IN-VIVO EVALUATION OF VARIATION IN HUMAN SKIN MOISTURE CONTENT AND TRANS EPIDERMAL WATER LOSS (TEWL) OF TOPICAL EMULSION CONTAINING CUMINUM CYMINUM (L.) SEED EXTRACT.

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ABSTRACT

Background: Dry skin disorders are growing now a days. Various types of topical formulations are available for the treatment but are expensive and carry risks of side effects. Objective: To evaluate the moisturizing and trans epidermal water loss reducing effects of topical emulsion as cream containing botanical seed extract of Cuminum cyminum which will be cost effective for the treatment of dry skin disorders. Methods: Formulations were prepared as control and experimental and applied to 11 human volunteers. Skin Moisture content and TEWL were measured and analyzed statistically, for a duration of 8 weeks. Results: With the help of ANOVA test it was found that there is significant increase in hydration level of the skin with both the formulations i.e. control and experimental by an increase in moisture content and a decrease in TEWL while this effect is more in case of experimental formulation. Conclusion: Results of the study shows that 4% Cuminum cyminum cream is cost effective and can be used in dry skin diseases. It will be the future horizon of this research.

Key Words: Trans epidermal water loss (TEWL), Moisture content of skin, Cream of Cuminum cyminum, Dry skin
INTRODUCTION

Investigation of the clinical aspects of natural topical agents is of great importance now a days. Skin aging is a complex process induced by constant exposure of skin to ultraviolet (UV) irradiation. Aging of the skin is characterized by irregular pigmentation, increased wrinkling, loss of elasticity, dryness and roughness etc. Recently, emulsions have established growing interest as a vehicle for the drug delivery to the body through different routes. The use of natural compounds in skin protection especially topical application of antioxidants indicates that they usefully decrease skin aging. UV generates reactive oxygen species leading to collagen deficiency and eventually skin wrinkling. Dryness of skin is symptom of various types of disorders. So, treatment of dryness is the use of skin moisturizing agents and to prevent the trans epidermal water loss. Topical formulations containing botanical extracts are of great importance in this regard. Furthermore, plant extracts are extensively used in topical and cosmetic formulations for different purposes such as fragrance, colorants, anti-irritant, anti-aging, skin moisturizers etc. As the use of natural products is growing day by day but they may induce allergy, contact dermatitis and phytophotodermatitis. Topical treatments are too a growing research area of formulators for skin diseases. Now-a-days herbal extracts are used in the cosmetic preparations for augmenting beauty and attractiveness. Herbal cosmetics can be classified on the basis of dosage form like- cream, powder, soaps, solutions etc. and according to part or organ of the body to be applied for like; cosmetics for skin, hair, nail, teeth and mouth etc. The use of cosmetics requires both efficacy as well as safety regarding skin irritation/skin sensitization. This is influenced by their formulation, nature of their use and quantity and quality of ingredients used. A number of synthetic and botanical compounds derived from natural sources, which are being investigated for their potential role in reducing melanin production and pigmentation. Cuminum cyminum is an annual herbaceous plant and has been recently reported that medicinal usage of Cumin seeds has also been widespread in diverse ethnomedical systems from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America, where these have remained as an integral part of their folk medicines. It is used for various purposes, its use as an antioxidant is of great value. Chemical constituents present in plants are responsible for a variety of activities. Cuminum cyminum contains a number of polyphenolic compounds. Numerous plant polyphenols are value-adding chemicals and are used as constituents in cosmetics, foods and pharmaceuticals. The prospective use of phenolic compounds for the introduction of new skin care cosmetics
(creams/emulsions) has been underlined. [11] Plant polyphenols can be used as sunscreen, whitening and anti-aging agents. [12]?

MATERIALS AND METHODS

Identification of plant

The identification of Cuminum cyminum was performed by Prof. Dr. Arshad at Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan. The specimen was deposited in the pharmacognosy section of Faculty of Pharmacy and Alternative Medicine.

Materials

Paraffin oil with dynamic viscosity of 110-230 mPas at 20°C and kinametic viscosity of 34.5 mm2/s at 40°C, and Ethanol were obtained from Merck (Germany). Abil- EM 90 (Cetyl Dimethicone copolyol with HLB 5) was purchased from Franken Chemical (Germany), distilled water and extract of Cuminum cyminum (ethanolic) was prepared in laboratory of Pharmacy department, the Islamia University of Bahawalpur, Pakistan.

Apparatus

Centrifuge Machine ( Hettich EBA 20, Germany), Cold Incubator (Sanyo MIR-153, Japan), Conductivity- Meter (WTW COND-197i, Germany), Digital Humidity Meter (TES Electronic Corp, Taiwan), Electrical Balance (Precisa BJ-210, Switzerland), Homogenizer (Euro-Star, IKA D 230, Germany), Hot Incubator (Sanyo MIR- 162, Japan), PH-Meter (WTW pH-197i, Germany), Refrigerator (Dawlance, Pakistan), Rotary evaporator (Eyela, Co. Ltd. Japan), water bath (HH .S21 4. China). Tewameter and Corneometer MPA 5 (Courage + Khazaka, Germany).

Extraction and Formulation Development

Extract of 500 g seeds of Cuminum cyminum was prepared by maceration method. To prepare the extract firstly, the dried seeds of cumin were powdered and then added into the solvent (80% ethanol and 20% distilled water to make total volume 500 ml). Extract was filtered using Whatman # 01 filter paper after 24 hours’ soaking. Evaporation of obtained filtrate was done at 40°C under vacuum by using a rotary evaporator. The evaporation process was continued till concentrate reduced to one third of the total volume used.
Oily phase used in the preparation (paraffin oil and Abel EM90) was heated up to 75°C±1°C. Meanwhile, aqueous phase (distilled water) was also heated up to 75°C±1°C. The Cuminum cyminum extract in 4% concentration by mass was added in the aqueous phase. 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 minutes until complete aqueous phase was added; 2 to 3 drops of rose water were added during this stirring time to give good fragrance to the cream. 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 minutes, and then the speed of the mixer was reduced to 500 rpm for further 5 minutes for complete homogenization; until the cream cooled to room temperature. And control formulation labelled as “Base” was prepared in the same way except the addition of seed extract.

Properties of formulation
Stability tests were performed at 8±0.1°C (in refrigerator), 25±0.1, 40±0.1 and 40±0.1°C (in incubator) with 75% relative humidity (RH). Physical characteristic (color, creaming and liquefaction), electrical conductivity and pH of formulations were noted at various intervals for 28 days.

Product evaluation on skin
Patch test (Burchard test)
Patch tests were conducted on the first day of skin testing, on the forearms of each volunteer. A 5cm X 4cm region on both the forearms was marked. Zero values for erythema and melanin were measured with the help of Mexameter. Small amount of base and formulation each were applied to the 5cm X 4cm marked regions separately on each forearm. Surgical dressing was employed to cover the regions after the application. After 24 hours, dressings were removed and the measurements of erythema and melanin were repeated on both forearms.

For this study 11 human volunteers were chosen whose ages were in between 25 and 40 years. Volunteers were examined for any serious skin disease or damage especially on cheeks and forearms. Every volunteer was provided with a volunteer protocol before the study. This protocol stating every volunteer signed the terms and conditions of the testing individually. One sided blind study was performed. Skin tests were performed at 25°C and 40% relative humidity conditions. On the first day, patch test was performed to determine any possible reactions to the creams, on the forearms of each volunteer. After 48 Hours each volunteer
was provided with two creams. One cream, the controlled formulation was labelled as “A” and the other one experimental was labelled as “B” containing the active ingredients. Application indication for each cream was marked with “right” or “left” indicating application of that cream to the respective cheek. Each volunteer applied the creams containing extract i.e. B and without extract i.e. A for 8 weeks of study period. Every individual was instructed to come for measurements of readings for skin on 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} week. After consecutive 4 weeks of measurement, volunteers were instructed to come after 15 days for 6\textsuperscript{th} week reading. In the same way after 6\textsuperscript{th} week, volunteers were instructed to come after 15 days for the 8\textsuperscript{th} week reading. At each reading time skin moisture content and Trans epidermal water loss were measured using corneometer and Tewameter.

**Study design**

A single blinded study was designed for the comparisons of two creams that is, the experimental formulation containing seed extracts of the stated plant and control formulation. Two formulations were named A (control formulation) and B (experimental formulation) and given to the volunteers with instructions of application. Results were measured in controlled room at 20 ± 1°C and 40±2% relative humidity. \[^7\]

**Ethical standards**

This study was approved by the board of advance study and research (BASR), The Islamia University of Bahawalpur and institutional ethical committee in compliance with NIH Principles of Laboratory Animal Care 1985. The reference no. is COSM -3986/14.

**Mathematical analysis**

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

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

Where:

A = Individual value of parameter i.e. skin moisture content and TEWL of 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th}, 6\textsuperscript{th} and 8\textsuperscript{th} week

B = Zero hour value of that parameter
**Statistical analysis:** The measured values obtained for different parameters (skin moisture content or TEWL) were analyzed using SPSS 17.0 on computer. The tools used for this purpose was Two-way ANOVA was applied for the variation between different time intervals. Two-way ANOVA is an extension of the paired t-test to more than two treatments. 5% level of significant was applied.

**RESULTS**
The developed cream was stable in all aspects including color, pH variation, and phase separation. Patch test results show no variation in erythema level of volunteers which is an evidence of safe concentration of cumin seed extract used with respect to skin irritation.

Figure 1 and Figure 2 depict the percentage change in moisture level of the skin at different time intervals after application of control and experimental formulations respectively.

**Figure 1:** Percentage of change in skin moisture content after application of control formulation

**Figure 2:** Percentage of change in skin moisture content after application of experimental formulation

Variation of skin TEWL is obvious from Figure 3 and Figure 4 after application of control and experimental formulations.
Figure 3: Percentage of change in skin TEWL content after application of control formulation

Figure 4: Percentage of change in skin TEWL content after application of experimental formulation

Statistical Evaluation: Results of ANOVA two way analysis for skin moisture content of volunteers after application of control and experimental formulations respectively shows that control formulation as well as experimental formulation produced significant effects with respect to increase in moisture level of skin at 5% level of significance. It was obvious from p-value calculated in case of control formulation, p<0.05, and also in case of experimental formulation, p<0.05.
To assess the TEWL statistically two way ANOVA was applied and results depict that in case of control formulation as well as in case of control formulation variation is significant i.e. p<0.05.
DISCUSSION
Finally it was concluded that both the formulation and base produced no skin irritation after performing patch test of 24 hours, so both emulsions can be used safely on human skin for in-vivo evaluation this was an evidence obtained after the measure of skin Erythema and Melanin level after patch test duration.

Skin Moisture Content
In this work, the effect of control and the experimental formulations on skin moisture content was examined. The change in skin moisture content was measured for 8 weeks at different time intervals in each individual. After application of control formulation it was found that there was increase in the moisture content of the skin of the healthy human volunteers throughout the study period. In case of experimental formulation there was also continuous increase in the moisture contents throughout the study period as evident from Figure 1 and Figure 2 respectively. Both the formulations show an enhancement of skin moisture level while it is obvious from Figure 2 that experimental formulation depicts more enhancement of moisture level and can be used to treat skin dryness disorders.

Flavonoids are present in various parts of plants that are responsible for tyrosinase inhibiting activity and parts of such plants can be utilized in hyperpigmentation skin diseases. Vitamin C is being marketed in cosmetic industry for the treatment of hyperpigmentation. [13] The vitamin C is a constituent of cumin seed extract[14], therefore the presence of stated constituents are responsible to decrease the melanin content. Other constituents present in the formulation are responsible for increased level of hydration of skin and formulation can be used in dry skin disorders and this will be the future horizon of this research.

Trans epidermal water loss (TEWL)
In this work, TEWL was constantly monitored throughout the study period of 8 weeks for both control and experimental formulations. It was found that there was a decrease in TEWL after the application of both control and experimental formulations throughout the study period, Figure 3and Figure 4 which ultimately increase the hydration level of skin. As two way ANOVA test results are an evident of the fact that experimental formulation can be used in skin diseases where TEWL increases i.e. dry skin disorders. This effect will be studied in diseased human subjects in future research.
CONCLUSION
It was concluded that both the base and the formulation prevent the transepidermal water loss, is due to a number of factors, such as both creams containing glycerin, a humectants, which creates a “reservoir” of moisture retaining ability in the skin. It moisturizes the full thickness of stratum corneum. Paraffin oil which has been used in both, the base and the formulation, forms an occlusive covering on the skin thus preventing TEWL. So due to moisture retaining properties; the formulation and the base enhanced the stratum corneum ability to attract, hold and redistribute water thus reducing the TEWL.

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REFERENCES