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Comparative Clinical Evaluation of Unani Formulations in the Treatment of Melasma

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ABSTRACT

Melasma is a cosmetic problem that sometimes cause great emotional suffering. One survey showed that melasma was associated with a significant impact on health related quality of life. It is defined as a disorder of pigment metabolism characterized by sharply demarcated, blotchy, brown maculae, usually symmetrical on the cheeks, forehead, sometimes on the upper lip and neck. In Unani Medicine, it is mentioned that *Kalf* is due to black bile homour and charred blood parts, which oozes out of capillaries and get accumulated under the skin. The present study was a Randomized, parallel group, comparative clinical trial. The present research was done to evaluate and to compare the clinical efficacy of a topical application made up of powdered cuttlefish bone and lemon juice along with oral *Cascuta reflexa* capsule which was compared with another group that is oral placebo with same topical application. The efficacy of the treatment was evaluated by MASI, skin color shades chart, Patient's Global Assessment, Patient's Satisfaction Index and DLQI. Assessment of safety was done by clinical assessment, patients' complains about any adverse effects i.e. itching, inflammation, rashes, eruptions etc., DLQI and laboratory parameters such as LFT, KFT, CBC and Hb%. At the end of the treatment all the efficacy parameters showed significant improvement when compared with baseline in both the groups; however the difference between the groups was not statistically significant. Thus, topical application had promising effects in melasma.

Key words- Kalf, Unani formulation, hyper pigmentation, MASI, cuttlefish bone

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INTRODUCTION

Melasma is a pigmentary skin discoloration usually occurring in yellowish brown patches or spots. These patches are rounded and oval with ill-defined margins[1]. It is defined as a disorder of pigment metabolism characterized by sharply demarcated, blotchy, brown maculae, usually in symmetrical distribution on the cheeks and forehead and sometimes on the upper lip and neck[2]. Lesions are irregular patches, usually involving the cheek, forehead, temples and upper lip[3]. Although melasma can affect all people, Asian and Hispanic females are most commonly effected[4]. Melasma is cosmetic problem that sometimes cause great emotional suffering[5].One survey showed that melasma was associated with a significant impact on health related quality of life (HRQoL)[6].

Factors involved in pathogenesis of the condition include genetic influence, exposure to UV radiation, pregnancy and hormonal therapy. Other factors implicated are phototoxic drugs, anticonvulsant medications and the use of certain cosmetics[7]. However, the actual pathogenicity of melasma is not fully understood. In Unani Medicine, it is mentioned that Kalf is due to melancholic morbidity and charred blood parts which oozes out of capillaries and get accumulated under the skin[8]. The disease course of melasma follows the pattern of worsening hyper pigmentation during the sunny season and spontaneous improvement during autumn and winter[9].

Cascuta reflexa belongs to the family Convulvulaceae. It is well known as *Aftimoon* in Unani medicine and it is an annual, parasitic, leafless plant[10, 11]. It is used for the treatment of skin disorders and melancholic disorders as blood purifier[10], bactericide[12], antisteroidogenic activities[13], analgesic[14], free redicals scavanging activities[15] and anti-inflammatory agent[16]. Its main chemical constituents are cuscutalin, cuscutin, amrabelin, dulcitol, luleolin, quercetin, luteolin, recins, etc.[11, 17, 18].

Lemon is a fruit of a small tree, about six meters in height from family Rutaceae. The tree is scientifically known as *Citrus limon* (Linn.)[19]. Pulp & juice have the actions like antioxidant[20], depigmentation[21], anti inflammatory[22], immune modulatory[23], antibacterial[24], antiscorbutic, rubefacient externally[25], etc., its main chemical constituents are citric acid, ascorbic acid, glucosides, geraniol, 1-linol, mineral salts, flavonoids, caffeic acid, citral, limonin, etc.,[10,11, 25],

Cuttlefish bone (*kaff-e-darya*) is the internal structure (*Os sepiae*) from cuttlefish *Sepia officinalis* Linn.[26]. It is a flat, broad, and oval in shape, represented by phragmocone with a broader and rounded oral end, called pro-ostracum, and a narrow, pointed aboral end called

rostrum. The shell is entirely dead and composed of calcareous rather than horny matte[27, 28]. Its chemical constituents are Calcium Carbonate, Calcium Phosphate, Calcium Sulphate, Silica, NaCl, $MgCl_2$, Iron, Iodine, etc.,[29, 30]. It is used as cleanser[31], antacid[32], astringent[26, 32], sedative[26], anti-inflammatory[33], promotes wound healing[34].

The present research was done to evaluate and to compare the clinical efficacy between two groups, one group was received topical application made up of powdered cuttlefish bone and lemon juice along with *Cascuta reflexa* capsule and the other group was received oral placebo with same topical application.

MATERIALS AND METHODS

This study was a Randomized, parallel group, comparative clinical trial carried out at Majeedia Hospital, Jamia Hamdard University, New Delhi, in accordance with the principles stated in the Declaration of Helsinki (2004). The protocol was approved by Institutional ethics committee for clinical trials in Unani drugs of Jamia Hamdard [DM/JH/FM/10 (iii)] at Jamia Hamdard. Patients were recruited Unani medical OPDs in Majeedia Hospital, New Delhi. Each participant was informed about the trial in accordance with Declaration of Helsinki. They were further given a description of anticipated risks and discomforts. Informed written consent was obtained from each participant in the prescribed format prior to perform the study related procedures (i.e. physical examination, laboratory screening and other investigational procedure) and before administration of any study related medication.

The study included either sex, satisfying the following criteria: Subjects with 18 to 50 years of age and both the sexes; Fitzpatrick skin types III, IV, V with otherwise healthy people with normal findings of lab parameters; participants who had given their written informed consent & agreed to follow the protocol voluntarily. Subjects who were excluded from this study: subjects with OCP and intrauterine devices; pregnant or lactating mother; outdoor workers and field workers; not willing to avoid cosmetics during period of study; not willing to sign informed consent form; diabetes mellitus, renal insufficiency, severe liver or heart diseases; neurological disorders and significant drug or alcohol abuse.

The patients who qualified for the study were randomized by using block randomization. The total of 40 patients, 20 in each group were randomly allocated to both groups (group A and group B). These blocks were covered in an envelope and numbered in a sequential order. When the patient had to include in the study, lowest sequential number was opened and assigned to

respected group. At first visit, patients were screened and examined for the baseline investigations and physical examinations.

The drugs were used in the study and their forms are as follows

Topical application:

Cuttlefish bone (*Os sepiae*) powder mixed and made paste with lemon juice. Dose topical application to melasma lesions twice daily for 56 days.

Internal drug:

Afteemoon (*Cuscuta reflexa*) -powder of dried water extract filled in capsules. Strength of the capsule is 0.31 g. Dose was two capsules once daily for 56 days.

Placebo capsules were identical with Afteemoon capsules and they were filled with equal weight of starch. Dose was two capsules once daily for 56 days.

Group A was received topical application twice daily with oral drug After moon 2 capsules once daily for 56 days while group B patients were received only topical application twice daily for 56 days.

Following outcome measures were used to evaluate the efficacy and safety of the treatment, which were done before and after the treatment. Clinical Assessment was made by s MASI (Melasma Area Severity Index), Melasma area severity index (MASI) is developed by Kimbrough-Green et al for the assessment of melasma[35]. The severity of the melasma in each of the four regions (forehead, right malar region, left malar region and chin) is assessed based on three variables: percentage of the total area involved (A), darkness (D), and homogeneity (H). MASI score is the most important among all, as almost every study on melasma after its introduction used MASI score to evaluate efficacy[36, 37]. Skin color shades chart[9] (This helps in identifying the differences in the skin colour).

The others are Patient's Global Assessment (PGA)[9], in which patient is asked to rate on a scale how they feel overall. Patient's Satisfaction Index (SI)[38] i.e. find out how much patients are satisfied with the treatment and Dermatological life Quality Index[39, 40] (DLQI) is most frequently used method in the evaluation of skin diseases in randomized controlled trials which consists of simple 10-question validated questionnaire which has been used in over 33 different skin conditions. Assessment of safety was done by clinical assessment, patients' complains of adverse effects, DLQI and safety laboratory parameters.

The results were presented as mean ± standard error they were analyzed by using paired-t test, Mann Whitney test & Wilcoxon signed rank test. Level of significance was taken at $P < 0.05$.

The analysis of the observational data was performed and presented in the form of graphs and tables by using Graphpad instate 3 and Microsoft® Excel (2007) software.

RESULTS AND DISCUSSIONS

There have been very few well-conducted trails in melasma and this makes the process of comparing treatment outcome between trails difficult. The high prevalence of melasma among female is a well-known fact. Out of total 40 patients, male patients in group A were 13 & female patients were 7. In group B, total number of male patients were 5 & female were 15 patients. The study revealed that about 60% of the patients were females. Earlier studies have also noted a female preponderance[41].

Results were given in Table 1. The results indicated that the MASI scores before treatment (BT) in the both groups were 12.105 ± 1.070 and 14.385 ± 1.401 respectively and There was no significant difference between them (P > 0.05). In group A, at the end of the treatment the MASI score was decreased by 21.07% while in group B, percentage change in MASI score at the end of the treatment was 18.67%. In group A, very significant improvement was found in MASI score when compared with baseline, (P < 0.01), while in group B, p value was found significant with P < 0.05 after treatment (AT). Both the groups had shown significant changes in MASI score but when comparing the both groups, changes were not significant. This shows that treatment is effective but role of oral drug over topical drug cannot be identified statistically.

Table 1. Results of outcome measures before treatment and after treatment in both groups (mean ± SEM)

MASI	Group A		Group B	
	BT	**AT**	**BT**	**AT**
	12.105 ± 1.07	9.555** ± 0.8678	14.385 ± 1.401	11.7* ± 1.172
Patient Satisfaction Index (SI)	0.3 ± 0.105	1.35*** ± 0.109	0.2 ± 0.092	1.25*** ± 0.160
Physician Global Assessment	4.55 ± 0.154	4.22* ± 0.156	4.35 ± 0.182	4.05* ± 0.154
Color Chart Assessment	8.2 ± 0.746	10.2*** ± 0.706	7.95 ± 0.742	9.4*** ± 0.626
DLQI score	4.8 ± 0.531	4.7† ± 0.436	5.05 ± 0.394	4.95† ± 0.303

†P> 0.05 (Not Significant) ; *P<0.05(Significant) ; **P<0.01(Very Significant) ; ***P<0.001 (Extremely Significant)

Patient Satisfaction Index (SI), in group A, at the end of treatment none of the patient was found not satisfied whereas in group B 7.5% patients were found 'not satisfied' with treatment at the end of the treatment. Hence, there was an extremely significant difference seen in the both groups at the end of the treatment when compared with baseline values. However, the results of the two groups when compared with each other were not found significant (P = 0.8049).

PGA assessment, a slight improvement occurred in the mean score of group A and group B, which was statistically significant. However, the difference between the groups was not significant ($P > 0.05$).

In colour chart assessment, at the end of the treatment change in colour of lesion was found extremely significant in group A and group B when compared with baseline ($P < 0.001$). The color chart was self-designed according to Munsell color system. Patients normal skin as well as the darkest part of lesional skin were compared with 30-coloured color chart to match the best possible grade. Both the groups showed significant changes in the color grades of lesional skin before and after treatment, although Mann-Whitney's test between the two groups was not significant.

DLQI scoring was done on baseline for before treatment score and after treatment. In group A, P value in Wilcoxon Matched Paired test (2-tailed) was found p=0.9515 considered as 'Not significant'. In group B, P value in Wilcoxon matched paired test was found P = 0.7019 which is again 'Not Significant'. When both groups were compared for AT score through Mann-Whitney test ($P > 0.05$) considered 'Not significant'.

DLQI, in this study is used as safety as well as efficacy parameter. The change in DLQI score in either group is statistically not significant showing that the quality of life had not changed much. Neither the treatment showed decrease in DLQI score statistically nor increase in DLQI score. Thus, the result of this scoring system is not only showed that improvement of the quality of life was not attended by the treatment but also showed that it was not deteriorated further. The mean of DLQI score after treatment in group A is 4.7 ± 0.435 and in group B is 4.95±0.303 which is in accordance with the range of mean of 110 studies on melasma i.e. 3.5 -11.5[40].

Table 2. Laboratory parameters: Group A (Mean ± SEM)

S. No.	Investigations	BT	AT	Wilcoxon Matched Paired test; 2-tailed
1.	Hb%	13.19 ± 0.358	13.29 ± 0.260	P = 0.6477†
2.	TLC	7855 ± 481.03	7660 ± 387.19	P = 0.7285†
3.	ESR	17.3 ± 2.279	12.55 ± 0.922	P = 0.0400*
4.	S. Bilirubin (T)	0.73 ± 0.037	0.77 ± 0.024	P = 0.2247†
5.	SGOT	28.1 ± 2.187	28 ± 1.492	P = 0.6215†
6.	SGPT	28.55 ± 1.797	33.35 ± 2.201	P = 0.0583†
7.	S. Alk. Phosp.	158.6 ± 8.770	169.1 ± 6.437	P = 0.2611†
8.	S. Creatinine	1.11 ± 0.048	1.09 ± 0.042	P = 0.5412†
9.	Blood Urea	17.7 ± 1.328	17.95 ± 1.127	P = 0.9563†
10.	S. Sodium	140.7 ± 1.707	142.05 ± 1.104	P = 0.2943†
11.	S. Potassium	4.4 ± 0.092	4.6 ± 0.205	P = 0.5678†
12.	S. Uric Acid	5.02 ± 0.277	5.06 ± 0.23	P = 0.7983†

†P> 0.05 (Not Significant); *P<0.05(Significant); **P<0.01(Very Significant); ***P<0.001 (Extremely Significant)

Laboratory parameters were also done for safety assessments which include Haemogram, LFT, KFT. All the parameters in both the groups have P > 0.05 when compared pre treatment and post treatment values (Wilcoxon Matched Paired Test) except ESR which was having significant difference (P = 0.04 in group A and P = 0.017 in group B). The decrease in ESR cannot be attributed to treatment as both the groups have almost same change. However, one group is topical which does not have systemic effect while another group have oral drug which may have systemic affects. Thus, change in ESR cannot be correlated.

Table 3. Laboratory Parameters: Group B (Mean ± SEM)

S. No.	Investigations	BT	AT	Wilcoxon Matched Paired test; 2-tailed
1.	Hb%	12.59 ± 0.468	12.66 ± 0.379	0.6477†
2.	TLC	7445 ± 563.75	6980 ± 374.07	0.3118†
3.	ESR	21.7 ± 2.560	14.1 ± 1.703	0.0172*
4.	S. Bilirubin (T)	0.695 ± 0.032	0.77 ± 0.036	0.0887†
5.	SGOT	35.75 ± 4.794	30.2 ± 1.677	0.6226†
6.	SGPT	37.95 ± 6.670	31.85 ± 3.183	0.8983†
7.	S. Alk. Phosp.	168.1 ± 10.065	173.8 ± 9.931	0.6215†
8.	S. Creatinine	1.06 ± 0.043	0.995 ± 0.028	0.1336†
9.	Blood Urea	15.95 ± 1.037	16.2 ± 0.917	0.8124†
10.	S. Sodium	139.05 ± 0.686	140.65 ± 1.294	0.2102†
11.	S. Potassium	4.3 ± 0.137	4.45 ± 0.143	0.8983†
12.	S. Uric Acid	4.915 ± 0.394	4.97 ± 0.351	0.2935†

†P> 0.05 (Not Significant); *P<0.05(Significant); **P<0.01(Very Significant); ***P<0.001 (Extremely Significant)

Thus, all the efficacy parameters had shown significant changes with respect to improvement in either groups but no patient was found to be completely cured by the treatment. This may be due to inadequate period of therapy. 8-weeks study is not sufficient to get complete cure with these drugs.

History of adverse effect on treatment shows that irritation is associated with topical application of drugs. 60% in group A and 80 % in group B complained of irritation on skin due to topical drug until washed off. Irritation may be due to cuttlefish, as its irritation property is mentioned in Unani literature and confirmed on modern parameters[42]. On comparing with 4% hydroquinone which is considered gold standard for melasma therapy, was found moderate to strong irritant[43,44]. 15% of patients in group A and 25% in group B also complained of redness which may be caused due to inflammation due to rubificient property of cuttlefish bone.

Patients also complained of hyper pigmentation but color chart assessment does not revealed it. However, in modern treatment measures with hydroquinone, chemical peeling and laser therapy had shown post inflammatory hyper pigmentation as an adverse effect[5, 38, 44]. Hydroquinone was also found to cause hypo pigmentation in some cases. In this respect, cuttlefish was found relatively better as no case of hypo pigmentation was recorded.

CONCLUSION

The present study reveals that treatment of melasma exhibit individual variations. However in general, the test drug evolved as highly promising solutions for this stubborn disease. The systemic drug is surmised to enhance the efficacy of topical drug, but this could not come out to be true.

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