Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.72121

Pectinase halophyte complex extract protects hairless mice skin damaged by UV-irradiation

Ji Hyeon PARK[1], Jae Young SHIN[2], Byoung Ok CHO[1], Suping HAO[1], Feng WANG[1], Yi Teak LIM[3], Da Jeong SHIN[4], Seon Il JANG[1,5]*

Abstract

The aim of this study was to evaluate the inhibitory effect and mechanism of collagen degradation by Pectinase-assisted Halophyte Complex Extract (PHCE) in hairless mice. Collagen expression *in vitro* was measured using Enzyme-linked Immunosorbent Assay (ELISA) and Western blotting. After collecting mice skin *in vivo*, histological analysis was performed with Hematoxylin & Eosin (H & E) and trichrome stain. Molecular expression was measured by western blot analysis. In HaCaT cells, the collagen expression level was increased compared to the positive control group. Epidermal thickness inhibition and collagen expression levels were also increased in the skin of a hairless mouse model. Western blot analysis confirmed the increase in collagen and elastin by inhibiting phosphorylation of NF-κB and decreasing the expression of matrix metalloproteinases-1 (MMP-1) and matrix metalloproteinases-9 (MMP-9). The skin protective effect of PHCE is considered to be due to inhibition of MMPs expression through inhibition of NF-κB activation. Therefore, PHCE can be considered as a candidate material as a food material that can improve skin damage caused by UV rays.

Keywords: red ginseng; halophyte; *Morus alba*; UV, collagen.

Practical Application: PHCE can be utilized as a natural product material that has side effects in the food or cosmetic industry to add protective effect to the skin.

1 Introduction

The skin is an organ that occupies the largest area in the human body. It protects the body from external stimuli such as pathogens, solar radiation. Ultraviolet rays (UV) emitted to the sun are an important factor in skin damage. UV-B with a wavelength of 280 nm to 300 nm causes various skin diseases such as skin damage and erythema, wrinkles, freckles, and loss of elasticity (Miyauchi et al., 1992). Collagen, the main extracellular matrix, is a representative fibrous protein present throughout the body, and there are 14 types, of which type I collagen is mainly present in the skin to maintain skin elasticity (Parvizi & Kim, 2010; Uitto et al., 1989).

UV-induced damage to the skin produces matrix metalloproteinases (MMPs) through cellular signaling pathways (Pittayapruek et al., 2016) When the skin is damaged by UV, it increases the expression of MMP-1, which initiates the degradation of type I collagen, and MMP-9, which further degrades collagen fragments (Kähäri & Saarialho-Kere, 1997). Expression of MMPs is through the NF- κB signaling pathway (Jesumani et al., 2019).

Red ginseng is the most widely used medicinal plant in oriental medicine and ginsenosides are known to have anti-inflammatory and antioxidant effects (Andrade et al., 2007). *Morus alba* is a medicinal plant widely used in oriental medicine with high content of bioactive compounds such as flavonoids and antioxidant effects (Doi et al., 2001). *Suaeda japonica* is a halophyte native to the coasts of Korea and Japan and is used as a food source. *S. japonica* has been reported to have antioxidant, anti-inflammatory and hepatoprotective effects (Cho et al., 2013). *Artemisia scoparia* is a perennial halophyte and has been used as herbal medicine in East Asia including Korea, China, and Japan (Yoon et al., 2006). *Triglochin maritimum* is a species plant in the arrowgrass family, and several studies on its antioxidant effects have been reported (Lee et al., 2018; Ding et al., 2021). *Limonium* tetragonum is a type of halophyte that grows wild in salt marshes in Korea. It contains myricetin, myricetin glycosides, tannins and caprolactam as active ingredients, and its antioxidant and hepatoprotective effects have been studied (Yang et al., 2014; Lee et al., 2011).

Recently, eco-friendly extraction technology has been required, and research on hydrolysis is being conducted to increase the extraction efficiency of plant extracts (Krakowska-Sieprawska et al., 2020). There have been reports of using pectinase to increase the extraction efficiency of phenolic compounds

Received 17 July, 2021
Accepted 25 Aug., 2021
[1] *Institute of Health Science, Jeonju University, Jeonju-si, Jeollabuk-do, Republic of Korea*
[2] *Department of Food Science and Technology, Chonbuk National University, Jeonju-si, Jeollabuk-do, Republic of Korea*
[3] *Jinandang Agricultural Corp., Jinan-gun, Jeollabuk-do, Republic of Korea*
[4] *Research Institute, Ato Q&A Co., LTD, Jeonju-si, Jeollabuk-do, Republic of Korea*
[5] *Department of Health Management, Jeonju University, Jeonju-si, Jeollabuk-do, Republic of Korea*
*Corresponding author: sonjjang@jj.ac.kr

from plant cell walls (Landbo & Meyer, 2001). Therefore, in this study, the protective effect of UV-induced skin damage in the red ginseng, *M. alba* and halophyte complex extract treated with pectinase was confirmed

2 Materials and method

2.1 Materials

Dulbecco's modified Eagle medium (DMEM), Penicillin/streptomycin antibiotics and fetal bovine serum were purchased from Gibco (Grand Island, NY, USA). Quanti-MAX™ WST-8 Cell Viability Assay Kit came from BIOMAX (Seoul, Korea). L-ascorbic acid and pectinase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Protease inhibitors was obtained from Cell Signaling Technology (Danvers, MA, USA). Radio-immunoprecipitation assay buffer (RIPA buffer) came from Thermo Scientific (Rockford, IL, USA). Procollagen Type I C-peptide (PIP) EIA Kit (Nojihigashi, Kusatsu, Japan). Collagen antibody was obtained from Invitrogen (Carlsbad, CA, USA). Elastin, MMP-1, MMP-9, NF-κB, P-NF-κB and HRP conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA., USA) and actin (Biosciences, Franklin Lakes, NJ, USA) antibodies were used in this study.

2.2 Plant material and enzyme extract

Red ginseng root was purchased from Jinandang Farming Association Corporation (Jinan-gun, Jeollabuk-do), *M. alba* was obtained from Jeonju, Jeollabuk-do, Korea. *S. japonica* and *L. tetragonum* used in the experiment were collected from Sinsido, Gunsan, Jeollabuk-do, Korea. *A. scoparia* was collected from Sohwangsagu, Uncheon-eup, Boryeong-si, Chungcheongnam-do, Korea and *T. maritimum* used in the experiment was collected from Simwon-myeon, Gochang-si, Jeollabuk-do. The ratio of the complex *M. alba*: Red ginseng: *A. scoparia: S. japonica: L. tetragonum: T. maritimum* was 3: 1: 1: 1: 1: 1. And Plant is extracted with 0.04% pectinase and hot water at 50 °C for 24 h, and then ethanol is added to make 70% ethanol to inactivate pectinase and extracted at 50 °C for 24 h. After that, PHCE was twice filtered using a 0.45m filter, concentrated in a rotary pressure reducer, freeze-dried, and stored at -20 °C.

2.3 Cell culture

HaCaT cell lines were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany), cells were cultured DMEM medium supplemented with 10% FBS, 1% penicillin/streptomycin in a 5% CO_2 incubator at 37 °C. The cells were not serum-starved for all experiments.

2.4 Cell toxicity

HaCaT cells (2 x 10^5 cells/mL) were cultured in 96-well plates for 24 h. PHCE was used to treat cells at various concentrations (0 μg/mL to 400 μg/mL). Plate was incubated for 20 hours in an incubator and then 10 μL of Quanti-Max™ was added. After 4 h of further incubation, the absorbance of each well was measured using a spectrophotometer (Tecan, Männedorf, Switzerland) at 450 nm. The absorbance of each well was consistent with HaCaT cell viability.

2.5 Animal experimental design

Hairless male mice of 5 weeks of age were purchased from Orient Bio Inc. I bought it from (Gwangju, Korea). The Mice were housed in a room that temperature of 23 ± 2 °C, the humidity of 50-60%, light and dark cycle of each 12 h. Mice were provided with a commercial standard laboratory diet and water ad libitum. All procedures performed complied with the Animal Care and Use Committee Guidelines Principles of the Animal Care and use Committee Guidelines of Jeonju University Veterinary Hospital. 1 week before the experiment, the mice were acclimatized to the laboratory environment. The number of mice was determined to be 5 per experimental group. The dorsal skin of mice was irradiated at UV with an LF-215.M lamp (emission peak 312 nm; Uvitec, Cambridge, UK) at an intensity of 300 mJ/cm for 3 weeks 3 times a week for 3 weeks. PHCE (100 mg/kg), PHCE (200 mg/kg), and L-ascorbic acid (AA) (20 mg/kg) were dissolved and administered orally to mice for 3 weeks. The vehicle was saline.

2.6 ELISA assay

The concentration of type I collagen was measured using culture medium supernatants of HaCaT cells treated with or without PHCE for 24 h after UV irradiation. Follow the kit manufacturer's described protocol without modification.

2.7 Protein extraction

In HaCaT cells (2 x 10^5 cells/mL), the culture medium of the cells pre-treated with PHCE for 1 h is removed and UV is irradiated with an intensity of 60 mJ/cm^2. After that, fresh medium and PHCE were injected and incubated for 24 h. Cells were washed twice with ice-cold PBS and centrifuged at 2000 rpm for 2 min at 4 °C. The supernatant was discarded and the cell pellet was suspended in 0.05 mL of ice-cold RIPA buffer and protease inhibitor solution. After that, centrifugation was performed at 12,000 rpm for 15 min at 4 °C and the supernatant was transferred to a new tube. The supernatant was stored at -80 °C for subsequent use. For protein extraction from skin tissue, after the animal experiment was over, the dorsal skin of hairless mice was obtained, and 0.2 g of each mouse tissue was put into 0.2 mL of RIPA buffer, protease inhibitor solution and homogenized. All homogenization was carried out by keeping the tube on ice. After that, centrifugation was performed at 4 °C for 15 min at 12,000 rpm. The supernatant was transferred to a new tube and stored at -80 °C for subsequent use.

2.8 Western blot analysis

30 μg protein was loaded on 7.5% or 10% SDS-PAGE gels and transferred to PVDF membranes. After blocking the protein with 5% skim milk (dissolved with 1%TBST (Tween 20 in 20 mmol/L TBS)), it was incubated overnight with a specific primary antibody. Membranes were washed 5 times with TBST, and secondary antibodies were incubated with 5% skim milk

for 2 h. Protein expression was detected and visualized using a chemiluminescence detection system. The density of each band in the immunoblot was analyzed using Image J analysis software (National Institutes of Health, Bethesda, MD, USA).

2.9 Histopathological examination

Cut the dorsal skin tissue fixed in 10% neutral buffered formalin, put it in a cassette, and wash 5 times with PBS 1X. After that, after dehydration in ethanol (60% ~ 100%) for 30 min, twice in xylene for 30 min each, embedd in paraffin and sectioning at 5 μm using a microtome. Tissues were used for H&E for epidermal thickness measurement and trichrome staining for collagen density analysis. Experiments did not modify the manufacturer's protocol. Histopathological changes were investigated by light microscopy (Leica, Wetzel, Germany).

2.10 Statistical analysis

Data are presented as mean ± SD. Statistically significant differences between each group were determined by one-way analysis of variance (ANOVA) followed by Turkey's test. $p < 0.05$ was considered statistically significant.

2.11 Ethical statement

The mice were managed and the experiments were performed according to the guidelines of the Jeonju University Animal Experiments and Use Committee with approval of the experiment (approval no. JJU-IACUC-2018-5) by Jeonju University.

3 Results

3.1 The protective effect of PHCE in UV-damaged cells

The cytotoxic effect of PHCE on HaCaT cells was tested. Cytotoxicity was measured after 24 h treatment of cells with various concentrations (0 to 400 μg/mL) of PHCE. In HaCaT cells, PHCE was confirmed to be non-toxic up to a concentration of 200 μg/mL (Figure 1A).

Before the in vivo experiment, we checked effect of PHCE on inhibition of collagen degradation in UV-stimulated HaCaT cells were evaluated. As a result of evaluating the expression level of collagen in the cell culture medium by ELISA experiment, it was shown that the expression level of collagen in the PHCE-treated group was increased compared to that of the non-treated group (Figure 1B). In addition, in western blot analysis, the expression level of collagen was significantly increased in the PHCE-treated group compared to the UV-irradiated group. (Figure 1C).

3.2 Effects of PHCE on morphological and histological change of UV irradiated hairless mice skin

After sacrificing the mice, tissues were obtained and H&E staining was performed to observe morphological and histological changes. As a result, UV irradiation caused erythema, wrinkles and keratinization of the skin. However, administration of PHCE suppressed skin damage compared to the untreated and AA-treated group (Figure 2A). Next, histological changes were observed through H&E staining. The results showed that UV irradiation increased the thickness of the epidermis. However, the administration of PHCE reduced the thickness of the epidermis compared to the untreated group. AA administration did not show a significant difference from the UV group (Figure 2B).

3.3 Effect of PHCE on collagen protection in UV irradiated hairless mice skin

Trichrome staining was performed to measure the collagen density in the skin of UV mice. UV irradiation significantly reduced collagen. However, it was confirmed that the mice in the group treated with PHCE had less collagen degradation compared to the untreated group. The effect was superior to that of the AA treatment group (Figure 3A). Western blotting was performed to further analyzed the mechanism of action of PHCE related to collagen protection and inhibition of MMPs in the skin of mice. According to this result, the skin of mice UV irradiated group and AA-treated group showed decreased collagen and elastin and increased MMP-1 and MMP-9. In contrast, in the skin of mice treated with PHCE, the increased expression levels of collagen and elastin. And MMP-1, MMP-9 were suppressed (Figure 3B).

3.4 Effect of PHCE on NF-κB phosphorylation in UV-irradiated hairless mice skin

To analyze the molecular mechanisms underlying the inhibitory effect of PHCE, evaluated the NF-κB pathway in UV-irradiated mouse skin. The phosphorylation of NF-κB

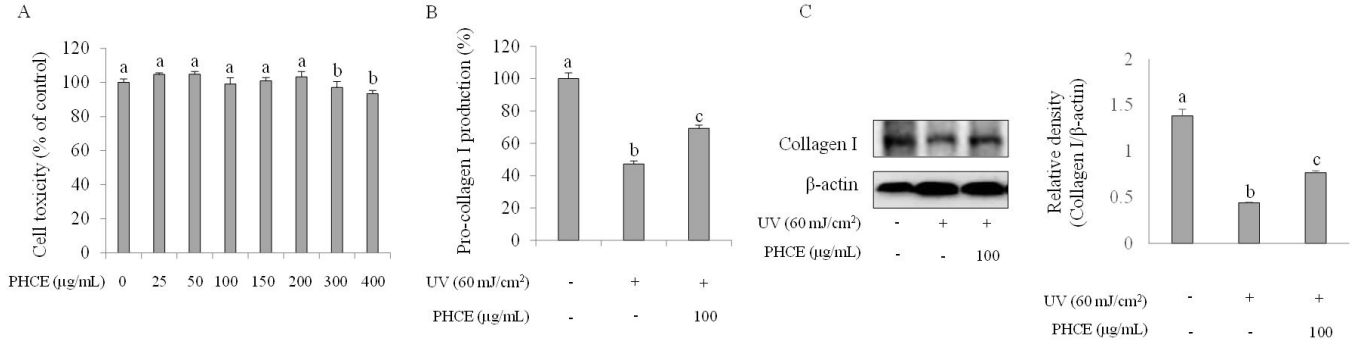

Figure 1. Assessment of cytotoxicity of PHCE (A), ELISA assay (B). Western blot analysis (C) was quantified using Image J software. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

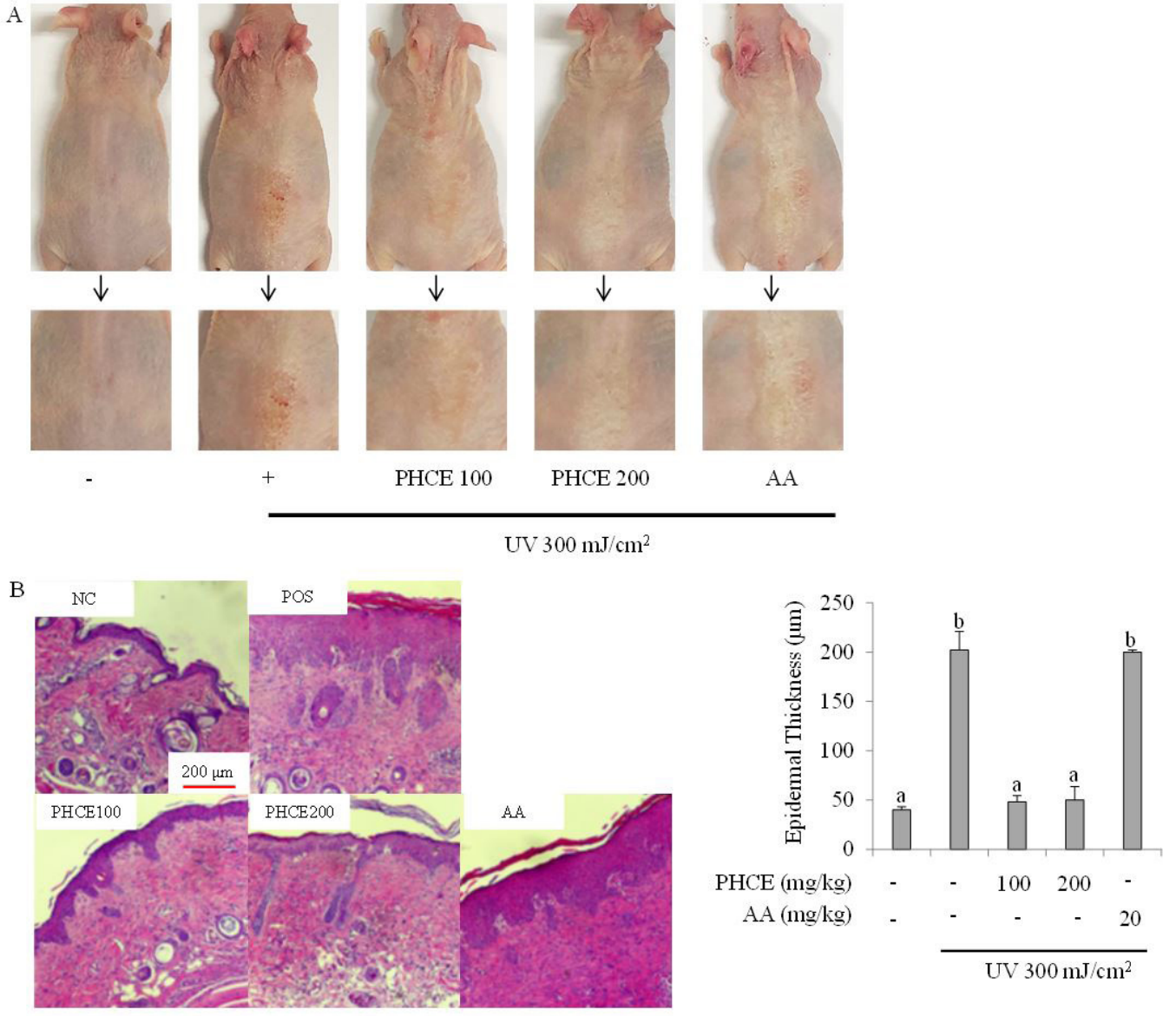

Figure 2. Skin changes were observed in various mice groups (A). Epidermal thickness was measured to H&E staining (B). Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$. NC, Normal Control; POS, Positive Control; AA, Ascorbic Acid; PHCE, Pectinase Halophyte Complex Extract.

was increased in the skin of mice irradiated with UV, but the phosphorylation of NF-κB was significantly decreased in the PHCE 200 mg/kg treatment group. AA-treated group did not reduce phosphorylation (Figure 4).

4 Discussion

In previous studies, the skin protection effect of red ginseng, *S. japonica, A. scoparia, T. maritimum, L. tetragonum* and *M. alba* was reported (Shin et al., 2021; Woo et al., 2017). There have also been reports that the extraction efficiency of bioactivity substances can be improved by decomposing pectin, the main component of plant cell walls, through enzyme-assist extraction (Heemann et al., 2019). Therefore, in this study, the effect of enzyme-assisted extraction using a mixture of red ginseng, *S. japonica*, *A. scoparia*, *T. maritimum*, *L. tetragonum* and *M. alba* was investigated. PHCE showed no toxicity at 200 μg/mL concentration, and cell viability was increased than non-pectinase treated extract in UV-irradiation (data not shown). Moreover, PHCE recovered collagen degradation caused by UVB irradiation in HaCaT cells.

Since the skin protection effect of PHCE was confirmed at the cell level, an experiment was conducted using hairless mice to check whether the same effect was exhibited in the *in vivo*.

When the skin is exposed to UV, collagen and elastin are broken down, which causes wrinkles and keratinization (Moloney et al., 1992). In addition, it induces the synthesis and

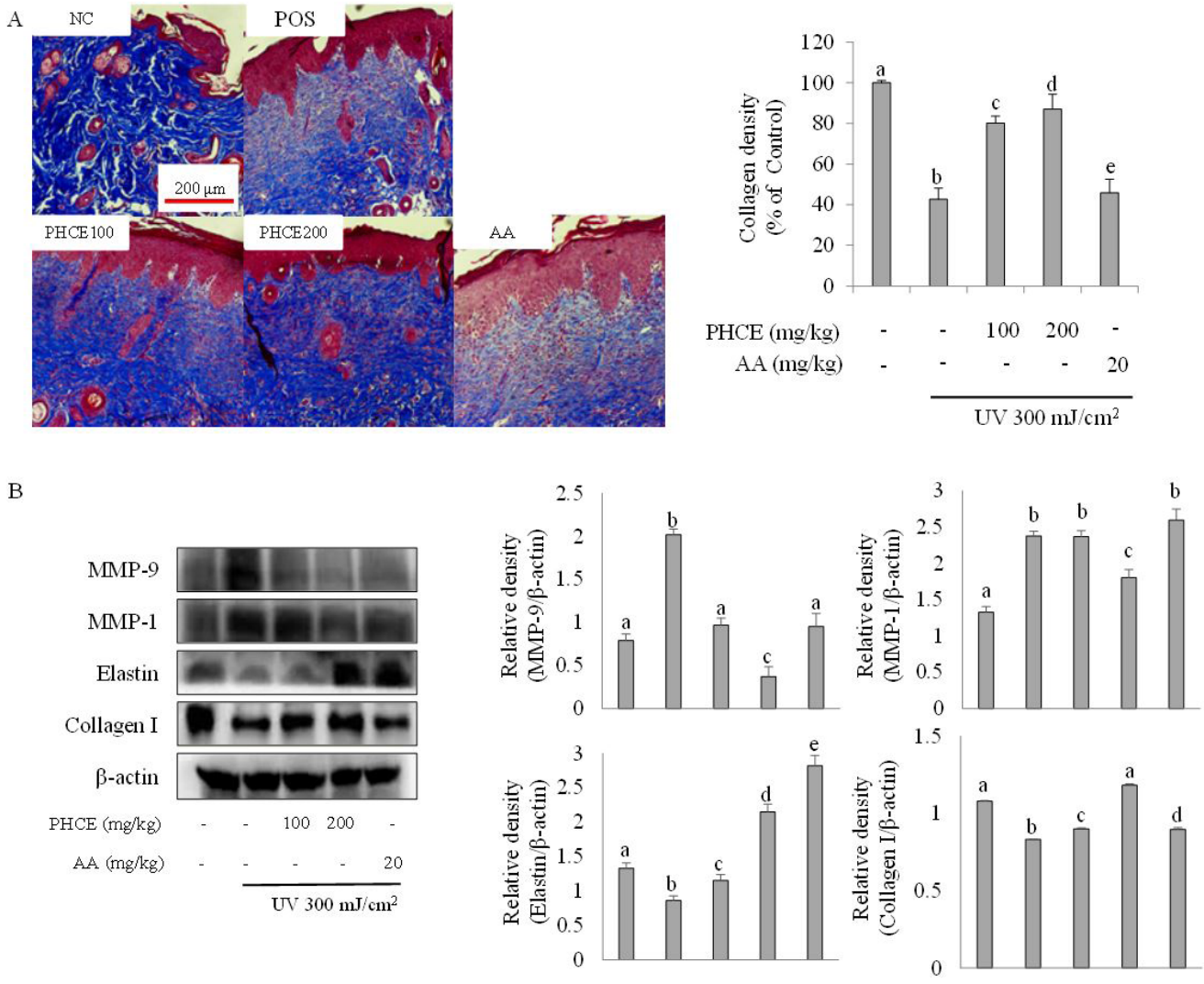

Figure 3. The density of collagen was measured by trichrome staining of the skin of mice (A). Expressions of MMP-1, 9, collagen and elastin were analyzed by western blot using skin protein extracts (B). Each bar represents the mean ± SD. Other lowercase letters indicate significant differences at $p < 0.05$.

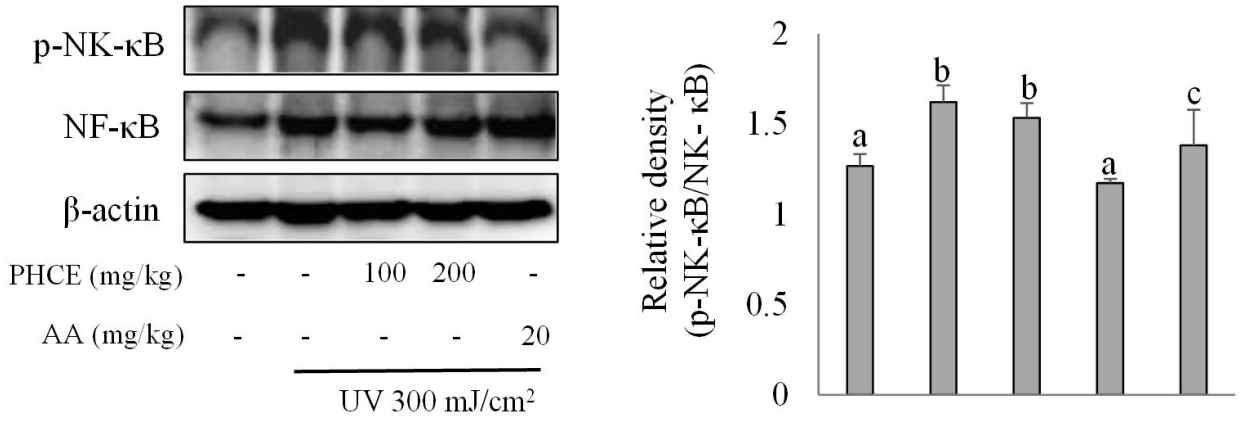

Figure 4. Phosphorylation of NF-KB was analyzed by Western blot experiment using skin protein extract. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

release of pro-inflammatory cytokines in keratinocytes, causing an acute inflammatory reaction accompanied by swelling and erythema in the skin (Lee et al., 2017). In the skin of hairless mice subjected to UV irradiation, erythema, swelling, wrinkles, and keratinization were reduced in the PHCE-treated group. However, in the AA-treated group, erythema decreased, but wrinkles and keratinization did not decrease. These results are considered to be similar to the results of previous studies showing that oral vitamin C supplementation lacks the protective effect of UV-induced skin damage (McArdle et al., 2002). Our results show that PHCE restores UV-degraded collagen and elastin in hairless mice. In addition, PHCE increased the expression of MMP1 and 9. The breakdown of collagen and elastin occurs by the expression of MMPs called collagenases (Brennan et al., 2003; Xiao et al., 2019). Therefore, it is considered that the skin protective effect of PHCE against photoaging is due to the suppression of the expression of MMPs.

Overexposure to UV causes the generation of intracellular ROS in the skin, which leads to the activation of MAPKs (Jesumani et al., 2019). Activation of MAPKs separates inactive NF-κB through binding to IκBα. And NF-κB separated from IκBα is translocated into the nucleus, causing transcription of MMPs genes. (Xiao et al., 2020). In this study, PHCE showed inhibition of the activation of NF-κB in the skin of UVB-irradiated mice skin. Therefore, it is presumed that the inhibitory effect of PHCE on collagen and elastin degradation is at least because it inhibits the activation of NF-κB, thereby inhibiting the transcription of MMPs genes. Current studies have only examined the effect of PHCE on NF-κB, the final signaling pathway for MMPs gene transcription. In order to clearly elucidate the mechanism of skin protection effect of PHCE from UV rays, it is necessary to investigate the effect of PHCE on ROS generation and upstream signaling pathways such as MAPKs in UV-irradiated skin.

5 Conclusions

In conclusion, PHCE can inhibit collagen/elastin degradation in UVB-irradiated HaCaT cells and mice skin. PHCE can also reduce erythema, swelling, wrinkles, and keratinization caused by UVB exposure. The skin protective effect of PHCE is considered to be due to inhibition of MMPs expression through inhibition of NF-κB activation. Therefore, PHCE can be considered as a candidate material as a food material that can improve skin damage caused by UV rays.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

BOC and SIJ designed the research. JHP, FW, SH, DJS and YTL performed experiments and analyzed the data. JHP prepared and wrote the manuscript. JYS, BOC and SIJ review and revised the manuscript.

Acknowledgements

This research was supported by a grant (grant No. RA201906-5-C4) from Jeonbuk Research & Development Program funded by Jeonbuk Province.

References

Andrade, E., de Mesquita, A. A., Claro Jde, A., de Andrade, P. M., Ortiz, V., Paranhos, M., & Srougi, M. (2007). Study of the efficacy of Korean Red Ginseng in the treatment of erectile dysfunction. *Asian Journal of Andrology*, 9(2), 241-244. http://dx.doi.org/10.1111/j.1745-7262.2007.00210.x. PMid:16855773.

Brennan, M., Bhatti, H., Nerusu, K. C., Bhagavathula, N., Kang, S., Fisher, G. J., Varani, J., & Voorhees, J. J. (2003). Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin. *Photochemistry and Photobiology*, 78(1), 43-48. http://dx.doi.org/10.1562/0031-8655(2003)078<0043:MMITMC>2.0.CO;2. PMid:12929747.

Cho, J.-Y., Yang, X., Park, K.-H., Park, H. J., Park, S.-Y., Moon, J.-H., & Ham, K.-S. (2013). Isolation and identification of antioxidative compounds and their activities from *Suaeda japonica*. *Food Science and Biotechnology*, 22(6), 1547-1557. http://dx.doi.org/10.1007/s10068-013-0250-2.

Ding, J., Wang, L., He, C., Zhao, J., Si, L., & Huang, H. (2021). Artemisia scoparia: Traditional uses, active constituents and pharmacological effects. *Journal of Ethnopharmacology*, 273, 113960. http://dx.doi.org/10.1016/j.jep.2021.113960. PMid:33636317.

Doi, K., Kojima, T., Makino, M., Kimura, Y., & Fujimoto, Y. (2001). Studies on the constituents of the leaves of *Morus alba* L. *Chemical & Pharmaceutical Bulletin*, 49(2), 151-153. http://dx.doi.org/10.1248/cpb.49.151. PMid:11217100.

Heemann, A. C. W., Heemann, R., Kalegari, P., Spier, M. R., & Santin, E. (2019). Enzyme-assisted extraction of polyphenols from green yerba mate. *Brazilian Journal of Food Technology*, 22, e2017222. http://dx.doi.org/10.1590/1981-6723.22217.

Jesumani, V., Du, H., Aslam, M., Pei, P., & Huang, N. (2019). Potential use of seaweed bioactive compounds in skincare: a review. *Marine Drugs*, 17(12), 688. http://dx.doi.org/10.3390/md17120688. PMid:31817709.

Kähäri, V. M., & Saarialho-Kere, U. (1997). Matrix metalloproteinases in skin. *Experimental Dermatology*, 6(5), 199-213. http://dx.doi.org/10.1111/j.1600-0625.1997.tb00164.x. PMid:9450622.

Krakowska-Sieprawska, A., Rafińska, K., Walczak-Skierska, J., & Buszewski, B. (2020). The influence of plant material enzymatic hydrolysis and extraction conditions on the polyphenolic profiles and antioxidant activity of extracts: a green and efficient approach. *Molecules*, 25(9), 2074. http://dx.doi.org/10.3390/molecules25092074. PMid:32365541.

Landbo, A. K., & Meyer, A. S. (2001). Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *Journal of Agricultural and Food Chemistry*, 49(7), 3169-3177. http://dx.doi.org/10.1021/jf001443p. PMid:11453748.

Lee, J. I., Kong, C.-S., Jung, M. E., Hong, J. W., Lim, S. Y., & Seo, Y. (2011). Antioxidant activity of the halophyte *Limonium tetragonum* and its major active components. *Biotechnology and Bioprocess Engineering*, 16(5), 992-999. http://dx.doi.org/10.1007/s12257-011-0213-5.

Lee, J. M., Yim, M.-J., Choi, G., Lee, M. S., Park, Y. G., & Lee, D.-S. (2018). Antioxidant and anti-inflammatory activity of six halophytes in Korea. *Natural Product Sciences*, 24(1), 40-46. http://dx.doi.org/10.20307/nps.2018.24.1.40.

Lee, S.-J., Lee, K. B., Son, Y. H., Shin, J., Lee, J.-H., Kim, H.-J., Hong, A. Y., Bae, H. W., Kwon, M. A., Lee, W. J., Kim, J. H., Lee, D. H., Jeong, E. M., & Kim, I. G. (2017). Transglutaminase 2 mediates UV-induced skin inflammation by enhancing inflammatory cytokine production. *Cell Death & Disease*, 8(10), e3148. http://dx.doi.org/10.1038/cddis.2017.550. PMid:29072680.

McArdle, F., Rhodes, L. E., Parslew, R., Jack, C. I., Friedmann, P. S., & Jackson, M. J. (2002). UVR-induced oxidative stress in human skin in vivo: effects of oral vitamin C supplementation. *Free Radical Biology & Medicine*, 33(10), 1355-1362. http://dx.doi.org/10.1016/S0891-5849(02)01042-0. PMid:12419467.

Miyauchi, H., Horio, T., & Asada, Y. (1992). The effect of ultraviolet radiation on the water-reservoir functions of the stratum corneum. *Photodermatology, Photoimmunology & Photomedicine*, 9(5), 193-197. PMid:1342187.

Moloney, S. J., Edmonds, S. H., Giddens, L. D., & Learn, D. B. (1992). The hairless mouse model of photoaging: evaluation of the relationship between dermal elastin, collagen, skin thickness and wrinkles. *Photochemistry and Photobiology*, 56(4), 505-511. http://dx.doi.org/10.1111/j.1751-1097.1992.tb02194.x. PMid:1454880.

Parvizi, J., & Kim, G. K. (2010). Collagen. In J. Parvizi & G. K. Kim (Eds.), *High yield orthopaedics* (Chap. 53, pp. 107-109). Philadelphia: W.B. Saunders. http://dx.doi.org/10.1016/B978-1-4160-0236-9.00064-X.

Pittayapruek, P., Meephansan, J., Prapapan, O., Komine, M., & Ohtsuki, M. (2016). Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *International Journal of Molecular Sciences*, 17(6), 868. http://dx.doi.org/10.3390/ijms17060868. PMid:27271600.

Shin, J. Y., Park, J. H., Che, D. N., Kang, H. J., Cho, B. O., Lim, Y. T., & Jang, S. I. (2021). Protective effects of halophyte complex extract against UVB-induced damage in human keratinocytes and the skin of hairless mice. *Experimental and Therapeutic Medicine*, 22(1), 682. http://dx.doi.org/10.3892/etm.2021.10114. PMid:33986847.

Uitto, J., Olsen, D. R., & Fazio, M. J. (1989). Extracellular matrix of the skin: 50 years of progress. *The Journal of Investigative Dermatology*, 92(4, Suppl.), 61S-77S. http://dx.doi.org/10.1038/jid.1989.34. PMid:2649616.

Woo, H., Lee, J., Park, D., & Jung, E. (2017). Protective effect of mulberry (*Morus alba* L.) extract against benzo[a]pyrene induced skin damage through inhibition of aryl hydrocarbon receptor signaling. *Journal of Agricultural and Food Chemistry*, 65(50), 10925-10932. http://dx.doi.org/10.1021/acs.jafc.7b04044. PMid:29231728.

Xiao, Z., Liang, P., Chen, J., Chen, M. F., Gong, F., Li, C., Zhou, C., Hong, P., Yang, P., & Qian, Z. J. (2019). A peptide YGDEY from tilapia gelatin hydrolysates inhibits UVB-mediated skin photoaging by regulating MMP-1 and MMP-9 expression in HaCaT cells. *Photochemistry and Photobiology*, 95(6), 1424-1432. http://dx.doi.org/10.1111/php.13135. PMid:31230361.

Xiao, Z., Yang, S., Chen, J., Li, C., Zhou, C., Hong, P., Sun, S., & Qian, Z.-J. (2020). Trehalose against UVB-induced skin photoaging by suppressing MMP expression and enhancing procollagen I synthesis in HaCaT cells. *Journal of Functional Foods*, 74, 104198. http://dx.doi.org/10.1016/j.jff.2020.104198.

Yang, M. H., Kim, N.-H., Heo, J.-D., Sung, S. H., & Jeong, E. J. (2014). Hepatoprotective effects of Limonium tetragonum, edible medicinal halophyte growing near seashores. *Pharmacognosy Magazine*, 10(39, Suppl. 3), S563-S568. http://dx.doi.org/10.4103/0973-1296.139783. PMid:25298675.

Yoon, W.-J., Lee, J.-A., Kim, J.-Y., Oh, D.-J., Jung, Y.-H., Lee, W.-J., & Park, S.-Y. (2006). Anti-oxidant activities and anti-inflammatory effects on *Artemisia scoparia*. *Korean Journal of Pharmacognosy*, 37(4), 235-240.