

쥐에서 자외선 B가 유도한 염증 반응에 대한 식물 추출물 함유 크림의 영향

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Effects of Plant Extract containing Creams on UVB Radiation-induced Inflammatory Responses in Mice

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요약: 자외선(UV)은 인체 피부 손상의 주된 요인이고 UV에 대한 피부의 초기 반응들은 홍반과 부종을 포함한다. 화장품에 사용할 효과적인 자외선 방어제를 발굴하기 위하여 세포수준의 효능평가법으로 다양한 식물 추출물들을 검색하였다. 시험된 총 38종의 식물 추출물들 중 제주조릿대(*Sasa quelpaertensis*), 접시꽃(*Althaea rosea*), 관중(*Dryopteris crassirhizoma*)에서 유래한 3종의 식물 추출물이 배양된 인체 표피 멜라닌세포에서 멜라닌 생성뿐만 아니라 UVB의 세포독성을 경감시켰다. 이 식물 추출물들의 항염증 작용은 동물 실험에서도 평가하였다. 대조 크림 혹은 식물 추출물을 1% 함유한 시험 크림을 C57BL/6 쥐의 귀에 혹은 SKH-1 무모쥐의 등에 UVB 조사 전과 후에 도포하였다. UVB 노출에 의한 영향으로서 부종 생성은 귀의 두께 변화를, 홍반 생성은 등 피부의 붉기의 변화를 측정하여 추정하였다. 3종류의 시험 크림 모두 두 실험 모두에서 항염증효과를 보였다. 제주조릿대, 접시꽃, 또는 관중 추출물을 함유한 크림은 자외선 조사 4일째 부종 반응을 각각 53.8%, 56.4%, 31.1% 경감시켰다. 또한 이 크림들은 자외선 조사 2일째 홍반 생성을 각각 45.7%, 34.1%, 20.5% 억제시켰다. 본 연구는 화장품에 함유된 특정 식물 추출물들이 자외선 과다노출로 야기되는 피부 염증을 완화시켜 줄 수 있음을 시사하였다.

Abstract: Ultraviolet radiation (UV) is a major cause of photodamages to human skin and the immediate responses of the skin to UV include the erythema and edema. In an attempt to find effective UV-protecting agents to be used in cosmetics, a number of plant extracts were screened in the cell-based assays. Among the total of 38 plant extracts tested, 3 plant extracts derived from *Sasa quelpaertensis*, *Althaea rosea*, and *Dryopteris crassirhizoma* attenuated the UVB-induced cytotoxicity as well as melanin synthesis in cultured human epidermal melanocytes. The anti-inflammatory effects of these plant extracts were further examined in animal models. A control or test cream containing 1% of a plant extract was topically applied to ears of a C57BL/6 mouse or the dorsal skin of a SKH-1 hairless mouse before and after the exposure to UVB. The change in ear thickness or dorsal skin redness due to UVB exposure was determined to monitor edema and erythema, respectively. All three test creams exhibited anti-inflammatory effects in both experiments. The creams containing *Sasa quelpaertensis*, *Althaea rosea* or *Dryopteris crassirhizoma* extract alleviated the UVB-induced edema response on day 4 by 53.8%, 56.4% and 31.1%, respectively. They also inhibited the erythema formation on day 2 by 45.7%, 34.1% and 20.5%, respectively. This study sug

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gests that the selected plant extracts formulated in cosmetics may attenuate skin inflammation caused by over-exposure to UV.

Keywords: inflammation, UVB, *Sasa quelpaertensis* Nakai, *Althaea rosea* Cavanilles, *Dryopteris crassirhizoma* Nakai

1. Introduction

The skin is an important organ providing a vast physiological barrier against mechanical, chemical and biological factors that may cause physiological disturbances or pathological events[1]. In addition, the skin provides a defense against ultraviolet radiation (UV) that causes the development of photoaging and photocarcinogenesis[2]. The structure of the skin comprises three major layers which from inside to outside are the hypodermis, dermis, and epidermis. Keratinocytes and melanocytes constitute about 95 % of the epidermis cells and the remainders are Merkel cells and Langerhan's cells[3]. Melanocytes, which are located at the stratum basale of the epidermis, synthesize melanins within membrane-bound organelles called melanosomes, and supply melanosomes loaded with melanins to the surrounding keratinocytes *via* dendrites[4]. In this way, melanocytes play a critical role in protecting the skin against UV radiation.

UV radiation consists of UVA (320 ~ 400 nm), UVB (280 ~ 320 nm) and UVC (200 ~ 280 nm). About 9 ~ 14 % of solar UVB reaches the depth of the melanocytes in the skin and UVB has a great potential to induce the inflammatory responses of the skin, observed as erythema and edema of sunburn reactions[3]. Sunburn reactions are known to be mediated by the p53 tumor suppressor which in turn is activated by DNA damages[5,6]. The p53 tumor suppressor causes cell cycle arrest, providing time for DNA repair or the removal of damaged cells, thus reducing the risk of skin cancer. Mutation of p53 may lead to cutaneous carcinogenesis. Overexposure to UV also causes the development of oxidative stress which is manifested as increased lipid peroxidation and the depletion of endogenous cutaneous anti-oxidants[7,8]. Previous studies have proven the preventive effects of exogenous anti-oxidants against photocarcinogenesis[9].

Cosmetics have become essential products in the pursuit of esthetic desires in modern society. Cosmetics have extended their role in preventing skin aging due to physiological and environmental factors. The main issues in this field include wrinkles and unwanted pigmentation, which are closely associated with the inflammation or oxidative stress due to overexposure to UV radiation[3,10]. Therefore there is current interest in identifying safer and more effective ingredients that might mitigate the vicious effects of UV radiation. In this regard, attractive are the plant extracts enriched with compounds that have UVB-shielding, anti-oxidative, and anti-inflammatory properties. Indeed, numerous plant extracts or constituents have previously been demonstrated to reduce inflammatory responses in animals and humans exposed to UVB[11,12]. The purpose of the present study was to examine the anti-inflammatory effects of cosmetic preparations which contained a selected plant extract, in animal models of erythema and edema due to UVB irradiation. This study focused on the extracts of *Sasa quelpaertensis* Nakai, *Althaea rosea* Cavanilles and *Dryopteris crassirhizoma* Nakai, which were selected because they had significantly reduced the UVB-induced cytotoxicity and melanin synthesis in cultured human epidermal melanocytes (HEMs).

2. Materials and Methods

2.1. Plant Materials

Samples of plant extracts used in the cell-based assay (Table 1) were purchased from Jeju bio-resource extract bank of Jeju Technopark (<http://www.jejuftp.or.kr>). The leaves, twigs or whole plants harvested in Jeju, Republic of Korea, were air-dried and extracted with 70 % (v/v) aqueous ethanol at room temperature. The extracted solutions were evaporated under reduced pressure to obtain crude extracts. Voucher specimens of

Table 1. Effects of Numerous Plants Extracts on Cell Viability and Melanin Synthesis under UVB

Plant name	Parts used	Cell viability under UVB (%)	Melanin synthesis under UVB (%)
Vehicle		76.0 ± 0.8	286 ± 13
<i>Acanthopanax koreanum</i> Nakai	Roots	61.4 ± 2.7	206 ± 5*
<i>Albizia julibrissin</i> Durazz	Leaves	62.4 ± 3.7	263 ± 13
<i>Althaea rosea</i> Cavanilles	Leaves	89.4 ± 5.2*	232 ± 30*
<i>Angelica keiskei</i> Koidzumi	Roots	72.0 ± 1.4	223 ± 8*
<i>Boehmeria platanifolia</i> Franch et Savatier	Leaves	67.7 ± 2.7	280 ± 13
<i>Clerodendron trichotomum</i> Thunberg	Leaves, twigs	22.0 ± 5.2	ND
<i>Cornus kousa</i> Buerger et Hanca		67.7 ± 3.5	217 ± 14*
<i>Cudrania tricuspidata</i> (Carriere) Breau	Leaves, twigs	55.0 ± 9.6	ND
<i>Daphniphyllum macropodum</i> Miquel	Leaves, twigs	83.7 ± 1.7*	250 ± 9
<i>Distylum racemosum</i> Siebold et Zuccarini	Leaves, twigs	65.0 ± 5.5	422 ± 52
<i>Dryopteris crassirhizoma</i> Nakai	Leaves, twigs	87.5 ± 1.4*	234 ± 5*
<i>Elaeagnus umbellate</i> Thunberg	Leaves	65.4 ± 1.9	349 ± 6
<i>Euonymus alatus</i> (Thunberg) Siebold	Leaves, twigs	76.0 ± 3.8	277 ± 18
<i>Euphorbia helioscopia</i> Linnaeus	Whole plants	57.0 ± 2.5	303 ± 15
<i>Eurya japonica</i> Thunberg	Leaves	66.6 ± 4.0	235 ± 15*
<i>Ficus erecta</i> Thunberg	Leaves	63.5 ± 2.4	259 ± 5
<i>Ficus erecta</i> Thunberg	Fruits	64.5 ± 3.4	228 ± 17*
<i>Hedera rhombea</i> Bean	Leaves	28.0 ± 2.8	ND
<i>Ligustrum obtusifolium</i> Siebold, et Zuccarini	Leaves, flowers	65.6 ± 2.6	263 ± 9
<i>Lindera erythrocarpa</i> Makino	Leaves	65.6 ± 4.1	257 ± 22
<i>Lindera obtusiloba</i> Blume	Leaves	65.6 ± 2.6	238 ± 11*
<i>Litsea japonica</i> Mirbel	Leaves	43.7 ± 3.3	286 ± 25
<i>Lonicera japonica</i> Thunberg	Leaves	64.5 ± 4.0	248 ± 16
<i>Mallotus japonicus</i> (Thunberg) Müller-Argoriensis	Leaves	59.3 ± 4.1	271 ± 29
<i>Melia azedarach</i> Linnaeus	Leaves	66.6 ± 3.9	259 ± 13
<i>Oxalis articulata</i> Sabigny	Whole plants	64.5 ± 3.2	246 ± 12*
<i>Petasites japonicas</i> (Siebold et Zuccarini) Maximowicz	Whole plants	80.8 ± 4.0	259 ± 5
<i>Punica granatum</i> Linnaeus	Leaves, twigs	40.0 ± 5.9	407 ± 62
<i>Quercus glauca</i> Thunberg	Leaves, twigs	84.0 ± 3.2	261 ± 24
<i>Quercus salicina</i> Blume	Leaves, twigs	10.0 ± 3.6	ND
<i>Rhododendron schlippenbachii</i> Maximowicz	Flowers	65.6 ± 7.2	304 ± 27
<i>Rhus javanica</i> Linnaeus	Leaves	65.6 ± 3.9	221 ± 18*
<i>Sasa quepaertensis</i> Nakai	Stems	92.0 ± 9.6*	204 ± 10*
<i>Smilax china</i> Linnaeus	Whole plants	76.0 ± 6.5	242 ± 23
<i>Smilax china</i> Linnaeus	Leaves, twigs	62.4 ± 1.9	238 ± 13*
<i>Tetragonia tetragonoides</i> (Pallas) Kuntze	Whole plants	60.0 ± 4.9	317 ± 35
<i>Wisteria floribunda</i> (willdenow) De Candolle	Leaves, twigs	93.0 ± 4.3	247 ± 29
<i>Zanthoxylum schinifolium</i> Siebold et Zuccarini	Leaves, twigs	67.7 ± 2.8	232 ± 8*

Data are presented as % of non-irradiated control cells. *, reduced cell death or melanin synthesis compared with the vehicle control (p < 0.05); ND, not determined due to cytotoxicity.

plants and detail information regarding the plant extracts are available at Jeju bio-resource extract bank.

2.2. Cell Based Assay : Effects of Plant Extracts on the Viability and Melanin Synthesis of HEMs Exposed to UVB

HEMs derived from moderately pigmented neonatal foreskins were obtained from Cascade Biologics (Portland, OR, USA). The cells were cultured at 37 °C in a humidified atmosphere containing 5 % CO₂ and 95 % air. The growth medium for HEMs was Medium 254 supplemented with Human Melanocyte Growth Supplement (Cascade Biologics, Portland, OR, USA), 100 U mL⁻¹ penicillin, 0.1 mg mL⁻¹ streptomycin and 0.25 µg mL⁻¹ amphotericin B). The cells were seeded on 12-well culture plates at a density of 5 × 10⁴ cells per well and grown in the growth medium for 48 h to reach 80 % confluency. Cells were pretreated with a test material (50 µg mL⁻¹) for 60 min and then exposed to UVB radiation (10 mJ cm⁻²), followed by incubation for an additional 48 h. The UVB treatment was conducted in a cell culture hood by using a UVB-18 lamp (ULTRA* LUM, Inc., Claremont, CA, USA)[13]. The UV intensity at the culture plate position was 80 µW cm⁻². Final dose of UV radiation was 10 mJ cm⁻². Cell viability was assayed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide[14]. Intracellular melanin was extracted with 0.1 M NaOH at 60 °C for 60 min and determined by the optical density at 490 nm[15]. The melanin content was normalized with respect to protein content, which was determined by Bio-Rad DC assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. Preparation of Creams Containing Plant Extracts

The extracts of *Sasa quepaertensis* Nakai, *Althaea rosea* Cavanilles and *Dryopteris crassirhizoma* Nakai were used as ingredients of cosmetic creams. The transmittance spectra of the plant extracts were recorded in the 200 ~ 400 nm range using a Shimadzu UV-1650PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The creams were manufactured by Dasso & Company Co. (Inchon, Korea) with and with-

out the inclusion of a plant extract. The cream had the following volumetric composition : Plant extract solution 10 %, moisturizer & emollient 18 %, anti-oxidant 0.1 %, viscosity increasing agent 3 %, wax 2.5 %, emulsifying agent 5 %, preservative 0.5 %, water 60.9 %. The control cream contained no plant extract and test creams A, B, and C contained *Sasa quepaertensis*, *Althaea rosea* and *Dryopteris crassirhizoma* extracts, respectively. Each plant extract was dissolved in propylene glycol at 10 % (w/v) and incorporated into a cream so that the final content of plant extract in the cream was 1 % (w/v).

2.4. Animal Experiments

Animal experiments were performed in accordance with the guideline of Kyungpook National University. The animals were maintained under controlled environmental conditions (23 ± 1 °C, 55 ± 5 % humidity, 12 h light/dark cycle) with free access to water and an *ad libitum* standard laboratory diet (Superfeed Co, Wonju, Kangwon, Korea). After an acclimation period, mice were randomly divided into 4 groups of 4 ~ 8 animals in each experiment.

2.4.1. Experiment 1 : Effects of the Creams on the Edema Induction by UVB in C57BL/6 Mice

A control or test cream was topically applied daily for a week to both ears of the 6-week-old female C57BL/6 mice (Daehan Biolink Co. Eumseong, Chungbuk, Korea) (100 mg per mouse per day). Twenty four hours after the last cream treatment, animals were anesthetized with a ketamine/rompun (5 : 1) mixture and the right ears were exposed to UVB (125 mJ cm⁻²) from a UVB lamp (Sankyo Denki Co., Hiratsuka, Kanagawa, Japan). The left ears were not exposed to UVB to provide non-irradiated controls. The creams were applied daily for an additional week. The thickness of both the irradiated and non-irradiated ears was measured using a digital caliper (Mitutoyo Co., Kawasaki, Kanagawa, Japan). The ear swelling response was calculated as the thickness of the right ear compared with the baseline value of the left ear.

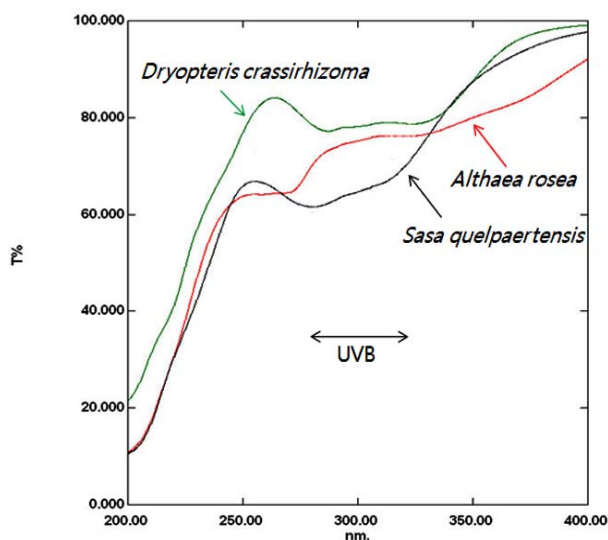


Figure 1. Transmittance spectra of the extracts of *Sasa quelpaertensis*, *Althaea rosea*, and *Dryopteris crassirhizoma* used in this study. Samples dissolved in 50 % ethanol at the concentration of $50 \mu\text{g mL}^{-1}$ were used for the spectrum measurement.

2.4.2. Experiment 2 : Effects of the Creams on the Erythema Induction by UVB in Hairless Mice

A control or test cream was topically applied twice a day for one week to two designated sites ($2 \times 2 \text{ cm}^2$ each) on the dorsal skin of the 6-week-old female SKH-1 hairless mice (Orient Bio, Inc., Gyeonggi-do, Korea) (100 mg per mouse per day). Twelve hours after the last cream treatment, the upper dorsal skin sites were exposed to UVB (250 mJ cm^{-2}). The lower dorsal skin sites were not exposed to UVB to serve as controls. The colors of both the irradiated and non-irradiated sites were measured using a spectrophotometer CR-10 (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan), in which the colors are described by L^* , a^* , and b^* values according to the Commission International de l'Eclairage color system. The a^* value from a reading was used as an index of skin redness.

2.5. Statistical Analysis

Data were presented as means \pm SEM of three or more independent experiments. Significant differences among the groups were determined by a one-way ANOVA. Duncan's multiple-range test was performed

if differences were identified between the groups at $p < 0.05$.

3. Results

HEMs were used for the preliminary screening of plant extracts that might affect the cellular response to UVB exposure. Melanocytes are advantageous in that they not only show viability loss but also increase melanin synthesis when overexposed to UVB, providing two indices of UVB-induced responses. A number of plant extracts were tested at $50 \mu\text{g mL}^{-1}$ by adding them to cultured cells 60 min before the UVB irradiation process (Table 1). Among the total of 38 plant extracts, only 5 extracts attenuated cell death due to UVB exposure, while several other extracts even promoted the cell death. In addition, a total of 13 plant extracts were observed to inhibit cellular melanin synthesis. There were 3 plant extracts which attenuated both the cell death and melanin synthesis due to UVB exposure. They were the extracts of *Sasa quelpaertensis*, *Althaea rosea* and *Dryopteris crassirhizoma*. The UV screening effects of these plant extracts were compared by measuring their transmittance spectra in UV range (Figure 1). The results showed that their UV screening effect decreased in the order: *Sasa quelpaertensis*, *Althaea rosea* and *Dryopteris crassirhizoma*.

In order to examine the potential anti-inflammatory effects of the creams containing a plant extract, two types of animal experiments were conducted according to the protocols summarized in Figure 2. These animal experiments were chosen because they are minimally invasive and provide reliable markers of inflammation such as edema and erythema of sunburn reactions. Creams A, B, and C contained 1 % of an extract derived from *Sasa quelpaertensis*, *Althaea rosea* and *Dryopteris crassirhizoma*, respectively.

First, the thickness of the UVB-irradiated ear was measured in comparison with the non-irradiated ear in a C57BL/6 mouse treated with a control or test cream that contained a plant extract. Creams were applied daily (100 mg per mouse per day) before and after UV exposure (125 mJ cm^{-2}). The final dose of a plant ex-

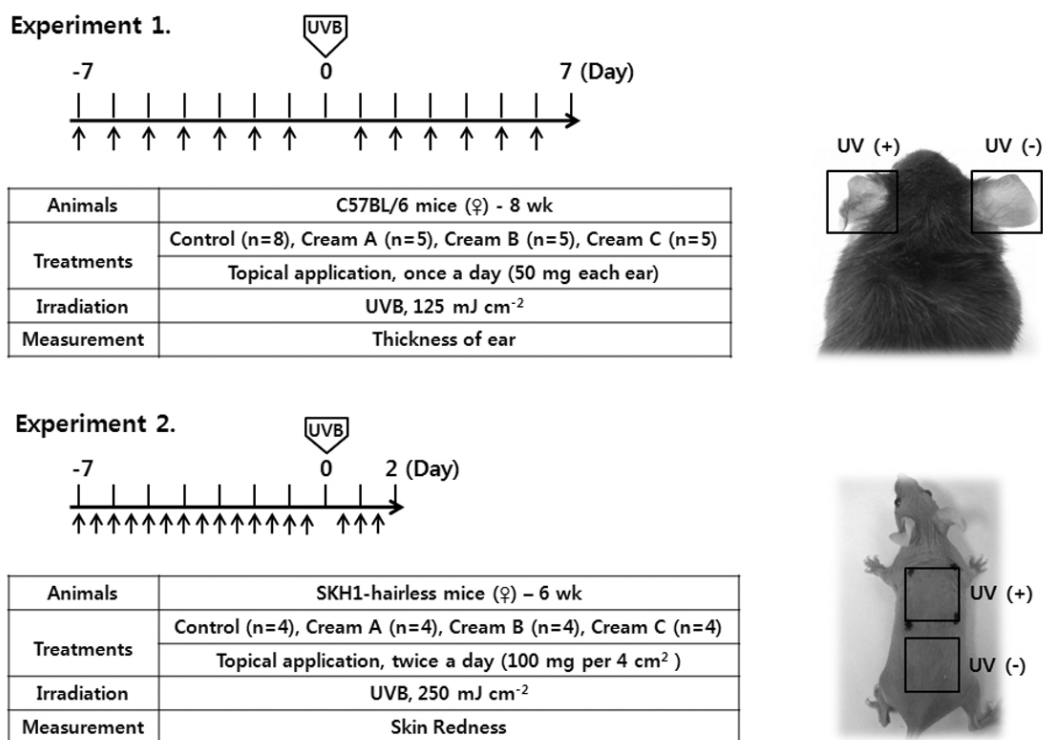


Figure 2. Protocols for animal experiments. UVB-induced edema and erythema were respectively investigated in C57BL/6 and SKH-1 hairless mice that were treated with a control or test cream according to the indicated schedules (arrows).

tract was thus 1.0 mg per mouse per day (50 mg per kg body weight per day). As shown in Figure 3A, the thickness of the UVB-irradiated ears of the mice increased gradually in a time dependent manner, whereas no significant changes were seen in the non-irradiated ears. The UVB-induced edema was estimated from the difference in the thickness of UVB-irradiated ears and non-irradiated ears, and the values were compared between control and test groups on day 1, 2, 3, and 4 (Figure 3B). The results showed that all three test creams reduced the edema induction by UVB compared with the control cream. For example, creams A, B and C alleviated edema response on day 4 by 53.8 %, 56.4 % and 31.1 %, respectively. Based on the results on day 4, creams A and B were considered to be more effective than cream C.

In the next experiment, the redness of the UVB-exposed dorsal skin site was measured in comparison with the non-irradiated skin site in a SKH-1 hairless mouse treated with a control or test cream. In this experiment,

the cream was applied twice daily (200 mg per mouse per day) and UVB was irradiated at the dose of 250 mJ cm⁻². As shown in Figure 4A, there was a great increase in the a^* value, an indication of redness, of the UVB-exposed sites while the values of the control sites remained virtually unchanged indicating erythema induction by UVB exposure. The increase of the a^* values due to UVB exposure was significantly less in mice treated with cream A, B and C, than in control mice on days 1 and/or 2 (Figure 4B). Creams A, B and C inhibited erythema formation on days 1 ~ 2 by 31.0 ~ 45.7 %, 26.1 ~ 34.1 % and 10.2 ~ 20.5 %, respectively. Thus cream A was considered to inhibit the erythema induction most effectively.

4. Discussion

Many plant extracts are rich in phenolic compounds or other secondary metabolites which can provide UVB-screening, anti-oxidative, and anti-inflammatory activities.

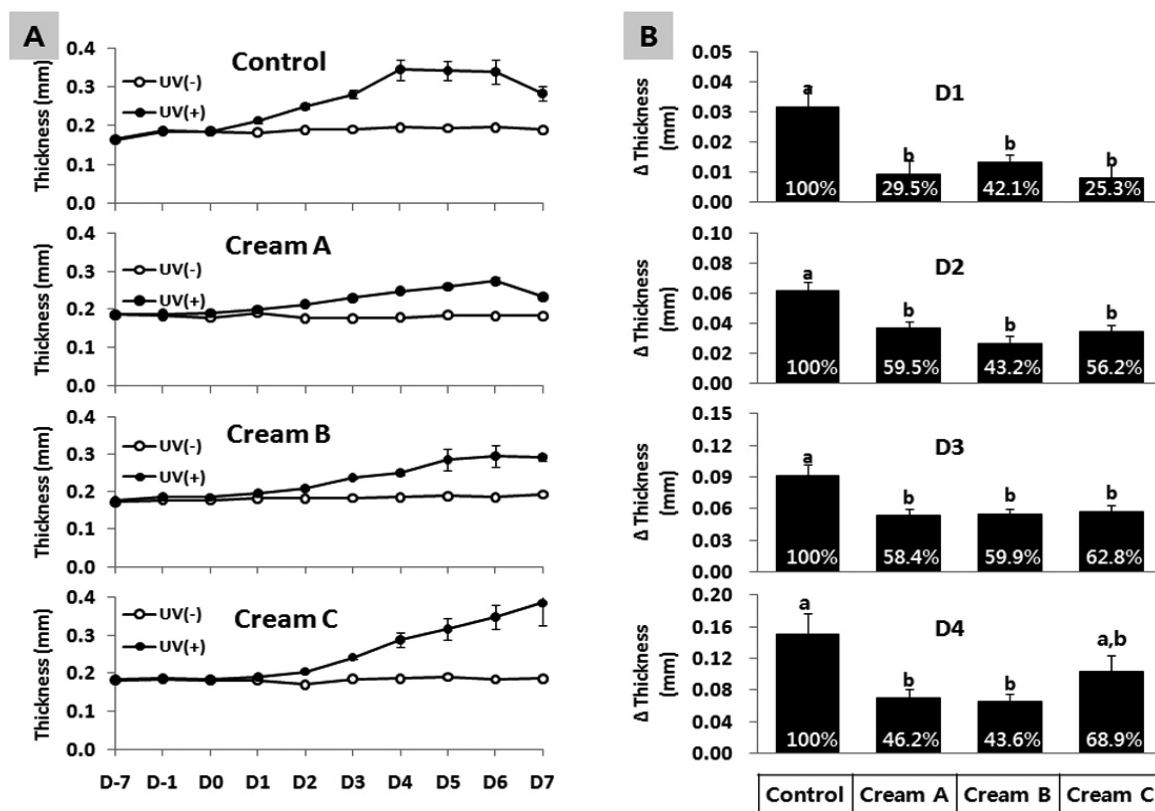


Figure 3. Effects of plant extract containing creams on the edema induction due to UVB exposure in C57BL/6 mice. Experimental protocol is shown in Figure 2 (Experiment 1). The thickness of both ears of an animal was measured before and after UVB-exposure of one ear (A). UVB-induced edema was estimated from the difference in the thickness of the UVB-irradiated ears and non irradiated ears, and the values were compared between the control and test groups on days 1, 2, 3, and 4 (B). The numbers on each column indicate relative edema values. Data not sharing the same letters (a or b) are significantly different from each other.

However, certain plant constituents can exhibit rather cytotoxic or pro-inflammatory effects in the absence and presence of irradiation with strong sun light. Thus, a high level of care must be taken in choosing the plant extracts for the use in cosmetics. The primary purpose of the current study was to choose plant extracts suitable for cosmetic use in terms of safety and efficacy. Preliminary screening tests at the cell level using HEMs led to the selection of three plant extracts that mitigated UVB-induced cytotoxicity and melanin synthesis (Table 1). The anti-inflammatory effects of the selected plant extracts were verified in animal models of the UVB-induced edema and erythema (Figures 3, 4).

Among the three plant extracts derived from *Sasa quelpaertensis*, *Althaea rosea* and *Dryopteris crassirhi-*

zoma, which were selected from the cell-based assays, *Sasa quelpaertensis* extract exhibited the most prominent and consistent anti-inflammatory effects in animal experiments (Figures 3, 4). *Sasa quelpaertensis* (Korean name, Jeju-Joritdae) is a type of bamboo widely distributed in Jeju island, Republic of Korea. Various types of bamboos have been used in traditional medicine in Asia for centuries, and modern studies have reported their anti-ulcer, anti-cancer and anti-oxidant effects[16-18]. Recent studies on *Sasa quelpaertensis* have revealed its anti-melanogenic affects[15,19] and hepatoprotective effects against alcohol intoxication[20]. *p*-Coumaric acid has been identified as a major active compound of *Sasa quelpaertensis*, providing anti-melanogenic and hepatoprotective effects[15,20]. *p*-Coumaric

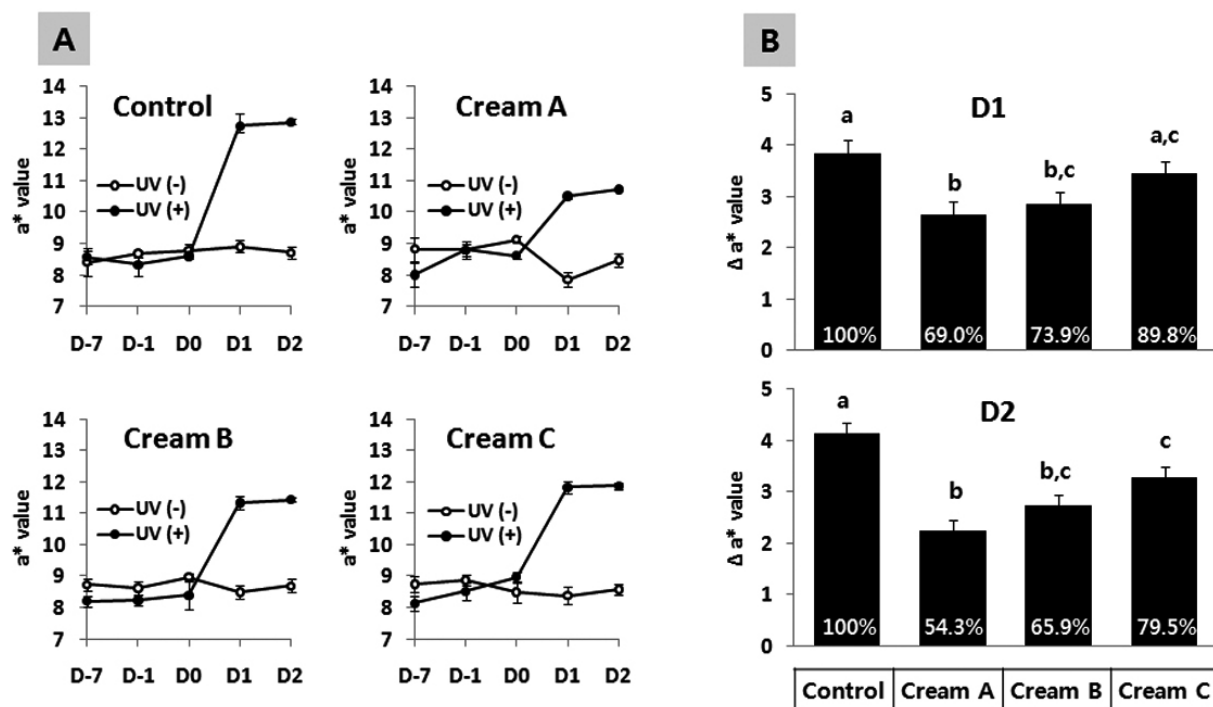


Figure 4. Effects of plant extract containing creams on the erythema induction due to UVB exposure in SKH-1 hairless mice. Experimental protocol is shown in Figure 2 (Experiment 2). The redness (a^* value) of two designated sites on the dorsal skin of an animal was measured before and after UVB-exposure of one specified site (A). UVB-induced erythema was calculated as the redness of the UVB-exposed sites minus the base line value of the control site, and the values were compared between the control and test groups on days 1, and 2 (B). The numbers on each column indicate relative erythema values. Data not sharing the same letters (a, b or c) are significantly different from each other.

acid has a high absorptivity of UVB and potent anti-oxidative properties as demonstrated in cells[21] and animal models[22]. Furthermore, *p*-coumaric acid increased the viability of HEMs under UVB[13] and prevented erythema induction in human skin exposed to UV[23]. Therefore it is tempting to speculate that *p*-coumaric acid might be the active compound responsible for the anti-inflammatory effects of the *Sasa quel-paertensis* extract against UVB-induced sunburn reactions of the skin.

Previous studies have shown that the flower of *Althaea rosea*, which contained various phenolic acids and flavonoids[24,25], exhibited analgesic and anti-inflammatory effects in animal models[26]. A number of studies have also demonstrated the anti-oxidative, anti-bacterial, and anti-cancer activities of the rhizome of *Dryopteris crassirhizoma*[27-29]. However, little is known regarding the constituents and bioactivities of the

leaves of *Althaea rosea* and the aerial parts of *Dryopteris crassirhizoma* used in the current study. Therefore further studies are warranted to identify the active constituents of these plants that provided anti-inflammatory effects against UVB irradiation.

5. Conclusion

In conclusion, this study suggests that some plant extracts formulated in cosmetic creams could help attenuate inflammation due to overexposure to UVB. The anti-inflammatory effects of the plant extracts might be attributed to the UVB-shielding and anti-oxidant effects of their constituents but further studies are needed to conclusively define the mechanism and active constituents.

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