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Alpha-pinene attenuates UVA-induced photoaging through inhibition of matrix metalloproteinases expression in mouse skin

Ramasamy Karthikeyan[a], Govindasamy Kanimozhi[a,b,*], Nirmal R. Madahavan[c], Balupillai Agilan[a], Muthusamy Ganesan[a], Nagarajan Rajendra Prasad[a,*], Pierson Rathinaraj[d]

[a] Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamilnadu, India
[b] Department of Biochemistry, Dharmapuram Gnanambigai Government College for Women, Mayiladuthurai 609001, Tamilnadu, India
[c] Department of Oral Pathology, Faculty of Dentistry, Rajah Muthiah Dental College & Hospital, Annamalainagar 608 002, Tamilnadu, India
[d] Center for Transdisciplinary Research and Innovation, Waikato Institute of Technology, Wintec Private Bag, Waikato Mail Centre, Hamilton, New Zealand

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ABSTRACT

Aim: The present study was designed to examine the role of alpha-pinene (AP) against skin photoaging in UVA-irradiated mice.

Materials and methods: Swiss albino mice were subjected to UVA-irradiation at the rate of 10 J/cm^2 per day for ten days, totally mouse received 100 J/cm^2. One hour prior to each UVA-exposure, the mouse skin was topically treated with AP (100 mg kg/b·wt). Biochemical methods were employed to study the status of antioxidant enzymes and lipid peroxidation. Histopathological observations were performed using hematoxylin and eosin (H & E) and Verhoeff van Gieson (VVG) staining in the mouse skin. The inflammatory and apoptotic protein expression was studied by immunohistochemical and Western blot methods. The mRNA expression of matrix metalloproteinases was determined by qRT-PCR and Western blot analysis.

Key findings: We found that AP pretreatment substantially ameliorated UVA-induced depletion of antioxidant enzymes and prevented UVA-induced lipid peroxidation in the mouse skin. Further, AP effectively inhibited UVA-induced activation of pro-angiogenic (iNOS and VEGF), inflammatory proteins (TNF-α, IL-6, and COX-2) expression and prevented the activation of NF-κB p65 in the mouse skin. Additionally, AP inhibited UVA-mediated apoptotic mediators (Bax, Bcl-2, caspase-3 and caspase 9) expression in the mouse skin. Moreover, AP inhibited mRNA expression of matrix metalloproteinases (MMP-13 and MMP-9) and tissue type IV collagenase (MMP-2) expression in the mouse skin. Histological studies showed that AP remarkably prevented the dermal tissue damage in UVA-irradiated mice.

Conclusion: Thus, AP treatment effectively prevented UVA-induced photoaging probably through its antioxidant property.

1. Introduction

Ultraviolet radiation (UVR) from the sunlight is closely linked to the pathogenesis of acute inflammation such as sunburn and photoaging in the skin tissue. The photoaging mechanism includes the activation of the proinflammatory signaling molecule, collagen degradation and decreased collagen synthesis [1]. The UVA (320–400 nm) radiation activates a wide range of signal transduction transcription factors to initiate the photoaging process. The NF-κB is the key transcription factor to modulate proinflammatory responses upon UVA exposure and its activation regulate several growth factors, including vascular endothelial growth factor (VEGF) and induced nitric oxide synthase (iNOS) which accelerate the pathogenesis of photoaging [2]. A number of in vivo studies clearly showed that UVA radiation generates wrinkling, sagging, laxity, patchy/mottled pigmentation, dryness, and long-term dermal structure deterioration. Further, UVA exposure induces a series of matrix metalloproteinases (MMPs) in the human skin. The cumulative actions of MMP-1, -2 and -9 resulted in the degradation of dermal extracellular matrix proteins. The UVA-induced activation of MMP-1 production has directly been involved in the degradation of type I collagen, the major component of the dermis. Gelatinases (MMP-2 and MMP-9) were reported to digest extracellular matrix (ECM) components such as collagen type I and IV. Apart from direct collagen degradation, UVA radiation negatively regulates collagen synthesis,

* Corresponding authors at: Department of Biochemistry & Biotechnology, Annamalai University, Annamalainagar 608 002, Tamilnadu, India.
E-mail addresses: gvkani@gmail.com (G. Kanimozhi), drprasadnr@gmail.com (N.R. Prasad).

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Table 1
The effect of AP on UVA-induced depletion of antioxidants and lipid peroxidation status in mice skin.

Group	TBARS (mmol per mg protein)	SOD* (Units per mg protein)	Catalase** (Units per mg protein)	GPx*** (Units per mg protein)	GSH (mg per g protein)
Control	0.53 ± 0.02[a]	0.48 ± 0.01[a]	2.31 ± 0.14[a]	0.92 ± 0.01[a]	0.75 ± 0.03[a]
AP	0.54 ± 0.02[a]	0.47 ± 0.03[a]	2.28 ± 0.21[a]	0.93 ± 0.03[a]	0.73 ± 0.02[a]
UVA	2.08 ± 0.77[b]	0.16 ± 0.02[b]	0.86 ± 0.24[b]	0.16 ± 0.01[b]	0.15 ± 0.01[b]
AP + UVA	0.79 ± 0.04[c]	0.32 ± 0.10[c]	1.87 ± 0.14[c]	0.76 ± 0.02[c]	0.56 ± 0.01[c]

Effect of AP and/or UVA-irradiation on enzymatic antioxidant status in mouse skin. The table represents the changes in the activities of SOD, CAT and GPx, GSH and lipid peroxidation. *Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in 1 min. **μmol of hydrogen peroxide consumed per minute. ***μg of glutathione consumed per minute. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c) differ significantly at $P \leq 0.05$ (DMRT).

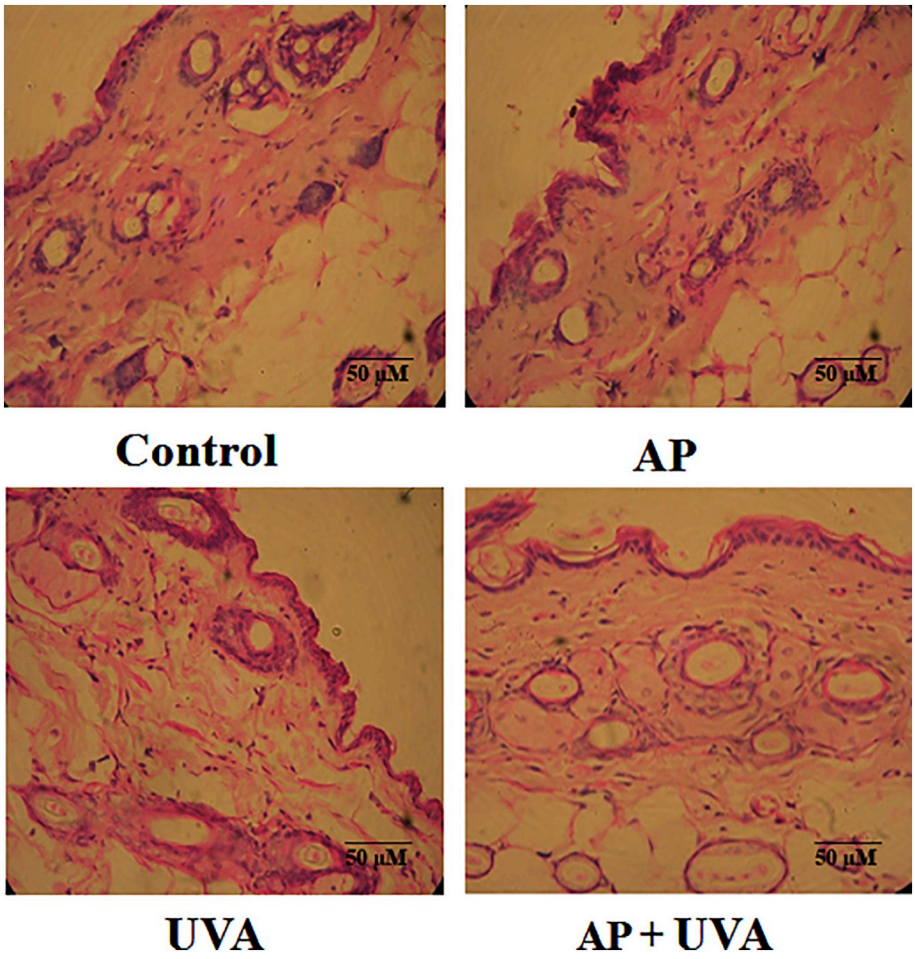

Fig. 1. Effect of AP on UVA-induced histological changes in the mouse skin. After 24 h from the last irradiation, animals were euthanized and skin biopsies were processed for histological observations using hematoxylin & eosin staining in mouse skin tissue section. UVA-exposed mouse skin showed clear dermal tissue damage (original magnification, 40×).

which progressively worse skin photoaging [3].

Solar UVA radiation exposure induces damage to the human skin via the generation of free radicals. The photoprotective agents are countermeasures that prevent UVA-induced cellular and molecular changes in the human skin. The currently available topical chemical photoprotective agents are bound to suffer due to toxicity and unwanted side effects [4]. The plant-derived natural products have attracted the researchers to develop potential antiphotoaging countermeasures due to their inherent antioxidant activity [5]. We earlier demonstrated that antioxidant phytochemicals such as caffeic acid, ferulic acid, linalool, and sesamol exerted good photoprotection in experimental models [6–9].

Essential oils are the highly concentrated essences of secondary metabolic processes of various plant parts like flowers, fruits, leaves, barks, and roots. Essential oils have long been used to treat skin inflammation, smooth wrinkles and make age marks vanish [10]. Essential oils possess a complex mixture of secondary metabolites. Terpenes, particularly monoterpenes, have been identified as the major constituents of essential oils [11,12]. Several investigations have proved that monoterpenes of essential oils exhibit strong antioxidant and antiinflammatory properties [13]. Alpha-pinene (AP) was reported as the dominant monoterpene in most of the essential oil [14]. The AP has been reported to disturb lipid arrangement in the intracellular region of the stratum corneum that ends up with an enhanced permeability of the skin [15]. The effective skin absorption of AP has well been documented [16]. Therefore, we hypothesized that the AP might

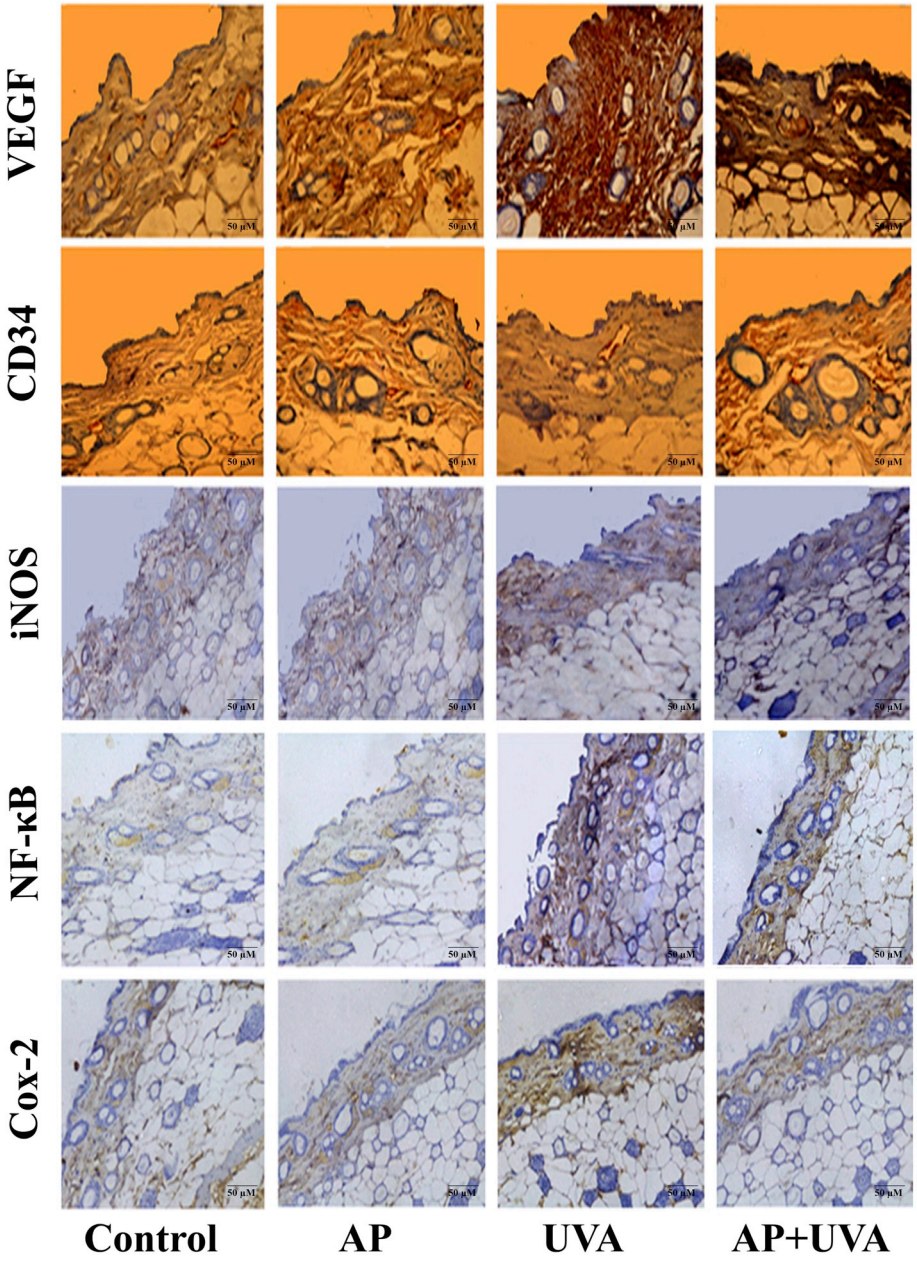

Fig. 2. Effect of AP on UVA-induced inflammatory markers COX-2, NF-κB, iNOS, VEGF and CD34 expression in mouse skin. Skin sections (5 μM thick) from different experimental groups were analyzed for inflammatory and angiogenic marker expression by immunohistochemistry. Photomicrographs show expression of COX-2 (1:100), NF-κB (1:100), iNOS (1:100), VEGF (1:100) and CD34 (1:100) in the mouse skin. Brown spot indicates the positive staining. The images were captured (40×) from each of three randomly selected in each group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

able to prevent UVA-induced photoaging mechanism in the skin. Till date, there were no reports available about the antiphotoaging property of AP in the literature. Recently, we reported the preventive role of AP against UVA-induced oxidative stress, DNA damage, and apoptosis in human skin epidermal keratinocytes [17]. In this study, we report the preventive role of AP against UVA-induced photoaging in the skin of experimental animals.

2. Materials and methods

2.1. Chemicals

Alpha-pinene, monoclonal antibodies such as cyclooxygenase-2 (COX-2), nuclear factor kappa B p65 (NF-κB p65), tumor necrotic factor-alpha (TNF-α), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF), MMP-1, MMP-9, Bax, Bcl-2, caspase-3, caspase-9, β-actin, anti-mouse and goat anti-mouse IgG-HRP polyclonal antibodies were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals, solvents, and other analytical grade chemicals were obtained from SD Fine Chemicals, Mumbai, India and Fisher Inorganic and Aromatic Limited, Chennai, India.

2.2. Experimental animals and study design

About 6 weeks old female Swiss albino mice (18–20 g) were housed at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, India. Mice were maintained in well-ventilated rooms (temperature 23 ± 2 °C, humidity 65–70% and 12 h light/dark cycle). All studies were conducted after approval from the Institutional Ethical Committee, Annamalai University (Reg No./160/1999/CPCSEA). Mice were divided into four experimental groups of 6 mice each group. The group I mice served as vehicle control, received 0.1% DMSO. Group II mice received the topical application with 200 μL of AP (100 mg/kg·b·wt) on the dorsal portion of the skin (area: 3 cm^2). Group III mice received 100 J/cm^2 of UVA-irradiation, (10 J/cm^2 exposure per day for ten days totally mouse received 100 J/cm^2) and Group IV mice received topical application of AP (100 mg kg/b·wt.) 1 h prior to each

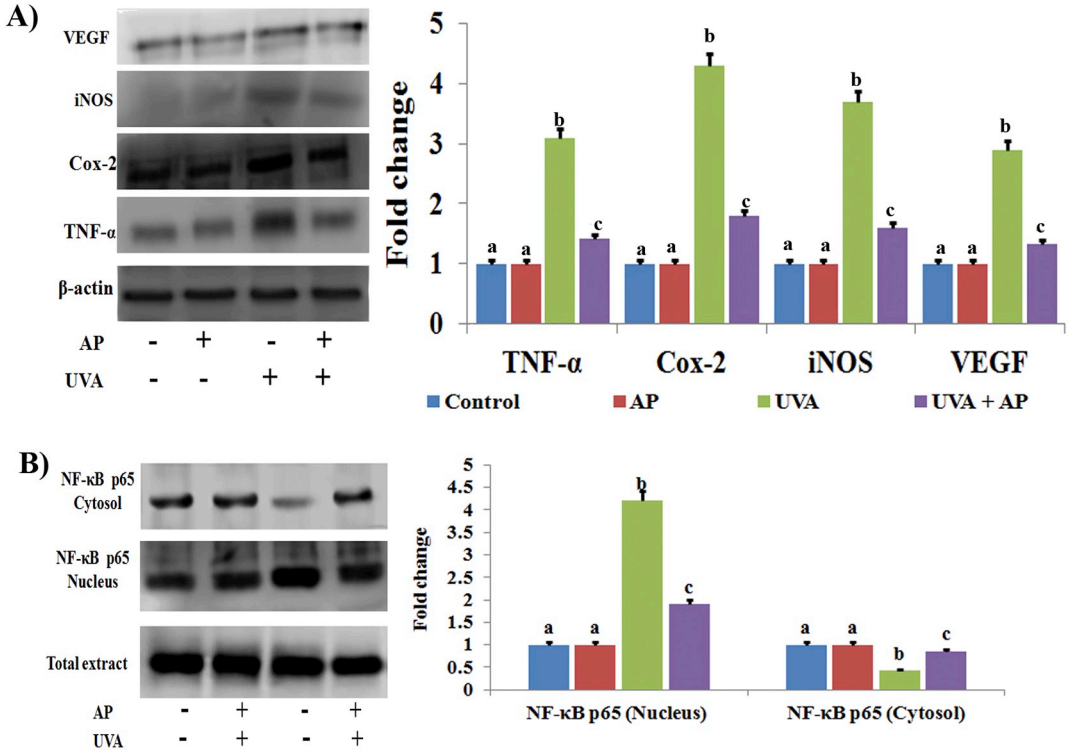

Fig. 3. A. Effect of AP on UVA-induced expression of VEGF, iNOS, TNF-α, and COX-2 in mouse skin by Western blot analysis. (B). AP on UVA-mediated NF-κB p65 nuclear translocation. Protein quantification was performed by densitometric analysis in Image Studio software (LI-COR Biotechnology, Lincoln, USA). Data were expressed as the ratio of the target protein to β-actin as the means ± S.D. $P \leq 0.05$ vs. non-irradiated control from three independent experiments. Values not sharing a common marking (a, b, c…) differ significantly at $P \leq 0.05$ (DMRT).

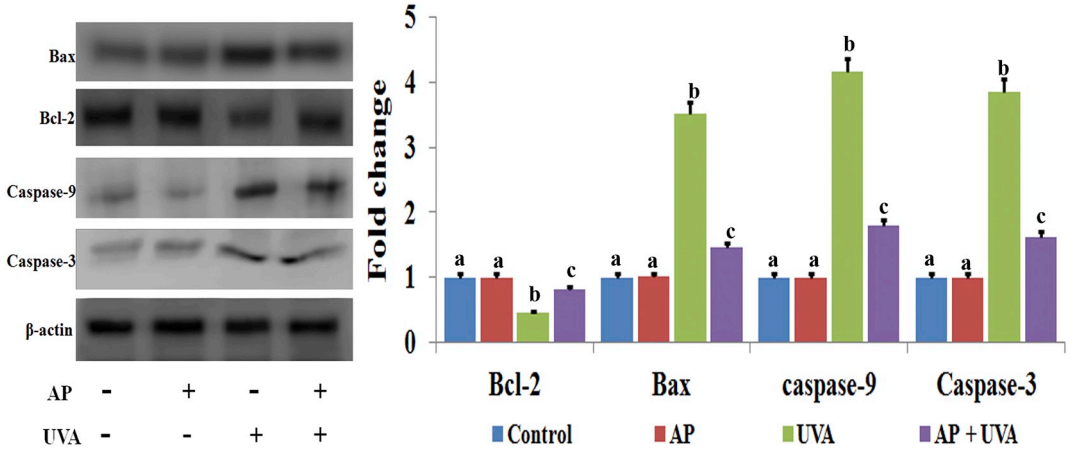

Fig. 4. Effect of AP on UVA-induced expression of Bax, Bcl-2, caspase-9, and caspase-3 in mouse skin by Western blot. Protein quantification was performed by densitometric analysis in Image Studio software (LI-COR Biotechnology, Lincoln, USA). Data were expressed as the ratio of the target protein to β-actin as the means ± S.D. $P \leq 0.05$ vs. non-irradiated control from three independent experiments. Values not sharing a common marking (a, b, c…) differ significantly at $P \leq 0.05$ (DMRT).

UVA exposure.

2.3. UVA-irradiation procedure for animals

Dorsal portion of mouse skin was shaved using an electric shaver (Philips, Mumbai, India) followed by applying the hair removal cream before three days of experimental initiation. The shaved dorsal skin was exposed to UVA-irradiation (365 nm). Animals were anesthetized with 100 mg/kg ketamine and 10 mg/kg of xylazine hydrochloride (intraperitoneal) and exposed to radiation. The dose rate was given as 10 J/cm^2 per day for ten days, totally mouse received 100 J/cm^2. After 24 h of last irradiation, mice were sacrificed and the skin was removed for various analyses.

2.4. Histopathology and immunohistochemistry studies

Mice skin tissue was fixed in 10% formalin immediately after removal. Formalin-fixed 5 μm thick sections were used for further studies. Hematoxylin and eosin (H & E) and Verhoeff van Gieson (VVG) were used to study tissue architecture and photoaging, respectively [18]. Immunohistochemical studies were performed using deparaffinized sections as described previously [19]. Briefly, sections were incubated with 1% H_2O_2 to stop the endogenous peroxidase activity. Then, sections were blocked for 1 h using 5% goat serum. Primary antibody

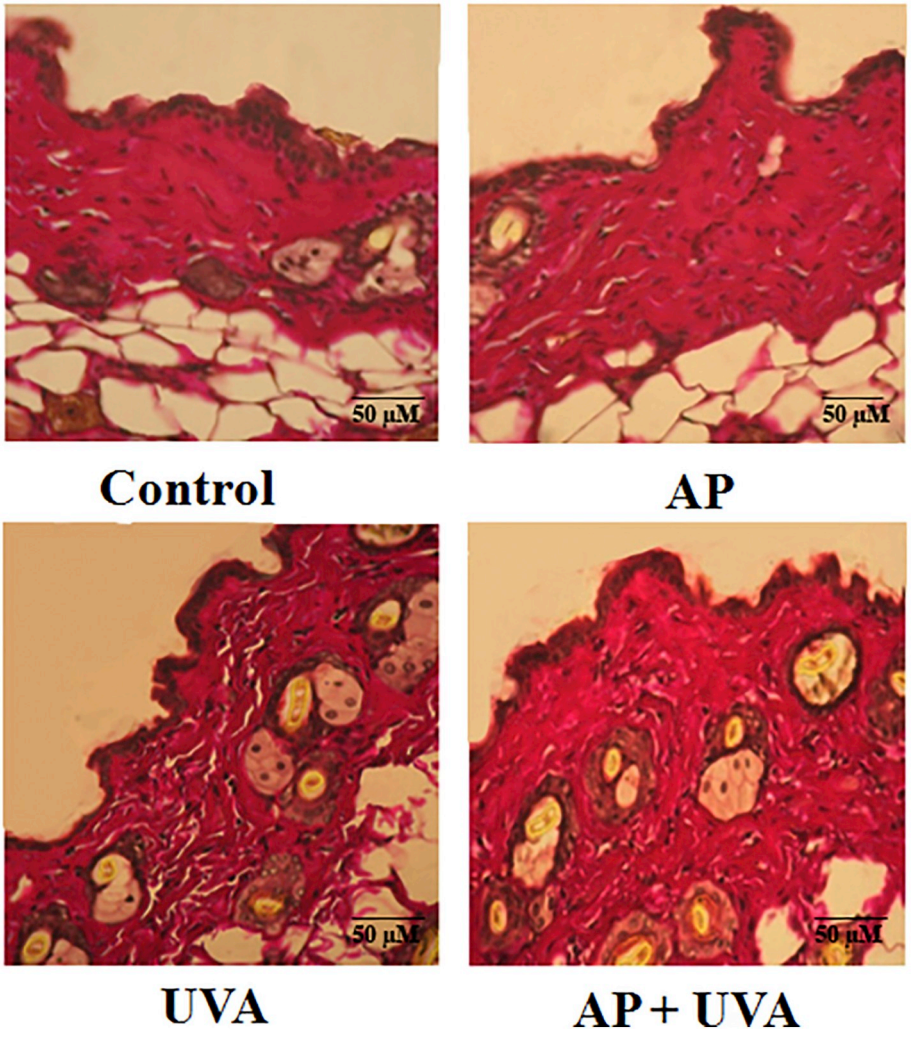

Fig. 5. Effect of AP on UVA-induced dermal collagen fiber density reduction the mouse skin. Verhoff van Gieson staining of mouse skin of UVA-irradiation displayed inappropriate structural density and unorganized arrangement of collagen in the dermis, whereas non-irradiated and AP mice skin tissue exhibits regular arrangement of collagen. Images captured under a light microscope (original magnification, 40×).

(1:100 dilution of VEGF, iNOS, CD34, NF-κB and COX-2 monoclonal antibody from R&D system, Shanghai, China) was added to the blocking solution. Then, the secondary antibody was added (1:200 dilution of biotinylated antimouse IgG) and counterstained with hematoxylin. The specimens were observed with a light microscope (40×). All histopathological changes were examined by a pathologist.

2.5. Estimations of lipid peroxidation and antioxidants

Lipid peroxidation was determined as described earlier by Niehaus et al. method [20]. Superoxide dismutase (SOD) activity was measured by Kakkar et al. method [21]. Catalase activity was performed based on Sinha method [22]. Rotruck et al. protocol was followed to determine glutathione peroxidase activity [23]. The total GSH content was quantified as described previously [24].

2.6. Western blot analysis

Proteins from the experimental mouse skin were isolated using the Trizol reagent method followed by the manufacturer's instruction containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, USA). Protein samples (50 µg from each sample) were boiled with loading buffer for 5 min, size-separated by 10% SDS-PAGE gel. After electrophoresis sample was blotted onto a nitrocellulose membrane using semi-dry apparatus (Bio-rad Laboratories, Munchen, Germany). The membrane was incubated with 5% BSA for overnight at 4 °C and the respective primary antibody was added (dilution 1:1000) and kept for 5–6 h at 37 °C. After washing thrice at 5 minute interval with TBST, the membrane was further incubated with secondary antibody coupled with horseradish peroxidase (dilution 1:2000) for 2 h at 37 °C. Finally, bands were detected using the enhanced chemiluminescence method (Bio-rad Laboratories, Munchen, Germany). The images were acquired by Image Studio software (LI-COR Biotechnology, Lincoln, USA) [25].

2.7. RNA preparation and qRT-PCR analysis

Total RNA was extracted using the Qiagen minicolumn RNA isolation kit according to the Manufacturer's protocol (Qiagen, Maryland, USA). Extracted RNA was preserved at −80 °C until used. Gene expression was carried out with Quantitative RT-PCR ReadyMix™ (Sigma Aldrich, St. Louis, USA) with respective forward and reverse primers using a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The following primer sequences were used for PCR reactions:

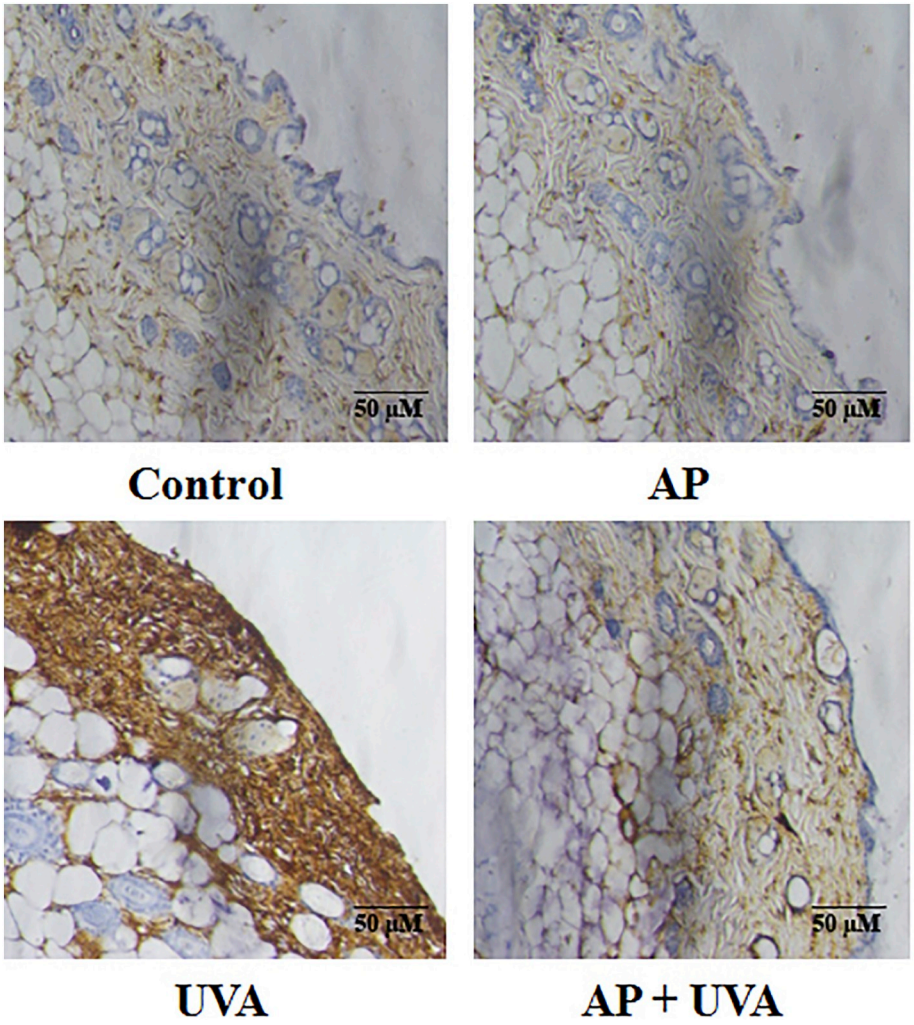

Fig. 6. Effect of AP on UVA-induced MMP-2 expression in the mouse skin. Photographs of UVA-exposed mouse skin exhibit increased MMP-2 expression observed under a light microscope (original magnification, 40×).

MMP-13	Forward-TGGACCTTCTGGTCTTCTGG
	Reverse-TCCTTGGAGTGATCCAGACC
MMP-9	Forward-AACACCACCGAGCTATCCAC
	Reverse-AGAGGAGTCTGGGGTCTGGT
GAPDH	Forward-AGAACATCATCCCTGCATCC
	Reverse-TGTTGAAGTCGCAGGAGACA

For quantitation, differences between treatments were analyzed by comparing mRNA levels to the control after normalization to GAPDH levels [26].

2.8. Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) on SPSS (Statistical Package for Social Sciences) and the group means were compared to Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the P value is < 0.05 levels.

3. Results

3.1. Effect of AP and/or UVA on antioxidants and lipid peroxidation status in the mouse skin

In this study, we noticed that UVA-exposure drastically decreased antioxidant enzymes such as SOD, CAT, GPx, and GSH in the mouse skin. Conversely, AP treatment prior to UVA-exposure significantly increased the SOD, CAT, GPx and GSH status compared to non-irradiated control. UVA exposure depletes the endogenous antioxidants and increases the radical generation; this ultimately damages the lipid-rich cellular membranes. Further, UVA-exposed mice showed increased lipid peroxidation levels when compared to non-irradiated control and AP alone treated mice. Treatment with AP prior to UVA-exposure significantly reduced the lipid peroxidation status when compared to UVA-exposed mice (Table 1).

3.2. Effect of AP on UVA-induced inflammatory protein expression

Immunohistochemistry studies showed that UVA-exposure to mouse significantly increased NF-κB and COX-2 expression when compared to the non-irradiated control mouse skin (Fig. 2). Meanwhile, the topical application of AP reduced NF-κB and COX-2 expression when compared to the UVA-exposed mice group. Further, we did not observe any significant changes in NF-κB and COX-2 expression in the topical application of AP alone treated groups compared to the non-irradiated control groups. This was further confirmed in Western blot analysis of COX-2 and the cytosolic and nuclear fraction of NF-κB p65 (Fig. 3).

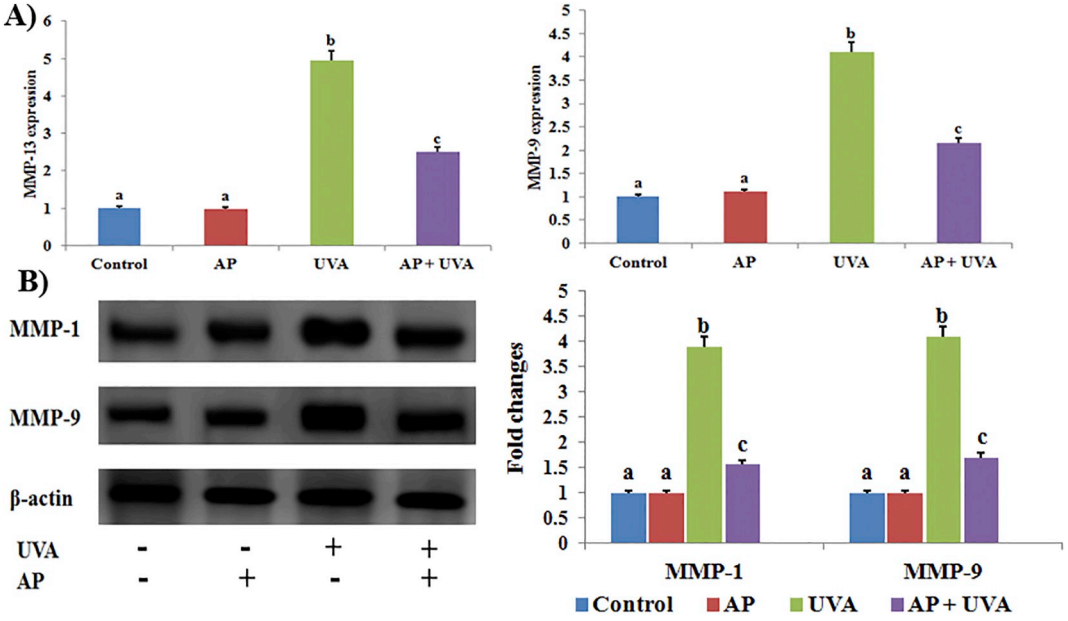

Fig. 7. (A). Effect of AP on UVA-induced photoaging by analyzing the mRNA expression of matrix metalloproteinases -13, & -9 by qRT-PCR. (B). The representative image shows the Western blot analysis of MMP-1 and MMP-9 expression. The protein quantification was performed by densitometric analysis in Image Studio software (LI-COR Biotechnology, Lincoln, USA). Data were expressed as the ratio of target proteins to β-actin as the means ± S.D. $P \leq 0.05$ vs. non-irradiated control from three independent experiments. Values not sharing a common marking (a, b, c…) differ significantly at $P \leq 0.05$ (DMRT).

3.3. Effect of AP on UVA-induced iNOS and VEGF expression in the mouse skin

We examined the effect of AP on UVA-induced proangiogenic protein expression. Immunohistochemical analysis of UVA-exposed mice showed a marked augment in the expression of iNOS and VEGF compared to non-irradiated control mice (Fig. 2). Meanwhile, topical application of AP significantly reduced iNOS and VEGF expression when compared to UVA-exposed mice. Further, we analyzed the blood vessel dilation using CD34 molecule expression by immunohistochemistry. The UVA-exposed mice exhibited a dramatic increase in blood vessel size compared to the non-irradiated control group. Conversely, the topical application of AP reduced the size of blood vessels compared to UVA-exposed mice. In addition, we noticed that the topical application of AP alone treatment did not produce any significant changes in the expression of iNOS and VEGF and further it was confirmed in Western blot analysis (Fig. 3).

3.4. Effect of AP on UVA-induced apoptotic protein expression

In this study, we assessed the preventive effect of AP on UVA-induced apoptosis. Western blot results revealed that UVA-exposure considerably increased Bax, caspase-9 and caspase-3 expression when compared to the non-irradiated control group. Pretreatment with AP inhibited Bax expression, thereby it prevents the UVA-induced activation of apoptosis initiator caspase-9 and the effector caspase-3 in the mouse skin. We did not observe any significant changes in Bax, Bcl-2, caspase-9 and caspase-3 expression in AP alone treated mouse skin compared to non-irradiated control group (Fig. 4).

3.5. AP inhibits UVA-induced MMPs expression in mouse skin

UVA-exposed mice showed severe dermal damage compared to non-irradiated mouse skin, while AP alone treatment did not cause any changes in the mouse skin. The VVG staining results revealed that UVA significantly reduced dermal collagen density (Fig. 5). As expected, the topical applications of AP prevented UVA-induced photoaging. The immunohistochemical analysis confirmed that the repeated short-term UVA-exposure induced overexpression of MMP-2 in mouse skin (Fig. 6). However, topical application of AP significantly inhibited the expression of MMP-2 in the mouse skin. There were no changes observed in AP alone treated mice. In order to find the mechanism of antiphotoaging effects of AP, we studied the mRNA expression of MMP-13 and MMP-9 by qRT-PCR. We found that the topical application of AP prior to UVA-exposure significantly prevented the mRNA expression pattern of MMP-13 and MMP-9 when compared to non-irradiated control mouse skin. This was also confirmed by MMP-1 and MMP-9 protein expression by Western blot analysis (Fig. 7A & B).

4. Discussion

Exogenous factors like UVA-irradiation can trigger a wide range of radicals; because there is a surplus of the chromophore in the skin cells, such as cytochromes, riboflavin, hemoglobin (erythrocytes), myoglobin (muscle), melanin and DNA [27]. The UVA exposure eventually leads to depletion of cellular antioxidant levels. In this study, the AP pretreatment significantly restored the levels of SOD, CAT, GPx, and GSH in UVA-exposed mouse skin. Similarly, it has already been reported that AP treatment (at 25 and 50 mg/L) increases the total antioxidant capacity in the cultured human lymphocytes [28]. Ben Ali et al. reported that AP isolated from the leaves of *Juniperus phoenicea* increased the activities of cellular enzymic and non-enzymic antioxidant system in Hcl/ethanol-induced ulcers in rats [29]. AP also increasing the total antioxidants capacity in primary rat neurons without any alterations in N2a cells [30]. Therefore, we state that free radical scavenging and antioxidant property of AP could be responsible for maintaining the cellular redox balance under UVA-induced oxidative stress.

Further, we evaluated the molecular mechanism of AP against UVA-induced apoptosis. UVA-induced apoptosis have primarily been activated by intrinsic apoptotic signaling pathway [31]. In this study, we noticed that AP treatment prior to UVA exposure significantly restored the levels of Bcl-2 thereby maintains Bax/Bcl-2 complex and mitochondrial membrane integrity resulted in attenuated activation of caspase-9 and caspase-3 in the mouse skin. Similarly, several natural phytochemicals reported preventing UV-induced apoptosis, primarily through the activation of antiapoptotic protein and inhibition of

proapoptotic Bax [32–34]. Hence, we conclude that AP prevents UVA-induced apoptosis by regulating Bax/Bcl-2 expression.

Acute effects of UVA exposure are both short-lived and reversible. UVA contributes 15–20% of the sunburn reaction, which is usually characterized by increased blood flow in the dermis [35]. Immunohistochemical studies using CD34 in the histological section of mouse skin confirmed the blood vessel dilation after UVA exposure. Further, we observed that overexpression of VEGF and iNOS in the mouse skin. It is due to changes in the superficial microvasculature during sunburns result in an activation of proangiogenic mediators [36]. However, topical application of AP effectively prevented sunburn reaction in UVA-exposed mouse skin dermis. Earlier, AP has been reported to inhibit the expression of iNOS at non-cytotoxic concentrations in human chondrocytes [37]. Mirian et al. has also found that AP exerts antiangiogenic property via inhibition of the release of VEGF [38]. UVA is known to cause cutaneous inflammation via activation of NF-κB, that triggers the secretion of VEGF and cytokine production TNF-α, IL-6 and COX-2. We observed that AP treatment before UVA exposure inhibited the nuclear translocation of NF-κB p65 thereby affected NF-κB expression which could be accounted for decreased expression of TNF-α, IL-6, and COX-2 in the mouse skin. Zhou et al. also clearly demonstrated that the AP mediated inhibition of nuclear translocation of NF-κB [39]. Similarly, AP exhibited antiinflammatory activity through the suppression of NF-κB and TNF-α in the allergic rhinitis mouse model [40]. On the same line, topical application of AP displayed significant antiinflammatory effects through inhibiting COX-2 overexpression in a xylene-induced ear edema model [41]. Hence, we understand that the antiinflammatory effect of AP in UVA-exposed mouse skin could be closely associated with inhibition of NF-κB and COX-2 expression.

The UVA radiation, due to its high penetration nature, reaches deeper parts into the skin and mostly induces profound alterations of the dermal connective tissue [42]. In this study, the H & E stained tissue sections of UVA-exposed mice showed deteriorated dermal structure (Fig. 1). However, there were no significant changes noticed in the epidermal region of UVA-exposed mouse skin. Previous reports also stated that exposure to UVA is not morphologically affected the epidermal structure and organization. Because the dermal fibroblasts are more sensitive to UVA than epidermal layer [43]. Further, we observed dermal collagen fiber density reduction in UVA-exposed mice. VVG stain results clearly showed that treatment of AP prior to UVA exposure significantly lowered the dermal damage and displayed structural density and regular arrangement of collagen in the dermis. Breaking down and/or degradation of collagen is predominantly regulated by a group of zinc-dependent proteolytic enzymes called matrix metalloproteinases (MMPs) [43]. MMPs are a family of 24 proteins. The MMP-1 (interstitial collagenase), MMP-2 and MMP-9 (gelatinases) are known to play important roles in UVR-induced photoaging [3]. To elucidate the mechanism of AP on UVA-induced photoaging, we have studied the mRNA expression of MMP-13 and MMP-9 in the mouse skin. The results demonstrated that the treatment of AP significantly decreased mRNA expression of MMP-13 and MMP-9. Inhibition of MMP-1 may have to be considered as the most important event because it degrades type I and III collagens. Incomplete degradation of type I and III collagens further activates MMP-9. The AP treatment has already reported inhibiting MMP-1 gene expression through inhibition of NF-κB and JNK activation in human chondrocytes [37]. Zeidán-Chuliá et al. analyzed different essential oils for the inhibition of MMPs in PMA induced models in HaCaT cells [44]. It has been found that AP and beta-pinene effectively inhibited MMP-9 and MMP-2 possibly through a mechanism based on their antioxidant potential. In this study, we noticed that UVA-exposure increased the expression of MMP-2 in the mouse skin. It has been well documented that UVA can directly activate MMP-2 [45]. Moreover, UVA-induced overexpression of IL-6 can activate MMPs via an autocrine stimulation [46]. Moreover, UVA-induced TNF-α also promotes the activation of MMP-2 via up-regulation of MMP-14 [47]. In this study, the topical application of AP significantly inhibited the MMP-2 expression in UVA-exposed mouse skin. Therefore, we conclude that the antiphotoaging mechanism of AP might be related to the inhibition of MMPs possibly through inhibition of NF-κB nuclear translocation.

5. Conclusion

The present study clearly demonstrated that AP prevents UVA-induced photoaging through maintaining intracellular antioxidants status, modulation of Bax/Bcl-2 expression, thereby hamper the activation of apoptotic mediators like caspases-3 expression. Further, AP effectively prevented the photoaging process by suppressing matrix metalloproteinases expression as well as by inhibiting UVA-induced activation of proangiogenic and inflammatory proteins expression. Therefore, the AP may be used as a potential photoaging countermeasure. However, the Sun Protection Factor (SPF) of AP needs to be explored before claiming it as a potential antiphotoaging agent.

Conflict of interest

The authors have declared having no conflict of interest.

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