Journal of Dermatologic Science and Cosmetic Technology 1 (2024) 100057

Contents lists available at ScienceDirect

Journal of Dermatologic Science and Cosmetic Technology

journal homepage: www.keaipublishing.com/en/journals/jdsct

Efficacy of topical *Raphanus sativus* seed powder mixed with honey versus hydroquinone 4 % cream in the treatment of melasma – A randomized controlled trial

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ARTICLE INFO

Keywords:
Hydroquinone
Chloasma
Mooli seed
Unani medicine
Modified Melasma Area and Severity Index

ABSTRACT

Background: Melasma is a prevalent skin disorder affecting a wide range of populations. In Unani system of medicine, a traditional healing system with a rich historical background, there exists a repository of therapeutic modalities for melasma, elucidated by esteemed scholars and practitioners. Despite its extensive utilization, the scientific substantiation supporting these interventions remains limited.

Objective: This study aims to scientifically evaluate and compare the safety and efficacy of topically applied *Raphanus sativus* seed powder and honey mixture versus hydroquinone (4 % w/w) cream for melasma treatment.

Methods: In this 8-week open-label, randomized controlled trial, 40 participants (20 per group), aged 18–65 years and diagnosed with melasma, successfully completed the study. The test group applied finely powdered *Raphanus sativus* seeds mixed with honey twice daily, while the control group used hydroquinone (4 % w/w) cream twice daily. Primary outcome measure was change in modified Melasma Area and Severity Index (*mMASI*) at each follow-up. Secondary measures examined subjective nuances using a 100 mm visual analogue scale (*VAS*) and recorded the dermatology life quality index (*DLQI*) at baseline and the conclusion of the study. Safety was ensured through comprehensive clinical reviews, with documentation of adverse events during bi-weekly follow-ups.

Results: Following the conclusion of the study, the mean difference in the *mMASI* score between the test and control groups was –0.22 (95 % CI: –0.75 to 0.30, $d = 0.27$, $p = 0.394$). Additionally, post-trial mean differences in VAS and DLQI scores between the test and control groups were determined as –5.25 (95 % CI: –10.65 to –0.15, $d = 0.62$, $p = 0.056$) and –0.10 (95 % CI: –1.53 to 1.33, $d = 0.04$, $p = 0.888$), respectively.

Conclusion: Based on the investigative findings, the test intervention involving *Raphanus sativus* and honey demonstrated therapeutic efficacy statistically comparable to conventional hydroquinone treatment for managing melasma, with no reported adverse reactions. Moreover, both groups exhibited statistically comparable improvements in the dermatology life quality index.

1. Introduction

Melasma, also known as chloasma characterized by hyperpigmentation of sun-exposed areas of the skin, is an acquired pigmentary disorder. It is primarily associated with pregnancy and the use of oral contraceptives.[1] The exact prevalence of melasma remains uncertain in South Asia, with estimates ranging from 0.25 % to 4 %. In India, melasma is one of the most common hyperpigmentary disorders,

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https://doi.org/10.1016/j.jdsct.2024.100057
Received 9 March 2024; Received in revised form 13 October 2024; Accepted 1 November 2024
Available online 4 November 2024

though exact prevalence rates differ from region to region.[2] Clinically, it manifests as brown, gray, or blue macules that coalesce to form irregular patches, lacking erythema or signs of irritation. Morphologically, melasma can be classified into three patterns: centrofacial, malar, or mandibular.[3] Centrofacial melasma is the most common type, impacting the forehead, nose, upper lip, chin, and medial cheeks.[4] Melasma is characterized by an upregulation of dermal and epidermal melanin production and retention.[5] Previous beliefs attributed hyperpigmentation of melasma solely to epidermal melanin, but recent research indicates that an increased number of melanocytes may also play a role in the epidermal hyperpigmentation seen in melasma.[1,6] Moreover, solar elastosis, indicative of cumulative sun exposure, is significantly associated with the pathogenesis of melasma.[1] Melasma can be triggered by the activation of estrogen receptors on melanocytes, which stimulates these cells to produce excess melanin. Furthermore, melasma has been associated with the use of photosensitizing and anticonvulsant medications, certain cosmetic products, and minor ovarian or thyroid abnormalities.[7]

Melasma management is complex and needs a protracted treatment regimen. Conventionally, multiple topical therapeutic options are available, including hypopigmenting agents such as hydroquinone, azelaic acid, and kojic acid with hydroquinone being the most frequently prescribed medication. It works by preventing the conversion of DOPA to melanin via the tyrosinase enzyme.[8] Hydroquinone monotherapy and triple combination (hydroquinone, tretinoin, and corticosteroid) are the most successful and well-established treatment options for melasma, whilst chemical peels, laser and light-based therapies are comparable to topical but have a greater risk of unpleasant effects.[9]

Conventional treatment options for melasma can pose adverse reactions. Hydroquinone may lead to dose and time-related effects, including erythema, stinging, and paradoxical postinflammatory hypermelanosis. Higher concentrations of hydroquinone (> 2 %) can cause "confetti-like" depigmentation and its long-term use may lead to ochronosis.[10] In addition, topical corticosteroids, used to reduce irritation, may cause skin lightening and, with prolonged use, lead to skin atrophy and other issues.[11] Given these potential side effects and limitations associated with conventional treatments, the exploration and development of alternative therapeutic options have become increasingly crucial. Researchers and clinicians are actively seeking safer and more effective approaches to address melasma, aiming to provide patients with improved outcomes and minimized risks of adverse reactions. Consequently, in this pursuit, the traditional system of medicine could have a crucial impact in this quest. Investigating ancient healing traditions like Unani medicine may unveil fresh therapeutic modalities and potential remedies for melasma.

The Unani system of medicine, originating in ancient Greece, draws upon the teachings of prominent figures such as Hippocrates and Galen, and was further developed by notable Arab and Persian physicians including Rhazes, Avicenna, Abulcasis, and Ibn Nafīs.[12] The description of melasma can be found extending as far back as the reports of Hippocrates (470–360 BC). Even the term "melasma" originates from the Greek word "melas," which means black.[13] Melasma is described extensively in classical Unani literature with the Arabic translated term '*Kalaf*'. Unani scholars have described the cause, pathology, diagnosis, and treatment of melasma based on the classical Unani principles. For the treatment, a number of single and compound drugs are recommended by Unani scholars. Among all those recommendations, *Tukhm-i Turb* (*Raphanus sativus*) with *'Asl* (honey) is widely described in Unani literature as an effective and potent medicine for melasma. Both possess skin cleansing (*Jāli*) action, which helps to reduce hypermelanosis.[14] It is also reported that *Raphanus sativus* and its bioactive components exhibit tyrosinase inhibitor and antioxidative activities.[15] Furthermore, honey exhibits antiseptic (*Dāfi-i Ta'affun*) and resolvent (*Muḥallil-i Awrām*) properties.[16]

Given these considerations, the present research has been meticulously designed to assess and compare the safety and efficacy of the formulation incorporating *Tukhm-i Turb* (*Raphanus sativus*) and *'Asl* (honey) with hydroquinone 4 % cream for the management of melasma.

2. Materials and methods

2.1. Trial design and setting

This prospective open label randomized controlled clinical trial was conducted from November 2022 to June 2023. The study was conducted at the Dept. of *Mu'ālajāt*, Luqman Unani Medical College Hospital and Research Centre (LUMCHRC), Vijayapur (Bijapur), Karnataka, India.

2.2. Participants

Individuals of any gender within the age range of 18–65 years, and those clinically diagnosed with bilateral facial melasma including epidermal and mixed type of melasma, were deemed eligible for inclusion in the study. Exclusion criteria comprised pregnant or lactating women, individuals with a documented history or current manifestation of skin allergies or hypersensitivity to hydroquinone, patients with a history or current presence of significant medical conditions, such as immunological disorders, ongoing malignancies, diabetes mellitus, and hypertension. Additionally, exclusion criteria encompassed individuals who had undergone topical or systemic treatment for scalp diseases within 15 days prior to baseline, those with a history of chemical peels and facial laser treatment within the past 9 months, and individuals utilizing sun protection creams.

2.3. Selection of the participants

Participants were chosen after undergoing a thorough screening process that included a detailed assessment of medical history, general physical examination, and systemic evaluation. Those who met the specified inclusion and exclusion criteria and underwent routine investigations were enrolled. Demographic information, comprehensive disease history, and results from physical and systemic examinations were recorded in the Case Report Form approved by the Institutional Ethics Committee (IEC).

2.4. Interventions

Study participants were randomized into two groups: the Unani group received a topical application of *Tukhm-i Turb* (*Raphanus sativus* seed) powder mixed with honey,[14] while the control group received topical hydroquinone (4 % w/w) cream.[17] Follow-up assessments were carried out bi-weekly over an 8-week duration. The concurrent use of other medications was prohibited throughout the study period.

2.4.1. Dosage regimen and quantitative description

Participants allocated to the Unani group were instructed to apply finely powdered *Tukhm-i Turb* (*Raphanus sativus* seed) with *'Asl* (honey) to the affected areas twice daily, ensuring adequate coverage of the skin lesion, for a duration of 8 weeks. The control group received instructions to apply hydroquinone 4 % cream topically twice daily to the affected skin area, also ensuring complete coverage of the lesion.

2.4.2. Qualitative testing and preparation of the test drug

High-quality *Tukhm-i Turb* (*Raphanus sativus* L.) and *'Asl* (honey) were obtained from a certified herbalist. Taxonomical identification of *Raphanus sativus* was conducted by Miss Pooja Desai, a botanist from SECAB's ARS Inamdar Arts, Science, and Commerce College for Women, Vijayapura, Karnataka, India. The identified botanical specimens were deposited in the Museum of LUMCHRC, Vijayapur (Bijapur), Karnataka, India, with herbarium file No. 04/HERB/LUMC/21 and voucher specimen number, DBARSI-BJP2103 for *Raphanus sativus* L. It

is also important to mention that the online database World Flora Online identifies *Raphanus sativus* L. as a synonym for *Raphanus raphanistrum* subsp. *sativus* (L.) Domin and designates the latter the accepted name.

Furthermore, *Tukhm-i Turb* underwent grinding with a mortar and was sieved through a no. 80 mesh to obtain a fine powder. This powder was provided to the participants with instructions to mix it in equal amounts with clarified honey and apply it to the affected skin.

2.4.3. Rationale for the type of control used

Hydroquinone 4 % is a globally recognized and extensively studied depigmenting agent, often considered the gold standard for treating melasma.[9] It functions by inhibiting melanin synthesis, specifically by preventing the conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) to melanin via tyrosinase inhibition.[18] Its selection as a control in this study is supported by its proven efficacy and mechanistic alignment with the condition under investigation.

2.5. Outcomes

2.5.1. Primary outcome measure

The primary outcome measure for this trial was the change in the modified Melasma Area and Severity Index (*mMASI*), evaluated at baseline and during each subsequent follow-up, occurring bi-weekly over an 8-week period. For the measurement of *mMASI*, the severity of melasma in four distinct facial regions (forehead, right malar, left malar, and chin) was assessed by considering two variables: the percentage of the total area involved (*A*) and the darkness (*D*).[19]

2.5.2. Secondary outcome measure

Secondary outcome measures included changes in patient's global assessment of melasma assessed through the 100 mm visual analogue scale (*VAS*) [20] and dermatology life quality index (*DLQI*).[21] All the secondary outcomes were evaluated at baseline and at the conclusion of the trial.

2.6. Safety and adverse event monitoring

To ensure participant safety, continuous monitoring and meticulous documentation of any adverse events were stringently enforced throughout the study. A dedicated provision was established to record all such events in the adverse event monitoring form, aligning with the regulatory requirements.

2.7. Laboratory investigations

Screening investigations were conducted to ensure participant eligibility. These included complete blood count (CBC) and random blood sugar (RBS) tests. These laboratory investigations were performed according to standard operating procedures (SOPs), and the results were recorded in the study case report form (CRF).

2.8. Sample size

The initial sample size estimate for the study was 40 participants. However, accounting for a 5 % allowance for potential dropouts, we increased it to 42 participants, with 21 in each group. This calculation was estimated on an anticipated difference in the mean modified Melasma Area and Severity Index (*mMASI*) of 1.5 between the test and control groups, coupled with a population standard deviation of 1.67. Consequently, the sample size was determined through the employment of the subsequent formulas: $n_1 = \kappa n_2$ *and* $n_2 = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 (1 + 1/\kappa)}{(\mu_T - \mu_C)^2}$, which took into account critical values for significance and power ($Z_{\alpha/2}$and Z_{β}) at an alpha level of 0.05 and 80% power. The population standard deviation (σ) was 1.67, the test-control allocation ratio (κ) was 1, and the anticipated difference in means between the two groups ($\mu_T - \mu_C$) was 1.5.[22]

2.9. Withdrawal criteria

The withdrawal criteria were explicitly defined to include voluntary withdrawal by the participant, non-attendance at the prescribed treatment sessions, or adverse effects necessitating additional treatment. The implications of withdrawal and the procedures to be followed were thoroughly explained to the participants during the informed consent process.

2.10. Randomization

A block randomization technique was employed, using four-person blocks prepared by the medical records department (MRD) of the institute. These blocks were placed in sequentially numbered, sealed, opaque envelopes to maintain allocation concealment. Although the trial was open-label, the investigators who assessed the outcomes were blinded to the treatment allocation to minimize bias.

2.11. Statistical methods

The collected data were input and organized using Microsoft Excel and analyzed using IBM SPSS Statistics v 25.0. Descriptive statistics, including proportions, means, and standard deviations (SD), were calculated. Inferential statistical methods were applied, such as the Chi-square test, Fisher's exact test, independent and paired samples *t*-tests, to analyze and interpret the data. A *p*-value less than 0.05 was deemed significant.

2.12. Informed consent

Written informed consent was obtained from the trial participants before administering the trial intervention. The trial subjects were afforded ample opportunity to discuss the aims and objectives of the study, the nature of the study, the intervention to be administered, and the method of treatment, all in their native language.

3. Results

3.1. Participant flow

A total of 46 patients were assessed for eligibility in the study, of which 42 met the stipulated inclusion criteria and were consequently enrolled. The remaining 4 individuals were excluded due to non-compliance with the specified criteria. Of the 42 enrolled participants, 40 successfully completed the course of treatment, while 2 participants were lost to follow-up, specifically one from the test group and one from the control group. The subsequent statistical analysis was performed on the 40 participants who adhered to and completed the prescribed therapy regimen, as illustrated in Fig. 1.

3.2. Clinical and demographic profile

The investigation engaged a cohort comprising 40 subjects, whose mean ± SD age was 32.70 ± 8.80 years, with the majority of 11 participants (27.5 %) falling within the 30–35 year age bracket. Within this sample, 22 participants (55.0 %) were identified as female, while 18 participants (45.0 %) were male, including 31 married individuals (77.5 %), with the remaining 9 cases (22.5 %) unmarried. In terms of dietary habits, 87.5 % adhered to a mixed diet pattern, whereas 12.5 % adhered to a vegetarian diet. Regarding socio-economic status, 45.0 % of participants were from the upper middle class, 37.5 % from the lower middle class, 10.0 % from the upper class, and 7.5 % from the upper lower class. Concerning family history, only 10.0 % exhibited a positive

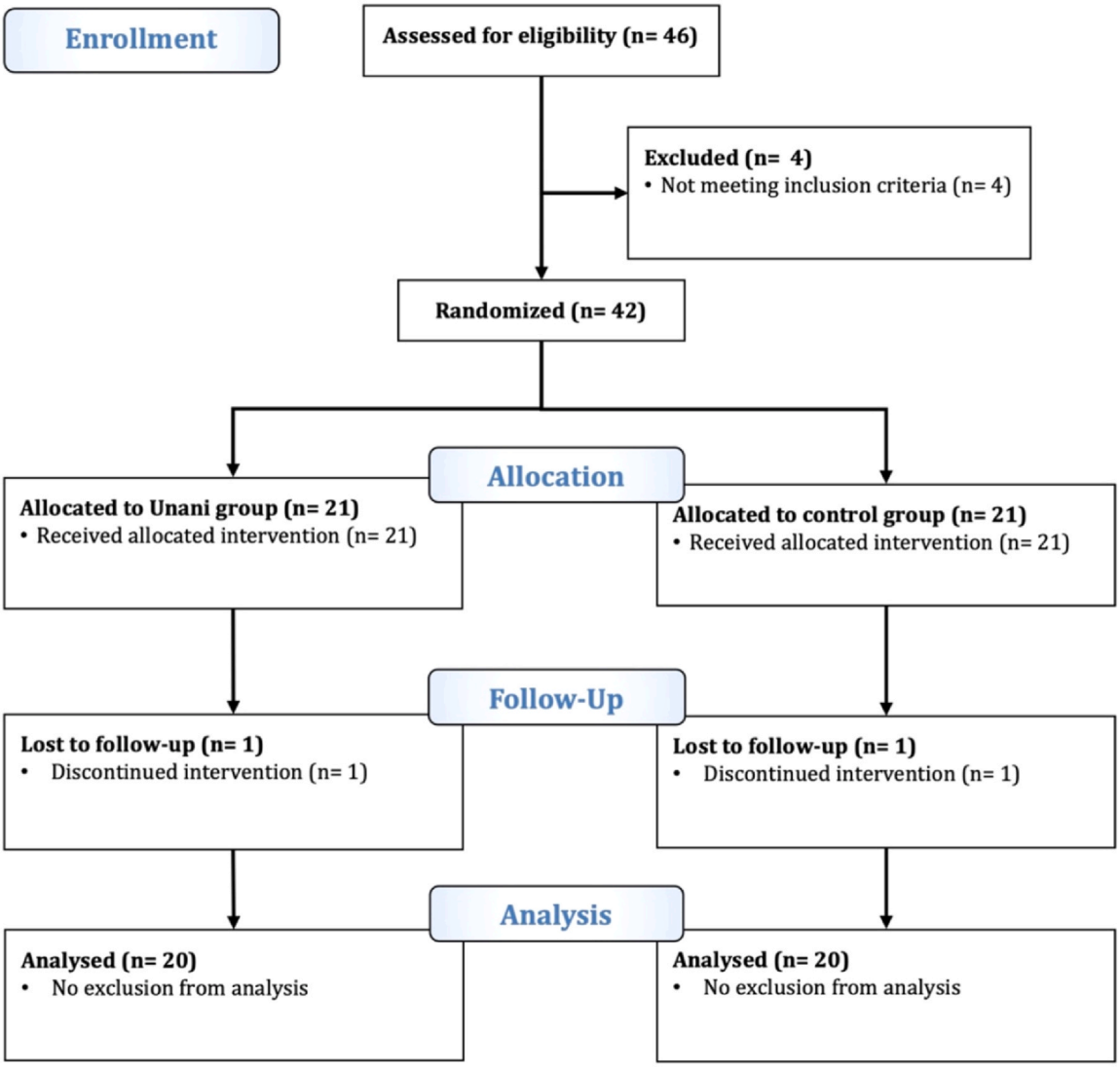

Fig. 1. CONSORT diagram of the participants.

Table 1
Baseline demographic and clinical profiles of the participants.

Characteristics		Unani group (n = 20)	Control group (n = 20)	*p*-Value
Age	mean ± SD, years	32.70 ± 8.80	34.15 ± 5.42	0.903*
Gender	Male, n, %	11 (55.0 %)	7 (35.0 %)	0.341#
	Female, n, %	9 (45.0 %)	13 (65.0 %)	
Marital status	Married, n, %	13 (65.0 %)	18 (90.00 %)	0.487#
	Unmarried, n, %	7 (35.0 %)	2 (10.0 %)	
Diet	Vegetarian, n, %	2 (10.00 %)	3 (15.0 %)	1.000#
	Mixed, n, %	18 (90.0 %)	17 (85.0 %)	
Socio Economic Status	Upper, n, %	3 (15.00 %)	1 (5.0 %)	0.733#
	Upper Middle, n, %	8 (40.0 %)	10 (50.0 %)	
	Lower Middle, n, %	7 (35.0 %)	8 (40.0 %)	
	Upper lower, n, %	2 (10.0 %)	1 (5.0 %)	
Family History	Present, n, %	2 (10.0 %)	2 (10.0 %)	1.000#
	Absent, n, %	18 (90.0 %)	18 (90.0 %)	

* Independent samples t-test: Between-group difference not significant ($p > 0.05$)
\# Fisher exact test: Between-group difference not significant ($p > 0.05$) in all the characteristics

family history, while the remaining 90.0 % had no familial predisposition to melasma. In summary, the baseline demographic and clinical characteristics showed comparable results between the Unani and control groups, with no statistically significant differences ($p > 0.05$) (Table 1).

3.3. Change in the modified Melasma Area and Severity Index (mMASI)

Within the test group, the baseline mean ± SD of the *mMASI* score was 8.44 ± 1.84. Follow-up assessments revealed a statistically significant reduction in *mMASI* scores over time. At the first follow-up, the

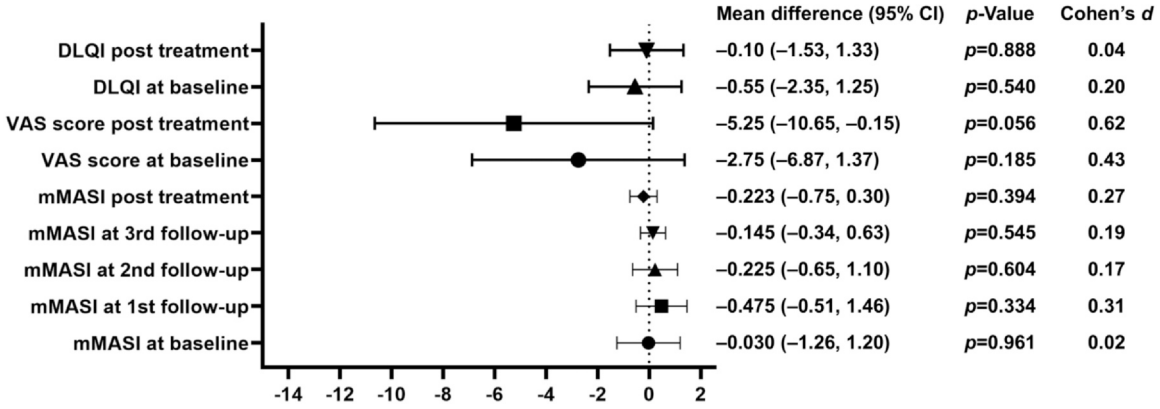

Fig. 2. Mean differences with 95 %CI between the Unani and control groups. The differences were not statistically significant ($p > 0.05$) for all outcomes, including mMASI, VAS, and DLQI scores. Effect sizes are indicated by Cohen's d: $d = 0.2$ for small effect, $d = 0.5$ for medium effect, and $d \geq 0.8$ for large effect.

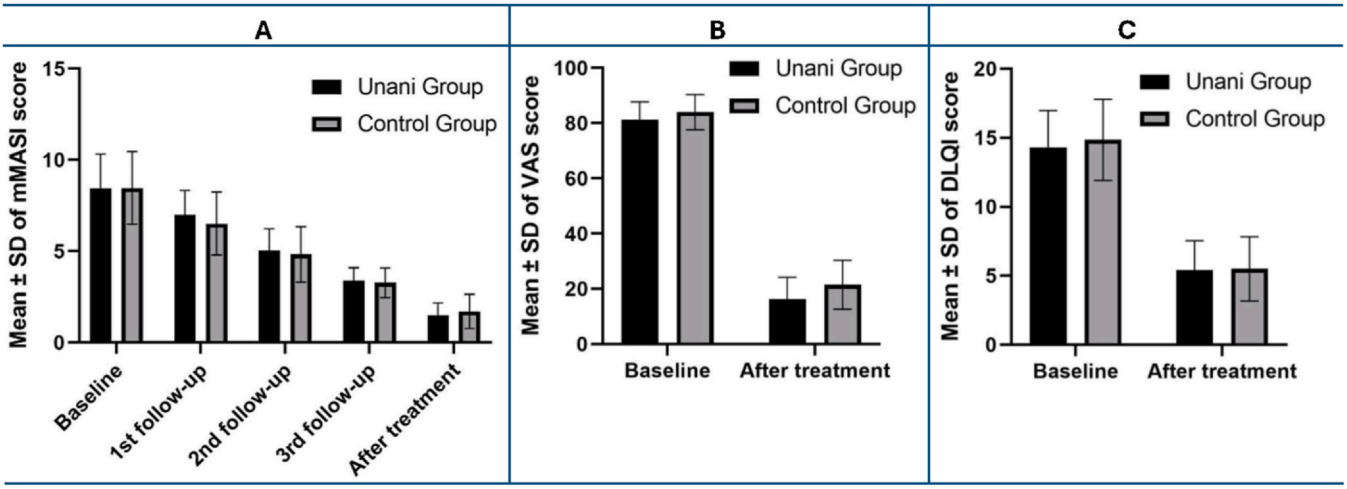

Fig. 3. Change in the mean ± SD of *mMASI* score (A), *VAS* score (B) and *DLQI* score (C); Between-group differences were not significant ($p > 0.05$) for all outcomes, including *mMASI* (A), *VAS* (B), and *DLQI* (C) scores. However, within-group differences were significant ($p < 0.05$) in both groups for *mMASI* (A), *VAS* (B), and *DLQI* (C) scores.

score decreased to 7.00 ± 1.33. This was followed by further reductions at the second follow-up (5.05 ± 1.18) and the third follow-up (3.41 ± 0.69). By the end of the trial, the score had significantly decreased to 1.49 ± 0.68 ($p < 0.0001$), indicating a significant therapeutic response (Fig. 2, 3, and 4).

In the control group, the baseline mean *mMASI* score was 8.47 ± 1.99. Successive evaluations revealed a steady decrease in *mMASI* scores: at the 1st follow-up, the score dropped to 6.52 ± 1.72; by the 2nd follow-up, it had significantly decreased to 4.83 ± 1.52; and at the 3rd follow-up, it further reduced to 3.27 ± 0.81. By the end of the trial, the *mMASI* score had markedly declined to 1.71 ± 0.94 ($p < 0.0001$), demonstrating substantial improvement over time (Fig. 3). Furthermore, the mean differences between the groups were calculated as –0.475 (95 % CI: –0.51 to 1.46, $d = 0.31$, $p = 0.334$) at the 1st follow-up, –0.225 (95 % CI: –0.65 to 1.10, $d = 0.17$, $p = 0.604$) at the 2nd follow-up, –0.145 (95 % CI: –0.34 to 0.63, $d = 0.19$, $p = 0.545$) at the 3rd follow-up, and –0.223 (95 % CI: –0.75 to 0.30, $d = 0.27$, $p = 0.394$) at the final follow-up (Fig. 2). These findings substantiate the efficacy of both therapeutic modalities in mitigating melasma symptoms, as measured by the *mMASI* score. While both interventions resulted in significant reductions in *mMASI* scores throughout the trial, the lack of statistical significance in the differences between the groups underscores the comparable efficacy of the experimental and control interventions.

3.4. Change in the VAS score from baseline to the end of the trial

In the test group, the baseline mean ± SD of the *VAS* score was recorded as 81.25 ± 6.46. Following the completion of the trial therapy, a significant reduction to 16.25 ± 7.92 was observed ($p < 0.0001$), indicating a substantial mitigation of subjective symptoms. Concurrently, in the control group, the baseline mean ± SD of the *VAS* score was 84.00 ± 6.40. At the completion of the trial, this score significantly decreased to 21.50 ± 8.90 ($p < 0.001$), reflecting a pronounced amelioration of subjective symptoms. Furthermore, the calculated mean difference between the groups following trial completion was –5.25 (95 % CI: –10.65 to –0.15, $d = 0.62$, $p = 0.056$), indicating a statistically insignificant difference (Figs. 2 and 3). The findings underscore the comparable efficacy of the test and control drugs in mitigating subjective symptoms, as indicated by the VAS score.

3.5. Change in the dermatology life quality index (DLQI)

In the test group, the baseline mean ± SD of the *DLQI* score was 14.30 ± 2.68. Following the completion of the trial therapy, a significant reduction to 5.40 ± 2.14 was observed ($p < 0.0001$), signifying a substantial improvement in the quality of life related to melasma. Likewise, in the control group, the mean ± SD of the *DLQI*

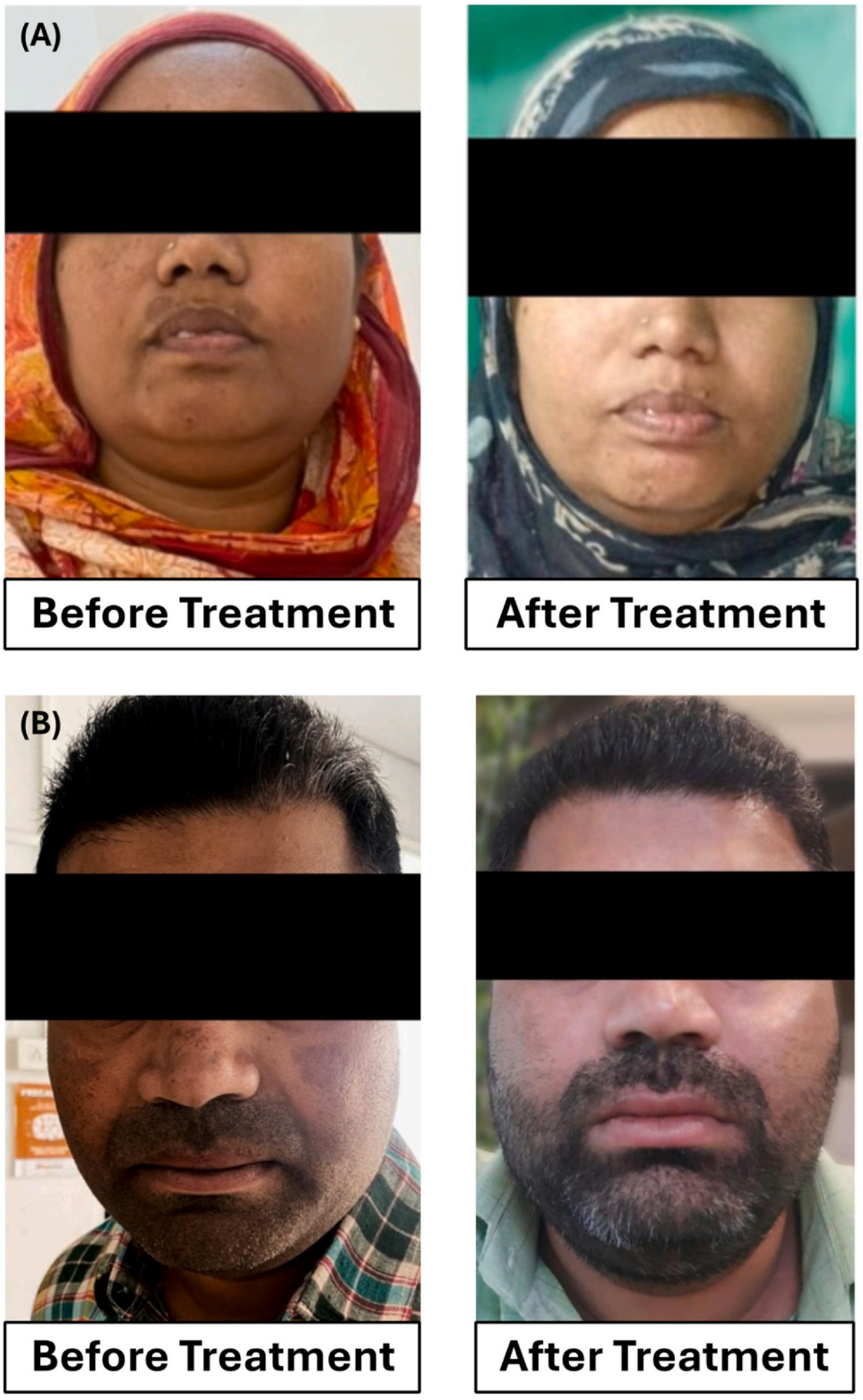

Fig. 4. Comparative before and after images showing the effects of Unani treatment over an 8-week period. (A) Female participant before and after treatment. (B) Male participant before and after treatment.

score was initially 14.85 ± 2.94 at baseline. By the conclusion of the trial, this score significantly decreased to 5.50 ± 2.33 ($p < 0.001$), indicating a significant positive impact on the participants' quality of life. The observed mean difference between the groups was –0.10 (95 % CI: –1.53–1.33, $d = 0.04$, $p = 0.888$) post-treatment completion (Figs. 2 and 3). These findings underscore the therapeutic benefits of both interventions on the quality of life among melasma patients. Both treatments yielded significant improvements in the quality of life, with no statistically significant difference between them, affirming their comparable efficacy.

3.6. Safety parameters

Over the entire course of the trial, participants demonstrated a conspicuous absence of adverse events, with no reported incidents. This observation attests to the favorable tolerability of both interventions within the study cohort.

4. Discussion

4.1. Overall evidence

The study aimed to assess the efficacy of topically applied *Tukhm-i Turb* (*Raphanus sativus*) seed powder mixed with *'Asl* (honey) in comparison to hydroquinone 4 % cream for managing *Kalaf* (melasma). Preliminary findings suggest that the test and control drugs exhibit comparable efficacy and safety in ameliorating overall disease severity and clinical features. Furthermore, the observed demographic findings had no statistical significance between the delineated groups. Through meticulous control for demographic variables, the study mitigates potential confounding variables and biases associated with demographic factors, thereby augmenting the reliability and validity of the observations and conclusions derived from the research.

The therapeutic effect of the experimental intervention might be attributed to its pharmacological properties as described in the Unani system of medicine, supported by contemporary scientific research. The *Hār Yābis Mizāj* (hot and dry temperament) of both *Tukhm-i Turb* and honey might be related to the observed response of the test drug and the reduction of hyperpigmentation.[16,23] Owing to their hot temperament, these substances are characterized by *Jālī* properties.[24] In the Unani medicine, *Jālī* drugs are primarily employed for skin cleansing which harbor potential to clear skin pores, thereby preserving skin integrity. This category of treatment exhibits keratolytic activity and facilitates the removal of dead skin and debris, promoting cleanliness in facial skin, hands, and other areas, and locally enhancing skin tone. The well-defined mechanism of action in Unani medicine entails the scraping of viscous fluid from skin pores, signifying that pores are effectively cleansed.[24] Furthermore, honey exhibits *Dāfi-i Ta'affun* (antiseptic) and *Muḥallil-i Awrām* (resolvent) properties.[16] These actions of the test drugs lead to the removal of the disease-causing substances.

Pharmacologically, an in vitro study conducted by Hamed *et al.* found that the seed extracts of *Raphanus sativus* demonstrate anti-tyrosinase activity.[25] Tyrosinase, acting as a rate-limiting enzyme in melanogenesis, plays a crucial role in melanin formation. Consequently, inhibition of tyrosinase activity can attenuate melanin synthesis, rendering tyrosinase inhibitors effective whitening agents.[26] Moreover, sulforaphane, a phytochemical of *Raphanus sativus* seed, has been reported to exhibit tyrosinase inhibiting activity in both in vitro and in vivo investigations. The mechanism by which sulforaphane suppresses tyrosinase activity involves the modulation of the phosphorylated mitogen-activated protein kinase pathways.[27] Therefore, the evidence suggests that *Raphanus sativus* seed and its bioactive components may inhibit tyrosinase activity and reduce melanin synthesis through the alteration of keratinocytes and melanocytes interaction. This alteration may lead to hypomelanosis and constriction of dermal vasculature.

Furthermore, *Raphanus sativus* seed and honey possess anti-inflammatory and antioxidant properties, which may contribute to the reduction of redness and other discolorations. These properties may also confer protection against damage from free radicals and UV radiation.[16,28] In addition to these effects, honey is known to have an exfoliating property that can facilitate the removal of dead skin cells. By eliminating the dull outer layer of skin cells, the complexion may gain increased brightness and luminosity.[16]

With respect to the pharmacological effects of hydroquinone in the management of melasma, a series of recent empirical studies have elucidated its capability in reducing the *mMASI*. Ibrahim *et al.* reported a statistically significant decrease in mean ± SD *mMASI* from 12.41 ± 3.92 to 5.74 ± 5.71 subsequent to a 12-week application of 4 % hydroquinone cream.[29] In a comparative study, Lima *et al.* assessed the efficacy and tolerability of 0.2 % thiamidol vis-à-vis 4 % hydroquinone in a cohort of 50 melasma patients, identifying a mean ± SD *mMASI* reduction from 7.2 ± 3.4 to 5.0 ± 3.1 over a 90-day treatment period.[30] Additionally, Shihab *et al.* observed that oral tranexamic acid, compared to placebo, combined with hydroquinone 4 % cream (evening) and broad-spectrum SPF 30 sunscreen (morning) in both groups, resulted in a mean ± SD *mMASI* reduction from 8.53 ± 2.04 to 7.6 ± 2.0 after three months, corresponding to a 10.9 % decrease.[31] A separate investigation by Bronzina *et al.*, involving 43 melasma-affected women treated with either new topical skin-lightening cosmetic product combination or 4 % hydroquinone cream, documented a mean *mMASI* decrement from 4.91 to 3.08 within the hydroquinone cream group following a 12-week treatment duration.[32]

In synthesizing the evidence from these scientific investigations, it is plausible to infer that the test drugs exhibit a therapeutic potential comparable to hydroquinone in reducing melasma severity. This similarity in pharmacological outcomes supports the potential equality of the test drugs and hydroquinone in the treatment of melasma, thus substantiating the exploration of the experimental interventions as alternative agents in the clinical management of melasma.

4.2. Strength of the study

The study was meticulously designed and executed to minimize the risk of bias and confounding variables. The findings are reported in accordance with the guidelines established by the CONSORT extension for herbal medicine interventions.[33] To circumvent potential bias, a comprehensive approach was adopted, incorporating both subjective and objective measures. The *mMASI* facilitated an objective assessment, while the *VAS* was utilized to capture subjective nuances. To further diminish the impact of both known and unknown confounding variables, a stringent randomization procedure was implemented, ensuring an equitable distribution of participants across various conditions. These methodological measures underscore the rigorous scientific investigation and the reliability of the findings of the study.

4.3. Study limitations

The findings of this study should be interpreted cautiously due to several limitations. The relatively small sample size may compromise statistical power. The absence of blinding introduces potential biases, impacting internal validity. The single-centric nature of the study may hinder the generalizability of findings to a broader population.

It is also important to note that the aroma of *Raphanus sativus* seeds is not particularly pleasant. Although the incorporation of honey mitigates the odor to some extent, it still persists and proves challenging for certain patients. Consequently, future research may explore the inclusion of additional agents in the formulation to effectively diminish the undesirable odor. These limitations highlight the need for future research with larger, diverse samples, a multi-centric design, and enhanced blinding procedures to validate or extend the current results.

5. Conclusion

The findings of this clinical trial demonstrate that the experimental intervention, *Tukhm-i Turb* (*Raphanus sativus*) seed powder with *'Asl* (honey), exhibits comparable efficacy to the standard treatment of hydroquinone 4 % for melasma. Moreover, both treatment groups exhibited statistically similar improvements in the dermatological quality of life and a favorable safety profile, underscoring the potential of *Tukhm-i Turb* with honey as an effective alternative treatment. Given these results, future studies should include larger, more diverse patient populations, a multi-centric design to account for regional variations, and enhanced blinding procedures to ensure robustness of the findings. Additionally, efforts should be made to address the persistent odor of

the formulation, which could improve patient compliance and acceptance.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

The research protocol of this study complied with the principles outlined in the Helsinki Declaration and received approval from the Institutional Ethics Committee of Luqman Unani Medical College, Hospital and Research Center, under protocol number BJP/LUMC/PG/IEC/04/2020–21/MOALAJAT/04. Furthermore, the trial was registered with the Clinical Trial Registry - India (CTRI) under registration number CTRI/2022/07/044080 to uphold transparency and adherence to both national and international guidelines.

CRediT authorship contribution statement

Arshan Horti: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Shaikh Nisar Ahmed Abbas:** Visualization, Validation, Resources, Methodology, Conceptualization. **Shavana Fathima:** Visualization, Validation, Resources, Conceptualization. **Mamadapur Saba Abdul Razzaq:** Visualization, Validation, Methodology, Conceptualization. **Arsheya Parvez Mistry:** Visualization, Validation, Formal analysis, Conceptualization. **Farooqui Shazia Parveen:** Visualization, Validation, Resources, Formal analysis. **Nazim Husain:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Data curation, Conceptualization.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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