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Full Length Research Paper

Evaluation for antierythmic and depigmenting effects of a newly formulated emulsion containing basil extract

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This study was designed to find out the effects of newly formulated topical cream (w/o emulsion) of basil (*Ocimum basilicum*) against its base as control on skin erythma and skin melanin. Concentrated basil extract was entrapped in the inner aqueous phase of w/o emulsion. Newly formulated and formerly evaluated base (containing no extract) and a formulation (containing 3% concentrated extract of basil) were applied. Both the base and formulation were applied to the cheeks of 11 healthy human volunteers for a period of 12 weeks. Skin erythma and melanin were determined every two weeks to measure any effect produced by these topical creams. The base showed insignificant ($p \geq 0.05$) whereas the formulation showed statistically significant decrease in skin erythma. Skin melanin content was significantly ($p \leq 0.05$) increased by the base but decreased by the formulation. The newly formulated cream of basil extract reduced skin melanin without causing any irritation and was found to be suitable for application on skin as determined by efficacy perception.

Key words: *Ocimum basilicum*, water-in-oil (w/o) emulsion, mexameter, skin whitening, anti erythmic.

INTRODUCTION

There has been a mounting curiosity in the study of medicinal plants as natural products and use of plants for the treatment of skin diseases is a common practice in different parts of the world traditionally (Ramesh and Satakopan, 2010). Natural products are important sources for biologically active drugs and many plants are used in the modern phytocosmetics (Saleh et al., 2009). Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the deterrence of various degenerative diseases and have potential benefits to the society (Lukmanul et al., 2008). The medicinal value of these plants depends on bioactive phytochemical constituents that produce definite physiological action in the human body. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins (Krishnaiah et al., 2009). Hyper pigmentation is the most common facial pigmentary disorder. It has been observed that a local increase in melanin synthesis or uneven distribution of melanin can cause local hyperpigmentation or spots. Whitening agents such as hydroquinone, arbutin, kojic acid, or azelaic acid are widely used in cosmetic products as active substances (Nakayama et al., 2000; Petit and Pie´rard, 2003). Plant extracts that have a good inhibitory effect on melanin formation may be a good choice for the cosmetic purposes of whitening facial skin and protection against skin darkening. In addition, they have relatively fewer side effects (Bernard and Berthon, 2000; Baurin et al., 2002).

Ocimum basilicum usually named common basil or sweet basil is an annual plant, with extraordinary medicinal properties and contains several antioxidant compounds. In traditional medicine, basil has been used as an antiseptic, preservative, sedative, diuretic and digestive regulator. It also has been recommended for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Ramesh and Satakopan, 2010). In this study a newly formulated water-in-oil (w/o) emulsion containing extract of basil was evaluated for effects on different

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parameters related to skin.

MATERIALS AND METHODS

Abil-EM90® was purchased from Franken Chemical (Germany), ethanol was taken from BDH England, paraffin oil was purchased from Merk KGaA Darmstadt (Germany). Basil (*O. basilicum*) leaves and flowers were collected from gardens of the Islamia University of Bahawalpur, Pakistan. The identification of the plant material was performed by Prof. Dr. Muhammad Arshad at Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur and a voucher specimen was preserved (voucher # OB-LF-4-11-21) at the herbarium for future reference. Mexameter® MPA 5 (Courage + Khazaka, Germany) was used for the measurement of skin erythma and skin melanin.

Detection of phenolics and flavonoids

Test for the presence of phenolic compounds in the extract was carried out by taking 1 ml of the extract. It was heated to remove the solvent and the residue was dissolved in some quantity of aqueous methanol. 0.5% ferric chloride solution was added to this test solution and a change in colour indicated the presence of phenolic compounds. Shinoda test was performed for flavonoids, few magnesium turnings and concentrated hydrochloric acid were added to the test solution, after few minutes the colour of solution turned crimson red indicating the presence of flavonoids in the extract (Prabhu et al., 2011).

Formulation of emulsions (creams)

Oil phase comprised of paraffin oil and surfactant (ABIL®-EM 90), and aqueous phase comprising of water was heated to 75±1°C and then treated with the extract. In the case of base no extract was added in the aqueous phase. W/O emulsions (base and formulation) were prepared by adding aqueous phase to the oily phase with continuous stirring at 2000 rpm by the mechanical mixer for 15 min until the addition of aqueous phase. Then the mixer speed was reduced to 1000 rpm for 5 min, and then further reduced to 500 rpm for 5 min for complete homogenization until the emulsions cooled to room temperature. In this study the products studied were found stable after evaluating for pH, electrical conductivity, centrifugation, phase separation, temperature stability tests at 8±0.1°C (in refrigerator), 25±0.1°C, 40±0.1°C and 40±0.1°C with 75% relative humidity and physical characteristics that is, color, creaming and liquefaction.

Product evaluation on skin

A total of 11 male volunteers with mean age of 48 years were selected for the study and consent forms were taken. The volunteers were examined by a doctor for skin and other diseases. The study was designed single blinded for the comparisons of two creams. The experiments were carried out on the cheeks of volunteers as cheeks are uniformly and more prone to ultraviolet (UV) radiations. Every volunteer applied creams at night on cheeks for the period of 12 weeks and came for measurement on 2nd, 4th, 6th, 8th, 10th and 12th week in the morning at 10 a.m. They were allowed to wash their faces with water and sit to become accustomed with the environment for 30 min before any measurements were taken. Values for different parameters were taken in controlled room temperature of 25±1°C and 45±2% relative humidity.

Skin compatibility evaluation

To assess skin compatibility, patch tests were performed on the both forearms of each volunteer on the first day of skin testing. A 5 × 4 cm region was marked on the forearms. The patch (Bandage disc) for the left forearm was saturated with 1.0 g of base while the patch for right forearm was saturated with 1.0 g of formulation. Each was applied to the marked regions separately on each forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 h and the forearms were washed with physiological saline (Hachem et al., 2002).

Panel test

Every individual was provided with a questionnaire prepared previously to test the sensory values of creams. This questionnaire consisted of seven parameters to be evaluated and every parameter was assigned 11 values from -5 to +5 indicating very bad to very good, respectively. The parameters were 1, ease of application; 2, spreadability; 3, sense just after application; 4, sense in long term; 5, irritation; 6, shine on skin; 7, sense of softness.

Ethical standards

This study was approved by the Board of Advanced Studies and Research, and its Ethical Committee for *in-vivo* studies (Reference No. 3715/Acad.), The Islamia University of Bahawalpur and was conducted according to the international guidelines of Helsinki Declaration.

Mathematical analysis

The percentage changes for the individual values of different parameters of volunteers were calculated by the following formula:

Percentage change = [(A – B) / B]*100 ….. (1)

Where; A = Individual value of any parameter of 2nd, 4th, 6th, 8th, 10th or 12th week, B = Zero hour value of that parameter.

Statistical analysis

The measured values obtained for skin erythma and melanin content were analyzed using SPSS 12.0 on the personal computer (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals while using a 5% level of significance for both skin parameters.

RESULTS AND DISCUSSION

Skin erythma

Formulations intended for topical applications must not exert any irritation or redness and to assess it, patch test was performed which indicated that both base and formulation did not provoke redness. In this study, it was found that there was a regular decline in values of erythma of skin throughout the study period after the application of base samples. Same was the case with

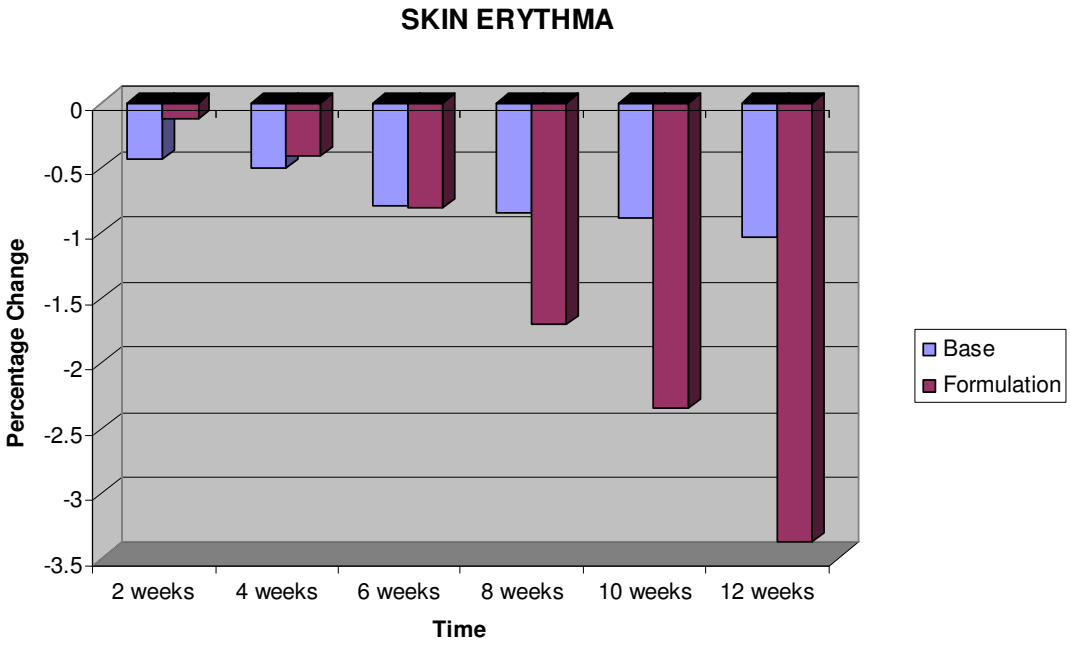

Figure 1. Percentage of changes in skin erythma after application of base and formulation.

formulation samples which also showed gradual decrease in skin erythma from beginning till the last week of study. The percentage changes determined after 2nd, 4th, 6th, 8th, 10th and 12th week and represented in Figure 1 indicate that there was more decline in erythma exhibited by the formulation.

However by applying ANOVA test it was found that the base produced insignificant ($p \geq 0.05$) effects on skin erythma with respect to time while formulation produced significant ($p \leq 0.05$) effects on skin erythma with respect to time. With the help of paired sample t-test, significant ($p \leq 0.05$) differences were observed between the skin erythma of base and the formulation from the start of study period. More decrease in erythma values as compared to the base can be due to anti-inflammatory properties exhibited by basil through inhibition of the key proinflammatory cytokines and mediators (Marwat et al., 2011). The decrease in skin erythma indicated that the formulation tend to soothe the skin.

Skin melanin

Skin melanin was determined at regular intervals of 2nd, 4th, 6th, 8th, 10th and 12th week and percentage of changes are represented in Figure 2. In this study, it was found that there was increase in skin melanin values after the application of base but in case of formulation there was gradual decrease in skin melanin content throughout the study period. With the help of ANOVA test, it was found that changes in skin melanin values produced by base and the formulation were significant ($p \geq 0.05$) with respect to time. By applying paired sample t-test it was found that there was significant ($p \geq 0.05$) variation in skin melanin with respect to base and formulation.

Melanin is the pigment responsible for the colour of skin in humans. Tyrosinase is known to be the key enzyme in melanin biosynthesis (Nerya et al., 2003). Over-activity of this enzyme leads to excessive production of melanin leading to hyper-pigmentation of the skin and under-activity leads to depigmentation. The decrease in skin melanin can be attributed to the phenolic compounds and flavanoids present in basil which include quercetin, isoquercetin, quercetin-diglycoside, kaempferol, dihydro kaempferol-glucoside, dihydroxy kaemnipferol-glycoside, caffeic acid, rosmarinic acid, rutin, carnosic acid, catechin, rutiniside, catechol derivatives (Jayasinghe et al., 2003). The tyrosinase inhibitory activity of the flavonoids might be due to chelating the active center of tyrosinase leading to reduced melanin production (Saewan et al., 2011).

Efficacy perception

The volunteers were asked to answer a questionnaire at the end of study in order to have information about their sense and perception regarding the cosmetic quality of the two formulations. Average points for the first question, that is, ease of application of creams were found to be 4.20 and 4.50 for the base and formulation, respectively indicating that base and formulation can be easily applied on the skin. Spread-ability got 4.0 for base and 4.30 for formulation which meant that the formulation spread on

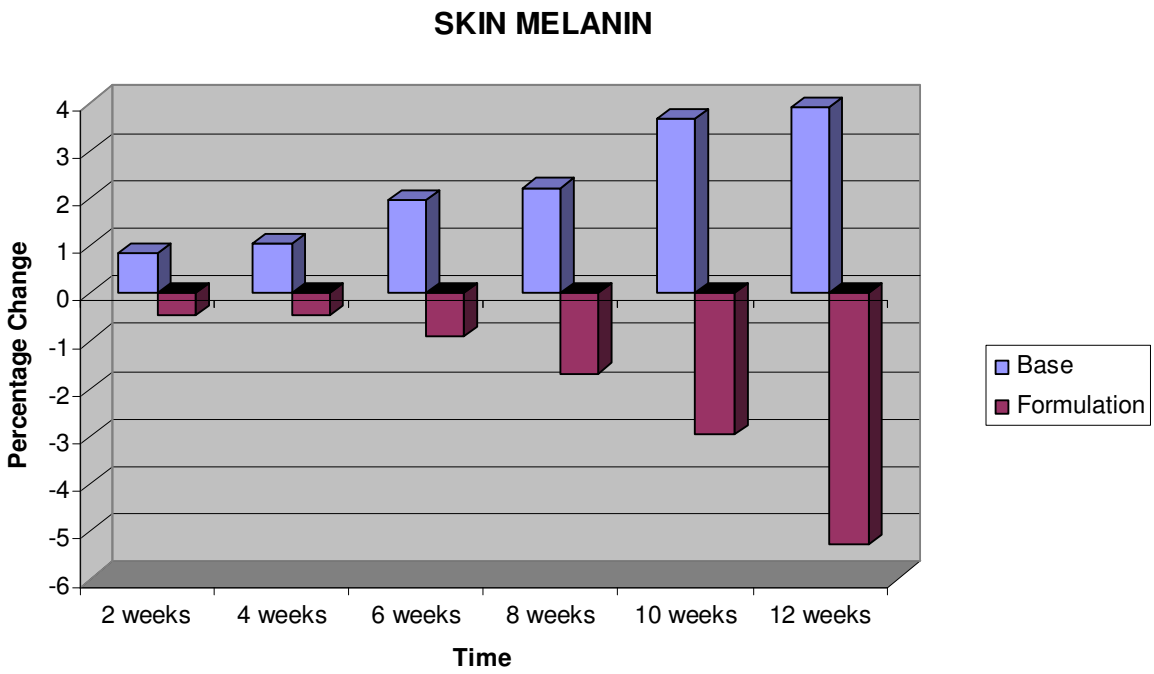

Figure 2. Percentage of changes in skin melanin after application of base and formulation.

skin better than the base. Average points for feel on application were 3.80 for the base and 3.50 for formulation which indicated that base was felt well on the skin than formulation. Average points for the sense in long-term application of creams were 3.60 and 3.70 for the base and formulation, respectively. This showed that formulation produced more pleasant feeling on application to skin than base. Irritation was assigned 0.00 point for both base and formulation. Shine on skin was 3.30 for the base and 3.20 for formulation. The formulation led to more softness of the skin than base as the average points were 4.40 for base and 4.70 for formulation. It was found from paired sample t-test that there was a non significant difference between the average points of sensitivity for base and formulation. It was concluded that there was no variation between base and formulation regarding the sensory evaluation. Both creams behaved similarly from the sensory point of view.

Conclusion

A stable topical emulsion containing basil (*O. basilicum*) exerts de-pigmenting/skin whitening properties as it significantly reduced skin melanin when applied topically. Further more reduction in skin erythma/redness showed that the formulation possess anti inflammatory effects. Sensory evaluation through a questionnaire suggested that the formulation had no harmful effects and can be applied safely. Conclusively, it can be used as cost effective topical skin whitening treatment.

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