Purpurogallin Protects Keratinocytes from Damage and Apoptosis Induced by Ultraviolet B Radiation and Particulate Matter 2.5

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Abstract

Purpurogallin, a natural phenol obtained from oak nutgalls, has been shown to possess antioxidant, anticancer, and anti-inflammatory effects. Recently, in addition to ultraviolet B (UVB) radiation that induces cell apoptosis via oxidative stress, particulate matter 2.5 (PM₂.₅) was shown to trigger excessive production of reactive oxygen species. In this study, we observed that UVB radiation and PM₂.₅ severely damaged human HaCaT keratinocytes, disrupting cellular DNA, lipids, and proteins and causing mitochondrial depolarization. Purpurogallin protected HaCaT cells from apoptosis induced by UVB radiation and/or PM₂.₅. Furthermore, purpurogallin effectively modulates the pro-apoptotic and anti-apoptotic proteins under UVB irradiation via caspase signaling pathways. Additionally, purpurogallin reduced apoptosis via MAPK signaling pathways, as demonstrated using MAPK-p38, ERK, and JNK inhibitors. These results indicate that purpurogallin possesses antioxidant effects and protects cells from damage and apoptosis induced by UVB radiation and PM₂.₅.

Key Words: Purpurogallin, Ultraviolet B radiation, Particulate matter 2.5, Oxidative stress, Human HaCaT keratinocytes

INTRODUCTION

Solar radiation is necessary for the synthesis of vitamin D in humans; however, ultraviolet (UV) radiation has been implicated in numerous skin disorders, including tumorigenesis (Feehan et al., 2016). UVB radiation has been shown to cause inflammation and skin cancer (Glady et al., 2018; Hosseini et al., 2018) through cellular DNA damage or immunosuppression (Nohynek and Schaefer, 2001). An abnormal increase in intracellular reactive oxygen species (ROS), including the hydroxyl radical, induces destruction of cytoskeleton and apoptosis (Kong et al., 2016; Zheng et al., 2018). Furthermore, excessive ROS levels strain the antioxidant defense systems, resulting in oxidative stress and skin damage (Zheng et al., 2014).

Recently, alongside UVB, particulate matter 2.5 (PM₂.₅) has become the focus of public health research, including research on skin hazards. PM₂.₅ represents outdoor air pollution and mainly consists of metals, allergens, toxic products of combustion of fossil fuels and endotoxins (He et al., 2016). PM was shown to damage the nervous system (Wang et al., 2017), respiratory epithelium (Liu et al., 2017), immune system (Castañeda et al., 2018) and cardiovascular system (Cao et al., 2016). As skin and keratinocytes form the outermost barrier directly facing harmful PM, the combined effects of UVB and PM₂.₅ on the skin are worth investigating.

Phenolic compounds were shown to possess antioxidant effects in cardiovascular diseases, anticancer activity, anti-platelet aggregation effects and anti-bacterial activity (Faggio et al., 2017). Furthermore, high levels of polyphenols promote collagen synthesis and protect human skin from photo-aging (Kang et al., 2018). Purpurogallin (PG), a natural phenol, suppressed delayed vasospasm, and protected cardiac and kidney cells (Zeng et al., 1992; Wu et al., 1996; Chang et al., 2014). Additionally, PG reduced inflammation in BV2 microglia cells and osteolytic diseases and showed antioxidant effects by scavenging hydroxyl radicals (Prasad and Laxdal, 1994; Park et al., 2013; Kim et al., 2018). UVB and PM₂.₅ aggravate the damage to keratinocytes and PG may possess cytoprotective effects. Hence, in this study,
we explored the antioxidant and cytoprotective effects of PG against UVB- and/or PM2.5-induced oxidative stress in HaCaT cells and investigated the underlying mechanisms.

**MATERIALS AND METHODS**

**Reagents and chemicals**

Purpureogallin (PG), Diesel particulate matter NIST SRM 1650b (PM2.5), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2',7'-dichlorofluorescein diacetate (DCF-DA), Primary antibodies anti-caspase-3, anti-caspase-9, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), Hoechst 33342, caspase inhibitor (Z-VAD-FMK), and p38 MAPK inhibitor (SB203580) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Diphenyl-1-pyrenylphosphine (DPPP) was purchased from Molecular Probes (Eugene, OR, USA). 5,5′,6,6′-tetrachloro-1',3',3′,tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was provided by Invitrogen (Carlsbad, CA, USA). SP600125 and U0126 were purchased from Tocris (Bristol, UK) and Calbiochem (La Jolla, CA, USA), respectively. Primary antibodies anti-Bax, anti-Bcl-2, anti-p38, and anti-PARP were purchased from Santa Cruz Biotechnology Inc (Dallas, TX, USA). Primary antibodies anti-ERK and anti-JNK were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-IgG secondary antibodies were purchased from Pierce (Rockford, IL, USA). All other chemicals and reagents were of analytical grade.

**Cell culture and UVB radiation**

Human HaCaT keratinocytes were provided by the AmorePacific Corporation (Yongin, Korea). Cells were maintained in Dulbecco’s modified Eagle medium (10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B) at 37°C in humidified atmosphere containing 5% CO2 (Life Technologies Co., Grand Island, NY, USA). The UVB energy spectrum (280-320 nm) was supplied by CL-1000M UV Crosslinker (UVP, Upland, CA, USA). UVB irradiation dose was 30 mJ/cm².

**Cell viability**

MTT assay was used to assess the cytotoxicity of PG and UVB. Cells (1.0×10⁵ cells/well) were plated into a 24-well plate. After 16 h incubation, the cells were exposed to 2.5, 5, or 10 μM PG or UVB radiation. MTT stock solution (2 mg/ml) was added and the cells were incubated for 4 h to yield formazan crystals, which were dissolved in dimethyl sulfoxide (DMSO). Finally, the absorbance was detected at 540 nm using a scanning multi-well spectrophotometer.

**DPPH radical detection**

PG (2.5, 5, or 10 μM) was mixed with 0.1 mM DPPH, shaken gently, and kept in the dark for 3 h. Residual DPPH was determined at 520 nm using a spectrophotometer.

**Determination of intracellular ROS**

The ability of PG to inhibit production of intracellular ROS induced by H2O2 (1 mM) or UVB radiation (30 mJ/cm²) was examined using DCF-DA. Cells (1×10⁵ cells/well) were seeded in a 96-well plate. After 16 h incubation, 2.5, 5, or 10 μM PG was added, the cells were incubated for 1 h, and treated with H2O2 or exposed to UVB radiation. DCF-DA (25 μM) was added to each well and cells were incubated for 10 min. Finally, the fluorescence of 2',7'-dichlorofluorescein was assessed using a LS-5B spectrofluorometer (Perkin-Elmer, Waltham, MA, USA).

*Fig. 1.* PG reduced ROS generation. (A) Chemical structure of purpureogallin. (B) The MTT assay was used to determine cell viability after treating HaCaT cells with PG (0, 2.5, 5, 10 μM) for 24 h. (C) DPPH radical scavenging activity of PG (0, 2.5, 5, 10 μM). (D) Hydroxyl radical scavenging potential of PG (2.5, 5, 10 μM). ROS generated by H2O2 or UVB were detected using the DCF-DA assay. (E) Intracellular ROS scavenging potential of PG (2.5, 5, 10 μM). ROS scavenging effect (%) was determined by flow cytometry. (F) Confocal microscopy and (G) flow cytometry were used for detecting intracellular ROS after DCF-DA staining. **p<0.05 vs. control cells,** p<0.05 vs. UVB-irradiated cells. (H) Confocal microscopy and (I) flow cytometry were used for detecting intracellular ROS after DCF-DA staining. **p<0.05 vs. control cells,** p<0.05 vs. UVB-irradiated cells.
Hydroxyl radical scavenging

Hydroxyl radical scavenging was assessed after the reaction of DMPO with hydroxyl radicals generated in the Fenton reaction (FeSO₄+H₂O₂). An electron spin resonance (ESR) spectrometer was used to detect the resultant DMPO/·OH adduct (Oh et al., 2016). Briefly, 0.3 M DMPO, 10 mM FeSO₄, 10 mM H₂O₂, and 10 μM PG (20 μl each, in phosphate buffer, pH 7.4) were used and the mixture was analyzed (recorded for 1 min). The ESR spectrometer parameters were as follows: central magnetic field 336.8 mT, power 1.00 mW, frequency 9.4380 GHz, modulation width 0.2 mT, amplitude 600, sweep width 10 mT, sweep time 0.5 min, gain 200, time constant 0.03 s and temperature 25°C.

Single-cell gel electrophoresis

The comet assay was used to measure oxidative DNA damage (Fernando et al., 2016). Cells (5×10⁴ cells/well) were seeded in medium with 10 μM PG in a 1 ml microtube for 30 min and treated with UVB (30 mJ/cm²) and/or PM₂.₅ (50 μg/ml) at 37°C. Data were collected after staining the cells with DCF-DA (25 μM) for 30 min at 37°C. Imaging analysis of intracellular ROS was conducted using a confocal microscope (Carl Zeiss, Oberkochen, Germany), whereas stained cells were counted using a flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Fig. 2. PG protected cells from UVB-induced damage to macromolecules. (A) The comet assay was used to detect DNA damage. (B) Confocal microscopy was used for detecting lipid peroxidation after DPPP (blue) staining. (C) Protein carbonylation was determined using a protein carbonyl ELISA kit. *p<0.05 vs. control, #p<0.05 vs. UVB-irradiated cells.

Additionally, cells (1.5×10⁵ cells/well) were seeded for 16 h, treated with 10 μM PG, and exposed to UVB (30 mJ/cm²) and/or PM₂.₅ (50 μg/ml) at 37°C. Data were collected after staining the cells with DCF-DA (25 μM) for 30 min at 37°C. Imaging analysis of intracellular ROS was conducted using a confocal microscope (Carl Zeiss, Oberkochen, Germany), whereas stained cells were counted using a flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Lipid peroxidation assay

Cells were plated on a four-well chamber slide in the presence of 10 μM PG exposed to UVB (30 mJ/cm²) and/or PM₂.₅

Fig. 3. PG protected cells from UVB-induced apoptosis. (A) HaCaT cell viability after UVB radiation was assessed using the MTT assay. (B) DNA fragmentation was assessed using a cellular DNA fragmentation ELISA kit. (C) Fluorescence microscopy detected apoptotic cells (arrows), stained with Hoechst 33342. The comet assay was used to detect DNA damage. (Fernando et al., 2016). Cells (5×10⁴ cells/well) were treated with 10 μM PG, and exposed to UVB (30 mJ/cm²) and/or PM₂.₅ (50 μg/ml) at 37°C. Data were collected after staining the cells with DCF-DA (25 μM) for 30 min at 37°C. Imaging analysis of intracellular ROS was conducted using a confocal microscope (Carl Zeiss, Oberkochen, Germany), whereas stained cells were counted using a flow cytometer (Becton Dickinson, Mountain View, CA, USA).
Protein oxidation was assessed using an Oxiselect™ Protein Carbonyl ELISA kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer’s instructions. Hoechst 33342 staining 

Mitochondrial membrane potential (Δψm) analysis

After treatment with 10 μM PG, the cells were exposed to UVB (30 mJ/cm²) or PM2.5 (50 μg/ml) for 5 h, stained with JC-1 (5 μM), and analyzed using confocal microscopy and high-performance flow cytometry.

Hoechst 33342 staining

Cells were treated with 10 μM PG for 1 h and exposed to UVB radiation (30 mJ/cm²) or PM2.5 (50 μg/ml) for 18 h. Additionally, after treatment with Z-VAD-FMK (30 μM), SB203580 (10 μM), SP600125 (10 μM), or U0126 (50 nM) for 1 h, the cells were treated with PG (10 μM) for 1 h and exposed to UVB radiation (30 mJ/cm²) for 18 h. The cells were stained with Hoechst 33342 (20 μM) and DNA-specific fluorescence was visualized using a fluorescence microscope equipped with a Cool SNAP-Pro color digital camera. Nuclear condensation levels were visualized using a fluorescence microscope.

Western blotting

Protein levels were analyzed as previously described (Cha et al., 2014). Membranes with proteins were sequentially incubated with the appropriate primary and secondary antibodies. Protein bands were detected by the Amersham ECL Plus Western Blotting Detection System (GE Healthcare Life Sciences, Amersham, UK).

Statistical analysis

All experiments were performed in triplicate. Data are represented as the mean ± standard error and were analyzed by the Sigma Stat (v12) software (SPSS, Chicago, IL, USA) using Tukey’s test and analysis of variance (ANOVA). p-values <0.05 were considered statistically significant.

RESULTS

PG attenuates UVB-induced ROS generation

In the MTT assay, PG (Fig. 1A) did not exhibit cytotoxicity to HaCaT cells under various concentrations (Fig. 1B). Cell viability in all treated groups was >96%, similar to control. DPPH
radical levels were significantly decreased in a dose-dependent manner in PG-treated groups (Fig. 1C). Hydroxyl radical scavenging potential of PG (10 μM) was evaluated using ESR spectrometry. In FeSO₄+H₂O₂ system, DMPO/·OH adducts were scavenged by PG (Fig. 1E). In addition, DCF-DA assay indicated that H₂O₂- and UVB-signal was reduced from 3366 to 2090 units by PG (Fig. 1D).

PG protects cellular macromolecules from UVB-induced damage

The protective effects of PG on UVB-induced DNA damage were evaluated by using the comet assay (Fig. 2A). The length of comet tails and percentage of tail fluorescence were significantly reduced in cells pretreated with PG compared to cells exposed to UVB (from 65 to 22%). Lipid peroxidation was analyzed using fluorescent DPPP oxide (Fig. 2B). The fluorescence intensity of DPPP oxide was higher in UVB-exposed cells, compared to that in cells treated with PG before UVB exposure. Furthermore, protein oxidation was assessed using the protein carbonylation assay (Fig. 2C). UVB irradiation significantly increased protein carbonylation, this effect alleviated by PG pretreatment. In general, these data indicate that PG effectively blocked UVB-induced damage to macromolecules, including DNA fragmentation and oxidation of proteins and lipids.

PG suppresses UVB radiation-induced apoptosis

Cell viability after UVB irradiation was also assessed (Fig. 3A). Compared to viability of control cells (100%), cell viability was reduced upon exposure to UVB (59%), an effect ameliorated by PG pretreatment (77%). These findings suggest that PG protected cells from UVB-induced cell death. Similarly, nuclear fragmentation of PG-pretreated cells was significantly reduced, indicating that PG alleviated UVB-induced DNA fragmentation (Fig. 3B). Additionally, Hoechst 33342-stained apoptotic cells in UVB-irradiated group (Fig. 3C) displayed massive nuclear condensation; however, formation of apoptotic bodies was reduced upon PG pretreatment. JC-1 staining was used to explore apoptosis caused by disrupted mitochondrial membrane potential, with red and green fluorescence representing aggregates and monomers of the JC-1 dye, respectively. Mitochondrial depolarization resulted in intense green fluorescence in UVB-exposed cells, which was attenuated by PG treatment (Fig. 3D). Flow cytometry was used to count apoptotic bodies (Fig. 3E). UVB-irradiated cells contained the highest fraction of apoptotic cells among cell groups tested; PG pretreatment decreased the percentage of apoptotic cells from 42 to 20%. These observations suggest that PG protects cells from UVB-induced apoptosis.

PG regulates UVB-induced cell death through caspase and MAPK signaling pathways

Western blotting was used to analyze the mechanisms of PG cytoprotective effects (Fig. 4A). Levels of pro-apoptotic protein Bax were increased by UVB and decreased by PG treatment. In contrast, the levels of anti-apoptotic protein Bcl-2
were decreased by UVB and increased by PG treatment. Formation of cleaved caspase-9 and caspase-3 was stimulated by UVB exposure but reduced in PG pretreated group. Similarly, UVB irradiation induced cleavage of PARP fragments, a target of caspase-3, whereas in PG-pretreated group, levels of cleaved PARP were notably lower than levels observed in the UVB-exposed group. Apoptotic bodies in cell groups pretreated with an irreversible caspase inhibitor (Z-VAD-FMK) and/or PG decreased considerably, and PG contributing to the effects of the caspase inhibitor (Fig. 4B). To further explore signaling pathways involved in PG-mediated modulation of apoptosis, p38 MAPK, JNK, ERK and their phosphorylated forms were detected by western blot (Fig. 4C). As expected, UVB irradiation increased the levels of phosphorylated p38 MAPK, JNK, and ERK, compared to levels in cells not exposed to UVB; however, this effect was reversed by PG pretreatment. In addition, apoptotic bodies were detected by Hoechst 3342 staining (Fig. 4D). Cells pretreated with p38 MAPK, JNK, and ERK inhibitors SB203580, SP600125, and U0126, respectively, also displayed a decreased number of UVB-induced apoptotic bodies, similar to cells pretreated with PG. Furthermore, PG enhanced the anti-apoptotic effects of these inhibitors. These results demonstrate that PG activates caspase and MAPK signaling pathways and prevents UVB-induced apoptosis by regulating apoptosis-associated proteins.

**PG attenuates PM<sub>2.5</sub>-induced oxidative stress and damage to cellular macromolecules**

DCF-DNA assay (Fig. 5A) and cell numbers (Fig. 5B) revealed that 10 μM PG reduced the levels of PM<sub>2.5</sub>-generated ROS. As for PM<sub>2.5</sub>-induced DNA damage, fluorescence and length of tails were significantly reduced in the PG pretreatment group (from 65 to 22%) (Fig. 5C). Additionally, cells pretreated with PG showed lower levels of protein carbonylation than PM<sub>2.5</sub>-exposed cells not treated with PG (Fig. 5D). PM<sub>2.5</sub> generated higher levels of DPPP oxide in cells not treated with PG, compared to PG-pretreated cells (Fig. 5E). These results suggested that PG suppressed PM<sub>2.5</sub>-induced ROS generation and protected cellular macromolecules from PM<sub>2.5</sub>-induced damage.

**PG blocks PM<sub>2.5</sub>-induced apoptosis**

Using JC-1 staining, normal mitochondrial polarization was observed in PM<sub>2.5</sub>-free cells, whereas PM<sub>2.5</sub>-exposed cells showed indications of mitochondrial depolarization (Fig. 6A). Intensity of red and green fluorescence in PG-pretreated cells suggested higher levels of normal mitochondrial polarization and a lower degree of mitochondrial depolarization, compared to mitochondria of the PM<sub>2.5</sub>-exposed group. Flow cytometry confirmed these observations (Fig. 6B). Additionally, Hoechst 33342 staining of apoptotic bodies indicated that PM<sub>2.5</sub>-exposed cell group presented the highest number of apoptotic cells, whereas cells pretreated with PG avoided apoptosis to a certain degree (Fig. 6C).

**PG protects cells against UVB- and PM<sub>2.5</sub>-induced apoptosis**

In order to confirm that PM<sub>2.5</sub> aggravates UVB-induced damage to keratinocytes, intracellular ROS levels were analyzed. Intracellular ROS levels were increased by UVB and/or PM<sub>2.5</sub>, whereas PG (10 μM) decreased ROS levels induced by UVB and/or PM<sub>2.5</sub> (Fig. 7A). Lipid peroxidation was investigated by DPPP staining. PM<sub>2.5</sub> combined with UVB induced a high degree of lipid peroxidation, outranking UVB irradiation alone, whereas lipid peroxidation induced by UVB and/or PM<sub>2.5</sub> in PG-pretreated cells was considerably lower (Fig. 7B), indicating that PG ameliorated lipid peroxidation induced by these two factors. UVB- and/or PM<sub>2.5</sub>-induced DNA damage was analyzed using the comet assay (Fig. 7C). Compared to UVB, PM<sub>2.5</sub> prolonged comet tails, whereas PG shortened comet tails in cells treated with UVB and/or PM<sub>2.5</sub>. Additionally, Hoechst 33342 staining indicated that PM<sub>2.5</sub> promoted UVB-induced apoptosis, whereas pretreatment with PG partially protected the cells from UVB- and/or PM<sub>2.5</sub>-induced apoptosis (Fig. 7D). Taken together, these results suggest that PG possesses cytoprotective effects against UVB- and/or PM<sub>2.5</sub>-induced oxidative damage and apoptosis.

**DISCUSSION**

Skin, the largest human organ, is susceptible to irritation and sunburns through UV exposure. Moreover, epidemiolog-
cal studies have indicated that UVB suppresses immune reactions, promotes ROS generation, and damages cell membrane proteins and lipids (Boakye et al., 2016). UVB-induced ROS production has also been reported to be a major cause of skin cancer, as it results in the formation of 8-hydroxy-2′-deoxyguanine (Agar et al., 2004). Additionally, PM2.5 (with particle diameter of <2.5 μm) can reach the lungs and stimulate ROS production in the skin, increasing oxidative stress (Piao et al., 2018). High ROS levels disrupt the normal function of endoplasmic reticulum, mitochondria, and lysosomes, leading to apoptosis.

This study evaluated the cytoprotective effects of PG on oxidative stress and apoptosis. Our results show that UVB and PM2.5 increase intracellular ROS production and cause DNA fragmentation, lipid peroxidation, and protein oxidation. In human HaCaT cells, UVB and PM2.5 exposure resulted in dysfunction of mitochondria and a high apoptosis index. Notably, PM2.5 aggravated UVB-induced skin damage (Fig. 7), increasing oxidative stress, damaging DNA, exacerbating lipid peroxidation, and increasing the number of apoptotic bodies. PG pretreatment reduced cellular ROS levels and consequently resulted in fewer apoptotic bodies.

As predominant epidermal cells, keratinocytes protect the organism from external hazards and are inevitably influenced by complex environmental conditions. Apoptotic factors, including caspases-3 and -9, are activated under harmful conditions. Caspase-9 is a necessary factor in initiating apoptosis (Würstle et al., 2012). Furthermore, caspase-9 can directly activate pro-caspase-3 (Yin et al., 2006), also a key factor of apoptosis (Brentnall et al., 2013). In turn, caspase-3 promotes PARP cleavage (Fulda and Debatin, 2006), preventing PARP from countering DNA damage. As an example, PARP-1 is a nuclear enzyme involved in DNA fragmentation stability and regulates transcription (Rajawat et al., 2017). Anti-apoptotic proteins are also important participants of apoptosis. Bax/Bcl-2 proteins contribute to changes in mitochondrial perme-
ability and ΔΨm loss (Dlugosz et al., 2006), which protect cells from apoptosis induced by pro-apoptotic Bax/Bak proteins (Reed, 2006). In this study, UVB increased the protein levels of Bax, cleaved caspase-9, cleaved caspase-3, and cleaved PARP and decreased Bcl-2 levels, contributing to apoptosis. In contrast, PG treatment suppressed the increase in protein levels of Bax, cleaved caspase-9, cleaved caspase-3, and cleaved PARP, while increasing Bcl-2 levels and decreasing the number of apoptotic bodies. In order to further explore these mechanisms, we used caspase inhibitor Z-VAD-FMK, which suppressed apoptosis induced by UVB. PG contributed to anti-apoptotic effects of caspase inhibitors, which suggests that PG protects cells from apoptosis through caspase signaling pathways, by regulating the levels of apoptosis-associated proteins.

Additionally, p38 MAPK was previously shown to degrade Bcl-2 (De Chiara et al., 2006) and activate Bax (Kim et al., 2006), resulting in mitochondrial apoptotic cell death (Lee et al., 2008). Therefore, in the current study, we detected the expression of MAPK signaling pathway-associated proteins, including p38, JNK, and ERK. Phosphorylated p38, JNK, and ERK were up-regulated by UVB-irradiation and down-regulated by PG pretreatment. Effects of MAPK signaling pathways were further explored using p38, JNK, and ERK inhibitors. PG pretreatment inhibited phosphorylation of p38, JNK, and ERK, similar to their inhibitors and contributed to reducing the number of apoptotic bodies.

Taken together, these results show that UVB irradiation and PM2.5 contribute to apoptosis and that PG treatment suppresses UVB-induced ROS generation, DNA damage, mitochondrial dysfunction, and protein oxidation. Furthermore, PG pretreatment inhibited UVB-induced cell apoptosis through caspase and MAPK signaling pathways by regulating key proteins participating in these pathways (Fig. 7E). These results suggest that PG could be of potential use in protecting the skin from UVB irradiation and PM2.5.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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