

Ribes fasciculatum var. *chinense* Attenuated Allergic Inflammation *In Vivo* and *In Vitro*

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Abstract

Ribes fasciculatum var. *chinense* MAX. (*R. fasciculatum*) has traditionally been used in Korea to treat inflammatory diseases. However, the exact mechanism that accounts for the anti-inflammatory effect of *R. fasciculatum* is not completely understood. We aimed to ascertain the pharmacological effects of *R. fasciculatum* on both compound 48/80- or histamine-induced scratching behaviors and 2, 4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD) in mice. Additionally, to find a possible explanation for the anti-inflammatory effects of *R. fasciculatum*, we evaluated the effects of *R. fasciculatum* on the production of inflammatory mediators in LPS-stimulated macrophage cells. Treatment of *R. fasciculatum* significantly reduced compound 48/80- or histamine-induced the pruritus in mice. *R. fasciculatum* attenuated the AD symptoms such as eczematous, erythema and dryness and serum IgE levels in AD model. Additionally, *R. fasciculatum* inhibited the production of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). The maximal rates of TNF- α and IL-6 inhibition by *R. fasciculatum* (1 mg/ml) were approximately 32.12% and 46.24%, respectively. We also showed that *R. fasciculatum* inhibited the activation of nuclear factor-kappa B in LPS-stimulated macrophages. Collectively, the findings of this study provide us with novel insights into the pharmacological actions of *R. fasciculatum* as a potential molecule for use in the treatment of allergic inflammatory diseases.

Key Words: *Ribes fasciculatum*, Inflammatory mediators, Nuclear factor-kappa B, Macrophage, Allergic inflammation

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by eczematous inflammation of the skin (Buske-Kirschbaum *et al.*, 2001). The incidence of this disease has increased steadily in recent years. AD is known to be the result of an immune system dysregulation, ultimately resulting in allergic inflammation (Gold and Kemp, 2005). Activation of macrophages is a hallmark of inflammation, especially in the AD skin where they can mediate chronic inflammation by producing cytokines. It has been previously reported that macrophages can be found in larger numbers in AD lesional skin (McCormick *et al.*, 2000). In response to various stimuli, macrophages generate a variety of cytokines, including interleukin (IL)-6, and tumor necrosis factor (TNF)- α . TNF- α has an important amplifying effect in asthmatic inflammation and stimulates airway epithelial cells to produce cytokines, includ-

ing IL-6, IL-8 and GM-CSF (Boero *et al.*, 2010). IL-6 is an important co-factor in IL-4 dependent IgE synthesis. The release of these cytokines may be of major importance in the development of a variety of inflammatory skin disorders (Trefzer *et al.*, 2003). Therefore, the inhibition of cytokine secretion can aid in the development of a useful therapeutic strategy for allergic inflammatory diseases such as AD. Nuclear factor-kappa B (NF- κ B) plays a crucial role in the regulation of many genes involved in immune and inflammatory responses (Tegeger *et al.*, 2001). In the nucleus, NF- κ B activates gene transcription; thus, NF- κ B plays a pivotal role in the regulation of immune and inflammatory responses, via control of the transcription of inflammatory cytokine genes (Gadaleta *et al.*, 2011). An increase in NF- κ B activity associated with the secretion of high levels of IL-6 and TNF- α has also been noted in the context of allergic inflammatory responses (Mukaida, 2000). The results of these studies demonstrated that NF- κ B activation

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and the subsequent activation of pro-inflammatory cytokine gene expression are critically important in the initiation and perpetuation of allergic inflammation. Traditional medicine has been the subject of increased interest for its potential in the treatment of inflammation. Although traditional herbal medicines have long been used effectively in treating diseases, the pharmacological mechanisms of action of most herbal medicines have not yet been elucidated. *Ribes fasciculatum* var. *chinense* MAX. (*R. fasciculatum*) is used in traditional oriental medicine for various medicinal purposes. It was reported that *R. fasciculatum* inhibited the nuclear factor of activated T cells (NFAT) transcription factor (Dat *et al.*, 2005). This result suggested that *R. fasciculatum* may be useful in the treatment of autoimmune diseases. Unfortunately, the exact mechanism that accounts for the anti-allergic and anti-inflammatory effects of the *R. fasciculatum* is still not understood. In this study, we attempted to ascertain the mechanisms underlying the pharmacological effects of *R. fasciculatum* on both compound 48/80- or histamine-induced scratching behaviors and 2, 4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis in mice. Additionally, to find a possible explanation for the anti-inflammatory mechanisms of *R. fasciculatum*, we evaluated the effects of *R. fasciculatum* on the production of inflammatory cytokines and activation of NF- κ B in LPS-stimulated macrophages.

MATERIALS AND METHODS

Reagents

Compound 48/80, LPS, avidin peroxidase (AP), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and DNCB were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagles Medium (DMEM) was purchased from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from JR Scientific, Inc. (Woodland, CA, USA). Anti-mouse TNF- α /IL-6, recombinant TNF- α /IL-6, biotinylated TNF- α /IL-6, anti-mouse IgE, recombinant IgE and biotinylated IgE were purchased from Pharmingen (San Diego, CA, USA). NF- κ B, and histone antibodies (Abs) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Animals

The original stock of male ICR mice (5 weeks, 25-30 g), BALB/c mice (5 weeks, 19-20 g) and SD rats (7 weeks, 250-300 g) were purchased from Orient Co., Ltd, a branch of Charles River Laboratories (Seoul, Korea). Animals were housed 10 per cage, allowed access to water and food ad libitum, and maintained at a constant temperature ($24 \pm 1^\circ\text{C}$) and humidity ($60 \pm 10\%$) under a 12-h light/dark cycle (light on 08:00:20:00 h). Animal experimental procedures were approved by the ethics committee of Daegu Haany University, Korea.

Preparation of *R. fasciculatum*

The dried of *R. fasciculatum* were purchased from the Human herb (Gyeongbuk, Korea). The roots (100 g) were chopped using a blender with 1 L of 70% ethanol solution under room temperature for 24 h and then concentrated under a vacuum. Then the extract solution obtained was filtered, concentrated

on a water bath under vacuo, frozen and lyophilized to yield ethanol extracts (yield: 5.83%). Dilutions were made in saline and filtered through 0.22- μm syringe filter.

Compound 48/80-induced systemic anaphylactic reaction

Mice were given an intraperitoneal injection of the mast cell degranulator compound 48/80 (8 mg/kg). *R. fasciculatum* dissolved in saline was administered orally 1 h before the injection of compound 48/80. Mortality was monitored for 23 min after induction of an anaphylactic reaction.

Scratching behavioral experiment

Before the experiment, the ICR mice (n=6) were put into acrylic cages (22 \times 22 \times 24 cm) for about 30 min for acclimation. The behavioral experiments were performed according to the method of Sugimoto *et al.* (1998). The rostral part of the skin on the back of mice was clipped, and compound 48/80 (50 $\mu\text{g}/\text{kg}$) or histamine (100 $\mu\text{g}/\text{kg}$) for each mouse was intradermally injected. The scratching agents were dissolved in tween 80 and then used. Control mice received a tween 80 injection in the place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage; and for the observation of scratching. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching for 30 min. *R. fasciculatum* (200 mg/kg) was orally administered 1 h before the scratching agents.

DNCB-induced atopic dermatitis

Experiments were conducted in accordance with a previously described protocol (Gao *et al.*, 2005). The dorsal skin of the BALB/c mice (n=6) was shaved and treated with a depilatory prior to the experiment. The mice were sensitized with 100 μl of 0.15% DNCB in acetone-olive oil (3:1) applied to the dorsal skin twice per week for 5 weeks. Control mice received vehicle (acetone/olive oil=3:1). After 3 weeks, *R. fasciculatum* (200 mg/kg) was orally administered 2 weeks until the end of the experiment.

Cell culture

Raw 264.7 cells, macrophage cell line, were grown in DMEM medium supplemented with 100 IU/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 10% heat-inactivated FBS at 37°C in 5% CO_2 .

MTT assay

To test the cell viability by each concentration of *R. fasciculatum*, the MTT colorimetric assay was performed. Briefly, cells (3×10^5 cells/well) were incubated with *R. fasciculatum* (0.01-1 mg/ml) for 12 h. After the addition of MTT solution, the cells were incubated at 37°C for 4 h. The crystallized MTT (formazan) was dissolved in dimethyl sulfoxide and measured the absorbance at 540 nm.

Assay of cytokines

Cytokine (TNF- α and IL-6) assay was performed by a modified enzyme-linked immunosorbent assay (ELISA). In this method, the wells of 96-well plates were coated with mouse monoclonal Abs specific for TNF- α and IL-6. The coated plates

Table 1. Effect of *R. fasciculatum* on compound 48/80-induced systemic anaphylactic reaction in mice

Dose (mg /kg) ^a	Compound 48/80 (8 mg/kg) ^b	Mortality (%) ^c
None (saline)	+	100.0
200	-	0.0
200	+	55.6*

^aThe groups of mice (n=18) were orally pretreated with saline or *R. fasciculatum* was given at various doses 1 h before the compound 48/80 injection. ^bThe compound 48/80 solution was intraperitoneally given to the groups of mice. ^cMortality (%) is presented as the 'Number of dead mice×100/Total number of experimental mice. The results were examined using Fisher's exact test. *p<0.01 as compared with compound 48/80-treated group.

were washed with PBS containing 0.05% Tween 20 prior to subsequent steps in the assay. All reagents used in this assay were incubated for 2 h at 37°C. Recombinant TNF-α and IL-6 were diluted and used as standards. The assay plates were sequentially exposed to biotinylated mouse TNF-α, IL-6, AP and ABTS substrate solution containing 30% H₂O₂. The absorbance values of the plates were recorded at 405 nm.

HPLC analysis

The analysis was carried out using a model 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was performed on Hypersil GOLD column (4.6×250 mm, 5 μm, Thermo Scientific). The mobile phase consisted of acetonitrile and acetic acid. The total running time was 60 min, and the flow rate was 1.0 ml/min. The UV detection wavelength was set at 360 nm.

RESULTS

Effect of *R. fasciculatum* on compound 48/80-induced systemic anaphylaxis

To assess the contribution of *R. fasciculatum* in anaphylactic reactions, the *in vivo* model of systemic anaphylaxis was used initially. As a non-immunologic stimulator, compound 48/80 (8 mg/kg) was used. After the injection of the compound 48/80, the mice were monitored for 23 min, after which the mortality rate was determined. As shown in Table 1, an oral administration of saline as a control induced a fatal reaction in 100% of each group. When the *R. fasciculatum* was orally administered before the compound 48/80 injections, the mortality was reduced (Table 1).

Effect of *R. fasciculatum* on histamine-induced scratching behavior in mice

In addition, we investigate the contribution of *R. fasciculatum* in histamine-induced scratching behavior. As shown in Fig. 1, orally administered *R. fasciculatum* inhibited the scratching behaviors by 46.11%. Terfenadine was used as a positive control in this study.

Effect of *R. fasciculatum* on compound 48/80-induced scratching behavior in mice

The anti-pruritic effects of *R. fasciculatum* were investigated on the compound 48/80-induced scratching behavior animal

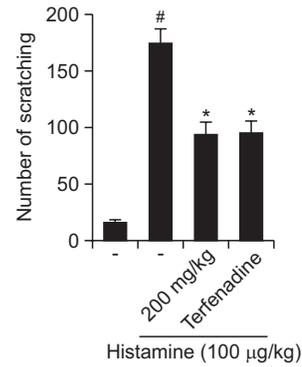


Fig. 1. Effect of *R. fasciculatum* on scratching behavior in ICR mice. *R. fasciculatum* (200 mg/kg) was orally administered 1 h before compound 48/80 (50 μg/kg) intradermally injection. Scratching behaviors was counted as one incident of scratching for 30 min. Statistical evaluation of the results was performed by ANOVA with a Tukey *post hoc* test. Each datum represents the means ± S.D. of experiments ([#]p<0.05 vs. control group, ^{*}p<0.05 vs. compound 48/80 or histamine-treated group).

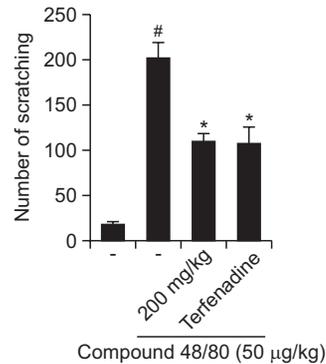


Fig. 2. Effect of *R. fasciculatum* on scratching behavior in ICR mice. *R. fasciculatum* (200 mg/kg) was orally administered 1 h before histamine (100 μg/kg) intradermally injection. Scratching behaviors was counted as one incident of scratching for 30 min. Statistical evaluation of the results was performed by ANOVA with a Tukey *post hoc* test. Each datum represents the means ± S.D. of experiments ([#]p<0.05 vs. control group, ^{*}p<0.05 vs. compound 48/80 or histamine-treated group).

model. When the *R. fasciculatum* was orally administered 1 h before compound 48/80 injections, the scratching behaviors was reduced. The inhibition rate of *R. fasciculatum* (200 mg/kg) was approximately 45.81% (Fig. 2).

Effect of *R. fasciculatum* on DNCB-induced atopic dermatitis and IgE levels in serum

In order to evaluate the regulatory effects of *R. fasciculatum* in an atopic dermatitis *in vivo* model, DNCB was administered to BALB/c mice. As shown in Fig. 3A, when mice were treated for 2 week with *R. fasciculatum*, the atopic dermatitis was recovered to a significant extent. To evaluate the effects of *R. fasciculatum* on IgE levels in serum, blood samples were collected. The levels of IgE were measured via ELISA. The results showed that IgE levels were increased as the result of DNCB exposure, but this phenomenon was significantly reduced in the *R. fasciculatum* group (Fig. 3B).



Fig. 3. Effect of *R. fasciculatum* on the DNCB-induced dermatitis and serum IgE level. (A) The BALB/c mice (n=6) were sensitized with 100 μ l of 0.1% DNCB in acetone-olive oil (3:1) or vehicle (acetone/olive oil=3:1) applied to the dorsal skin twice each week for a total period of 5 weeks. After 3 weeks, *R. fasciculatum* (200 mg/kg) was orally administered 2 week prior to the end of the experiment. (B) Blood samples were collected and then levels of serum IgE in the indicated groups were measured using ELISA method. Statistical evaluation of the results was performed by independent *t*-test. Each datum represents the means \pm S.D. of three independent experiments ([#]*p*<0.05 vs. control group, ^{*}*p*<0.05 vs. DNCB -treated group).

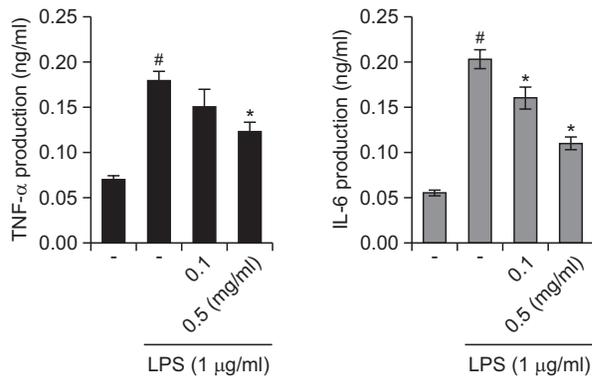


Fig. 4. Effects of *R. fasciculatum* on the production of inflammatory cytokines in LPS-stimulated Raw 264.7 cells. Cells were pre-treated with *R. fasciculatum* (0.1-0.5 mg/ml) for 1 h and then stimulated LPS (1 μ g/ml) for 12 h. The levels of inflammatory cytokines (TNF- α and IL-6) were measured from cell supernatant using ELISA. Statistical evaluation of the results was performed by independent *t*-test. All data were represented in the mean \pm S.D. of triplicate determinations from triplicate separate experiments ([#]*p*<0.05 vs. control, ^{*}*p*<0.05 vs. LPS alone).

Effect of *R. fasciculatum* on the TNF- α and IL-6 production in LPS-stimulated macrophage

In an effort to determine the molecular mechanism of *R. fasciculatum*, the macrophage cell line, Raw 264.7, was employed in this study. We determined whether *R. fasciculatum* modulates the LPS-induced production of TNF- α and IL-6. The levels of TNF- α and IL-6 in culture supernatants were measured via ELISA. As is shown in Fig. 4, the production of TNF- α and IL-6 in response to LPS was inhibited as the result of pre-treatment with *R. fasciculatum* in a dose-dependent manner. The maximal rates of TNF- α and IL-6 inhibition

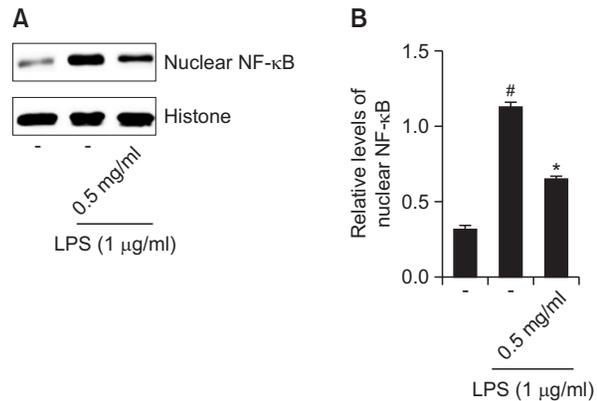


Fig. 5. Effect of *R. fasciculatum* on the NF- κ B activation in the nuclei of LPS-stimulated Raw 264.7 cells. Cells were pre-treated with *R. fasciculatum* (0.5 mg/ml) for 1 h and then stimulated with LPS for 2 h. (A) Nuclear extracts were prepared as described in the *Materials and Methods* section and evaluated for RelA/p65 via Western blot analysis. (B) The relative levels of NF- κ B were represented. Statistical evaluation of the results was performed by independent *t*-test. All data were represented in the mean \pm S.D. of triplicate determinations from triplicate separate experiments ([#]*p*<0.05 vs. control, ^{*}*p*<0.05 vs. LPS alone).

by *R. fasciculatum* (1 mg/ml) were approximately 32.12% and 46.24%, respectively. Additionally, we observed that *R. fasciculatum* did not affect cell viability (data not shown).

Effect of *R. fasciculatum* on NF- κ B activation in the nuclei of LPS-stimulated RAW 267.4 cells

As the suppression of NF- κ B activation has been linked with anti-inflammation, we speculated that the effects of *R. fasciculatum* might be mediated, at least in part, via the suppression

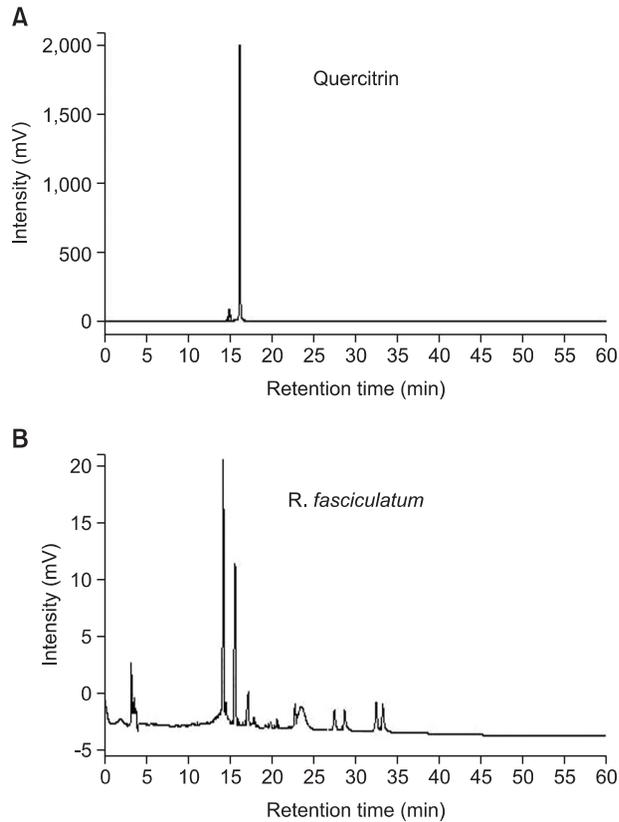


Fig. 6. HPLC chromatogram of *R. fasciculatum*. The retention time of quercitrin was specified on the chromatogram for the comparison. The mobile phase was 0.1% aqueous acetic acid (A) and acetonitrile (B) with a gradient program as follows: 0-5 min; isocratic 95% A; 5-60 min, linear gradient elution, 95-0% A; at a flow rate of 1 mL/min. The sample injection volume was 20 μ L and the peak was monitored at a wavelength of 360 nm.

of NF- κ B activation. Additionally, because NF- κ B activation requires the nuclear translocation of the RelA/p65 subunit of NF- κ B, we evaluated the effects of *R. fasciculatum* on the nuclear pool of RelA/p65 protein via western blot analysis. In LPS-stimulated cells, the levels of Rel/p65 were increased, but *R. fasciculatum* reduced these enhanced nuclear levels of Rel/p65 (Fig. 5A). The relative levels of NF- κ B (in nucleus) were represented in Fig. 5B.

Qualitative analysis of *R. fasciculatum* by HPLC

To confirm the constituents of *R. fasciculatum*, an HPLC analysis was performed. HPLC measurement of *R. fasciculatum* demonstrated various chromatographic peaks. Comparing the chromatographic peaks of *R. fasciculatum* with reference chromatographic peaks, quercitrin was identified (Fig. 6).

DISCUSSION

In this study, we demonstrated the molecular mechanisms of action of *R. fasciculatum* on allergic inflammation *in vivo* and *in vitro*. The findings of this study show that *R. fasciculatum* attenuated the compound 48/80- or histamine-induced scratching behaviors and inhibited DNCB-induced atopic der-

matitis under *in vivo* conditions. Additionally, *R. fasciculatum* inhibited the production of TNF- α and IL-6 and activation of NF- κ B in LPS-simulated macrophages.

AD is a chronic inflammatory skin disease and is characterized by erythema, edema, and scaling (Leung and Bieber, 2003). Generally, steroid therapy is very important in the treatment of AD, but it cannot be administered over a long-term, owing to its deleterious side-effects. Therefore, several researchers have attempted to find a new drug, which is effective in the treatment of AD (Shiohara *et al.*, 2004). AD is characterized by a potent skin inflammation associated with an elevated level of IgE against many types of allergens (Allam and Novak, 2006; Brennkmeijer *et al.*, 2008). On the basis of the results of these studies, we aimed to evaluate the effects of *R. fasciculatum* on DNCB-induced allergic reactions *in vivo*. The findings of this study revealed that *R. fasciculatum* significantly reduced DNCB-induced atopic dermatitis. Additionally, *R. fasciculatum* caused a reduction in serum IgE levels induced by DNCB. These results demonstrate the potential effect of *R. fasciculatum* on anti-allergic responses via the regulation of IgE levels.

In pathological skin conditions, histamine is involved in the induction of itching and edema (Minami and Kamei, 2004). This study focused on the manner in which *R. fasciculatum* regulates histamine-induced scratching behaviors in mice. We showed that *R. fasciculatum* inhibited the histamine-induced scratching behaviors in mice. Compound 48/80 is a well-known histamine releaser (Kim *et al.*, 2003). Antihistamines decreased the release of inflammatory mediators from inflammatory cells and the activation of eosinophils in an *in vitro* study (Assanasen and Naclerio, 2002). Thus, compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylactic reaction. The results demonstrated that *R. fasciculatum* attenuated compound 48/80-induced scratching behavior and anaphylactic reaction in mice. These results indicate that *R. fasciculatum* regulates the allergic response *in vivo*.

In inflammatory processes, inflammatory cytokines recruit activated immune and inflammatory cells to the site of lesions, thereby amplifying and perpetuating the inflammatory state. Macrophages are important effector cells in allergic inflammatory diseases, such as asthma and AD. Inflammatory cytokines derived from macrophages play an important role in the development of inflammatory reactions. To gain further insights into the mechanisms underlying *R. fasciculatum*-mediated inhibition of LPS-induced inflammatory mediators (TNF- α and IL-6), we examined the regulatory effect of *R. fasciculatum* on intracellular signaling molecules involved in the LPS signaling pathways in macrophages. In this research, we demonstrated that *R. fasciculatum* inhibited the secretion of TNF- α and IL-6 in LPS-simulated macrophages. The maximal inhibition rates of TNF- α and IL-6 production by *R. fasciculatum* were approximately 32.12% and 46.24%, respectively. These results demonstrate that *R. fasciculatum* exerts an anti-inflammatory effect via the regulation of inflammatory cytokine production.

Cytokine production is associated with increased activation of the gene transcriptional regulator, NF- κ B (Gillmore and Garbati, 2011). After a variety of stimuli, the I κ B proteins are phosphorylated, and degraded, allowing NF- κ B to translocate into the nucleus where it can bind to specific DNA sequences located in the promoter regions of target genes and activate gene transcription, thereby indicating its pivotal

role in the regulation of inflammatory responses, via control of the transcription of inflammatory cytokine genes. Based on this, inhibition of NF- κ B activation has been suggested as an anti-inflammatory treatment strategy in AD. Therefore, we attempted to determine whether the anti-inflammatory effect of *R. fasciculatum* is via the regulation of NF- κ B activation. The results demonstrated that *R. fasciculatum* inhibited the NF- κ B translocation into nucleus in the LPS-stimulated RAW 264.7 cells. Therefore, we hypothesized that *R. fasciculatum* might exert anti-inflammatory effects via NF- κ B activation. Although *R. fasciculatum* attenuated the activation of NF- κ B, the effect of *R. fasciculatum* on the pathways involving NF- κ B (phosphorylation of I κ B- α and IKK activation) was not determined. Therefore, further studies will be necessary in order to clarify more precisely the role of *R. fasciculatum* in the NF- κ B pathway.

The chemical constituents of *R. fasciculatum* are catechin, sarmentosin, quercitrin and so on (Dat *et al.*, 2005). It was reported that these compounds regulated the allergic inflammation. For example, quercitrin attenuated the degranulation of mast cell, histamine release and allergic anaphylaxis reaction in mice (Cruz *et al.*, 2008). Shimamura *et al.*, also showed that catechin may be useful in the treatment of atopic dermatitis via suppression of TNF- α production (Hisano *et al.*, 2003). Although we confirmed that quercitrin is one of compounds from *R. fasciculatum* in this study, the pharmacological effects of these compounds were not investigated. Therefore, further studies will be necessary to determine the effect and mechanism of these compounds on allergic reaction.

In conclusion, *R. fasciculatum* can regulate the allergic response *in vivo*, including in compound 48/80- or histamine-induced scratching behaviors and DNCB-induced atopic dermatitis. Additionally, we demonstrated in this study that the anti-inflammatory activities of *R. fasciculatum* could be attributed, at least in part, to the inhibition of inflammatory cytokine production (TNF- α and IL-6). These effects of *R. fasciculatum* are caused by the inhibition of LPS-induced activation of NF- κ B in LPS-stimulated macrophages. These results provide experimental evidence demonstrating that *R. fasciculatum* may prove useful in the treatment of allergic inflammatory diseases.

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REFERENCES

- Allam, J. P. and Novak, N. (2006) The pathophysiology of atopic eczema. *Clin. Exp. Dermatol.* **31**, 89-93.
- Assanasen, P. and Naclerio, R. M. (2002) Antiallergic anti-inflammatory effects of H1-antihistamines in humans. *Clin. Allergy Immunol.* **17**, 101-139.
- Boero, S., Silvestri, M., Ullmann, N. and Rossi, G. A. (2010) Modulation by flunisolide of tumor necrosis factor- α -induced stimulation of airway epithelial cell activities related to eosinophil inflammation. *J. Asthma* **47**, 381-387.
- Brennkmeijer, E. E., Spuls, P. I., Legierse, C. M., Lindeboom, R., Smitt, J. H. and Bos, J. D. (2008) Clinical differences between atopic and atopiform dermatitis. *J. Am. Acad. Dermatol.* **58**, 407-414.
- Buske-Kirschbaum, A., Geiben, A. and Hellhammer, D. (2001) Psychobiological aspects of atopic dermatitis: an overview. *Psychother. Psychosom.* **70**, 6-16.
- Cruz, E. A., Da-Silva, S. A., Muzitano, M. F., Silva, P. M., Costa, S. S. and Rossi-Bergmann, B. (2008) Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. *Int. Immunopharmacol.* **8**, 1616-1621.
- Dat, N. T., Cai, X. F., Shen, Q., Lee, I. S. and Kim, Y. H. (2005) New inhibitor against nuclear factor of activated T cells transcription from *Ribes fasciculatum* var. chinense. *Chem. Pharm. Bull.* **53**, 114-117.
- Gadaleta, R. M., Oldenburg, B., Willemsen, E. C., Spit, M., Murzilli, S., Salvatore, L., Klomp, L. W., Siersema, P. D., van Erpecum, K. J. and van Mil, S. W. (2011) Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF- κ B signaling in the intestine. *Biochim. Biophys. Acta* **1812**, 851-858.
- Gao, X. K., Fuseda, K., Shibata, T., Tanaka, H., Inagaki, N. and Nagai, H. (2005) Kampo medicines for mite antigen-induced allergic dermatitis in NC/Nga mice. *Evid. Based Complement. Alternat. Med.* **2**, 191-199.
- Gilmore, T. D. and Garbati, M. R. (2011) Inhibition of NF- κ B signaling as a strategy in disease therapy. *Curr. Top. Microbiol. Immunol.* **349**, 245-263.
- Gold, M. S. and Kemp, A. S. (2005) Atopic disease in childhood. *Med. J. Aust.* **182**, 298-304.
- Hisano, M., Yamaguchi, K., Inoue, Y., Ikeda, Y., Iijima, M., Adachi, M. and Shimamura, T. (2003) Inhibitory effect of catechin against the superantigen staphylococcal enterotoxin B (SEB). *Arch. Dermatol. Res.* **295**, 183-189.
- Kim, M. S., Na, H. J., Han, S. W., Jin, J. S., Song, U. Y., Lee, E. J., Song, B. K., Hong, S. H. and Kim, H. M. (2003) Forsythia fructus inhibits the mast cell mediated allergic inflammatory reactions. *Inflammation* **27**, 129-135.
- Leung, D. Y. and Bieber, T. (2003) Atopic dermatitis. *Lancet* **361**, 151-160.
- McCormick, T. S., Stevens, S. R. and Kang, K. (2000) Macrophages and cutaneous inflammation. *Nat. Biotechnol.* **18**, 25-26.
- Minami, K. and Kamei, C. A. (2004) A chronic model for evaluating the itching associated with allergic conjunctivitis in rats. *Int. Immunopharmacol.* **4**, 101-108.
- Mukaida, N. (2000) Interleukin-8: an expanding universe beyond neutrophil chemotaxis and activation. *Int. J. Hematol.* **72**, 391-398.
- Shiohara, T., Hayakawa, J., Mizukawa, Y. (2004) Animal models for atopic dermatitis: are they relevant to human disease? *J. Dermatol. Sci.* **36**, 1-9.
- Sugimoto, Y., Umakoshi, K., Nojiri, N. and Kamei, C. (1998) Effect of histamine H1 receptor antagonists on compound 48/80-induced scratching behavior in mice. *Eur. J. Pharmacol.* **351**, 1-5.
- Tegeeder, I., Pfeilschifter, J. and Geisslinger, G. (2001) Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J.* **15**, 2057-2072.
- Trefzer, U., Hofmann, M., Sterry, W. and Asadullah, K. (2003) Cytokine and anti-cytokine therapy in dermatology. *Expert Opin. Biol. Ther.* **3**, 733-743.