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**NATURAL DRUGS**

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**FORMULATION AND CHARACTERIZATION OF A CREAM CONTAINING  
EXTRACT OF FENUGREEK SEEDS****MUHAMMAD K. WAQAS<sup>1</sup>, NAVEED AKHTAR<sup>1</sup>, MAHMOOD AHMAD<sup>1</sup>, GHULAM MURTAZA<sup>1\*</sup>,  
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**Abstract:** Fenugreek seeds possess antioxidant effects and contain a mucilage which has emollient properties. It can also produce skin healing, whitening, moisturizing, skin soothing and antiwrinkle effects. The purpose of study was to formulate a stable W/O emulsion containing fenugreek seeds extract using liquid paraffin oil. Fenugreek seeds extract, which was obtained by concentrating methanolic extract of fenugreek seeds, was entrapped in the inner aqueous phase of W/O emulsion. A base containing no active material and a formulation containing concentrated extract of fenugreek (in a concentration of 4%) in the internal aqueous phase (W/O emulsion) were prepared and stored at different accelerated conditions for a period of four weeks to predict the stability of these creams. It was found that both, the base and the formulation, were stable at all the accelerated conditions regarding color, liquifaction and phase separation. However, insignificant changes in the pH of base and significant changes in the pH of the formulation were observed with the passage of time. Both the base and the formulation were applied to the cheeks of human volunteers for six weeks and various parameters of the skin were evaluated every week to measure any effect produced by these creams. All the effects of base were statistically significant except the sebum contents and pH, which changed but insignificantly. A significant decrease in skin melanin and erythema was produced by the formulation. An insignificant decrease in TEWL was observed for the formulation.

**Keywords:** fenugreek seeds extract, melanin, erythema, skin moisture, skin sebum, TEWL (trans epidermal water loss)

An emulsion is a system in which one fluid is dispersed in another with which it is immiscible. Macroscopic separation of the phases is prevented by the addition of a suitable surfactant (1). There has been renewed interest in the emulsion as a vehicle for delivering drugs to the body as it has been found to have several advantageous characteristics, frequently enhancing the bioavailability of the drug substance. In an emulsion the therapeutic properties and spreading ability of the constituents are increased (2). Water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications (3). Additional value can be given to these formulations by including plant extracts with specific cosmetic effects. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients (4).

An extract of fenugreek can be obtained by macerating the powdered seeds in methanol (80%)

and filtering and concentrating it on a rotary evaporator. Extract so obtained is rich in polyphenols, galactomannans and flavonoids that have some cosmetic benefit on the skin due to their antioxidant and emollient properties (5). Fenugreek seeds extract is usually used to treat severe skin inflammation, chapped lips and skin aging (6). Paraffin oil has been used in this study that consists chiefly of a mixture of hydrocarbons belonging to the methane series. It occurs as a colorless, oily, transparent, tasteless, non-fluorescent liquid, odorless when cold, but having a faint petroleum odor when heated (7). ABIL EM 90 has been used as an emulsifying agent, which is nonionic surfactant and makes possible a dispersion of aqueous droplets within an oil phase. It is a clear, colorless liquid, available in various viscosities which functions as antifoaming agent and emollient (8). The aim of this study was to measure the effects of a W/O cream of fenugreek

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seeds extract on different physiologic functions of skin like melanin, erythema, skin moisture, skin sebum and TEWL.

## MATERIALS AND METHODS

### Materials and apparatus

Extract of fenugreek seeds (purchased locally) and distilled water were prepared in the laboratory of Pharmacy Department, The Islamia University of Bahawalpur. Paraffin oil was obtained from Merck (Germany). Abil-EM 90 was purchased from Franken Chemical (Germany), Lemon oil was obtained from Chemoflor Manufacturing Corp., Pakistan.

### Preparation of base

In this study, W/O emulsion was prepared by the addition of aqueous phase to the oily phase with continuous agitation. To prepare the base, an oily phase that consisted of paraffin oil (16%) and surfactant ABIL-EM 90 (3.5%) was heated up to  $75 \pm 1^\circ\text{C}$ . At the same time, aqueous phase consisting of 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 complete aqueous phase was added; 2 to 3 drops of lemon oil 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 to 1000 rpm for homogenization, for a period of 5 min, and then the speed of the mixer was reduced to 500 rpm for further 5 min for complete homogenization, until the emulsion cooled to room temperature.

### Preparation of formulation

Oily phase that consisted of paraffin oil and surfactant (ABIL-EM 90), was heated up to  $75 \pm 1^\circ\text{C}$ . At the same time, aqueous phase consisting of water was heated to the same temperature and then the fenugreek seeds extract (4%) were added. After that, aqueous phase was added to the oil phase drop by drop. Then, the procedure was continued exactly as described above for preparation of the base.

### Characterization of emulsions and stability tests

Emulsion was analyzed to assure the formulation of desired properties by physical analysis, types of emulsions, pH determination, electrical conductivity and centrifugation tests.

Stability tests were performed under different conditions for emulsions to note the effect of these

conditions on the storage of emulsions. These tests were performed on samples kept at  $8 \pm 0.1^\circ\text{C}$  (in refrigerator),  $25 \pm 0.1^\circ\text{C}$  (in incubator),  $40 \pm 0.1^\circ\text{C}$  (in incubator) and  $40 \pm 0.1^\circ\text{C}$  (in incubator with 75% relative humidity, RH). Physical characteristic of simple emulsions, i.e. color, creaming and liquefaction, were noted at various intervals for 28 days.

### Product evaluation on skin

Eleven human volunteers were selected whose ages were between 25 and 35 years. Male volunteers were included in this work. Prior to the tests, a cosmetic expert examined the volunteers for any serious skin disease or damage especially on cheeks and forearms. Each volunteer was provided with two creams. One cream was the base and the other one was the formulation containing the active ingredients. Each cream was marked with "right" or "left" indicating application of that cream to the respective cheek. The volunteers themselves, as instructed, applied the creams for 6 weeks. Every individual was instructed to come after every week for the dermatological tests.

### Dermatological tests

Melanin/erythema content, skin moisture content, skin sebum content, trans epidermal water loss (TEWL) are also determined after 1, 2, 3, 4, 5 and 6 weeks.

### Mathematical and statistical analysis

The percentage changes for the individual values of different parameters of volunteers, taken every week, were calculated by the following formula;

$$\text{Percentage change} = [(A - B) / B] \times 100$$

where, A = individual value of any parameter of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, or 6<sup>th</sup> week. B = zero hour value of that parameter.

The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, elasticity and pH) were analyzed statistically using SPSS 12.0 (paired sample *t*-test for variation between the two preparations; two-way ANOVA for variation between different time intervals).

## RESULTS AND DISCUSSION

### Stability testing

In this study, the base and the formulation were divided into four samples separately and these samples were kept at different storage conditions i.e. at  $8^\circ\text{C}$  in refrigerator, at  $25^\circ\text{C}$ ,  $40^\circ\text{C}$  and at  $40^\circ\text{C} + 75\%$  RH in stability chambers. These samples under dif-

Table 1. The pH values of base and formulation kept at 8°C, 25°C, 40°C and 40°C + 75% RH (B = base, F= formulation, RH = relative humidity).

Time*	8°C		25°C		40°C		40°C + 75% RH	
	B	F	B	F	B	F	B	F
0 h	5.38	5.89	5.38	5.89	5.38	5.89	5.38	5.89
12 h	5.27	5.78	5.53	5.93	5.41	5.72	5.17	5.69
24 h	5.89	5.81	5.64	5.86	5.66	5.64	5.36	5.72
36 h	5.96	5.84	5.47	5.61	5.83	5.80	5.51	5.79
48 h	5.67	5.74	5.81	5.71	5.43	5.42	5.24	5.61
72 h	5.77	5.69	5.62	5.76	5.48	5.29	5.38	5.62
7 d	5.28	5.90	5.83	5.86	5.21	5.67	4.86	5.55
14 d	5.55	5.81	5.80	5.72	4.94	5.65	4.90	5.34
21 d	5.34	5.80	5.22	5.71	4.66	5.72	4.26	5.57
28 d	5.20	5.24	4.60	5.70	4.31	5.10	4.03	5.26

\* h = hours, d = days

ferent storage conditions were observed for a period of 28 days at definite time intervals. The samples were observed with respect to change in color, liquefaction and phase separation.

#### Color

No change in the color of base and formulation at the end of observation periods may be attributed to different factors contributing to the emulsion stability, such as the components of oil phase, i.e. paraffin oil which is a colorless, transparent, tasteless, non-fluorescent liquid; and is a mixture of hydrocarbons (7), Abil-EM90 which is a clear, colorless and nontoxic liquid emulsifier (8), as the active ingredient, i.e. fenugreek extract containing polyphenols (9). Polyphenols are well documented to have microbicide activities against huge number of bacteria (10). Thus, they may protect the formulation components from microbial growth of those organisms which might produce such substances, which are able to change the color of the formulation during the storage time.

#### Liquefaction

No liquefaction was observed in any of the sample of base and formulation kept at 8°C and 25°C during the whole observation period of 28 days. Slight liquefaction was observed in the sample of base kept at 40°C on 28<sup>th</sup> day. Liquefaction was also observed in the sample of base kept at 40°C + 75% RH from 21<sup>st</sup> day of observation but there was no further increase in liquefaction till the end of the study period. On the other hand, a slight liquefaction

was observed in formulation samples kept at 40°C + 75% RH on 28<sup>th</sup> day of observation period.

#### Phase separation

The samples of base were stable at 8°C and 25°C but slight separation was observed at 40°C and 40°C+ 75% RH on 28<sup>th</sup> day of observation. In the case of formulation, no phase separation was observed in any of the samples kept at 8°C, 25°C, 40°C and 40°C+ 75% RH up to the observation period of 28 days. This indicated that the formulation was relatively more stable than base at higher temperatures considering phase separation as a parameter of stability. Depending on conditions, emulsions may be more stable at lower temperature due to increased phase viscosity (11).

#### Centrifugation test

No phase separation on centrifugation was seen in any of the samples kept under different storage conditions, i.e. 8°C, 25°C, 40°C and 40°C + 75% RH up to 28<sup>th</sup> day of observation. This indicated that the emulsions were stable at all the storage conditions for 28 days. It may be said that proper homogenization speed during emulsion formulation prevented the base and the formulation breakage during stress testing (12).

#### Electrical conductivity

In this study, conductivity test was performed for all the samples of base and the formulation kept under different storage conditions up to a period of 28 days at definite time intervals. No electrical con-

ductivity was seen in any of the samples of base and formulation kept under different storage conditions up to 28<sup>th</sup> day of observation.

### pH

The pH of human skin typically ranges from 4.5 to 6.0 (21) and 5.5 is considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH close to this range. In this study, the pH of freshly prepared base and formulation was 5.38 and 5.89, respectively, which is within the range of skin pH. The pH values of the samples of base kept under the mentioned storage conditions were found to be increasing gradually in the 1<sup>st</sup> week and then it started to decline continuously till 28<sup>th</sup> day with some variations. At the end of the study, the pH values of the samples of base at 8°C, 25°C, 40°C and 40°C + 75% RH were 5.20, 4.60, 4.31 and 4.03, respectively, whereas the pH values of the samples of formulation kept under the same conditions showed gradual reduction with slight variations with time and were 5.24, 5.70, 5.10 and 5.26 at 28<sup>th</sup> day, respectively, as represented in Table 1.

By using two-way analysis of variance (ANOVA) technique at the 5% level of significance, it was found that the change in pH of different samples of base was insignificant at different levels of time and temperature but there was a significant difference in change of pH of different samples of formulation at different levels of time and temperature. When LSD test was applied to check the individual average effects of the pH of the samples of base at different temperatures with the passage of time by taking the average pH values at zero hour at different temperatures as a standard, it gave insignificant changes except for 3<sup>rd</sup> and 4<sup>th</sup>

week, where differences were significant. Again, when LSD test was applied to check the individual average effect of the pH of the samples of formulation at different temperatures with the passage of time by taking the average pH values at zero hour at different temperatures as a standard, it gave significant changes from 48<sup>th</sup> hour till the end of study period except the 7<sup>th</sup> day. From LSD test it was concluded that there was insignificant change in pH of the samples of base under different storage conditions but significant changes were observed in pH of the samples of formulation under the same storage conditions with the passage of time. The decrease in pH of the formulation at different storage conditions might be due to the oxidation of paraffin oil which produces aldehydes and organic acids (8).

### Dermatological tests

#### Melanin

In this study, the effect of the base and the formulation on the production of skin melanin was examined. The amount of melanin was measured for 6 weeks at different time intervals for each individual after application of base and formulation. It was found that the base increased the melanin contents in the skin irregularly till the end of 6<sup>th</sup> weeks, while the formulation increased the melanin contents in 1<sup>st</sup> and 2<sup>nd</sup> week but then decreased it gradually throughout the study period, as shown in Figure 1. With the help of ANOVA test it was found that the base and formulation produced insignificant effects on skin melanin content in the volunteers. With the help of paired sample *t*-test, no significant differences were observed between the melanin effects of base and the formulation throughout the study period.

This showed that the two creams, the formulation and the base, have different effects on melanin but these differences are statistically insignificant for 6 weeks. It was concluded that the decreased skin melanin content after application of formulation may be attributed to antioxidant activity due to the presence of flavonoids (13). The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet oxygen quenchers and metal chelators (14).

#### Erythema

In this study, irritation was constantly monitored every week for the base and the formulation throughout the period of application. It was found that erythema contents increased in an irregular pattern after the application of base throughout the

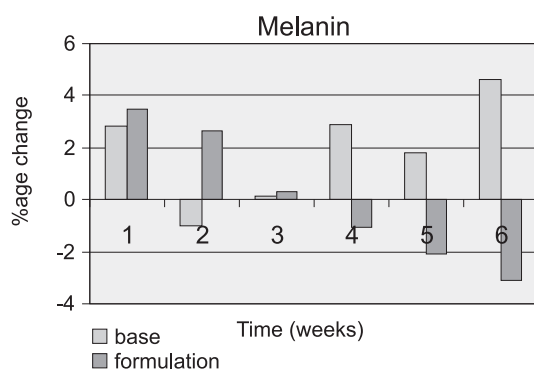


Figure 1. Percentage of change in skin melanin content after application of base and formulation

study period, whereas after the application of formulation, erythema contents were slightly increased during 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week and then gradually decreased till 6 week, as shown in Figure 2. With the help of ANOVA test it was found that the base and formulation produced insignificant effects on the skin erythema at different time intervals and with the help of paired sample *t*-test it was evident that there was no significant variation in irritation with respect to the base and formulation throughout the study period. It was concluded that the base increased while formulation slightly decreased the erythema contents of skin at the end of the study period and the overall effect of formulation on skin erythema was insignificant, so the formulation can be used safely without any significant skin irritation.

**Skin moisture content**

It was found that there was a slight decrease in moisture value in the 1<sup>st</sup> week after the application of

the base and a very slight increase was observed at 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> week, while after the application of formulation there was the increase in moisture contents during the whole study, as shown in Figure 3. With the help of ANOVA test it was found that the base showed insignificant change with respect to the basic values, whereas the formulation showed significant variation throughout the study period of 6 weeks. With the help of paired sample *t*-test it was evident that significant differences in the moisture values were observed after application of both base and formulation. The significant increase in moisture after application of formulation may be due to the fact that fenugreek extract contains mucilaginous polysaccharide that is a natural effective ingredient for improving skin hydration, possibly through a humectant mechanism. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin (5).

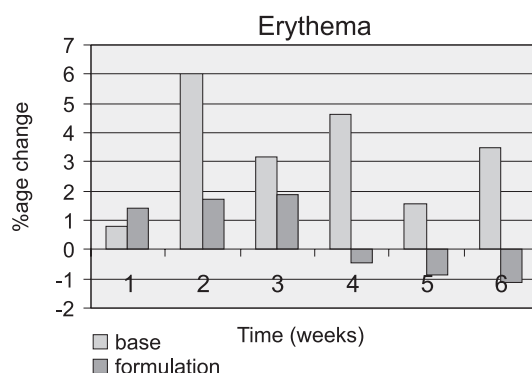


Figure 2. Percentage of change in skin erythema content after application of base and formulation

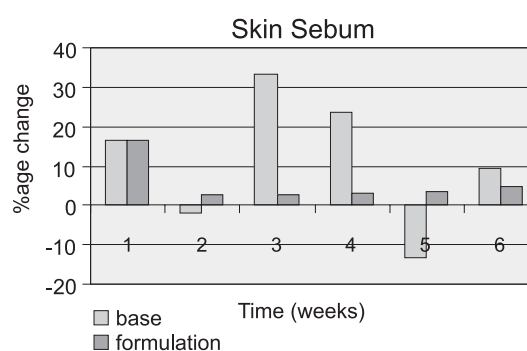


Figure 4. Percentage of change in skin sebums content after application of base and formulation

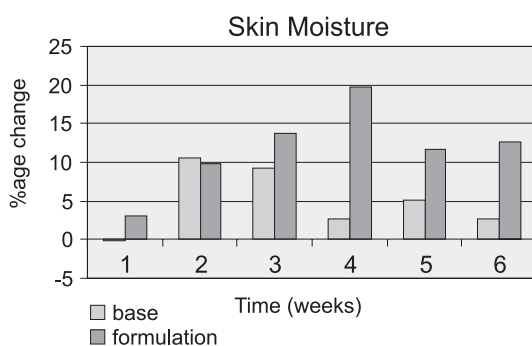


Figure 3. Percentage of change in skin moisture content after application of base and formulation

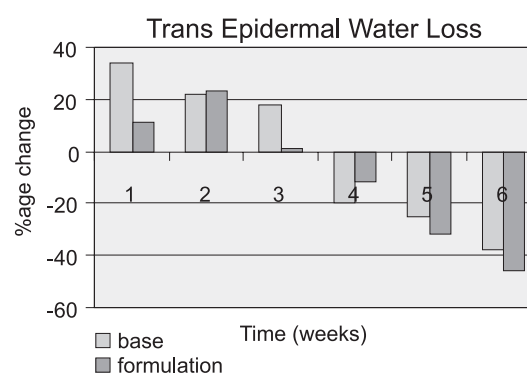


Figure 5. Percentage of change in trans epidermal water loss after application of base and formulation

### Skin sebum content

The effects of the base and formulation on the sebum contents of human cheeks were investigated. Sebum was measured every week in all the individuals. It was found that the base decreased sebum contents in 2<sup>nd</sup> and 5<sup>th</sup> week of study period but increased in 1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 6<sup>th</sup> week. The formulation showed a gradual increase in sebum contents in a regular manner as the study continued from the 1<sup>st</sup> to 6<sup>th</sup> week, as shown in Figure 4. With the help of ANOVA test it was evident that there was an insignificant effect of the base and formulation on the skin sebum throughout the study period. By applying LSD test it was evident that insignificant changes in sebum contents were observed at different time intervals after application of base and formulation. With paired sample *t*-test it was found that the base and formulation showed insignificant variations throughout the study period. It is concluded that an increase in sebum contents after the application of base may be attributed to the oily nature of W/O emulsion having a thick viscous oily liquid, i.e. the paraffin oil (7). Galactomannans constitute an extracellular deposition of endosperm of fenugreek seeds and serve as energy reserves used during germination. They have emollient properties that relieve dryness and provide a soothing membrane that covers the skin. This provides the protection of the skin hydration, and produce softening effects to skin (15).

### Trans epidermal water loss (TEWL)

It was found that there was an increase in TEWL values after the application of base having the greatest value after 1<sup>st</sup> week then gradual reduction. After the formulation there was an increase in TEWL after 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week but a decrease in the remaining period of study, as shown in Figure 5. With the help of ANOVA test, it was found that changes in TEWL produced by both formulation and base were significant. By applying LSD test, it was found that in both the cases, i.e. the base and formulation, the changes in TEWL values became significant after 5<sup>th</sup> and 6<sup>th</sup> week of application. With the help of paired sample *t*-test it was found that there was significant variation in TEWL with respect to the base and formulation in 2<sup>nd</sup> week of study, while insignificant for other periods. The endosperm of fenugreek seeds is rich in galactomannans which have emollient properties that relieve dryness and provide a soothing membrane (15). Emollient helps to prevent dryness while moisturizing and softening the skin. Applying an emol-

lient provides a surface film of lipid and restores some of the barrier function. This oily layer also helps to trap water under the stratum corneum, reducing epidermal water loss and making the skin softer and suppler (16).

### CONCLUSION

It can be concluded that a stable W/O formulation using extract of fenugreek seeds and paraffin oil can be formulated and used to reduce skin melanin contents and to increase skin moisture contents without causing any skin irritation.

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