Physalis peruviana L. inhibits ovalbumin-induced airway inflammation by attenuating the activation of NF-κB and inflammatory molecules

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Abstract. *Physalis peruviana* L. (PP) is well known for its various properties, including its antioxidant property. In our previous study, the protective effects of PP against cigarette smoke-induced airway inflammation were confirmed. The purpose of the present study was to evaluate the anti-inflammatory effect of PP against ovalbumin (OVA)-induced airway inflammation. Treatment with PP inhibited the numbers of eosinophils and the levels of inflammatory cytokines, including interleukin (IL)-4, IL-5 and IL-13, in the bronchoalveolar lavage fluid (BALF) of animal models with OVA-induced allergic asthma. PP also significantly decreased the production of total immunoglobulin E in the serum. Lung sections stained with hematoxylin and eosin revealed that the influx of inflammatory cells was decreased in the lungs of mice treated with PP compared with cells in the OVA group. The increased expression levels of monocyte chemoattractant protein-1 (MCP-1) and T cell marker KEN-5 were also reduced following PP treatment in the lung tissues compared with those in the OVA group. The PAS staining results showed that PP attenuated the overproduction of mucus in the lung. Additionally, western blot analysis revealed that PP significantly downregulated the activation of nuclear factor-κB/p38 mitogen-activated protein kinase/c-Jun N-terminal kinase, and upregulated the expression of heme oxgenase-1 in the lungs. In an *in vitro* experiment, PP effectively reduced the levels of LPS-stimulated MCP-1 in a concentration-dependent manner. Taken together, these results indicate that PP has considerable potential in the treatment of allergic asthma.

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Abbreviations: OVA, ovalbumin; BALF, bronchoalveolar lavage fluid; IL-5, interleukin 5; IL-13, interleukin 13; IgE, immunoglobulin E; MCP-1, monocyte chemoattractant protein-1; AHR, airway hyperresponsiveness; NF-κB, nuclear factor-κB; IκB, inhibitor of NF-κB; MAPKs, mitogen-activated protein kinases; HO-1, heme oxygenase-1; PP, *Physalis peruviana* L.

Key words: *Physalis peruviana* L., airway inflammation, eosinophil, Th2 cytokines, immunoglobulin E, nuclear factor-κB

Introduction

Allergic asthma, a chronic airway inflammatory disease, is a serious public health issue, and the prevalence of asthma has increased considerably worldwide (1). Generally, the major characteristics of allergic asthma are an airway inflammatory response, mucus overproduction, obstruction and airway remodeling, which are closely associated with high levels of Th2-type cytokines, including interleukin (IL)-5/IL-13, eosinophil influx and serum immunoglobulin E (IgE) production (2-4). The increased level of monocyte chemoattractant protein (MCP-1) is closely associated with inflammatory cell influx in the pathogenesis of allergic asthma (5-7). Nuclear factor-κB (NF-κB) is critical for the regulation of Th2 cytokine production, Th2 cell differentiation and mucus overproduction (8). It is also well documented that mitogen-activated protein kinases (MAPKs) are important in the activation,

proliferation and migration of inflammatory cells, and the activation of MAPKs is significantly higher in the lungs of allergic asthma animals compared with those in normal controls (9,10). Heme oxygenase-1 (HO-1) is an antioxidant protein that has anti-inflammatory properties, and there is considerable evidence for its protective effect against ovalbumin (OVA)-induced airway inflammation (11).

Natural compounds have attracted attention due to their potent anti-inflammatory effects and minimal side-effects for the treatment of chronic inflammatory diseases, including allergic asthma (12,13). Cape gooseberry [*Physalis peruviana* L. (PP)] is a species within the Solanaceae family, which has potent antioxidant activity and has a variety of biological effects, including antimycobacterial, anticancer and anti-inflammatory activities (14-16). The levels of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages were found to be effectively downregulated by total extract from the calyces of PP (17). In our previous study, a methanol extract of PP markedly reduced the degree of inflammatory cell recruitment, including inflammatory cytokines and chemokines, which are considered important indicators of the progression of airway inflammatory in chronic obstructive pulmonary disease (COPD)-like models in animals (18). Therefore, the results from previous studies suggest the possibility that treatment with PP may effectively attenuate the inflammatory response in the lung tissues of allergic asthma animal models. However, to the best of our knowledge, no previous studies have investigated the anti-inflammatory activity of PP in a mouse model of OVA-induced allergic asthma. Therefore, in the present study, the ability of PP to ameliorate pathological phenotypes, including airway inflammation and mucus hypersecretion, was evaluated in an OVA-induced asthma model.

Materials and methods

Preparation of PP. The fresh *P. peruviana* plant was collected from the forest hills of the Katu Village, Lore Lindu National Park (Central Sulawesi, Indonesia). The collected plant sample was identified by the Center for Pharmaceutical and Medical Technology (Tangerang, Indonesia), and authentication was confirmed by the Herbarium Bogoriense (Bogor, Indonesia). Voucher specimens were recorded as KRIB 0049496 and PMT 1884, which have been deposited in the herbarium of the Korea Research Institute of Bioscience and Biotechnology (Cheongju, Korea) and at the Center for Pharmaceutical and Medical Technology and Herbarium Bogoriense (18). Following drying and grinding of the leaves of the plant. A total of 150 g of powder was added to 150 ml of methanol, and extraction was performed by maceration at room temperature for 18 h. The extract was filtered and concentrated using a rotary evaporator (Laborota 4000; Heidolph, Jakarta, Indonesia) under reduced pressure, thereby obtaining 7.05 g of PP methanolic extract. In the following experiments, the extract was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml and then diluted to various concentrations prior to use.

Mouse model of airway inflammation induced by OVA. The experimental procedure was performed according to the methods of Jeon *et al* (19). Briefly, healthy female BALB/c mice (n=24; 6-weeks old; body weight, 16-18 g) were obtained from Koatech Co. (Pyeongtaek-si, Korea) and were acclimated to a specific pathogen-free condition (22-23˚C and 55-60% humidity) with free access to food and water at least 1 week prior to the experiments. Mice were immunized twice intraperitoneally on days 0 and 14 with 0.2 ml of a vehicle solution containing 30 μg OVA and 3 mg Alums (cat. no. 77161; Thermo Fisher Scientific, Inc., Waltham, MA, USA). At 7 days following the second immunization, OVA inhalation was performed on three consecutive days using a nebulizer (NE-U12; OMRON Corp., Tokyo, Japan) (1% OVA: alum-free saline solution, 60 min/day, day 21-23). The oral administration of PP or dexamethasone (DEX) was administered for six consecutive days (day 18-23). The mice were randomly divided into four groups as follows: Normal control group (NC), OVA (OVA sensitization/challenge) group, DEX (OVA sensitization/challenge + oral gavage of 1 mg/kg of DEX) group, and PP5 (OVA sensitization/challenge + oral gavage of 5 mg/kg of PP) group. The experimental protocol is presented in Fig. 1. The experimental procedures of the present study were performed in accordance with procedures approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology (KRIBB-AEC-18054) and in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and Korean National Laws for Animal Welfare.

Determination of inflammatory cell counts. To count the numbers of inflammatory cells, bronchoalveolar lavage fluid (BALF) collection was performed according to the protocol described by Lee *et al* (20). Briefly, the collection of BALF was performed 48 h following the final administration of DEX and PP by tracheal cannulation and infusion with 700 μl of ice-cold PBS (total volume, 1.4 ml). To distinguish the different cells, 0.1 ml BALF was centrifuged at 264 x g for 5 min at room temperature, and the numbers of eosinophils and macrophages were counted using a Diff-Quik® staining kit according to the manufacturer's protocol (IMEB, Inc., Deerfield, IL, USA) and light microscope (magnification, x400).

Measurement of inflammatory cytokines in the BALF and IgE in the serum. The levels of inflammatory mediators in the BALF, including IL-4, IL-5 and IL-13, were evaluated using ELISA kits according to the manufacturer's protocol. Blood samples were collected from the mice 48 h following the final administration of PP and DEX, and serum was prepared. Total IgE levels in the serum were determined using an ELISA kit according to the manufacturer's protocol (IL-4, IL-5 and IL-13 ELISA kits, cat. nos. M4000B, M5000 and M1300CB; R&D Systems, Inc., Minneapolis, MN, USA; and IgE ELISA kit, cat. no. 432404; BioLegend, Inc., San Diego, CA, USA).

Western blot analysis. The lung tissues were homogenized in CelLytic™ MT cell lysis reagent (cat. no. c3228; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) prior to protein quantification. The protein concentration was determined with the Pierce BCA Protein assay kit (cat. no. 23225; Thermo Fisher Scientific, Inc.). Total protein was separated by 10-12% SDS-PAGE (protein quantity, 20-50 μg), and the samples were

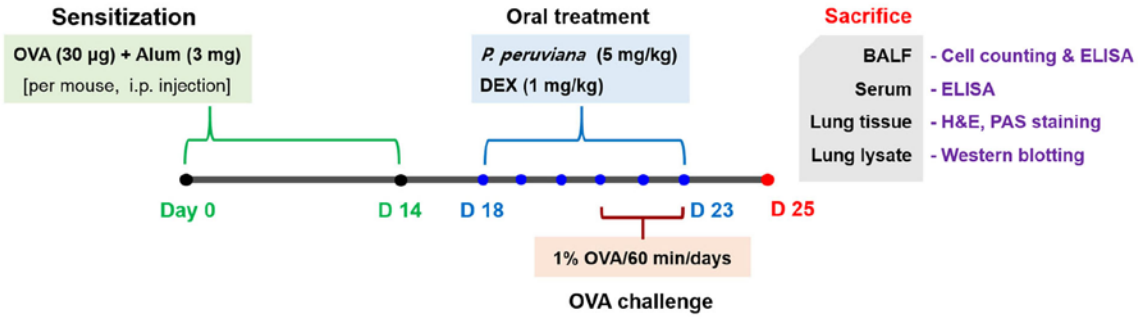

Figure 1. Experimental procedure for the allergic asthma model and administration of PP or DEX. BALB/c mice were divided into four groups (n=6 in each group). The mice were orally administered with PP (5 mg/kg) or DEX (1 mg/kg) between days 18 and 23. On days 21-23, the mice were exposed to 1% OVA using a nebulizer for 1 h each day. On day 25, the mice were sacrificed, and the BALF, serum and lung tissues were acquired. PP, *Physalis peruviana* L.; DEX, dexamethasone; OVA, ovalbumin; BALF, bronchoalveolar lavage fluid; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; D, day.

transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk solution for 1 h and incubated overnight at 4˚C with primary antibodies. The primary antibodies and dilution rates were as follows: Anti-phosphorylated (p)-p38 (cat. no. 9211), anti-p-extracellular signal-regulated kinase (ERK; cat. no. 4370), anti-p-NF-κB p65 (cat. no. 3033) and anti-β-actin (cat. no. 4967) at 1:1,000 dilutions (Cell Signaling Technology, Inc., Danvers, MA, USA), anti-MCP-1 (cat. no. sc-28879), anti-p38 (cat. no. sc-7149), anti-ERK (cat. no. sc-154), anti-p-c-Jun N-terminal kinase (JNK; cat. no. sc-6254), anti-JNK (cat. no. sc-474), anti-NF-κB p65 (cat. no. sc-372), anti-inhibitor of NF-κB (IκB-α; cat. no. sc-1643) and anti-KEN-5 (cat. no. sc-59373) at 1:1,000 dilutions (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and anti-HO-1 (cat. no. PA5-27338; Thermo Fisher Scientific, Inc.) at 1:1,000 dilution. The membranes were washed four times with TBST for 10 min and incubated with HRP-conjugated secondary antibodies (goat anti-mouse, cat. no. 115-035-003; goat anti-rabbit, cat. no. 111-035-003: 1:2,000 dilution; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) at room temperature for 2 h. Finally, the membranes were developed using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.). The LAS-4000 luminescent image analyzer was used to visualize all bands, and Fuji Multi Gauge version 3.0 (both Fujifilm, Tokyo, Japan) was used to assess the band density.

Histological analysis. Following collection of the BALF, the lung tissues were removed and fixed in 10% (v/v) neutral-buffered formalin solution. For histological examination, the lung tissues were embedded in paraffin and sectioned at a thickness of 4 μm. The lung sections were then stained with hematoxylin (cat. no. 3580; BBC Biochemical Inc., Mount Vernon, WA, USA) and eosin (cat. no. 6766007; Thermo Fisher Scientific, Inc.) solutions (H&E) and a periodic acid-Schiff (PAS) stain solution kit (cat. no. K7308; IMEB, Inc., San Marcos, CA, USA). The degree of inflammatory cell influx and mucus production was visualized using a light microscope (Leica Microsystems, Ltd., Milton Keynes, UK; magnification, x100 and x200).

Cell culture. RAW264.7 mouse macrophages were obtained from the American Type Culture Collection (Manassas, VA, USA) and grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) and a 1% (w/v) antibiotic-antimycotic solution (Invitrogen; Thermo Fisher Scientific, Inc.) and incubated in a 5% CO_2 incubator at 37˚C. To determine the levels of MCP-1, the RAW 264.7 cells were seeded at 2.5x105 cells per well in a 6-well plate and incubated with 10% DMEM for 24 h. Following treatment with PP (5, 10, 20 and 40 μg/ml) for 1 h, the cells were incubated with LPS (200 ng/ml) for 20 h in a 5% CO_2 incubator at 37˚C. The quantity of MCP-1 in the supernatants was determined using an ELISA kit (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis. The values are expressed as the mean ± standard deviation. The statistical significance was determined by one-way analysis of variance followed by a multiple comparison test with Dunnett's adjustment using SPSS ver. 12.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significance difference.

Results

Treatment with PP reduces the number of eosinophils in the BALF of an OVA-induced airway inflammation animal model. The numbers of inflammatory cells, including eosinophils and macrophages, were significantly upregulated in the BALF of the OVA-sensitized/challenged mice compared with those in the NC group (Fig. 2A and B). However, these levels were markedly downregulated in the PP-treated mice compared with those in the OVA group. The mice treated with DEX, which was used a positive control, also exhibited a decrease in the numbers of eosinophils and macrophages in the BALF compared with those in the OVA-challenged mice. The effects of PP were similar to those of DEX treatment at 1 mg/kg.

Treatment with PP decreases the levels of Th2 cytokines in the BALF. As Th2 cytokines are involved in the airway inflammatory response in allergic asthma (21-23), the production of Th2 cytokines, including IL-4, IL-5 and IL-13, was examined by ELISA. The OVA-challenged mice exhibited a significant increase in IL-4 and IL-5 production compared with the NC mice. (Fig. 3A and B). However, this level was significantly downregulated in the PP treatment group. Similar to the results obtained for IL-4 and IL-5, the PP-treated mice exhibited a marked reduction in the level of IL-13 compared with the OVA-challenged mice (Fig. 3C). The inhibitory

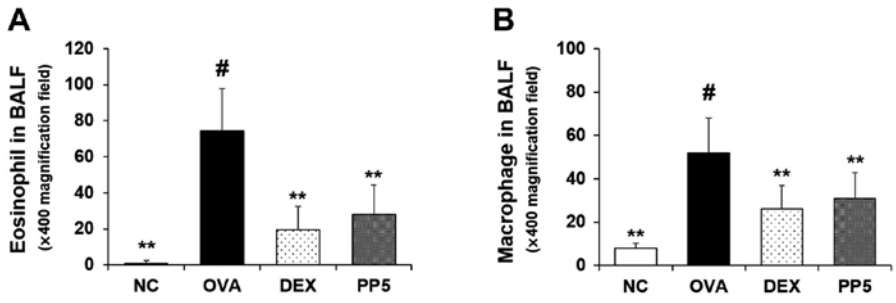

Figure 2. PP decreases the numbers of inflammatory cells in the BALF of mice with OVA-induced airway inflammation. Diff-Quik® staining was used to determine the different cells in the BALF. (A) Eosinophil and (B) macrophage counts were determined. Data are expressed as the mean ± standard deviation (n=6). NC, normal control mice; OVA, mice administered with OVA; DEX, mice administered with DEX (1 mg/kg) + OVA; PP5, mice administered with PP (5 mg/kg) + OVA. #P<0.05, vs. NC group; **P<0.01, vs. OVA group. PP, *Physalis peruviana* L.; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA; BALF, bronchoalveolar lavage fluid.

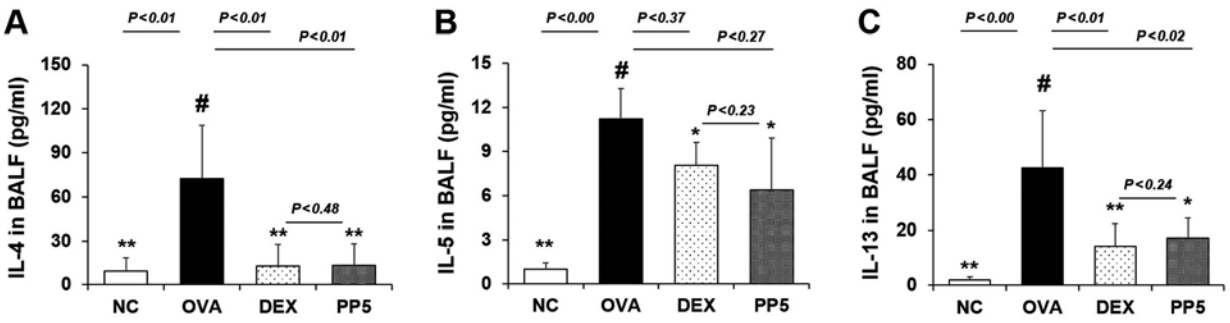

Figure 3. PP inhibits the production of inflammatory cytokines in the BALF of mice with OVA-induced airway inflammation. BALF inflammatory cytokines (A) IL-4, (B) IL-5 and (C) IL 13 were determined using ELISA kits. The absorbance was measured at 450 nm with a microplate reader. #P<0.05 vs. NC group; *P<0.05 and **P<0.01, vs. OVA group. PP, *Physalis peruviana* L.; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA; IL, interleukin; BALF, bronchoalveolar lavage fluid.

rates of IL-4 were 82.1% (DEX, 1 mg/kg) and 81.4% (PP, 5 mg/kg). The inhibitory rates of IL-5 were 31.3 % (DEX, 1 mg/kg) and 43.2% (PP, 5 mg/kg). The inhibitory rates of IL-13 were 66.7% (DEX, 1 mg/kg) and 59.6% (PP, 5 mg/kg). The inhibitory effect of 5 mg/kg PP on Th2 cytokines was similar to that of 1 mg/kg DEX.

Treatment with PP attenuates the production of IgE in the serum. ELISA was performed to evaluate the effect of PP on the production of IgE. The results revealed that the production of IgE in the serum was higher in the OVA-challenged mice compared with that in the NC mice (Fig. 4). However, the increased level of IgE was significantly reduced in the PP-treated mice compared with that in the OVA-challenged mice.

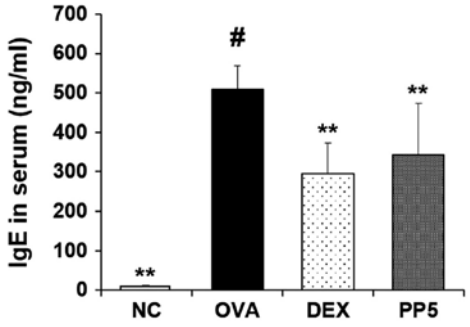

Figure 4. PP downregulates the level of IgE in the serum of mice with OVA-induced airway inflammation. Serum levels of IgE were evaluated using ELISA kits. The absorbance was measured at 450 nm with a microplate reader. #P<0.05, vs. NC group; **P<0.01, vs. OVA group. PP, *Physalis peruviana* L.; IgE, immunoglobulin E; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA.

Treatment with PP inhibits the levels of inflammatory cell influx and mucus hypersecretion in the lungs. H&E staining was performed to determine whether PP affects the inflammatory cell recruitment induced by OVA. In the OVA-challenged mice, cell infiltration into the peribronchial lesions in the lungs was markedly increased (Fig. 5A). However, this cell infiltration was notably downregulated in the PP-treated mice. In order to assess the effect of PP on mucus production, PAS staining was performed. The results revealed the presence of mucus hypersecretion in the bronchial airways of the OVA-challenged mice (Fig. 5B). Notably, this level was reduced in the lungs of mice in the PP treatment group compared with that in the lungs of the OVA-challenged mice. The effect of 5 mg/kg PP was similar to that of treatment with 1 mg/kg DEX. Western blot analysis was performed to determine the expression levels of MCP-1 and KEN-5 in the different groups. The expression levels of MCP-1 and KEN-5 were markedly enhanced in the OVA-challenged mice, whereas these levels were lower in the PP group, as in the DEX reference drug group (Fig. 5C-E). These results indicate that PP may be useful in airway inflammation via the suppression of inflammatory cell recruitment, chemokine production and mucus production.

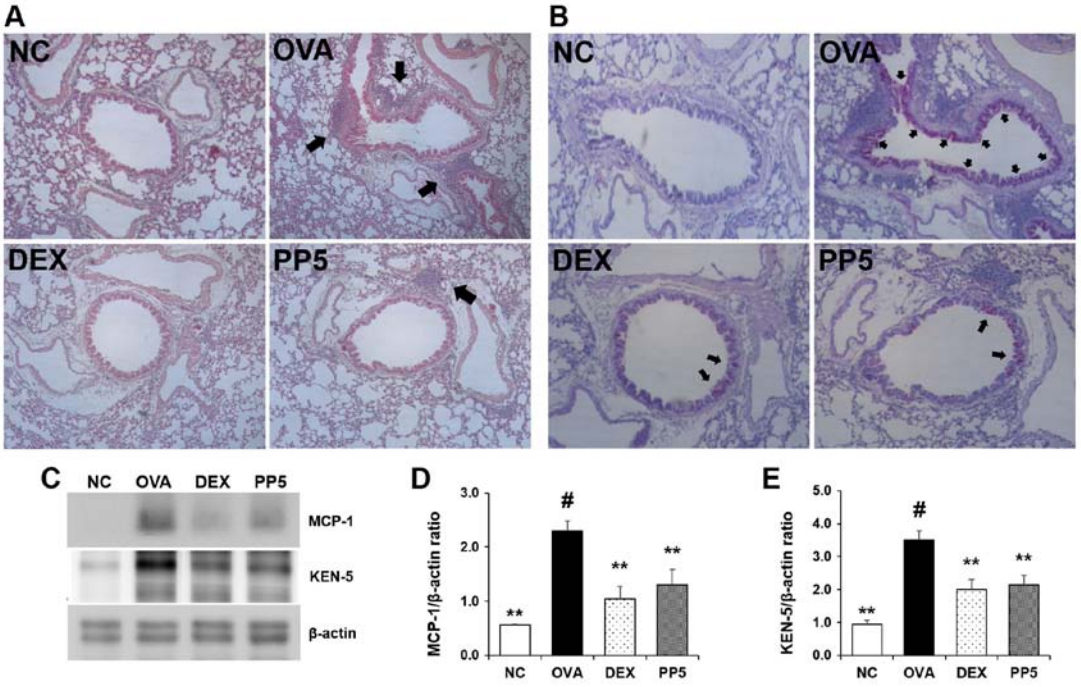

Figure 5. PP reduces the recruitment of inflammatory cells and the production of mucus in the lungs of mice with OVA-induced airway inflammation. (A) Levels of inflammatory cell influx were confirmed with hematoxylin and eosin staining (peribronchial lesion, magnification, x100). The arrow indicates the influx of inflammatory cells (B) Periodic acid-Schiff staining was used to assess mucus production (peribronchial lesion, magnification, x200). The arrow indicates the production of mucus. (C) Western blot analysis was used to determine the protein expression levels of MCP-1 and KEN-5 in the lung tissue samples. Quantitative analysis of (D) MCP-1 and (E) KEN-5 was performed by densitometric analysis. $^{\#}$P<0.05, vs. NC group; **P<0.01 vs. OVA group. PP, *Physalis peruviana* L.; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA; MCP-1, monocyte chemoattractant protein-1.

Treatment with PP reduces the activation of NF-κB in the lungs. It has been reported that NF-κB is activated following the activation of its inhibitor IκB (24,25). Upregulation in the phosphorylation of IκB and NF-κB has been identified in the lung samples of OVA-induced allergic asthma mice (26). To evaluate the effect of PP on the OVA-induced NF-κB signaling pathway, the level of IκB phosphorylation was investigated using western blot analysis. As shown in Fig. 6A and B, the level of IκB phosphorylation was significantly increased compared with that in the NC mice. However, treatment with PP effectively downregulated this level. The increase in OVA-induced NF-κB phosphorylation was significantly suppressed by PP treatment (Fig. 6A and C).

Treatment with PP inhibits the activation of p38 and JNK in the lungs. The levels of MAPK activation were subsequently evaluated, and it was confirmed that the activation of p38, JNK and ERK were upregulated in the lungs of the OVA-challenged mice (Fig. 7A). Notably, treatment with PP effectively inhibited the activation of p38 and JNK induced by OVA in mice. By contrast, the activation of ERK was not significantly affected by PP treatment.

Treatment with PP leads to the induction of HO-1 in the lungs. Given the importance of HO-1 in inflammatory lung diseases (27,28), the present study evaluated the effect of PP on the induction of HO-1. As shown in Fig. 8A and B, marginal increases in the expression of HO-1 were observed in the OVA and DEX groups compared with that in the NC group. These increases were not statistically significant (NC, vs. OVA group, P<0.09; NC, vs. DEX group, P<0.07). No significant difference was observed between the OVA and DEX group (P<0.32). Of note, the expression of HO-1 was significantly upregulated in the PP-treated group compared with that in the NC, OVA and DEX groups (NC, vs. PP group, P<0.01; OVA, vs. PP group, P<0.01; DEX, vs. PP group, P<0.01).

Inhibitory effect of PP on LPS-stimulated inflammatory molecule in RAW264.7 macrophages. It was confirmed that PP can regulate the recruitment of macrophages and the production of MCP-1 in an OVA-induced animal model (Fig. 2B and 5C). Therefore, the present study subsequently evaluated the effect of PP on the production of MCP-1 in activated macrophages. As shown in Fig. 9, there was a significant increase in MCP-1 in LPS-stimulated RAW264.7 macrophages, whereas pretreatment with PP effectively decreased the levels of MCP-1.

Discussion

Airway inflammation and mucus hypersecretion are the major characteristic of allergic asthma. Accordingly, the aim of the present study was to evaluate the protective effects of PP against the progression of these characteristics in an experimental animal model of OVA-induced allergic asthma. The results demonstrated that PP ameliorated airway inflammation and the overproduction of mucus, and indicated that the improvement effects were correlated with the inactivation of NF-κB/p38/pJNK and the induction of HO-1.

Airway inflammation is regarded as a typical feature of inflammatory lung diseases, including allergic asthma (29). Eosinophil accumulation is the prominent characteristic of the airway inflammatory response and eosinophil-derived

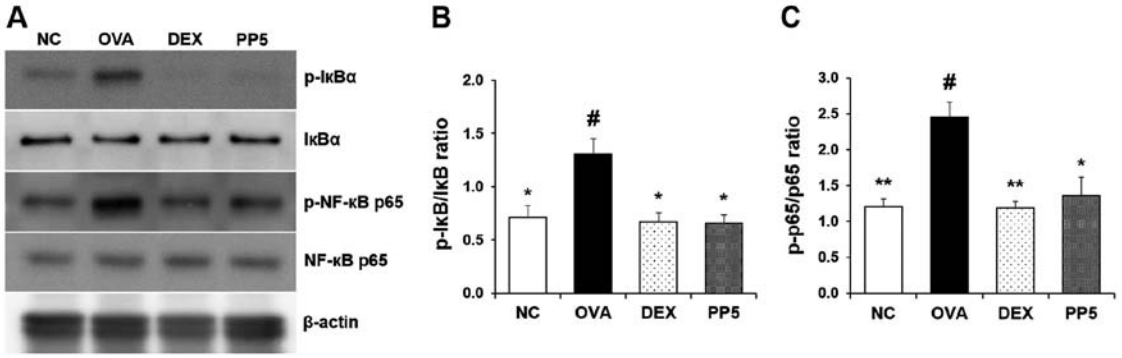

Figure 6. PP inhibits the activation of NF-κB in the lungs of mice with OVA-induced airway inflammation. (A) Levels of NF-κB and IκB activation were determined by western blot analysis. Quantitative analysis of (B) p-IκB and (C) p-p65 was performed by densitometric analysis. #P<0.05, vs. NC group; *P<0.05 and **P<0.01, vs. OVA group. PP, *Physalis peruviana* L.; NF-κB, nuclear factor-κB; IκB, inhibitor of NF-κB; p-, phosphorylated; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA.

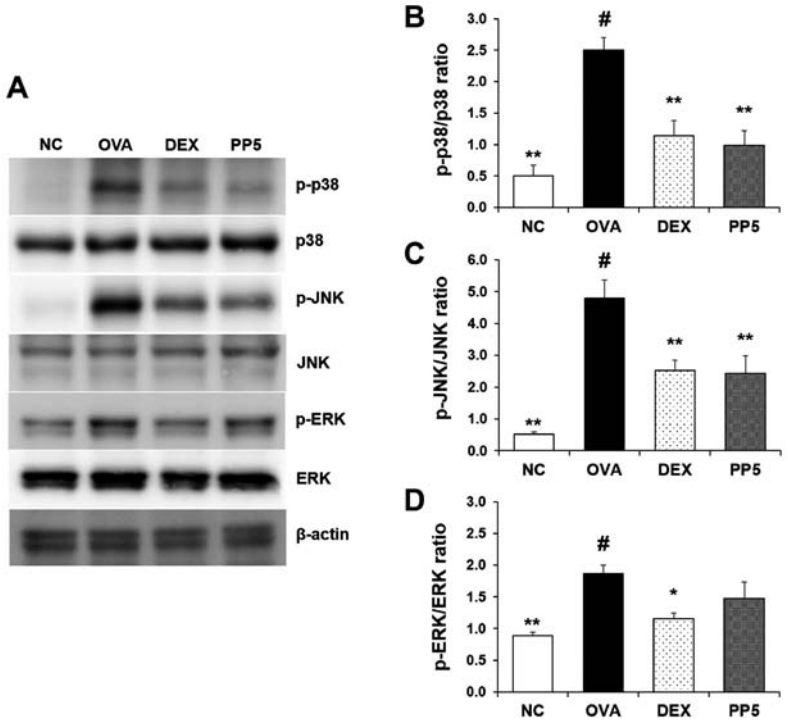

Figure 7. PP downregulates the activation of p38 mitogen-activated protein kinases in the lungs of mice with OVA-induced airway inflammation. (A) Levels of p38, ERK and JNK activation were determined by western blot analysis. Quantitative analysis of (B) p-p38, (C) p-JNK and (D) p-ERK was performed by densitometric analysis. #P<0.05, vs. NC group; *P<0.05 and **P<0.01, vs. OVA group. PP, *Physalis peruviana* L.; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; p-, phosphorylated; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA.

inflammatory mediators and cytokines lead to mucus hypersecretion and bronchoconstriction (30-32). Macrophages are important in the airway inflammatory response and contribute to the process of tissue remodeling in allergic asthma (33,34). The increased levels of eosinophils and macrophages in the BALF are the main features of the OVA-induced allergic asthma animal model (8). Based on this, the present study investigated the inhibitory effects of PP on the OVA-induced eosinophil and macrophage recruitment. The results showed that PP had regulatory effects on the recruitment of inflammatory cells, including eosinophils and macrophages, in the allergic asthma animal model (Fig. 2A and B).

The abnormal Th2 type response is also a key feature of the allergic reaction (4,35). Th2-derived IL-5 is responsible for the maturation, survival and recruitment of eosinophils (21). It is well known that IL-13 stimulates the B lymphocyte-derived production of IgE (22) and has a primary stimulatory action in the pathophysiology of allergic asthma by causing mucus hypersecretion (36). Therefore, regulation of the abnormal production of Th2-derived IL-5 and IL-13 are important in the alleviation of the allergic response. In the present study, it was confirmed that PP treatment effectively inhibits the OVA-induced production of IL-5 and IL-13 (Fig. 3). These findings suggest that PP has an immunoregulatory effect in an allergic asthma model.

It is well documented that IgE amplifies the allergic response, and its immunomodulatory functions include the activation of mast cells and the increase of B cell-derived

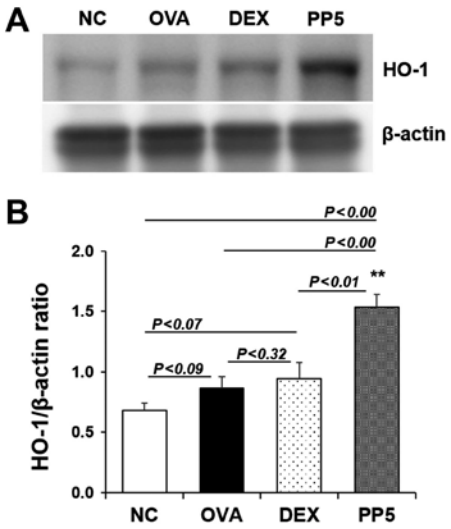

Figure 8. PP upregulates the expression of HO-1 in the lungs of mice. (A) Expression levels of HO-1 were examined by western blot analysis. (B) Quantitative analysis of HO-1 was performed by densitometric analysis. **P<0.01, vs. NC group. PP, *Physalis peruviana* L.; HO-1, heme oxygenase-1; NC, normal control group; OVA, ovalbumin; DEX, dexamethasone + OVA; PP5, 5 mg/kg PP + OVA.

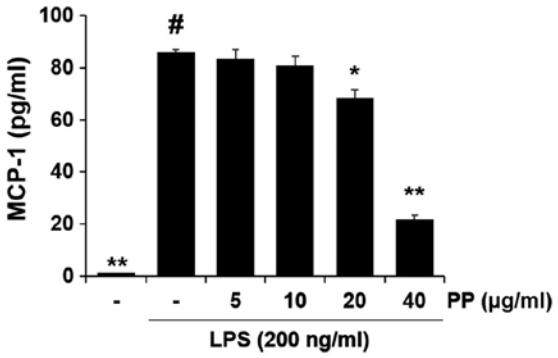

Figure 9. PP downregulates the production of MCP-1 in LPS-stimulated RAW264.7 macrophages. Supernatant levels of MCP-1 were evaluated using ELISA kits. #P<0.05, vs. NC group; *P<0.05 and **P<0.01 vs. LPS group. PP, *Physalis peruviana* L.; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1.

allergen uptake for antigen presentation (37). IgE also stimulates inflammatory cells to produce the inflammatory molecules histamine and leukotrienes, which cause inflammatory cell influx and mucus hypersecretion (38). Therefore, it is important to control the levels of IgE to prevent or treat allergic asthma. The present study focused on the inhibitory effect of PP on the overproduction of IgE. The ELISA results show that PP can act as a potent inhibitor of IgE (Fig. 4).

Macrophage activation is not only associated with the airway inflammatory response but also with airway remodeling (39), and cell-derived MCP-1 acts as a potent eosinophil chemoattractant (40,41). Therefore, the regulation of macrophage activation and overproduction of MCP-1 is an alternative therapeutic strategy for treating allergic asthma. In a previous study, PP was shown to exert anti-inflammatory effects on LPS-stimulated macrophage cells by reducing NO (17). In our previous study, PP was shown to inhibit the production of MCP-1in a COPD animal model (18). In the present study, it was confirmed that PP reduced the number of inflammatory cells, including eosinophils and macrophages, in the BALF of OVA-induced allergic asthma mice (Fig. 2). Based on previous results and those of the present study, it was expected that PP exerts an inhibitory effect on OVA-induced inflammatory cell influx and the overexpression of MCP-1. As expected, PP treatment decreased the levels of inflammatory cell influx, T cell marker expression and the expression of MCP-1 compared with the OVA group (Fig. 5A and C) and effectively downregulated the production of MCP-1 in LPS-stimulated RAW264.7 macrophages (Fig. 9), indicating that PP may be a potent inhibitor of MCP-1 in airway inflammatory diseases, including allergic asthma.

The original function of mucus is to protect the lungs against foreign particles (42). However, the overproduction of mucus interrupts the airflow and leads to airway obstruction (43). Goblet cell hyperplasia is responsible for the overproduction of mucus and airway hyper-responsiveness (AHR), which are the major hallmarks of allergic asthma (44). In the present study, the PAS staining results revealed that treatment with PP ameliorated OVA-induced mucus hypersecretion (Fig. 5B).

In the inflammatory response, IκBα is phosphorylated and rapidly degraded, and this event leads to the phosphorylation of NF-κB and its nuclear translocation, and eventually induces the production of inflammatory molecules (4,45,46). Downregulation of the activation of NF-κB is closely associated with the amelioration of airway inflammation by suppressing inflammatory cell recruitment, expression of inflammatory molecules and AHR in OVA-challenged lungs (1,4,45). Therefore, NF-κB is a promising molecular target in the treatment of allergic asthma. Castro *et al* reported that PP leads to the downregulation of NF-κB-dependent inflammatory molecules, including tumor necrosis factor-α, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) in an *in vivo* model (17). In our previous study, the regulatory effect of PP on NF-κB-dependent molecules, including iNOS, COX-2 and MCP-1, were observed in a COPD-like animal model (18). In present study, the regulatory effect of PP on the OVA-induced expression of MCP-1 was confirmed (Fig. 4C). Therefore, it was expected that PP can regulate the activation of NF-κB. In the present study, it was found that the NF-κB signaling pathway was activated in the OVA group, leading to the phosphorylation of IκB and NF-κB. However, treatment with PP significantly suppressed the phosphorylation of IκB and NF-κB (Fig. 6). These results suggest that suppression of the NF-κB signaling pathway is closely associated with the protective effect of PP in an allergic asthma animal model.

The MAPK signaling pathway is also considered to be linked with the inflammatory response (47). It has been shown that MAPKs are crucial in the pathophysiology of asthma by controlling inflammatory molecules; their activation has been detected in *in vitro* and *in vivo* asthma models, and inhibition of their activation inhibits the airway inflammatory response (6,48). The results of the present study revealed that treatment with PP decreased the OVA-induced activation of MAPKs (Fig. 7). In particular, the levels of p38 and JNK activation were markedly downregulated following administration with PP in the lungs of the OVA-induced mice. These findings suggest that PP treatment disrupts the activity of p38 and JNK.

Previously *in vivo* experiments demonstrated that treatment with antioxidants ameliorate allergic airway inflammation by upregulating the expression of HO-1 in a mouse model of asthma, and increased levels of HO-1 in the group treated with OVA and antioxidant were higher compared with those in normal control or OVA-treated groups in a concentration-dependent manner (1,27,28). Based on these results, the present study examined the effect of PP on the induction of HO-1. The results showed that the effects on the expression of HO-1 in the NC, OVA and OVA + PP administration group were similar to those of previous studies (1,27,28). In particular, treatment with 5 mg/kg PP markedly increased the expression of HO-1 compared with that in the NC, OVA or DEX groups. This result suggests that the upregulation of HO-1 may be associated with an improved airway inflammatory response in the OVA-induced allergic asthma model (Fig. 8). Therefore, PP may be considered as an essential herbal plant for the prevention of airway inflammation.

In conclusion, the results of the present study demonstrated a significant reduction in the eosinophil count of BALF samples from the PP-treated group. Similarly, PP suppressed the lung infiltration of inflammatory cells. The results also showed significant alleviation in the levels of IL-4, IL-5 and IL-13 in the BALF and in the level of IgE in serum samples of the PP-treated group. Furthermore, PP downregulated the levels of mucus hypersecretion. These effects were associated with the inhibited activation of NF-κB/p38-pJNK MAPK and upregulation of the expression of HO-1. These findings provide evidence that PP possesses potent anti-inflammatory effects and exerts protective effects against OVA-induced asthmatic symptoms.

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Availability of data and materials

All data generated and/or analyzed during the present study are included in this published article.

Authors' contributions

HAP and OKK performed the experiments and contributed to the interpretation of the results. HWR contributed to the extraction of *P. peruviana* L. JHM, MWP and MHP contributed to the acquisition of data. JHP, SC, IP and PY made substantive intellectual contribution to the published study. SRO provided conceptual advice. JWL designed the study and wrote the manuscript. KSA supervised the project and was involved in revising it critically for important intellectual content. All authors discussed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

All experiments were approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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