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RESEARCH PAPER

DIMINUTION OF ULTRAVIOLET IRRADIATION INDUCED DETRIMENTAL EFFECTS ON MICE SKIN BY HERBAL COSMECEUTICAL

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The present study was aimed to formulate and evaluate the efficacy of polyherbal cosmeceutical that comprised of plant extracts rich in polyphenolic constituents based on the hypothesis that topical polyphenolic constituents rich plant extracts supplementation modulates the antioxidant network in skin and that bolstering the antioxidant capacity protects against oxidative damage by solar-stimulated UV irradiation. A herbal cosmeceutical comprising of plant extracts rich in polyphenolic constituents was formulated and evaluated for its antioxidant and antiaging potential. Group-I of mice was the un-irradiated control (neither UV-exposure nor any treatment received) Group-II was irradiated control, comprised of mice which received only UV-exposure of 5 minutes twice a day. Group-III received both UV-exposure and treatment of formulation prepared with potent plant extract concentrations four hour prior to UV-exposure. The degree of protection was quantified using biochemical tests and moisture content estimations. The result of biochemical tests, lipid peroxidation (assessed in terms of MDA) and glutathione estimation showed that prepared herbal formulation topical treatment was capable to reduce the effects of UV light induced photoaging on the mice skin by decreasing MDA level from 918±6.94 to 226 (±9.17) nm/gm of tissue (P<0.01) and by increasing GSH level from 66±3.97 to 206±8.27 µg/gm (P<0.01) compared to UV irradiated control group. Moisture content was also found to be increased from 520±5.54 to 649±3.86 (weight in mg) in prepared herbal formulation treated mice groups after 7 days treatment. Results based on lipid peroxidation, glutathione estimation & moisture content determination indicated a photo-protective effect of prepared formulation on the skin after UV exposure.

Key words: Cutaneous photoaging, Polyherbal cosmeceutical, UV radiation, Photoprotection.

INTRODUCTION

Extrinsic ageing is characterized by chronologically premature and coarse skin wrinkling, elastosis, irregular skin pigmentation, telangiectasia and roughness. The most notable cause of extrinsic ageing is chronic exposure to UV radiation *i.e.* the sun (Nicol and Fenske, 1993; Fulton, 1997; Korac and Khambholja, 2011). The severity of extrinsic ageing is dependent upon ethnicity, eye and hair colour and certain genetic factors, such as the individuals' tendency to form nevi and freckles. Photodamage is independent of chronological age, and is limited to those areas, which are exposed to the sun (Taylor *et al* 1990).

It has been revealed that after UV exposure various biochemical processes take place in the skin, whose end products are engaged in

photoaging (Ryu *et al* 1997). To understand the complex phenomena of photoageing; primarily due to generation of free radicals, it is necessary to appreciate the role the antioxidant enzymes as well. These antioxidants act in concert to minimize the damage caused by ROS within the cell (Steenvoorden and van Henegouwen, 1997).

In order to combat various deleterious effects produced by ROS generated from ultraviolet radiation, human body has built-in antioxidant mechanism to suppress uncontrolled free radicals but unfortunately these defense mechanisms may at times fail due to overwhelming production of toxic radicals, the depletion of compounds used in the skin's defence system, or a combination of both (Kligman, 1989). So, to overcome this problem, the development of a novel formulations that can minimize the effects of ROS and delay cutaneous aging has been the quest of the cosmeceutical industries (Milbury *et al* 2006).

Since decades, plants are well known for their vital pharmacological effects due to their constituents (Dahiya, 2007; 2008; Dahiya and Kumar, 2008; Agarwal *et al* 2015; Degirmenci *et al* 2016; Dahiya *et al* 2017; Oloke *et al* 2017; Dahiya and Singh, 2017; Senthil Kumar *et al* 2017; Viana *et al* 2017). Many plant constituents have been reported to retard the oxidation process in their natural environment and in formulation (Emran *et al* 2012; Shrestha *et al* 2016; Gajendiran *et al* 2016). Natural anti-oxidants occur in all plant parts *e.g.* wood, bark, stems, pods, leaves, fruits, roots, flowers and seeds. These are usually phenolic or polyphenolic compounds (Kim *et al* 1997a; Grant, 1999). Typical compounds that possess anti-oxidative activities including tocopherol, flavonoids, cinnamic acid derivatives, phosphatides and polyfunctional organic acids can inhibit mutagenesis and carcinogenesis in addition to retarding ageing. Thus, topical delivery of these agents is an attractive alternative and using optimized delivery of antioxidants will ensure their place in the cosmetic scientist's armamentarium for the next millennium (Monagas *et al* 2007).

In the past decade, the antioxidant activity of various plant phenolic, flavanoids, carotenoids, catechins, polyphenolic compounds in plant extracts have demonstrated the ability to suppress UV-induced adverse effects (Kim *et al* 1997b; Stahl and Sies, 2007).

It has been decided to explore the topical antioxidant efficacy of certain herbal extracts comprising these and similar phytoconstituents and looking to phytochemical profile, *Tagetes erectus, Aloe vera, Areca catechu, Daucus carota, Prunus amygdalus* have been chosen for the present investigation in order to develop an ideal cosmetic product.

MATERIAL AND METHODS

Collection and identification of plant material

Flowers of *Tagetes erectus*, roots of *Daucus carota*, seeds of *Areaca catechu* and kernels of *Prunus amygdalus* were collected from the local market in the month of October-November. The plant material was authenticated on the basis of taxonomic characters and by direct comparison with the herbarium specimens available at the Meseum-cum herbarium of Department of Botany, Dr. H.S. Gour University, Sagar where the voucher specimen of the plants has been deposited.

Preparation of selected herbs

The plant materials were cleaned by dusting, shifting and blowing to remove surface contaminants. The flowers heads of *T. erectus* and cut roots of *D. carota* were spread in layers over clean polythene sheets in shady and airy room with proper air circulations.

The plant materials were frequently turned over to facilitate drying to avoid any microbial growth and other degradation. After drying, the materials were powdered in a mixer/grinder and sieved through 40 mesh size and the coarse powder thus obtained were stored in a well stoppered, labelled container for further studies.

Preparation of plant extracts

Extracts of *T. erectus* and *D. carota* were prepared using 60% ethanol by maceration followed by Soxhlet extraction. The dried flower powder of *T. erectus* were kept in 60% ethanol for 7 days for maceration. The solvent used for maceration was reused for Soxhlet extraction and that was continued till the material was exhausted. The extract was then concentrated to obtain the syrupy extract, which was then lyophilized in lyophilizer to get dry extract.

The dried root powder of *D. carota* was processed by the same procedure as applied for *T. erectus* using 60% ethanol. The ethanol extract was obtained by the maceration followed by Soxhlet extraction using the macerate. The syrupy ethanol extract was lyophilized to get dry extract.

Seeds of *A. catechu* were crushed into powder and sieved through Sieve no. 40. One kg of this powder was macerated for 7 days using 80% methanol (2 litre). The macerated material was reextracted with the same solvent by Soxhlet extraction method. The saturated solvent was evaporated to get syrupy extract. The syrupy extract was lyophilized to obtain dry extract of *A. catechu*.

Kernels of *P. amygdalus* were kept in vessel containing water for 24 h. The outer coat of kernels was removed and kernels were crushed, blended with minimum volume of water to form paste. Aloe gel used in formulation was received as a gift sample from Sapna Herbal Products, New Delhi.

Preparation of herbal cosmeceutical formulation

Three grams of *T. erectus* ethanol extract (60%), 2 g *P. amygdalus* paste, 3 g Aloe gel , 4 g of *D. carota* ethanol extract (60%) and 4 g of *A. catechu* methanol extract (80%) were taken and incorporated into cream base comprising 60 g PEG 400 and 40 g PEG 4000 respectively.

Weighed quantity of PEG 4000 was heated on a water bath to 50-52°C for melting and then weighed quantities of the extracts were added to it one by one and stirred until all ingredients were mixed thoroughly. Subsequently, weighed quantity of PEG 400 was incorporated into prepared mixture consisting of PEG 4000 and the plant extracts and the mixture was further stirred constantly until it congealed to form homogenous cream.

Selection of animals

Female Lacca mice weighing 15-25 g were used. The animals were housed in plastic bottom cages where they were allowed free access to standard animal feed and water. The hairs on demarcated area of approximately 4 cm^2 on the dorsal surface of mice were removed using a rose anne french hair removing cream. The mice were observed for 48 h and those showing any abnormal hair growth or any reaction to the cream were excluded. A hair removing cream was preferred over a shaving blade to minimize free radical production due to trauma from the blade.

UV light exposure conditions and development of photo ageing

Mice were divided into groups of 24 animals each. The first group of 24 animals, neither received UV exposure nor any treatment, was the un-irradiated control. This set of animals were kept to account for any oxidative stress contributed by the depilatory (hair removing cream) which was applied every third day on all animals of all groups. The second group of 24 animals receiving only UV exposure of 5 sec twice a day was treated as irradiated control. The further groups each consisting of 24 mice received both UV radiations and formulations treatment. The treatment was given 4 h prior to UV exposure.

Treatment protocol

All animals were kept inside the solar simulator (designed in our laboratory, fitted with UV lamp) at a distance of 40 cm from the UV light source and UV exposure was controlled by the time of exposure. Exposure were given daily for 5 sec. For the mice receiving topical applications of drugs, the dorsal skin was treated with drugs 4 h prior to each UV radiation exposure. The formulations were delivered in a dose of 2 mg/cm^2 area of the skin.

Statistical analysis

All observations were presented as mean±SD. The data were analyzed by Mann-Whitney's test and $P \leq 0.05$ was considered as statistically significant.

Biochemical investigation

Lipid peroxidation

One gram sample of treated skin was taken. To this, 9 ml of 1.15% KCl was added and homogenized while keeping temperature of the tissue below 5°C to prevent free radical generation due to heat production. Homogenization was continued until a viscous, turbid mixture with no solid particles was formed.

To a sample (0.2 ml) of 10% (*w/v*) tissue homogenate, 0.2 ml of 8.1% sodium lauryl sulphate and 1.5 ml of 20% acetic acid solution (pH adjusted to 3.5 with sodium with sodium hydroxide) were added. Then 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA) was added. The mixture was made upto 40 ml with distilled water and then heated on a water bath at 95°C for 60 min. After cooling with tap water 1.0 ml of distilled water and 5.0 ml mixture of *n*-butanol and pyridine (15:1) were added to the above mixture which was shaken vigorously. After centrifugation at 4000 rpm for 10 min, absorbance of the organic layer was recorded at 532 nm. The levels of lipid

peroxidase were expressed as nanomoles (nmole) MDA (malondialdehyde) per gm of tissue. The observation were recorded for herbal formulation (Ohkawa *et al* 1979).

Estimation of GSH

Glutathione reduces H_2O_2 directly to water or react directly with the free radicals by radical transfer process which yield thiol radicals. This thiol radical or the sulfhydryl group present in glutathione forms a colored complex with DTNB which is measured spectrophotometrically at 412 nm (Ellman, 1959).

The promptly excised skin was kept in chilled 0.9% NaCl. After washing with saline, 500 mg of skin tissue was homogenized with 5 ml of 10% TCA using Teflon glass tissue homogenizer (Remi India) and centrifuged at 3000 rpm (4°C) for 10 min. The supernatant diluted 10 fold in phosphate buffer and kept on ice was used for study of glutathione.

The reaction mixture contained 2 ml. Phosphate buffer (5.225 g in 100 ml DW, pH adjusted to 8.4), 100 μl of sample and 500 μl of 0.002% DTNB (2 mg DTNB in 1% sodium citrate). To this reaction mixture 400 μl of distilled water was added and absorbance measured at 412 nm. Changes in absorbance was measured and the level of GSH was expressed as μg/gm of tissue.

Moisture content

The moisture content of the skin is greatly influenced by ground substances and the loss of water may be responsible for wrinkling and laxity of the skin accompanying cutaneous ageing. The moisture holding capacity of ground substances gradually decreases with the advancing age resulting in skin ageing (Jung *et al* 1997). As moisture content of the skin is a vital parameter for skin health, therefore, it was planned to evaluate the effects of herbal formulation for their moisturizing property on mice skin.

For measuring the moisture content, the fresh skin samples were weighed accurately. The samples were kept for drying until a constant weight was observed which was found to be 24 h on an average. Moisture content was calculated by the following formula:

Moisture content (%) = {(Wt. of skin before drying - Wt. of skin after drying)/Wt. of skin before drying} ×100

RESULT AND DISCUSSION

In the present biochemical investigation, the elevated level of end product lipid peroxidation in mice skin exposed to UV radiations were observed.

The increase in MDA level in skin tissue suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Pretreatment with prepared herbal formulation (2 mg/cm^2/day) significantly reversed these changes by significantly decreasing MDA level to 226 (±9.17) nmol/gm compared to UV-irradiated control that was 918 (±6.94) nmol/gm of tissue in 15 days treatment period. Prepared herbal formulation was even found to be more effective in decreasing MDA level to 226 (±9.17) in comparison to marketed Formulation which could decrease MDA level to 399 (±6.25) compared to UV-irradiated control that was 918 (±6.94) nmol/gm of tissue in 15 days treatment period (**Table 1**, **Figure 1**).

Table 1. Malondialdehyde levels upon chronic UV exposure of mice skin with formulated herbal cosmeceutical and marketed formulation

S. No.	Group	Nanomoles MDA/gm of tissue±SD. (n=6)			
		3 day	7 day	11 day	15 day
I	Un-irradiated control	198±3.40	210±3.95	213±7.8	224±9.1
II	Irradiated control	398±7.42	696±10.15	802±5.27	918±6.94
III	Prepared herbal cosmeceutical formulation	188±3.31	368±8.27	398±7.61	226±9.17*
IV	Marketed formulation	218±3.32	379±7.52	441±5.25	399±6.25*

*Values are significant ($p<0.05$) as compared to irradiated control

The level of glutathione was significantly low in UV induced oxidative stress mice when compared to un-irradiated control. The mice group pretreated with prepared formulation showed remarkable increase in GSH level. The elevation in GSH from 66 (±3.97) to 206 (±8.27) μg/gm GSH of tissue could be observed in formulation treated mice skin after 15 days of

treatment period (**Table 2,** , **Figure 2**). Further, prepared herbal formulation was found to be more effective in increasing GSH level to 206 (±8.27) in comparison to marketed formulation which could increase GSH level to 159 (±2.86) compared to UV-irradiated control.

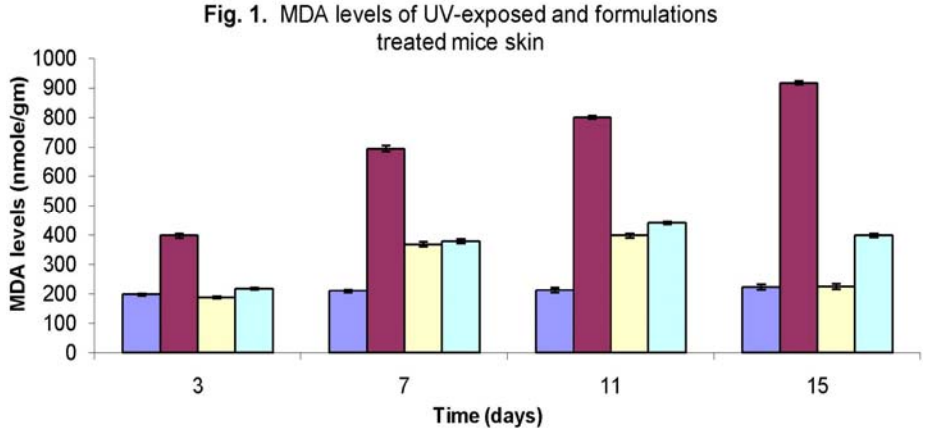
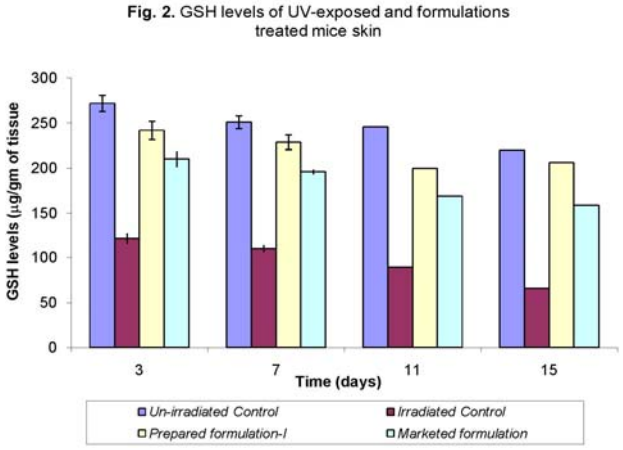


Table 2. GSH levels upon chronic UV exposure of mice skin with prepared formulated herbal cosmeceutical and marketed formulation

S. No.	Group	*μ*g GSH /gm of tissue±SD (n=6)			
		3 day	7 day	11 day	15 day
I	Un-irradiated control	272±8.62	251±9.92	246±8.83	220±7.18
II	Irradiated control	121±9.21	110±4.31	89±6.0	66±3.97
III	Prepared herbal cosmeceutical formulation	242±8.76	229±2.81	200±9.99	206±8.27*
IV	Marketed formulation	210±5.34	196±3.07	169±8.78	159±2.86*

*Values are significant (p<0.05) as compared to irradiated control



The moisture content of the skin is greatly influenced by ground substances and the loss of water may be responsible for wrinkling and laxity of the skin accompanying cutaneous ageing.

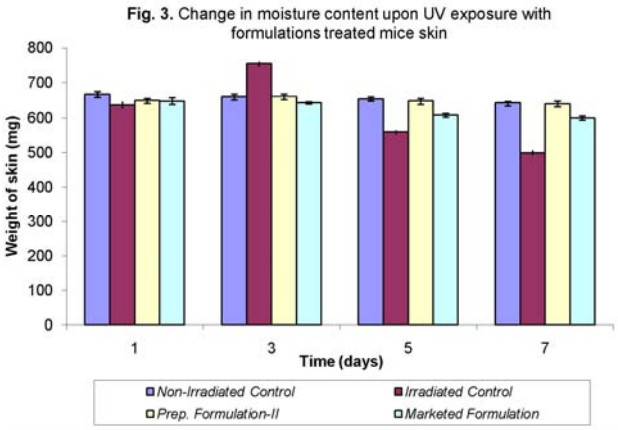
The moisture holding capacity of ground substances gradually decreases with the advancing age and the effect could be worsen with elevated level of free radicals which resulting in skin premature ageing. Present study also showed the same effect by decreasing Weight of irradiated mice skin from 653±4.32 to 520 (±5.54) (in mg).

The prepared formulation could retain moisture as the weight retained from 520 (±5.54) to 649 (±3.86) (in mg) in formulation treated mice skin group in 7 days of treatment period while marketed formulation could increase up to 600 (±7.52) mg within same 7 days treatment duration (**Table 3,** , **Figure 3**).

Table 3. Moisture content of mice skin upon UV exposure with prepared formulated herbal cosmeceutical and marketed formulation

S. No.	Group	Weight of skin in mg±SD (n=4)			
		1 day	3 day	5 day	7 day
I	Un-irradiated control	697±7.87	689±7.54	683±6.79	653±4.32
II	Irradiated control	656±10.66	634±10.29	580±7.19	520±5.54
III	Prepared herbal cosmeceutical formulation	671±8.39	682±7.40	640±8.78	649±3.86*
IV	Marketed formulation	647±8.63	681±8.81	608±10.21	600±7.52*

*Values are significant (p<0.05) as compared to irradiated control



The data clearly indicated that the formulation comprising of plant extract capable of scavenging the free radicals by showing the decrease and increase of MDA and GSH level respectively and also enhanced the moisture content in prepared formulation treated mice group as compared to irradiated control mice group. This may be possible because of presence of polyphenolic constituents especially flavonoids, carotenoids and phenolic compounds in chosen plant extracts in prepared formulation. Therefore being rich in phenolic, flavonoids, carotenoids, catechins and polyphenolic compounds, these plant extracts considered as a value-added by product for elaborating topical antioxidant ingredient. Further bioactivity guided fractionation studies for isolation and identification of active principles responsible for antioxidant activity and moisture retained properties is underway.

CONCLUSION

It is concluded from present findings that prepared cosmeceutical formulation may also have a role in preventing the adverse effects of photo-aging that may be due to antioxidant potentials against UV-induced oxidative stress and thus, it may contribute as a new photo protective cosmetic formulation for protection from UVB induced skin damage provided further bioactivity guided fractionation studies for isolation and identification of active principles in individual plant extract responsible for anti-oxidant and moisture retained properties should be explored and verified.

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