# *Hippophae rhamnoides* oil-in-water (O/W) emulsion improves barrier function in healthy human subjects

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**Abstract**: This study aimed to investigate the changes in skin barrier function in human subjects, following long-term topical application of *Hippophae rhamnoides* oil-in-water (O/W) emulsion whereas effects were measured using non-invasive probes like tewameter and corneometer.

For this purpose, two stable oil-in-water (O/W) emulsions were formulated one with 5% *Hippophae rhamnoides* extract and other without extracts. Thirteen healthy, male subjects with a mean age  $27 \pm 4.8$  years were enrolled after their informed consents. The subjects were instructed to apply either the active formulation or the base formulation over 84 days while they were not known with the contents of either formulation. Biometrological measurements of skin hydration and transepidermal water loss (TEWL) were performed on both sides of the face in each volunteer at baseline and on day 07, 14, 21, 28, 42, 56, 70 and 84.

The statistical analysis revealed formulation with 5% plant extract was superior compared to placebo (base formulation) as formulation with extract have shown extremely significant improvements in skin hydration (p=0.0003) and TEWL (p=0.0087) throughout treatment course. Moreover, a significant (p $\leq$ 0.05) correlation between the active formulation and the improvement of the skin barrier functions was observed. The active formulation found to be superior to that of placebo.

Results affirmed that future studies are necessary to clinically evaluate the active formulation hence it can be proposed that *Hippophae rhamnoides* emulsion could be an alternative pharmacological tool in treating barrier compromised conditions of skin.

Keywords: Corneometer, Tewameter, Hippophae rhamnoides.

### INTRODUCTION

Botanical extracts have been used by cosmetic science in order to beautify and preserve the physiological poise of the human skin. Various plant products depicted in scientific literature showing diverse actions on the skin, such as moisturizing (Futrakul, Kanlayavattanakul, Krisdaphong, 2010; Akhtar et al., 2011), antioxidant (Jorge et al., 2011), sunscreen (Kale et al., 2010) and depigmentation (Nguyen and Kim, 2011). The cosmetic formulation with high amounts of botanical extracts is characterized as a market fashion in cosmetics and perfumery. This reality is a result of both the consumer search for natural products with confirmed efficacy as well as the economic curiosity of the industry for such components. The pleasing features of cosmetic ingredients are safety, efficacy, innovation, formulation stability, easy metabolism in skin, and cost effectiveness, which has augmented the demand and use of herbal cosmetics (Ashawat et al., 2009). Moreover, in contrast to synthetic cosmetic formulations, products with plant botanicals are placid and bio-degradable, as well having a low toxicity (Chanchal and Swarnlata, 2008). Skin Aging is a complex phenomenon that arises as a result of numerous factors. To avoid aging, it is indispensable to

protect the skin from damage. Pre-cautions should be taken against environmental factors. Products with sun block activity should be used on regular basis. Loss of moisture in the stratum corneum (SC water content) should be prevented and the skin moisture level should be restored. The enhance stratum corneum moisture level provides a smoother and more even appearance of skin. Moisturizers shield the skin against external effects by forming a thin film on the skin (Hekimoglu, 1997).

Development in biometrological probes have made it easier to determine the changes in skin barrier function non-invasively. The current study has been aimed to assess the changes in skin barrier function following twelve weeks treatment with *Hippophae rhamnoides* loaded (O/W) emulsions.

#### MATERIALS AND METHODS

#### **Materials**

For the formulation of emulsions (used in the study) *Hippophae rhamnoides* berries were taken from Pak Sea Buckthorn International Skardu, Pakistan. The identification of berries was performed at Chulistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. The specimen was deposited in the Pharmacognosy section of Faculty of Pharmacy.

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Paraffin oil with dynamic viscosity of 110-230 mPas at 20°C and kinametic viscosity of 34.5 mm<sup>2</sup>/s at 40°C, was obtained from Merck (Germany). Stearic acid, Tween80, Span20, Bees wax and Cetomacrogol were taken from Merck Germany. The apparatus employed were Corneometer MPA 5 and TEWA Meter MPA 5 (Courage + Kazaka, Germany).

### Preparation of emulsions

In this study, O/W emulsions were formulated by the continuous addition of aqueous phase to the oily phase after heating both the phases on water bath at 75°C. Few drops of sea blue were used as fragrance. Composition of emulsion is summarized in table 1. These emulsions were found stable after assessing for pH, centrifugation (for phase separation), electrical conductivity, temperature stability tests at  $8\pm0.1^{\circ}$ C,  $25\pm0.1^{\circ}$ C,  $40\pm0.1^{\circ}$ C and  $40\pm0.1^{\circ}$ C with 75% Relative Humidity, Physical nature i.e. color, creaming and liquefaction and rheological characteristics.

 Table 1: Composition of emulsions (% w/w)

Sr. No.	Composition	Base	Active Formulation
01	Liquid Paraffin	24	24
02	Stearic Acid	5	5
03	Span 20	2	2
04	Bees wax	7	7
05	Cetomacrogol	5	5
06	Tween 80	6	6
07	Plant Extract	Nil	5
08	Preservative	1	1
09	D/W	50	45

### Subjects

In this study 13 healthy subjects (Mean age  $27 \pm 4.8$ ) were enrolled after their informed consents. All volunteers completed the study successfully. None of them has pathological condition on their cheeks specified for applying the emulsions. They were sufficiently informed about the use of products. Moreover, they were informed not to use any other skin products specially moisturizers throughout the study period of 12 weeks. Furthermore they were requested not to alter their dietetic practice during the study to abolish the effects of such changes on study results.

# Study Design

Mono-centric, placebo controlled study was conducted to assess the effects of newly formulated O/W emulsions for the improvement in skin epidermal function (Moisturizing effects). This study was conducted during the months of August-December and an expert investigator ensured the proper handling of biometrology measurements, allowing the experimentation with uniform results. Tests were carried out on right and left cheeks of volunteers.

### Ethical considerations

This study was approved by the Board of Advance Studies and Research (BASR) and institutional ethical review committee in accordance to the principles of Helsinki Declaration and was consistent with Good Clinical Practice guidelines (No.942/Acad). All volunteers were included in this study after taking written informed consents.

### Instrumental measurements

Bio-metrologically, Corneometer and Tewameter with noninvasive probes were used in this study for the assessment of skin epidermal function. The Corneometer® (Courage & Khazaka, Germany) measures the electrical capacitance of the stratum corneum expressed in arbitrary units (a.u) ranges from 0-120 a.u (Mahmood and Akhtar, 2012). The Corneometer can evaluate the hydration level of the stratum corneum to a depth of about 0.1 mm. The probe was applied to the skin and the results were shown digitally in arbitrary units within 3 seconds of applying the probe. Tewameter® (Courage & Khazaka, Germany) measures the transepidermal water loss (TEWL) which is based on the diffusion in an open chamber and is measured as  $g/m^2/h$ .

# Mathematical analysis

Percent changes indicate the difference from baseline values at different time intervals. It can be calculated by the following formula

Change (%) = 
$$[(Tx - T0)/T0] \times 100$$

Where

T0 = Baseline values.

Tx is the value obtained at the dermatological tests on day 07, 14, 21, 28, 42, 56, 70 and 84. Experimental error is expressed in the as the standard error of measurement (SEM).

# STATISTICAL ANALYSIS

The measured values were analyzed using statistical Graphpad prism software version 5. Paired sample t-test for variation between the active formulation and base. ANOVA with Dunnett Multiple Comparisons Post Test was used to find the correlation between baseline values compared with different time intervals values. P-values of less than 5% were considered statistically significant while P<0.01 considered for very significant effects.

#### Single application closed patch test

A 5 × 4 cm region was marked on the inner forearms. Area of the inner forearm specified for the test was shaved 4 hour before baseline assessment and before application of formulations. Right forearm was specified for base while left forearm was specified for active formulation. A small amount of each formulation was applied, wrapper it with cotton bandage (like patch), covered with adhesive tape. After 24 hour, the patches were detached, and the skin was gently cleansed with normal saline. After an hour, COLIPA visual scoring method (CVSM) was used for inclusion/exclusion of volunteers on the basis of sensitivity (Basketter *et al.*, 1995). COLIPA grading is shown in table 2.

**Table 2**: Grading for irritant patch test

Grading	Description of skin response	
+4	Intense erythema	
+3	Intense erythema with edema	
+2	Erythema	
+1	Mild erythema	
0.5	Doubtful erythema	
0	No visible reaction	

## RESULTS

None of the 13 volunteers described any sensitivity or side effects following the patch test. The percent changes for the epidermal function parameters following 12 weeks treatment with placebo and active formulation of *Hippophae rhamnoides* along with statistical analysis, have been shown in fig. 1 and 2.

In case of TEWL, when we checked placebo side of the cheek, it was noticeable that placebo treatment has shown mean initial decrease in TEWL i.e. -4.66 % after 1<sup>st</sup> week and the effect remained uncertain after 12<sup>th</sup> week up to -0.93%, with a maximum mean reduction in the 10<sup>th</sup> week i.e. -4.89% compared to baseline TEWL values of 13 volunteers.

Different to placebo treatment effects, some excellent improvement in barrier function has been observed after treatment with *Hippophae rhamnoides* containing emulsion i.e. -7.04% mean initial reduction in TEWL after 1<sup>st</sup> week treatment, -12.26% reductions after 1 month, and -18.77 % reductions in TEWL at the end of study period to that of baseline values. When one way analysis of variance, ANOVA (Kruskal-Wallis test) applied, there was significant (P<0.0001) different between active formulation and base. When Dunn's multiple comparison test applied there were insignificant (P<0.05) results between various time intervals.

In case hydration level of the *stratum corneum*, when we checked placebo side of the cheek then it was noticeable that placebo treatment has shown very slight initial increase in hydration level i.e. 5.5% after 1<sup>st</sup> week and the effect remained gradually increased till 12<sup>th</sup> week up to 7.10%, compared to baseline hydration level of 13 volunteers. When analysis of variance, ANOVA (Kruskal-Wallis test) applied, there was significant (P<0.05) different between active formulation and base. When Dunn's multiple comparison test applied there were insignificant (P<0.05) results between various time intervals except the 1<sup>st</sup> and 3<sup>rd</sup> week, 1<sup>st</sup> and 10<sup>th</sup> week where significant results obtained.

### DISCUSSION

Aging of the skin is a complex phenomenon that happens as a result of numerous factors. To stop aging, it is necessary to guard the skin from damage. Protection should be taken against environmental factors. The moisture of the skin should be restored by preventing the loss of moisture in the stratum corneum layer. The increase in the moisture of stratum corneum supplies a smoother and more even look. Moisturizers protect the skin against external effects by forming a thin film layer on the skin (Ozgen OZER, 2009).

Trans-epidermal water loss is the outward diffusion of water through the skin. An increase in TEWL indicates a damaged water barrier. TEWL analysis allows parametric assessment of the effect of barrier emulsions against irritants and description of skin functionality. The barrier functions restoration ability of Hippophae rhamnoides containing emulsion may be attributed to its polyunsaturated fatty acid (linoleic acid) as it is described in the literature that oils containing linoleic acid are believed good for reducing TEWL and restoring skin barrier function (Barkat et al., 2011). In case of reduction of TEWL by base (placebo), the Paraffin oil may be attributed as it is described that mineral oil (paraffin oil) reduces TEWL by as much as 30% (Naveed et al., 2012). A regular increase in skin moisture was found after application of the Hippophae rhamnoides containing emulsion throughout 12 weeks of study period. Sharma et al., 2008 and Colven and Pinnell, 1996 reported that Vitamin C have the ability to stimulate dermal fibroblasts for the synthesis of collagen, as the collagen intensity is increased, the hydration level also enhanced (Sharma et al., 2008; Colven and Pinnell, 1996). As the vitamin C content of Hippophae rhmanoides varies from 28 to 2500 mg/ 100 g so the emulsion produced a significant increase in hydration level of the stratum corneum (Barkat et al., 2011). In case of increase in hydration level of the stratum corneum by base (placebo), the mineral oil (Paraffin oil) may be attributed as it is described in the literature that vehicles like mineral oils (paraffin oil) exhibit occlusive softening effect on the human skin (Ozgen OZER, 2009).



**Fig 1**: Percentage of Change in Values of Trans Epidermal Water Loss (TEWL) after Application of Base and Formulation



Fig 2: Percentage of Changes in Values of Stratum Corneum water level after Application of Base and Formulation

# CONCLUSION

Anticipation of skin aging and preventing the changes in the look of skin with aging is the important objectives of topical emulsions development. The use of emulsions which are topically applied and contain plant botanicals that influence the skin's biological function has significantly increase in recent years. Moisturizers are used to keep the moisture of the skin in balance and protect it against external consequences. From this study, it was concluded that the quantitatively measured values of hydration level of the stratum corneum and TEWL following long term treatment with *Hippophae rhamnoides* containing emulsion lead us to conclude that the results are promising enough and future studies are necessary to evaluate these formulations in situations with compromise skin barrier clinically.

## REFERENCES

Akhtar N, Zaman SU, Khan BA, Haji MSK, Ahmad M, Rasool F, Mahmood T and Rasul A (2011). Evaluation

of various functional skin parameters using a topical cream of calendula officinalis extract. *Afr. J. Pharm. Pharmacol.*, **5**: 199-206.

- Ashawat MS, Banchhor M, Saraf S and Sara S (2009). Herbal cosmetics: trends in skin care formulation. *Pharmacogn. Rev.*, **3**: 82-89.
- Barkat AK, Naveed A, Tariq M, Haji MSK, Shahiq UZ, Akhtar R, Muhammad I, Atif A, Salimullah K and Mughal Q (2011). *Afr. J. Pharm. Pharmacol.*, 5: 1092-1095.
- Basketter D, Baverel M and Diembeck W (1995). Cosmetic product test guidelines for the assessment of human skin compatib- ility. In: COLIPA Symposium on Alternatives to Animal Testing, Brussels, pp.110-117
- Chanchal D and Swarnlata S (2008). Novel approaches in herbal cosmetics. J. Cosmet. Dermatol., 7: 89-95.
- Colven RM and Pinnell SR (1996). Topical vitamin C in aging. Clinic. *Dermatol.*, 14: 227-234.
- Futrakul B, Kanlayavattanakul M and Krisdaphong P (2010). Biophysic evaluation of polysaccharide gel from durian's fruit hulls for skin moisturizer. *Int. J. Cosmet. Sci.*, **32**: 211-215.
- Hekimoglu S and Hıncal A (1997). Skin aging and a look at cosmetics. Ankara: Dawn Printing, Turkey, pp.29-40.
- Jorge ATS, Arroteia KF, Lago JC, Rocha DS, Gesztesi J and Moreira PL (2011). A new potent natural antioxidant mixture provides global protection against oxidative skin cell damage. *Int. J. Cosmet. Sci.*, **33**: 113-119.
- Kale S, Sonawane A, Ansari A and Ghoge P (2010). Formulation and *in-vitro* determination of sun protection factor of *Ocimum basilicum* leaf oils sunscreen cream. *Int. J. Pharm. Pharm. Sci.*, **2**: 147-149.
- Mahmood T and Akhtar N (2012). Short-term study of human skin irritation by single application closed patch test: assessment of four multiple emulsion formulations loaded with botanical extracts. *Cutan. Ocular. Toxicol.*, **32**: 35-40.
- Nguyen DH, Lee JE and Kim EK (2011). Identification of depigmenting components from *Nigella glandulifera* Freyn. *Korean J. Chem. Eng.*, **28**: 1070-1073.
- Ozgen O. Anti-Aging Cosmeceutics for Facial Skin Care in Aging. *Turkiye. Klinikleri. J. Med. Sci.*, **29**: 40-43.
- Sharma SR, Poddar R, Sen P and Andrews JT (2008). Effect of vitamin C on collagen biosynthesis and degree of birefringence in polarization sensitive optical coherence tomography (PS-OCT). *Afr. J. Biotechnol.*, 7: 2049-2054.