dermatology life quality index (DLQI) and melasma quality of life (MQOL). Wood's lamp examination was conducted to differentiate the types of melasma. The temperament was assessed on the basis of standard questionnaire developed by Central Council for Research in Unani Medicine, New Delhi (Anonymous, 2016). MASI score was calculated to measure severity of the illness. DLQI, specific to dermatologic diseases and MQOL, a disease-specific QoL tool, were measured to observe the negative impact of melasma on QoL of the participants (Jiang *et al.*, 2018). The questionnaires for assessment of DLQI (Ali *et al.*, 2013) and MQOL have been presented in Table 1 and Table 2 respectively.

The statistical analyses were performed using Microsoft excel 2013. The data were analysed retrospectively. The continuous variables were expressed in mean

Table 1: Questionnaire for assessment of DQLI

1.	How much itchy, sore, painful or stinging has been your skin over last week?
2.	How much embarrassed or self-conscious you have been due to your skin, over last week?
3.	How much has your skin interfered with you when going shopping, home, garden over last week?
4.	How much was your skin influenced by clothes you wore over last week?
5.	How much has your skin affected any social or leisure activities?
6.	How much has your skin made it difficult to do any sport, over last week?
7.	Your skin prevented you from working or studying, over last week?
8.	You skin created problems with your partner or friends or relatives, over last week?
9.	How much has your skin caused any sexual difficulties, over last week?
10.	How much of a problem has the treatment for your skin been?

(**Scoring:** Very much=3, a lot=2, a little=1, and not at all=0)

Table 2: Questionnaire for assessment of MQOL

1.	Its appearance on your skin condition
2.	Frustration due to the appearance of your skin condition
3.	Embarrassment about the appearance of your skin condition
4.	Feeling depressed about your skin condition
5.	The effects of your skin condition on your interactions with others (e.g.: interactions with family, friends, close relationships, etc.)
6.	The effects of your skin condition on your desire to be with people
7.	Your skin condition making it hard to show affection
8.	Skin discoloration making you feel unattractive to others
9.	Skin discoloration making you feel less vital or productive
10.	Skin discoloration affecting your sense of freedom

(Each question scored 1-7)

and standard deviation whereas categorical data were measured in percentage and frequency.

Calculation of MASI: The MASI developed by Kimbrough-Green *et al.* was measured by subjective assessment of three factors: area (A) of involvement, darkness (D), and homogeneity (H) (Ali *et al.*, 2013). MASI is the sum of the severity rating for darkness (D) and homogeneity (H) multiplied by the numerical value of the areas involved (A) and the percentages of the four facial areas. The face was divided into 4 areas – forehead (f), right malar region (mr), left malar region (ml) and chin (c) corresponding to 30%, 30%, 30% and 10% of the face respectively. Darkness, homogeneity and areas involved are scored as per Table 3. The MASI ranges between 48 and 0.

(**MASI** = 0.3 (Df + Hf) Af + 0.3 (Dmr + Hmr) Amr + 0.3 (Dml + Hml) Aml + 0.3 (Dc + Hc) Ac).

Observations and Results

The present study focused on clinico-demographic profile of the participants. There were 71 (81%) female and 17 (19%) male participants. The average age of the participants (n= 88) was 39 ± 8.1 years (average age: male = 36.2 (±8.3) years; female = 39 (±7.7) years). In this study, the majority of the participants (i.e. 44 participants) were in the age group of 30–40 years. In addition, the average age of onset of the illness was 34 ± 8.1 years and the average age of onset in male and female was 31.6 (±8.1) years and 34.1 (± 8) years respectively.

Table 3: Scoring scale for assessment of MASI

S. No.	Scoring for Darkness (D)	Scoring for Homogeneity (H)	Scoring for Area Involved (A)
1.	Score 0 = Normal skin colour without any hyperpigmentation	Score 0 = Normal skin colour without any hyperpigmentation	Score 0 = no involvement
2.	Score 1 = Specks of involvement	Score1 = Barely visible hyperpigmentation	Score 1 = < 10%,
3.	Score 2 = Small patchy areas of involvement <1.5 cm in diameter	Score 2 = Mild hyperpigmentation	Score 2 = 10-29%,
4.	Score 3 = Patches of involvement >2 cm in diameter	Score 3 = Moderate hyperpigmentation	Score 3 = 30-49%,
5.	Score 4 = Uniform skin colour without any clear areas	Score 4 = Severe hyperpigmentation	Score 4 = 50-69%,
6.			Score 5 = 70-89%,
7.			Score 6 = 90-100%.

The study showed that the chronicity of melasma ranged between 6 months and 20 years with an average chronicity of illness of 5.2 (± 4.2) years. The majority of the participants (37%) had chronicity between 3 to 5 years. Besides, 31 participants (35%) had a positive family history of melasma. Out of 88 participants, 79 participants (90%) were married.

In this study we observed that the temperament of 37 (42%), 34 (37%) and 17 (19%) participants was sanguine, phlegmatic and bilious respectively. It was also observed that 44 participants (50%) had dermal type of melasma followed by 23 participants (26%) of mixed type of melasma and 21 participants (24%) of epidermal type of melasma. The clinico-demographic profile of the participants has been shown in Table 4.

Table 4: Clinico-demographic profile of the participants (n= 88)

Variables	Data
Gender	
Male, n (%)	17 (19%)
Female, n (%)	71 (81%)
Average age (years)	
Total Participants	39 ± 8.1
Male	36.2 (±8.3)
Female	39 (± 7.7)

Variables	Data
Age groups (years)	
18-30, n (%)	13 (35%)
30-40, n (%)	44 (50%)
40-50, n (%)	22 (25%)
50-55, n (%)	9 (10%)
Average age of onset (years)	
Total participants	34 ± 8.1
Male	31.6 (±8.1)
Female	34.1 (± 8)
Average chronicity of melasma (years)	5.2
Male	4.5
Female	5.3
Positive family history of melasma, n (%)	31 (35%)
Male	3 (4%)
Female	28 (31%)
Marital status	
Married, n (%)	79 (90%)[9]
Unmarried, n (%)	09 (10%)
***Mizāj* (temperament)**	
Damawī, n (%)	37 (42%)
Balghamī, n (%)	34 (37%)
Safrawi, n (%)	17 (19%)
Dietary habit	
Vegetarian, n (%)	33 (37.5%)[9]
Non vegetarian, n (%)	55 (62.5%)
Types of melasma	
Dermal, n (%)	44 (50%)
Epidermal, n (%)	21 (24%)
Mixed, n (%)	23 (26%)
MASI score (Mean ± SD)	
Total participants	17.84 ± 9.13
Male	18.19± 9.14
Female	17.75 ± 9.13
Mean DLQI score	
Total participants	20.3
Male	19.9
Female	20.4
Mean MQOL score	51.4
Total participants	49.8
Male	51.9
Female	

The present study showed that history of sun exposure in 12 participants (14%), OCP in 21 participants (24%), pregnancy in 13 participants (15%), amenorrhea in 12 participants (16%) and liver and endocrinal disorders in 5 participants (6%) were present as probable risk and contributing factors. Besides, 25 participants (28%) had observed menopause. Table 5 displays the participants with their risk factors.

Table 5: Distribution of participants according to risk factors

S.No.	Risk factors	Number of participants
1.	Sun-exposure, n (%)	12 (14%)
2.	Oral contraceptive, n (%)	21 (24%)
3.	History of pregnancy, n (%)	13 (15%)
4.	Amenorrhea	12 (16%)
5.	Menopause	25 (28%)
6.	Liver and endocrinal disorders	5 (6%)

It was also observed that centro-facial pattern of melasma was present in 41 participants (45%) followed by malar pattern in 36 participants (41%) and mandibular pattern in 11 participants (12.5%). The classification of the participants as per pattern of melasma is displayed in Table 6. Moreover, figures 1 to 3 show the patterns of melasma found in the participants.

Table 6: Distribution of participants according to pattern of melasma

S. No.	Types of melasma	Number of participants	
		Female (N=71)	Male (N=17)
1.	Centro-facial, n (%)	36 (51%)	5 (29%)
2.	Malar pattern, n (%)	28 (39%)	8 (47%)
3.	Mandibular pattern, n (%)	7 (10%)	4 (23.5%)

Furthermore, we observed that 70 participants had a positive treatment history for melasma as 52 (59%), 11 (12.5%) and 7 (8%) participants had taken allopathic, homeopathic and ayurvedic treatment respectively. In this study, melasma was observed in the participants having III (light brown), IV (medium brown) and V (dark brown) Fitzpatrick types of skin. Out of the 88 participants, 60 (68%) and 21 (24%) had IV and III Fitzpatrick types of skin. Only 7 participants (10%) had V Fitzpatrick type of skin.

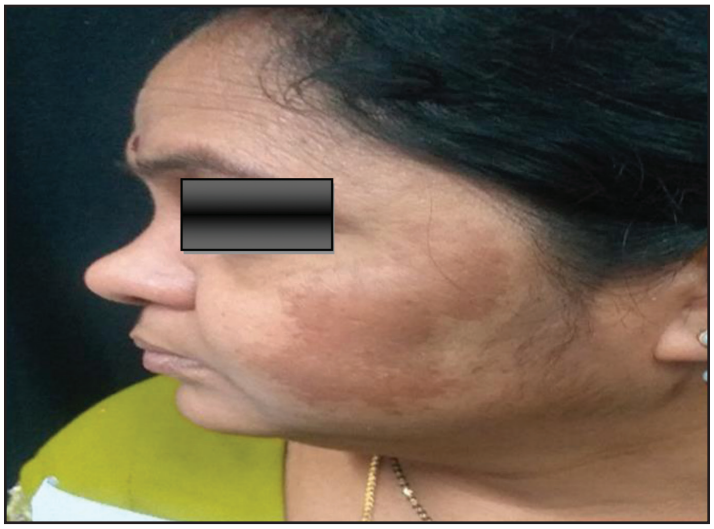

Figure 1: Mandibular pattern of melasma

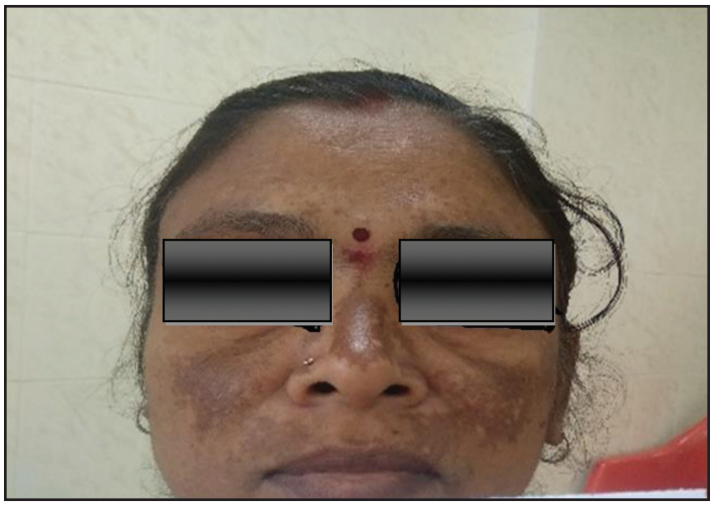

Figure 2: Malar pattern of melasma

Figure 3: Centro-facial pattern of melasma

In this study, the average MASI score was 17.84 (ranged between 40.8 and 1.8). The participants had mean DLQI of 20.3 and mean MQOL of 51.4.

Discussion

Melasma is an acquired, asymptomatic and hyper-pigmented lesion on facial skin. It is observed in all ethnic and population groups including Asian (Charupalli *et al.*, 2018; Handel *et al.*, 2014). Its impact on QoL demands urgent remedy and instigate to have current understanding about the disease.

The demographic, epidemiological and clinical profile of melasma was observed in this study. The study showed that the average age of the participants was 39 ± 8.1 years with an average age 39 ± 7.7 years in female and 36.2 ± 8.3 years in male. Acher and Rathi (2011) in their study reported that the average age of patients with melasma was 33.45 years.

In addition, the majority of the participants belonged to 30–40 years of age group. The similar observation was reported in a previously published study (Arora *et al.*, 2017). In another study, 48.33% patients were in the age group of 30–39 years (Kumar & Sharma, 2018).

The present study showed that melasma was predominant in female participants (almost 81%) with male to female ratio of 1:4. Similar finding was observed in several studies (Ali *et al.*, 2013; Arora *et al.*, 2017). Moreover, Sivayathorn *et al.* reported a female to male ratio of 6:1 in a Malaysian population and 24:1 in Indonesian population (KrupaShankar *et al.*, 2014). Melasma is common in female because of hormonal activities which is considered to be one of the risk factors for the development of melasma.

In our study, the average age of onset of the illness was 31.6 ±8.1 years in males and 34.1 ± 8 years in females. Whereas the mean age of onset was reported 24.78 years in males and 30.80 years in females in a study conducted by Pawar *et al.* (Pawar et al., 2015). Moreover, Sarkar *et al.* reported similar mean age of onset in males and females.

In the present study, the average chronicity of melasma was 5.2 (± 4.2) years. In addition, the majority of the participants (i.e. 37%) had chronicity between 3 to 5 years. Similar observation was reported in previous studies (Arora *et al.*, 2017). In a study, it was reported that about 43% of the patients had a disease chronicity of >3 years (KrupaShankar *et al.*, 2014).

Besides, we observed a positive family history of melasma in 31 participants (35%). In another study, a positive family history was observed in 16.67% of patients (Pawar *et al.*, 2015). The study conducted by Achar and Rathi showed a positive family history in 33.33% patients (Achar & Rathi, 2011). In another study, out of 60 participants, a positive family history of melasma in either

parents or any of the siblings was present in 18 patients (Kumar & Sharma, 2018). In this study, it was found that 79 (90%) participants were married. Marriage has not been associated with melasma in any of the previous studies.

In this study, we observed that the temperament of the participants was sanguine, phlegmatic and bilious except melancholic. Unani literature says that melasma was common in individuals of melancholic temperament (Khan, 1906). Our observation does not correspond with the observation reported in classical literature.

Histologically, melasma is classified on the basis of excessive melanin deposition in the epidermis (epidermal type, 70%), dermal macrophages (dermal type, 10%), or both (mixed type, 20%) (Filoni *et al.*, 2019). The present study demonstrated that the participants had dermal, epidermal and mixed types of melasma. The similar observation was reported in previous studies (Filoni *et al.*, 2019).

In this study we found that sun exposure, OCP, pregnancy, amenorrhea, liver and endocrinal disorders were present as probable risk and contributing factors. It is reported that melasma is common during reproductive period of female. In addition, we found history of taking oral contraceptive pills in the participants. It is reported that female hormones may play a role in the acceleration and aggravation of melasma. The exogenous hormonal therapy (progestogen-estrogen combinations) for ovulation control, dysmenorrhea, infertility, and endometriosis has added another cause for melasma (Hammer, 1968). Development of melasma during third trimester of pregnancy is more likely to be associated with circulating female hormones (Filoni *et al.,* 2019). According to epidemiological data, 14.5% to 56% of pregnant women and 11.3% to 46% of individuals who take oral contraceptives suffer from melasma in different countries (Filoni *et al.*, 2019). In our study, there were 25 participants (28%) in menopause. It has been reported that almost 10% participants start melasma after menopause (Filoni *et al.*, 2019). An association between thyroid hormone and melasma has also been reported in several previous studies (Śakmak *et al.*, 2015).

Melasma is classified into three types: centro-facial, malar, and mandibular patterns (Filoni *et al.,* 2019). In this study we observed that centro-facial was the commonest clinical pattern of melasma followed by malar pattern and mandibular pattern. In a study it was reported that malar pattern melasma was predominant in the southern region of India to a greater extent when compared with the northern region (KrupaShankar *et al.*, 2014).

Furthermore, we observed that a positive treatment history was present in 70 participants. They had taken allopathic, homeopathic and ayurvedic treatment but response of the treatment was poor. This shows that the treatment of melasma is difficult and relapse of melasma is common. In this study, melasma was observed in the participants having III (light brown), IV (medium brown)

and V (dark brown) Fitzpatrick types of skin. The same observation has been reported in previous studies (Pawaskar *et al.*, 2007).

In this study mean MASI score of the total participants was 17.84 (± 9.13) with a range between 40.8 and 1.8. Arora *et al.* reported in their study mean MASI score as 4.7 and Sarkar *et al.* reported it as 20.0±7.5. In another study, MASI score ranged between 4.5 and 38.6 with mean MASI score of 16.94 (Pawar *et al.,* 2015). Another study showed the mean MASI score 12.1±6.5 (median 10.8) (Harumi & Goh, 2016). Our observation is almost similar to the observation reported by Sarkar *et al.* (2016).

The present study showed that the QoL of the affected individuals was significantly impacted by melasma. We observed significantly higher mean DLQI (20.3) and mean MQOL (51.4) in the participants of this study. QoL includes physical health, interpersonal relationships, and social well-being. A few previous studies also demonstrated that melasma had a negative impact on QoL of the patients (Jiang *et al.,* 2018). Improvement in QoL of the patients is one of the objectives of the proper management of melasma. Moreover, we observed that negative impact on QoL is independent of severity of melasma or MASI score. We classified the participants into mild (a MASI score of 0–16.9), moderate (a MASI score of 17–32.9) and severe (a MASI score of 33–48) cases of melasma and found that mean DLQI and mean MQOL were almost comparable among the three groups. But in a study it was reported that the patients with severe disease had greater impairment of QoL (Ali *et al.,* 2013). In another study, significant positive correlation between MQOL and salary per month was reported (Coban, 2018).

This is a retrospective cross-sectional study. This study had several limitations. The study had small sample size and there was no control group to compare the association of the probable risk factors with the disease. The possibility of bias could not be ruled out in this study. The observations may further be confirmed by another prospective case-control and/or cohort study.

Conclusion

We studied age of onset, gender predilection, risk factors, clinical patterns and impact on QoL with regard to melasma in this study. We need these information for comprehensive understanding of melasma. The literature shows that melasma is a common, acquired, chronic disfiguring and cosmetic skin disorder. Prolonged treatment, lack of definitive therapies and higher rate of relapse are the imminent challenges in its treatment. Our findings may be essential for developing strategy for better prevention and management of melasma.

Moreover, negative impact of melasma on quality of life of the participants has been observed in the study. The patient may suffer from interpersonal,

psychological, social and emotional stress despite the severity of the disease. The aim of the treatment should include improvement in the QoL of the patient as a strategy for proper management of the disease. The role of stress in inducing melasma has already been established.

This is an observational study. Despite several limitations, it provides current trends of epidemiological and clinical profile of melasma. It may seek attention of the researchers and academia for utilization of the data in the prevention and management of melasma.

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सारांश

कलफ़ (मेलाज़्मा) का क्लिनिको–एपिडेमियोलॉजिकल अध्ययन एवं जीवन की गुणवत्ता पर इसका प्रभाव

इफ़रा अब्दुल क़य्यूम, *मोहम्मद नवाब,एम.एच. काज़मी

सारांश

परिचयः *कलफ़* (मेलाज़्मा) एक लक्षणहीन कॉस्मेटिक विकार है जिसका प्रभावित व्यक्तियों के जीवन की गुणवत्ता पर नकारात्मक प्रभाव पड़ता है। सम्पूर्ण थेरेपी की कमी और पुनरावृत्ति की उच्च दर मेलाज़्मा के उपचार में प्रमुख चुनौतियां हैं।

उद्देश्यः इस अध्ययन का उद्देश्य *कलफ़* (मेलाज़्मा) की जनसांख्यिकीय, एपिडेमियोलॉजिकल और नैदानिक अभिलक्षणों का अध्ययन करना और व्यक्ति के जीवन की गुणवत्ता पर इसके प्रभाव का अवलोकन करना था।

सामग्री और विधिः हमने रा.त्व.रो.यू.चि.अ.सं., हैदराबाद के बाह्य रोगी विभाग में आने वाले 18 से 55 वर्ष की उम्र के किसी भी लिंग के मेलाज़्मा के 88 रोगियों पर एक क्रॉस सेक्शनल अध्ययन किया। सम्मुख इण्टरव्यू और नैदानिक परीक्षण में पूर्व निर्धारित प्रारूप पर डाटा एकत्रित किया गया और पूर्वव्यापी रूप से विश्लेषण किया गया।

अवलोकन और परिणाम : हमने मेलाज़्मा के रोगियों की औसत आयु (39 ± 8.1 वर्ष), रोग शुरुआत पर औसत आयु (34 ± 8.1 वर्ष), महिला से पुरूष अनुपात (4:1) और रोग की औसत दीर्घकालिकता (5.2 ± 4.2 वर्ष) का अवलोकन किया। हमने रोगियों में मीन एमएएसआई (17.84), मीन डीएलक्युआई (20.3) और मीन एमक्यूओएल (51.4) पाया।

चर्चाः अध्ययन से पता चला कि 30–40 वर्ष की आयु की महिलाओं में मेलाज़्मा सामान्य था। सबसे आम नैदानिक पैटर्न चेहरे पर केंद्रित था। सूर्य अनावरण, गर्भावस्था, मौखिक गर्भनिरोधक गोलियों का सेवन एवं लंबे समय तक रक्तस्राव संभावित योगदान कारक थे। उच्च मीन डीएलक्युआई और मीन एमक्युओएल ने रोग की गंभीरता के फर्क के बावजूद रोगियों के जीवन की गुणवत्ता पर मेलाज़्मा का नकारात्मक प्रभाव दिखाया।

निष्कर्षः निष्कर्ष के तौर पर यह अध्ययन मेलाज़्मा के क्लिनिको–एपिडेमियोलॉजिकल के लक्षणों के वर्तमान ज्ञान की जानकारी प्रदान करता है। रोगियों के जीवन की गुणवत्ता पर इसका नकारात्मक प्रभाव होता है।

शब्दकुंजीः त्वचा विज्ञान, अतिवर्णकता, एमएएसआई, मेलाज़्मा, गर्भावस्था, यूनानी, *कलफ़*

Immuno-modulatory Action of Unani Formulation against HBV Induced Compensated Cirrhosis of Liver

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Abstract

Chronic inflammation almost always precedes and accompanies fibrotic changes and the medicines that target the inflammatory cascade of different cytokines such as tumour necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), different interleukins, etc. typically have immune-modulator and anti-fibrotic activity. Thus, measuring these cytokines serum levels in the cirrhotic individuals and targeting them as a parameter in the improvement of fibrosis could be a strategy in the treatment of HBV induced cirrhosis of liver besides the control of replicating HBV. To evaluate the immuno-modulatory action of Unani treatment, present case series was carried out in 7 patients with HBV induced compensated cirrhosis of liver where patients were treated with a set of Unani treatment for a period of 6–8 months. Estimation of serum levels of IFN-γ and TNF-α was carried out as a prime investigation in addition to evaluation of HBV DNA quantitative, liver function test and fibroscan of liver. Observed significant results substantiate the immuno-modulatory as well as beneficial potential of Unani medicines in the treatment of compensated cirrhosis of liver.

Keywords: Cirrhosis of liver, Unani medicine, IFN-γ, TNF-α, fibrosis

Introduction

Cirrhosis of the liver is the fourth cause of death in adults in Western countries, with complications of portal hypertension being responsible for most casualties. Chronic liver disease (CLD) affects more than 29 million people in Europe and over 300 million people worldwide (Blachier, 2013). The main causes of CLD are alcohol abuse, chronic viral hepatitis (hepatitis B virus and hepatitis C virus), and metabolic factors (non-alcoholic fatty liver disease). Mortality in CLD is primarily due to complications of liver cirrhosis and hepatocellular carcinoma (HCC), which is considerably more prevalent in the patients with cirrhosis (Rosselli, 2013; Annalisa, 2017).

Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury that leads to portal hypertension and end stage liver disease (Schuppan, 2008). Cirrhosis is a common pathological consequence of chronic liver disease. Hepatitis B virus (HBV) is one of the etiologies of liver cirrhosis in India. Cirrhosis has two phases, compensated and decompensated (Annalisa, 2017; Schuppan, 2008). Compensated cirrhosis means that the liver is still functioning relatively well. At this stage, the early symptoms may still be vague and some people may be unaware that they have cirrhosis (Annalisa, 2017). Indeed, even after the onset of cirrhosis, the disease can remain asymptomatic, or 'compensated', for a long

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time. Nevertheless, during this time, portal hypertension progressively develops (non-bleeding esophageal varices) and usually accompanied by a decline in hepatocellular function (D'Amico, 2006).

HBV is a stealth non-cytopathic virus in the liver. The recurrent and chronic hepatitis observed due to HBV infection is rather immune mediated due to activation of T cells and release of cytokines such as tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), various interleukins, etc. (Attallah *et al.*, 2016). Studies have shown that serum levels of TNF are significantly higher in patients with cirrhosis than in those without cirrhosis, reaching the highest levels in decompensated cirrhosis. It has also been shown that plasma levels of TNF-α correlate with the severity of hepatic encephalopathy (HE) in fulminant hepatic failure (Odeh, 2004). While, IFN-γ is a major factor, reflecting hepatic dysfunction regardless of underlying disease more than liver inflammation parameters. Various studies support the hypothesis that liver damage against HBV infection is due to an immune-mediated destructive mechanism rather than direct cytopathic effect of the virus itself (Attallah et al; 2016).

Thus, the effective inhibition of HBV can partially stop or reverse liver fibrosis in the patients with chronic hepatitis and liver cirrhosis, and the anti-fibrotic strategy focusing on the regulation of hepatic extracellular matrix is still required. Modern anti-fibrotics are still in phase 3 clinical trials while modern antivirals do not fulfil all the clinical needs due to their questionable efficacy, adverse effects and resistance (Siddiqui, 2016; Ansari, 2019).

Unani Medicine has been found to enhance the degradation of collagens in fibrotic liver, regeneration of liver tissue, and has a good action against persistent immune attacks (hepatocyte) in liver of the patients with chronic hepatitis B. However, there are no high quality clinical evidences which can demonstrate if the combination of Unani medicines acting as antiviral and anti-fibrotic therapy can improve the reversion of the cirrhosis due to HBV (Siddiqui, 2016).

The present case series tried to preliminarily substantiate the immunomodulatory action of Unani treatment in improving the serum levels of TNF-α and IFN-γ whose increased levels are strongly linked with advancement in cirrhotic changes.

Material and Methods

Informed consent was taken from the patients. Human data included in this case series was obtained in compliance with the Declaration of Helsinki as revised in 1983.

Intervention

1. *'Araq-i-Mako* (*Solanum nigrum*) + *'Araq-i-Kāsnī* (*Cichorium intybus*) + *'Araq-i-Biranjāsif* (*Achillea millefolium*) 150 ml, twice daily

2. *Sharbat-i-Jigrīn* 25 ml, twice daily / Capsule *Jigrīnā* 2 capsules, thrice daily

3. *Ḥabb-i-Ḥiltīt* 3 pills, twice daily after meals

4. *Sharbat-i-Buzūrī Mu'tadil* 25 ml, twice daily/ *Banādiq al-Buzūr* 2 tablets, thrice daily

5. *Ma'jūn Dabīd al-Ward*, 10 g twice daily

6. Decoction of *Shāhitara* (*Fumaria officinalis*), *Sarphokhā* (*Tephrosa purpurea*), *Chirā'ita* (*Swertia chiraita*), *Gul-i-Mundī* (*Sphaeranthus indicus*), and *Ṣandal Surkh* (*Pterocarpus santalinus*) each 5 g soaked in 300 ml of water for overnight and boiled for 10 minutes till decoction became half of its original volume, filtered and ingested empty stomach in the morning daily

7. Diet: Avoidance of oily, spicy, fatty, red-meat diet. Advise for intake of soft diet, oatmeal and barley water daily

All the patients in the case series received these drugs for a period of 6–8 months.

Case Presentation

Case 1: A 50-year Indian male was admitted in Majeedia Unani Hospital, Jamia Hamdard, New Delhi on Jan 17, 2015 with chief complains of (1) loss of appetite, (2) mild pain in abdomen, (3) yellow coloration of eye, skin and urine, (4) general weakness, and (5) breathlessness while walking for 3–4 months. He was a diagnosed case of HBV induced compensated cirrhosis of the liver and was taking treatment from a reputed tertiary care liver-specialty hospital in New Delhi for 3 years.

Case 2: A 42-year old Indian male was admitted in Majeedia Unani Hospital, Jamia Hamdard, New Delhi on March 9, 2015 with chief complains of (1) yellow coloration of the eye ×15 days, (2) loss of appetite ×15 days, (3) pain in upper abdomen ×15 days, (4) on and off fever × 2 months, (5) itching all over body × 2 months, and (6) headache × 2 months. He reported positive history of hepatitis B in wife. He was a diagnosed case of hepatitis B for 5 years and was not taking any treatment. He was diagnosed as HBV induced compensated cirrhosis of liver with anaemia, splenomegaly, cholelithiasis and right renal cyst.

Case 3: A 40-year old Indian male was admitted in Majeedia Unani Hospital, Jamia Hamdard, New Delhi on February 13, 2015 with chief complains of (1) jaundice × 8 days, (2) fever × 1 month, (3) pain in abdomen × 2 months, and (4) loss of appetite × 2 months. He had positive history of hepatitis B in siblings. He was diagnosed as HBV induced compensated cirrhosis of liver, gallbladder edema, and periportal lymphadenopathy.

Case 4: A 35-year old Indian female was admitted in Majeedia Unani Hospital, Jamia Hamdard, New Delhi on March 6, 2014 with chief complains of (1) general weakness, (2) heaviness in the abdomen, (3) easy tiredness, (4) joint pain, and (5) recurrent dark colored urine from 8–10 months. She was diagnosed as a case of HBV induced compensated cirrhosis of liver with gross splenomegaly, esophageal varices grade-II, diabetes mellitus T-2 and dilation of portal vein.

Case 5: A 53-year old Indian female was admitted in Majeedia Unani Hospital, Jamia Hamdard, New Delhi on December 16, 2014 with chief complains of (1) fatigue, (2) jaundice, (3) on and off fever, (4) joint pain, and (5) heaviness in the abdomen for 2 years. She was diagnosed with HBV induced compensated cirrhosis of liver with cholelithiasis.

Case 6: A 40-year old obese Indian female came to OPD of Majeedia Unani Hospital, Jamia Hamdard, New Delhi on October 15, 2015 with chief complains of (1) mild to moderate pain in abdomen, (2) loss of appetite, (3) nausea after taking meals, (4) bloating and tightening of abdomen frequently, (5) general weakness, and 6) negative feeling toward life from 3 years. The patient was taking on and off treatment for HBV induced cirrhosis of livers for 3 years. She was also diagnosed with hypertension, splenomegaly and grade-I esophageal varices.

Case 7: A 47-year old Indian male came to OPD of Majeedia Unani Hospital, Jamia Hamdard, New Delhi on April 15, 2015 with chief complains of (1) general weakness × 6 months, (2) yellow discoloration of urine × 1 year, (3) nausea after taking meals ×1 year, (4) heaviness of abdomen × 1 year, (5) joint pain × 1 year, and (6) headache × 1 year. He was diagnosed with HBV induced cirrhosis of liver with splenomegaly. He took modern antivirals for 2 months and thereafter left taking medicine 3 months ago due to feverish feeling and no symptomatic improvement.

Outcome Measures

Serum TNF-α, IFN-γ, HBV DNA and SGPT, and Fibroscan of the liver was performed at the baseline and end of the treatment. TNF-α and IFN-γ were used as a prime investigation to evaluate the immuno-modulatory action of the Unani treatment. [Normal range of Serum TNF-α (1.7-8.1 pg/mL through chemiluminescence), IFN-γ (<0.1 pg/mL through ELISA), HBV DNA (<20 IU/mL undetectable through real time PCR) and SGPT (<40U/mL through automated biochemistry analyzer), and Fibroscan of liver in NABL laboratories].

Statistical Analysis

Normal-distributed continuous variables were calculated as mean ± standard deviation (SD). They were compared by Wlicoxan signed rank test through

GraphPad Prism, version 7.00 for Windows created on March 31, 2016. Differences were considered significant when the p value was less than 0.05. Test results were ranked as: ns - Non significant p>0.05, *p < 0.05 significant, **p < 0.01 very significant, ***p < 0.001 extremely significant.

Results

Effect of Unani treatment on Serum TNF-α, IFN-γ, HBV DNA and SGPT, and Fibroscan of liver is demonstrated in Table 1 below:

Table 1: Effect of test drugs on investigations

S. No.	TNF-α (pg/ mL)		IFN-γ (pg/ mL)		Fibroscan (E median)		HBV DNA (IU/ mL)		SGPT (U/ mL)	
	Base-line	After treat-ment*	Base-line	After treat-ment*	Base-line	After treat-ment*	Base-line	After treat-ment*	Base-line	After treat-ment**
Case 1	18.1	8.41	46.1	23.4	E (42,0) CAP (188)	E (26,0) CAP (176)	22700	<20	14	49
Case 2	16.22	8.0	42.0	19.6	E (45,0) CAP (148)	E (24,0) CAP (122)	221000	1027	2043	53.81
Case 3	17.3	10.6	39.1	21.2	E (39,0) CAP (171)	E (20,0) CAP (140)	188115	<20	1271	34.1
Case 4	17.7	10.71	46.6	25.1	E (50,0) CAP (166)	E (35,0) CAP (176)	1200	51	204	60
Case 5	14.31	6.36	35.3	20.4	E (21,0) CAP (135)	E (13,0) CAP (134)	91300	<20	105	25.6
Case 6	14.9	7.3	25	20.1	E (21,8) CAP (302)	E (15,0) CAP (305)	506	120	64	25
Case 7	16.7	8.1	25.1	19.0	E (24,6) CAP (205)	E (19,1) CAP (175)	1037	<20	304	45

** p<0.01 very significant; ** p < 0.001 extremely significant.*

Discussion

In chronic HBV infection, repeated immune attacks through T cells trigger production of inflammatory cytokines including IFN-γ, TNF-α, interleukin (IL) 2, etc. which produces repeated inflammation of the hepatocyte. Chronic inflammation in the liver potentiates the formation of the fibrous tissue leading to formation of nodules which consequently advances to compensated or decompensated cirrhosis of the liver and hepatocellular carcinoma, and their complications (Schiff, 2007).

Unani Medicine has immense potential for the treatment of chronic diseases including chronic liver diseases. The diverse actions of a single Unani drug such as antiviral, antifibrotic, immuno-modulatory, anti-oxidant and anti-inflammatory nature made Unani drugs most suitable to be used in the treatment of HBV induced cirrhosis of liver. In this particular case series, we primarily evaluated immunomodulatory action of a set of Unani drugs in reducing the serum levels of IFN-γ and TNF-α which potentiate immune mediated inflammation in the liver. We observed that Unani treatment reduced serum levels of both IFN-γ and TNF-α in the seven patients of HBV induced compensated cirrhosis of liver, very significantly at $p<0.01$. The observed immuno-modulatory effect of the Unani treatment could be due to immuno-modulatory action of the constituents in the above mentioned Unani treatment, evidenced in various animal models (Nancollas, 1995; Amirghofran, 2000; Kumar, 2003).

Significant reduction ($p<0.01$) in 'E' median in fibroscan was observed in our seven cases. The observed decrease in fibrosis in fibroscan of the liver could be due to antioxidant effect of the constituents of Unani treatment. An antioxidant which may have exerted a preventive effect on hepatocyte injury may also be antifibrogenic directly (Ansari, 2019). Based on this principle, various constituents such as *Shāhitara* (*Fumaria officinalis*), *Sarphokā* (*Tephrosia purpurea*), *Chirā'ita* (*Swertia chiraita*), *Gul-i-Mundī* (*Sphaeranthus indicus*), *Ṣandal Surkh* (*Pterocarpus santalinus*), *Mako* (*Solanum nigrum*) and *Kāsnī* (*Cichorium intybus*) evidenced as potential antioxidants (inhibiting ROS generation), could be the plausible mechanism of reduction of fibrosis in the fibroscan and liver regenerative effect (Shirwaikar, 2006; Kshirsagar, 2015; Miniaev, 1987; Arulmozhi, 2010; Rafiquzzaman, 2013; Siddiqui, 2015). These drugs have also substantiated their antiviral effect in various *in-vitro, in-vivo* and clinical studies which could be the possible antiviral effect observed against HBV in our cases (Siddiqui, 2015; Zhou *et al.*, 2015).

Conclusion

The present case series preliminarily substantiates the immuno-modulatory and antifibrotic potential of Unani treatment in HBV induced compensated

cirrhosis of liver. However, large randomized controlled clinical and rigorous pharmacological studies should be performed to prove their relative efficacy in reversing, controlling and halting advanced outcomes of the disease such as decompensated cirrhosis and hepatocellular carcinoma.

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सारांश

एचबीवी प्रेरित क्षतिपूर्ति यकृत सिरोसिस के विरूद्ध यूनानी मिश्रण की इम्यूनोमॉड्यूलेटरी क्रिया

शबनम अंसारी, मोहम्मद मआज़, आसिम अली ख़ान, अज़हर जबीन, *शाह आलम

सारांश

दीर्घकालिक इन्फ्लामेशन लगभग हमेशा फाइब्रोटिक परिवर्तन के पहले से होता है और इसके साथ–साथ रहता है। जो औषधियां विभिन्न साइटोकिन्स जैसे ट्यूमर नेक्रोसिस फैक्टर अल्फा (टीएनएफ– α), इंटरफरॉन–गामा (आईएफएन– γ), विभिन्न इंटरल्यूकिन्स इत्यादि के इन्फ्लामेटरी कैस्केड को लक्षित करती हैं उनमें आमतौर पर रोगप्रतिरोधक–मॉड्यूलेटर और एंटी–फाइब्रोटिक गतिविधि होती है। इस प्रकार सिरोसिस वाले व्यक्तियों में इन साइटोकिन्स सीरम स्तरों को मापना और उन्हें फाइब्रोसिस के सुधार में एक पैरामीटर के रूप में लक्षित करना एचबीवी की प्रतिकृति के नियंत्रण के अलावा यकृत के एचबीवी प्रेरित सिरोसिस के उपचार में एक रणनीति हो सकती है। वर्तमान केस श्रृंखला यूनानी उपचार की इम्यूनो–मॉड्यूलेटरी क्रिया का मूल्यांकन करने के लिए एचबीवी प्रेरित क्षतिपूर्ति यकृत सिरोसिस के 7 रोगियों में की गई जिसमें रोगियों का उपचार 6–8 महीने की अवधि के लिए कुछ विशेष यूनानी औषधियों द्वारा किया गया। आईएफएन–γ और टीएनएफ–α के सीरम स्तरों का आकलन एचबीवी डीएनए मात्रात्मक, लीवन फंक्शन टेस्ट और यकृत का फाइब्रोस्केन के मूल्यांकन के अतिरिक्त एक प्रमुख जांच के रूप में किया गया। प्राप्त महत्वपूर्ण परिणाम एचबीवी प्रेरित क्षतिपूर्ति यकृत सिरोसिस के उपचार में यूनानी चिकित्सा की इम्यूनो–मॉड्यूलेटरी के साथ–साथ लाभकारी क्षमता को सिद्ध करते हैं।

शब्दकुंजी: यकृत सिरोसिस, यूनानी चिकित्सा, आईएफएन–γ, टीएनएफ–α, फाइब्रोसिस

Effect of *Ḥabb Muṣaffī-i-Khūn, Iṭrīfal Shāhitara* and Eczenil Ointment in a Case of *Qūbā al-Badan* (*Tinea Corporis*)

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Abstract

Tinea corporis is a superficial infection of skin of trunk, arms and legs of humans commonly caused by three microorganisms, e.g. *Trichophyton*, *Microsporum* and *Epidermophyton*. However, *Trichophyton rubrum* is the most common cause. According to the philosophy of Unani Medicine, it is an inflammation of skin due to the accumulation of *Akhlāṭ Fāsida* and *Ajsām Khabītha*. Tinea infection is diagnosed on the basis of its clinical appearance and confirmed by microscopic or culture test. In this case study, an 18-year old female student suffering from the disease for last 8 months was given Unani pharmacopoeial formulations *Ḥabb Muṣaffī-i-Khūn* and *Iṭrīfal Shāhitara,* and a market product eczenil ointment. These Unani medicines showed remarkable effect in decreasing the inflamed margin within a 21-day treatment. Further control studies may be designed to evaluate the effect of these medicines in the treatment of the disease in a larger sample size.

Keywords: *Qūbā*, *Tinea corporis*, Ringworm, Unani Medicine

Introduction

A common skin infection caused by fungus is called ringworm (dermatoophytosis). It is called 'ringworm' because it causes ring like circular red and itchy rashes on the skin. In Unani Medicine (UM), it is known as *Qūbā* (Havlickova *et al.*, 2008). It is a fungal infection of the skin caused by 40 types of fungi and can affect multiple areas at a time (Parveen *et al.,* 2019). Risk factors include using public showers, contact sports such as wrestling, excessive sweating, contact with animals, obesity, and poor immune function (Havlickova *et al.*, 2008). Generally superficial fungal infections are due to different genera of dermatophytes: *Trichophyton*, *Microsporum* and *Epidermophyton* (Kaur *et al.*, 2019; Jegadeesan *et al.,* 2017). It has been classified according to its disease patterns not due to the agents that cause dermatophytosis/ringworm/*Qūbā*. Fungal infection of the arms, legs and trunk is called *Tinea corporis* (Parveen *et al.,* 2019). *Qūbā* is a disease condition in which skin becomes rough along with other symptoms including itching, scaling, dryness and sometime fish like scales shed off from the site of lesion (Parveen *et al.*, 2019). Many Unani scholars described this disease condition in their treatises, e.g. Abu al-Hasan Ali bin Sahl Rabban Tabari (770-850 AD) in *Firdaus al-Ḥikmat* (Tabari, 1981), Abu Bakr Muhammad ibn Zakariyya al-Razi (850-923 AD) in 23rd volume of *Al-Ḥāwī fi'l-Ṭibb* (Razi, 1994) and *Kitāb al-Manṣūrī fi'l-Ṭibb* (Razi, 1991), Abu al-Hasan Ahmad bin Mohammad Tabari in *Al-Mu'ālajāt al-Buqrāṭiya* (Tabari, 1997), Abu Mansur al-Hasan ibn Nuḥ Qumri (d. 980-990) in *Kitaāb al-Ghinā wa al-Munā fi 'Ilm al-Ṭibb* (Qumri,

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1930), Ali ibn al-'Abbas al-Majusi (982–994) in *Kitāb Kāmil al-Ṣinā'a al-Ṭibbiyya* (Majoosi, 1889), Ibn Sina (980-1037) in *Al-Qānūn fi'l-Ṭibb* (VIth volume) (Ibn Sina, 1903) and Zayn al-Din Sayyed Isma'il ibn Husayn Jurjani (1040–1136) in *Zakhīra Khawārizm Shāhī* (Jurjani, 2010). Later on many Mughal and Indian Unani scholars also referred them in their treatises, e.g. Muhammad Akbar Arzani (d. 1722) in *Ṭibb-i-Akbar* (Arzani, 1903), Hakim Mohammad Azam Khan (1814-1902) in *Iksīr-i-A'zam* (Khan, 1873) and *Rumūz-i-A'zam* (Khan, 1903), Allama Mohammad Kabiruddin in *Sharḥ-i-Asbāb Urdu Tarjuma-i-Kabīr*, vol. III (Kabiruddin, 1916).

According to UM, the causes of *Qūbā* are *Ḥiddat* (excessive heat), *Qalawiyat* (alkalinity) and *Sawdāwiyat* (coldness & dryness) in the humour of the affected part. In *Qūbā,* either *Dam* or *Sawdā*' becomes *Raqīq* (liquefied) and slightly basic in nature which increases the penetrating power of humour into the skin. *Qūbā* appears on the skin and causes roughness, desquamation, shedding, itching and sometime hotness at the site (Khan, 1873; Majoosi, 1889; Ibn Sina, 1903; Arzani, 1903; Khan, 1903; Kabeeruddin, 1916; Qumri, 1930; Tabari, 1981; Razi, 1991; Razi, 1994; Tabari, 1997; Jurjani, 2010).

Modern medicine gives it different names according to the site of appearance in the body. Accordingly, contemporary Unani scholars have given different names to the disease considering the site affected, e.g. *Qūbā* at the arm is named as *Qūbā al-Badan* (*Tinea corporis*) (Ahmad, 2004).

Unani scholars recommended the treatment of *Qūbā* by *Faṣd* (venesection), a component of *'Ilāj bi'l-Tadbīr* (regimen therapy), *Istifrāgh-i-Mādda* (evacuation of causative matter) and *Ghassāl Adwiya* (drugs of irrigation) with *Muṣaffiyāt-i-Dam* (blood purifiers) for systemic and local application (Khan, 1873; Majoosi, 1889; Ibn Sina, 1903; Arzani, 1903; Khan, 1903; Kabiruddin, 1916; Qumri, 1930; Tabari, 1981; Razi, 1991, 1994; Tabari, 1997; Jurjani, 2010; Ahmad, 2004).

Objective of the study

The objective of this case presentation is to showcase the efficacy of Unani pharmacopoeial formulations *Ḥabb Muṣaffī-i-Khūn* and *Iṭrīfal Shāhitara* along with market product *Eczenil* ointment, a Unani topical medicine, in a case of *Qūbā* (*Tinea corporis*).

Case presentation

An 18-year old female student presented to the General Outpatient Department at Regional Research Institute of Unani Medicine, Aligarh with complaint of reddish lesions on the left arm with severe itching for the last 8 months. The patient had the history of taking allopathic treatment from private practitioners in her

locality. The physician prescribed her antifungal, antiallergic systemic medicines and lotion for local application. But she did not get any remarkable relief in the sign and symptoms. She did not have history of diabetes, hypertension and any other systemic illness. No family history of ring worm was found. A photograph of the affected part of the patient was taken before starting the treatment as shown in Figure 1. The patient was investigated for blood sugar fasting, haeamogram, liver function test, kidney function test, lipid profile, urine and stool for routine and microscopic examinations before and after 21 days of treatment.

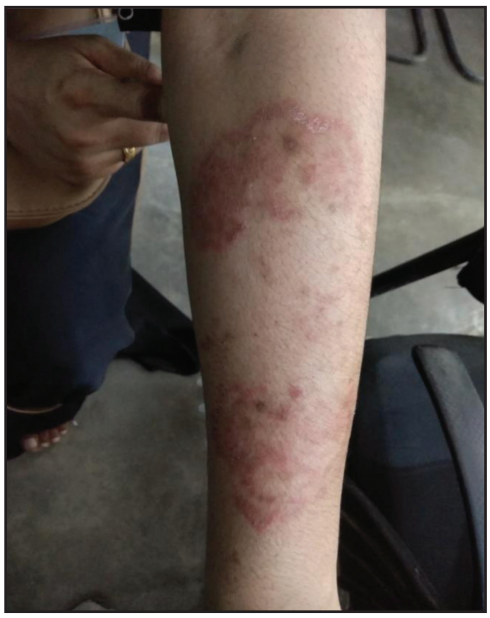

Figure 1: Before treatment

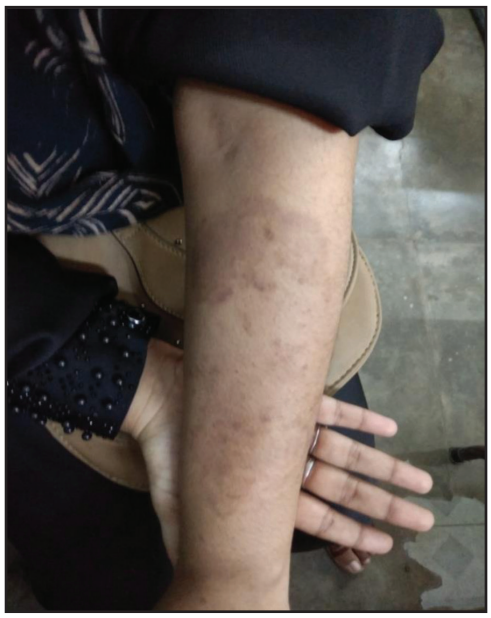

Figure 1a: After treatment of 15 days

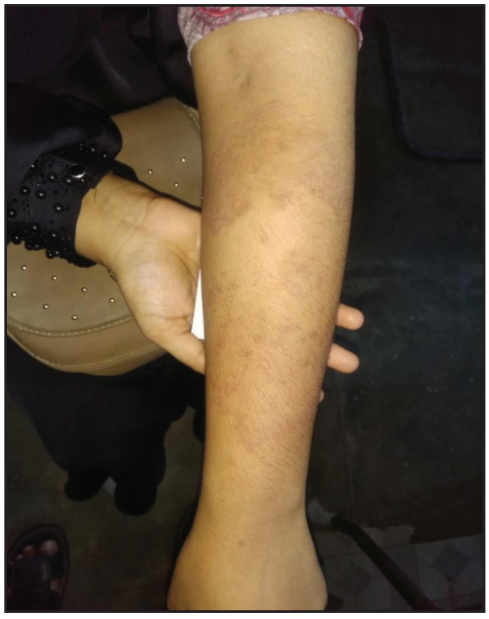

Figure 1b: After treatment of 21 days

The patient was given Unani pharmacopoeial medicine *Ḥabb Muṣaffī-i-Khūn* (Anonymous, 2011) in the dose of two tablets twice daily with *Iṭrīfal Shāhitara* (Anonymous, 2006) 7 g with lukewarm water on empty stomach in the morning and evening with the instruction not to take any meal upto 1 hour after taking the medicines. The action of the drug *Ḥabb Muṣaffī-i-Khūn* is *Muṣaffī-i-Dam* (blood purifier) and is useful in *Fasād-i-Dam*. It is a broad term in Unani system of medicine which stands for chronic abnormality of blood which affects the nutrition of the skin and produces various changes in it, e.g. pigmentation, discoloration, melasma and *Ātishak* (soft chancre/chancroid) (Anonymous, 2011). The actions of *Iṭrīfal Shāhitara* are *Muṣaffī-i-Dam* (blood purifier) and *Mulayyin* (laxative). The therapeutic uses are *Fasād-i-Dam*, *Ātishak* (soft chancre/chancroid), *Ṣudā'* (headache), *Duwār* (giddiness) and *Khārish* (itching) (Anonymous, 2006).

The propriety product of Unani Medicine *Eczenil* ointment (E-102) was prepared as per the standard method of ointment preparation mentioned in Unani Pharmacopoeia of India. *Eczenil* ointment was mixed with coconut oil in 1:1 ratio and applied on the affected part of the body. The patient was advised to apply ointment from the outer margins of the affected part and come to the centre. The composition of propriety product *Eczenil* ointment is as follows. Each 50 g contains:

S. No.	Unani name	Scientific/English name	Quantity
1.	*Murdārsang*	Lead oxide/Plumbioxidum	5 g
2.	*Tinkār*	Ore-borax / Sodium biborate	5 g
3.	*Kibrīt*	Purified Sulphur	5 g
4.	*Kāfūr*	*Cinnamomum camphora* (L.) J. Presl	5 g
5.	*Rawghan-i-Nīm*	Oil of *Azadirachta indica* L.	10 g
6.	Petroleum jelly	Petroleum jelly	10 g
7.	*Mom Zard*	*Cera alba* (Bee wax)	10 g

The patient was advised to avoid sore, junk and fast foods, any kind of pickles and citrus item in the food.

Results

The first follow-up was done after seven days of treatment. The patient informed that she had strictly followed advice regarding diet and medicine. She felt mild relief in itching and coolness at the affected site. On second follow-up on 15th day, all the margins of the affected part were vanished and no sign of inflammation was seen. Only slight black marks were present at the affected site

as shown in Figure 1a. On third follow-up on 21st day, the patient felt better, no itching and burning was felt at the site. The photograph was taken as shown in Figure-1b. The photographs are self-explanatory to showcase the beneficial effect of Unani dugs without any adverse reactions. No significant changes were observed in haemogram, liver function test, kidney function test, lipid profile, urine and stool for routine and microscopic examination as compared to the baseline.

Conclusion

Qūbā (*Tinea corporis*) is a superficial dermatophyte infection characterized by either inflammatory or non-inflammatory lesions on skin regions other than the scalp, groin, palms, and soles. Unani principle-based combination of pharmacopoeial and propriety medicines showed positive effect in the case of *Qūbā* (*Tinea corporis*). Oral and local use of Unani medicines showed good results without any adverse effect on the body. These results give an idea for effective, cheap and safe management of *Tinea corporis*. Unani pharmacopoeial medicines effectively counter the disease without any adverse reaction. However, more rigorous and larger studies are needed to confirm the therapeutic efficacy of *Ḥabb Muṣaffī-i-Khūn*, *Iṭrīfal Shāhitara* and Eczenil ointment for *Tinea corporis*.

Conflict of Interest: The authors report no conflicts of interest. There were no funding sources for this study.

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सारांश

कूबा अल–बदन (टीनिया कॉर्पोरिस) के एक रोगी में *हब्ब–ए–मुसफ़्फ़ी–ए–खून, इतरीफल शाहितरा* और एक्ज़ेनिल मरहम का प्रभाव

***मिस्बाहुद्दीन अजहर, ज़मीर अहमद, मुस्तहसन**

सारांश

टीनिया कॉर्पोरिस मनुष्यों के ट्रंक, बाहों और पैरों की त्वचा का सतही संक्रमण है जो आमतौर पर तीन सूक्ष्मजीवों – ट्रायकॉफ़ायटन, माइक्रोपोरम और इपीडरमोफ़ायटन – के कारण होता है। इन में ट्रायकॉफ़ायटन रूब्रम सबसे आम कारण है। यूनानी चिकित्सा के तत्त्वज्ञान के अनुसार यह *अख्लात फासिदा* और *अजसाम खबीसा* के संचय के कारण त्वचा की सूजन है। टीनिया संक्रमण का निदान इसके नैदानिक लक्षण के आधार पर किया जाता है और सूक्ष्म या संस्कृति परीक्षण द्वारा इस की पुष्टि की जाती है।

इस केस अध्ययन में 8 महीनों से बीमारी से पीड़ित एक 18 वर्षीय महिला छात्र को यूनानी भेषजकोषीय मिश्रण *हब्ब–ए–मुसफ़्फ़ी–ए–खून, इतरीफल शाहितरा* और एक बाजार उत्पाद एक्ज़ेनिल मरहम दिया गया। इन औषधियों ने 21 दिनों के उपचार में ही सूजन मार्जिन को कम करने में उल्लेखनीय प्रभाव दिखाया। इस रोग के उपचार में इन औषधियों के प्रभाव का मूल्यांकन करने के लिए आगे एक बड़े आकार के नमूने पर नियंत्रण अध्ययन डिजाइन किया जा सकता है।

शब्दकुंजीः *कूबा*, टीनिया कॉर्पोरिस, रिंगवार्म, यूनानी चिकित्सा

Therapeutic Evaluation of *Marham Dākhliyūn* in Vaginal Candidiasis

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Abstract

Vaginal candidiasis is a major problem of growing concern, worldwide. It is so common that 75% women have at least one episode during their lifetime and nearly 45% of women experience two or more. Fortunately, few are plagued with a chronic, recurrent infection. In spite of the prevalence of this disease at mass level, the choice of treatment available in western medicine is comparatively few. Unani Medicine claims to possess a number of effective and safe therapeutic agents that are commonly used in the management of vaginal candidiasis. *Marham Dākhliyūn*, an important patent and proprietary preparation of Oeba India Pharmaceuticals, is one such drug frequently used in candidiasis without any reported serious side effect. In the present study, an attempt has been made to evaluate its efficacy in the management of vaginal candidiasis. A standard controlled single blind clinical trial was conducted. The patients were divided into two groups after confirming the diagnosis by clinical and microbiological examination. The patients in group I, serving as standard control group, were administered Clotrimazole, 100 mg in the form of vaginal pessary daily at bedtime for 7 nights. The test drug was locally applied over vaginal mucosa and fornices in a dose of 5 g at bedtime for 14 days in the patients of group II serving as test group. The patients treated with test drug showed 86% reduction in important clinical features of candidiasis. The findings suggested that the test drug is effective in the treatment of candidiasis.

Keywords: Candidiasis, Moniliasis, *Sayalān al-Raḥim*, *Marham Dākhliyūn*

Introduction

Candidiasis is the most opportunistic mycosis in the world (Panda, 2000). It is a fungal infection of vagina caused by a Gram positive yeast-like fungus *Candida albicans*. It is present in the vagina in about 20% of women without having any symptom (Dutta, 2003). Vaginal candidiasis is a major problem of growing concern, worldwide. It is so common that 75% women have at least one episode of vaginal candidiasis during their lifetime. Nearly 45% of women experience two or more episodes. Fortunately, few are plagued with a chronic, recurrent infection (Berek, 2007). Since the advent of antibiotics, the incidence of clinical vulvo-vaginal candidiasis has increased. Its incidence is higher in pregnant than in non-pregnant women. The increased incidence in pregnancy is due to increased glycogen content of the vaginal epithelium. For the same reason, it is also common in diabetic patients (Masani, 1982). It thrives on carbohydrate and likes an acidic medium (pH 4.0-5.5). This explains why the patient's symptoms are temporarily relieved by bathing or douching with one per cent

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sodium bicarbonate solution and during menstruation when the vagina is more alkaline (Kumar & Malhotra, 2008). The microorganism invades on skin and mucus membrane of the organs and survives in the favourable conditions like damp and cold (Berek, 2007). Small numbers of candida commonly occur as normal components of the flora of the human skin, vagina and intestinal tract. It grows excessively and leads to disease only if there are general or local changes that favour the development of the fungus. General factors which favour candidiasis include debility, undernutrition, malignant disease, immunological deficiency state and diabetes. Therapy with antibiotics, steroids and antimitotic agent may play a part. Local factors favouring excessive growth include maceration, moisture and the presence of other eruptions, chiefly eczema of the intertriginous sites (Read *et al.*, 1984). The patient complains of thick curdy vaginal discharge with intense vulvo-vaginal pruritus, vaginal irritation and dysuria (Padubidri & Daftary, 2004).

Unani Medicine postulates that it occurs following the changes in the quality or/and quantity of phlegm (*Balgham*) in the body especially in pelvic region. Phlegm is described to be synthesized in liver and used by the organs as it is one of the important components of humour necessary to maintain the homeostasis (Zeenat & Hasan, 2016). However in case its quality or quantity is compromised anyhow it becomes a source of diseases and may also serve as a medium for infection (Ahmad *et al.*, 2011). In Unani literature, vaginal candidiasis has been further described to be a type of *Sayalān al-Raḥim* characterized by excessive uterine discharge. It has been described that *'Ufūnat* (infection) in the uterus leads to weakening of *Quwwat Hāḍima* (digestive faculty) of *'Urūq-i-Ḥayḍ* and predominance of *Akhlāṭ Arba'a* are responsible for *Sayalān al-Raḥim* (Ibn Sina, 2007). Majusi (1889) described that *Ḍu'f-i-Quwwat Jādhiba* (weak retensive power), excess of waste in the body and predominance of *Akhlāṭ Arba'a* are the causative factor of *Sayalān al-Raḥim*. While some other Unani physicians have described that *Sayalān al-Raḥim* is caused by *Ḍu'f-i-Quwwat Jādhiba* of *Raḥim* along with predominance of *Akhlāṭ Arba'a* and accumulation of waste material in the body (Ibn Hubal, 2007; Jurjani, 1903; Kabiruddin, 2003).

Globally, candidiasis is an extremely common infection and is associated with important public health problem but has received only scant attention. In spite of the prevalence of this disease at mass level, the choice of treatment available in western medicine is comparatively few. Even the drugs available for the purpose are not devoid of toxic effects (Rang *et al.*, 2007) and often fail to cure the disease completely. Oral agents are more convenient, less messy, but may have systemic toxicity, e.g., ketoconazole produces hepatotoxicity (Kumar & Malhotra, 2008). Amphotericin B has a wide antifungal activity but it is highly toxic and a variety of reactions may develop after its intravenous use (Satoskar *et al.*, 2001). Clotrimazole is effective against many fungi. Given parenterally,

it is more toxic than other imidazole (Satoskar *et al.*, 2001). Clotrimazole is well-tolerated by most patients. Local irritation with stinging and burning sensation occurs in some cases. No systemic toxicity is seen after topical use (Tripathi, 2008). Fluconazole may cause nausea, gastrointestinal disturbances and abnormalities of liver enzymes (Satoskar *et al.*, 2001). Such a situation warrants some alternative arrangement for the treatment of vaginal candidiasis having potential of leading to diverse complications. Unani Medicine claims to possess a number of safe and effective drugs that can be used in the management of vaginal candidiasis, vaginitis and *Sayalān al-Raḥim*. However, many important Unani drugs used extensively in candidiasis since ancient times have still not been scientifically evaluated on specific parameters for their efficacy and safety. *Marham Dākhliyūn*, an important patent and proprietary preparation of Oeba India Pharmaceuticals, is also one such drug mainly described to be effective in *Sayalān al-Raḥim* and inflammation of uterus, commonly being used in the management of candidiasis successfully since long without any reported serious side effect. Therefore, the present study was designed to evaluate the efficacy of *Marham Dākhliyūn* in the patients suffering from candidiasis (*Sailanur Raham*).

Methods

Marham Dākhliyūn, an important patent and proprietary preparation of Oeba India Pharmaceuticals, was procured from local agency at Malegaon. The ingredients of *Marham Dākhliyūn* are given in Table 1.

Table 1: Ingredients of *Marham Dākhliyūn*

S. No.	Name of Ingredients	Scientific Name	Quantity (Each 20 g contains)
1	*Aspghol Musallum*	*Plantago ovata*	0.325 g
2	*Alsī*	*Linum usitatissimum*	0.325 g
3	*Tukhm-i-Khaṭmī*	*Althea officinalis*	0.325 g
4	*Tukhm-i-Kanawcha*	*Phyllanthus maderaspatensis*	0.325 g
5	*Tukhm-i-Methī*	*Trigonella foenum-graecum*	0.325 g
6	*Murdār Sang*	Monoxide of lead	0.156 g
7	*Rowghan-i-Arandī*	*Ricinus communis*	6.506 g
8	*Rowghan-i-Kunjad*	*Sesamum indicum*	6.596 g
9	*Mom*	Wax	5.204 g

The patients visiting the OPD of Department of Ilmul Qabalat wa Amraz-e-Niswan, Mohammadia Tibbia College and Assayer Hospital, Mansoora, Malegaon

during 2011–13 were screened for the presence of *Candida albicans* on the basis of clinical signs and symptoms. The diagnosis of screened patients was however confirmed after pathological investigations.

After taking informed consent, sixty diagnosed patients of 18–45 years of age were included in the study and were informed about the disease, examination to be performed and type of treatment. The patients suffering from any other local or systemic diseases were excluded from the study. The permission of Institutional Ethics Committee (IEC) was taken prior to the initiation of the clinical trial. The patients were divided with the help of computer randomized tables/numbers into two groups of 30 patients each (Table 2). The patients in group I, serving as standard control group, were administered Clotrimazole 100 mg in the form of vaginal pessary daily at bedtime for 7 nights to make the study comparable. While the patients in group II, serving as test group, were treated with the test drug *Marham Dākhliyūn,* 5 g per vaginally, once a day at bedtime for two weeks. Abstinence was advised and no concomitant therapy was allowed during the treatment.

Table 2: Treatment Schedule

Group	Drug Treatment	Dose	Duration
Group I	Clotrimazole	100 mg x HS	7 days
Group II	*Marham Dākhliyūn*	5 g x HS	14 days

The patients were advised for weekly follow-up. They were carefully interviewed at each visit and their statement about the amount, consistency and odour of vaginal discharge, pruritus, burning in vagina and vulva (soreness of vulva and vagina) and lower abdominal pain was recorded. Each patient underwent per vaginal examination in lithotomy position after general and systemic examination. Inflammation and tenderness of vagina was recorded by per speculum examination (Wasim *et al.*, 2016).

Before and after the treatment, specific investigations, such as wet swab test of vaginal discharge, was done to confirm the diagnosis and used as important objective parameters for the assessment. This is the most efficient and cost-effective way to diagnose candidiasis. Wet swab is made quickly and easily. Vaginal discharge was collected by a pipette or swab from the posterior vaginal fornix using Cusco's speculum in lithotomy position and a small amount of secretion was placed on a clean glass slide. Then a drop of 10% KOH was added to it and examined under a microscope (HPF 45^{X}). KOH destroys white blood cells and bacteria and balances the epithelial cells leaving other than *Candida albicans*. The presence of candida was recorded in the case record form. On every follow-up, the clinical features were graded on point scales and the changes were recorded in CRF (Wasim *et al.*, 2016).

Scoring system for overall evaluation of each patient was done. The vaginal discharge was graded (Zeenat & Hasan, 2016) as none (-) for no discharge, mild (slightly) (+) for normal moistness of vagina without staining or moistening the underclothes, moderate (profuse) (++) for undeniably soiled the underclothes that require changing and washing frequently and severe (markedly profuse) (+++) that requires the wearing of some extra absorbent pad. Pruritus (Akhyani *et al*., 2005) and burning micturition and dysurea were classified as none (-), mild (+), moderate (++) and severe (+++). Lower abdominal pain was assessed by visual analogue scale (Lin *et al*., 2005) as none (-), mild (+), moderate (++) and severe (+++). Tenderness and congestion of vaginal wall, redness of vaginal mucous membrane, low backache, local soreness and dyspareunia were also graded as none (-), mild (+), moderate (++) and severe (+++). Small number of candida commonly occur as normal components of the flora of the human skin, vagina and intestinal tract (Read *et al*., 1984). Presence of number of candida was graded as 0–8, 8–15, 15–30 and more than 30 for absent (-), slightly (+), moderate (++) and bulk (+++) respectively (Wasim *et al*., 2016). The pathological status of each patient was expressed by a scoring system. Scores of 0, 1, 2 and 3 were given for the parameters graded as -, +, ++ and +++. The score for all parameters observed in each patient were then added up. The percentage decrease in scores was determined by comparing the baseline and post treatment. Finally, recorded findings were statistically analyzed using Chi square test to determine the significance.

Results

The test drug was studied in the management of vaginal candidiasis by observing clinical features and laboratory investigations. The findings were tabulated, analyzed and compared with the standard drug (Table 3). Abnormal vaginal discharge was found in 100% of the patients included in each group on the day of registration, while it remained only in 16.66% and 13.33% of the patients and on the basis of percentage, improvement was observed in 83.33% and 86.66% of the cases in group I and II, respectively. Pruritus vulva on day zero was found in 93.33% and 100% of the patients in group I and II, respectively, whereas after treatment it was reduced and found only in 3.57% and 6.66% of the patients and improvement was observed in 96.42% and 93.33% of the cases, respectively. Prior to the treatment local soreness was found in 63.33% and 66.66% of the cases in group I and II, respectively, whereas after treatment it was reduced and found only in 10.52% and 5% of the cases and improvement was observed in 89.47% and 95% of the cases, respectively.

Before the treatment, dyspareunia was found in 60% and 50% of the cases in group I and II, respectively, whereas after treatment it endured only in 5.55% and 6.66% of the cases and improvement was observed in 94.44% and 93.33%

of the cases, respectively. Prior to the treatment, low backache was found in 56.66% and 63.33% of the patients in group I and II, respectively, whereas after treatment it has totally disappeared and 100% improvement was observed in group I and it was reduced and found only in 5.26% and improvement was observed in 94.73% of the cases in group II. On day zero, pain in lower abdomen was found in 50% and 43.33% of the patients in group I and II, respectively, whereas after treatment it has totally disappeared and 100% improvement was observed in each group. Prior to the treatment, burning micturition and dysuria were found in 66.66% and 73.33% of the cases in group I and II, respectively, whereas after treatment it has totally disappeared and 100% improvement was observed in each group.

Table 3: Effect of standard and test drugs on clinical features

Clinical Features	Group I			Group II		
	Baseline No (%)	Post Treat-ment No (%)	Improve-ment No (%)	Baseline No (%)	Post Treat-ment No (%)	Improve-ment No (%)
Abnormal vaginal discharge	30 (100)	5 (16.66)	25 (83.33)	30 (100)	4 (13.33)	26 (86.66)
Pruritus vulva	28 (93.33)	1 (3.57)	27 (96.42)	30 (100)	2 (6.66)	28 (93.33)
Local soreness	19 (63.33)	2 (10.52)	17 (89.47)	20 (66.66)	1 (5)	19 (95)
Dyspareunia	18 (60)	1 (5.55)	17 (94.44)	15 (50)	1 (6.66)	14 (93.33)
Low backache	17 (56.66)	0 (0)	17 (100)	19 (63.33)	1 (5.26)	18 (94.73)
Pain in lower abdomen	15 (50)	0 (0)	15 (100)	13 (43.33)	0 (0)	13 (100)
Burning micturition & dysuria	20 (66.66)	0 (0)	20 (100)	22 (73.33)	0 (0)	22 (100)
Redness of vaginal mucous membrane	30 (100)	4 (13.33)	26 (86.66)	30 (100)	2 (6.66)	28 (93.33)
Tenderness of vagina	30 (100)	5 (16.66)	25 (83.33)	30 (100)	4 (13.33)	26 (86.66)
Candida in slide	30 (100)	5 (16.66)	25 (83.33)	30 (100)	4 (13.33)	26 (86.66)

On pretreatment examination, redness of mucous membrane of the vagina and vulva was found in all the patients included in the study, whereas after

treatment it was found reduced and only 13.33% and 6.66% of patients were found affected. The findings indicated improvement in 86.66% and 93.33% of the cases in respective group. Prior to the treatment, tenderness of vaginal wall on day zero was found in all the patients included in the study, while after treatment it decreased significantly and was found in 16.66% and 13.33% of the cases indicating an improvement in 83.33% and 86.66% of the patients in group I and II, respectively. On pretreatment investigation, candida in slide was found in 100% of the patients in each group, whereas after treatment it decreased significantly and was found only in 16.66% and 13.33% of the patients and an improvement was observed in 83.33% and 86.66% of the cases in respective groups.

Relief in clinical symptoms along with reduction in microbiological count was considered as the criteria of efficacy. The cases having relief from abnormal vaginal discharge along with absence of candida in slide after treatment were rated as cured. While the patients having no relief in abnormal vaginal discharge and candida were found in slide after treatment, were rated as not cured. Complete cure was observed in 83.33% and 86.66% patients in group I and II, respectively, whereas 16.66% and 13.33% cases were not cured, respectively.

Discussion

The findings in respect of different parameters of this study indicated that there was no statistical difference between the two groups suggesting that both the drugs produced almost equal degree of response. The findings of the study suggested that local application of the test drug is equally effective as the local administration of Clotrimazole. Although in Unani Medicine both local and oral treatment is suggested simultaneously to treat the patients of vaginal candidiasis with the aim to improve the quality of phlegm that has undergone through derangement and to improve the local pathology. But only local treatment is also advised commonly to treat such a condition (Zeenat & Hasan, 2016). The present study indicated that only local application is also sufficient to cure the majority of the patients. About 13.33% of the patients were not cured with the test drug probably because few patients may have required the local as well as the systemic intervention as the only local treatment may not be sufficient to negotiate the severity of the disease. The result is in consonance with the Unani principle of treatment that suggests that the disease arises following the disturbance in the quality, quantity or composition of the phlegm (Majoosi, 2005). Candidiasis may develop as sequelae of systemic derangement of phlegm but it may also develop because of its local overthrow that is frequently compounded with other factors that trigger the pathology. The cure rate of only 86% may be because of the demand of the nature of pathological condition for systemic intervention.

Since the candidiasis is a disease of *Khilṭ Balgham* having the symptoms of cold and wet temperament, it is obvious to treat the disease by the drugs that have hot and dry temperament. Therefore, few ingredients having hot and dry temperament (Ghani, 2011; Hakeem, 1999) were included in *Marham Dākhliyūn*.

Most of the ingredients of *Marham Dākhliyūn* have been reported in classical Unani literature as *Qābiḍ* (astringent), *Muḥallil* (resolvent), *Muḥallil-i-Awrām* (antiinflammatory), *Mujaffif* (dessicative), *Mulaṭṭif* (demulscent), *Munḍij* (concoctive), *Musakkin* (analgesic), *Rādi*‘ (repellent), *Murakhkhī* (emollient) and effective in phlegmatic derangement (Ghani, 2011; Hakeem, 1999; Khan, 1313H; Nabi, 2007). They have also been reported in ethnopharmacological literature as astringent, demulscent, emollient, useful in backache, inflammation and in relieving the inflammation of mucous membrane of urogenital tract. Pessaries of *Trigonella foenum-graecum* seeds are therapeutically used in leucorrhoea. *Murdār Sang* is a powerful local astringent (Anonymous, 1969; Anonymous, 2000; Nadkarni, 1954; Nadkarni, 1954). The anticandidal activity of *Linum usitatissimum* oil has also been reported in an experimental study (Kaithwas *et al*., 2011). Due to these medicinal properties, *Marham Dakhliyn* produces constriction in the vaginal wall, absorbs the vaginal secretions and resolves the discharge and inflammatory condition.

The study clearly showed that the test drug is very effective in candidiasis which is proved by decrease in the amount of abnormal vaginal discharge, pruritus, local soreness, dyspareunia, low backache, pain in lower abdomen, burning micturition and dysuria, redness of mucous membrane of vagina and vulva, tenderness and congestion of vaginal wall, and candida in slide. The efficacy of the test drug may be attributed to its anticandidal activity which was almost equal to that of Clotrimazole. Thus, the study validated the therapeutic application of the test drug *Marham Dakhliyn* in the management of vaginal candidiasis.

Conclusion

In the light of above findings and discussion, it can be concluded that Unani drug *Marham Dākhliyūn*, a proprietary preparation of Oeba India Pharmaceuticals, possesses significant effect against vaginal candidiasis. Therefore, it may be used in the patients suffering from candidiasis (*Sayalān al-Raḥim*) and associated condition.

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सारांश

वजाइनल कैंडिडियासिस में *मरहम दाखलियून* का चिकित्सीय मूल्यांकन

वसीम अहमद, फ़रह नाज़, *फहमीदा ज़ीनत, अज़हर हसन

सारांश

वजाइनल कैंडिडियासिस विश्व भर में एक प्रमुख चिंताजनक समस्या है। यह इतनी सामान्य है कि 75% महिलाओं के जीवनकाल में कम से कम एक बार घटित होती है और लगभग 45% महिलाओं के जीवनकाल में दो या दो से अधिक बार घटित होती है। भाग्यवश उनमें से कुछ ही जटिल, आवर्तक संक्रमण से ग्रस्त हैं। बड़े पैमाने पर इस रोग की व्यापकता के बावजूद, एलोपैथिक चिकित्सा में उपचार का विकल्प तुलनात्मक रूप से बहुत कम है। यूनानी चिकित्सा कई प्रभावी एवं सुरक्षित चिकित्सीय औषधियों का दावा करती है जो आमतौर पर वजाइनल कैंडिडियासिस के उपचार में उपयोग की जाती हैं। ओबा इंडिया फार्मास्यूटिकल्स की एक महत्वपूर्ण पेटेंट एवं स्वामित्व औषधि *मरहम दाखलियून* एक ऐसी औषधि है जिसका उपयोग अक्सर कैंडिडियासिस में किया जाता है और जिसका कोई गंभीर दुष्प्रभाव नहीं देखा गया है। वर्तमान अध्ययन में वजाइनल कैंडिडियासिस के उपचार में इसकी प्रभावकारिता का मूल्यांकन करने का प्रयास किया गया है। एक मानक नियंत्रित एकल ब्लाइंड नैदानिक परीक्षण किया गया। नैदानिक और सूक्ष्मजीवविज्ञान परीक्षण द्वारा रोगनिदान की पुष्टि के बाद रोगियों को दो समूहों में विभाजित किया गया। मानक नियंत्रण समूह के रूप में समूह I के रोगियों को क्लोट्रिमाज़ोल वजाइनल पेसरी के रूप में 100 मि.ग्रा. 7 रातों तक सोते समय दी गई। परीक्षण समूह के रूप में समूह II के रोगियों को परीक्षण औषधि वजाइनल म्यूकोसा और फोर्निक्स पर 5 ग्राम की मात्रा में 14 दिनों तक सोते समय बाहरी रूप से लगाई गई। परीक्षण औषधि से उपचारित रोगियों ने कैंडिडियासिस के महत्वपूर्ण नैदानिक लक्षणों में 86% कमी दर्शाई। निष्कर्षों से पता चला कि कैंडिडियासिस के उपचार में परीक्षण औषधि प्रभावी है।

शब्दकुंजी: कैंडिडियासिस, मोनिलियासिस, *सयलान अल–रहिम, मरहम दाखलियून*

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