

The improvement on the skin surface by a new type of dermocosmetic loaded plant extract: A split face skin topographic study

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Abstract: Various extrinsic and intrinsic origins slant skins and pledge evident vicissitudes of the skin surface. We explored the effects of dermocosmetic loaded medical *Cannabis* seed extract and evaluate the improvement on the skin surface in Asian and male volunteers in a split face topographic study. Dermocosmetic and base (without extract) fabricated were directed to apply by volunteers (Asian male) on their right and left cheek, respectively, in the split face skin topographic study up to three months. Efficacy of dermocosmetic versus base was assessed by non-invasive diagnostic technique focusing on skin texture parameters (energy, contrast and variance) and surface evaluation of the living skin (SELS), SER (skin roughness), SEsc (skin scaliness), SEsm (skin smoothness), SEw (skin wrinkles). Unlike base, dermocosmetic showed significant effects on skin texture parameters (energy, variance and contrast) and SELS (SER, SEsc, SEsm and SEw) parameters in Asian male volunteers when ANOVA applied. The level of significance was 5%. Dermocosmetic ultimately improved on skin surface and advocacies for anti-aging effects on skin appearance.

Keywords: Dermocosmetic, skin, surface, plant extract.

INTRODUCTION

Aging of the skin is a complex phenomenon with morphological and chemical changes that may be remarkably accentuated by solar UV radiation (Wulf *et al.*, 2004). Exposure to solar UV radiation initiates skin dryness, wrinkling, scaling, hypopigmentation, hyperpigmentation, and cancer (Nichols and Katiyar 2010) by oxidative impairment (González *et al.*, 2008; Ali and Akhtar, 2015) collagen insufficiency and ultimately skin roughness (Ichihashi *et al.*, 2003). The intrinsic and extrinsic aging of the face will lead to in skin elasticity and visible wrinkle formation (Williams *et al.*, 2009). Thus, scavenging reactive oxygen species are thought to be one mechanism of action underlying skin protective effects of antioxidants incorporated topically (Heinrich *et al.*, 2006). *C. sativa* (Cannabaceae) is inborn to Central Asia like India, Pakistan, Sri Lanka and China (Zuardi *et al.*, 2006) and has been used for medicinal purposes for many years. Medical cannabis is of pharmaco-economic significance all over the world which is assessed at \$100-2000 million annually (Radwan *et al.*, 2008). Phytochemistry of medical *Cannabis* characterize the chemical classes like phenolic compounds, Phyto-cannabinoids, mono- and sesquiterpenes, hydrocarbons, steroids, flavonoids, nitrogenous compounds, sugars and amino acids, among others (Elsohly and Slade, 2005; Baker *et al.*, 2003). Medical *Cannabis* became widely used for the treatment of cramps, asthma, and dysmenorrhea. Medical Cannabis has various effects on skin diseases (Ali and

Akhtar, 2015; Baker *et al.*, 2003). In *Cannabis* control law in Japan, Cannabis seeds have been omitted from legal regulations (Yotoriyama *et al.*, 2005). *C. sativa* has been reported free radical scavenging activity against several ROS species, and its anti-aging ROS activities have been proposed safe for cosmetic use (Abrams and Guzman, 2015; Ali and Akhtar, 2015) but it needs investigation due to scarce data (Stokes *et al.*, 2000).

We investigated the effects of dermocosmetic loaded medical *Cannabis* seeds extract and evaluate improvement on skin surface in Asian and male volunteers using a split face skin topographic study.

MATERIALS AND METHODS

Subjects

Eleven Asian male volunteers were selected with an age between 20 to 35 years. All healthy volunteers with no known and any dermatological problem to substance in products were included. Declaration of Helsinki was followed in this split face skin topographic study. All volunteers previously were informed signed consent before the start of this study from. The exclusion measures were as presence of, any dermatitis and/or other dermal or allergic problems, barbed men, extraordinary hairs on the cheeks, any type of smokers and previous therapy of skin with any cosmetic and dermal products such as sunscreens, moisturizers or anti-aging cosmetics or transdermal preparations. All the volunteers were allowable to rinse normally, but were educated not to treat any other skin care products on cheeks 24 h before the beginning and throughout the test study period. Moreover,

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solar exposure and dressing of occlusive clothes were forbidden on the measurement site.

Plant identification and ethical standards

Seeds of *C. sativa* were obtained from Islamabad, Pakistan. Identification of the plant (*C. sativa*) was executed by taxonomist Dr. Muhammad Arshad (late) at the Cholistan Institute of Desert Studies (CIDS), the Islamia University of Bahawalpur, Bahawalpur- Pakistan. The specimen (voucher Number: CS-SD-01-11-32) was placed in the Herbarium of the Islamia University of Bahawalpur. The legal and ethical aspects of study (Ref. No. 3715/Acad) was approved by the Advanced Study and Research Board (ASARB), the Islamia University of Bahawalpur and the Institutional Ethical Committee, Faculty of Pharmacy and Alternative medicines, the Islamia University of Bahawalpur, Bahawalpur- Pakistan.

Preparation of the crude extract

The crushed seeds (40 g weighed for each sample) were extracted with solvent-aqueous methanol (methanol: water, 80:20 v/v) (1L) - for 6 hours at room temperature in a mechanical mixer (Euro-Star, IKA D 230, Germany). The extract by filtering through Whatman No. 1 filter paper was removed from the residues. The residues with the same fresh solvent were extracted twice and extracts combined. The combined extracts were concentrated using a rotary evaporator (Eyela, Co. Ltd. Japan) under reduced pressure at 45°C and freed of solvent up to one tenth. The concentrated extract obtained was kept in a refrigerator (- 4 °C) for further experiments.

Determination of anti-oxidant activities

Free scavenging activities of the plant extract alone and after addition in the product were assessed. The free radical scavenging of H-donor capability was evaluated by consuming a methanol solution of DPPH, a durable nitrogen-centered free radical. The DPPH discloses maximum absorbency at 517nm, which decreases in the existence of H-donor molecules. The DPPH stable free radical was used to determine free radical scavenging of extract. In 5µl of plant extract (aqueous methanolic), included DPPH to create the volume up to 100µl in 96 well plates. All the contents were mixed and incubation was carried out at 37°C for 30 minutes. Optical density was measured at 517nm. Ascorbic acid was treated 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 (Ali *et al.*, 2013). Experiments were done in triplicates. The results were taken as the mean and standard error of mean of three independent experiments.

% DPPH scavenging activity = $(100 - OD \text{ of the test sample} / OD \text{ of controlled} \times 100)$

Fabrication of the products

A dermocosmetic was fabricated by 14% Paraffin oil (Merck, Darmstadt, Germany), 2% Abil EM 90 (Franken

Chemicals, GMBH, Germany), 3% *C. sativa* seeds extract, 1% fragrance and rest of deionized water. Heated oily phase and aqueous phase were mixed using Homogenizer (Euro-Star, IKAD 230, Germany) by adding of plant extract and fragrance. The same method was adopted to prepare the base without the extract

Skin surface topographic evaluations

For skin topography, Visioscan® VC 98 (Courage and Khazaka, Germany) was used which is a special UVA-light video camera with high resolution to study the skin parameters directly. The images display the skin structure and dryness level, very remarkably. The camera can be connected to the computer by the digitization unit Video Digitizer VD 300 via FireWire port (Courage and Khazaka, Germany).

All measurements were performed by the author according to manufacturer's instructions. Only normal cleansing products were allowed to use by volunteers before the study and during the treatment period. Two products (base and dermocosmetic) were given to each volunteer. Guidelines were given to the volunteers about the correct use of products. Measurements of skin parameters which include texture parameters (energy, contrast and variance) and SELS, that is, SEr (skin roughness), SEsc (skin scaliness), SEsm (skin smoothness), SEw (skin wrinkles) parameters were ensured every month up to end study period of three months. Approximately 500 mg of both dermocosmetic and bases were instructed to apply to the cheeks twice daily (mornings, 7:00-9:00; evenings, 19:00-21:00) over a 12-week period at homes by the volunteers. Before all measurements, volunteers remained in the experimental room for at least 15 min in order to tolerate full skin adjustment to room temperature. All measurements were made in a draught-free room, with controlled temperature (18.0-20.6°C) and relative humidity (50-65%).

STATISTICAL ANALYSIS

Skin parameters values (texture parameters and surface evaluation of living skin) of the right and left cheek of the volunteers were calculated at zero h, 1st, 2nd and 3rd months. 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 products. The level of significance was 5%.

RESULTS

Anti-oxidant activity of plant extract and after addition of plant extract of the product was found to be 87% and 79% respectively. Percentage of change in the texture parameter (energy, contrast and variance) values of volunteers after the application of base and

dermocosmetic taken by Visioscan® VC 98 of the study period was shown in fig. 1. Energy values were increased in this study resulting for dermocosmetic and significant effects were produced by volunteers at all reading intervals. And same time, base effects were statistically insignificant. The dermocosmetic also showed significant effects when paired sample t-test was applied for variation between the two products with respect to time.

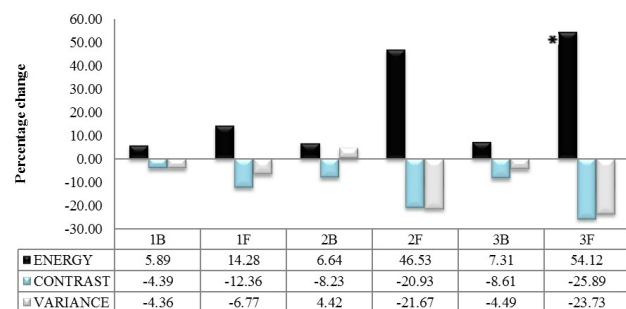


Fig. 1: Percentage of change with time in the texture parameter (energy, contrast and variance) values of Asian volunteers after application of the base and dermocosmetic. Here 1B= application of base after one month, 1F= application of dermocosmetic after one month, 2B, application of base after two months, 2F= application of dermocosmetic after two months, 3B= application of base after three months, 3F= application of dermocosmetic after three months.

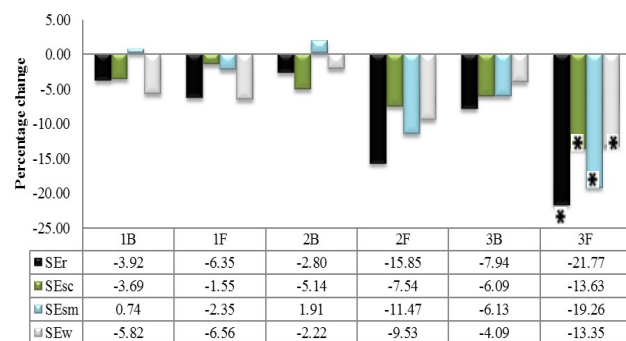


Fig. 2: Percentage of change with time in the SELS parameter values of Asian volunteers after application of the base and dermocosmetic. Here 1B= application of base after one month, 1F= application of dermocosmetic after one month, 2B, application of base after two months, 2F= application of dermocosmetic after two months, 3B= application of base after three months, 3F= application of dermocosmetic after three months.

Decrease in the variance and contrast values resulting for dermocosmetic was statistically significant at all reading intervals but base produced insignificant effects. The dermocosmetic also showed significant effects when paired sample t-test was applied for variation between the two products up to three months.

Percentage of change in the SELS (SEr, SEsc, SEsm and SEw) parameter values of volunteers after the application

of base and dermocosmetic measured by Visioscan® VC 98 of the study period are shown in fig. 2. Fig. 3 showed images of the right cheek of a human subject before application (A) and 3 months after application (B) obtained from Visioscan® VC 98 depicting improvement in skin surface. In this study, it was found that the base produced statistically insignificant effects on the SELS (SEr, SEsc, SEsm and SEw) parameter of skin and the dermocosmetic produced significant effects on the SELS (SEr, SEsc, SEsm and SEw) parameter of skin at all reading intervals when ANOVA two way- analysis was performed. When paired sample t-test was applied for SEr, SEsc, SEsm and SEw significant effects were observed for dermocosmetic with respect to time.

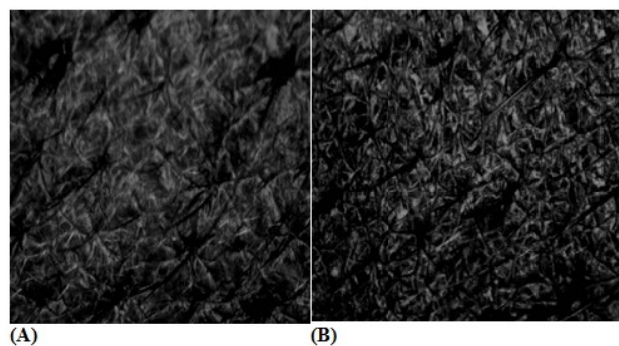


Fig. 3: Images of the right cheek of an Asian volunteer before application (A) and 3 months after application (B).

DISCUSSION

Phenolic compounds widely appearing in the plant kingdom have been stated to impel strong antioxidant activity (Stokes *et al.*, 2000) and it is prone that antioxidant activity of the extract may be due to presence of these compounds (Ali and Akhtar, 2015). The difference in absorbency made by reduced DPPH was expended to assess the ROS inhibition capacity of the plant extract and product loaded CS extract. The result behaved strong antioxidant activities. The parameters i.e., energy, contrast and variance investigate changes in colors of neighbored pixel (Visioscan® VC 98 and the Software SELS, 2009). Energy is increased with rise in skin hydration level and more image homogeneity. Young, energetic, elastic skin and hydrated has a high energy value evaluated and compared to aged skin with sundry wrinkles (Ali *et al.*, 2014). Our findings in increase of energy parameter indicated an over-all indication over the status of skin predicting the moisturizing product or anti-aging product of *Cannabis* seeds extract.

Variance represents the average of a local variance over an amount of pixels of image by visioscan. The tangible quantity of the pixel is equated to the average. High roughness of skin surface results high variance (Debowska *et al.*, 2005). This investigation unveils the

reduction in variance supports the less skin surface roughness.

Contrast specifies the difference between gray levels of the two neighbored pixel of image by Visioscan. The higher the different measurements of two neighbors results the contrast is higher (Debowska *et al.*, 2005). Reduction of contrast showed improvement and smoothness in skin surface. The SELS parameters embody the physiological state of the quantitative skin topography. They purposely differ from the conventional roughness parameters.

SEr (surface evaluation roughness) depicts the proportion of dark pixels of image SEsm (surface evaluation smoothness) is the index of smoothness and is equated from the mean width and depth of wrinkles. SEsc (surface evaluation scaliness) is the index of scaliness of skin and depicts the level of dryness of the skin. SEw (surface evaluation wrinkles) depicts aging and is equated from the proportion of horizontal and vertical wrinkles (Ali *et al.*, 2014; Debowska *et al.*, 2005). It was observed that the values of SEr, SEsc, SEsm and SEw were fallen gradually for the dermocosmetic produced on volunteers. The dermocosmetic depicted reduction in mean values of SEsm in contrast to SEr which showed that the dermocosmetic held skin anti-aging signs. The low SEsc value relates to high skin moisture resulted in lowering values for SEsc as treatment with moisturizing or anti-aging products. Low SEw value depicted that there were less wrinkles present on the skin which revealed that the dermocosmetic lessened the wrinkles of skin surface. This is associated with the collagen efficiency which has persuasive correlation with trans-epidermal water loss (Trojahn *et al.*, 2015). Greater epidermal water loss piloted to less water retention by the collagen and degraded its efficiency. Collagen is a protein in the body's connective tissues. Elastin maintains the body's natural elasticity. Elastin halts skin from sagging and aids in the skin healing after a wound (Ishii *et al.*, 2008). Our findings were directly connected to reduction in appearance of aging signs.

The development in skin surface parameters can be credited to the radical scavenging capacity of the dermocosmetic loaded extract of *Cannabis* seeds. DPPH assay is suitable for evaluating the radical scavenging activity and the correlation analysis showed that the antioxidants in cannabis belonged to phenolic (Tennstedt and Saint-Remy, 2011). It is evident that cannabisin B and N-trans-caffeoyltyramine are principal antioxidant compounds in cannabis seeds extract (Chen *et al.*, 2012). High quantities of unsaturated fatty acids are present in *Cannabis* seeds. It is evident that linoleic acid, alpha- and gamma- linolenic acids (identical expression omega-3 and omega-6 acids) shows skin effects. Specifically, polyunsaturated fatty acids (PUFA) play importance role

in skin appearance, elasticity and barrier functions changes. It integrates with cell membrane fluidity with direct relevance to proper functions including metabolic activity. It has been proved that the omega-3 unsaturated fatty acids impede the expression of matrix metalloproteases type 1 (MMP-1) after ultraviolet induction. MMP-1 intricate in the destruction of structural skin proteins specially collagen and elastin (Baker *et al.*, 2003). These results defense the statement of maintenance of skin vitality by collagen safety. The activities of omega-3 and omega-6 fatty acids which are both compounds of the *Cannabis* provide reinforcement of skin barrier function. All of these investigations collectively support our findings to be a good anti-aging product.

CONCLUSION

Topical supplementation of this valuable plant seeds extract positively improve on skin surface. It could consequently reduce the signs of skin aging. Future studies are required to be conducted to un-reveal the skin anti-aging and cancer mechanism of *Cannabis* constituents.

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