Protective effects of compounds from *Garcinia mangostana* L. (mangosteen) against UVB damage in HaCaT cells and hairless mice

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Received March 13, 2017; Accepted September 26, 2017

DOI: 10.3892/ijmm.2017.3188

Abstract. Ultraviolet B (UVB) radiation causes alterations in the skin, such as epidermal thickening, wrinkle formation and inflammation. Therefore, preventing UVB-induced skin damage can promote general health among the human population. *Garcinia mangostana* L. (mangosteen) is a fruit that has become a popular botanical dietary supplement because of its perceived role in promoting overall health. The present study investigated the photoprotective effects of α-, β-, γ-mangostins and gartanin against UVB radiation using the HaCaT immortalized human keratinocyte cell line as an *in vitro* model and hairless mice as an *in vivo* model. UVB radiation increased the expression of matrix metalloproteinase (MMP)-1 and -9 and decreased the mRNA expression levels of involucrin, filaggrin and loricrin in HaCaT cells; however, these changes were attenuated by pretreating the cells with α-, β-, γ-mangostins and gartanin. Among these compounds, α-mangostin exhibited the greatest effects in reducing UVB-induced skin wrinkles, inhibited epidermal thickening in hairless mice *in vivo*. Exposure to UVB radiation increased the expression of MMPs and pro-inflammatory cytokines and activated mitogen-activated protein kinases in hairless mice, but these changes were attenuated by α-mangostin. The authors suggested that α-mangostin exerts anti-wrinkle and anti-aging properties.

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Key words: *Garcinia mangostana* L., ultraviolet B, HaCaT cell, hairless mice, anti-wrinkle

Introduction

Extrinsic aging is caused by environmental oxidative factors primarily resulting from exposure to ultraviolet (UV) radiation (1,2). Skin degeneration by UV radiation is a cumulative process, and the degeneration rate depends on the frequency, duration and intensity of solar exposure, as well as the natural protection provided by skin pigmentation (2). UVB radiation penetrates the superficial layers of the skin down to the basal layer of the epidermis, where it generates harmful reactive oxygen and nitrogen species (ROS and RNS, respectively) and subsequently induces inflammation and sunburn and precipitates skin aging (3).

Oxidative stress plays a synergistic role in UV-induced skin damage. ROS can be induced by UV radiation, and the accumulation of ROS eventually causes inflammation and wrinkle formation in the skin (4). Increased ROS levels induce the production of matrix metalloproteinases (MMPs) and lead to skin damage, subsequently causing skin photoaging (5). Thus, MMPs are used as major markers of UVB-induced wrinkle formation and skin inflammation; additionally, their expression can result in increased skin aging, including the formation of deep wrinkles and thickening of the dermis and epidermis (6).

UVB-induced inflammatory responses involve an increase in pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, all of which accelerate skin damage and result in MMP activation (7,8). There is considerable evidence that UV-induced oxidative stress mediates the phosphorylation or activation of protein kinases such as mitogen-activated protein kinases (MAPKs) through a series of cascades (9). UVB-induced MAPK phosphorylation has been implicated in various skin diseases, including skin cancer and photoaging (10).

Garcinia mangostana (*G. mangostana*) L. (mangosteen) is native to Southeast Asia, and its fruits, which are famous for their remarkably pleasant flavor, have become a popular botanical dietary supplement because of their perceived

role in promoting overall health (11,12). Various parts of *G. mangostana*, mostly the fruit hull, bark and roots, have been used in Southeast Asia for hundreds of years in herbal medicine to treat a wide variety of medical conditions, including diarrhea, infected wounds and abdominal pain (11,13). Mangosteen fruits are rich in phenolic acids, xanthones, anthocyanins and condensed tannins, including α-mangostin, β-mangostin, γ-mangostin and gartanin (14,15). The most abundant xanthones in mangosteen fruits are α-mangostin and γ-mangostin, also other xanthones in mangosteen include β-mangostin, gartanin and among others (15). α-mangostin is the major compound that used as traditional medicine for various activities containing anti-inflammatory, antioxidant and antibacterial effects (16,17). Gartanin is a polyphenolic xanthones was observed to have anticancer activities on human bladder cancer cells (18). To the best of the authors' knowledge, anti-wrinkle activity of *Garcinia mangostana* L. extracts (GME) and its compounds by oral administration in hairless mice was not studied.

In a previous study, mangosteen was reported to contain a variety of bioactive compounds with potential applications as therapeutic agents or functional food additives (19,20). In the current study, the authors isolated α-mangostin, β-mangostin and γ-mangostin, as well as gartanin from GME and investigated whether these compounds could protect skin from UVB-induced damage.

Materials and methods

Preparation of α-mangostin, β-mangostin, γ-mangostin and gartanin. The compounds α-mangostin, β-mangostin, γ-mangostin and gartanin, which were used in the present *in vitro* study, were obtained from a previous study (21). Large-scale isolation of α-mangostin for the *in vivo* experiments was conducted as follows: Dried pericarps from *G. mangostana* (7.29 kg) were extracted three times with ethanol at room temperature to obtain 351 g of solid extract. This extract was suspended in water and then aliquoted into hexane, chloroform, ethyl acetate, n-butanol and water, resulting in hexane-soluble (12 g), chloroform-soluble (213 g), ethyl acetate-soluble (15 g), n-butanol-soluble (81 g) and aqueous (126 g) extracts. The chloroform-soluble extract (200 g) was chromatographed on a silica gel column (10x60 cm, 2,000 g) using a gradient mixture of hexane and EtOAc (100:0 to 2:1) followed by a gradient mixture of $CHCl_3$ and MeOH (20:1 to 1:1) to produce 21 fractions (GMC-A to GMC-U). The GMC-I fraction yielded 45 g of α-mangostin. The structures of α-mangostin, β-mangostin, γ-mangostin and gartanin are presented in Fig. 1.

Cell culture and UVB irradiation. An immortalized, non-tumorigenic human keratinocyte cell line (HaCaT) was maintained in Dulbecco's modified Eagle's medium (Life Technologies, Gibco-BRL Products, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics at 37˚C in a humidified incubator containing 5% CO_2. Cells ($1x10^4$) were seeded, allowed to adhere for 24 h, and treated with various concentrations of GME and the purified compounds prior to exposure to UVB radiation at a dose of 20 mJ/cm^2. Control cells received neither GME nor purified compounds and were not exposed to UVB radiation. Immediately following UVB irradiation, the cell viability was assessed by incubating the cells with MTS (Promega, Madison, WI, USA) for 1 h, and the MTS reduction to formazan was measured according to the manufacturer's instructions. The absorbance of the samples was measured at 490 nm using a microplate fluorimeter (Molecular Devices, LLC, Sunnyvale, CA, USA).

Determination of MMP-1 and MMP-9 secretion using enzyme-linked immunosorbent assay (ELISA). The MMP-1 and MMP-9 levels in the culture media of HaCaT cells ($5x10^4$) exposed to UVB radiation were determined using human total MMP-1 and MMP-9 ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, HaCaT cells were seeded in 96-well plates and treated with GME or the purified compounds. Following exposure to UVB radiation, the cell culture supernatants were collected and centrifuged at 189 x g for 5 min. The levels of MMP-1 and MMP-9 were quantified using colorimetric analysis using a microplate fluorimeter (Molecular Devices, LLC).

Experimental animals. HR-1 hairless male mice (weighing 20-23 g, 6 weeks old; Center Laboratory Animal Inc., Seoul, Korea) were stabilized for one week before the study. The animals were maintained at a temperature of 24˚C and 50% relative humidity with 12 h light/dark cycles and provided free access to food and water. All animal procedures were performed in accordance to the guidelines for the Care and Use of Laboratory Animals developed by the Institute of Laboratory Animal Resources of the National Research Council, and were approved by the Institutional Animal Care and Use Committee of Daejeon University in Daejeon, Korea. The mice were divided into control (n=5), UVB-irradiated vehicle (n=5) and UVB-irradiated α-mangostin (n=5) groups. The mice from the α-mangostin group were orally administered 0.1 ml water containing 100 mg/kg α-mangostin daily prior to UVB irradiation. Normal drinking water was supplied to the animals in the vehicle group, whereas the unexposed control group was not UV irradiated and received untreated water.

UVB irradiation. Mice were subjected to UVB irradiation using an UVM-225D Mineralight UV Display lamp (UVP, Inc., Upland, CA, USA), which emitted radiation at a wavelength of 302 nm. The UV intensity was measured using an HD2102-2 UV meter (Delta Ohm, Padova, Italy). For the *in vivo* experiments, UVB radiation was applied to the backs of the mice three times a week for 12 weeks. The radiation dose was progressively increased from 60 mJ/cm^2 per exposure at week 1 (one minimal erythematous dose = 60 mJ/cm^2) to 90 mJ/cm^2 per exposure at week 7.

Histological examination. Isolated mouse skin tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and sliced into 5 μm sections, which were stained with hematoxylin and eosin. The epidermal thickness was measured under light microscopy with an eyepiece micrometer.

Antioxidant enzyme activities. Superoxide dismutase (SOD) and catalase (CAT) activities were measured using a colorimetric

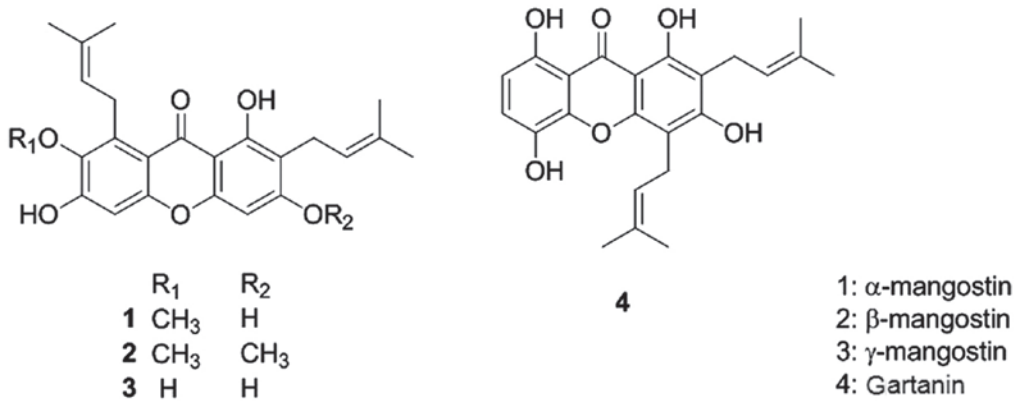

Figure 1. Chemical structures of α-mangostin, β-mangostin, γ-mangostin and gartanin isolated from *Garcinia mangostana* L. (mangosteen).

assay kit (SOD: cat. no. 706002, CAT: cat. no. 707002; Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocol. For protein extraction, skin tissue was homogenized in cold lysis RIPA buffer (Thermo Fisher Scientific, San Jose, CA, USA). The absorbance was measured at 450 and 540 nm to determine the SOD and CAT activity, respectively, using a plate reader (Molecular Devices, LLC).

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from skin tissue from UVB-irradiated mice using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. RT-qPCR was performed using TaqMan assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) specific for involucrin, loricrin, filaggrin, IL-1β, IL-6 and TNF-α on a QuantStudio™ 6 Flex Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Each sample was assayed in triplicate, and the relative mRNA expression levels were calculated using the ΔΔCt method and normalized to the β-actin mRNA levels in each sample (22).

Determination of MMP-1 and MMP-9 secretion by ELISA. MMP-1 and MMP-9 levels in the skin tissue after UVB irradiation were determined using total MMP-1 (cat. no. DMP100) and MMP-9 (cat. no. DMP900) ELISA kits according to the manufacturer's instructions. The levels of MMP-1 and MMP-9 were quantified by colorimetric analysis.

Western blotting. The protein content in the supernatant was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). The protein lysate was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel, and the proteins were transferred to polyvinylidene difluoride membranes and then blocked with blocking buffer (ATTO, Tokyo, Japan), which were incubated with specific primary antibodies targeting MMP-1 (1:1,000 dilution, ab137332; Abcam, Cambridge, MA, USA), MMP-9 (1:1,000 dilution, cat. no. 3852), p-ERK (1:1,000 dilution, cat. no. 4370), ERK (1:1,000 dilution, cat. no. 9102), p-p38 (1:1,000 dilution, cat. no. 9211), p38 (1:1,000 dilution, cat. no. 9212), p-JNK (1:1,000 dilution, cat. no. 9251), JNK (1:1,000 dilution, cat. no. 9252), and β-actin (1:1,000 dilution, cat. no. 4970) (all from Cell Signaling Technology, Beverly, MA, USA) at 4˚C overnight. The blots were washed for 10 min three times with phosphate-buffered saline (PBS) containing 0.1% Tween-20 and then incubated for 2 h with the appropriate anti-rabbit secondary antibody (1:5,000 dilution, SC-2004; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Protein bands were detected using an enhanced chemiluminescence solution (Bio-Rad) western blot detection system (LAS-4000; Fuji, Tokyo, Japan).

Statistic analysis. All measurements were completed in triplicate, and the data are presented as the mean ± standard error of the mean. The results were subjected to analysis of variance using Tukey's multiple comparison test to analyze the differences, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Protective effects of GME and purified compounds against UVB-induced damage in HaCaT cells. The authors investigated the effect of GME and the purified compounds on HaCaT cell proliferation after UVB exposure. Cell viability was reduced to 51.7% by UVB irradiation in untreated cells but increased to 82.6% (Fig. 2A) in the presence of α-mangostin.

Effects of GME and purified compounds on UVB-induced secretion of MMP-1 and MMP-9. The authors evaluated the effect of GME and the purified compounds on UVB-induced MMP expression in HaCaT cells. UVB radiation resulted in a marked increase in MMP-1 and MMP-9 levels compared with non-irradiated cells (Fig. 2B and C). The ELISA analysis revealed that GME and the individual compound treatments reduced the MMP-1 and MMP-9 protein levels in the culture media of HaCaT cells in a dose-dependent manner.

Effects of GME and compounds on involucrin, filaggrin, and loricrin levels in UVB-irradiated HaCaT cells. The results indicated that the expression of involucrin, filaggrin and loricrin was decreased in UVB-irradiated HaCaT cells compared with non-irradiated cells. As presented in Fig. 3, the mRNA expression levels of involucrin, filaggrin and loricrin in UVB-irradiated cells treated with GME or the purified

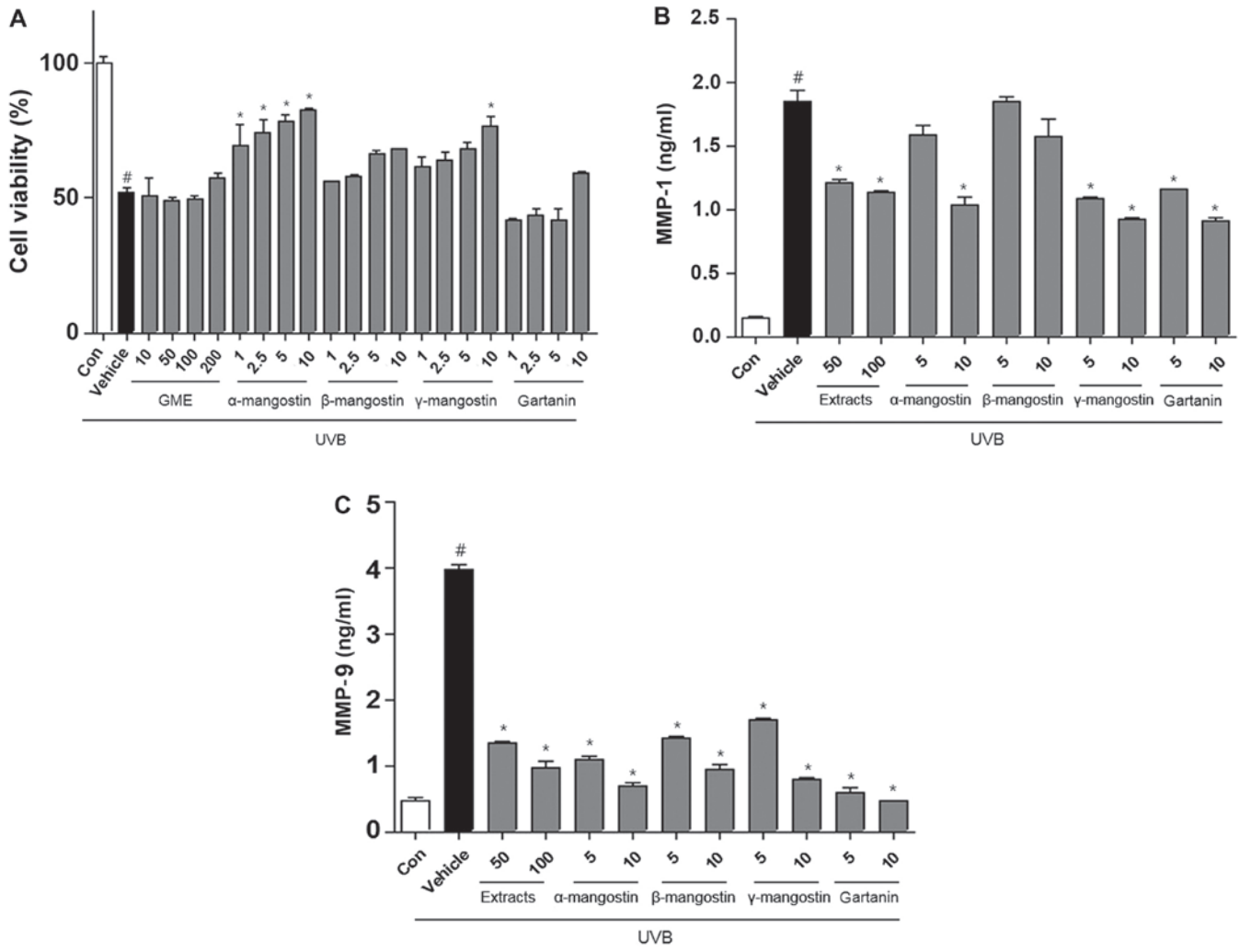

Figure 2. Effects of GME and the purified compounds on cell viability and MMP expression in HaCaT cells subjected to UVB irradiation. (A) HaCaT cells were pretreated with the indicated concentrations for 24 h followed by UVB irradiation of 20 mJ/cm^2. The expression levels of (B) MMP-1 and (C) MMP-9 in the culture medium of UVB-irradiated HaCaT cells were analyzed using ELISA. Concentration of GME is μg/ml, and other compounds are μM. Data are presented as mean ± standard error of the mean. $^{\#}$P<0.05 vs. control group; *P<0.05 vs. vehicle group. GME, *Garcinia mangostana* L. extracts; UVB, ultraviolet B; MMP, matrix metalloproteinase; Con, control; ELISA, enzyme-linked immunosorbent assay.

compounds were increased compared to the untreated cells exposed to UVB.

α-mangostin inhibits UVB-induced wrinkle formation in hairless mice. Because α-mangostin was the most effective compound in the *in vitro* studies, the authors investigated the effect of α-mangostin on UVB-induced wrinkle formation by repeatedly exposed hairless mouse skin to UVB for 12 weeks. Histological evaluation shows that this exposure induced skin wrinkles, which was blocked by α-mangostin (Fig. 4). To evaluate the effect of α-mangostin on skin thickening, the epidermal thickness was measured. The epidermal thickness of the UVB-irradiated α-mangostin-treated group was 118.7 vs. 55.0 μm in the UVB-irradiated vehicle-treated group.

Effects of α-mangostin on the activity of antioxidant enzymes in UVB-irradiated hairless mice. To investigate whether the radical-scavenging activity of α-mangostin was mediated by antioxidant enzymes, the activities of well-known antioxidant enzymes were examined in hairless mice exposed to UVB radiation. The SOD activity was decreased in the UVB-irradiated hairless mice compared to unexposed control mice (Fig. 5) but was enhanced by α-mangostin treatment in a dose-dependent manner. Furthermore, compared with the control group, the UVB-irradiated group exhibited decreased CAT activity, but treatment with α-mangostin ameliorated this loss of CAT activity. These results indicated that α-mangostin could protect the activities of antioxidant enzymes, which scavenge free radicals, and thereby further inhibit UVB-induced oxidative stress.

Effects of α-mangostin on involucrin, filaggrin, and loricrin expression in UVB-irradiated hairless mice. The authors indicated that the expression of involucrin, filaggrin and loricrin decreased in the UVB-irradiated group compared to the unexposed control group. As shown in Fig. 6, the mRNA expression levels of involucrin, filaggrin and loricrin in the UVB-irradiated hairless mice treated with α-mangostin were increased compared to the UVB-irradiated vehicle-treated mice.

Effect of α-mangostin on the release of inflammatory cytokines in hairless mice. To assess the regulatory effect of

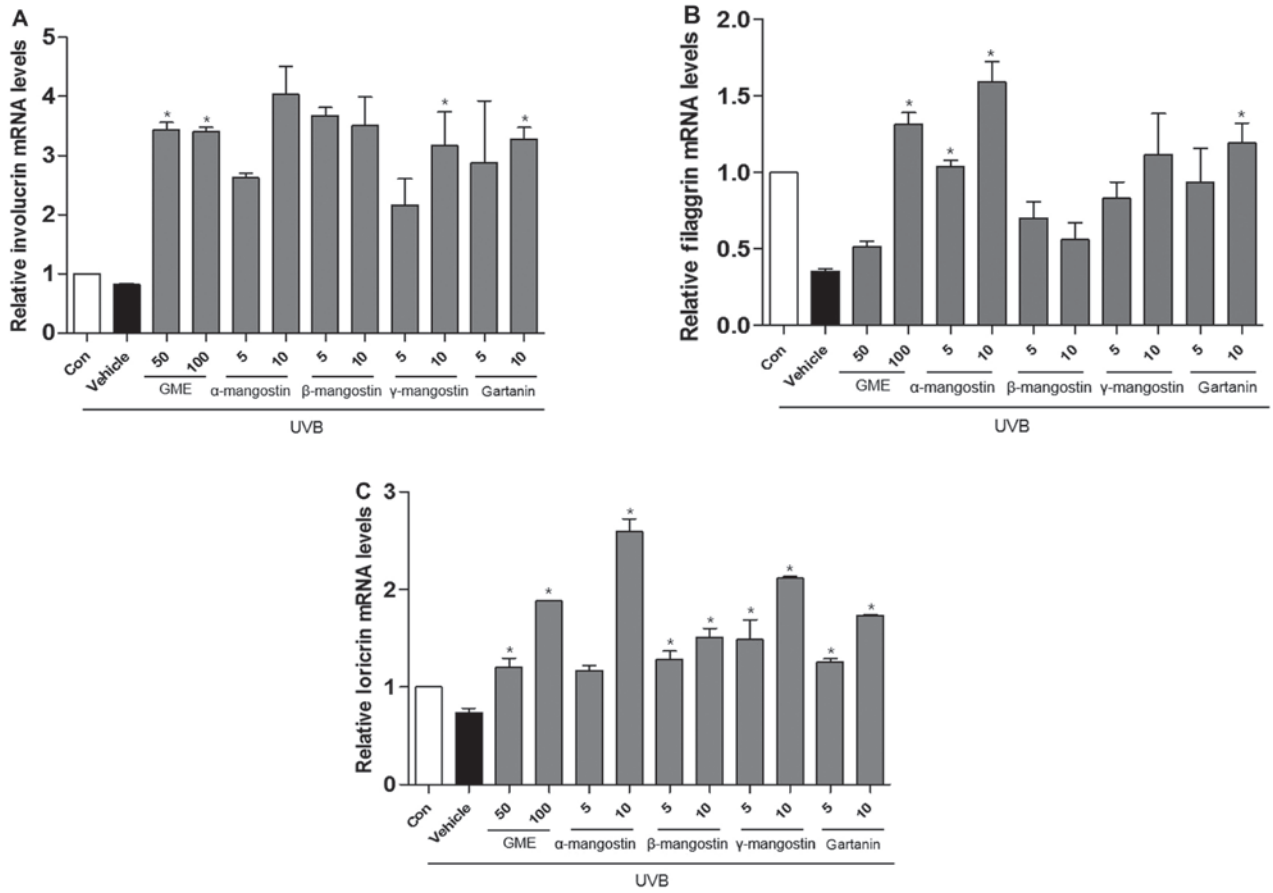

Figure 3. Effects of α-mangostin on (A) involucrin, (B) filaggrin and (C) loricrin expression in UVB-irradiated HaCaT cells. Concentration of GME is μg/ml, and other compounds are μM. Data are presented as mean ± standard error of the mean. *P<0.05 vs. vehicle group. GME, *Garcinia mangostana* L. extracts; UVB, ultraviolet B; Con, control.

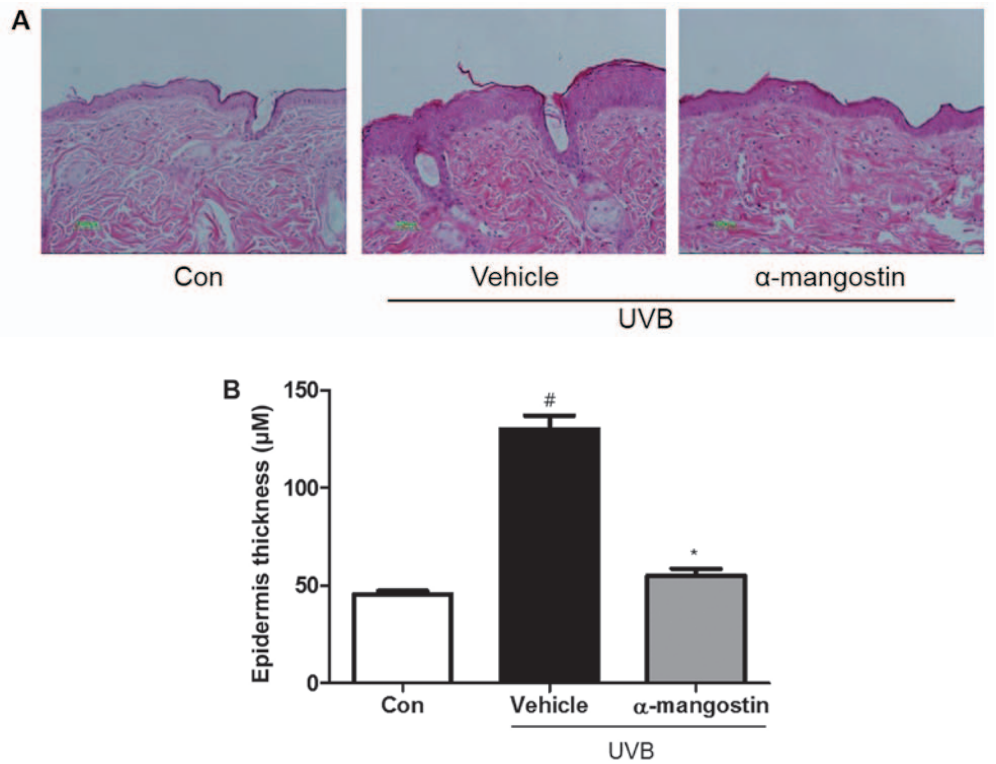

Figure 4. Effect of α-mangostin treatment on UVB-induced skin thickening in hairless mice. (A) Hematoxylin and eosin staining of UVB-irradiated hairless mouse skin. Original magnification, x200. (B) Dorsal skin epidermal thickness. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. vehicle group. UVB, ultraviolet B; Con, control.

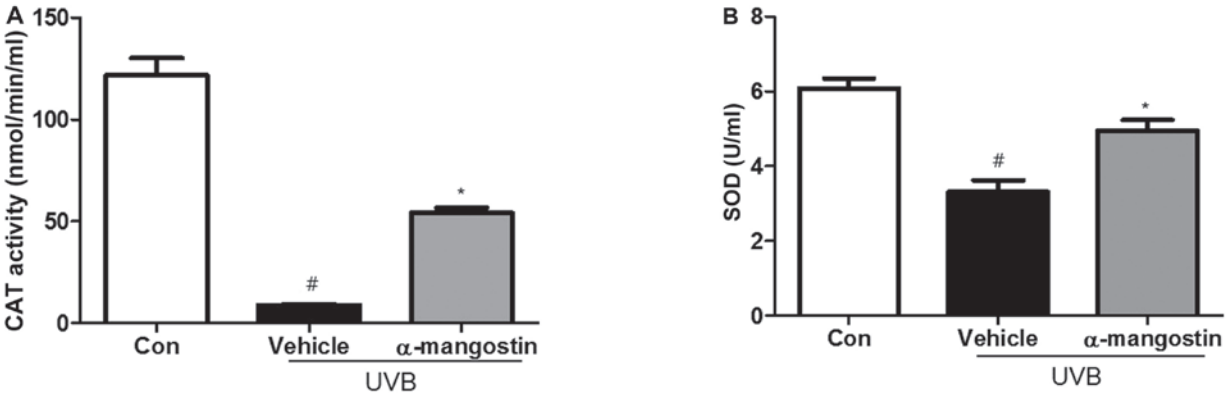

Figure 5. Effects of α-mangostin on the activity of (A) CAT and (B) SOD in hairless mouse skin exposed to UVB radiation. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. vehicle group. CAT, catalase; SOD, superoxide dismutase; UVB, ultraviolet B; Con, control.

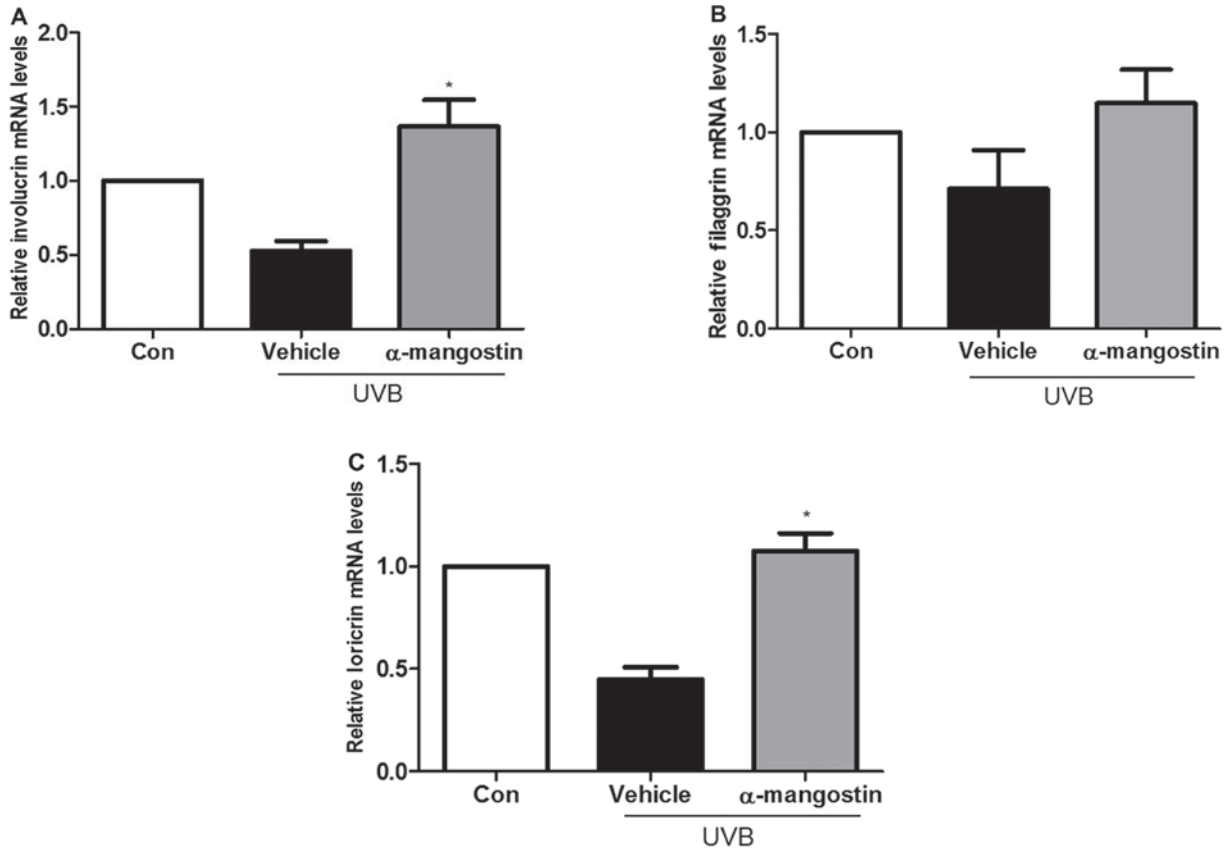

Figure 6. Effects of α-mangostin on (A) involucrin, (B) filaggrin and (C) loricrin expression in UVB-irradiated hairless mouse skin. Data are presented as mean ± standard error of the mean. *P<0.05 vs. vehicle group. UVB, ultraviolet B; Con, control.

α-mangostin on the production of pro-inflammatory cytokines in UVB-irradiated hairless mice, the mRNA expression of pro-inflammatory cytokines was investigated. As presented in Fig. 7, the IL-1β, IL-6 and TNF-α mRNA levels were increased in mice exposed to UVB compared to the unexposed control; however, this increase was suppressed by treatment with α-mangostin. This suggested that α-mangostin exerted a photoprotective effect by reducing the inflammatory response to UVB radiation.

Effects of α-mangostin on MAPK phosphorylation in UVB-irradiated hairless mice. Using western blotting, the authors assessed the effect of oral administration of α-mangostin on the activation of MAPK family proteins and downstream MMP-1 and MMP-9 proteins in hairless mouse skin exposed to UVB irradiation (Fig. 8A). UVB irradiation increased the skin expression of MMP-1 and MMP-9 levels in hairless mice. Treatment with α-mangostin reduced the expression of MMP-1 and MMP-9 (Fig. 8B and C). The authors examined whether α-mangostin could suppress the UVB-induced phosphorylation of MAPKs, including ERK, p38 and JNK. As presented in Fig. 8D, UVB irradiation resulted in the phosphorylation of ERK, p38 and JNK, whereas treatment of mice with α-mangostin prior to UVB

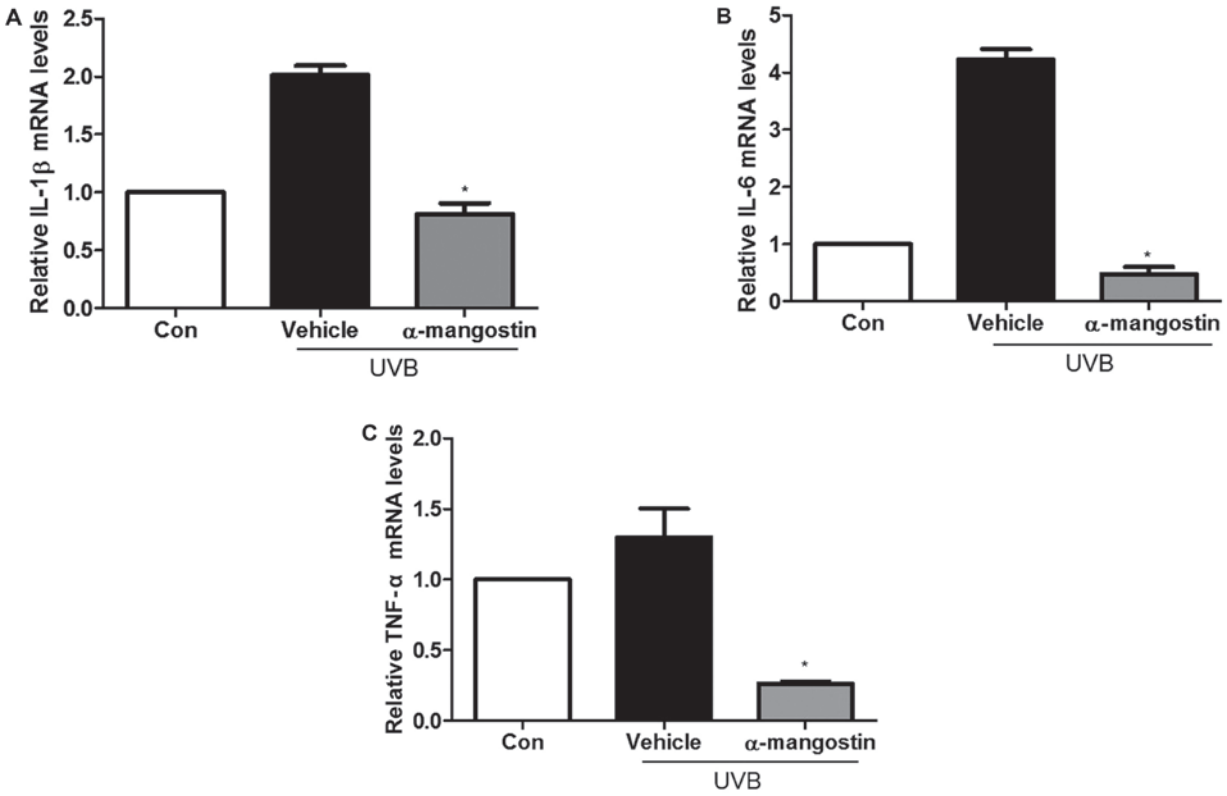

Figure 7. Effects of α-mangostin on pro-inflammatory cytokines in UVB-irradiated hairless mouse skin. The mRNA expression levels of (A) IL-1β, (B) IL-6 and (C) TNF-α were determined using reverse transcription-quantitative polymerase chain reaction. Data are presented as mean ± standard error of the mean. *P<0.05 vs. vehicle group. UVB, ultraviolet B; Con, control; IL, interleukin; TNF-α, tumor necrosis factor-α.

irradiation inhibited the UVB-mediated phosphorylation of these MAPKs.

Discussion

Acute UV exposure on human skin causes sunburn, altered pigmentation, inflammation, immune suppression, and dermal connective tissue damage (3). UV irradiation of the skin enhances collagenase activity and contributes to wrinkle formation via collagen degradation in the dermal extracellular matrix (ECM) (23). The authors investigated the effects of UVB exposure on skin aging in a hairless mouse model, specifically, its effects on the development of skin wrinkles. It was identified that extended UVB radiation induced skin wrinkles in hairless mice after 12 weeks.

Previous pharmacological studies of *G. mangostana* suggested numerous beneficial effects of the mangosteen, such as anti-acne, anti-adipogenic, antibacterial, anticancer, anti-obesity and antioxidative effects (21,24,25). Thus, the effects of α-mangostin on skin aging were investigated in the same hairless mouse model, especially regarding the development of skin wrinkles.

UVB-induced wrinkle formation is mediated by a complex mechanism involving ECM damage, cell death and the consequent inflammatory responses in both the epidermis and dermis (26). Histological and ultrastructural studies have revealed that photodamaged skin is associated with increased epidermal thickness and alterations in connective tissue organization (27). Epidermal thickness is often used as a parameter to quantitatively evaluate skin photoaging because epidermal hypertrophy is thought to cause wrinkle formation (28). The thickness of the basal membrane in photodamaged skin is increased, possibly reflecting damage of basal keratinocytes. Furthermore, the distribution of melanocytes along the basal membrane is irregular, and these cells vary widely in size, dendricity and pigmentation (29). Also, a cornified cell envelope, which is composed of involucrin, loricrin and filaggrin is a component of differentiated epidermal keratinocytes and corneocytes, and it is important in the skin barrier and moisture (30). The correct formation of the cornified cell envelope is essential for the barrier function of the skin then barrier inhibits the TEWL and associated loss of solutes (31). In the current study, the mRNA expression levels of involucrin, filaggrin and loricrin in UVB-irradiated cells treated with GME or the purified compounds were increased compared to cells only receiving UVB irradiation. In addition, the epidermal thickness of the dorsal skin of the mice was increased upon UVB exposure but was significantly reduced in mice administered α-mangostin prior to UVB exposure.

UV irradiation of the skin increases the levels of hydrogen peroxide, other ROS, and antioxidant enzymes (32). Increased ROS production alters the structure and function of multiple genes and protein, leading to skin damage (11). Although ROS are part of normal regulatory circuits and the cellular redox state is tightly controlled by antioxidants, an increased ROS load and the loss of cellular redox homeostasis can promote carcinogenesis and photoaging (33). In the present study, the results revealed that α-mangostin could protect the activities of antioxidant enzymes, which scavenge free radicals, thereby further inhibiting UVB-induced oxidative stress.

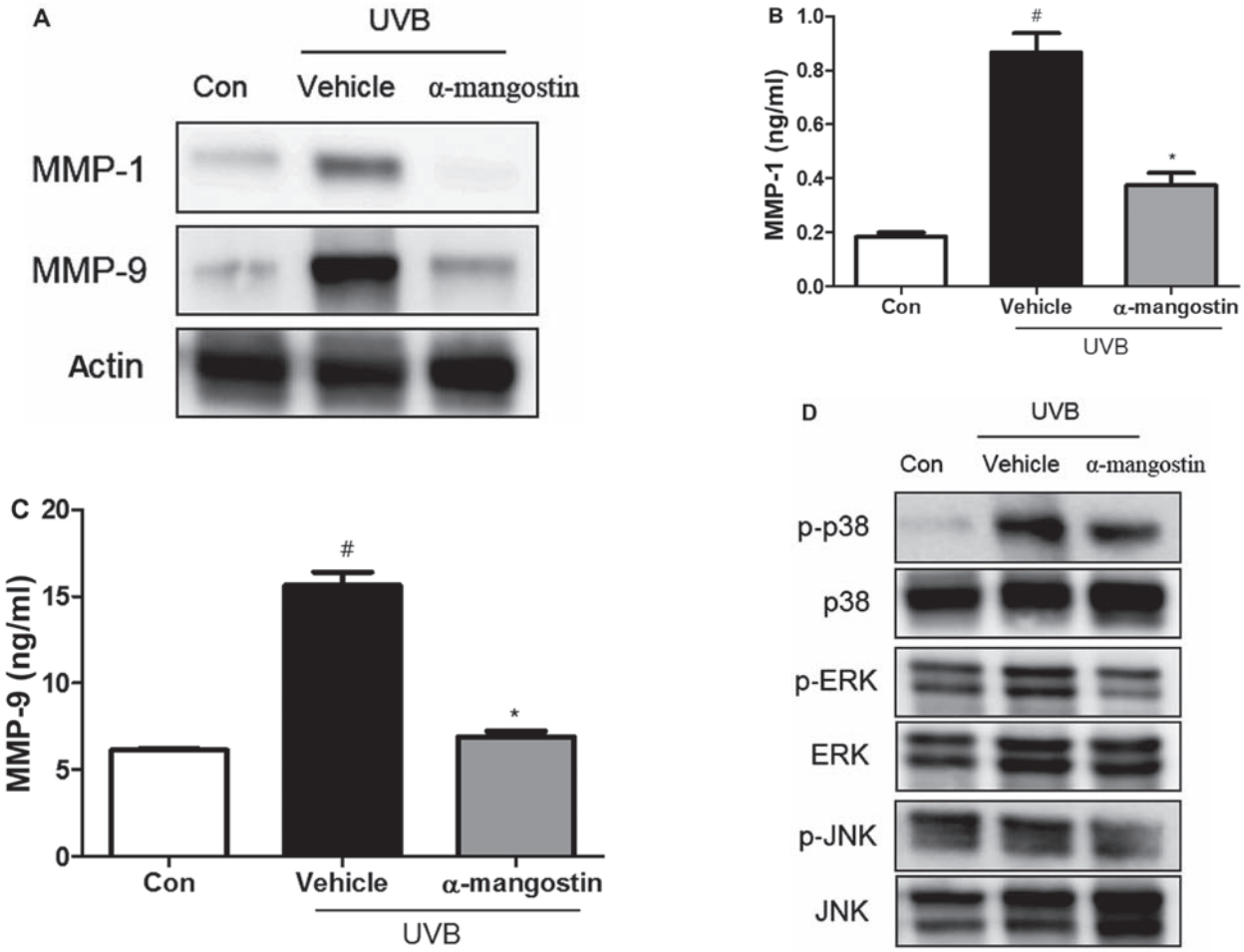

Figure 8. Inhibitory effects of α-mangostin on UVB-induced (A) MMP-1 and MMP-9 protein levels and (B) inhibition of the UVB-induced MMP-1 and (C) MMP-9 expression by ELISA assay kit. (D) ERK1/2, JNK and p38 phosphorylation in HaCaT cells. The phospho-ERK1/2, phospho-p38, phospho-JNK and β-actin protein levels were determined using western blotting. Data are presented as mean ± standard error of the mean. #P<0.05 vs. control group; *P<0.05 vs. vehicle group. UVB, ultraviolet B; MMP, matrix metalloproteinase; Con, control; ELISA, enzyme-linked immunosorbent assay.

Upregulated MMP-1 expression following UV irradiation results in collagen degradation, the histopathological hallmark of photoaging (34). A wealth of evidence has indicated that MMP induction plays a major role in the pathogenesis of photoaging (35). MMP-1 initiates the degradation of type I and III collagens and further degrades the collagen fragments generated by collagenases (6). Chronic UV irradiation of human skin results in elevated MMP expression, leading to marked degenerative changes in the upper dermal connective tissue via degradation of elastic and collagen fibers (36). In the current study, treatment with either GME or the purified compounds reduced the MMP-1 and MMP-9 expression levels.

UV radiation stimulates and activates various cells to produce and release cytokines, which likely play a significant role in the process of photoaging (37), and produces free radicals that release pro-inflammatory cytokines and growth factors, which activate proteases that degrade collagen and elastin. This eventually results in structural and functional changes in the ECM (38). UVB radiation triggers cutaneous inflammatory responses by directly inducing epidermal keratinocytes to produce specific pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6 and cyclooxygenase-2 (39,40). In the current study, the effect of GME was examined and purified compounds on HaCaT cells as well as of α-mangostin on UVB-induced pro-inflammatory cytokine production in UVB-irradiated hairless mouse skin; the results indicated that the inhibitory effect of α-mangostin on the production of inflammatory mediators was accompanied by reduced mRNA expression levels of IL-1β, IL-6 and TNF-α.

UVB irradiation has been shown to stimulate ROS production and activate MAPKs (41), including ERK1/2, JNK and p38 MAPK (42). In the study, α-mangostin inhibited the UVB-induced activation of JNK, p38 and ERK.

In conclusion, GME and the purified compounds from *G. mangostana* L. could effectively reduce skin damage. Specifically, α-mangostin ameliorated wrinkling processes induced by UVB irradiation of a hairless mouse model, as indicated by histological examination, increased SOD and CAT activities, and decreased MMP expression; furthermore, α-mangostin could reduce the protein levels of pro-inflammatory cytokine and multiple MAPKs.

Acknowledgements

The present study was supported by grants from the Korea Institute of Oriental Medicine (grant no. K15301) and the National Research Foundation of Korea funded by the Korean Government (grant no. NRF-2015R1A2A2A01006736).

References

1. Helfrich YR, Sachs DL and Voorhees JJ: Overview of skin aging and photoaging. Dermatol Nurs 20: 177-183, quiz 184, 2008.
2. Kammeyer A and Luiten RM: Oxidation events and skin aging. Ageing Res Rev 21: 16-29, 2015.
3. Dupont E, Gomez J and Bilodeau D: Beyond UV radiation: A skin under challenge. Int J Cosmet Sci 35: 224-232, 2013.
4. Wenk J, Brenneisen P, Meewes C, Wlaschek M, Peters T, Blaudschun R, Ma W, Kuhr L, Schneider L and Scharffetter-Kochanek K: UV-induced oxidative stress and photoaging. Curr Probl Dermatol 29: 83-94, 2001.
5. Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ and Fisher GJ: Matrix-degrading metalloproteinases in photoaging. Invest Dermatol Symp Proc 14: 20-24, 2009.
6. Amano S, Ogura Y, Akutsu N, Matsunaga Y, Kadoya K, Adachi E and Nishiyama T: Protective effect of matrix metalloproteinase inhibitors against epidermal basement membrane damage: Skin equivalents partially mimic photoageing process. Br J Dermatol 153 (Suppl 2): 37-46, 2005.
7. Brink N, Szamel M, Young AR, Wittern KP and Bergemann J: Comparative quantification of IL-1beta, IL-10, IL-10r, TNFalpha and IL-7 mRNA levels in UV-irradiated human skin in vivo. Inflamm Res 49: 290-296, 2000.
8. Campanini MZ, Pinho-Ribeiro FA, Ivan AL, Ferreira VS, Vilela FM, Vicentini FT, Martinez RM, Zarpelon AC, Fonseca MJ, Faria TJ, *et al*: Efficacy of topical formulations containing Pimenta pseudocaryophyllus extract against UVB-induced oxidative stress and inflammation in hairless mice. J Photochem Photobiol B 127: 153-160, 2013.
9. Son Y, Cheong YK, Kim NH, Chung HT, Kang DG and Pae HO: Mitogen-activated protein kinases and reactive oxygen species: How can ROS activate MAPK pathways? J Signal Transduct 2011: 792639, 2011.
10. Bickers DR and Athar M: Oxidative stress in the pathogenesis of skin disease. J Invest Dermatol 126: 2565-2575, 2006.
11. Obolskiy D, Pischel I, Siriwatanametanon N and Heinrich M: *Garcinia mangostana* L.: A phytochemical and pharmacological review. Phytother Res 23: 1047-1065, 2009.
12. Kinghorn AD, Chai HB, Sung CK and Keller WJ: The classical drug discovery approach to defining bioactive constituents of botanicals. Fitoterapia 82: 71-79, 2011.
13. Pedraza-Chaverri J, Cárdenas-Rodríguez N, Orozco-Ibarra M and Pérez-Rojas JM: Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol 46: 3227-3239, 2008.
14. Zadernowski R, Czaplicki S and Naczk M: Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). Food Chem 112: 685-689, 2009.
15. Han AR, Kim JA, Lantvit DD, Kardono LB, Riswan S, Chai H, Carcache de Blanco EJ, Farnsworth NR, Swanson SM and Kinghorn AD: Cytotoxic xanthone constituents of the stem bark of *Garcinia mangostana* (mangosteen). J Nat Prod 72: 2028-2031, 2009.
16. Gopalakrishnan C, Shankaranarayanan D, Kameswaran L and Nazimudeen SK: Effect of mangostin, a xanthone from *Garcinia mangostana* Linn. in immunopathological and inflammatory reactions. Indian J Exp Biol 18: 843-846, 1980.
17. Ibrahim MY, Hashim NM, Mariod AA, Mohan S, Abdulla MA, Abdelwahab SI and Arbab IA: α-Mangostin from *Garcinia mangostana* Linn: An updated review of its pharmacological properties. Arab J Chem 9: 317-329, 2016.
18. Liu Z, Antalek M, Nguyen L, Li X, Tian X, Le A and Zi X: The effect of gartanin, a naturally occurring xanthone in mangosteen juice, on the mTOR pathway, autophagy, apoptosis, and the growth of human urinary bladder cancer cell lines. Nutr Cancer 65 (Suppl 1): 68-77, 2013.
19. Pothitirat W, Chomnawang MT, Supabphol R and Gritsanapan W: Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia 80: 442-447, 2009.
20. Naczk M, Towsend M, Zadernowski R and Shahidi F: Protein-binding and antioxidant potential of phenolics of mangosteen fruit (*Garcinia mangostana*). Food Chem 128: 292-298, 2011.
21. Pothitirat W, Chomnawang MT, Supabphol R and Gritsanapan W: Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods. Pharm Biol 48: 182-186, 2010.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
23. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S and Voorhees JJ: Mechanisms of photoaging and chronological skin aging. Arch Dermatol 138: 1462-1470, 2002.
24. Balunas MJ, Su B, Brueggemeier RW and Kinghorn AD: Xanthones from the botanical dietary supplement mangosteen (*Garcinia mangostana*) with aromatase inhibitory activity. J Nat Prod 71: 1161-1166, 2008.
25. Sakagami Y, Iinuma M, Piyasena KG and Dharmaratne HR: Antibacterial activity of alpha-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. Phytomedicine 12: 203-208, 2005.
26. Matsuda M, Hoshino T, Yamakawa N, Tahara K, Adachi H, Sobue G, Maji D, Ihn H and Mizushima T: Suppression of UV-induced wrinkle formation by induction of HSP70 expression in mice. J Invest Dermatol 133: 919-928, 2013.
27. Rittié L and Fisher GJ: UV-light-induced signal cascades and skin aging. Ageing Res Rev 1: 705-720, 2002.
28. Gilchrest BA: Skin aging and photoaging. Dermatol Nurs 2: 79-82, 1990.
29. Hendi A, Brodland DG and Zitelli JA: Melanocytes in long-standing sun-exposed skin: Quantitative analysis using the MART-1 immunostain. Arch Dermatol 142: 871-876, 2006.
30. Lee WJ, Park KH, Cha HW, Sohn MY, Park KD, Lee SJ and Kim DW: The expression of involucrin, loricrin, and filaggrin in cultured sebocytes. Ann Dermatol 26: 134-137, 2014.
31. Hänel KH, Cornelissen C, Lüscher B and Baron JM: Cytokines and the skin barrier. Int J Mol Sci 14: 6720-6745, 2013.
32. D'Orazio J, Jarrett S, Amaro-Ortiz A and Scott T: UV radiation and the skin. Int J Mol Sci 14: 12222-12248, 2013.
33. Burke KE: Photoaging: The role of oxidative stress. G Ital Dermatol Venereol 145: 445-459, 2010.
34. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S and Voorhees JJ: Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 337: 1419-1428, 1997.
35. Zhuang Y and Lyga J: Inflammaging in skin and other tissues - the roles of complement system and macrophage. Inflamm Allergy Drug Targets 13: 153-161, 2014.
36. Chung JH and Eun HC: Angiogenesis in skin aging and photoaging. J Dermatol 34: 593-600, 2007.
37. Kondo S: The roles of cytokines in photoaging. J Dermatol Sci 23 (Suppl 1): S30-S36, 2000.
38. Fisher GJ: The pathophysiology of photoaging of the skin. Cutis 75 (Suppl 2): 5-9, 2005.
39. Jung E, Lee J, Baek J, Jung K, Lee J, Huh S, Kim S, Koh J and Park D: Effect of *Camellia japonica* oil on human type I procollagen production and skin barrier function. J Ethnopharmacol 112: 127-131, 2007.
40. Urbanski A, Schwarz T, Neuner P, Krutmann J, Kirnbauer R, Köck A and Luger TA: Ultraviolet light induces increased circulating interleukin-6 in humans. J Invest Dermatol 94: 808-811, 1990.
41. Peus D, Vasa RA, Beyerle A, Meves A, Krautmacher C and Pittelkow MR: UVB activates ERK1/2 and p38 signaling pathways via reactive oxygen species in cultured keratinocytes. J Invest Dermatol 112: 751-756, 1999.
42. Roux PP and Blenis J: ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68: 320-344, 2004.