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Original Article

Rosae multiflorae fructus extract and its four active components alleviate ovalbumin-induced allergic inflammatory responses via regulation of Th1/Th2 imbalance in BALB/c rhinitis mice

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ABSTRACT

Background: *Rosae Multiflorae* fructus has potent antioxidative, analgesic, and anti-inflammatory properties.
Purpose: We investigated the immunomodulatory effect of *Rosae Multiflorae* fructus extract (RMFE) on allergic inflammation in an allergic rhinitis (AR) mouse model.
Methods: Mice were sensitized and intranasally challenged with ovalbumin (OVA), the Th1/Th2-related cytokines and histopathology were examined after RMFE treatments. Primary cell culture from spleen and NALT was performed to evaluate RMFE effect on Th1/Th2 responses. Four active components of RMFE were determined using HPLC and then tested the inhibition on Th2 response.
Results: Oral administration of RMFE inhibited the accumulation of eosinophils in nasal lavage fluid (NALF) and the nasal mucosa, goblet cells in the nasal epithelium, and mast cells in the respiratory region of the nasal cavity. Thus, the swelling of the nasal epithelium, nasal-associated lymphoid tissue (NALT), and lung tissue were ameliorated. Furthermore, the RMFE suppressed Th2-related cytokines, such as IL-4, IL-5, and IL-13 in NALF, NALT, and splenocytes, whereas the Th1-associated cytokine IL-12 was up-regulated by RMFE. We also revealed the active components of RMFE, such as ellagic acid, hyperoside, isoquercitrin, and miquelianin. They may inhibit IL-4 secretion in allergic responses.
Conclusion: RMFE may have therapeutic potential for treating AR by modulating the relationships between Th1/Th2 responses.

Introduction

Allergic rhinitis (AR) is a common allergy-mediated inflammatory disease characterized by nasal itching, sneezing, and nasal congestion. AR is also an important risk factor for asthma (Bachert et al., 2008). Epidemiological data showed that AR affects up to 50% of the world's population (Strachan et al., 1997). Over 500 million people suffer from AR worldwide, and the incidence is increasing (Brozek et al., 2010).

Abbreviations: AR, allergic rhinitis; Dex, Dexamethasone; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; Ig, immunoglobulin; IL, interleukin; IFN, interferon; NALF, nasal lavage fluid; NALT, Nasal-associated lymphoid tissue; OVA, ovalbumin; PBS, phosphate-buffered saline; RMF, Rosae Multiflorae fructus; RMFE, Rosae Multiflorae fructus extract; Th, Type helper T cell

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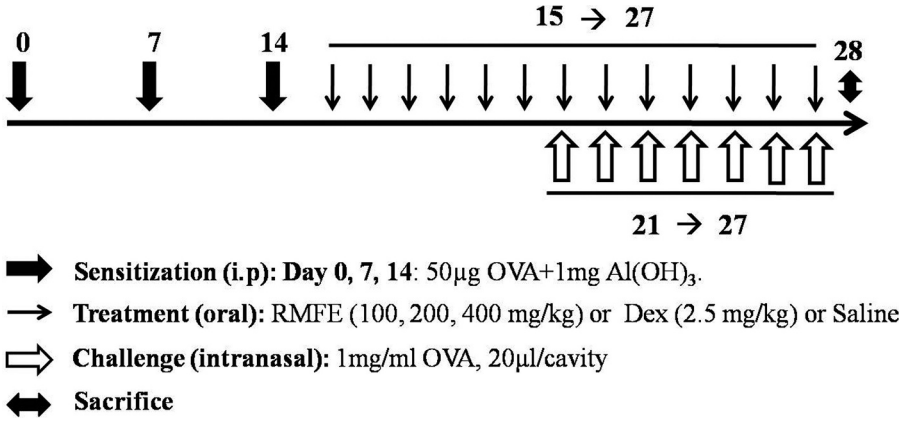

Fig. 1. Experimental protocol for allergic rhinitis mouse model and treatment with RMFE. Mice were sensitized on days 0, 7 and 14 and challenged on days 21 - 27 with ovalbumin (OVA). Mice in the Naive group were sensitized and challenged with saline. Mice in the RMFE or Dex groups were administered orally once daily at 100, 200, 400 mg/kg RMFE or 2.5 mg/kg Dex for 11 days, respectively.

AR is characterized by an imbalance of the effects of $CD4^+$ T cell subsets and an accumulation of eosinophils and mast cells in the nasal mucosa. In general, $CD4^+$ T cell subsets can be classified into type 1 helper T (Th1), Th2, Th17, and regulatory T cells, based on the expression of specific cell surface markers. In particular, Th2-associated immune responses, which can produce IL (interleukin) – 4, IL-5, and IL-13, have been known to cause various allergic disorders, including AR (Ishigame et al., 2009; Mo et al., 2011; Quan et al., 2012). In other words, the dominance of Th2-mediated immune responses reduces the population of other $CD4^+$ T cell subsets, especially Th1 immune responses, resulting in a Th1/Th2 immune imbalance (Malmhall et al., 2007). Thus, it may be possible to treat allergic disorders, such as AR, atopic dermatitis, allergic asthma, and food allergies, by regulating the Th1/Th2 immune balance or specifically suppressing Th2 immune responses.

It is well-acknowledged that anti-histamines, anti-leukotriene, and intranasal corticosteroids are current AR treatments that only ameliorate allergic symptoms (Jung et al., 2013; Takahashi et al., 2009). Additionally, corticosteroids do not address all issues and in all patients (Alvarez et al., 1992). The long-term use of steroids is also often accompanied by undesirable adverse effects, such as headache, throat irritation, and nasal dryness, particularly when high doses are used (Lipworth and Jackson, 2000). Hence, it is necessary to identify novel therapeutic agents that are both safe and effective for AR treatment. Herbal extracts have been selected for development of novel therapeutics because they have no undesirable negative side effects.

Rosa multiflora Thunberg, commonly known as "Multiflora Rose," is native to Korea, Japan, and China. *Rosae Multiflorae* fructus (RMF) hips have been used as a traditional medicine for rheumatism, joint inflammation, edema, and beriberi (Cheng et al., 2016). In a previous study, we revealed for the first time that RMF attenuated symptoms of allergic asthma induced by ovalbumin (OVA) through inhibiting the degranulation of mast cells (Song et al., 2016). RMF is rich in quercetin glycosides (Takagi et al., 1976a), multiflorins (Takagi et al., 1976b), carotene, vitamin E, essential fatty acids and minerals (Huang et al., 2001). It has not been determined yet, however, which components have anti-allergic effects.

In this study, we investigated the anti-allergic effect of RMF extract (RMFE) in an OVA-induced AR mouse model through regulating Th2-associated immune responses. We also investigated which RMFE-derived components have anti-allergic activities against IL-4 production induced by OVA in splenocytes.

Materials and methods

Animals

Male BALB/c mice, 6 weeks of age, were obtained from Damool Science (Dae-jeon, Korea). The mice were housed under average temperature (23 ± 3 °C) and relative humidity (50 ± 10%) with 12-h light/dark cycles. All procedures of experiments were approved by and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School (CBN 2016–37).

Preparation of RMFE

The RMFE (HU028) used in this study was provided by Huons. Co. Ltd. Briefly, RMF was collected at the Back-Jock Mountain and provided by a RMF-producing group (Janghowon, Icheon, Korea). Raw RMF material was extracted with 10 ± 2 x volumes of distilled water by heating (85 ± 5 °C) for 8 ± 2 h; and the filtrate was collected using cartridge filter. This process was repeated. Next, the filtrate was evaporated to yield over 20 brix extract. The RMFE (specimen number: KFRI-SL-2157-W) was mixed with dextrin at a ratio of 1:1 and then dried using spray dry with an inlet air temperature of 180 °C and an outlet air temperature of 95 °C. After filtering, the RMFE powders were kept at room temperature until needed and managed with specimen number (HU028) at both the Korea Food Research Institute and Huons. Co. Ltd.

The yield of RMFE was 60 ± 10% (g RMFE/g RMF). The RMFE was standardized to contain 1.34 mg/g (mg miquelianin/g RMFE) (content range of ± 20%) of miquelianin. For the AR mouse model treatment, the RMFE powder was dissolved in saline to concentrations of 100, 200, and 400 mg/kg and the solution was prepared fresh daily before each treatment.

AR model establishment and treatment

Mice were divided into six groups ($n = 6$ each group): (1) Naive group; (2) OVA group; (3) RMFE100 group; (4) RMFE200 group; (5) RMFE400 group, and (6) Dex group. The procedures for establishing an OVA-induced AR mouse model and treatment were established as described in Fig. 1. Briefly, mice were sensitized on days 0, 7, and 14 with an intraperitoneal injection of 200 μl saline and 50 μg OVA (Grade V, Sigma Aldrich, St. Louis, MO, USA) and 1 mg aluminum hydroxide (Thermo Scientific, Rockford, MD, USA). On days 21–27, the mice received an intranasal challenge with 20 μl of 1 mg/ml OVA in each nasal cavity. On days 15–27, the mice in the RMFE and Dex groups were orally administered once daily with RMFE and Dex at doses of 100, 200, 400 mg/kg and 2.5 mg/kg, respectively; the OVA groups were given saline. Mice were treated one hour before the intranasal challenge of OVA. The nasal symptoms were evaluated by counting the number of nasal rubbing and sneezing events for 20 min after the last OVA intranasal challenge. The mice were sacrificed 24 h after the last OVA challenge. This animal study was repeated 3 times.

Nasal lavage fluid collection and cell count

Twenty-four hours after the last OVA challenge, the mice were sacrificed, blood samples were harvested by cardiac puncture and centrifuged to obtain serum. Nasal lavage fluid (NALF) was collected via an 18-gauge catheter. The trachea was partially resected, a catheter was inserted from the trachea into the nasopharynx, and the nasal passages were gently perfused with 1 ml phosphate-buffered saline (PBS). The NALF was centrifuged at 10000 RPM/min for 10 min at 4 °C. The supernatant was stored at −70 °C for enzyme-linked immunosorbent assay (ELISA) analysis. The cell pellets were resuspended in PBS, and the total cell numbers were counted in a hemocytometer.

To determine differential cell counts, 150 μL NALF was centrifuged onto slides using a cytospin device (Centrifuge 5403, Eppendorf, Hamburg, Germany) (1000 rpm, 10 min, 4 °C). Diff-Quik Staining reagent (1-5-1 Wakinohama-Kaigandori, Chuo-Ku, Kobe, Japan) was used for the cell staining according to the manufacturer's instructions.

Histological examination

To allow examination of the nasal cavity, the heads were fixed in 10% neutral buffered formalin for 3 days and decalcified in an ethylenediaminetetraacetic acid decalcifying solution for 5 days at room temperature. The specimens were then embedded in paraffin and sectioned at 5 μm thickness. Nasal tissues were stained with hematoxylin and eosin (H&E) (Sigma Aldrich, St. Louis, MO, USA) for general morphological observation, periodic acid-Schiff (PAS) (Sigma Aldrich) to assess goblet cell hyperplasia, and Giemsa (Sigma Aldrich, St. Louis, MO, USA) to assess eosinophil and mast cell infiltration. The eosinophils and mast cells were counted and epithelial damage was analyzed in randomly selected high-power fields at 400 × magnification.

For examining the lung tissue, the middle lobe of the lung was removed and fixed in 10% neutral-buffered formalin. The specimen was dehydrated with ethyl alcohol and xylene. Tissues were embedded in paraffin and sliced at a thickness of 5 μm. The lung sections were stained with H&E to identify general morphology.

Primary cell culture from spleen or NALT

Spleen and nasal-associated lymphoid tissue (NALT) were prepared by aseptically removing the tissues from each mouse and then homogenized the tissues to single cells. Splenocytes were collected and treated with red blood cell lysing buffer (Sigma Aldrich, St. Louis, MO, USA). The cells were adjusted to 5×10^6 cell/ml in RPMI 1640 medium (Welgene Inc., Daegu, Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 g/ml streptomycin and 5×10^{-5} M 2-ME (Welgene Inc., Daegu, Korea); the cells were then cultured in OVA (200 μg/ml/well). The plates were incubated at 37 °C for 72 h in a humidified incubator with 5% (v/v) CO_2 and 95% (v/v) air. NALT were collected and treated with 1 mg/ml collagenase (Sigma Aldrich, St. Louis, MO, USA) in RPMI 1640 medium and then incubated in shaking incubator at 37 °C for 30 min. After centrifuging at 1200 rpm for 5 min, NALT were collected in Hank's balanced salt solution (Welgene Inc., Daegu, Korea) supplemented with 5% FBS and 5 mM EDTA in a shaking incubator at 37 °C for 5 min. After centrifuging, NALT were collected in PBS (Welgene Inc., Daegu, Korea) for 10 min. The NALT cells were seeded at 1×10^6 cell/ml and cultured in RPMI 1640 medium with concanavalin A (2 μg/ml/well). The plates were incubated at 37 °C for 48 h in a humidified incubator with 5% (v/v) CO_2 and 95% (v/v) air.

Measurement of immunoglobulins and cytokine levels using ELISA

The NALF was centrifuged to collect the supernatant. Blood was collected from the vena cava, and serum was obtained by centrifugation and stored at −70 °C until use. The levels of OVA specific Ig (immunoglobulin) E, anti-OVA IgG1, anti-OVA IgG2a were quantified using ELISA kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturers' instructions. Cytokine assay kits (BD Biosciences, San Diego, CA, USA) were used to measure cytokine levels (IL-4, IL-5, IL-13, interferon (IFN)-γ, IL-12) and NFκBp65, IκBα according to manufacturer protocols. Briefly, supernatants and standard solutions were transferred to 96-well plates pre-coated with monoclonal antibodies to each of the target cytokines and then incubated at room temperature for 2 h. After thorough washing with the washing buffer included in the kit, a horseradish peroxidase-conjugated secondary antibody was added to each well, and incubation was continued at room temperature for 2 h. After removal of the secondary antibody, the substrate solution for the enzymatic reaction was added, and samples were incubated for another 30 min in the dark. The reaction was terminated by addition of the stop solution, and absorbance was measured at 450 nm in a microplate reader (Molecular Devices, Inc., Sunnyval, CA, USA).

HPLC analysis

High-performance liquid chromatography (HPLC) analysis was carried out with an Agilent 1260 Infinity series liquid chromatography system (Agilent, Santa Clara, CA, USA) equipped with a multi-wavelength detector (Agilent G4212B 1260 DAD, Agilent, Santa Clara, CA, USA). Samples were injected to 1 μg/ml by autoinjector into the Agilent G1329B 1260 ALS (Agilent, Santa Clara, CA, USA), and a GL Science Inertsil ODS-3 column (250 × 4.6 mm) (GL Science, Tokyo, Japan) was used to separate compounds at 30 ~ 35 °C in a column adapter (Agilent G1316A 1260 TCC, Agilent, Santa Clara, CA, USA). The used solvents were a mixture of 0.1% phosphoric acid in water (A) and acetonitrile (B) in gradient mode (eluent A: 5 to 100% in 42 min); the flow rate was 1 ml/min. The UV wavelength for detection was 254 nm. As reference compounds used in this study, the ellagic acid (purity: > 95%) was purchased from Sigma Aldrich (St. Louis, MO, USA) and the hyperoside (purity: 95.03%), isoquercitrin (purity: 90.81%), and miquelianin (purity: 96.07%) were purchased from HWI analytic GmbH (Ruelzheim, Germany).

Statistical analysis

The results were performed using Graph Pad Prism software (v5.0, La Jolla, CA, USA). Data were expressed as means ± SEM. The differences among the groups were analyzed by one-way ANOVA, followed by Tukey's test. Statistical significance was considered at $p < 0.05$.

Results

Effect of RMFE on the nasal symptoms of rhinitis induced by OVA

To analyze the anti-allergic effect of RMFE, we established the OVA-induced AR mouse model and counted the frequencies of nosal rubbing and sneezing for 20 min after an OVA intranasal challenge on day 27. The results showed that OVA-induced AR mice had markedly increased rubbing and sneezing scores compared to those in Naive group. RMFE administration significantly alleviated AR symptoms compared to that in the OVA group. Similarly, the Dex treatment also displayed an inhibition effect on nasal symptoms in the AR mouse model (Fig. 2A, B).

Effect of RMFE on the infiltration of differential inflammatory cells in NALF

To determine the effect of RMFE on nasal symptoms in the AR model, the number of total cells and differential inflammatory cells in NALF were measured. Mice in OVA group had significantly elevated cell numbers, including macrophages, neutrophils, and especially eosinophils, compared to the Naive group. By contrast, RMFE treatments

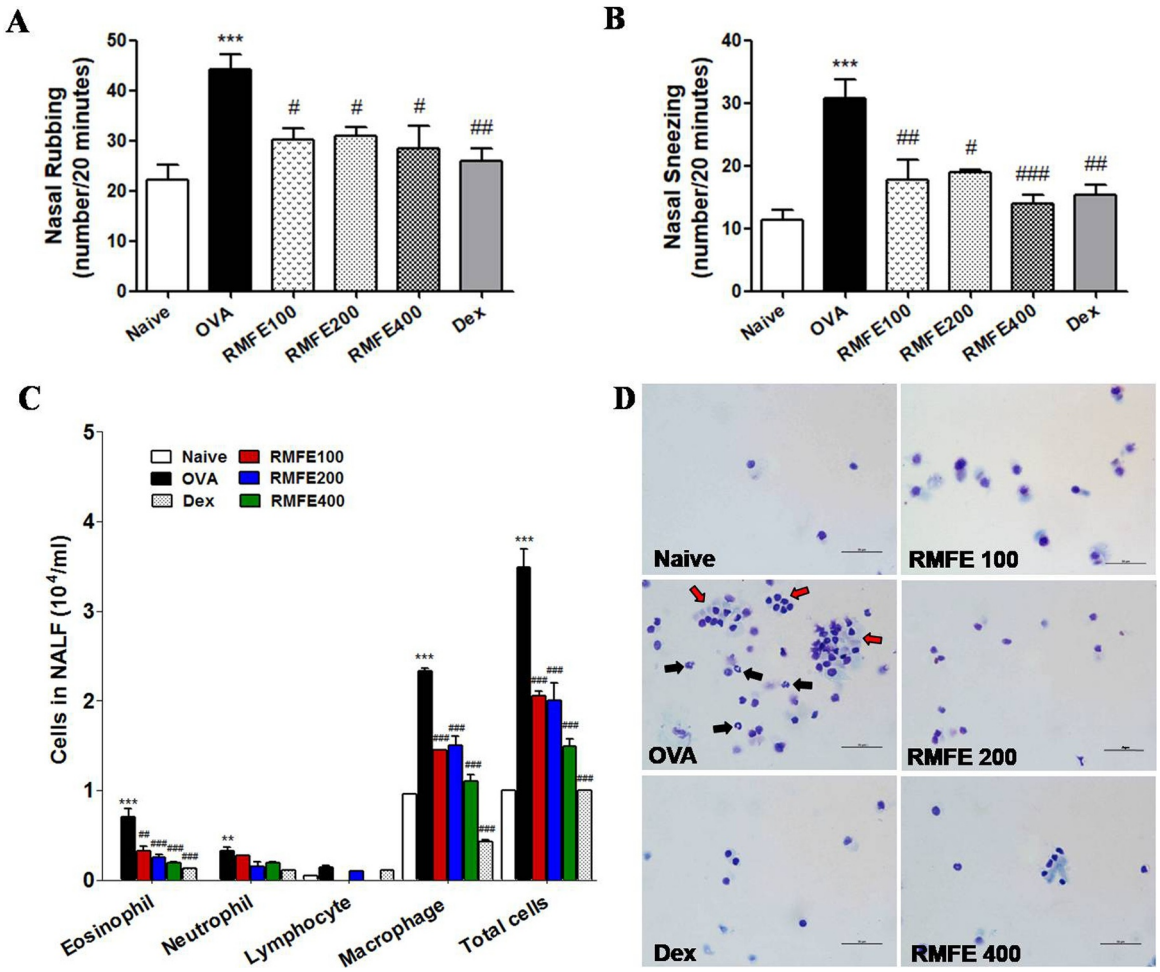

Fig. 2. Effect of RMFE on nasal symptom scores, and number of differential cells and total cells in NALF. (A) Rubbing and (B) Sneezing scores. Rubbing and sneezing events were counted for 20 min after the OVA challenge on day 27. (C) The number of differential cells and total cells in NALF. (D) NALF was collected 24 h after the last OVA challenge; the cells were isolated by cytospin and stained with Diff-Quick. Black arrows indicate eosinophils. Red arrows indicate ciliated epithelium cells that were caused loss from inflammatory tissue. The values represent the mean ± S.E.M (n = 6/group). Significant differences at $^{***}p < 0.001$, $^{**}p < 0.01$ compared with the Naive group. $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with the OVA group. Scale bar: 50 μm.

(100, 200, and 400 mg/kg) significantly inhibited the increase of these cell numbers when compared to those in OVA-induced AR mice; Dex treatment (2.5 mg/kg) showed similar results. Neutrophil and lymphocyte numbers, however, did not differ significantly among groups (Fig. 2C). Furthermore, in the OVA group, the respiratory mucosa was damaged, resulting in an exacerbated loss of ciliated cells (epithelial cells were indicated by the red arrows). However, RMFE and Dex treatments markedly ameliorated epithelial shedding from nasal epithelium layer (Fig. 2D).

Effect of RMFE on nasal thickness, goblet cell hyperplasia, eosinophils and mast cells infiltration in nasal mucosa and on lung inflammation

The nasal mucosa thickness of the OVA group was significantly increased compared with that in the Naive group. The RMFE and Dex treatments had markedly decreased mucosal edema compared to the OVA group (Fig. 3A). H&E staining was performed to assess nasal mucosa histopathological changes. There were no morphological abnormalities in the nasal mucosa of the Naive group. In the OVA group, the nasal septum showed inflammatory cell accumulation in the subepithelium, as well as significantly increased subepithelial thicknesses as compared with that in the Naive group (double-headed arrow, Fig. 4A.OVA). The subepithelial layers of nasal mucosa in RMFE and Dex groups were considerably thinner than those in OVA-induced AR mice (Fig. 4A.Dex, RMFE 100–400).

There was a significantly higher number of goblet cells in the nasal epithelium in OVA-induced AR mice compared to that in the Naive group (Fig. 3B). Consequently, PAS staining showed mucus hypersecretion as a violet color in the nasal mucosa columnar epithelium (black arrow, Fig. 4B). Dex- and RMFE-treated mice, however, showed significantly inhibited mucus overproduction in nasal columnar epithelium compared with that observed in the OVA-induced AR mice (Fig. 4B. Dex, RMFE 100–400).

In the OVA group, the number of eosinophils in the subepithelium significantly increased compared to that in the Naive group and markedly decreased in RMFE and Dex treatments (Fig. 3C). Giemsa staining also revealed that the administration of RMFE and Dex suppressed eosinophilic infiltration, which was observed as fewer red eosinophil cytoplasm in the nasal subepithelium (Fig. 4C).

Similarly, in OVA-induced AR mice, the number of mast cells and degranulated mast cells in the nasal mucosa was significantly higher than that in the Naive group, whereas RMFE and Dex treatments resulted in significantly fewer mast cells and less mast cell degranulation present in the nasal mucosa than OVA-induced AR mice (Fig. 3D).

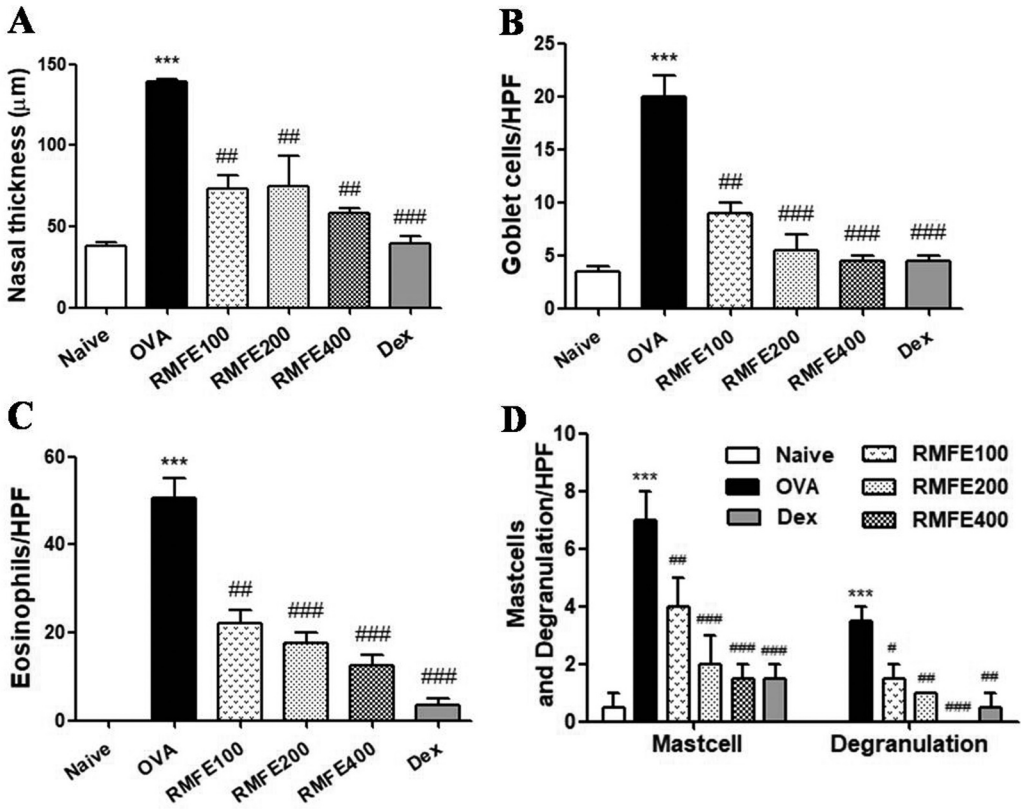

Fig. 3. Effects of RMFE on epithelial thickness of nasal septum and the number of inflammatory cells in the nasal mucosa. (A) Epithelial thickness of nasal septum. The epithelium of OVA group was significantly thicker than that of the Naive group; the epithelial swelling was ameliorated by RMFE and Dex treatment. (B) The number of goblet cells, (C) eosinophils, and (D) mast cells in the epithelium and mast cell degranulation of the OVA group were significantly increased compared to the Naive group and markedly decreased by RMFE and Dex treatment. The values represent the mean ± S.E.M (n = 6/group). Significant differences at $^{***}p < 0.001$ compared with the Naive group. $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with the OVA group.

Giemsa staining was performed; blue and black arrows indicate mast cells in which the cytoplasm was stained dark blue. Histological examination revealed that in OVA-induced AR mice, mast cells infiltrated to the respiratory region (figure not shown), olfactory region (blue arrow, Fig. 4D.OVA) and nasal-associated lymphoid tissue region (black arrow, Fig. 4E.OVA) of the nasal cavity. Respiratory region, olfactory region and NALT region in RMFE and Dex treatments showed no presence of mast cells (Fig. 4D, E. Dex, RMFE 100–400).

We next examined whether RMFE has a protective effect on lower airway inflammatory processes. The lung of the Naive group showed delicate alveoli and bronchioles structures, as well as a thin alveolar epithalamium. The OVA-induced AR mice showed increased inflammatory cell infiltration into alveoli (black arrow, Fig. 4F.OVA) and thicker alveolar epithelial and bronchial walls (double-headed arrow, Fig. 4F.OVA). The RMFE and Dex treatments provided protection against damage to the alveoli and bronchioles (Fig. 4F. Dex, RMFE 100–400).

Effect of RMFE on systemic allergic responses induced by OVA

OVA-specific antibody levels were regulated via T helper cytokines; we examined the levels of anti-OVA IgE, anti-OVA IgG1 and anti-OVA IgG2a in serum to determine the effect of RMFE on inflammatory responses. In this study, the data showed a significant up-regulation of anti-OVA IgE, anti-OVA IgG1 levels and down-regulation of anti-OVA IgG2a in OVA group compared with those in the Naive group. RMFE administration, however, dose-independently and significantly down-regulated the levels of anti-OVA IgE, anti-OVA IgG1 and up-regulated anti-OVA IgG2a in serum compared with those in the OVA group. Dex-treated mice also showed significantly attenuated serum levels of all three antigen-specific antibodies compared to the OVA group (Fig. 5A–C).

We investigated the cytokine patterns in splenocytes isolated from OVA-induced AR mice. IL-4, IL-5, and IL-13 levels were used as a measure of Th2-related cytokines. All the Th2-related cytokines (IL-4, IL-5, and IL-13) showed increased levels in the OVA group compared with those in the Naive group and were significantly inhibited by oral administration of RMFE (Fig. 5D–F). Levels of the Th1-related cytokine IL-12, however, was not found to significantly differ among the groups (Fig. 6H). These results indicate that the administration of RMFE specifically inhibits the Th2-associated systemic immune responses, resulting in improvement of AR symptoms.

Effect of RMFE on Th2-associated cytokines in NALF and NALT

We examined the relative levels of IL-4, IL-5, IL-13, and IL-12 cytokines in NALF to investigate the effect of RMFE in the OVA-induced AR mouse model. In the OVA group, the levels of IL-4, IL-5, and IL-13 were significantly higher than those in the NALF of the Naive group (Fig. 6A–C), while IL-12 level in the NALF was significantly decreased compared to those in the Naive group (Fig. 6G). In contrast, RMFE treatment markedly reduced IL-4, IL-5 and IL-13 levels at all doses compared with levels observed in the OVA-induced AR mice (Fig. 6A–C). In addition, RMFE-treated mice had significantly elevated IL-12 cytokine level at 200 mg/kg and 400 mg/kg concentrations (Fig. 6G). Similarly, Dex-treated mice demonstrated significantly decreased levels of IL-4, IL-5, and IL-13 compared to the Naive group (Fig. 6A–C).

Furthermore, we investigated cytokines production in NALT isolated from OVA-induced AR mice. In the OVA group, levels of Th2-related cytokines, such as IL-4, IL-5, and IL-13, were increased, whereas administration of RMFE significantly reduced the upregulation of Th2-associated cytokines in NALT (Fig. 6D–F). Levels of the Th1-related cytokine IL-12, however, were increased by RMFE in NALT (Fig. 6I). These results indicate that the oral administration of RMFE regulates the Th1/Th2 balance by enhancing the Th1 response and suppressing

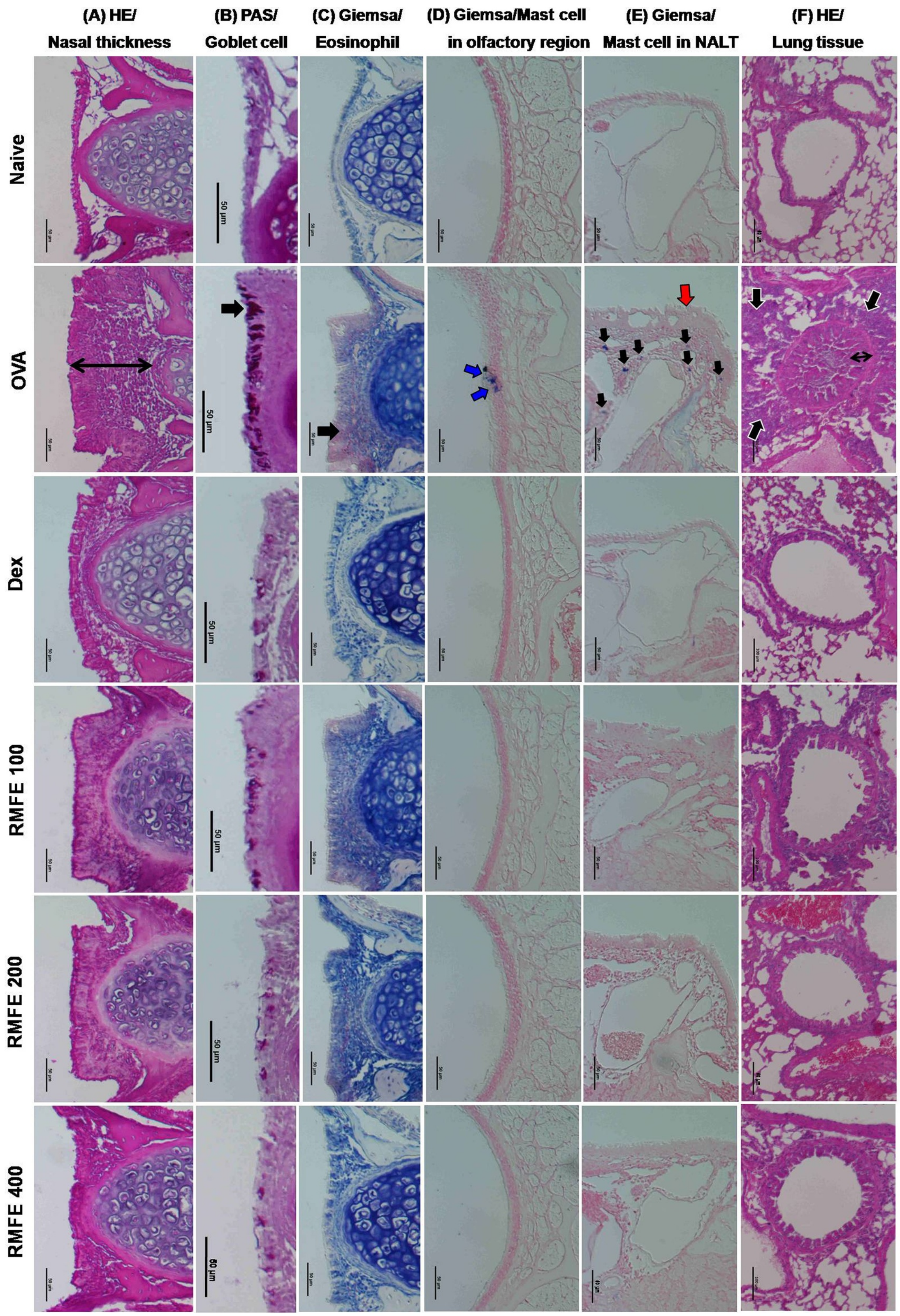

Fig. 4. Effects of RMFE on histological changes of nasal mucosa and lung tissue. (A) HE staining of nasal mucosa showed that the epithelium was thicker in the OVA group than in the Naive group; the epithelium swelling was ameliorated by RMFE. (B) Goblet cells staining (Periodic Acid-Schiff stain). Black arrow indicates goblet cells that were stained violet. The secretory hyperplasia in the epithelium of OVA group was overexpressed and was suppressed by RMFE. (C) Eosinophils staining (Giemsa stain). The cytoplasm of the eosinophils was stained red. The number of eosinophils in the epithelium of OVA group increased; the number of cells was markedly decreased by RMFE. (D, E) Mast cell staining (Giemsa). Black and blue arrows indicate mast cells stained dark blue; (D) Mast cells infiltrated to the epithelium of olfactory region (blue arrows); (E) Mast cells infiltrated to the nasal-associated lymphoid tissue (NALT) (black arrows). Polypoid lesions were characterized by the presence of eosinophils and mast cells. This indicates that mast cell infiltration induced NALT polypoid lesions in the OVA group (red arrows). (F) Lung tissue (HE). Black arrows indicate leukocyte infiltration in peribronchial and perivascular tissues in the OVA group. RMFE strongly reduced the number of inflammation cells present in the lung tissue in a concentration-dependent manner. Scale bar: 50 µm in A-E, 100 µm in F. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

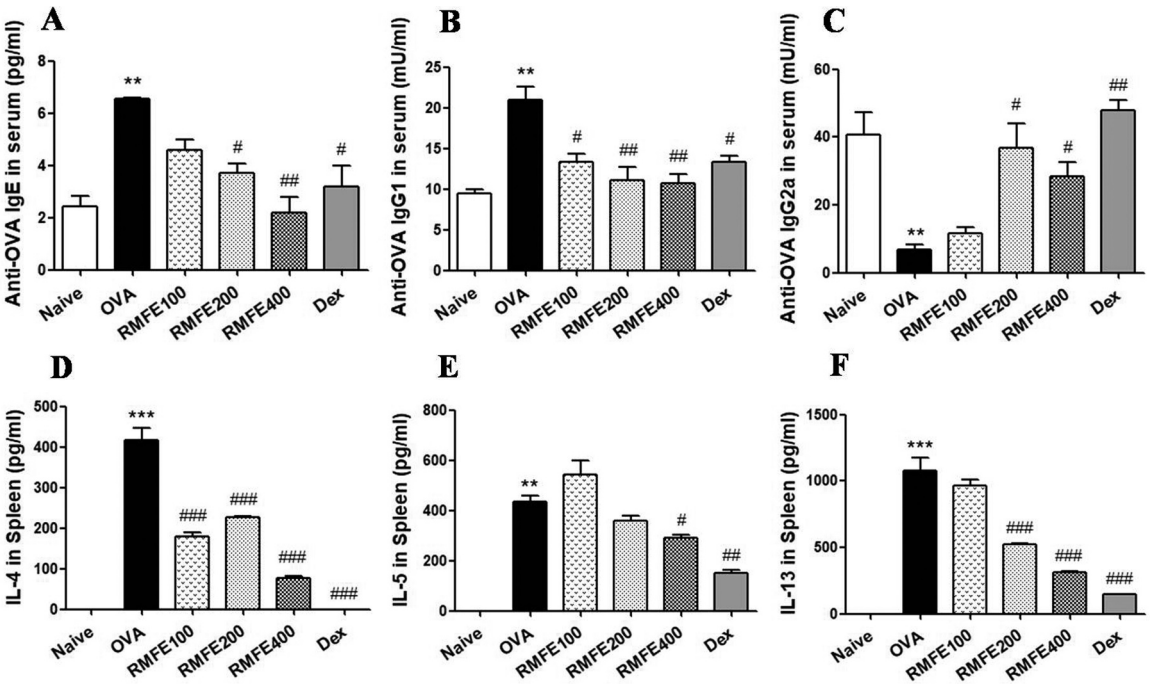

Fig. 5. Effects of RMFE on systemic allergic responses induced by OVA. (A, B, C) RMFE treatment significantly reduced anti-OVA IgE, anti-OVA IgG1 and elevated anti-OVA IgG2a levels in serum. (D, E, F) The Th2-related cytokines in splenocytes (IL-4, IL-5, and IL-13) showed increased patterns following AR and were significantly inhibited by oral administration of RMFE. The values represent the mean ± S.E.M (n = 6/group). Significant differences at $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}P < 0.05$ compared with the Naive group. $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ compared with the OVA group.

the Th2 response in abnormal Th2 dominant (allergic) responses of peripheral lymph nodes.

Effect of RMFE on NFκBp65/IκBα

To investigate whether the NFκBp65/IκBα relate to the balance of Th1/Th2 responses in RMFE treatments, the NFκBp65 and IκBα levels were detected. We found that OVA group increased the expression of NFκBp65 and decreased IκBα level in NALF compared to Naive group. However, in RMFE-treated mice, NFκBp65-free level was inhibited and IκBα was up-regulated compared to OVA group (Fig. 7).

Active components of RMFE

To investigate the active components of RMFE, the RMFE was analyzed by HPLC. The results showed main four peaks (P1, P2, P3, and P4), which were identified as follows; P1, ellagic acid; P2, hyperoside; P3, isoquercitrin; and P4, miquelianin (Fig. 8A). We then investigated the effects of ellagic acid, hyperoside, isoquercitrin, and miquelianin (Fig. 8B) on OVA-induced IL-4 production in splenocytes. All four components inhibited IL-4 secretion (Th2-associated main cytokine) (Fig. 8C). Their inhibitory rates (IC_{50}) were 1.54, 115, 3.68, and 14.2 ug/ml, respectively. Thus, the results suggest that four compounds (ellagic acid, hyperoxide, isoquercitrin, and miquelianin) are active components of RMFE and can attenuate the allergic responses by inhibiting Th2-related cytokine.

Discussion

The sensitization to allergies is triggered by the penetration of allergens into epithelial cells. The antigen-presenting cells digest the allergens and present particles or peptides of allergens to naive $CD4^+$ T cells, and the cells are then differentiated into allergen-specific Th2 cells, which lead to the production of cytokines, such as IL-4, IL-5, and IL-13 (Eigenmann, 2002). Th2-mediated cytokines can promote IgE production by B cells, and the IgEs bind to high-affinity receptors (FcεRI) on the surfaces of mast cells and basophils (Galli et al., 2008). This state is called the sensitization phase.

The AR response has been divided into two phases: early and late (Galli et al., 2008). Early-phase responses occur within minutes after allergen re-exposure. When they are cross-linked by cognate allergen, mast cells immediately secrete histamine, which induces early-phase responses including sneezing, itching and watery nasal discharge (Galli et al., 2008; Skoner, 2001; Wallace et al., 2008). Late-phase responses of AR occur 6–10 h after allergen re-exposure (Galli et al., 2008). Inflammatory cells such as eosinophils, infiltrate in the nasal cavity at the late-phase response. Eosinophils-derived mediators induce epithelial damage, resulting in nasal mucosal swelling (Galli et al., 2008; Skoner, 2001; Wallace et al., 2008).

In the present study, we investigated the anti-allergic effects of RMFE, using an OVA-induced AR mouse model. To evaluate the contributions of RMFE in sensitization phase, AR early-phase, and AR late-phase, we demonstrated the levels of Th2-mediated cytokines and OVA-specific IgE in the sensitization phase, the nasal symptoms and degranulation of mast cells in the AR early-phase, and the infiltration of eosinophils, goblet cells, and mast cells in the AR late-phase under AR disease progression.

In allergic responses, including the sensitization phase, Th2 immune responses play a potential role via production of Th2-related cytokines and activation of IgE-producing B cells. Among Th2-associated cytokines, IL-4 and IL-5 cytokines have been reported to regulate the growth and differentiation of eosinophils (Kim et al., 2013) and IL-13 causes sub-epithelial fibrosis and mucus metaplasia, and induced infiltration of eosinophils and macrophages, results in nasal and airway hyper-responsiveness (Mauad et al., 2007; Zhu et al., 1999). In this study, the administration of RMFE effectively inhibited Th2-mediated cytokine (IL-4, IL-5, and IL-13) production in an OVA-induced AR mice (Fig. 5.D–F, Fig. 6.A–F). Furthermore, we revealed that treatment with RMFE suppressed the secretion of IL-4, IL-5, and IL-13 *in vitro* experiments using splenocytes (data not shown).

Allergic inflammation is characterized by a predominant Th2 response, while the Th1 response is believed to prevent this process

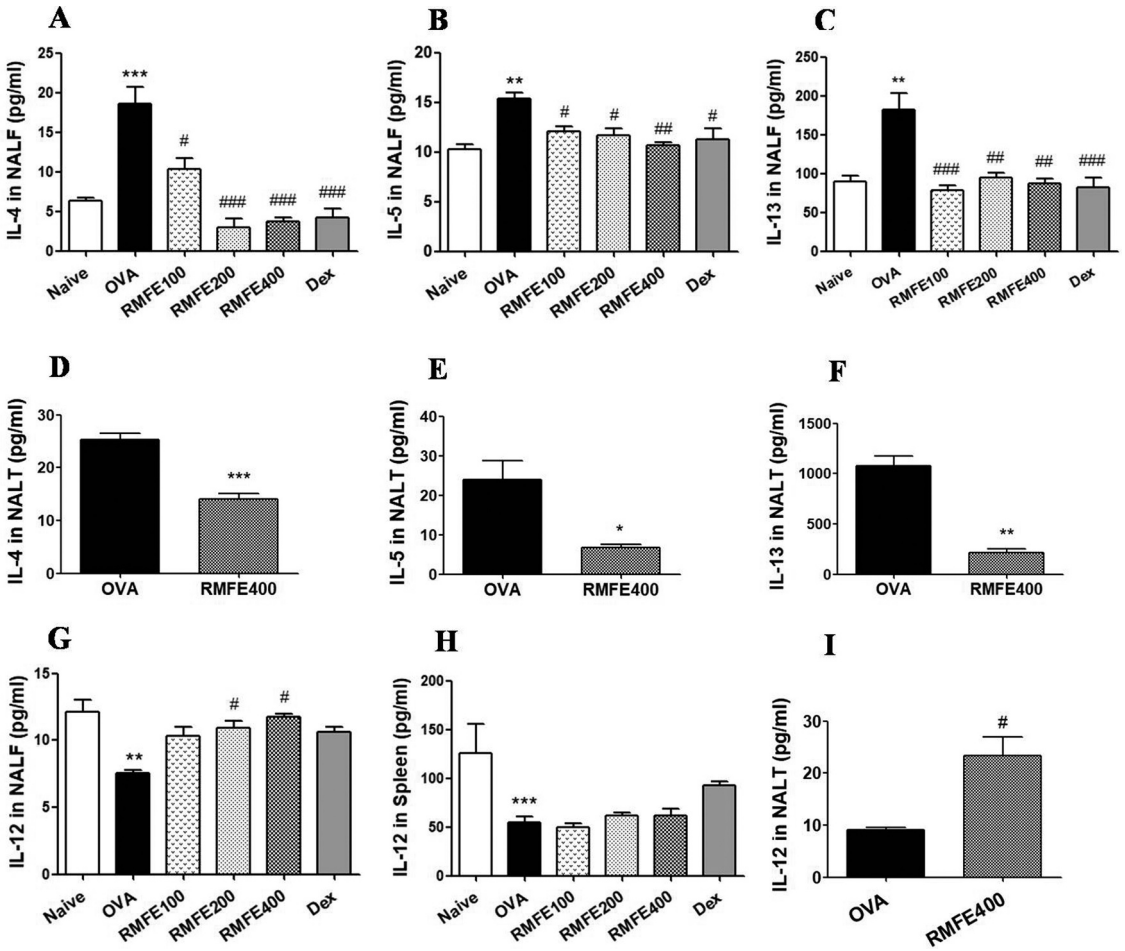

Fig. 6. Effects of RMFE on the Th1 and Th2 cells responses in NALF, NALT and splenocytes. The concentrations of IL-4, IL-5, IL-13 in NALF (A, B, C) and NALT (D, E, F) and the concentration of IL-12 in splenocytes, NALF, and NALT (G, H, I) were determined using ELISA kits. Oral administration of RMFE resulted in the down-regulated levels of IL-4, IL-5, IL-13 cytokines and the up-regulated level of IL-12 cytokines in both NALF and NALT. The values represent the mean ± S.E.M ($n = 6$/group). Significant differences at $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}P < 0.05$ compared with the Naive group. $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ compared with the OVA group.

(Pawankar et al., 2011). Previous studies have shown that regulation of Th1/Th2 cytokines can inhibit allergic inflammation in OVA-sensitized mice (Lee et al., 2012; Ma et al., 2013). Th1-associated cytokines play an important inhibitory role on the recruitment of eosinophils and inhibit the synthesis of IgE and the differentiation of precursor cells to Th2 cells (Fulkerson et al., 2004; Roever et al., 2003). Among Th1-related cytokines, IFN-γ or IL-12 promotes Th1 differentiation and prevents Th2 development in OVA-induced AR mice (Billiau and Matthys, 2009); they also influence neutrophil survival and epithelial integrity (Luo and Loison, 2008; Soyka et al., 2012). Thus, increasing levels of IFN-γ or IL-12 have been used to explain the anti-allergic effects of therapeutic agents. Our findings showed that RMFE-treated mice had down-regulated Th2-related cytokines (IL-4, IL-5, IL-13) (Fig. 6A–C) and up-regulated Th1-related cytokine level (IL-12) in NALF (Fig. 6G) and NALT (Fig. 6I) compared with OVA-induced AR mice. These findings demonstrated that RMFE could attenuate the development of allergic inflammation responses by regulating the Th1/Th2 immune balance.

AR induced by nasal allergen exposure results in IgE-mediated inflammation of the membranes lining the nose. In this study, we investigated the levels of OVA-specific IgE and OVA-specific IgG1 as Th2-related immunoglobulins in serum isolated from an OVA-induced AR mice. Our results showed that the administration of RMFE significantly reduced the levels of both OVA-specific IgE and IgG1 in sera (Fig. 5A, B). We also examined whether RMFE treatment directly inhibited IgE production by B cells; the RMFE did not inhibit IgE levels in B cells (data not shown). Therefore, we suggest that the levels of the OVA-specific IgE and IgG1 reduced by RMFE in AR model mice were affected by the Th2 immune response suppression by RMFE, because RMFE directly suppressed the production of Th2-mediated cytokines *in vitro* experiments. Besides, OVA-specific IgG2a, a Th1-related production, significantly up-regulated in RMFE-treated mice (Fig. 5C), as a result in predominance of Th1 immune response.

A diagnosis of AR is mainly based on nasal symptoms, such as sneezing and watery nasal discharge, and the detection of serum antigen-specific IgE. Therefore, we analyzed the rubbing and sneezing frequencies, as well as IgE levels, to assess the severity of early-phase allergic reactions in an AR mouse model. Mice challenged intranasally with OVA had an increased frequency of sneezing and rubbing. Clinical symptoms were clearly ameliorated with RMFE in a dose-independent manner (Fig. 2A, B), indicating that RMFE treatment has an inhibitory effect on early-phase responses in OVA-induced AR mice. In addition, the degranulation of mast cells, which cause the release of histamines, leukotriens, and tryptases, is important in the early-phase of AR. In a previous study, we revealed that the administration of RMFE effectively ameliorated the symptoms of allergic asthma by regulating the degranulation of mast cells *in vivo* and *in vitro* (Song et al., 2016). In this study, we confirmed that the administration of RMFE attenuated the symptoms of AR, and the inhibitory effect of RMFE on degranulation of mast cells (Fig. 3D) could contribute to improve AR.

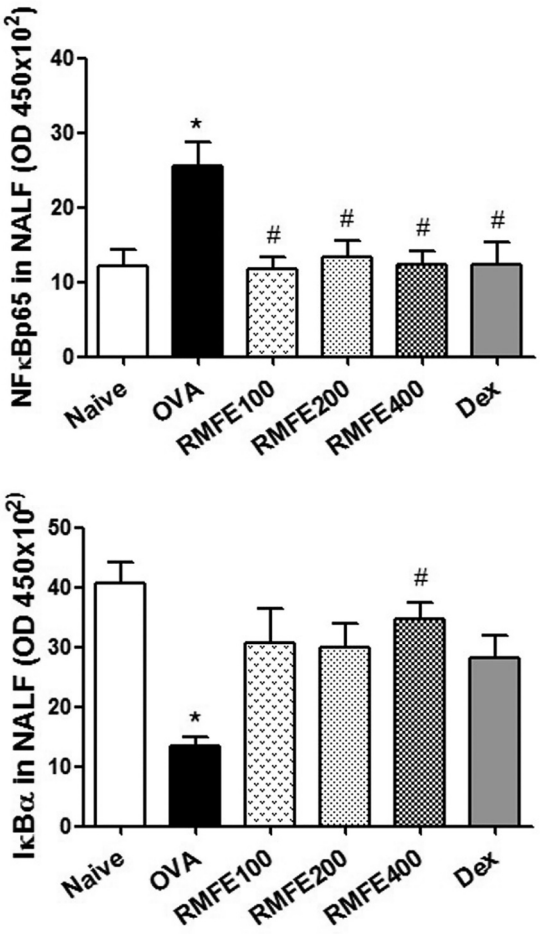

Fig. 7. Effects of RMFE on the activation of NFκBp65. RMFE reduced the NFκB active form at all doses and increased the IκBα subunit at dose 400 mg/kg. The values represent the mean ± S.E.M ($n = 6$/group). Significant differences at $^{*}p < 0.05$ compared with the Naive group. $^{\#}p < 0.05$ compared with the OVA group.

In late-phase responses of AR, infiltration of various inflammatory cells was occurred, resulting in induction of airway and nasal inflammation. In the present study, the OVA-induced AR mice showed severe inflammation through increasing inflammatory cells infiltration such as macrophages and eosinophils in NALF and nasal mucosa. The administration of RMFE showed less apparent inflammatory cells, especially eosinophilic infiltration in NALF (Fig. 2C, D) and nasal mucosa (Fig. 4C), and the swelling of nasal epithelium (Fig. 4A. RMFE 100–400) as well as lung tissue (Fig. 4F.RMFE 100–400) were clearly ameliorated by RMFE. Furthermore, the overexpression of mucus-secreting goblet cells in the respiratory epithelium (Fig. 4B. RMFE 100–400) was also significantly inhibited by RMFE.

In addition, RMFE at high doses (200 and 400 mg/kg) inhibited mast cells infiltration as well as mast cells degranulation in the respiratory region, olfactory region and nasal-associated lymphoid tissue region of the nasal cavity (Fig. 4D, E). Polypoid lesions were characterized by the presence of eosinophils and mast cells. These indicated that mast cells infiltration into the NALT region may be induced NALT polypoid lesions in the OVA group (red arrow, Fig. 4E. Dex) and were ameliorated by RMFE. In fact, activation and infiltration of mast cells can contribute to sneezing and rubbing in mice (Galli et al., 2008; Skoner, 2001; Wallace et al., 2008). Hence, the suppression of rubbing and sneezing symptoms in RMFE-treated mice may have resulted in the inhibition of mast cell accumulation and mast cell degranulation in the nasal mucosa.

In late-phase responses of AR, the infiltration of various inflammatory cells finally induces airway and nasal inflammation. Transcription factor NFκB plays an important role in regulating different Th1 and Th2 cells, therefore, we focused on the activation of NFκBp65 as the main inflammatory factor and examined whether the administration of RMFE regulates the activation of NFκBp65 in the OVA-induced AR mouse model. A reduction of NFκBp65 active form in the RMFE at all doses and an increase of IκBα subunit in RMFE at dose 400 mg/kg was observed. These data suggested that RMFE could control the Th1/Th2 balance via inhibiting NFκBp65 activation (Fig. 7).

In the present study, we revealed that four components identified in RMFE inhibited IL-4 production induced by OVA in splenocytes (Fig. 8). Ellagic acid, naturally found in various fruits, has been suggested to be a potential target inhibitor to ameliorate airway inflammatory disorders, such as asthma and COPD (Ha and Rogers, 2016). Ellagic acid inhibited hyperplasia of goblet cells in a dose-dependent manner, resulting in the blocking of mucin synthesis (Alves Cde et al., 2013). Furthermore, ellagic acid reduced both the levels of Th2-associated cytokines, such as IL-4, IL-5, and IL-13, and eosinophilic inflammation in a mouse model of asthma (Zhou et al., 2014). Isoquercitrin, a flavonoid found in a wide variety of plants, is linked to the 3-O-glucose of quercetin. It presented anti-inflammatory and anti-allergic properties (Gabor, 1986). Isoquercitrin also demonstrated anti-asthmatic activity by inhibiting carbachol- and leukotriene D4-induced contraction in guinea pig airways (Fernandez et al., 2005; Rogerio et al., 2007). In particular, isoquercitrin modified by enzyme ameliorated the symptoms of Japanese cedar pollinosis *in vivo* and in clinical trials (Hirano et al., 2009). The hyperoside, well-known as a plant flavonoid, has been reported to have antioxidant and anti-inflammatory effects (Ku et al., 2015; Ye et al., 2017). Recently, it was reported that the administration of hyperoside attenuated OVA-induced allergic airway inflammation through inhibition of NFκB and activation of Nrf2 in a mouse model of allergic asthma (Ye et al., 2017). Quercetin 3-O-glucuronide, called miquelianin, has been known to have antioxidant activity and neuroprotective effects (Yuan et al., 2017; Zheleva-Dimitrova et al., 2012). The physiological properties of miquelianin are not yet known, unlike those of ellagic acid, isoquercitrin, and hyperoside. In this study, we revealed the anti-allergic effect of miquelianin through inhibition of IL-4 production for the first time. Thus, we suggested that miquelianin may be able to ameliorate various allergic disorders, such as AR, allergic asthma, atopic dermatitis, and food allergies. In further studies, we will investigate the anti-allergic mechanism of miquelianin on a variety of allergic diseases.

The IC_{50} of each compound for IL-4 inhibition was 1.54 μg/ml (ellagic acid) < 3.68 μg/ml (isoquercitrin) < 14.2 μg/ml (miquelianin) < 115 μg/ml (hyperoside). In RMFE, however, each component was not included in sufficient concentration to inhibit IL-4 production (e.g. the miquelianin was included in 0.13% in RMFE). Nevertheless, the RMFE was effective enough to inhibit IL-4 levels. Thus, we assumed that these components exhibited synergistic effects in the RMFE.

Conclusion

Collectively, the present study has demonstrated that oral administration of RMFE at a high-dose (400 mg/kg) significantly attenuated nasal inflammation in an OVA-induced AR murine model by suppressing accumulation of inflammation cells, such as eosinophils, macrophages, and mast cells and by ameliorating goblet cell hyperplasia in nasal mucosa. Further, the immunological protective effect of RMFE was found to be through regulating the imbalance of Th1, Th2-related cytokines and inhibiting expression of serum anti-OVA IgE, and anti-OVA IgG1 levels. Furthermore, we investigated four active compounds of RMFE, including ellagic acid, hyperoxide, isoquercitrin, and miquelianin, that may attenuate the allergic responses by inhibiting Th2-related cytokines. These results suggest that RMFE may be a promising therapeutic agent for AR via controlling Th1/Th2 cytokine productions.

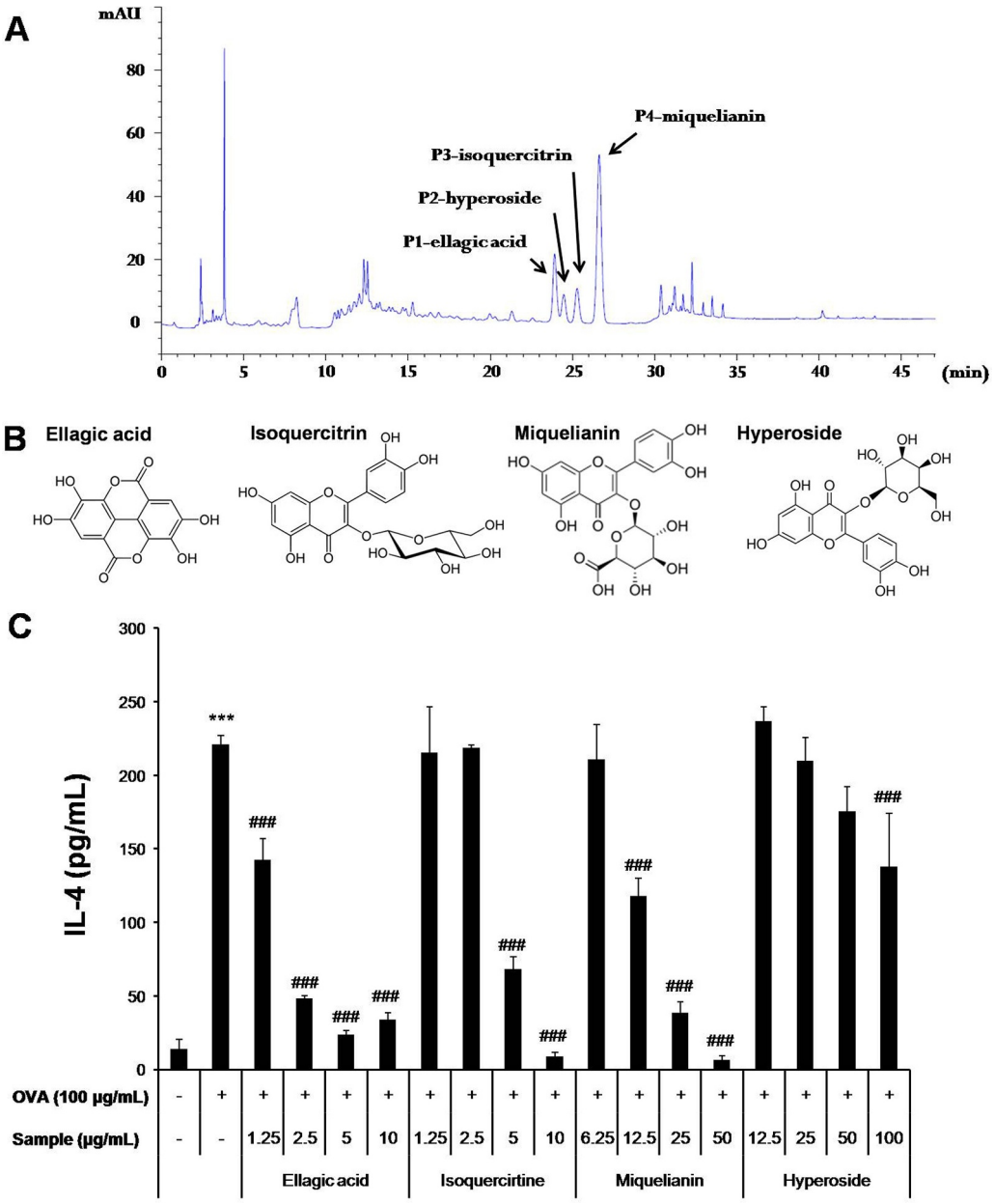

Fig. 8. HPLC chromatograms of RMFE compounds and effects on OVA-induced IL-4 production in splenocytes (A) The pattern of RMFE: P1, ellagic acid; P2, hyperoxide; P3, isoquercitrin; and P4, miquelianin. (B) Chemical structure of RMFE compounds. (C) The effects of four compounds on OVA-induced IL-4 production in splenocytes. All of four components inhibited IL-4 secretion. The values represent the mean ± S.E.M (n = 6/group). Significant differences at $^{***}p < 0.001$ compared with the Naive group. $^{###}p < 0.001$ compared with the OVA group.

Acknowledgments

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

Alvarez, J., Surs, W., Leung, D.Y., Ikle, D., Gelfand, E.W., Szefler, S.J., 1992. Steroid-resistant asthma: immunologic and pharmacologic features. J. Allergy Clin. Immunol. 89, 714–721.

Alves Cde, F., Angeli, G.N., Favarin, D.C., de Andrade, E.L., Chica, J.E., Faccioli, L.H., da Silva, P.R., Rogerio Ade, P., 2013. The effects of proresolution of ellagic acid in an experimental model of allergic airway inflammation. Mediat. Inflamm., 863198 2013.

Bachert, C., Jorissen, M., Bertrand, B., Khaltaev, N., Bousquet, J., 2008. Allergic Rhinitis and its impact on asthma update (ARIA 2008). Belg. Persp. B-ENT 4, 253–257.

Billiau, A., Matthys, P., 2009. Interferon-gamma: a historical perspective. Cytokine Growth Factor Rev. 20, 97–113.

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, 466–476.

Cheng, B.C., Yu, H., Guo, H., Su, T., Fu, X.Q., Li, T., Cao, H.H., Tse, A.K., Wu, Z.Z., Kwan, H.Y., Yu, Z.L., 2016. A herbal formula comprising Rosae Multiflorae Fructus and

Lonicerae Japonicae Flos, attenuates collagen-induced arthritis and inhibits TLR4 signalling in rats. Sci. Rep. 6, 20042.
Eigenmann, P.A., 2002. T lymphocytes in food allergy: overview of an intricate network of circulating and organ-resident cells. Pediatr. Allergy Immunol. 13, 162–171.
Fernandez, J., Reyes, R., Ponce, H., Oropeza, M., Vancalsteren, M.R., Jankowski, C., Campos, M.G., 2005. Isoquercitrin from Argemone platyceras inhibits carbachol and leukotriene D4-induced contraction in guinea-pig airways. Eur. J. Pharmacol. 522, 108–115.
Fulkerson, P.C., Zimmermann, N., Brandt, E.B., Muntel, E.E., Doepker, M.P., Kavanaugh, J.L., Mishra, A., Witte, D.P., Zhang, H., Farber, J.M., Yang, M., Foster, P.S., Rothenberg, M.E., 2004. Negative regulation of eosinophil recruitment to the lung by the chemokine monokine induced by IFN-gamma (Mig, CXCL9). Proc. Natl. Acad. Sci. U. S. A. 101, 1987–1992.
Gabor, M., 1986. Anti-inflammatory and anti-allergic properties of flavonoids. Prog. Clin. Biol. Res. 213, 471–480.
Galli, S.J., Tsai, M., Piliponsky, A.M., 2008. The development of allergic inflammation. Nature 454, 445–454.
Ha, E.V., Rogers, D.F., 2016. Novel therapies to inhibit mucus synthesis and secretion in airway hypersecretory diseases. Pharmacology 97, 84–100.
Hirano, T., Kawai, M., Arimitsu, J., Ogawa, M., Kuwahara, Y., Hagihara, K., Shima, Y., Narazaki, M., Ogata, A., Koyanagi, M., Kai, T., Shimizu, R., Moriwaki, M., Suzuki, Y., Ogino, S., Kawase, I., Tanaka, T., 2009. Preventative effect of a flavonoid, enzymatically modified isoquercitrin on ocular symptoms of Japanese cedar pollinosis. Allergol. Int. 58, 373–382.
Huang, T.K., Ding, Z.Z., Zhao, S.X., 2001. Compendium of Modern Material Medical China Mecico Pharmaceutical Science &Technology. Publishing House, Beijing, pp. 2473–2474.
Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., Sudo, K., Nakae, S., Sasakawa, C., Iwakura, Y., 2009. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30, 108–119.
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, 254–260.
Kim, Y.H., Park, C.S., Lim, D.H., Son, B.K., Kim, J.H., Ahn, S.H., Bochner, B.S., Na, K., Jang, T.Y., 2013. Antiallergic effect of anti-Siglec-F through reduction of eosinophilic inflammation in murine allergic rhinitis. Am. J. Rhinol. Allergy 27, 187–191.
Ku, S.K., Zhou, W., Lee, W., Han, M.S., Na, M., Bae, J.S., 2015. Anti-inflammatory effects of hyperoside in human endothelial cells and in mice. Inflammation 38, 784–799.
Lee, M.Y., Shin, I.S., Lim, H.S., Shin, H.K., 2012. A water extract of Samchulkunbi-tang attenuates airway inflammation by inhibiting inos and MMP-9 activities in an ovalbumin-induced murine asthma model. BMC Complement. Altern. Med. 12, 257.
Lipworth, B.J., Jackson, C.M., 2000. Safety of inhaled and intranasal corticosteroids: lessons for the new millennium. Drug. Saf. 23, 11–33.
Luo, H.R., Loison, F., 2008. Constitutive neutrophil apoptosis: mechanisms and regulation. Am. J. Hematol. 83, 288–295.
Ma, C., Ma, Z., Liao, X.L., Liu, J., Fu, Q., Ma, S., 2013. Immunoregulatory effects of glycyrrhizic acid exerts anti-asthmatic effects via modulation of Th1/Th2 cytokines and enhancement of CD4(+)CD25(+)Foxp3+ regulatory T cells in ovalbumin-sensitized mice. J. Ethnopharmacol. 148, 755–762.
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, 1007–1013.
Mauad, T., Bel, E.H., Sterk, P.J., 2007. Asthma therapy and airway remodeling. J. Allergy Clin. Immunol. 120, 997–1009 quiz 1010-1001.
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, 279–286.
Pawankar, R., Mori, S., Ozu, C., Kimura, S., 2011. Overview on the pathomechanisms of allergic rhinitis. Asia Pac Allergy 1, 157–167.
Quan, S.H., Zhang, Y.L., Han, D.H., Iwakura, Y., Rhee, C.S., 2012. Contribution of interleukin 17A to the development and regulation of allergic inflammation in a murine allergic rhinitis model. Ann. Allergy Asthma Immunol. 108, 342–350.
Roever, A.C., Heine, G., Zuberbier, T., Worm, M., 2003. Allergen-mediated modulation of CD23 expression is interferon-gamma and interleukin-10 dependent in allergic and non-allergic individuals. Clin. Exp. Allergy 33, 1568–1575.
Rogerio, A.P., Kanashiro, A., Fontanari, C., da Silva, E.V., Lucisano-Valim, Y.M., Soares, E.G., Faccioli, L.H., 2007. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflamm. Res. 56, 402–408.
Skoner, D.P., 2001. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. J. Allergy Clin. Immunol. 108, S2–S8.
Song, C.H., Bui, T.T., Piao, C.H., Shin, H.S., Shon, D.H., Han, E.H., Kim, H.T., Chai, O.H., 2016. Rosae multiflorae fructus hot water extract inhibits a murine allergic asthma via the suppression of Th2 cytokine production and histamine release from mast cells. J. Med. Food. 19, 853–859.
Soyka, M.B., Wawrzyniak, P., Eiwegger, T., Holzmann, D., Treis, A., Wanke, K., Kast, J.I., Akdis, C.A., 2012. Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN-gamma and IL-4. J. Allergy Clin. Immunol. 130, 1087–1096 e1010.
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, 161–176.
Takahashi, Y., Kagawa, Y., Izawa, K., Ono, R., Akagi, M., Kamei, C., 2009. Effect of histamine H4 receptor antagonist on allergic rhinitis in mice. Int. Immunopharmacol. 9, 734–738.
Takagi, S., Yamaki, M., Masuda, K., Kubota, M., 1976a. On the constituents of the fruits of *Rosa multiflora* Thunb. I. Yakugaku Zasshi 96, 284–288.
Takagi, S., Yamaki, M., Masuda, K., Kubota, M, 1976b. On the constituents of the fruits of *Rosa multiflora* Thunb. II. Yakugaku Zasshi 96, 1217–1222.
Wallace, D.V., Dykewicz, M.S., Bernstein, D.I., Blessing-Moore, J., Cox, L., Khan, D.A., Lang, D.M., Nicklas, R.A., Oppenheimer, J., Portnoy, J.M., Randolph, C.C., Schuller, D., Spector, S.L., Tilles, S.A., 2008. The diagnosis and management of rhinitis: an updated practice parameter. J. Allergy Clin. Immunol. 122, S1–84.
Ye, P., Yang, X.L., Chen, X., Shi, C., 2017. Hyperoside attenuates OVA-induced allergic airway inflammation by activating Nrf2. Int. Immunopharmacol. 44, 168–173.
Yuan, Z., Luan, G., Wang, Z., Hao, X., Li, J., Suo, Y., Li, G., Wang, H., 2017. Flavonoids from Potentilla parvifolia Fisch. and their Neuroprotective effects in Human Neuroblastoma SH-SY5Y Cells *in vitro*. Chem. Biodivers. 14.
Zheleva-Dimitrova, D., Nedialkov, P., Girreser, U., Kitanov, G., 2012. Benzophenones and flavonoids from Hypericum maculatum and their antioxidant activities. Nat. Prod. Res. 26, 1576–1583.
Zhou, E., Fu, Y., Wei, Z., Yang, Z., 2014. Inhibition of allergic airway inflammation through the blockage of NF-kappaB activation by ellagic acid in an ovalbumin-induced mouse asthma model. Food. Funct. 5, 2106–2112.
Zhu, Z., Homer, R.J., Wang, Z., Chen, Q., Geba, G.P., Wang, J., Zhang, Y., Elias, J.A., 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J. Clin. Invest. 103, 779–788.