Effect of pre-treatment of almond oil on ultraviolet B–induced cutaneous photoaging in mice

Yasmin Sultana, PhD,[1] Kanchan Kohli, PhD,[1] M Athar, PhD,[2] R K Khar, PhD,[1] & M Aqil, PhD[1]

[1]Departments of Pharmaceutics and [2]Medical Elementology and Toxicology, Hamdard University, New Delhi, India

Summary

Background Ultraviolet (UV) radiation has been implicated in photoaging and various types of skin carcinomas. Although the human skin has evolved several defense mechanisms to survive the insults of actinic damage like keratinization, melanin pigmentation, etc., it is still subjected to the harmful effects of sunlight.
Aims In this study, the role of almond oil in reducing the degradative changes induced in skin upon exposure to UV radiation was investigated.
Methods Mice were divided in four groups of 20 animals. Group I was the control group. Group II was negative control, which received almond oil treatment alone. Group III was exposed to UV radiation only and Group IV received both UV treatment and almond oil treatment. Visible skin grading assessed the changes based on a rating scale, biochemical tests (glutathione estimation and lipid peroxidation), and histopathologic studies.
Results Upon exposure of mice to UV radiation, it was found that pronounced visible skin changes were seen after 12 weeks of exposure. The results of the biochemical tests, glutathione estimation, and lipid peroxidation showed that almond oil reduced the effect of UV light–induced photoaging on the skin. Histopathologic studies also indicated a photoprotective effect of almond oil on the skin after UV exposure.
Conclusions It was concluded that topical almond oil is capable of preventing the structural damage caused by UV irradiation and it was also found useful in decelerating the photoaging process.

Keywords: almond oil, cutaneous photoaging, photoprotective effect, ultraviolet light

Introduction

Primary factors contributing to wrinkled, spotted skin include chronologic aging, exposure to the sun (photoaging), and loss of subcutaneous support. Other factors that contribute to aging of the skin include stress, gravity, daily facial movement, obesity, smoking, and even sleep position. Smoking can produce free radicals – oxygen molecules that are overactive and unstable.

Correspondence: Dr Yasmin Sultana, PhD, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India.
E-mail: yas2312003@yahoo.com

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Photoaging is significantly different from chronologic aging in both clinical and histologic appearance.[1] However, unlike chronologic aging, which depends on the passage of time per se, photoaging depends primarily on the degree of sun exposure and skin pigment. Our current knowledge of cellular and molecular mechanisms that bring about chronologic aging and photoaging reveals that chronologic aging and photoaging share fundamental molecular pathways.[2]

Although sunscreens are useful in protecting skin against UV-catalyzed degradation, they suffer drawback of incomplete spectral protection and toxicity.[3] Antioxidants work on three levels to combat free radicals. They stop free radicals from forming both before and after insult by

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the sun and also may correct some sun damage. Many topical antioxidants have been reported previously for supplementing sunscreen protection and providing additional anticarcinogenic protection, like vitamins C and E, selenium, zinc, silymarin, soy isoflavones, polypodium leucotomos, and tea polyphenols.[4–7] Almond oil has been used as a lipophilic vehicle to reduce UV-catalyzed degradation of retinyl palmitate to the less active *cis*-isomers.[8]

Almond oil has been extensively used in traditional medicine to maintain the elasticity of the skin and its youthful appearance.[9] The objective of this study was to evaluate the photoprotective effect of almond oil in mice against UV radiation–induced chronic skin damage. The degree of protection is quantified using biochemical, histopathologic, and visual perception methods.

Methods

Almond oil was a gift sample from Hamdard (Wakf) Laboratories, India, and all other chemicals and reagents were purchased at the highest purity available.

Animals

The studies were carried out on healthy adult Swiss female mice of the Jamia Hamdard Animal House weighing approximately 25–30 g. The animals were kept in the group of five in polypropylene/plastic cages housed in an air-conditioned room. They were fed with the standard pellet diet (Gold Mohar, Lipton India Ltd, Bombay, India) and had *ad libitum* access to water.

Light exposure conditions and development of photoaging

Mice were divided into groups of 20 animals each. The first group of 20 animals, which received no treatment, was the control group. The second group of 20 animals, which received almond oil treatment, was the negative control. The third group of 20 animals received UV treatment only, and the fourth group of 20 animals received both UVB and almond oil treatment.

Treatment protocol

The hairs on the dorsal side of the mice were removed by using an electric clipper followed by the application of a depilatory cream.

Animals of Groups III and IV were kept inside the UV chamber (designed in our laboratory, fitted with UV lights) at a distance of 30 cm from the UV light source (a bank of six Toshiba fluorescent sunlamps [FLSE lamps]) and UV exposure was controlled by the time of exposure. Exposures were given daily for 15 min for the first week and the exposure time was increased by 15 min every week. At the end of 12th week, when pronounced wrinkles were developed on the skin of the mice, the exposure time was 3 h, which was continued till the end of the experiments. For the mice receiving topical treatment of almond oil, the dorsal skin was treated with almond oil 4 h prior to each UV radiation exposure. The test formulations were delivered to 3-cm^2 areas of the skin outlined by adhesive tape using micropipette for delivering almond oil. The oil was then spread evenly over the dorsal skin with the flat edge of the disposable pipette tip. The erythema produced upon exposure of the skin to UV light were noted in the different groups of animals (where 0 is no erythema, 1 is mild, 2 is moderate, and 3 is severe erythema) and is given in Table 1.

Table 1 Erythema produced on exposure of skin of mice to ultraviolet B light.

	Mean (± SEM) of erythema produced in mice (n = 20)				
Group	Zero week	Third week	Sixth week	Ninth week	Twelfth week
I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
II	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
III	0 (0)	2.62 (0.50)	2.71 (0.46)	2.80 (0.40)	2.90 (0.30)
IV	0 (0)	1.09 (0.30)	1.05 (0.22)	0.66 (0.48)	0 (0)

Group I, normal mice; Group II, almond oil–treated mice; Group III, UVB treatment; Group IV, almond oil and UVB treatment.

Visible skin grading

Mice were observed for skin wrinkling (UVB radiation–induced event) and grading of the skin was done on the basis of wrinkling, where 0 is normal, 1 is mild, 2 is moderate, and 3 is maximum visible skin change. The visual observations were done for skin elasticity/wrinkling based on the rating scale and are given in Table 2.

Biochemical tests

Six animals of each group were taken and animals were killed by cervical dislocation. Glutathione estimation and lipid peroxidation assay were done on skin homogenate.

Estimation of reduced glutathione[10]

Intracellular-reduced glutathione was estimated in the homogenates of skin of normal (Group I), UV-exposed

Table 2 Skin wrinkling of the mice exposed to ultraviolet B treatment.

Group	Mean (± SEM) of skin wrinkling produced in mice (n = 20)				
	Zero week	Third week	Sixth week	Ninth week	Twelfth week
I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
II	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
III	0 (0)	2.45 (0.51)	2.80 (0.41)	2.90 (0.30)	3.00 (0)
IV	0 (0)	1.55 (0.51)	1.15 (0.36)	0.95 (0.22)	0 (0)

Group I, normal mice; Group II, almond oil–treated mice; Group III, UVB treatment; Group IV, almond oil and UVB treatment.

(Group III), and UV-exposed and almond oil–treated mice (Group IV). The homogenate was obtained by centrifuging aliquot drawn out from each sample at 400 × g for 54 min. To the homogenate, 1.8 mL of 1 g/L EDTA and 3 mL of precipitating reagent (1.67 g metaphosphoric acid, 0.20 g EDTA, and 30 g NaCl in 100 mL of double distilled water) were added. After mixing, the sample was allowed to stand for 5 min before being filtered. Two milliliters of the filtrate was added to 4 mL of disodium hydrogen phosphate buffer (0.1 M, pH 7.4) and 1 mL of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) reagent (40 mg of DTNB in 100 mL of 10 g/L sodium citrate). A blank was prepared by adding 0.8 mL of EDTA solution, 4 mL of disodium hydrogen phosphate and 1 mL of DTNB reagent. The result was expressed as μmol glutathione/g of tissue (mg protein).

The absorbance was read within 5 min at 412 nm against a reagent blank with no homogenate using DU-64 Beckman spectrophotometer, Beckman Coulter, American Laboratory Trading, Groton, CT. The total glutathione content was calculated using the formula described by Ellman.[10] The content "Co" of glutathione is given by

$$Co = AD/E$$

where A is absorbance at 412 nm, E is molar extinction coefficient, and D is dilution factor.

Lipid peroxidation assay

Enzymatic and nonenzymatic lipid peroxidation was assessed by estimating 2-thiobarbituric acid reacting substance, i.e. malonaldehyde (MDA).[11]

The reaction mixture in 2 mL contained 2 mg of microsomal protein in 1.5 mL of phosphate buffer (0.1 M, pH 7.4) with EDTA (0.1 mM). For the enzymatic and nonenzymatic lipid peroxidation studies, the final concentrations of NADPH and iron in incubation media were 0.4 mM and 2.5 mM, respectively. The reaction mixture was incubated aerobically at 37 °C for 1 h, with constant shaking in metabolic shaker. Reaction was terminated by the addition of 0.3 mL of HCl (5 N) and 0.625 mL of 40% trichloroacetic acid. The samples were then transferred into centrifuge and 0.625 mL of 2% neutralized thiobarbituric acid solution was added and the tubes were incubated at 90 °C for 20 min in a temperature-controlled water bath. After incubation, the samples were placed in an ice bath for 5 min and then centrifuged at 10 000 × g for 5 min in Sorvall RC 5B (GMI, Inc., Bamsey, MN) refrigerated super speed centrifuge. The amount of malondialdehyde formed in each of the samples was assessed by measuring the topical density of the supernatant at 532 nm using a Beckman DU-64 spectrophotometer. The results were expressed as nmol MDA formed/hr/mg protein at 37 °C by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results of glutathione estimation and lipid peroxidation assay are given in Tables 3 and 4.

Table 3 Results of glutathione estimation.

Group	Mice treated with formulation	Mean of glutathione content (μmol/g)	± SEM
I	Normal mice	5.45×10^{-4}	0.200
II	Mice treated with almond oil	5.84×10^{-4}	0.320
III	UV-treated mice	1.68×10^{-4}	0.520
IV	UV- and almond oil–treated mice	3.52×10^{-4}	0.600

Table 4 Results of lipid peroxidation assay.

Group	Mice treated with formulation	n moles of MDA/mg of protein, thiobarbituric acid reactive substances (TBARS) mean (± SEM)
I	Normal mice	28.27 ± 1.547
II	Mice treated with almond oil	28.27 ± 0.001
III	UV-treated mice	327.04 ± 0.169
IV	UV- and almond oil–treated mice	56.15 ± 0.043

Histologic studies

Female albino mice were exposed to UV radiation for 12 weeks and skin biopsies (2 × 10 mm) from the dorsal side of normal mice (Group I), UVB-exposed mice (Group III) after 12 weeks, and almond oil–treated and UVB-exposed mice (Group IV) after 12 weeks were taken for histologic evaluation. Skin biopsies were fixed in 10% buffered

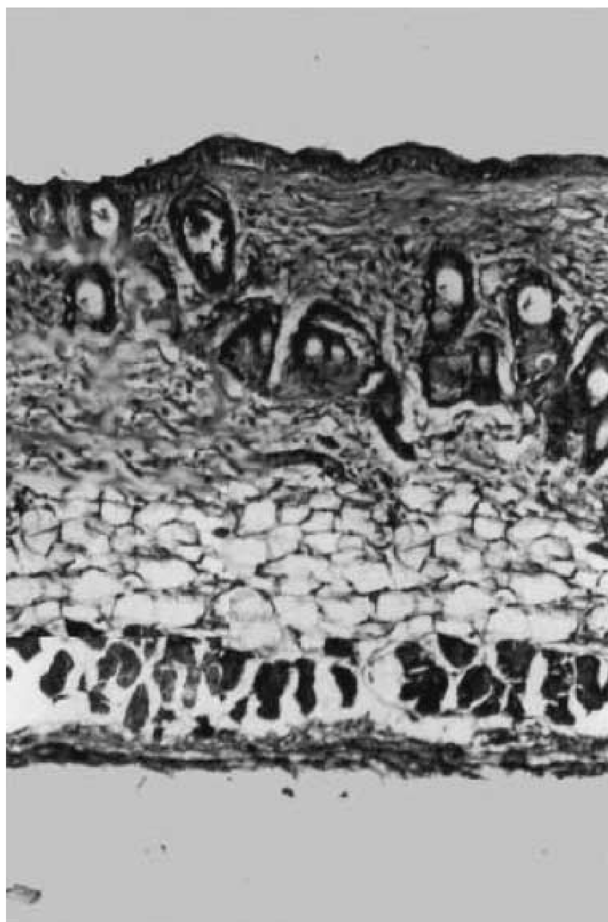

Figure 1 The photographs showing histology of Group I (normal) mice (H&E ×80 magnification). Normal skin of mice revealing normal various layers of skin – dermis, stratum corneum, stratum luciderm, and stratum granulosum – shows normal connective tissue in collagen fibers along with various glands.

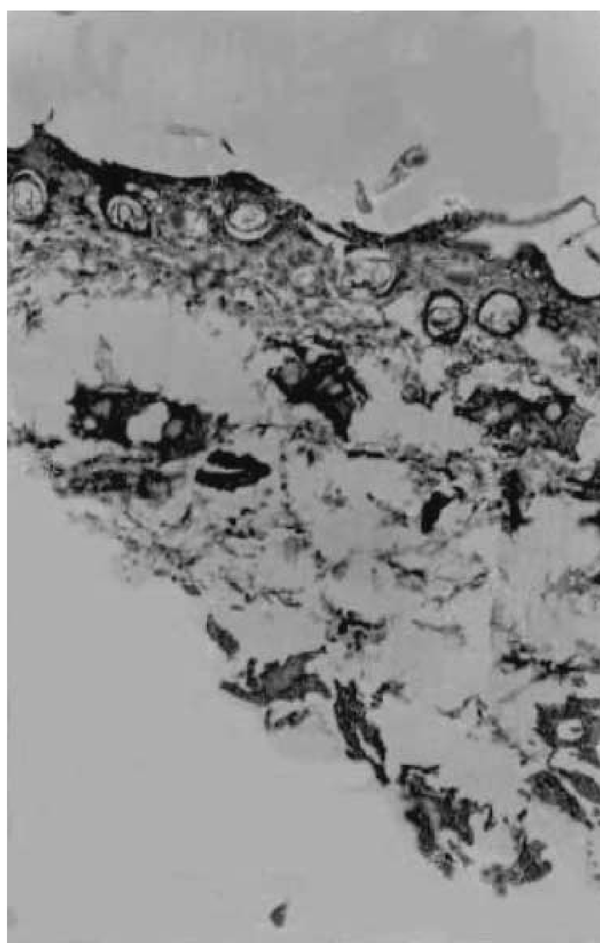

Figure 2 The photographs of Group III mice after 12 weeks of exposure to UV radiation (H&E ×100 magnification). Section reveals severe dysplasia and dyshesive changes in the epidermal layer. At places there is complete absence of epidermal layer of the skin. There is marked elastosis with accumulation of elastotic material in the dermal layer along with basement membrane of the epidermis.

formalin, embedded in paraffin, and sectioned at 6–10 microns. Sections were then stained with hematoxylin and eosin stain (H&E ×80 magnification). The photographs of groups I, III, and IV mice after 12 weeks of exposure to UV radiation are given in Figures 1–3.

Results and discussion

Ultraviolet C does not reach the earth's surface because it is absorbed by the ozone layer of the atmosphere. UVB reaches us in significant amounts; it produces acute sunburn and is carcinogenic. UVA is more insidious; it penetrates deeper into the skin and breaks down collagen. Chronic UV irradiation of both human and mouse skin causes the production of large quantities of thickened, twisted, abnormal elastic fibers that results in elastosis. This is associated with a substantial loss of collagen and with greatly increased amounts of glycosaminoglycans, the ground substance. In addition, dermal blood vessels become dilated and distorted and eventually are nearly destroyed.[12–17] In humans, the manifestation of these connective tissue changes resulting from similar chronic UV exposure are yellowed, leathery, wrinkled, sagging skin, a condition that is known as photoaging.[18] These abnormalities have been thought to be irreversible. It was found that appreciable repair could occur after the application of almond oil. Mice were chosen as the experimental animal as it suffers actinic damage similar to that of humans.[19]

Sweet almond oil is an extremely popular oil, sought after for its rich concentration of oleic and linoleic

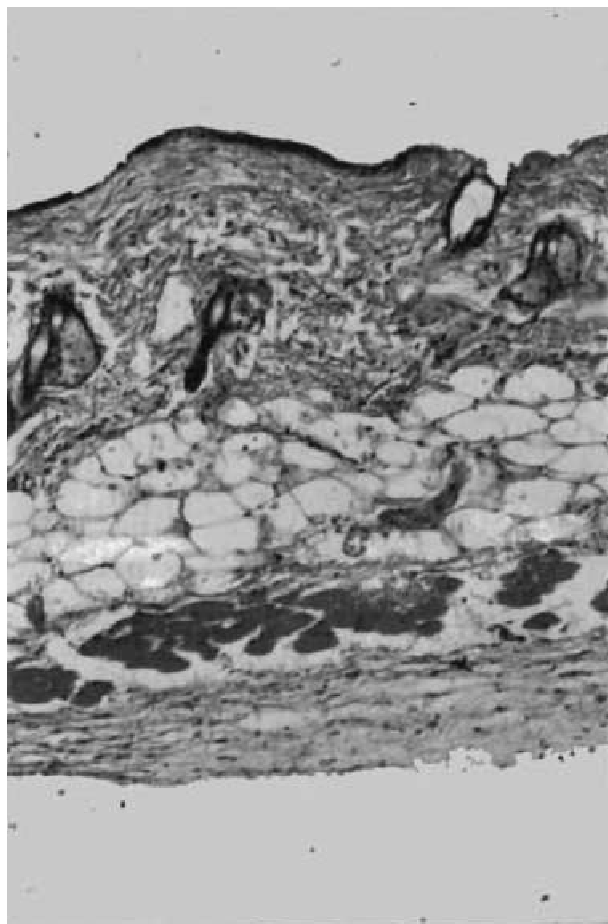

Figure 3 The photographs of Group IV mice after 12 weeks of exposure to UV radiation (H&E ×80 magnification). Skin of mice revealed well-marked regular epidermal layers like stratum corneum, stratum luciderm, and stratum granulosum. Dermal layer shows well brought out papillary layer revealing collagen fibrils, comprising of reticular and elastic fibers.

acids.[20] The average composition of fatty acids is: oleic acid, 64–82%; linoleic acid, 8–28%; and palmitic acid, 6–8%. Almond oil is rich in β-sitosterol, squalene, and α-tocopherol, and it is one of the best sources of vitamin E, which protect healthy cells from destruction by free radicals. Almond oil is a good source of manganese, an essential trace metal used as a skin supplement for suppressing premature aging, irritation, and blemishes. Manganese also triggers energy production and activates certain skin enzymes.

Mice were exposed to UV light and exposure time was increased every week by 15 min; no well-defined erythema was noted till the exposure time reached 2 h, 45 min. When exposure time was increased to 3 h, pronounced erythema developed and shrinkage of the mice skin was noted. Wrinkles developed in 12 weeks' time. Mice developed visible skin changes, histologic alterations, and tumors (rare) (Tables 1 and 2).

The results of the biochemical tests (glutathione estimation and lipid peroxidation) showed that almond oil reduces the effect of UV light–induced aging on the skin. Glutathione levels were reduced in the skin of UV-exposed mice, and after treatment with almond oil, glutathione levels again increased (Table 3).

It was found that levels of thio-barbituric acid reactive substances (TBARS) significantly increased upon exposure of mice to ultraviolet light, and almond oil applied topically was effective in reducing this to normal levels (Table 4).

Normal skin of mice revealing normal various layers of skin, epidermis (stratum corneum, stratum luciderm, and stratum granulosum), dermis and normal connective tissue in collagen fibers along with various glands. There is presence of smooth muscles (Fig. 1).

After 12 weeks of exposure of Group III to UV radiation, a section revealed severe dysplasia and dyshesive changes in the epidermal layer. At places there was complete absence of epidermal layer of the skin. There was marked elastosis with accumulation of elastotic material in the dermal layer along with basement membrane of the epidermis (Fig. 2). It was found that in the animals of Group IV in 12 weeks, skin of mice revealed well-marked regular epidermal layers like stratum corneum, stratum luciderm, and stratum granulosum (Fig. 3). Dermal layer showed well brought out papillary layer revealing collagen fibrils, comprising of reticular and elastic fibers. The reticular layer revealed bundles of collagen fibers as well as smooth muscle fibers, thereby revealing total reversibility of skin. After the cessation of irradiation, a broad band of new normal collagen was produced subepidermally, pushing downward the old elastotic tissue.

Almond oil contains several double bonds, which could interfere with light-induced degradation Additionally, UV-exposed and almond oil–treated mice showed a lower degree of histologic parameters of photoaging damage, including dermal elastosis and skin tumors, compared with mice treated with UV radiation alone. The results of this study illustrate that almond oil treatment helps to ameliorate and to partially inhibit some of the histologic damage associated with photoaging of skin and appears to contribute to a decrease in the prevalence of UVB-induced skin tumors in mice.

Conclusion

On the basis of visible changes, biochemical tests, and histologic studies, it can be concluded that almond oil treatment revealed photoprotective effects on the skin.

Further work is needed to establish the efficacy of almond oil in the treatment of aging of the skin in the humans.

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