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Effects of *Emblica Officinalis* Extract Cream on Human Skin Trans-epidermal Water Loss Measured with Non Invasive Probe

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Abstract

The purpose of this study was to assess hydroalcoholic *Emblica officinalis* fruit extract cream on human skin trans-epidermal water loss (TEWL). Ten male volunteers were examined in this study. Measurements were made on cheeks. Creams with different concentrations of ABIL® EM 90 and liquid paraffin containing 3% hydroalcoholic Emblica officinalis fruit extract were developed and tested for stability studies for a period of 28 days. Selected cream and its base were further assessed in different conditions at 8 ℃ (in refrigerator), 25 ℃ (in incubator), 40 ℃ (in incubator) and 40 ℃ (in incubator) with 75% relative humidity (RH) for color, odor, thickness, grittiness liquefaction, phase separation, and pH at different time intervals. Trans-epidermal water loss (TEWL) was monitored every week by non-invasive bio-instrument Tewameter MPA 5. All measurements were made statistically by ANOVA and t-paired test. Significant decrease on skin TEWL was produced.

Keywords: Hydroalcoholic *Emblica officinalis* fruit extract, Facial cream, Biophysical parameter

1. Introduction

Human skin is the largest and the outermost organ responsible for regulatory and multiple defensive functions. The skin barrier function resides almost entirely in the epidermis the skin superficial layer – stratum corneum (SC) lies in epidermis particularly plays important role of skin barrier function [1]. This skin barrier function reflects the ingression of xenobiotics and egression of water residing in the outer 15μm [2]. It is not only responsible for control of trans-epidermal water loss (TEWL) but also has influential resistant of skin reactivity to external factors [3]. This skin barrier stops the organism from loss of vital components such as ions, water and serum proteins but it is not completely impermeable for directly applied chemicals on the skin surface. This phenomenon is used in topical dermatological therapy especially important for researchers [1].

Emulsions are a unique group of Skin care formulations, because they must fulfill the exacting criteria, attractive appearance to the original formulations, retain this appeal during storage, give an acceptable feeling during use and, most essentially, provide long term agreeable effects to the skin properties [4]. Emulsions are disperse systems comprising two insoluble or slightly soluble phases. The phase with larger amount is called external or continuous phase and the smaller amount phase is called internal or dispersed phase. Emulsions are of different categories according to the size of dispersed phase: (i) macroemulsion, (ii) microemulsion, (iii) colloid and (iv) latex [5]. The formation, stability, structure and rheology of emulsions have been subjects of recent years [6].

Emblica officinalis Gaertn (Euphorbiaceae), common name Amla, grows in tropical and subtropical parts of China, India, Indonesia and the Malay Peninsula. All parts of the plant are used for medicinal purpose. The fresh (or) the dry fruit is used in traditional medicines for the treatment of diarrhea, jaundice, fever and

inflammations, the pulp of the fruit is smeared on the head to dispel headache and dizziness and leaves are for fever and inflammatory treatments by rural populations in its growing areas. It has been repoted that plant has potent anti-microbial anti-oxidant, adaptogenic, hepatoprotective, anti-tumour and anti-ulcerogenic activities in the fruits of *Emblica officinalis* [7]. Emblica officinalis has long been reported to treat a number of disorders like, stomach ulcers, inflammatory diseases, to inhibit tumor growth, liver diseases, in diabetes, and geriatric complaints. *Emblica officinalis* has also been reported to use for skin disorders and beauty care [8].

The target of this study is to formulate agreeable, acceptable and pleasant cream of hydroalcohilic *Emblica officinalis* fruit extract with assessment of trans-epidermal water loss (TEWL) by a non-invasive bioinstrumentation.

2. Materials and Methods

2.1 Preparation of Hydro-Alcoholic Extracts

Air dried and crushed by grinder of *Emblica officinalis* (1 Kg) was macerated in hydro alcoholic mixture (1 Litre) for 48 hours. The materials were shaken after 12 hours for 10 minutes. The macerated material was filtered through several layers of muslin cloths for course filtration and then whatman No.1 filter paper. The filtrate so obtained was evaporated under reduced pressure at 40 ℃ in a Rotary vacuum evaporator. The process of evaporation was continued till concentrate was reduced to one third of the starting volume. The Brownish colored extract so obtained was collected in stoppered glass tubes and stored in refrigerator until used.

2.2 Preparation of Creams

Our approach was to prepare W/O emulsion by the combination of aqueous phase to the oily phase with continuous agitation.

2.2.1 Preparation of Base

Oily phase comprising of paraffin oil (16%) and ABIL®-EM 90 (4%) and then aqueous phase comprising distilled water (QS) were weighed carefully and heated separately up to 75 ℃ at the same time. After heating, aqueous phase was mixed to the oily phase drop by drop with continuous stirring by the mechanical mixer at 2000 rpm for 15 minutes until complete aqueous phase was added; 2 to 3 drops of fragrance 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.

2.2.2 Preparation of Formulation

Oily phase comprising of paraffin oil (16%) and ABIL®-EM 90 (4%) and then aqueous phase comprising distilled water were weighed carefully and heated separately up to 75 ℃ at the same time. During this, *Emblica officinalis* fruit extract (3%) was added in it. After heating, aqueous phase was mixed to the oily phase drop by drop with continuous stirring by the mechanical mixer at 2000 rpm for 15 minutes until complete aqueous phase was added; 2 to 3 drops of fragrance 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.

2.3 Study Design

For application of formulation, 10 male volunteers were selected whose ages were in between 25 and 35 years in this work. Each Volunteer was examined for any serious skin disease or damage especially on cheeks and forearms. Each volunteer was provided with a volunteer protocol before the study for authentication. No volunteer had knowledge about the contents of the formulations. Skin tests were performed at 25 ℃ and 40% relative humidity conditions. Before application of formulation a patch test was performed on forearms of the volunteers for 24 hours to check any irritation in the formulation. Each volunteer on the second day was provided with formulation and volunteers were instructed properly about the application of formulation. Each individual was instructed to come for measurements of readings for skin TEWL every week.

2.4 Mathematical and Statistical Analysis

The percentage changes for the individual values of biophysical parameter, taken every week, of volunteers were calculated. The measured values obtained for skin TEWL effects were analyzed using SPSS 12 on the PC computer (ANOVA) for variation between different time intervals and level of significance was 5 %.

3. Results and Discussion

3.1 In-Vitro Evaluation of Creams

Stability of the formulation was evaluated using different conditions of storage i.e.8 ℃, 25 ℃, 40 ℃ and 40 ℃ ± 75 (relative humidity). In this study no liquefaction and phase separation were observed in the formulation samples throughout the study period of 28 days even at elevated temperatures. Abil ®EM 90 is a lipophilic surfactant and it has been found that lipophilic surfactants are more stable at elevated temperatures. Physical characteristics of cream such as color, appearance, thickness and grittiness were observed during this study period. Furthermore pH of the formulation was 4.49, considered as normal skin pH range i.e.4 to 6.5[9].

3.2 In-Vivo Characterization of the Formulation for TEWL Effects

The intact skin reflects a functional barrier to the uncontrolled loss of water and other materials from the organism [10]. Skin has a special composite structure and its epidermal barrier, stratum corneum, is the rate-limiting unit for the permeation of exogenous substances. The permeability barrier status is affected by various external and internal factors like climatic conditions, physical stressors, and concerned skin and systemic diseases. Today, different non-invasive approaches are used to monitor the skin barrier physical properties and internal factors such as climate, physical stressors, and a number of skin and systemic diseases. Today, different non-invasive approaches are used to monitor the skin barrier physical properties in vivo. The quantification of biophysical parameter trans-epidermal water (TEWL) loss is crucial for the fundamental examination of the epidermal barrier status [1]. TEWL changes are concerned with stratum corneum water binding capacity [11]. Healthy stratum corneum typically has a water content of 10–20%. Increasing stratum corneum hydration can progressively reduce its barrier efficiency. Stratum corneum is extremely hygroscopic: it can pick up 500% of its dry weight in less than 1h following immersing in water, swelling vertically to 4–5 times its original width [12]. In this study, it was found that there was increase in TEWL values after the application of base during 2nd, 6th and 8th week and decreased during 1st, 3rd and 4th week and after formulation there was increase in TEWL after 6th and 8th week but decrease in 1st, 2nd, 3rd and 4th week of study (Figure 1 & 2). With the help of ANOVA test, it was found that changes in TEWL produced by formulation and base were significant during whole the study of 8 weeks by applying LSD test, it was found that the change in TEWL is significant on 3rd week in case of base. While in case of formulation the change in TEWL is significant on 1st week. With the help of paired sample t-test it was

found that there was insignificant variation in TEWL with respect to base and formulation during whole the study period of 8 weeks.

Decrease in TEWL contents in first four weeks in case of formulation are due to the presence of ascorbic acid in the Emblica officinalis. More recently, the role of ascorbic acid in the formation of stratum corneum barrier lipids has been discovered [13]. The data obtained show that the Emblica fruit contains ascorbic acid (0.40%, w/w), and that the Ayurvedic method of processing enhances the healthy characteristics of the fruit thanks to a higher content of ascorbic acid (1.28%, w/w) [14]. Ascorbic acid concentration in total skin ranges from 0.4 to 1 mg/100 g of wet-tissue weight. Ascorbic acid is distributed in all layers of the skin. Topical application of ascorbic acid provides photo-protection and prevents inflammation and UVB-induced immune-suppression [13].

Studies have also reported that antioxidant approaches prevent damage to stratum corneum lipids and proteins which are directly in contact to pro-oxidative climatic conditions. Moreover, some applied antioxidants gather in the outermost layer of epidermis, stratum corneum, play an important role in restoring the permeability barrier of skin and against UV-induced photo damage in skin [15]. Significant results obtained after topical application of formulation demonstrates the effectiveness of the formulations topically applied against lipid peroxidation on human stratum corneum and it is because of *Emblica officinalis* contain a profile of potent antioxidants such as low molecular weight (<1000) hydrolyzable gallotannins comprising emblicanin A, emblicanin B, punigluconin and pedunculagin, isolated from solvent extracts of Emblica fruits [16].

4. Conclusion

Significant TEWL effects related skin barrier function was observed in Emblica officinalis fruit extract cream after application by non-invasive instrumentation, Tewameter. Above mentioned results seems to promote the benefits of Emblica. The Formulation was observed to decrease TEWL significantly which shows that the formulation has anti-wrinkle affects. Since Transepidermal water loss is involved in aging so this formulation can be used as anti-aging product.

5. Conflict-Of-Interest Policy

Authors do not have any commercial affiliations, or potential conflicts of interest associated with this work submitted for publication.

6. Ethical Standards

In this study, the clinical research using human subjects was conducted in accordance with the ethical considerations for human subjects approved by Board of Advance Studies and Research. Prior to the tests, the volunteers were examined by a dermatologist for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was signed with a volunteer protocol stating the terms and conditions of the testing. Volunteers were not informed about the contents of formulations. All the skin tests were done at 25 ℃ and 40% relative humidity conditions. On the first day, patch test (i.e. skin sensitivity test) was performed on the forearms of each volunteer to determine any possible reactions to the topical cream.

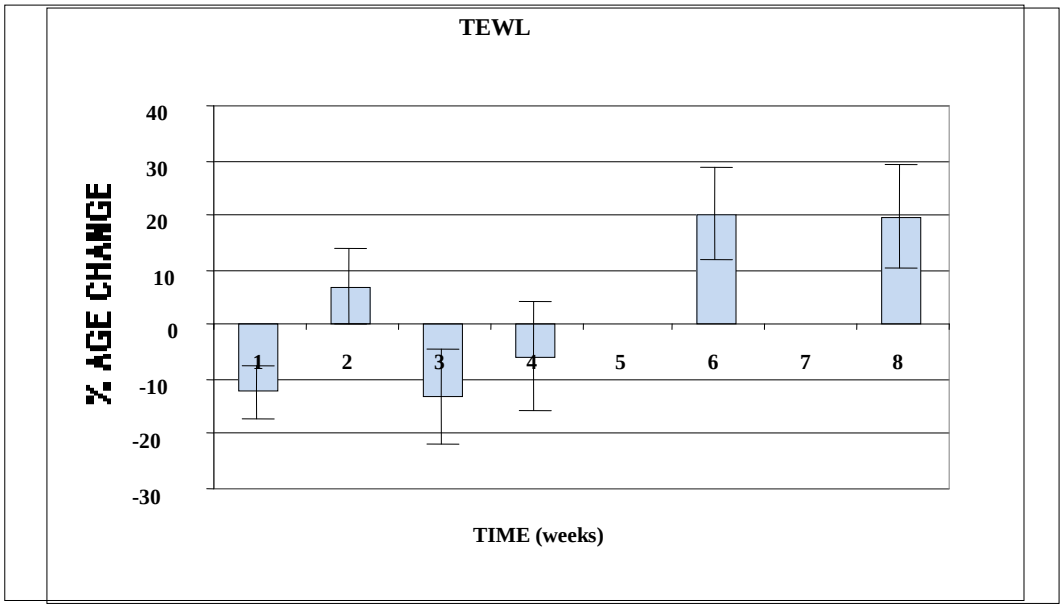

Fig 1: Percentage change in values of trans-epidermal water loss after application of base

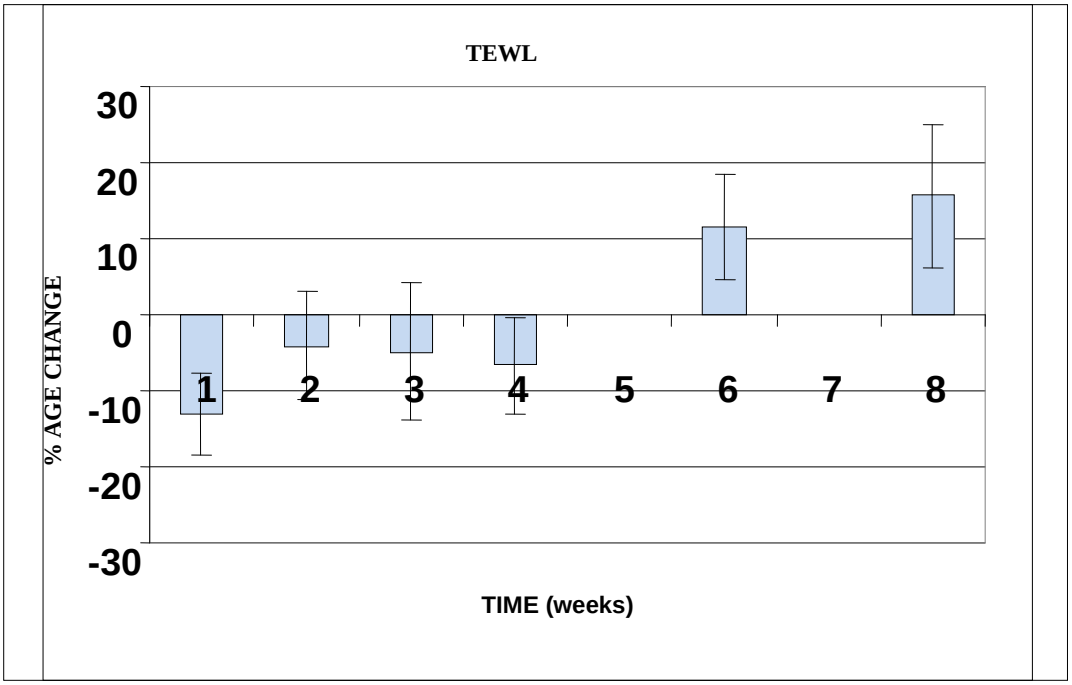

Fig 2: Percentage change in values of trans-epidermal water loss after application of formulation

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