International Journal of Advanced Smart Convergence Vol.8 No.1 113-125 (2019)
http://dx.doi.org/10.7236/IJASC.2019.8.1.113

IJASC 8-1-14

The Effects of Reducing Skin Wrinkles and Improving Skin Elasticity from Korean Radish Extract

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Abstract

The radish skin and radish greens are an edible part of the radish. But they are removed before eating the radish and used as a byproduct or an animal feed material because of their tough and rough texture. This study was conducted to investigate the effect of supercritical heat-treated radish-extract on UV-induced HRM-2 wrinkled mouse animal model on anti-aging wrinkles. Supercritical heat-treated radish-extract was applied on the back of seven-weeks old HRM-2 mice. The effect of HRE on skin thickness, elasticity and wrinkle formation of the mice was observed by using UVB lamp to induce melanogenesis and wrinkle formation. As the result, increased depth of wrinkles was observed in the negative control group in comparison to the normal group. In contrast, decreased depth of wrinkles was observed in the radish-extract-free group compared to the negative control group. In the study of the effect of radish-extract on wrinkle-formation related gene expression and protein what protein expression, MMP-2 and MMP-9 gene expression significantly increased in the negative control group compared to the normal group. The gene expression reduced in dependence to the mass of radish-extract treated. Similar to quantitative results of mRNA expression, the expression of MMP-2 protein increased as a result of UVB-irradiation. The MMP-2 expression was inhibited in dependence to the mass of radish-extract treated. In conclusion, the supercritical heat-treated radish-extract has an effect on improving skin wrinkles not only when it is applied to the skin but also when orally ingested. Thus, it can be effectively used as a composition to health functional products. Therefore we can also conclude that radish a food that does not show any side-effects even upon long-term intake can reduce wrinkle formation as well as improve skin elasticity when taken regularly for a long period.

Keywords: *Korean radish, Epidermal growth factor, Anti-wrinkle effect, Anti-aging effect, UVB*

1. Introduction

Wrinkles can be defined as the folding of the skin, which is caused by reduced tension and elasticity of the skin. Wrinkles are initially fine lines, but gradually become larger and deeper. Wrinkles are formed by the changes in physical properties (including elasticity) of the skin due to changes in the properties and quantities

Manuscript Received: Feb. 3, 2019 / Revised: Feb. 9, 2019 / Accepted: Feb. 13, 2019
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of dermal extracellular matrix proteins or formed by the reduction of skin moisture and subcutaneous fat. Wrinkles begin to appear from around 20 years of age and become more severe with age. Since wrinkles are formed more quickly in body areas which are more often used with exercise, the face that uses a lot of muscles to change facial expressions is prone to wrinkles. Skin aging represented by wrinkles can be roughly divided into two types. The first type is intrinsic aging which refers to a phenomenon of aging that is unavoidable with age. The clinical features of intrinsic aging are relatively mild, and include fine wrinkle lines, dry skin, reduced skin elasticity, and the like. The second type is photoaging which results from a combination of intrinsic aging and the effects of ultraviolet rays and can be prevented by avoiding exposure to ultraviolet rays. It is mainly observed in skin areas exposed to sunlight for a long time, including the face, the back of the hand, the back of the neck, etc. Regarding its clinical features, its symptoms are more severe and observed earlier than those of intrinsic aging[1-4]. For example, irregular pigmentation occurs in the skin exposed to sunlight, hyperpigmentary diseases such as solar lentigo increase, the skin becomes very rough and dry, and the skin elasticity decreases so that the skin becomes sagged in severe cases. Wrinkles caused by photoaging are also thicker and deeper than those caused by intrinsic aging, and involve fine lines. The skin is exposed to ultraviolet rays during walking outside in daily life, even thought the amount of ultraviolet rays to which the skin is exposed is small. It is known that when the skin is exposed to ultraviolet rays, the synthesis of collagen in the skin decreases in proportion to the amount of ultraviolet rays irradiated, and the expression of matrix metalloproteinases (MMPs), which are enzymes that degrade extracellular matrices including collagen, increases. The degradation of matrix proteins by MMPs is a kind of skin damage caused by sunlight. The process of wound healing, including the synthesis of new collagen, continues, but the wound healing process is not always perfect. For this reason, if the damage continues for a long time, skin aging, including wrinkles, gradually becomes more severe[5, 6, 7]. Due to ozone layer destruction caused by environmental pollution, the amount of ultraviolet rays reaching the ground is increased, and the region of ultraviolet rays is also changed. Thus, photoaging is gradually increasing, and the risk of photoaging is expected to increase further in the future. In order to prevent damage from being caused by ultraviolet rays, UV blocking agents have been used. However, it has recently been reported that the UV clicking agents themselves induce mutations which may cause skin cancer. In addition, when the skin is suddenly exposed to sunlight, appropriate amounts of irritation alleviating agents and antioxidants are used to calm the skin. However, this method is not sufficiently effective in soothing the skin damaged by ultraviolet rays or providing anti-inflammatory activity. Since this skin damage caused by ultraviolet rays is ultimately related to skin aging, a method capable of protecting the skin from ultraviolet rays is one of the concerns of the cosmetic industry. Accordingly, many studies have been conducted to reduce skin wrinkles and improve skin elasticit[8, 9, 10].

The radish is a vegetable of the family Cruciferae containing volatile sulfur-compounds that cause its unique spiciness. The spiciness peculiar to the radish is caused by the production of thiocyanate and isothiocyanate released enzymatically from the thioglucoside contained in the radish as a result of the enzyme glucosidase activity when the radish is cut to break cells. The radish contains a larger amount of free amino acids, sugars, calcium, phosphorus, etc. than other vegetables. The root of radishes contains sugar components like glucose and fructose and other ingredients, such as coumaric acid, caffeic aid, ferulic acid, phenylpyruvic acid, gentidin acid, hydroxyl benzoic acid, and a variety of amino acids. Particularly, it has the content of vitamin C amounting up to 20 to 25 mg and becomes an important source of vitamin C in winter. According to the ancient medicinal records, the root of radish, *nabok*, has the curative effects on phlegm, coughing, dysentery, etc. and eliminates food poisoning associated with fish, shellfish, and noodles. Diastase contained in the radish is used to promote digestion, neutralize the effects of food poisoning, and ease a hangover, and rapine is known as an antibiotic component against germs, fungus, parasites, etc[11,12].

Radish skin and radish greens are an edible part of the radish. But they are removed before eating the radish and used as a byproduct or an animal feed material because of their tough and rough texture. The research have conducted studies on a processing method capable of further increasing the physiological activity of radish, and as a result, have found that processed radish products including by-products may be effectively used to reduce skin wrinkles and improve skin elasticity[13,14].

2. Experiment Materials

2.1 Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM) [Daegu, Korea], foetal bovine serum (FBS) (WelGene Co., Korea), streptomycin and penicillin (Lonza,MD, USA), TRIZOL® reagent (Invitrogen, Carlsbad, CA, USA), oligodT (Bioneer oligo synthesis), MITF, TYR, TRP-1, TRP-2 and β-actin primers were obtained from Bioneer (Daejeon, Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma-Aldrich. Antibodies for MITF, TYR, TRP-1 and TRP-2 were obtained from Santa Cruz (Santa Cruz biotechonology, Inc, Texas, USA). Tyrosinase from mushroom and L-3,4-dihydroxyphenylalanine (L-DOPA) were purchased from Sigma (St. Louis, MO, USA). All other reagents were of local analytical grade.

2.2 Heat Treatment of Radish and Preparation of Extract

Radish was purchased from an agricultural and marine products wholesale market in Korea, and the whole plant, including leaves and peels, were used after washing.

A heat-treatment device (Jisco, Seoul, Korea) was used, which was designed and constructed such that it could resist a pressure of 10 kg/cm^2 or higher. The whole radish sample was placed in an inner container which was then placed in an outer container containing a predetermined amount of water. Then, the radish sample was heated at a predetermined temperature for a predetermined time so as to prevent the sample from being carbonized by direct heat transfer. During the heat-treatment process, the radish sample could be treated with steam. The heat-treatment temperature was set at 110, 120, 130, 140 and 150°C, and the heat-treatment time was set at 6 hours.

2.3 Preparation of Heat-Treated Radish Extract (HRE)

The heat-treated radish sample was cooled, and then ground using a grinder, and a 10-fold volume (v/v) of distilled water was added thereto, followed by extraction for 14 to 16 hours. The extract was filtered, and then freeze-dried and used.

2.4 Preparation of Heat-Treated Radish Supercritical Extract (HO)

The heat-treated radish was dried in a well-ventilated place for 24 hours, and then ground to a size of 200 mesh or less. The ground material was placed in a supercritical fluid extractor and extracted with supercritical CO_2 by use of butylene glycol as a co-solvent at an extraction temperature of 40 to 80°C and an extraction pressure of 200 to 500 bar. The collected extract was freeze-dried and used.

3. Experimental Method

3.1 Preparation and Treatment of Test Animals

The Seven-week-old HRM-2 mice were purchased, acclimated in an animal room for one week, and then used in the experiment. The test animals were housed at a temperature of 22°C and a humidity of 50% with 12-hour light and 12-hour dark cycles and were allowed access to feed and water *ad libitum*. After one week of

the acclimation, the sample prepared in Example 1 was administered orally to the mice once a day or applied to the right portion of the two equally divided portions of the back of each HRM-2 mouse. Then, the mice were irradiated with UVB, and the effect of the sample on skin aging was evaluated. The test animals were grouped as shown in Table 1, and the number of the test animals per group was five.

Table 1. Experimental design

Group	Treatment
Normal	HRM-2 without any treatment
Negative control	HRM-2 + UVB irradiation vehicle
Positive control	HRM-2 + UVB irration + 20 μl 0.01% sunblock
Test group 1 (HRE 300 mpk)	HRM-2 + UVB irration + 300 mg/kg HRE oral administration
Test group 2 (HRE 150 mpk)	HRM-2 + UVB irration + 150 mg/kg HRE oral administration
Test group 3 (1% HO oint)	HRM-2 + UVB irration + 20 μl 1% HO ointment
Test group 4 (0.5% HO oint)	HRM-2 + UVB irration + 20 μl 0.5% HO ointment

3.2 UVB irradiation and induction of photo aging

HRM-2 mice were irradiated at the dorsal skin with UVB lamp (15 W, maximum wave length 312 nm; UV intensity 100 μW cm $^{-2}$, Ieda Boeki Co., Tokyo, Japan). To evaluate the effects of positive control (0.01% sunblock) and KRG on wrinkle formation and pigmentation, they were applied daily, 5-10 min before exposure of mice to UVB radiation. HRM-2 mice were irradiated with 100 mJ/cm^2 UVB radiation (1 minimal erythematic dose = 100 mJ/cm^2) daily for the first week and then UVB radiation was increased to 200 mJ/cm^2 from 2-5 weeks and mice were monitored 3 times in a week. Dietary intakes and body weight were taken at regular intervals every week till 12 weeks[15-19].

3.3 Body Weight and Food Intake

In order to examine the effects of sample treatment and UVB irradiation on the food intake and body weight of the HRM-2 mice, the body weight and the food intake were measured and recorded at 10:00 every Thursday during the test animal treatment process.

3.4 Skin Wrinkle Evaluation of the Effect of Radish Extract on UVB Irradiation

The degree of skin aging induced by UVB was measured by observing wrinkle formation. To evaluate the formation of wrinkles, each HRM-2 mouse was anesthetized by intraperitoneal injection of chloral hydrate (body weight 0.1 ml/7% CH / 25 g mouse) at 5th week. Exposure to UVB and samples treatment was same as described in the previous section. Skin wrinkles were measured using at 3rd, 4th and 5th weeks using DETAX System II (MIXPAC) and Double-Stick Disc (3M, Germany) after UVB irradiation. Double-Stick Disc (sprayed with DETAX System II) was attached to mouse skin and removed after 2-3 minutes. Disc wrinkles were evaluated according to the scoring system presented by [13]. According to this evaluation, grade 0 is defined as the absence of wrinkles, grade 1 as several shallow wrinkles, grade 2 as some wrinkles, and grade 3 as some deep wrinkles. For visual analysis of skin wrinkles, after disc removal, skin was cleaned with 70% ethanol and photographed with USB Digital Microscope (x400, CE FOROHS, China) [20- 25].

3.5 Evaluation of the Effect of Radish Extract on UVB Irradiation-induced Expression of wrinkle-related gene and protein

To this end, at 5 weeks after the start of UVB irradiation in the experiment, the mice were sacrificed, and the back skin tissue irradiated with UVB was dissected. To the dissected back tissue, 500 μl of Trizol was added. The cells were lysed using a homogenizer, and 100 μl of chloroform ($CHCl_3$) was added thereto, followed by mixing for 15 seconds. The resulting solution was left on ice for 15 minutes, and then centrifuged at 13,000 rpm. About 200 μl of the supernatant was collected and mixed with 200 μl of 2-propanol. The mixture solution was shaken slowly and left on ice for 15 minutes. The resulting solution was centrifuged again at 13,000 rpm, washed with 80% EtOH, and then dried in a vacuum, thereby extracting RNA. 3 μg of the extracted total RNA was denatured at 75°C for 10 minutes, and then 2.5 μl of 10 mM dNTPs mix, 1 μl of random sequence hexanucleotides (25 pmole/ 25 μl), 1 μl of RNase inhibitor (20 U/ μl) as RNA inhibitor, 1 μl of 100 mM DTT, and 4.5 μl of 5 × RT buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$) were added there to, followed by addition of 1 μl of M-MLV RT (200 U/μl), and distilled water treated with DEPC (diethyl pyrocarbonate) was added to a final volume of 20 μl. 20 μl of the reaction mixture was stirred well, and then subjected to centrifugal sedimentation at 2,000 rpm for 5 seconds and incubated on a heating block at 37°C for 60 minutes, thereby synthesizing first-strand cDNA. The synthesized cDNA was left at 95°C for 5 minutes to inactivate M-MLV RT. Using the synthesized cDNA, RT-PCR was performed. The primer sequences used in the PCR are shown in Table 3 below, and an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, USA) was used in the PCR. The Taqman probe (FAM dye-labeled, ABi, USA) was used, and the Mouse GAPDH probe set [Endogenous Control (VIC/MGB Probe, Probe Limited) from Applied Biosystems (4352339E)] was used for the internal standard. They were reacted as the final primer concentration reached 200 nM. For real-time quantitative PCR conditions, predenaturation was conducted for 2 minutes at 50 °C and for 10 minutes at 94 °C, and 40 cycles of denaturation were conducted for 15 seconds 95 °C and for 1 minute at 60°C[13, 14].

Table 2. RT PCR

Primer	Sequences	
MMP-2	**Forward 5'-CAG GGA ATG AGT ACT GGG TCT ATT-3'**	**SEQ ID NO: 1**
	Reverse 5'-ACT CCA GTT AAA GGC AGC ATC TAC-3'	**SEQ ID NO: 2**
MMP-9	**Forward 5'-AAT CTC TTC TAG AGA CTG GGA AGG AG-3'**	**SEQ ID NO: 3**
	Reverse 5'-AGC TGA TTG ACT AAA GTA GCT GGA-3'	**SEQ ID NO: 4**

3.6 Histological observation of skin

The skin tissues extracted from each experimental group were fixed in 10% formalin solution for 48 hours, and then Hematoxylin and Eosin staining (H&E) was done according to[22] for epidermal thickness. For collagen visualization, Masson's Trichome (M-T) staining was performed according to established protocols[23].

3.7 Statistical analysis

Data were presented as mean ±SEM. One way ANOVA, Dunnet's test and un-paired students T test were applied for the statistical evaluation of data or where specifically otherwise indicated. Statistical analyses with ***$p < 0.001$, **$p<0.05$ and *$p<0.01$ were considered significant.

4. Result and Discussion

4.1 Body Weight and Food Intake

In order to examine the effects of sample treatment and UVB irradiation on the food intake and body weight of the HRM-2 mice, the body weight and the food intake were measured and recorded at 10:00 every Thursday during the test animal treatment process as described in figure 1. Figure 1 is a graph showing changes in the body weight with the passage of time, and Figure 2 graphically shows food intake and food efficiency ratio for 5 weeks. Food efficiency ratio (FER %) means the ratio of the gain in body weight for 5 weeks to the food intake for 5 weeks. Figure 1 shows that the significant difference in body weight between the groups by irradiation with UVB or treatment with the sample was not observed. In addition, the food intake in the negative control group slightly decreased compared to that in the normal group, and the food efficiency ratio increased, but this increase was not significant. The food intake in the group administered orally with the radish extract or in the oint-applied group was similar to that in the normal group, but the food efficiency ratio in all the groups significantly decreased to that in the negative control group.

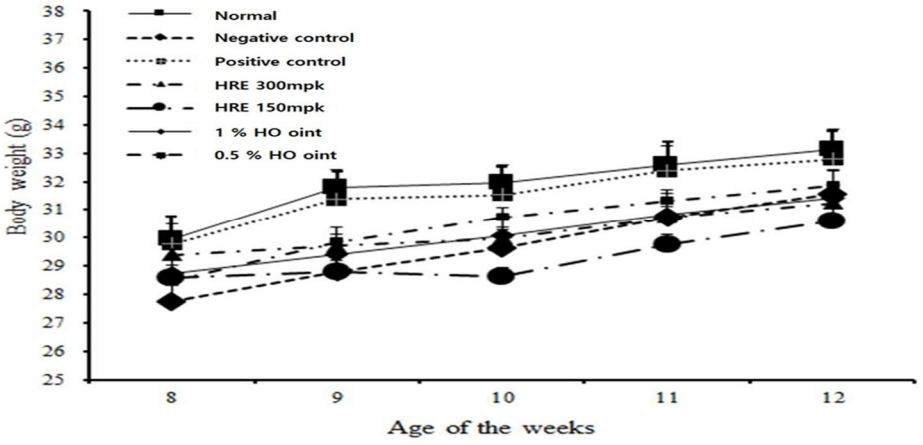

Figure 1. The changes in the body weight.

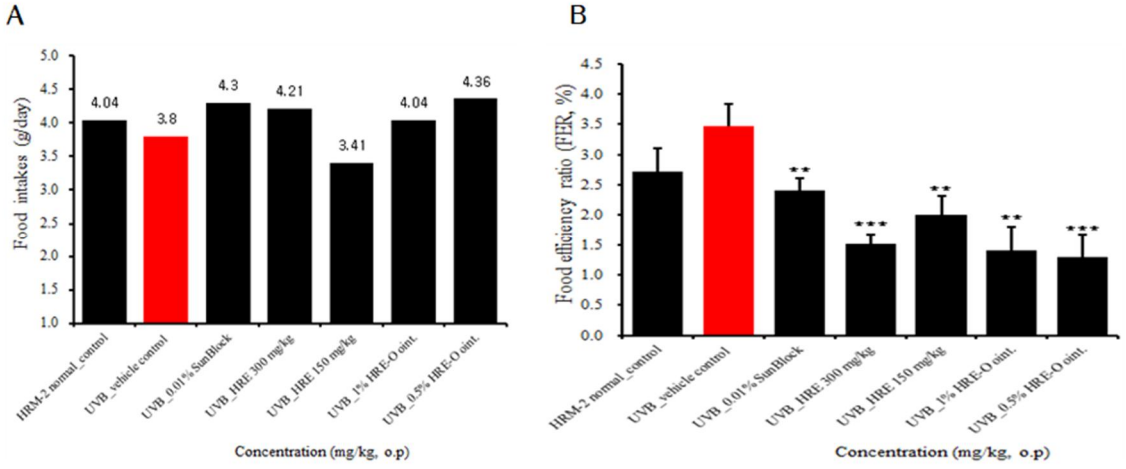

Figure 2. The Change of food intake and food efficiency ratio. Values are expressed as mean ± SEM (n=5).

Exposure to artificial UVB serves as a stress to animal skin resulting in loss of appetite, therefore we evaluated the dietary intake and dietary efficiency of HRM-2 mice exposed to UVB for 5 weeks and given sunblock and HRE orally. As can be seen in Figure 1, there was no significant change in the body weights of

normal, control UVB or the treatment groups. In addition food intake was also found to be significantly increased for positive control and HRE treated groups as shown in Figure. 2. This proves that although UVB acted like stress inducer in mice, HRE treatment was effective in securing the mice from loss of appetite and weight.

4.2 Evaluation of the Effect of Radish Extract on Wrinkle Formation Induced by UVB Irradiation

In order to evaluate the effect of the radish extract on wrinkle formation induced by UVB irradiation, the depth of skin wrinkles was measured using the skin dermobella 3D at 3rd, 4th and 5th after the start of the experiment Figure 3 is a graph showing the results of the measurement. As can be seen therein, the depth of wrinkles was increased in the negative control group compared to that in the negative control group due to UVB irradiation. However, the depth of wrinkles in the group treated with the radish extract was decreased compared to that in the negative control group.

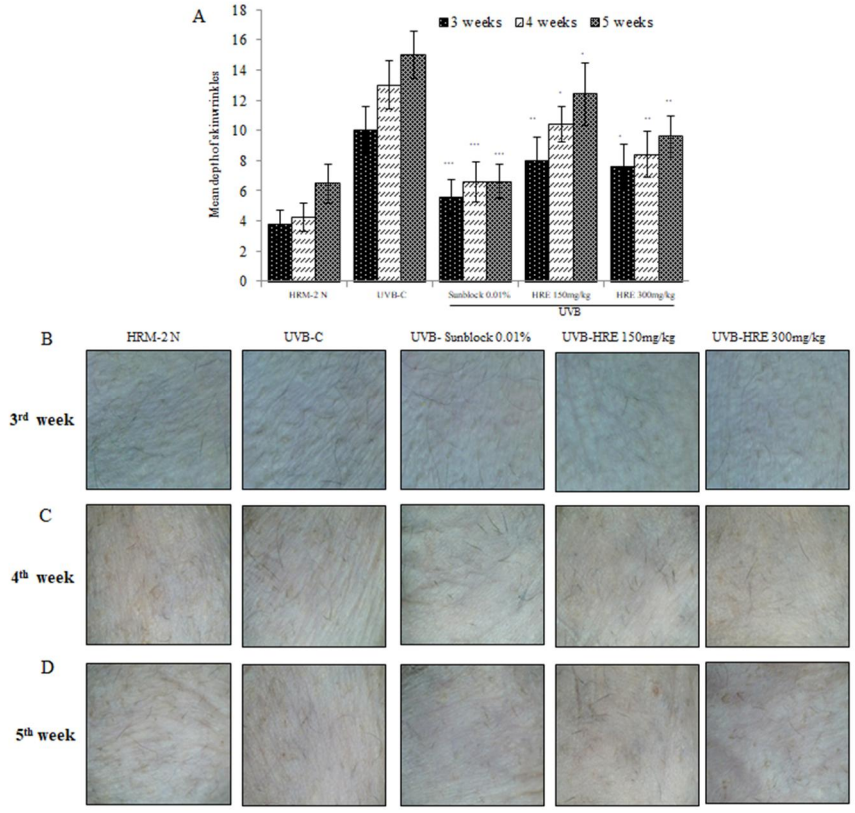

Figure 3. The Analysis of skin dermobella taken from the dorsal skin of hairless mice after UVB-irradiation. Effects of HRE extract on UVB-induced mean depth of skin wrinkle. Values are means±S.E.M. for 5 mice. *Significantly different from UVB/vehicle control treatment ($p<0.05$). **Significantly different from UVB/vehicle control treatment ($p<0.01$). ***Significantly different from UVB/vehicle control treatment ($p<0.001$). ## : $p<0.01$, ### : $p<0.001$ compared with normal group.

4.3 Evaluation of Evaluation of the Effect of Radish Extract on UVB Irradiation-Induced Expression of Wrinkle-Related Gene and Protein

The radish extract had the effect of reducing the depth of wrinkles induced by photo aging. Thus, the expression levels of wrinkle-related genes and protein by UVB irradiation were measured so that quantitative

evaluation could be made Figure 4 graphically shows the results of the RT PCR. As can be seen therein, the expression levels of MMP-2 and MMP-9 genes were significantly increased in the negative control group compared to the normal group due to UVB irradiation, but the expression levels of the genes were significantly decreased by treatment with the radish extract. In addition, the expression levels of the genes decreased dependently on the amount of radish extract used. The expression level of MMP-2 protein in the dissected back skin tissue was measured by ELISA using a mouse MMP-2 ELISA (R & D System, USA) kit according to the manufacturer's manual. As can be seen in Figure 5, similarly to the quantitative results for mRNA, the expression level of the MMP-2 protein was also significantly increased by UVB irradiation, and the radish extract the expression of the MMP-2 protein in a manner dependent on the amount of radish extract used.

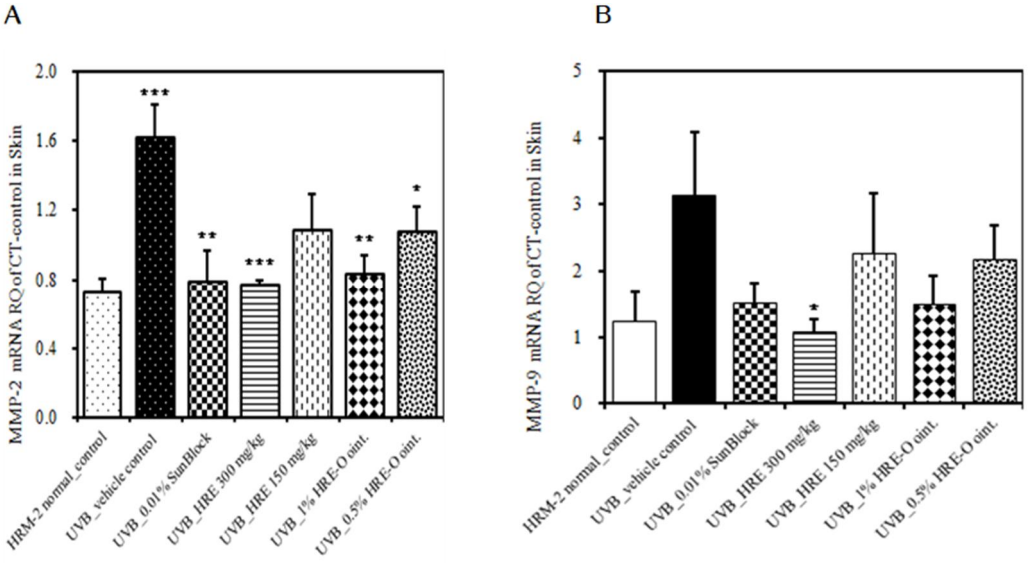

Figure 4. The Effects of HRE extract on MMP-2 and MMP-9 mRNA gene expression in animal skins exposed to UVB. 5 weeks after the initiation of UVB irradiation(the stage of hyperpigmen- tation); The expression of MMP-2 and MMP-9 mRNA levels in each sample was analyzed by real-time PCR and the relative quanyitatives (RQ) of MMP-2 and MMP-9 mRNA was normalized to the quantity of GAPDH. The amount of SYBR Green was measured at the end of each cycle. The cycle number at which the emission intensity of the sample rises above the baseline is referred as to the RQ (relative quantitative) and is proportional to the target concentration. Real time PCR was performed in duplicate and analyzed by a Applied Biosystems 7500 Real-Time PCR system. Values are expressed as means±S.E. from two-independent experiments(* p<0.05, ** p<0.01, *** p<0.001). ### : p<0.001 compared with normal group.

4.4 Effect of Radish Extract on Melanogenesis in Skin Damage Induced Conditional Model by UVB Irradiation

In order to confirm the whitening efficacy of Radish extracts, the difference in the formation of melanin at 1st , 3rd , and 5th weeks of UVB irradiation was examined by dividing the dorsal skin of HRM-2 mice into left(untreated) and right (UVB and samples treated sections). As can be seen from Figure 5A, during the 1st week of exposure of mice to UVB with the treatment of sunblock and HRE, there was no significant decrease in production of melanin by both HRE doses. Then, in the 3rd week, there was a minor decrease in

melanin production in positive control sunblock treated group and HRE groups when compared to control UVB group as can be seen in Figure 5B. Then finally at 5th week as it is clearly evident from Figure 5C, the positive sunblock treated group and both HRE treated groups showed significant decrease in melanin production. These results clearly indicate that HRE can be effectively used as skin whitening agent.

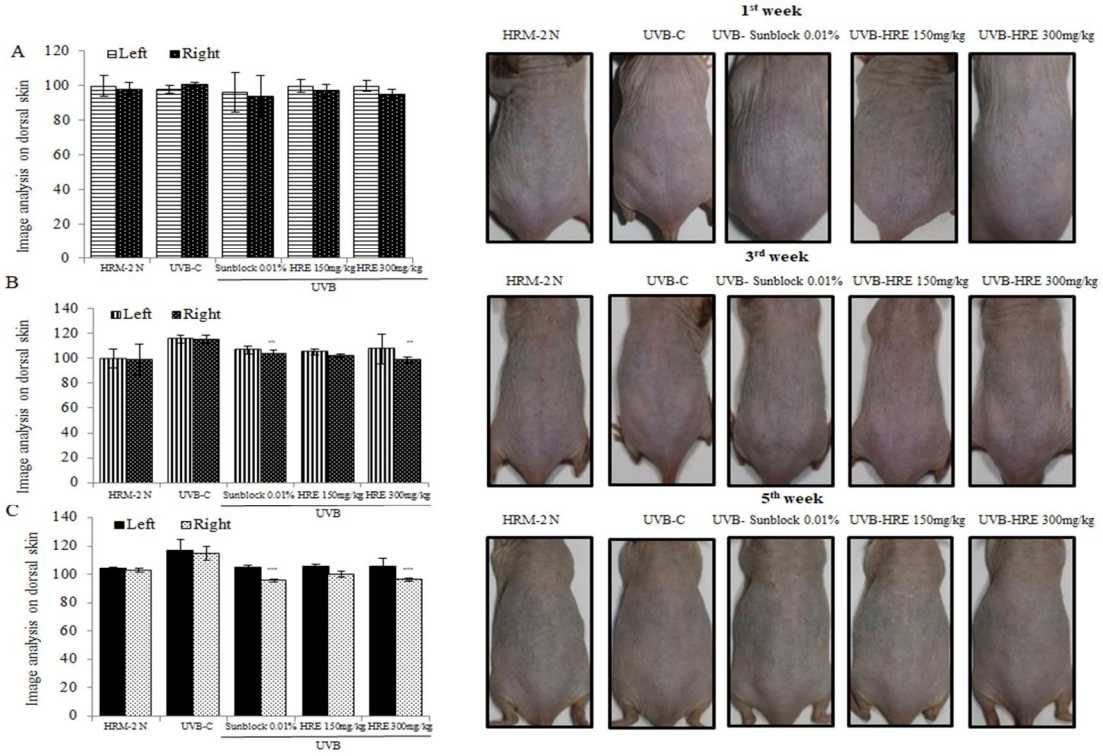

Figure 5. Effects of Radish extract on Melanogenesis in Skin Damage Induced Conditional Model by UVB Irradiation. In order to confirm the whitening efficacy of HRE, the difference in the formation of melanin at 1st , 3rd , and 5th weeks of UVB irradiation was examined by dividing the dorsal skin of HRM-2 mice into left (untreated) and right (treated) sides. Images were taken with digital camera as described in material and methods section. (A) No significant reduction was found in the melanin production in any groups in 1st week. (B) Minor reduction was found in the melanin production in HRE group in 3rd week. (C) Significant reduction was found in the melanin production in both HRE groups in 5th week .Values in bar graphs are ± SEM from three -independent experiments. *** p<0.001 and **p<0.05 when compared with UVB control. HRM-2 N= normal mice, UVB-C= HRM-2 mice control (exposed to UVB).

4.5 Decrease in epidermal thickness and increase in collagen by Radish Extract in Skin Damage Induced Conditional Model by UVB Irradiation.

Ultraviolet radiations can cause increase in epidermal thickness causing the skin to become thicker and rough. Moreover continuous exposure to UVB can degrade the MMP's that causes a loss in collagen which is very essential to good skin health [22]. As shown in Figure 6A, the epidermal thickness caused by UVB was potently reduced by the positive sunblock treated group and both HRE treated groups. Moreover it can be seen in Figure 6B that the epidermal thickness as visualized by H&E staining was also clearly decreased by the positive sunblock treated group and both HRE treated groups. The M-T staining was done to visualize the matrix components in the skin of HRM-2 mice. As shown in Figure 6C, the intensity of M-T stain is

reduced in control UVB group as a result of degradation in collagen fibers but with the treatment of mice with sunblock and both HRE, the intensity of staining is dramatically increased when compared to UVB control group. These results clearly show that Korean Radish extract can be safely and efficaciously used for reducing skin wrinkles and improving skin elasticity effects.

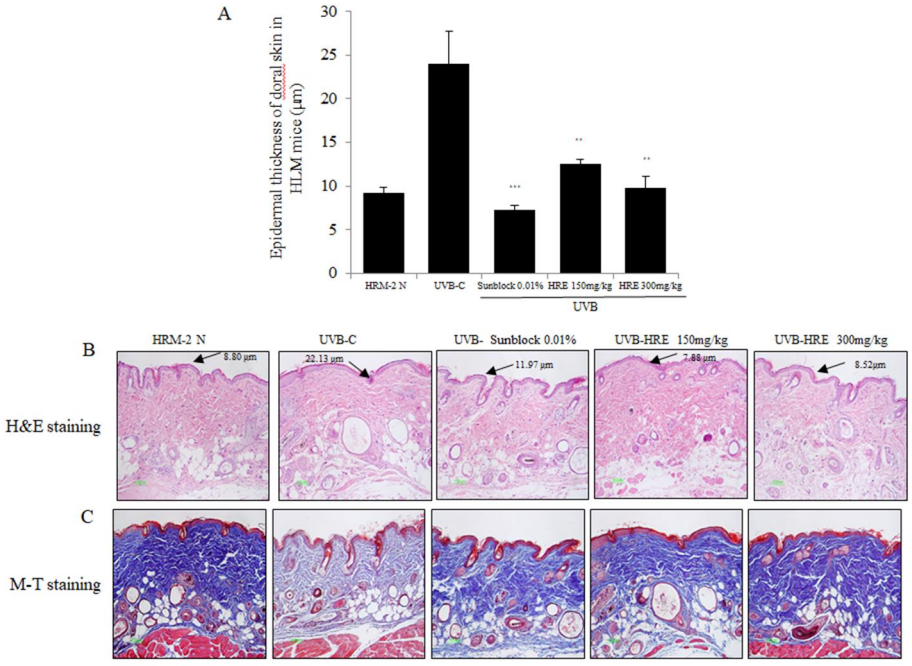

Figure 6. Effects of Radish extract on epithelial thickness and collagen fiber changes in HRM-2 mice UVB irradiated mice. To evaluate the effects of KRG on epidermal thickness and amount of collagen fibers, HRM-2 mice were irradiated with UVB to induce photo aging for 5 weeks along with positive control and KRG administration and application. After termination of 5 weeks, skin tissues were stained with H & E to measure epithelial thickness using the microscope as given in materials and methods section. (A&B) Significant reduction was found in the epidermal thickness of KRG treated groups. Values in bar graphs are ± SEM from three -independent experiments. *** $p<0.001$ and **$p<0.05$ when compared with UVB control. (C) The intensity of M-T staining was decreased in the UVB-control group compared to the normal group suggesting that collagen fiber degradation progressed and wrinkle formation accelerated. However, the amount of collagen fibers in HRE and positive control group were increased indicating that HRE reduced the amount of collagen degradation.

5. Conclusion

Skin aging can be roughly classified into two types. One is intrinsic or endogenous aging, which is an inevitable aging phenomenon with the increasing age of human. Clinical features of this type of aging are relatively mild, including fine lines, dry skin, and reduced elasticity. The second is photo aging or exogenous aging, which refers to the aging phenomenon observed in the skin exposed to sunlight for a long time. In this case the element responsible is UVB via sunlight. This type of photo aging can be prevented by use of commercially available sunblock application before going into sun and also minimizing the exposure time under the sun. The clinical features of exogenous aging are deleterious like very rough, dry skin with reduced

elasticity, deep wrinkles formation with severe skin sagging. It also makes skin very easily prone to pigmentation diseases like solar lentigo and so on. Moreover when the skin is excessively exposed to sunlight that contain UVB, the amount of extracellular matrix metalloproteinases (MMP's) increases that cause the degradation of matrix proteins and among them mainly collagen. Since collagen is globally accepted element for skin elasticity and aging, its degradation causes early winkles formation and reduction in skin elasticity. After exposure to UVB, increased MMPs degrade collagen and other substrate proteins. This is a sort of wound that the skin receives by the sun's rays, and our body makes efforts to heal wounds by synthesizing new collagen. However, since the process of wound healing is not always perfect, if the skin is continuously exposed to UVB, it becomes more aged clinically, including wrinkles[17-25].

Radish skin and radish greens (mucheong) are an edible part of the radish. But they are removed before eating the radish and used as a by product or an animal feed material because of their tough and rough texture. The research have conducted studies on a processing method capable of further increasing the physiological activity of radish, and as a result, have found that processed radish products including by-products may be effectively used to reduce skin wrinkles and improve skin elasticity. In order to examine the effects of sample treatment and UVB irradiation on the food intake and body weight of the HRM-2 mice, the body weight and the food intake were measured. These results clearly shows that the significant difference in body weight between the groups by irradiation with UVB or treatment with the sample was not observed. In addition, the food intake in the negative control group slightly decreased compared to that in the normal group, and the food efficiency ratio increased, but this increase was not significant. The food intake in the group administered orally with the radish extract or in the oint-applied group was similar to that in the normal group, but the food efficiency ratio in all the groups significantly decreased to that in the negative control group. In order to evaluate the effect of the radish extract on wrinkle formation induced by UVB irradiation, the depth of skin wrinkles was measured using the skin dermobella 3D at 3rd, 4th and 5th after the start of the experiment. As the results, the depth of wrinkles was increased in the negative control group compared to that in the negative control group due to UVB irradiation. However, the depth of wrinkles in the group treated with the radish extract was decreased compared to that in the negative control group. The radish extract had the effect of reducing the depth of wrinkles induced by photoaging. Thus, the expression levels of wrinkle-related genes and protein by UVB irradiation were measured so that quantitative evaluation could be made. As the result, the expression levels of MMP-2 and MMP-9 genes were significantly increased in the negative control group compared to the normal group due to UVB irradiation, but the expression levels of the genes were significantly decreased by treatment with the radish extract. In addition, the expression levels of the genes decreased dependently on the amount of radish extract used. Similarly to the quantitative results for mRNA, the expression level of the MMP-2 protein was also significantly increased by UVB irradiation, and the radish extract the expression of the MMP-2 protein in a manner dependent on the amount of radish extract used. In order to confirm the whitening efficacy of Radish extracts, during the 1st week of exposure of mice to UVB with the treatment of sunblock and HRE, there was no significant decrease in production of melanin by both HRE doses. Then, in the 3rd week, there was a minor decrease in melanin production in positive control sunblock treated group and HRE groups when compared to control UVB group. Then finally at 5th week as it is clearly evident from the result, the positive sunblock treated group and both HRE treated groups showed significant decrease in melanin production. These results clearly indicate that HRE can be effectively used as skin whitening agent. Ultraviolet radiations can cause increase in epidermal thickness causing the skin to become thicker and rough. Moreover continuous exposure to UVB can degrade the MMP's that causes a loss in collagen which is very essential to good skin health. As the result, the epidermal thickness caused by UVB was potently reduced by the positive sunblock

treated group and both HRE treated groups. Moreover it showed that the epidermal thickness as visualized by H&E staining was also clearly decreased by the positive sunblock treated group and both HRE treated groups. The M-T staining was done to visualize the matrix components in the skin of HRM-2 mice. the intensity of M-T stain is reduced in control UVB group as a result of degradation in collagen fibers but with the treatment of mice with sunblock and both HRE, the intensity of staining is dramatically increased when compared to UVB control group. These results clearly show that Korean Radish extract can be safely and efficaciously used for reducing skin wrinkles and improving skin elasticity effects.

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