



Ethanol extract of *Dryopteris crassirhizoma* alleviates allergic inflammation via inhibition of Th2 response and mast cell activation in a murine model of allergic rhinitis

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ABSTRACT

Ethnopharmacological relevance: *Dryopteris crassirhizoma* (DC) is used as a traditional herbal remedy to treat various diseases, the tapeworm infection, common cold, and cancer in Korea, Japan, and China. DC also has the antioxidant anti-inflammatory and antibacterial activities. However, the anti-allergic inflammatory effect of DC and some of its mechanisms in allergic rhinitis model are unknown well.

Aim of this study: The purpose of this study is to investigate the anti-allergic inflammatory effect of DC on the allergic rhinitis model, mast cell activation and histamine release.

Materials and methods: Allergic rhinitis was induced in BALB/c mice by sensitization and challenge with ovalbumin (OVA). Different concentration of DC and dexamethasone was administrated by oral gavage on 1 h before the OVA challenge. Mice of the control group were treated with saline only. Then mice were evaluated for the presence of nasal mucosa inflammation, the production of allergen-specific cytokine response and the histology of nasal mucosa.

Results: DC significantly ameliorated the nasal symptoms and the inflammation of nasal mucosa. DC also reduced the infiltration of eosinophils and mast cells in these tissues and the release of histamine in blood. Meanwhile, DC evidently inhibited the overproduction of Th2 cytokines and increased the Th1 and Treg cytokines in nasal lavage fluid by OVA. DC also reduced the levels of OVA-specific IgE, IgG1 and IgG2a in blood.

Conclusions: This study suggests that DC has a significant anti-allergic inflammatory effect in the nasal cavity. DC may have the therapeutic effect of allergic rhinitis.

1. Introduction

Allergic rhinitis (AR) is a common inflammatory disease of the nasal mucosa that is characterized by developing several nasal symptoms including sneezing, nasal itching, rhinorrhea, and nasal congestion. AR is one of the multiple risk factors for the development of asthma both

for adults and children (Guerra et al., 2002; Wright et al., 1994). The prevalence of AR is increasing worldwide. Epidemiologic data show that AR affects up to 50% of the world's population (Strachan et al., 1997).

AR belongs to one of the chronic inflammatory airway diseases characterized by intense eosinophil infiltration, mucus hypersecretion,

Abbreviations: DC, *Dryopteris crassirhizoma*; AR, Allergic rhinitis; Dex, Dexamethasone; OVA, Ovalbumin; Con, Control; NALF, Nasal lavage fluid; RPMCs, Rat peritoneal mast cells; HE, Hematoxylin and eosin; PAS, Periodic acid-Schiff; I.P., Intraperitoneal; I.N., Intranasal; P.O., Per oral; Th1, T helper type 1; Th2, T helper type 2; Treg, Regulatory T cell; DC-100, mice were received 100 mg/kg bodyweight DC dissolved in saline; DC-200, mice were received 200 mg/kg bodyweight DC dissolved in saline; DC-400, mice were received 400 mg/kg bodyweight DC dissolved in saline

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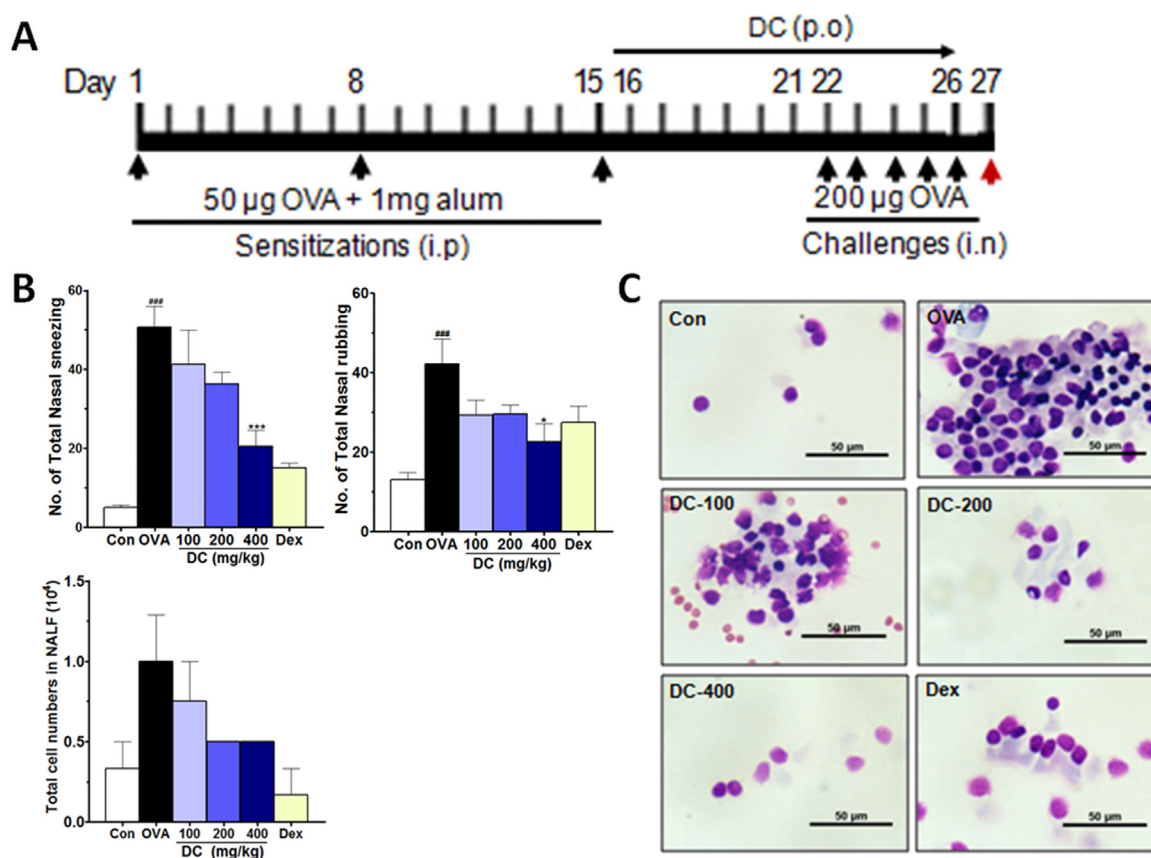


Fig. 1. Experimental protocol for AR mouse model and effect of DC on the nasal symptoms and the nasal inflammation in OVA-induced AR mouse model. (A) Time schedule of the OVA-induced AR mouse model and treatment with DC. (B) The frequencies of total sneezing and rubbing numbers during 5 days were evaluated by counting that occurred in the 20 min after the OVA challenge without anesthesia and total cell numbers in NALF. (C) Cytospin cell preparations were made by NALF onto clean glass slides and stained with Diff-Quik (Red arrows indicate the eosinophils). Scale bar = 100 µm. (*** $P < 0.001$ significantly different from the value of control group (DC: *Dryopteris crassirhizoma*, P.O: per oral, I.N: intranasal). ** $P < 0.01$ significantly different from the value of OVA group).

and airway remodeling, which is orchestrated mainly by antigen-specific T helper type 2 (Th2) cells and their cytokines including IL-4, IL-5, and IL-10 (Liu et al., 2010). AR is caused by allergic mediators such as inflammatory cytokines and histamine from mast cells (Howarth et al., 2005). At the beginning of the AR reaction, mast cells combine with immunoglobulin (Ig) E receptors on the mast cell surface, which can trigger the release of inflammatory cytokines and histamine (Church and Levi-Schaffer, 1997). AR has long been considered to result from the up-regulation of Th2 cells and a relative lack of Th1 cells (Malmhall et al., 2007). In recent years, the discovery of regulatory T (Treg) cells introduced complexity into the existing Th1/Th2 balance paradigm and expanded our understanding of the pathogenesis of AR (Wang et al., 2014). Treg cells represent a unique subpopulation of CD4 + T cells, which can inhibit T cell proliferation and an autoimmune response (Wing et al., 2006). In adults, it has been shown that AR has a major impact on asthma morbidity and that treatment of AR help improves asthma control (Brozek et al., 2010). Even though there are available multiple treatments for disease as medical treatment, immunotherapy or surgery, the rates of side effects and recurrence are high after stopping medication (Jeon et al., 2007). Herbal medicines are generally recognized to be safe because they are often perceived as “natural” (Markman, 2002). Hence, many studies aiming to find an appropriate treatment for AR has focused on traditional medicines, which have fewer side effects and are more stable than the currently used drugs (Jung et al., 2013).

Dryopteris crassirhizoma Nakai (DC), a species in the genus *Dryopteris* (family *Dryopteridaceae*), is used as a traditional herbal remedy for various diseases and widely distributed in Asia, especially, to treat tapeworm infestation, common cold, and cancer in Korea, Japan and

China (Chang et al., 2010). And it was formerly used as an anti-helminthic herbal remedy against *Dipyllobothrium latum* (Shinozaki et al., 2008) and an anti-infection agent to treat the flu (Yang et al., 2013), and treatment for respiratory tract infections, uterine bleeding, mumps and feverish illnesses (Ha et al., 2013). Previous studies have shown that the rhizome of DC has antioxidant, anticancer, anti-inflammatory and antibacterial activities (Ha et al., 2013). These activities are mediated by active components such as phloroglucinol derivatives (albaspidin, aspidin, flavaspidic acids, and dryocrassin), triterpenes such as acylphloroglucinols, and dimethylflavanones such as desmethoxymatteucinol, matteucinol, and methoxymatteucin (Chang et al., 2006; Lee et al., 2009, 2008; Liang et al., 2010). Further assays for genetic toxicity demonstrated that DC was devoid of its genotoxic effect under the experimental conditions (Hwang et al., 2013). However, thus far, the pharmacological effect of DC on allergic disorders and its mechanisms are unknown well. To the best of our knowledge, the current studies have investigated the effect of DC on the ovalbumin (OVA)-induced AR model and further clarified its mechanism of DC on anti-allergic inflammatory response involved.

2. Materials and methods

2.1. Preparations of *Dryopteris crassirhizoma*

The DC (KFRI-SL-2021) was deposited at Herbarium, Korea Food Research Institute and provided from the division of nutrition and metabolism research. The DC used in this study was purchased from the Kyeong-Dong Oriental Pharmacy Market (Seoul, Korea). The underwent reflux extraction twice in 95% ethanol. The ethanol extract was dried

under a vacuum in a rotary evaporator. The concentrated extract was finally lyophilized, yielding a dried powder that was kept at 4 °C until needed. The dried ethanol extract was dissolved in saline prior to use.

2.2. Animals

Specific pathogen-free male 5-week-old BALB/c mice, weighing approximately 20 g, were purchased from Damool Science (Dae-jeon, Korea). They were housed 5 per cage in a laminar air-flow cabinet maintained at 23 ± 2 °C at a relative humidity of $55 \pm 10\%$ with a 12 h dark/light cycle throughout the study. All animal experiments were performed in compliance with NIH guideline for the care and use of laboratory animals and were approved by the Animal Research Committee of Chonbuk National University.

2.3. Murine AR model and treatment

The DC ethanol extract was dissolved in saline at three dose levels (100, 200, 400 mg/kg body weight) and administered by a single oral gavage. The schedule for allergen sensitization and the intranasal challenge is summarized in Fig. 1A.

Briefly, the mice were divided randomly into six groups ($n = 5$ per group), namely control group, OVA group, 100 mg/kg of DC (DC-100), 200 mg/kg of DC (DC-200), 400 mg/kg of DC (DC-400) and 2.5 mg/kg of Dexamethasone (Dex) group. Sensitization was performed on days 1, 8 and 15 by intraperitoneal injection of 50 µg of OVA (Grade V, Sigma, St. Louis, MO, USA) emulsified in 1 mg of alum (Imject Alum; Pierce, Rockford, IL, USA) in a total volume of 200 µl. On days 22–26, mice were challenged by intranasal instillation of OVA (10 mg/ml solution, 20 µl/nostril). From days 16–26, mice were received DC (100, 200, 400 mg/kg body weight) and Dex (2.5 mg/kg) dissolved in saline in a total volume of 200 µl by orally 1 h before every OVA challenge a day for 11 consecutive days. Mice of the control group were treated with saline only (NaCl 0.9%; B. Braun Medical BV, Oss, The Netherlands) without sensitization and challenge. Mice were sacrificed 24 h after the last challenge for further analysis.

2.4. Evaluation of nasal symptoms

After the last OVA challenge, mice were placed into observation cages and nasal symptoms were evaluated by counting the frequencies of nasal rubbing and sneezing that occurred in the 20-min observation period to evaluate early allergic responses.

2.5. Analysis of nasal lavage fluid (NALF) and blood sample collection

24 h after the last OVA challenge, blood was collected from the orbital venous plexus of anesthetized mice 24 h after the last challenge. The samples were centrifuged ($1000 \times g$, 10 min, 4 °C) to isolate the serum and stored at -80 °C until analysis. After sacrifice, the NALF was obtained immediately after the blood collection. The nasal cavity was gently perfused with 1 ml of saline from the choana to the nostril and the NALF was centrifuged and the supernatants of NALF were stored at -80 °C. The cell pellets were resuspended in 1 ml of saline. The total NALF cells were counted double-blindly using a hemocytometer. Total cell numbers were conducted using trypan blue staining and standard with light microscopy. Cytospin cell preparations were made by NALF onto clean glass slides for 10 min (150 µl, 4 °C), and staining with Diff-Quik (1–5–1 Wakonohama-Kaigandori, Chuo-Ku, Kobe Japan).

2.6. Histopathological examination of nasal tissues

After performing the NALF, the heads of mice were removed and then fixed in 10% paraformaldehyde 3 days, followed by decalcification in an ethylenediamine triacetic acid decalcifying solution for 5 days,

and the specimens were embedded in paraffin wax. Nasal tissues were coronally sectioned into 5 µm slices, and stained with hematoxylin and eosin for general morphology, periodic acid-Schiff for goblet cell hyperplasia and sirius red staining for eosinophil and giemsa staining for epithelial mast cell infiltrations. Subsequently, the number of differential inflammatory cells were counted and the epithelial damage was evaluated in randomly selected high-power fields at $400 \times$ magnification.

2.7. Histamine assay

The level of histamine was measured from serum according to the manufacturer's specifications using the histamine assay kit (R&D Systems Inc, USA) following the manufacturer's instruction.

2.8. In vitro study with compound 48/80-induced mast cell activation

Rats were anesthetized and intraperitoneally injected with 50 ml of saline and gentle kneading of the abdominal region for 90 s. After gentle massaging, the cavity was carefully opened and the fluid was aspirated by Pasteur pipette. Next, peritoneal lavage was collected and centrifuged ($1000 \times g$, 10 min, at 4 °C). The supernatant was discarded and the cells were collected and resuspended in 7 ml HEPES. Rat peritoneal mast cells (RPMCs) were preincubated with different concentrations (10, 20, 40 mg/ml) of DC at 37 °C for 10 min, and then incubated with compound 48/80 (final concentration, 5 µg/ml) at 37 °C for 15 min. After incubation, the RPMCs were placed between slides and cover slip and observed under a high-power microscope. Then the RPMCs degranulation rate was counted under the high-power fields at $400 \times$ magnification.

2.9. Ultra performance liquid chromatography-quadrupole-time of flight (UPLC-Q-TOF) mass spectrometry (MS)

To identify chemical constituents of DC, chemical constituents from 70% ethanolic extract of DC were analyzed by an ultra performance liquid chromatography-quadrupole-time of flight (UPLC-Q-TOF) MS (Waters Corp., Milford, MA, USA). The extract was injected into an Acquity UPLC BEH C18 column (2.1×100 mm, 1.7 µm; Waters Corp.) at a column temperature of 40 °C. Mobile phase consisted of water with 0.1% formic acid (FA) and acetonitrile with 0.1% FA at a flow rate of 0.35 ml/min for 9 min. The eluents were ionized by electrospray ionization (ESI) with positive or negative mode and analyzed using a Q-TOF MS. The scan range of TOF MS data was from 50 to 1500 m/z with a scan time of 0.2 s. The capillary voltage was set at 3 or 2.5 kV for positive mode and negative mode, respectively, while the sample cone voltage was 40 V. The desolvation flow rate was 900 l/h at a temperature of 400 °C and source temperature set to 100 °C. Leucine-enkephalin ($[M + H] = m/z$ 556.2771) was used as a reference for lock mass at a frequency of 10 s. The MS/MS spectra were obtained using collision energy ramps from 20 to 45 eV. Metabolites were identified by Unifi software with various LC/MS databases.

2.10. Statistical analysis

Each experiment was repeated three times with 5 animals per group. Data are expressed as mean $\pm$ SEMs. Statistical comparisons were performed using one-way ANOVA, followed by the Fisher test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effects of DC on nasal symptoms in a murine AR model

To determine the effect of DC on nasal symptoms, the frequencies of nasal rubbing and sneezing behaviors were evaluated for 20 min after

the OVA challenge. As shown in Fig. 1B, intranasal OVA challenge to the OVA-sensitized mice induced the rubbing and sneezing responses. However, DC and Dex groups were significantly decreased the nasal rubbing and sneezing frequencies compared to those in the OVA group (Fig. 1B). These findings suggested that DC treatment efficiently improved OVA-induced allergic nasal symptoms.

3.2. Effects of DC on the infiltration of inflammatory cells in NALF

To examine the effect of DC on inflammation in the nasal cavity of AR murine model, total cell numbers in NALF were counted. OVA group showed significantly increased total cell numbers in NALF than the control group, and most of these cells were epithelial cells and a few eosinophils were shown. (Fig. 1B–C). However, the DC group markedly decreased and this suppressive of the effect exerted similar to Dex group (Fig. 1B–C).

3.3. Effects of DC on histopathological changes in nasal mucosa tissues

To explore whether DC administration was able to prevent the inflammatory cells infiltration in nasal mucosal tissues, we next examined the histological changes of the OVA-induced AR model including mucosal thickness, eosinophil and mast cell infiltration and goblet cell hyperplasia from mice of each group. As shown in Fig. 2A–B, the OVA group caused remarkably histological alterations such as epithelial disruption and edema, infiltration of mast cells. Also, the mucosal thickness of nasal septum was significantly higher in OVA-induced AR mice than control mice (Fig. 2A). Fig. 2C–D showed that the OVA group induced significantly increased the eosinophil infiltration and goblet cell hyperplasia. As expected DC oral administration notably mitigated these histopathological changes, such as DC treatment improved less epithelial disruption, and inhibited the infiltration of eosinophils, mast cells and the hyperplasia of goblet cells and these effects were more obvious in a high dose of DC group. These results suggested that DC has anti-inflammatory effects by attenuating the inflammatory cells infiltration in nasal tissues.

3.4. Effects of DC on levels of Th1, Th2 and Treg cytokines in NALF

Further, we determined the effect of DC on Th1/Th2/Treg allergic responses in OVA-induced AR model, the levels of secreted cytokines in NALF were measured. First, we examined the secretion of the inflammatory cytokines (Th2-related cytokines) IL-4 and IL-5 using ELISA. OVA group showed increased in the levels of IL-4 and IL-5 in NALF, respectively. However, DC treatment significantly decreased the cytokine levels, respectively (Fig. 3A). In addition, the anti-inflammatory cytokines (Th1-related cytokines) such as IFN- γ and IL-12 levels were assayed. Secretion of IFN- γ induced by OVA was significantly increased by DC and Dex treatment (Fig. 3B). While the production of Treg cytokines IL-10 and TGF- β 1 were measured. The levels of IL-10 and TGF- β 1 were significantly decreased in the OVA group (Fig. 3C). In contrast, DC treatment was markedly diminished (Fig. 3C). These results were consistent the reduced infiltration of inflammatory cells in NALF of the DC treatment mice via modulating the Th1/Th2/Treg cytokines.

3.5. Effect of DC on serum levels of OVA-specific immunoglobulins

In order to examine whether the DC affected the OVA-specific immunoglobulins production in the AR model, the serum levels of OVA-specific IgE, IgG1 and IgG2a were measured. They were significantly higher in the OVA group compare to the control group. However, DC and Dex groups were markedly inhibited the levels of OVA-specific IgE, IgG1 and IgG2a (Fig. 3, D–F). These results indicated that DC might have anti-allergic activity via suppression of allergic mediator productions.

3.6. Effect of DC on compound 48/80-induced RPMCs degranulation

In this study, we used RPMCs to evaluate the anti-allergic inflammatory effect of DC in vitro, RPMCs were treated with compound 48/80 and morphological changes of RPMCs were observed. As shown in Fig. 4A, RPMCs stimulated with compound 48/80 clearly showed extensive degranulation processes and multiple granules extruding from the RPMCs. However, in RPMCs treated with DC at doses of 10, 20 and 40 mg/ml for 15 min prior to stimulation with compound 48/80, these degranulations were suppressed and their morphology was similar to those of saline-treated RPMCs (Fig. 4A). Compound 48/80 group induced highly RPMCs degranulation rate, however, DC stimulated mast cell showed significantly prevented the RPMCs degranulation rate (Fig. 4B).

3.7. Effects of DC on level of histamine in serum

To explore whether DC inhibited histamine release from mast cell, OVA-induced histamine level in serum were measured. As shown in Fig. 4C, the OVA group showed that histamine release significantly increased as compared to the control group. However, DC and Dex groups significantly decreased the OVA-induced histamine levels in serum (Fig. 4C). Therefore, these results suggest that the inhibition of histamine release is associated with the anti-allergic effect of DC.

3.8. Active components of DC

To investigate the main components of ethanol extract of DC, the DC was analyzed by UPLC-Q-TOF MS. The results showed main four peaks in negative mode (Fig. 5A) and eight peaks in positive mode (Fig. 5B), which were identified as follows; caffeic acid 3-glucoside, chlorogenic acid, isoquercetin, pinellin acid, procyanidin B, quercetagenin dehydrate, asteric acid, isoquercetin, skimming/protein, pravastatin, 4-methylumbelliferyl-hexoside, and Gabexate/5-((6,7,8-trimethoxyquinazolin-4-yl) amino) pentan-1-ol.

4. Discussion

In the present study, the effect of DC on OVA-induced AR mouse model was investigated. These data demonstrated that DC has a significant anti-allergic inflammatory effect in nasal airways resulting from the down-regulated the inflammatory cells, the adjusted Th1/Th2/Treg cytokines balance, the inhibited mast cell degranulation and histamine release, and the decreased the serum concentration of OVA-specific IgE, IgG1 and IgG2a levels. In addition, DC significantly inhibited AR symptoms such as sneezing and nasal rubbing.

As well known, airway inflammation present in asthma and rhinitis mouse model is characterized by the infiltration of inflammatory cells such as eosinophils, neutrophils, and lymphocytes. The allergic response in the nasal mucosa challenged with OVA in this study includes a late phase response characterized by the recruitment of eosinophils, basophils, and T cells secreting Th2 cytokines (Broide, 2010). OVA sensitization and challenge in mouse model led to increasing the levels of histamine and antigen-specific IgE in serum and the infiltration of inflammatory cells in epithelium and sub-epithelium of the nasal mucosa (Bahekar et al., 2008). In the present study, DC treatment significantly reduced the number of eosinophils, neutrophils, and lymphocytes in NALF. It also inhibited the infiltration of eosinophils, mast cells, goblet cells, and epithelial cells extensive into nasal mucosa. These results suggested that DC may have anti-inflammatory activities in OVA-induced allergic rhinitis.

OVA is an antigen that has been widely used as a sensitizer in studies of AR (Kool et al., 2008). Aluminum hydroxide can induce a Th2 immune response in mice without significant toxicity (Bahekar et al., 2008). Based on the previous models reported and our experience, we prepared OVA with aluminum hydroxide and the dose was adjusted

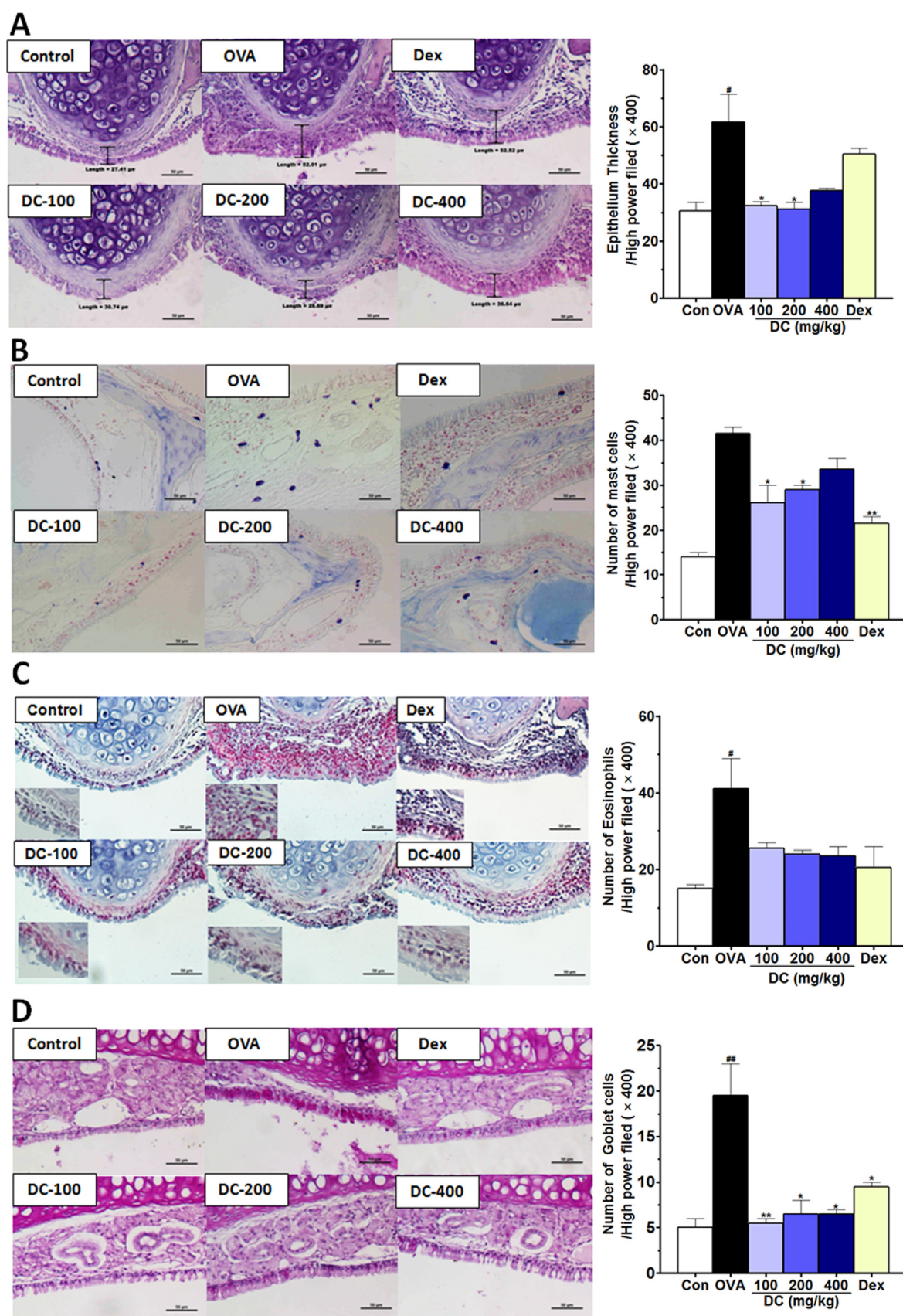


Fig. 2. Effect of DC on the infiltration of inflammatory cells in AR nasal mucosa tissues. (A) Structure of nasal septum and epithelium thickness of mucosa. H-E staining. (B, C) Infiltration of eosinophils and mast cells. Sirius Red and Giemsa staining. (D) Goblet cells hyperplasia. PAS staining. The epithelium thickness and inflammatory cells were counted. ([#] $P < 0.05$, ^{##} $P < 0.01$, significantly different from the value of the control group. ^{*} $P < 0.05$, ^{**} $P < 0.01$, significantly different from the value of OVA group). Scale bar = 50 μ m.

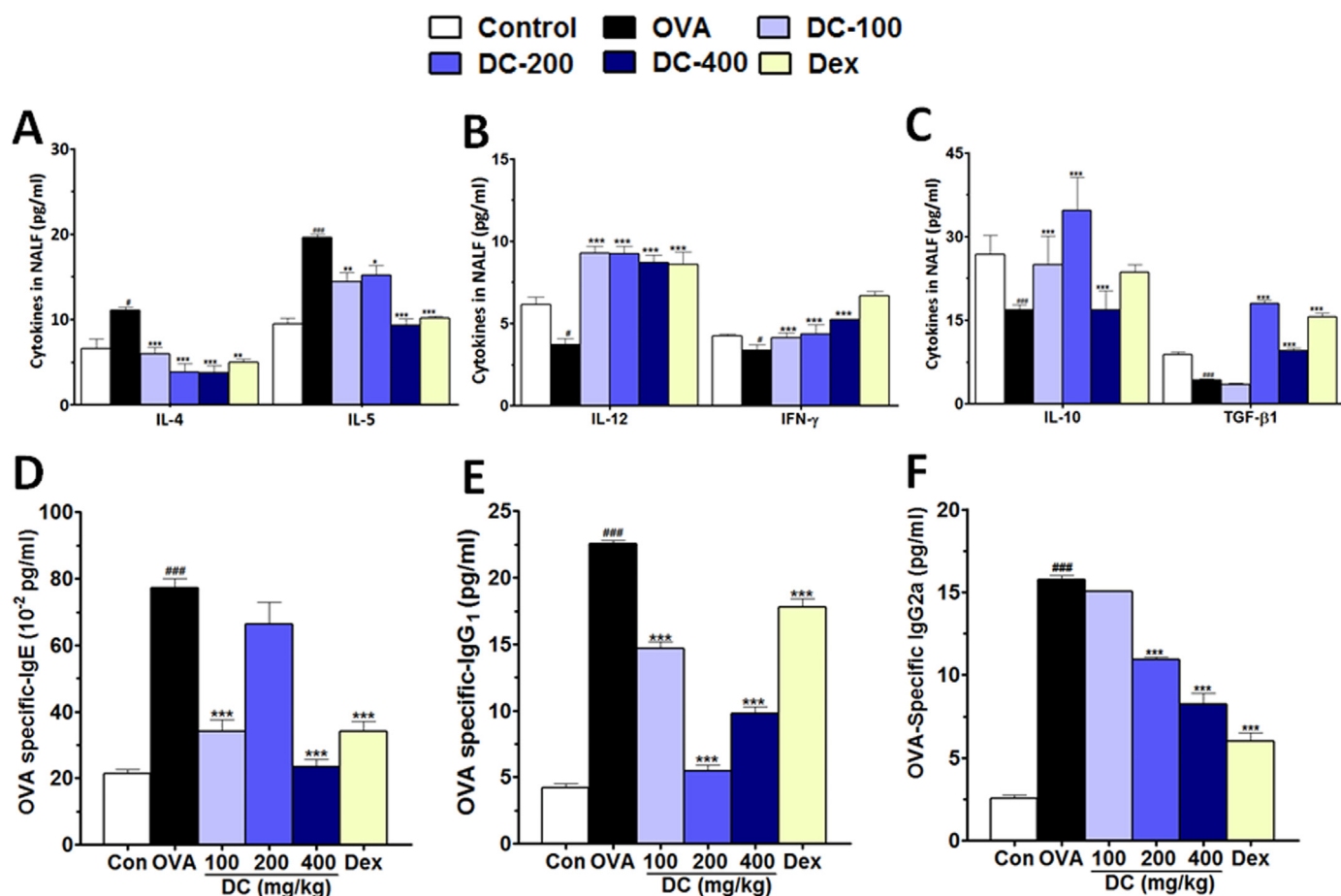


Fig. 3. Effect of DC on the levels of Th1/Th2/Treg cytokines in NALF and OVA-specific immunoglobulins in serum. (A–C) The levels of IL-4, IL-5, IL-10, IL-12, IFN- γ , and TGF- β 1 were determined in NALF. (D–F) The measurement of OVA-specific IgE, IgG1 and IgG2a in serum. ([#] $P < 0.05$, ^{###} $P < 0.001$, significantly different from the value of control group. ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$, significantly different from the value of OVA group).

according to repeated sensitization and challenge principles to sensitize and challenge mice to the allergen in order to induce AR. This allows adequate time for the development of OVA-specific IgE in these animals. In this study, the AR model was developed in mice by using the method and timeline that were modified from previously successful allergy model development (Mo et al., 2011; Sookrung et al., 2008).

In the immune system, Th1 and Th2 cells influence the production of IgE. Th2 cells secrete IL-4 and IL-5, the characteristic factors leading to enhanced IgE production, eosinophilia and atopic disease (Fallon et al., 2002). In contrast, Th1 cells secrete IL-12 and IFN- γ , which are responsible for the suppression of IgE synthesis as well as the inception of IgE-mediated allergies (Chung, 2001). Reduced serum levels of OVA-specific IgE may be associated with enhanced production of Th1 cytokines, decreased synthesis of Th2 cytokines, or both in some instances, as both Th1 and Th2 cytokines were down-regulated (Borchers et al., 2009). The present study demonstrated that IL-4 and IL-5 secretions were significantly lower in the DC and Dex groups than in the OVA group, supporting a reduced Th2 response, meanwhile, DC group up-regulated in Th1 representative factors IFN- γ and IL-12. This suggests DC instates Th1/Th2 equilibrium mainly through the inhibition of the Th2 response with promoting a Th1 response. Treg cells could potentially suppress IgE production and directly or indirectly suppress the activity of effector cells, including eosinophils, basophils, and mast cells of allergic inflammation (Ozdemir et al., 2009). Tregs are the mediators of immunological tolerance and possess anti-inflammatory capabilities (Sakaguchi et al., 2008). Tregs play a central role in the regulation of autoimmune, infectious, and allergic diseases by cell-to-cell contact-dependent inhibition and by the secretion of anti-inflammatory

cytokines such as IL-10 and TGF- β (Taylor et al., 2005). IL-10 and TGF- β decrease IgE production and inhibit the production of Th2 cytokines such as IL-4 and IL-5 (Larche et al., 2006). In the present study, the production of IL-10 and TGF- β 1 were significantly reduced in the OVA group compared with the control group, whereas the levels of IL-10 and TGF- β 1 were markedly increased in the DC group. Results from this study, we speculated that inhibition of nasal tissue inflammation by DC administration may be associated with the induced activity of Tregs and Th1 response and inhibited Th2 response. In particular, to investigate the any impact of the DC treatment in the process of OVA sensitization and Th2 polarization, we did experiments with DC-treated and DC-non-treated mice splenocytes and challenged with OVA. Splenocytes isolated from the mice were cultured with OVA for 72 h and then the supernatant used to measure Th2-mediated main cytokine IL-4. As a result, OVA group (OVA sensitization) increased IL-4 secretion and Dex group decreased IL-4 production in splenocytes. However, DC group was not changed IL-4 secretion compared with OVA group (Not shown data). Therefore, this result indicated that DC did not affect the phase of sensitization and immunization by OVA. So, we thought that the DC, could inhibit Th2-related immune response, affected after the sensitization of OVA.

In recent years, Th2 cells have been known as the dominant modulators in regulated downstream cell differentiation and proliferation including B cells class switch to secreting IgE, IgG1, and eosinophils (Hsieh et al., 2010). After the establishment of the systemic IgE-mediated response, continuous exposure to the same allergen at a specific site may lead to the development of Th2-dominant local allergic reactions, such as skin or nasal symptoms (Shimizu et al., 2000; Tsunematsu

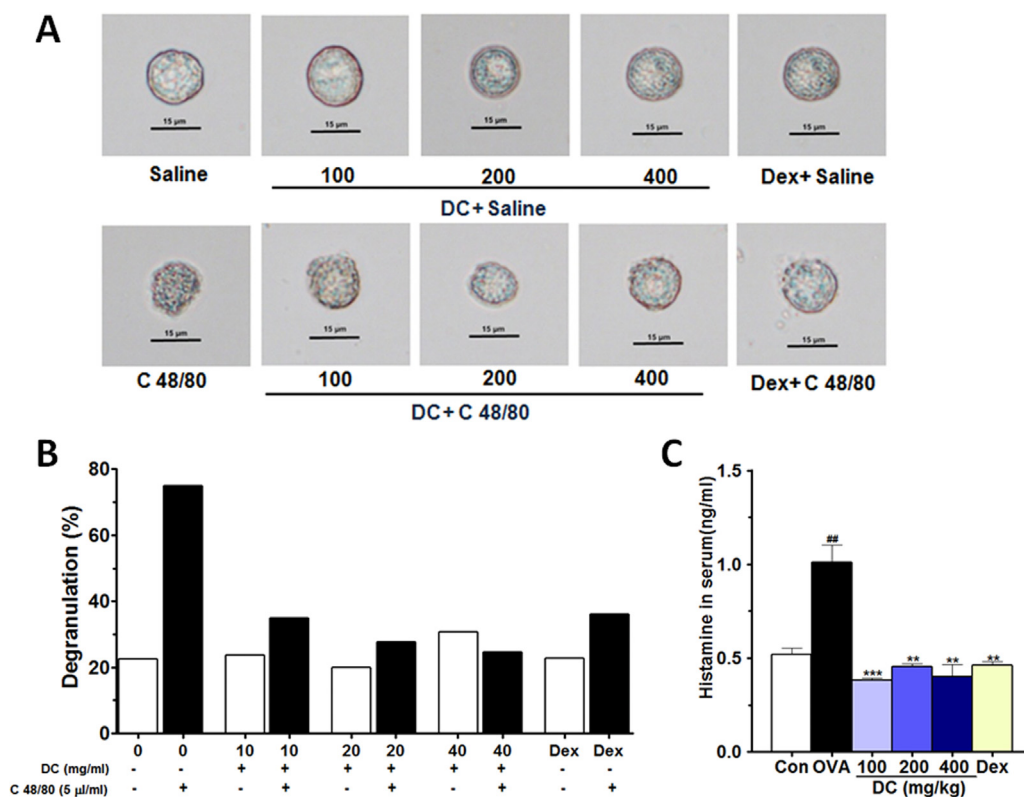


Fig. 4. Effect of DC on the compound 48/80 (C48/80)-induced mast cell degranulation and histamine release in serum. (A) Morphologies of compound 48/80-stimulated RPMCs pretreated with DC. (B) Mast cell degranulation rate. (C) Histamine release from serum. ($^{##}P < 0.01$, significantly different from the value of the control group. $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the value of OVA group).

et al., 2007). We showed that repeated topical intranasal OVA application caused typical AR symptoms, whereas DC strongly reduced nasal symptoms, serum levels of histamine and OVA-specific immunoglobulins. Our result suggested that DC may have an anti-allergic effect by preventing the production of allergic mediators including immunoglobulins and histamine.

Glucocorticoids are used as mainstay therapy for allergic disease; however, treatment is not always effective, and there are side effects (Adcock et al., 2008; Ulrich et al., 2008). Thus, the development of more targeted anti-inflammatory therapies may be beneficial for the treatment of AR. Although modes of delivery of our interventions are not directly comparable and Dex is currently the most relevant approach to therapy, our study provides proof-of-principle data that DC has the potential to suppress allergic inflammation. Here we demonstrate that DC was as effective as glucocorticoid therapy. In the present study, mice treated with DC or Dex displayed a significant reduction in the severity of the inflammatory lesions induced by OVA challenge. Dex has previously been shown to inhibit inflammation in OVA exposure models of allergic asthma (Ulrich et al., 2008). Here we confirm the anti-inflammatory actions of Dex after acute exposure to OVA. Dex suppressed eosinophil recruitment, mucus hypersecretion, mast cell infiltration, and Th2/Treg and immunoglobulin productions in NALF or blood after OVA exposure. The anti-inflammatory effect of DC was similar to Dex. However, its effect on cytokines production was not as dose-dependent manner. Dex treatment completely suppressed, this partial reduction in cytokines and immunoglobulin productions by DC treatment was clearly therapeutically beneficial.

Among effective cells, mast cells are one of the most important cells in allergic responses, such as AR (Ito et al., 2012). Mast cells are responsible for a variety of allergic disorders including allergic rhinitis, dermatitis, asthma, and food allergies, as well as catastrophic anaphylactic reactions (Hollander et al., 2003). Among the inflammatory mediators released from mast cells, histamine is one of the most potent vasoactive mediators implicated in the acute phase of immediate hypersensitivity (Cook et al., 2002).

Mast cells are major immune cells involved in allergic diseases with

versatile physiological functions and are important for allergic responses to the stimuli-induced release of histamine (Bischoff, 2007; Mekori and Metcalfe, 2000; Prussian and Metcalfe, 2003). Compound 48/80 induces signal transduction pathways, leading to exocytosis and of mast cells causing histamine release (Kim et al., 2005). Although several traditional herbal medicines have been shown to have inhibitory effects on allergic inflammation, there is little information about the effect of DC on RPMCs. Therefore, in this study, the effect of DC on RPMCs degranulation in vitro, RPMCs were treated with compound 48/80 and morphological changes of RPMCs were observed. DC treatment evidently inhibited the RPMCs degranulation stimulated with compound 48/80. These results clearly indicated that allergic reactions were suppressed by DC according to the decreased histamine level from mast cells.

Natural products contribute new strategy to novel drug development efforts, therefore, played a significant role in drug discovery and development for the treatment of some inflammatory diseases (Camateros et al., 2007). DC was safe after single oral administration to both male and female Sprague-Dawley rats up to 2000 mg/kg (Hwang et al., 2013). Therefore, 400 mg/kg as the maximal dose of oral DC was selected. Actually, no deaths or significant adverse events were noted in the DC treated groups. Isoquercitrin which is one of the components of DC suppressed the PMACI-mediated activation of NF- κ B in the human basophil cells and present the potential therapeutic use of isoquercitrin for the treatment of inflammatory and allergic reactions (Li et al., 2016). Procyanidins are widely found in plants and have anti-inflammatory activities. Procyanidin B2 inhibits production of proinflammatory cytokines in macrophages (Tanaka et al., 2017). Chlorogenic acid protects against hydrogen peroxide induced oxidative stress in MC3T3-E1 cells through the Nrf2-signaling pathway (Tanaka et al., 2017). Isoquercetin ameliorates via anti-oxidative, anti-inflammatory, and anti-apoptotic effects in cerebral impairment (Ma et al., 2018). Isoquercetin, a novel extracts of plants and fruits could protect neuronal cells from neurotoxicity and neuro-inflammation (Lu et al., 2016). Based on these reports, the DC is believed to have an anti-allergic inflammatory effect because it contains the active components such as

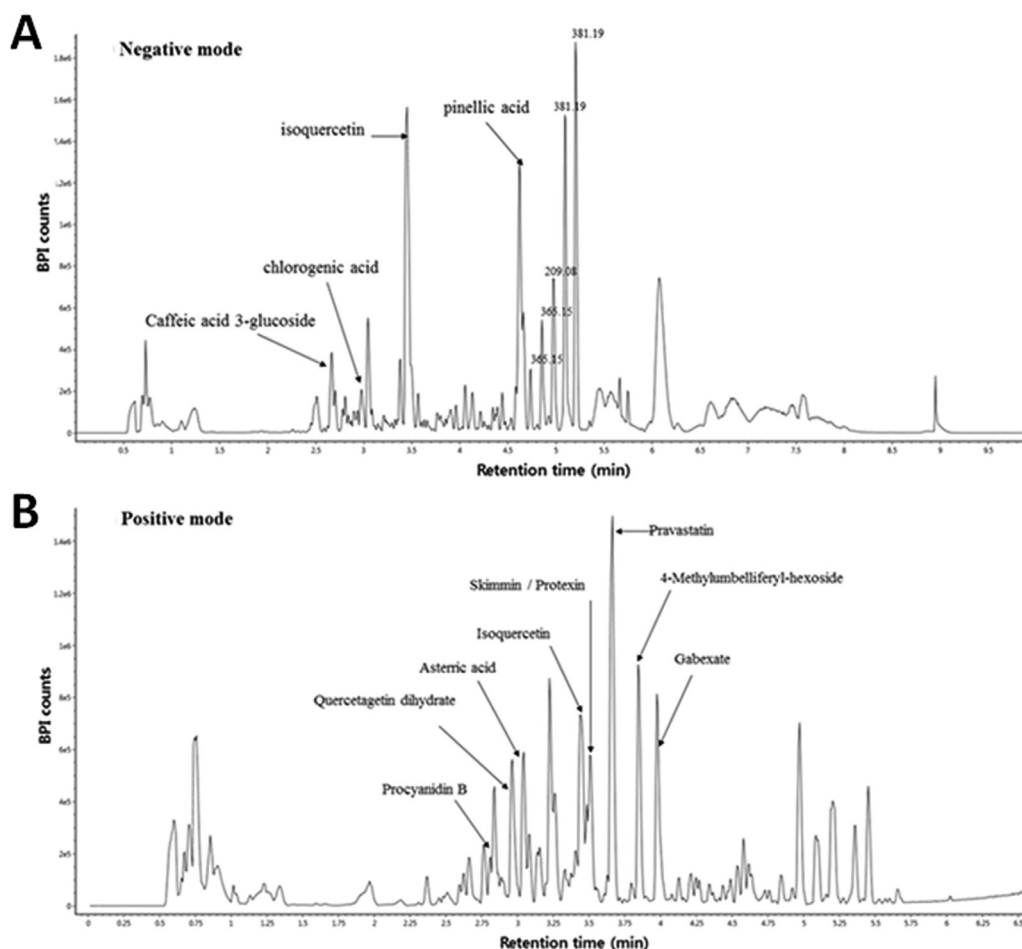


Fig. 5. Chemical profiles of DC by UPLC-Q-TOF MS. (A) Negative mode. (B) Positive mode. The main components of DC were identified as follows; caffeic acid 3-glucoside, chlorogenic acid, isoquercetin, pinelllic acid, procyanidin B, quercetagenin dihydrate, asterric acid, isoquercetin, skimming/protexin, pravastatin, 4-methylumbelliferyl-hexoside, and Gabexate/ 5-((6,7,8-trimethoxyquinazolin-4-yl) amino) pentan-1-ol.

isoquercitrin and procyanidin B.

5. Conclusion

In summary, the results of this study demonstrated that DC exerts anti-allergic inflammatory effects, particularly on nasal tissues in a mouse model of rhinitis and that its mechanisms mainly include the induction of Treg and Th1 cytokines and inhibition of Th2 cytokines and histamine release. Meanwhile, these results provide underlying mechanistic evidence for the clinical use of DC to treat the allergic rhinitis.

Conflict of interest statement

The authors have declared that there is no conflict of interest.

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References

Adcock, I.M., Ford, P.A., Bhavsar, P., Ahmad, T., Chung, K.F., 2008. Steroid resistance in asthma: mechanisms and treatment options. *Curr. Allergy Asthma Rep.* 8 (2), 171–178.

Bahekar, P.C., Shah, J.H., Ayer, U.B., Mandhane, S.N., Thennati, R., 2008. Validation of guinea pig model of allergic rhinitis by oral and topical drugs. *Int Immunopharmacol.* 8 (11), 1540–1551.

Bischoff, S.C., 2007. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat. Rev. Immunol.* 7 (2), 93–104.

Borchers, A.T., Selmi, C., Meyers, F.J., Keen, C.L., Gershwin, M.E., 2009. Probiotics and immunity. *J. Gastroenterol.* 44 (1), 26–46.

Broide, D.H., 2010. Allergic rhinitis: Pathophysiology. *Allergy Asthma Proc.* 31 (5), 370–374.

Brozek, J.L., Bousquet, J., Baena-Cagnani, C.E., Bonini, S., Canonica, G.W., Casale, T.B., van Wijk, R.G., Ohta, K., Zuberbier, T., Schunemann, H.J., Global, A., Asthma European, N., Grading of Recommendations Assessment, D., Evaluation Working, G., 2010. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. *J. Allergy Clin. Immunol.* 126 (3), 466–476.

Camateros, P., Tamaoka, M., Hassan, M., Marino, R., Moisan, J., Marion, D., Guiot, M.C., Martin, J.G., Radzioch, D., 2007. Chronic asthma-induced airway remodeling is prevented by toll-like receptor-7/8 ligand S28463. *Am. J. Respir. Crit. Care Med.* 175 (12), 1241–1249.

Chang, S.H., Bae, J.H., Hong, D.P., Choi, K.D., Kim, S.C., Her, E., Kim, S.H., Kang, C.D., 2010. *Dryopteris crassirhizoma* has anti-cancer effects through both extrinsic and intrinsic apoptotic pathways and G0/G1 phase arrest in human prostate cancer cells. *J. Ethnopharmacol.* 130 (2), 248–254.

Chang, X., Li, W., Koike, K., Wu, L., Nikaido, T., 2006. Phenolic constituents from the rhizomes of *Dryopteris crassirhizoma*. *Chem. Pharm. Bull.* 54 (5), 748–750.

Chung, F., 2001. Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-gamma. *Mediat. Inflamm.* 10 (2), 51–59.

Church, M.K., Levi-Strauss, F., 1997. The human mast cell. *J. Allergy Clin. Immunol.* 99 (2), 155–160.

Cook, E.B., Stahl, J.L., Barney, N.P., Graziano, F.M., 2002. Mechanisms of antihistamines and mast cell stabilizers in ocular allergic inflammation. *Curr. Drug Targets Inflamm. Allergy* 1 (2), 167–180.

Fallon, P.G., Jolin, H.E., Smith, P., Emson, C.L., Townsend, M.J., Fallon, R., Smith, P., McKenzie, A.N., 2002. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity* 17 (1), 7–17.

Guerra, S., Sherrill, D.L., Martinez, F.D., Barbee, R.A., 2002. Rhinitis as an independent risk factor for adult-onset asthma. *J. Allergy Clin. Immunol.* 109 (3), 419–425.

- Ha, H., Shim, K.S., An, H., Kim, T., Ma, J.Y., 2013. Water extract of *Spatholobus suberectus* inhibits osteoclast differentiation and bone resorption. *BMC Complement Altern. Med* 13, 112.
- Hollander, C., Nystrom, M., Janciauskiene, S., Westin, U., 2003. Human mast cells decrease SLPI levels in type II - like alveolar cell model, in vitro. *Cancer Cell Int.* 3 (1), 14.
- Howarth, P.H., Persson, C.G., Meltzer, E.O., Jacobson, M.R., Durham, S.R., Silkoff, P.E., 2005. Objective monitoring of nasal airway inflammation in rhinitis. *J. Allergy Clin. Immunol.* 115 (3Suppl 1), S414–S441.
- Hsieh, C.C., Hsiao, H.B., Lin, W.C., 2010. A standardized aqueous extract of *Anoectochilus formosanus* modulated airway hyperresponsiveness in an OVA-inhaled murine model. *Phytomedicine* 17 (8–9), 557–562.
- Hwang, Y.H., Ha, H., Ma, J.Y., 2013. Acute oral toxicity and genotoxicity of *Dryopteris crassirhizoma*. *J. Ethnopharmacol.* 149 (1), 133–139.
- Ito, T., Smrz, D., Jung, M.Y., Bandara, G., Desai, A., Smrzova, S., Kuehn, H.S., Beaven, M.A., Metcalfe, D.D., Gilfillan, A.M., 2012. Stem cell factor programs the mast cell activation phenotype. *J. Immunol.* 188 (11), 5428–5437.
- Jeon, S.G., Lee, C.G., Oh, M.H., Chun, E.Y., Gho, Y.S., Cho, S.H., Kim, J.H., Min, K.U., Kim, Y.Y., Kim, Y.K., Elias, J.A., 2007. Recombinant basic fibroblast growth factor inhibits the airway hyperresponsiveness, mucus production, and lung inflammation induced by an allergen challenge. *J. Allergy Clin. Immunol.* 119 (4), 831–837.
- Jung, H.W., Jung, J.K., Park, Y.K., 2013. Comparison of the efficacy of KOB03, ketotifen, and montelukast in an experimental mouse model of allergic rhinitis. *Int Immunopharmacol.* 16 (2), 254–260.
- Kim, S.H., Choi, C.H., Kim, S.Y., Eun, J.S., Shin, T.Y., 2005. Anti-allergic effects of *Artemisia iwayomogi* on mast cell-mediated allergy model. *Exp. Biol. Med.* 230 (1), 82–88.
- Kool, M., Soullie, T., van Nimwegen, M., Willart, M.A., Muskens, F., Jung, S., Hoogsteden, H.C., Hammad, H., Lambrecht, B.N., 2008. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J. Exp. Med.* 205 (4), 869–882.
- Larche, M., Akdis, C.A., Valenta, R., 2006. Immunological mechanisms of allergen-specific immunotherapy. *Nat. Rev. Immunol.* 6 (10), 761–771.
- Lee, H.B., Kim, J.C., Lee, S.M., 2009. Antibacterial activity of two phloroglucinols, flavaspidic acids AB and PB, from *Dryopteris crassirhizoma*. *Arch. Pharm. Res.* 32 (5), 655–659.
- Lee, J.S., Miyashiro, H., Nakamura, N., Hattori, M., 2008. Two new triterpenes from the Rhizome of *Dryopteris crassirhizoma*, and inhibitory activities of its constituents on human immunodeficiency virus-1 protease. *Chem. Pharm. Bull.* 56 (5), 711–714.
- Li, L., Zhang, X.H., Liu, G.R., Liu, C., Dong, Y.M., 2016. Isoquercitrin suppresses the expression of histamine and pro-inflammatory cytokines by inhibiting the activation of MAP kinases and NF-kappaB in human KU812 cells. *Chin. J. Nat. Med.* 14 (6), 407–412.
- Liang, Y.H., Wang, W., Yu, S.W., Ye, M., He, X.H., Gong, N.B., Lu, Y., Khan, I.A., Guo, D.A., 2010. A new chirate type triterpenoid from the rhizomes of *Drynaria fortunei*. *Fitorapia* 81 (8), 988–991.
- Liu, Y., Lu, X., Yu, H.J., Hua, X.Y., Cui, Y.H., Huang, S.K., Liu, Z., 2010. The expression of osteopontin and its association with Clara cell 10 kDa protein in allergic rhinitis. *Clin. Exp. Allergy* 40 (11), 1632–1641.
- Lu, T., Zhang, C., Chai, M., An, Y., 2016. Isoquercetin ameliorates tunicamycin-induced apoptosis in rat dorsal root ganglion neurons via suppressing ROS-dependent endoplasmic reticulum stress. *Biomed. Pharmacother.* 80, 343–351.
- Ma, C., Jiang, Y., Zhang, X., Chen, X., Liu, Z., Tian, X., 2018. Isoquercetin ameliorates myocardial infarction through anti-inflammation and anti-apoptosis factor and regulating TLR4-NF-kappaB signal pathway. *Mol. Med Rep.* 17 (5), 6675–6680.
- Malmhall, C., Bossios, A., Pullerits, T., Lotvall, J., 2007. Effects of pollen and nasal glucocorticoid on FOXP3+, GATA-3+ and T-bet+ cells in allergic rhinitis. *Allergy* 62 (9), 1007–1013.
- Markman, M., 2002. Safety issues in using complementary and alternative medicine. *J. Clin. Oncol.* 20 (18 Suppl), 39S–41S.
- Mekori, Y.A., Metcalfe, D.D., 2000. Mast cells in innate immunity. *Immunol. Rev.* 173, 131–140.
- Mo, J.H., Kang, E.K., Quan, S.H., Rhee, C.S., Lee, C.H., Kim, D.Y., 2011. Anti-tumor necrosis factor-alpha treatment reduces allergic responses in an allergic rhinitis mouse model. *Allergy* 66 (2), 279–286.
- Ozdemir, C., Akdis, M., Akdis, C.A., 2009. T regulatory cells and their counterparts: masters of immune regulation. *Clin. Exp. Allergy* 39 (5), 626–639.
- Prussin, C., Metcalfe, D.D., 2003. 4. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* 111 (2 Suppl), S486–S494.
- Sakaguchi, S., Yamaguchi, T., Nomura, T., Ono, M., 2008. Regulatory T cells and immune tolerance. *Cell* 133 (5), 775–787.
- Shimizu, T., Hirano, H., Majima, Y., Sakakura, Y., 2000. A mechanism of antigen-induced mucus production in nasal epithelium of sensitized rats. A comparison with lipopolysaccharide-induced mucus production. *Am. J. Respir. Crit. Care Med.* 161 (5), 1648–1654.
- Shinozaki, J., Shibuya, M., Masuda, K., Ebizuka, Y., 2008. Dammaradiene synthase, a squalene cyclase, from *Dryopteris crassirhizoma* Nakai. *Phytochemistry* 69 (14), 2559–2564.
- Sookrung, N., Indrawattana, N., Tungtrongchitr, A., Karuhassuwan, C., Chaisri, U., Chaicumpa, W., 2008. A murine model of allergy caused by American cockroach (CR), *Periplaneta americana*. *Asian Pac. J. Allergy Immunol.* 26 (2–3), 143–149.
- Strachan, D., Sibbald, B., Weiland, S., Ait-Khaled, N., Anabwani, G., Anderson, H.R., Asher, M.I., Beasley, R., Bjorksten, B., Burr, M., Clayton, T., Crane, J., Ellwood, P., Keil, U., Lai, C., Mallol, J., Martinez, F., Mitchell, E., Montefort, S., Pearce, N., Robertson, C., Shah, J., Stewart, A., von Mutius, E., Williams, H., 1997. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the international study of asthma and allergies in childhood (ISAAC). *Pediatr. Allergy Immunol.* 8 (4), 161–176.
- Tanaka, S., Furuya, K., Yamamoto, K., Yamada, K., Ichikawa, M., Suda, M., Makabe, H., 2017. Procyanidin B2 gallates inhibit IFN-gamma and IL-17 production in T cells by suppressing T-bet and RORgammat expression. *Int. Immunopharmacol.* 44, 87–96.
- Taylor, A., Verhagen, J., Akdis, C.A., Akdis, M., 2005. T regulatory cells and allergy. *Microbes Infect.* 7 (7–8), 1049–1055.
- Tsunematsu, M., Yamaji, T., Kozutsumi, D., Murakami, R., Kimura, S., Kino, K., 2007. Establishment of an allergic rhinitis model in mice for the evaluation of nasal symptoms. *Life Sci.* 80 (15), 1388–1394.
- Ulrich, K., Hincks, J.S., Walsh, R., Wetterstrand, E.M., Fidock, M.D., Sreckovic, S., Lamb, D.J., Douglas, G.J., Yeadon, M., Perros-Huguet, C., Evans, S.M., 2008. Anti-inflammatory modulation of chronic airway inflammation in the murine house dust mite model. *Pulm. Pharmacol. Ther.* 21 (4), 637–647.
- Wang, S.B., Deng, Y.Q., Ren, J., Xiao, B.K., Liu, Z., Tao, Z.Z., 2014. Exogenous interleukin-10 alleviates allergic inflammation but inhibits local interleukin-10 expression in a mouse allergic rhinitis model. *BMC Immunol.* 15, 9.
- Wing, K., Fehervari, Z., Sakaguchi, S., 2006. Emerging possibilities in the development and function of regulatory T cells. *Int Immunol.* 18 (7), 991–1000.
- Wright, A.L., Holberg, C.J., Martinez, F.D., Halonen, M., Morgan, W., Taussig, L.M., 1994. Epidemiology of physician-diagnosed allergic rhinitis in childhood. *Pediatrics* 94 (6 Pt 1), 895–901.
- Yang, Q., Gao, L., Si, J., Sun, Y., Liu, J., Cao, L., Feng, W.H., 2013. Inhibition of porcine reproductive and respiratory syndrome virus replication by flavaspidic acid AB. *Antivir. Res.* 97 (1), 66–73.