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A Clinical, Prospective, Randomized, Double-blind Trial Comparing the Efficacy of a Combination *vs*. Control as an Oral Intervention for Chloasma

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Research Article

Abstract

Chloasma is an acquired, chronic hypermelanosis that remains difficult to improvement. The combination of collagen peptide (CP), soy peptide (SP) and an aqueous extract of *Chrysanthemum morifolium* (AECM) has been proven to have antioxidant and anti-melanogenesis activity *in vitro* and in an animal model. In this study, we evaluated the efficacy of the combination versus a control as an oral intervention for chloasma by a non-invasive clinical method. Sixty-two subjects were recruited and randomly assigned to two groups: test group and control group. The test group was administered the combination every day for 60 days according to the manufacturer's instructions. Two types of methods — an instrument-based measurement and clinical grading — were conducted at each time point to evaluate the improvement in the chloasma. The instrumental results revealed a significant decrease in skin yellowness at 60 days compared with the control group, and the clinical-grading results were in good agreement. Additionally, after administration of the combination, skin hydration exhibited an increasing trend compared with the control group. No side effects were observed during the study. The combination was effective in reducing the hyperpigmentation of subjects with chloasma. The combination oral intake might prevent chloasma from worsening.

Keywords: Chloasma; Combination oral drink; Peptide; *Chrysanthemum morifolium*; Clinical study.

1. Introduction

Chloasma is a common, acquired, chronic, and symmetrical hypermelanosis characterized by irregular light to dark brown patches of hyperpigmentation on sun- exposed areas, predominantly on the face [1-3]. Although the etiology is unknown, several etiogenic factors have been implicated, including genetic factors, UV exposure, pregnancy, oral contraceptives, etc. The sun exposure is the most important factor, and is present in all patients, who improve or worsen with sun exposure influence [4]. Therapy remains a challenge for chloasma [5,6]. All topical treatments have been associated with side effects, including allergy and contact dermatitis, depigmentation of the surrounding normal skin and post-inflammatory hyperpigmentation. Thus, the oral intake of antioxidants, such as vitamins C and E and grape seed extract, has recently attracted substantial attention for the intervention of chloasma as these antioxidants might prevent UV-induced melanogenesis and reduce hyperpigmentation [7-9]. An aqueous extract of Chrysanthemum morifolium (AECM) has been found to contain flavonoids and exhibit anti-melanogenesis properties [10]. Our previous study revealed that AECM extract enhanced the antioxidant and anti-melanogenic efficacy of a peptide mixture (PM), which contained soy peptide (SP) and collagen peptide (CP); in a UV-irradiation model [11]. The purpose of this study is to clinically evaluate the efficacy of the formula containing AECM extract, SP and CP as a healthy food supplement in the intervention of facial chloasma. We seek to assess the clinical response objectively by evaluating the chloasma area using the Melasma Area and Severity Index (MASI) and the skin color using a Skin Tone Color Scale, Spectrophotometer readings and image analysis.

2. Materials and Methods

2.1 Product administration and application

Combination drink

Participants opened a small package and combined the included powder with cool water or fruit juice. It was recommended to drink the combination after a meal, 1 time/day. A sunscreen product (Amway, Guangzhou, China) with SPF30/PA+++ was provided and used to prevent the effects of sunlight. Every morning, the subjects applied the sunscreen to his or her face, reapplying before going outside.

2.2 Subject recruitment and preparation

A total of 62 Chinese adult subjects aged 18-65 years with diagnosed chloasma were recruited and randomly assigned to the test (n=32) or control

Table 1. Subject demographics.

Age	30-39	40-49	50-59	60-69	Total
Test group	10	11	10	2	33
Control group	5	15	6	3	29

(n=29) group (Table 1). Participants in the test group received the combination drink for chloasma intervention. Additionally, subjects in both test group and control group were provided with usage instruction for the assigned sunscreen product.

2.3 Inclusion criteria

All subjects were required to meet all of the following criteria to be eligible for enrollment: Chinese; age 18-65 years; confirmed chloasma diagnosed by a dermatologist; in generally good health during the study; willing to maintain the original skin care regimen, including product type and usage habit, during the test period; and willing and able to participate as evidenced by signing of an informed consent form.

2.4 Exclusion criteria

Those who met any of the following criteria were excluded from participation: pregnant or lactating women; currently taking medication (e.g. corticotherapy or aspirin); history of dermatological problems that may affect the results; any skin or dermatological medical procedures currently or within the recent 3 months; participation in another clinical study; consumption of oral products that would have affect the skin; alcoholic or smoker; regular diarrhea; or allergy to the product.

2.5 Subject instruction

The following test instructions were given to all participants: Need to maintain original skin care regimen, including product type and usage habit, during the test period; must not apply any products to the face on the day of the test, although cleansers and soaps are allowed; and should not attend other clinical studies or conduct any medical treatment on the testing area during the test.

2.6 Ethical considerations

All of the test methods were noninvasive and approved by the Institutional Review Board. All subjects provided written informed consent to participate in the study.

2.7 Testing environment

During the test period, the lab temperature and humidity were controlled at 21 ± 1°C and 50 ± 5% RH, respectively.

Skin color of the chloasma-affected area measured by spectrophotometer

The L*, a* and b* color space was a color-opponent space, and the three coordinates of L*, a* and b* represented the lightness of the color (L*=0 indicates black and L*=100 indicates diffuse white), its position between red/magenta and green (negative values of a* indicate green, whereas positive values indicate magenta), and its position between yellow and blue (negative values of b* indicate blue, and positive values indicate yellow), respectively. A SP64 Sphere Spectrophotometer (X-Rite, Incorporated) was used to measure the reflectivity of the measured surface to different light waves in the visible spectrum, which was described by a spectrum curve; in this test, we detected L* and b* values.

Photography and image analysis for skin color

In-house developed software was used for facial skin image analysis to determine skin color. Facial skin images were captured by using a Visia-CR imaging system (Canfield, USA) and by using in-house software, color calibration, regions of interest (ROIs) management on the forehead and cheeks, color information (L*, a*, b*) acquisition of each ROI were processed. In this study, we analyzed L* and b* values via image analysis.

MASI grading by a dermatologist to evaluate the chloasma area and severity

The Melasma Area and Severity Index (MASI) were used as a clinical-grading system to grade the chloasma area, severity and evenness; a higher score indicated more serious illness. Dermatologists evaluated the darkness (D), area (A) and homogeneity (H) of chloasma on the forehead (F), left cheek (ML), right cheek (MR), and lower face (C). The MASI score was the sum of the scores determined for the forehead, left cheek, right cheek and lower face scores. The MASI score was calculated as follows:

MASI=0.3 (DF+HF) AF+0.3 (DMR+HMR) AMR+0.3 (DML+HML) AML+0.1 (DC+HC) AC

Clinical grading of skin color with skin tone color scale

The Skin Tone Color Scale (Hadatone Checker, Daiich Sankyo Healthcare) was used for the evaluation. The Skin Tone Color Scale Bar is composed of five different hue plastic bars, 1YR, 3YR, 5YR, 7YR and 9YR. Chromaticity (C) was fixed as C4. Nineteen kinds of value (V) color charts from 4.0-8.5 with increments of 0.25 were attached to each bar. Higher value indicates lighter skin tone. Dermatologist measured chloasma affected skin color with rough adjustment of value using the 5YR bar that was the middle of the hue range. Second, hue was determined with a fixed V. Finally, a precise V was adjusted with the determined hue. The value of the chosen color chart indicated the darkness of the affected skin.

Skin hydration measured by a corneometer

Skin hydration was determined using the internationally recognized Corneometer method,

which is based on capacitance. This measurement is based on the completely different dielectric constant of water and other substances, which are mostly <7. The measuring capacitor revealed changes in the capacitance that correlated with the skin hydration of the samples.

2.8 Data analysis

The product effect (PE%) was calculated as follows:

PE (%)=(Treatment Effect - Miscellaneous Effect) * 100

Treatment Effect (TE)=Test group @ Time point/ Test group @ Baseline; Miscellaneous Effect (ME)=Control group @ Time point/Control group @ Baseline

2.9 Statistical analysis

Statistical analysis was performed using a 2-tailed, paired t-test at a 0.05 confidence level (CL).

3. Results

3.1 Skin color measured by spectrophotometer

Using Spectrophotometer, we measured L* and b* values to evaluate the skin color. Compared with the control group, the chloasma area of the test group did not show a significant improvement in L* value at all-time points. However, b* value of the chloasma area decreased significantly at day 20 (PE -3.25%) and day 60 (PE -3.88%) (Table 2).

3.2 Photography and image analysis for skin color

The image analysis results showed that the cheek did not exhibit significant improvement in L* value in the test group compared with the control group. In contrast, b* value of the cheek area was significantly decreased on days 20, 40 and 60 (PE: -5.16%, -2.17% and -4.36%, respectively). Because the cheek was the most common area affected by chloasma, the image results for the cheek are useful for evaluating the chloasma treatment efficacy (Table 3). The significant improvement in skin color measured by Spectrophotometer was in agreement with the significant improvement in skin color measured by imaging analysis based on b* value.

3.3 MASI grading

The MASI grading results showed that after consuming the product for 60 days, a significant decrease (PE -61.52%) occurred in the MASI value, indicating a decrease in the severity of chloasma (Table 4).

3.4 Clinical grading of skin color with skin tone color scale

The results showed a significant improvement in color tone on day 40 (PE 2.11%) and day 60 (PE 5.07%), indicating that the chloasma color lightened (Table 5).

3.5 Skin hydration measured by a corneometer

After 60 days of product use, no significant

Table 2. Skin color L* and b* analysis by Spectrophotometer measurement of test group and control group.

Spectrophotometer		Baseline	Day 20	Day 40	Day 60
b*	Test group	18.3	17.73	18.21	18.18
	Control group	17.46	17.44	17.53	18
	PE (%)	-	-3.25*	-0.94	-3.88*
L*	Test group	58.11	57.66	58.55	58.43
	Control group	56.59	57.05	57.14	57.44
	PE (%)	-	-1.64	-0.28	-1.01

"*": Compared with Control group, significant difference based on a 95% CL

Table 3. Skin color L* and b* results obtained via image analysis of test group and control group.

Image analysis (cheek)		Baseline	Day 20	Day 40	Day 60
b*	Test group	25	24.96	26.05	26.96
	Control group	24.09	25.26	25.47	27.02
	PE (%)	-	-5.16*	-2.17*	-4.36*
L*	Test group	69.47	69.17	69.76	69.98
	Control group	67.85	68.57	68.49	69.07
	PE (%)	-	-1.99	-1.01	-1.5

"*": Compared with Control group, significant difference based on a 95% CL

Table 4. The result of MASI grading using a clinical-grading method.

MASI grading		Baseline	Day 20	Day 40	Day 60
MASI	Test group	10.68	12.05	13.86	10.88
	Control group	12	15.3	15.46	16.03
	PE (%)	-	-5.34	24.58	-61.52*

"*": Compared with Control group, significant difference based on a 95% CL

Table 5. Effect of the product effect with the control at different time points based on a skin tone color scale.

Clinical grading		Baseline	Day 20	Day 40	Day 60
Skin Tone Color Scale	Test group	5.88	6.05	6.19	6.26
	Control group	5.71	5.88	5.88	5.79
	PE (%)	-	-0.36	2.11*	5.07*

"*": Compared with Control group, significant difference based on a 95% CL

Table 6. Effect of the product on hydration measured by a Corneometer compared with the control at different time points.

Corneometer.		Baseline	Day 20	Day 40	Day 60
Skin hydration	Test group	47.68	50.24	56.55	59.04
	Control group	41.81	43.22	45.27	48.43
	PE (%)	-	0.75	11.58*	7.67

"*": Compared with Control group, significant difference based on a 95% CL

improvement in the skin hydration value was observed. However, we did find an increasing trend of skin hydration from day 20 to day 60 and some positive results on day 40 (Table 6).

4. Discussion

Chloasma appears as a symmetrical, blotchy, brownish pigmentation on the face among Asians and can lead to considerable embarrassment and distress [8]. The pigmentation is the result of the overproduction of melanin from the pigment cells, melanocytes, because of sun exposure, medication, hepatic dysfunction and other causes [1-4]. The available therapies, such as chemical peels and laser and intense pulsed light treatments, are neither satisfactory nor safe [9-12]. It has been reported that some nutrients, such as vitamins A, E and C and herbal extracts, such as pycnogenol, exhibit skin-lightening effects because of their antioxidant effects [13,14]. Thus, the importance of a dietary source of photoprotection has attracted great interest. In the search to find a new intervention based on the relationship between food intake and improvement in the appearance of chloasma, the oral administration of a peptide combination was studied clinically. The combination of CP, SP and AECM was demonstrated to exert antioxidant and anti-melanogenesis activity in our previous study [11]. However, there are few clinical trials evaluating the effects of CP on the skin biophysical improvement especially on the skin hydration. Matsumoto et al. [15] demonstrated daily ingestion of CP for 6 weeks improving the skin hydration improvement efficacy on 25 Japanese women with dry and rough skin, however different with subjects in our study of whom skin hydration didn't have much improvement space. In another clinical study, Sumida et al. evaluated the effect of daily ingestion of CP for 60 days in 20 healthy Japanese women in comparison with a placebo group (n=19). The water absorption ability of the stratum corneum improved in the test group, but this improvement was not statistically significant [16]. Clinical trials performed in human subjects were critical to determine the efficacy profile of the oral administration of chloasma treatment. The objective assessment of the extent and severity of skin pigmentation disorders benefited from a series of non-invasive methods [17-20]. In this study, we used four complementary methods. The subjective assessment of chloasma was achieved using MASI and clinical grading. The specific pigmentation-dependent color was determined by instrument-based measurements and imaging analysis. Participants were instructed to ingest the peptide combination for 60 days and the chloasma improvement effect was evaluated at 20, 40 and 60 days. No side effects were observed at any time. Our results showed that the Spectrophotometer results of the test group revealed a significant decrease in b* value on day 60 (↓3.88%) compared with the control group. Image analysis of skin color of the cheek area also exhibited significant decreases in b* value on day 20 (↓5.16%), day 40 (↓2.17%) and day 60 (↓4.36%) compared with the control group, which showed less dramatic improvement. Additionally, after consuming the product for 60 days, there was significant decrease in the MASI value, indicating an improvement in the severity of the chloasma. The skin color grading results showed significant improvements in color tone in the test group on day 40 (↑2.11%) and day 60 (↑5.07%) compared with the control group, which exhibited less improvement. After consuming the combination, skin hydration showed a statistically significant improvement on day 40 (↑11.58%), but only increasing trend on day 20 and 60. However, further studies are required to verify not only the significance of the difference relative to the control group, but also the skin hydration measurement method selection. Tagami [21] reported that Corneometer measurement is less sensitive to evaluate the hydrated skin surface but they are more sensitive for the evaluation of dry skin conditions as noted in various skin diseases.

5. Conclusion

In conclusion, after consuming the peptide combination for 20, 40 and 60 days, chloasma improved greatly based on the obvious decrease in b* value of the pigmentation area. The significant improvement in skin color measured by Spectrophotometer and image analysis was in agreement with the improvement in

the MASI and clinical grade based on a Skin Tone Color Scale. The results were particularly clear after 60 days. Additionally, based on the increasing trend of skin hydration, the skin barrier might be repaired. This effect might result from the patient metabolizing the absorbed product. The data, which were measured using several methods, confirmed that the peptide combination was effective in the treatment of chloasma. The work described here indicates that this novel therapy consisting of long-term oral administration of a moderate dose of a peptide combination is safe and effective in the treatment of chloasma and that the underlying mechanism might be related to its effects in improving the patients' antioxidant function.

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