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ORIGINAL ARTICLE

Dictamnus dasycarpus Turcz., Root Bark Alleviates Oxazolone-Induced Atopy-Like Dermatitis in Mice

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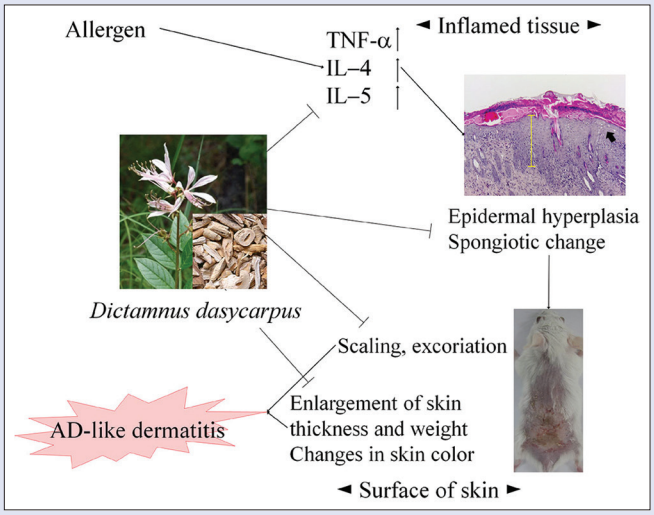
ABSTRACT

Background: *Dictamnus dasycarpus* Turcz., is one of the most frequently used herbal medicine to treat dermatosis associated with psoriasis, pruritus, scabies, and eczema. **Objective:** We investigated the anti-inflammatory effects of *D. dasycarpus* extract (DDE) using a mouse model with atopic dermatitis (AD)-like dermatitis. **Materials and Methods:** The therapeutic effects of DDE on skin lesion, tone of color, and inhibitory effects on histopathological changes and cytokine production in skin tissues were assessed in mice with AD-like dermatitis induced by oxazolone. **Results:** Topical application of DDE alleviated skin lesions such as erythema, scaling and excoriations, and ameliorated erythema and the melanin index. In addition, DDE effectively prevented skin enlargement induced by oxazolone, while also preventing epidermal hyperplasia, spongiotic change, and hyperkeratosis and reducing the production of tumor necrosis factor-alpha and interleukin (IL)-4 and IL-5 in inflamed tissues. Finally, DDE did not affect changes in body weight and spleen–body weight ratio relative to dexamethasone. **Conclusion:** These results indicate that *D. dasycarpus* can be used as a topical agent for inflammatory skin diseases with relative safety.

Key words: Atopic dermatitis, dermatosis, *Dictamnus dasycarpus*, herbal medicine, inflammation, traditional medicine

SUMMARY

- *Dictamnus dasycarpus* extract (DDE) effectively inhibited skin enlargement and ameliorated skin lesions of atopic dermatitis
- DDE prevented epidermal hyperplasia, spongiotic changes, and hyperkeratosis
- DDE effectively reduced the production of tumor necrosis factor-alpha, interleukin (IL)-4, and IL-5.



Abbreviation used: HM: Herbal medicine; DNFB: Dinitrofluorobenzene, ICAM-1: Intercellular adhesion molecule-1, AD: Atopic dermatitis, DDE: *Dictamnus dasycarpus* extract, AOO: Vehicle composed of acetone and olive oil, DEX: Dexamethasone, CBA: Cytometric bead array.

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INTRODUCTION

Herbal medicines (HMs), including traditional Chinese Medicine in China, Korean Medicine in Korea, and Kampo Medicine in Japan, have long been used for the treatment of dermatosis. HMs have primarily been orally administered for the past few centuries; nevertheless, many therapists in East Asia have recommended topical application of HMs for patients with dermatosis because of their therapeutic efficacies and relative safety.[1,2]

The root bark of *Dictamnus dasycarpus* Turcz., is one of the most frequently used HMs for the treatment of dermatoses such as psoriasis, pruritus, scabies, and eczema. According to the encyclopedia of traditional medicine, topical application of *D. dasycarpus* extract (DDE) can kill worms, arrest itching, and clear away heat and eliminate dampness.[3] We previously reported the anti-inflammatory effects of D. *dasycarpus* on dinitrofluorobenzene (DNFB)-induced contact dermatitis (CD) in mice.[4,5] In these studies, we found that DDE effectively inhibited ear swelling and reduced the levels of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in inflamed tissues.[4] In addition, DDE inhibited intercellular adhesion molecule-1 (ICAM-1) expression in skin tissues and HaCaT keratinocytes.[5]

Atopic dermatitis (AD), which is often referred to as atopic eczema, is a chronic inflammatory skin disease characterized by mild-to-severe

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erythema, scaling, and excoriations that reflect severe itch. The histopathological abnormalities associated with AD are epidermal hyperplasia, hyperkeratosis, and spongiosis, as well as immune cell infiltration, all of which are stage dependent.[6]

Based on these findings, we examined the effects of DDE on skin lesions and histopathological abnormalities in mice with AD-like dermatitis. Specifically, the effects of DDE on skin lesions; skin thickness; tone of color; histopathological changes such as epidermal hyperplasia, hyperkeratosis, and spongiosis; and cytokine production in mice with AD-like dermatitis induced by oxazolone were evaluated in the present study.

MATERIALS AND METHODS

Preparation of *Dictamnus dasycarpus* extract

The root bark of *D. dasycarpus* was purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Korea). Fifty grams of *D. dasycarpus* root bark was immersed in 500 ml of methyl alcohol and sonicated for 15 min, after which they were extracted for 24 h. Following extraction, the supernatant was transferred and *D. dasycarpus* was again extracted with 500 ml of methanol for 24 h. The two extracts were subsequently combined and filtered through Whatman No. 20 filter paper, after which they were condensed using a rotary evaporator (EYELA, Tokyo, Japan). The condensed extract was then lyophilized using a freeze dryer (Labconco, Kansas City, MO, USA). Finally, 2.8 g of lyophilized powder was obtained (yield, 5.6%). The extract of *D. dasycarpus*, root bark (DDE, Voucher No. MH2010-010) and specimen were deposited at the Division of Pharmacology, School of Korean Medicine, Pusan National University.

Animals

Six-week-old male Balb/c mice were obtained from Samtako (Incheon, Korea). All mice used in this experiment were housed in cages under specific conditions, including a 12 h light/dark cycle and specific pathogen-free conditions. In addition, the mice were provided with free access to standard rodent feed and water. All animal experiments were approved by our Animal Care and Use Committee and were conducted according to the institutional guidelines (PNU-2016-1301).

Induction of atopic dermatitis and experimental schedule

AD was induced using a slightly modified version of the method described by Man *et al.*[7] Briefly, 5% oxazolone (20 μL, Sigma, St. Louis, MO, USA) in vehicle composed of acetone and olive oil (4:1, AOO) was applied onto the dorsum of each ear on day 1 (sensitization). The mice were then treated by application of 0.1% oxazolone (50 μL) in vehicle onto the shaved backs of each mouse every 2 days for challenge (seven times) as shown in Figure 1.

For topical treatment with drugs, dexamethasone (DEX) and DDE were dissolved in ethanol, filtered using a syringe filter (0.45 μm), and finally diluted in vehicle (AOO: ethanol, 4:1). DDE (50, 150, or 500 μg/day) was topically applied onto the backs of mice for seven consecutive days. The dosages of DDE were determined based on the previous studies.[4,5] In this experiment, 50 μl/day of DDE (1, 3, and 10 mg/ml, corresponding to 0.1, 0.3, and 1% [w/v], respectively) was topically applied. Naïve animals were treated with vehicle (n = 6), while control animals cytotoxic T lymphocyte (CTL) were sensitized and challenged with oxazolone in AOO, then painted with vehicle (n = 8). DDE-treated animals were subsequently sensitized and challenged with oxazolone and then painted with 50, 150, or 500 μg/day of DDE (n = 8). DEX-treated animals were sensitized and challenged with oxazolone, after which they were painted with 125 μg/day of DEX. DEX was used as a positive control. The experimental procedures are summarized in Figure 1.

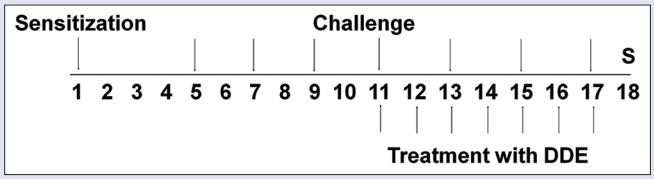

Figure 1: Experimental schedule. Mice in all experimental groups except the normal group were sensitized by application of 5% oxazolone onto the dorsum of each ear on day 1 and then challenged by application of 0.1% oxazolone onto their shaved backs every other day (seven times). The mice in the *Dictamnus dasycarpus* extract-treated groups were topically treated with 50, 150, or 500 μg/day of *Dictamnus dasycarpus* extract for 7 days from day 11–17 (n = 8). The dexamethasone group was topically treated with 125 μg/day of dexamethasone (n = 6). All animals were sacrificed on day 18. S indicates sacrifice

Observing skin lesions and measurement of erythema and melanin index

At the end of the experiment, mice were sacrificed with CO_2 and skin lesions were observed using a digital camera (Olympus, Tokyo, Japan). Next, the erythema and melanin indexes were measured using a dermospectrophotometer (DSM II, Cortex Technology, Hadsund, Denmark).

Measurement of dorsal skin thicknesses and weights

Mice were sacrificed with CO_2, after which ear pieces (5 mm in diameter) obtained via dermal punch were weighed using a microbalance, and the thicknesses of earpieces were measured with a digimatic caliper (Mitutoyo, Kanagawa, Japan).

Tissue preparation and staining

Obtained tissues were fixed in 4% formalin for 24 h and then dehydrated using ethyl alcohol. Next, all tissues were soaked in xylene and finally embedded in paraffin. Skin tissues (4 μm) were subsequently resected, after which the sections were stained with hematoxylin and eosin and observed using a light microscope (100×).

Evaluation of epidermal hyperplasia

To evaluate epidermal hyperplasia, five nonoverlapping fields per slide were randomly selected and captured with a light microscope. The height from the basal lamina to the top of the stratum granulosum was quantified to evaluate the epidermal thickness. Five lengths were used to calculate the mean epidermal thicknesses of each tissue slide.

Measurement of cytokine production

Cytokine levels in skin tissues were evaluated using a mouse Th1/Th2 cytometric-bead array kit (BD Biosciences, San Jose, CA, USA). Briefly, to obtain tissue lysates, resected inflamed tissues were lysed using protein extraction solution (Intron Bio, Daejeon, Korea) and a homogenizer (Next Advance, NY, USA). Next, 50 μg aliquots of lysates were used to evaluate the levels of TNF-α, interleukin-4 (IL-4), IL-5, and IFN-γ.

Measurement of body and spleen weights

Body weights were measured on days 1 and 18 using an electronic scale (CAS, Gyeonggi, Korea). Spleen weights were measured on day 18

using a microbalance (Sartorius, Gyeonggi, Korea). The effects of DDE on changes in spleen weights are presented as the spleen–body weight ratio.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test using Prism 5 for Windows, version 5.01 (GraphPad Software Inc, San Diego, California, USA). All data are presented as the means ± standard deviation. $P < 0.05$ was considered statistically significant.

RESULTS

Dictamnus dasycarpus extract alleviated skin lesions in mice with atopic dermatitis-like dermatitis

Repeated application of oxazolone induced AD-like skin lesions such as moderate erythema, scaling, and excoriations in the CTL group [Figure 2b]. Treatment with DDE alleviated these abnormalities [Figure 2].

Dictamnus dasycarpus extract lowered erythema and the melanin index on the skin surface

A marked increase in erythema index and a significant increase in the melanin index were observed in the CTL group. DDE treatment reduced the erythema index significantly, while treatment with 500 μg/day of DDE lowered the melanin index significantly. Conversely, DEX did not affect the erythema or melanin index [Figure 3].

Dictamnus dasycarpus extract prevented enlargement of skin thicknesses and weights

At the end of the experiment, the thicknesses and weights of dorsal skins were evaluated. In the CTL group, topical application of oxazolone elevated the levels of skin thickness and weight by more than two times relative to the normal group. In addition, treatment with 100 and 500 μg/day of DDE effectively inhibited enlargement of skin thickness [Figure 4a], while 500 μg/day of DDE inhibited weight gain of skin significantly [Figure 4b]. DEX was more effective than DDE [Figure 4].

Dictamnus dasycarpus extract prevented epidermal hyperplasia, spongiotic changes, and hyperkeratosis in inflamed tissues

The effects of DDE on epidermal hyperplasia, one of the major features of AD, spongiosis and a hallmark of skin inflammation, and hyperkeratosis were investigated. Marked increases in epidermal thickness (yellow bars) and hyperkeratosis with exudate were observed in CTL mice. In addition, spongiotic changes were observed [Figure 5a and b]. Treatment with more than 150 μg/day of DDE inhibited epidermal hyperplasia, spongiotic changes, and hyperkeratosis in inflamed tissues [Figure 5a]. Treatment with

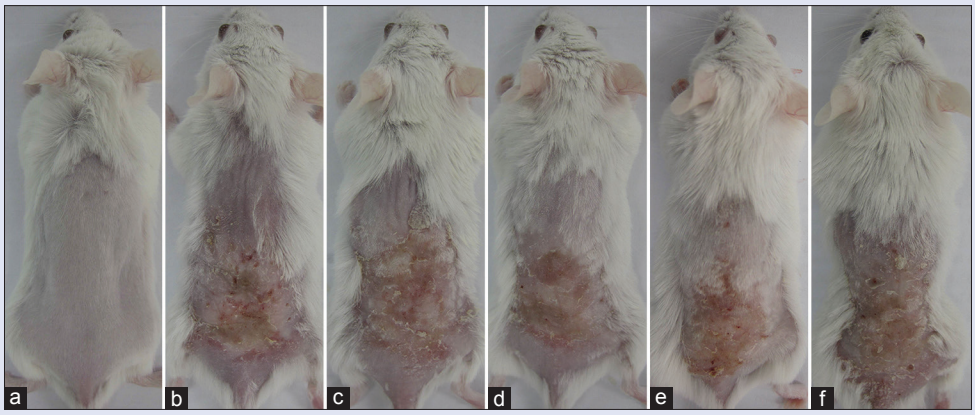

Figure 2: Effect of Dictamnus dasycarpus extract on skin lesions in mice with atopic dermatitis-like dermatitis. (a) NOR; (b)Control; (c) 50 μg/day of *Dictamnus dasycarpus* extract; (d) 150 μg/day of *Dictamnus dasycarpus* extract treated; (e) 500 μg/day of *Dictamnus dasycarpus* extract; (f) 125 μg/day dexamethasone

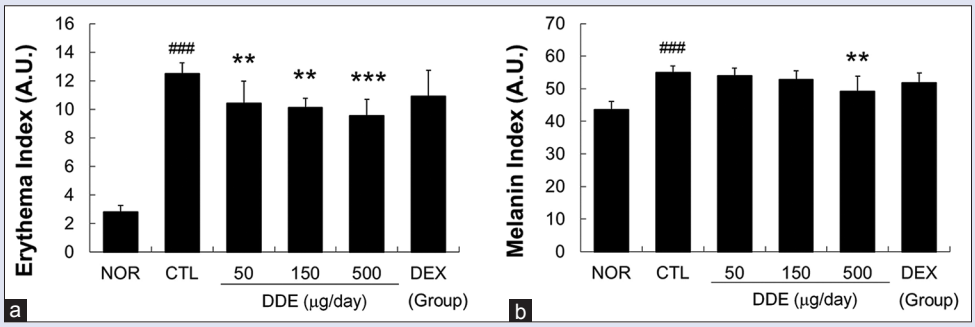

Figure 3: Effect of *Dictamnus dasycarpus* extract on erythema and melanin index on the skin surface. The erythema and melanin index were measured using a dermospectrophotometer on day 18. (a) Erythema index; (b) melanin index. NOR, nontreated normal mice; CTL, nontreated mice with atopic dermatitis-like dermatitis; DEX, 125 μg/day of dexamethasone-treated mice with atopic dermatitis-like dermatitis. All values are presented as the means ± standard deviation. $^{###}P < 0.001$ versus NOR group, and $^{**}P < 0.01$ and $^{***}P < 0.001$ versus cytotoxic T lymphocyte group

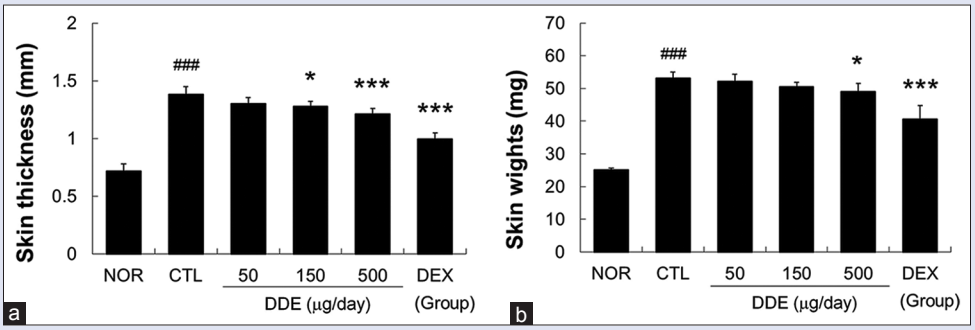

Figure 4: Effect of *Dictamnus dasycarpus* extract on skin thicknesses and weights in mice with atopic dermatitis-like dermatitis. The skin thicknesses and weights were measured on day 18. Abbreviations are the same as in Figure 3. (a) Skin thickness; (b) skin weight. All values are presented as the means ± standard deviation. $^{###}P < 0.001$ compared to the NOR group, and $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to the control group

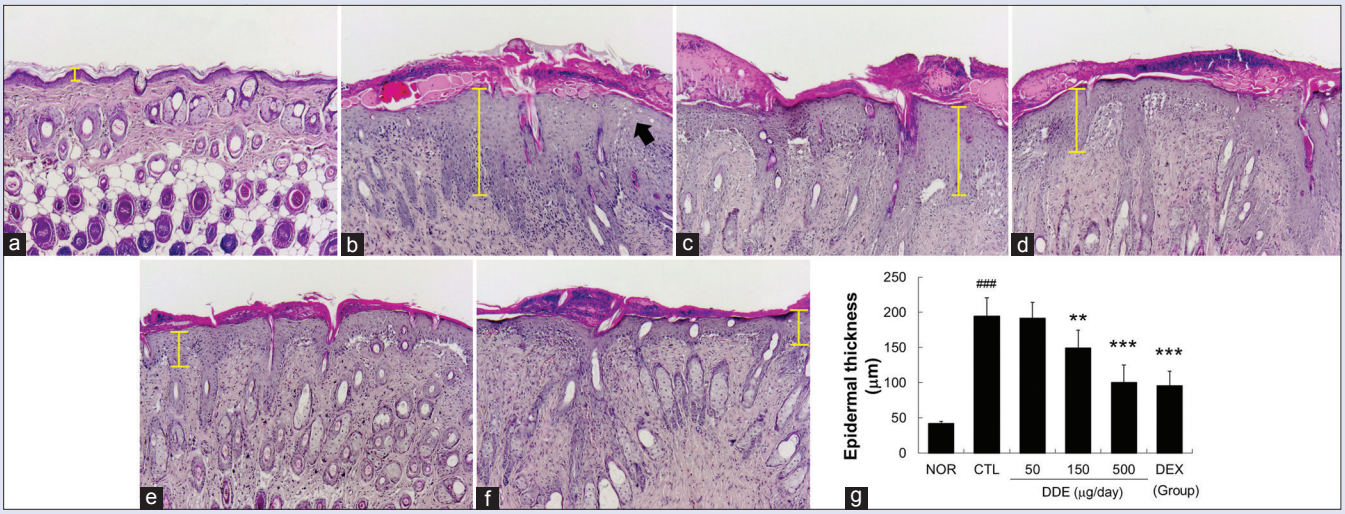

Figure 5: Effect of *Dictamnus dasycarpus* extract on histopathological abnormalities in inflamed tissues. Skin tissue was observed under a light microscope (×100). Yellow bars indicate the epidermis. Filled arrows indicate spongiotic areas (a-f). (a) NOR; (b) Control; (c) 50 μg/day of *Dictamnus dasycarpus* extract; (d) 150 μg/day of Dictamnus dasycarpus extract treated; (e) 500 μg/day of *Dictamnus dasycarpus* extract; (f) 125 μg/day of dexamethasone. The epidermal thicknesses were evaluated using a quantitative method. All values are presented as the means ± standard deviation (g). $^{###}P < 0.001$ Compared to the NOR group, and $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the CTL group. DDE: *Dictamnus dasycarpus* extract, CTL: Control, DEX: Dexamethasone

DDE effectively inhibited epidermal hyperplasia compared to the CTL mice [Figure 5b]. DEX treatment was most effective among all experimental groups [Figure 5].

Dictamnus dasycarpus extract lowered the production levels of tumor necrosis factor-alpha, interleukin-4, interleukin-5, and interferon-gamma in inflamed tissues

Elevated levels of TNF-α, IL-4, IL-5, and IFN-γ production were observed in CTL mice [Figure 6]. Topical application of DDE effectively lowered the TNF-α level compared to that in the CTL group [Figure 6a], while treatment with 500 μg/day of DDE significantly lowered the levels of IL-4 and IL-5, respectively [Figure 6b and c]. DEX inhibited the production of TNF-α, IL-4, and IL-5 significantly [Figure 6a-c], while IFN-γ production was not changed by DDE or DEX [Figure 6d].

Dictamnus dasycarpus extract did not affect body weights or spleen/body weight ratio in mice with atopic dermatitis-like dermatitis

The effects of DDE on body weights were investigated, and enlargement of the spleen was then estimated based on the spleen/body weight ratio. We found that DDE did not affect body weight gain, but that DEX inhibited body weight gain significantly [Figure 7a]. The spleen/body weight ratio in the CTL group was elevated compared to the NOR group. The DDE group showed no change in spleen/body weight ratio; however, spleen body weight ratio was significantly decreased in the DEX group [Figure 7].

DISCUSSION

Recently, *D. dasycarpus* was reported to be one of the most frequently used medicinal plants for skin diseases in Taiwan.[8-10] Chien *et al.* reported that *D. dasycarpus* was the most frequently used Chinese HM for the treatment of urticaria in Taiwan during 2009.[9] This frequency

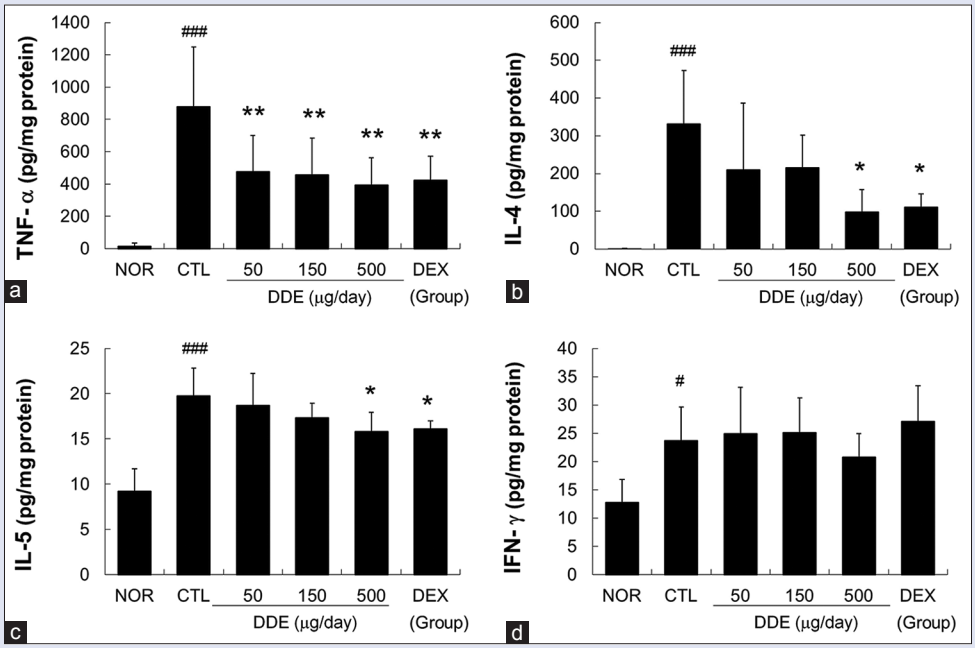

Figure 6: Effect of *Dictamnus dasycarpus* extract on production levels of cytokines in inflamed tissues. The cytokine levels in inflamed tissues were evaluated using the cytometric bead array method. (a) Tumor necrosis factor-alpha; (b) interleukin-4; (c) interleukin-5; (d) interferon-gamma. All values are presented as the means ± standard deviation. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ compared to the NOR group, and $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the CTL group. DDE: *Dictamnus dasycarpus* extract, CTL: Control

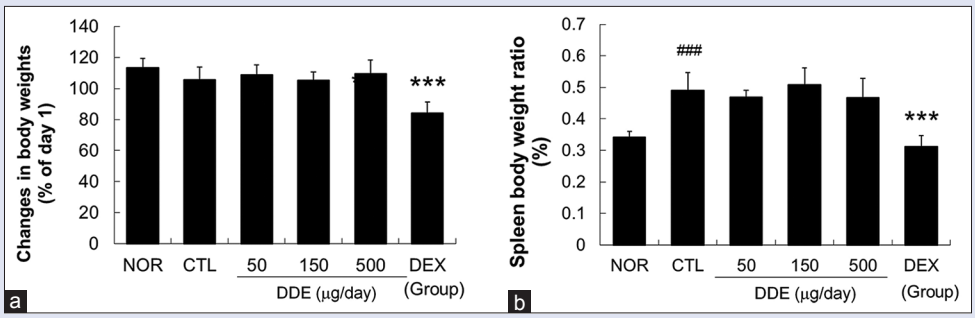

Figure 7: Effects of *Dictamnus dasycarpus* extract on spleen/body weight ratio in mice with atopic dermatitis-like dermatitis. Body weights were measured at the beginning (day 1) and end (day 18) of the experiment. Changes in body weights were based on the average weights on day 18 and expressed as percentages of weight on day 1 (a). Spleen weights were also measured on day 18, at which time the spleen/body weight ratio was calculated (b). All values are presented as the means ± standard deviation. $^{\#\#\#}P < 0.001$ compared to the NOR group and $^{***}P < 0.001$ compared to the CTL group. DDE: *Dictamnus dasycarpus* extract, CTL: Control

is quite similar to that observed in the study by Chen *et al.*, even if the criteria are extended into allergic skin diseases.[10]

We investigated the topical efficacies of *D. dasycarpus* because previous studies showed that it was used as a cleansing agent for psoriasis, pruritus, scabies, and eczema, in addition to its frequent use for skin diseases. Moreover, Jiang *et al.* have reported that 70% ethanol extract from *D. dasycarpus* inhibited the systemic anaphylactic shock and scratching behavior induced by compound 48/80 in a dose-dependent manner.[11] In our previous study, *D. dasycarpus* inhibited ear swelling, epidermal hyperplasia, immune cell infiltration, cytokines such as TNF-α and IFN-γ, and chemokine production via suppression of ICAM-1 expression in CD mice.[4,5] Recently, decoction of *D. dasycarpus* was shown to ameliorate skin lesions and inhibits inflammatory reactions in mice with CD.[12]

Conversely, the pathophysiology of AD is conventionally characterized as Th2-skewing reactions of T lymphocytes. For this reason, different animal models were used to investigate CD and AD in the laboratory. We used oxazolone to induce AD-like dermatitis in mice. In our model, repeated application of oxazolone induced moderate erythema, scaling, and excoriations on the skin surface, as well as epidermal hyperplasia, spongiotic changes, and hyperkeratosis in inflamed tissues. In addition, production of TNF-α, a proinflammatory cytokine, as well as Th2-skewing cytokines, IL-4 and IL-5, and the Th1-skewing cytokines, INF-γ and IL-2, were elevated in inflamed tissues.

We had compared the differences in skin lesions, histopathological abnormalities, and cytokine productions between CD and AD animal model. In our preliminary experiment, topical application of DNFB induced marked scale, crust, and erythematous eruption, while Ox induced erythematous eruption and mild scale and crust. Histopathological examination revealed that Ox induced marked hyperplasia in the dermis

and epidermis, mild hyperkeratosis, and mild infiltration of immune cells compared to histopathological abnormalities by DNFB.

Above all, these two animal models differ in terms of cytokine profile. In the CD animal model induced by DNFB used in previous study, TNF-α and IFN-γ were mainly elevated and levels of IL-4 and IL-5 were not. On the other hand, in the animal model of AD-like dermatitis used in this experiment, levels of IL-4, IL-5, and TNF-α were elevated and IFN-γ level was not. Considering that human AD is now understood to be a much more heterogeneous disease with additional activation of the Th22, Th17/IL-23, and Th1 cytokine pathways,[13] our model seems to mimic chronic AD of humans, which is mainly induced via Th2 and additionally induced via Th1-skewing reactions.

In the present study, topical application of DDE alleviated skin lesions such as scaling, excoriations, and erythema [Figures 2 and 3]. In addition, DDE effectively inhibited increased skin thickness and weight [Figure 4]. These results imply that *D. dasycarpus* can be used as an external agent to treat AD.

We had shown similar results in previous study;[12] however, methanol extract was more effective than decoction of *D. dasycarpus*. These results seem to be due to differences in the active ingredients according to the extraction method, rather than differences in animal models. Methanol or ethanol extracts of *D. dasycarpus* contain considerably different components compared to decoction, and methanol or ethanol extracts of *D. dasycarpus* have a higher probability of containing components that are likely to be bioactive.[14,15]

Chronic and repeated inflammatory reactions lead to thickening of the skin of AD patients, which is one of the evaluation criteria for AD and psoriasis and that it is closely related to epidermal and dermal hyperplasia.[16] In the present study, histopathological observation revealed that topical application of DDE effectively inhibited epidermal hyperplasia [Figure 5]. These results are in accordance with the effects on skin thickness and weight as shown in Figure 4. In addition, DDE ameliorated both scaling on the surface and hyperkeratosis in the epidermis [Figures 2 and 5], implying that DDE can ameliorate scaling via inhibition of hyperkeratosis in inflamed tissues.

In skin inflammation, TNF-α, which is a major proinflammatory cytokine, was mainly produced by keratinocytes and T lymphocytes, and the serum level of TNF-α tends to be elevated in AD patients.[17] In the present study, TNF-α levels in the inflamed tissue were significantly reduced by DDE. In addition, DDE inhibited spongiotic change, which is a hallmark of skin inflammation, in the epidermis [Figures 5 and 6]. These results indicate that DDE can act as an anti-inflammatory agent in inflamed AD tissue.

Th2-skewing cytokines such as IL-4, IL-5, and IL-13 are elevated in patients with extrinsic types of AD[18] and can be used as diagnostic indices and therapeutic targets.

On the other hand, unlike elevated levels of IFN-γ in CD patients, IFN-γ levels in AD patients are generally normal. Even IFN-γ has been proposed as a therapeutic agent for AD patients.[19,20] Our results showed that levels of IL-4 and IL-5 were reduced by DDE treatment and that DDE did not affect IFN-γ in inflamed tissue [Figure 6]. These findings imply that the anti-inflammatory mechanism of DDE in AD-like dermatitis is closely related to the regulation of Th2-skewing reactions.

Our previous results showed that DDE reduced IFN-γ production in CD mice.[4] This discordance seems to be caused by the different animal models used. In the CD animal model generated using DNFB, the IFN-γ levels were highly elevated. Conversely, IFN-γ levels in the AD-like dermatitis model generated using oxazolone in the present study were only slightly elevated. In addition, DEX and DDE did not affect IFN-γ levels [Figure 6d]. Considering these phenomena, it is difficult to evaluate the anti-inflammatory effects related to the Th1-skewing reaction using our AD-like dermatitis model.

In our previous study, we had shown inhibitory effects on degranulation of mast cells via preventing p38 phosphorylation and overexpression of ICAM-1 via the regulation of NF-κB pathway in HaCaT keratinocytes. These mean the anti-inflammatory effects of D. *dasycapus* and these mechanisms seem to have been applied similarly to the results of this study. In particular, the ability to inhibit epidermal hyperplasia similar to that in CD means that DDE can effectively block the accumulation of keratinocyte by TNF-α or other stimuli in the AD-like dermatitis model.

Taken together, these results imply that DDE can be recommended as a new therapeutic agent that can relieve various symptoms of AD-like dermatitis and we newly elucidate that the therapeutic mechanism of DDE is associated with the inhibition of Th2-skewing cytokines such as IL-4 and IL-5.

Corticosteroids such as DEX and hydrocortisone are frequently and repeatedly used in inflammatory skin diseases, despite adverse reactions to its use. Among various adverse reactions of corticosteroids, general immune suppression can give rise to enhanced susceptibility to pathogens. As shown in Figure 7, DEX treatment lowered the spleen–body weight ratio significantly, while DDE did not [Figure 7b]. These results imply that DDE did not exert extensive suppressive effects against immune reactions and that the therapeutic mechanisms of DDE are somewhat different from those of DEX.

CONCLUSIONS

In this study, DDE effectively reduced the production of TNF-α, IL-4, and IL-5 in inflamed tissues, preventing epidermal hyperplasia, spongiotic change, and hyperkeratosis. Finally, DDE effectively inhibited skin enlargement and ameliorated erythema, scaling, and excoriations, implying that it can be used topically for AD patients with relative safety.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gu S, Yang AW, Li CG, Lu C, Xue CC. Topical application of Chinese herbal medicine for atopic eczema: A systematic review with a meta-analysis. Dermatology 2014;228:294-302.
2. Kim JH, Kim H. Combination treatment with herbal medicines and Western medicines in atopic dermatitis: Benefits and considerations. Chin J Integr Med 2016;22:323-7.
3. Kim CM, Shin MG, An DG, Lee KS. The Encyclopedia of Oriental Herbal Medicine. Seoul: Jeongdam; 1997. p. 1648-51.
4. Kim H, Kim M, Kim H, Lee GS, An WG, Cho SI, *et al.* Anti-inflammatory activities of *Dictamnus dasycarpus* Turcz. Root bark on allergic contact dermatitis induced by dinitrofluorobenzene in mice. J Ethnopharmacol 2013;149:471-7.
5. Han HY, Ryu MH, Lee G, Cheon WJ, Lee C, An WG, *et al.* Effects of *Dictamnus dasycarpus* Turcz. Root bark on ICAM-1 expression and chemokine productions *in vivo* and vitro study. J Ethnopharmacol 2015;159:245-52.
6. Wollenberg A, Bieber T. Atopic dermatitis: From the genes to skin lesions. Allergy 2000;55:205-13.
7. Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR, *et al.* Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: Structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. J Invest Dermatol 2008;128:79-86.
8. Lin YH, Chen YC, Hu S, Chen HY, Chen JL, Yang SH, *et al.* Identifying core herbal treatments for urticaria using Taiwanng Taiwanal treatments for Yang SH, 9-Ethnopharmacol

2013;148:556-62.

9. Chien PS, Tseng YF, Hsu YC, Lai YK, Weng SF. Frequency and pattern of Chinese herbal medicine prescriptions for urticaria in Taiwan during 2009: Analysis of the national health insurance database. BMC Complement Altern Med 2013;13:209.
10. Chen HY, Lin YH, Huang JW, Chen YC. Chinese herbal medicine network and core treatments for allergic skin diseases: Implications from a nationwide database. J Ethnopharmacol 2015;168:260-7.
11. Jiang S, Nakano Y, Rahman MA, Yatsuzuka R, Kamei C. Effects of a *Dictamnus dasycarpus* T. Extract on allergic models in mice. Biosci Biotechnol Biochem 2008;72:660-5.
12. Yang B, Lee HB, Kim S, Park YC, Kim K, Kim H, *et al.* Decoction of *Dictamnus dasycarpus* Turcz. Root bark ameliorates skin lesions and inhibits inflammatory reactions in mice with contact dermatitis. Pharmacogn Mag 2017;13:483-7.
13. Brunner PM, Guttman-Yassky E, Leung DY. The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies. J Allergy Clin Immunol 2017;139:S65-76.
14. Chang JB, Lane ME, Yang M, Heinrich M. A hexa-herbal TCM decoction used to treat skin inflammation: An LC-MS-based phytochemical analysis. Planta Med 2016;82:1134-41.
15. Lv M, Xu P, Tian Y, Liang J, Gao Y, Xu F, *et al.* Medicinal uses, phytochemistry and pharmacology of the genus *Dictamnus* (*Rutaceae*). J Ethnopharmacol 2015;171:247-63.
16. Al-Waili NS. Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: Partially controlled, single-blinded study. Complement Ther Med 2003;11:226-34.
17. Pellegrino M, Minervini B, Musto P, Matera R, Greco A, Checchia de Ambrosio C, *et al.* Tumor necrosis factor-alpha and interleukin-1 beta. Two possible mediators of allergic inflammation. Minerva Pediatr 1996;48:309-12.
18. Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. J Dermatol Sci 2010;58:1-7.
19. Noh G, Lee KY. Pilot study of IFN-gamma-induced specific hyposensitization for house dust mites in atopic dermatitis: IFN-gamma-induced immune deviation as a new therapeutic concept for atopic dermatitis. Cytokine 2000;12:472-6.
20. Lee JH, Noh G. Polydesensitisation with reducing elevated serum total IgE by IFN-gamma therapy in atopic dermatitis: IFN-gamma and polydesensitisation (PDS). Cytokine 2013;64:395-403.