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Anti-Apoptotic and Anti-Inflammatory Activities of Edible Fresh Water Algae *Prasiola japonica* in UVB-Irradiated Skin Keratinocytes

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Abstract: Skin is the outer tissue layer and is a barrier protecting the body from various external stresses. The fresh water green edible algae *Prasiola japonica* has antiviral, antimicrobial, and anti-inflammatory properties; however, few studies of its effects on skin-protection have been reported. In this study, *Prasiola japonica* ethanol extract (Pj-EE) was prepared, and its skin-protective properties were investigated in skin keratinocytes. Pj-EE

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inhibited ROS production in UVB-irradiated HaCaT cells without cytotoxicity. Pj-EE also suppressed the apoptotic death of UVB-irradiated HaCaT cells by decreasing the generation of apoptotic bodies and the proteolytic activation of apoptosis caspase-3, -8, and -9. Moreover, Pj-EE downregulated the mRNA expression of the inflammatory gene cyclooxygenase-2 (COX-2), the pro-inflammatory cytokine genes interleukin (IL)-1β, IL-8, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, and the tissue remodeling genes matrix metalloproteinase (MMP)-1, -2, -3, and -9. The Pj-EE-induced anti-inflammatory effect was mediated by suppressing the activation of nuclear factor-kappa B (NF-κB) signaling pathway in the UVB-irradiated HaCaT cells. Taken together, these results suggest that Pj-EE exerts skin-protective effects through anti-oxidant, anti-apoptotic, and anti-inflammatory activities in skin keratinocytes.

Keywords: Green Algae; *Prasiola japonica*; Anti-Oxidant; Anti-Apoptotic Effect; Anti-Inflammatory Effect; Keratinocytes.

Introduction

Skin is the soft, outermost tissue that covers and protects the body from environmental stress including ultraviolet (UV) radiation, harmful chemicals, and infectious microbes, and the failure of skin to function as a barrier causes detrimental skin conditions such as oxidative stress, inflammation, cancer, and aging (D'Orazio *et al.*, 2013; Martinez *et al.*, 2018). Among these detrimental factors, UV radiation is considered one of the most critical risk factors for skin aging because it induces skin damage, wrinkles, and hyperpigmentation (Rittie *et al.*, 2002; Wu *et al.*, 2017). UVB irradiation has the largest effect on the outer layer of the skin and causes various types of skin damage and aging-associated processes such as oxidative stress, cell death, and inflammation (Che *et al.*, 2017; Hong *et al.*, 2018; Jeong *et al.*, 2018). UVB-mediated skin damage and aging are triggered by various biological processes including: (1) the regulation of genes associated with skin tissue remodeling, such as matrix metalloproteinases (MMPs) and collagens, (2) the expression of inflammatory genes such as cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines, (3) the activation of intracellular signaling pathways such as mitogen-associated protein kinases (MAPKs) and activator protein-1 (AP-1), and (4) the proteolytic activation of apoptosis caspases such as caspase-3, -8, and -9 (Sitailo *et al.*, 2002; Darwiche *et al.*, 2005; Kim *et al.*, 2018; Park *et al.*, 2018).

Green algae are the most diverse group of algae, consisting of more than 7,000 species growing in a variety of habitats, including fresh water and marine habitats, and are characterized by chlorophylls a and b as the main photosynthetic pigments. Green algae are considered a rich natural source of pharmaceuticals, nutraceuticals, and cosmeceuticals (Chalamaiah *et al.*, 2018; Saini *et al.*, 2018). The fresh water green algae *Prasiola japonica* contains a variety of constituents including loliolide, glucitol, mannose, mannitol, 1,6-dihydro-6-oxo-3-pyridinecarboxamide, 1-hydroxy-2-propanone, diisopropylamine, and methyl pyrazine (Akoto *et al.*, 2008). Dried green algae have been traditionally used for skin care and nutritional supply for woman in childbed. Several studies have reported that

constituents of *P. japonica*, in particular loliolide, have antiviral, antimicrobial, and anti-inflammatory properties (Cheng *et al.*, 2010; Chung *et al.*, 2016; Sun *et al.*, 2016); however, the effects of *P. japonica* on skin damage and aging are unknown. In this study, *P. japonica* ethanol extract (Pj-EE) was prepared, and the skin-protective effects of Pj-EE and the underlying molecular mechanism were investigated by evaluating its anti-oxidant, anti-apoptotic, and anti-inflammatory activities in UVB-irradiated skin keratinocytes HaCaT cells.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), penicillin, streptomycin, and L-glutamine were purchased from GIBCO (Grand Island, NY, USA). Sodium dodecyl sulfate (SDS), 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), 4′,6-diamidino-2-phenylindole (DAPI), bovine serum albumin (BSA), sodium azide, propidium iodide, and fluorescein isothiocyanate–Annexin V were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylidene fluoride (PVDF) membranes and enhanced chemiluminescence (ECL) reagents were purchased from Bio-Rad (Hercules, CA, USA). Antibodies for Western blotting specific for each target protein were purchased from either Cell Signaling Technology (Beverly, MA, USA) or Santa Cruz Biotechnology (Santa Cruz, CA, USA). TRI reagent® was purchased from Molecular Research Center Inc. (Cincinnati, OH, USA). MuLV reverse transcriptase (RT) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Primers specific for each target for semi-quantitative RT polymerase reaction (PCR) and quantitative real-time PCR were synthesized at Bioneer Inc. (Daejeon, Korea).

Preparation of Pj-EE

Prasiola japonica used in the experiment was supplied by the Prasiola japonica Research Center (Samcheok, Korea). Samples were cut into 2 × 2-cm pieces and then extracted with 70% ethanol at room temperature for 24 h. Samples and solvents were extracted at a ratio of 1:20 (w/v). After completion of the extraction, the filtrate was filtered through 110-nm filter paper (No. 2, Advantec, Toyo Co., Tokyo, Japan), and the filtrate was concentrated using a vacuum concentrator (Eyela New Rotary Vacuum Evaporator, Rikakikai Co., Tokyo, Japan). The concentrated samples were dried using a vacuum freeze dryer (Eyela FD1, Rikakikai Co.), and the yield of the dried samples was measured. The final weight of the extract was 2.752 g (original sample: 44.87 g) with a yield of 6.13%. The dried samples were stored in a −20°C freezer until use.

Cell Culture

HaCaT human keratinocytes (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/ml), streptomycin

(100 mg/ml), and L-glutamine (2 mM) at 37°C in a 5% CO_2 humidified incubator. To maintain fresh cells, the cells were split and given fresh medium two times per week.

Cell Viability Assay

HaCaT cells were either treated with Pj-EE (0–400 μg/ml) for 24 h or irradiated with UVB (30 mJ/cm^3) for 10 s (Bio-Link BLX-312, Vilber Lourmat, France) in the absence or presence of Pj-EE (0–100 μg/ml) and further incubated for 24 h. The cell viability determined using a conventional MTT assay as previously described (Gerlier and Thomasset, 1986). Briefly, 100 μl of HaCaT cells in culture media were mixed with 10 μl of MTT solution (10 mg/ml in PBS, pH 7.4) and incubated at 37°C for 4 h. The mixture was then incubated with a 15% SDS solution at 37°C for 24 h. The absorbance of the culture media was measured at 570 nm using a SpectraMax 250 microplate reader.

DAPI Staining

HaCaT cells were treated with Pj-EE (0–200 μg/ml) for 30 min and irradiated with UVB (30 mJ/cm^2). The cells were washed twice with PBS and fixed with 1 mL of 3.7% paraformaldehyde in PBS for 10 min. The cells were washed twice with PBS, stained with DAPI reagent (1 μl/ml) for 30 min, and then washed twice with PBS. The cells were then covered with a cover slip on a rectangular glass slide using mounting solution and left to dry at room temperature for 24 h. Samples were visualized using a Nikon Eclipse Ti fluorescence microscope (Nikon, Tokyo, Japan).

ROS Generation Assay

The ROS level was determined using H2DCFDA, as described previously (Szychowski *et al.*, 2016). Briefly, HaCaT cells were pre-treated with Pj-EE (0–200 μg/ml) for 30 min then irradiated with UVB (30 mJ/cm^2) and incubated with H_2DCFDA (10 μM) at 37°C for 30 min. The cells were then washed three times with PBS, and the fluorescence was detected using a fluorescent microscope. The nucleus of cells were also stained with DAPI.

Flow Cytometry Analysis

Apoptosis of HaCaT cells was analyzed using flow cytometry. HaCaT cells were irradiated with UVB (30 mJ/cm^2) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 30 min. The cells were then washed twice with cold PBS and resuspended in 1× binding buffer (1% BSA, 0.1% sodium azide). Next, 100 μl of cell suspension (10^5 cells) was mixed with 10 μl of H_2DCFDA (10 μM) for ROS determination or 10 μl of propidium iodide and 5 μl of fluorescein isothiocyanate–Annexin V for apoptotic cell analysis, and the cells were incubated for 15 min at room temperature in the dark. Finally, 400 μL of 1× binding buffer was added, and the fluorescence was assessed using a Guava easyCyte flow cytometer (Millipore, Billerica, MA, USA).

Western Blot Analysis

HaCaT cells were irradiated with UVB (30 mJ/cm^2) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 24 h. Whole cell lysates were prepared as previously described (Yi *et al.*, 2014a), subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto polyvinylidene fluoride membranes. Pro- and cleaved forms of caspase-3, -8, and -9, as well as the total and phosphorylated forms of IκBβ, p65, and p50, were detected using antibodies specific for each target and visualized using ECL reagents.

Semi-Quantitative RT-PCR and Quantitative Real-Time PCR

HaCaT cells irradiated with UVB (30 mJ/cm^2) were treated with Pj-EE (0–200 μg/ml) for 6 h. Total RNA was isolated from the cells using TRI reagent® according to the manufacturer's instructions, and cDNA was synthesized using 1 μg of total RNA and MuLV RT

Table 1. Primer Sequences Used in This Study

Name		Sequences (5′ to 3′)
		Semi-quantitative RT-PCR
MMP-1	F	CCCAGCGACTCTAGAAACACA
	R	CTGCTTGACCCTCAGAGACC
MMP-9	F	GCCACTTGTCGGCGATAAGG
	R	CACTGTCCACCCCTCAGAGC
COX-2	F	CAAAAGCTGGGAAGCCTTCT
	R	CCATCCTTCAAAAGGCGCAG
IL-1β	F	CCGACCACCACTACAGCAAG
	R	GGGCAGGGAACCAGCATCTT
IL-8	F	AAGGTGCAGTTTTGCCAAGG
	R	CAACCCTCTGCACCCAGTTT
GAPDH	F	CACTCACGGCAAATTCAACGGCAC
	R	GACTCCACGACATACTCAGCAC
		Quantitative real-time PCR
MMP-2	F	GTCCCTACCGAGTCTCTTCT
	R	TTTTTAAGTTTCCGCTTCTG
MMP-3	F	GGTTGGACCTACAAGGAGGC
	R	GGTTCATGCTGGTGTCCTCA
IL-6	F	TACCCCCAGGAGAAGATTCC
	R	TTTTCTGCCAGTGCCTCTTT
TNF-α	F	GAAAGCATGATCCGGGACGT
	R	GATGGCAGAGAGGAGGTTGA
IFN-γ	F	CCAACGCAAAGCAATACATGA
	R	CCTTTTTCGCTTCCCTGTTTTA
GAPDH	F	CAATGAATACGGCTACAGCAAC
	R	AGGGAGATGCTCAGTGTTGG

according to the manufacturer's instructions. Semi-quantitative RT-PCR and quantitative real-time PCR were conducted using primers specific for each target as previously described (Baek *et al.*, 2016; Yang *et al.*, 2017), and the primer sequences used in this study are listed in Table 1.

Statistical Analysis

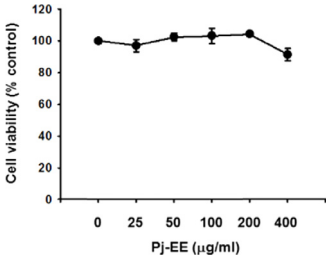
All results are expressed as the mean ± standard deviation (SD) of at least three independent experiments. For the statistical analyses, the data were compared between experimental groups using the Mann–Whitney test or analysis of variance. $P < 0.05$ was considered statistically significant, and the statistical comparisons were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

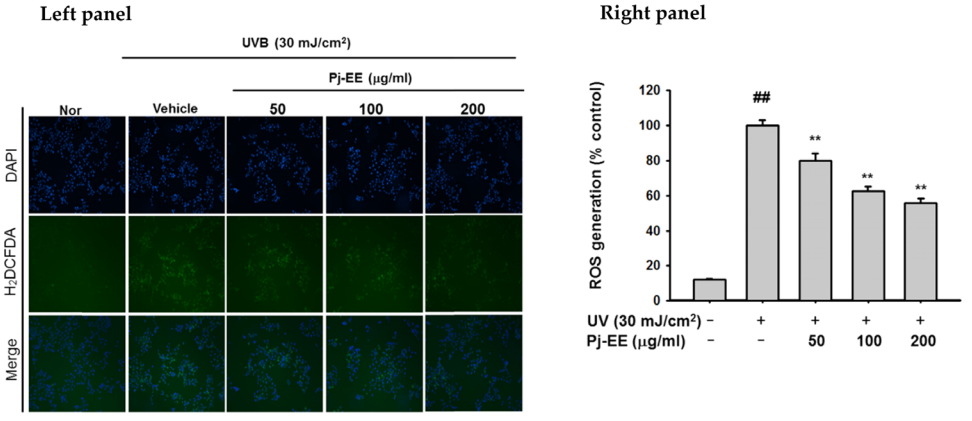
Anti-oxidant Effects of Pj-EE

UV radiation triggers the damage of cutaneous tissues by activating several deleterious factors including oxidative stress, apoptotic cell death, and skin aging (Amaro-Ortiz *et al.*, 2014); therefore, many efforts have been made to develop agents to prevent UV-induced skin damage and aging. In this study, Pj-EE was prepared, and its skin-protective effects were explored in skin keratinocyte HaCaT cells exposed to UVB.

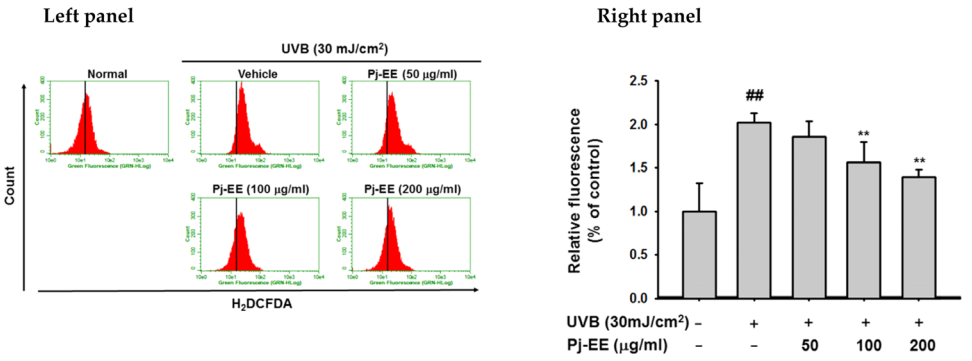
Even if agents have pharmacological activity, they are not able to be used as therapeutic drugs if they are cytotoxic. Therefore, the cytotoxic effects of Pj-EE in skin keratinocytes were first examined using a conventional MTT assay. Pj-EE did not show notoal cytotoxicity at concentrations from 25 μg/ml to 400 μg/ml (Fig. 1A). Although Pj-EE did not exhibit statistically significant cytotoxicity in HaCaT cells up to 400 μg/ml, the cell viability was slightly decreased at 400 μg/ml Pj-EE. Therefore, Pj-EE-mediated anti-oxidant effects were evaluated at doses up to 200 μg/ml. Oxidative stress in skin keratinocytes is a critical factor that induces skin damage and causes aging (Rinnerthaler *et al.*, 2015; Lephart, 2016), and UV radiation is the leading deleterious factor that induces oxidative stress in skin keratinocytes (Panich *et al.*, 2016). Oxidative stress is induced by an imbalance between reactive oxygen species (ROS) generation and the anti-oxidant systems that suppress the generation of ROS; hence, the generation of ROS is a key factor in the induction of oxidative stress (Al-Gubory *et al.*, 2010; Kozina *et al.*, 2012). Therefore, the anti-oxidant effects of Pj-EE were investigated in UVB-irradiated skin keratinocyte HaCaT cells through ROS assays. Oxidative stress was induced in the HaCaT cells by irradiating the cells with UVB (30 mJ/cm^3), and the ROS level was determined using H_2DCFDA (Szychowski *et al.*, 2016). Pj-EE inhibited ROS generation in the UVB-irradiated HaCaT cells as assessed by confocal microscopy (Fig. 1B Left and Right panels) and flowcytometry (Fig. 1C Left and Right panel) in a dose-dependent manner. These results confirm a previous study that showed Pj-EE protects against oxidative in HaCaT cells by scavenging



(A)



(B)



(C)

Figure 1. Anti-oxidant effects of Pj-EE in HaCaT cells. (A) HaCaT cells were treated with the indicated concentration of Pj-EE (0–400 μg/ml) for 24 h, and the cell viability was determined using a conventional MTT assay. (B and C) HaCaT cells treated with the indicated concentration of Pj-EE (0–200 μg/ml) for 30 min were irradiated with UVB (30 mJ/cm^3) and incubated with H_2DCFDA (10 μM) and DAPI (1 μM) for 30 min. The fluorescence of the cells was visualized using a fluorescent microscope (B Left panel) or flowcytometric analysis (C Left panel) and their quantitative results were measured by ImageJ (Right panels). $^{\#\#}P < 0.05$ compared with the untreated group and $^{**}P < 0.01$ compared with the vehicle control.

the radical cation ABTS (Park *et al.*, 2018) and suggest that Pj-EE has an anti-oxidant effect in skin keratinocytes that are damaged by UVB radiation.

Anti-Apoptotic Effects of Pj-EE

Oxidative stress induces apoptotic cell death (Kagan *et al.*, 2002; Sinha *et al.*, 2013; Elswefy *et al.*, 2016). Because Pj-EE exhibited anti-oxidant effects in UVB-irradiated HaCaT cells (Fig. 1), the anti-apoptotic effects of Pj-EE was also investigated in HaCaT cells. Pj-EE significantly protected against UVB-induced apoptotic death of HaCaT cells in a dose-dependent manner (Fig. 2A). Apoptotic bodies are generated in cells following UVB exposure (Han *et al.*, 2015); therefore, the anti-apoptotic effects of Pj-EE were further examined by quantifying the apoptotic bodies generated by UVB irradiation in HaCaT cells. As expected, Pj-EE markedly reduced the number of apoptotic bodies in UVB-irradiated HaCaT cells in a dose-dependent manner (Fig. 2B left and right panels). This anti-apoptotic effect of Pj-EE was also confirmed using flow cytometry analysis (Fig. 2C). These results strongly indicate that Pj-EE has a protective activity against the UVB-induced apoptotic death of skin keratinocytes.

Caspases are a group of proteases that play a critical role in programmed cell death, including apoptosis, necrosis, and pyroptosis, and in inflammatory responses (Li and Yuan, 2008; Shalini *et al.*, 2015). Among these caspases, caspase-8 and -9 initiate apoptosis, and caspase-3 executes apoptosis (Li and Yuan, 2008; McIlwain *et al.*, 2015; Shalini *et al.*, 2015; McArthur and Kile, 2018). Therefore, the molecular mechanism of Pj-EE's anti-apoptotic effects was investigated in HaCaT cells by examining the proteolytic cleavage of apoptosis-related caspases. Both the expression of the pro-forms of caspase-3, -8, and -9 and the proteolysis-mediated activation of these caspases were induced in UVB-irradiated HaCaT cells, and Pj-EE reduced the proteolytic activation of these caspases (Fig. 2D). These results suggest that Pj-EE anti-apoptotic effects in UVB-damaged skin keratinocytes by suppressing the activation of apoptotic caspases such as caspase-3, -8, and 9.

Anti-Inflammatory Effects of Pj-EE

Inflammation is an innate immune response to protect the body from invading pathogens and intracellular stress signals. However, chronic inflammation, defined as repeated and long-term inflammation, is considered a major risk factor in inducing tissue damage (Janeway and Medzhitov, 2002; Yi *et al.*, 2016, 2018), and inflammation is a hallmark of oxidative stress (Hussain *et al.*, 2016; McGarry *et al.*, 2018). In cutaneous tissues, inflammation causes remodeling of the tissue architecture and skin damage, leading to aging (Kumari and Pasparakis, 2017; Ferrucci *et al.*, 2018; Zhang and Duan, 2018). Therefore, the anti-inflammatory effects of Pj-EE were investigated. In HaCaT cells irradiated with UVB to induce inflammation (Hruza and Pentland, 1993; Ryser *et al.*, 2014; Lee *et al.*, 2017), the mRNA expression of the inflammatory enzyme COX-2 and the pro-inflammatory cytokines IL-1β and IL-8 was upregulated (Fig. 3A), which was markedly decreased by Pj-EE. Moreover, Pj-EE significantly inhibited mRNA expression of other

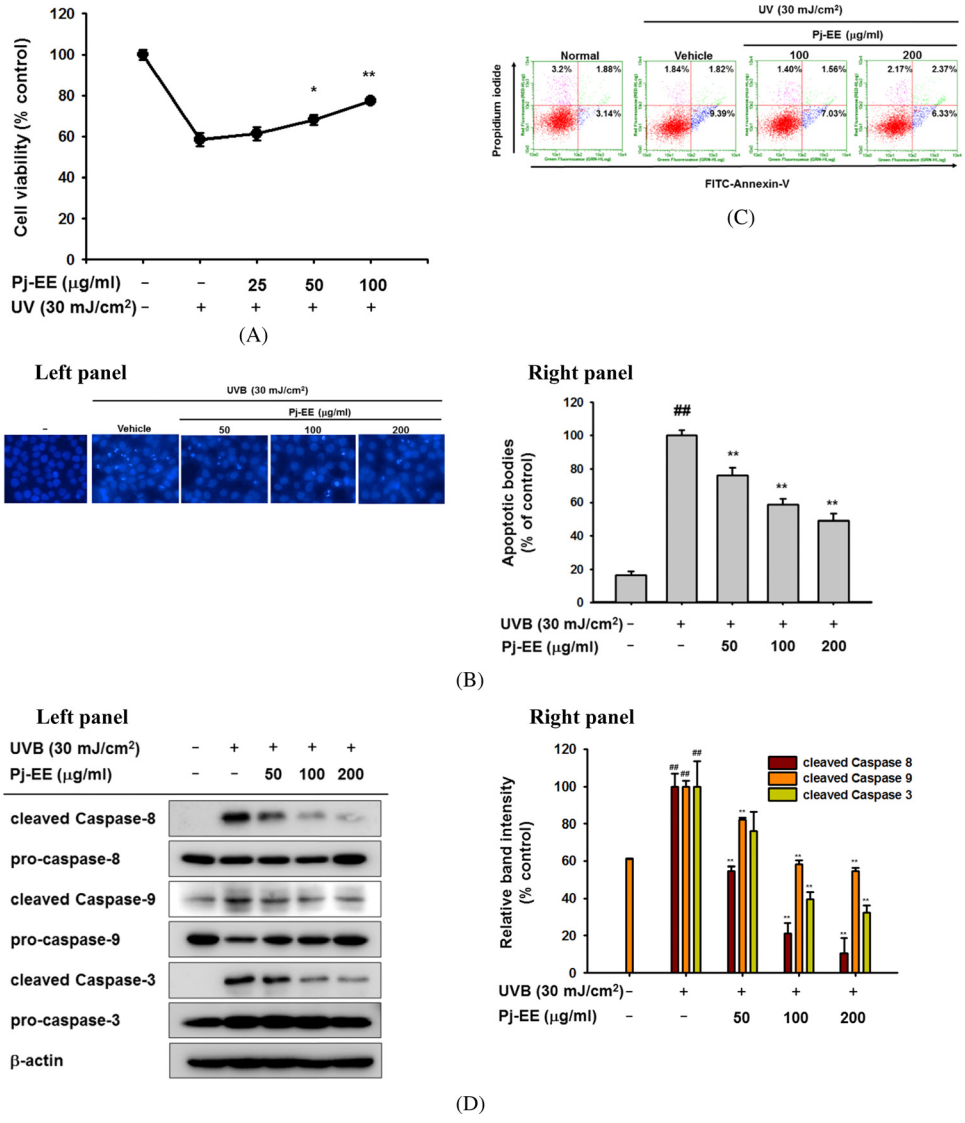


Figure 2. Anti-apoptotic effects of Pj-EE in HaCaT cells. (A) HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–100 μg/ml) and further incubated for 24 h. The cell viability was determined using a conventional MTT assay. (B) HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 30 min. Apoptotic bodies were imaged using a fluorescent microscopy (Left panel) and quantified (Right panel). (C) HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 30 min. Apoptotic cell death was determined using flow cytometry analysis. (D) HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 24 h. The pro- and cleaved forms of caspase-3, -8, and -9 were detected using Western blot analysis with β-actin as an internal control (Left panel). Quantification of band intensity was measured by Image J (Right panel). $^{\#\#}P < 0.05$ compared with the normal group and $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle control.

types of pro-inflammatory cytokines, such as IL-6, TNF-α, and IFN-γ in a dose-dependent manner (Figs. 3B–3D). COX-2 induces an inflammatory response by producing the inflammatory mediator PGE_2 (Ricciotti and FitzGerald, 2011; Norregaard *et al.*, 2015), and IL-1β, IL-6, TNF-α, and IFN-γ are the most critical pro-inflammatory cytokines produced by cells during the inflammatory responses (Schuerwegh *et al.*, 2003; Wojdasiewicz *et al.*, 2014; Yi *et al.*, 2017). Moreover, IL-8 is the cytokine overexpressed in active psoriatic dermatitis (Grossman *et al.*, 1989; Sauder *et al.*, 1990) and is involved in the pathogenesis of atopic dermatitis (Kimata and Lindley, 1994). Therefore, Pj-EE-mediated inhibition of COX-2, IL-1β, IL-8, IL-6, TNF-α, and IFN-γ mRNA expression strongly indicates that Pj-EE suppresses the inflammatory responses induced in skin keratinocytes by UVB radiation.

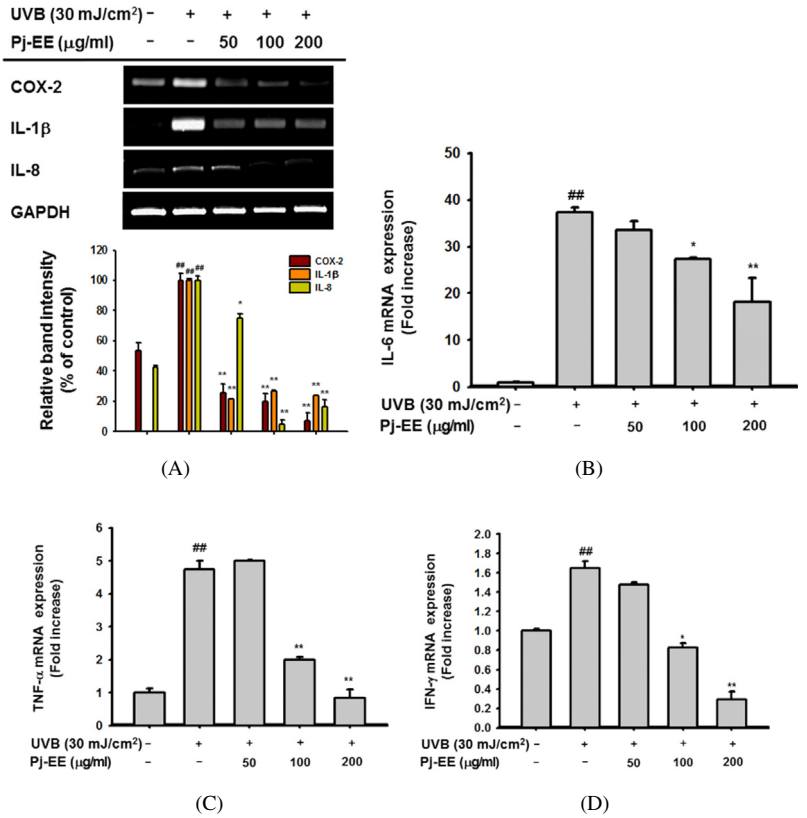


Figure 3. Anti-inflammatory effects of Pj-EE in HaCaT cells. HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 6 h. mRNA expression of COX-2, IL-1β, and IL-8 was determined using semi-quantitative RT-PCR (A), and mRNA expression of IL-6 (B), TNF-α (C), and IFN-γ (D) was determined using quantitative real-time PCR. mRNA expression of MMP-1 and MMP-9 was determined using semi-quantitative RT-PCR (E), and mRNA expression of MMP-2 (F) and MMP-3 (G) was determined using quantitative real-time PCR. GAPDH was used as an internal control. Quantification of band intensity was measured by Image J (E bottom panel). $^{\#\#}P < 0.05$ compared with the normal group and $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle control.

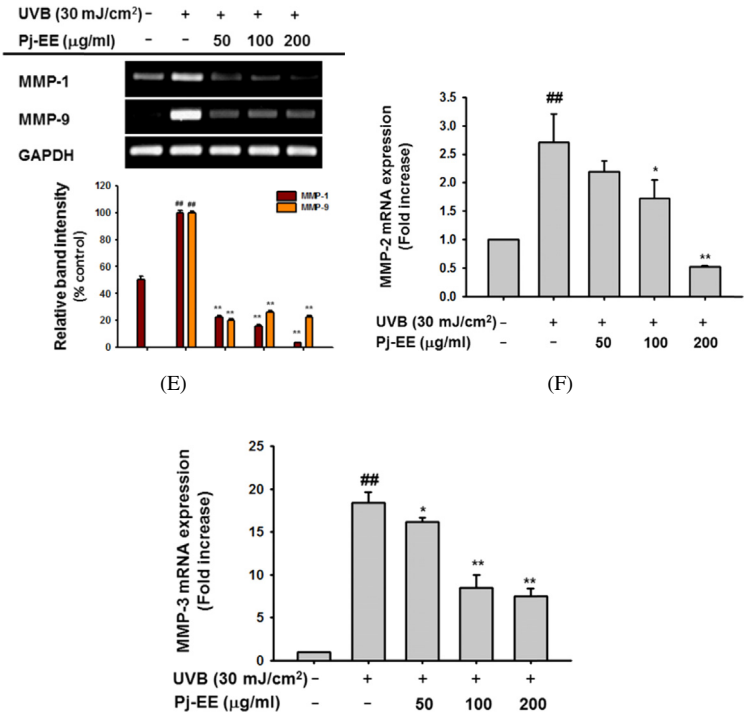


Figure 3. (*Continued*)

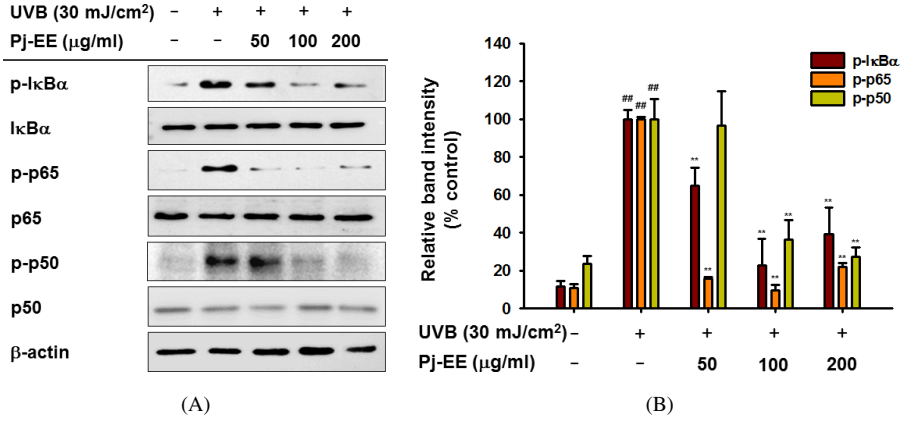


Figure 4. Anti-inflammatory effects of Pj-EE through suppression the NF-κB signaling pathway in HaCaT cells. (A) HaCaT cells were irradiated with UVB (30 mJ/cm^3) for 10 s in the absence or presence of Pj-EE (0–200 μg/ml) and further incubated for 24 h. The total and phosphorylated forms of IκBα, p65, and p50 proteins in the whole cell lysates were visualized using Western blot analysis with β-actin as an internal control. (B) Quantification of band intensity was measured by Image J. $^{\#\#}P < 0.05$ compared with the normal group and $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle control.

Matrix metalloproteinases (MMPs) are a group of endopeptidases responsible for degrading extracellular matrix proteins and bioactive molecules, and expression of MMP-1, -2, -3, and -9 are upregulated in the UVB-induced inflammatory response of skin keratinocytes (Oh *et al.*, 2013; Chung *et al.*, 2018; Hong *et al.*, 2018; Lee *et al.*, 2018). The mRNA expression of MMP-1, -2, -3, and -9 were increased in UVB-irradiated HaCaT cells, which was dramatically decreased by Pj-EE (Figs. 3E–3G), indicating that Pj-EE inhibits inflammatory responses by suppressing mRNA expression of inflammatory MMPs in UVB-irradiated HaCaT cells. Taken together, these results strongly suggest that Pj-EE has anti-inflammatory effects by suppressing mRNA expression of inflammatory enzymes, pro-inflammatory cytokines, and inflammatory MMPs in UVB-irradiated skin keratinocytes.

Anti-Inflammatory Effects of Pj-EE through Suppression of the NF-κB Signaling Pathway

Several signaling pathways are activated during inflammatory responses, of which the NF-κB signaling pathway is one of the most critical (Byeon *et al.*, 2012; Yu *et al.*, 2012; Yang *et al.*, 2014; Yi *et al.*, 2014b). Moreover, the NF-κB signaling pathway is activated in skin keratinocytes during UVB-induced inflammatory responses (Li *et al.*, 2018; Nan *et al.*, 2018). Therefore, the effects of Pj-EE on the activation of the NF-κB signaling pathway during UVB-induced inflammatory responses were investigated. Phosphorylation of IκBα and the NF-κB transcription factors p65 and p50 was increased in UVB-irradiated HaCaT cells (Figs. 4A and 4B), which was markedly reduced by Pj-EE. However, this extract did not suppress NF-κB activity under normal or overexpression conditions with MyD88 and TRIF, which are adaptor molecules mediating TLR4 signaling cascade, as assessed by NF-κB luciferase assay (data not shown). This suggests that Pj-EE has an anti-inflammatory effect by inhibiting the activation of the inflammatory NF-κB signaling pathway during only UVB-induced inflammatory responses in skin keratinocytes.

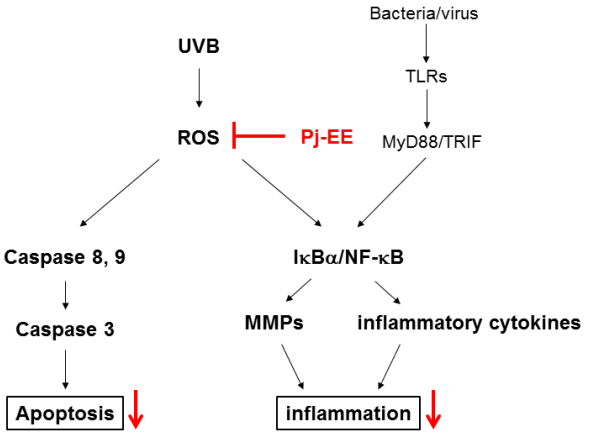


Figure 5. Putative inhibitory pathway of Pj-EE's anti-apoptotic and anti-inflammatory activities.

Conclusion

In this study, we explored the skin-protective effects of Pj-EE in skin keratinocyte HaCaT cells. Pj-EE ameliorated the oxidative stress induced in HaCaT cells by UVB radiation, which not only prevented apoptotic death by inhibiting the activation of apoptosis caspases but also reduced inflammatory responses by suppressing the expression of MMPs and inflammatory genes via deactivation of the NF-κB signaling pathway, as summarized in Fig. 5. In conclusion, our findings demonstrate that Pj-EE has skin-protective effects by suppressing oxidative stress, apoptotic cell death, and inflammatory responses in UVB-irradiated skin keratinocytes and also provides insight to developing promising remedies for skin diseases and aging.

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