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Original Research Article

Skin Depigmentation Activity of *Crocus sativus* Extract Cream

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Abstract

Purpose: *To determine the antioxidant activity of Crucus sativus extract and its effect on human skin using a non-invasive probe mexameter.*
Methods: *The antioxidant activity of C. sativus extract was determined using DPPH method. Water in oil (w/o) topical cream of C. sativus extract (3 %) was formulated and compared with the base (cream without extract). Both creams (formulation and base) were applied to the cheeks of 10 healthy human volunteers for a period of 8 weeks. Melanin and erythema values of skin were measured with a mexameter.*
Results: *The antioxidant activity of the extract was 81 %. Change in the levels of skin melanin and erythema was -24.04 ± 3.23 and -13.57 ± 2.28, respectively, indicating that unlike the base, the formulation containing Crocus sativus extract produced significant ($p \leq 0.05$) depigmentation and anti erythemic effect on human skin.*
Conclusion: *Application of the formulation containing 3 % C. sativus extract to human skin may be useful in the management of melanoma. However, further studies are still required to ascertain this.*

Keywords: *Antioxidant, Crocus sativus, Cream, Skin, Melanin, Erythema, Depigmentation*

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INTRODUCTION

Water-in-oil (W/O) emulsions offer a series of significant benefits compared to traditional oil-in-water (O/W) emulsions. By forming an occlusive layer on the skin, they are efficient in reducing water loss from the skin by evaporation. For this reason they are widely used in formulations targeting the needs of consumers with dry skin. Their excellent water repellency makes them a very attractive formulation basis for sun care and color cosmetics formulations. Despite these attractive benefits, the use of w/o emulsions has been limited due to issues associated with stability and a heavy skin feel resulting from high oil content [1]. Nowadays, water in oil emulsions are employed more widely in the treatment of dry skin and for emollient application.

Emulsions of plant extracts containing antioxidants have high potential as topical applications in the management of certain skin disorders, hence antioxidants of many plants are already being incorporated into heterogeneous system to achieve desirable cosmetic effects [2]. *Crocus sativus* is a perennial low growing bulbous plant globular corm which produces 6 to 9 sessile leaves surrounded in lower part by 4 to

5 broad membranous scales. Many chemical substances are present in saffron as carotenoids, flavonoids, carbohydrates, mucilage, monoterpenoids and 31 compounds have been isolated from the petals of saffron [3]. The antioxidant activity of *Crocus sativus* is due to the presence of carotenoids, phenolics and flavonoids [4,5].

The use of cosmetics can be safe if, no kind of contact dermatitis is seen after their application to skin. Environmental conditions such as changes in temperature and humidity, and consumer misuse are also vital factors in the effective handling of cosmetic products. Saffron also produces antiaging effects by decreasing melanogenesis by inhibiting the activity of tyrosinase [6]. Monoterpenoids, quercetin, kaempferol, and other phenolic components of saffron are responsible for this inhibition of melanogenesis.

EXPERIMENTAL

Materials

Dried stigmas of *Crocus sativus linnus* were used as plant material. The plant part was collected in the month of February from Kashmir. The identification was done by Saeed Raza Peerzada, Assistant Professor, at Cholistan Institute of Desert Studies at The Islamia University of Bahawalpur, Bahawalpur Pakistan; voucher no. 233 was assigned to it and kept in the herbarium, of the Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, under voucher number 169.

For the preparation of emulsion, the extracts of dried stigma of *Crocus sativus* were used. ABIL-EM90 (emulsifying agent) was purchased from Franken Chemical (Germany). Paraffin oil and ethanol were purchased from Merk KgaA Darmstadt, Germany. Distilled water was taken from the Department of Pharmacy, IUB, Pakistan.

Preparation of *Crocus sativus* dried stigma extract

Extract of 5 g dried stigmas of *Crocus sativus* was prepared by maceration. First, it was powdered and then was introduced into beaker contained 70 % ethanol and 30 % distilled water to make the final volume 500 mL. The glass beaker was sealed with aluminium foil and kept at room temperature in dark for fourteen days. Maceration was done in the dark to prevent possible photodegradation. The beaker was shaken for 10 min after every 24 h. Then filtration of macerated extract was done through several layers of muslin cloth. The filtrate initially obtained was then passed through filter paper (Whatman no. 1) for final filtration to remove fine undissolved particles. Evaporation of filtrate was done at 40 ^{o}C under vacuum using a rotary evaporator. The evaporation process was continued till concentrate reduced to one third of the total volume used. The reddish coloured extract so obtained was collected in glass beaker and was sealed with aluminium foil and stored in a refrigerator at 8 ^{o}C.

Preparation of water-in-oil emulsion

Emulsion (w/o) of the extract was made by the addition of aqueous phase to oily phase with continuous agitation. The oily phase used in the preparation was paraffin oil and surfactant (ABIL®EM 90) was heated up to 75 ± 1 ^{o}C, while the aqueous phase which was distilled water was heated to the same temperature. After heating, aqueous phase was added to the oily phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for 15 min until all the aqueous phase was added; 2 to 3 drops of rose water (fragrance) were added during this stirring time to give good fragrance to the emulsion. After the complete addition of the aqueous phase, the speed of the mixer was reduced steadily over the next 15 to 20 min while cooling the emulsion to room temperature to achieve complete homogenization.

Preparation of emulsions

The oily phase, paraffin oil and surfactant (ABIL®EM 90), was heated up to 75 ± 1 ^{o}C. At the same time, the aqueous phase (water) was heated to the same temperature and then the extract added to it. The aqueous phase was added to the oil phase drop by drop while stirring at 2000 rpm with a mechanical mixer for 15 min until the aqueous phase was completely added, 2 to 3 drops of rose water were added while stirring to give the formulation a good fragrance.

After complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization, for another period of 5 min, thereafter 500 rpm for 5 min for complete homogenization; the emulsion was then allowed cool down to room temperature [7].

Determination of antioxidant activity

The constant 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity. A range of concentrations of

Table 1: Composition of emulsion (cream) formulations

Emulsion phase	Ingredient	Emulsion base (g)	Extract emulsion (g)
Oily	Paraffin oil	14	14
	ABIL®EM 90	2.5	2.5
Aqueous	Extract	-	3
	Distilled Water (q.s)	100	100

plant extract in relevant solvent was added at an equal volume from 10 to 90 µL of ethanolic DPPH (100 µM) to a total volume of 100 µL in 96-well plates. The contents were mixed and incubated for 30 min. The absorbance was measured at 517 nm. Vitamin C was used as the standard antioxidant. The experiments were carried out in triplicates. The decrease in absorbance means the increase in radical scavenging activity which was calculated using equation 1.

$$SA\ (\%) = \{100-(A_t/A_c)\}100 \quad \text{.....(1)} \quad [8]$$

where SA is scavenging activity, A_t is the absence of test compounds and A_c is the absence of control.

Depigmentation and antierythemic study

Ten healthy human volunteers (all males) were chosen whose ages were in between 25 and 40 years. They were examined for any serious skin disease especially on cheeks and forearms. Each volunteer was provided with a volunteer protocol before the study, and signed the terms and conditions of the testing individually. They were not informed about the contents of the formulations. Before application of formulation, a patch test was performed on forearms of the volunteers for 48 h to rule out allergic reaction to any of the contents of the formulation. Each volunteer on the second day was provided with the formulation and the volunteers instructed properly on the application of formulation. Each individual was instructed to return for measurements of skin sebum production over a period of 8 weeks.

Ethical approval

This study was approved by the Board of Advanced Study and Research (BASR), The Islamia University of Bahawalpur, and the institutional ethical committee, Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, in compliance with NIH Principles of Laboratory Animal Care, 1985. The ethical committee gave a letter of recommendation for using the humans in this study, whose reference no. is Pharm 1713.

Burchard (Patch) test

Patch test was done on the forearm of each volunteer on the first day of skin testing. The bandage disc (Patch) was saturated with 1 g of base and 1 g of formulation containing Crocus sativus extracts and applied on the left and right forearm of the volunteer, respectively. The patch covered the area of 5 × 4 cm on the forearm and after the application of base and formulation it was then enclosed with the help of surgical dressing. After 48 h the patch was removed and the forearm was washed with normal saline solution after which erythema (redness of skin) was determined by using score on a scale with 4 points from 0-3, where 0 is for absence of erythema, 1 for mild erythema, 2 for moderate erythema and 3 for severe erythema.

Panel test

A proforma sheet was given to every volunteer to test the sensory values of cream. Seven parameters; ease of application, spreadability, sense just after application, sense in long turn, irritation, shine on skin and sense of softness were noted in this form for evaluation. Each parameter was assigned 11 values from –5 to +5, indicating very bad to very good. Each volunteer was requested to complete this form after week 8. Percent change in parameter values was computed weekly for each volunteer, as in eq 2.

$$C\ (\%) = \{(A - B)/B\}100 \quad \text{.....} \quad (2)$$

where C is the change, A is the individual value of any parameter (from 1st to 8th week) and B is the zero hour value of that parameter

Statistical analysis

The measured values obtained for skin melanin production and skin erythema (irritation) were analyzed using SPSS 15.0. Paired sample t-test was used for the variation between two preparations and two-way ANOVA for variation between different time intervals; $p < 0.05$ was set as the level of significance.

RESULTS

Antioxidant activity of extract

Antioxidant activity of extract with different solvent was determined. The antioxidant activity of extract was 81 % using 70 % ethanol. The result of the patch test is shown in Table 1. No severe erythema was shown by any volunteer, mild erythema shown by 1 and 2 volunteer, moderate erythema was shown by 2 and 3 volunteers and no erythema was shown by 14 and 12 volunteer for both base and formulation, respectively.

Skin melanin content

During the *in vivo* study for 8 weeks, changes in melanin by the base and formulation were observed. However, changes in melanin were not prominent but extract cream enormously decreased the melanin of skin in 8th weeks. Statistical analysis showed change in melanin contents of skin by formulation was highly significant ($p < 0.05$) except in 8th week.

Skin erythema level

There were irregular effects of base on erythema level while formulation decreased the erythema level steadily in the period of 8 weeks. It was concluded from results of ANOVA test base showed insignificant effects with respect to time. While effects of formulation on skin erythema were significant with respect to time. A significant variation was shown with base and formulation on 4th and 6th weeks, when compared with paired sample t-test. Erythema contents were decreased by the formulation due to antioxidant and anti-inflammatory effects of Crocus sativus, exhibited by mainly Crocin, β-carotene and safranal [10]. Antioxidants of *Crocus sativus* inhibit the expression of markers of inflammation, tumor necrosis factor (TNF) and interleukin (IL) [11].

DISCUSSION

The patch test of cosmetic preparation is used to check the compatibility of formulation with the human skin before its application. In this study no skin irritation was observed after the application of formulation on the cheeks of human volunteers. So this formulation was considered compatible and relatively safe for human use. Another important parameter which must be observed during the formulation of cosmetic product is that the formulation must have aesthetic appeal in all aspects of sensory evaluation. For this purpose panel test was performed. Panel test was done by both base and formulation. The calculations of panel test results showed that the formulation was more aesthetic in all sensory evaluations than the base.

The color of skin and protection form UV radiation is dependent on a pigment known as melanin present in dermis of skin. Pigmentation of skin occurred mainly due to increased melanogensis by melanocytes stimulated by exposure to UV light [12].

Melanocytes produced melanin in skin as a mixture of two pigments eumelanin and phaeomelanin which are (brown black) and (red yellow) respectively [13]. Melanogenesis is accomplished by a series of oxidative reactions controlled by various enzymes. Tyrosinase is the main catalyst for this phenomenon [14].

During the *in vivo* study of two months, change in melanin was observed by base and formulation. Change in melanin was not prominent in the base but formulation enormously decreased the melanin of skin in 8th weeks. The results of applied statistics showed the insignificant results of change in melanin by base and significant results of formulation. Insignificant effects were shown when base and formulation were tested with paired sample t-test except in the 8th week,

Table 2: Volunteer's self-assessment of irritation/itching after the application of base and formulation (**Note:** the values indicate number of volunteers)

Cream type	Absence of erythema	Mild erythema	Moderate erythema	Severe erythema
Cream base	7	2	1	0
Extract cream	8	1	1	0

Table 3: Change (%) in skin melanin content after application of base and formulation (mean ± SEM)

Cream type	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
Cream base	-0.30 ± 0.86	-0.39 ± 1.03	-0.25 ± 0.89	1.49 ± 0.67	2.46 ± 0.80	0.97 ± 1.36
Extract cream	-3.047 ± 0.81	-5.61 ± 1.29	-10.48 ± 1.75	-14.93 ± 2.63	-19.36 ± 2.40	-24.04 ± 3.23

Table 4: Percent (%) change in values of skin erythema after application of base and formulation. Values of skin erythema (mean ± SEM)

Cream type	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
Cream base	6.07 ± 6.05	6.80 ± 6.26	6.01 ± 6.37	6.96 ± 6.86	9.73 ± 8.93	5.35 ± 6.55
Extract cream	-2.56 ± 1.26	-4.78 ± 1.22	-5.89 ± 1.30	-8.76 ± 1.24	-11.76 ± 1.11	-13.57 ± 2.28

The results of formulation were significant due to presence of strong antioxidants in it. Antioxidant activity is mainly exhibited by monoterpenoids, crocin, quercetin, kaempferol, and by other phenolic components of *Crocus sativus*. The mode of action of these compounds to reduce skin melanin is by inhibiting the activity of tyrosinase [15].

Cosmetics are safe if, no kind of contact dermatitis is seen after their application to skin. Contact dermatitis not only causes but is an active part of cosmetics. Environmental conditions, temperature humidity and consumer misuse are also vital factors for contact dermatitis. Skin is irritated by chemicals due to their toxic effects to skin cells and blood vessels. During this study of two months, changes in erythema by base and formulation were observed. It was found to be irregular effects of base on erythema level. On the other hand, formulation decreased the erythema level regularly in the period of two months. Insignificant effects with respect to time, while effects of formulation on erythema were significant with respect to time. A significant variation was shown with base and formulation on 4th and 6th weeks, when compared with paired sample t-test. The formulation brought about a significant decrease in erythema. Therefore, formulation would safe to skin and is unlikely to cause any significant irritation to skin. The decrease in erythema by the formulation is likely due to the antioxidant and anti-inflammatory effects of compounds in *Crocus sativus*, mainly Crocin, picrocrocin, β- carotene and safranal. Antioxidants of saffron inhibit the expression of markers of inflammation tumor necrosis factor (TNF) and interleukin (IL).

CONCLUSION

A cream formulation containing *Crocus sativus* extract has significant depigmentation and anti-erythema effects on human skin due apparently to reduced melanin and erythema levels in the skin. *Crocus sativus* exhibits skin depigmentation effect due probably to the presence of strong antioxidants in the plant.

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