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ORIGINAL CONTRIBUTION

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A double-blind, placebo-controlled randomized trial of skin-lightening cream containing lycopene and wheat bran extract on melasma

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Abstract

Objective: Melasma is an acquired hyperpigmentation disorder, and reactive oxygen species play important role in regulating melanin synthesis. Lycopene is one of the most effective oxygen neutralizers among tomato-derived carotenoids. Also, hydroquinone is a compound that has been used for the treatment of hyperpigmentation by mechanism of tyrosinase inhibition and can be found in wheat.

Methods: An appropriate cream formulation containing 0.05% tomato lycopene and 3.45% wheat bran extract was prepared, and physicochemical characterization was performed. The prepared formulations were applied twice a day for three months in combination with SPF = 30 sunscreen by 22 patients diagnosed with melasma. MASI score in two groups was evaluated at weeks 0, 3, 6, 9, and 12 and 1 month after the treatment.

Results: The prepared formulation shows smooth and homogeneous appearance with suitable spreadability and viscosity. The MASI score of intervention group from the sixth week until the end of the treatment was significantly decreased compared to the onset of the treatment ($P < .05$), and the mean difference of the MASI score and the rate of skin discoloration in intervention group (0.53 ± 0.47 and 3.73 ± 1.90, respectively) were significantly higher than in placebo group (0.14 ± 0.20 and 0.91 ± 0.07, respectively; $P < .05$). Size of melasma during the study was decreased significantly from 6.59 ± 3.47 to 5.97 ± 3.83 ($P < .05$).

Conclusion: The data of mean difference of the MASI score indicated skin improvement in intervention group. Meanwhile, no recurrence was observed one month after the end of the treatment. These data suggest that the prepared formulation containing lycopene and wheat bran extract is safe and could be promising as an efficacious cosmetic treatment.

KEYWORDS

formulation, lycopene, melasma, skin lightening, wheat bran extract

1 | INTRODUCTION

Hyperpigmentation occurs when excessive melanin deposits in the skin. This can make spots or patches on the skin which appear darker than surrounding areas. Melasma is an acquired hypermelanotic condition presenting with light- to dark-brown–colored irregular macules on sun-exposed areas of skin.[1] Multiple etiological factors have been implicated in the pathogenesis of melasma such as chronic ultraviolet (UV), genetic factors, sex hormones, and side effects of the drug.[2] Pigmentation disorders such as melasma affect the quality of life. In fact, healthy natural skin is one of the most important aspects of feeling well and confident. The prevalence of mental disorders among patients with dermatological disorders varies from 30 to 60%.[3] For effective treatment of melasma, the active ingredients can be inserted in to several dosage forms. Also, nowadays, herbal medicines are very popular due to their ability to treat various types of disease with less toxicity.[4] A wide range of preparations are available for lightening skin discolorations. Among them, there are variety of formulations composed of substances of plant origin.[5] Topical treatment of melasma is mainly aimed at protecting the light and disrupting the enzymatic activity of pigment production by melanocytes. Recently, studies have been conducted on the protective effects of carotenoids and phytochemical micronutrient against UV ray to prevent skin damage. Lycopene is one of the most effective neutralizers of active oxygen among tomatoes' carotenoids and its derivatives.[6] Its pigment-reducing effect mainly attributed to the reactive oxygen species (ROS)–scavenging properties.[7]

The other method for the treatment of melasma can be inhibiting the activity of tyrosinase enzyme. The hydroquinone in wheat bran extract binds to histidine in active site of tyrosinase enzyme, which impairs the function of the enzyme and generally reduces the pigmentation on skin.[7,8]

2 | METHODS

2.1 | Preparation of formulation

Cream formulation containing 0.05%w/w tomato lycopene and 3.45%w/w wheat bran extract (LWB cream) was prepared as described. The oil-soluble ingredients including stearic acid (17%), potassium hydroxide (0.5%), sodium carbonate (0.5%), and lycopene were taken into porcelain dish and melted at 70°C. Glycerin (6%), water, and wheat bran extract were heated at same degree separately. Ascorbic acid (2%) was added to the aqueous phase in the last 2 minutes. The aqueous phase was added to the oil phase with continuous stirring by an agitator at 70°C, until the cream was formed, and then, it was allowed to cool down at room temperature.[9] Blank formulation was prepared same as above except no active ingredients were included.

2.2 | Physicochemical characterization

Physical appearance, color, texture, phase separation, and homogeneity of formulation were evaluated by visual observation. Type of emulsion was determined by dilution test. To do this, the emulsion is diluted with either oil or water and stability of the preparation evaluated in each case based on the principle that an emulsion can be diluted with its external phase.[10]

pH of preparation was measured by pH meter. Viscosity of cream was determined by Brookfield viscometer using spindle 64 at 10 rpm at room temperature.

To test the spreadability of the formulation, 1 g cream was applied in between of two glass slides and 100 g weight was placed on the glass slides for 5 minutes to compress the sample to uniform thickness. The required time to separate two slides was taken as a measure of spreadability according to the following equation, where S is spreadability (g.cm/s), m is weight placed on upper slide in g, l is length of slides in cm, and t is time in s.[10]

$$S = \frac{m \times l}{t}$$

Stability evaluation of formulation was performed using cooling and heating tests. To perform cooling test, the formulation was stored at −20°C for 48 hours and then changed to room temperate for another 48 hours and this test was performed for six periods. To perform heating test, the formulation was stored at 45°C and room temperature every 48 hours for six cycles.[10]

2.3 | Clinical trials

All participants were informed about the study protocol, and they declared their consent officially. At last, twenty-two female patients diagnosed with melasma were randomly included in two groups of intervention and placebo (Figure 1).[11]

Subjects were not eligible in case of pregnancy, lactation, using topical treatment for melasma in last three months, history of lycopene or wheat bran extract allergy, and corticosteroids and anti-seizure drug administration.

Simple randomization method using shuffled cards (22 cards including equal number of A and B) was done by a person who had no involvement with patient care. All investigators, treating clinicians, and participants were blinded to the allocation.

The patient's history was recorded at the beginning of the study, and the distribution, homogeneity, and melasma type (dermal, epidermal, and mixed) were examined using a wood's lamp. Clinical assessment of severity of melasma was recorded by melasma area and severity index (MASI score).[12]

$$\text{MASI score} = [0.3A(D+H)]_{\text{forehead}} + [0.3A(D+H)]_{\text{right malar}} + [0.3A(D+H)]_{\text{left malar}} + [0.1A(D+H)]_{\text{chin}}$$

D: darkness score

H: homogeneity

A: area of involvement

The patients received the previously coded formulation twice daily for 12 weeks and have been evaluated at the weeks 3, 6, 9, and

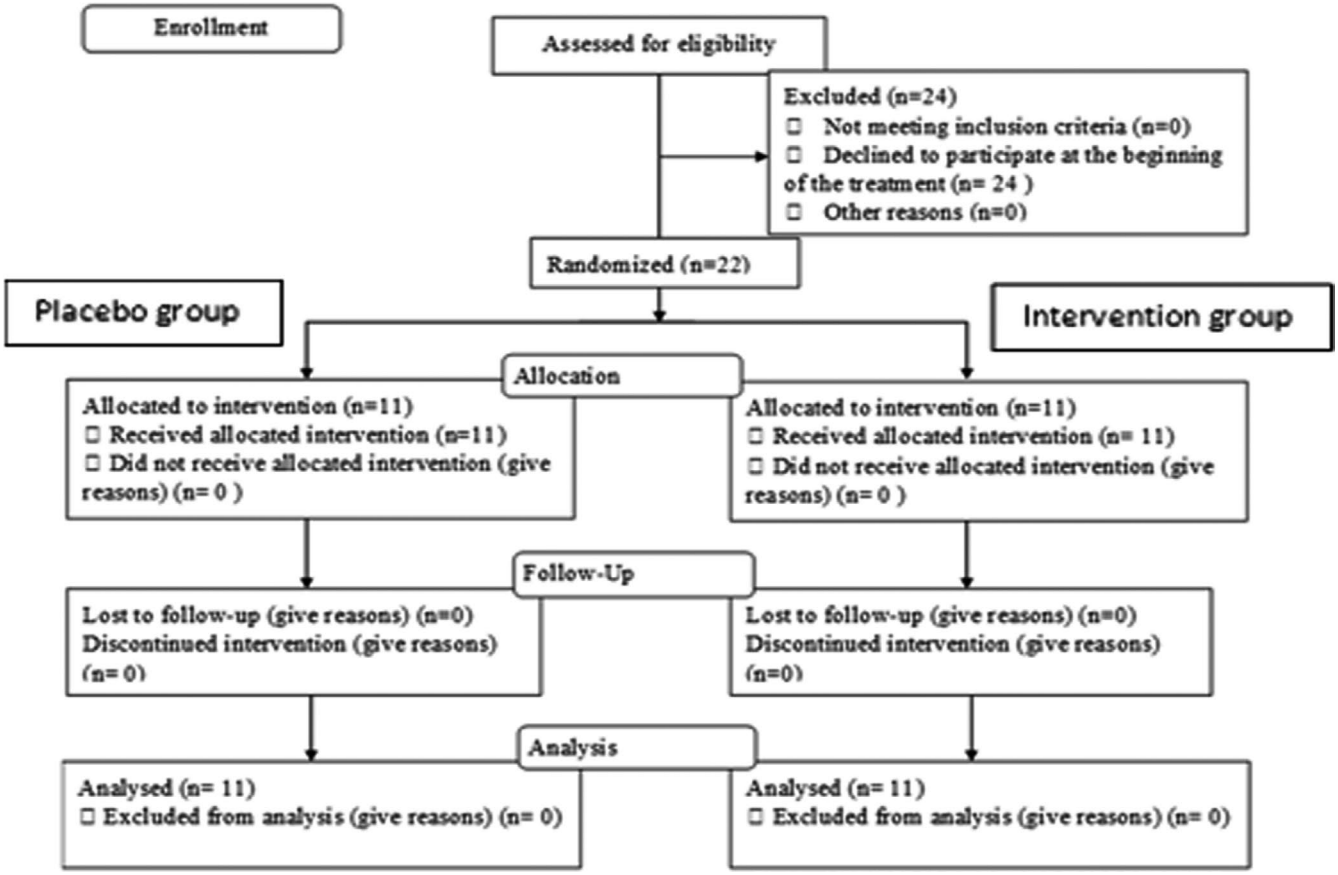

FIGURE 1 CONSORT flowchart of study screening and enrollment

12 of the treatment and one month after the end of the treatment (week 16). All participants received SPF = 30 sunscreen and were advised to apply it.

The lesions of each patient were pictured before the treatment, at the weeks 3, 6, 9, and 12 of the treatment, and four weeks after the end of the treatment in a location with same brightness and distance with fixed camera.

Discoloration rate of skin was measured by comparing the photographs of skin, were taken during the study (0 is the score for the least and 10 is given to the most changed sample). And area of melasma was measured at the start and the end of the treatment, using the photographs were taken during the study using graph paper.

The patient's satisfaction of treatment was scored from zero to ten (the highest satisfaction was indicated by 10, and no satisfaction was indicated by 0) at the end of study.

2.4 | Statistical analysis

The mean and standard deviation were used in quantitative variables. Independent samples *t* test and paired samples *t* test were performed in order to analyze the results. A *P*-value of .05 or less was considered to indicate significance. All analyses were conducted by SPSS software version 20.

TABLE 1 Physicochemical characteristics of LWB cream (mean ± SD, n = 3)

Parameters	Characteristics	
Organoleptic properties	Appearance	Creamy
	Homogeneity	Homogeneous and smooth
pH	After preparation	6.13 ± 0.01
	After 3 mo of storage	6.78 ± 0.01
Viscosity (cps)		10 690 ± 130
Spread ability (gcm/s)		14.22 ± 0.11

3 | RESULTS

3.1 | Physicochemical characterization of cream

Physicochemical characterization of the preparation is shown in Table 1. Type of emulsion was considered as oil in water according to the dilution test, and the preparation revealed suitable stability after performing heating and cooling tests.

3.2 | Clinical trials

The patients' basic characteristics are shown in Table 2. At the onset of the study, the mean size of melasma was 5.36 ± 4.08 (mm^2) in the placebo group and 6.59 ± 3.47 (mm^2) in the intervention group. All aforementioned parameters including patients' age, type of melasma, duration of melasma involvement prior to the beginning of the study, and the mean area of melasma at the onset of the study showed insignificant differences between both groups of placebo and intervention (independent samples *t* test, $P > .05$).

After twelfth week, the mean area of melasma was 5.32 ± 4.06 (mm^2) in the placebo group and 5.97 ± 3.83 (mm^2) in the intervention group which is not statistically significant difference between the both groups (independent samples *t* test, $P > .05$). The mean area of melasma in the twelfth week of treatment was not significantly different compared to that of the start of the treatment in the control group (paired samples *t* test, $P > .05$), but there was a significant decrease in mean area of melasma in the intervention group during the treatment (paired samples *t* test, $P < .05$). At the end of the treatment, the mean of lesion discoloration rate (blind investigators' assessment) in the intervention group (3.73 ± 1.90) was significantly higher than the placebo group (0.91 ± 0.07; independent samples *t* test, $P < .05$) (Figures S1A, S1B).

At the onset of the study, the week 0, the mean of MASI score was 8.86 ± 8.05 in the placebo group and 12.06 ± 8.54 in the intervention group, which showed no significant difference between the two groups (independent samples *t* test, $P > .05$).

MASI score reductions (ΔMASI), defined as amount of reduction in MASI score at any of the intervals compared to before the treatment (week 0), are shown in Table 3.

ΔMASI in intervention group were significantly higher than ΔMASI of placebo group in intervals of weeks 6, 9, 12, and 16 (independent samples *t* test, $P < .05$).

In addition, in intervention group, the MASI score significantly declined in the sixth week compared to third week (paired samples *t* test, $P < .05$) and in twelfth week compared to ninth week (paired samples *t* test, $P < .05$), but no significant difference was seen in third week compared to week zero (paired samples *t* test, $P > .05$), ninth week compared to sixth week (paired samples *t* test, $P > .05$), and one month after the treatment compared to twelfth week (paired samples *t* test, $P > .05$; Table 3).

TABLE 2 Patients' basic characteristics

Characteristics	LWB cream	Placebo cream	*P*-value
Age, y, mean ± SD	34.7 ± 10.2	33.5 ± 7.8	.314
Type of melasma, percent (number): Epidermal	27.27 (3)	36.36 (4)	.312
Dermal	45.45 (5)	36.36 (4)	
Combination	27.27 (3)	27.27 (3)	
Duration of involvement, wk, mean ± SD	255 ± 140	166 ± 69	.326

In placebo group, no significant difference was seen between the MASI score of each interval compared to the previous one during the study (Table 3).

It should be also noted that during the course of the 12-week treatment, no symptoms of skin irritation such as skin flushing, itching, rash, or swelling were either observed or reported.

At the end of the study, the mean satisfaction was 6.18 ± 2.18 in intervention group which was significantly higher than that of placebo group (4.36 ± 1.50; independent samples *t* test, $P < .05$).

4 | DISCUSSION

Herbal cosmetics have much popularity among the consumers. Most of lightening products' formulations are oil-in-water emulsion, which increases the medication compliance as they satisfy consumers' sensory expectations.[13] The dilution test confirmed that the prepared cream containing tomato lycopene and wheat bran extract was oil-in-water emulsion. The other characterization that is evaluated for skin products such as organoleptic properties, pH, viscosity, spreadability, and stability was desirable. The study by Djiobie Tchienou, GE (2017), stated that spreadability had important role in regulating the effective dosage for topical treatment and cream spreadability between 0.9 and 31.02 (g cm/s) could be considered as suitable spreadability.[14] The spreadability of LWB cream was 14.22 ± 0.11 (g cm/s) which was desirable to apply on the skin. The present research is the first clinical study to assess the effect of skin-lightening cream containing lycopene and wheat bran extract for the treatment of melasma. It was performed through the investigation of twenty-two female patients assigned to placebo and intervention groups. The patients' MASI score, area of melasma, and lesion discoloration rate were examined in specified intervals during the study. Higher ΔMASI and discoloration rate, and decreased area of melasma were achieved in intervention group after 12 weeks of twice-daily application of LWB cream. Also, one month after the treatment (week 16), no significant statistical difference was observed compared to the twelfth week, which indicates the absence of recurrence. These findings of MASI score determination and standard photography showed a marked improvement of the hyperpigmented lesions. The use of cream was well tolerated and did not induce any side effects.

The relatively small sample size is a limitation of this study. Besides, different percent of dermal and epidermal type in each group makes the comparison somehow confusing because it is well known that dermal type of melasma hardly responds to the treatment.

Zhang Q et al. (2019) used a polyherbal cream for the treatment of melasma, and the patients in this study were divided into three groups: A (received polyherbal cream), B (received arbutin cream), and C (received placebo cream). They used cream, twice daily for twelve weeks, and at the end of study, the MASI score of group A showed significant difference compared to that of B and C groups.[15] Khan BA et al. (2013) also conducted a study and evaluated the

TABLE 3 Melasma area and severity index (MASI score) and MASI score reductions (ΔMASI) in groups of intervention and placebo during the study (mean ± SD)

	Intervention group		Placebo group	
Times (wk)	MASI score	ΔMASI	MASI score	ΔMASI
0	12.06 ± 8.54	-	8.86 ± 8.05	-
3	12.06 ± 8.54	0	8.86 ± 8.05	0
6	11.86 ± 8.66	0.20 ± 0.22	8.82 ± 8.08	0.04 ± 0.09
9	11.77 ± 8.71	0.29 ± 0.24	8.80 ± 8.10	0.06 ± 0.12
12	11.53 ± 8.87	0.53 ± 0.47	8.72 ± 8.13	0.14 ± 0.20
16	11.53 ± 8.87	0.53 ± 0.47	8.72 ± 8.13	0.14 ± 0.20

effect of two cream formulations containing either *Hippohae rhamnoides* or *Cassia fistula* methanolic extract on the treatment of melasma. They concluded that after twelve weeks, improvement in the intervention groups was significant.[16]

5 | CONCLUSION

The results of present study showed that the patients' MASI score and area of melasma were declined significantly in intervention group during the treatment. No significant differences were observed in placebo group regarding the abovementioned parameters. Lesion discoloration in intervention group was dominant compared to the placebo group. Patients' satisfaction in the intervention group was also significantly higher than placebo group. No side effects were observed during the study. Overall, the LWB cream could be an effective and safe therapy for melasma and it is recommended to apply for a longer period of time.

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CONFLICT OF INTERESTS

The authors report no conflict of interests.

AUTHORS' CONTRIBUTION

N.B, M.A.M, and M.S conceived and designed the study. M.S acquired data. N.B, M.A.M, M.S, and S.M.L analyzed and interpreted data. M.S drafted the manuscript. N.B critically revised the manuscript.

ETHICAL STATEMENTS

The clinical study was performed in full compliance with local regulatory principles of Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS) (approval ID: IR.AJUMS.REC.1398.307). The study was undertaken at Dermatology Clinic of Ahvaz Imam Khomeini hospital. This trial is registered with the Iranian Clinical Trials Registry (IRCT20190930044932N1).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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