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*Full Length Research Paper*

# *In vivo* skin irritation potential of a cream containing *Moringa oleifera* leaf extract

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**The aim of the present study was to evaluate the skin irritation potential of a cream containing *Moringa oleifera* leaf extract. Skin irritation potential of a cream containing *M. oleifera* leaf extract (3%) versus base was investigated by performing an *in-vivo* visual scoring (skin irritation), patch test and erythema index for long term study by using non-invasive instrumental assessment Mexameter in 11 volunteers in a single blinded 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 under a draught-free room, with controlled temperature (18.0 to 20.6°C) and relative humidity (55 to 65%). No serious adverse effects were observed. The *M. oleifera* leaf extract cream was not-irritant according to 48 h semi-occluded patch test. There was a significant decrease in skin erythema and base showed insignificant results when applied ANOVA. The results suggested that the *M. oleifera* leaf extract cream was very well accepted by all volunteers and decreased erythema content. Additionally, product can be regarded as safe for topical application.**

**Key words:** *Moringa oleifera*, extract, cream, skin irritation potential, mexameter.

## INTRODUCTION

Natural remedies have been used widely in cosmetics and pharmaceuticals for improving skin appearance and skin conditions in which photo-toxicity, inflammation, psoriasis, alopecia areata and atopic dermatitis are the most prominent.

Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio (Aburjai and Natsheh, 2003). However, plant extracts also used in topical and cosmetic formulations as fragrance, colorants, anti-irritant and anti-aging etc. Natural products may induce allergic and irritant contact dermatitis and phyto-photo-dermatitis (Almeida et al., 2008).

Members of the Ranunculaceae, Euphorbiaceae and Asteraceae (Compositae), Umbelifereae, Rutaceae and Moraceae, Lauraceae, Magnoliaceae and Jubulaceae plant families are especially involved in irritant contact dermatitis, phyto-photo-dermatitis and allergic contact dermatitis (Christopher, 1997; Rates, 2001). Moreover, UV irradiation is one of the major causes to make morphological and ultra-structural changes in human skin (Svobodova et al., 2006).

Phyto-photo-protectives which include antioxidants, phenolic acids, flavonoids and high molecular polyphenols have been incorporated in topical formulations against UV mediated oxidative damage and offers a simple approach to build up the endogenous protection systems which are omnipresent in plants. But it is most important point to explore those natural products that can aggravate skin adverse effects such as phyto-photo-dermatitis, allergic and irritant contact dermatitis (Almeida et al., 2008) and a number of skin diseases are believed to be associated with oxidative stress including psoriasis, acne and cutaneous vasculitis (Rates, 2001).

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Evaluation of skin irritation potential is a foremost interest in safety measurement of cosmetic formulations, when long-term use of these formulations is expected. Non-invasive biophysical tools have been operated earlier to measure skin irritation potential of cosmetic formulations (Mahmood and Akhtar, 2012). *Moringa oleifera* (Moringaceae) pan-tropical species (Iqbal and Bhanger, 2006); bioactive compounds such as gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin; carotene, vitamin C, vitamin B, vitamin A, phenolics, carotenoids etc have been reported (Manguro and Lemmen, 2007; Singh et al., 2009). *M. oleifera* leaf extract have been identified as potent anti-oxidant (Iqbal and Bhanger, 2006). Leave are used as anti-inflammatory, 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). The aim of the present study was to evaluate the skin irritation potential of an extract of *M. oleifera* leaves in the form of topical application.

## MATERIALS AND METHODS

*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.

### 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.

Abil EM 90 was procured from Franken Chemicals Germany, Paraffin oil from Merck Germany, Methanol and Phosphoric acid from BDH England. Deionized water was obtained in the Pharmaceutical Labs of Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

### Preparation of the active cream

An active cream was prepared by an anionic hydrophilic colloid (14% Paraffin oil), 2.5% Abil EM 90, 3% *M. oleifera* leaves aqueous methanolic extract, 0.2% phosphoric acid, 1% fragrance and rest of deionized water. Heated oily phase and aqueous phase were mixed using homogenizer (Euro-Star, IKAD 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 an age between 20 to 35 years. All subjects were healthy males with no known dermatological diseases or allergy to substance in active creams. 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 active creams 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 erythema measurements (EI) were performed with reflectance spectrophotometer, a Mexameter from Courage and Khazaka Electronics GmbH, Cologne Germany. The Mexameter was calibrated according to guidelines of manufactures. 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 at 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.

### Skin compatibility by evaluation of primary skin irritation

For primary irritation potential of creams, patch tests were accomplished on both forearms of each volunteer on the first day of skin assessment. A 5 × 4 cm area was marked on the forearms. The patch (Bandage disc) for the left forearm was drenched with 1.0 g of base while the patch for right forearm was drenched with 1.0 g of active cream with surgical dressing after application on marked areas. The patches were removed after 48 h and the forearms were observed for any skin irritation by an experience dermatologist and also using Mexameter. The quantification of the skin irritation given through a numeric scale was used to quantify the skin irritation (visual scoring). The average irritant score of the active cream calculated from the average of the quotations obtained for each volunteer, allowing ranking from "non-irritant to very irritant".

The reactions were evaluated according the following arbitrary scale. No erythema: 0, Light erythema (hardly visible): 1, Clearly visible erythema: 2, Moderate erythema: 3, Serious erythema (dark red with possible formation of light eschars): 4, No edema: 0, Very light edema (hardly visible): 1, Light edema: 2, Moderate edema (about 1 mm raised skin): 3, Strong edema (extended swelling even beyond the application area): 4, index of average irritation was classified according amended Draize system: Non-irritating, 0.5 to 2.0: slightly irritating, 2.0 to 5.0: moderately irritating, 5.0 to 8.0 (highly irritating).

### Erythema index in long term study

*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 erythema was done every second week up to the end of 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.

#### Efficacy perception – subjective analysis

To assess the effectiveness of the two creams, that is, base and active cream tested in this study, the volunteers were asked to answer a questionnaire consisting of seven parameters after three months from the beginning of the study. 1. Ease of application; 2. Spreadibility; 3. Sense just after application; 4. Sense on long term; 5. Irritation; 6. Shine on skin; 7, Sense on softness.

#### Ethical standards

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

#### Statistical analysis

Skin erythema contents after application of base and after application of active cream were compared at same time intervals (that is, 0 h readings of skin erythema after application of base were compared with 0 h readings of skin erythema after application of active cream, 48 h readings of skin erythema after application of base were compared with 48 h readings of skin erythema after application of active cream). Different parameters of sensory evaluation (that is, ease of application, spreadability, sense just after application, sense in long term, irritation, shine on skin and sense of softness) were compared for base and active cream; after their application on the cheeks of human volunteers. Paired sample t-test was applied to calculate for base and active cream. The erythema values of the right and left cheek of the volunteers were calculated at 0 h, $2^{nd}$, $4^{th}$, $6^{th}$, $8^{th}$, $10^{th}$ and $12^{th}$ 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 active creams. The level of significance was 5%.

## RESULTS AND DISCUSSION

### Skin compatibility by evaluation of primary skin irritation

Patch testing after a single application is a widely used procedure to evaluate acute irritant reactions (Gaspar et al., 2008). It was found by performing patch testing on forearms of volunteers for 48 h for both the base and active cream that erythema level after application of base was slightly decreased while the erythema level after application of active cream was pronouncedly decreased after 48 h (data not shown).

But with paired sample t-test, it was evident that the effects of active cream and base were insignificant regarding the skin Erythema even though the active cream decreased the skin erythema more than the base. Initially, evaluation of irritancy testing was based on visual scoring only. This type of evaluation, although subjective, can be a sensitive, reliable and reproducible method.

The possible irritating power of the *Moringa* leaf extract was evaluated according to single application, 48 h semi-occluded patch test. Neither erythema nor edema after the application was observed (Table 1). Finally, it was concluded that both the active cream and base produced no skin irritation after performing patch test of 48 h, so both emulsions can be used safely on human skin for *in-vivo* evaluation.

### Erythema index for long term study

In this study, it was found that there were slight variations observed in erythema values of base till 12 weeks. However, in active cream, it was found that there was gradual decrease in erythema values to $12^{th}$ weeks (Table 2 and Figure 1). With the help of ANOVA test, it was found that changes in erythema values produced by active cream were significant and base were 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 erythema values except $2^{nd}$, $4^{th}$, and $6^{th}$ weeks. Anti-oxidants and phenolic compounds have been used in dermatology to approach widely for skin disorders in recent few years (Nichols and Katiyar, 2010; Ali et al., 2012). The inflammatory reaction following acute UV irradiation and the degenerative progressions associated to chronic UV radiation skin exposure are largely mediated by the overproduction of ROS and by impairment of the antioxidant endogenous system (Almeida et al., 2008; Bissett, 2009). Most of the polyphenols play a vital role to protect skin against UV-induced disorders. UV-induced skin inflammation, oxidative stress and DNA damage with a focus on mechanisms underlying the photo-protective effects of these polyphenols (Nichols and Katiyar, 2010).

Phenolic antioxidants present in *Moringa* leaves reduce free-radical damage, thereby preventing impairment at the cellular level. They inhibit inflammation, which leads to collagen efficiency, and they offer protection against photo-damage and skin cancer. Several phenolic compounds were identified in *M. oleifera* leaf extract obtained under optimized conditions, namely, phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (myricetin, kaempferol, quercetin and rutin) (Sultana and Anwar, 2008). DPPH scavenging activity has been found 91% for these phenolic compounds, and thus their accepted input to the free radical scavenging activity of the whole extract. In fact, a preventive effect

**Table 1.** Values and classification of average irritation indexes.

| Volunteer | Erythema | Edema | Total reading 48 h |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 |
| Total irritation | | | 0 |
| Irritation index | | | 0.00 |
| result | | | Non irritant |

**Table 2.** Percentage of change in the erythema values of volunteers after the application of base and active cream.

| Time | 2nd week | 4th week | 6th week | 8th week | 10th week | 12th week |
|---|---|---|---|---|---|---|
| Base | -1.05 | -1.17 | -1.06 | -1.51 | -0.67 | -0.85 |
| Active cream | -6.09 | -7.05 | -8.14 | -11.5 | -12.65 | -15.13 |

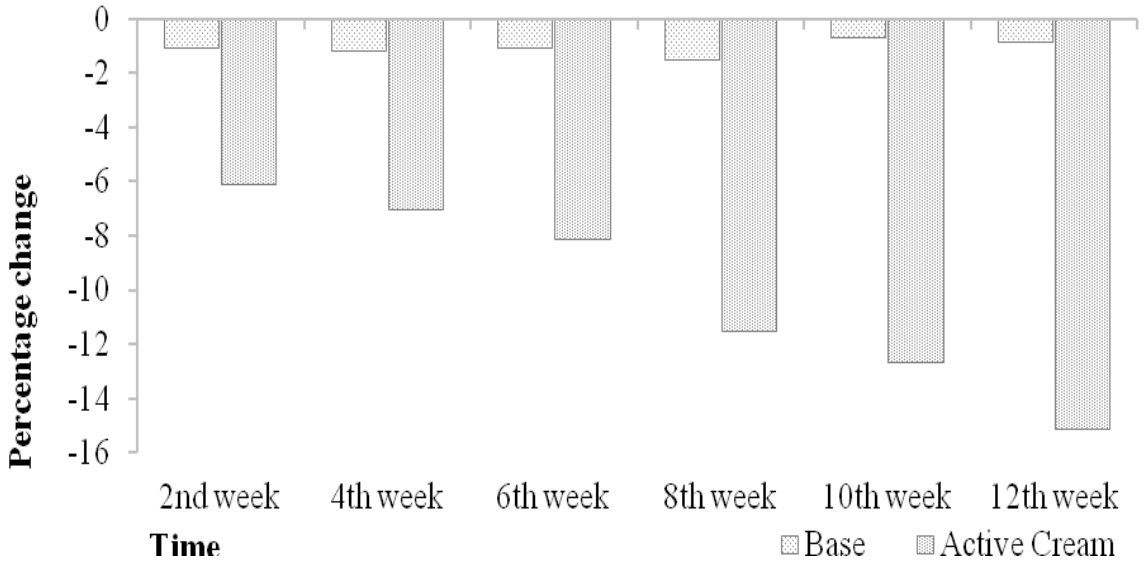


**Figure 1.** Percentage of change in the erythema values of volunteers after the application of base and active cream.

against photo-oxidative stress induced by UVA radiation has been depicted for rutin. Despite the absence of reports of adverse effects of *M. oleifera* leaves or of the phenolic compounds found in its composition, safety cannot be buried and appropriate tolerance investigations should be performed.

**Efficacy perception – subjective analysis**

Average points for each parameter were shown in Table 3

**Table 3.** Average values ± SEM for panel test.

| Variable | Average point for base ± SEM | Average points for active cream ± SEM |
|---|---|---|
| Ease of application | 4.07 ± 0.05 | 4.21 ± 0.12 |
| Spreadability | 4.17 ± 0.08 | 4.34± 0.06 |
| Sense just after application | 3.95 ± 0.07 | 3.03 ± 0.08 |
| Sense in long term | 4.06 ± 0.08 | 4.04 ± 0.11 |
| Irritation | 0.00 ± 0.00 | 0.00 ± 0.000 |
| Shine on skin | 4.14 ± 0.09 | 4.07 ± 0.04 |
| Sense of softness | 4.45 ± 0.08 | 4.60 ± 0.09 |

for both base and active cream. From paired sample t-test, non-significant difference between the average points for base and active cream were observed which showed that there was no variation between base and active cream.

## Conclusions

In conclusion, the optimized *M. oleifera* leaf extract presents attractive features that could be applicable for topical application in the prevention and treatment of oxidative stress-mediated diseases and photo-aging. Furthermore, the good skin tolerance found after a single application under patch test reinforces its accepted awareness as topical antioxidant, after inclusion in appropriate and secure topical bases.

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