Biomedical Research 2013; 24 (1): 127-130 ISSN 0970-938X

Effect of *Moringa oleifera* on undesireble skin sebum secretions of sebaceous glands observed during winter season in humans

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Abstract

The present study was planned to test the efficacy of *Moringa oleifera* on undesirable skin sebum secreted by sebaceous glands during winter season in humans. For this purpose, an extract of *Moringa* leaves was prepared due to strong antioxidant activity. 3% *Moringa* leaves extract was incorporated in active cream and the base was without extract. The study was conducted during the winter season (October to January). A total of eleven healthy male volunteers with aged 20 to 35 years were 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. Also, the antioxidant activity of the plant extract alone and after addition in the creams was assessed using the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The instrumental measurements were carried out with photometric device (Sebumeter) with relative humidity (55–65%). The *Moringa oleifera* cream significantly reduces undesirable skin sebum during winter season in humans when applied ANOVA and paired t-sample test. Treatment with *Moringa oleifera* showed reduction of undesirable skin sebum secretions secreted by sebaceous glands during winter season in humans.

Keywords: *Moringa oleifera*; Skin Sebum; Winter; Sebumeter.

Accepted December 03 2012

Introduction

Moringa oleifera (Moringaceae), a pan-tropical species that is identified by such regional names as benzolive, kelor, mulangay, marango, mlonge, drumstick tree, saijhan, nébéday and sajna. *Moringa oleifera*, native of the western and sub-Himalayan tracts, Pakistan, India, Asia Minor, Arabia and Africa and is one of the potent antioxidants thorourghly researched plants [1]. Bioactive compounds such as carotene, vitamin C, vitamin A, tocopherols, phenolics, caortenoids etc have been reported. Leaves of *Moringa oleifera* are reported potent antioxidants than other parts [2]. Leave 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; leaf juice is believed to control glucose levels, applied to reduce glandular [3,4].

Some botanical compounds are considered useful to reduce sebum [5]. Sebum is a complicated mixture of lipids including wax esters, triglycerides, cholesterol esters, squalene, and free fatty acids. Under physiological conditions, sebaceous lipids play a role to the integrity of the skin, affect inflammatory processes, transport antioxidants to the skin surface, and have innate antimicrobial activity [6]. Protection of skin against undesirable effects of seasonal changes is a vital function of cosmetics or external preparations [7]. Oily skin is a common condition affecting men and women, characteristically between puberty and about 60 years of age. Gernerally, men exhibit a higher rate of sebum secretion compared to women. In humans, the highest rate of sebaceous glands, with 300–1500 glands ⁄cm^2, is found on the face [8]. Although excessive sebum production has minimal physical impact on body function, chronic oily skin can cause significant concern for people who have the condition. Oily skin appears greasy and shiny, adds to the growth of acne, and is commonly accompanied by large pores on the face.The outcomes of surplus sebum may be accompanying with adverse psychological and social effects resulting from associated acne and the appearance of skin oiliness and shine. Numerous findings have projected 66% to 75% aged 15–20 years are affected.

Surveys have also originated that sufferers feel unpleasant, rough and irritated by the state [9,10]. Also, a number of skin diseases are thought to be connected with oxidative stress, including psoriasis, acne and cutaneous vasculitis [11].

Therefore, the present study was planned to test the efficacy of *Moringa oleifera* grown to reduce undesirable skin sebum secretions from this region during winter season in humans.

Materials and Methods

Plant Material and identification

Moringa oleifera leaves were collected during July 2010 in Dera Ghazi Khan, Pakistan and dried at room temperature for 4 weeks. The identification of the plant (*Moringa oleifera*) was performed at the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. The specimen (voucher Number: MO-LE-09-10-31) was deposited in the Herbarium of The Islamia University Bahawalpur.

Materials

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

Preparation of the Extract

The air-dried ground (80 mesh) plant material (40 g for each sample) was extracted with each of the solvent - aqueous methanol (methanol: water, 80:20 v/v) (1L) – for 6 hours 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 upto 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 $^{\circ}$C), until used for analyses.

Free radical scavenging activities

The free radicals scavenging activity of The H-donor ability was assessed by using an methanol solution of DPPH, a stable nitrogen-centered free radical. The DPPH shows maximum absorbency at 517 nm, which decreases in the presence of H-donor molecules. The DPPH stable free radical was used for the determination of free radical scavenging activity of extract [12]. In 5 miroleter of aqueous ethanolic plant extract, added DPPH to make the volume upto 100 µl in 96 well plates. Mixed the contents and incubated at 37°C for 30 minutes and measured the optical density at 517 nm. Ascorbic acid was used as a standard. Ascorbic acid had a strong antioxidant property that's why it was used as standard to evaluate the antioxidant activity of substances [13]. Experiments were done in triplicates. Results were taken as mean and standard error of mean of three independent experiments.

% DPPH sacavanging activity = (100 - OD of test sample/ OD of controlled x100)

Preparation of the formulation

An active cream stabilized by an anionic hydrophilic colloid (14% Paraffin oil), was developed, based on 2.5% Abil EM 90, 3% *Moringa oleifera* leaves extract, 1% fragrance, 0.2% phosphoric acid and rest of deionized water. Oily phase and aqueous phase were heated upto 75 ± 5°C on water bath. With the help of homogenizer with speed of 2000 rpm for 15 minutes, both phases were mixed contineously by further addtion of extract, fragrance and phosphoric acid (pH adjuster) in aquoeus phase. The speed of homgenizer was reduced at 1000 rpm for 5 minutes and it further reduces to 500 rpm till creams were cooled. Oily phase comprises of paraffin oil and ABIL - EM 90 (emulsifier). The same method was adopted to prepare the base without extract.

Subjects

Eleven healthy males with an age between 20 to 35 years, with no known dermatological diseases or allergy to substances in topical formulations, joined in the single blind study, in accordance with Declaration of Helsinki. Informed consent was signed before start of this study from all volunteers. Additionally, solar exposure and use of occlusive clothes on the test area were forbidden.

Instrumental assessment

Non-invasive biophysical measurements were also performed. The sebum level of both cheeks were determined with a photometric device sebumeter SM 815 (Courage + Khazaka,Germany). Sebum was collected with special opaque plastic tape (64 mm^2) by pressing onto the skin for 30 s with a slight pressure. The resulting increase in transparency of the tape was measured and the displayed values tally to the sebum amount on the skin surface in µg sebum/cm^2.

Study protocol

Study was carried out during the winter season (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 subjects were allowed only the use of regular cleansing products. Each volunteer was then given two creams, a active cream having the extract of the plant and a base without the extract. The volunteers were educated about the appropriate use of the creams. Measurements of skin sebum production were done every second week up to end study period three months. Approximately 500 mg

of both base and active cream were instructed to apply to the cheeks twice daily (mornings, 7:00–9:00; evenings, 19:00–21:00) over a 12 weeks period at home by the volunteers. The area around the eyes was omitted.

Ethical standards

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

Mathematical and statistical analysis

The sebum values of the right and left cheek of the volunteers were calculated at zero hour, 2nd week, 4th week, 6th week, 8th week, 10th week and 12th week. The data obtained was then analyzed by the SPSS 17.0 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

The antioxidant ability of the plant extract and cream containing MO extract were measured by DPPH assay. The antioxidant activity of plant extract and after addition of plant extract of the cream was found to be 91% and 85% respectively.

In this present investigation, effects produced on volunteers by both base and active cream were assessed for skin sebum level (figure 1). Sebum measurements were carried out for 12 weeks. It was found from our results the base has a variable increasing tendency on skin sebum but in active cream, it was found that sebum contents decreased regularly in the study.

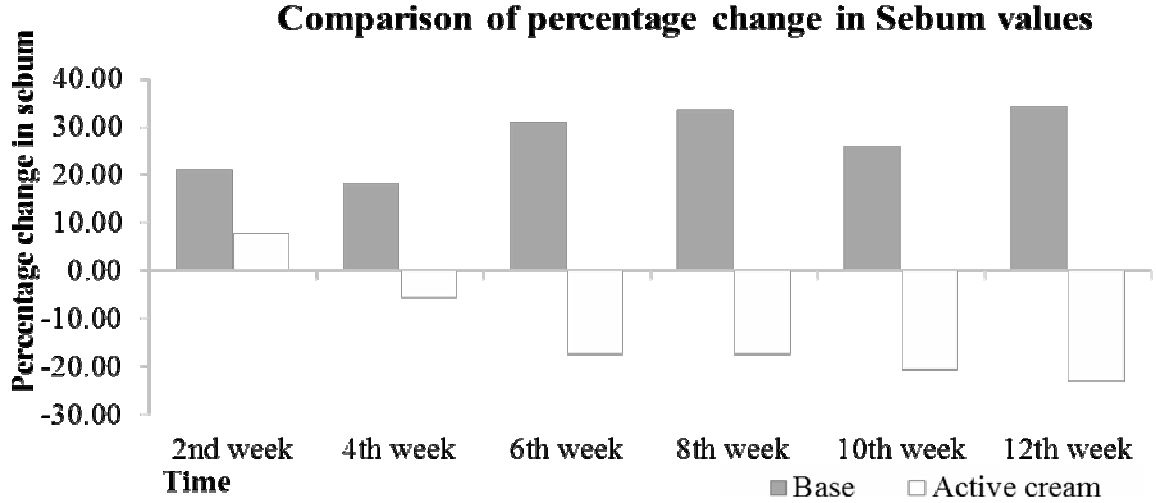

Figure 1. *Percentage of change in the Sebum values of volunteers after the application of Base and Active cream.*

After applied ANOVA test, it was found that there was an insignificant effect of base with respect to time while in case of active cream it was evident that active cream containing *Moringa oleifera* has significant effect with respect to time**.** When the paired sample t-test was applied it was found that the base and active cream showed significant effects produced in volunteers with respect to time.

Discussion

The DPPH assay is extensively used for the extent of free radical scavenging capacity. The antioxidant potential of the extract is correlated to flavanoids, tannins, proteins and reducing sugars. The phenolic compounds are reported as scavengers of free radicals [14]. Phenolic compounds pretend to be antioxidants and the activity of the extract may be reasonable response to these compounds [15]. The cream containing MO leaves extract illustrated lower H-donor potential. The formulation components present in the reaction mixture resulted lower H-donor potential in active cream. Since the DPPH scavenging is measured by spectroscopy, the formulation components may interfere with the antioxidant measurement [16].

It is reported that sebum secreted by sebaceous glands is coordinated by estrogens and androgens (sex hormones), corticosteroids (hormones of the adrenal cortex) and others. androgens receptors and 5 α-reductase converts testosterone into dihydrotestosterone (the most active form stimulating sebum secretion). It has been reported that androgens receptors are present in various parts of skin areas. The reasons behind to increase the sebum secretion are: (1) dihydrotestotesterone DHT is a testosterone metabolite synthesized with the use of 5 α-reductase type I (2) progesterone is an inhibitor of 5α-reductase. This

hormone increases seborrhea by stimulating the division of sebocytes [6] and (3) estrogen causes reduction in sebum secretion [10].

Botanical compounds founds in *Moringa* leaves have the potential to inhibit the 5 α-reductase. Epigallocatechin gallate, myrecetin, querticin, rutin, morin, toxifolin, chrysin, baicalein, fisetin, biochanin A, genistein, kempferol, emodin anthraquinone, caffeic acid phenethyl ester and octyl and dodcyl gallates are examples of phenolic compounds which have inhibitory activity against 5 α-reductase [3,17]. It is assumed that continuous reduction of sebum by application of active cream in human cheeks in our results reported was due to presence of phenolic compounds in *Moringa oleifera* in active cream *Moringa* leaves are reported to rich in phenolic compounds [18]. Myricetin, Quercetin, Kampeferol [19], gallic acid, syringic acid, and rutin [18] have been identified in *Moringa* leaves. Inhibitory activity of 5-α-reductase of these compounds lead to reduced sebum content when applied topically.

Conclusion

In conclusion, the *Moringa oleifera* presents interesting features that could be relevant for topical application in undesirable skin sebum during winter season and eventually unpleasant oiliness. A number of skin diseases are believed to be associated with oxidative stress, including psoriasis, and acne. Hence, it is suggested that *Moringa oleifera* may speculate the results against acne vulgaris, acne rosocca, acne infantum, acne tarda and others.

Acknowledgements

The authors wish to thank Higher Education Commission of Pakistan for providing financial support to conduct the study. The authors also acknowledge the moral support given by the Chairman and Dean of the faculty of pharmacy & alternative medicine, The Islamia University of Bahawalpur, Pakistan.

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