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Zingiber mioga Extract Improves Moisturization and Depigmentation of Skin and Reduces Wrinkle Formation in UVB-Irradiated HRM-2 Hairless Mice

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Abstract: Here, we investigated the effects of *Zingiber mioga* extracts (FSH-ZM) on the moisturization and depigmentation of skin as well as wrinkle formation in UVB-irradiated HRM-2 hairless mice. The mice were divided into six groups as follows: normal control (NC), UVB-irradiated control (C), positive control 1 (PC1, L-ascorbic acid 200 mg/kg b.w.), positive control 2 (PC2, Arbutin 200 mg/kg b.w.), Z100 (FSH-ZM 100 mg/kg b.w.), and Z200 (FSH-ZM 200 mg/kg b.w.). The experiment spanned a period of 6 weeks. We found that FSH-ZM led to an increase in the expression of hyaluronan synthase 2, fibrillin-1, and elastin mRNAs, and showed improved skin hydration in HRM-2 hairless mice compared to that in the UVB-irradiated control group. Furthermore, FSH-ZM also inhibited the expression of inflammatory cytokines and wrinkle forming factors generated by UVB and reduced the formation of wrinkles in the test group relative to that in the control group by increasing collagen synthesis. Moreover, we found that FSH-ZM decreased the expression of melanogenesis factors, which improved depigmentation in UVB-irradiated hairless mice. These results suggest that *Zingiber mioga* can potentially be utilized to develop products aimed at improving skin moisturization and depigmentation and reducing wrinkle formation.

Keywords: *Zingiber mioga*; moisturizing; wrinkle improvement; whitening

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1. Introduction

In recent years, there has been increased consumer interest and desire for improved skin health. Accordingly, considerable research studies into the role of food and cosmetics as natural materials that delay natural endogenous skin aging and exogenous skin aging caused by environmental factors such as ultraviolet (UV) rays and air, gave been undertaken [1]. One of the factors involved in skin aging include photoaging due to the repeated exposure to UV rays, which results in decreased moisturization of the skin, decreased elasticity, wrinkle formation, and pigmentation (e.g., erythema) [2].

Skin tissue is composed of several layers (i.e., epidermis, dermis and subcutaneous fat), with keratinocytes and melanocytes being distribute within the epidermis. The dermis is composed of fibrous and matrix components, 90% of which is collagen present as a fibrous component [3]. In order to maintain health skin, its sufficient moisturization is vital. The skin epidermis, which acts as a physical barrier by preventing the loss of moisture in the body, upon damaged by UV rays is unable to prevent the loss of moisture, causing the skin to become dry, wrinkled, and rough [4].

The level of moisturization in the skin is determined both by hyaluronic acid in the stratum corneum, and the activity of hyaluronan synthase (HAS) which maintains the concentration of hyaluronic acid in the skin [5]. In addition, the action of reactive oxygen species (ROS), caused by continuous UV exposure, not only causes inflammatory reactions,

but also promotes the activity of a matrix metalloproteinase (MMP), known as collagenase, which inhibits collagen synthesis in the dermis and promotes wrinkle formation [6,7]. In response to these UV exposures, the body activates a defense mechanism by increasing tyrosinase activity which leads to melanin production. Melanin plays a crucial role in skin protection by removing ROS [8]. Conversely, excessive melanin production is known to cause melanoma, blemishes, spots, and freckles [9]. Therefore, the development of functional health foods and cosmetics that help in the suppression of skin aging and pigmentation due to UV rays, is mUch needed.

Hairless mice are commonly used in skin studies. In this work, we used hairless HRM-2 mice that can synthesize melanin pigments, and are also routinely used in melanin synthesis, skin sensitivity, irritation, long-term subcutaneous absorption, and collagen synthesis experiments [10,11].

Zingiber mioga (ZM) belongs to the family of ginger and is distributed across East Asia (i.e., Korea, China, and Japan). Recently, it has been reported that ginger extract contains phenolic acids such as pyrogallol p-hydroxy bezoic acid, *p*-coumaric acid, and ferulic acid [12]. Among these, p-coumaric acid is present in free or combined form in plants and mUshrooms such as fruits, vegetables, and grains [13]. In the ginger family, it was reported that the Zingiber officinale species contained coumaric acid [14]. However, few studies on the content or related activity of *p*-coumaric acid in ZM have been performed.

Therefore, in this study, we evaluated the efficacy of ZM for skin moisturization and depigmentation, and wrinkle improvement in vivo, and assessed the feasibility of its utilization in the development of functional foods and cosmetics for skin health.

2. Materials and Methods

2.1. Preparation of Materials

The ZM hot water extract (FSH-ZM, Z) was supplied by Frombio (Suwon, Korea). ZM was produced in Jeju, Korea, and the materials to be used in the experiment were cut, dried, and extracted for 5–8 h at 85 °C, followed by filtration, concentration, and lyophilization. L-ascorbic acid and arbutin (Sigma-Aldrich, St. Louis, MO, USA) were used as a positive control.

2.2. High-Performance Liquid Chromatography (HPLC) Analysis

The ZM hot water extract was mixed with chloroform and water mixture.

After collecting the filtered water layers, and 10 µL of the new samples were injected into the HPLC equipment (Shimadzu, Kyoto, Japan). An eclipse plus phenyl-hexyl column (4.6 × 250 mm, 5 µm, Agilent Technologies, Palo Alto, CA, USA) was used for separation. The column temperature was 30 °C, the flow rate was 1.0 mL/min. The mobile phase consisted of 0.1% phosphoric acid (A) and acetonitrile with 0.1% phosphoric acid (B). HPLC gradient conditions were as follows: 0–30 min (10% B), 30.5–40 min (90% B), and 40.05–50 min (10% B). The detection wavelength was set at 310 nm.

2.3. Animal Treatment and UVB Exposure

All animal experiments were approved by the Institutional Animal Care and Use Review Committee of Kyung Hee University (KHUASP[SE]-18-015). Male HRM-2 mice (4 weeks old, 20 ± 1 g) were purchased from Saeronbio, Inc. (Uiwang, Korea). The housing facility was maintained at 23 ± 2 °C, 50–60% relative humidity with a 12h light/dark cycle. The experimental mice were acclimated for one week and provided with AIN93G diet and fresh water before starting the experiment. A total of 36 mice were randomly assigned randomly to six groups with six animals per group: normal control (NC), UVB-irradiated control (C), positive control 1(PC1, L-ascorbic acid 200 mg/kg b.w.), positive control 2 (PC2, Arbutin 200 mg/kg b.w.), Z100 (FSH-ZM 100 mg/kg b.w.), and Z200 (FSH-ZM 200 mg/kg b.w.). All experimental diets were based on the AIN93G diet and provided to mice ad libitum.

We used a UVB lamp (5 Sankyo Denky G5T5 lamps, Sankyo Denki Co., Yokohama, Japan) for chronic UVB exposure of mice. The dorsal regions of the mice were irradiated by UV ray three times a week with a minimum erythema dose (MED) set at 150 mJ/cm^2. UVB

irradiation was set to 1MED at 1 week, 2 MED at 2 weeks, 3 MED at 3 weeks, and 4 MED at 4–6 weeks, to induce skin dryness, wrinkles, and melanin production. The experiment was conducted for 6 weeks, following which blood and skin tissues of the mice were collected for analysis.

2.4. Real-Time Polymerase Chain Reaction (RT-PCR)

Skin tissues were lysed using a QIAzol® Lysis Reagent (Qiagen, Gaithersburg, MD, USA). Subsequent RNA extraction, quantification, cDNA synthesis, and RT-PCR were performed in our conventional protocol [15]. The gene sequences were designed using Primer express program of applied biosystems (Thermo Fisher Scientific, Waltham, MA, USA) and shown in Table 1. As an internal control, glyceraldehyde 3-phophate dehydrogenase (GAPDH), a housekeeping gene commonly used in mRNA and DNA experiments, was used [16]. All data were calculated as delta delta CT values in the Bio-rad CFX manager 3.1 program (Bio-Rad Laboratories, Hercules, CA, USA).

Table 1. Primer sequences used in real-time PCR-based quantification of mRNA.

Gene	Murine Sequences (Forward)	Murine Sequences (Reverse)
HAS2	F 5′-TGGCTGTGTCCAGTGCATAAG-3′	R 5′-CACAAATTCATGCAGCAAGGA-3′
Fibrillin-1	F 5′-ACAATTGTTCACCGAGTCGATCT-3′	R 5′-ACTGTACCTGGGTGTTGCCATT-3′
Elastin	F 5′-TGGTGACATGATCCCTCTCTCTT-3′	R 5′-CCAGGGTGTCCCAGATGTG-3′
MAPK8 (JNK)	F 5′-AGGGAATGCTGGGATCGTTA-3′	R 5′-ACCCAGTGGTGCATGTTGAA-3′
AP-1	F 5′-TGGCTGTGTCCAGTGCATAAG-3′	R 5′-CACAAATTCATGCAGCAAGGA-3′
MMP1	F 5′-GACCGTTCTATTCCTCAGTGCAA-3′	R 5′-CCCGGTGACACACAAAGACA-3′
MMP3	F 5′-TTACGTGGCAAGTGAGGGTTT-3′	R 5′-TGTCCAGATGCACTTCTTGTTTG-3′
MMP9	F 5′-TGGTGACATGATCCCTCTCTCTT-3′	R 5′-CCAGGGTGTCCCAGATGTG-3′
Procollagen	F 5′-TTACGTGGCAAGTGAGGGTTT-3′	R 5′-TGTCCAGATGCACTTCTTGTTTG-3′
Collagen type I	F 5′-GACCGTTCTATTCCTCAGTGCAA-3′	R 5′-CCCGGTGACACACAAAGACA-3′
IL-1β	F 5′-AGTTGACGGACCCCAAAAGA-3′	R 5′-GGACAGCCCAGGTCAAAGG-3′
IL-6	F 5′-CCACGGCCTTCCCTACTTC-3′	R 5′-TGGGGAGTGGTATCCTCTGTGA-3′
TNF-α	F 5′-CACAAGATGCTGGGACAGTGA-3′	R 5′-TCCTTGATGGTGGTGCATGA-3′
CREB	F 5′-TATTGAGGTCCGGTGAGATTCC-3′	R 5′-CCAGTTGTGAGTGCTGGAGTAAA-3′
MITF	F 5′-ATTCTCAAGGCCTCTGTGGACTAC-3′	R 5′-AGGTCCTTAGCTCGTTGCTGTT-3′
Tyrosinase	F 5′-TATCCTTCTGTCCAGTGCACCAT-3′	R 5′-CACGGTCATCCACCCCTTT-3′
TRP1	F 5′-ATGCGGTCTTTGACGAATGG-3′	R 5′-CGTTTTCCAACGGGAAGGTA-3′
GAPDH	F 5′-CATGGCCTTCCGTGTTCCTA-3′	R 5′-GCGGCACGRCAGATCCA-3′

HAS2, hyaluronic acid synthase2; MAPK8, mitogen-activated protein kinase; AP-1, activator protein 1, MMP, matrix metalloproteinase; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; CREB, cAMP response element binding protein; MITF, microphthalmia-associated transcription factor; TRP1, tyrosinase-related protein 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

2.5. Western Blotting

Skin tissues (50 mg) were homogenized using 0.5 mL of cell lysis reagent (Sigma-Aldrich) with protease inhibitor Cocktail (Thermo Fisher Scientific). The following protein quantification, loading, and transfer were performed in our conventional method [15]. After protein transfer, a blocking step was performed, and primary antibodies such as ß-actin, AP-1 (Abcam, 1:1000), COL1A1, MMP1, MMP3, MMP9, CREB, and MITF (Cell Signaling Technology, 1:1000) were exposed to the membrane. ß-actin was used as a control. After that, the secondary antibody attachment, visualizing step, and analysis were also performed in the conventional method [15].

2.6. Measurement of Skin Hydration

Skin hydration was measured using Howskin (Innoinsight Inc., Yongin, Korea) to determine the moisture content of the skin. The values were measured by placing the electronic terminal of the device on the back of the mouse. The value measured with a simple button on the device can be sent to the mobile phone and checked. This analysis was performed on the sixth week.

2.7. Measurement of Antioxidant Activity

The blood samples of the experimental animals were centrifuged (14,000 rpm at 4 °C for 20 min). The serum samples, thus obtained, were used to measure the antioxidant activity. The activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were measured by ELISA (Biovision Inc., Moutain View, CA, USA) according to the specifications of the manufacturer.

2.8. Morphological Observation of Skin

We used digital microscopy (DG-3x, Scalar Co., Tokyo, Japan) for the morphological observations of the skin. Using DG-3x (Tokyo, Japan) camera, close-angle photo of the dorsal region of the mice were taken and wrinkles visible on the skin were observed.

2.9. Hematoxylin and Eosin (H&E) Staining

Hzematoxylin and eosin (H&E) staining was performed to measure skin wall thickness and wrinkles. After collecting the skin tissues of the experimental animals and fixing them in 10% (*v*/*v*) formalin, paraffin blocks were prepared, sliced to a thickness of 5 µm, and attached to slides coated with saline. Additionally, after deparaffinization using xylene, hydrolysis was performed in 100% alcohol, and the tissue samples were stained with H&E, and observed under a microscope (Nikon elipse TS100, Tokyo, Japan).

2.10. Fontana-Masson Staining

To detect melanin formation in the skin of HRM-2 hairless mice, Fontana-Masson staining was performed. After collecting the skin tissues of the experimental animals and fixing them in 10% (*v*/*v*) formalin overnight at room temperature, tissues were stained to detect melanin using a Fontana-Masson staining kit (American Mastertech, Inc., Lodi, CA, USA). Sliced skin samples were stained with ammoniacal silver solution for 1 h at 60 °C and incubated in 0.1% (*v*/*v*) gold chloride followed by 5% (*v*/*v*) sodium thiosulfate treatment. Melanin spots were observed under a microscope (Nikon).

2.11. Statistical Analysis

All data are presented as mean ± standard deviation (SD). We analyzed the significance of results with Duncan's mUltiple-range test after confirming the normality and homoscedasticity and conducting a one-way ANOVA using the SPSS statistical program. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. HPLC Analysis of ZM Extract

The HPLC analysis of the ZM extract revealed one peak, which matched that of *p*-coumaric acid (a standard), with retention times of approximately 27.079 min. Furthermore, the ZM extract contained 0.025 mg/g *p*-coumaric acid (Figure 1).

3.2. Effects of FSH-ZM on Body Weight and Organ Weights of UVB-Irradiated HRM-2 Mice

Table 2 shows the effects of FSH-ZM on weight gain, food intake, food efficiency rate (FER), and organ weights. There were no significant differences in the aforementioned factors in all animals.

3.3. Effects of FSH-ZM on mRNA Expressions Related to Skin Moisturizing in UVB-Irradiated HRM-2 Mice

The mRNA expression of HAS2 was significantly decreased (64.8%) in the C group compared with that in the NC group and significantly increased (127.7%) in the PC1 group compared to the C group. Compared with that in the C group, the groups receiving diet containing Z showed significantly increased (Z100: 47.5% and Z200: 89.7%) mRNA expression of HAS2 in a dose dependent manner. The mRNA expression of fibrillin-1 was significantly decreased (80.5%) in the C group compared with that in the NC group

and significantly increased (189.8%) in the PC1 group compared with that in the C group. Compared with that in the C group, the groups receiving the diet containing Z showed significantly increased (Z100: 99.1% and Z200: 129.5%) mRNA expression of fibrillin-1; although no significant difference was observed between the Z100 and Z200 groups. The mRNA expression of elastin was significantly decreased (76.8%) in the C group compared with that in the NC group, and significantly increased by two times in the PC1 group compared to the C group. When compared with that in the C group, the groups receiving the diet containing Z showed a significantly increased (Z100: 90.6% and Z200: 148.6%) mRNA expression of elastin, but there was no significant difference between the Z100 and Z200 groups. In the PC2 group, there were no significant differences in the levels of mRNA expression of HAS, fibrillin-1, and elastin compared with that in the C group ($p < 0.05$; Figure 2A–C).

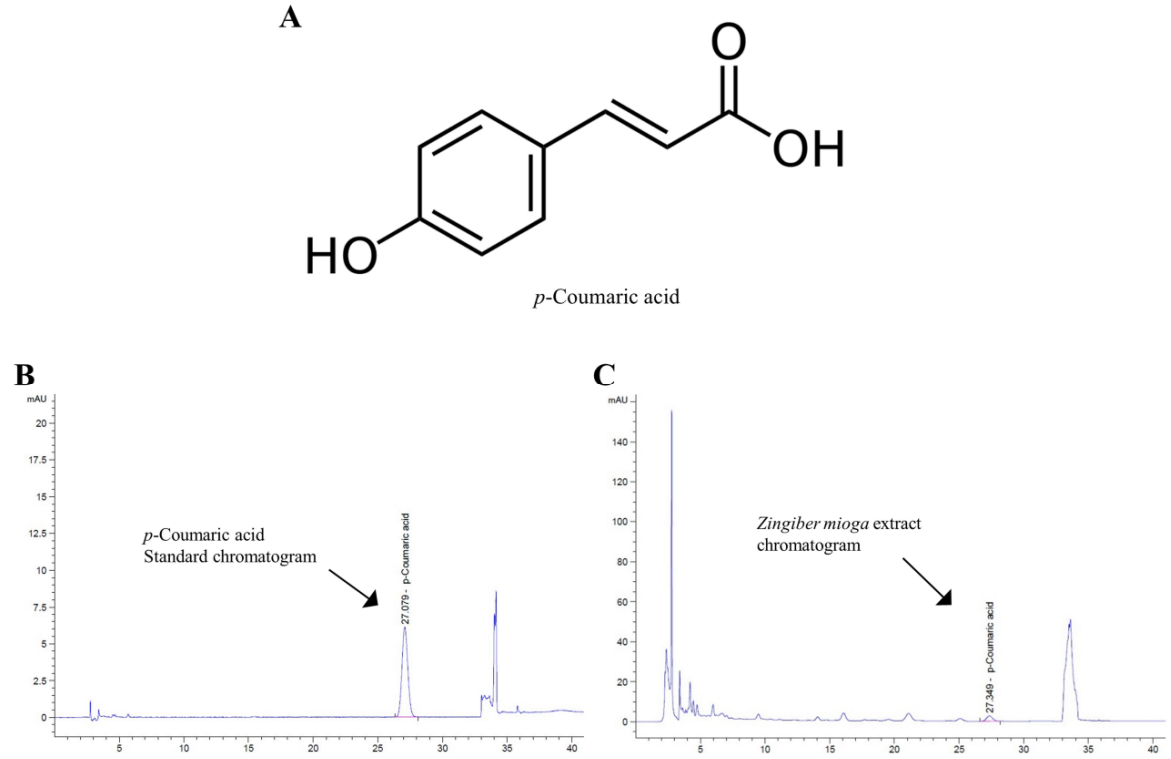

Figure 1. HPLC chromatograms of p-coumaric acid in the ZM extract at 310 nm. (**A**) p-coumaric acid structure, (**B**) p-coumaric acid standard chromatogram, (**C**) ZM extract chromatogram. The peaks appeared with retention times of approximately 27.079 min.

Table 2. Effects of *FSH-ZM* on body weight and organ weights of HRM-2 mice with or without UVB-irradiation.

	UVB Irradiation					
Groups	***NC***	***C***	***PC1***	***PC2***	***Z100***	***Z200***
Weight gain (g) *	5.68 ± 2.33 ^{ns}	6.65 ± 1.05	5.11 ± 0.60	5.75 ± 0.96	5.82 ± 1.17	5.15 ± 1.69
Food intake (g/day/mouse)	3.44 ± 0.16 ^{ns}	3.47 ± 0.15	3.47 ± 0.05	3.43 ± 0.16	3.44 ± 0.14	3.39 ± 0.14
FER **	3.75 ± 0.54 ^{ns}	4.35 ± 0.69	3.35 ± 0.39	3.81 ± 0.64	3.84 ± 0.77	3.45 ± 1.13
Tissue weight (g)						
Liver	1.81 ± 0.04 ^{ns}	1.82 ± 0.05	1.79 ± 0.04	1.80 ± 0.06	1.77 ± 0.02	1.79 ± 0.04
Kidney	0.45 ± 0.02 ^{ns}	0.46 ± 0.02	0.47 ± 0.04	0.47 ± 0.05	0.45 ± 0.02	0.46 ± 0.03
Spleen	0.15 ± 0.05 ^{ns}	0.16 ± 0.03	0.18 ± 0.07	0.16 ± 0.05	0.14 ± 0.01	0.13 ± 0.02

Values are presented as mean ± standard deviation (n = 6), and different superscript letters indicate significance at $p < 0.05$. NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200 mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w.; ns, not significant. * Weight gain (g/6 weeks) = final body weight (g)–initial body weight (g). ** FER (Food efficiency rate) = weight gain (g)/total food consumption (g) × 100.

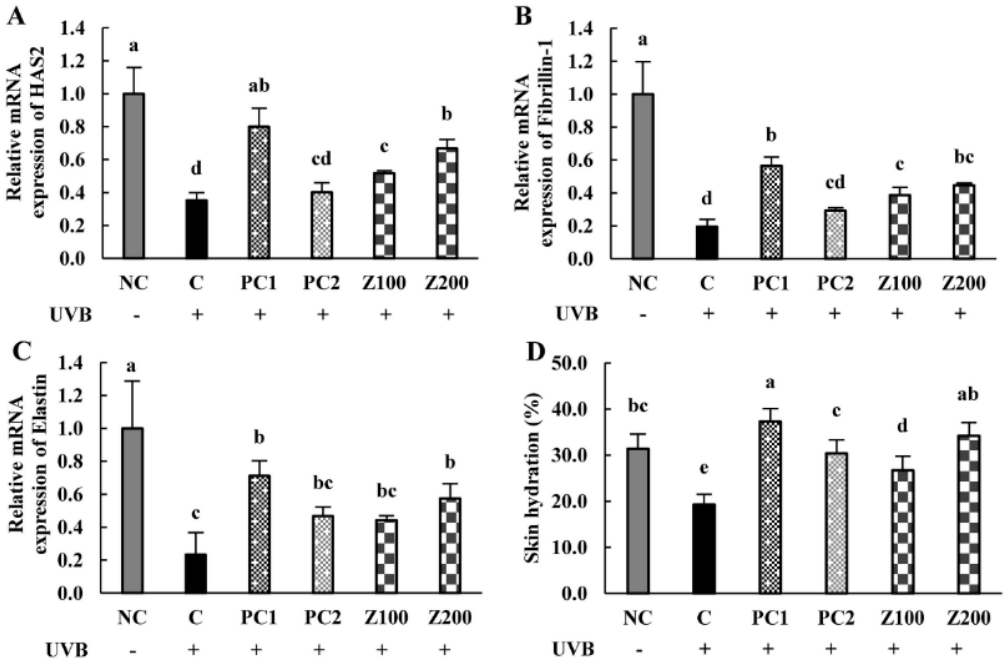

Figure 2. Effects of FSH-ZM on the expression of mRNAs related to skin moisturizing and skin hydration content in skin from the back of UVB-irradiated HRM-2 hairless mice. (**A**) The expression of HAS2 mRNA. (**B**) The expression of Fibrillin-1 mRNA. (**C**) The expression of Elastin mRNA. (**D**) Skin hydration. NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w. Values are presented as mean ± standard deviation (n = 6), and different superscript letters indicate significance at $p < 0.05$.

3.4. Effects of FSH-ZM on Skin Hydration in UVB-Irradiated HRM-2 Mice

We found that the moisture content of skin was significantly decreased in the C group (19.33 ± 2.16%) compared with that in the NC group (31.33 ± 3.27%) and significantly increased in the PC1 and PC2 groups (37.33 ± 2.73% and 30.33 ± 2.94%, respectively) compared with that in the C group. Compared with the C group, the groups receiving the diet containing Z showed significantly increased in the moisture content of skin in a dose-dependent manner (26.67 ± 3.08% and 34.17 ± 2.93%; $p < 0.05$; Figure 2D).

3.5. Effects of FSH-ZM on Wrinkle Formation and Skin Thickness in UVB-Irradiated HRM-2 Mice

Wrinkle formation was significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 and PC2 groups compared to the C group. Compared with that in the C group, the groups receiving the diet containing Z showed significantly decreased wrinkle formation in a dose-dependent manner (Figure 3A).

Skin thickness was significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 and PC2 groups compared to the C group. Compared with that in the C group, the groups receiving the diet containing Z showed significantly decreased skin thickness in a dose-dependent manner (Figure 3B).

3.6. Effects of FSH-ZM on mRNA and Protein Expressions Related to Wrinkle Formation in UVB-Irradiated HRM-2 Mice

The mRNA or protein expressions of the MAPK8, AP-1, MMP1, MMP3, and MMP9 were significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 and PC2 groups compared to the C group. In comparison with the C group, the groups receiving the diet containing Z decreased the mRNA or protein expression of the MAPK8, AP-1, MMP1, MMP3, and MMP9 in a dose-dependent manner ($p < 0.05$). On the other hand, the mRNA or protein expression of procollagen and collagen type I (COL1A1) were significantly decreased in the C group compared with that in the NC

group and significantly increased in the PC1 group compared to the C group. Compared with that in the C group, the groups receiving the diet containing Z increased the mRNA or protein expression of the procollagen and collagen type I (COL1A1) in a dose-dependent manner ($p < 0.05$; Figure 4).

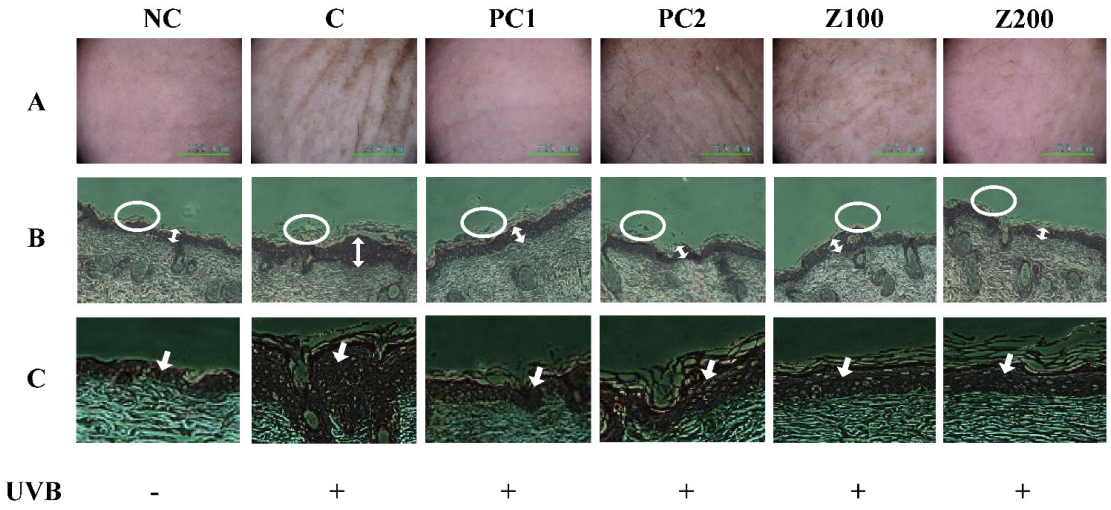

Figure 3. Effects of FSH-ZM on wrinkle suppression and skin thickening in skin from the back of UVB-irradiated HRM-2 hairless mice. (**A**) The wrinkle photography. (**B**) H&E staining. (**C**) Fontana–Masson staining. NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200 mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w.

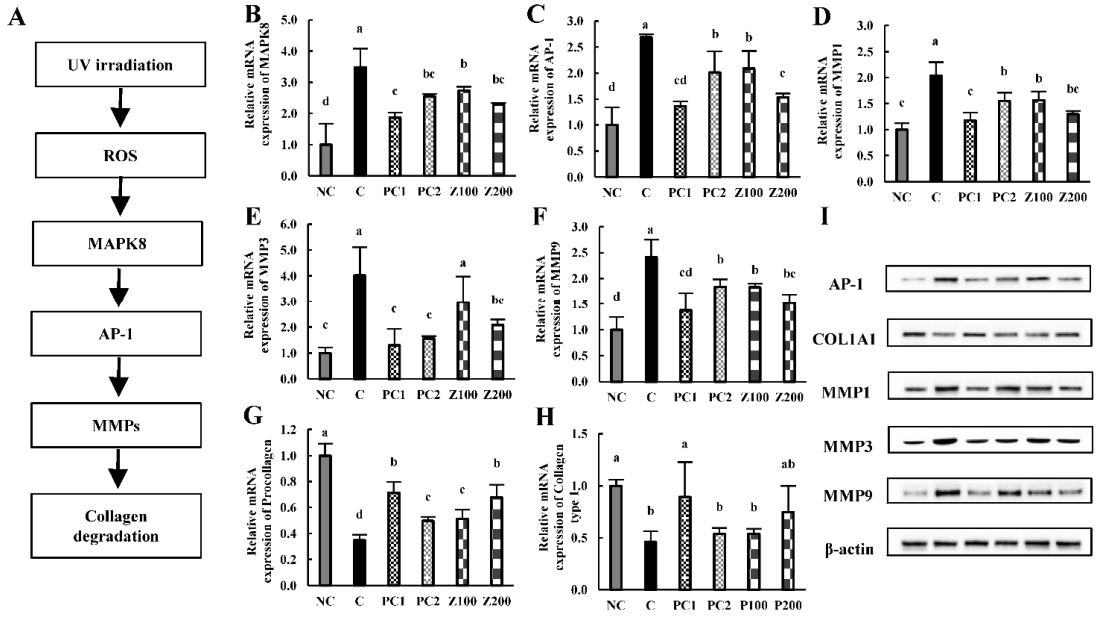

Figure 4. Inhibitory effects of FSH-ZM on mRNA and protein expression related to wrinkle formation in skin from the back of UVB-irradiated HRM-2 hairless mice. (**A**) The mechanism of wrinkle formation. (**B**) The expression of MAPK8 mRNA. (**C**) The expression of AP-1 mRNA. (**D**) The expression of MMP1 mRNA. (**E**) The expression of MMP3 mRNA. (**F**) The expression of MMP9 mRNA. (**G**) The expression of Procollagen mRNA. (**H**) The expression of Collagen type I mRNA. (**I**) Protein expressions. NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w. Values are presented as mean ± standard deviation (n = 6), and different superscript letters indicate significance at $p < 0.05$.

3.7. Effects of FSH-ZM on mRNA Expressions Related to Pro-Inflammatory Cytokines in UVB-Irradiated HRM-2 Mice

The mRNA expressions of the IL-1β, IL-6, and TNF-α were significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 group compared with that in the C group. In comparison with the C group, the groups receiving the diet containing Z showed decreased mRNA expression of IL-1β, IL-6, and TNF-α in a dose-dependent manner ($p < 0.05$; Figure 5A).

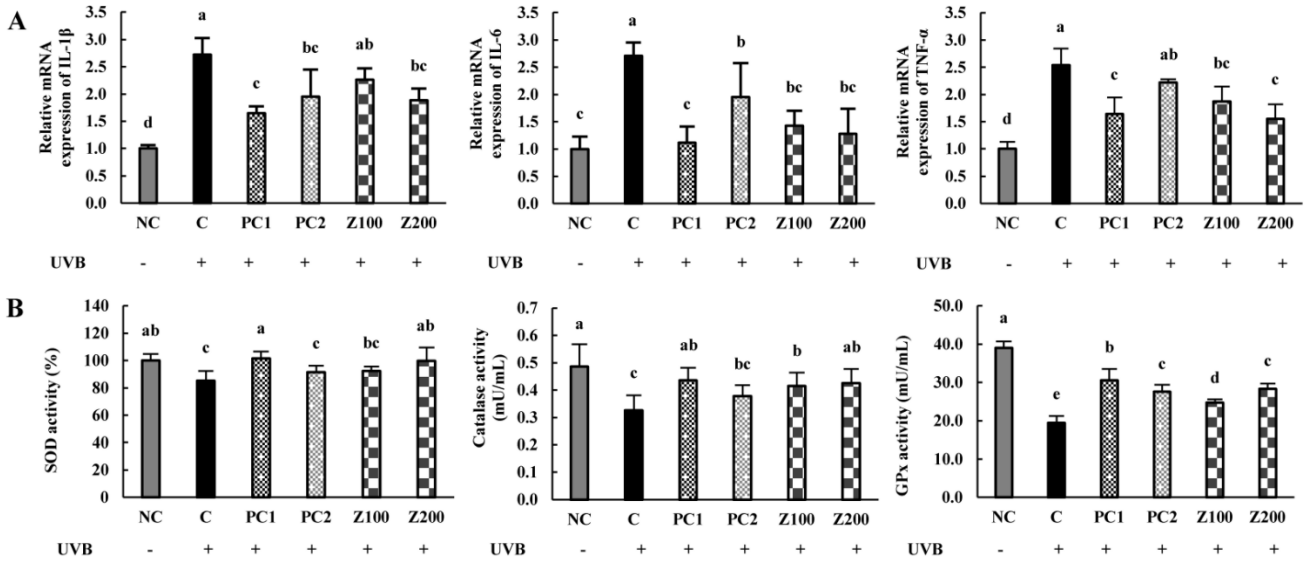

Figure 5. Effects of FSH-ZM on pro-inflammatory cytokine levels and antioxidant activity in skin from the back of UVB-irradiated HRM-2 hairless mice. (**A**) mRNA expressions (IL-1β, IL-6, TNF-α). (**B**) ELISA (SOD, catalase, GPx). NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w. Values are presented as mean ± standard deviation (n = 6), and different superscript letters indicate significance at $p < 0.05$.

3.8. Effects of FSH-ZM on Antioxidant Activity in UVB-Irradiated HRM-2 Mice

SOD activity was significantly decreased in the C group (85.27 ± 7.00%) compared with that in the NC group (100.00 ± 4.67%) and significantly increased in the PC1 group (101.58 ± 4.93%) compared with that in the C group. In comparison with the C group, the Z groups (Z100: 92.48 ± 3.00%, Z200: 99.73 ± 9.72%) showed an increase in the SOD activity in a dose-dependent manner and showed significance only for the Z200 group ($p < 0.05$). The catalase activity was significantly decreased in the C group (0.33 ± 0.05 mU/mL) compared to the NC group (0.49 ± 0.08 mU/mL) and significantly increased in the PC1 group (0.44 ± 0.05 mU/mL) compared with that in the C group. Compared with the C group, the Z groups (Z100: 0.41 ± 0.05 mU/mL, Z200: 0.43 ± 0.05 mU/mL) showed significantly increased catalase activity, but there was no significant difference between Z100 and Z200 ($p < 0.05$). The GPx activity was significantly decreased in the C group (19.42 ± 1.85 mU/mL) compared to the NC group (39.03 ± 1.69 mU/mL) and significantly increased in the PC1 group (30.56 ± 2.95 mU/mL) compared to the C group. Compared with that in the C group, the Z groups (Z100: 24.75 ± 0.83 mU/mL, Z200: 28.35 ± 1.31 mU/mL) showed a significant increase in the GPx activity in a dose-dependent manner ($p < 0.05$; Figure 5B).

3.9. Effects of FSH-ZM on Skin Pigmentation in UVB-Irradiated HRM-2 Mice

Skin pigmentation was significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 group compared to the C group. Additionally, when compared with the C group, the Z100 and Z200 groups showed significantly decreased pigmentation in a dose-dependent manner (Figure 3C).

3.10. Effects of FSH-ZM on mRNA and Protein Expressions Related to Melanogenesis in UVB-Irradiated HRM-2 Mice

The mRNA and protein expression of the CREB and MITF were significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 and PC2 groups compared with that in the C group. In comparison with the C group, the groups receiving the diet containing Z significantly decreased the mRNA and protein expression of the cAMP response element binding protein (CREB) and microphthalmia-associated transcription factor (MITF) in a dose-dependent manner ($p < 0.05$). Furthermore, the mRNA expression of the tyrosinase and tyrosinase-related protein 1 (TRP1) were significantly increased in the C group compared with that in the NC group and significantly decreased in the PC1 and PC2 groups compared to the C group. Compared with that in the C group, the groups receiving the diet containing Z showed a significant decrease in the mRNA expressions of tyrosinase and TRP1 in a dose-dependent manner ($p < 0.05$; Figure 6).

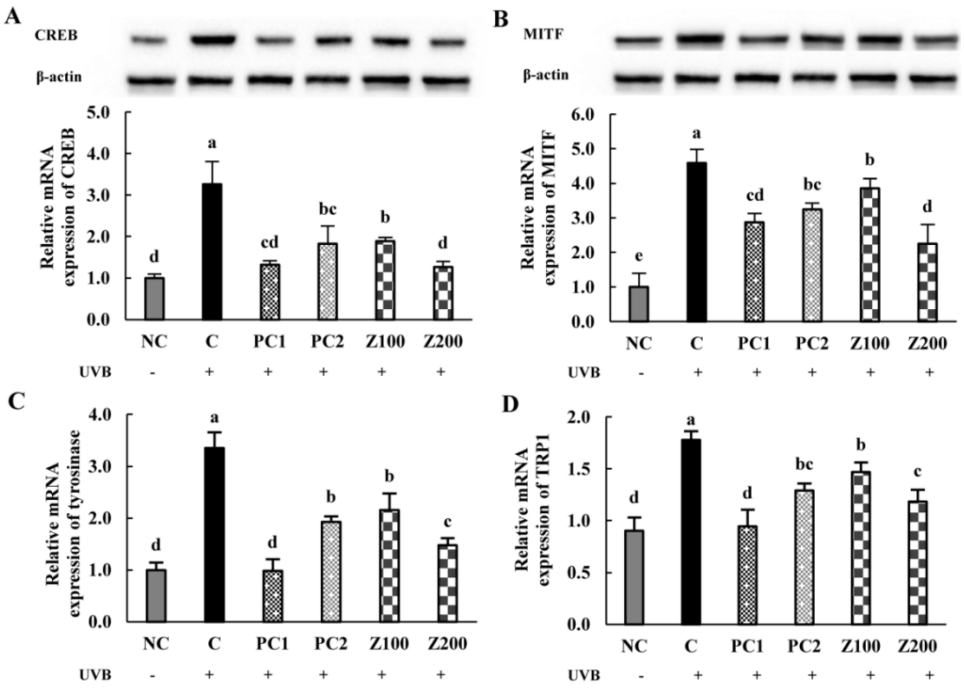

Figure 6. Inhibitory effects of FSH-ZM on the expressions of mRNA and proteins related to melanogenesis in skin from the back of UVB-irradiated HRM-2 hairless mice. (**A**) The expression of CREB at mRNA and protein level. (**B**) The expression of MITF at mRNA and protein level. (**C**) The expression of tyrosinase mRNA. (**D**) The expression of TRP1 mRNA. NC, normal control; C, UVB-irradiated control; PC1, positive control 1, L-ascorbic acid 200 mg/kg b.w.; PC2, positive control 2, Arbutin 200 mg/kg b.w.; Z100, FSH-ZM 100 mg/kg b.w.; Z200, FSH-ZM 200 mg/kg b.w. Values are presented as mean ± standard deviation (n = 6), and different superscript letters indicate significance at $p < 0.05$.

4. Discussion

Due to climate change and the destruction of the ozone layer, the amount of UV radiation on the earth's surface is progressively increasing; this intensified UV radiation has raised concerns about skin health, globally [17]. UV rays reduce skin moisture, cause wrinkles, pigmentation, and the loss of skin fiber [18]. Long-term exposure to UV light can cause skin cancers such as melanoma, and the use of some drugs to treat skin cancers such as advanced melanoma can cause a variety of side effects [19]. Therefore, there is a growing interest in the development of functional health foods and cosmetics that can help prevent UV ray induced skin damage [1].

For centuries, ginger (*Zingiber officinale* Roscoe, *Zingiberacae*) has been a crucial ingredient in East Asian markets, found in Ayurvedic and Tibb-Unani herbal medicines for the treatment of asthma, stroke, rheumatism, catarrh, nervous diseases, constipation, gingivitis,

and diabetes [20–22]. *Zingiber mioga* (ZM), a member of the ginger family, is a perennial plant and is grown in small quantities in Jeju and the southern coast of Korea [23]. Its bud has a peculiar smell and has been used as an ingredient in spices, herbs and pickles for a long time [24]. In addition, ZM's young buds contain zingerene, zingerone, shogaol, β-phellanderene, aframodial, and galanal A/B, shown to be effective in the treatment of leucorrgea, menstrual irregularity, odynolysis, eye inflammation, sputum, and heart disease [25,26]. Moreover, several flavonoids (e.g., rutin, pentahydroxyflavone, and galangin), anthocyanin compounds (e.g., delphinidine glucoside and delphinidin), [8]-gingerol, and oleamide have been known to be present in ZM [27]. Rutin, a flavonoid present in ZM, has been shown to be effective in preserving phospholipid membranes in skin fibroblasts destroyed after UV irradiation [28]. As a result of measuring the content of *p*-coumaric acid, which was selected as a candidate among the active substances of ZM, 0.025 mg/g of *p*-coumaric acid was detected in the ZM extract, although not mUch. *p*-coumaric acid has been reported to have antimicrobial, antiviral, and anti-cancer activity, and is also used as a hypopigmenting agent [29–32]. Vitamin C has been used in a wide variety of skin studies. Vitamin C is known to enhance collagen formation [33], remove oxidants [34], inhibit melanogenesis [35], interact with cell signaling pathways [36], inhibit winkles [37], inhibit skin surface roughness [38], and delay skin aging [39]. Also, arbutin has been studied for skin depigmentation [40]. Although there are many different studies of ZM, no animal studies have yet been conducted in skin experiments.

In this study, we investigated whether ZM extracts play a role in skin moisturizing, wrinkle suppression, and skin depigmentation in vivo. Z increased the HAS2 mRNA expression, which maintains the concentration of hyaluronic acid, and elastin and fibrillin-1 mRNA expression, which maintain the skin barrier and elasticity, compared with the UVB-irradiated control group. UV irradiation of the skin has been reported to cause inflammation and inhibit hyaluronic acid synthesis [41]. In another recent skin study, we found that lamb placenta increased mRNA expression of HAS2, elastin, and fibrillin-1 in UVB-irradiated animal models [42]. Additionally, Z increased skin hydration content compared with the UVB-irradiated control group. Moisture content plays an important role in all aspects of skin aging. Recently, *Ultica thunbergiana* also increased skin moisture content in the background of UVB-irradiation in an in vivo study [43].

In living cells, UV irradiation regulates nuclear factor-kappa B (NF-κB) expression by activating mitogen-activated protein kinase (MAPK) and generating ROS [44]. The MAPK pathways regulate the transcription factor activating protein 1 (AP-1), a heterodimer consisting of c-Fos and c-Jun, thereby upregulating MMPs in the skin [45,46]. Z reduced the mRNA or protein expression of MAPK, AP-1, MMP1, MMP3, and MMP-9 compared to the UVB-irradiated control group. Moreover, Z increased the mRNA or protein expression of procollagen and collagen type I compared to the UVB-irradiated control group. More than 90% of collagen, which is the most abundant in the dermis, exists in the form of collagen type I produced from procollagen [47]. Among the MMPs, MMP-3 has a substrate specificity for collagen type IV, and MMP-2 has substrate specificity for collagen type IV as well as gelatin. MMP-2 and MMP-9 are known as gelatinases A and B, but MMP-3 acts as a promotor for MMP-1 and MMP-9. As MMP-1 activity leads to MMP-9 activity through MMP-2 activity, it is pertinent to identify MMP-1, MMP-3, and MMP-9 in this study [48].

UVB stimulates ROS production after reaching the skin epidermis, causing oxidative damage to cell membrane lipids and promoting the release of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α [2,49]. These inflammatory reactions can cause skin disease such as psoriasis, wrinkles and atopic dermatitis [50]. In addition, they are known to increase the expression of MMPs, reduce collagen synthesis, promote degradation, reduce skin elasticity, and promote skin aging [51]. Z reduced the mRNA expression of IL-1β, IL-6, and TNF-α compared to that in the UVB-irradiated control group, and increased the activities of SOD, catalase, and GPx compared to that in the UVB-irradiated control group. Oxidative stress is caused by overloading free radicals or insufficient antioxidants and increases during exposure to infection from contaminants, inflammation, sunlight and

radiation. These oxygen radicals damage cellular DNA and proteins, causing a variety of cancers, including skin cancer. Some phytochemicals have been shown to maintain the homeostasis of the redox state of intracellular enzymes [52–55].

Melanin has important physiological functions such as protecting skin from UV damage by absorbing UV light and removing ROS [56]. However, excessive melanin production can lead to pigmentation, post-inflammatory hyper pigmentation, solar lentigo, and melanoma. To prevent these stresses, tyrosinase inhibitors and melanogenesis inhibitors have been developed [57,58]. Melanin pigments are synthesized as either red based eumelanine or brown based pheomelanin, which begins with the activation of tyrosinase in melanocytes. In addition, the amount of L-tyrosine and L-DOPA increases, leading to the increase in the levels of tyrosinase-related protein 2 (TRP-2) and TRP-1 through the synthesis of L-dopaquinone [59–61]. The expression of tyrosinase and TRP-1 gene is activated by MITF, which is activated by cyclic adenosine monophosphate (cAMP). Thus, the down-regulation of melanocyte migration in melanocytes leads to the mechanism of depigmentation and down-regulation of melanin synthesis [62,63]. Z reduced the mRNA or protein expressions of CREB, MITF, tyrosinase, and TRP-1 compared to the UVB-irradiated control group. These results are in line with the recent findings that Bamboo stems or the flower extracts from *Paeonia decomposita* and *Paeonia ostii* inhibit melanogenesis through the CREB-mediated pathway [64,65].

5. Conclusions

ZM increased the production of hyaluronic acid by increasing the expression of genes such as HAS2, a skin moisturizing factor, and regulated the expression of genes involved in collagen synthesis in the dermal tissue. In addition, it inhibited the occurrence of pigmentation by affecting the expression of CREB and MITF, which are involved in melanin pigment production. The results of this study, thus, prove that ZM extract helps in improving the moisturization and depigmentation of skin, as well as in reducing wrinkle formation in vivo. In particular, *p*-coumaric acid, a bioactive substance candidate for ZM, was detected in ZM extract, and the effect of ZM on the reduction of skin moisturization, wrinkles, and pigmentation caused by UV rays may have been the effect of *p*-coumaric acid.

Therefore, extracts of *Zingiber mioga* could potentially be used in the development of nutraceutical, medical, and cosmetic products aimed at improving general skin health. However, more studies are needed to evaluate the safety and efficacy of these extracts in human models.

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