

Topical application of cream containing enriched-schizandrin from Schisandra chinensis fruit exert anti-photoaging efficacy in ultraviolet B- irradiated hairless mice

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Research Article

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Abstract

Excessive ultraviolet (UV) B irradiation induced skin photoaging. The aim of this study was to evaluate the anti-photoaging efficacy of enriched-schizandrin from *Schisandra chinensis* fruit in UVB-induced hairless mice. The cream containing enriched-schizandrin from *S. chinensis* fruit manufactured with another cosmetic ingredient. UVB-photoaged hairless mice topically applied with the cream once a day for 5 weeks. Application of the cream showed normal changes of body weight and food efficiency in the UVB-photoaged hairless mice. The cream application also was decreased interleukin (IL)-1 β , matrix metalloproteinase (MMP)-2 and MMP-9 mRNA expressions and then it inhibited MMP-2 protein expression in UVB-photoaged hairless mice. Furthermore, the cream application inhibited epidermal wrinkle formation and decreased wrinkle depth and it restored to wrinkle thickness and collagen degradation of skin in UVB-photoaged hairless mice. Therefore, the cream could recover photoaging generated by UVB irradiation via downregulation of IL-1 β , MMP-2, MMP-9 mRNA expressions and suppression of expression of MMP-2 proteins.

Summary

The cream containing enriched-schizandrin from *S. chinensis* was prepared and observed the protective efficacy of the cream on UVB-photoaged mice. Our results showed that the cream containing enriched-schizandrin conducted protective roles in UVB-photoaged skin damages such as expression of MMP-2, MMP-9 mRNA, IL-1 β proteins. Therefore, enriched-schizandrin will be a potential ingredient in cosmetics with beneficial effects on photoaging skin.

Introduction

Human skin consists of epidermis on the outside and the dermis below, and skin aging show a quite different appearance depending on dermis or epidermis (Rinnerthaler et al. 2015). Intrinsic aging is generated by a result of genetic factors and showed physical changes occurring during the aging process, whereas extrinsic aging is aging process accelerated by environmental factors (Farage et al. 2008).

The skin is the first defense line protecting various damages of body by chemicals, infections and ultraviolet (UV) radiation (Hwa et al. 2011). UVB irradiation penetrates the human skin and products intracellular reactive oxygen species (ROS), which consequently generates cellular oxidative stress and skin aging (Subedi et al. 2017). Especially in the last decade, profound suspicions arose that ROS are the most important factors that are generating aging (Lapointe and Hekimi 2010).

The skin of extrinsic aging gets thicker skin and changes its composition (Farage et al. 2013). The extrinsic aging dramatically disrupted several collagens in aging skin (Talwar et al. 1995). This disruption is further accelerated by elastases after inflammation or UV exposure and by the activation of matrix

metalloproteases (MMPs). (Labat-Robert et al. 2000). Especially MMP1, 2, 3 and 9 are deeply related with degradation of extracellular matrix (Birkedal-Hansen et al. 1993).

The efficacy of tretinoin in photoaging was first demonstrated in animal model of photoaging (Kligman et al. 1984). The treatment of 0.1% tretinoin cream in UV photoaged skin inhibited collagen degradation by blockade of interstitial collagenase and gelatinases synthesis (Fisher et al. 1996). Now, only two topical retinoids (tretinoin and tazarotene) among the many topical agents have obtained U.S. Food and Drug Administration approval for photoaging treatment (Antoniou et al. 2010).

Schizandrin is a lignin compound isolated from *Schisandra chinensis* fruit and shows several activities including anti-inflammatory and anticancer (Li et al. 2018). In addition, schizandrin improved cerebral ischemia reperfusion injury to give neuroprotective effect (Wang et al. 2019). In this study, we investigated the anti-photoaging efficacy of cream containing enriched-schizandrin from *S. chinensis* fruit on photoaging-mediated skin damage generated by skin inflammation and MMPs in UVB-photoaged mice.

Metherials And Methods

Reagents and equipment

All reagents obtained from Sigma-Aldrich (St. Louis, MO, USA). A Alliance e2695 XE high-performance liquid chromatography (HPLC) (Waters, Tokyo, Japan) was used for identification of enriched-schizandrin. Wakosil-II C₁₈ column analytical column (250 × 4.6 mm, CH₃CN : Water = 50 : 50, 1.0 mL/min, 40 °C and diode array detector 254 nm) were used. All chemicals used analytical grade quality using commercially.

Preparation of enriched-schizandrin

To obtain enriched-schizandrin, distilled water (DW), propanediol and 70% ethanol used as solvent. Each three *S. chinensis* dried fruit (600 g) were extracted with DW (2 L), propanediol (2 L) and 70% ethanol (2 L) with sonication of 20 kHz for 2 hrs, 3 hrs and 4 hrs at 20°C, respectively. The extracts by 70% ethanol with sonication of 20 kHz for 4 hrs among various extract conditions showed the highest schizandrin by HPLC analysis (Fig. 1A). The extracts possessing the highest schizandrin was used next experiment.

Preparation of cream

The composition of cream containing enriched-schizandrin from *S. chinensis* showed in Table 1. First, a agents were mixed in a tank using a Homo Disper (1,200 rpm and 65°C; premix, japan). B agents added in the mixture until the mixtures reaches uniform phase stage completely. Then C agents dissolved in a tank by a Homo Disper (400 rpm and 75°C). The A, B and C mixtures emulsified in a tank by the Homo Disper (3,500 rpm and 75°C) and those cooled into 50°C. The emulsified mixtures mixed with D agents and then cooled into 30°C. The vehicle made with same composition of cream except for enriched-

schizandrin. SunBlock made with same composition of cream using retinoic acid as a substitute for enriched-schizandrin.

Table 1
Formulation of cream for anti-photoaging efficacy

Agent	Composition (%)
A Glycin	6.0
1,3-Butylene glycol	4.0
Carbopol 940 (2%)	0.4
Didosium EDTA	0.02
Distilled water	65.46
B Potassium hydroxide (KOH) 85%	0.12
C Cetyl ethylhexanoate	7.0
Liauid paraffin KF-70	10.0
Cetyl alcohol (Kacol 6098)	1.6
Arlacel 165	0.3
Cetyl stearyl alcohol (Lanette O Gesch)	0.8
Glycerin mono stearate (K.M. #205C)	2.5
D 1,2-Hexanediol	0.8
Enriched-schizandrin	1.0

Animal experiment

The female HRM-2 hairless mice (Seven-weeks old) obtained from Central Lab Animal Inc (Seoul, Korea). They were keep in the animal care facility of Daejeon University. The animal experiments conducted according to protocols approved by the Animal Care Committee of the Institute of Daejeon University, South Korea (No. KW-160919-1). All animal studies conducted in accordance with regulatory standards and guidelines.

The mice were divided into a normal group (n = 5), UVB-vehicle group (n = 5), UVB-SunBlock (positive control) group (n = 5), and UVB-SC (experimental group) group (n = 5). The minimal erythema dose (MED) on mice skin was irradiated. The mice irradiated UVB using a UVB lamp (Ieda Boeki, Tokyo, Japan). The first MED (1 minute 70 seconds with 100 mJ/cm²) of UVB per day for the first week was irradiated on the back of mice at a distance of 15 cm. Second MED (3 minute 40 seconds with 200 mJ/cm²) of UVB between two and three weeks was irradiated three times per week at a distance of 15 cm.

The cream of 0.2 mL applied topically on the skin of mice daily for 5 weeks. The images of morphology recorded under digital camera (NX100; Samsung, Giheung, Korea). Body weight and food efficiency ratio recorded every week.

Wrinkle measurement

To observe the wrinkle improvement of HRM-2 skin generated by UVB irradiation, the mice were anesthetized using ethyl ether. The wrinkles of skin in mice observed using a Dermobella wrinkle analyzer (Chowis, Seongnam, Korea) at 3, 4 and 5 weeks. The analyzer equipped with skin measurement lens using 5-mega pixel image camera and various image modes. The analyzer used measurement values from 0 to 100. The measurement values reconverted into wrinkle index from 0 to 30. The formula is following to:

$$\text{Wrinkle index} = \sum (L_i \times D_i) / \text{Image size}$$

L_i is length of wrinkle recognized in i .

D_i is average depth of wrinkle recognized in i .

Wrinkles were photographed at digital microscope of 400 × magnification.

Quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Quantitative RT-PCR conducted to observe the anti-photoaging efficacy of enriched-schizandrin on inflammatory cytokine gene expression [interleukin (IL)-1 β] and MMP mRNA expressions [MMP-2 and MMP-9] from skin tissue. Total cellular RNA extracted in skin tissue using the phenol-chloroform method (RNAzolB; Tel-Test Inc., Friendswood, TX, USA). Total RNA (3 μ g) were used for cDNA synthesis using the ReverTraAce- α -cDNA synthesis kit (Toyobo Co., Osaka, Japan). The 7500 Fast RT-PCR system (Applied Biosystems, Foster City, CA, USA) used for quantitative RT-PCR with the following primer sequences. IL-1 β , 50-CAACCAACAAGTGATATTCTCCATG-30 and 50-AGATCCACACTCTCAGCTGCA-30; MMP-2, 50-CAGGGAATGAGTACTGGGTCTATT-30 and 50-ACTCCAGTTAAAGGCAGCATCTAC-30; MMP-9, 50-AATCTCTTCTAGAGACTGGGAAGGAG-30 and 50-AGCTGATTGACTAAAGTAGCTGGA-30.

The TaqMan probe contained carboxyfluorescein dye (Applied Biosystems) and the probe used to observe mRNA gene expression. Mouse glyceraldehyde-3-phosphate dehydrogenase probe set (4352339E, VIC/MGB Probe, Probe Limited; Applied Biosystems) used as internal standard. The final concentration of primer used in quantitative RT-PCR was 200 nM. The standard PCR conditions were 2 min at 50 °C, 10 min at 94 °C, then 40 cycles for 1 min at 94 °C and 1 min at 60 °C. The number of cycles in which the emission intensity of the sample rises above the baseline represents the relative quantity (RQ) and is proportional to the target concentration.

The analysis of RT-PCR conducted according to the Applied Biosystems 7500 Fast RT-PCR system user manual. The relative quantitative value (RQ) of the target group calculated by quantitative RT-PCR.

Enzyme-Linked Immunosorbent Assay (ELISA)

The expression levels of MMP-2 protein in dorsal skin tissues extracted from HRM-2 mice observed using MMP-2 ELISA kit (R&D System Inc., Minneapolis, MN, USA). MMP-2 coated antibody (100 μ L) were dispensed into each wells and incubated at 4 °C for 16 hrs. The plate was washed with washing buffer prior to the addition of assay diluent (200 μ L) and a 1 hr incubation at room temperature (RT). After diluting the standard solution and diluting the supernatant 20 times, the plate washed and and supernatant (100 μ L) was added to the well and incubated for 2 hrs at RT. The plate washed, working detector (100 μ L) added to wells, and it incubated for 1 hr at RT. After another washing, substrate solution (100 μ L) added to wells prior to incubation in a dark room for 30 min at RT. Finally, stop solution (50 μ L) added to the wells. The absorbance obtained at 450 nm using a microplate spectrophotometer.

Histological analysis

Histological analysis conducted to observe epidermal thickness and collagen fiber analysis. Dorsal skin of each group obtained after UVB irradiation for 5 weeks. The dorsal skin fixed with 10% formalin and embedded in paraffin. Sections of dorsal skin were stained with hematoxylin and eosin (H&E) and Masson's trichrome. After staining of sections, the changes of epidermal thickness and collagen fibers observed in the stained sections. Epidermal thickness and collagen fibers were observed at 100 \times magnification under a digital microscope.

Statistical analysis

Results expressed as means \pm standard deviation (SD). The data were analyzed using ANOVA and Duncan's multiple range tests. The $p < 0.05$, < 0.01 and < 0.001 showed significant difference.

Results

Changes of body weight and food efficiency

The mouse applied topically with the cream for 5 weeks. The body weight and food efficiency of all group obtained at every day. As the results, body weight of 3 groups didn't exhibit significant difference except for UVB-Vehicle control (Fig. 2B). The food efficiency showed similar pattern with body weight (Fig. 2C). Therefore, these results showed that mice applied the cream containing enriched-schizandrin didn't effect on side reaction of body growth.

Effect of cream containing enriched-schizandrin on IL-1 β , MMP-2, MMP-9 mRNA expressions of and expression of MMP-2 proteins in mice

The effects of cream containing enriched-schizandrin on mRNA expression of IL-1 β investigated. The IL-1 β mRNA expression increased under UVB irradiation (Fig. 3A). However, application of the cream containing enriched-schizandrin decreased mRNA expression of IL-1 β (Fig. 3A).

To observe the effect of the cream containing enriched-schizandrin on MMPs, MMP-2, MMP-9 mRNA expressions and MMP-2 protein expression investigated. MMP-2, MMP-9 mRNA expressions and MMP-2 protein expression increased in the UVB-vehicle group compared to the normal control group (Fig. 3B-3D). Application with the cream containing enriched-schizandrin suppressed the mRNA and protein expressions relative to UV-vehicle group with significant difference.

Effect of cream containing enriched-schizandrin on wrinkle formation in UVB-induced photoaging mice

The effect of the cream containing enriched-schizandrin on wrinkle formation observed in UVB-induced mice. The hairless mice irradiated with UVB for 5 weeks. The effect on formation of dorsal skin wrinkles and wrinkle depth observed (Fig. 4A and 4B). The increased wrinkle formation and profound wrinkle depth showed in the UVB-vehicle group. The profound wrinkle depth by UVB irradiation restored in mice applied cream containing enriched-schizandrin (Fig. 4A and 4B).

Effect of cream containing enriched-schizandrin on epidermal thickness and collagen degradation in UVB-induced photoaging mice

Histological analysis on dorsal skin of mice done to observe the anti-photoaging effects of the cream containing enriched-schizandrin on epidermal thickness and collagen degradation. H&E staining showed significantly increased epidermal thickness in UVB irradiation (Fig. 5A and 5B). Mice applied cream containing enriched-schizandrin exhibited decreased epidermal thickness (Fig. 5A and 5B). Masson's trichrome staining used to identify collagen fibers. Masson's trichrome staining showed significantly decreased collagen fibers in the UVB-vehicle group compared to the normal group (Fig. 4A). However, application of cream containing enriched-schizandrin showed significantly increased collagen fibers in the UVB-SC group compared to the normal group (Fig. 4A). These results suggest that the cream containing enriched-schizandrin inhibits epidermal thickness and collagen degradation by UVB irradiation.

Discussion

S. chinensis Fructus (SCF) is the dry fruit of *S. chinensis* (Turcz.) Baill and mainly used for the treatment of insomnia, palpitation and dysphoria in traditional Chinese medicine (Lu and Chen 2009). SCF mainly include polysaccharides, lignans and volatile oils (Cheng et al. 2014). Many studies have reported the properties of SCF including anti-HIV effects, anti-tumor effects and hepatoprotective effects (Xu et al. 2015). In addition, one of the components of the SCF, schizandrin, is reported many therapeutic effects,

including anti-inflammatory antioxidant and anticancer effects (Xu et al. 2019). In this study, enriched-schizandrin from SCF was prepared in 70% ethanol with sonication of 20 kHz for 4 hrs and its anti-photoaging efficacy was observed in UVB-induced hairless mice. The cream containing enriched-schizandrin inhibits UVB-photoaged wrinkle formation reducing expressions of IL-1 β , MMP-2, MMP-9 mRNA and MMP-2 protein.

Natural products as the cosmetics' components are often associated with good quality, marked activity and safety (Koch et al. 2019). In general, cosmetics containing plant extracts have a positive effect on the skin and ameliorate skin damage, erythema and lipid peroxidation under UV exposure without side effect (Arct and Pytkowska 2008). In this study, the cream containing enriched-schizandrin from SCF was prepared and side reaction of the cream application observed in change of body weight and food efficiency. Mice applied topically the cream for 5 weeks didn't generated side effect on body weight and food efficiency in 3 groups except for UVB-Vehicle control.

UVB radiation generally induces acute inflammatory responses by stimulating the release of proinflammatory cytokines (IL-1 α , IL-6, and IL-8) and leads extracellular matrix remodeling proteins including matrix metalloproteinases (MMPs) (Finkel and Holbrook 2000). Finally, these events eventually lead to photoaging of skin (Chung et al. 1996). In our result, UVB-irradiation increased expressions of IL-1 β mRNA, MMP-2, MMP-9 and MMP-2 proteins. The application of cream containing enriched-schizandrin decreased them in UVB-photoaged mice.

Continuous UVB exposure generated wrinkle formation (Kang et al. 2018). In Fig. 4, UVB irradiation for 5 weeks in mice increased depth of skin wrinkle. The topical application of the cream for 5 weeks decreased depth of skin wrinkle. Therefore, application of the cream in mice exert anti-wrinkle effect.

Photoaged skin showed increased epidermal thickness and collagen degradation by increasing MMPs (Lee et al. 2014). In Fig. 5, UVB irradiation for 5 weeks in mice increased skin thickness and collagen degradation, the cream containing enriched-schizandrin decreased thickness and collagen degradation in UVB-photoaged mice skin. From these results, we condensed that the cream containing enriched-schizandrin may be a protective candidate for photoaged skin.

Declarations

Funding Information

The authors have declared no funding.

Ethics statement

The animal experiments conducted according to protocols approved by the Animal Care Committee of the Institute of Daejeon University, South Korea (No. KW-160919-1). This article does not contain any studies with human participants performed by any of the authors.

Authors' contributions

MY Yun: In vitro and vivo laboratory work and study design;HJ Choi: manuscript preparation and data analysis; HJ Choi:data analysis and writing. All authors read and approved the final manuscript.

Availability of data and material

All the data involved in this study have been included within the article.

Conflict of interest

No conflict of interest.

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Figures

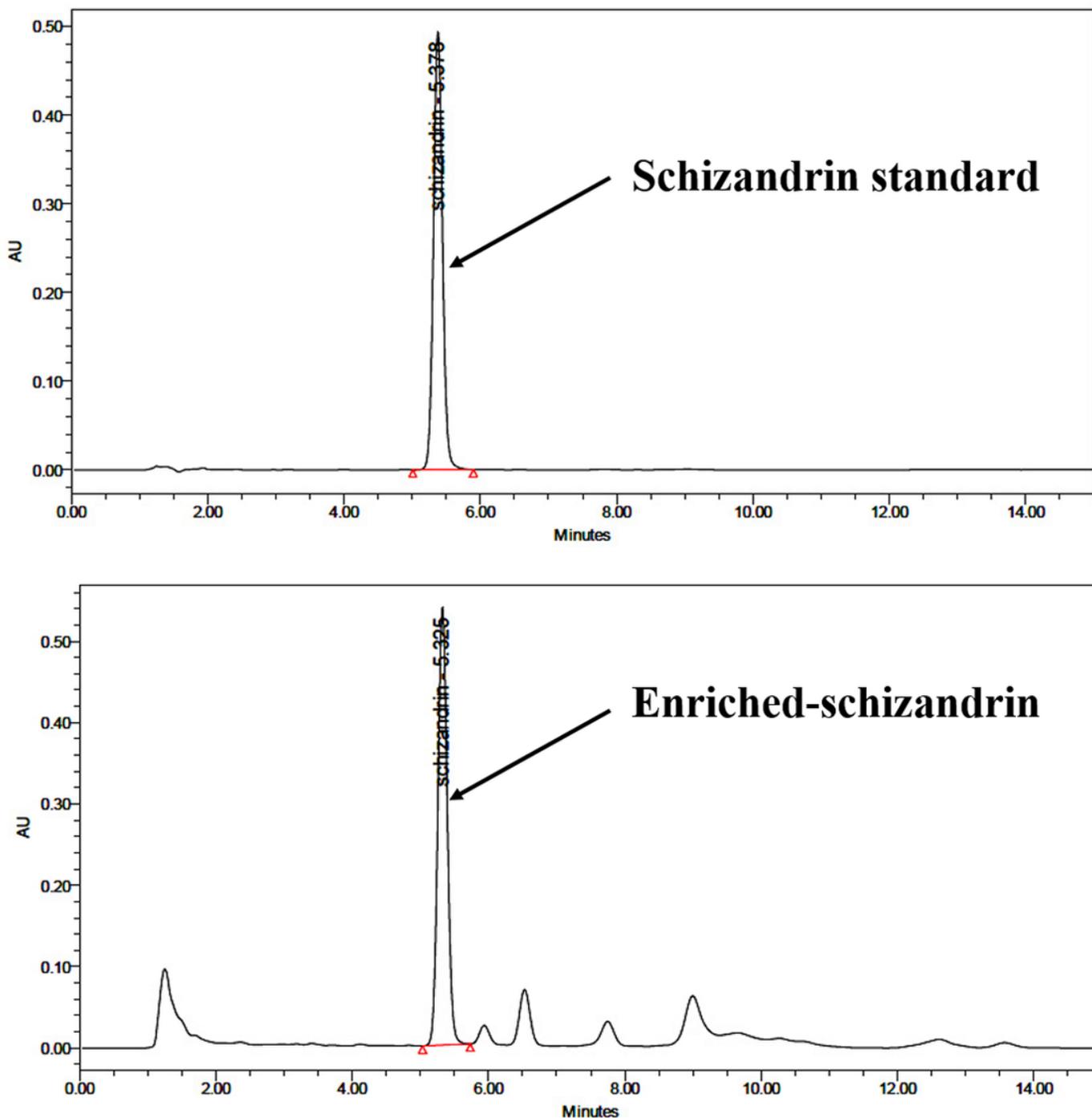


Figure 1

HPLC analysis of enriched-schizandrin from *S. chinensis*. The enriched-schizandrin prepared by 70% ethanol with sonication of 20 kHz for 4 hrs and analyzed by HPLC. HPLC analysis conducted under

Alliance e2695 XE HPLC with Wakosil-II C18 column analytical column (250 × 4.6 mm, CH₃CN : Water = 50 : 50, 1.0 mL/min, 40 °C and diode array detector 254 nm).

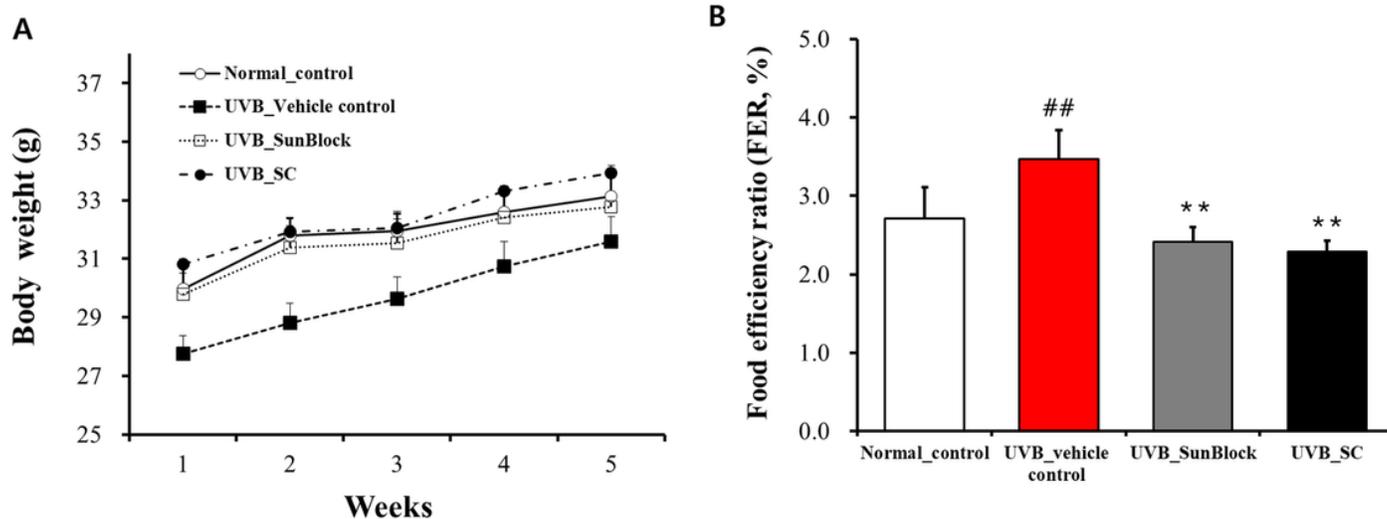


Figure 2

Body weight (A) and food efficiency ratio (B) in HRM-2 hairless mice applied the cream containing the enriched-schizandrin from *S. chinensis* topically for 5 weeks. UVB-vehicle (negative) control group; positive control (SunBlock), cream application (experiment) group. SC, cream containing enriched-schizandrin from *S. chinensis*. Values are expressed as means \pm S.D. from two-independent experiments (n =5). **Significantly different from UVB_Vehicle treatment (p<0.01).

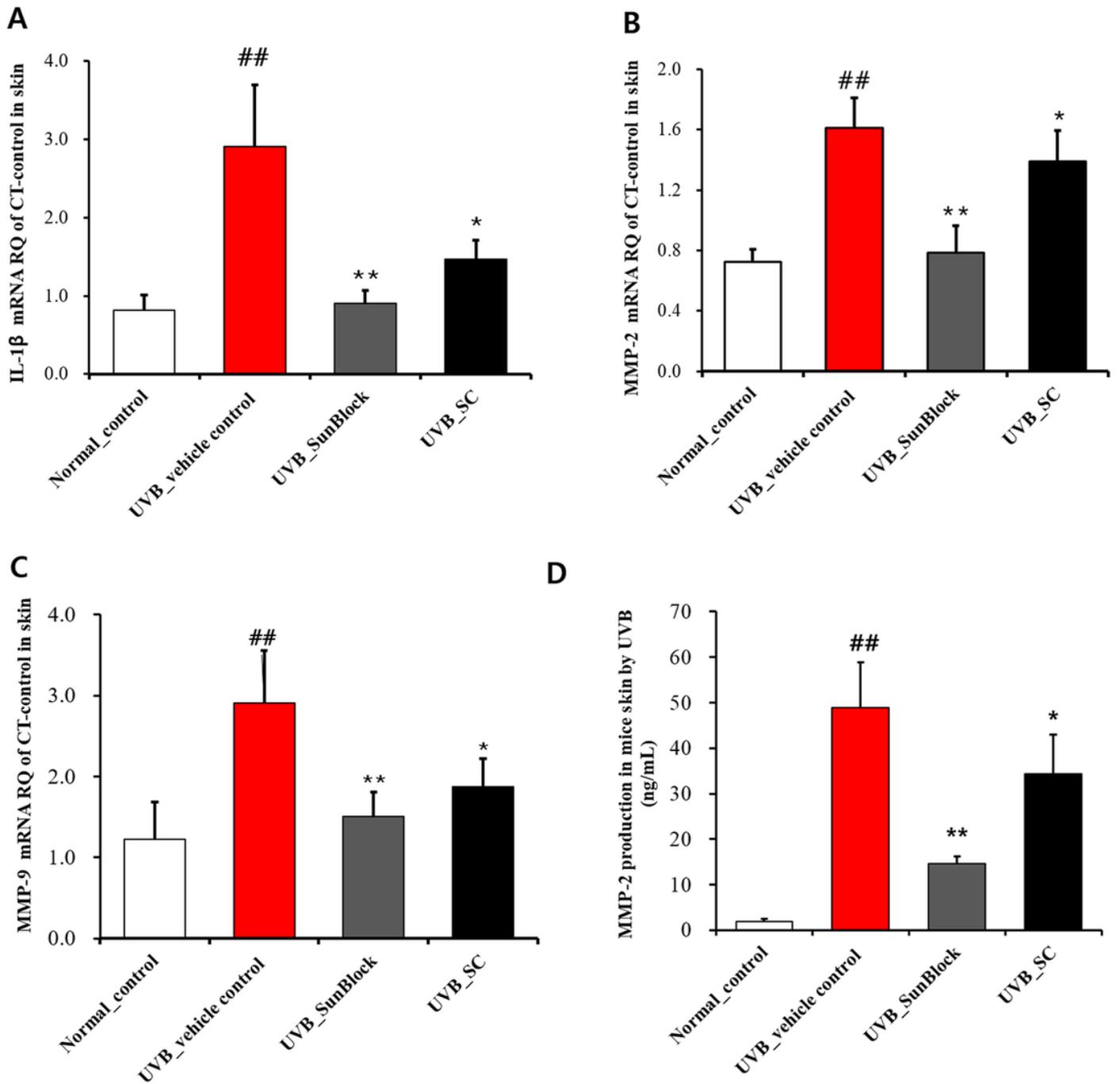


Figure 3

Effects of the cream containing enriched-schizandrin from *S. chinensis* on expression of cytokine and proteins in UVB-induced hairless mice. (A) mRNA expression of IL-1 β ; (B) mRNA expression of MMP-2; (C) mRNA expression of MMP-9; (D) MMP-2 proteins expression. Values are expressed as means \pm S.D. from two-independent experiments (n =5). ##Significantly different from normal_control (p<0.01). ###Significantly different from normal_control (p<0.001). *Significantly different from UVB_vehicle control (p<0.05). **Significantly different from UVB_vehicle control (p<0.01).

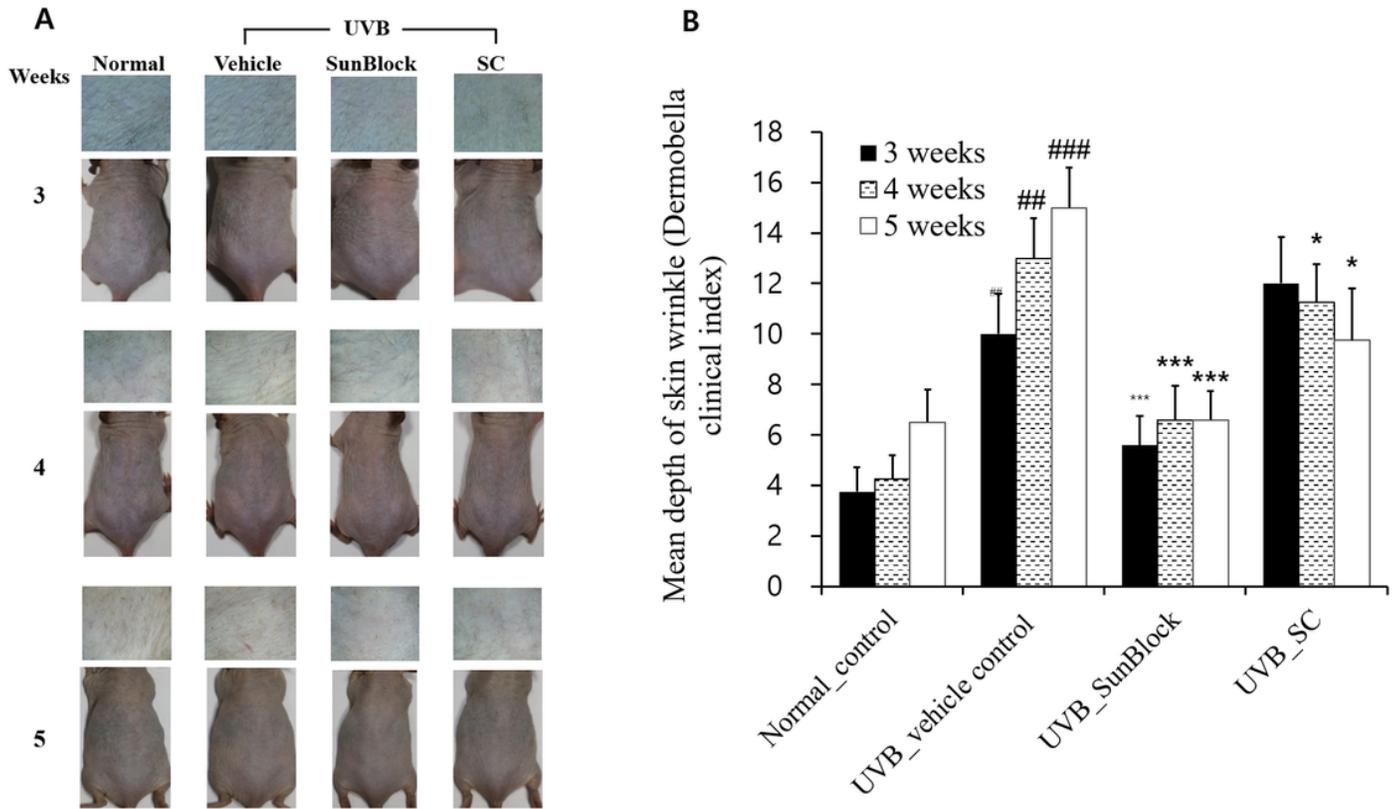


Figure 4

Effect of the cream containing the enriched-schizandrin from *S. chinensis* on UVB-induced wrinkle formation in hairless mice. (A) Features of dorsal skin of hairless mice exposed to UVB; (B) Mean of skin wrinkle depth. Values are expressed as means \pm S.D. from two-independent experiments (n =5). ##Significantly different from normal_control (p<0.01). ###Significantly different from normal_control (p<0.001). *Significantly different from UVB_vehicle treatment (p<0.05). **Significantly different from UVB_vehicle control (p<0.01). ***Significantly different from UVB_vehicle control (p<0.001).

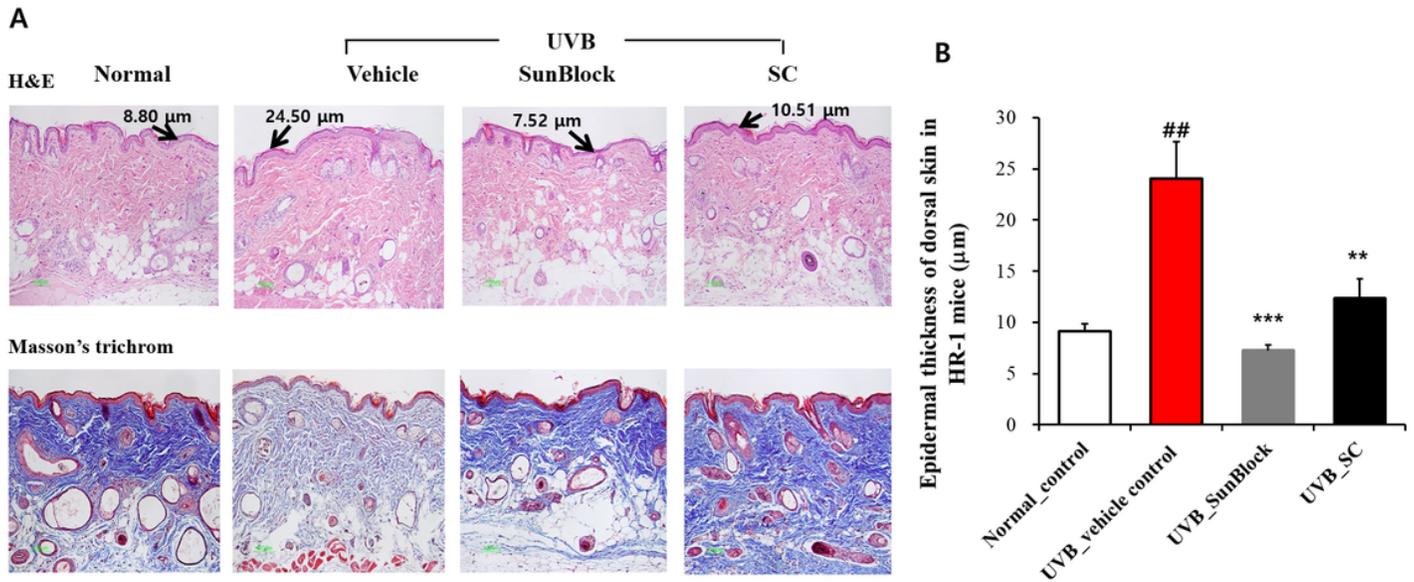


Figure 5

Histological analyses of epidermal thickness and collagen degradation in hairless mouse skin. (A) H&E stained sections; (B) Epidermal thickness; (C) Collagen fibers were stained using Masson's trichrome stain. Values are expressed as means \pm S.D. from two-independent experiments ($n = 5$). ##Significantly different from normal_control ($p < 0.01$). ***Significantly different from UVB_vehicle control ($p < 0.001$).