

# Antiallergic effect of *Ostericum koreanum* root extract on ovalbumin-induced allergic rhinitis mouse model and mast cells

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## Summary

**Objective:** In the present study, the anti-allergic effect of OR extract was evaluated on an ovalbumin (OVA)-induced allergic rhinitis in mice and rat peritoneal mast cells (RPMC).

**Methods:** Balb/c mice were systemically sensitized to OVA followed by intraperitoneal and nasal allergen challenges. We investigated the effect of OR extract on allergic symptoms, serological marker production and histological changes of the nasal mucosa in a mouse model of allergic rhinitis. We observed mast cell degranulation and detected the production of histamine and inflammatory cytokines by ELISA.

**Results:** Compared to the OVA-control group, oral administration of OR extract at doses of 50 and 100 mg/kg significantly ( $p < 0.001$ ) decreased the serum levels of histamine, OVA-specific IgE and Th2 cytokine, IL-4 as well as increasing Th1 cytokine, IFN- $\gamma$ . Oral administration of OR extract also attenuated disease progression as determined by nasal symptoms and histological changes of the nasal mucosa in OVA-sensitized mice. Furthermore, treatment with OR extract at doses of 0.2, 0.5 and 1 mg/mL in RPMC significantly ( $p < 0.01$ ,  $p < 0.001$  and  $p < 0.001$ , respectively) decreased compound 48/80-induced histamine release and suppressed mast cell degranulation. Treatment with OR extract in RPMC also inhibited PMA/A23187-induced production of

inflammatory cytokines such as TNF- $\alpha$  and IL-6. The mechanism of action underlying OR extract in allergic inflammation appears to be inhibition of the phosphorylation of ERK1/2 and p38 MAPK, in addition to blocking of the NF $\kappa$ B pathway.

**Conclusions:** These results indicate that OR extract has the potential to be a source of anti-allergic agents for use in allergen and/or mast cell-mediated diseases including allergic rhinitis. (*Asian Pac J Allergy Immunol* 2011;29:338-48)

**Key words:** Antiallergy, allergic rhinitis, mast cell, *Ostericum koreanum*, ovalbumine

## Introduction

Allergic rhinitis (AR) is characterized by inflammation of the nasal mucosa with hypersensitivity resulting from seasonal or perennial responses to specific environmental allergens and it exhibits symptoms like nasal rubbing, sneezing, rhinorrhea, lacrimation, nasal congestion and obstruction and, less frequently, cough.<sup>1,2</sup> AR is frequently associated with chronic airway diseases, such as bronchial asthma, which is also caused by hypersensitivity to antigens resulting in greater local inflammation as well as bronchoconstriction, vasomotor change and mucus hypersecretion.<sup>3</sup> Among immune cells, the mast cell play a critical role in the pathogenesis of allergic responses in AR. Mast cells express the immunoglobulin Fc epsilon receptor I (Fc $\epsilon$ RI) that triggers specific antigens to IgE. After IgE-dependent stimulation, mast cells release allergic mediators such as histamine,  $\alpha$ -hexosaminidase, cytokines, chemokines and arachidonic acid derivatives, mediating acute and chronic inflammation.<sup>4</sup> Therefore, treatment options for AR consist of allergen avoidance, symptomatic treatment and allergen immunotherapy to further improve the control of allergic responses.<sup>5,6</sup> A better therapeutic agent is required, as the current treatment of AR is limited to antihistamines, nasal corticosteroids, antileukotrienes, and antiallergen

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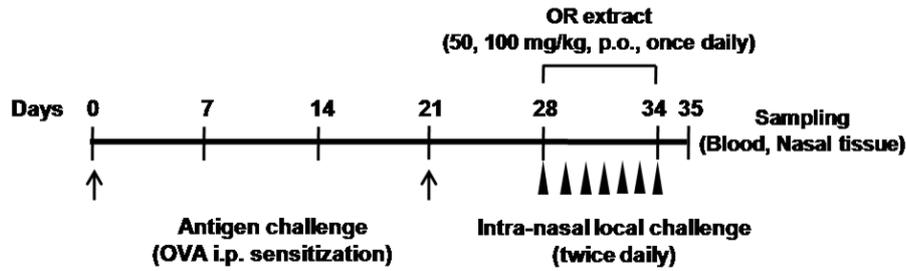
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**Figure 1.** Schematic diagram of ovalbumin (OVA)-induced allergic rhinitis mouse model.

immunotherapy. Since these therapies are still not perfect, it is important to continue to study the pharmacology of this disease as part of the search for better drugs.<sup>7,8</sup>

Recently, the rising interest in medicinal plants and their bioactive compounds for treatment of allergic diseases has led to increased attention of their potential safety and efficacy. Of them, the roots of *Ostericum koreanum* Maximowicz (*Osterici Radix*, Umbelliferae) have traditionally been used as herbal medicine to treat colds, headache, neuralgia and arthralgia in the past.<sup>9,10</sup> *Osterici Radix* (OR) is also known to have various pharmacological effects such as antimicrobial,<sup>11</sup> antioxidant,<sup>12</sup> antiinflammatory<sup>13</sup> and antitumor activities.<sup>14</sup> However, little is known about the pharmacological effect of *Osterici Radix* on allergic responses.

In this study, we evaluated an *Osterici Radix* extract on allergic responses in ovalbumin-induced allergic rhinitis mice and on mast cell-mediated allergic inflammatory reactions in rat peritoneal mast cells. We further identified the mechanism of action of this extract as the MAPK and NFκB signaling pathways in mast cells.

## Methods

### Plant material

Roots of *O. koreanum* (*Osterici Radix*) were collected from Bongwha Alpine Medicinal Plant Experiment Station (BAMPES; Gyeongbook, South Korea) in October 2007 and authenticated by Professor Y.-K. Park, a medical botanist in the Department of Herbology, College of Oriental Medicine, Dongguk University. A voucher specimen was deposited at the Herbarium of BAMPES, with the registration number, NK-2007-10. *Osterici Radix* (OR; 1.5 kg) was cut into small pieces, pulverized and extracted three times with 4.5 L of methanol at room temperature for 24 h. The supernatant was collected by filtration and the solvent was evaporated under vacuum at 40°C (yield; 155 g).

The lyophilized OR extract was dissolved in saline solution to create a stock.

### Animals

Male Balb/c mice (10 weeks old) were purchased from SLC, Inc. (Shizuoka, Japan). They were housed for 1 week in a room with controlled temperature and humidity and given free access to food and water before the experiments were performed. The mice were treated according to the ethical guidelines of the Animal Center, Dongguk University. The Animal Studies Committee of Dongguk University approved the experimental protocol.

### OVA-induced allergic reaction in mice

Male Balb/c mice (10 weeks old) were divided into four groups and given free access to a standard laboratory diet and water during the experimental period. As shown Figure 1, OVA solution (0.5 mg/ml in saline) and Al(OH)<sub>3</sub> (20 mg/mL in saline) were mixed in a 1:1 ratio (v/v) and administered by intraperitoneal injection at a dose of 0.1 mL/mouse. For the second immunization, an OVA solution (0.4 mg/mL in saline; antigen challenge) was injected into a footpad (0.02 mL/mouse) on day 21. In addition, a local challenge was performed twice daily everyday from day 28 to a day 34 by administration of an OVA solution (4 mg/mL in saline) into both nasal cavities using a micropipette. OR extract (50 and 100 mg/kg, once daily) was administrated orally once daily at doses of 50 or 100 mg/kg of body weight for 7 consecutive days to the OVA-sensitized mice until the assay date. Normal and OVA-sensitized control mice were given saline solution alone according to the same schedule.

Blood samples were taken from each mouse by cardiac puncture under anesthesia with isoflurane 24 h after the oral administration of OVA, and the mice were then killed. Serum was prepared and frozen at -70°C prior to analysis. Nasal tissue was removed and examined for histopathological changes.

**Serological markers in sera**

The serum was analyzed for the detection of OVA-specific IgE and cytokines (IL-4 and IFN- $\gamma$ ) using a commercial available ELISA kits (R&D Systems Inc. MN, USA) according to the manufacturer's instructions.

**Evaluation of Nasal symptoms**

On day 34 of treatment, the animals were placed into an observation cage for about 10 min for acclimatization. Then, after nasal instillation of 20  $\mu$ L of OVA (4 mg/mL in saline) into the bilateral nasal cavities, the animals were placed into observation cages (60x40x30 cm, one animal/cage), and the numbers of sneezes and nasal rubbing movements were counted for 30 min by two blinded observers who were unaware of sample identity.<sup>15</sup>

**Preparation of rat peritoneal mast cells**

Primary mast cells were harvested from a peritoneal lavage of male Wister rats (Japan SLC Ltd.) as previously described<sup>16</sup> and purified to greater than 90% purity by centrifugation on a percoll density gradient. The collected rat peritoneal mast cells (RPMC) were washed with Tyroid buffer (NaCl 137 mM, KCl 2.7 mM, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O 0.4 mM, MgCl<sub>2</sub>·6H<sub>2</sub>O 1 mM, NaHCO<sub>3</sub> 12 mM, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.8 mM) and then incubated with 0.5 mL thyroid buffer in culture plates (2x10<sup>5</sup> cells/mL) for further experiments. RPMC preparations were approximately 95% pure as assessed by toluidine blue staining and at least 98% of these cells were viable as assessed by trypan blue exclusion.

**RPMC viability assay**

The cell viability was determined by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich Inc., St. Louis, MO, USA) reduction assay. Briefly, RPMC were incubated overnight in 24-well plates at a density of 2x10<sup>5</sup> cells per well, and then washed with phosphate-buffered saline (PBS). The cells were treated with different concentrations (0.2 ~1 mg/mL) of OR extract for 24 h and then exposed to MTT (0.5 mg/mL) at 37°C for 4 h. After the culture supernatants were removed, the resulting dark blue crystals were dissolved with dimethyl sulfoxide (DMSO). Absorbance values were read at 540 nm on an automated SpectraMAX 340 (Molecular Devices, Sunnyvale, CA, USA). All determinations were confirmed by replication in at least three independent experiments.

**RPMC degranulation and histamine assays**

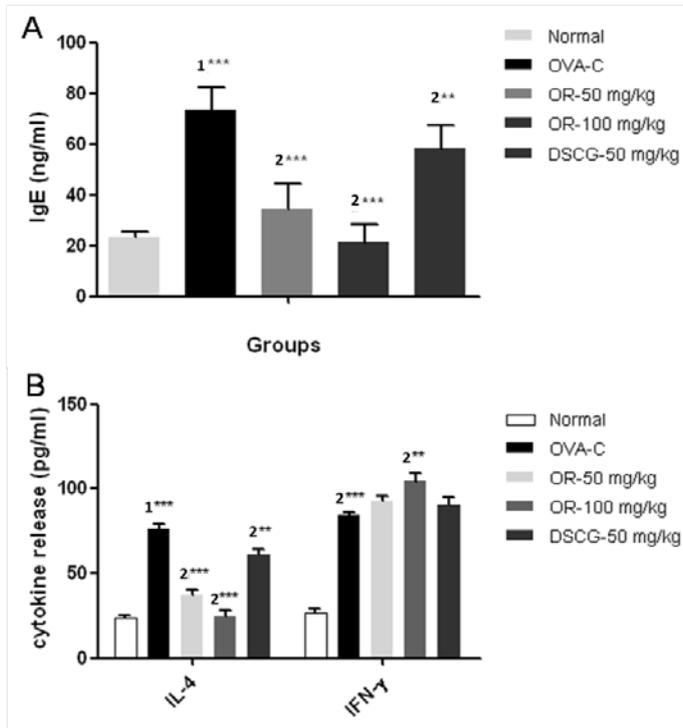
RPMC was preincubated with different concentrations (0.2~1 mg/mL) of OR extract at 37°C for 30 min, and then incubated with compound 48/80 (final concentration, 0.5  $\mu$ g/mL) at 37°C for 15 min. After incubation, the cells were fixed in 2% glutaldehyde, stained with toluidine blue (0.1% w/v, pH 3.0), placed between slides, cover slips, and observed under a high-power microscope. After incubation, the amount of histamine in the culture supernatant was also measured by a commercially available histamine enzyme immunoassay (EIA) kit (Cayman Chemical Co., MG, USA). The inhibition percentage of histamine release was calculated as previously described (Song et al., 2010).

**Cytokine assays**

The concentration of cytokines, TNF- $\alpha$  and IL-6 in each culture supernatant was measured using commercially available ELISA kits (eBioscience, San Diego, CA, USA). In brief, RPMC (2x10<sup>5</sup>/mL) was preincubated with different concentrations (0.2~1 mg/mL) of OR extract for 30 min and then incubated with phorbol 12-myristate 13-acetate (PMA; 25 nM) and calcium ionophore A23187 (1  $\mu$ M) for 24 h. The cell culture supernatant was collected and ELISA assays were performed. The cytokine concentrations were calculated according to a standard curve using each of the recombinant cytokines in the ELISA kits.

**Reverse transcription-polymerase chain reaction (RT-PCR) analysis**

RT-PCR for the detection of the expression of cytokine mRNA was performed. Briefly, after PMA/A23187 stimulation for 6 h, the total RNA was isolated using Trizol™ (GibcoBRL) and reverse transcribed into cDNA. Each transcript was identified using specific forward and reverse primers as per the manufacturer's instructions (Promega, Madison, WI, USA). GAPDH expression was included as an internal, housekeeping control gene. Ethidium bromide-stained reaction products were separated by electrophoresis on a 1% agarose gel in 1 x TAE and visualized by UV transillumination. Images were captured by a Kodak EDAS 290 camera system (Kodak, Rochester, NY, USA). The primers used in these experiments were designed to span introns, thereby allowing differentiation between amplified genomic DNA and cDNA PCR products. Primer sequences were as follows: GAPDH as an internal control for PCR, 5'-GAC ATC ATA CTT GGC AGG-3' (sense), 5'-CTC GTG



**Figure 2.** Effects of OR extract on serum levels of OVA-specific IgE and cytokines in OVA-sensitized mice. A. IgE and B. IL-4 and IFN- $\gamma$  were measured in sera of the mice by ELISA. Three independent experiments were performed ( $n=10$  per group), and the data shown indicate the means  $\pm$  SDs. A difference was considered statistically significant when  $*P<0.05$ ,  $**P<0.01$  and  $***P<0.001$  vs. normal<sup>1</sup> or OVA-sensitized control group<sup>2</sup>. OVA-C, OVA control group; OR, OR extract-administrated group; and DSCG, sodium cromoglycate-administrated group.

GAG TCT ACT GGT-3' (anti-sense); TNF- $\alpha$ , 5'-TAG CCC ACG TCG TAG CAA AC-3' (sense), 5'-CTG GGG GCT CTG AGG AGT AG-3' (anti-sense); and IL-6, 5'-AAC CTT TCC AAA GAT GGC TGA A-3' (sense), 5'-CAG GAA CTG GAT CAG GAC TTT-3' (anti-sense). The band intensity was quantified by densitometric analysis (Digital Image Analysis System; GenoMx<sup>TM</sup> Vision, BioGenex, San Ramon, CA).

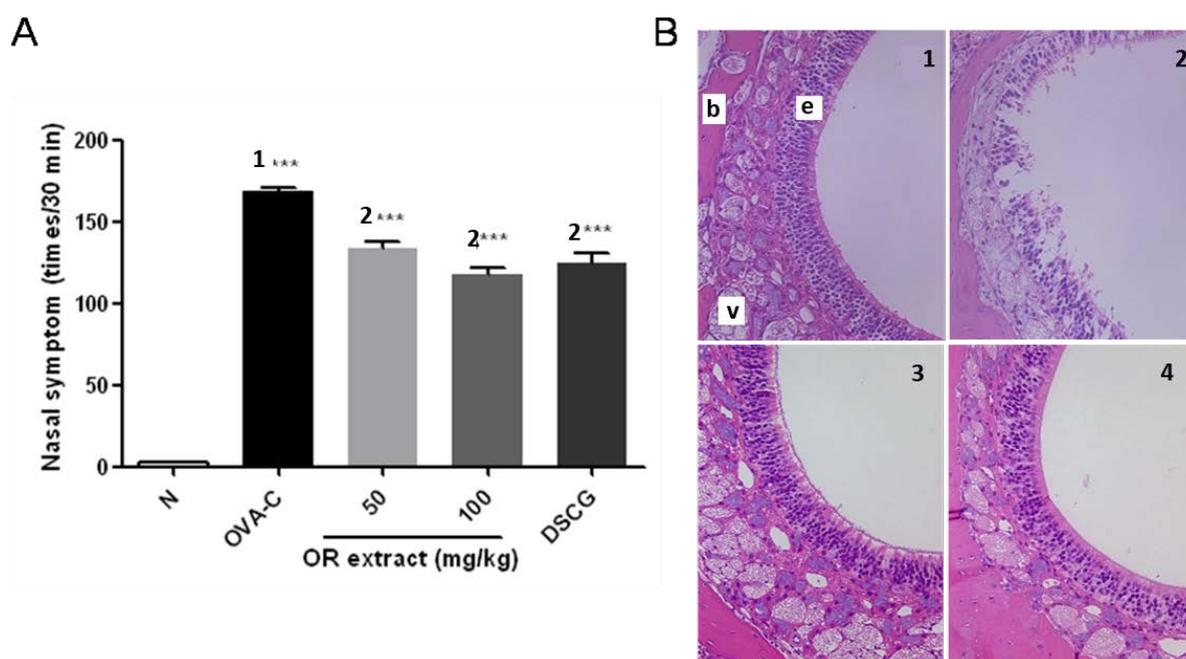
#### Western blot analysis

After PMA/A23187 stimulation for 30 min, cells were washed twice with ice-cold PBS, scraped off with a rubber policeman, and centrifuged at 5,000 rpm for 5 min at 4°C. Cell pellets were re-suspended in an appropriate volume of lysis buffer (1% Triton X-100, 1% deoxycholate and 0.1% NaN<sub>3</sub>) and incubated for 30 min on ice. Lysates were then

centrifuged at 12,000 rpm for 10 min at 4°C and collected for further analysis. For nuclear extraction, cells were washed twice with cold PBS and lysed with NE-PER<sup>TM</sup> nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL, USA). After centrifugation at 12,000 rpm for 10 min, the supernatant was stored at -80°C until use. Protein concentrations of samples were determined by Bradford assay (Bio-Rad, Hemel, Hempstead, UK) using samples equilibrated to 2 mg/mL with lysis buffer. Fifty  $\mu$ g of protein was separated on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA). Each membrane was incubated for 1 h with 5% skimmed milk in TBS-T buffer (0.1 M Tris-HCl [pH 7.4], 0.9% NaCl, 0.1% Tween-20) to block non-specific binding, and was then incubated with primary antibodies that recognized  $\beta$ -actin (1:5000, Sigma-Aldrich), and the phospho- or total forms of MAPKs (1:1000, Cell Signaling Technology), I $\kappa$ B $\alpha$  and p65 NF $\kappa$ B (1:1000, Cell Signaling Technology). Each protein was detected by using a chemiluminescence detection system according to the instructions of the manufacturer (ECL, Amersham, Beshire, UK). The band intensity was quantified by densitometric analysis (Digital Image Analysis System; GenoMx<sup>TM</sup> Vision, BioGenex, San Ramon, CA).

#### HPLC analysis

HPLC analysis for identification of the standards was performed on a Waters HPLC system (Dionex Corp., CA, USA) equipped with 486 UV detector and Varian column oven, 717 autosampler. A sample (100 mg) was dissolved in 1 mL of 100% methanol for pattern analysis using an HPLC system (Dionex Corp.) with a photodiode array detector. Chromatographic separation was carried out using an Akso Nobel, KT100- 5C<sub>18</sub> column (4.6 mm  $\times$  250 mm, 5  $\mu$ m; Waters) at room temperature with an injection volume of 10  $\mu$ L with the following solvent ratios for the mobile phase, where solvent A is 35% methanol in water containing 0.05% formic acid and solvent B is 65% methanol in water; A : B = 100 : 0 (0 min)  $\rightarrow$  60 : 40 (30 min)  $\rightarrow$  25 : 75 (30 min)  $\rightarrow$  75 : 25 (5 min)  $\rightarrow$  0 : 100 (10 min). The detection wavelength was 254 nm with a flow of 1 mL/min at 30°C. Peak analysis and assignments were performed using standards including oxypeudandin, bisabolangelone, imperatorin, osthol and isoimperatorin, which were identified in accordance with their UV spectra and retention time on the HPLC chromatogram.



**Figure 3.** Effects of OR extract on clinical scores and histological changes of nasal mucosa in OVA-sensitized mice. A. Nasal symptoms were measured by clinical scoring. Three independent experiments were performed ( $n=10$  per group) and the data shown indicate the means  $\pm$  SDs. A difference was considered statistically significant when  $^*P<0.05$ ,  $^{**}P<0.01$  and  $^{***}P<0.001$  vs. normal<sup>1</sup> or OVA-sensitized control group<sup>2</sup>. OVA-C, OVA control group; and DSCG, sodium cromoglycate-administrated group. B. The histological changes of nasal mucosa were observed by microscope after H&E staining. 1. normal, 2. OVA-C, 3. OR extract-administrated group, and 4. DSCG-administrated group. b, bone; v, blood vessel; and e, epithelium.

### Statistical analysis

All statistical analyses were performed using GraphPad Prism 4.0a for the Macintosh (GraphPad Software, San Diego, CA, USA). Data include the means  $\pm$  SDs of three samples and are representative of three independent experiments. Differences between two means were analyzed by a Student's *t*-test. Values of  $p < 0.05$  were determined to be significant.

### Results

#### Effect of OR extract on serological marker levels in OVA-sensitized mice

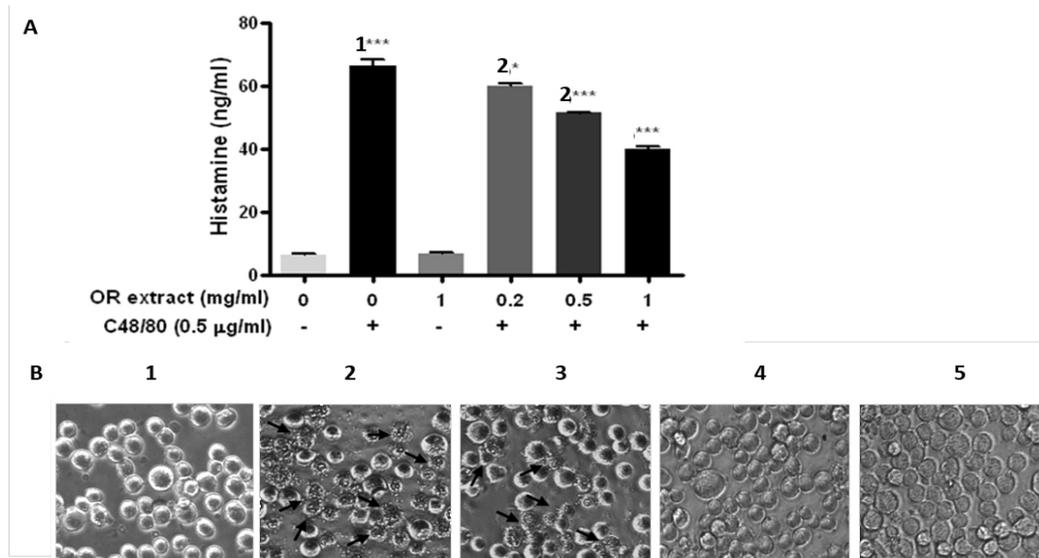
To demonstrate the effect of OR extract on OVA-induced allergic responses in mice, the levels of OVA-specific IgE, IL-4 and IFN- $\gamma$  were measured in sera of OVA-sensitized mice by ELISA. OVA-specific IgE levels were increased by sensitization (Figure 2A). Oral administration of OR extract significantly inhibited OVA-specific IgE production in the OVA-sensitized mice ( $p < 0.001$ ) as compared to control mice given OVA alone. Further, the levels

of the Th2-type cytokine, IL-4 was significantly increased in sera of OVA-sensitized mice ( $p < 0.001$ ), while the production of the Th1 cytokine, IFN- $\gamma$  was significantly decreased compared with that for normal mice ( $p < 0.001$ ; Figure 2B). Oral administration of OR extract at a dose of 100 mg/kg significantly inhibited IL-4 production ( $p < 0.001$ ), compared with OVA-sensitized mice, and enhanced IFN- $\gamma$  production ( $p < 0.01$ ).

#### Effect of OR extract on nasal symptom changes in OVA-sensitized mice

We evaluated the effect of OR extract on nasal symptoms by scoring the numbers of sneezing and nasal rubbing movements for 30 min in OVA-sensitized mice. OR extract significantly inhibited the nasal symptom at doses of 50 and 100 mg/kg ( $p < 0.001$ ; Figure 3A). Similar results were obtained with sneezing.

We next investigated the effect of OR extract on histological changes in the nasal mucosa by evaluation of H&E stained tissue sections. In the OVA-sensitized control mice, histological observation revealed epithelial disruption, mucosa edema, goblet



**Figure 4.** Effects of OR extract on histamine release and degranulation in compound 48/80-stimulated peritoneal mast cells. A. Histamine concentrations were measured in culture medium by ELISA. Three independent experiments were performed, and the data shown indicate the means  $\pm$  SDs. A difference was considered statistically significant when  $^*P < 0.05$ ,  $^{**}P < 0.01$  and  $^{***}P < 0.001$  vs. normal<sup>1</sup> or compound 48/80 (C48/80) alone<sup>2</sup>. B. Mast cell degranulation (arrow) was observed by microscope 15 min after compound 48/80 treatment (x100). 1. normal, 2. C48/80 alone, 3. C48/80+OR extract 0.2 mg/ml, 4. C48/80+OR extract 0.5 mg/ml, and 5. C48/80+OR extract 1 mg/ml.

cell hyperplasia in the nasal mucosa (Figure 3B). However, OR extract at doses of 50 and 100 mg/kg protected nasal mucosa against damage and greatly reduced mucosa edema, especially the OR extract at dose of 100 mg/kg.

#### **Effect of OR extract on compound 48/80-induced histamine release in RPMC**

To evaluate the effect of OR extract on mast cell-mediated allergic responses, we studied compound 48/80 or PMA/A23187-treated peritoneal mast cells. In compound 48/80-treated mast cells, histamine release significantly increased as compared to normal cells. However, OR extract significantly decreased compound 48/80-induced release of histamine ( $p < 0.01$ ; Figure 4).

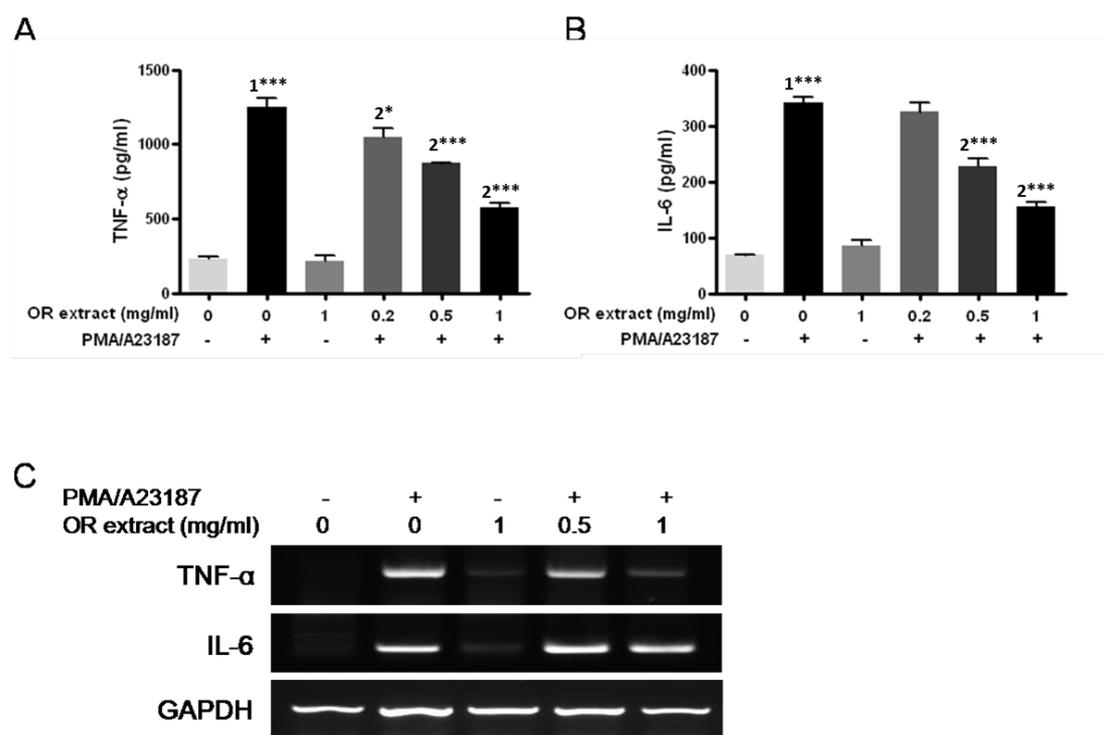
We next observed the degranulation in peritoneal mast cells under the microscope. Figure 4B shows that the cytoplasm of normal mast cells is dominated by closely packed secretory granules. Mast cells stimulated with compound 48/80 clearly showed extensive degranulation processes and the presence of multiple granules extruding from the cells. However, in mast cells treated with OR extract at doses of 0.5 and 1 mg/ml for 15 min prior to stimulation with compound 48/80, there was inhibition of this degranulation and their morphology was similar to that of normal cells.

#### **Effect of OR extract on PMA/A23187-induced cytokine release in RPMC**

Additionally, we examined the effects of OR extract on the secretion and mRNA expression of inflammatory cytokines, such as TNF- $\alpha$  and IL-6 in peritoneal mast cells using ELISA and RT-PCR. PMA/A23187-induced release of TNF- $\alpha$  and IL-6 was significantly decreased by OR extract at concentrations of 0.2, 0.5 and 1 mg/mL in peritoneal mast cells (Figure 5A,B). Moreover, OR extract suppressed PMA/A23187-induced mRNA expression of both cytokines (Figure 5C). These results suggest that OR extract had immunosuppressive effects on mast cells and meaningfully inhibited the antigen-induced mRNA expression and production of inflammatory cytokines related to allergic reactions.

#### **Effect of OR extract on MAPK/NF $\kappa$ B pathways in RPMC**

MAPK and NF $\kappa$ B, are important signaling pathways that control the synthesis and release of pro-inflammatory mediators by activated mast cells during the inflammatory response. Therefore, to investigate the effect of OR extract on signaling pathways in mast cells, the phosphorylation of three MAPK proteins, namely extracellular signal-related kinase 1/2 (ERK1/2), p 38 MAP kinase (p38) and c-Jun NH<sub>2</sub>-terminal kinase (JNK), in addition to nuclear



**Figure 5.** Effects of OR extract on inflammatory cytokine production in PMA/A23187-stimulated peritoneal mast cells. A,B. TNF- $\alpha$  and IL-6 concentrations were measured in culture medium by ELISA. Three independent experiments were performed and the data shown indicate the means  $\pm$  SDs. A difference was considered statistically significant when \* $P$ <0.05, \*\* $P$ <0.01 and \*\*\* $P$ <0.001 vs. normal<sup>1</sup> or PMA/A23187 alone<sup>2</sup>. C. The mRNA levels of each cytokine were measured by RT-PCR. GAPDH expression was used as an internal control. The figures show representative results of three independent experiments.

translocation of NF $\kappa$ B p65 subunit was analyzed by Western blot analysis. PMA/A23187 stimulation rapidly induced the phosphorylation of ERK 1/2, p38 MAPK and JNK within 15 min in peritoneal mast cells (Figure 6A). However, OR extract at a concentration of 1 mg/mL suppressed the phosphorylation of ERK1/2 and p38 MAPK in PMA/A23187-stimulated cells, while the non-phosphorylated forms of the MAPK molecules remained the same. Moreover, the cells treated with OR extract attenuated the phosphorylation of I $\kappa$ B $\alpha$  in the cytosol and the nuclear levels of NF $\kappa$ B p65 subunit in PMA/A23187-stimulated cells. These results indicate the potential role of NF $\kappa$ B in the suppression of inflammatory mediators including inflammatory cytokines by OR extract in allergic inflammatory response by mast cell activation.

#### HPLC analysis

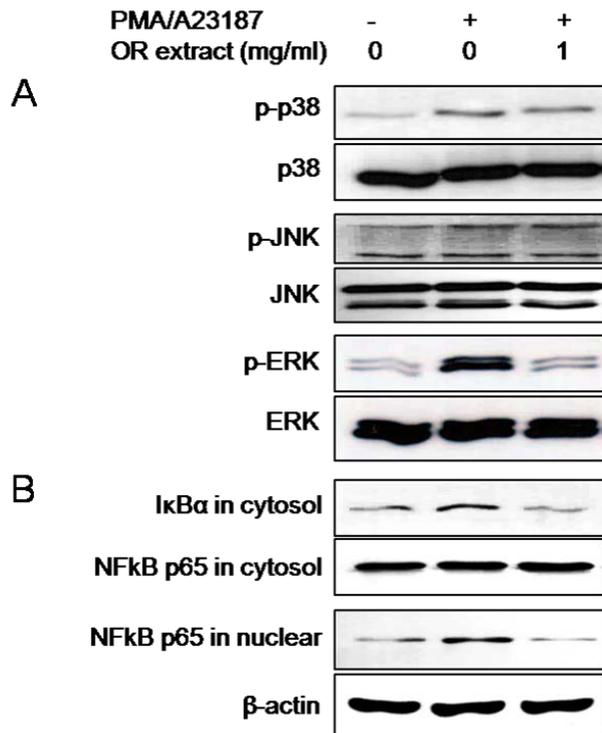
HPLC analysis of OR extract, identified the compounds, bisabolangelone (116.25 mg/g) and isoimperatorin (13.96 mg/g) based on their retention times of 19.2 and 26.0 min, respectively (as compared to retention times of standards) from the

HPLC chromatogram and by their UV spectra (Figure 7).

#### Discussion

Treatment strategies for allergic rhinitis aim at either reducing the effect of local release of chemical mediators from activated mast cells and eosinophils, or the release of mediators from tissue.<sup>17</sup> The most important pharmacological agents used to treat allergic rhinitis include H1 receptor antagonists, corticosteroids, anti-leukotrienes, mast cell stabilizers, decongestants and intranasal anticholinergic agents.<sup>7,8</sup> Although several novel drugs are under development for treatment of allergic rhinitis, problems including side effects and low clinical efficacy remain because many drugs used to manage rhinitis are administered over a period of a few weeks to months.

Dietary supplementation with complementary/alternative medicines including herbal medicines improves multi-factorial diseases including allergic diseases and is considered as a general treatment paradigm for allergic rhinitis.<sup>18</sup> It is of great interest



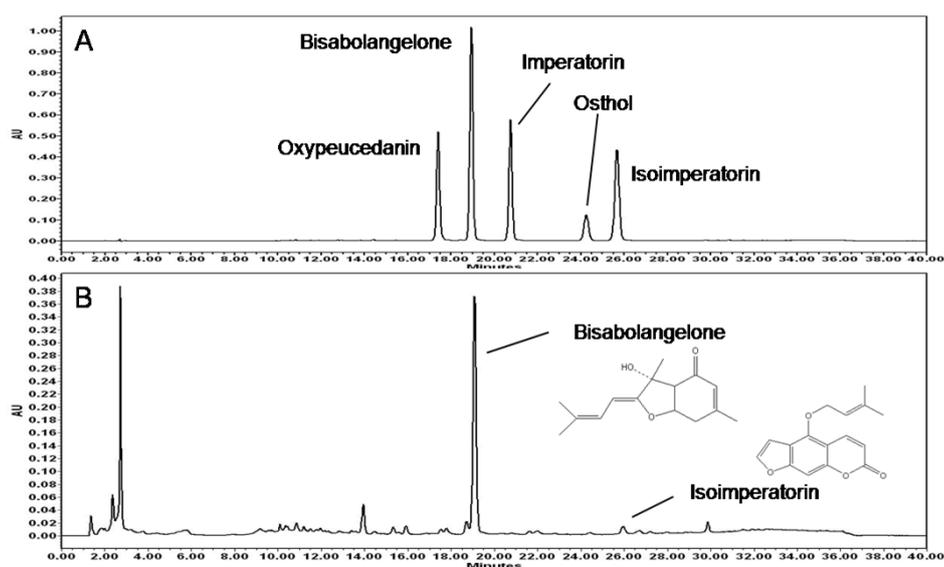
**Figure 6.** Effects of OR extract on MAPKs phosphorylation in PMA/A23187-stimulated peritoneal mast cells. Cells were pretreated with different concentrations of OR extract for 30 min and stimulated with PMA/A23187 for 15 min for MAPKs and for 30 min for NF- $\kappa$ B/I- $\kappa$ B. A. The cellular proteins from the cells were used for the detection of phosphorylated or total forms of three MAPK molecules, ERK1/2, p38 MAPK and JNK. B. NF- $\kappa$ B and I- $\kappa$ B were detected in cytosol or nucleus of the cells. The figures show representative results of three independent experiments. The ratio of mean densitometry analysis for each MAPK molecule was quantified and expressed in bar charts.

to the general public, medical and nutritional experts, and health and food researchers to know the bioactive capacities, including immune modulation and constituents, in the foods and medicines we consume. Among various herbal medicines produced in Traditional Korean Medicine, *Osterici radix* is (KFDA, SFP1-002) one of the most promising candidates with anti-allergic and anti-inflammatory effects in chronic allergic diseases. In the present study, we confirmed that *Osterici radix* extract has a good therapeutic effect on allergic responses in OVA-sensitized/challenged mice and can control mast cell activation via inhibition of histamine release in addition to blocking ERK1/2, p38 MAPK and NF $\kappa$ B pathways.

OVA sensitization and challenge in the present in vivo model led to an increase in histamine and OVA-specific IgE titers in sera, increased IL-4 release in nasal lavage and infiltration of inflammatory cells in the epithelium and sub-epithelium of the nasal mucosa.<sup>17</sup> Hence, the model exhibits the characteristic pathology of rhinitis and its symptoms, such as sneezing, nasal rubbing, congestion and edema. It is believed that nasal symptoms are mediated not only by histamine but also prostaglandins, leukotrienes and typtase from activated mast cells, eosinophils and basophils.<sup>19</sup> In the present study, oral administration of OR extract significantly inhibited rhinitis symptoms including clinical scores, morphological changes of nasal mucosa and histamine release in OVA-sensitized/challenged mice. These results demonstrated that OR extract can improve rhinitis symptoms in a similar way to sodium cromoglycate. Oral administration of sodium cromoglycate (DSCG) used in the treatment of rhinitis and asthma as an anti-inflammatory agent also reduces rhinitis symptoms. Sodium cromoglycate acts by preventing extracellular calcium influx into the cytoplasm of mast cells and maintains their physiological integrity, thereby preventing release of different mediators including histamine.<sup>20</sup> The present study is in agreement with previous studies showing that sodium cromoglycate has a weak effect on compound 48/80-induced scratching behavior in mice or antigen-induced rhinitis symptoms.<sup>21</sup>

Mast cells play a central role in the immediate type of allergic responses through the release of numerous mediators such as histamine and cytokines via de-granulation. Mast cell de-granulation can be elicited by the synthetic compound 48/80 which has been used as a direct reagent to study the mechanism of anaphylactic shock.<sup>22</sup> Therefore, mast cell de-granulation is considered as a major target for effective anti-allergic drugs. In the present study, OR extract attenuated compound 48/80-induced histamine release in peritoneal mast cells by inhibiting their de-granulation. These results demonstrate that OR extract shows marked mast cell stabilization and antihistamine activities against compound 48/80-induced allergic response in mast cells.

The existence of Th1/Th2 subsets in Th lymphocytes is due in part, to their cytokine secretion patterns. Type I allergic responses are



**Figure 7.** HPLC pattern analysis of OR extracts. A. The pattern of standard compounds. B. The pattern of OR extract.

characterized by an elevation of IgE production and mast cell de-granulation involving the release of histamine and other mediators of allergy.<sup>23</sup> Cytokines, such as IL-4, IL-5, IL-6 and TNF- $\alpha$ , enhance IgE synthesis, while IFN- $\gamma$ , IFN- $\alpha$  and transforming factor (TGF)- $\beta$  inhibit IgE production.<sup>23</sup> Therefore, production of IgE is significantly dependent on the ratio in which the cytokines IL-4 and IFN- $\gamma$  are elicited after exposure to an allergen. In this study, OR extract significantly inhibited the production of IL-4 and slightly increased IFN- $\gamma$  release in the sera of OVA-sensitized/challenged mice. These results demonstrate that OR extract can inhibit IgE production by the regulation of IL-4/IFN- $\gamma$  ratio in allergic responses. Osterici radix has been reported to inhibit the production of Th1 cytokines such as IL-4, IL-5 and IL-13 measured in broncho-alveolar lavage (BAL) fluid of OVA-induced allergic asthma mice.<sup>24</sup>

MAPKs, such as ERK1/2, JNK and p38 MAPK, play an important role in regulating cell growth/differentiation as well as cytokine responses.<sup>25</sup> Thus, the expression of inflammatory cytokines, TNF- $\alpha$ , IL-6 and IL-8 is regulated by MAPKs in mast cells. In this study, OR extract significantly inhibited PMA/A23187-induced phosphorylation of ERK1/2 and p38 MAPK which are responsible for the inhibition of inflammatory mediator production in activated mast cells. These results indicate that OR extract has an anti-inflammatory effect through

blocking of the ERK1/2 and p38 MAPK signals which might be involved in the inhibition of inflammatory cytokine production in PMA/A23187-stimulated mast cells. The activation of the NF $\kappa$ B pathway results in the phosphorylation, ubiquitination and proteasome-mediated degradation of the I $\kappa$ B proteins, followed by nuclear translocation.<sup>26</sup> Therefore, blocking the NF $\kappa$ B transcriptional activity in the mast cell nucleus can suppress the expression of inflammatory mediators, including cytokines and adhesion molecules. In this study, OR extract inhibited the degradation of I- $\kappa$ B $\alpha$  from NF- $\kappa$ B in cytosol and nuclear translocation of NF- $\kappa$ B in PMA/A23187-stimulated mast cells. These results indicate that OR extract could prevent the nuclear localization step of NF- $\kappa$ B, which is a mechanism for controlling the NF- $\kappa$ B pathway activation in mast cells, thereby inhibiting the expression of the inflammatory cytokines, TNF- $\alpha$  and IL-6.

Osterici radix long has been used for the treatment of cold, headache, neuralgia and arthralgia in traditional Chinese medicine and has various actions, including anticancer,<sup>14</sup> anti-inflammation<sup>13</sup> and anti-allergic<sup>24</sup> and anti-oxidative effects.<sup>27</sup> It has been reported that Osterici radix contains several compounds such as oxypeudanol,<sup>14</sup> bisabolangelone,<sup>28</sup> imperatorin, isoimperatorin<sup>29</sup> and 11-hydroxy-sec-O-glucosylhamaudol.<sup>30</sup> Herbal extracts possess multiple compounds that have different biological activities. So, it is difficult to verify a main biological

active compound indicating the efficacy of herbal extracts. Therefore, we are now trying to find the main active compound in OR extract and further to evaluate its anti-allergic properties. We are currently exploring whether bisabolangelone is the main biologically active compound in OR extract by investigating its anti-inflammatory effects in mouse macrophages.<sup>28</sup>

In conclusion, our observations suggest that OR extract has effective anti-allergic properties, improving rhinitis symptoms, inhibiting histamine and IL-4 release in OVA-induced allergic rhinitis mice and inhibiting mast cell de-granulation in compound 48/80-stimulated mast cells. Furthermore, OR extract has anti-inflammatory activity by suppressing the production of inflammatory cytokines, TNF- $\alpha$  and IL-6, via blocking the MAPKs/NF- $\kappa$ B pathways in PMA/A23187-stimulated mast cells. This suggests that OR extract might be useful for the treatment of inflammatory allergic diseases including rhinitis. Although OR extract has shown its therapeutic potential against mast cell-mediated allergic response in an acute in vivo model, further studies could be conducted on the evaluation of OR extract in chronic allergic responses mediated by multiple pathways.

### Conflicts of interest

The authors declare that they have no conflicts of interest.

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