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ORIGINAL ARTICLE

The Possible Pre- and Post-UVA Radiation Protective Effect of Amaranth Oil on Human Skin Fibroblast Cells

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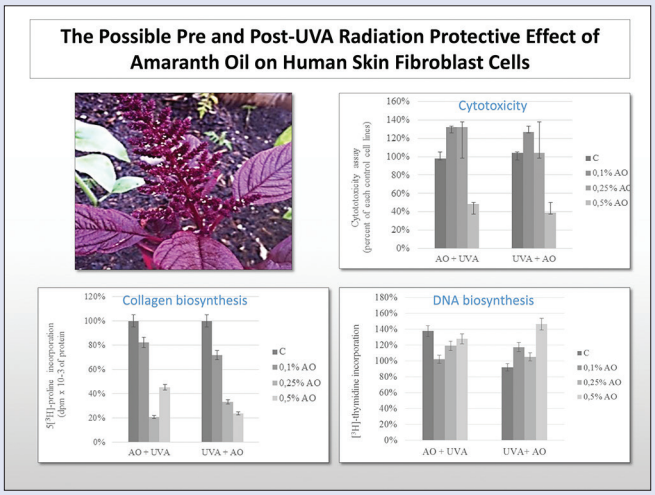
ABSTRACT

Background: The health effects of Amaranth Oil (AO) are attributed to its specific chemical composition. That makes it an outstanding natural product for the prevention and treatment of ultraviolet (UV) irradiation-related pathologies such as sunburn, photoaging, photoimmunosuppression, and photocarcinogenesis. Most of the studies are taken on animal model, and there is a lack of research on the endogenous effect of AO on fibroblast level, where UVA takes it harmful place. **Objective:** The aim of this study was evaluation if AO can protect or abolish UVA exposure effect on human skin fibroblast. **Materials and Methods:** The 0.1% AO, 0.25% AO, and 0.5% AO concentration and irradiation for 15 min under UVA-emitting lamp were studied in various condition. In all experiments, the mean values for six assays ± standard deviations were calculated. **Results:** Pretreatment with various concentrations of AO was tested. The highest concentration of AO where cell survival was observed was 0.5%. Cytotoxicity assays provided evidence for pre- and post-UVA protective effect of 0.1% AO among three tested concentrations. The results also provide evidence that UVA has inhibitory effect on collagen biosynthesis in confluent skin fibroblast, but presence of 0.1% AO abolishes pre- and post-UVA effect comparing to other used AO concentration. The assessment results on DNA biosynthesis show the significant abolished post-UVA effect when 0.1% and 0.5% of AO were added. **Conclusion:** AO gives pre- and post-UVA protection in low concentration. This provides the evidence for using it not as a main protective factor against UV but as one of the combined components in cosmetic formulation.

Key words: Amaranth Oil, collagen, fibroblast, *in vitro*-ultraviolet A protective effect

SUMMARY

- The recommended Amaranth Oil (AO) concentration in cosmetic formulation is between 0.1 and 5%
- Pretreatment with various concentrations of AO suggests to use the highest 0.5% concentration of AO in human skin fibroblast cultures
- The 0.1% of AO in fibroblast cultures, protects and abolishes effect of ultraviolet A (UVA) exposure
- UVA has inhibitory effect on collagen biosynthesis in skin fibroblast, but presence of 0.1% AO abolishes pre- and post-UVA effect
- The abolished post-UVA effect occurs when 0.1% and 0.5% of AO were added on DNA biosynthesis.



Abbreviations used: AO: Amaranth Oil.

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INTRODUCTION

The commercially photoprotective products with appropriate sun protection factor (SPF) values are more efficient in solar erythema and sunburn protection, which are caused by high-energy ultraviolet (UV) photons produced by UVB radiation. However, their efficacy in preventing photoaging and skin cancer depends on their ability to block low-energy UVA radiation. It is also general thinking that commercial sunscreens give good UVA and UVB protection. However, the photostability of the sunscreen in the UVA range is not always adequate. Most sunscreens offer good protection against UVB, whereas the UVA photostability of some products decreases during UV exposure.[1] Ideal sunscreening agents should be safe, chemically inert, nonirritating, nontoxic, photostable, and able to provide complete protection to the skin against damage from solar radiation. Although considered safe, sunscreening agents are not free from adverse effects.[2] Phototoxic and photoallergic reactions accompany in increased use of cosmetic products containing of synthetic UV filters, for example,. contact dermatitis, allergic, irritant, and phototoxic and photoallergic reactions. Since a photoprotective cosmetic formulation is applied on large skin surfaces, they may cause the penetration and bioaccumulation of significant quantities of UV chemical absorbers. These percutaneous absorption

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processes may cause various adverse health effects such as endocrine disruption activity, decrease in superoxide dismutase activity, and even mutagenic and carcinogenic effects.[3] There have been also reports of increased incidence of melanoma as a result of sunscreen use. The reason for this may be because sunscreens absorb UVB almost completely but transmit large quantities of UVA.[2,4] Effects of UVA manifest usually after a long duration of exposure, even if doses are low. The UV generation of ROS and reactive nitrogen species can outcome in the structural and functional alteration of cutaneous proteins, for example, collagen, elastin, and glycosaminoglycans, which may contribute to photoaging.[5] It has been postulated that UVA upregulates the formation of matrix metalloproteinases (MMPs), enzymes that degrade the matrix protein's elastin and collagen. Degradation in these proteins can result in marked reduction in skin elasticity and increased wrinkling. UVA radiation also produces nuclear structural to the DNA damage,[2,6] impairs the immune system, and leads to cancer. It has been linked to 67% of malignant melanoma.[6]

Numbers of conventional and novel herbal cosmetics are useful to treat UV-damaged skin. The steady increase in the incidence of melanoma, nonmelanoma cutaneous neoplasia and preneoplasic disorders has contributed to the demand for more effective protection from the sun.[1,7-9] Although modern sunscreens containing UV filters are highly efficient to protect the skin from the deleterious effects of the sun, herbal sunscreens are rapidly replacing them due to mentioned above associated side effects with UV filters.[1]

Some researchers have found that some plant oils contain natural sunscreens. For example, sesame oil resists 30% of UV rays, whereas coconut, peanut, olive, and cottonseed oils block out about 20%.[10] Plant oils have already received much attention as source of antioxidants, but the unique composition of Amaranth Oil (AO) makes it an outstanding natural product for the prevention and treatment of UV irradiation-related pathologies such as sunburn, photoaging, photoimmunosuppression, and photocarcinogenesis The diverse health effects of AO is attributed to its specific chemical composition, i.e., a high level of linoleic acid (up to 50%), tocopherols/tocotrienols and squalene (up to 8%).[11]

The efficacy of sunscreen products has been recognized as an important public health issue. Adequate methods for assessment of the level of protection should be developed and standardized. While the SPF COLIPA testing method *in vivo* has been used for years, preference should be given to *in vitro* testing methods as *in vivo* methods raise ethical concern. The *in vitro* screening methods may represent a fast and reasonable tool reducing the number of *in vivo* experiments and risks related to UV exposure of human subjects.[12]

Due to searching of a new nontoxic, compatible with the skin, natural sun filters and post-UV exposure ingredients with proven endogenous protective properties, we wanted to asses if AO abolishes effect of UVA radiation.

MATERIALS AND METHODS

Amaranth Oil characteristics

AO was a gift from Amaranth Bio Company (San Diego, CA, USA). Gifted AO had a clean yellow-goldish color. Its sensory characteristics are produced by cold-pressing using organic amaranth grain. The oil was obtained from *Amaranthus cruentus* seeds. Relative density at 25°C: 0.91–0.98; refraction index at 25°C: 1.20–1.60; melting point: −27°C; heavy metals: maximum 20 ppm; preservatives: none; saponification value: 165–190; acid number: maximum 30 mg KOH/g; peroxide number: maximum 10 mmol/kg. Fatty acid profile: palmitic 16%–22%, oleic 20%–26%, linoleic 40%–50%, and stearic 2%–5%. Squalene: 4%–8%. Vitamin E: 5–10 mg/100 g. AO can be stored for up to 12 months after production. It is recommend to store it in closed containers, away from light, and at a temperature of 5–10°C.

Cell lines and culture

All studies were performed on human skin fibroblasts which were purchased from the American Type Culture Collection (Rockville, MD, USA). The fibroblast cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C in a 5% CO_2 incubator. Fibroblast cells were incubated for 24 h in DMEM without glucose and glutamine with varying concentrations of AO and irritated with UVA (365 nm). First, 0.1 ml stock solution of AO was prepared in 9.9 ml of ethanol (v/v). Stock solution was diluted in growth medium to final concentration of 0%, 0.1%, 0.25%, 0.5%, and 1%. Human skin fibroblasts were exposed to above prepared concentrations of AO for 24 h without refreshing the medium. The maximal concentrations of AO were determined by a cell viability study. The highest concentration of AO where cell survival was observed was 0.5%.

Cytotoxicity assay

Toxicity of tested substances was determined by the method of Carmichael *et al*.[13] Fibroblast cells were maintained as described above in 24-well plates. Cells were incubated for 24 h with AO before and after irritation of UVA, medium was discarded, and the cells were rinsed 3 times with phosphate-buffered saline (PBS). Then, the cells were incubated for 4 h in 1 ml of PBS with 25 μl of MTT (5 mg/ml). Medium was removed from the wells, and the cells were lysed 1 ml of DMSO with 20 μl of Sorensen's buffer (0.1 mol/l glycine with 0.1 mol/l NaCl, pH 10.5). The absorbance was measured at the wavelength of 570 nm. Component-treated cells were calculated as a percent of each control cell lines.

[^{3}H]-thymidine incorporation/DNA biosynthesis assay

To examine the effect of different concentration of AO before and after irritation with UVA on the fibroblast cells proliferation, the cells were plated in 24-well tissue culture dishes at 1×10^5 cells/well with 1 ml of growth medium. After 48 h, the plates were incubated with 0.5 μCi of [^{3}H] thymidine for 24 h at 37°C. Cells were rinsed 3 times with PBS, solubilized with 1 ml of 0.1 mol/l sodium hydroxide containing 1% SDS, then scintillation fluid "Ultima Gold XR" was added, and incorporation of the tracer into DNA was measured in scintillation counter.

Collagen biosynthesis

Incorporation of radioactive precursor into proteins was measured after the labeling of confluent cells cultured in growth medium with 5[^{3}H]-proline (5 μCi/ml and 28 Ci/mM) for 24 h. Incorporation of tracer into collagen was determined by digesting proteins with purified *Clostridium histolyticum* collagenase, according to the method of Peterkofsky *et al*.[14] Results are shown as combined values for cell plus medium fractions.

Statistical analysis

In all experiments, the mean values for six assays ± standard deviations (SD) were calculated. The results were submitted to the statistical analysis using the Student's *t*-test accepting $P < 0.05$ as statistically significant.

RESULTS

Cytotoxicity assay results/cell viability

The pre-UVA protective effect of different concentration of AO was tested under condition, when fibroblast was incubated for 24 h with 0.1% AO, 0.25% AO, and 0.5% AO, then irradiated for 15 min under UVA-emitting lamp. To assess the post-UVA protective effect of different concentration of AO, fibroblast was irradiated for 15 min under UVA-emitting lamp, then incubated for 24 h with 0.1% AO, 0.25% AO, and 0.5% AO.

Preincubation of the fibroblasts with 0.1% and 0.25% concentrations of AO statistically increased ($P < 0.05$) cell proliferation by 35% (132%± SD, $n = 6$) in both groups when compared to control cell line. In contrast to the 0.5% AO concentration which statistically ($P < 0.05$) inhibited cell formation by 50% (48%± SD , $n = 6$). This provides evidence for pre-UVA protective effect of 0.1% AO [Figure 1]. The 15 min UVA irritation did not inhibit cell formation when 0.1% and 0.25% AO concentrations were added straight after. Results were significant ($P < 0.05$) for increased cell formation when 0.1% AO was used, and results were significant ($P < 0.05$) for inhibition of cell formation when 0.5% AO was used. This provides evidence for post-UVA protective effect of 0.1% AO concentration [Figure 2].

Collagen biosynthesis results

The pre- and post-UVA effect of 0.1%, 0.25%, and 0.5% of AO on collagen synthesis in human skin fibroblast was measured. To assess the activity of AO on collagen synthesis in human skin fibroblasts, cells were incubated for 24 h in 0.1%, 025%, and 0.5% AO for 24 h and irradiated with UVA for 15 min after incubation, as well as irradiated with UVA for 15 min before 24 h incubation in AO.

In control cultures, collagen biosynthesis was intensive. Exposure of the fibroblasts to different concentrations of the AO led to the inhibition of the newly synthesized collagen by 7.64% (98.59% ± SD; $n = 6$), 83.91% (22.32 ± SD; $n = 6$), and 59.12% (47.11% ± SD; $n = 6$), respectively, when compared with the control value (106.23% ±10.93, $n = 6$). It has been shown that AO significantly ($P < 0.05$) affects collagen synthesis in 0.25 and 0.5% concentration [Figure 2].

The results show that UVA significantly affects collagen synthesis in both cases of pre- and post-presence of AO. The results provide evidence that UVA has an inhibitory effect on collagen biosynthesis in confluent skin fibroblast, but presence of 0.1% AO abolishes pre- and post-UVA effect comparing to other used AO concentration with control group. In 0.1% AO + UVA group, the collagen biosynthesis decreased only by 18% (82%± SD, $n = 6$) and by 28% (72%± SD, $n = 6$) in UVA + 0.1% AO group comparing to control group (c). In contrast, 0.25% AO collagen biosynthesis decreased by 79% (21%± SD, $n = 6$) in 0.25% AO + UVA group and by 67% (33%± SD, $n = 6$) UVA + 0.25% AO group. The results were significant [Figure 3].

[^{3}H]-thymidine incorporation/DNA biosynthesis assay

To assess the activity of AO on DNA biosynthesis in human skin fibroblasts, cells were incubated for 24 h in 0.1%, 025% and 0.5% AO for 24 h, and irradiated with UVA for 15 min after incubation, as well as irradiated with UVA for 15 min before 24 h incubation in AO.

In control cultures, DNA biosynthesis was intensive. Exposure of the fibroblasts to different concentrations of the AO led to the inhibition of the synthesized DNA by 22% (78 ± SD; $n = 6$), 18% (82%± SD; $n = 6$), and increased DNA biosynthesis by 5% (105%± SD; $n = 6$), respectively, when compared with the control value [Figure 4].

The assessment results of pre- and post-UVA effect of different concentration of AO on DNA biosynthesis in human skin fibroblast show the significant abolished post-UVA effect when 0.1% and 0.5% of AO were added [Figure 5].

DISCUSSION

Many liquid oils, extracted from fruit and vegetable seeds, are light, low in viscosity, and less occlusive than hydrocarbon oils. Their penetrating and carrying properties, as well as their natural content of tocopherols, carotenoids, and essential fatty acids, make them highly valuable. Several natural-based sunscreen lotions, including the oils of almond, avocado, coconut, cottonseed, olive, peanut, sesame, and soybean, have been reported to have UV filters.[15]

Amaranth grains contain only 1%–7.7% lipids, but these lipids are extremely valuable because of the presence of ingredients such as squalene, unsaturated fatty acids, Vitamin E as tocopherols, tocotrienols, and phytosterols, which are not seen together in other common oils (e.g.: Olive oil).[16]

While much research on plant oils is focused on the effects of UVB radiation, there are not much studies on their effect under UVA

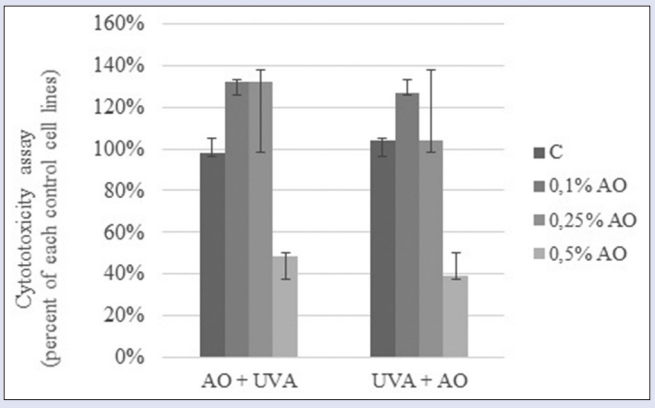

Figure 1: Cytotoxicity assay results in confluent human skin fibroblasts incubated with different concentrations of Amaranth Oil with pre- and post-irritation of ultraviolet A. Component-treated cells were calculated as a percent of each control cell lines. Error bars represent ± standard deviation, $n = 6$

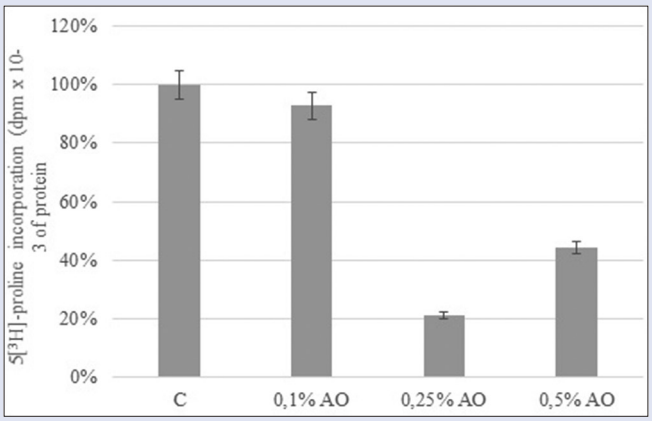

Figure 2: Collagen biosynthesis measured as 5[3H]-proline incorporation into proteins susceptible to the action of bacterial collagenase in confluent human skin fibroblasts incubated for 24 h with different concentrations of Amaranth Oil. Error bars represent ± standard deviation, $n = 6$

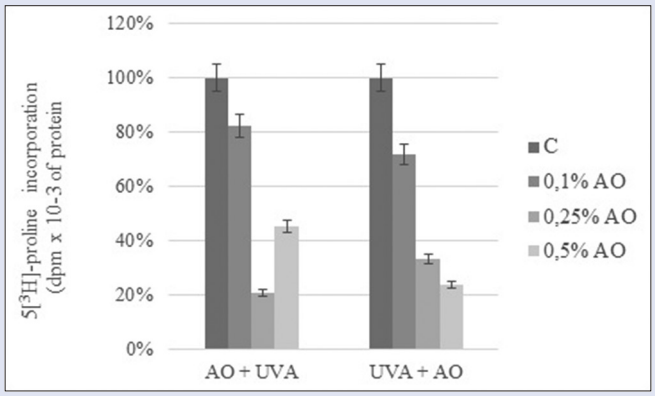

Figure 3: Collagen biosynthesis measured as 5[3H]-proline incorporation into proteins susceptible to the action of bacterial collagenase in confluent human skin fibroblasts incubated for 24 h with different concentrations of Amaranth Oil with pre- and post-ultraviolet A irritation. Error bars represent ± standard deviation, *n* = 6

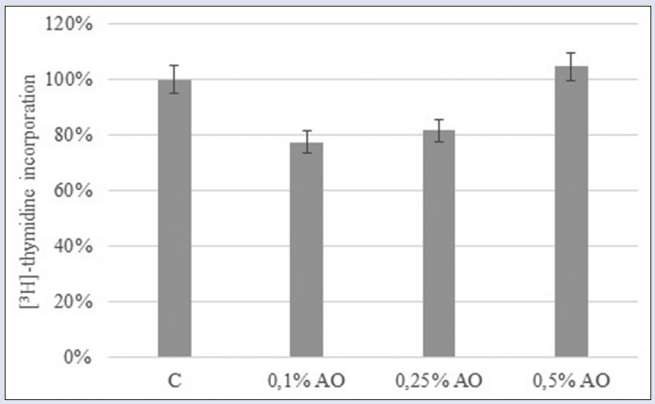

Figure 4: DNA biosynthesis measured as [3H]-thymidine incorporation into DNA in human skin fibroblasts (control) incubated with different concentrations of Amaranth Oil without the presence of ultraviolet A. Error bars represent ± standard deviation, *n* = 6

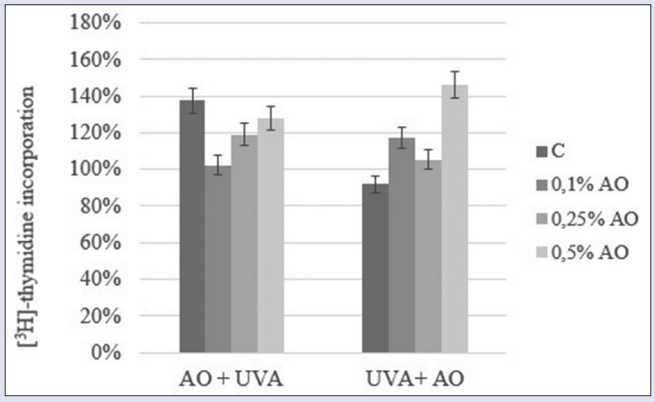

Figure 5: DNA biosynthesis measured as [3H]-thymidine incorporation into DNA in human skin fibroblasts (control) incubated with different concentrations of Amaranth Oil in pre- and post-presence of ultraviolet A. Error bars represent ± standard deviation, *n* = 6

condition. The aim of our study was to compare pre- and post-UVA properties of different concentrations of AO on human skin fibroblasts. The cytotoxicity, collagen, and DNA biosynthesis under different condition were studied. Our research confirms both protective and abolishing effect of AO when UVA exposure occurs.

The recommended AO concentration in cosmetic formulation is between 0.1 and 5%. Our pretreatment with various concentrations of AO suggested to use the highest 0.5% concentration of AO in human skin fibroblast cultures. The addition of 0.1% of AO suggests its protective and abolishing effect when UVA exposure occurs.

The early sign of skin aging is due to wrinkle formation. The collagen is the main component of the skin dermis, and its reduction result in aging of the skin. The collagen is the chief structural unit of the extracellular matrix (ECM). The type I collagen helps in the maintenance of the skin dermis structure.[5,17] Aging and irradiation accelerate the degradation of the ECM, resulting in a decrease in dermal collagen and an increase in the level of the matrix MMP-1, which cleaves interstitial collagen leads the skin appear to be aged.[5,18-20] The presented research results show that exposure of human skin fibroblast to UVA exposure is less harmful on collagen formation when 0.1% AO concentration is used.

The assessment results of pre- and post-UVA effect of different concentration of AO on DNA biosynthesis in human skin fibroblast show the significant abolished post-UVA effect when 0.1% and 0.5% of AO were added. The sunscreen products available in the market contain UV absorbers that have been controlled at protecting against UV-induced sunburn and DNA damage. Since the biological endpoint for the determination of the SPF is the UV erythema. The SPF label is the indicator only for a protection against erythemally-effective solar UV, largely confined to the UVB, and partially short-wavelength UVA radiation.[5] There is a continuous need of quantitative determination of different parameters, such as SPF, protection against UV radiations, to support the efficacy and safety of the products.[5,15]

There are many factors affecting the UV protective properties such as the use of different solvents, in which the sunscreens are dissolved; the combination and concentration of the sunscreens; the type of emulsion; the effects and interactions of vehicle components such as esters, emollient and emulsifiers used in the formulation; the interaction of the vehicle with the skin; the addition of other active ingredients; and the pH system and the emulsion rheological properties, among other factors, which can increase or decrease UV absorption of each sunscreen.[15] Natural oil and AO represent good quality ingredient in the above-mentioned specification.[1,9-10,16]

CONCLUSION

Our study shows that AO gives acceptable pre- and post-UVA protection in low concentration. This provides the evidence for using AO not as a main protective factor against UV but as one of the combined components in cosmetic formulation. It was shown that using only one natural component is not enough for skin protection. Combination of several different natural substances is a right solution.[10] The SPF value for the AO should be a measure to support the efficacy and safety of the cosmetic products also more detailed studies on collagen 1 and MMP-1 activity should be taken. This can provide an evidence for using AO in antiaging cosmetic creams as a wrinkle reducing and natural SPF component.

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Conflicts of interest

There are no conflicts of interest.

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