A thesis

For the Degree of Master of Science in Medicine

Fermented fish oil derived from mackerel protects skin cell damage by UVB-induced oxidative stress

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Fermented fish oil derived from mackerel protects skin cell damage by UVB-induced oxidative stress

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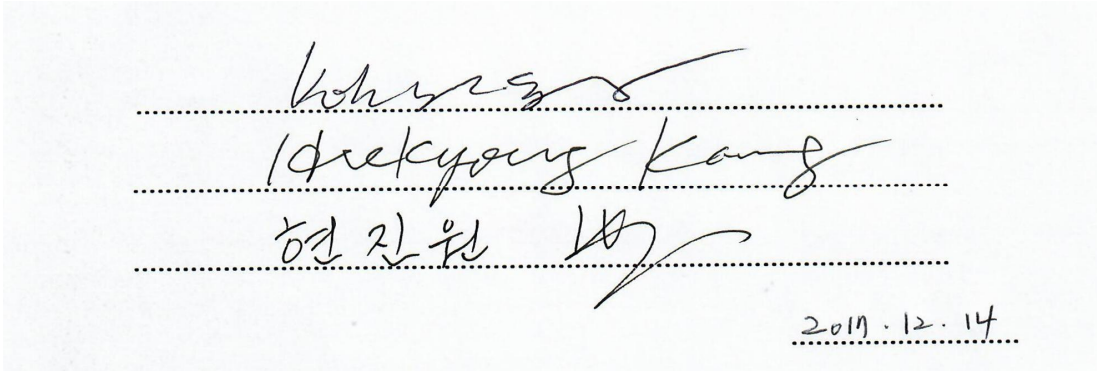

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Abstract

Ultraviolet B (UVB) radiation causes skin diseases by oxidative stress. This study investigated the protective effect of mackerel-derived fermented fish oil (FFO) against UVB radiation-induced oxidative stress in human HaCaT keratinocytes and mouse skin tissue. HaCaT cells were exposed to 30 mJ/cm^2 of UVB radiation for 24h with or without FFO treatment, which showed cytoprotective effects by scavenging UVB-induced intracellular reactive oxygen species. FFO treatment attenuated UVB-induced oxidative modifications including lipid peroxidation, protein carbonylation, and DNA damage. FFO treatment also reduced UVB-induced apoptosis by reducing apoptotic bodies, DNA fragmentation, caspase activation ,and proapoptotic protein expression. UVB radiation activated mitogen-activated protein kinases, phosphorylated (phospho)-extracellular signal-regulated kinase, phospho-c-Jun N-terminal kinase, and phospho-p38. FFO had a more cytoprotective effect than docosahexaenoic acid, the main component of fish oil, against UVB exposure-induced cell damage. Furthermore, the cytoprotective effect of FFO was evident in both UVB-exposed HaCaT cell and mouse models. Overall, these results demonstrate that FFO protects the skin against UVB-induced oxidative stress through antioxidant effects. FFO may be developed as a preventive/therapeutic drug against UVB-induced skin damage.

Keywords: Fermented fish oil, Oxidative stress, Skin damage, UVB; Cytoprotection

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This study was submitted in journal as '*Molecular nutrition & food research 1613-4125*'

1. Introduction

Solar ultraviolet (UV) radiation on the earth's surface consists of approximately 90 - 99% UVA and 1 - 10% UVB rays [1]. UVB rays (280 - 320 nm) pass through the ozone layer, can penetrate the basal cell layer of epidermal cells, and play a crucial role in the induction of skin cancer by various mechanisms [1]. Recent studies have shown that following UVB exposure, reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion, and peroxide radical are produced because of increased levels of oxidative stress [2, 3]. During metabolism in aerobic cells, significant amounts of electrons in the inner mitochondrial membrane pass through the transport chain. During this time, ROS levels increase in the inner mitochondrial membrane, and oxidative stress such as UVB further increases ROS levels in the mitochondria [4]. Oxidative products such as cyclobutane pyrimidine dimer, pyrimidine-pyrimidone (6-4), and 8-hydroxyguanine in DNA, protein carbonyl, and lipid hydroperoxide have been proven to rise because of direct absorption of UVB photons or UVB-induced oxidative stress [5].

The fermentation process forms a deformable chemical structure or produces a new substance [6]. Recently, numerous scientists have focused their attention on fermented foods, which are gaining recognition as an important biological response modifier. For instance, butyrate, a short-chain fatty acid generated when microbes ferment gut dietary fiber, enhances the programmed cell death of colon cancer cells [7], and cultured milk products reduce the risk of bladder cancer [8]. Currently, there has been a growing trend in the use of fermented foods to develop remedies for skin diseases that act by upregulating the endogenous antioxidant system. In a recent study, fermented food such as milk or papaya enhanced the antioxidant capacity of the skin and the expression of major skin genes while promoting

skin anti-aging effects [9, 10]. Fermented milk significantly attenuated UVB-induced erythema formation, dryness, and epidermal proliferation of mouse skin via downregulating the cyclobutane pyrimidine dimers and upregulated mRNA levels of xeroderma pigmentosum complementation group A, which is involved in DNA repair [9]. Furthermore, fermented milk significantly suppressed the ratio of IL-10/IL-12 and IL-10/IFN-γ mRNA level, suggesting it has anti-inflammatory effects [9].

This study was designed to evaluate the potential protective role of fermented fish oil (FFO) derived from mackerel against UVB-induced skin cell damage in vitro and in vivo by assessing its antioxidant properties.

2. Materials and Methods

2.1 Cell culture and UVB irradiation

Human keratinocytes (HaCaT cells) obtained from Amore Pacific Co., (Yongin, Republic of Korea), were cultured in DMEM containing 10% heat-inactivated fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2.The UVB source was a CL-1000M UVC ross linker (UVP, Upland, CA, USA),which was used to deliver anenergy spectrum of UVB radiation (280 - 320 nm; peak in tensity, 302nm), and the cells were exposed to 30 mJ/cm^{2}of UVB radiation.

2.2 Cell viability

Cells were treated with FFO (Choung Ryong Fisheries Co., Jeju, Republic of Korea) at 10, 20, 30, 40, and 50 μg/mL or exposed to UVB (30 mJ/cm^2).After incubation for 24h at 37°C,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St Louis, MO, USA) was added to each well and incubated for 4 h. The formazan crystals formed in each well were subsequently dissolved in DMSO, and the absorbance at 540 nm was measured using a scanning multi-well spectrophotometer [11].

2.3 Intracellular ROS detection

Cells were treated with FFO (10, 20, 30, 40, and 50 μg/mL) for 24 h or FFO (20 μg/mL) for 1 h and then exposed to UVB (30 mJ/cm^2) for 24h. After treatment with 25 μM 2′,7′-dichlorodihydrofluoresceindiacetate (DCF-DA,Sigma-Aldrich) solution for 10min, the DCF fluorescence was detected using a Perkin Elmer LS-5Bspectro fluorometer (PerkinElmer, Waltham, MA, USA) and a confocal microscope using the LSM5PASCAL

software (CarlZeiss,Jena,Germany) [12].

2.4 Detection of superoxide anion

The superoxide anion was produced using the xanthine/xanthine oxidase (Xanthie/XO) system, which was reacted with a nitrone spin trap, 5,5-dimethyl-1-pyrroline N-oxide (DMPO, Cayman Chemical Co., Ann Arbor, MI, USA). The DMPO/•OOH adducts were detected using a JES-FA electron spin resonance (ESR) spectrometer (JEOL, Tokyo, Japan) [13]. Briefly, ESR signaling was recorded after 20 μL Xanox (0.25 unit/mL) was mixed with 20 μL each of Xan (5 mM), DMPO (1.5 M), and FFO (20 μg/mL).

2.5 Detection of hydroxyl radical

The hydroxyl radical was generated via the Fenton reaction (hydrogen peroxide plus iron(II) sulfate; H_2O_2+$FeSO_4$) and reacted with DMPO. The result an tDMPO/•OH adducts were detected using an ESR spectrometer. The ESR spectrum was recorded after a phosphate buffer solution (pH 7.4) was mixed with 0.2 mL each of DMPO (0.3 M), $FeSO_4$(10mM),H_2O_2(10mM), and FFO(20μg/mL).

2.6 Lipid peroxidation assay

Diphenyl-1-pyrenylphosphine (DPPP) reacts with lipid hy-droperoxides to generate a fluorescent product, DPPP oxide, thereby indicating oxidative lipid damage. Cells were in-cubated with 20 μM diphenyl-1-pyrenylphosphine (DPPP, Invitrogen, Carlsbad, CA, USA) for 30 min in the dark [14]. Images of DPPP fluorescence were captured using a confocal microscope. In addition, a commercial enzyme immune assay kit (Cayman Chemical) was used to detect 8-isoprostane, a marker of lipid peroxidation.

2.7 Protein carbonyl formation

The extent of protein carbonyl for-mation was determined using an Oxiselect^{TM}proteincarbonylELISAkit(CellBiolabs,SanDiego,CA,USA).

2.8 Single-cell gel electrophoresis (comet assay)

The degree of oxidative DNA damage was assessed using a comet assay [15]. The cell suspension was mixed with 1% low-melting agarose, and then it was immersed in lysis solution on an agarose-coated slide for 1 h at 4°C. The slides were subsequently subjected to gel electrophoresis for DNA unwinding and the expression of alkali-labile damage, stained with ethidium bromide, and then observed using a fluorescence microscope and image analyzer (Komet 5.5, Kinetic Imaging Ltd, Wirral, UK).

2.9 Detection of mitochondrial membrane potential

Cells were treated with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra ethyl benzimidazolo carbocyanine iodide (JC-1, Thermo Fisher Scientific, Inc., Waltham, MA, USA), a lipophilic cationic fluorescent dye that enters the mitochondria where it emits a fluorescence that changes from green to red as the membrane potential increases. The cells were then incubated for an additional 30 min at 37°C, and the stained cells were mounted on the chamber slide in mounting medium. Images were captured using the confocal microscope using the LSM 5 PASCAL software (Carl Zeiss).

2.10 Western blot analysis

The protein lysates were elec-trophoresed on 12% SDS-polyacrylamide gels, transferred onto membranes, incubated with an appropriate primary anti-body, and then further incubated with secondary IgG-horseradish peroxidase conjugates. Protein bands were visualized by developing the blots using an

enhanced chemiluminescence western blotting detection kit (Amersham, Buckinghamshire, UK). The antibodies used were as follows: anti-B-celllymphoma-2(Bcl-2), anti-Bcl-2-associated X protein(Bax) antibodies (Santa Cruz Biotechnology ,Dallas, TX, USA), and anti-caspase-9 and-3 (Cell Signaling Technology, Beverly, MA, USA).

2.11 Nuclear staining with Hoechst 33342

The DNA-specific fluorescent dye Hoechst 33342 (Sigma-Aldrich) was added to each well, and the degree of nuclear condensation in the stained cells was de-termined by visualization using a fluorescence microscope equipped with a Cool SNAP-Pro color digital camera.

2.12 DNA fragmentation

Cellular DNA fragmentation was assessed by ana-lyzing cytoplasmic histone-associated DNA fragments using a kit from Roche Diagnostics (Portland, OR, USA).

2.13 Treatment of animals and histological analysis

Male, 7-week-old HR-1 hairless mice (Orient Bio Inc., Gyeonggi-do, Republic of Korea) were maintained at 25 - 28 C under a 12-h light/dark cycle and were provided a standard diet and water ad libitum. All experimental procedures were conducted in accordance with the Guidelines for the care and use of laboratory animals at Jeju National University (Jeju, Republic of Korea, permission number: 2017-0026). The mice were randomly divided into the following four groups (n = 5/group): normal control, UVB (100 mJ/cm^2)-treated, FFO(2mg/mL) and UVB (100mJ/cm^2)-treated, FFO(20mg/mL) and UVB (100mJ/cm^2)-treated groups. Following each daily UVB exposure, 200μL of FFO or PBS was applied to the dorsal skin of each mouse for 2 weeks.

The mice were euthanized 24 h after the last UVB exposure. For the histopathological analysis, skin sections were fixed in 10% neutral-buffered formalin solution and routinely processed for paraffin embedding. Sections (5-μm) of paraffin-embedded skin tissue were deparaffinized and then stained with hematoxylin and eosin (H&E). The height of the epidermis (from the stratum basal to the stratum corneum) were measured in approximately 10 randomly chosen fields taken from five representative sections per group, followed by microscopic evaluation at ×100 optical magnification using a digital camera (DP72, Olympus, Center Valley, PA, USA). The heights of the epidermis samples were measured using the Cellsens standard software (Olympus).

2.14 Statistical analysis

All measurements were performed in triplicate, are expressed as the means ± SEM. The results were subjected to an analysis of variance (ANOVA) using Tukey's test to analyze the differences between means. In all cases, a $p < 0.05$ was considered statistically significant.

3. Results

3.1 FFO scavenges ROS and absorbs UVB

The 10, 20, 30, 40, and 50 μg/mL FFO-treated cells did not exhibit any signs of cytotoxicity and showed a > 95% viability (Fig. 1A). The intracellular ROS generation at the FFO concentration range tested was measured in UVB-exposed cells. As shown in Fig 1B, all the tested concentrations of FFO significantly decreased the ROS levels in UVB-exposed cells, more than the positive control, N-acetyl cysteine (NAC), did. Based on these results, 20 μg/mL was selected as the optimal FFO concentration for all subsequent experiments. FFO 20 μg/mL reduced the UVB-induced intracellular ROS increase, as measured using confocal microscopy after staining with DCF-DA (Fig. 1C).

Next, we determined whether FFO directly quenched ROS using an ESR analysis. In the Xan/Xanox system, the DMPO/·OOH produced from superoxide anion yielded a signal value of 1839 compared to the control value of 387 while FFO reduced the signal value to 1450 (Fig. 1D). In addition, in the Fenton reaction, DMPO/·OH produced from the hydroxyl radical yielded a signal value of 1322 compared to the control value of 25 while FFO reduced the signal value to 470 (Fig. 1E). Furthermore, FFO absorbed the UVB wavelength, showing a maximum peak at 296 nm, which is in the UVB region (280 - 320 nm, Fig. 1F).

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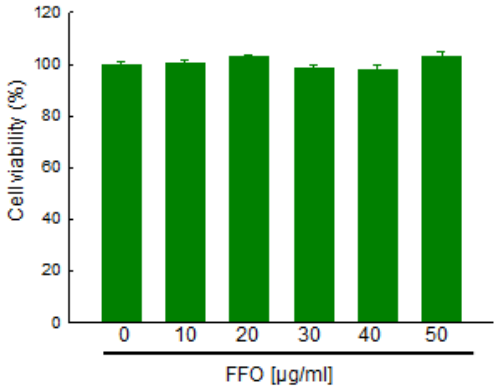

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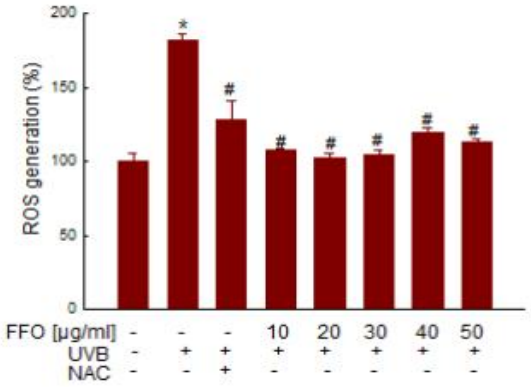

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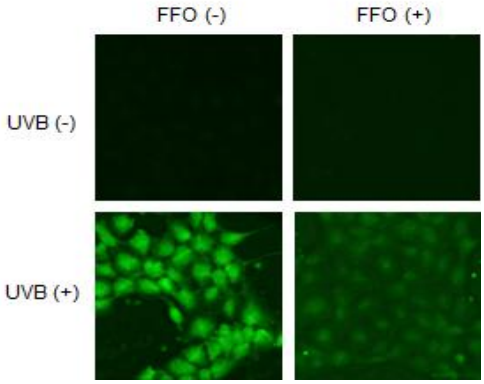

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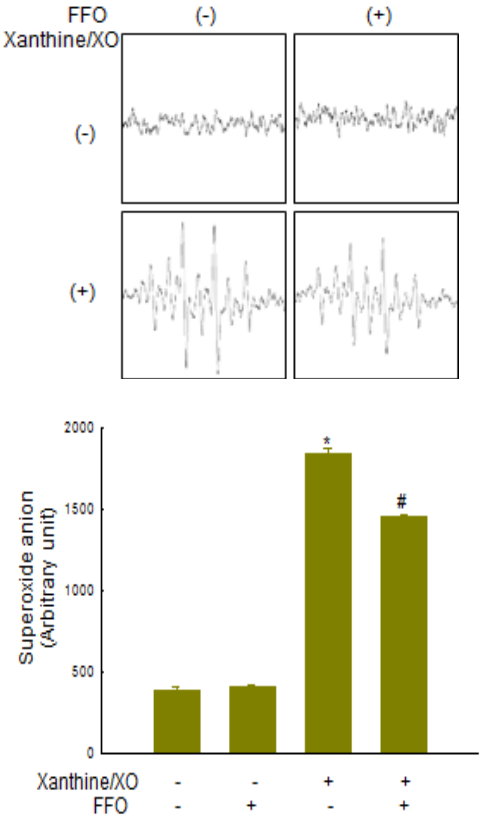

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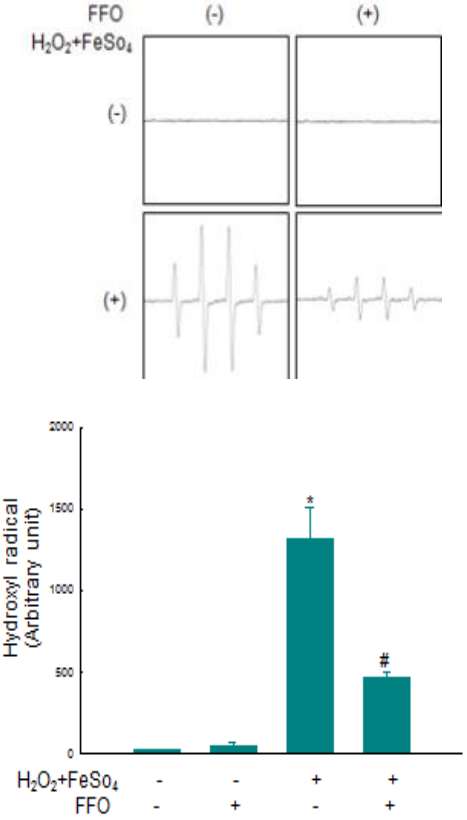

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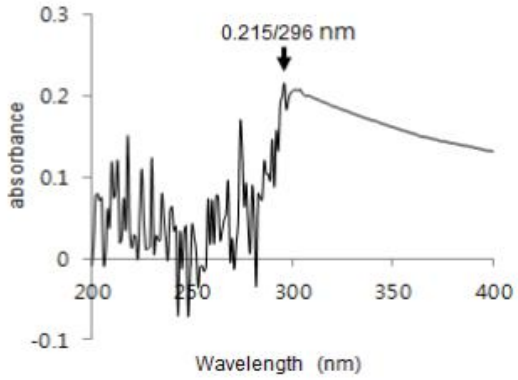

Figure 1. Fermented fish oil scavenges UVB-induced intracellular reactive oxygen species. (A) Viability of HaCaT cells treated with FFO at the indicated concentrations was detected using MTT assays. (B) Intracellular ROS levels in FFO-treated and UVB-irradiated cells were assessed using spectrofluorometer after DCF-DA treatment. $*p < 0.05$ and $^{\#}p<0.05$compared to control and UVB-irradiated cells, respectively.(C) IntracellularROSlevelsofFFO-pretreated(20μg/mL) and UVB-irradiated cells was assessed using confocal microscopy after DCF-DA staining. Representative images are shown, and green fluorescence indicates ROS level. (D) Superoxide anion was evaluated using xanthine/xanthine oxidase (Xanthine/XO) system using ESR spectrometer. $*p < 0.05$ and $^{\#}p<0.05$ compared to control and superoxideanion, respectively. (E)Hydroxyl radical was estimated using Fentonreaction ($FeSO_4$+H_2O_2system) using ESR spectrometer. $*p < 0.05$ and $^{\#}p<0.05$comparedtocontrolandhydroxyl radical, respectively. (F) UVB absorption spectra of FFO were determined by UV scanning at 200 - 400 nm. Arrow indicates absorbance peak at 291 nm.

3.2 FFO suppresses oxidative cellular damage by UVB in HaCaT cells

Intracellular ROS, one of the most destructive products generated in skin cells damaged by exposure to UVB radiation, damages cellular macromolecules such as protein, DNA, and lipids [16]. The DPPP oxide fluorescence intensity, a specific detector of lipid peroxidation, was more markedly increased in UVB-exposed cells than in the control group cells. However, this fluorescence intensity was decreased in cells pretreated with FFO (Fig. 2A). The 8-isoprostane, which can be used as a specific indicator of lipid peroxidation, was significantly increased in UVB-exposed cells while the levels were decreased by FFO pretreatment (Fig. 2B).

FFO significantly inhibited protein carbonyl formation, which is a marker of oxidative protein modification, in UVB-irradiated cells compared to that in untreated UVB-irradiated cells (Fig. 2C). When oxidative DNA damage was detected using the comet assay, FFO was shown to have significantly decreased the tail length of DNA in UVB-irradiated cells compared to that of the untreated UVB-irradiated cells (Fig. 2D).

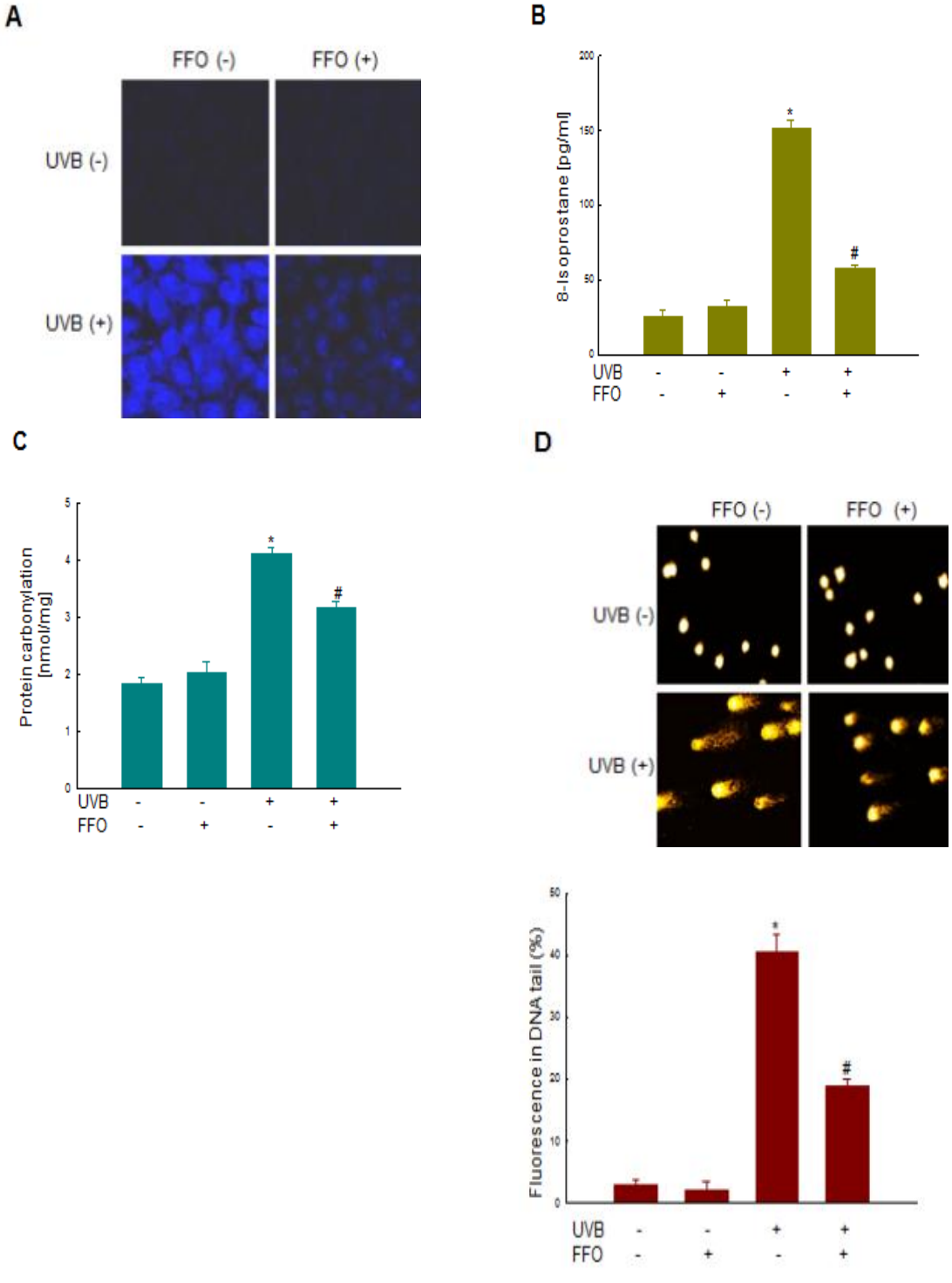

Figure 2. Fermented fish oil protects HaCaT cells against UVB-induced oxidative lipid, protein, and DNA damage. Cells were pre-treated with FFO for 1 h and then exposed to UVB (30 mJ/cm^2) radiation. (A) Lipid peroxidation was assessed using fluorescence microscopy after DPPP staining. (B) Lipid peroxidation was assessed by measuring 8-isoprostane levels in conditioned medium. $*p < 0.05$ and $^{\#}p<0.05$ compared to control and UVB-irradiated cells. (C) Level of protein carbonyl was assessed using protein carbonyl ELISA kit. $*p < 0.05$ and $^{\#}p<0.05$ compared to contro land UVB-irradiated cells, respectively. (D) Comet assay was used to assess oxidative DNA damage. Representative images and percentages of cellular fluorescence in comet tails are shown. $*p < 0.05$ and $^{\#}p<0.05$ compared to control and UVB-irradiated cells, respectively.

3.3 FFO attenuates UVB-induced apoptosis

The assessment of cell viability using an MTT assay to determine whether FFO inhibits UVB-induced cell death revealed that FFO restored the cell viability decreased induced by UVB compared to that of the untreated UVB-irradiated cells (Fig. 3A). During UVB-induced cell death, the mitochondrial membrane potential was disrupted from the polarized form (red fluorescence) to the depolarized form (green fluorescence) as detected using JC-1 staining. However, FFO inhibited the UVB-induced mitochondrial membrane potential disruption compared to that in the untreated UVB-irradiated cells (Fig. 3B). Two typical proteins, Bcl-2 and Bax, which are members of a family of proteins involved in apoptosis, play an important role in inhibiting or promoting apoptosis, respectively and regulating cytochrome c release in the mitochondrial membrane [17]. FFO treatment increased Bcl-2 expression and decreased that of Bax in UVB-irradiated cells (Fig. 3C). Furthermore, FFO treatment decreased the expression of activated cleaved-caspase-3 and cleaved-caspase-9 induced by UVB (Fig. 3D). FFO treatment was also confirmed to decrease DNA fragmentation and apoptotic cell bodies, which were increased by UVB (Fig. 3E and F).

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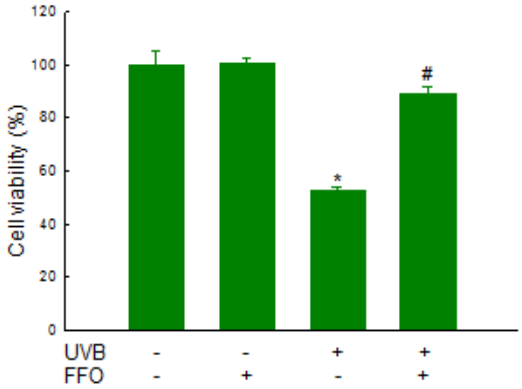

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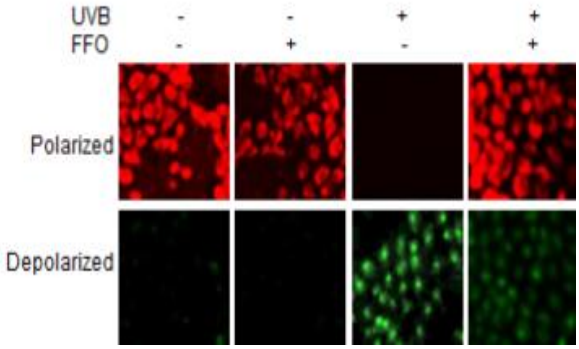

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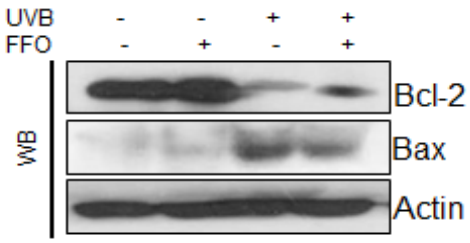

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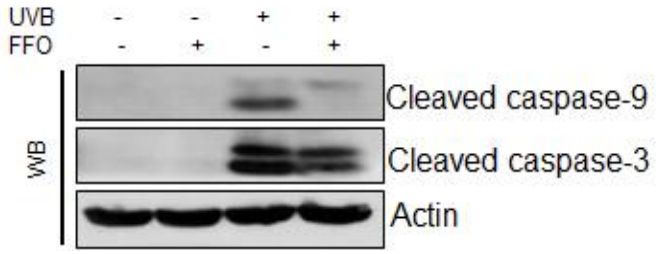

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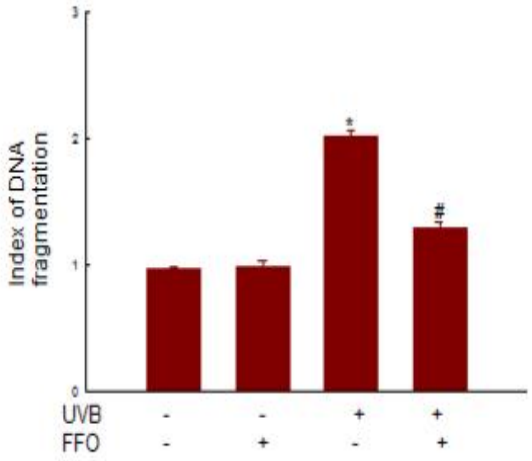

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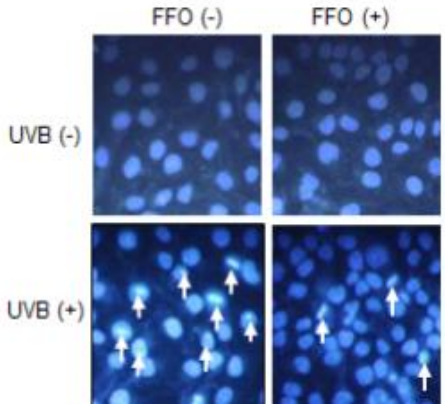


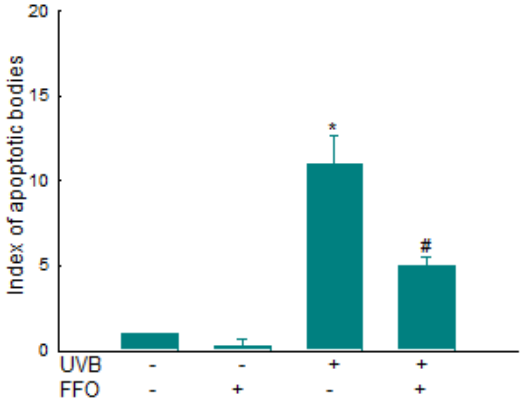
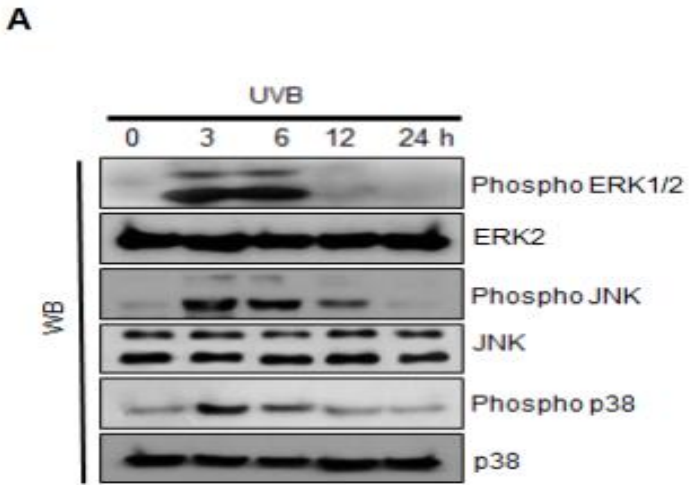


Figure 3. Fermented fish oil reduces mitochondria-mediated apoptosis of UVB-irradiated HaCaT cells. (A) Cells were pre-treated FFO for 1 h, and then exposed to UVB radiation (30 mJ/cm^2). Cell viability was measured using MTT assay. *$p<0.05$ and $^{\#}p<0.05$ compared to control and UVB-irradiated cells, respectively.(B) Mitochondrial depolarization was measured using confocal microscopy with JC-1 staining. Polarization and depolarization are indicated in red and green, respectively. Expression levels of (C) Bcl-2, Bax, (D) cleaved caspase-3, and -9 proteins were monitored using western blot analysis. (E) DNA fragmentation was assessed using DNA fragmentation ELISA kit and (F) apoptotic body formation (arrows) was assessed using fluorescence microscopy after Hoechst 33342 staining. *$p < 0.05$ and $^{\#}p<0.05$ compared to control and UVB-irradiated cells, respectively.

3.4 FFO enhances cell viability by attenuating mitogen activated protein kinase (MAPK) signaling pathway

The active (phospho) forms of mitogen-activated protein kinase (phospho-MAPK), phospho- extracellular signal-regulated kinase (ERK), phospho-c-Jun N-terminal kinase (JNK), and phospho-p38 protein, which are a group of apoptosis-related signaling proteins, were significantly expression 3 h after UVB irradiation (Fig. 4A). Treatment with the ERK inhibitor U0126, JNK inhibitor SP600125, and p38 inhibitor SB203580 increased the cell viability reduced by UVB exposure. However, cells treated with FFO and each inhibitor showed a more enhanced cell viability than that the cells treated with each inhibitor alone (Fig. 4B). Furthermore, the protective effect of FFO against UVB-induced apoptosis was consistent with the effects illustrated in Fig. 4B (Fig. 4C).



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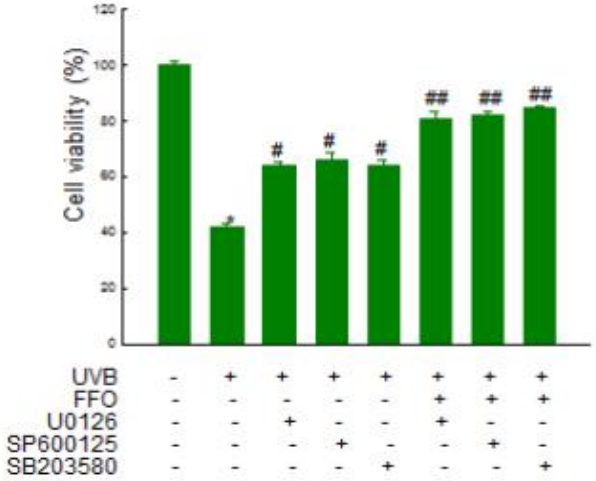

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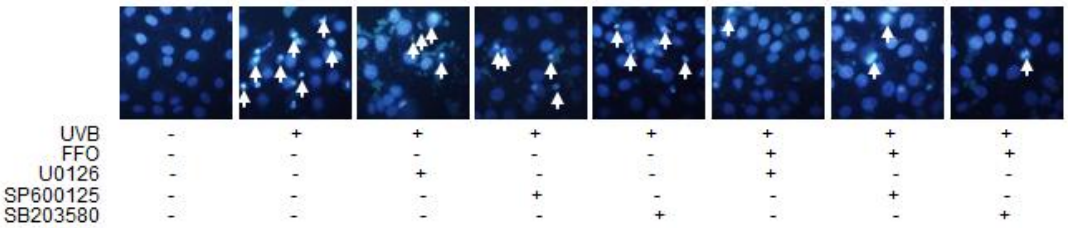


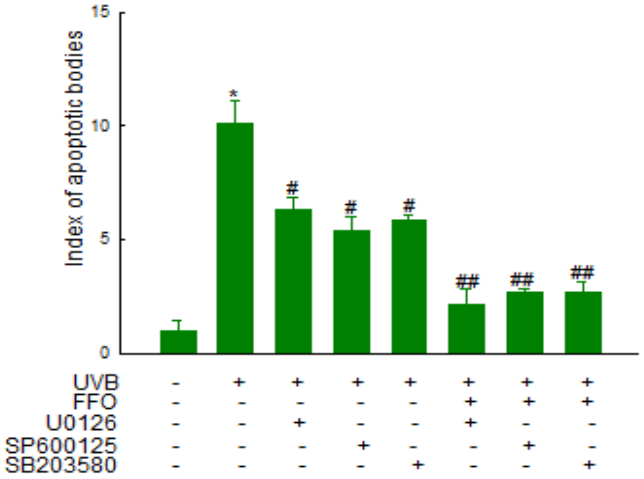
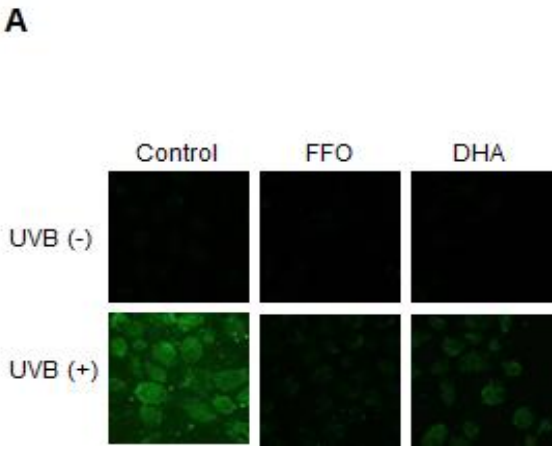


Figure 4. Fermented fish oil inhibits MAPK-mediated HaCaT cell death induced by UVB radiation. (A) Expression levels of phospho-JNK, phospho-ERK, and phospho-p38 were monitored using western blot analysis. Cells were pre-treated with MAPK inhibitors (JNK, ERK, and p38 inhibitors: SP600125, U0126, and SB203580, respectively) and FFO, followed by UVB (30 mJ/cm^2)irradiated, and then cytotoxicity and apoptosis were assessedusing (B) MTT assay and (C) staining with Hoechst 33342. Arrows indicate apoptotic bodies. $*p < 0.05$, $^{\#}p<0.05$, and $^{\#\#}p<0.05$ compared to control, UVB-irradiated, and inhibitor-treatedcells, respectively.

3.5 FFO shows more cyto protection than docosahexaenoic acid (DHA), the main component of fish oil, against UVB-induced apoptosis

Recent studies have shown that docosahexaenoic acid (DHA) activates peroxisome proliferator-activated receptor (PPARγ) and expression of its target catalase, thus inhibiting ROS-mediated activation of Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) and IL-6 expression [18]. DHA-induced PPARγ activation and catalase expression may underlie the antioxidant and anti-inflammatory effects of DHA. We compared DHA and FFO to confirm their intracellular ROS scavenging and cell protective effect against UVB. The FFO-pretreated group was more protected against UVB-induced cell damage than the DHA-pretreated group was as shown by the results of ROS level determination (Fig. 5A), cell viability (Fig. 5B), and intracellular apoptotic body generation (Fig. 5C).



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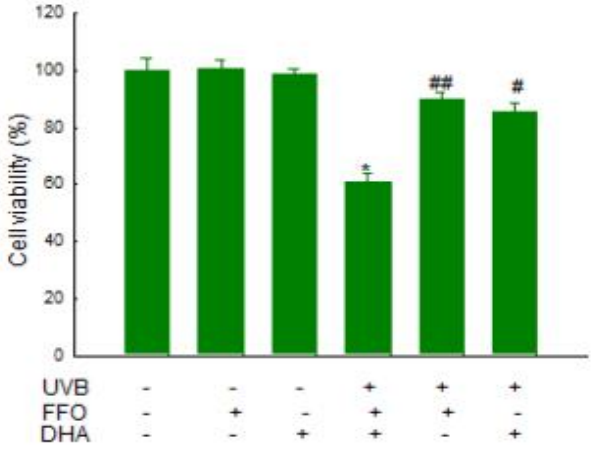

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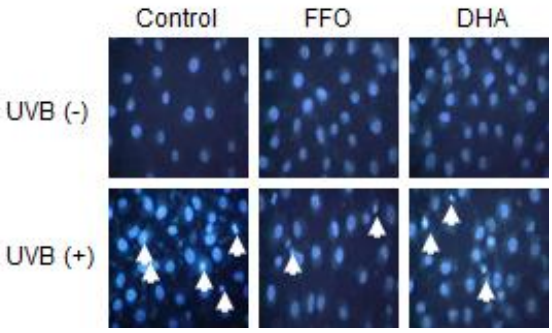


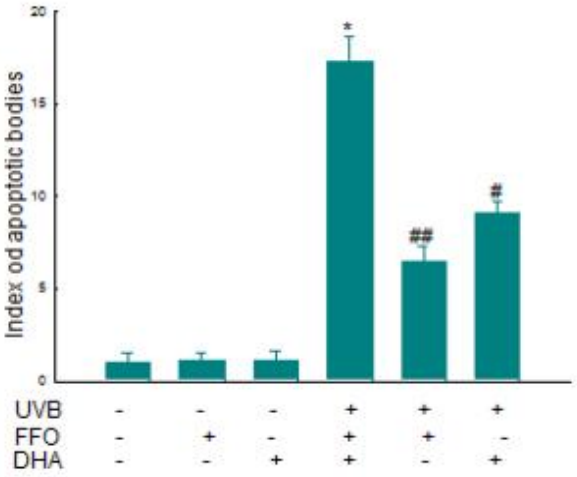
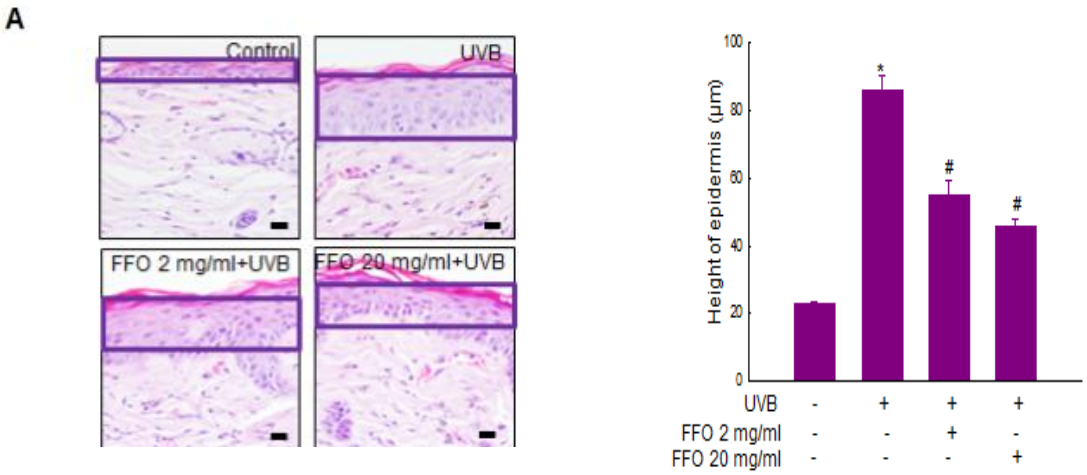


Figure 5. Fermented fish oil decreases UVB-induced apoptosis more than that by docosahexaenoic acid. (A) Intracellular ROS induced by UVB radiation. ROS level was measured using DCF-DA in FFO- or DHA-pretreated and UVB-irradiated cells. Cell viability and apoptotic bodies induced by UVB irradiation of FFO- or DHA-pretreated cells were monitored using (B) MTT assay and (C) microscopy after Hoechst 33342 staining, respectively. Arrows indicate apoptotic bodies. $^{*}p < 0.05$ and $^{\#}p < 0.05$ compared to control and UVB-irradiated cells, respectively.

3.6 FFO protects mouse skin against UVB damage

Histopathological analysis has also provided important evidence for the protective effects of FFO on UVB radiation. In the skin sections of the normal controls showed a normal histological architecture and epidermal height whereas that of UVB-irradiated mice exhibited a severely hyperkeratotic epidermis (Fig. 6A). Compared with the UVB-treated group, the FFO-treated epidermal height was reduced dose-dependently (Fig. 6A). In addition, FFO-treatment decreased the apoptosis-related proteins, Bax, active caspase-3, and active caspase- 9 in the UVB-irradiated group, whereas the reduced anti-apoptotic protein Bcl-2 level was increased (Fig. 6B).



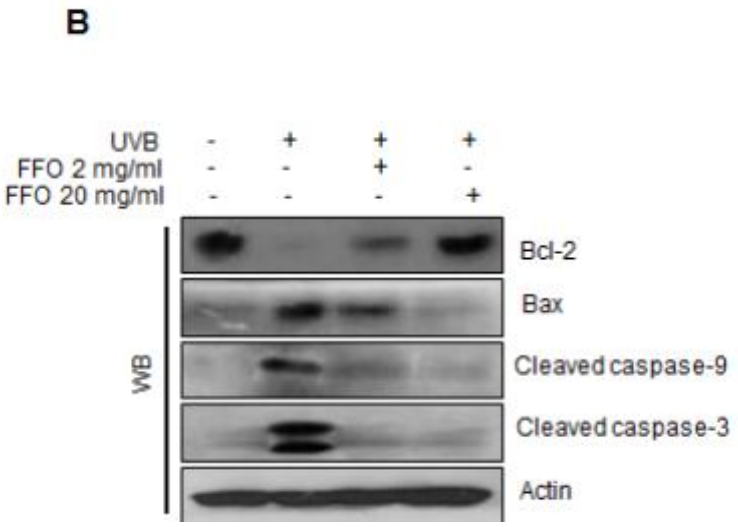


Figure 6. Fermented fish oil inhibits UVB-induced keratosis in dorsal mouse skin. (A) Normal control, UVB irradiation, FFO (2 mg/mL), and FFO (20 mg/mL) plus UVB radiation groups were assessed to determine the height of the mouse skin epidermis. Representative images from each group and graph of semi-quantified epidermis height of mouse skin samples are shown. $^{*}p < 0.05$ and $^{\#}p < 0.05$ compared to normal control and UVB-irradiated groups, respectively. Scale bars, 20 μm. (B) Expression levels of Bcl-2, Bax, cleaved caspase-3, and -9 proteins were monitored using western blot analysis.

4. Discussion

Cell damage induced by UVB can cause numerous harmful reactions such as erythema, immunosuppression, pigmentation, loss of hair growth, and premature aging [19, 20]. Keratinocytes are the main cell types in the basal layer of the skin and scalp. UVB is mainly absorbed in the epidermal basal cell layer and induces intracellular molecular damage to DNA, lipid, and protein, leading to cell death. In this study, experiment focused on elucidating the biological mechanism underlying the protective effects of FFO against UVB-induced cellular damage. Recently, has reported that FFO alleviated the allergic inflammatory response by increasing the expression of the immune-suppressive cytokines transforming growth factor (TGF)-β and IL-10 to activate $CD4^{+}CD25^{+}Foxp3^{+}T$ cells, which down regulate the progression of immune disorders [21]. Fish oil contains a variety of substances such as stearic acid, eicosatrienoic acid, linolenic acid, eicosapentaenoic acid (EPA), and DHA [22]. Especially, EPA and DHA mainly exhibit protective effects against oxidative stress-induced cell damage [23, 24]. FFO contains more than twice the concentration of EPA and DHA [21] that unfermented fish oil does, suggesting that fermentation converts the chemical structure of some constituents of fish oil to generate new substances with antioxidant activity mediated via ROS scavenging.

This study has demonstrated that FFO removed ROS such as the superoxide anion and hydroxyl radical and, thereby, attenuated ROS production by UVB exposure. FFO exhibits ROS scavenging and UVB absorbing effects that protect HaCaT keratinocytes and mouse skin tissue against UVB-induced oxidative stress that leads to lipid peroxidation, oxidative DNA damage, and protein carbonylation. The UVB-induced

oxidative stress leads to mitochondria-mediated apoptotic cell death, which occurs by disruption of the mitochondrial membrane potential, alterations in the expression levels of Bcl-2 and Bax, and activation of caspases; however, these effects were significantly attenuated by FFO.

UVB irradiation mediates apoptosis by oxidative stress-dependent activation of upstream MAPKs in skin cells [24, 25]. Thus, this result determined whether FFO protects against UVB-mediated apoptosis of skin cells. The present study demonstrated that UVB induced the phosphorylation of MAPKs (ERK, JNK, and p38), which led to apoptotic cell death, whereas the FFO- and MAPK inhibitor-treated cells exhibited enhanced cell viability, suggesting that the protective effects of FFO were mediated by the inhibition of MAPK-induced apoptosis. In addition, FFO showed a more protective effect against UVB-induced cell death than DHA, a main active component of FFO, did and the more potent effect of FFO may be due to the generation of potent antioxidants in the fish oil during fermentation. In addition, our in vivo results demonstrate that FFO prevented the induction of a severe hyperkeratotic epidermis by UVB radiation, and decreased apoptosis-related proteins such as Bax, active caspase-9, and activated caspase-3.

In summary, these results suggest that FFO could be used as a therapeutic agent to protect the skin from the harmful effects of UVB irradiation by its antioxidant effect. These results provide an experimental platform for further studies to examine the bioavailability and photo protective activity of FFO in vitro and in vivo and determine the underlying mechanism.

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6. Abstract in Korea

발효어유 (FFO) 는 고등어 생선으로부터 유래된 물질로서 자외선 B의 조사에 의한 산화적 스트레스로 인해 손상된 인간 피부세포와 쥐 피부 조직을 보호하는 효과가 있다. 인간 각질 세포 에 발효어유를 전 처리 한 그룹 과 그렇지 않은 그룹을 자외선 (B 30 mJ/cm^2) 조사 24시간동안 노출 시킨 후 비교한 결과 발효어유 전처리 그룹의 세포내 활성 산소 종 제거능이 증가 하였고, 각 세포분자인 단백질, 지질, 유전물질 등 의 산화적 손상을 측정 한 결과 자외선 B에 의한 세포분자 손상이 감소하였다. 또 한, 자외선 B로 유도되는 세포사멸 연관 신호 단백질인 MAPK p-JNK, p-p38, ERK 가 발효어유에 의해서 감소하였다. 생선의 구성물질인 DHA 또한 자외선 B로부터 야기되는 세포손상에 대해 보호효과를 보이나, 발효어유는 DHA 와는 별개의 구조로서 인간 피부세포와 쥐 피부 조직에서 항산화적인 효과를 입증한다. 향 후 발효어유는 자외선 B로부터 손상되는 피부손상을 보호할 수 있는 치료제로서 개발 가능하다.

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