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Instrumental Evaluation of the Depigmenting Efficacy of an Oral Supplementation Containing Peptides and Chrysanthemum Extract for the Treatment of Melasma

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Abstract: The aim of this study was to assess the efficacy of an oral supplement (CP) containing collagen peptide, soy peptide, and chrysanthemum extract in Chinese female adult volunteers with melasma. The approval of the Institutional Ethics Committee of the third affiliated hospital, Sun-Yat Sen University, was obtained before the study. A signed consent was obtained from each volunteer prior to study to enable the volunteer to appreciate the aim of the study and the consequences of her consent. Sixty-two female volunteers aged 30–60 years were included in the study, and were randomized into a treatment group or a placebo group. The skin tone of the pigmented spots was evaluated using Chromameter, and pigment density was evaluated using Mexameter before and after the treatment. Significant changes in skin tone parameters of L value and ITA° (individual typology angle) were detected in the lesion area after the treatment ($P < 0.01$). When compared with placebo group, the treatment group achieved significant improvement in the brightness of the pigmented spots at the 45 and 60-day time points. A significant decrease in the level of melanin was observed in the treatment group when compared with the placebo group ($p < 0.01$). All data demonstrated through non-invasive in vivo instrumental measurement that daily oral intake of CP had clinical efficacy of reducing melasma severity.

Keywords: melasma; oral intake; clinical efficacy; spectrophotometric evaluation

1. Introduction

Melasma is a common symmetrical hypermelanosis characterized by irregular light-to-dark brown patches of hyperpigmentation in sun-exposed areas, predominantly on the face [1,2]. Sun exposure is known to affect melasma severity significantly. The condition worsens in patients with intense sun exposure [3]. Therapy remains a challenge for melasma, and topical treatments are the mainstay but may include allergic and contact dermatitis, depigmentation of surrounding normal skin, and postinflammatory hyperpigmentation [4]. Thus, the oral intake of antioxidants such as Vitamin C, E and grape seed extract have recently attracted much attention in the treatment of melasma, with expectation to prevent UV-induced melanogenesis and/or to reduce hyperpigmentation [5–8].

Soy peptide and collagen peptide have been used as important active components in medicinal and food industries because of their excellent bioactivity, good biocompatibility, and penetrability [9]. Our previous study confirmed that soy peptide had synergistic antioxidant activities with collagen

peptide [10]. The mixture of soy peptide and collagen peptide showed good antioxidant activity in the senescent mouse model [5]. *Chrysanthemum morifolium* extract has been found to contain flavonoids and showed various bioactivities [11]. Our previous study showed that *Chrysanthemum morifolium* extract enhanced the antioxidant and anti-melanogenic efficacy of peptide mixture of collagen peptide and soy peptide in UV-irradiative mouse model [10]. Therefore, we optimized the ratio of soy peptide, collagen peptide and chrysanthemum extract to design a novel oral supplement (CP) to treat melasma.

The purpose of this study was to clinically evaluate the safety and efficacy of CP as a healthy food supplement in the treatment of facial melasma. We assessed the clinical response objectively by evaluating the skin color using the instrumental methods. A patient quality of life assessment was carried out at baseline and during follow-up time points, and no adverse events were reported.

2. Materials and Methods

2.1. Subjects

This was a double-blinded and placebo-controlled clinical trial performed between April 2014 and July 2014 in Guangzhou. Sixty-two healthy female volunteers between 20 and 60 years of age, who were clinically diagnosed with melasma by dermatologists, were voluntarily enrolled in the study. The study was performed in the third affiliated hospital, Sun-Yat Sen University in accordance with Good Clinical Practices, and in compliance with local regulatory requirements. The appropriate national authorities and local institutional review boards approved the protocol before study commencement with IRB number GDIRB[2014]8-2. All of the volunteers provided written informed consent before admission to the study. Volunteers were excluded from the study if they had any of the following conditions: alcoholic, smoker, pregnant, lactating, under medication (e.g., corticotherapy, aspirin), experiencing regular diarrhea, allergic to test product, having a history of dermatological conditions, receiving dermatological procedures, attending another clinical study in the past three months, or consuming oral products that would have effects on skin.

2.2. Test Product

The test product used in this study was a supplement composed of collagen peptide derived from a special hydrolysis of fish skin collagen, soy peptide derived from soy protein, and the aqueous extract of *Flos Chrysanthemi Alba* from Hangzhou of China. The product was provided by Amway (China) R&D center (Shanghai, China), commercially available under the name Xiyan®.

2.3. Study Design

Volunteers were instructed to take 10 g of CP per day along with the breakfast or lunch for 60 days. Each volunteer underwent a dermatological examination with Wood's light to detect facial hyperpigmentation at the study baseline (D0), and at 30 (D30), 45 (D45), and 60 (D60) days during the course of study. Skin colorimetric measurements and melanin detection were taken at each time point. For enhancing the compliance of the subjects, mobile phone text message was used before each hospital visit.

2.4. Reflectance Spectrophotometric Evaluation

A tristumulus colorimeter (Chromameter® from Minolta) was used to measure color parameters of L*a*b* in CIELAB color space. Measurements were made on the melasma lesion site as well as on an adjacent normal- skin site [12]. The L*a*b* color space is a comprehensible system, which includes all perceivable colors, with the L* parameter expressing color brightness (varying between a value of 100 for a white surface and 0 for a black surface), the a* parameter representing colors along a red-green axis, and the b* parameter indicating colors along a yellow-blue axis. The average values of the three chromametric parameters, as well as the individual typological angle or ITA° [ITA°= {arctangent (L* – 50)/b*}180/π] were used to describe skin color changes during the course of study. The ITA°

characterizes the lightness of skin pigmentation (innate or acquired) with an ITA° > 55° corresponding to a very fair skin, and an ITA° < 10° a very dark one.

The melanin contents were evaluated using a Mexameter® in order to calculate and compare erythema and melanin numeric scores recorded at the D0, D30, D45, and D60. A melanin contrast index was calculated to present the difference between lesion area and control area as follows.

$$\text{Melanin Contrast Index} = (\text{Melanin Value}_{Controlsite} - \text{Melanin Value}_{Lesion})/\text{Melanin Value}_{Controlsite}$$

2.5. Statistical Analysis

All of the results are expressed as mean ± standard deviation (SD). For each parameter, the results obtained at each time point after treatment (30, 45 and 60 days) were compared to those obtained at the study baseline using Student's *t*-test with a two-tailed significance level of 5%. For the inter-group comparison, the results were analyzed using a two-way ANOVA test. The analysis was performed by using the statistical package in Microsoft Excel.

3. Results

Table 1 summarizes the response rate of the colorimetric parameters L*, a*, b*, and ITA° between the baseline (D0) and the final visit (D60). Among the 31 subjects who completed the study in the treatment group, 30 subjects finished with lighter skin (increased L* and ITA°), only 1 subject with darker lesion area skin to inclusion.

Table 1. Response rate of L*, a*, b* parameters and ITA° between the baseline (D0) and the final visit (D60). Percentage of volunteers (n = 31) presenting a positive, stationary or negative evolution between the baseline and the final visit of the study (delta D60–D0) for the 3 chromametric coordinates: L* (from dark to pale), a* (green to red) and b* (blue to yellow) as well as for the individual typological angle or ITA°.

Group	Parameter	Percentage of Patients (Increase, %)	Percentage of Patients (Decrease, %)
Treatment group (n = 31)	L*	96.77	3.23
	a*	48.39	51.61
	b*	67.74	32.26
	ITA°	96.77	3.23
Placebo group (n = 31)	L*	61.29	38.71
	a*	41.94	58.06
	b*	83.87	16.13
	ITA°	51.61	48.39

Differences of color parameters measured between baseline and the day-30, 45, or 60 time point were calculated to compare treatment efficacy within and between the study groups. Average changes in L*a*b* and ITA° observed in the 62 volunteers are shown in Table 2. Statistically significant changes ($p < 0.05$) of parameters L* and ITA° were noted for the treatment group during the study, indicating a lighter facial skin tone when compared to the study baseline. However, there was no significant differences of parameters L* and ITA° observed for placebo group ($p > 0.05$).

Colorimetric analysis showed that hyperpigmentation reduction of the spot pigmentation area at D45 and D60 as compared to D0 in the treatment group had significant differences when comparing with the placebo group in the average L* and ITA° values (Figure 1, Supplementary Figure S1).

These results were confirmed by the data recorded using a Mexameter. The melanin contrast index in the lesion area in the treatment group decreased significantly when comparing with the index in the placebo group after 45 or 60 days of study (Figure 2).

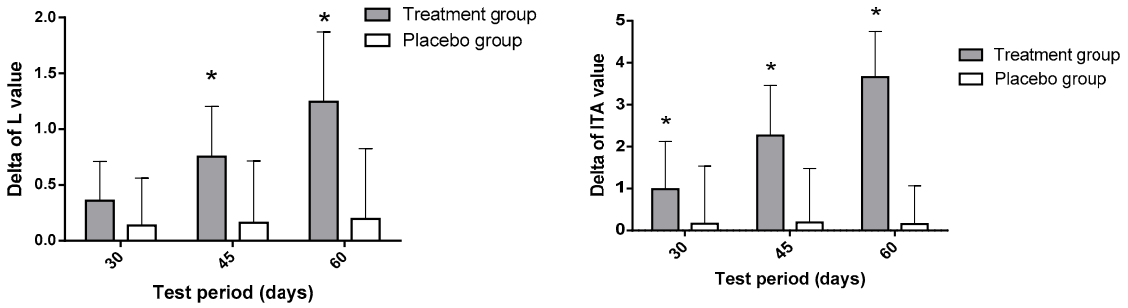

Figure 1. Average L and ITA value changes in the lesion area in both of product and control group.

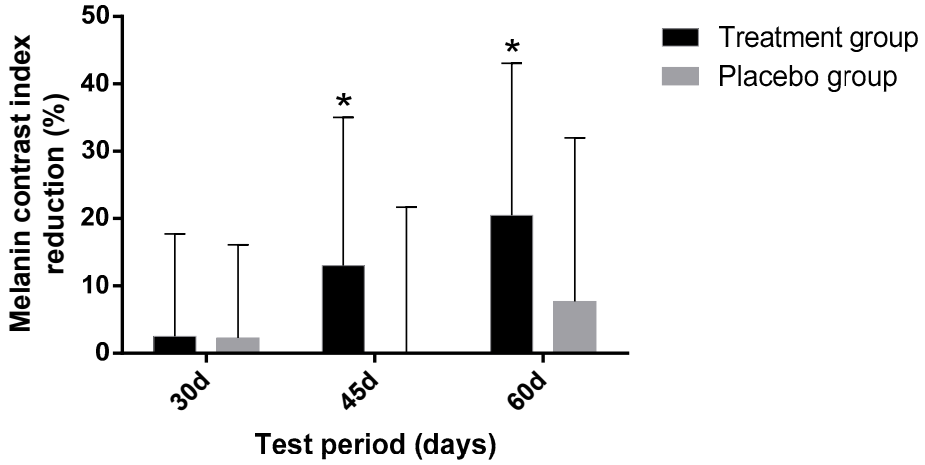

Figure 2. Reduction in Melanin contrast index in the lesion area in both of product and control group (%).

The area of melasma decreased after CP or placebo intake, but no statistical significance was observed between these groups at all time points (Figure 3).

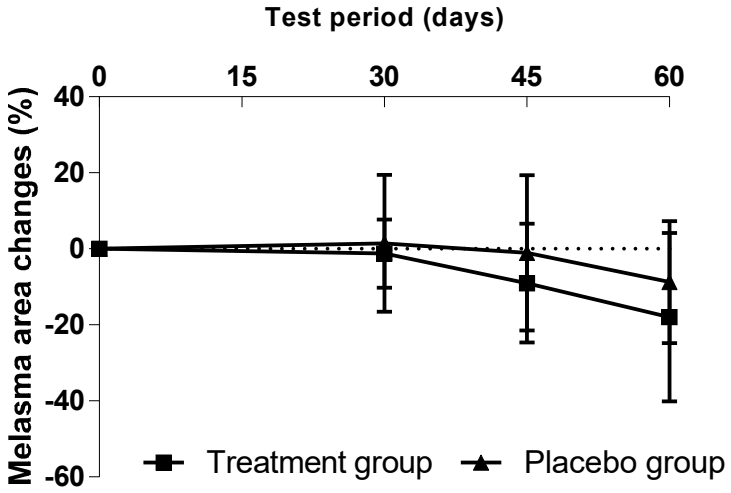

Figure 3. The melasma area changes in product and control group (%).

No CP intake-related adverse side effects were observed on the skin of all the candidates.

Table 2. Average differences in colorimetric parameters L*, a*, b*, and ITA° between baseline (D0) and the 30, 45, and 60 days visits in the lesion area.

Group	Evaluation Time (n = 31)	Parameter	Variation (Delta)	p-Value
Treatment group (n = 31)	30 days (D30–D0)	L*	0.359	0.001
		a*	0.153	0.461
		b*	0.388	0.009
		ITA°	0.986	0.000
	45 days (D45–D0)	L*	0.754	0.000
		a*	0.184	0.328
		b*	0.267	0.144
		ITA°	2.265	0.000
	60 days (D60–D0)	L*	1.246	0.000
		a*	−0.020	0.914
		b*	0.413	0.075
		ITA°	3.660	0.000
Placebo group (n = 31)	30 days (D30–D0)	L*	0.138	0.081
		a*	−0.098	0.618
		b*	0.467	0.002
		ITA°	0.161	0.518
	45 days (D45–D0)	L*	0.163	0.112
		a*	−0.012	0.956
		b*	0.538	0.000
		ITA°	0.188	0.554
	60 days (D60–D0)	L*	0.196	0.093
		a*	−0.198	0.281
		b*	0.736	0.000
		ITA°	0.154	0.662

4. Discussion

Melasma is the most common pigmentary disorder among Asians. It has been reported in 50–70% of pregnant women and in non-pregnant women who are taking birth control pills [5]. Sun exposure together with the endocrine disorders, genetic factors, medications, nutritional deficiency, and hepatic dysfunction are risk factors for melisma [6]. Recently, increasing effort has been devoted to reveal the relationship between food intake and skin condition, which led to the modern concept of "skin care from within" [5,13]. It has been reported that some nutrients, such as vitamin A, E, C, as well as the herbal extracts, such as pycnogenol, orange extract and grape seeds extract exhibited skin lightening effects due to their antioxidant effects [5,6,14]. In this respect, the importance of the dietary source for photoprotection has attracted a great interest.

As important raw materials, soy peptide and collagen peptide are commonly used in the medicine and food industries. It has been reported that the benefits to skin increased markedly when taking soy peptide and collagen peptide together in comparison to the intake of collagen peptide alone [15]. The collagen hydrolysate rich in oligopeptide possesses good in vitro free radical scavenging/antioxidant activity and tyrosinase inhibitory activities [16,17]. Dietary supplementation with collagen peptide was able to counteract human oxidative stress by increasing the serum status of GSH-Px and decreasing the serum MDA level [9]. *C. morifolium* extract was characterized by a high content of antioxidant natural compounds such as flavonoids and anthocyanin [18]. *C. morifolium* extract has been shown to have several biological applications, and the antioxidant and tyrosinase inhibitory effects are already well known in literature.

In this study, we investigated if the oral intake of CP supplement, a mixture containing soy peptide, collagen peptide, and chrysanthemum extract, could reduce facial hyperpigmentation of Chinese women with melasma in a 60-day study. The previous in vivo test models have proven that CP supplement intake can counteract skin hyperpigmentation through the inhibition of melanin overproduction caused by repeated UV exposure [10]. To this purpose, the increase of melanin content in lesion area has been monitored by objective instrumental method (reflectance spectrophotometry) in subjects with melasma before and after oral supplementation of 45 or 60 days. Results showed that significant variation of melanin content between lesion area and spotless skin sites, used as control,

was observed after supplementation with CP. In addition, CP improved the ITA° value, L* value, and size measurements at the different visit days of the study, as compared with those at the start. No adverse side effects related to CP intake were observed on the skin of all the candidates throughout the study period. Taken together, these results indicated that daily supplementation with CP successfully and safely improved facial melasma hyperpigmentation.

Melasmas were aggravated in a few women during the study period, especially in the placebo group. These women reported an exacerbation of melasma following the high intensity of sun exposure. It was strongly suggested that the aggravation of melasmas was due to the sun exposure. A sunscreen should be used to protect against ultraviolet impacts during the study period. UV radiation is an important causative factor in melasma, inducing ROS formation and melanin pigment biosynthesis in the skin [19,20]. Our previous study demonstrated that the CP exerted photoprotective and antioxidant activity both in UV-induced mice model and in vitro study [10]. The number and size of DOPA-positive melanocytes were found to be significantly decreased in the group treated with CP against UBV-induced skin response, suggesting CP inhibited UVB-induced pigmentation because of its antioxidant and tyrosinase inhibitory effects [10]. In this study, oral intake of CP reduced melasma severity, which was consistent with our previous results in mice.

Regarding to the measurement of melasma, clinical trials performed in human subjects were critical to determine the efficacy profile of the oral administration. The objective assessment of the extent and severity of skin pigmentation disorders benefited from a series of non-invasive methods [21,22]. In this study, we used instrumental non-invasive methods to assess the melasma changes after treatment. The specific pigmentation-dependent color was measured by using the skin reflectance instruments of Chromameter and Mexameter. Nowadays, research interest focuses on the use of non-invasive clinical tools for the characterization and monitoring of skin diseases. Skin reflectance instruments have already been demonstrated to be useful for the in vivo non-invasive visualization of pigmentary skin disorders, and give the prospective possibility of application in nutricosmetic application for therapeutic follow-up and management in melasma patients [6,23].

5. Conclusions

Taken together, oral intake of CP effectively reduced the hyperpigmentation of women with melasma. CP supplement can be considered as a safe and useful nutricosmetic product for reducing melasma severity.

Supplementary Materials: The following are available online at www.mdpi.com/2079-9284/4/4/42/s1, Figure S1: Before- and after-clinical photographs of two subjects.

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Author Contributions: M.G., Y.C. and R.L. designed the study. M.G. and Y.C. performed the experiments. M.G., J.K., D.Q., Y.L. and J.D. prepared the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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