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A Thesis for the Degree of Master of Science

**Development of 7,3',4'-Trihydroxyisoflavone, an
Enzyme-Converted Soy Isoflavone of Daidzein, as Anti-
Atopic Cosmetic Material**

콩 이소플라본 다이드제인의 효소전환체 7,3',4'-
트리하이드록시이소플라본을 활용한 아토피 개선
기능성 화장품 소재 개발

August, 2017

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ABSTRACT

Atopic dermatitis (AD) is characterized by chronic inflammatory skin lesions. It commonly occurs in childhood and infancy when the immune system is not sufficiently developed. Although many studies are ongoing, the cause and mechanism of AD have not been defined clearly. Despite its growing prevalence, therapeutic treatments remain limited. It is generally accepted that topical steroid treatment is crucial for the management of AD, but it cannot be used for long periods because a lot of side-effects including liver and kidney toxicity, diabetes, skin thinning, and drug resistance are frequently observed. Also, cosmetic therapy for AD care is only focused on skin hydration.

Thus, there is great need for development of new and effective approach for AD care. For cosmeceutical care of AD, the integrated research should be conducted including functional effect, constant effect in cosmetic formulation, and also satisfy the clinical safety.

Phytochemical as natural immune modulators from herbal or plant may be useful for treating AD symptoms. Among the phytochemicals, soy isoflavone could be the new cosmeceutical materials from natural resources for AD care. This study examined the anti-atopic effect of soy isoflavone, 7,3',4'-trihydroxyisoflavone (7,3',4'-THIF), a metabolite of Daidzein. 'Enzyme-converted' means enzymatic structure conversion of isoflavone selectively to enhance its functional effect. We found that 7,3',4'-THIF suppresses the atopic marker; thymus and activation regulated chemokine (TARC/CCL17), Macrophage-Derived Chemokine(MDC/CCL22) in HaCaT cell line. And we confirmed that the anti-atopic effect is also available in cosmetic formulation in NC/Nga mice model induced by *Dermatophagoides-farina* extract(DFE). Histological analysis demonstrated that 7,3',4'-THIF decreased DFE-induced eosinophil and

mast cell infiltration into skin lesions. We also found that 7,3',4'-THIF significantly reduced the DFE-induced increase in serum IgE and MDC level. We confirmed the liver and kidney toxicity compared to dexamethasone. These results suggest that 7,3',4'-THIF might be a cosmeceutical material candidate for the treatment of AD.

Keywords: Soybean; Isoflavone; Daidzein; 7,3',4'-trihydroxyisoflavone; Dexamethasone; Atopic dermatitis; NC/Nga; DFE; Enzyme Conversion; Physicochemical stability; Cosmetic material; Topical application; TARC(CCL17); MDC(CCL22); IgE; Student ID: 2015-20517

CONTENTS

ABSTRACT	i
CONTENTS	iv
I . INTRODUCTION	1
II . MATERIALS AND METHODS	5
1. Chemicals and reagents	5
2. Sample preparation	6
3. Cell culture and treatments	6
4. Cell viability	7
5. Sample optimization	8
6. Animals and treatments	9
7. Evaluation of clinical symptoms	11
8. Measurement of kidney and liver toxicity	11
9. Measurement of TARC, MDC and IgE	11
10. Histological examination	12
11. Statistical analysis	13

III. RESULTS.....	14
1. 7,3',4'-THIF decreased DFE-induced TARC, MDC expression in HaCaT cell line	14
2. Physicochemical stability of 7,3',4'-THIF and sample optimization	18
3. Topical application of 7,3',4'-THIF decreased DFE-induced AD symptoms in NC/Nga mice	23
4. 7,3',4'-THIF decreased DFE-induced infiltration of eosinophils and mast cells into skin lesions in NC/Nga mice	29
5. Pre-clinical liver and kidney changes of 7,3',4'-THIF compared to Dexamethasone	34
6. 7,3',4'-THIF attenuates DFE-induced increases in IgE, and MDC levels in NC/Nga mice	37
IV. DISCUSSION.....	40
V. CONCLUSION.....	44
VI. REFERENCES.....	46
VII. 국문 초록.....	49

I. INTRODUCTION

Atopic dermatitis (AD) is an inflammatory skin disease related to immune disorder. The first symptom of which mainly occurs in childhood. It is accompanied by itching, dry skin, chronic dermatitis and characteristic eczema. Although a lot of research is going on all over the world, specific cause and mechanism of AD has not yet been clarified [1]. In general, it is presumed that skin barrier abnormality based on environmental, genetic, and immunological factors are main causes of AD. The most common cause is the involvement of allergic reactions to antigens due to deterioration of skin defense function [2]. Antigens are allergens that cause allergies, such as house dust mites and fungi [3].

Acute skin lesions of AD have very itchy rashes and inflammation. In addition, infiltration of immune cells such as mast cells and eosinophils is observed, which also contributes to the inflammatory

response [4]. In skin lesions of AD, inflammatory cytokines and chemokines are locally overexpressed. Cytokines such as TNF- α in cells (keratinocytes, mast cells and dendritic cells) bind to receptors in vascular endothelial cells and activate the cell signaling system. These processes cause inflammatory cells to leak out of the blood vessels and infiltrate into tissues, which react with chemoattractant cytokines and chemokines. In particular, chemokine receptor (CCR) 4 ligands such as thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) promote inflammatory infiltration of Th2 lymphocytes and ultimately increase IgE expression in lesional tissues [4-6].

The treatment of AD consists of the 3-step. First stage is caring without drug, the second step is topical application of the anti-inflammatory agent such as steroids to the lesion, and the third step is administration of drug to complement the immune control function.

Depending on the patient's symptoms and conditions, step-by-step therapy should be applied [7].

Steroids are anti-inflammatory drugs that are essential in AD. It is effective for the treatment of acute AD, for a short period of time, by inhibiting the progress of inflammation. So it is widely used for the management of AD [8]. However, due to serious side effects such as liver and kidney function, diabetes, skin thinning, immunodeficiency, and drug resistance, other alternative therapies are needed [9]. In order to solve this problem, new drug such as nonsteroidal local ointment have been developed recently [10]. However, many side effects have been reported on these nonsteroidal medicines also. As these steroids and synthetic AD treatments exhibit various side effects and limitations, the value of natural active substances that are considered to be relatively safe is newly emerging. Many studies are being actively carried out to improve AD without side effects by appropriately using immune-

modulators derived from natural substances with high safety [11].

7,3',4'-THIF is a rare isoflavone derived from soy. The physiological activity of 7,3',4'-THIF has not been actively studied yet. 7,3',4'-THIF can be obtained by Enzyme-Conversion from Daidzein, which is a major soy isoflavone, along with Genistein [12]. The administration effect of 7,3',4'-THIF on the improvement of AD has been studied [13]. However, the effect of 7,3',4'-THIF as a topical agent for external use has not been studied until now. In this study, we investigated the effect of external application of 7,3',4'-THIF on the improvement of AD. In addition, we want to find a way to stably add 7,3',4'-THIF to cosmetic formulations, and finally, we intend to develop 7,3',4'-THIF as an anti-atopic functional cosmetic material.

II. MATERIALS AND METHODS

1. Chemicals and reagents

7,3',4'-THIF was obtained from Daidzein (Xi'an Rongsheng Biotechnology Co., Ltd, China) by enzyme converting process [12]. Dexamethasone was bought from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified eagle medium (DMEM) was purchased from Welgene (Gyeongsan, Korea). Fetal bovine serum (FBS) was bought from Sigma-Aldrich (St.Louis, MO). 3-[4,5-dimethyliazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) powder was purchased from USB Co. (Cleveland, OH). Penicillin-Streptomycin Solution was purchased from Mediatech, Inc. (Manassas, VA). Protein assay reagent kits were obtained from Bio-Rad Laboratories (Hercules, CA). Cosmetic solvents for topical application were bought from Shinjin Cosmetic co. (Seoul,

Korea). *Dermatophagoides farina* body extract (DFE)-AD cream was purchased from Biostir Inc. (Hiroshima, Japan)

2. Sample preparation

7,3',4'-THIF was provided by Dr. Byung Gee Kim (Seoul National University, Seoul, Korea). 7,3',4'-THIF was converted from Daidzein (Xi'an Rongsheng Biotechnology Co., Ltd, China) by enzyme converting process [12]. 7,3',4'-THIF was gained after purification, elution, and drying process. The purity of 7,3',4'-THIF was measured by HPLC more than 98%. All samples (7,3',4'-THIF, Daidzein, Dexamethasone) were dissolved in PEG300, 1,3-Butyleneglycol (7:3) for animal treatment and in dimethyl sulfoxide (DMSO) for cell treatment.

3. Cell Culture and treatments

Human keratinocytes (HaCaT; kindly provided by Dr. Zigang Dong, Hormel Institute, University of Minnesota, Minneapolis, MN, USA) were cultured at 37°C in a 5% CO₂ atmosphere in DMEM supplemented

with 10% FBS, 2mM L-glutamine and penicillin/streptomycin. Cell cytotoxicity was measured by MTT assay. The cells were cultured in 96-well plates at a density of 5×10^4 cells/well, and incubated at 37°C in a 5% CO₂ atmosphere prior to serum deprivation for 24 h. Various concentrations of samples (7,3',4'-THIF, Daidzein) were added to the wells for 22 h.

4. Cell viability and measurement of TARC and MDC Level

The cell cytotoxicity was measured using the MTT assay. HaCaT were cultured in the 96 well plates at a density of 5×10^4 cells/well and incubated in DMEM-10% FBS containing penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere. Cells were starved in serum-free DMEM for 24 h. The cells and each sample were incubated for 22 h at 37 °C, followed by treatment with MTT solution for 2 h. The medium was removed and formazan crystals were dissolved by the addition of dimethyl sulfoxide (DMSO). The absorbance at 570 nm was then

measure using a microplate reader (Molecular Devices, CA).

HaCaT cells were seeded at a density of 1×10^6 cells/well in a 6 well plate for sandwich ELISA after incubation for 48 h, the cells were stimulated with 10ng/ml TNF- α /IFN- γ in the presence or absence of 7,3',4'-THIF or Daidzein for the indicated period of time. TARC and MDC levels were measured using ELISA kits according to the manufacturer's instructions.

5. Sample optimization

Among the major solvents (Glycerin, 1,3-butylene glycol, Propylene glycol, PEG300, PEG400, Ethanol) used in cosmetics, suitable solvent were selected to ensure solubility in the effective concentration range. To improve the unstable temperature and light stability of the 7,3',4'-THIF, a selection of solvents was carried out. The temperature stability was measured under time-dependent four temperature conditions of 5 °C, 25 °

C, 40 ° C and 50 ° C. The light stability measurement was carried out under sun-light exposed condition. It was also time-dependent. To optimize the solubility, temperature and light stability, optimal solvent combination was selected by changing the blending ratio. To measure the temperature and light stability, the degree of degradation of 7,3',4'-THIF was measured by measuring the absorbance at 340 nm [14]. The absorbance at 340 nm was measured using a microplate reader (Molecular Devices, CA).

6. Animals and treatments

Three-week-old NC/Nga male mice were purchased from SLC Japan (Tokyo, Japan). Mice were housed in individual ventilated cages under specific pathogen-free conditions at 22°C with a 12-h light-dark cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Seoul National University, Korea [15].

The overall experimental design is depicted in Fig. 3A. After

one week of acclimation, mice were divided into the following nine groups (n=8 per group): (1) Control (Non-induction) + Vehicle, (2) *Dermatophagoides farina* extract (DFE) + Vehicle (3) DFE + 7,3',4'-THIF 800 nmols in Vehicle, (4) DFE + 7,3',4'-THIF 200 nmols in Vehicle, (5) DFE + 7,3',4'-THIF 50 nmols in Vehicle. (6) DFE + Daidzein 800 nmols in Vehicle. (7) DFE + Daidzein 200 nmols in Vehicle. (8) DFE + Daidzein 50 nmols in Vehicle. (9) DFE + 0.1% Dexamethasone (510 nmols) in Vehicle. To induce AD-like symptoms and skin lesions, DFE was applied to the dorsal skin and the back of both ears of NC/Nga mice. To disrupt the skin barrier, a day after complete dorsal hair removal (approximately 4 cm²), and 200 μL of 4% (w/v) sodium dodecyl sulfate were topically applied to the shaved dorsal skin surface 1 h before DFE-AD cream application. DFE-AD cream was applied twice per week for 3 weeks (100 mg per mouse per application) [15].

All samples (7,3',4'-THIF,Daidzein,Dexamethasone) dissolved in Vehicle (PEG300:1,3-BG 7:3) and were topically applied to the dorsal skin, face, and back of both ears, from 50 nmols to 800 nmols, five times a week for 3 weeks. At the end of experiments, animals were anesthetized with 2% isoflurane.

7. Evaluation of Clinical Symptoms

Scratching time was observed for 10 min per mouse to evaluate itching severity. Epidermal thickness was measured with Vernier Calipers.

8. Measurement of kidney and liver change

Kidney and liver were collected on the last day of the experiment (day 21). The relative weight change of kidney and liver were compared by measuring the kidney and liver weight per bodyweight.

9. Measurement of serum MDC and IgE

Blood and dorsal skin were collected on the last day of the experiment

(day 21) and stored until use. Serum MDC and IgE levels were measured using an enzyme-linked immunosorbent assay ELISA kit (R&D system, USA) according to the manufacturer's instructions.

10. Histological examining

To investigate epidermal thickness, Hematoxylin and eosin staining was performed. Mouse skin samples were fixed with 10 % neutral-buffered formalin, and embedded in paraffin. Serial sections (4 μm) were mounted onto slides. After deparaffinizing, skin sections were re-hydrated and stained with Hematoxylin solution for 5 minutes. And then, slides were washed and stained in counterstain in eosin Y solution for 30 seconds. Next, the slides were dehydrated through 95% alcohol and washed in absolute alcohol, 5 minutes each. Lastly, they were incubated in xylene overnight to clear of any water and then dry them. Skin sections were examined at 400 \times magnification using an Olympus AX70 light microscope (Tokyo, Japan). To detect eosinophil and mast cell

infiltration, the dorsal skin of each mouse was also prepared on the last day of the experiment (day 21) as described above. Deparaffinized skin sections were stained with Congo Red (CR) and Toluidine Blue (TB), respectively. The number of eosinophils and mast cells per 0.025 mm² skin was counted at 400× magnification. Tissue sections were examined using an Olympus AX70 light microscope (Tokyo, Japan) [16].

11. Statistical analysis

Statistical analyses were performed using one-way ANOVA followed by Duncan and *p* values of less than 0.05 were considered statistically significant.

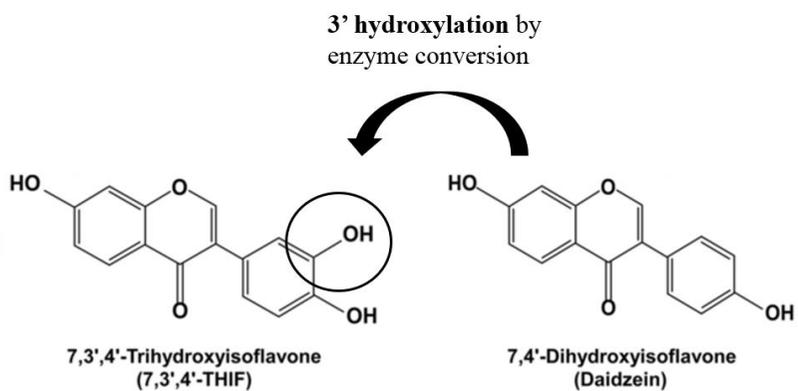
III. RESULTS

1. 7,3'4'-THIF decreased TNF- α /IFN- γ induced TARC, MDC expression in HaCaT cell line.

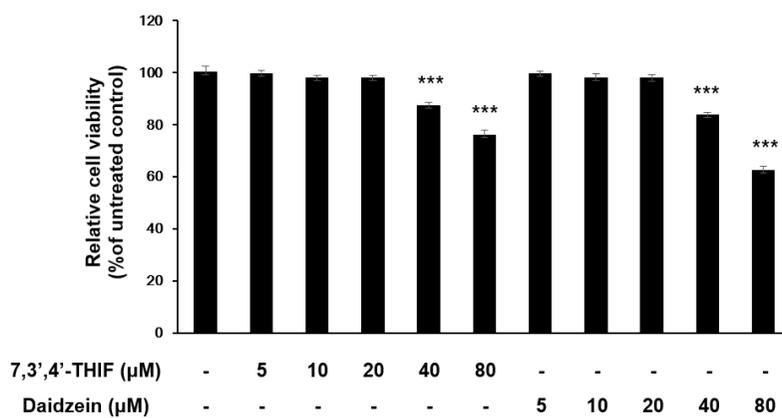
To determine cytotoxicity of 7,3'4'-THIF, cell viability assay (MTT assay) was performed (Fig. 1B). To investigate anti-atopic effects of 7,3'4'-THIF, HaCaT cells were treated by TNF- α /IFN- γ cocktail inducer, followed by 7,3'4'-THIF. TARC and MDC expression level were measured by ELISA assay (Fig. 1C, 1D). There was more significant decrease of TARC and MDC level in 7,3'4'-THIF than that of Daidzein.

Figure 1

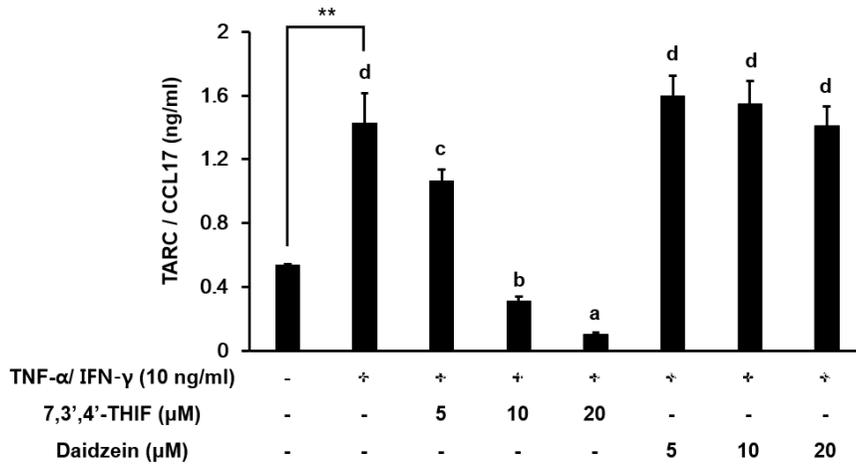
A



B



C



D

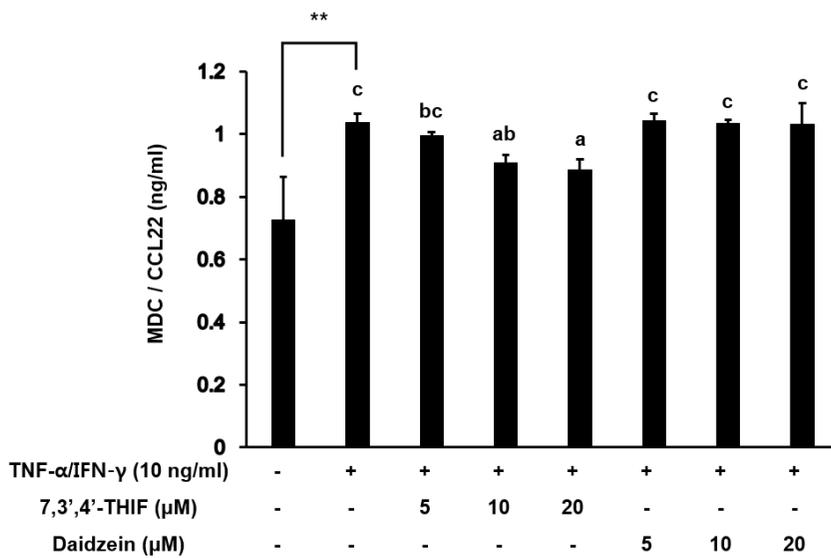


Figure 1. Effects of 7,3',4'-THIF and Daidzein on atopic chemokine production in HaCaT Cell line.

(A) 7,3',4'-THIF was produced by enzyme-converting process from Daidzein. (B) Cell viability of HaCaT was constant up to 20 μ M 7,3',4'-THIF and Daidzein. (C, D) 7,3',4'-THIF decreased the production of atopic chemokine TARC (CCL17) and MDC (CCL22) in HaCaT cells. The cells were pretreated with TNF- α /IFN- γ cocktail for 1h, and treated with each sample at the indicated concentrations for 1 h. Data represent the means \pm SEM (n=3).

2. Physicochemical property of 7,3',4'-THIF and sample optimization.

Solubility, light stability and temperature stability were measured in 7 kinds of cosmetic solvents (Glycerin, 1,3-butylene glycol, Propylene glycol, PEG 300, PEG 400, Ethanol, DW) for 7,3',4'-THIF (Table. 1). The solubility of 7,3',4'-THIF in each solvent was measured in order to select a solvent which can ensure an effective concentration (20 mM) (Fig. 2A).

Table 1. Confirmation of dissolution of 7,3',4'-THIF in cosmetic solvent by concentration interval

Conc.	Glycerin	1,3-BG	PG	PEG300	PEG400	EtOH	DW
10 mM	-	O	Δ	O	O	O	-
20 mM	-	O	-	O	O	O	-
40 mM	-	ΔΔΔ	-	O	ΔΔΔ	ΔΔΔ	-
80 mM	-	Δ	-	ΔΔΔ	ΔΔ	Δ	-

* Temperature: 25°C

* O : 100% ΔΔΔ : 80-99% ΔΔ : 50-79% Δ : 20-49% - : 0-19%

The temperature stability measurement was carried out on three solvents (PEG300, 1,3-Butylene glycol, Ethanol) which can ensure solubility (20 mM). The temperature stability was measured under four

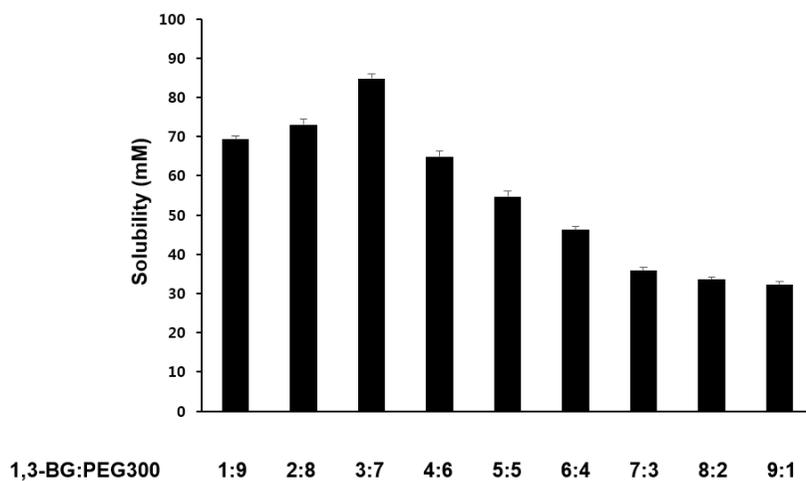
temperature conditions of 5°C, 25°C, 40°C and 50°C. To measure the temperature stability, the degree of degradation of 7,3',4'-THIF was measured by measuring the absorbance at 340 nm (Fig. 2B).

The light stability measurement was also carried out on three solvents (PEG300, 1,3-Butylene glycol, Ethanol) which can secure a solubility (20 mM) (Fig. 2C).

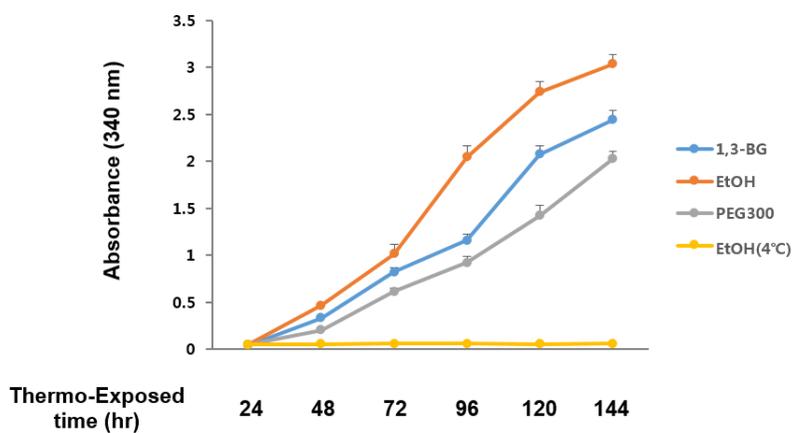
In order to find a solvent mixing ratio optimized for improving the temperature and light stability while satisfying the solubility to the effective concentration of 7,3',4'-THIF, the changes in physicochemical stability of isoflavones were measured according to the ratio of PEG 300 (the most stable solvent in temperature stability) and 1,3-butylene glycol (the most stable solvent in light stability). The solvent ratio of PEG 300 to 1,3-butylene glycol was 9: 1 ~ 1: 9, and the solubility, temperature and light stability of each sample were tested (Fig. 2D).

Figure 2

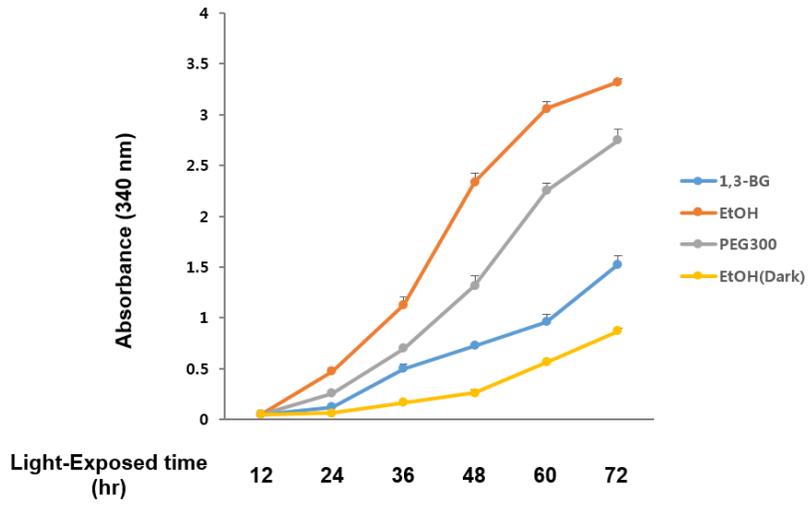
A



B



C



D

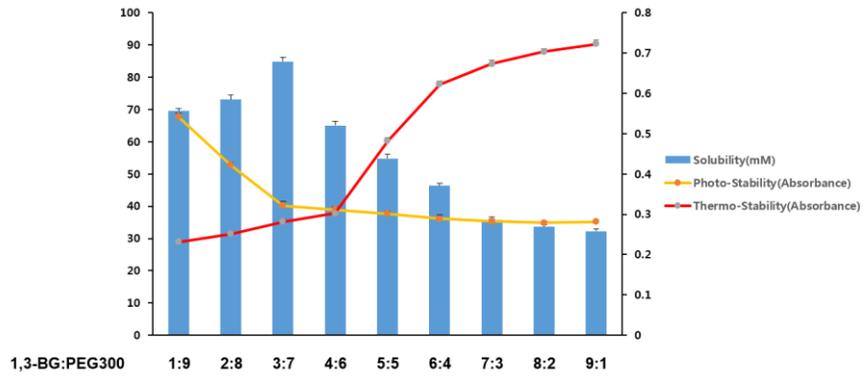


Figure 2. Solubility, thermo-stability, light-stability and solvent optimization of 7,3',4'-THIF.

(A) Solubility of 7,3',4'-THIF in individual solvents was highest in PEG300. (B) As a result of measurement and analysis, temperature stability was higher in the order of PEG 300, 1,3-butylene glycol and Ethanol. (C) The light stability was higher in the order of 1,3-butylene glycol, PEG300 and ethanol. (D) The blending ratio of PEG 300: 1,3-butylene glycol 7: 3 was selected. The formulation ratio of PEG 300: 1,3-butylene glycol satisfies both temperature and light stability, and also ensure a maximum solubility (80 mM) in consideration of the blending limit (~ 5%) of PEG 300 recommended by the industry. Data represent the means \pm SEM (n=3).

3. Topical application of 7,3',4'-THIF decreased DFE-induced AD symptoms in NC/Nga mice.

To investigate a topical effect of 7,3',4'-THIF on AD symptoms, DFE-induced NC/Nga mouse model was used. In comparison of control group, DFE-induced group showed AD-like symptoms such as erythema, excursion, keratinization and dryness were observed. Topical application of 800nmol or 0.1% Dexamethasone significantly lowered AD symptoms in comparison to the control group. The 7,3',4'-THIF-treated groups showed dose-dependent improvement (Fig. 3A).

Also, it was confirmed that the external application method of 7,3',4'-THIF using cosmetic solvent reduces epidermal thicknesses and scratching in NC / Nga atopic mouse model. Dorsal epidermal thickness of NC/Nga mice was also increased in the DFE-induced groups and the thickness was decreased in 7,3',4'-THIF and Dexamethasone group. On the other hand, there was no significant change in Daidzein group (Fig

3C). Dorsal skin samples were prepared and stained with hematoxylin and eosin (H&E). It is shown in (Fig. 3B).

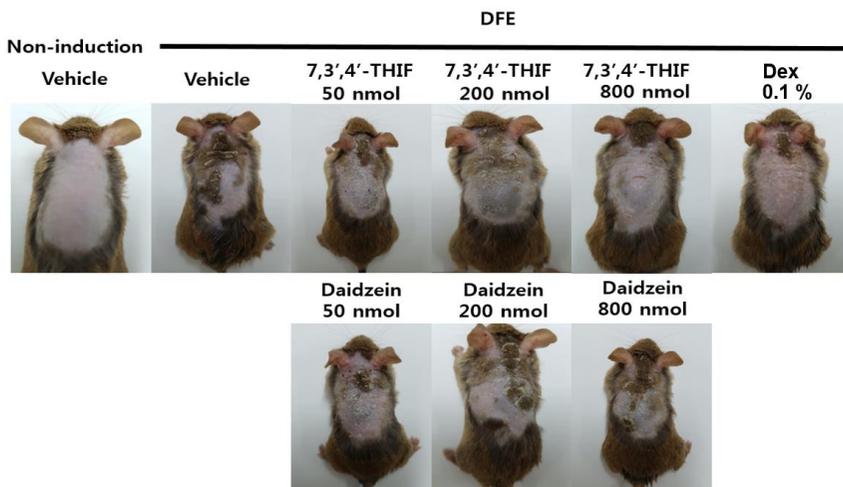
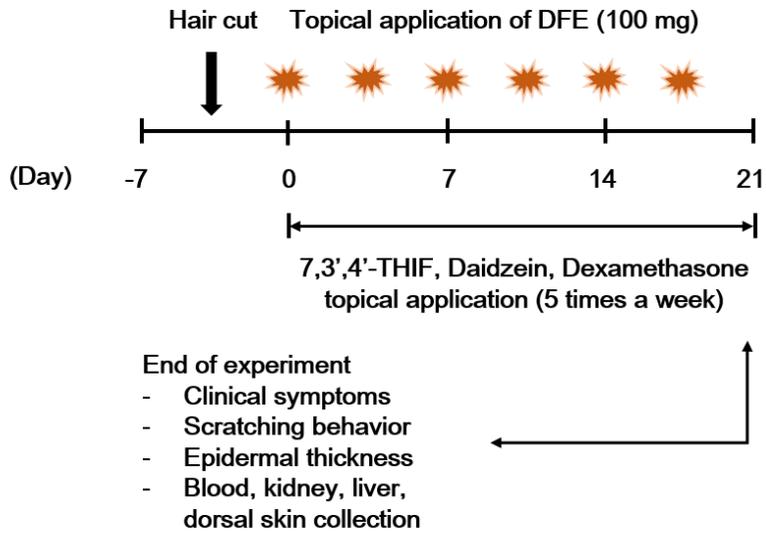
Scratching time was also increased in the DFE-induced groups.

In scratching, 7,3',4'-THIF, Daidzein and Dexamethasone were effective in decreasing scratching, but the 7,3',4'-THIF group showed the best efficacy (Fig. 3D).

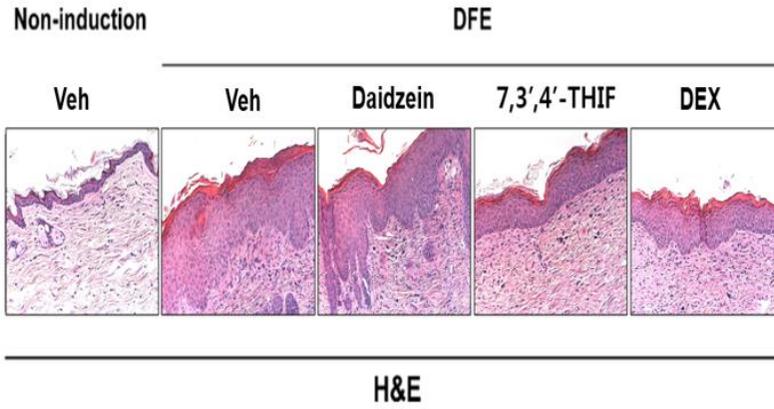
Figure 3

A

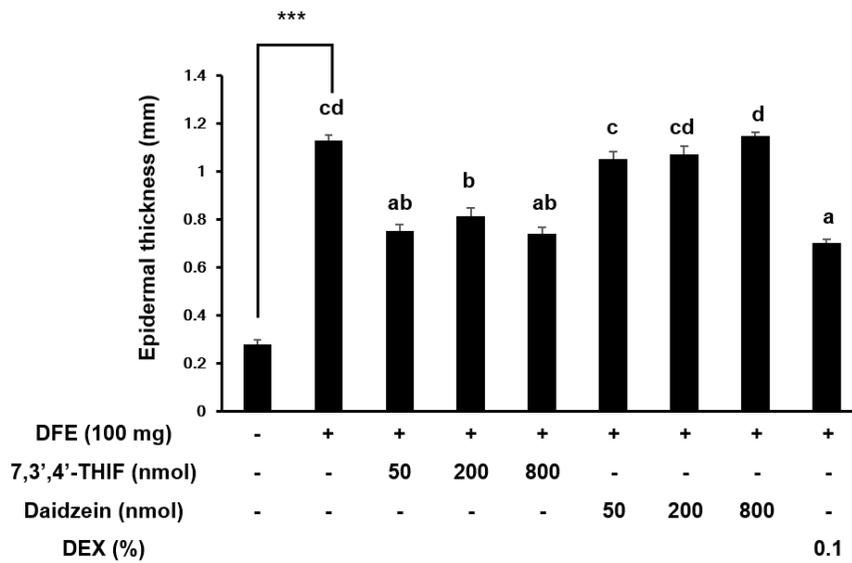
Schedule of experiment



B



C



D

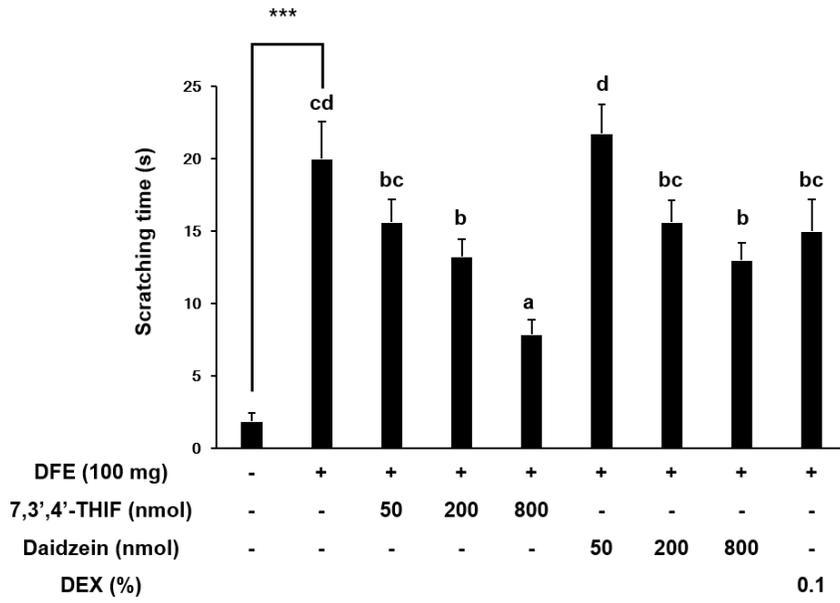


Figure 3. Effect of 7,3',4'-THIF on DFE-induced AD symptoms in NC/Nga mice.

(A) Topical application of 7,3',4'-THIF showed less severe AD-like symptoms than Daidzein group. (B, C) Treatment of 7,3',4'-THIF and Dexamethasone markedly attenuated the DFE- induced increase in dorsal thickness compared to Daidzein. (D) Scratching time was significantly decreased in 7,3',4'-THIF group. Data represent the means \pm SEM (n=6-8).

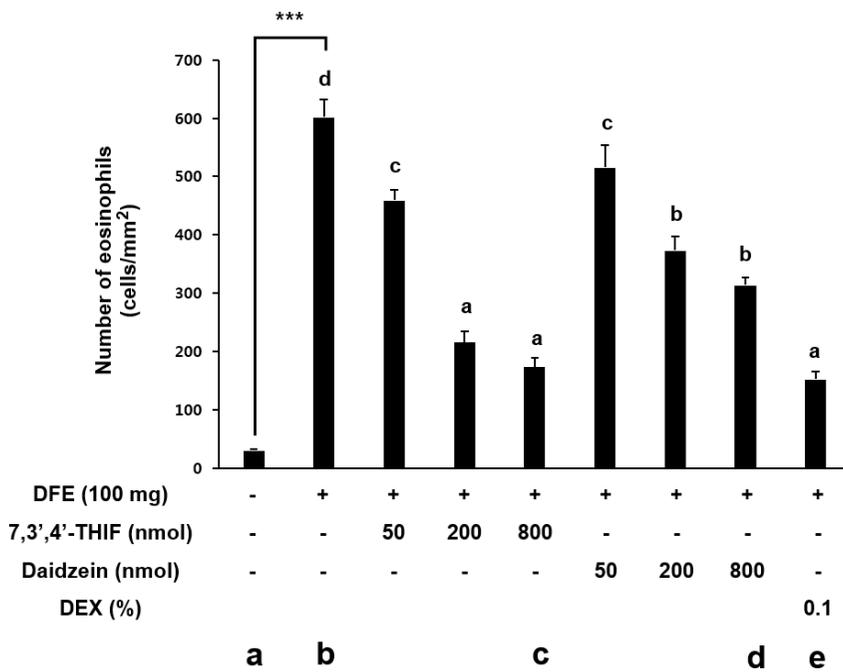
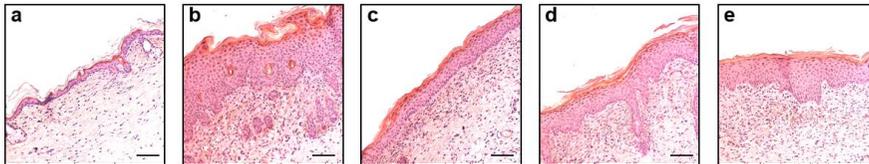
4. 7,3',4'-THIF decreased DFE-induced infiltration of eosinophils and mast cells into skin lesions in NC/Nga mice

To investigate whether topical application of 7,3',4'-THIF suppresses infiltration of eosinophils and mast cells in DFE-induced skin lesions, dorsal skin tissue were stained with Congo Red (CR) and Toluidine Blue (TB). The number of eosinophils in skin lesion was significantly increased in the DFE-induced group compared to the control group (Fig. 4A).

The number of eosinophils in the 7,3',4'-THIF and 0.1% Dexamethasone group was observed to be dramatically decreased (Fig. 4A). In addition, the number of mast cells in skin lesion was also increased in the DFE-induced group compared to control group. In 7,3',4'-THIF and 0.1% Dexamethasone group, there was the highest decrease of mast cell infiltration and it showed dose-dependent decrease. (Fig. 4B).

Figure 4

A



B

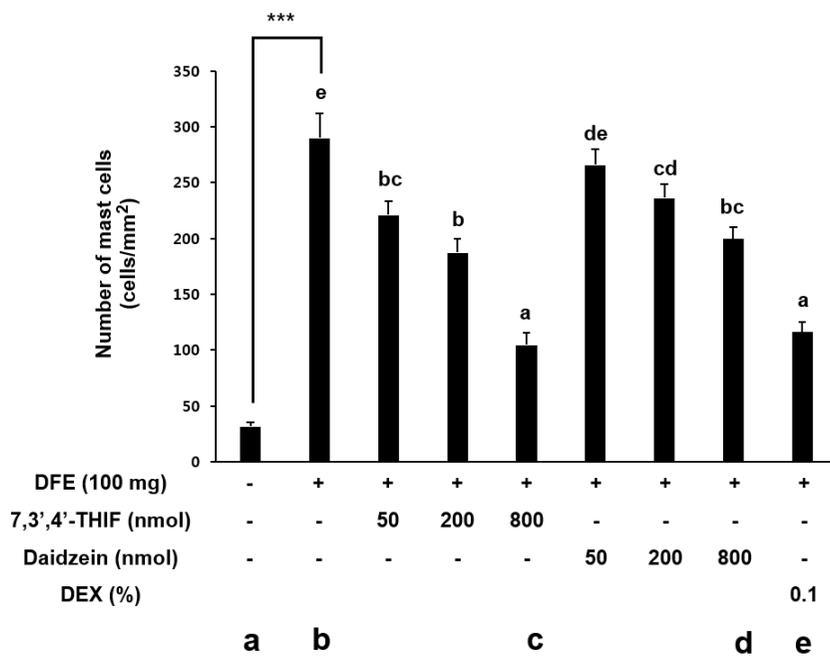
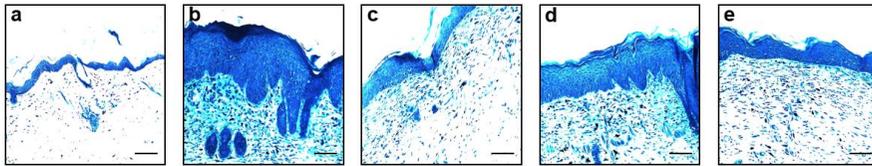


Figure 4. Reduction effect of 7,3',4'-THIF on DFE-induced infiltration of eosinophils and mast cells into skin lesions in NC/Nga mice

Treatment of 7,3',4'-THIF markedly attenuated the DFE- induced infiltration of eosinophils and mast cells into skin lesions.

(A) Images show the histological features of skin lesions. To identify eosinophils, skin lesions were stained with Congo Red (CR). (B) The numbers of eosinophils of skin lesion in 1 mm² sections were measured. Data represent the mean values \pm SEM ($n = 6$). (C) To identify mast cells, skin lesions were stained with Toluidine Blue (TB). (D) The numbers of mast cells of skin lesion in 1 mm² sections were measured. Data represent the mean values \pm SEM ($n = 6$).

Skin lesions were evaluated under a microscope at 400x magnification. Scale bar: 50 μ m. (a) Untreated control group; (b) DFE-treated group; (c) DFE plus high-dose 7,3',4'-THIF (800 nmols/treat);

(d) DFE plus high-dose Daidzein (800 nmols/treat) (e) DFE plus 0.1%

Dexamethasone.

5. Pre-clinical liver and kidney changes of 7,3',4'-THIF compared to Dexamethasone.

To investigate the safety and toxicity of 7,3',4'-THIF, Daidzein, and Dexamethasone, liver and kidney changes were observed. As a result, it was confirmed that the weight of liver per body weight were abnormally increased in the Dexamethasone group. On the other hand, there were no significant changes in liver weights per body weight in the 7,3',4'-THIF and Daidzein groups at all concentrations (Fig. 5A). In addition, it was also confirmed that the weight of kidney per body weight were significantly increased in the Dexamethasone group. However, there were no significant changes in kidney weights per body weight in the 7,3',4'-THIF and Daidzein groups at all concentrations (Fig. 5B).

Figure 5. Pre-clinical effect of 7,3'4'-THIF, Daidzein and Dexamethasone on liver and kidney changes

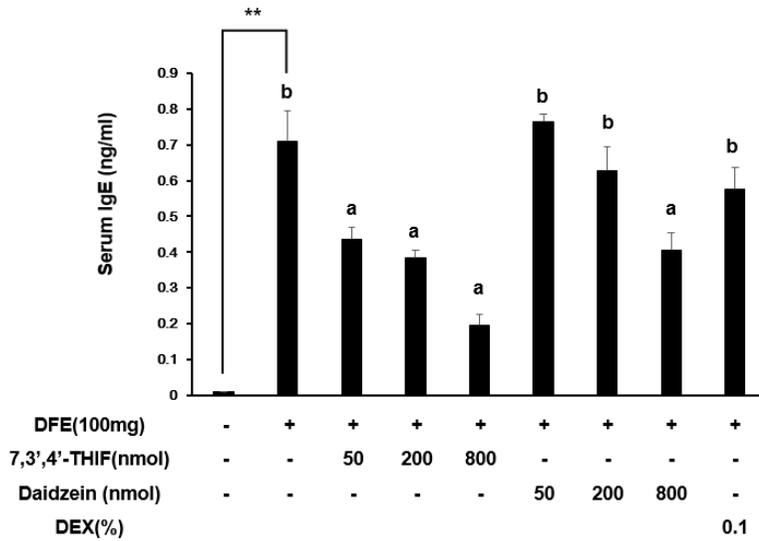
(A) Abnormal liver weight changes were observed in the dexamethasone group. (B) The weight of the kidneys was significantly increased in the Dexamethasone group. Data represent the mean values \pm SEM (n = 8). $P < 0.05$, compared with DFE-treated mice.

6. 7,3',4'-THIF attenuates DFE-induced increases in IgE, and MDC levels in NC/Nga mice.

To investigate the effect of 7,3',4'-THIF in IgE and MDC levels in DFE-induced NC/Nga mice, serum samples were collected on the final day of the experiment (Day 21). Serum IgE levels in the DFE-induced group were increased dramatically compared to that of the control group. And topical application of 7,3',4'-THIF showed a significant reduction in IgE levels (Fig. 6A). Serum MDC levels in the DFE-induced group were also increased compared to the control group. MDC levels in 7,3',4'-THIF groups were lower than in the DFE-induced group. But in MDC levels, the decrease was the highest in the Dexamethasone group (Fig. 6B).

Figure 6

A



B

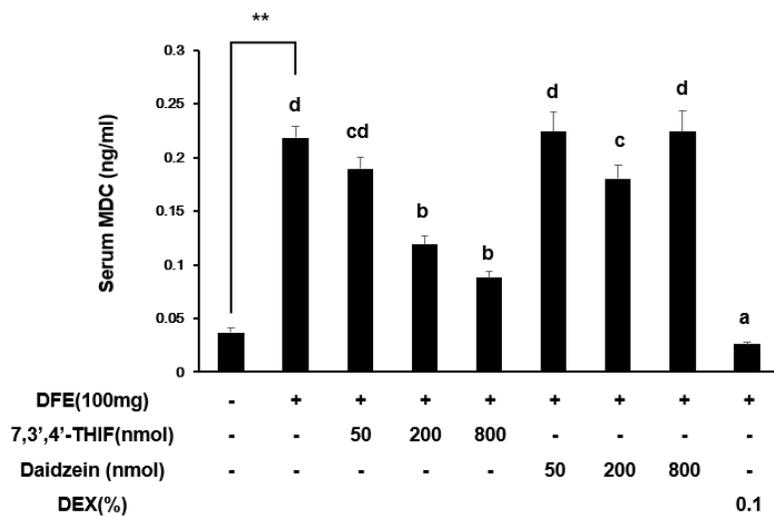


Figure 6. Effect of 7,3',4'-THIF on DFE-induced increases IgE and MDC levels in serum.

(A, B) 7,3',4'-THIF decreased the levels of serum MDC and IgE. MDC and IgE level were measured by ELISA. Data represent the mean values \pm SEM ($n = 6-8$). Mean values with letters (a–d) within a graph are significantly different from each other at $p < 0.05$. TARC: thymus- and activation-regulated chemokine; MDC: macrophage-derived chemokine.

IV. DISCUSSION

In preventing or improve AD by using functional materials, there are pharmacological differences between the dosing and topical methods. The dosing regimen requires high-dose prescriptions to reach the skin tissue (inside to outside). Also, topical steroid use is known to have serious adverse effects on other tissues and organs [8, 10].

In a dietary model, there was previous study that had confirmed the anti-atopic function of 7,3',4'-THIF [13]. However, detailed pharmacological mechanism of 7,3',4'-THIF on the skin tissue is unclear. Therefore, in order to improve and prevent AD at the early symptom stage by using 7,3',4'-THIF, studies on the external use and dermatological mechanism of 7,3',4'-THIF are needed.

7,3',4'-THIF has a remarkably low solubility in water and is insoluble in main solvents used for medicines, health functional foods

and cosmetics [17]. In addition, 7,3',4'-THIF has low physicochemical stability, such as oxidation and temperature and light stability, and their bio-availability and usage are limited [18]. Therefore, in order to utilize 7,3',4'-THIF as a food, medicine, and cosmetic composition, there is a need for a method for enhancing the solubility and physicochemical stability up to the effective concentration, by utilizing the blending ratio of an appropriate solvent.

In this study, we aimed to obtain physicochemical stability by using a solvent optimized for 7,3',4'-THIF and confirmed anti-atopy effect as an externally applied method of 7,3',4'-THIF to skin lesions.

HaCaT is a skin cell of epidermis which is the first skin tissue damaged by AD-induced scratching. Th2 chemokines such as TARC and MDC are involved in inflammatory cell infiltration and ultimately affects the expression of IgE [4, 5]. In this study, we confirmed that 7,3',4'-THIF reduced TNF- α /IFN- γ induced TARC and MDC expression in HaCaT.

The physicochemical stability of the functional material is important. In particular, since 7,3',4'-THIF has a structurally unstable energy level, it is necessary to utilize an appropriate vehicle to secure the physicochemical stability of such a material while ensuring effective concentration [19]. Therefore, in this study, we have investigated methods for improving the solubility, optical stability, and temperature stability of 7,3',4'-THIF in cosmetic solvents.

It was confirmed that the external application method of 7,3',4'-THIF using cosmetic solvent reduces skin thickness and scratching in NC/Nga atopic mouse model. Serum analysis showed that IgE and MDC also decreased in the group treated with 7,3',4'-THIF. In the case of Dexamethasone, serum MDC was significantly decreased, while IgE level showed a relatively small decrease. IHC analysis showed that the inflow of mast cells and eosinophils was reduced in the 7,3',4'-THIF group.

The most important requirement for 7,3',4'-THIF to be applied as an atopic cosmetic material is safety. Therefore, we checked the weight change of kidney and liver, as a basic phenotype. As a result, there was no significant change in 7,3',4'-THIF and Daidzein group, but Dexamethasone group showed significant increase in weight and kidney weight.

V. CONCLUSION

Over the past several decades, the physiological activities of isoflavones such as Genistein and Daidzein and their metabolites have been extensively studied. However, these isoflavone metabolites have been difficult to be industrially produced due to technical problems related to production. Recently, however, the isoflavone metabolite has been produced through mass production technology through bio-conversion and enzyme-conversion technology, and the production cost has reached industrialization level [12]. However, despite the various efforts to utilize isoflavones, the solubility and stability of the material was very low, making it difficult to utilize it industrially [17-19].

Therefore, in this study, we investigated the anti-AD effect of Daidzein metabolite, 7,3',4'-THIF, which is known to have excellent anti-inflammatory activity. And, at the same time, we have selected a

solvent mixture that can improve the physicochemical stability of the material and secure preclinical safety. Through these processes, we developed 7,3',4'-THIF as an anti-atopic cosmetic material.

It is hard to compare natural immune modulators such as 7,3',4'-THIF with medicines in terms of efficacy. However, since natural materials have few side effects, compared to chemical drugs, it is an appropriate method for early symptoms of atopy rather than severe. Therefore, cosmetic products containing natural anti-atopic functional materials such as 7,3',4'-THIF will be a good therapy in early stage of AD.

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VII. 국문 초록

아토피는 피부장벽파괴와 염증을 동반하는 면역성 피부 질환이다. 면역체계가 충분히 형성되지 않은 영유아기에 주로 발생하며, 그 원인과 작용기전이 아직 명확히 밝혀지지 않는 않지만, 전세계적으로 많은 연구들이 진행 중이다. 일반적인 치료법으로 화학합성 스테로이드제가 사용되고 있지만 심각한 부작용으로 인해 다른 대체요법이 필요한 실정이다. 본 연구의 목적은 천연물 유래의 면역조절제를 적절히 활용하여 부작용 없이 아토피를 개선하고자 하는데 있다. 따라서 본 연구에서는 콩 유래 이소플라본 대사체 7,3',4'-trihydroxyisoflavone의 화장품 및 외용제로써의 아토피 피부염 개선 효능을 규명하고, 그 활용방안에 대해 논의하고자 한다.

본 연구의 목적은 7,3',4'-trihydroxyisoflavone의 아토피 관련 마커 저해효능을 피부 유래 세포주 (HaCaT)

에서 확인하고, 집먼지 진드기 (Dermatophagoide farina extract)로 유도 된 NC/Nga 아토피 마우스 모델에서 7,3',4'-trihydroxyisoflavone 과 그 전구체 Daidzein, 그리고 화학스테로이드인 Dexamethasone의 아토피피부염 개선 효능을 비교 및 확인하는 것이다.

관련하여 아토피 마커인 TARC, MDC의 발현량을 세포수준에서 확인하고 또한 NC/Nga 마우스 모델에서 혈청 분리 후 측정하였다. 또한 등 피부층 두께 및 혈중 IgE 농도의 증감을 확인하고, 전임상적 안전성을 확인하기 위해 신장 및 간의 무게를 측정하였다. 그 결과, HaCaT 세포주 및 NC/Nga 마우스 모델에서 7,3',4'-trihydroxyisoflavone의 아토피 개선 효능 및 전임상적 안정성을 확인 하였다. 또한 기능성 화장품 및 외용제로의 적용을 위해 7,3',4'-trihydroxyisoflavone의 이화학적 특성을 분석하고 그 활용 가능성에 대해 논의하고자 한다.

본 연구 결과를 통해 7,3',4'-trihydroxyisoflavone은 천연 물 유래의 아토피성 피부염 개선을 위한 기능성 화장품 소재로 활용 가능할 것이다.