Dietary *Foeniculum vulgare* Mill extract attenuated UVB irradiation-induced skin photoaging by activating of Nrf2 and inhibiting MAPK pathways

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Background: *Foeniculum vulgare* Mill (FV) has long been prescribed in traditional medicine due to its antioxidant anti-inflammatory properties. However, little research has been done on the use of FV to alleviate changes in UVB-induced photoaging

Purpose: This study was to investigate the photoprotective effects and mechanism of FV in vitro and in vivo.

Methods: The anti-photoaging effect of FV was assessed in normal human dermal fibroblasts (NHDFs) in vitro. The secretion of reactive oxygen species (ROS), lactate dehydrogenase (LDH), GSH, matrix metalloproteinases (MMPs), procollagen type I, IL-6 and transforming growth factor-β1 (TGF-β1) were measured by kits. Additionally, the level of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), p-ERK and p38 were evaluated by western blotting. In vivo, H&E and Masson’s trichrome staining were employed. The expression of MMP-1, procollagen type I, TGF-β1 and elastin were measured by western blot.

Results: FV significantly increased the production of collagen, elastin and TGF-β1 levels, while blocked matrix metalloproteinases (MMPs) production in UVB irradiation induced hairless mice, which were consistent with the result in NHDFs. Furthermore, FV dose-dependently decreased the production of ROS and LDH by promoting the nuclear amount of Nrf2 and enhancing the expression of cytoprotective antioxidants such as GSH. FV also significantly quenched UVB-induced phosphorylation of ERK and p38 in NHDFs.

Conclusion: Our results indicate that FV is a potential botanical agent for the treatment of skin damage induced by UV irradiation.

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1. Introduction

Ultraviolet (UV) light, consisting of UVA (320–400 nm), UVB (280–320 nm) and UVC (100–280 nm), is one of the most common factors leading to DNA damage and inflammatory responses, consequently resulting in various cutaneous lesions such as photoaging and photocarcinogenesis (Scharffetter–Kochanek et al., 2000). Chronic exposure to UV damages the integrity of the extracellular matrix (ECM) in skin tissues, which is responsible for skin wrinkling, laxity, dryness and thickness (Uitto, 2008). ECM alterations were observed in aged skin along with abnormal matrix metalloproteinase (MMP) production (Dong et al., 2008). MMPs, which are zinc-dependent endopeptidases, play a pivotal role in collagen degradation in the ECM, which provides structural support to the dermis (Fineschi et al., 2007).

DNA damage and oxidative stress biomarkers are considered as key biological events of UV irradiation in human skin cells. Accumulation of ROS could regulate the expression of redox sensitive genes and transcription factors (McMillan et al., 2008). Investigations have shown that ROS may upregulate the oxidative stress marker, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Hseu et al., 2012). On stimulation, Nrf2 translocates into the nucleus...
and binds to the antioxidant response element (ARE), initiating the expression of detoxification enzymes and antioxidants, such as NAD(P)H quinone oxidoreductase-1 (NQO-1) and glutathione (Kaspar et al., 2009). The antioxidant enzymes are essential for protecting skin from oxidative stress induced skin damages. Therefore, increasing the antioxidant capacity of skin cells by antioxidant supplements could be a valuable strategy for preventing UVB-induced oxidative stress and skin damage.

UV induced ROS in turn stimulates many cascades, including mitogen-activated protein kinases (MAPKs) and activated protein 1 (AP-1). This ultimately accelerates MMP secretion and collagen fragmentation (Ba et al., 2008). Thus, antioxidants that scavenge and quench ROS have been proposed as photoprotective agents. The antioxidant gallic acid inhibits UV-induced human skin aging through MAPK signaling pathways (Hwang et al., 2014a). UV stimulates the secretion of pro-inflammatory mediators and cytokines, such as nuclear factor-κB (NF-κB) and IL-6, resulting in MMP overexpression (Pillai et al., 2005). On the other hand, transforming growth factor-β1 (TGF-β1) is a major regulator of procollagen type I synthesis (Quan et al., 2004). Accordingly, increased MMP-1 and decreased procollagen type I are the most notable features of UV-induced photaged skin. Thus, agents that decrease the production of MMP-1 and increase the synthesis of procollagen type I are potential candidates for the treatment of skin photocaging.

Applications of botanical agents in skin care products have recently increased due to their antioxidant anti-inflammatory properties (Afaq and Mukhtar, 2006). Foeniculum vulgare Mill (FV) has long been prescribed in traditional medicine. A DPPH radical scavenging assay on Foeniculum vulgare Mill seed extracts indicated that Foeniculum vulgare Mill had antioxidant activity (Goswami and Chatterjee, 2014). Oral administration of a methanolic extract of Foeniculum vulgare Mill fruit reduced the malondialdehyde level, suggesting that this extract possessed anti-inflammatory effects in animals (Kang et al., 2013). However, little research has been done on the use of FV to alleviate changes in UVB-induced photocaging in vitro and in vivo. In this study, we evaluated the protective effect of FV extract against UVB-induced photocaging in normal human fibroblasts and hairless mice (HR-1). Our results showed that FV inhibited MMP production and collagen degradation in vitro and in vivo, suggesting that FV may be useful in protecting against skin damage caused by UV irradiation.

2. Materials and methods

2.1. Chemicals

ELISA kits for MMP-1, IL-6, and TGF-β1 were purchased from R&D Systems (R&D Systems, Inc., Minneapolis, MN, USA), while ELISA kits for procollagen type I were purchased from Takara (Takara, Shiga, Japan). The LDH level was measured using an LDH cytotoxicity assay kit (Roche Diagnostics, Mannheim, Germany). Total GSH content was determined using a GSH assay kit (Cayman Chemical Co, Ann Arbor, MI). DMEM, FBS and penicillin streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). FV was purchased from mountain rose herbs company (Eugene, USA). Unless otherwise mentioned, solvents were purchased from Samchun Chemicals (Seoul, Korea), and inorganic salts were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

The dried seeds of Foeniculum vulgare Mill (10 g) were powdered and extracted three times with 1000 ml of ethyl alcohol (50%) for 24 h at room temperature. The ethyl alcohol (50%) was filtered and subjected to vacuum evaporation at 38°C.

2.3. HPLC analysis

High performance liquid chromatography (HPLC) was performed on a Dionex Chromelon TM chromatography data system with P580 and UVD100 detectors (Thermo Fisher Scientific Inc., Waltham, MA, USA). Chromatographic separation was performed on a Waters Sunfire C18 column (250 × 4.6 mm, 5-μm particle size). Elution was performed with a methanol/acetonitrile (3:1) gradient containing 1% formic acid. The gradient was linearly increased from 10% to 90% methanol over 35 min. The injection volume was 10 μl and the flow rate was 1 ml/min.

2.4. Cell culture, UVB irradiation and sample treatment

Normal human dermal fibroblasts (MCTT Core, Inc., Seoul, Korea) were cultured with DMEM (supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin). The cells were maintained at 37 in a humidified atmosphere containing 5% CO2. Then, sub-confluent cells were subjected to irradiation supplied by a Bio-Link BLX-312 (Vilber Lourmat GmbH, France) with 144 mJ/cm2 for NHDFs. Experimental group cells were treated with FV (1, 10 and 100 μg/ml; complete dissolve in DMSO), control cells were treated with vehicle. Normal cells were treated with the same dose of vehicle without UVB exposure. After 72 h, supernatants were harvested to assess the production of MMP-1, MMP-3, TGF-β1 and IL-6. In the case of RT-PCR, cells were harvested 24 h after UVB irradiation. No more than 10 cell passages were used in the experiments.

2.5. MTT assay

Cell viability tests were performed as described previously (Hwang et al., 2014a). In brief, MTT (100 μg/ml) was added and incubated for another 2 h after 72 h of sample treatment, then DMSO was added in order to dissolve the formazan crystals. The absorbance was read at 570 nm using a microplate reader (Molecular Devices E09090; San Francisco, CA, USA).

2.6. Measurement of ROS scavenging ability

Following UVB (144 mJ/cm2) irradiation and sample treatment for 24 h, NHDFs were stained with 30 μM 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich) for 30 min at 37°C. The cells were then rinsed twice with PBS and subjected to a multi-mode microplate reader (Molecular Devices Filter Max F5; Sunnyvale, CA, USA).

2.7. Monitoring of lactate dehydrogenase (LDH) release

The LDH level in the culture medium was measured using an LDH cytotoxicity assay kit (Roche Diagnostics, Mannheim, Germany). This method is based on the LDH-catalyzed reduction of pyruvate to lactate by NADH. Briefly, equal amounts of culture supernatant were mixed with fresh LDH buffer containing NADH. After a 30 min incubation at room temperature, the absorbance was measured with an ELISA microplate reader at 490 nm.

2.8. Determination of intracellular GSH

Total GSH content in the culture medium was determined using a GSH assay kit (Cayman Chemical Co, Ann Arbor, MI). The kit uses an enzymatic recycling method involving glutathione reductase to quantify GSH. The sulfhydryl group of GSH reacts with DTNB (5, 5′-dithiobis-2-nitrobenzoic acid, Ellman’s reagent) and produced a yellow-colored compound, 5-thio-2-nitrobenzoic acid (TNB). The
absorbance was measured with at 405 nm. The rate of TNB production indicates the concentration of GSH in the sample based on the standard curve provided with the assay kit.

2.9. Preparation of cytosolic and nuclear extracts

After exposure to UVB, NHDFs were treated with FV (10 and 100μg/ml) for 2 h. The cytosolic and nuclear portions of the cells were separated with a commercial kit (NE-PER nuclear and cytoplasmic extraction reagents; Pierce).

2.10. Measurement of LDH, GSH, MMP-1, MMP-3, IL-6, procollagen type I and TGF-β1

We measured the concentrations of LDH, GSH, MMP-1, MMP-3, IL-6, procollagen type I and TGF-β1 in the medium with commercially available kits according to the manufacturer’s instructions. Each sample was analyzed in triplicate.

2.11. Reverse transcription (RT)-PCR

At the indicated time, RNA from UVB-irradiated NHDF cells was isolated using Trizol reagent according to the manufacturer’s instructions (Invitrogen Life Technologies, Carlsbad, CA). RT-PCR were performed in a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA) according to our previous research (Hwang et al., 2014a). PCR products were stained with ethidium bromide and separated by 2.0% agarose gel. Each experiment was repeated at least three times.

2.12. Animal UV irradiation and sample treatment

The animal experimental protocol for this study [KHUASP(SU)-12-09] was approved by the Institutional Animal Care and Use Committee of Kyung Hee University. Seven-week-old male albino hairless mice (HR-1) (20–27 g) were obtained from Central Lab Animals, Inc. (Seoul, Korea). UVB irradiation was performed as described previously (Hwang et al., 2014a). Hairless mice were randomly divided into 4 groups of 5 mice per cage: Normal (without UVB irradiation); Control (UVB irradiation); FV 0.1% (UVB irradiation + 0.1% FV); FV 1% (UVB irradiation + 1% FV). Hairless mice underwent oral administration for 10 weeks and body weight was measured once a week (Table 1 and 2).

2.13. Wrinkle measurement

Light-bodied silicone (SilfloR, Flexico, Colchester, UK) was introduced to obtain replicas of the dorsal skin. The visiometer technique was introduced to detect changes in the transparency of thin silicone replicas. Photos were obtained with a CCD video camera and analyzed using Skin Viscometer SV 600 software (Courage & Khazaka, Cologne, Germany). The R1, R2, R3, R4, and R5 values were obtained as previously described (Hwang et al., 2014a).

2.14. Histological analysis

Biopsies (dorsal skin) were obtained, fixed in 4% paraformaldehyde, dehydrated in ethanol and then embedded in paraffin. Approximately 10-μm-thick sections were deparaffinized and stained with hematoxylin-eosin (H&E) and Masson’s trichrome staining. Stained slides were then photographed using a light microscope (ZEISS Observer D2, Germany).

2.15. Western blot analysis

Total cell lysates were assayed by Western blot according to our previous research (Hwang et al., 2014a). The antibodies Nrf2, Histone, ERK, phosphor-ERK, JNK, phosphor-JNK, p38, phosphor-p38, anti-rabbit-HRP, anti-goat-HRP and anti-mouse-HRP were from Cell Signaling Technology (Danvers, USA), and β-actin, MMP-1, procollagen type I, TGF-β1 and elastin were purchased from Santa Cruz Biotechnology (Dallas, USA). Each experiment was repeated at least three times.

2.16. Statistical analysis

The data are presented as means ± SD values of three independent experiments in triplicate. Statistical analysis was performed using One-way ANOVA test. Statistical significance was set at p < 0.05.

3. Results

3.1. Analysis of extracts from FV

To prepare FV samples, we extracted the dried seeds of FV (10 g) with 50% ethyl alcohol, obtaining crude product (1.28 g) with a 12.8% yield. As shown in Fig. 1, we detected the main compounds chlorogenic acid (44.1%), ferulic acid (36.1%) and rutin (12.4%), which comprised about 92.6% of the FV extract.

3.2. Inhibition of wrinkle formation and collagen digestion in UVB-exposed hairless mouse skin

Skin wrinkle formation was measured in hairless mice chronically exposed to UVB radiation for 8 weeks. Integumentary images and cutaneous characteristics of wrinkles were taken and analyzed by digital and CCD optical camera (Fig. 2). Long-term UVB irradiation accelerated wrinkle formation in mouse skin, rendering the skin rough and scaly (Fig. 2), which is indicative of skin phototoxing. When we administrated FV (0.1% and 1%) to hairless mice, wrinkle formation was noticeably attenuated and dorsal skin attributes improved.

H&E stained slides were photographed (ZEISS Observer D2, Germany) and skin epidermal thickness was measured (Axio Vision Rel.4.8). As shown in Fig. 3, the epidermal thickness of the dorsal skin increased 56.5% due to chronic UVB exposure, compared with that of the normal group. Topical application of FV (0.1% and 1%) substantially decreased the epidermal thickness of the dorsal skin, which was 27.8% and 25.6%, respectively, of the thickness of the UVB-irradiated control group. Therefore, topical treatment with FV diminished roughness and wrinkle formation caused by chronic UVB irradiation.

Histological staining with Masson-trichrome for dermal collagen fibers showed that chronic UVB radiation damaged cellular
Fig. 1. HPLC results. (A) FV seed extract; (B) Chlorogenic acid standard; (C) Ferulic acid standard; (D) Rutin standard.
Table 2
Body weight gain, food intake, and food efficiency ratio.

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal</th>
<th>UVB</th>
<th>FV 0.1%</th>
<th>FV 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g)</td>
<td>3.05 ± 1.46</td>
<td>2.95 ± 1.33</td>
<td>2.75 ± 1.32</td>
<td>4.75 ± 2.32</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>153.56 ± 3.20</td>
<td>156.70 ± 4.50</td>
<td>158.72 ± 7.10</td>
<td>179.00 ± 6.24</td>
</tr>
<tr>
<td>FER (%)</td>
<td>1.99 ± 1.42</td>
<td>1.89 ± 1.26</td>
<td>1.73 ± 1.38</td>
<td>2.65 ± 1.68</td>
</tr>
</tbody>
</table>

Normal: control diet only; UVB: UVB irradiation + control diet; FV 0.1%: UVB irradiation + diet containing 0.1% FV; FV 1%: UVB irradiation + diet containing 1% FV.

FER (food efficiency rate) = gain of body weight (g)/amount of food intake (g) × 100.

Fig. 2. Wrinkle measurement and analysis of skin replicas. Photographs of replicas: normal (without sample treatment), control (UVB irradiation without sample treatment), FV 0.1% (UVB irradiation with 0.1% of FV) and FV 1% (UVB irradiation with 1% of FV). Arbitrary units (R1 – R5) were assigned to each sample based on depth measurements of the furrows according to shadow size and brightness: skin toughness (R1), longest of the five distances (R2), average maximum distance (R1) derived from each of the five parts of the line (R3), mean area surrounded by a horizontal line drawn at the highest crest and furrow profile (R4), and mean deviation of the furrow profile from the middle line (R5). All data are shown as the mean ± SD of at least three independent experiments performed in triplicate. # p < 0.05, ## p < 0.01, compared to the control; * p < 0.05, compared to the group receiving only UVB radiation.
collagen density in the dermis (Fig. 4). In contrast, there was heavier staining of collagen fibers in FV (0.1% and 1%) treated hairless mice exposed to UVB, compared with that of the UVB-irradiated control group (Fig. 4), indicating that FV promoted the production of collagen.

3.3. Suppression of MMP-1 production and increased production of procollagen type I, elastin and TGF-β1 in UVB exposed hairless mouse skin

As the key structural proteins of ECM, procollagen type I and elastin were degraded by MMP-1 and stimulated by TGF-β1. In our study, UVB irradiation caused an 87.6% increase of MMP-1 in the UVB-irradiated group. During FV administration (0.1% and 1%), the MMP-1 level was alleviated in a dose-dependent manner (34.2% and 72.6% respectively) compared with that of the UVB-irradiated control group (Fig. 5).

Conversely, decreased procollagen type I caused by UVB was reinstated by 63.6% and 136.4% with 0.1% and 1% FV, respectively. The decrease in TGF-β1 was also significantly restored by topical application of FV (1%), which increased TGF-β1 by 132.6% compared with that of the UVB-irradiated control group. Similarly, UVB reduced elastin by 69.2%, and 1% FV increased the impaired elastin by 93.3% compared with that of the UVB-irradiated group.

All in vivo data indicated that FV was a good botanical agent using protecting skin from UVB induced photoaging. Furthermore, we explored the mechanism of FV acted in vitro.

3.4. Toxicity analysis of FV extract on NHDFs

MTT analysis was performed to investigate the cytotoxic effects of FV on UVB-irradiated NHDFs. Exposure of cells to UVB caused damage, but this was not statistically significant. FV was not cytotoxic to cells treated with the indicated concentrations (1, 10 and 100 μg/ml) (Fig. 6(A)).
3.5. Cytoprotective effects of FV on UVB-irradiated NHDFs

It is well known that UV induces ROS, causing oxidative stress, which is harmful to cells. NHDFs exposure to UVB showed significant increase of ROS generation, whereas this trend was reduced by 35.9% when treated with FV (100 μg/ml) in UVB-induced cells (Fig. 6(B)). Besides, exposure of NHDFs to UVB markedly elevated the release of LDH, whereas FV (1, 10, 100 μg/ml) treatment reduced LDH release in a dose-dependent manner (Fig. 6(C)). Moreover, due to UVB irradiation, the intracellular GSH was remarkably depleted, whereas FV treatment restored GSH in a dose-dependent manner. Specifically, FV (100 μg/ml) restored GSH level almost close to the control values (Fig. 6(D)).

3.6. Promotion of Nrf2 nuclear translocation in UVB-irradiated NHDFs

To assess the FV-mediated nuclear translocation of Nrf2, both cytosolic and nuclear Nrf2 proteins were assessed in UVB-irradiated NHDFs. As shown, the nuclear amount of Nrf2 was significantly increased by UVB stimulation. Simultaneously, FV promoted the expression of Nrf2 in a dose dependent manner in nucleus (Fig. 7).

3.7. Inhibition of MMPs and IL-6 production and promotion of procollagen type I and TGF-β1 in UVB-irradiated NHDFs

MMP is a major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin (Hwang et al., 2014a). As shown in Fig. 8, UVB led to an abnormal increase in MMP-1 and MMP-3 secretion. When treated with FV extract, this trend was remarkably altered. Specifically, FV quenched MMP-1 secretion by 26.4% at 100 μg/ml, and suppressed MMP-3 production by 55.6% at 100 μg/ml in NHDFs (Fig. 8(A) and (B)). At the same time, UVB irradiation also resulted in severe irritation of the pro-inflammatory cytokine IL-6. In contrast, treatment with FV significantly calmed the activation of IL-6 protein, which showed an inhibition rate of 53.8% at 100 μg/ml (Fig. 8(D)).

This study attempted to confirm whether FV restored the UVB-induced collagen and TGF-β1 breakdown in NHDFs. As expected, FV dose-dependently enhanced the secretion of procollagen type I and TGF-β1, which were dampened by UVB irradiation. Specifically, FV promoted the production of procollagen type I and TGF-β1 by 191.7% and 394.5%, respectively, at 100 μg/ml when compared with UVB-irradiated control cells (Fig. 8(C) and (E)).

3.8. Blocking the mRNA expression of MMP-1 and enhancing procollagen type I levels in UVB-irradiated NHDFs

We measured MMP-1 and procollagen type I mRNA levels in UVB-induced NHDFs. As shown in Fig. 9, the low basal mRNA level of MMP-1 in quiescent fibroblasts was elevated due to UVB stimulation. Consistent with the ELISA results (Fig. 8), MMP-1 transcript expression was inhibited during FV exposure when compared with that of UVB-irradiated control cells (Fig. 9). Conversely, the mRNA level of procollagen type I was blocked in the UVB-induced NHDFs. FV treatment increased the expression of procollagen type I by 37.6% at 100 μg/ml, compared with that of UVB-irradiated control cells.

3.9. Alleviation of MAPK signaling pathway phosphorylation in UVB-irradiated NHDFs

The MAPK signaling pathway was shown to play an important role in regulating the expression of MMP-1 (Hwang et al., 2014a). To investigate the underlying mechanism of FV’s action, we assessed the phosphorylation rate of the MAPKs by western blotting. As shown in Fig. 10, UVB dramatically initiated the phosphorylation of ERK (p-ERK) and p38 (p-p38) and FV quenched UVB-induced p-ERK and p-p38. Specifically, FV (100 μg/ml) inhibited 58.4% of p-ERK and 36.8% of p-p38 in UVB-irradiated NHDFs.
Fig. 5. Protein expression of MMP-1, procollagen type I, TGF-β1 and elastin in the dorsal skin of hairless mice. Signal intensities were quantified and normalized to the corresponding value of β-actin. Values are reported as mean ± SD #p < 0.05 versus normal control, ∗ p < 0.05 versus UVB-irradiated control. All data are shown as the mean ± SD of at least three independent experiments performed in triplicate. # p < 0.05, ## p < 0.01, compared to the control; ∗ p < 0.05, ∗∗ p < 0.01, ∗∗∗ p < 0.001, compared to the group receiving only UVB radiation.

Fig. 6. Effects of FV on cell viability in NHDFs. NHDFs were irradiated or non-irradiated with 144 mJ/cm², followed by treatment with the indicated concentrations of FV (1, 10 and 100 μg/ml) for 72 h. (A) Cell viability; (B) ROS production; (C) LDH release; (D) GSH secretion. All data are shown as the mean ± SD of at least three independent experiments performed in triplicate. # p < 0.05, compared to the control; ∗ p < 0.05, compared to the group receiving only UVB radiation.
4. Discussion

Pharmacological research indicates that solar UV irradiation plays a key role in skin carcinogenesis and photoaging. Chronic solar UV irradiation results in oxidative stress, inflammation, direct and ROS-mediated DNA damage, and dysregulation of cellular signaling pathways, all of which promote skin cancer (Afaq et al., 2005). Polyphenolic compounds that possess antioxidant properties are the most potential for therapeutic agents for a variety of diseases. FV has been widely used in traditional folkloric medicine. In this study, three polyphenols, including chlorogenic acid, ferulic acid and rutin, were identified as the predominant active compounds in FV. Chlorogenic acid were reported to prevent photoaging by suppressing MMP expression through MAP kinase pathway (Chiang et al., 2011). There are also reports about ferulic acid and rutin on inhibition of UVB-induced matrix metalloproteinases in mouse skin (Staniforth et al., 2012), (Hwang et al., 2014b). Consistent with these reports, FV exhibited good protective effect against UV-induced skin damage in hairless mice (Fig. 2, 3 and 4). It should be noted that these polyphenolic compounds are largely broken down by bowel flora and absorbed as smaller phenolic molecules (Senger et al., 2016), which are capable of both directly scavenging free radical and indirectly increasing endogenous cellular antioxidant defenses via activation of the Nrf2 transcriptional pathways, as well as potentially via multiple other mechanisms (Satoh et al., 2014). However, the activation of Nrf2 pathway represents a major mechanism of action for FV, particularly in the skin photoaging.

Histological and ultrastructural studies showed that enhanced epidermal thickness and alterations of connective tissue organization are common features in photodamaged skin (Cho et al., 2007). Collagen and elastin are mainly responsible for dermal strength and resiliency (Fisher, 2005). UV radiation initiates the activation of MMPs, which attack and degrade collagen and elastin, resulting in skin photoaging (Quan et al., 2009). The inhibition of MMPs is thought to be an effective strategy for the prevention of UV-induced photoaging. In this study, UVB radiation accelerated collagen degradation in NHDFs and hairless mice via activated collagenolytic MMP-1. FV (1%) treatment quenched the production of MMP-1 by 72.6%, whereas FV (1%) restored collagen and elastin protein loss by 136.4% and 69.2% respectively in mouse skin (Fig. 5). On this basis, FV executed its photoprotective effects not only through inhibition of MMP-1 expression, but also via promotion of structure related proteins in the ECM. Our results supported that FV could be used in the preparation of skin care food to repair and regenerate the essential proteins in photoaged skin.

UV irradiation induces MMPs and pro-inflammatory cytokines in NHDFs. Cytokines such as IL-6 accelerated the secretion of MMP-1 (Hwang et al., 2013). On the other hand, TGF-β1 promoted procollagen type I synthesis. Moreover, IL-6 restrained the TGF-β1 pathway by inducing MMP-1 production in dermal fibroblasts (Luckett and Gallicco, 2007) and impairing procollagen type I synthesis, thereby leading to collagen loss in the dermis (Zhong et al., 2011). Thus, prevention of inflammation using anti-inflammatory compounds appears to be one strategy for ameliorating photoaging. Our results showed that UVB irradiation provoked the
secretion of IL-6 in NHDFs, which subsided after FV treatment (Fig. 8(D)). Meanwhile, FV promoted the expression of TGF-β1 (Fig. 8(E)). It is possible that FV elevated the level of procollagen type I partly by enhancing TGF-β1 pathway; the underlying mechanisms and principles are unclear and require further study.

ROS was reported to be related to MMP production and collagen fragmentation induced by UVB in fibroblasts (Bae et al., 2008). Therefore, control of ROS levels appears to be a primary mechanism by which botanical compounds with antioxidative activity could be particularly effective. Ethanol extracts from Dalbergia odorifera protected skin keratinocytes against UVB-induced photoaging by suppressing the production of reactive oxygen species (Ham et al., 2015). Topical application of patchouli alcohol inhibited UV-induced reactive oxygen species that led to photoaging in mouse skin (Feng et al., 2014). In our study, FV scavenged the elimination of ROS, which explained the decreased LDH and increased intracellular GSH levels in NHDFs (Fig. 6(B), (C) and (D)). These protective properties of FV appeared to be accomplished by upregulation of cellular antioxidant genes, which mediated by the activation of the Nrf2/ARE signaling pathways in UVB irradiated NHDFs (Fig. 7). Due to UVB stimulation, Nrf2 rapidly translocates into nucleus and transactivates the ARE in the promoter region antioxidant genes (Rotblat et al., 2012). Since the photoprotective effect that FV exhibited must be a fundamental biological process, several mechanisms have been proposed, such as modulation of downstream cellular anti-inflammatory or anti-immunosuppressive activities; these include activation of MAPK signaling pathways (Afq and Katiyar, 2011). It has been reported that activation of Nrf2 at serine and threonine by MAPKs facilitate the dissociation of Nrf2 from Keap-1, and subsequently translocate to the nucleus (Sahu et al., 2015). In this work, we focused on MAPK signaling as a possible mechanism, based on our previous work (Hwang et al., 2014a). MAPK pathway is associated with a variety of biological responses, and is a critical axis to relay extracellular signals to the nucleus and further trigger target gene expression (33). Our results indicated that UV irradiation-induced ROS initiated the components of the MAPK pathways. As expected, FV decreased the abnormal phosphorylation of ERK and P38 (Fig. 10), and inhibited the excess production of MMPs (Fig. 8(A) and (B)). Accordingly, our results indicated that FV protected skin from UVB irradiation.
induced photoaging through activation of Nrf2 and inhibition of MAPK pathways. However, the coordination of Nrf2 and MAPK at the skin level is a reasonable mechanism that warrants further research.

5. Conclusions

In this study, we demonstrated that FV prevented chronic UVB radiation-induced photoaging in fibroblasts and in hairless mice. The protective effect exhibited by FV was associated with the inhibition of MAPK signaling pathways and activation of Nrf2 pathway. Based on our present results, we suggest the potential of FV for the prevention of skin damage caused by solar radiation.

Conflicts of interest

The authors have no conflicts of interest to declare.

References


