Pinus densiflora extract protects human skin fibroblasts against UVB-induced photoaging by inhibiting the expression of MMPs and increasing type I procollagen expression

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ABSTRACT

Exposure to ultraviolet (UV) light can cause skin photoaging, which is associated with upregulation of matrix metalloproteinases (MMPs) and downregulation of collagen synthesis. It has been reported that MMPs, especially MMP-1, MMP-3 and MMP-9, decrease the elasticity of the dermis by degrading collagen. In this study, we assessed the effects of Pinus densiflora extract (PDE) on photoaging and investigated its mechanism of action in human skin fibroblast (Hs68) cells after UVB exposure using real-time polymerase chain reaction, Western blot analysis, and enzymatic activity assays. PDE exhibited an antioxidant activity and inhibited elastase activities in vitro. We also found that PDE inhibited UVB-induced cytotoxicity, MMP-1 production and expression of MMP-1, -3 and -9 mRNA in Hs68 cells. In addition, PDE decreased UVB-induced MMP-2 activity and MMP-2 mRNA expression. Moreover, PDE prevented the decrease of type I procollagen mediated by exposure to UVB irradiation, an effect that is linked to the upregulation and downregulation of Smad3 and Smad7, respectively. Another effect of UV irradiation is to stimulate activator protein 1 (AP-1) activity via overexpression of c-Jun/c-Fos, which, in turn, upregulates MMP-1, -3, and -9. In this study, we found that PDE suppressed UV-induced c-Jun and c-Fos mRNA expression. Taken together, these results demonstrate that PDE regulates UVB-induced expression of MMPs and type I procollagen synthesis by inhibiting AP-1 activity and restoring impaired Smad signaling, suggesting that PDE may be useful as an effective anti-photoaging agent.

1. Introduction

The process of skin aging can be divided into chronological (intrinsic) and photoaging (extrinsic). Chronological aging is caused by the aging process and is highly correlated with genetic factors. Photoaging, in contrast, is mainly due to exposure to UV short wavelength light (UVB) and is characterized by severe wrinkling, pigmentary changes and collagen degradation [1,2].

UV irradiation causes marked changes in skin collagenous tissues because of the breakdown of collagen, a major component of the extracellular matrix (ECM), which also includes type I collagen, elastin, proteoglycans, and fibronectin [3–5]. The disarrangement and fragmentation of these proteins by UV irradiation advance the skin aging process. UV irradiation also causes the generation

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of reactive oxygen species (ROS) and up-regulates the mitogen-activated protein kinase (MAPK) cascades, which in turn regulate AP-1 [6]. Increased AP-1 activity induces the expression of MMPs, namely MMP-1, -3, and -9 in human skin [7] and inhibits type-I procollagen [8,9]. The transcriptional activity of AP-1, a heterodimer comprised of c-Fos and c-Jun, is dependent on c-Fos and c-Jun expression levels [8]. UV irradiation also changes transforming growth factor beta (TGF-β)/Smad signaling, which regulates ECM tissue genesis and metabolism through type I collagen production. TGF-β inhibits growth of epidermal keratinocytes and stimulates growth of human dermal fibroblasts [10]. In addition, TGF-β induces synthesis and secretion of major ECM proteins collagen and elastin [11] and inhibits expression of certain enzymes involved in the breakdown of collagen, including MMP-1 and MMP-3. The actions of TGF-β are up-regulated by Smad3 and antagonized by Smad7 [12,13].

Pinus densiflora, which belongs to the family *Pinaceae*, is commonly distributed in East Asian countries such as China and Korea and widely consumed as a dietary supplement or food to promote health [14]. *P. densiflora* is known to contain numerous proanthocyanidins, which are the major polyphenols in red wine and have potent antioxidant activity [15,16]. It has been reported that PDE inhibits both tyrosinase activity and L-DOPA oxidation [17]. However, it is not known whether PDE exerts antiphotoaging effects on human fibroblasts. Therefore, we investigated whether PDE affects UVB-induced expression of MMPs and type I procollagen, as well as activity of elastase in human fibroblast cells, Hs68. We found that PDE protects the cells from UVB-induced photodamage by suppressing the underlying mechanisms of damage.

2. Materials and methods

2.1. Chemicals

Gelatin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Coomassie blue R-250, TritonX-100, isopropanol, methanol, trichloroacetic acid (TCA), ascorbic acid, elastase substrate IV, porcine elastase, HCl, sodium dodecyl sulfate (SDS), Trizma base, acetic acid, ursolic acid and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Fetal bovine serum (FBS), Anti–anti, trypsin-EDTA, and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco (Invitrogen, Carlsbad, CA, USA).

2.2. Preparation of plant extract

The extracts from *P. densiflora* stem bark were purchased from Korea Plant Extract Bank, Korea Research Institute of Bioscience & Biotechnology (Daejon, Korea). According to extraction method of the supplier, the plant material was washed, sliced and completely dried in a hot-air oven at 70 °C. The MeOH solution was filtered and evaporated under vacuum to give a MeOH extract. The plant extract was dissolved in dimethylsulfoxide (DMSO) and used as sample for screening tests.

2.3. Cell culture

Hs68 cells were kindly gifted by Dr. J. K. Hwang (Yonsei University, Seoul, Korea). The cells were grown in DMEM supplemented with 1% Anti–anti and 10% FBS in 5% CO_2 at 37 °C. The cells were subcultured following trypsinization and used for experiments between the 15th and 25th passages.

2.4. UVB irradiation

Hs68 cells were treated with various concentrations (10 or 50 μg/mL) of PDE in serum-free medium for 24 h and then exposed to UVB light (100 mJ/cm^2) with a 312-nm UVB light source (VL-6.LM; Vilber Lourmat, Marne-la-Vallée Cedex 1, France) for 38 s. According to Rigel et al. [18], average daily UVB radiation exposure received by study volunteers (4 high school students at Riverdale Country School, New York, NY) was 8.01 mJ/cm^2/day. In this experiment, we used UVB radiation at 100 mJ/cm^2, which is equivalent to approximately 12.5 days of sun exposure. After UVB irradiation, the cells were cultured in serum-free medium for 24 h.

2.5. Cell viability

Cell viability was determined using the MTT colorimetric assay. Hs68 cells cultured in 6-well plates (4×10^6 cells/well) were treated with various concentrations of PDE for 24 h and exposed to UVB (100 mJ/cm^2), then incubated for 24 h in serum-free medium. Cells were then incubated with 1 mg/mL MTT for 2 h at 37 °C. MTT/formazan was extracted by overnight incubation at 37 °C with 1 mL extraction buffer [10% triton X-100, 89% isopropanol and 11% 1 N HCl] and optical densities at 570 nm were measured.

2.6. Diphenylpicrylhydrazyl (DPPH) radical scavenging activity

Reaction mixtures containing a methanol solution of 200 μM DPPH (100 μL) and serial dilutions of different concentrations of PDE (ranging from 50 to 500 μg/mL) were placed in a 96-well microplate at room temperature in the dark for 30 min. After incubation, the absorbance was read at 517 nm by an ELISA reader (TECAN M200, Salzburg, Austria). Ascorbic acid was measured as a positive control. Scavenging activity was determined by the following equation:

$$\% \, \text{scavenging activity} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100$$

2.7. Measurement of elastase activity

Elastase inhibition was investigated using elastase from porcine pancreas. Elastase (500 U) was dissolved in 5 mL

of 10 mM Tris buffer solution (pH 6.0) and 5 mg elastase substrate IV was dissolved in 5 mL of 100 mM Tris buffer solution (pH 8.0). To measure elastase activity, 100 μL of 100 mM Tris buffer solution (pH 8.0), 25 μL of elastase substrate IV solution, 50 μL of sample solution, and 25 μL of elastase solution were dispensed into each well of a 96-well plate and incubated for 20 min at room temperature. Elastase activity was quantified by measuring light absorbance at 405 nm by a microplate reader (TECAN M200, Salzburg, Austria). Each assay was carried out in triplicate. The inhibition rate of elastase was calculated by the equation:

$$\text{Inhibition}(\%) = \left[1 - \frac{(C - D)}{(A - B)}\right] \times 100$$

where A indicates the absorbance with enzyme but without sample, B indicates the absorbance without enzyme or sample, C indicates the absorbance with enzyme and sample, and D indicates the absorbance without enzyme but with sample.

2.8. Western blotting for MMP-1 and type I procollagen

Cells were harvested and lysed with RIPA lysis buffer (Cell Signaling, Beverly, MA, USA.). Protein concentration in the cells was measured using Bradford reagent (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard. Cell lysates containing equal amounts of total protein were separated by electrophoresis on SDS-polyacrylamide gel and then transferred to a PVDF membrane (Hybond-P, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). The membranes were blocked with 5% nonfat dry milk in PBS-T and subsequently incubated with primary antibodies against MMP-1 and type I procollagen were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The MMP-1 antibody is able to detect a pro form of MMP-1. Specific reactive bands were detected with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz, CA, USA) and the immunoreactive bands were visualized by the enhanced chemiluminescence (ECL) detection system (Millipore Corporation, Bedford, MA, USA).

2.9. Zymography for MMP-2

MMP-2 activity in cell culture medium was evaluated by gelatin zymography. After UVB irradiation, Hs68 cells were cultured for 24 h. The total medium of each well was collected and precipitated with 20% TCA. Equal amounts of each sample were mixed with zymography sample loading buffer (Bio-Rad, Hercules, CA, USA) and separated by electrophoresis through a 10% SDS-polyacrylamide gel containing 0.1% gelatin. The gels were washed with 2.5% Triton X-100 on an orbital shaker for 1 h at room temperature to remove the SDS and incubated in 50 mL reaction buffer (50 mM Tris–HCl, pH 7.5, 10 mM CaCl$_2$ and 0.15 M NaCl) at 37 °C for 24 h. The bands were visualized by staining with 0.1% Coomassie brilliant blue R-250 followed by destaining with 50% methanol-acetic acid in water. The area of light translucent zones over the blue background

was determined by a densitometric program to estimate gelatinase activity

2.10. RNA isolation and cDNA synthesis and real-time quantitative PCR

Total RNA was prepared by an RNeasy Mini kit (QIAGEN) according to the manufacturer's instructions. The integrity of RNA was assessed by an automated microfluidics-based system (Bioanalyzer 2100, Agilent, Palo Alto, CA). First strand cDNA was synthesized with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) and real-time PCR was performed using iCycler iQ Real-Time Detection System (Bio-Rad). PCR reactions were carried out with iQ SYBR Green Supermix (Bio-Rad). Specific primer pairs (Genotech, Daejeon, Korea) are as follows: MMP-1 forward 5′-GCA TAT CGA TGC TGC TCT TTC-3′, MMP-1 reverse 5′-GAT AAC CTG GAT CCA TAG ATC GTT-3′, MMP-2 forward 5′-GGA GGA GAA GGC TGT GTT-3′, MMP-2 reverse 5′-TAA AGG CGG CAT CCA CTC-3′, MMP-3 forward 5′-CAA AAC ATA TTT CTT TGT AGA GGA CAA-3′, MMP-3 reverse 5′-TTC AGC TAT TTG CTT GGG AAA-3′, MMP-9 forward 5′-ATG TAC CCT ATG TAC CGC TTC-3′, MMP-9 reverse 5′-GTG TGG TGG TGG TTG GAG-3′, c-Jun forward 5′-AAT AAC ACA GAG AGA CAG ACT TG-3′, c-Jun reverse 5′-CTT GGA TAC CCT TGG CTT TAG-3′, c-Fos forward 5′-GTG TGT ATT GTT CCC AGT GA-3′, c-Fos reverse 5′-AGT TAA TGC TAT GAG AAG ACT AAG G-3′, Smad3 forward 5′-GGC TGC TCT CCA ATG TCA-3′, Smad3 reverse 5′-CAC TCT GCG AAG ACC TCC-3′, Smad7 forward 5′-TTC CCT CCA AGA AGG ATT TG-3′, Smad7 reverse 5′-ACG AGT GTA TGA GTT GTA GAA G-3′, β-actin forward 5′-GCG AGA AGA TGA CCC AGA T-3′ and β-actin reverse 5′-ATC ACG ATG CCA GTG GTA-3′, the latter two of which were used as an internal control. Amplification of real-time PCR was performed according to the protocols of Jung et al. [37], with modification, Briefly. The reaction was carried out at 95 °C for 3 min and followed by 39 cycles of amplification (95 °C for 10 s, 58 °C for 10 s, 72 °C for 30 s). A melt curve was produced to confirm a single gene-specific peak and detect primer/dimer formation by heating the samples from 65 to 95 °C in 0.5 °C increments with a dwell time at each temperature of 10 s while continuously monitoring fluorescence. The *mRNA* levels of specific genes were normalized to those of β-actin.

2.11. Enzyme-linked immunosorbent assay (ELISA)

Hs68 cells were cultured in a 6-well plate (4×10^6 cells/well) and pretreated with PDE (10 or 50 μg/mL) for 24 h. The cells were washed with PBS, irradiated with UVB (100 mJ/cm^2) through a thin layer of PBS, and then incubated with serum-free DMEM. After 24 h, Pro-MMP-1 in culture medium was quantified by using a human MMP-1 ELISA kit (QIA55; Merck & Co. Inc., Whitehouse Station, NJ, USA) according to the manufacturer's instructions.

2.12. Statistics

Statistical analyses were performed using GraphPad Prism software (version 5.0 GraphPad Software, USA). The significance of differences between groups was evaluated
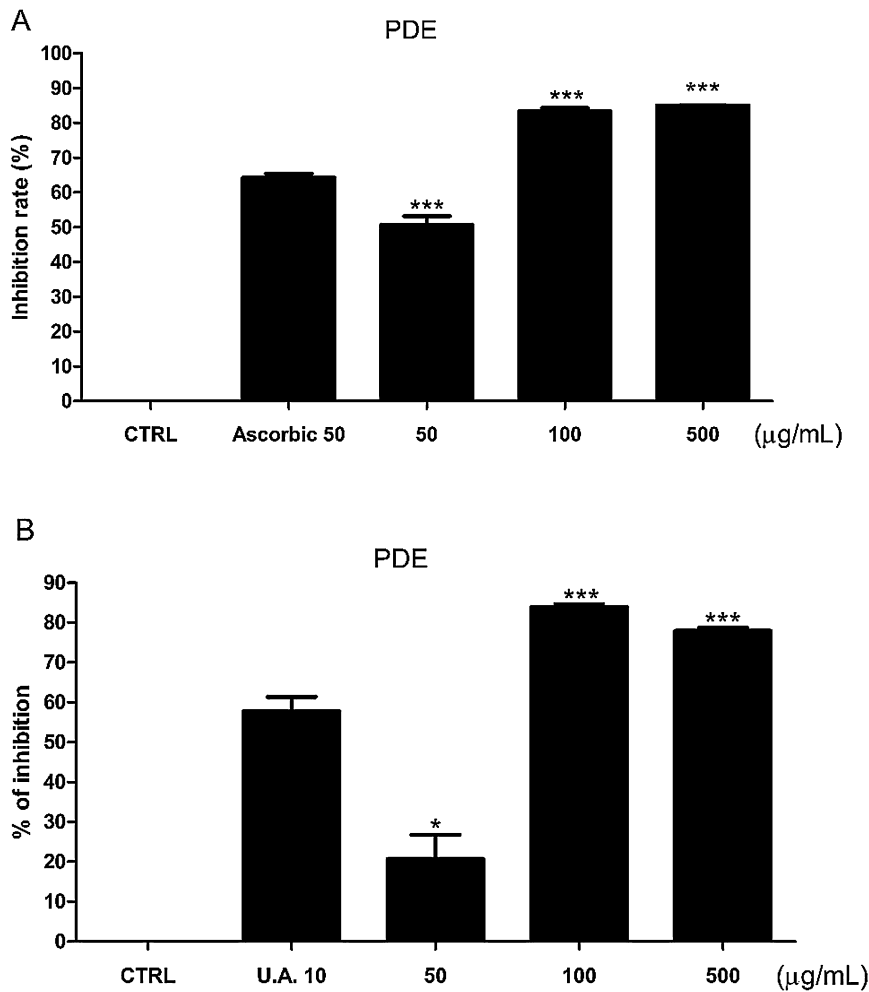
Fig. 1. Antioxidant effect and elastase inhibition of *Pinus densiflora* extracts (PDE). (A) DPPH radical scavenging activity of PDE. (B) The inhibition (%) of porcine elastase activity of PDE. Ursolic acid was used as positive control. (n = 3; significant difference versus CTRL: * <0.01, *** <0.0001) Ascorbic: Ascorbic acid, U.A.: Ursolic acid).

by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test or Student's *t*-test and values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Antioxidant and elastase inhibition effects of PDE

3.1.1. Scavenging of DPPH radicals

The free radical scavenging activity of PDE (50–500 μg/mL) and ascorbic acid (50 μg/mL) is shown in Fig. 1A. PDE induced DPPH radical scavenging activity in a dose-dependent manner.

3.1.2. Effects of PDE on elastase activity

We found that PDE inhibited elastase activity (range: 20.7 ± 10.5% at 50 μg/mL to 77.9 ± 1.5% at 500 μg/mL) and that the inhibitory effect elicited by PDE at 100 μg/mL

(83.9 ± 1.3%) was more significant than that elicited by ursolic acid (positive control) at 10 μM (57.8 ± 8.7%) (Fig. 1B). Elastase inhibitors have anti-wrinkle function, and ursolic acid is one of the most commonly used elastase inhibitors [19].

3.2. Effects of PDE on cell viability in UVB-exposed Hs68 cells

The cytotoxic effects of PDE and UVB on Hs68 cells were measured by MTT assay. Treatment of Hs68 cells with PDE at various concentrations from 50 to 200 μg/mL for 24 h exhibited that cell viability was not significantly reduced (Fig. 2A) at PDE levels up to 200 μg/mL. To examine the cytotoxic effects of UVB, Hs68 cells were exposed to UVB irradiation with various UVB energy sources within a range of 20 to 200 mJ/cm^2. At 24 h after UVB exposure, cell viability was significantly reduced in a dose-dependent manner
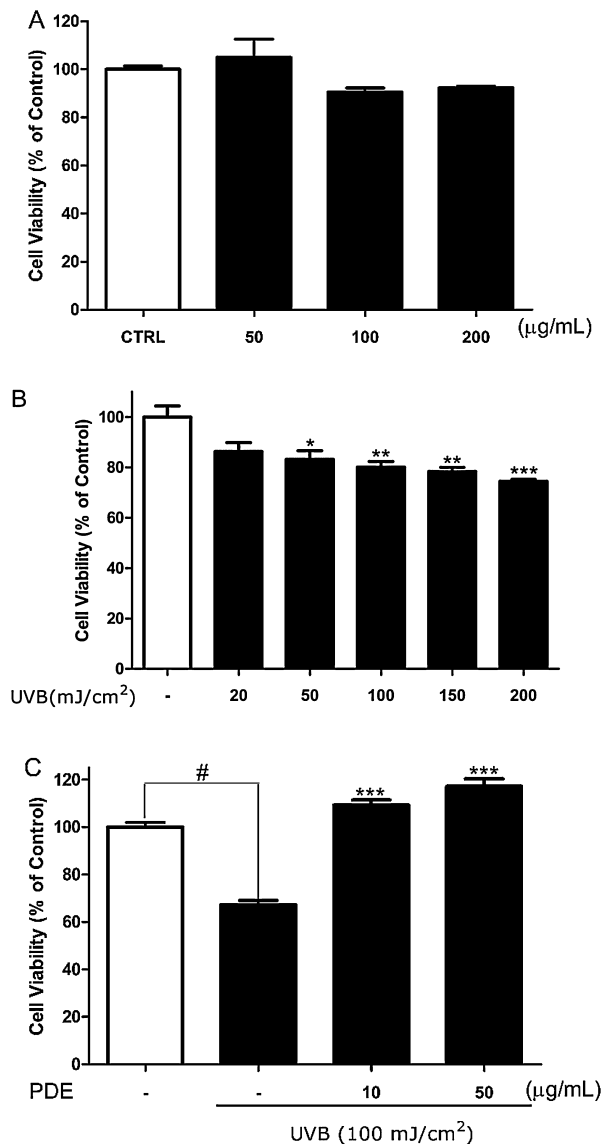
Fig. 2. Effects of PDE on Hs68 cell viability. (A) Hs68 cells were treated with various concentrations of PDE for 24 h and cytotoxicity was determined by MTT assay. (B) Cell viability of Hs68 cells exposed to various UVB irradiation intensities prior to 24 h incubation. (C) Protective effects of PDE against UVB-induced cell death. Hs68 cells were treated with different doses of PDE for 24 h and then the cells were exposed to UVB (100 mJ/cm²) irradiation. After UVB exposure, fresh media was added. At 24 h after UVB irradiation, percent cell viability was assessed by MTT assay. Results are expressed as the mean ± SD (% control) of three independent experiments. (* $P < 0.05$, ** $P < 0.005$ and *** $P < 0.0001$ versus UVB-treated control. Values determined by one-way analysis of variance and subsequently applying Tukey's test; # $P < 0.01$ versus control group by the student's t-test)

(Fig. 2B). The protective effects of PDE against UVB-induced cytotoxicity in Hs68 cells were then investigated. When UVB-exposed Hs68 cells were treated with PDE at various concentrations, cell viability was substantially increased (Fig. 2C).

3.3. Effects of PDE on UVB-induced MMP-1 production, Type I procollagen protein expression and MMPs mRNA expression

To evaluate the effects of PDE on MMP-1 production in UVB-exposed Hs68 cells, cells were exposed to UVB (100 mJ/cm²) and secreted MMP-1 was measured by using an MMP-1 ELISA assay kit. PDE reduced UVB-induced MMP-1 production by 32% at 10 μg/mL and 73% at 50 μg/mL compared with the UV-induced control (Fig. 3A). To investigate the effects of PDE on UVB-mediated expression of MMPs, MMP-1, -3 and -9 *mRNA* levels were measured by real-time PCR. PDE (10 or 50 μg/mL) dramatically attenuated MMP-1, -3 and -9 *mRNA* levels (Fig. 3B–D). Moreover, PDE treatment suppressed UVB-induced up-regulation of MMP-1 protein and increased UVB-induced down-regulation of type I procollagen protein in the cells (Fig. 3E).

3.4. Effects of PDE on UVB-induced MMP-2 secretion and mRNA expression

MMP-2 is a UVB-inducible MMP that plays an important role in photoaging. Fig. 4A and B shows that UVB irradiation led to the activation of MMP-2 in Hs68 cells and that this increase was inhibited by treatment of the cells with PDE. In addition, UVB-mediated induction of MMP-2 *mRNA* levels in Hs68 cells was also decreased by treatment with PDE (Fig. 4C).

3.5. Effects of PDE on UVB-induced expression of c-Jun, c-Fos, Smad3, and Smad7

To determine whether PDE inhibited UVB-induced expression of MMPs in Hs68 cells by blocking AP-1 signaling, we examined the effects of PDE on UVB-induced expression of c-Jun and c-Fos. Fig. 5 shows that UVB irradiation induced expression of c-Jun and c-Fos in Hs68 cells, an effect that was inhibited by PDE treatment (Fig. 5A and B). To determine whether PDE increases the expression of type I procollagen by blocking the effects of UVB irradiation on the TGF-β/Smad pathway, Smad3 and Smad7 *mRNA* levels in UVB-irradiated Hs68 cells were determined by real-time PCR analysis. *mRNA* levels of Smad3 were increased by 1.7-fold at 10 μg/mL and 1.2-fold at 50 μg/mL, meaning that this effect did not occur in a dose dependent manner. Smad7 levels were reduced by 2-fold at 10 μg/mL in PDE-treated cells compared with the UV-irradiated control cells (Fig. 5C, D).

4. Discussion

MMPs play an essential role in the physiological mechanism of skin photoaging induced by UV irradiation. UVB exposure causes connective tissue damage of the skin due to overexpression of MMPs [20]. MMP-1 initially cleaves types I, III, VII, VIII, and X collagens, which are then further degraded by MMP-2 and -9 [21,22]. The gelatinase activity of MMP-2 and -9 play a critical role in the formation of wrinkles by UV irradiation [23], while MMP-3 activates proMMP-1. Therefore, topical MMP inhibitors may
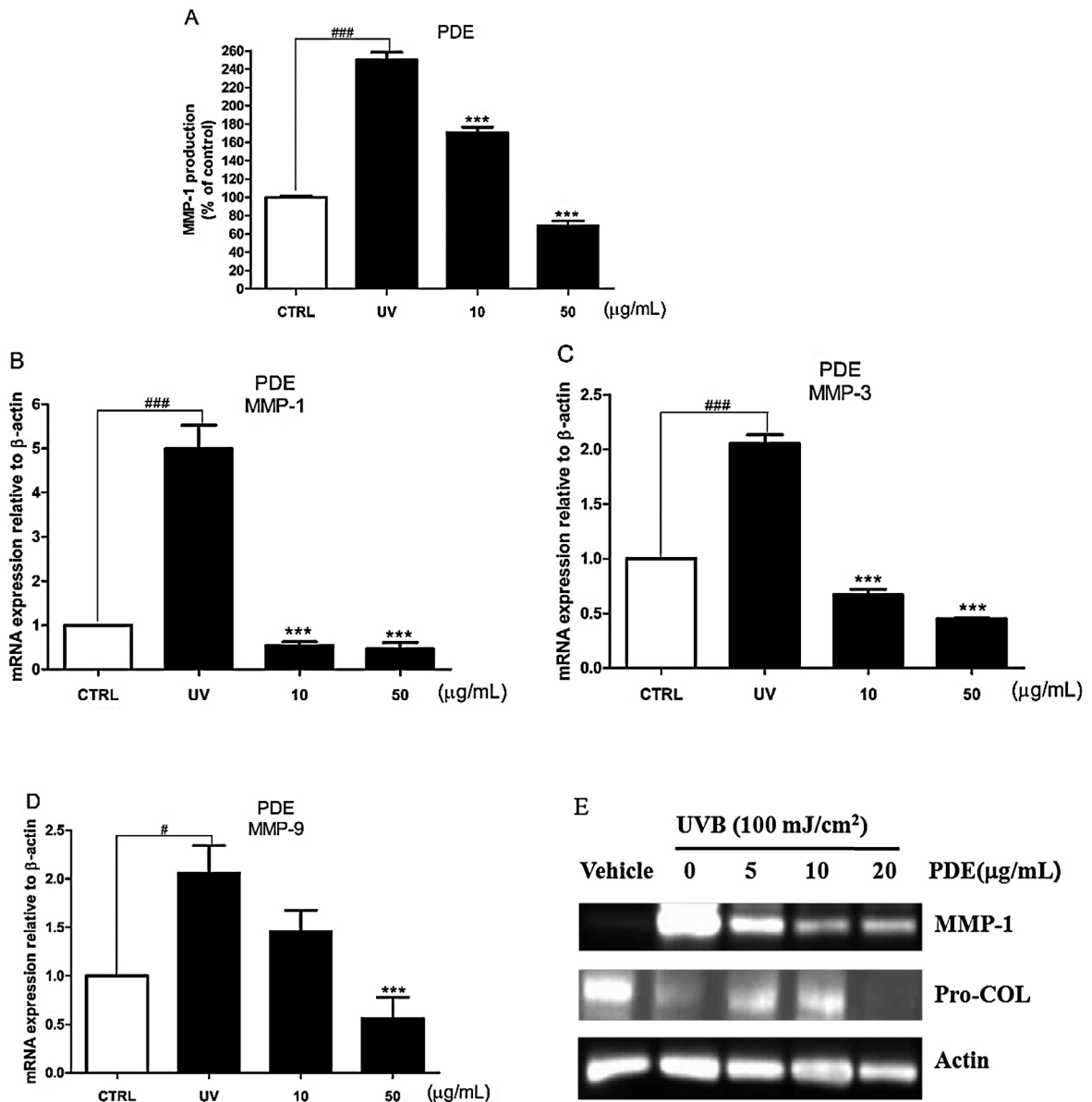
Fig. 3. Effect of PDE on MMP-1 production, MMPs *mRNA* expression and type 1 pro-collagen protein in UVB-induced Hs68 cells. Hs68 cells were treated with various concentrations of PDE for 24 h and then exposed to UVB irradiation (100 mJ/cm^2). At 24 h after UVB exposure, (A) release of MMP-1 into the culture media was determined by ELISA and (B–D) the expression of MMP-1, -3, -9 *mRNA* was measured by real-time PCR. β-Actin was used as an internal control for real-time PCR. (E) The expression of MMP-1 and type 1 pro-collagen (Pro-COL) protein was determined by Western blotting. Data are shown as the mean ± SD of three independent experiments ($^#$ $P < 0.05$ and $^{###}$ $P < 0.001$ versus control group by student's *t*-test; *** $P < 0.0005$ versus UVB-treated control by one-way analysis of variance and subsequently applying Tukey's test).

be effective at preventing UVB-induced formation of wrinkles. In this study, we found that PDE significantly inhibited the expression of MMP-1, -2, -3, and -9 mRNA induced by UVB irradiation (Figs. 3B–D and 4C). In addition, PDE reduced UVB-induced overexpression of MMP-1 protein (Fig. 3A and E) and activation of MMP-2 (Fig. 4A and B). However, PDE does not enhance MMP-9 activity (data not shown). Previous studies have reported that irradiation of human fibroblast cells with UVB causes a decrease in type I procollagen expression, resulting in photoaging of the skin

[24]. In this study, we also found that type I procollagen was downregulated by UVB irradiation in Hs68 cells but recovered when the cells were treated with PDE (Fig. 3E). Taken together, these results suggest that PDE potentially affects anti-photoaging by downregulating MMPs and upregulating collagen synthesis.

Elastin also plays an important role in the ECM of dermis; elastin degradation leads to line and wrinkle formations in the skin [25]. It has been shown that damage to the elastic fiber network in the skin of hairless mice
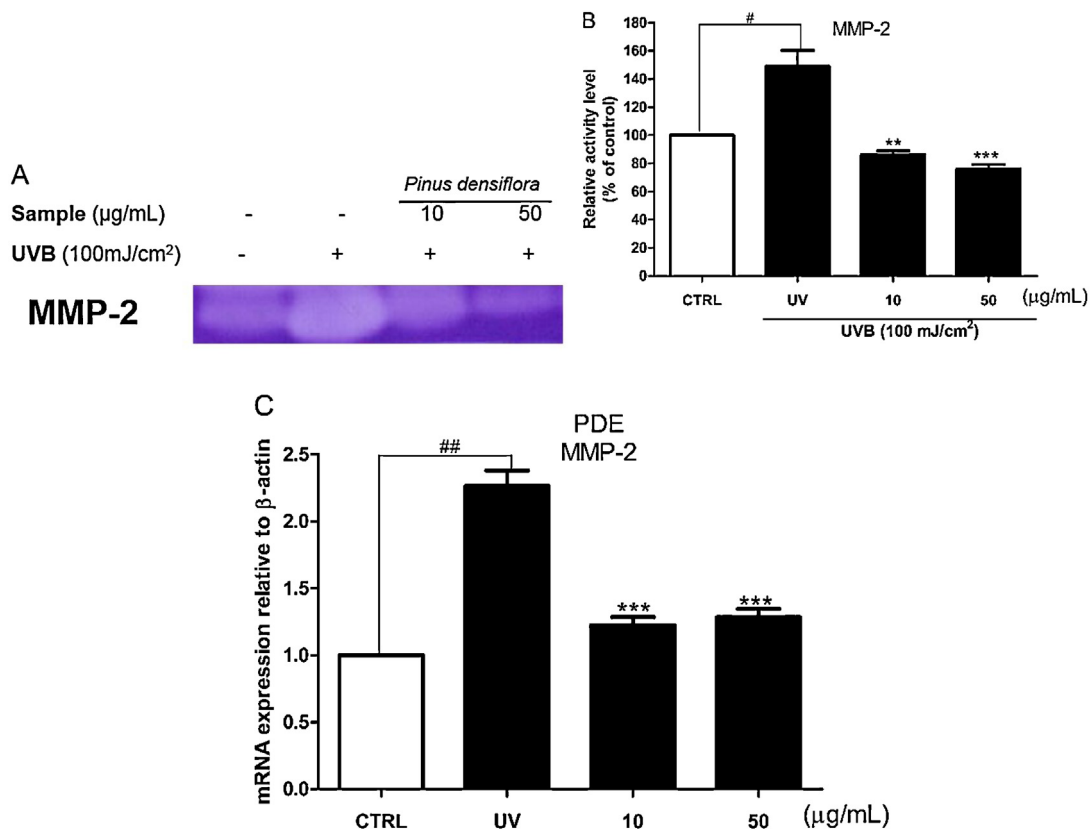
Fig. 4. Effect of PDE on UV-induced expression of MMP-2 in human fibroblasts. (A and B) Zymography assay showing the effect of PDE on MMP-2 activity in the culture medium of human fibroblasts. (C) Quantitative MMP-2 *mRNA* expression was analyzed by real-time PCR. The data represent mean ± SD ([#] $P < 0.05$ and [##] $P < 0.01$ versus control group by student's *t*-test; [**] $P < 0.005$ and [***] $P < 0.001$ versus UVB-treated control by one-way analysis of variance and subsequently applying Tukey's test).

was responsible for wrinkling of UVB-exposed skin [19]. Therefore, agents that inhibit elastase activity are an ideal candidate for the treatment or prevention of skin photoaging. Based on this evidence, we investigated the inhibitory effects of PDE on elastase activity. The results showed that treatment of Hs68 cells with PDE (100 μg/mL) inhibited elastase activity more than treatment with ursolic acid did (Fig. 1B), indicating that PDE prevents photoaging via elastase inactivation.

UV irradiation stimulates cell surface growth factor receptors, cytokines, and mitogen activated protein kinases (MAPKs), which in turn regulate AP-1. Increased AP-1 activity downregulates type I procollagen and upregulates MMP-1. MMP-1 gene expression is regulated by c-Jun and c-Fos, components of the AP-1 heterodimer complex [6,22]. Here, we found that PDE suppressed UV-induced c-Jun and c-Fos mRNA expression (Fig. 5A and B), which may inhibit AP-1 activity and MMP-1 expression. UV irradiation also alters TGF-β/Smad signaling, which modulates ECM metabolism and tissue genesis via the production of type I collagen [12,13]. TGF-β binds to the TGF-β receptor complex, thereby regulating cellular functions. This receptor-ligand binding causes the activation of Smad2 and Smad3, which induces the expression of TGF-β target genes. Smad7 antagonizes TGF-β signaling by

suppressing Smad2 and Smad3. UV irradiation increases Smad7 *mRNA* and protein levels, which consequently impairs TGF-β/Smad signaling [6,22]. Smad3 sends a TGF-β signaling cascade from the cell-surface receptor to the procollagen gene promoter in human dermal fibroblasts [26,27]. In our current study, PDE restored expression of Smad3 and Smad7 in UVB-irradiated fibroblasts (Fig. 5C and D), indicating that PDE restores impaired TGF-β/Smad signaling via regulating the expression of Smad3 and Smad7.

Crude extracts of plant materials rich in phenolic content are of interest to the cosmetic industry because of their activities of anti-oxidation and antiphotoaging by inhibiting the activation of MMPs. Some studies have shown that polyphenol-rich extracts of *C. arabica* [28] and *Terminalia catappa* L. [29] prevented skin cells from UVB-induced photoaging by inhibiting the activation of MMPs. In addition, the polyphenol epigallocatechin-3-gallate (EGCG) in green tea prevents UVB-induced expression of MMP-1, -8, and MMP-13 [30] and that equol from soy inhibits UV-induced expression of MMP-1 and type I procollagen [31,32]. This report is the first to demonstrate that PDE is a potent antioxidant, as shown by its ability to scavenge DPPH free radicals, and inhibits photo-induced expression and secretion of MMPs. According to the previous studies,
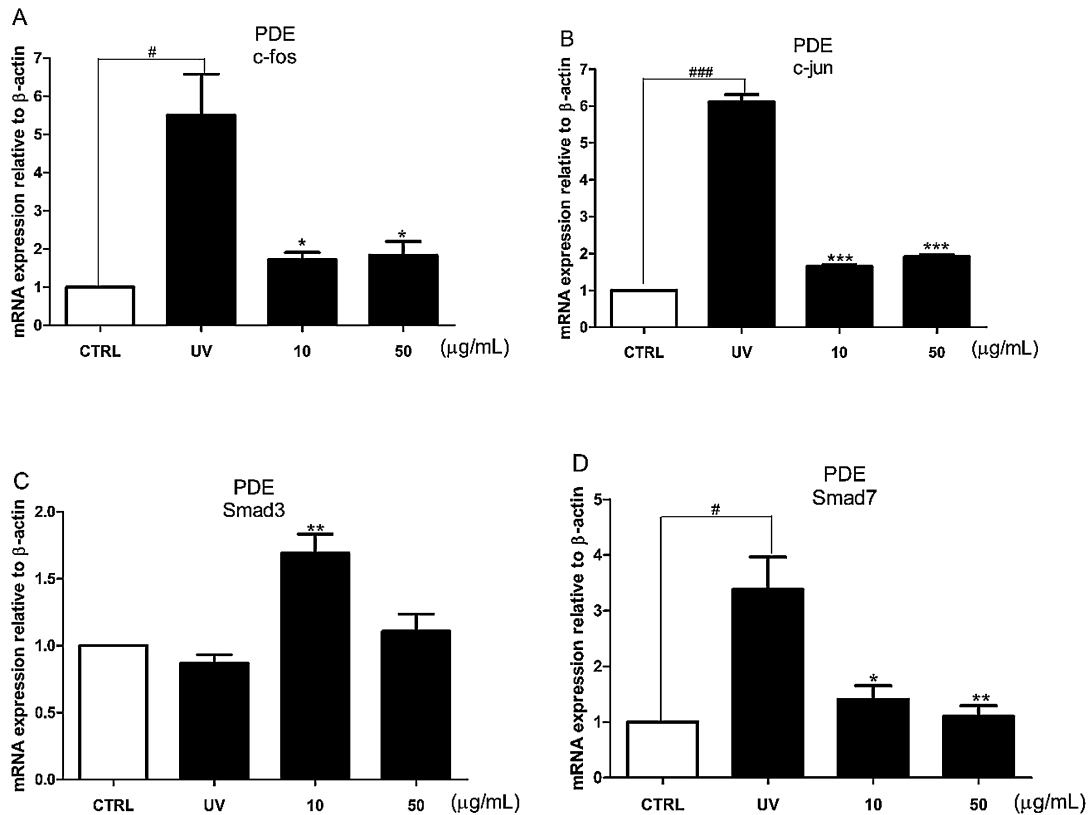
Fig. 5. Effect of PDE on expression of c-Jun, c-Fos, Smad3, and Smad7. Quantitative measures for (A) c-Fos *mRNA* expression, (B) c-Jun *mRNA* expression, (C) Smad3 *mRNA* expression and (D) Smad7 *mRNA* expression in Hs68 cells irradiated with UVB were determined by real-time PCR. The data represent mean ± SD ([#] $P < 0.05$ and [###] $P < 0.005$ versus control group by student's *t*-test; [*] $P < 0.05$, [**] $P < 0.005$ and [***] $P < 0.0001$ versus UVB-treated control by one-way analysis of variance and subsequently applying Tukey's test).

the stem bark of *P. densiflora* contains catechin, which shows the excellent anti-oxidant property and inhibits the activities of elastase and MMPs [33–36], suggesting that catechin might be an important antiphotoaging component in PDE.

Taken together, these findings show that PDE exerts antiphotoaging effects in fibroblasts, thus showing promise as a way to prevent and treat skin photoaging. However, further studies, particularly in animal models, are necessary to explain PDE's anti-aging properties and characterize the underlying mechanisms in vivo.

5. Conclusion

We determine that PDE scavenged DPPH radicals, inhibited elastase activity, altered TGF-β/Smad signaling and attenuated UVB-induced overexpression of MMP-1, 2, 3, and 9, thereby increasing type I procollagen expression. PDE, therefore, could be a promising agent for the prevention of photodamage.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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