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*Full Length Research Paper*

# Moisturizing effect of cream containing *Moringa oleifera* (Sohajana) leaf extract by biophysical techniques: *In vivo* evaluation

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**The purpose of this present investigation is to evaluate the moisturizing effects of cosmetic creams containing extract of *Moringa oleifera* leaves (Sohajana) by non-invasive bioengineering techniques. The study was conducted during the winter months (October to January). A total of eleven healthy male volunteers with ages between 20 and 35 years contributed to accomplish this single blind study. The active cream and base were applied twice daily to the face (cheeks) for a period of 12 weeks. The instrumental measurements were carried out with tewameter and corneometer under a draught-free room, with controlled temperature (18.0 to 20.6°C) and relative humidity (55 to 65%). The results recommended that the assessed outcome was very well accepted, and improved skin hydration and skin barrier function by the test product. There was a significant decrease in skin transepidermal water loss (TEWL) and increase in skin hydration achieved when ANOVA and paired t-sample test were applied.**

**Key words:** *Moringa oleifera*, extract, cream, corneometer, tewameter, moisturizer.

## INTRODUCTION

Approximately every human being faces dry skin during life. There are occasional episodes in many peoples, and chronic difficulty like xerosis, that is, itching, happens in some people. Dry skin represents dry, scaly or rough look with the possible presence of cracking, reddening or itching and less flexibility than normal skin (Flynn et al., 2001). Human skin retains its appearance and function by equilibrium between the water content of stratum corneum and surface lipids (Dal' Belo et al., 2006). The skin acts has a distinctive barrier to the entrance of hazardous materials and prevents loss of water (Hadgraft and Lane, 2009).

The "bricks and mortar" model advocates that it functions as an active membrane. Water barrier function smashes up when intercellular lipids misplaces ensuing repairment of stratum corneum triggers to initiate. Intercellular lipids contain ceramides, fatty acids and cholesterol (Iwaskai, 2010).

This barrier function of skin is disturbed by various external factors such like that ultraviolet radiation, air humidity and hormones. Numerous soaps, topical irritants and detergents may also disrupt the skin surface lipids. Consequently, dry skin is particularly observed in atopic dermatitis. A healthy stratum corneum naturally has a water content of 10 to 20% (Dal'Belo et al., 2006). Ultra violet radiation acts as an initiator for several skin disorders like wrinkling, scaling, dryness, mottled pigment abnormalities including, photo-aging, hypopigmentation, hyperpigmentation, and skin cancer; though many environmental and genetic factors contribute to the development of various skin diseases (Nichols and katiyar, 2010). Reactive oxygen species (ROS) are

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generated and can mediate damage to cellular proteins, lipids and saccharides, and eventually possible production of various skin disorders (Neelum et al., 1999). UVA (ultra violet A) can produce structural damage to DNA, impair the immune system, and lead to cancer. UVB (ultra violet B) generates direct and indirect undesirable biological consequences: initiation of ornithine decarboxylase activity, the production of pyrimidine photoproducts, motivation of DNA synthesis, cell cycle growth arrest, free radical formation in the skin, photoaging and photocarcinogenesis. It provokes free radical production and induces a significant decrease in skin antioxidants, impairing the skin's ability to protect itself against the free radicals generated after sunlight exposure (Alena and Daniela, 2006; Masamitsu et al., 2000).It is perceptive that the topical application of antioxidants may neutralize some of the resulting free radicals, and consequently lessen or prevent destruction of skin barrier function, dryness and other related problems by absorbing UV radiation and act as a sunscreen (Nichols and katiyar, 2010). The antioxidant activity of herbal phenolics, namely phenolic acids and flavonoids has attained considerable attention (Alena and Jitka, 2003). Moisturizers containing antioxidants and phenolic compounds are the bastion of management for dry skin, daily safeguarding of normal skin, and adjunctive therapy related many skin diseases (Dal'Belo et al., 2006). However, the moisturizing treatment engrosses: (1) enhance water content; (2) decrease TEWL; (3) refurbishing the skin barrier; and (4) reinstating the lipids' ability to attract, grasp and redistribute water (Flynn et al., 2001).

*Moringa oleifera* (Moringaceae) is well known as kelor, benzolive, marango, mlonge, mulangay, sohajana, saijhan, drumstick tree, nébéday and sajna. It is a pantropical specie (Iqbal and Bhanger, 2006). Bioactive compounds such as carotene, vitamin C, vitamin B, vitamin A, phonolics, caortenoids etc, have been reported. Its leaves are used as purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh (Anwar et al., 2007). However, action of *M. oleifera* leaves as moisturizing effects is scarce.

Experimental research to assess the moisturizing effect of *M. oleifera* leaf extracts are obligatory to endorse. Objective methodologies are considered suitable to establish and to elucidate the mechanisms of action of substances that progress skin hydration. Among these are non-invasive skin bioengineering techniques, which are often employed as they permit assessment of cosmetic products under authentic conditions of use.

## MATERIALS AND METHODS

### Materials

*M. oleifera* leaves were gathered during July 2010 in Dera Ghazi Khan, Pakistan, and air dried at room temperature for a period of 4 weeks.

Abil EM90 was procured from Franken Chemicals Germany, Paraffin oil from Merck Germany, Methanol and Phosphoric acid from BDH England. Deionized water was obtained from the Pharmaceutical Labs of Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

### Identification of plant

The identification of the plant (*M. oleifera*) was executed at the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. The specimen (voucher Number: MO-LE-09-10-31) was placed in the Herbarium of The Islamia University Bahawalpur.

### Preparation of the extract

The air-dried ground (80 mesh) plant material (40 g) was extracted with each of the solvent - aqueous methanol (methanol: water, 80:20 v/v) (1L) – for 6 h at room temperature in mechanical mixer (Euro-Star, IKA D 230, Germany). The extract was separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent up to one tenth under reduced pressure at 45°C, using a rotary evaporator (Eyela, Co. Ltd. Japan). The concentrated extract was stored in a refrigerator (- 4°C), until used for analyses.

### Preparation of the formulation

An active cream was prepared by an anionic hydrophilic colloid (14% Paraffin oil), 2% Abil EM 90, 3% *M. oleifera* leaves extract, 0.2% phosphoric acid, 1% fragrance and rest of deionized water. Heated oily phase and aqueous phase were mixed using homogenizer (Euro-Star, IKA D 230, Germany) by addition of phosphoric acid, extract and fragrance. Base was prepared without extract. The same method was adopted to prepare the base without extract.

### Subjects

Eleven subjects were selected with ages between 20 and 35 years. All subjects were healthy males with no known dermatological diseases or allergy to substance in formulations. Declaration of Helsinki was followed in this blind study. Informed consent was signed before start of this study from all volunteers. The exclusion criteria were as follows: presence of any dermatitis and/or other skin or allergic diseases, smokers and previous treatment of forearms' skin with cosmetic formulations such as sunscreens, moisturizers or anti-ageing cosmetics. During the test period, the subjects were allowed to wash normally, but were instructed not to use any other skin care products on their arms. The volunteers were asked not to apply any topical products on cheeks 24 h before the beginning and throughout the test period. Additionally, solar exposure and use of occlusive clothes on the test area were forbidden.

### Instrumental assessment

Non-invasive bioengineering measurements were performed. The trans-epidermal water loss (TEWL) measurements were performed with a tewameter MPA 5 from Courage and Khazaka Electronics

GmbH, Cologne Germany. The tewameter was calibrated according to guidelines of manufactures. The water content of the stratum corneum was measured with a skin capacitance meter (Corneometer MPA 5, Courage and Khazaka Electronic GmbH, Cologne, Germany). The device represents the values in arbitrary units by measuring the water content of the superficial epidermal layers down to a depth of about 0.1 mm. All measurements were made in a draught-free room, with controlled temperature (18.0 to 20.6°C) and relative humidity (50 to 65%).

### Study protocol

Physical stability was evaluated by exposing the creams at 8, 25 and 40°C with 75% RH (relative humidity) to storage for a period of two months. Physical characteristics of creams, that is, color, creaming, liquefaction, centrifugation and pH were noted at various intervals for a period of 2 months.

*In vivo* investigations have been carried out during the winter months (October to January). All instrumental measurements were done by the author according to manufacturer's instructions. Two weeks before study begin and during the treatment period, the volunteers permitted only the use of normal cleansing products. Each volunteer was then handed two creams, an active cream containing the extract of the plant and a base without the extract. The volunteers were well-informed about the correct use of the creams. Measurements of skin water content and TEWL were done every second week up to end study period of three months. Approximately 500 mg of both active cream and base were instructed to apply to the cheeks twice daily (mornings, 7:00 to 9:00; evenings, 19:00 to 21:00) over a 12 weeks period at home by the volunteers. The area around the eyes was omitted. Before all measurements, volunteers remained in the room for at least 15 min in order to tolerate full skin adjustment to room temperature.

### Ethical standards

The approval of this study was taken from the Board of the Advanced Study and Research (BASR), the Islamia University, Bahawalpur and the Institutional Ethical Committee, Faculty of Pharmacy and Alternative medicine, The Islamia University, Bahawalpur.

### Mathematical and statistical analysis

The TEWL and skin hydration values of the right and left cheek of the volunteers were calculated at zero hour, 2nd, 4th, 6th, 8th, 10th and 12th week. SPSS 17.0 was used for data analysis on the computer by using the two-way ANOVA for variation between different time intervals and the paired sample t-test for the variation between the two formulations. The level of significance was 5%.

## RESULTS AND DISCUSSION

In this study, it was found that there was increase in TEWL values after the application of base during 2nd week, and then decrease in values was noted at 4th week. After 4th week, TEWL was increased till 10th week and then slight reduction was observed after 12th week. However, in active cream, it was found that there was decrease in TEWL values continuously to 12th week (Figure 1). With the help of ANOVA test, it was found that changes in TEWL produced by active cream were significant and base was insignificant with respect to time. When the paired sample t-test was applied, it was found that the base and active cream showed significant variations regarding the TEWL values except 2nd and 4th, 6th and 8th weeks.

However, there was increase in hydration values after the application of base during $2^{nd}$, $4^{th}$ and $6^{th}$ week then decrease in values was noted after $8^{th}$, $10^{th}$ and $12^{th}$ week. However, in active cream, it was found that there was increase in skin hydration values continuously to $12^{th}$ week (Figure 2). With the help of ANOVA test, it was found that changes in skin hydration values produced by active cream was significant and base was insignificant with respect to time. When the paired sample t-test was applied, it was found that the base and active cream showed significant variations regarding the skin hydration values except 2nd and 10th weeks.

The quantification of biophysical parameter trans-epidermal water loss (TEWL) is vital for the fundamental assessment of the epidermal barrier status (Daleski et al., 2009). TEWL changes are involved with stratum corneum water binding capability. In general, there is a correlation between stratum corneum (SC) hydration and TEWL values, so lower TEWL (intact epidermal barrier function) corresponds to normal hydration state of the horny skin layer status (Daleski et al., 2009). The assessment combined TEWL and capacitance is suitable to evaluate skin susceptibility (Wang et al., 2003).

Decrease in TEWL in active cream is due to presence of antioxidants and phenolic compounds in *M. oleifera* extract. The binding of water in the stratum corneum can become compromised. In this case, it is beneficial to lower the trans-epidermal water loss by spreading over occlusive films (Aburjai and Natsheh, 2003). It has been reported that *M. oleifera* leaves contain ascorbic acid (vitamin C), and ascorbic acid content is 106.95 mg/100 g (Anwar et al., 2007; Singh et al., 2009). More recently, the function of ascorbic acid in the configuration of stratum corneum barrier lipids has been revealed. 0.4 to 1 mg/100 g of wet-tissue weight of ascorbic acid concentration was found in total skin ranges (Shapiro and Saliou, 2001). Ascorbate regularizes epidermal lipid profiles (in particular, glucosphingolipids and ceramides) in refurbished epidermis. Also, it is revealed that ascorbic acid gives photo-protection and prevents inflammation, and UVB-induced immune-suppression when applied topically (Shapiro and Saliou, 2001). In addition, better skin moisturizing effects have resulted in the form of decrease in TEWL produced by active cream in volunteers. *M. oleifera* leaves are rich in phenolic compounds. Total phenolic contents of *M. oleifera* leaf aqueous methanolic extract has been reported 12.2 ± 0.28 (GAE g/100 g of DW) (Sultana et al., 2001). Phenolic compounds are currently being investigated for therapeutic use topically (Epstein, 2009). *M. oleifera* leaves contain quercetin-3-O-glucoside and quercetin-3-

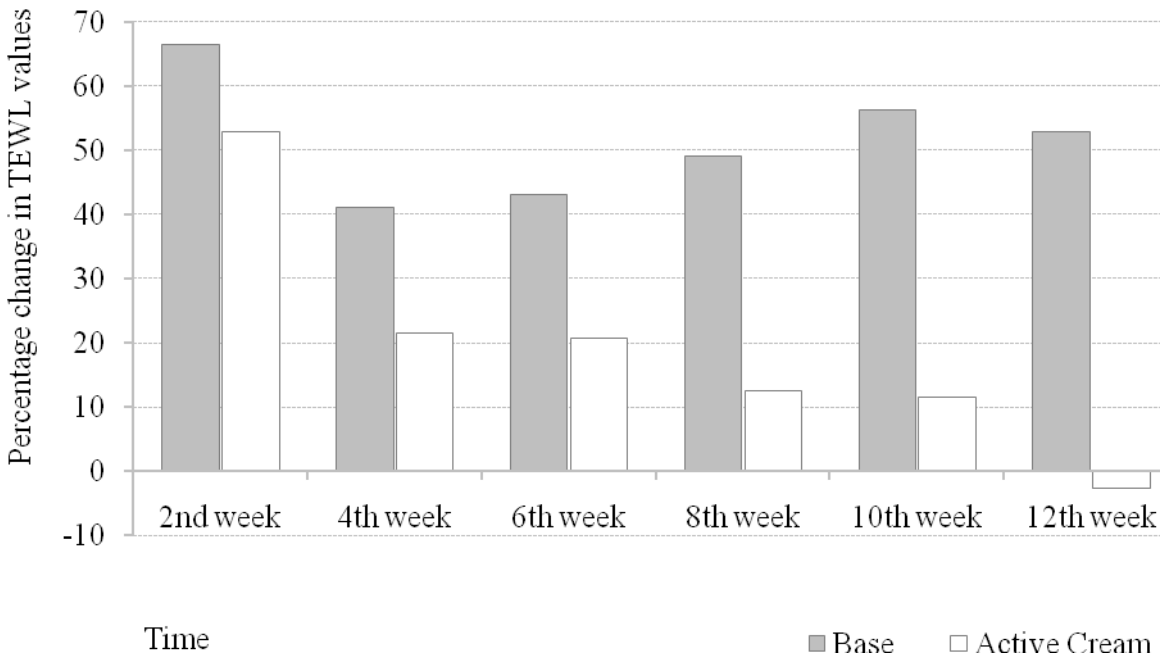


**Figure 1.** Percentage change in the TEWL values of volunteers after the application of base and active cream.

O-(6"-malonyl-glucoside), lower amounts of kaempferol-3-O-glucoside, kaempferol-3-O-(6"-malonyl-glucoside, 3-caffeoylquinic acid and 5-caffeoylquinicacid (Bennet et al., 2003). Other phenolic compounds which may collaborate in prevention UV radiation and dryness include: Kaempferol 3-O-rhamnoside, kaempferol, syringic acid, gallic acid, rutin and quercetin 3-O-glucoside (Manguro and Lemmen, 2007). The high-performance liquid chromatography (HPLC) analysis indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin) (Verma et al., 2009). HPLC and MS/MS analysis was carried out, which showed the presence of gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin. The LE (aqueous extract of leaves) was higher in total phenolics content (105.04 mg gallic acid equivalents (GAE)/g), and total flavonoids content (31.28 mg quercetin equivalents (QE)/g) than other parts of *M. oleifera* and showed better antioxidant activity (85.77%), anti-radical power, reducing power (ascorbic acid equivalents (ASE)/ml), inhibition of lipid per-oxidation, protein oxidation, OH-induced deoxyribose degradation, and scavenging power of superoxide anion and nitric oxide radicals (Singh et al., 2009; Nichols and Katriyar, 2010). Phenolic antioxidants present in *M. oleifera* leaves reduce free-radical damage, thereby preventing impairment at the cellular level. They inhibit inflammation, which leads to collagen efficiency, and may offer protection against skin photo-damage.

It is reported that *M. oleifera* leaf extract has a rich composition in the amino acids protein, and in particular amino acids such as present histidine, arginine, threonine, serine, glycine and alanine, which may improve water retention in the stratum corneum (Anwar et al., 2007). Increase in hydration level in our results may have a possibility to some extent due to enhance water retention in stratum corneum by amino acids.

*M. oleifera* leaves contain vitamin A (Anwar et al., 2007; Nambiar and Parnami, 2008). Vitamin A, its derivatives, and beta-carotene (pro vitamin A) have been well-liked additives in cosmetics for years. Beta-carotene has been revealed to have topical photo-protective outcomes in an increase of protein and collagen as well as DNA content and increased epidermal thickening (Lupo, 2001).

Vitamin B is a group of water-soluble nutrients. It functions as a humectant and increases the water content. Humectants can attract water into the stratum corneum to soften the skin; this is effective as a moisturizer in cosmetics (Lupo, 2001; Anwar et al., 2007). Our findings that increase in skin hydration values produced by active cream in volunteers may be associated with the presence of vitamin B in *M. oleifera* leaves.

Including all of these, moisturizing creams provide water directly to the skin from their water phase. The lipids in the creams may also form a film at the skin surface which reduces TEWL and increase skin hydration so that the water is put together. Lipids may also access into smashed skin and influence the barrier recovery (Loden et al., 1999).

Patients with atopic skin show a defective barrier function both in rough and in clinically normal skin, with

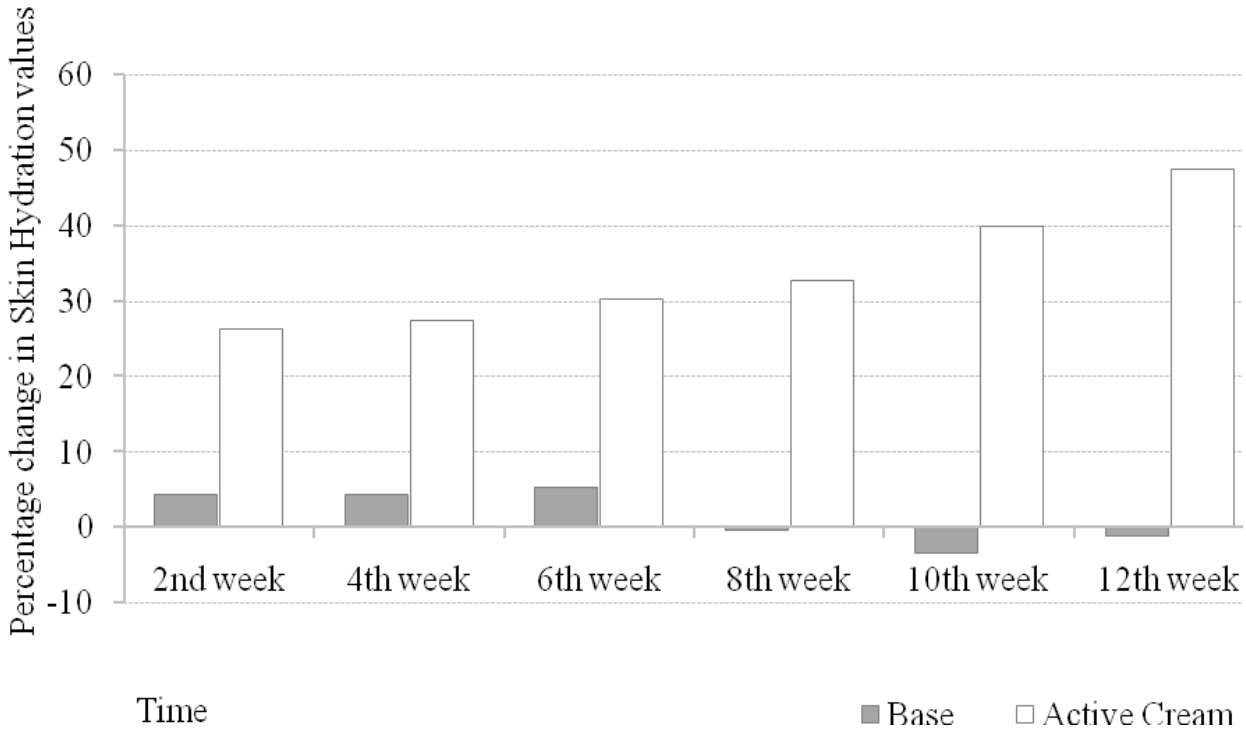


**Figure 2.** Percentage of change in the skin hydration values of volunteers after the application of base and active cream.

an increasing risk of developing contact dermatitis. Moisturizing creams are often used in the treatment of dry skin. The mechanism and the clinical relevance need further investigation.

## CONCLUSIONS

Cream containing *M. oleifera* leaves extract showed efficacy in improving skin moisture by preventing UV radiation (phenolic compounds), when evaluated. After application of *M. oleifera* leaf extract, only cream supplemented antioxidants, phenolic compounds, vitamin A and Vitamin B improved the water content of the stratum corneum and were found significantly effective. Thus, *M. oleifera* leaves extract is a natural effective ingredient for improving skin hydration, which can be used in moisturizing cosmetic formulations and also to complement the treatment of dry skin.

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