Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan

Formulation development of a cream containing fennel extract: *in vivo* evaluation for anti-aging effects

A. Rasul[1], N. Akhtar[1], B. A. Khan[1], T. Mahmood[1], S. Uz Zaman[1], H. M. Shoaib Khan[1]

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Akhtar Rasul, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
akhtarasul@gmail.com

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This study was aimed to formulate and evaluate anti-aging effects of a topical cream (w/o emulsion) containig extract of Fennel (*Foeniculum vulgare*) versus its base. Formulation containing 4% concentrated extract of *Foeniculum vulgare* was developed by entrapping in the inner aqueous phase of w/o emulsion and base contained no extract. Both the base and formulation were stored under different storage conditions to predict their stability. The formulation and base were evaluated for effect on skin moisture and transepidermal water loss (TEWL). The base showed insignificant while the formulation showed significant effects on skin moisture and TEWL. The parameter volume and surface evaluation of living skin (SELS) parameters SE*r*, SE*sc*, SE*sm*, SE*w* were also evaluated and showed a significant ($p \leq 0.05$) decline. The texture parameter energy showed a significant increase proving that the formulation possesses potential anti-aging effects.

1. Introduction

Water in oil (w/o) emulsions are widely used for the treatment of dry skin and in emollient applications (Magdy 2004). There is a rising curiosity in natural antioxidants present in plants. Many active compounds are secluded from natural herbs and extracts and used in cosmetics as potential antioxidants (Akhtar et al. 2011).

Fennel (*Foeniculum vulgare* Mill), belonging to the family Apiaceae is a plant that has a long history of herbal uses. Fennel seeds are traditionally used as analgesic, anti-inflammatory, carminative, antispasmodic and diuretic agents. There has been significant interest in the potential antioxidant activities of fennel seed extracts (Farooq et al. 2009).

Aging of the skin is generally associated with increased wrinkling, drooping and laxity, but taking into consideration the primary reasons for these changes, it is important to discriminate between the effects of biological aging and environmental factors, such as exposure to the sun (Jenkins, 2002). Skin aging is a complex process involving several environmental factors, most important of which is UV light from sun. Beside other factors about 80% of the facial wrinkling is considered due to the UV light. Photo-damaged skin is characterized by irregular pigmentation, loss of elasticity, increased roughness, deep wrinkling and dryness (Fischer et al. 1997; Kligman et al. 1986).

The antioxidative activity of *Foeniculum vulgare* provides the basis for cosmetic use of this plant. In this study we formulated a w/o emulsion of containing extract of *Foeniculum vulgare* and studied its stability over a 8 weeks study period. The emulsion was also evaluated for different parameters related to skin aging.

2. Investigations, results and discussion

In this study, base and formulation were divided in to four samples separately and these samples were kept at 8 °C in a refrigerator, at 25 °C, 40 °C and at 40 °C + 75% RH (relative humidity) in stability chambers. They were observed organoleptically with respect to change in color, liquefaction and phase separation for a period of 56 days at definite time intervals.

2.1. Color

Freshly prepared base and formulation were white and creamy. There was no change in color of any sample of base and formulation under different storage conditions up to end of the observation period of 56 days. This shows that both base and formulation were stable at different storage conditions up to 56 days.

2.2. Liquefaction

No liquefaction was observed in any of the samples kept at 8 °C and 25 °C during the whole observation period of 56 days. A slight liquefaction was observed in the sample of base and formulation kept at 40 °C and 40 °C + 75% RH on the 48st and 56th day of observation. The rate of creaming is inversely proportional to the viscosity of the dispersion medium according to the Stokes' law. So as creaming increases, the viscosity of the base and formulation gradually decreases with increasing temperature resulting in liquefaction (Swarbrick 2004).

2.3. Phase separation

The samples of base and formulation were stable at 8 °C, 25 °C, but a slight phase separation in the sample of base occurred at 40 °C and 40 °C + 75% RH on 56th day of observation but the formulation was stable at 40 °C and 40 °C + 75% RH on 56th day of observation. This may be due to the conditions of storage.

Table 1: 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

	Values of pH (mean ± SEM)			
	Storage conditions			
Cream	8 °C	25 °C	40 °C	40 °C + 75%RH
Base	5.51 ± 0.056	5.63 ± 0.052	5.56 ± 0.049	5.67 ± 0.030
Formulation	5.76 ± 0.048	5.87 ± 0.041	5.98 ± 0.029	6.01 ± 0.039

The emulsions can be more stable at lower temperature due to increased phase viscosity (Derrick 2000).

2.4. Electrical conductivity test

Electrical conductivity values of base and formulation of fresh creams and samples kept at different storage conditions for 56 days have been determined. No electrical conductivity was found in any sample of base and formulation throughout the study period. This indicated that creams were stable at different storage conditions.

2.5. pH tests

In this study, the pH of freshly prepared base and formulation was 5.74 and 5.83 respectively, which is within the range of skin pH. The pH of human skin ranges from 4.5 to 6.5 (Jennifer, 2006), with 5.5 being the average pH of the skin.
The pH values of the samples of base and formulation kept at different storage conditions was found to be gradually increasing. By using two-way analysis of variance (ANOVA) technique at 5% level of significance, it was found that the change in pH of different samples of base and formulation was significant at different levels of time and temperature. The pH values of the samples of base and formulation kept at different storage conditions i.e. 8 °C, 25 °C, 40 °C and 40 °C + 75% RH are shown in Table 1.

2.6. Skin moisture

Skin moisture content was measured before application of creams (0 hour readings) and then at 2nd, 4th, 6th, 8th, 10th and 12th week of study period by Corneometer MPA 5 (Courage and Khazaka GmbH). The percent changes in the values for 11 volunteers were calculated and given in Fig. 1. In this study base improved the moisture content of the skin to some extent but there was a regular increase in the skin moisture contents after the application of formulation throughout the study period.
With the help of ANOVA two way analysis it was found that the base produced insignificant effects and the formulation produced significant ($p \leq 0.05$) effects on moisture contents with respect to time. With the help of paired sample t-test it was evident that significant differences in the moisture values were observed after application of formulation throughout the study period. The improvement in the skin moisture content after the application of formulation is due to linoleic acid present in Fennel (Gurdip 2006). Linoleic acid and α-linolenic acids are essential fatty acids which improve skin physiology by affecting barrier permeability to repair it (Kraft and Lynde 2005).

2.7. Transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) is the outward transmission of water through skin. An increase in TEWL reveals an impairment of the water barrier. Transepidermal water loss (TEWL) was measured before application of creams (0 hour readings) and then at 2nd, 4th, 6th, 8th, 10th and 12th week of study period by Tewameter MPA 5 (Courage and Khazaka GmbH). The percent changes in the values for 11 volunteers were calculated and given in Fig. 2.
In this study the base showed an irregular pattern in the values of TEWL of skin but there was a regular decrease in the skin transepidermal water loss after the application of formulation.
With the help of the ANOVA test, it was found that changes in TEWL produced by base and formulation were insignificant with respect to time. With the help of the paired sample t-test it was found that there was a significant variation in TEWL with respect to base and formulation. The basic mechanism of TEWL reduction is unknown but it is scientifically proven that flavanoids mediate cutaneous blood flow which may contribute to a better skin appearance (Shoaib et al. 2010).

2.8. Volume and energy

The parameter volume calculates the virtual amount of liquid (mm^3) needed to fill the depths in image. If the surface is smoother, less virtual liquid is needed. The base produced statistically insignificant ($p \leq 0.05$) effects on the parameter volume while the formulation produced significant effects when ANOVA two way analysis was performed. A decrease in the values of was observed for the formulation. The formulation showed significant effects when the paired sample t-test was

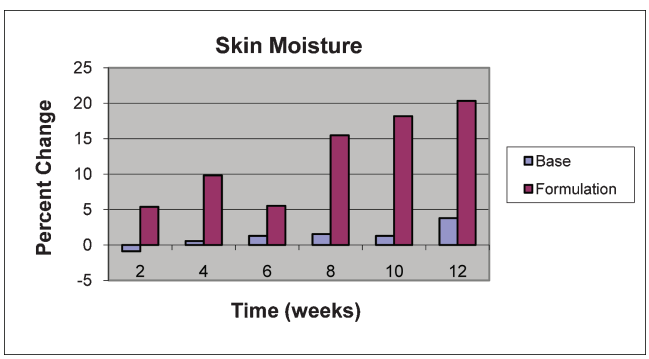

Fig. 1: Percentage of change in skin moisture content after application of base and formulation

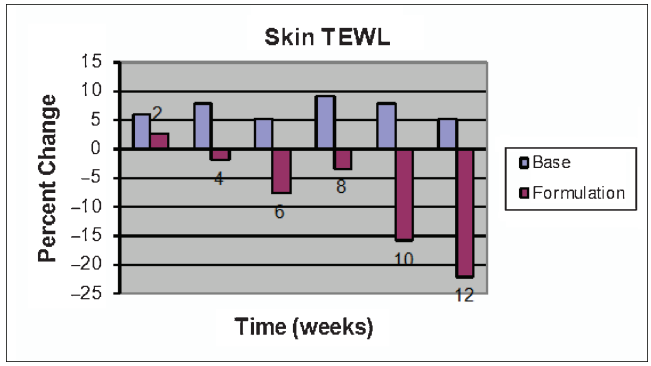

Fig. 2: Percentage of change in TEWL content after application of base and formulation

Table 2: SELS parameters values (mean ± SEM)

Parameter	Cream	O hour	4th week	8th week	12th week
SEr	Base	3.08 ± 0.189	3.08 ± 0.188	3.08 ± 0.190	3.08 ± 0.190
	Formulation	3.03 ± 0.177	2.99 ± 0.173	2.94 ± 0.174	2.90 ± 0.174
SEsc	Base	1.67 ± 0.051	1.67 ± 0.054	1.67 ± 0.052	1.67 ± 0.050
	Formulation	1.67 ± 0.050	1.63 ± 0.049	1.59 ± 0.050	1.54 ± 0.048
SEsm	Base	100.23 ± 4.92	100.23 ± 4.92	100.03 ± 4.90	99.88 ± 4.89
	Formulation	100.23 ± 4.92	98.29 ± 5.01	95.96 ± 4.93	93.04 ± 5.03
SEw	Base	60.52 ± 2.78	60.52 ± 2.78	60.51 ± 2.77	60.50 ± 2.78
	Formulation	60.46 ± 2.74	60.40 ± 2.75	59.21 ± 2.73	58.17 ± 2.73

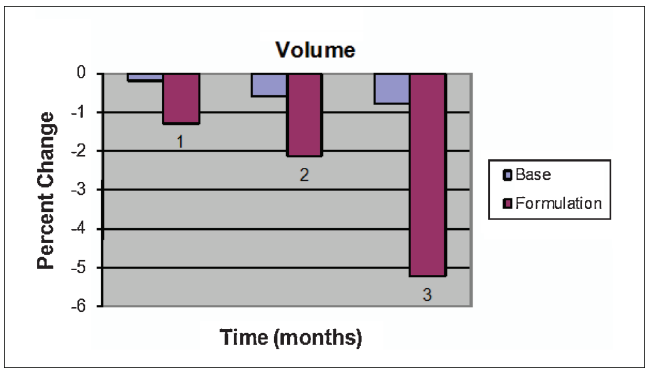

Fig. 3: Percentage of change in volume after application of base and formulation

applied. This is also supported by the results of parameter skin rouhness as less rough skin would need less amount of virtual liquid (Khazaka 2000). The percentage changes in the values of volume are represented in Fig. 3.

In this study increase in the energy values for both base and formulation was statistically significant with respect to time but percent changes were less for the base as compared to the formulation which are presented in Fig. 4. This is supported by the values obtained for skin moisture by Corniometer MPA 5 as highly hydrated elastic skin has higher energy values than skin with less moisture and more wrinkles (Khazaka 2000).

2.9. Surface Evaluation of living skin (SELS)

SELS parameters SE*r*, SE*sc*, SE*sm* and SE*w* were measured before application of creams (0 hour readings) and then at 4th, 8th and 12th week of study period by Visioscan VC 98/software SELS 2000 (Courage and Khazaka GmbH). The percent changes in the values for 11 volunteers were calculated and given

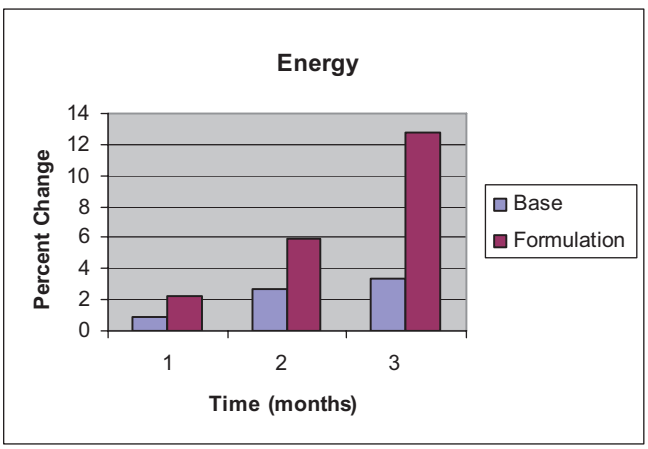

Fig. 4: Percentage of change in energy after application of base and formulation

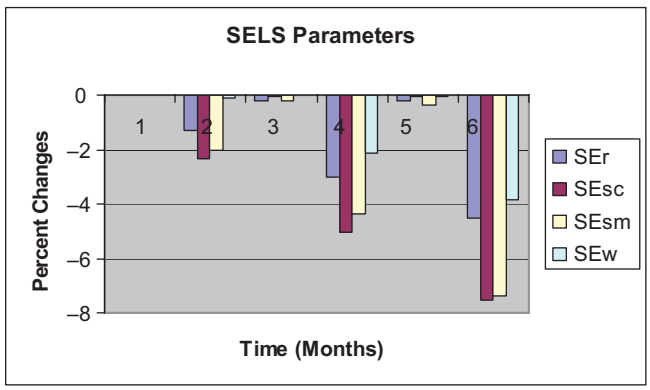

Fig. 5: Percentage of Change in mean VC 98 units of SELS parameters after Application of Base and Formulation. 1 = Base values after one month, 2 = Formulation values after one month, 3 = Base values after two months, 4 = Formulation values after two months, 5 = Base values after three months, 6 = Formulation values after three months

in Fig. 5. The values (Mean ± SEM) of different SELS parameters SE*r*, SE*sc*, SE*sm* and SE*w* measured before application of creams (0 hour readings) and then at 4th, 8th and 12th week of study period are given in Table 2.

2.9.1. Skin roughness (SEr)

SEr is the index of skin roughness and calculates the proportion of dark pixels (Kajimoto et al. 2001). In this study it was found that the base produced statistically insignificant ($p \leq 0.05$) effects on the roughness parameter of skin and the formulation produced significant effects when ANOVA two way analysis was performed. A gradual decrease in the values of roughness were observed for the formulation. When the paired sample t-test was applied significant effects were obtained. Lower values indicate less roughness od skin (Khazaka 2000).

2.9.2. Skin scaliness (SEsc)

SEsc is the index representing scaliness of skin (Kajimoto et al. 2001). The base produced statistically insignificant ($p \leq 0.05$) effects on the skin scaliness while the formulation produced significant effects when ANOVA two way analysis was performed. A gradual decrease in the values of scaliness were seen. In the paired sample t-test was applied significant effects were observed for the formulation. The formulation increased moisture content as is also supported by the values obtained by Corniometer MPA 5 for skin hydration. The smaller *SEsc* value corresponds to higher skin moisture (Hiroshi et al. 2008).

2.9.3. Skin smoothness (SEsm)

SEsm is the index of smoothness and is calculated from mean depth and width of wrinkles (Kajimoto et al. 2001). In this study

it was found that both base and formulation produced statistically significant ($p \leq 0.05$) effects on the skin smoothness. A decrease in the values of the parameter *SEsm* was observed for the formulation. In the paired sample-t-test significant effects were seen for the formulation which showed a decrease in mean values of skin smoothness which indicates that the formulation possess anti-aging properties as after treatment with moisturizing or anti-aging formulations the values for *SEsm* go down (Khazaka 2000).

2.9.4. Skin wrinkles (SEw)

SEw is the index of skin wrinkles and indicates the fineness of skin texture in both vertical and horizontal directions and number and width of wrinkles (Kajimoto et al. 2001). The base produced statistically insignificant ($p \leq 0.05$) effects on the skin wrinkles while the formulation produced significant effects when ANOVA two way analysis was performed. A decrease in the values of the parameter *SEw* was observed for the formulation. The formulation showed a significant effects when paired sample t-test was applied. Higher values for the parameter *SEw* indicates that there are more wrinkles present on the skin. The formulation showed a decrease in mean values of skin wrinkles which indicates that the formulation reduces the fine wrinkles and improves the appearance of skin by increasing the elasticity of connective tissues (Arslan et al. 1989). The improvement in skin surface parameters can be due to the presence of polyphenols and flavanoids in fennel. Polyphenols are potent antioxidant compounds which scavenge free radicals and contribute to anti-aging mechanisms (Senthilmohan et al. 2003).

2.10. Conclusion

This study depicts that fennel (*Foeniculum vulgare*) extract possesses potent anti-aging properties when applied topically and it can be concluded that a stable topical emulsion containing fennel extract can be formulated. The formulation was observed to cause an increase in skin moisture content. Highly hydrated skin showed increasing energy values. The decrease in volume, SELS parameters and TEWL showed that the formulation possesses anti-wrinkle effects. Furthermore the formulation showed no harmful effects and it can be used as cost effective topical anti-aging treatment.

3. Experimental

3.1. Material

Distilled water and ethanolic extract of *Foeniculum vulgare* was prepared in the laboratory of the Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. Paraffin oil was obtained from Merck (Germany), Abil-EM 90 (Cetyl dimethicone copolyol with HLB 5) was purchased from Franken Chemical (Germany).

3.2. Apparatus

Cold Incubator (Sanyo MIR-153, hot incubator (Sanyo MIR-162, Japan), Centrifuge machine (Hettich EBA 20, Germany), conductivity-meter (WTW COND-197i, Germany), Corneometer MPA 5, TEWA meter MPA 5 and Visioscan VC 98 (Courage + Khazaka, Germany), digital humidity meter (TES Electronic Corp, Taiwan), electrical balance (Precisa BJ-210, Switzerland), homogenizer (Euro-Star, IKA D 230, Germany), pH-Meter (WTW pH-197i, Germany), refrigerator (Dawlance, Pakistan), rotarvapor (Eyela, Co. Ltd. Japan), and UV spectrophotometer-16 (Shimadzo Japan).

3.3. Methods

3.3.1. Identification of plant material

Fennel *(Foeniculum vulgare)* seeds were purchased from local market of Bahawalpur, Pakistan. The identification of the seeds was performed at Cholistan Institute of Desert studies, The Islamia University of Bahawalpur and a voucher specimen was preserved (Voucher # FV-SD-4-11-20) at the herbarium of Pharmacognosy Section, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur for future reference.

3.3.2. Preparation of crude extract

Foeniculum vulgare seeds (200 g) were finely grinded and extracted at room temperature with 1 L of 95% ethanol for 48 h. The beaker was sealed with aluminium foil and shaken for 10 min after every 12 h. Finally the macerated material was filtered through several layers of muslin cloth for coarse filtration. Approximately 800 ml coarse filtrates were then filtered through a Whattman # 01 filter paper. The filtrates so obtained were evaporated under reduced pressure at 40 °C in a Rotavapor. The process of evaporation was continued until the concentrate reduced was to one third of the initial volume of the solvent used. The obtained extract was stored in freezer at 0 °C.

3.3.3. Antioxidant activity

The antioxidant activity of *Foeniculum vulgare* seed extract was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) which is a stable free radical (Akhtar et al. 2010a). Vitamin C was used as standard and the activity of free radicals was calculated in % inhibition according to the following relation:

$$Percent\ Inhibition = \frac{(\text{A control} - \text{A test})}{\text{A control}} \times 100 \quad (1)$$

The free radical scavenging activity of *Foeniculum vulgare* seeds extract was 91% in comparison to the standard.

3.3.4. Preparation of formulations

Different formulations with varying the concentrations of Parrafin oil, Abil EM 90 and water were prepared in this study. The oil phase comprising paraffin oil and surfactant (ABIL- EM 90) was heated up to 75 ± 1 °C, water heated of the same temperature, and then extract was added. In case of base no extract was added in the aqueous phase. W/O emulsions were prepared by adding the aqueous phase to the oily phase under continuous stirring at 2000 rpm by the mechanical mixer for 15 min until the aqueous phase was added completely. Then, the mixer speed was reduced to 1000 rpm for 5 min, and then the mixer speed further was reduced to 500 rpm for a period of 5 min for complete homogenization until the emulsion cooled to room temperature.

Stability tests were performed at 8 ± 0.1 °C (in refrigerator), 25 ± 0.1 °C, 40 ± 0.1 °C and 40 ± 0.1 °C (in incubator) with 75% relative humidity (RH). Physical characteristics (color, creaming and liquefaction), electrical conductivity and pH of formulations were noted at various time intervals for 8 weeks. Among the various formulations tested for stability, following formula was found to be stable and chosen for further study.

Formula of base:
Paraffin oil 14%
Abil® EM 90 2.5%
Distilled water q.s 100%

Formula of active formulation:
Paraffin oil 14%
Abil® EM 90 2.5%
Plant extract 4%
Distilled water q.s 100%

3.3.5. Study protocol

A single blinded study was designed for the comparisons of two creams (Akhtar et al. 2010b). Eleven male volunteers with a mean age of 45 years having no skin or other diseases were selected for the study and consent was taken. The volunteers were not informed about the contents of the creams. Patch test was performed to determine any possible reactions of creams, on forearms of each volunteer on the first study day. After 48 h, each volunteer was provided with the two creams. One cream was the base (B) and other was the active formulation (A). Each volunteer applied the cream for the period of 12 weeks. Every volunteer was instructed to come for measurement after 2, 4, 6, 8, 10 and 12 weeks. Results were measured in a controlled room at 20 ± 1 °C and 40 ± 2% relative humidity.

3.3.6. Patch tests

Patch tests were performed on the forearms of each volunteer. The patch (Bandage disc) for the right forearm was saturated with 1.0 g of base while the patch for left forearm was saturated with 1.0 g of formulation. Each was separately applied to marked regions 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. After 48 h, scores were recorded for the presence of erythema (skin redness)

Table 3: Score given by volunteers to base and formulation on the basis of itching/irritation*

Score		3	2	1	0
No of volunteer	Base	0	1	4	7
	Formulation	0	1	2	8

* No severe erythema occurred in any of the volunteers, mild erythema occurred in one and one volunteers, moderate erythema occurred in four and two volunteers, whereas no erythema occurred at all in seven and eight volunteers for both base and formulation, respectively

using a scale with 4 points from 0 to 3. The score given by volunteers is presented in Table 3.

3.3.7. 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 (Helsinki Declaration).

3.3.8. Statistical analysis

The percentage changes with respect to zero hour/initial values of volunteers for different parameters, taken at 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} or 12^{th} week were calculated. The measured values obtained for different parameters (skin moisture, TEWL and SELS) were analyzed using SPSS 12.0 on a computer (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals. 5% level of significance was applied.

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