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ORIGINAL ARTICLE

Decoction of *Dictamnus Dasycarpus* Turcz. Root Bark Ameliorates Skin Lesions and Inhibits Inflammatory Reactions in Mice with Contact Dermatitis

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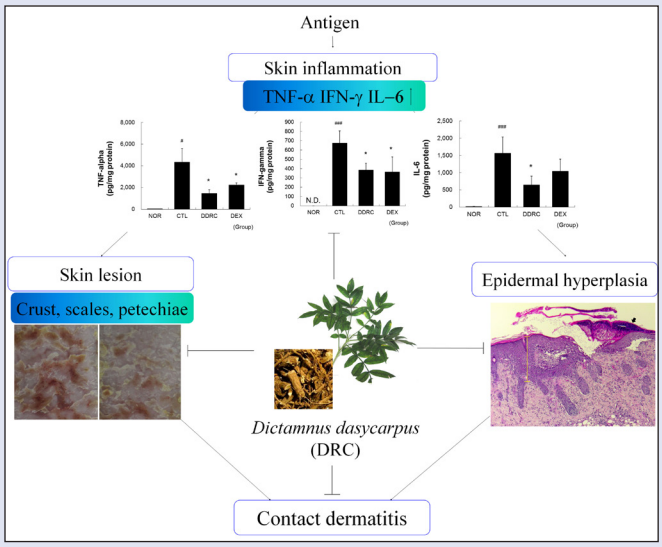
ABSTRACT

Background: The root bark of *Dictamnus dasycarpus* Turcz. (Dictamni Radicis Cortex) has been widely used to treat skin diseases in Korea, and its anti-inflammatory efficacies were recently reported. **Objective:** The paper aims to investigate the inhibitory effects of decoction of Dictamni Radicis Cortex (DDRC) in mice with contact dermatitis (CD). **Materials and Methods:** We investigated the effects of DDRC on skin lesion characteristics such as crust, scales, incrustation and petechiae, the erythema and melanin indexes, skin thickness, histopathologic changes, and cytokine production in 1-fluoro-2,4-dinitrofluorobenzene (DNFB)-induced CD mice. **Results:** Topical application of DDRC ameliorated crust, scales, incrustation, and induced by DNFB. In addition, DDRC lowered the erythema index significantly ($P < 0.05$). DDRC effectively inhibited enlargement of skin thickness ($P < 0.05$). Histopathologic observation showed that DDRC inhibited epidermal hyperplasia, hyperkeratosis, and spongiotic changes. Finally, DDRC decreased production levels of IFN-γ, TNF-α and IL-6 induced by repeated application of DNFB ($P < 0.05$). **Conclusion:** These data suggest that DDRC can be used in the treatment of inflammatory skin diseases including CD. Moreover, these results are closely related to the decreasing production of TNF-α IFN-γ and IL-6 in inflamed tissues.

Key words: *Dictamnus dasycarpus*, traditional Chinese medicine, inflammation, contact dermatitis

SUMMARY

- DDRC ameliorated skin lesions such as crust, scales, incrustation and petechiae, and lowered erythema index on skin surface in CD mice
- DDRC inhibited enlargement of dorsal skin and prevented epidermal hyperplasia, hyperkeratosis, and spongiotic changes in inflamed tissues
- DDRC reduced the levels of TNF-α, IFN-γ, and IL-6 in inflamed tissues of CD mice
- DDRC did not affect spleen/body weight ratio in CD mice.



Abbreviations used: DDRC: decoction of Dictamni Radicis Cortex, CD: contact dermatitis, DNFB: 1-fluoro-2,4-dinitrofluorobenzene, AOO: acetone and olive oil, DEX: dexamethasone, CBA: cytometric bead array

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INTRODUCTION

Contact dermatitis (CD), which affects roughly 20% of the population in the United States, is the most general form of occupational skin disease.[1,2] CD has great socio-economic impact and can lead to decreased work performance and quality of life.[3] CD is a T-cell-mediated type IV hypersensitivity in which exposure to allergens or sensitization is followed by inflammation on the second exposure, and therefore, allergic reaction occurs.[4] Skin diseases including CD can be caused by both external and internal factors. External factors can be work environment, cosmetics, or other personal care products, while internal factors are psychological and genetic.[3,5] Although external factors are the major cause of CD, psychological and genetic factors also play a role by enhancing contact hypersensitivity.[5] Although the exact cause of allergic reaction is unknown, several of its mechanisms have been elucidated. Type IV hypersensitivity is highly associated with mast cells, which act as effector cells by secreting pro-inflammatory substances such as histamines, interleukin (IL), tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma.[4-6] CD results in increased dermal weight and

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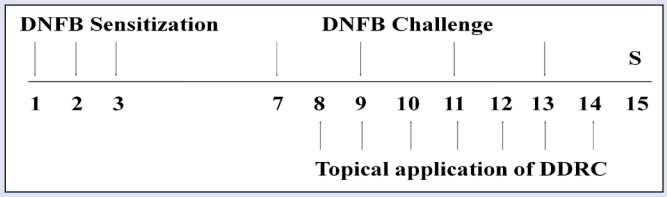

Figure 1: Experimental design. The experimental groups, except for the nasign is summarized in nged with DNFB, after which they were p, and 3. Mice were challenged by DNFB on days 7, 9, 11, and 13. The nare challenged by DNFB on days 7, 9, 11mmarized in nged witDDRC and DEX groups were topically treated with DDRC (60 μg/day) or DEX (30 μg/day) on seven consecutive days. All animals were sacrificed on day 15

thickness, symptomatic hyperplasia, edema, spongiosis, and scale,[6] and can also develop as a chronic disease if not properly treated.[7]

There are a wide variety of herbs clinically used in eastern countries to treat skin diseases, many of which have been tested because of their relatively low price and safety.[8] The root bark of *Dictamnus dasycarpus* Turcz. (Dictamni Radicis Cortex) has been used in the treatment of pruritus vuluae, eczema, and scabies because of its ability to arrest itching, clear away heat, and eliminate dampness.[9] Hence, it is used in Korean medicine to treat skin diseases. Based on this background, decoction of Dictamni Radicis Cortex (DDRC) has long been used in Korea.[10] Other effects of *D. dasycarpus* on neuroprotection, anti-inflammation, and insecticide have also been reported.[6,11]

For these reasons, we investigated the effects of DDRC on dorsal skin thickness, as well as characteristics of skin lesions such as crust, scale, incrustation, petechiae and pigmentation, erythema and melanin index, histopathologic changes in skin tissue, and cytokine productions in mice with CD induced by repeated application of DNFB. Spleen/body weight ratio was also measured since the spleen is associated with immune response.

MATERIALS AND METHODS

Preparation of DDRC

The root bark of *Dictamnus dasycarpus* Turcz. (Dictamni Radicis Cortex) was purchased from Hwalim Medicinal Herbs (Pusan, Korea) and authenticated by Prof. Jung-Hun Kim, School of Korean Medicine, Pusan National University. Briefly, 50 g of Dictamni Radicis Cortex was boiled in 1 L of distilled water for 3 h. After 3 h of extraction, the extract was filtered through Whatman filter paper No. 20 and evaporated under reduced pressure using a vacuum evaporator (Eyela, Japan). The condensed extract was subsequently lyophilized using a freeze dryer (Labconco, Kansas City, MO, USA). Finally, 5.77 g of lyophilized powder was obtained (yield, 11.54%). The decoction of Dictamni Radicis Cortex (DDRC, Voucher No. MH2013-040) was deposited at the Division of Pharmacology, School of Korean Medicine, Pusan National University.

Animals

Male 6-week-old Balb/c mice were purchased from Samtaco (Incheon, Korea). Mice were housed under specific pathogen-free conditions with a 12-h light/dark cycle and free access to standard rodent food and water. All animal experiments were approved by our Animal Care and use Committee and conducted according to institutional guidelines (PNU-2012-0140).

Induction of CD and experimental design

CD was induced using our method of standardization as previously described.[6] Briefly, mice were sensitized by painting 30 μL of DNFB (0.1%, v/v) in acetone:olive oil (AOO, 4:1) onto the dorsum of both ears for three consecutive days. Four days after sensitization, each mouse was challenged by painting 60 μL of DNFB (0.2%, v/v) in AOO onto the shaved back of each mouse every 2 days. For topical application of the drugs, DDRC and DEX were dissolved in ethanol, filtered using a 0.45 μm pore size syringe filter, and finally diluted in AOO (ethanol: AOO, 1:4). DDRC solution (1 mg/mL, 0.1%, w/v) was applied onto the shaved back for 7 days. Naïve animals were treated with vehicle (n = 4), whereas control animals (CTL) were sensitized and challenged with DNFB in AOO, then painted with vehicle (n = 6). DDRC-treated animals were sensitized and challenged with DNFB, after which 60 μg/day of DDRC was applied (n = 6). Dexamethasone (DEX) treated animals were sensitized and challenged with DNFB, after which they were painted with 30 μg/day of DEX (n = 4) as a positive control. The experimental design is summarized in Figure 1.

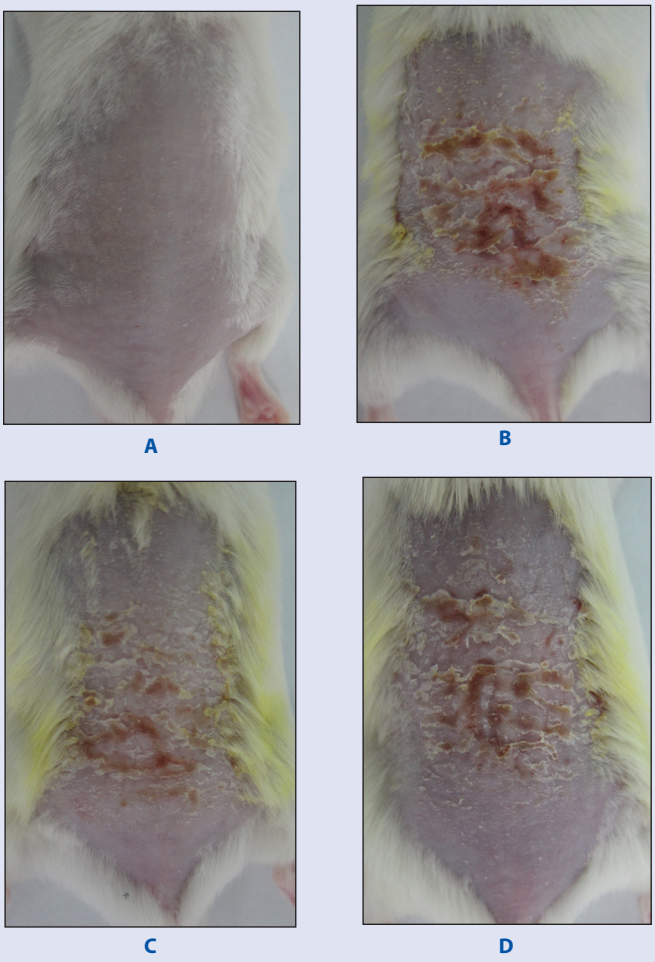

Figure 2: Effect of DDRC on skin lesion in CD mice. The skin surfaces were captured using a digital camera on day 15. (A) NOR; (B) CTL; (C) DDRC (60 μg/day); (D) DEX (30 μg/day)

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 dermo-spectrophotometer (DSM II, Cortex Technology, Hadsund, Denmark).

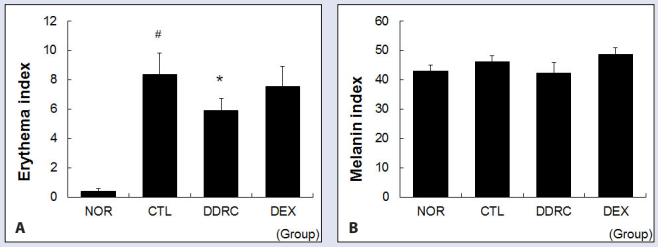

Figure 3: Effect of DDRC on erythema and melanin indexes on skin surface in CD mice The erythema and melanin indexes were measured using a dermo-spectrophotometer on day 15. (A) Erythema index; (B) melanin index. NOR: nontreated normal mice, CTL: nontreated CD mice, DDRC: 60 μg/day of DDRC-treated CD mice, DEX: 30 μg/day of dexamethasone-treated CD mice. All values are presented as the mean ± SD. #$P < 0.05$ vs. nontreated normal mice (NOR). *$P < 0.05$ vs. nontreated CD mice (CTL)

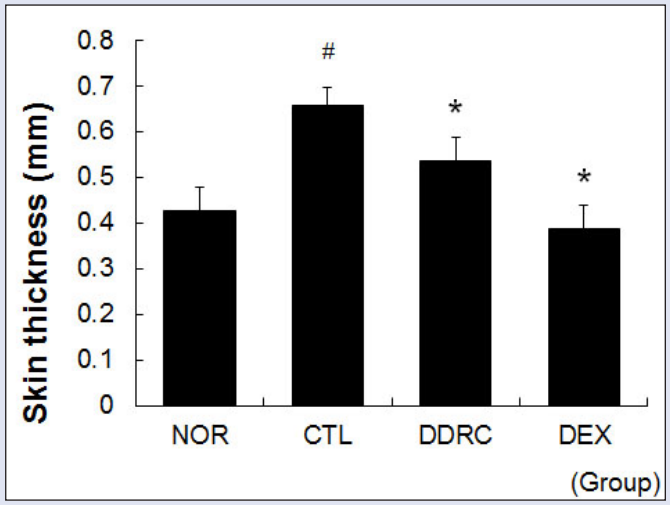

Figure 4: Effect of DDRC on skin thickness in CD mice. The skin thickness was measured using Vernier calipers on day 15. Abbreviations are the same as in [Figure 3]. All values are presented as the mean ± SD. #$P < 0.05$ vs. nontreated normal mice (NOR). *$P < 0.05$ and vs. nontreated CD mice (CTL)

Measurement of dorsal skin thicknesses

After observing skin lesions and measuring the erythema and melanin index, the dorsal skins were resected and their thicknesses measured using Vernier calipers (Mitutoyo, Tokyo, Japan).

Histopathologic examination

Tissues obtained from experimental animals were fixed in 4% neutral formalin overnight, then washed, dehydrated with ethyl alcohol sequentially, treated with xylene and embedded in paraffin. Skin tissues (4 μm) were then resected, after which sections were stained with hematoxylin and eosin (H and E) and observed using a light microscope (200x).

Measurement of cytokine production

Cytokine levels in inflamed tissues were measured according to the cytometric bead array (CBA) method using a Mouse Inflammation CBA kit (BD Biosciences, San Jose, CA, USA). At the final step of the experiment, resected dorsal skin tissues were lysed and homogenized with protein extraction solution (Intron Bio, Daejeon, Korea) using a bullet blender (Next Advance, NY, USA) to obtain tissue lysates. Fifty micrograms of each lysate was then used to measure the levels of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10). All experimental procedures were conducted according to the manufacturer's protocols.

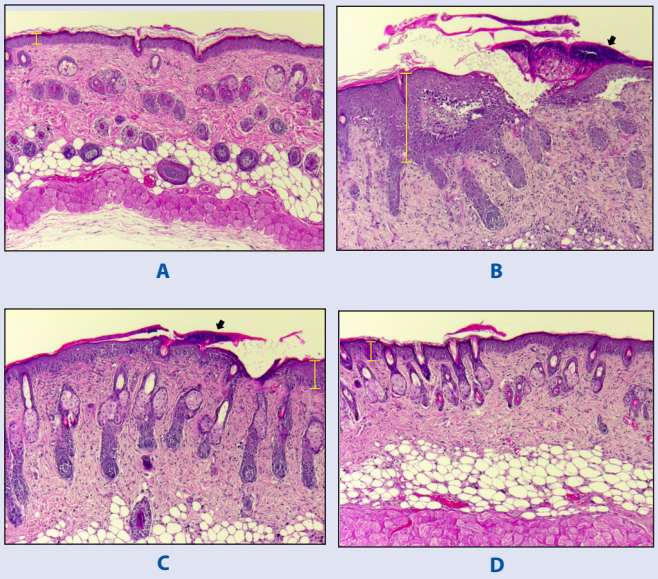

Figure 5: Effects of DDRC on histopathologic changes in CD mice. Skin tissues were stained with H and E and observed using a light microscope. (A) NOR; (B) CTL; (C) 60 μg/day of DDRC; (D) 30 μg/day of DEX. Yellow bars indicate the vertical length of the epidermis. Filled arrows indicate the hyperkeratotic area. All observations were made at a magnification of 200×

Statistical analysis

A Student's t-test was used for all statistical comparisons, and Prism 5 version 5.01 (Graph Pad Software Inc., La Jolla, CA, USA) was used for all analyses. All data are presented as the mean mean ± SD. *P* less than 0.05 were considered significant.

RESULTS

DDRC ameliorated skin lesions in CD mice

DNFB treatment induced skin lesions of CD such as crust, scale, incrustation, petechiae, and pigmentation in the CTL group [Figure 2B]. Treatment with DDRC ameliorated these symptoms and decreased the crust, scale, and incrustation [Figure 2C].

DDRC lowered erythema index on skin surface in CD mice

A marked increase in erythema index was observed in the CTL group. Treatment with DDRC reduced the erythema index significantly, while DEX did not [Figure 3A]. Conversely, neither DDRC nor DEX affected the melanin index [Figure 3B].

DDRC inhibited enlargement of dorsal skin in CD mice

The inhibitory effects of DDRC on swelling and enlargement of skin tissues were evaluated by measuring the effects on skin thicknesses in mice with CD. Repeated application of DNFB increased the levels of skin thickness to more than twice those observed in the normal group. These increases were effectively inhibited by DDRC and DEX [Figure 4].

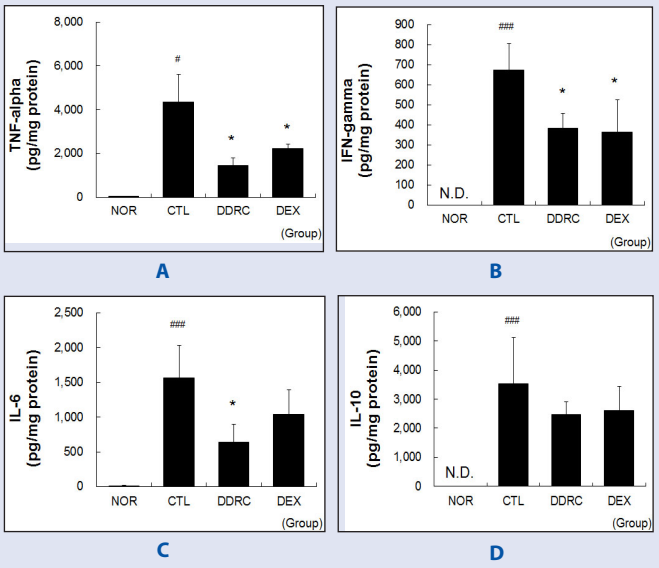

Figure 6: Effects of DDRC on cytokine production in CD mice. The levels of TNF-α, IFN-γ, IL-6 and IL-10 in skin tissues were measured using the cytometric bead array method. A total of 50 μg of tissue lysates were used to measure the cytokine levels. Abbreviations are the same as those used in [Figure 3]. (A) TNF-α; (B) IFN-γ; (C) IL-6; (D) IL-10. All values are presented as the mean ± SD. #$P < 0.05$ and ###$P < 0.001$ vs. nontreated normal mice (NOR). *$P < 0.05$ vs. nontreated CD mice (CTL)

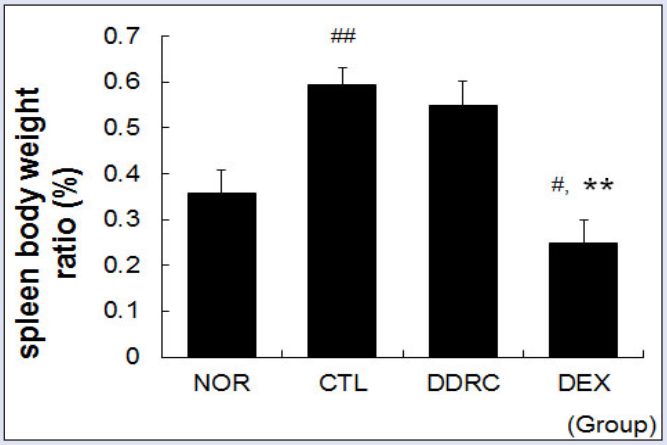

Figure 7: Effects of DDRC on spleen/body weight ratio in CD mice. Body weights and spleen weights were measured on day 15, at which time the spleen/body weight ratio was calculated. Abbreviations are the same as in [Figure 3]. All values are presented as mean ± SD. #$P < 0.05$ and ##$P < 0.01$ vs. nontreated normal mice (NOR). p **$P < 0.01$ vs. nontreated CD mice (CTL)

DDRC prevented epidermal hyperplasia, hyperkeratosis, and spongiotic changes in inflamed tissues

There were no abnormal changes in the normal group [Figure 5A]. Repeated painting with DNFB induced significant epidermal hyperplasia (yellow bar), as well as hyperkeratosis (filled arrow), which are hallmarks of skin inflammation. In addition, spongiotic changes were observed in the CTL group [Figure 5B], while DDRC treatment inhibited epidermal hyperplasia, hyperkeratosis, and spongiotic changes [Figure 5C]. Treatment with DEX most effectively prevented abnormal changes in inflamed tissues of CD mice [Figure 5D].

DDRC reduced the levels of TNF-α, IFN-γ, and IL-6 in inflamed tissues of CD mice

Marked increases in TNF-α, IFN-γ, IL-6 and IL-10 production were observed in the CTL group. These increases in TNF-α, IFN-γ, and IL-6 production were effectively reduced by topical application of DDRC. Neither DDRC nor DEX affected the IL-10 level [Figure 6].

DDRC did not affect spleen/body weight ratio in CD mice

The effects of DDRC on enlargement of the spleen were estimated based on the spleen/body weight ratio. The spleen/body weight ratio in the CTL group doubled compared with the NOR group. The DDRC group showed no change in spleen/body weight ratio in CD mice. However, when compared with the CTL and NOR groups, this ratio was significantly decreased in the DEX group [Figure 7].

DISCUSSION

In this experiment, sensitization with DNFB and increased concentration of DNFB for challenge led to increased susceptibility to CD. Sensitization of the ears induced activation of immune cells, so synthesis of pro-inflammatory factors such as cytokines and chemokines could occur. The immune cells activated in the initiation phase then circulate throughout the body with lymph and blood circulation.[12] During the elicitation phase, effector T cells from lymph nodes and blood were recruited at the challenged site.[4] The animal model used in this experiment was designed to mimic human CD, especially allergic contact dermatitis. For this reason, mice were sensitized on days 1, 2, and 3 (initiation phase) and challenged on days 7, 9, 11, and 13 (elicitation phase) on different sites (ear and shaved back).

Dorsal skin appearance data show that both DDRC and DEX relieved crust, scales, incrustation, and petechiae compared with the control group [Figure 2]. The erythema index was distinctly higher in all challenged groups than in the normal group, and only DDRC led to a significant reduction in the erythema index [Figure 3A]. Conversely, the melanin index remained unchanged [Figure 3B]. In addition, the control group had the thickest skin due to inflammation, whereas the DDRC and DEX groups had thinner skin than the control group, with the skin of the DEX group being slightly thinner than that of the normal group [Figure 4]. Although these data indicate that DDRC and DEX significantly relieved skin inflammation, the results observed for DEX indicate that corticosteroid use results in a thinner skin barrier. This results in aesthetic and functional problems, with vessels underneath the skin becoming more visible. Moreover, the thinner skin barrier makes it more vulnerable to other foreign substances. Notably, topical application of corticosteroids to relieve allergic reaction has been shown to result in skin atrophy,[13] as has steroid injection.[14] Therefore, patients with dermal inflammation may need to compromise or change their treatment to alternative or complementary agents.

The control groups had thicker layers because of their inflammatory response, which was a result of keratinocyte proliferation.[15] In addition, repeated application of DNFB induced spongiotic changes, which are a hallmark of inflammatory skin diseases and large vesicles in control group [Figure 5B]. The histopathologic changes observed in the present study indicate that both DDRC and DEX prevented epidermal hyperplasia, hyperkeratosis, and spongiotic changes induced by DNFB [Figure 5]. In CD, keratinocytes can be activated by TNF-α and IFN-γ and play a major role as hapten-presenting cells associated with $CD8^+$ T cells.[4,16] In addition,

IL-6 is known to induce the proliferation and migration of keratinocytes, leading to skin diseases such as psoriasis.[17] Once activated, keratinocytes secrete cytokines including IL-1, IL-3, IL-6, IL-8, and TNF-α.[18,19] As shown in Figure 6, there were marked increases in TNF-α, IFN-γ, IL-6, and IL-10 in the control group. DDRC had an inhibitory effect on TNF-α, IFN-γ, and IL-6 production, indicating that DDRC acts as an anti-inflammatory agent in skin tissue. Considering the proliferative effect of TNF-α, IFN-γ, and IL-6, these data indicate that DDRC can inhibit epidermal hyperplasia via regulation of pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 in inflamed tissues.

The spleen/body weight ratio of the control group was almost double that of the normal group. The DDRC group showed almost the same level as that of the control group, but the DEX group showed a reduced weight ratio relative to the normal group, indicating that corticosteroids led to immune suppression Figure 7. Based on these data, the continuous use of corticosteroid can lead to immune suppression and bring other unexpected side effect. It has also been reported that dexamethasone induces spleen atrophy via the apoptosis of splenocytes.[20] Conversely, DDRC did not affect spleen/body weight ratio, implying that the action mechanisms of DDRC are somewhat different from those of corticosteroids, especially in the framework of general immune weakness.

Taken together, these data indicate that DDRC can prevent epidermal hyperplasia, hyperkeratosis, and spongiotic changes via regulation of the production levels of pro-inflammatory cytokines. In addition, these anti-inflammatory actions of DDRC inhibit increases in skin thickness and ameliorate skin lesions and the erythema index.

CONCLUSIONS

We demonstrated the anti-inflammatory effects of DDRC in CD mice in this study. DDRC inhibited the production of TNF-α, IFN-γ, and IL-6, resulting in reduced epidermal hyperplasia, hyperkeratosis, and spongiotic changes. These anti-inflammatory reactions of DDRC finally prevented enlargement of skin thickness and ameliorated skin lesions. However, in contrast to dexamethasone, DDRC had no effect on the spleen/body weight ratio. Overall, these results indicate that DDRC can be used to treat patients with inflammatory skin diseases with relative safety.

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Conflict of interest

There are no conflicts of interest

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