EFFECT OF TOPICALLY APPLIED OLIVE OIL ON THE SKIN OF MICE AFTER THE PROLONGED EXPOSURE TO UVB

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Abstract. Since olive oil has been reported to be a potent antioxidant, we examined its effects on Swiss albino mice. Olive oil was applied topically before repeated exposure of the mice to UVB. Histological data indicated that penetration of the olive oil into the skin of the mice was directly correlated with the time of exposure to UVB radiation. Additional experiments will be required to demonstrate the ability of olive oil to inhibit DNA damage induced by UVB.

Key words: Olive oil, Swiss albino mice, UVB radiation.

INTRODUCTION

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main parts of wavelengths, ultraviolet (45%), visible (5%), and infrared (50%) [5]. Ultraviolet (UV) light region occurs between 100–400 nm. According to the international commission on illumination, UV radiation is divided into three categories depending on the wavelength: long wave UVA (315–400 nm), medium wave UVB (280–315 nm), and short wave UVC (100–280 nm) [5, 6, 8]. Intensive or extensive exposure to UVA can burn sensitive skin, and if prolonged, it can damage underlying structures in the corium and cause premature photoaging of the skin [5]. After UVA exposure reactive oxygen species (ROS) are generated and they can mediate the damage to cellular proteins, lipids and saccharides; also, ROS may produce structural damage to DNA, impair the immune system, which will lead to cancer. The exposure to UVA has been linked to 67% of malignant melanoma [1, 2, 12].

Some natural agents, particularly plant and vegetable extracts (green tea, olive oil, rapeseed oil and safflower oil) have widely been considered to have

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antioxidant and free radical scavenging capabilities. UV induced carcinogenesis was suppressed when olive oil, green tea polyphenol fractions were topically applied to the mouse skin or were ingested orally in the drinking water [14, 15]. Olive oil packed with antioxidants protects human from developing cancer and helps to prevent premature aging. Laboratory studies show that mice that received topical olive oil treatment after being exposed to UV rays developed fewer skin cancer related tumors [4]. Study of methylene blue penetration into skin using a digital analysis method for color images is very important for effective photodynamic therapy for several diseases [7].

This work aimed to examine the effects of time variation on the topical application of olive oil on the skin of Swiss albino mice after UVB exposure.

MATERIAL AND METHODS

15 Swiss albino mice of either sex, 6–8 weeks of age, weighing 25–30 g were used. The animals were housed in standard cages, maintained at room temperature, and fed with regular food. The study protocol with the experimental animals was carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University (Appendix 2, Guiding Principles for Biomedical Research Involving Animals, 2011).

The mice used in this experiment were divided as followings: 1) 5 mice were treated with olive oil (extra-virgin olive oil, 99% purity, was a gift from DHC Co., Tokyo, Japan) and left in the normal environment for two hours; 2) 10 mice were treated with olive oil, and were divided into 2 groups: 5 mice were exposed to UVB for one hour and 5 mice were exposed to UVB for two hours.

The hair from the dorsum of the mice was removed using an electric razor. Prior to shaving, the mice were anaesthetized with ether (Sigma-Aldrich); the animals were treated with olive oil in conjugation with the methylene blue (MB, Sigma-Aldrich) and then exposed to UV radiation: 630 nm, total output power of 65 mW, 220 V, Photon Company, Egypt. The lamp used in this experiment, which emitted the total spectrum of ultraviolet radiation was: 1% UVC, 53% UVB, and 46% UVA. The distance between the exposure unit and the back of the exposed animals was approximately 22 cm and this minimized the variation in the intensity of UV exposure at the level of the dorsum of the animals.

The treated skins collected from each animal were cut immediately into pieces and frozen at –30°C in the cryostat to prepare frozen cross-sections which were picked up on slides. Then, the slides were left out to dry and were stained with eosin as a counter stain for better visualization of the morphology of the skin layers. The sections were photographed using Olympus light microscopy BX41 attached with digital camera Olympus U-CMAD3 (Japan) and analyzed with the imaging software (image analyzer,

Image J Software Math work Software Inc., USA). All images were evaluated with regard to the depth of MB staining which penetrated into the skin layers (Fig. 2). All images were collected under the 400× original magnification [7].

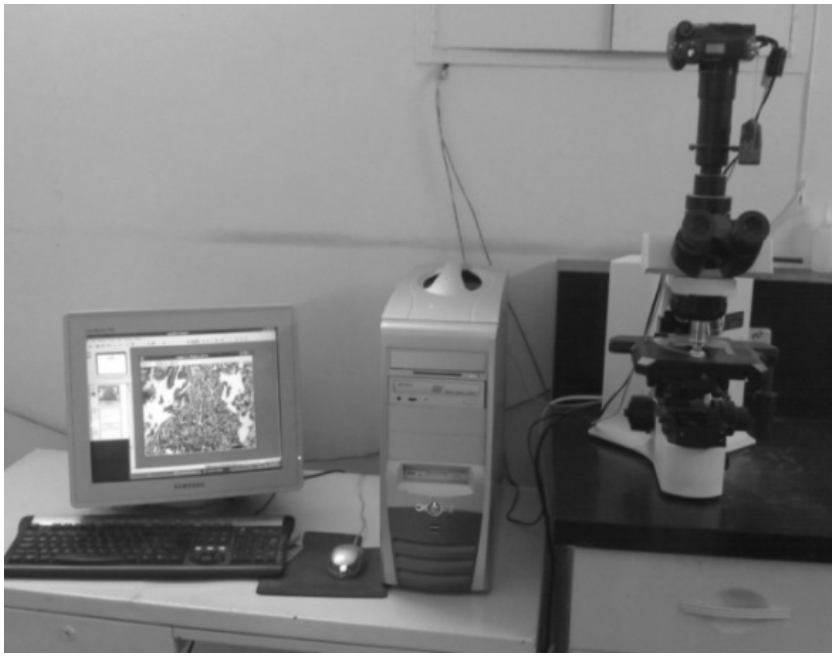

Fig. 1. Photograph of the image analyzer: light microscope and PC to visualize and analyze the tissue sections.

RESULTS

Figure 2 shows the comparison between the studied groups according to the amount of methylene blue transported through the skin. We can notice that with increasing the time of exposure to UVB, the amount of the penetration of MB also increased (indicated by the amount of the absorption of the olive oil through the skin) and these results were confirmed by the histopathological study. Figure 3 demonstrates frozen section of the skin mice treated with olive oil only. One can notice the poor penetration of the MB through the skin. Figure 4 illustrates frozen section micrograph of mice skin treated with olive oil, and exposed to UVB radiation for 1 hour, while figure 5 shows frozen sections of the mice skin treated with olive oil, and exposed to UVB radiation for 2 hours. The penetration of MB increased gradually and the penetration of the olive oil increased also with increasing the time of exposure.

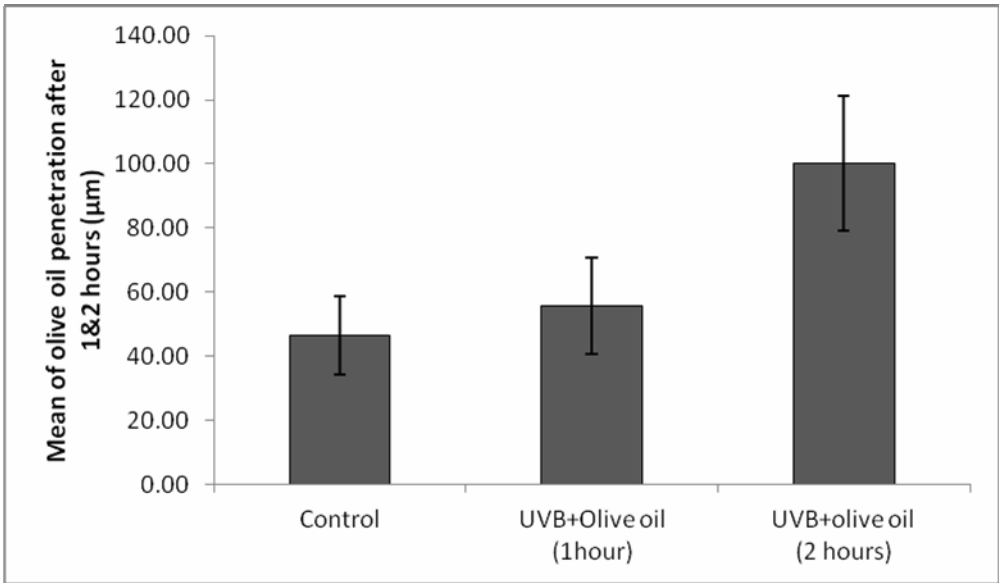

Fig. 2. Comparison between the studied groups according to the penetration of MB. Examination was carried out with the light microscope using control and treated skin samples for 1 and 2 hours, respectively (n = 12).

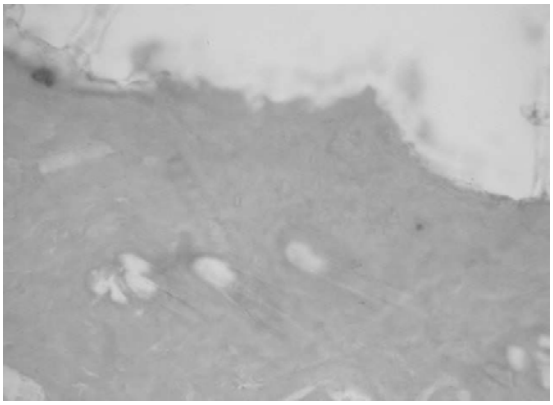

Fig. 3. Micrograph of the frozen section in case of normal skin of the mice. The absence of the MB in the skin layers can be noticed (400×).

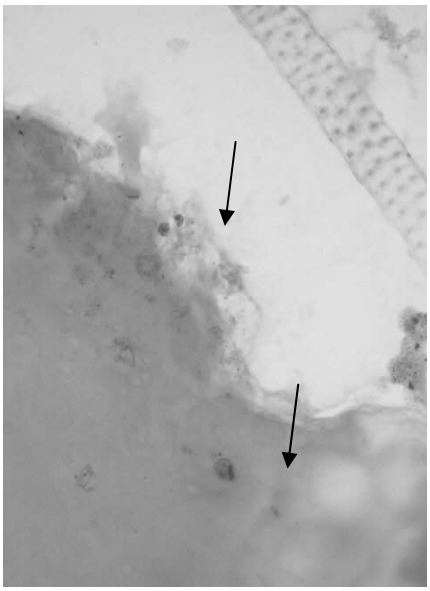

Fig. 4. Micrograph of the frozen section of mice skin treated with olive oil and exposed to UVB radiation for about 1 hour. Note the penetration of MB stain (arrows) both in epidermis and dermis of the skin layers (40×).

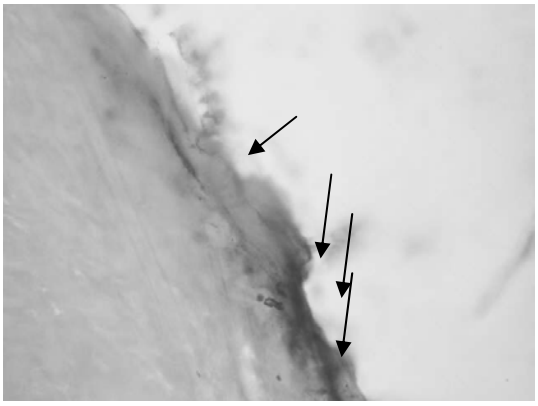

Fig. 5. Frozen sections of the mice skin treated with olive oil, and exposed to UVB radiation for about 2 hours. Note the spread of MB stain (arrows), both in epidermis and dermis of skin layers (400×).

DISCUSSION

UV light energetic photons transmitted through the skin layers can be absorbed by the cellular molecules, such as chromophores or photosensitizers and the major chromophoric aminoacids present in proteins are tryptophan, tyrosine, and phenylalanine. UVB radiation was experimentally demonstrated to cause DNA damage, mostly by formation of dimeric photoproducts between adjacent pyrimidine bases [3]. Some studies revealed that UVB damage can be reduced by following a diet that is high in omega-9 (olive oil) and omega-3 (oily fish) fatty acids and dietary antioxidants such as carotenoids, vitamin C, E, selenium, flavonoids, and polyphenols whilst low in red meat, saturated fat and dairy [3]. Carotenoids such as beta-carotene, lutein and lycopene, found in red/orange/yellow fruits and vegetables, are particularly effective. Wilhelm Stahl, a German scientist, has shown that carotenoids, when eaten habitually over a long period, protect against sunburn and increase the capacity of the skin to reflect UV rays, improving skin protection [3]. Studies also link beta-carotene with reduced risk of melanoma and revealed that lutein may be able to protect the skin against UVB [9]. Olive oil contains phenolic compounds such as hydroxytyrosol and oleuropein, which have been shown to act as antioxidants [9, 12, 11]. It has been shown that hydroxytyrosol in olive oil is highly protective against DNA damage caused by the prolonged exposure to UVB [2]. In this study we evaluated the spreading of olive oil into the skin of Swiss albino mice exposed to UVB radiation. The olive oil infiltrated the skin of the mice in time dependent manner in the presence of UVB radiation. Kimura *et al*. reported that olive oil prevented chronic UVB-induced skin damage and carcinogenesis; their findings suggested that the preventive actions of the oil on chronic UVB-induced skin damage, tumor incidence and tumor growth may be due to inhibition of the expression of several genes [10].

CONCLUSION

Olive oil is known to be a potent protective against the damage induced by UVB radiation. In our study we presented the spreading of the olive oil in the skin of Swiss albino mice after the exposure to UVB and the penetrance of the oil was time dependent. Further studies are also needed to illustrate the best protection of the oil for use in the community.

REFERENCES

1. AFAG, F., H. MUKHTAR, Effects of solar radiation on cutaneous detoxification pathways, *J. Photochem. Photobiol.* 2001, **63**, 61–69.

2. AFAG, F., H. MUKHTAR, Photochemopreventation by botanical antioxidants, *Skin Pharmacol. Appl. Skin Physiol*. 2002, **15**, 297–306.
3. SVOBODOVA, A., D. WALTEROVA, J. VOSTALOVA, Utraviolet light induced alteration to the skin, *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Republic*. 2006, **150**, 25–28.
4. BUDIYANTO, A., N.U. AHEMD, A. WU, O. NIKAIDO, T. OSAWATA, M. UEDA, M. ICHIHASHI, Protective effect of topically applied olive oil against photocarcinogenesis following UVB exposure of mice, *Carcinogenesis*, 2000, **21**, 2085–2090.
5. CLYDESDALE, G.J., G.W. DANDIE, H.K. MULLER, Ultraviolet light induced injury: immunological and inflammatory effects, *Immunol. Cell Biol*., 2001, **79**, 547–568.
6. DUTHIE, M.S., I. KIMBER, M. NORVAL, The effects of ultraviolet radiation on the human immune system, *Br. J. Dermatol*., 1999, **140**, 995–1009.
7. GENINA, E.A., A.N. BASHKATOV, V.V. TUCHIN, In vitro study of methylene blue diffusion through the skin tissue, *Proc. of SPIE*, 2002, **4609**, 29–36.
8. HUSSEIN, M.R. Ultraviolet radiation and skin cancer. Molecular mechanisms, *J. Cutan. Pathol*., 2005, **32**, 191–205.
9. LITRIDOU, M., J. LINSSEN, H. SCHOLS, M. BERGMANS, M. POSTHUMUS, M. TSIMIDOU, D. BOSKOU, Phenolic compounds in virgin olive oils: Fractionation by solid phase extraction and antioxidant activity assessment, *J. Sc. Food. Agric*., 1997, **74**, 169–174.
10. KIMURA, Y., M. SOSHIYUHI, Olive leaf extract and its main component prevent chronic UVB radiation-induced skin damage and carcinogenesis in hairless mice, *J. Nutrition*, 2009, **70**, 1541–1555.
11. MANNA, C., P. GALLETTI, V. CUCCIOLLA, O. MOLTEDO, A. LEONE, V. ZAPPIA, The protective effect of the olive oil polyphenol (3,4 dihydrophenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells, *J. Nutr*., 1997, **127**, 286–292.
12. TRAUNTINGER, F., Mechanisms of photodamage of the skin and its functional consequences for skin ageing, *Clin. Exp. Dermatol*., 2001, **26**, 573–577.
13. VISIOLI, F., G. BELLOMO, C. GALLI, Free radical-scavenging properties of olive oil polyphenols, *Biochem. Biophys. Res. Commun*., 1997, **247**, 60–64.
14. WANG, Z.Y., R. AGARAWAL, D.R. BICKERS, H. MUKTAR, Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols, *Carcinogenesis*, 1991, **12**, 1527–1530.
15. WANG, Z.Y., M.T. HUANG, T. FERRARO, C.Q. WONG, Y.R. LOU, K. REUHL, M. IATROPOULOS, C.S. YANG, A.H. CONNEY, Inhibitory effects of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice, *Cancer Res.*, 1992, **52**, 1162–1170.