Formulation and in vivo evaluation for anti-aging effects of an emulsion containing basil extract using non-invasive biophysical techniques

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ABSTRACT

Background and the purpose of study: Skin aging is a complex process induced by constant exposure to ultraviolet (UV) irradiation and damages human skin. UV generates reactive oxygen species leading to collagen deficiency and eventually skin wrinkling. Basil contains a number of phenolics and flavonoids which possess antioxidant properties. The aim of this study was to formulate and investigate the antiaging potential of a cream containing Basil extract.

Methods: A single blinded study was conducted using non-invasive methods. Formulation containing 3% of the concentrated extract of Basil was developed by entrapping in the inner aqueous phase of w/o emulsion and base contained no extract. Both creams were stored at different storage conditions of 8°C, 25°C, 40°C and 40°C+ 75% relative humidity to predict their stabilities. The formulation and base were evaluated for their effects on various skin parameters i.e., moisture and trans epidermal water loss (TEWL), volume, energy and surface evaluation of the living skin (SELS).

Results: Significant effects (p≤0.05) were observed for both creams in the case of TEWL. The base showed insignificant (p≤0.05) while formulation showed significant effects on skin moisture. Volume, SELS SEr (skin roughness), SEsc (skin scaliness), SEsm (skin smoothness), SEw (skin wrinkles) parameter showed significant decline while texture parameter of ‘Energy’ showed significant increase.

Conclusion: The results statistically indicated that the active formulation containing extract of Basil exert antiaging effects when applied topically.

Keywords: Ocimum basilicum, Surface evaluation of living skin (SELS), Transepidermal water loss (TEWL), w/o emulsion.

INTRODUCTION

Skin aging is a complex process induced by constant exposure to ultraviolet (UV) irradiation. UV generates reactive oxygen species leading to collagen deficiency and eventually skin wrinkling (1, 2). Aging of the skin is characterized by irregular pigmentation, increased wrinkling, loss of elasticity, dryness and roughness (3). Recently, emulsions have established growing interest as a vehicle for the drug delivery to the body. The use of natural compounds in skin protection especially topical application of antioxidants indicates that they usefully decrease skin aging (4). Basil (Ocimum basilicum) is an annual plant, with surprising medicinal properties and contains several antioxidant compounds. Basil in traditional medicine, has been used for the treatment of coughs, headaches, constipation and fever (5).

In this study a w/o emulsion containing extract of basil was formulated and its effects on different parameters related to skin aging was evaluated.

MATERIAL AND METHODS

Preparation of the Crude Extract

Basil leaves and flowers collected and identified at Cholistan Institute of Desert Studies of the Islamia University of Bahawalpur, Pakistan (voucher # OB-LF-4-11-21) were shade dried and finely grounded. Four hundreds gm of the powdered material was extracted at room temperature with 2000 ml of 95% ethanol for 48 hrs. Then the glass beaker was sealed with aluminium foil and shaken for 10 min after every 12 hrs. It was filtered through a Whatman # 01 filter paper, evaporated under reduced pressure at 40 °C in a Rotavapor (Eyela, Co. Ltd. Japan) till the concentrate was reduced to the one third of the initial volume of the solvent used and stored in freezer at 8 °C in refrigerator till further analyses.

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The presence of phenolic compounds and flavonoids was tested according to the methods in the literature (6). The change in colour indicated the presence of phenolics and flavonoids in the extract. The antioxidant activity of the extract was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) which is a stable free radical, and vitamin C as standard (7). The antioxidant activity of basil extract was found to be 88% in comparison to the standard.

**Preparation of Creams (Emulsions)**

Different formulations containing different concentrations of Parrafin oil (Merck, Germany), Abil EM 90 (Franken Chemicals Germany) and distilled water were prepared in this study. Oil phase comprised of paraffin oil and surfactant (ABIL EM 90) heated up to 75°C±1°C. Aqueous phase comprising of water was heated to the same temperature and then treated with the extract. In the case of base no extract was added to the aqueous phase. W/O emulsions were prepared by addition of aqueous to the oily phases with continuous stirring first at 2000 rpm by the mechanical mixer (Euro-Star, IKA D 230, Germany) for 15 min then 1000 rpm for 5 min, and thereafter 500 rpm for 5 minutes for complete homogenization by the mechanical mixer (Euro-Star, IKA D 230, Germany) and finally the emulsion was cooled to room temperature. Creams were divided into four parts and stability tests were performed at 8°C±0.1°C in refrigerator (Dawlance, Pakistan) and at 25°C±1°C, 40°C±1°C and 40°C±1°C in incubator with 75% relative humidity (RH). Electrical conductivity using conductivity meter (WTW COND-197i, Germany), and pH using pH meter (WTW pH-197i, Germany) of formulations were noted at various time intervals for 8 weeks (8). The formula which was found to be most stable among different formulations and was selected for further studies is given in table 1.

**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.), of the Islamia University of Bahawalpur and was conducted according to the guidelines of Helsinki declaration.

**Study protocol**

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 design single blinded for the comparisons of two creams. was Patch test was performed to determine any possible irritation caused by creams. 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 morning at 10 a.m. They were allowed to wash their faces with water and, sit to become accustomed with the environment for 30 minutes before taking any measurements. Values for different parameters were taken in controlled room temperature of 25±1°C and 45±2% relative humidity.

**Biophysical techniques**

Skin microrelief parameters were evaluated using Visio Scan® VC98 (Courage and Khazaka, Germany). The skin moisture was determined with a skin capacitance meter (Corneometer® MPA 5) and transepidermal water loss (TEWL) was determined by an evaporimeter (Tewameter® MPA 5, Courage and Khazaka, Germany).

**Statistical analyses**

The percentage of changes with respect to initial values/zero hour of volunteers for different parameters, taken at 2nd, 4th, 6th, 8th, 10th and 12th week were calculated. The measured values were analyzed using SPSS 12.0 on computer.

**RESULTS AND DISCUSSION**

**Physical tests**

In this study, the pH of freshly prepared base and formulation were 5.59 and 5.68 respectively, which were within the range of skin pH (9). By applying ANOVA, it was found that the change in pH of different samples of base and formulation was not significant at different time intervals and temperature. The pH values are shown in table 2. The colors of freshly prepared base and active formulation were white and light green respectively. There was no change in color of any sample of base and formulation at different storage conditions. No change are in color may be attributed to different factors that related to emulsion stability including the components of oil phase, paraffin oil and Abil-EM90 which are colorless, transparent and non toxic liquids (10). No electrical conductivity was found in any sample of base and formulation throughout the period of the study. This is because the emulsion is of w/o type and oil being the continuous phase contributes to no passage of current. There was no liquefaction in any of the samples kept at 8°C and 25°C. The samples were stable at 8°C, 25°C, but slight phase separation in the sample of base occurred at 40°C and 40°C+75% RH on 56th day of observation whereas the formulation was stable. This may be due to the antimicrobial properties of basil which protects the emulsion from microbial contamination and degradation (11).

**Skin Moisture and TEWL**

The skin moisture content and TEWL were measured before application of creams (0 hour readings) and
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In this study base improved the moisture content of the skin to some extent but there was regular increase after the application of formulation throughout the study period. The base showed irregular pattern in the values of TEWL of skin but there was regular decrease in the skin TEWL after application of the formulation throughout the study period. By applying ANOVA two way analysis it was found that the base had insignificant and the formulation had significant (p≤0.05) effects on moisture content and TEWL with respect to time. By the use of paired sample t-test it was evident that significant differences in the moisture and TEWL values were observed after application of formulation throughout the study period. The enhancement in the skin moisture content after application of formulation is due to flavonoids present in basil which implies that treatment with flavonoids result in increase of extracellular collagen. Collagen synthesis ultimately leads to increased skin moisture and more elastic skin (12). TEWL is the outward transmission of water through skin. An increase in TEWL reveals an impairment of the water barrier. The mechanism of TEWL decline is not known but flavonoids intervene cutaneous blood flow which may contribute towards a better skin appearance (13).

Table 1. Formula of creams.

| Cream            | Paraffin oil | Abil® EM 90 | Plant Extract | Distilled water |
|------------------|--------------|-------------|---------------|-----------------|
| Base             | 14 %         | 2.5 %       | Nil           | q.s 100 %       |
| Active formulation | 14 %         | 2.5 %       | 3 %           | q.s 100 %       |

q.s. = Quantity sufficient

| Table 2. Average pH values of base and formulation kept at 8°C, 25°C, 40°C and 40°C + 75% RH for a period of 8 weeks. |
|---------------------------------------------------------------|
| **Cream** | **Storage condition** | **Values of pH (mean±SD)** |
|-----------|------------------------|---------------------------|
|           | 8°C                    | 25°C                      | 40°C                      | 40°C + 75% RH |
| Base      | 5.48±0.046             | 5.47±0.043                | 5.49±0.039                | 5.52±0.033    |
| Formulation | 5.26±0.113             | 5.31±0.085                | 5.39±0.074                | 5.30±0.107    |

Table 3. SELS parameters values (mean±SD).

| Parameter | Cream | O hour | 1 Month | 2 Months | 3 Months |
|-----------|-------|--------|---------|----------|----------|
| SEr       | Base  | 4.06±0.088 | 4.05±0.087 | 4.05±0.090 | 4.05±0.087 |
|           | Formulation | 4.05±0.087 | 4.01±0.085 | 3.96±0.085 | 3.83±0.082 |
| SEsc      | Base  | 1.75±0.051 | 1.75±0.051 | 1.74±0.052 | 1.74±0.051 |
|           | Formulation | 1.74±0.050 | 1.71±0.051 | 1.69±0.055 | 1.67±0.056 |
| SEsm      | Base  | 109.99±4.92 | 109.99±4.92 | 109.64±4.90 | 108.82±4.93 |
|           | Formulation | 110.01±4.78 | 108.73±4.82 | 107.72±4.67 | 103.03±4.24 |
| SEw       | Base  | 72.84±1.55 | 72.84±1.56 | 72.84±1.55 | 71.93±2.01 |
|           | Formulation | 72.74±1.53 | 71.94±1.54 | 70.96±1.66 | 69.51±1.65 |

SEr ;skin roughness, SEsc ;skin scaliness, SEsm; skin smoothness, SEw ;skin wrinkles.
Figure 1. Percentage of change in skin moisture content after application of base and formulation.

Figure 2. Percentage of change in TEWL after application of base and formulation.

Figure 3. Percentage of change in volume after application of base and formulation.
require less amount of virtual liquid (14). The percentage of changes in the values of volume is represented in figure 3.

Energy parameter describes the level of hydration of the skin. In this study increase in the energy values for formulation was statistically significant at all reading intervals but base produced insignificant effects. Percentage of changes are represented in figure 4. By treatment with moisturizing and anti-aging formulations, the energy values increased, which is supported by the values obtained for skin moisture by Corneometer® MPA 5 as highly hydrated elastic skin has higher energy values compared to the dry skin with less moisture (14).

**Surface Evaluation of Living Skin (SELS)**

The values of different SELS parameters SEr, SEsc, SEsm and SEw measured by Visioscan® VC 98/ software SELS 2000 before application of creams and at 1st, 2nd and 3rd months of the study period are given in table 3. The percent changes in the values for 11 volunteers were calculated and given

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**Figure.** Percentage of change in energy after application of base and formulation.

**Figure 5.** Percentage of change in mean VC 98 units of SELS parameters after application of base and formulation.

1B; Base values after one month, 1F; Formulation values after one month, 2B; Base values after two months, 2F; Formulation values after two months, 3B; Base values after three months, 3F; Formulation values after three months
in figure 5.

SEr is the roughness parameter which calculates the proportion of dark pixels. SESm is the index of smoothness and is calculated from the mean width and depth of wrinkles. SEsc is the index of scaliness of skin which shows the level of dryness of the skin. SEw identifies aging including wrinkles and is calculated from the proportion of horizontal and vertical wrinkles (15).

In this study it was found that the base produced statistically insignificant (p<0.05) effects on the roughness parameter of skin and the formulation produced significant effects at all reading intervals when ANOVA two ways analysis was performed. When paired sample t-test was applied, significant effects were observed for active formulation. Gradual decrease in the values of roughness, scaliness, smoothness and wrinkles were observed for the formulation. The formulation showed decrease in mean values of skin smoothness in contrast to skin roughness which indicates that the formulation possess anti-aging properties. The smaller SEsc value corresponds to higher skin moisture as treatment with moisturizing or anti-aging formulations resulted in lower values for SEsc (14). Lower values for the parameter SEw indicate that there were less wrinkles present on the skin which indicates that the formulation reduced the fine wrinkles. This is directly related to the loss of collagen which has strong relation with transsepidermal water loss. Greater epidermal water loss leads to less water retained by the collagen and results in collagen degeneration (16). The decrease in TEWL as measured by Tewameter® support the development of collagen which ultimately lead to less wrinkles. The improvement in skin surface parameters can be attributed to the phenolic compounds and flavanoids present in Basil which include quercetin, isoorceutin, kaempferol, caffeic acid, rosmarinic acid, rutin, catechin, ferulic acid, rutiniste and apigenin (17).

CONCLUSION

From the results of this study it appears that Basil (Ocimum basilicum) possesses potential anti-aging properties when applied topically and stable topical emulsions containing Basil extract can be formulated. The active formulation was found to have skin moisturizing effects as it increased skin moisture content. Highly hydrated skin showed increasing energy values. The decrease in Volume, SELS parameters and TEWL showed that the formulation possess anti-wrinkle affects. This study also depicts that non invasive biophysical techniques are a valuable tool for assessment of anti aging effects of topical skin applications. Furthermore the formulation showed no harmful effects and it can be used as cost effective topical anti-aging treatment. Future studies are required to be conducted to un-reveal the anti-aging mechanism of basil constituents.

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