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Preventive effects of tamarind seed coat extract on UVA-induced alterations in human skin fibroblasts

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Synopsis

One of the most damaging actions on skin is from solar radiation, particularly from its ultraviolet (UV) component, through the formation of oxidative species. Thus, an antioxidant strategy that prevents the formation of these oxidants could form the basis of an efficacious cutaneous protectant. Many herbal materials contain antioxidant polyphenols, and this study assessed the possibility that tamarind seed coat extract could fulfill this role. An alcoholic extract of the tamarind (*Tamarindus indica* L.) seed coat showed stronger antioxidant activity (2,2-diphenyl-1-picrylhydrazyl inhibition, EC_{50} = 12.9 μg/ml) than L-ascorbic acid (EC_{50} = 22.9 μg/ml) and α-tocopherol (EC_{50} = 29.3 μg/ml). In cultured fibroblasts taken from human skin, hydrogen peroxide (100–1000 μM) damaged 62–92% of the cells compared to only 35–47% when the cells were preincubated in extract (200 μg/ml) for 24 h. UVA (40 J/cm^2) irradiation of human fibroblasts damaged 25% of the cells but the death rate was reduced to 10% with extract. UV irradiation increased the proportion of cells arrest in G_0/G_1 phase (from 59% to 78%) but this was largely prevented by the extract (64%), according to flow cytometry. Intracellular total glutathione of UVA-irradiated cells pretreated with the extract increased to 10–25% compared to the non-pretreated group at 24–72 h after irradiation. Fibroblasts typically increased matrix metalloproteinase-1 secretion after photodamage, and this is prevented by the extract. This is the first report showing that tamarind seed coat extract is an antioxidant and can protect human skin fibroblasts from cellular damage produced by UVA and thus may form the foundation for an antiaging cosmetic.

INTRODUCTION

Solar ultraviolet (UV) radiation is composed of UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm) and of these, UVA is able to penetrate the reticular dermis, thereby accelerating skin aging. This influences fibroblast functions including in-

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creased matrix metalloproteinase-1 (MMP-1) secretion and decreased type I procollagen expression level (1–3). UV exposure causes formation of reactive oxygen species (ROS) that activate the signal transduction pathways involved in MMP-1 and procollagen expressions, and also growth, differentiation, senescence, and/or damage of fibroblasts (4–6). ROS-induced cell damage correlates with cell-cycle arrest (7–9). Nevertheless, skin possesses extremely efficient antioxidant defenses such as superoxide dismutases, glutathione peroxidases, glutathione reductase, and catalase. However, excessive exposure to UV can overwhelm the defense and cellular macromolecule repair capacities, leading to an accumulation of oxidative damage to cells. Therefore, exogenous antioxidants could moderate the free-radical burden, thereby ameliorating the process of skin aging and damage.

Plant phenols and polyphenols constitute an important group of naturally occurring antioxidants because the phenolic group can accept electrons from ROS, which benefits to cutaneous function. Thus, they promote procollagen production and inhibit metalloproteinases, hence preventing proteolytic degradation of extracellular matrix (1,10–12). Tamarind (*Tamarindus indica* L.) grows widely throughout tropical climates and its seed coat is an industrial waste. Its polyphenol content includes catechins, 2-hydroxy-3', 4'-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate and 3,4-dihydroxyphenyl acetate, which are antioxidants *in vitro* (13,14) as well as have additional health benefits (13,15,16). Likewise, tamarind seed coat may also protect skin against UV-induced damage but such an effect has not yet been identified.

Thus, we aimed to show that the tamarind seed coat extract containing phenolic compounds could reduce UVA-induced alterations of fibroblast viability and functions. The extract was tested on (i) oxidative damage in fibroblasts created by hydrogen peroxide (H_2O_2) and (ii) irradiated fibroblasts for cell cycle, and levels of total glutathione, type I procollagen, and MMP-1. The data suggest that tamarind seed coat extract has a UV-protectant action.

MATERIALS AND METHODS

PREPARATION OF TAMARIND SEED COAT EXTRACT

Ripe tamarind seeds were purchased from a local market in Phetchabun Province, Thailand. The seeds were heated in a hot air oven at 140°C for 45 min, cooled, and cracked to separate the outside brown layer. Only brown-red seed coats were collected and then ground into fine powder. For seed coat extraction, 70% ethanol was used. A Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) column was used to remove tannin, and the extract was dried under vacuum and then stored in a tight Amber glass bottle at 4°C for further studies.

QUANTIFICATION OF TOTAL PHENOLIC COMPOUNDS IN THE EXTRACT

The amount of total phenolic compounds was determined by Folin–Ciocalteu assay. The Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) is a mixture of phosphomolybdate

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and phosphotungstate used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants. The reagent reacts with phenols and nonphenolic reducing substances to form chromogens that were measured spectrophotometrically.

An aliquot of the samples (40 μl) was mixed with 1.8 ml Folin–Ciocalteu reagent previously diluted with distilled water (1:10). The solution was incubated at 25°C for 5 min before adding 1.2 ml of 15% sodium carbonate solution in distilled water. After incubation at room temperature for 30 min, the absorbance at 760 nm was measured. The amount of total phenolic compounds was calculated as gallic acid equivalents from the calibration curve. The result was shown in gallic acid equivalents (g/100 g sample).

FREE RADICAL SCAVENGING ACTIVITY ON DPPH

The antioxidant activity of tamarind seed coat extract was measured in terms of hydrogen-donating or radical-scavenging ability, using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH; Sigma-Aldrich Chemie, Steinheim, Germany). The assay is based on hydrogen atom- or electron-donating ability measured as bleaching of the purple-colored methanolic DPPH.

In a 96-well plate, 75 μl of various concentrations of the extract (0.5–1000 μg/ml in methanol) was added, followed by 150 μl of 0.2 mM methanolic DPPH. After incubation for 30 min at room temperature, the absorbance was measured spectrophotometrically at 515 nm against a methanolic DPPH blank (without the test sample). L-ascorbic acid (POCH SA, Slaskie, Poland) and α-tocopherol (Sigma-Aldrich) were used as antioxidant standards. The radical-scavenging activity was calculated as a percentage of DPPH decoloration using the following equation:

$$\%\text{Free-radical scavenging} = [1 - (A_{(\text{sample})}/A_{(\text{blank})})] \times 100$$

where $A_{(\text{sample})}$ is an absorbance intensity of sample solution and $A_{(\text{blank})}$ an absorbance intensity of blank solution. EC_{50}, the equivalent concentration to give the 50% effect, was determined by log-probit analysis using 10 different final concentrations of the samples. The study was performed in triplicate.

CYTOTOXICITY OF THE EXTRACT TO HUMAN SKIN FIBROBLASTS

Cell isolation and cultivation. Fibroblasts were obtained from the eyelid of a woman, aged 65 years, after routine plastic surgery. The procedure was approved by the ethical committee of Naresuan University. The dermis layer of human excess surgery skin tissue was cut into small pieces by a surgical blade. Four to five skin disks were then placed in a culture dish and subsequently incubated at 37°C with a humid atmosphere containing 5% CO_2 for 30 min. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM; low glucose; Sigma-Aldrich) and 10% fetal bovine serum (FBS; Cultilab, Campinas, São Paulo, Brazil), and 1% stock penicillin/streptomycin (GIBCO/Invitrogen Corporation, Grand Island, NY) was added to each flask. After incubation, the fibroblast

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cells migrated from the original attachment site. The cells were cultured in DMEM/FBS in air/5% CO_2 and temperature of 37°C.

Cell treatment. To determine the cytotoxicity of the extract, we transferred the cell suspension (not exceeding eight passages) from a 175-cm^2 flask into a 96-well plate (1×10^4 cells per well). After 24 h incubation, cells were exposed to 50–200 µg/ml extract for 24 h. The control cells were cultured in DMEM without extract. The viability of cells was determined by 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT; Boehringer Mannheim, Mannheim, Germany) assay (17,18). Briefly, following incubation, the old medium was replaced with 200 µl serum-free DMEM and 50 µl XTT-labeling mixture was added. The samples were further incubated for 4 h. The intensity of dye was measured with a spectrophotometer at 490 nm. The fraction of viable cells was calculated by subtracting the optical density fraction of treated cells from the untreated cells. These determinations were performed in triplicate.

EFFECTS OF THE EXTRACT TO H_2O_2-DAMAGED FIBROBLASTS

A suspension of fibroblasts from the primary cultures (not exceeding eight passages) was transferred from a 175-cm^2 flask into a 24-well plate (1×10^5 cells per well) and then incubated at 37°C under 5% CO_2 for 24 h. After treatment with 200 µg/ml tamarind seed coat extract, cells were treated with various concentrations (100–1000 µM) of H_2O_2 to induce oxidative stress in fibroblast (19). The control cells were not exposed to H_2O_2 or the extract. The viability of cells was determined by XTT assay. The cytotoxicity of H_2O_2 on cells was quantified and expressed as percentage of cell damage relative to 0% damage calculated from the number of control cells. The study was performed in triplicate.

DETERMINATION OF PREVENTIVE EFFECTS OF THE EXTRACT TO UVA-INDUCED CELL ALTERATIONS

UVA irradiation. The UVA source was generated by a Honle F-lamp with a H-1 band-pass filter (320–400 nm) and the output intensity measured by a meter with UVA probe (Honle, Gräfelfing, Germany). A suspension of fibroblasts from the primary cultures (not exceeding eight passages) was transferred from a 175-cm^2 flask into 12-well plates (2×10^5 cells per well). There were three treatment groups: (1) no extract, no UVA irradiation (control group); (2) no extract plus UVA irradiation; and (3) extract pretreatment plus UVA irradiation group.

For group 3 cells, tamarind seed coat extract was added directly to the medium (200 µg/ml) and all the groups were similarly incubated for 24 h (37°C/5% CO_2) after which they were 80–90% confluent. To avoid UV being absorbed or creating adjuncts, all media were replaced by phosphate-buffered saline (PBS; without extract). Some of the plates (groups 2 and 3) were UVA irradiated (40 J/cm^2) after which the PBS in all the plates was replaced by culture media. After this, the cells of each group were studied as follows:

Cell viability: After 24 h of irradiation, the XTT assay was used to assess cell viability. The optical density of the control group was adjusted to 100%.

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Cell-cycle analysis: After 72 h of irradiation, the proportions of cells at cell-cycle stages G_1, S, and G_2 were determined by the DNA stain, propidium iodide (PI; Sigma-Aldrich). Cells were detached by trypsinization and washed twice with PBS containing 2 mM EDTA (Sigma-Aldrich). The cells were fixed overnight with absolute ethanol (−20°C) and then stained with a solution containing 10 μg/ml PI, 10 μg/ml RNAse (GIBCO/Invitrogen Corporation), and 2 mM EDTA in PBS. After 20 min incubation at room temperature in the dark, fluorescent cells were sorted in a flow cytometry system equipped with a 488-nm argon laser (model FACScalibur, Becton Dickinson, Franklin Lakes, NJ). Cell-cycle data were analyzed using CellQuest Pro software.

Total glutathione content: After 6, 24, 48, and 72 h of irradiation, cells were collected and lysed with T-PER lysis buffer (Pierce Biotechnology, Rockford, IL) for 20 min, and the resultant samples were centrifuged (5000g/5 min) to collect the supernatant. Total glutathione (GSH/GSSG) used was a commercial kit (Dojindo Molecular Technology, Kumamoto, Japan). To each well of 96-well plates, 20 μl enzyme solution, 140 μl coenzyme solution, and 20 μl of either GSH standard solution or the test supernatant were incubated at 37°C for 10 min. 5-5'-Dithio-bis(2-nitrobenzoic acid) solution (20 μl) was then added, and the samples were further incubated at 37°C for 20–40 min. Absorbance was measure at 415 nm on a microplate reader (Spectra Count; PerkinElmer, Waltham, MA), and GSH content read off a calibration curve.

MMP-1 and type I procollagen content: An enzyme-linked immunosorbent assay was used to quantitate human MMP-1, pro- and active forms, and newly synthesized type I procollagen in fibroblast cell culture supernatants. After 24–72 h of irradiation, cell-free supernatants were collected and stored at −80°C until used. The amount of MMP-1 and type I procollagen was measure by using a commercial human MMP-1 EIA kit (RayBiotech, Norcross, Germany) and a commercial human procollagen type I C-peptide EIA kit (Takara Bio, Shiga, Japan), respectively. The levels of MMP-1 and type I procollagen were normalized against a standard dose–response curve based on the absorption at 450 nm using a microplate reader. The experiments were performed in triplicate.

STATISTICAL ANALYSIS

All quantitative data were expressed as means of samples for each treatment. Student's unpaired *t*-test was used for comparison between two groups. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

CHARACTERISTICS OF THE TAMARIND SEED COAT EXTRACT AND TOTAL PHENOLIC CONTENT

The crude extract from tamarind seed coat was light-brown powder. The percent yield of the extract obtained was 22.0 ± 0.8% w/w. As determined by Folin–Ciocalteu assay, the amount of the phenolic compounds was 58.0 ± 0.3 g gallic acid equivalents per 100 g extract.

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FREE RADICAL SCAVENGING ACTIVITY OF THE EXTRACT

Several assays including DPPH, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), and ferric reducing antioxidant power have been used to measure antioxidant capacities in plant extract (20–22). Most assays are based on the ability of the sample to scavenge a synthetic colored radical or to reduce the redox-active compounds. Among them, the DPPH oxidative assay is convenient in its application and thus is used worldwide in the quantification of radical-scavenging capacity (23,24).

In this study, the antioxidant activity of the extract was measured in terms of radical-scavenging ability, using DPPH. Such activity is inversely proportional to EC_{50} value, which was calculated from the nonlinear regression of the percentage of radical-scavenging activity against the sample concentration (Fig. 1). The lower EC_{50} value indicates the higher antioxidant activity. According to the obtained results, the EC_{50} value of tamarind seed coat extract was 12.9 μg/ml whereas those of L-ascorbic acid and α-tocopherol were 22.9 and 29.3 μg/ml, respectively. This result accords with previous studies (13,25) indicating strong antioxidant activity of tamarind seed coat extract, probably residing in its phenolic compounds (14,26).

TOXICITY OF THE EXTRACT TOWARD HUMAN SKIN FIBROBLASTS

To determine the cytotoxicity of tamarind seed coat extract, fibroblast viability was tested at various extract concentrations (50–200 μg/ml) for 24 h. Viability was better than 90% (Fig. 2). Moreover, even at highest concentration tested (200 μg/ml), changes in fibroblast appearance could not be detected (data not shown). Thus, the extract at the concentration of 200 μg/ml was used for further studies.

EXTRACT REDUCES H_2O_2-INDUCED CELL DAMAGE

H_2O_2 is an ROS that readily diffuses into cells and is far more stable than superoxide and hydroxyl radicals. H_2O_2 can be converted into highly reactive hydroxyl radical in the presence of reduced transition metal such as ferrous or cuprous ions (27). As an oxidant, H_2O_2 damaged primary human skin fibroblasts in a concentration-dependent manner (62–92%, 100–1000 μM) (Fig. 3). The tamarind seed coat extract could reduce the

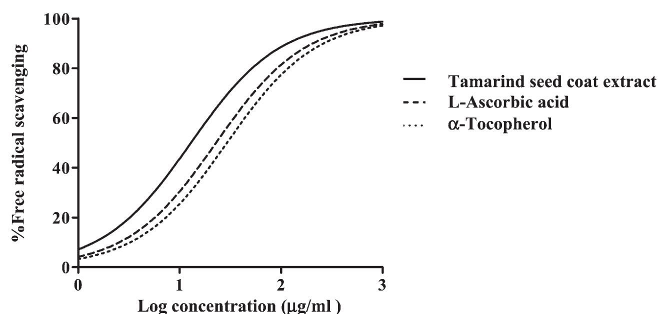

Figure 1. Free radical scavenging activity of tamarind seed coat extract, L-ascorbic acid, and α-tocopherol.

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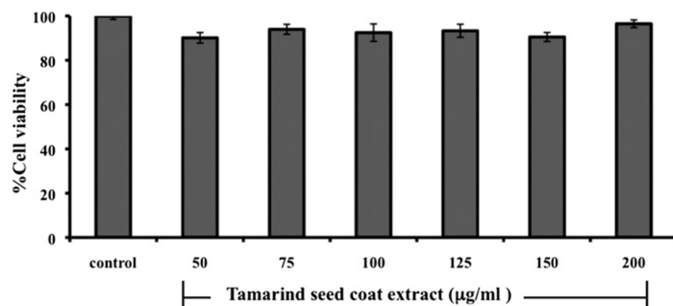

Figure 2. Effect of tamarind seed coat extract on viability of human skin fibroblasts. Data are expressed as percentage of control (untreated cells), and each bar represents mean ± SD of triplicate study.

amount of cell damaged caused through H_2O_2 by 25–45%. The preventive action of the extract may occur from free-radical scavenging activity of polyphenols contained in the extract. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen donors or electron acceptors and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (28). Another possible action underlying the antioxidative properties of the extract is the ability of polyphenols to alter peroxidation by modification of the lipid packing order and to decrease membranes fluidity (29). These changes can sterically hinder diffusion of free radicals and restrict peroxidative reactions. These actions can protect cell damage from membrane lipid peroxidation and apoptosis.

PREVENTIVE EFFECT OF THE EXTRACT TO UVA-INDUCED CELL ALTERATIONS

UVA radiation has long been known to generate an oxidative stress in cells irradiated in culture, and this has been linked to many studies that show that both endogenous anti-

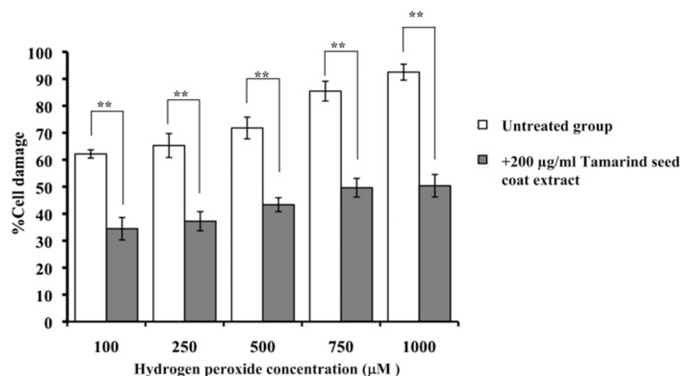

Figure 3. Preventive effect of tamarind seed coat extract on cell damage in H_2O_2-induced fibroblasts. Data are expressed as percentage of cell damage relative to 0% damage calculated from the number of cells that were not exposed to H_2O_2, and each bar represents mean ± SD of triplicate study. Significantly different from the untreated group; $^{**}p < 0.01$ (Student's t-test).

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oxidant pathways and added antioxidants can protect against the damage that arises from exposure to UVA (30–33). Endogenous antioxidant defense molecules such as glutathione are depleted in skin and skin cells by UVA radiation and this may lead not only to apoptosis in human keratinocytes (34) but also to the induction of the interstitial collagenase, MMP-1, in cultured skin fibroblasts (35,36). This study investigated the preventive effects of tamarind seed coat extract on skin fibroblasts after UVA irradiation, in terms of levels of MMP-1, type I procollagen, and antioxidant defense molecules such as total glutathione.

The XTT assay showed that viability of fibroblasts was decreased (~25%) at 24 h after UVA exposure. However, this was almost completely reversed by the extract at concentration used (200 μg/ml) (Fig. 4B). Under UVA, many of the fibroblasts became more rounded whereas cultures pretreated with extract showed a greater proportion of spindle-shaped fibroblasts resembling the controls (Fig. 4A). We then assessed more subtle changes in the cultured fibroblasts by studying their replicative ability after radiation. After radiation, there was a clear induction in the number of cells in the arrested G_0/G_1 phase (from 59% to 78%, $p < 0.01$) (Fig. 5) and a corresponding reduction in the G_2/M (mitotic) phase (from 16% to 9%, $p < 0.05$). The obtained results coincide with the previous study indicating increase in G_0/G_1 proportion and decrease in G_2/M proportion in low-dose UVA-irradiated fibroblasts (37). The arrest in the G_0/G_1 suggests that fibroblasts were in the resting state; cell synthesis and proliferation were inhibited. Interestingly, pretreatment of UV-irradiated cells with the extract showed a cell-cycle profile very similar to the nonirradiated controls. These observations indicate that tamarind seed coat extract is effective in the prevention of UVA-induced fibroblast cell arrest in G_0/G_1. Further studies should be performed to clarify preventive mechanism of the extract, particularly a p53-dependent cell-cycle arrest.

Glutathione is an important cell antioxidant and crucial in the regeneration of other endogenous antioxidants, and thus its level is a sensitive indicator of oxidative stress (38,39). In healthy cells, ~95% of the intracellular glutathione is present in its reduced form (GSH) because of an efficient pathway to re-reduce the oxidized form (GSSG). After 6 h of UVA irradiation, total glutathione of UVA-irradiated group was markedly increased to 25% ($p < 0.05$), compared to the control that was set 100% (Fig. 6). This implies that one of the cellular responses against UV exposure causing high-level ROS is the induction of detoxifying system level. However, the accumulation of ROS resulted in low level of total glutathione as a result in 72 h incubation of UVA-irradiated group. Interestingly, pretreatment with the extract restored the level of total glutathione to the level found in the control group. The extract may suppress the formation of radicals and protect against cell damages and/or function alterations. Moreover, the extract may have capacity to improve the activity of glutathione and/or increase the level of intracellular glutathione. The mechanism underlying the extract-induced total glutathione level/activity is needed to clarify in the future.

Crucial functions of skin fibroblasts are the biosynthesis and secretion of type 1 procollagen and MMP-1, and these were measured in the cell supernatant by enzyme immunoassay at 24, 48, and 72 h after UVA irradiation. There was a clear increase in secretion of MMP-1, which had normalized at 72 h after radiation (Fig. 7A). In contrast, the extract prevented this increased MMP-1 secretion. In contrast to MMP-1, radiation had essentially the reverse effect on procollagen biosynthesis (Fig. 7B). However, this

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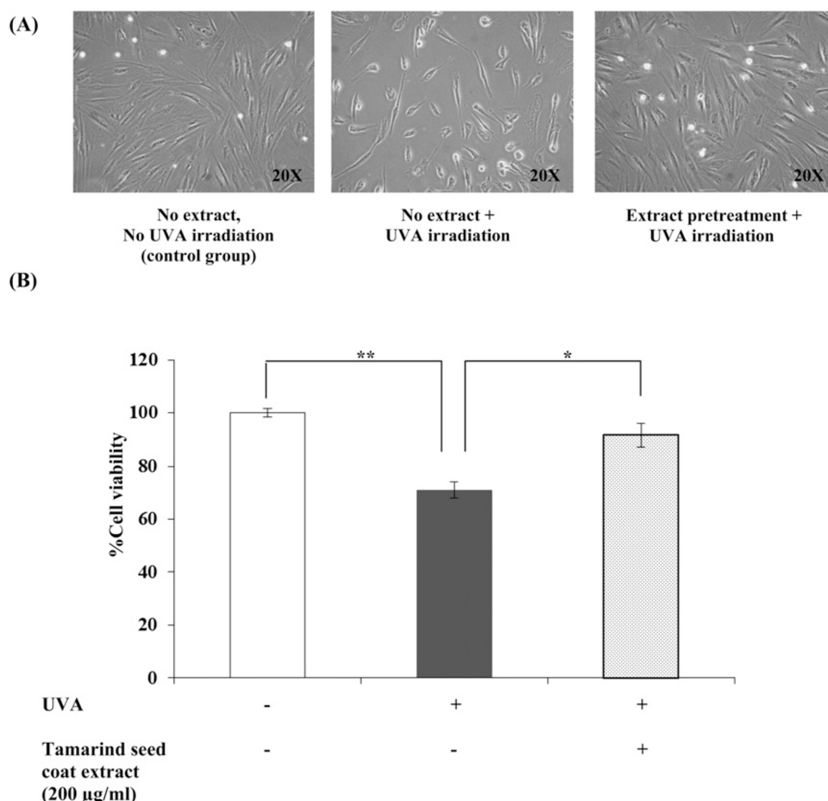

Figure 4. (A) Morphology of fibroblasts under 20X microscope and (B) viability of UVA-irradiated fibroblasts pretreated with 200 µg/ml tamarind seed coat extract at 24 h after UVA exposure. Data are expressed as percentage of control (no extract, no UVA irradiation), and each bar represents mean ± SD of triplicate study. Significantly different between two group; $*p < 0.05$, $**p < 0.01$ (Student's *t*-test).

depression persisted at 72 h after radiation and the extract seemed effective only at the later time. Both the increased secretion of MMP-1 and the reduced procollagen are controlled by the same transcription factor, AP-1, the different time courses suggest other inactions. Some differences may be ascribed to affect cell growth rate and cell death.

UVA-induced skin aging correlates with the loss of dermal connective tissue. It has been reported that the loss of type I collagen in photodamage skin is due to the progressive increase in MMP production and hence increased degradation of collagen (40–42), which is not compensated by procollagen production (43,44). Our results correlate with previous study indicating induction of MMP-1 and reduction of procollagen production by UVA-irradiated fibroblasts (3, 36, 44–45). In addition, pretreatment with the extract (200 µg/ml) provided protection against promoted MMP-1 activity. Such inhibition of MMP-1 induction in irradiated fibroblasts, at least partially, by antioxidant effect of the extract against oxidative damage through glutathione induction consequently results in lower MMP-1 secretion found in the extract-treated group. The oxidative stress has numerous effects on cell signaling, particularly the

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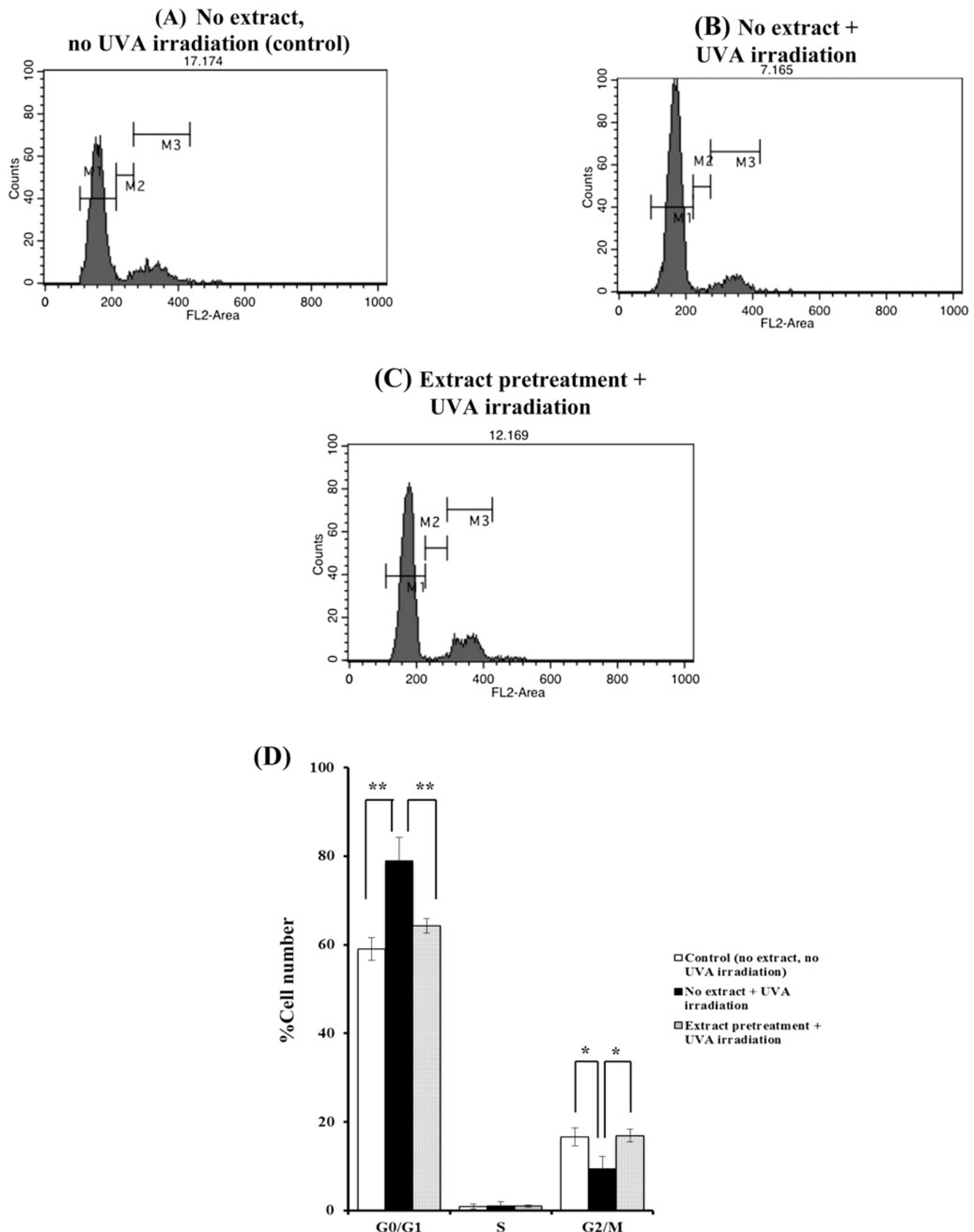

Figure 5. (A) Histogram of flow cytometric data of fibroblasts in control (no extract, no UVA irradiation); (B) no extract plus UVA irradiation; (C) 200 μg/ml extract pretreatment plus UVA irradiation; and (D) percentage of cell number in control, no extract plus UVA irradiation and extract pretreatment plus UVA-irradiated group at different stages of the cell cycle at 72 h after UVA exposure. Each bar represents mean ± SD of triplicate study. Significantly different between two group; $*p < 0.05$, $**p < 0.01$ (Student's *t*-test).

activation of inflammatory response, nevertheless our tamarind seed coat extract has the potential of protecting cutaneous fibroblasts of intact skin exposed to UV radiation.

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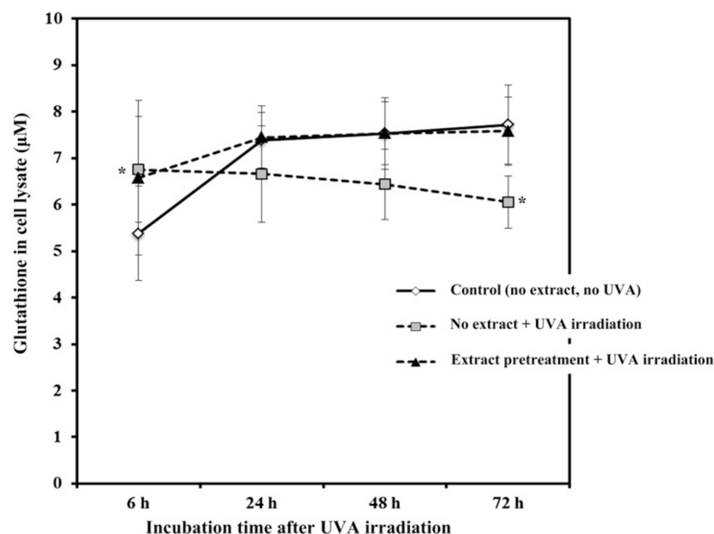

Figure 6. Effect of tamarind seed coat extract on intracellular glutathione level of UVA-irradiated fibroblasts. Fibroblasts were pretreated with 200 μg/ml tamarind seed coat extract for 24 h before irradiation. Each point represents mean ± SD of triplicate study. Significantly different when compared to control (no extract, no UVA irradiation); $*p < 0.05$ (Student's *t*-test).

CONCLUSIONS

Nowadays, botanical extract is playing an increasingly important role in cosmetics. Our study revealed the preventive effects of the tamarind seed coat extract on UVA-induced damage/alterations of fibroblasts, which have a major function in maintaining the structural integrity of dermal extracellular matrix by continuously secreting precursors such as procollagen. We have found that the extract could prevent cell damage from H_2O_2 and UVA exposure. Pretreatment with extract could restore glutathione level and markedly inhibited the MMP-1 induction in UVA-irradiated fibroblasts. The extract was also capable of restoring G_0/G_1 arrest with increasing G_2/M phase in UVA-irradiated cells. All findings suggest that the tamarind seed coat extract has a potential to prevent skin alterations. Clinical studies in human subjects will be needed to determine the efficacy of the extract on UV-induced skin damage.

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GLOSSARY

AP-1: The activator protein 1 (AP-1) is a transcription factor, which stimulates gene transcription in response to various stimuli including cytokines, growth factors, and stress. In dermal fibroblasts, it regulates transcription of MMP and procollagen genes.

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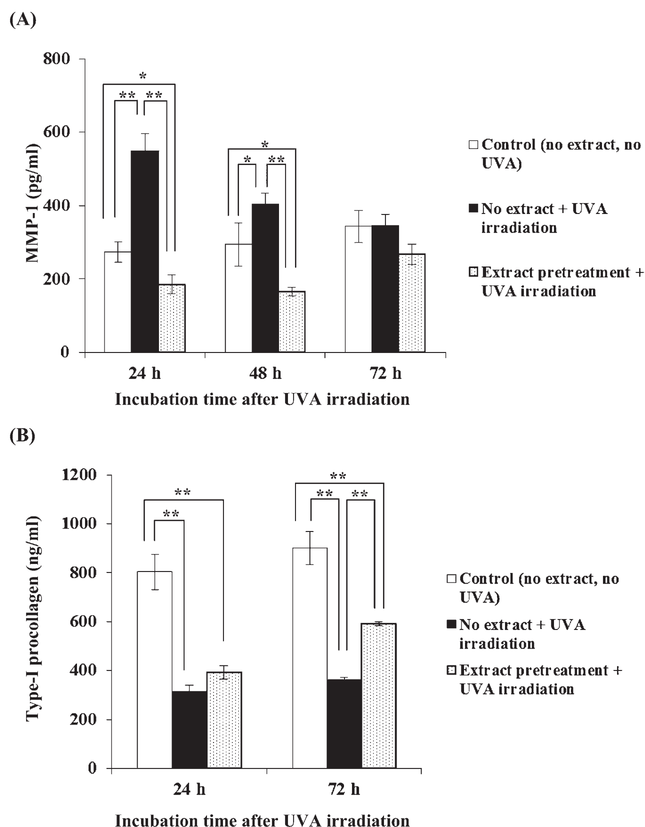

Figure 7. (A) Effects of tamarind seed coat extract on MMP-1 and (B) type I procollagen production by UVA-irradiated fibroblasts. Fibroblasts were pretreated with 200 μg/ml tamarind seed coat extract for 24 h before irradiation. Each bar represents mean ± SD of triplicate study. Significantly different between two group; $*p < 0.05$, $**p < 0.01$ (Student's *t*-test).

DPPH: This assay is based on the measurement of scavenging ability of antioxidant test substances toward the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). Hydrogen atom or electron-donating ability of the test substances is spectrophotometrically measured from the bleaching of the purple-colored methanol solution of DPPH.

Fibroblasts: These are the major cells found in the dermal skin. They are responsible for synthesis of extracellular matrix (ECM) components including collagen, elastin, and proteoglycans. Moreover, they secrete matrix metalloproteinases (MMPs), the enzymes that break down collagen and other proteins that comprise the dermal ECM.

G_0/G_1 phase: In normal skin, some 30% of basal cells are preparing for division. Following mitosis, a cell enters the G_1 phase, synthesizes RNA and protein, and grows in size. Later, DNA is synthesized (S phase) and chromosomal DNA is replicated. A short post-synthetic (G_2) phase of further growth occurs before mitosis (M). Some basal cells remain inactive in a so-called G_0 phase but may reenter the cycle and resume proliferation.

MMP-1: Matrix metalloproteinase-1 (MMP-1) is one of catalytic MMPs enzyme produced by fibroblasts. It is also known as fibroblast collagenase. Its major function is to initiate cleavage of fibrillar collagen (type I and III in skin). Once cleaved by MMP-1, collagen can be further degraded by elevated levels of MMP-3 and MMP-9.

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REFERENCES

(1) D. He, J. Sun, X. Zhu, S. Nian, and J. Liu, Compound K increases type I procollagen level and decreases matrix metalloproteinase-1 activity and level in ultraviolet-A-irradiated fibroblasts, *J. Formos. Med. Assoc.,* **110,** 153–160 (2011).

(2) N. Buechner, P. Schroeder, S. Jakob, K. Kunze, T. Maresch, C. Calles, J. Krutmann, and J. Haendeler, Changes of MMP-1 and collagen type Iα1 by UVA, UVB and IRA are differentially regulated by Trx-1, *Exp. Gerontol.,* **43,** 633–637 (2008).

(3) H. Watanabe, T. Shimizu, J. Nishihira, R. Abe, T. Nakayama, M. Taniguchi, H. Sabe, T. Ishibashi, and H. Shimizu, Ultraviolet A-induced production of matrix metalloproteinase-1 is mediated by macrophage migration inhibitory factor (MIF) in human dermal fibroblasts, *J. Biol. Chem.,* **279,** 1676–1683 (2004).

(4) G. Jenkins, Molecular mechanisms of skin ageing, *Mech. Ageing Dev.,* **123,** 801–810 (2002).

(5) E. C. Naylor, R. E. B. Watson, and M. J. Sherratt, Molecular aspects of skin aging, *Maturitas*, **69,** 249–256 (2011).

(6) T. M. Hagen, Oxidative stress, redox imbalance, and the aging process, *Antioxid. Redox. Signal.,* **5,** 503–506 (2003).

(7) P.-J. Chua, G. W.-C. Yip, and B.-H. Bay, Cell cycle arrest induced by hydrogen peroxide is associated with modulation of oxidative stress related genes in breast cancer cells, *Exp. Biol. Med.,* **234,** 1086–1094 (2009).

(8) M. Li, L. Zhao, J. Liu, A.-L. Liu, W.-S. Zeng, S.-Q. Luo, and X.-C. Bai, Hydrogen peroxide induces G2 cell cycle arrest and inhibits cell proliferation in osteoblasts, *Anat. Rec.,* **292,** 1107–1113 (2009).

(9) F. Hazane, S. Sauvaigo, T. Douki, A. Favier, and J.-C. Beani, Age-dependent DNA repair and cell cycle distribution of human skin fibroblasts in response to UVA irradiation, *J. Phytochem. Photobiol.,* **82,** 214–223 (2006).

(10) T. Pluemsamran, T. Onkoksoong, and U. Panich, Caffeic acid and ferulic acid inhibit UVA-induced matrix metalloproteinase-1 through regulation of antioxidant defense system in keratinocyte HaCaT cells, *Photochem. Photobiol.,* **88,** 961–968 (2012).

(11) J. Viyoch, S. Buranajaree, F. Grandmottet, S. Robin, D. Binda, C. Viennet, N. Waranuch, and P. Humbert, Evaluation of the effect of Thai breadfruit's heartwood extract on the biological functions of fibroblasts from wrinkles, *J. Cosmet. Sci.,* **61,** 311–324 (2010).

(12) J. Kim, J. S. Hwang, Y. K. Cho, Y. Han, Y. J. Jeon, and K. H. Yang, Protective effects of (-)-epigallocatechin-3-gallate on UVA- and UVB-induced skin damage, *Skin Pharmacol. Appl. Skin Physiol.,* **14,** 11–19 (2001).

(13) G. Pumthong, Antioxidative activity of polyphenolic compounds extracted from seed coat of *Tamarindus indicus* Linn. PhD Thesis, Chiangmai Mai University, Thailand, 1999.

(14) T. Tsuda, M. Watanabe, K. Ohshima, A. Yamamoto, S. Kawakishi, and T. Osawa, Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.), *J. Agric. Food Chem.,* **42,** 2671–2674 (1994).

(15) W. Aengwanich, M. Suttajit, T. Srikhun, and T. Boonsorn, Antibiotic effect of polyphenolic compound extracted from tamarind (*Tamarindus indica* L.) seed coat on productive performance of broilers, *Int. J. Poult. Sci.,* **8,** 749–751 (2009).

(16) T. Komutarin, S. Azadi, L. Butterworth, D. Keil, B. Chitsomboon, M. Suttajit, and B. J. Meade, Extract of the seed coat of *Tamarindus indica* inhibits nitric oxide product by murine macrophages in vitro and in vivo, *Food Chem. Toxicol.,* **42,** 649–658 (2004).

(17) S. Intaraprasit, A. Faikrua, A. Sittichokechaiwut, and J. Viyoch, Efficacy evaluation of the fibroblast-seeded collagen/chitosan scaffold on application in skin tissue engineering, *ScienceAsia,* **38,** 268–277 (2012).

(18) P. Inpanya, A. Faikrua, A. Ounaroon, A. Sittichokechaiwut, and J. Viyoch, Effects of the blended fibroin/aloe gel film on wound healing in streptozotocin-induced diabetic rats, *Biomed. Mater.,* **7,** 035008 (2012). doi: 10.1088/1748-6041/7/3/035008.

(19) K. Tanaka, N. Fujita, M. Yoshioka, and N. Ogawa, Immunosuppressive and non-immunosuppressive immunophilin ligands improve H_2O_2-induced cell damage by increasing glutathione levels in NG108-15 cells, *Brain Res.,* **889,** 225–228 (2001).

(20) M. I. Gil, F. A. Tomás-Barberán, B. Hess-Pierce, and A. A. Kader, Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California, *J. Agric. Food Chem.,* **50,** 4976–4982 (2002).

(21) L. P. Leong and G. Shui, An investigation of antioxidant capacity of fruits in Singapore markets, *Food Chem.,* **76,** 69–75 (2002).

(22) C. Guo, J. Yang, J. Wei, Y. Li, J. Xu, and Y. Jiang, Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay, *Nutr. Res.,* **23,** 1719–1726 (2003).

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(23) A. Floegel, D.-O. Kim, S.-J. Chung, S. I. Koo, and O. K. Chun, Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, *J. Food Compos. Anal.,* **24**, 1043–1048 (2011).

(24) J. Deng, W. Cheng, and G. Yang, A novel antioxidant index (AAU) for natural products using the DPPH assay, *Food Chem.,* **125**, 1430–1435 (2011).

(25) N. Lourith, M. Kanlayavattanakul, and S. Chanpirom, Free radical scavenging activity of tamarind seed coat and its cosmetic application, *J. Health Res.,* **23**, 159–162 (2009).

(26) Y. Sudjaroen, R. Haubner, G. Würtele, W. E. Hull, G. Erben, B. Spiegelhalder, S. Changbumrung, H. Bartsch, and R. W. Owen, Isolation and structure elucidation of phenolic antioxidants from tamarind (*Tamarindus indica L.*) seeds and pericarp, *Food Chem. Toxicol.,* **43**, 1673–82 (2005).

(27) W. Dröge, Free radicals in the physiological control of cell function, *Physiol. Rev.,* **82**, 47–95 (2002).

(28) C. A. Rice-Evans and N. J. Miller, Antioxidant activities of flavonoids as bioactive components of food, *Biochem. Soc. Trans.,* **24**, 790–795(1996).

(29) A. Arora, T. M. Byrem, M. G. Nair, and G. M. Strasburg, Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids, *Arch. Biochem. Biophys.,* **373**, 102–109 (2000).

(30) A. Tarozzi, A. Marchesi, S. Hrelia, C. Angeloni, V. Andrisano, J. Fiori, G. Cantelli-Forti, and P. Hrelia, Protective effects of cyanidin-3-O-β-glucopyranoside against UVA-induced oxidative stress in human keratinocytes, *Photochem. Photobiol.,* **81**, 623–629 (2005).

(31) R. P. Singh, S. Sharad, and S. Kapur, Free radicals and oxidative stress in neurodegenerative diseases: Relevance of dietary antioxidants, *J. Indian Acad. Clin. Med.,* **5**, 218–225 (2004).

(32) A. Chiu and A. B. Kimball, Topical vitamins, minerals and botanical ingredients as modulators of environmental and chronological skin damage, *Br. J. Dermatol.,* **149**, 681–691 (2003).

(33) A. Svobodová, J. Psotová, and D. Walterová, Natural phenolics in the prevention of UV-induced skin damage. A review, *Biomed. Pap.,* **147**, 137–145 (2003).

(34) Y. Y. He, J. L. Huang, D. C. Ramirez, and C. F. Chignell, Role of reduced glutathione efflux in apoptosis of immortalized human keratinocytes induced by UVA, *J. Biol. Chem*, **278**, 8058–8064 (2003).

(35) L. Yin, A. Morita, and T. Tsuji, Skin aging induced by ultraviolet exposure and tobacco smoking: Evidence from epidemiological and molecular studies, *Photodermatol. Photoimmunol. Photomed.,* **17**, 178–183 (2001).

(36) M. Wlaschek, G. Heinen, A. Poswig, A. Schwarz, T. Krieg, and K. Scharffetter-Kochanek, UVA-induced autocrine stimulation of fibroblast-derived collagenase/MMP-1 by interrelated loops of interleukin-1 and interleukin-6, *Photochem. Photobiol.,* **59**, 550–556 (1994).

(37) Z. Liu, H. Chen, H. Yang, J. Liang, and X. Li, Low-dose UVA radiation-induced adaptive response in cultured human dermal fibroblasts, *Int. J. Photoenergy* **2012**, 1–11 (2012).

(38) F. Afaq and H. Mukhtar, Effects of solar radiation on cutaneous detoxification pathways, *J. Photochem. Photobiol. B.,* **63**, 61–69 (2001).

(39) C. L. Hammond, T. K. Lee, and N. Ballatori, Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes, *J. Hepatol.,* **34**, 946–954 (2001).

(40) T. Quan, Z. Qin, W. Xia, Y. Shao, J. J. Voorhees, and G. J. Fisher, Matrix-degrading metalloproteinases in photoaging, *J. Investig. Dermatol. Symp. Proc.,* **14**, 20–24 (2009).

(41) M. Brennan, H. Bhatti, K. C. Nerusu, N. Bhagavathula, S. Kang, G. J. Fisher, J. Varani, and J. J. Voorhees, Matrix mettalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin, *Photochem. Photobiol.,* **78**, 43–48 (2003).

(42) G. J. Fisher, H. C. Choi, Z. Bata-Csorgo, Y. Shao, S. Datta, Z. Q. Wang, S. Kang, and J. J. Voorhees, Ultraviolet irradiation increases matrix metalloproteinase-8 protein in human skin in vivo, *J. Invest. Dermatol.,* **117**, 219–226 (2001).

(43) T. Quan, T. He, S. Kang, J. J. Voorhees, and G. J. Fisher, Ultraviolet irradiation alters transforming growth factor beta/smad pathway in human skin in vivo, *J. Invest. Dermatol.,* **119**, 499–506 (2002).

(44) L. Yin, A. Morita, and T. Tsuji, The crucial role of TGF-b in the age-related alterations induced by ultraviolet A irradiation, *J. Invest. Dermatol.,* **120**, 703–705 (2003).

(45) X. Z. Song, J. P. Xia, and Z. G. Bi, Effects of (-)-epigallocatechin-3-gallate on expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in fibroblasts irradiated with ultraviolet A, *Chin. Med. J. (Engl.)*, **117**, 1838–1841 (2004).

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