

## Anti-allergic effects of *Perilla frutescens* var. *acuta* Kudo 30% ethanol extract powder

Hyun-A Oh<sup>1,2</sup>, Sung-Hoon Kim<sup>1</sup>, Wung-Seok Cha<sup>3</sup>, Hyung-Min Kim<sup>2</sup> and Hyun-Ja Jeong<sup>4,\*</sup>

<sup>1</sup>Cancer Preventive Material Development Research Center, Department of Pharmacology, College of Oriental Medicine, Kyung Hee University, Seoul, 130-701, Republic of Korea; <sup>2</sup>Department of Pharmacology, Institute of Oriental Medicine, College of Oriental Medicine, Kyung Hee University, Seoul, 130-701, Republic of Korea; <sup>3</sup>Department of Korean Medical History, College of Korean Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea; <sup>4</sup>Biochip Research Center, Hoseo University, 165, Sechul-ri, Baebang-myun, Asan, Chungnam, 336-795, Republic of Korea

Received for publication December 30, 2009; accepted August 24, 2010

### SUMMARY

*Perilla frutescens* var. *acuta* Kudo (PF) is a traditional Korean medicinal herb for allergic reaction regulation. In the present study, we investigated the effect of 30% ethanol extract powder of PF (EPPF) and rosmarinic acid (RA), the active compound of EPPF on various allergic reactions using *in vivo* and *in vitro* models. EPPF and RA significantly inhibited compound 48/48-induced systemic anaphylactic reaction and histamine release ( $P < 0.05$ ). In addition, EPPF and RA significantly inhibited passive cutaneous anaphylaxis (PCA) in a dose-dependent manner ( $P < 0.05$ ). These effects were stronger than those of disodium cromoglycate, the reference drug tested. EPPF and RA also significantly inhibited production of inflammatory cytokines, tumor necrosis factor- $\alpha$  interleukin (IL)-6, and vascular endothelial growth factor on the PCA reaction and phorbol 12-myristate 13-acetate and calcium ionophore A23187-stimulated human mast cell line, HMC-1 cells ( $P < 0.05$ ). Moreover, EPPF and RA showed an inhibitory effect on lipopolysaccharide (LPS)-induced IL-4 production from whole spleen cells. Finally, EPPF and RA significantly decreased IL-4-dependent IgE production by LPS-stimulated whole spleen cells ( $P < 0.05$ ). In conclusion, these results indicate that EPPF has potent anti-allergic activities.

**Key words:** *Perilla frutescens* var. *acuta* Kudo; rosmarinic acid; Allergic reactions; Histamine; Inflammatory cytokines; IgE; Anti-allergic effect

### INTRODUCTION

In general, mast cell-mediated allergic reaction is elicited by various stimulators including compound 48/80, concanavalin A and anti-IgE (Paul *et al.*, 1993). Among the preformed and newly synthesized

allergic substances released on degranulation of mast cells, histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen *et al.*, 1996). Compound 48/80, the best-known polybasic histamine releaser, was discovered in the search for hypotensive agents. Its hypotensive effect was shown by Paton to be the result of histamine release (Paton and Webster, 1985).

The secretory responses of mast cells can be

\*Correspondence: Hyun-Ja Jeong, Biochip Research Center, Hoseo University, 165, Sechul-ri, Baebang-myun, Asan, Chungnam, 336-795, Republic of Korea. E-mail: hjjeong@hoseo.edu

induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen (Kim and Lee, 1999; Jeong *et al.*, 2002). It has been established that anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) as a typical *in vivo* model for immediate hypersensitivity in anaphylactic reactions. Rat's skins are useful sites for studying PCA (Shine *et al.*, 1997). Although mast cells also store small amounts of cytokines in their granules (Gordon and Galli, 1990), these cells dramatically increase their production of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, vascular endothelial growth factor (VEGF) and other cytokines after their surface Fc $\epsilon$ R I are cross-linked with specific antigen (Gurish *et al.*, 1991; McPherson *et al.*, 2009; Textor *et al.*, 2007).

IL-4 is a cytokine produced predominantly by activated CD4 TH2 cells and involved in the proliferation and differentiation of activated B cells. On resting B cells, IL-4 increases the expression of MHC class II antigens and IgE receptors; on activated B cells, it increases the expression of IgG1 and IgE. In addition, IL-4 affects many other cells, including granulocytes, fibroblasts, endothelial cells, and certain thymocytes, as well as B cells (Snapper and Mond, 1993; Strober *et al.*, 1991; Keithley *et al.*, 1990; Takahashi *et al.*, 1992; Croft and Swain, 1991). The *Perilla frutescens* var. *acuta* Kudo (PF) well known as 'So-Yeop' in Korea, has been used for the management of the common cold with cough and nausea and bronchial asthma, and it still occupies an important place in traditional Korean medicine (Yook and Ann, 1975). The leaves of PF are primarily used to treat affective disorders such as depression and anxiety (Takeda *et al.*, 2002). Previously, we reported that aqueous extract of PF inhibits mast cell-mediated immediate-type allergic reactions *in vivo* and *in vitro* (Shin *et al.*, 2000). In this study, we investigated the effect of 30% ethanol extract powder of PF (EPPF) and rosmarinic acid (RA), the active compound of EPPF on various allergic reactions using *in vivo* and *in vitro* models.

## MATERIALS AND METHODS

### Materials

Compound 48/80, phorbol 12-myristate 13-acetate (PMA), A23187, lipopolysaccharide (LPS), disodium cromoglycate (DSCG), anti-DNP IgE antibody, DNP-human serum albumin (HSA), o-phthalaldehyde (OPA), avidin peroxidase, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) tablets substrate (ABTS), and other reagents were purchased from Sigma (St. Louis, MO, USA). The  $\alpha$ -minimal essential medium was purchased from Flow Laboratories (Irvine, UK). Fetal bovine serum (FBS), iscove's modified dulbecco's medium (IMDM), RPMI 1640, and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). Anti-human TNF- $\alpha$ /IL-6/VEGF antibody (Ab), biotinylated antihuman TNF- $\alpha$ /IL-6/VEGF Ab, recombinant human (rh) TNF- $\alpha$ /IL-6/VEGF, anti-mouse TNF- $\alpha$ /IL-6/VEGF/IL-4/IgE Ab, biotinylated antimouse TNF- $\alpha$ /IL-6/VEGF/IL-4/IgE Ab, recombinant mouse (rm) TNF- $\alpha$ /IL-6/VEGF/IL-4/IgE were purchased from Pharmingen (San Diego, CA, USA).

### Culture of HMC-1 cells

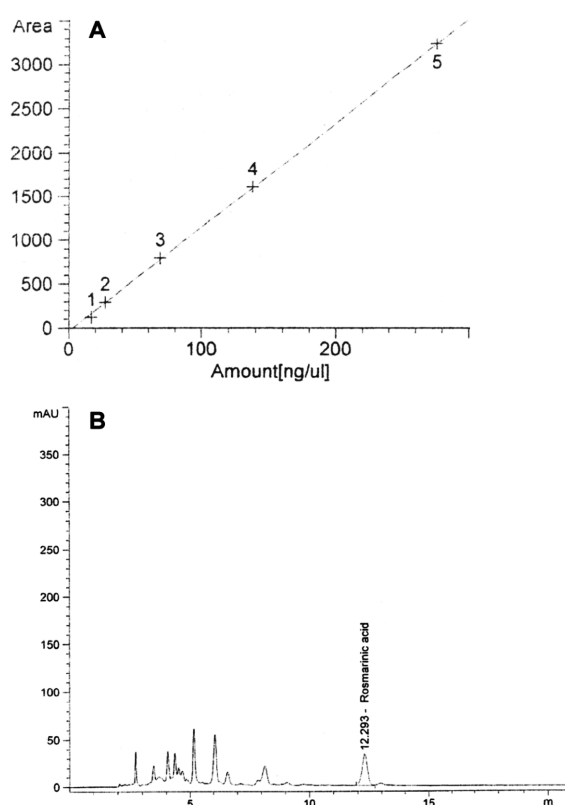
Human leukemic mast cell line (HMC-1) was grown in IMDM supplemented with 100 unit/ml penicillin, 100 mg/ml streptomycin and 10% heat-inactivated FBS at 37 °C, 5% CO<sub>2</sub> and 95% humidity. HMC-1 cells ( $3 \times 10^5$  cells/ml) were treated with EPPF (0.01 - 1.0 mg/ml) or RA (100 mM) for 1 h prior to stimulation with PMA and calcium ionophore A23187 (PMACI) incubated for 2 h or 8 h.

### Preparation of EPPF

For all experiments, PF was extracted twice overnight with 30% ethanol at 80 °C. The crude extracts were filtered and concentrated *in vacuo* at 55 °C. It was lyophilized and reduced to powder. The yield of dried extract from starting materials was about 20% (w/w). The EPPF was dissolved in distilled water (DW) and filtered with 0.22  $\mu$ m syringe filter.

### HPLC analysis

HPLC analysis for EPPF and its active chemical constituent, RA, was performed on an Agilent 1100 HPLC system (Agilent Company, USA) combined with a quaternary pump, onlinedegasser, diode array detector (DAD), and column thermostat at 30 °C, using a SP-C18 MG column (250 mm × 4.6 mm i.d., 5 μm, Agilent, USA). The mobile phases used were solvent A (acetonitrile) and B (2% acetic acid) as a ratio of 22:78 (v/v), delivered at a flow rate of 1.0 mL/min. Products were detected at 320 nm. Data collection and analysis were conducted using ChemStation software (Agilent Technologies, Wilmington, DE). The standard, RA (Sigma Chemicals Co., MO, USA), was injected to the HPLC analysis system three times to determine the precision of the developed HPLC method. The standards and peak areas were plotted against the quantities (g)



**Fig. 1.** Standard calibration curve of RA (A) and HPLC chromatograms of EPPF (B).

of the analysis injected. The standard curves were used to determine recovery of RA (Fig. 1A). In the experimental conditions employed, the HPLC analysis of RA showed at approximately 12.0 min. Results on the chemical composition of EPPF showed that the RA was ranged of between 9.03 - 9.11 mg/g (Fig. 1B).

### Animals

The original stock of male ICR mice (6 weeks, 25 - 30 g) and male SD rats (7 weeks, 250 - 300 g) were purchased from the Dae-Han experimental Animal Center (Eumsung, South Korea). The animals were maintained at the College of Oriental Medicine, Kyung Hee University. The animals were housed five to ten per cage in a laminar air-flow room maintained at a temperature of  $22 \pm 1$  °C and relative humidity of  $55 \pm 1\%$  throughout the study. No animal was used more than once. All protocols were approved by the Institutional Animal Care and Use Committee of College of Oriental Medicine, Kyung Hee University.

### Preparation of rat peritoneal mast cells (RPMCs)

RPMCs were isolated as previously described (Jeong *et al.*, 2006). In brief, rats were anesthetized by ether and injected with 30 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM  $\text{NaHCO}_3$ , 2.7 mM KCl, 0.3 mM  $\text{Na}_2\text{HPO}_4$ ) containing 0.1% gelatin into the peritoneal cavity, and the abdomen was gently massaged for about 2 min. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated with Pasteur pipette. Thereafter, peritoneal cells were sedimented at  $150 \times g$  for 10 min at room temperature and resuspended in tyrode buffer B. To separate mast cells from the major components of rat peritoneal cells, i.e., macrophages and small lymphocytes, suspended peritoneal cells were layered on 2 ml of 22.5% metrizamide (density, 1.12 g/ml) and centrifuged at  $400 \times g$  for 10 min at room temperature. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet

were washed and resuspended in 1 ml of tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5.6 mM glucose, 0.1% bovine serum albumin) containing calcium. Mast cells were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

#### **Compound 48/80-induced systemic anaphylactic reaction**

Mice were given an intraperitoneal injection of the mast cell degranulator compound 48/80 (8 mg/kg). EPPF and RA dissolved in DW was administered orally 1 h before the injection of compound 48/80. Mortality was monitored for 23 min after induction of an anaphylactic reaction. The blood was collected into eppendorf tubes and the sera were separated from blood by centrifugation at 1,500 × g for 15 min at 4 °C. The collected sera were assayed for histamine concentration.

#### **Histamine assay**

RPMCs suspensions (2 × 10<sup>5</sup> cells/ml) were incubated for 30 min at 37 °C before the addition of compound 48/80 for stabilization. The cells were incubated with the EPPF or RA for 1 h min, and then incubated for 25 min with compound 48/80 (6 µg/ml). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4 °C. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400 × g for 5 min at 4 °C. The histamine content was measured by the OPA spectrofluorometric procedure. The fluorescent intensity was measured at 460 nm (excitation at 355 nm) using a spectrofluorometer.

#### **PCA reaction**

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE Ab followed by an injection of DNP-HSA into the mice tail vein 48 h later. The DNP-

HSA was diluted in phosphate-buffered saline (PBS). The mice were injected intradermally with 100 ng of anti-DNP IgE Ab into each of three dorsal skin sites that had been shaved. The sites were outlined with a water insoluble red marker. 48 h later, each mouse received an injection of 200 µl of the 1:1 mixture of 1 mg/ml DNP-HSA in PBS and 4% evans blue via the tail vein. EPPF and RA were orally administered to mice 1 h before the challenge. The mice were sacrificed 40 min after the intravenous challenge. The dorsal skin of the mouse was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 0.5 ml of 1 N KOH and 4.5 ml of a mixture of acetone and phosphoric acid (with a ratio of 13:5). The absorbent intensity of the extraction was measured at 620 nm in a spectrofluorometer, and the amount of dye was calculated with the evans blue measuring-line. The blood was collected into eppendorf tubes and the sera were separated from blood by centrifugation at 1,500 × g for 15 min at 4 °C. The collected sera were assayed for inflammatory cytokines.

#### **Enzyme-linked immunosorbent assay (ELISA)**

A modified enzyme-linked immunosorbent assay (ELISA), as previously described (Jeong *et al.*, 2009), was used to measure the cytokine on tissue protein. The ELISA was performed by coating 96-well plates with 6.25 ng/well of capture Ab. Before the subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% tween-20 (PBST). All reagents and coated wells used in this assay were incubated for 2 h at room temperature. The standard curve was generated from known concentrations of cytokine, as provided by the manufacturer. After exposure to the medium, the assay plates were exposed sequentially to each of the biotin-conjugated secondary antibodies, and avidin peroxidase, and ABTS substrate solution containing 30% H<sub>2</sub>O<sub>2</sub>. The plates were read at 405 nm. Appropriate specificity controls were included, and all samples were run in duplicate.

Production of TNF- $\alpha$ , IL-6, VEGF, IL-4, and IgE were divided according to the total protein. Protein was determined using a bicinchoninic acid (Sigma, St. Louis, USA).

### Preparation of mouse whole spleen cells

The spleen was excised from the mice under chloroform anesthesia. Whole spleen cells were treated with lysis buffer (0.155 M NHCl, 10 mM KHCO<sub>3</sub>, 0.1 mM EDTA·2Na, pH 7.5) to lyse red blood cells. The cells washed three times in the culture medium, RPMI 1640-10% (v/v) FBS, to remove the treated reagents. The viability of the whole spleen cells was above 95% by Trypan blue staining. Mouse spleen cell was cultured at 37 °C under 5% CO<sub>2</sub> and 95% air in 4-well tissue culture dishes (Nunc, Roskilde, Denmark). RPMI 1640 medium supplemented with 10% FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, 1 × 10<sup>-5</sup> M 2-ME, 100 U/ml penicillin G, and 50 mg/ml streptomycin was used as a culture medium. LPS (4 mg/ml) and EPPF (0.01 - 1 mg/ml), RA, or DSCG were added to the cultured medium at the start and IL-4 (100

U/ml) on Day 1. On Day 8, the supernatants were harvested for IgE assay. For IL-4 assay, the cells were pre-treated with EPPF (0.01 - 1 mg/ml) or RA (100 mM) for 1 h before LPS (10 mg/ml) for 24 h.

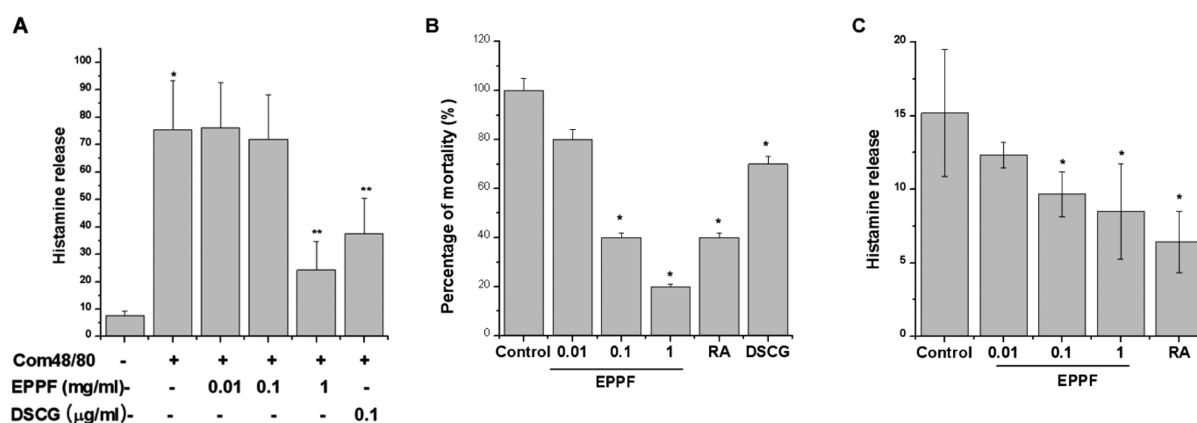
### Statistical analysis

The experiments shown are a summary of the data from at least three experiments and statistical analyses were performed using SPSS statistical software (SPSS 11.5, USA). Treatment effects were analyzed by one-way ANOVA, offered by Tukey's multiple range tests, and  $P < 0.05$  was used to indicate significance.

## RESULTS

### Effects of EPPF on compound 48/80-induced histamine release and systemic anaphylactic reaction

To investigate regulatory effects of EPPF on histamine release from mast cells, we measured histamine release. The inhibitory effects of EPPF on compound 48/80-induced histamine release from RPMCs were shown in Fig. 2A. DSCG, known as



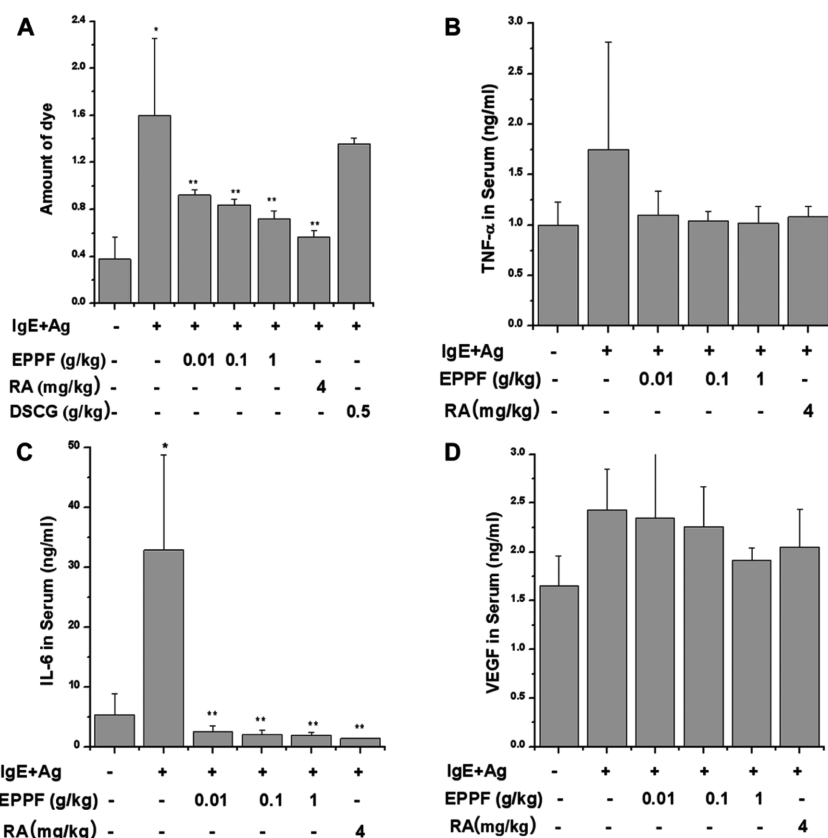
**Fig. 2.** Effects of EPPF on compound 48/80-induced histamine release and systemic anaphylactic reaction. RPMCs ( $2 \times 10^5$  cells) were preincubated with various concentrations of EPPF or DSCG at 37 °C for 1 h prior to incubation with compound 48/80. Histamine release was assayed (A). \* $P < 0.05$ ; significantly different from the unstimulated cells. \*\* $P < 0.05$ ; significantly different from the compound 48/80. Group of mice were orally pretreated with 200  $\mu$ l saline, EPPF (0.01 - 1 g/kg), RA (4 mg/kg), or DSCG (0.5 g/kg) 1 h before ( $n = 5$  group) the compound 48/80 injection. The compound-48/80 solutions were intra-peritoneally given to the group of mice. Mortality (%) within 1 h following the compound 48/80 injections is presented as the No. of dead mice  $\times 100$ /total No. of experimental mice (B). Serum was isolated from blood and then assayed about histamine (C). \* $P < 0.01$ ; significantly different from the compound 48/80.

an anti-histamine drug, was used as a reference drug. EPPF dose-dependently inhibited compound 48/80-induced histamine release at concentrations of 0.01 - 1 mg/ml. At the concentration of 1 mg/ml, the inhibition rate reached up to about 74.11%. In particular, EPPF significantly inhibited the compound 48/80-induced histamine release at concentrations of 1 mg/ml. To assess the contribution of EPPF and RA in anaphylactic reactions, the *in vivo* model of systemic anaphylactic reaction was used. After the injection of the compound 48/80, the mice were monitored for 23 min, after which the mortality rate was determined. As shown in Fig. 2B, an oral administration of DW as a control induced a fatal reaction in 100% of each group. When the EPPF, RA, or DSCG were orally administered

for 1 h before the compound 48/80 injections, the mortality was reduced. The inhibitory effect of EPPF and RA was higher than that of DSCG. EPPF also inhibited the compound 48/80-induced histamine release in a dose-dependent manner on sera (Fig. 2C).

#### Effect of EPPF on PCA in mice

We examined the anti-allergic activity of EPPF by using PCA in the rat dorsal skin model. Anti-DNP IgE was injected into dorsal skin sites followed 48 h later with an injection of DNP-HAS containing 4% Evans blue. EPPF (0.01 - 1 g/kg), RA (4 mg/kg), or DSCG (0.5 g/kg) were orally administered 1 h before injection of antigen. The cutaneous anaphylactic reaction was best visualized by extravasation of the



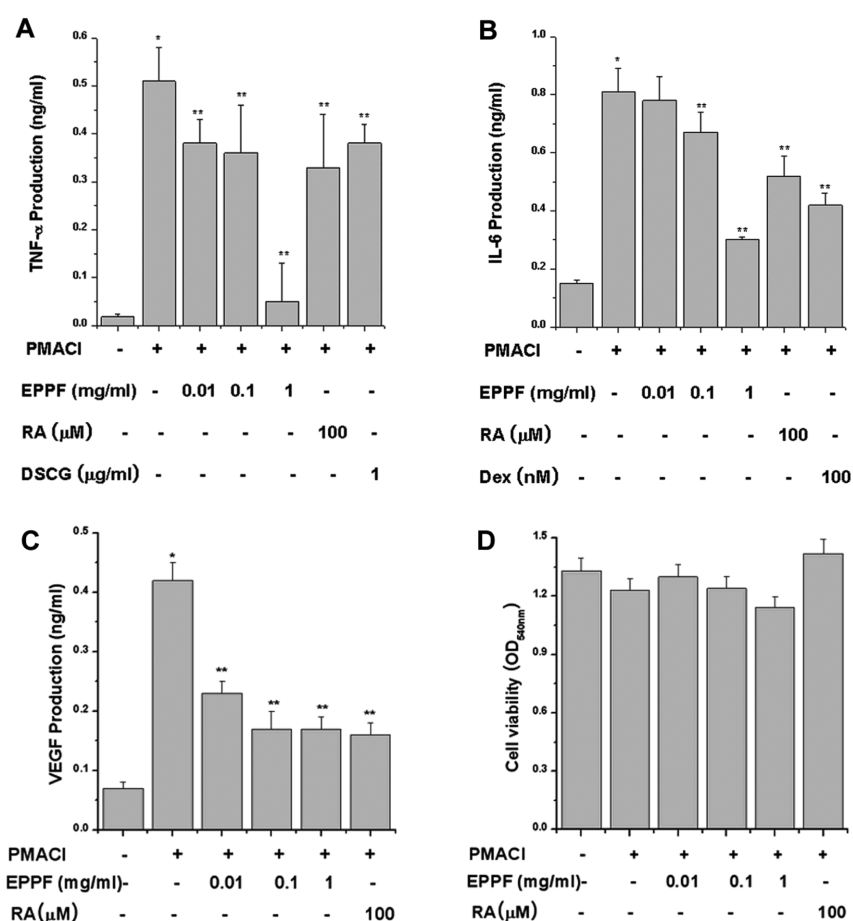
**Fig. 3.** Effect of EPPF on PCA in mice. EPPF, RA, or DSCG was orally administered 1 h prior to the challenge with antigen (n = 5). Each amount of dye is presented as the mean  $\pm$  S.D. (A). The sera were assayed for TNF- $\alpha$  (B), IL-6 (C), and VEGF (D). \* $P$  < 0.05; significantly different from the control value. \*\* $P$  < 0.05; significantly different from the stimulated group.

evans blue dye. We found that EPPF and RA significantly suppressed the mast cell-dependent PCA reaction (Fig. 3A,  $P < 0.05$ ). The ability of EPPF and RA to influence anaphylactic reaction-induced TNF- $\alpha$ , IL-6, and VEGF production was investigated in serum. As shown in Fig. 3B-D, EPPF and RA also inhibited anti-DNP IgE-induced TNF- $\alpha$ , IL-6, and VEGF production in sera.

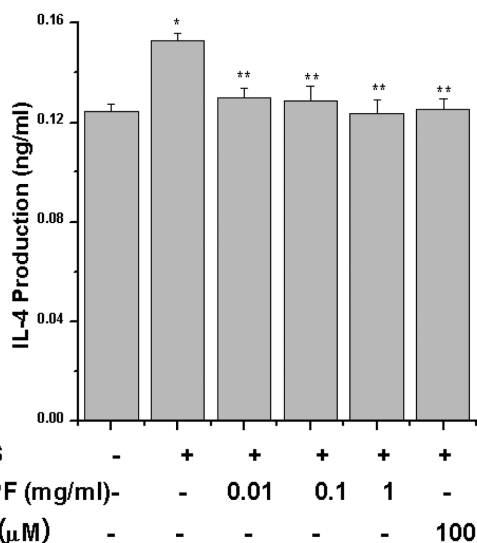
#### Effects of EPPF on PMACI-induced TNF- $\alpha$ , IL-6, and VEGF production on HMC-1 cells

In order to confirm the inhibitory effect of EPPF on PMA (50 nM) plus calcium ionophore A23187 (1

mM) (PMACI)-induced TNF- $\alpha$ , IL-6, and VEGF production, the dose response of EPPF (0.01 - 1 mg/ml) was measured in HMC-1 cells. The cells were pre-treated with EPPF or RA (100  $\mu$ M) for 1 h before PMACI-stimulation for 8 h. DSCG and dexamethasone was used as reference drug. Culture supernatants were assayed for TNF- $\alpha$ , IL-6, and VEGF protein levels using the ELISA method. EPPF and RA significantly inhibited PMACI-induced TNF- $\alpha$ , IL-6, and VEGF production in a dose-dependent manner (Fig. 4A-C,  $P < 0.01$ ). The maximal inhibition rate of TNF- $\alpha$ , IL-6, and VEGF production by EPPF (1 mg/ml) were about 93.66%, 77.20%, and 72.72% respectively.



**Fig. 4.** Effects of EPPF on PMACI-induced TNF- $\alpha$ , IL-6, and VEGF production on HMC-1 cells. Cells were pretreated with EPPF or RA for 1 h and then challenged with PMA (50 nM) plus A23187 (1  $\mu$ M) for 8 h. TNF- $\alpha$ , IL-6, and VEGF concentrations were measured from cell supernatants using the ELISA method (A-C). Cells were then collected and assessed for viability using MTT (D). Values are the mean  $\pm$  S.D. of duplicate determinations from three separate experiments. Dex, Dexamethasone. \* $P < 0.01$  compared to no stimulation. \*\* $P < 0.01$ ; significantly different from the stimulated group.



**Fig. 5.** Effect of EPPF on IL-4 production from whole spleen cells. Whole spleen cells ( $3 \times 10^5$  cells) were treated with EPPF (0.01 - 1 mg/ml) or RA (100 μM) for 1 h and then stimulated with LPS for 24 h. The culture supernatants were assayed for IL-4 concentrations by ELISA. Each datum is presented as the mean  $\pm$  S.D. of three independent experiments. \* $P < 0.05$  compared to no stimulation. \*\* $P < 0.05$ ; significantly different from the stimulated group.

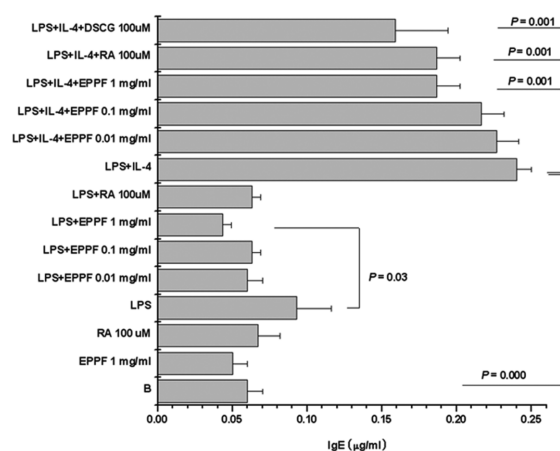
Cell cytotoxicity by EPPF was not observed up to 1 mg/ml (Fig. 4D).

#### Effect of EPPF on IL-4 production from whole spleen cells

We examined the inhibitory effect of EPPF on the LPS-induced IL-4 production from whole spleen cells. Culture supernatant was assayed for IL-4 by ELISA method. Pretreatment of EPPF inhibited LPS-induced IL-4 production (Fig. 5). The maximal inhibition rate by EPPF (1 mg/ml) was about 101.75%,

#### Effect of EPPF on IgE production from whole spleen cells

IL-4 can induce IgE class switching in mouse and human activated B cells (Coffman *et al.*, 1993). In the supernatants of LPS-stimulated whole spleen cells in the presence of IL-4 and EPPF, RA, or DSCG, IgE was assayed on Day 8. EPPF dose-



**Fig. 6.** Effect of EPPF on IgE production from whole spleen cells. Whole spleen cells ( $1 \times 10^6$  cells) were cultured in the various conditions, EPPF (0.01 - 1 mg/ml), RA (100 μM), or DSCG (100 μM) at indicated concentrations, and for 7 days as described under Materials and Methods. The culture supernatants were assayed for IgE concentrations by ELISA. Each datum is presented as the mean  $\pm$  S.D. of three independent experiments.

independently inhibited the LPS plus IL-4-dependent IgE production by whole spleen cells (Fig. 6). DSCG also showed inhibitory effect at the dose of 100 mM.

## DISCUSSION

This study has shown that EPPF inhibited compound 48/80-induced allergic response and anti-DNP IgE-induced PCA reaction in murine model by oral administration. EPPF also inhibited TNF- $\alpha$ , IL-6, VEGF, IL-4, and IgE production.

Using *in vivo* and *in vitro* model, we show that EPPF and RA inhibits mast cell-mediated allergic responses. Mice were orally administered EPPF or RA, which inhibited the compound 48/80 or IgE-mediated anaphylactic reaction. Compound 48/80 has been reported to increase the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This report indicates that the membrane permeability increase may be an essential trigger for the release of mediators from mast cells



(Tasaka *et al.*, 1986). Hence, it can be hypothesized that EPPF might act on the lipid bilayer membrane such that it prevents perturbation induced by compound 48/80. DSCG prevents release of the mediators of type I allergic reaction as a mast cell stabilizer. The drug does not inhibit the binding of IgE to mast cells nor the interaction between cell-bound IgE and specific antigen; instead, DSCG suppresses the release of substances (e.g., histamine, SRS-A) (Cook *et al.*, 2000). Our results suggest that EPPF and RA might play a role as a mast cell stabilizer on PCA.

The intracellular calcium pathways are critical to the degranulation of mast cells. The inhibition of intracellular  $Ca^{2+}$  elevation could lead to an inhibition of calcium-dependent protein kinase C, which is critical in mast cell activation (Saito *et al.*, 1996). Increased  $Ca^{2+}$  induces the release of inflammatory mediators including TNF- $\alpha$  and IL-6 (Jeong *et al.*, 2002). Intracellular  $Ca^{2+}$  plays an important role in the expression of TNF- $\alpha$ , IL-6, and VEGF. Mast cell-derived pro-inflammatory cytokines, especially TNF- $\alpha$ , IL-6, and VEGF have a critical biological role in the allergic reaction. The spectrum of cytokines produced by HMC-1 cells with PMACI stimulation supports the well-recognized role of mast cells immediate hypersensitivity. TNF- $\alpha$ , IL-6, and VEGF play a major role in triggering and sustaining the inflammatory allergic response in mast cells (Hide *et al.*, 1997; Mican *et al.*, 1992; Lee *et al.*, 2008). These reports indicate that the reduction of proinflammatory cytokines from mast cells is one of the key indicators of reduced allergic inflammatory symptoms. This study has demonstrated that EPPF and RA inhibited the production of TNF- $\alpha$ , IL-6, and VEGF in PCA reaction and PMACI-stimulated HMC-1 cells. This suggests that the anti-allergic effect of EPPF was the result of its ability to decrease the level of TNF- $\alpha$ , IL-6, and VEGF production from mast cells.

IL-4 (TH2 cytokine) can induce IgG1 (IgG4) and IgE class switching in mouse and human activated B cells (Coffman *et al.*, 1993). The effects of IL-4 on

the stimulation of IgG secretion *in vitro* are somewhat more complex. In this study, EPPF and RA inhibited the IL-4 and IgE production from whole spleen cells. Recently, it was found that a hypothalamic pituitary-adrenal axis of the endocrine system plays an important role in the regulation of Th 1/Th2 balance can shift the balance to the Th2 side (Rook *et al.*, 1994). The effect of EPPF may decrease Th2 activity and IL-4 production by Th2 cells. Thus, it may be concluded from the results of the present study that EPPF possess anti-allergic activity by inhibition of Th2 activity.

In conclusion, our data indicated that EPPF and its active compound, RA inhibited systemic anaphylactic reaction induced by compound 48/80 *in vivo*. The main factors of allergic reaction, histamine, TNF- $\alpha$ , IL-6, VEGF, IL-4, and IgE were inhibited by EPPF and RA. Overall our findings suggest that EPPF has various regulatory effects, which might explain its beneficial effect in the regulation of various allergic diseases.

## ACKNOWLEDGEMENTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. 2009-0063466).

## REFERENCES

- Coffman RL, Lebman DA, Rothman P. (1993) Mechanism and regulation of immunoglobulin isotype switching. *Adv. Immunol.* **54**, 229-270.
- Cook EB, Stahl JL, Barney NP, Graziano FM. (2000) Mechanisms of antihistamines and mast cell stabilizers in ocular allergic inflammation. *Curr. Drug. Targets. Inflamm. Allergy.* **1**, 167-180.
- Croft M, Swain S. (1991) B-cell response to fresh and effector T Helper cells. *J. Immunol.* **146**, 4055-4064.
- Gordon JR, Galli SJ. (1990) Mast cell as a source of both preformed and immunologically inducible TNF- $\alpha$ /cachectin. *Nature.* **346**, 274-276.
- Gurish MF, Ghildyal N, Arm J, Austen KF, Avraham S, Reynolds DS, Stevens RL. (1991) Cytokine mRNA are preferentially increased relative to secretory

- granule protein mRNA in mouse bone marrow-derived mast cells that have undergone IgE-mediated activation and degranulation. *J. Immunol.* **146**, 1527-1533.
- Hide I, Toriu N, Nuibe T, Inoue A, Hide M, Yamamoto S, Nakata Y. (1997) Suppression of TNF- $\alpha$  secretion by azelastine in a rat mast (RBL-2H3) cell line: evidence for differential regulation of TNF- $\alpha$  release, transcription, and degranulation. *J. Immunol.* **159**, 2932-2940.
- Jeong HJ, Hong SH, Lee DJ, Park JH, Kim KS, Kim HM. (2002) Role of Ca<sup>2+</sup> on TNF- $\alpha$  and IL-6 secretion from RBL-2H3 mast cells. *Cell. Signal.* **14**, 633-639.
- Jeong H J, Moon PD, Kim SJ, Seo JU, Kang TH, Kim JJ, Kang IC, Um JY, Kim HM, Hong SH. (2009) Activation of hypoxia-inducible factor-1 regulates human histidine decarboxylase expression. *Cell. Mol. Life Sci.* **66**, 1309-1319.
- Jeong HJ, Lee SA, Moon PD, Na HJ, Park RK, Um JY, Kim HM, Hong SH. (2006) Alginic acid has anti-anaphylactic effects and inhibits inflammatory cytokine expression via suppression of nuclear factor-kappaB activation. *Clin. Exp. Allergy.* **36**, 785-794.
- Keithley EM, Krekorian TD, Sharp PA, Harris JP, Ryan AF. (1990) Comparison of immune-mediated models of acute and chronic otitis media. *Eur. Arch. Otol. Rhinolaryngol.* **247**, 247-251.
- Kim HM, Lee YM. (1999) Role of TGF- $\beta$  1 on the IgE-dependent anaphylaxis reaction. *J. Immunol.* **162**, 4960-4965.
- Lee KS, Kim SR, Park SJ, Min KH, Lee KY, Choe YH, Park SY, Chai OH, Zhang X, Song CH, Lee YC. (2008) Mast cells can mediate vascular permeability through regulation of the PI3K-HIF-1 $\alpha$ -VEGF axis. *Am. J. Respir. Crit. Care Med.* **178**, 787-797.
- McPherson VA, Sharma N, Everingham S, Smith J, Zhu HH, Feng GS, Craig AW. (2009) SH2 domain-containing phosphatase-2 protein-tyrosine phosphatase promotes Fc( $\epsilon$ )RI-induced activation of Fyn and Erk pathways leading to TNF( $\alpha$ ) release from bone marrow-derived mast cells. *J. Immunol.* **183**, 4940-4947.
- Mican JA, Arora N, Burd PR, Metcalfe DD. (1992) Passive cutaneous anaphylaxis in mouse skin is associated with local accumulation of interleukin-6 mRNA and immunoreactive interleukin-6 protein. *J. Allergy. Clin. Immunol.* **90**, 815-824.
- Paton DM, Webster DH. (1985) Clinical pharmacokinetics of H1-receptor antagonists (the antihistamines). *Clin. Pharmacokinet.* **10**, 477-497.
- Paul WE, Seder RA, Plaut M. (1993) Lymphokine and cytokine production by Fc  $\epsilon$ RI<sup>+</sup> cells. *Adv. Immunol.* **53**, 1-29.
- Petersen LJ, Mosbech H, Skov P. (1996) Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: characterization of factors influencing histamine releasability. *J. Allergy. Clin. Immunol.* **97**, 672-679.
- Rook GA, Hernandez-pando R, Lightman SL. (1994) Hormones, peripherally activated prohormones and regulation of the Th 1/Th2 balance. *Immunol. Today.* **15**, 301-303.
- Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, Iikura Y, Awaji T, Tsujimoto G, Yanagida M, Uzumaki H, Takahashi G, Tsuji K, Nakahata T. (1996) Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. *J. Immunol.* **157**, 343-350.
- Shin BK, Lee EH, Kim HM. (1997) Suppression of L-histidine decarboxylase mRNA expression by methyleugenol. *Biochem. Biophys. Res. Commun.* **232**, 188-191.
- Shin TY, Kim SH, Kim SH, Kim YK, Park HJ, Chae BS, Jung HJ, Kim HM. (2000) Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by *Perilla frutescens*. *Immunopharmacol. Immunotoxicol.* **22**, 489-500.
- Snapper CM, Mond JJ. (1993) Towards a comprehensive view of immunoglobulin class switching. *Immunol. Today.* **14**, 15-17.
- Strober W, Harriman GR, Kunimoto DR. (1991) Early steps of IgA B-cell differentiation. *Immunol. Res.* **10**, 386-388.
- Takahashi M, Kanai N, Watanabe A, Oshima O, Ryan AF. (1992) Lymphocyte subsets in immune-mediated otitis media with effusion. *Eur. Arch. Otorhinolaryngol.* **249**, 24-27.
- Takeda H, Tsuji M, Matsumiya T, Kubo M. (2002) Identification of rosmarinic acid as a novel antidepressive substance in the leaves of *Perilla frutescens* Britton var. *acuta* Kudo (*Perillae Herba*). *Nihon Shinkei Seishin Yakurigaku Zasshi.* **22**, 15-22.

Tasaka K, Mio M, Okamoto M. (1986) Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. *Ann. Allergy.* **56**, 464-469.

Textor B, Licht AH, Tuckermann JP, Jessberger R, Razin E, Angel P, Schorpp-Kistner M, Hartenstein

B. (2007) JunB is required for IgE-mediated degranulation and cytokine release of mast cells. *J. Immunol.* **179**, 6873-6880.

Yook CH, Ann DK. (1975) *Herbology*, KO Moon, Seoul, p.212.