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Full Length Research Paper

In-vivo study of stratum corneum water content and transepideramal water loss using a newly formulated topical cream

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This study was purposed to determine the effects of newly formulated topical skin-care cream (w/o emulsion) of *Hippophae rhmanoides* versus its vehicle (Base) as control on stratum corneum (SC) water content and transepidermal water loss (TEWL) . Concentrated *H. rhamnoids (sea Buckthorn)* fruit extract was entrapped in the inner aqueous phase w/o emulsion. Newly formulated and previously evaluated base (containing no active material) and a formulation (containing 1% concentrated extarct of *H. rhmanoides)* were applied. Both the base and formulation were applied to the cheeks of 21 healthy human volunteers for a period of 8 weeks. SC water content and TEWL were monitored every week to measure any effect produced by these topical creams. The vehicle (base) showed insignificant (p 0.05) effects whereas the formulation showed statistically significant (p 0.05) decrease in TEWL. SC water content was significantly (p 0.05) increased by the formulation. The newly formulated cream of *H. rhmanoides* fruit extract, applied is suitable for improvement and quantitative monitoring of skin hydration level (SC water content/ moisturising effects) and reducing TEWL in people with dry skin.

Key words: *Sea Buckthorn*, skin, Stratum corneum (SC), water content and transepidermal water loss (TEWL).

INTRODUCTION

Hippophae rhmanoides (Sea Buckthorn) is a deciduous, plant with numerous greenish- yellow flowers and bright orang, globular, ellipsoid fruit which belongs to family Elaeagnaceae (Heber, 2007) . It is native to Europe, India, Nepal, Bhutan, Pakistan and Afghanistan. *H. rhmanoides* shrub is 2 m tall with 2 to 6 cm long leaves (Rizvi et al., 2007) . *H. rhmanoides* juice is an important source of some valuable chemicals such as vitamin C, toco-pherolmacrotrients, organic acids and polyunsaturated fatty acids (Zeb, 2006). *H. rhmanoides* has been used for the treatment of radiation damage, inflammation and burns in Chinese folk medicines (Negi et al., 2005) . *H. rhmanoides* oil extracts have also been used in the treatment of skin disorders such as eczema,psoriasis, lupus erythematosus and dermatosus (Guliyev et al., 2004).

The main advantage of applying topical emulsions is that they increase the solubility and bioavailability of therapeutic drugs as well as the ability to favor the topical transport of hydrophilic solute. Topical emulsions also avoid gastrointestinal environment and first pass effect (Marti-Mestres et al., 2002).

The epidermis especially the *Stratum corneum* is concerned with environmental protection of organism, the dermis together with hypodermis is considered essential for protecting the skin from mechanical stress (Escoffier et al., 1989) . Exposure to ultraviolet (UV) radiations results in skin damage through several mechanisms such

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Table 1. Score given by volunteer to base and formulation on the basis of itching/irritation[a].

Score		0	1	2	3
No of volunteer	Base	16	3	2	0
	Formulation	14	4	3	0

[a]No severe erythema occurred in any of volunteer; mild erythema occurred in 2 and 3 volunteers; moderate erythema occurred in 3 and 4 volunteers, whereas no erythema occurred in 16 and 14 volunteers for both base and formulation, respectively.

as collegenase production, thymine dimer formation and enhancing inflammatory reaction. Antioxidants protect human skin from free radicals produced by UV radiations in the untimely stages of revelation (Bauman, 2005).

MATERIALS AND METHODS

Materials

For the formulation of emulsions /Creams (Applied in the study) *H. rhamnoides* berries were purchased from Pak Sea Buckthorn International Skardu, Pakistan. ABIL-EM90 was purchased from Franken Chemical (Germany) and Methanol, n. Hexane and paraffin oil were purchased from Merk KGaA Darmstadt (Germany). Ethanol was taken from BDH England.

Apparatus

The apparatus used includes Corneometer MPA 5 and TEWA Meter MPA 5 (Courage + Khazaka, Germany), SPSS 12. Corneometer was used for SC water content measurement while TEWA meter was used for TEWL measurement.

Emulsions (Creams)

In this study the products studied were newly formulated W/O emulsions (base and formulation) which were found stable after evaluating for pH, electrical conductivity, centrifugation, phase separation, temperature stability tests at 8 ± 0.1°C (in refrigerator), 25 ± 0.1°C, 40 ± 0.1°C and 40 ± 0.1°C with 75% RH (in incubator) and Physical characteristics, that is, color, creaming and liquefaction.

Study design for product evaluation on skin

One-sided blind study was designed with placebo control in the month of August to September. 21 healthy human volunteers who signed the informed consent, with age range of 20-35 years were selected. Male volunteers were included in this work as they were easily available with regular under control observations. All the skin tests were performed at 21±01°C and 40±2% relative humidity conditions. The experiments were carried out on the cheeks of volunteers as cheeks are uniformly and more prone to UV radiations. On the first day, patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. Each volunteer was provided with two creams. One cream was Base and the other one was formulation containing the active ingredients. Each cream was marked with “right” or “left” indicating application of that cream to the respective cheek. The creams were applied by the volunteers themselves as instructed for 60 days. Every individual was instructed to come on 1st, 2nd, 3rd, 4th, 6th and 8th week for the skin measurements.

Burchard tests (Patch tests)

On the first day of skin testing, patch tests were performed on the both forearms of each volunteer. A 5 X 4 cm region was marked on the forearms. 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 applied to the 5 X 4 cm marked regions separately 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 (Hachem et al., 2002). After 48 h, scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0 to 3.0 stands for absence of erythema, 1 for mild erythema, 2 for moderate erythema while 3 stands for severe erythema.Each volunteer was asked to note their irritation/itching towards the patches and then assign a score from the same scale. Average score with respect to volunteers is given in Table 1.

Panel test

Every individual was provided with a Performa prepared previously to test the sensory values of creams. This Performa consisted of seven parameters to be evaluated and every parameter was assigned 11 values from –5 to +5 indicating very bad to very good, respectively. This Performa was asked to be completed independently by each individual at the end of study period. From the average reply of volunteers it was concluded that base and formulation were felt well on the skin, produced pleasant feeling on application to skin and no irritation on the skin in both cases i.e. base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was more for formulation. This was expected since the formulation contained essential fatty acids. Similarly, the formulation led to more softness of the skin than base.

It was found from paired sample t-test that there was an insignificant difference between the average points of sensitivity for base and formulation. It was concluded that there was no immense variation between base and formulation regarding the sensory evaluation. Both of the creams have similar performance from the sensory of view.

Mathematical analysis

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

Table 2. *Percentage of change in SC water content after application of base and formulation*.

	Values of SC water content (Mean ± SEM)					
Time (week)	**1st**	**2nd**	**3rd**	**4th**	**6th**	**8th**
Base	-0.88±1.57	-3.38±2.03	-6.57±3.56	-2.15±2.56	-3.55±3.90	-2.29±6.63
Formulation	13.16±3.07	22.36±4.17	31.02±4.70	59.69±10.68	61.62±9.43	62.79±12.72

*Percent change values are the average of 21 volunteers calculated by using the formula; Percentage Change = [(A – B) / B]*100. Where, A = Individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th or 8th week; B = Zero hour value of that parameter.

Table 3. *Percentage of Change in Values of transepidermal water loss (TEWL) after application of base and formulation*.

	Values of TEWL (Mean ± SEM)					
Time (week)	**1st**	**2nd**	**3rd**	**4th**	**6th**	**8th**
Base	-5.33±12.25	9.38±13.02	10.68±10.52	15.07±13.65	-0.52±9.17	11.24±8.13
Formulation	-9.66±4.94	-18.89±5.59	-28.68±6.62	-39.61±8.22	-46.31±8.75	-52.03±6.39

*Percent change values are the average of 21 volunteers calculated by using the formula; Percentage Change = [(A – B) / B]*100. Where, A = Individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th or 8th week; B = Zero hour value of that parameter.

2Percentage Change = [(A – B) / B]*100

where A = Individual value of any parameter of 1st, 2nd, 3rd, 4 th, 6th, or 8th week; B = Zero hour value of that parameter.

Statistical analysis

The measured values obtained for SC water content and TEWL were analyzed using SPSS 12.0 on the personal computer (paired samples t-test for variation between the two preparations; two-way analysis of variance (ANOVA) for variation between different time intervals while using a 5% level of significance for both skin parameters.

RESULTS

Stratum corneum (SC)

The percent change occurred in the SC water content before and after applications of base and formulation have been presented in Table 2.

Transepidermal water loss (TEWL)

The percent changes occurred in the values of TEWL before and after applications of base and formulation have been given in Table 3.

DISCUSSION

Stratum corneum (SC) water content

In this study, it was found that there was an irregular decline in SC water content values throughout the study period after the application of base samples. In case of formulation samples, it was found that there was gradual increase in SC water content from the 1st week up to the 8th week of study.

With the help of ANOVA test, it was found that the base produced insignificant (p 0.05) effects on SC water content with respect to time while formulation produced significant (p 0.05) effects on moisture contents with respect to time.

With the help of paired sample t-test, significant (p 0.05) differences were observed between the SC water content of base and the formulation from the 2nd week of study period.

Vitamin C has the advantage of stimulating dermal fibroblasts for the synthesis of collagen. As the collagen level is increased, the hydration level also improved (Sharma et al., 2008; Colven and Pinnell, 1996). The vitamin C concentration in *H. rhmanoides fruit* ranges from 28 to 2500 mg/ 100 g (Zeb, 2006) so the formulation produced a significant (p 0.05) increase in SC water content.

Transepidermal water loss (TEWL)

In this study, it was found that there were variations in TEWL values after the application of base. On the 1st and 6th week it was decreased while on the 2nd, 3rd, 4th and 8th week it was increased. In case of formulation there was gradual decrease in TEWL throughout the study period.

With the help of ANOVA test, it was found that changes in TEWL values produced by base were insignificant (p 0.05) with respect to time. Whereas applying ANOVA test

to the formulation it was concluded that the changes in TEWL values were significant (p 0.05) with respect to time.

With the help of paired sample t-test it was found that there was insignificant (p 0.05) variation in TEWL with respect to base and formulation for the 1st three weeks while from 4^{th} to the 8^{th} week of study significant (p 0.05) differences were observed between the TEWL values of base and the formulation.

Oils having linoleic acid are considered good for reducing TEWL and restoring skin barrier function. *H. rhmanoides* oils made up of about 12.4% linoliec acid (POINT OF INTEREST, 2009). So the formulation reduces TEWL significantly.

Conclusion

In conclusion, a stable topical cream (W/O emulsion) containing *H. rhamnoids* fruit extract can produced a pronounced increase in moisture content of the skin showing that the formulation has skin moisturizing effects. The formulation was observed to decrease TEWL significantly which shows that the formulation has anti-wrinkle affects. Since both of the aforementioned parameters are involved in aging so this formulation can be used as anti-aging product.

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