

Research Article

Anti-Aging Effects of *Hippophae rhamnoides* Emulsion on Human Skin

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Abstract

Purpose: This study aimed to evaluate the effects of topically applied water-in-oil (w/o) emulsion of *Hippophae rhamnoides* using standard R cutometer parameters.

Methods: A w/o emulsion of 1 % hydro-alcoholic extract of *H. rhamnoides* (formulation) and placebo control (base) were used in the study. Eleven healthy male volunteers with a mean age of 24.5 years were selected after obtaining informed consent. The subjects were assigned to blindly use either the formulation or the base for 7 consecutive weeks. The skin mechanical parameters determined with a cutometer, were R0 (first maximum amplitude), R2 (gross-elasticity), R6 (viscoelasticity), R7 (biological elasticity) and R8 (total recovery). In addition, the antioxidant activity of the formulation was evaluated by 1, 1-diphenil-2-picrylhydrazyl (DPPH) method.

Results: Topical application of 1 % organic extract emulsion of *H. rhamnoides* improved most of the biomechanical parameters evaluated when compared to the base group ($p < 0.05$). However, skin extensibility and firmness of the active formulation- and base-treated groups (R0) were not different ($p < 0.01$). Of note, a significant correlation between the active formulation and the improvement of the skin mechanical parameters was observed. The active formulation was found to be superior than the placebo control.

Conclusion: The topical antioxidant emulsion of *H. rhamnoides* significantly improved skin biomechanical parameters after 7 weeks of treatment. The data obtained suggest that *H. rhamnoides* could be an alternative pharmacological tool for treating age-related loss of skin elasticity.

Keywords: Skin elasticity, Skin aging, Emulsion, Cutometer, *Hippophae rhamnoides*

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INTRODUCTION

The gradual physiologic tone-down of human skin may be attributed to the changes that occur with normal aging process. The main age-related changes in human skin include roughness (dryness) and wrinkling [1]. However, the most significant age-related change in human skin is decline in its elasticity [2]. The epidermis, especially the stratum corneum, is responsible for protection of the organism against external environment. In addition, the dermis and the hypodermis, jointly, are considered essential for protecting the skin from mechanical stress [3].

The human skin is rich in elastic fiber network. From the deeper dermis, fibers rise-up toward the dermis, dividing repeatedly, and thus becoming thinner [4]. Exposure to UV radiations results in skin damage through several mechanisms such as collagenase production, thymine dimer formation and enhancing inflammatory reaction. Antioxidants protect human skin from free radicals produced by UV radiations. Free radical activation results in the production of collagenases which cause degradation of collagen by kinase pathways; antioxidants block these pathways, thus preventing photoaging [5].

Decreased skin elasticity is considered to be involved in promoting wrinkle formation [6]. Human skin is viscoelastic in nature, and elastic components include collagen and elastic fibers while the viscous nature of skin is due to friction between cells and collagen bundles [7]. Immediate deformation or strain and delayed strain or distension observed in human skin are considered to be related with actinic aging due to UV radiations. Intraepidermal elasticity is considered to be related to sweat. Age-related changes in collagen fibers are variable, however, and the average network gradually shrinks with advancing age as the net amount of collagen is reduced [8].

Novel topical dermatologic or Cosmeceutical agents are needed to treat cutaneous hyperpigmentation (melasma), acne, inflammation, dry skin and aging since most currently available products typically contain synthetic agents which have significant disadvantages. There is a need to find new compounds from natural sources, specifically, plants that would be capable of countering aging conditions, and developing them into dermatologic/cosmeceutical products. The present study was aimed to evaluate the effect of a cream incorporating *Hippophae rhamnoides* extract on skin biomechanical properties using a suction, non-invasive device.

EXPERIMENTAL

Subjects

A total of 11 male healthy human volunteers with mean age of 24.5 years were enrolled in the study after securing their informed consent. They were free of any skin disease on their cheeks and were restricted from using any other topical agent on the test area during the period of the study. The study was approved by the Board of Advanced Study and Research (BASR) of The Islamia University of Bahawalpur as well as by the institutional ethical committee in compliance with Helsinki Declaration of ethical principles for medical research. The Reference No is 1663.

Extract preparation

500g of the finally grinded *H.rhamnoides* fruit was macerated by a mixture of two liters of analytical grade methanol and Distilled water in a ratio of 1:1 in a Glass beaker. The beaker was shaken for 10 minutes after every 12 hours. The macerated plant material was filtered through 16 layers of muslin cloth for coarse filtration. The coarse filtrate was then filtered through a Whatman # 01 filter paper in order to get particle free extracts and evaporated under reduced pressure at 40 °C in a Rotary vacuum evaporator.

Cream Preparation

Water-in-oil cream (emulsion) was used in this study. The oily phase consisting of 16% paraffin oil and 5 % ABIL[®]- EM 90 (emulsifying agent) was heated up to 70±1°C. The aqueous phase, distilled water was heated to the same temperature and then 1% *H.rhamnoides* extract was added to it. The aqueous phase was then added to the oily phase drop by drop and stirred until all aqueous phase was added; lemon oil was added during this stirring time to give good fragrance. Placebo was also prepared by the same method but contained no *H.rhamnoides* extracts [9].

Antioxidant activity of *Hippopae rhamnoides*

The free radical scavenging activity of *Hippopae rhamnoides* was determined using DPPH (1, 1-diphenil-2-picrylhydrazyl) method [10]. Equal volumes of the (0.5 µL) extract DPPH (6 x 10⁻³M) in absolute ethanol and maintained at room temperature for 20 min. The absorption of the mixture was measured spectrophotometrically (UV-Visible Spectrophotometer 1601, Shimadzu, Japan) at 517 nm. Vitamin C was used as standard. The activity of free radicals was calculated as % inhibition as in Eq 1.

$$\% \text{ Inhibition} = 100(A - C)/A \dots\dots\dots (1)$$

Where A is the absorbance of the extract mixture and C is the absorbance of control.

The free radical scavenging activity of *Hippopae rhamnoides* was 80% in comparison to the standard. It may due to the presence of natural antioxidants in *Hippophae rhamnoides* such as Vitamin C, Flavonoids, Carotenes and Tocopherols [11].

Measurement of skin biomechanical parameters

The equipment used for the determination of skin biomechanical parameters was a non-

invasive skin meter (Cutometer[®] MPA 580, Courage + Khazaka, Germany) which offers four different measurement modes [11]. However, in the present study, we used the measurement mode with constant negative pressure fixed at 350 mbar was used. In this mode, the skin was drawn across the aperture of the 2 mm probe during the measurement period at a constant negative pressure of 350 mbar. Thereafter, the negative pressure was switched off and the skin returned to its original shape. A typical skin deformation curve is presented in Figure 1. The curve illustrates out the viscoelastic features of the skin.

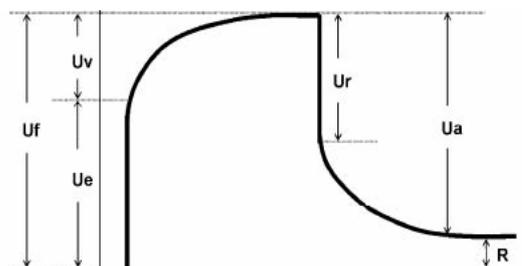


Figure 1: Typical skin deformation curve obtained by the Cutometer.

Ue: Skin extensibility, *Uv:* Delayed distension, *Uf:* Final deformation, *Ur:* Immediate retraction, *Ua:* Total recovery, *R:* Residual deformation at the end of measuring cycle

Study protocol for cream evaluation on skin

A one-sided blind study was designed with placebo control between August and September, 2010. Prior to the tests, the volunteers were examined by a cosmetology expert for any serious skin disease or damage especially on cheeks and forearms. All the skin tests were performed at 21 ± 1 °C and 40 ± 2 % relative humidity [7].

Patch test (Burchard test)

The experiments were carried out on the cheeks of volunteers as cheeks are more uniformly exposed to UV radiations. On the first day, the patch test (Burchard test) was performed on the forearms of each volunteer

to determine any possible reactions to the emulsions. In this test, 5 x 4 cm region was marked on the forearms. The patch (bandage disc) for the right forearm was saturated with 1 g of base while the patch for left forearm was saturated with 1 g of the extract formulation. Each was applied to the marked region separately on each forearm. and covered with surgical dressing after application. The patches were removed after 48 h, the forearms washed with physiological saline [12] and scores recorded for the presence of erythema (skin redness) using a scale of 4 points, ranging from 0 to 3; where 0 stands for absence of erythema, 1 for mild erythema, 2 for moderate erythema and 3 for severe erythema. Each volunteer was asked to note the degree irritation/itching and assign a score from the same scale. The mean scores by the volunteers are given in Table 1. On the third day, each volunteer was provided with two creams. One was the base and the other the formulation containing the extract. Each cream was marked with "right" or "left" indicating application of the cream to the particular cheek. The creams were applied by the volunteers themselves as instructed for 60 days. Each individual was instructed to come back on weeks 0, 1, 2, 3, 6 and 7 for skin measurements.

Skin elasticity measurements

The mechanical properties of the skin were assessed with a Cutometer having a 2 mm probe. Three suction cycles each of 18 s at 350 mBar followed on each occasion by a release cycle of 2 s were applied. In the elastometric evaluation, the skin surface was aspirated from the depression induced by the machine into the 2 mm aperture of the cutometer. The depth of skin penetration by the probe was measured by an optic sensor. Cutaneous elasticity reflects the skin potential capacity (in mm) for retraction. A typical graph (Figure 1) was obtained that shows the deformation curve of skin undergoing suction, and includes the following components: (a) immediate deformation or skin elasticity (Ue), i.e., an elastic component which is related to

the part of curve that ascends rapidly, and is reversible to deformation; (b) delayed distension or visco-elasticity (Uv), i.e., a plastic component, which corresponds to the part of the curve that ascends slowly and is not completely reversible to deformation; (c) final deformation or extension (Uf); (d) immediate retraction (Ur); (e) total recovery or pliability (Ua); and (f) lasting deformation at the end of the measuring cycle (R).

Percentage change in the individual values of R parameters, taken every week, for the volunteers was calculated as in Eq 2.

$$\% \text{ Change} = \{(A - B) / B\} * 100 \dots\dots\dots (2)$$

Where: A is the individual value of any parameter in weeks 1 - 7 and B is zero hour value of the parameter.

Statistical analysis

Two-way ANOVA for variation between different time intervals was performed using the SPSS 12.0 software. Student's t-test was used to determine the significance of differences between the active formulation and the base. $P < 0.05$ was considered as statistically significant.

RESULTS

Patch test

No severe erythema occurred in any of volunteer, mild erythema occurred in 2 and 1 volunteers, moderate erythema occurred in 2 each volunteers, whereas no erythema occurred in 7 and 8 volunteers for both Base and Formulation, respectively.

R0 parameter

Following application of the base (control), R0 values showed a variable tendency to increase and decrease up to the end of the study period of 7 weeks; on the other hand,

following application of the active formulation, R0 values tend to increase regularly, as shown in Figure 2.

Table 1: Score given by volunteer to Base and Formulation on the basis of itching/irritation

Cream type	Score			
	0	1	2	3
	No. of volunteers indicating irritation/itching			
Base	7	2	2	0
Formulation	8	2	1	0

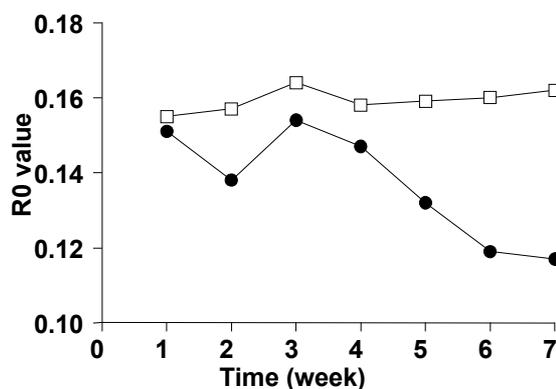


Fig. 2: Mean values of R0 parameter for all volunteers versus time following application of base (●) and active (□) formulations

When ANOVA test was used to analysis the data there was, no statistically significant differences ($p < 0.01$) between active formulation and base. On the other hand, when paired t-test was applied to determine the significance of differences between the active formulation and base, a positive relation was found from week 2 - 7.

R2 parameter

R2 (U_a/U_f or U_a+R) represents the gross elasticity of the skin. The skin is 100 % elastic if R2 reaches 1 [7]. As Fig 3 shows, R2 parameter for the active formulation increased throughout the study period except in week 2. On the other hand, there was irregular variation in R2 values for the base. When ANOVA was applied, insignificant ($p >$

0.05) results were noticeable for base and the active formulation with respect to time. When paired t-test was applied, a statistical significant ($p < 0.05$) relation was found in 2nd, 3rd, 6th and 7th week of the study.

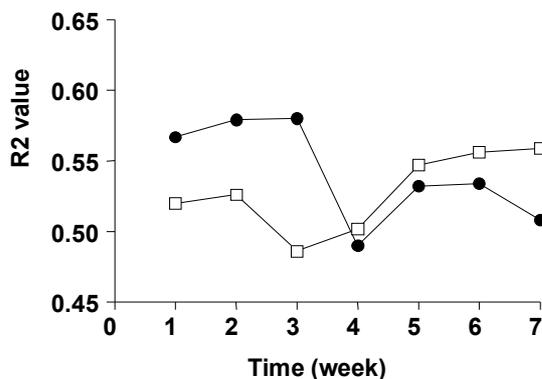


Fig. 3: Mean values of R2 parameter for all volunteers versus time following application of base (●) and active (□) formulations

R6 parameter

R6 (U_v/U_e) is the viscoelastic ratio of the skin, i.e., the ratio between the viscoelastic properties of the skin and immediate distension [9]. Fig 4 shows there was overall decline in the value of R6 parameter for the active formulation over the study period while variable decline occurred for the control (base). When ANOVA was applied, statistically significant ($p < 0.05$) results were evident with the active formulation. When results were compared by paired t-test to determine the significance of differences between the active formulation and base, significant effects were produced by the formulation from the 2nd week upto the end of study period of 7 weeks.

R7 parameter

R7 (U_r/U_f) which is independent of skin thickness, measures the ability of the skin to regain its initial position after deformation. The parameters, R7 and R2, are used as the main parameters to assess skin elasticity and aging [13]. Fig 5 indicates that the active formulation enhanced R7 throughout the

study period except in week 1 unlike the base (control) which exhibited a decrease in R7 value. When ANOVA was applied, statistically significant ($p < 0.05$) results were produced by the formulation while insignificant effects were produced by the base over the time. When paired t-test was applied, a statistical significant ($p < 0.05$) relation was found in 1st, 2nd and 7th week of the study period.

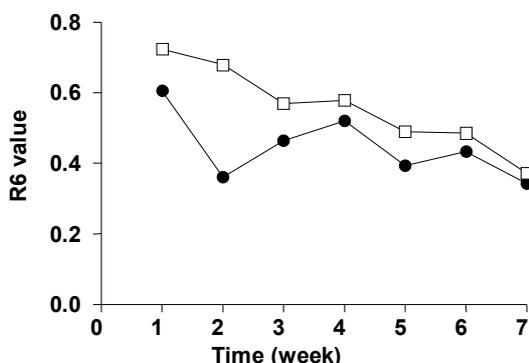


Fig. 4: Mean values of R6 parameter for all volunteers versus time following application of base (●) and active (□) formulations

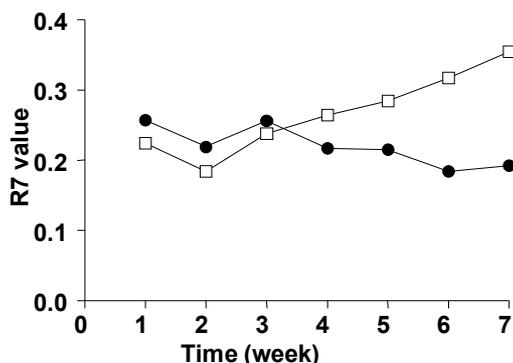


Figure 5: Mean values of R6 parameter for all volunteers versus time following application of base (●) and active (□) formulations

R8 parameter

R8 (Ua) represents viscopart, i.e., the area under the suction part of the deformation curve [2]. Elderly skin usually shows a high R8 value. Fig 6 shows that R8 value

decreased throughout study period except in week 6; in contrast, the base (control) exhibited constant R8 value all through.

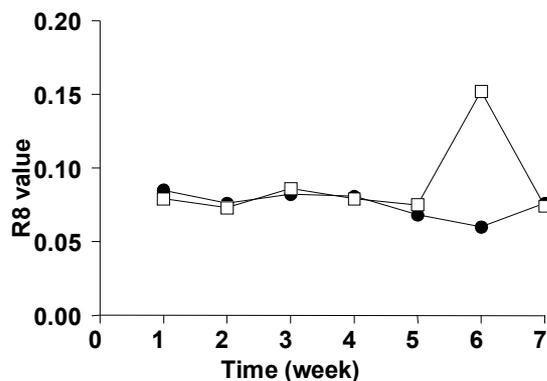


Fig. 6: Mean values of R6 parameter for all volunteers versus time following application of base (●) and active (□) formulations

DISCUSSION

Topically applied emulsions (creams) used on the skin can be helpful for aging skin conditions. Skin is a complex amalgam of tissues whose mechanical characteristics depend on the interdependence of the constituent parts. In the present study, we investigated the effects of topically applied *Hippophae rhamnoides* extract on the mechanical properties of human skin. Overall, the mechanical properties of human skin represented by R parameters were improved, except for R8. These improvements might be due to the presence of antioxidants such as carotene, especially β carotene, and tocopherols. This plays an important role in the prevention of aging as it has been established that seeds and berries of *Hippophae rhamnoides* have sufficient amounts of tocopherols/vitamin E. Furthermore, increase in skin hydration improves viscous resistance against deformation and vitamin C has the advantage of stimulating dermal fibroblasts for the synthesis of collagen [15]. As collagen level is increased, hydration level also improves. Vitamin C concentration in *Hippophae rhamnoides* extract ranges from 28 - 2500 mg/100 g [16].

Another possible reason is the increased expression of cell surface whereby integrins promotes collagen contraction. This mediates interaction between fibroblasts and extracellular matrix proteins present in the dermis [17].

This mechanism may supports the possibility that *Hippophae rhamnoides* extract alters the mechanical properties of skin. Topical application of a *Hippophae rhamnoides* extract caused a significant increase in Uf (final deformation, extension or R0) value and a tendency to increase the Ue (immediate deformation or skin elasticity) value. Ue and Uf values, which represent skin distensibility, declined significantly in proportion to aging. These results show that *Hippophae rhamnoides* extract promotes improvement in skin mechanical properties against aging.

For skin measurements, this work also indicates that cutometer can be used with high reliability on normal skin for testing topical emulsions, and that cutometer alone is sufficient for the evaluation of skin elasticity.

CONCLUSION

Our findings suggest that Cutometer is a valuable piece of equipment for the evaluation of effects of topically applied formulations on skin mechanical properties. Furthermore, topical application of *Hippophae rhamnoides* extract demonstrated significant improvement in facial skin mechanical parameters, indicating that the extract possesses anti-aging characteristics.

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REFERENCES

1. Yoon HS, Baik SH, Oh CH. Quantitative measurement of desquamation and skin elasticity in diabetic patients. *Skin Res Technol.* 2002; 8: 250-254.
2. Dobrev H. Use of Cutometer to assess epidermal hydration. *Skin Res Technol.* 2000; 6: 239-244.
3. Escoffier C, Rigal J, Rochefort A, Vasselet R, Leveque JL, Agache PG. Age-related mechanical properties of human skin: An in vivo study. *J Invest Dermatol.* 1989; 93: 353-357.
4. Montagna W, Carlisle K. Structural changes in aging human skin. *J Invest Dermatol.* 1979; 73: 47-53.
5. Baumann L. How to prevent photoaging. *J Invest Dermatol.* 2005; 125: 56-62.
6. Fujimura T, Haketa K, Hotta M, Kitahara T. Loss of skin elasticity precedes to rapid increase of wrinkle levels. *J Dermatol Sci.* 2007; 47: 233-239.
7. Wissing SA, Muller RH. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity-in vivo study. *Euro J Pharm & Biopharm.* 2003; 56:67-72.
8. Takema Y, Imokawa G. The effects of UVA and UVB irradiation on the viscoelastic properties of hairless mouse skin in vivo. *Dermatol.* 1998; 196: 397-400.
9. Barkat AK, Naveed A, Tariq M, Mughal Q, Shahiq UZ. Formulation and pharmaceutical evaluation of a w/o emulsion of *Hippophae rhamnoides* fruit extract. *J Pharm Res.* 2010; 3: 1342-1344.
10. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 1958; 181: 1199 – 1200.
11. Cua A B, Wilhelm K P, Maibach H I. Elastic properties of human skin: relation to age, sex, and anatomical region. *Arch Dermatol Res.* 1990; 282: 283-288.
12. Barkat AK, Naveed A, Tariq M, Haji MS, Mughal Q, Tariq S. Effects of Antioxidants and Flavonoids of Sea Buckthorn on Skin Whitening and Skin Erythema. *Asian J Chem.* 2011; 23: 903-906.
13. Akhtar N, Khan BA, Mahmood T, Parveen R, Qayum M, Anwar A et al. Formulation And Evaluation Of Anti Sebum Secretion Effects Of Sea Buckthorn W/O Emulsion. *J Pharm Biol Sci.* 2010; 2: 13-17.
14. Sungyeon A, Seunghun K, Haekwang L, Seongjoon M, Ihseop C. Correlation between a Cutometer® and quantitative evaluation using Moire topography in age-related skin elasticity. *Skin Res Technol.* 2007; 13: 280-284.
15. Draaijers LJ, Botman YAM, Tempelman FRH, Kreis RW, Middelkoop E, Zuijlen P PM. Skin elasticity meter or subjective evaluation in scars: a reliability assessment. *Burns.* 2004; 30: 109-114.
16. Zeb A. Chemical and Nutritional constituents of Sea Buckthorn juice. *Pak J Nutr.* 2006; 3: 99-106.

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17. *Tsutomu F, Kkazue T, Sshigeru M, Ttakashi K, Ttomohiko S, Yyoshinori T. Treatment of human skin with an extract of Fucus vesiculosus hanoesi ts thicknessa nd mechanical properties. J Cosmet Sci. 2002; 53: 1-9.*