Petasites japonicus-propolis mixture attenuates airway inflammation in a mouse model of PM_{10} and ovalbumin-induced respiratory disease

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Abstract. Airway inflammation driven by particulate matter (PM) exposure underlies diseases such as asthma and allergic rhinitis. Although conventional anti-inflammatory therapies exist, they often cause significant side effects. Natural plant extracts offer non-toxic alternatives with comparable efficacy. The present study evaluated the effects of *Petasites japonicus*-propolis (PJP) mixture in a mouse model co-exposed to PM (intranasal) and ovalbumin (OVA; intraperitoneal) over 30 days. PJP was administered orally at 50, 100 or 200 mg/kg daily for 9 days. PJP reduced sneezing and nasal rubbing. Serum levels of total IgE and OVA-specific IgG_1 were decreased by PJP. In addition, bronchoalveolar lavage fluid and nasal lavage fluid showed lower histamine and IL-4 concentrations. In lung tissue, PJP reduced the epithelial thickness and inflammatory cell infiltration (goblet cells, eosinophils and mast cells). At the molecular level, PJP downregulated suppression of tumorigenicity 2, IL-33, TNF-α and IL-4 expression, and inhibited NF-κB phosphorylation. PJP attenuated PM/OVA-induced airway inflammation by suppressing NF-κB signaling and associated cytokine responses, highlighting its potential as a therapeutic candidate for inflammatory respiratory diseases.

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Introduction

Airway inflammation is a central feature in the pathogenesis of respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis, and allergic rhinitis, all of which contribute significantly to global morbidity (1). Allergic asthma and rhinitis alone affect approximately 300 million people worldwide, with projections suggesting an additional 100 million cases by 2030 due to escalating environmental exposures (2). Among various environmental triggers, particulate matter (PM)-originating from both anthropogenic sources (e.g., vehicle exhaust, industrial emissions) and natural phenomena (e.g., wildfires, dust storms)-has emerged as a potent inducer of airway inflammation and systemic injury (3,4).

Upon inhalation, PM can disrupt pulmonary homeostasis by promoting allergen-specific immunoglobulin production, complement activation, and recruitment of inflammatory cells such as neutrophils, eosinophils, mast cells, and macrophages (5,6). These immune responses contribute to disease-specific pathologies, including bronchoconstriction and airway hyperresponsiveness in asthma, mucus hypersecretion in chronic bronchitis, and histamine-mediated nasal symptoms in allergic rhinitis (7-9). Although inhaled corticosteroids, leukotriene modifiers, antihistamines, and bronchodilators can attenuate these inflammatory pathways. However, their long-term use is often limited by side effects such as mucosal dryness, drowsiness, and high cost. Moreover, these treatments do not address the underlying oxidative stress and cytokine-mediated tissue damage (10,11). Given these limitations, there is growing interest in phytochemicals that offer targeted anti-inflammatory action with improved safety and affordability.

Natural products have gained increasing attention as accessible, non-toxic alternatives to conventional therapies while often offering comparable efficacy (12). Notably, combinations of natural compounds frequently exhibit

enhanced pharmacological activities compared to a single agent (13). Among these, *Petasites japonicus* (butterbur), a perennial herb in the Asteraceae family, has long been used in East Asia for its antipyretic, antitussive, and wound-healing properties and for treating migraines, allergies, bronchial asthma, and gastrointestinal ulcers (14). Recent studies have further demonstrated butterbur's anti-tumor, anti-allergic, anti-inflammatory, neuroprotective, and anti-asthmatic effects (15). Propolis is a natural substance used by bees to protect their hives. It is an extract of a mixture of tree resin, honey, pollen, and their own enzymes. Propolis is known to have a variety of pharmacological properties, including anti-oxidant, anti-inflammatory, antibacterial, wound healing, and immunomodulatory effects (16-18). However, the therapeutic potential of a *P. japonicus*-propolis (PJP) mixture remains unexplored, especially in the setting of PM_{10}-induced airway inflammation.

Thus, this study aimed to evaluate the efficacy of PJP extract in a mouse model of airway inflammation exacerbated by particulate matter exposure. We focused on its ability to modulate the IL-33/ST2/NF-κB signaling pathway. IL-33, an alarmin cytokine released during cellular stress or damage, can bind to the ST2 receptor to activate NF-κB signaling, thereby driving the production of pro-inflammatory cytokines and chemokines that recruit immune cells to the airway (19). Our previous *in vitro* studies demonstrated that a 1:1 mixture of P. japonicus and propolis exhibited synergistic anti-inflammatory and antioxidant effects in particulate matter-exposed cell models (20,21). Based on these findings, we sought to investigate whether this synergistic effect would be replicated *in vivo*. By targeting this axis, PJP might offer a novel, natural strategy for mitigating environmentally induced airway inflammation.

Materials and methods

Reagents. Ovalbumin (OVA, A5503), particulate matter 10 (PM_{10}, MRMCZ110), aluminum hydroxide (239186), dexamethasone (Dex, D4902) and decalcifying solution (HS-105) were sourced from Sigma-Aldrich (St. Louis, MO, USA). H&E staining kit (ab245880) and periodic acid-Schiff (PAS, ab150680), Picro-Sirius Red stain kit (ab150681) and rabbit-specific HRP/DAB detection IHC kit (ab64261) were obtained from Abcam (Cambridge, UK). Toluidine blue O (198161) was sourced from Sigma-Aldrich (St. Louis, MO, USA). Mouse IgE ELISA Kit (EMIGHE) and anti-ST2 antibody (PA5-28383) were acquired from Thermo Fisher Scientific (Rockford, IL, USA). Anti-Ovalbumin IgG1 (mouse) ELISA Kit Cayman (500830) (Ann Arbor, MI, USA) and, IL-4 ELISA kits (M4000B) were sourced from R&D system (Minneapolis, MN, USA). Histamine ELISA kit (ENZ-KIT140A) was obtained from Enzo life sciences (Farmingdale, NY, USA)., Donkey anti-rabbit IgG secondary antibody (31458) was obtained from Thermo Fisher Scientific (Rockford, IL, USA). Goat anti-Mouse IgG(H+L)-HRP (SA001) was sourced from GenDEPOT (Katy, TX, USA)., IL-33 (88513) was obtained from Cell Signaling (Danvers, MA, USA)., NF-κB (sc-8008), p-NF-κB (sc-136548), and β-actin (sc-8432) were acquired from Santa Cruz Biotechnology (Dallas, TX, USA).

Preparation of P. japonicus propolis extract. In this study, *P. japonicus* was sourced from (Jinan-gun, South Korea) and propolis powder was obtained from Unique Biotech Co., Ltd. (Iksan-si, South Korea). Identification and authentication of the plant were conducted by Professor Hong-Jun Kim (College of Oriental Medicine, Woosuk University, Jeonbuk, South Korea). A voucher specimen (#2024-02-01) was placed in the Department of Health Management, College of Medical Science, Jeonju University. *P. japonicus* samples were ground using a blender and then extracted with 15 times their volume of 70% ethanol at 50˚C under reflux for 6 hours. The resulting extract was filtered through a cartridge filter using a Rotavapor R-210 (BUCHI Labortechnik AG, Flawil, Switzerland). The filtered extract was then concentrated using a vacuum rotary evaporator and subsequently lyophilized with a freeze dryer. The *P. japonicus* extract was then mixed with the propolis powder (1:1). The prepared sample (PJP) was stored at -20˚C to maintain its stability until use.

Animal studies and experimental design. Animal studies were designed and conducted using adult male BALB/c mice (6 weeks old, approximately 20 g) obtained from Han-il Laboratory Animal Center (Wanju, South Korea). Mice were housed under controlled environmental conditions (temperature: 22±2˚C, humidity: 50-60%, 12/12 h light/dark cycle) with ad libitum access to a standard laboratory diet and water. The present study was approved by Institutional Animal Care and Use Committee (IACUC) of Jeonju University. The health status of each of the animals was monitored daily. Humane experimental endpoints were established as a 20% or greater loss of body weight, a decreased appetite for more than two consecutive days, dyspnea, increased heart rate, self-mutilation, jaundice, persistent diarrhea or vomiting, or a decreased response to external stimuli. No animals reached these endpoints, and no mortality or euthanasia occurred during the study. Mice were acclimatized for one week before they were used for experiments. The total duration of the animal experiment, including the one-week acclimatization period and treatment schedule, was 38 days. The sample size of six mice per group was determined based on previous studies using PM_{10}/OVA-induced airway inflammation models (22), which commonly employ five to six animals per group to detect biologically meaningful differences in inflammatory markers with approximately 80% statistical power. This number balances scientific rigor with ethical considerations following the 3Rs principle (Reduction) to minimize animal use while ensuring robust and reproducible results. While we acknowledge that the limited sample size may affect statistical power and generalizability, the current findings offer meaningful preliminary insights. Future studies employing larger sample sizes are planned to validate and expand upon these results. The PM_{10}/OVA-induced airway inflammation model used in this study closely mimics key pathological features of human allergic asthma, such as airway hyperresponsiveness, eosinophilic inflammation, and mucus hypersecretion. After acclimation, mice were randomly assigned to six groups, with each group consisting of six mice. Group 1 (negative control group) was exposed to, sensitized, treated, and challenged with saline. Group 2 (positive control group) was exposed to 100 μg of PM_{10}, sensitized with 50 μg of OVA and 1 mg of alum and

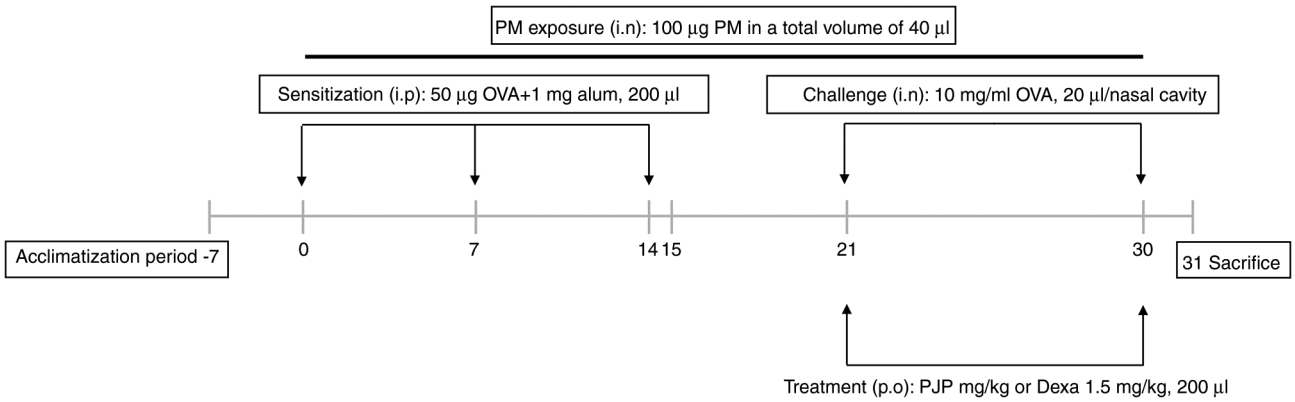

Figure 1. Schematic representation of the experimental protocol to evaluate the effects of PJP on the OVA-PM-induced respiratory disease mouse model. i.p, intraperitoneal; i.n, intranasal; p.o, per os; OVA, ovalbumin; alum, aluminum hydroxide; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dexa, dexamethasone.

challenged with 10 mg/ml of OVA, and treated with saline. Group 3 (PJP-50 group) was exposed to 100 μg of PM_{10}, sensitized with 50 μg of OVA and 1 mg of alum, challenged with 10 mg/ml of OVA, and treated with PJP at 50 mg/kg. Group 4 (PJP-100 group) was exposed to 100 μg of PM_{10}, sensitized with 50 μg of OVA and 1 mg of alum, challenged with 10 mg/ml of OVA, and treated with PJP at 100 mg/kg. Group 5 (PJP-200 group) was exposed to 100 μg of PM_{10}, sensitized with 50 μg of OVA and 1 mg of alum, challenged with 10 mg/ml of OVA, and treated with PJP at 200 mg/kg. Group 6 (dexamethasone group) was exposed to 100 μg of PM_{10}, sensitized with 50 μg of OVA and 1 mg of alum, challenged with 10 mg/ml of OVA, and treated with dexamethasone at 1.5 mg/kg. All test compounds were administered via oral gavage. Groups 2-6 were exposed to 100 μg PM_{10} in a total volume of 40 μl daily via intranasal instillation and also sensitized on days 0, 7, and 14 with an intraperitoneal injection of 50 μg of OVA and 1 mg of alum adjuvant solution. From day 15 to day 30, mice were treated with either saline, 50-200 mg/kg PJP, or 1.5 mg/kg dexamethasone. On days 21 and 30, all mice except those in the control group received an intranasal challenge of 20 μl of OVA solution (10 mg/ml). On day 30, rubbing and sneezing were recorded for 15 min for each mouse. Throughout the study, no noticeable adverse effects related to PJP administration, such as body weight loss or reduced appetite, were observed. Mice were sacrificed 24 h after the last OVA challenge (day 31). Mice were deeply anesthetized with inhalation of isoflurane at 2-6% for induction and maintained at 1-3% during sample collection. Euthanasia was performed by cervical dislocation under deep anesthesia. Death was confirmed by the absence of respiratory movement and heartbeat. Blood samples were collected from their orbital venous plexus 24 h after the last OVA challenge (day 31). Required mouse samples were then collected and stored at -80˚C until use. A schematic of the experimental overview is shown in Fig. 1.

Evaluation of nasal symptoms. After the final OVA challenge on day 30, each mouse's behavior was recorded with a camera for 15 min to monitor frequencies of nasal rubbing and sneezing. The scoring of sneezing and nasal rubbing was performed by observers blinded to the experimental groups to prevent bias.

Collection of serum, nasal lavage fluid (NLF), and bronchoalveolar lavage fluid (BALF). Serum, NLF, and BALF were collected using previously established methods (23). At the end of the experiment, mice were anesthetized with a 2-6% isoflurane for induction and maintained at a 1-3% concentration. Blood samples (600-800 μl per animal, collected once) were collected from the orbital venous plexus. Subsequently, mice were euthanized through cervical dislocation and, after confirming the absence of respiratory and heartbeat, the trachea was opened, and 1 ml of sterile saline was injected into the nasal cavity using a small tube to collect NLF. BALF was obtained by inserting an 18-gauge catheter into the partially exposed trachea and infusing 1 ml of saline, which was then gently aspirated. The collected fluid was transferred to a microcentrifuge tube for centrifugation. The supernatant obtained and serum were used for enzyme-linked immunosorbent assays (ELISAs).

Histopathology. Nasal and lung tissues were collected immediately after euthanasia on day 31. The tissues were carefully dissected and rinsed with cold saline to remove blood prior to fixation. Nasal and lung tissues were fixed in 10% formalin at 24±2˚C for three days. Nasal tissues were then decalcified using a decalcifying solution (HS-105) for histopathological examination. After fixation, tissues were embedded in paraffin blocks and sectioned at a thickness of 5 μm. The tissue sections were stained with hematoxylin and eosin (H&E), Sirius red, Toluidine blue and periodic acid-Schiff (PAS) according to the manufacturer's guidelines to analyze general morphology, goblet cell hyperplasia, and mast cell infiltration. The severity of lung inflammation was assessed using a five-point scoring system as previously described (24): 0 for normal, 1 for a few cells, 2 for a ring of cells one-cell-layer deep, 3 for a ring of cells two-to four-cells deep, and 4 for a ring of cells deeper than four-cells deep. Areas of PAS-positive regions were measured using Fiji software.

Evaluation of immunoglobulin and cytokines. Levels of OVA-specific IgE and OVA-specific IgG1 in serum were quantified using commercial ELISA kits following the manufacturer's instructions. Similarly, levels of IL-4 and histamine in both NLF and BALF were measured using ELISA kits according to the manufacturer's guidelines.

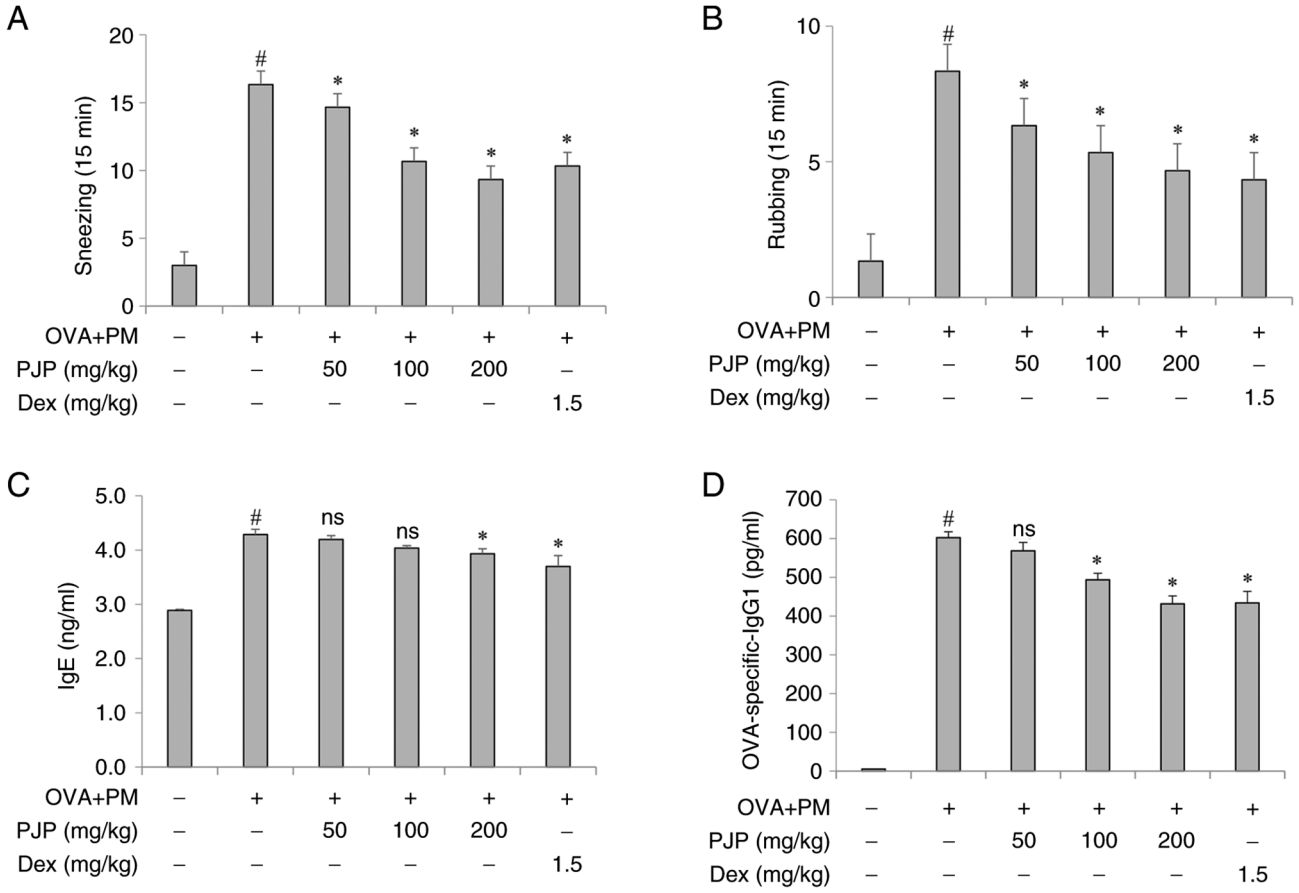

Figure 2. PJP reduces nasal allergy symptoms and the levels of OVA-specific immunoglobulins in serum. (A) Number of sneezing events and (B) nasal rubbing were measured for 15 min following the final OVA challenge on day 30. (C) Serum levels of OVA-specific IgE. (D) Serum levels of OVA-specific IgG1. Each bar represents the mean ± SEM (n=6). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. OVA + PM group. ns, not significantly different from the OVA + PM group; OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone.

Immunohistochemistry. Nasal tissue samples were collected and fixed in 10% formalin at 24±2˚C for three days. The nasal tissues were then decalcified using a decalcifying solution for histopathological examination. After fixation, the tissues were embedded in paraffin blocks and sectioned at a thickness of 4.5 μm. These sections were analyzed using a rabbit-specific HRP/DAB detection IHC kit.

Western blotting. Lung tissues were homogenized, sonicated, and lysed using radioimmunoprecipitation assay buffer. Protein quantification was performed using the Bradford method, with bovine serum albumin as the standard. Protein samples (50 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. These membranes were then blocked with freshly prepared 5% skim milk at 22±2˚C for 1 h. Each membrane was subsequently incubated overnight on an orbital shaker with a primary antibody specific to the target protein. After washing, the membranes were incubated with a secondary antibody for approximately 2 h at 22±2˚C. Signals were detected and visualized using an enhanced chemiluminescence detection system (Alliance version 15.11; UVITEC). Bands were analyzed using Image J software (NIH, Bethesda, MD, USA).

Statistical analysis. All data are presented as mean ± standard error of the mean (SEM) (n=6). The Shapiro-Wilk test was used to assess the normality of the data, and the results indicated that the data did not meet the assumption of normality. Therefore, non-parametric statistical analyses were performed. Group comparisons were conducted using the Kruskal-Wallis test, followed by Dunn's multiple comparison test for post hoc analysis. Statistical analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, USA), and a P-value of <0.05 was considered statistically significant.

Results

PJP reduces nasal symptoms of mice with PM_{10}/OVA-induced respiratory disease in mice. Exposure to PM_{10} and OVA throughout the experimental period led to a significant increase in the frequency of nasal rubbing and sneezing in mice of the positive control group compared to the negative control group. However, treatment with PJP at various doses resulted in a decrease in these symptoms. In particular, mice administered PJP at 200 mg/kg exhibited approximately a 42.9% reduction in sneezing and a 44.5% reduction in nasal rubbing compared to the positive control group. These effects were comparable to those observed in the dexamethasone-treated group (1.5 mg/kg), with no significant differences between the PJP 100 or 200 mg/kg groups and the dexamethasone group (Fig. 2A and B).

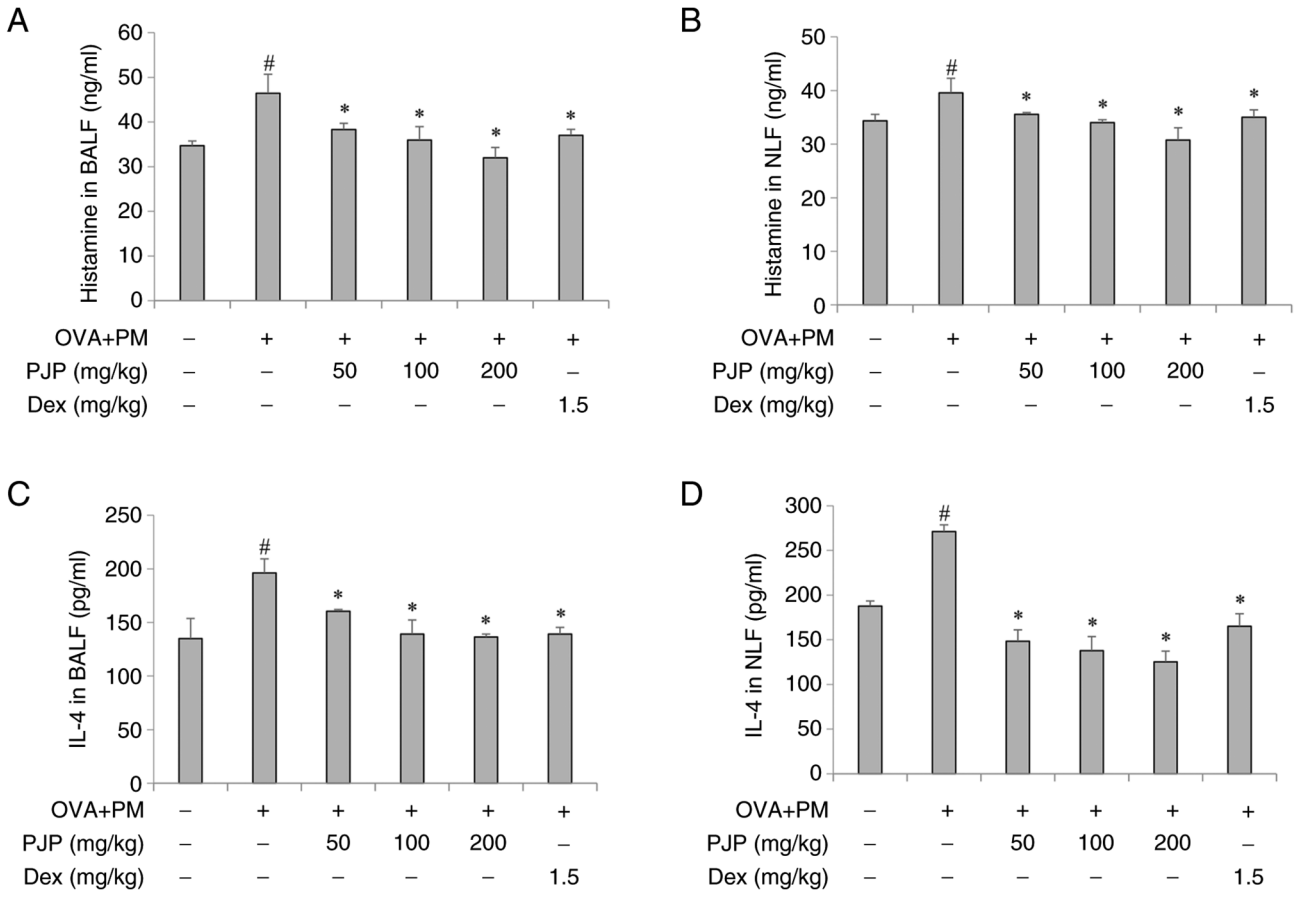

Figure 3. PJP reduces histamine and IL-4 levels in NLF and BALF. (A) Levels of histamine in BALF. (B) Levels of histamine in NLF. (C) Levels of IL-4 in BALF. (D) Levels of IL-4 in NLF. Each bar represents the mean ± SEM (n=6). #P<0.05 vs. normal group. *P<0.05 vs. OVA + PM group. OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; NLF, nasal lavage fluid; BALF, bronchoalveolar lavage fluid.

PJP reduces immunoglobulin levels in serum of PM_{10}/OVA-induced respiratory disease in mice. Effects of PJP on immunoglobulin levels in mice with respiratory disease were assessed. Exposure to PM_{10} and OVA throughout the experimental period significantly increased serum levels of OVA-specific IgE and IgG1 in the positive control group compared to the negative control group. Administration of various doses of PJP resulted in a decrease in these immunoglobulin levels. In particular, mice administered 200 mg/kg of PJP showed approximately 9.3 and 28.3% decrease in serum OVA-specific IgE and IgG1 levels, respectively, compared to the positive control group. There was no significant difference in these levels between mice treated with PJP at 100 mg/kg or 200 mg/kg and those treated with dexamethasone at 1.5 mg/kg (Fig. 2C and D).

PJP reduces inflammatory mediators and histamine release in BALF and NLF of PM_{10}/OVA-induced respiratory disease in mice. Effects of PJP on inflammatory mediators and histamine release in BALF and NLF were investigated. Exposure to PM and OVA throughout the experimental period significantly increased levels of IL-4 and histamine in the BALF and NLF of the positive control group compared to the negative control group. Treatment with PJP at various doses resulted in a decrease in histamine levels in both BALF and NLF, with significant reductions observed at all doses. Interestingly, PJP at 200 mg/kg reduced histamine release by approximately 31.3 and 22.4% in BALF and NLF, which was more effective than the reduction achieved by dexamethasone at 1.5 mg/kg (Fig. 3A and B). In addition, PJP treatment significantly decreased IL-4 secretion in both BALF and NLF. PJP at 200 mg/kg reduced IL-4 levels in BALF by approximately 30.4% compared to the positive control group. Furthermore, in NLF, PJP at 200 mg/kg reduced IL-4 secretion by approximately 53.8% more than the reduction observed with dexamethasone at 1.5 mg/kg (Fig. 3C and D).

PJP reduces inflammatory mediators in lung tissues of PM_{10}/OVA-induced respiratory disease in mice. Effects of PJP on inflammatory mediator expression in lung tissues were examined. Exposure to PM_{10} and OVA throughout the experimental period significantly increased the expression of ST2, IL-33, TNF-α, and IL-4 in lung tissues of the positive control group compared to the negative control group. Treatment with various doses of PJP resulted in a reduction in the expression of these mediators, with the greatest decreases observed as follows: 52.8% for ST2, 45.7% for IL-33, 41.2% for TNF-α, and 11.1% for IL-4. These effects were comparable to those observed in the dexamethasone-treated group (1.5 mg/kg) (Fig. 4).

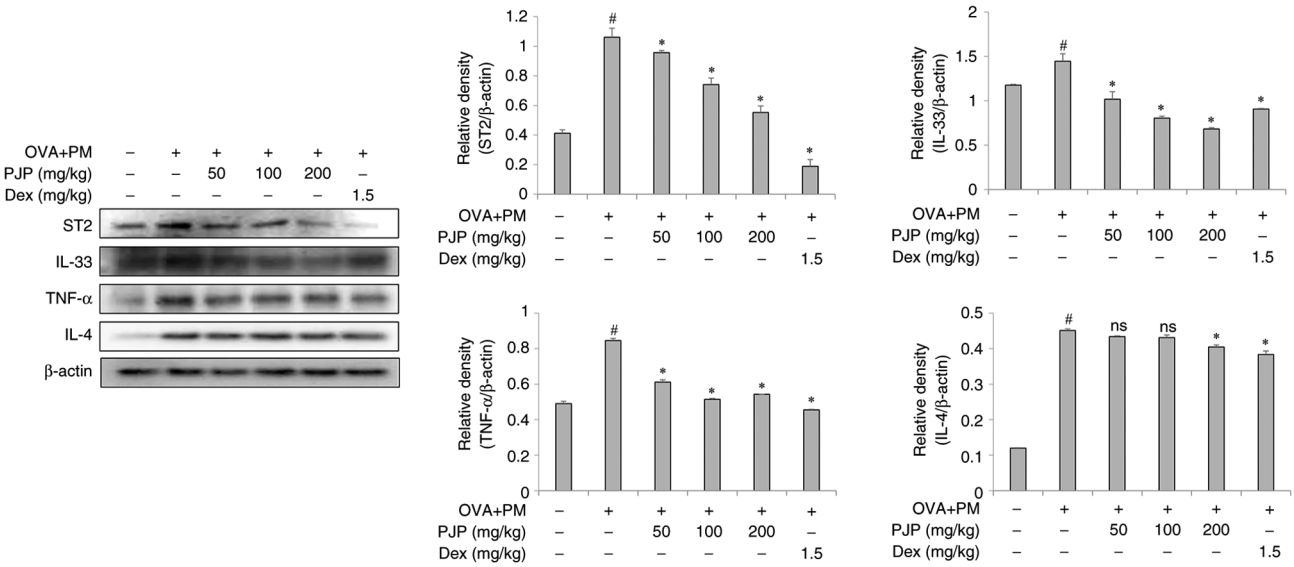

Figure 4. Inhibitory effects of PJP on ST2, IL-33, TNF-α and IL-4 expression in the lung tissue of OVA-PM-induced respiratory disease mice, analyzed by western blotting. Each bar represents the mean ± SD (n=6). #P<0.05 vs. normal group. *P<0.05 vs. OVA + PM group. ns, not significantly different from the OVA + PM group; OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; ST2, suppression of tumorigenicity 2.

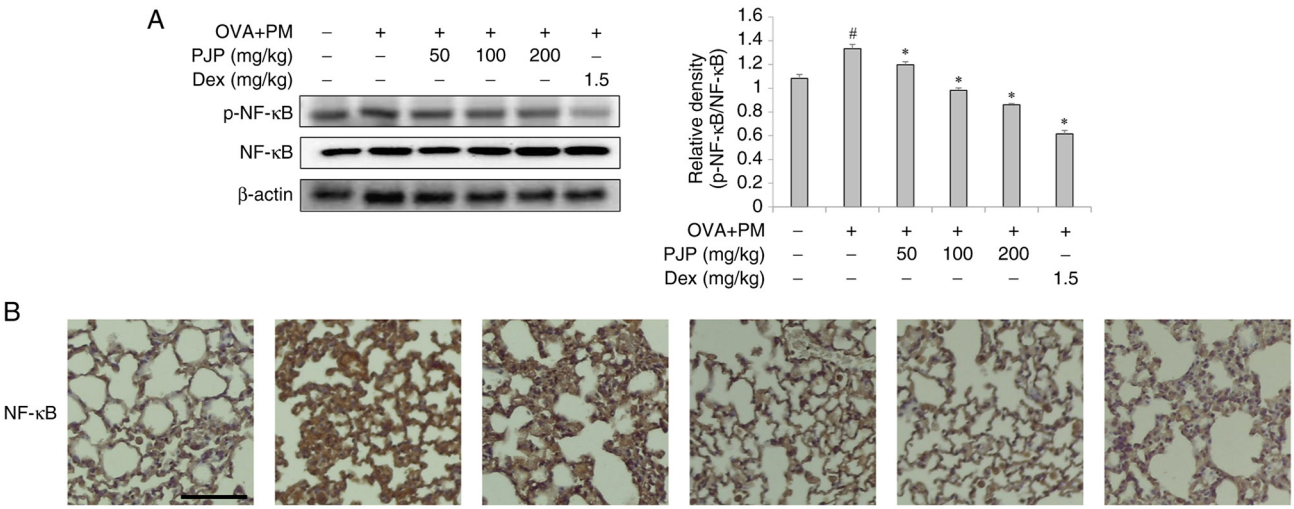

Figure 5. Effects of PJP on NF-κB phosphorylation in lung tissue. (A) Phosphorylation of NF-κB in lung tissue was analyzed by western blotting. The protein levels of NF-κB and p-NF-κB were semi-quantified and compared among groups. (B) Immunohistochemical analysis of NF-κB levels in lung tissue observed at a magnification of x100. Positive staining indicates the localization and activation of NF-κB in the tissue sections. Scale bar, 200 μm. Each bar represents the mean ± SEM (n=6). #P<0.05 vs. normal group. *P<0.05 vs. OVA + PM group. OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; p-, phosphorylated.

Effect of PJP on NF-κB signaling pathway in lung tissues. Effects of PJP on activation of the MAPK/NF-κB signaling pathway were analyzed. Both western blot and IHC analysis of NF-κB phosphorylation in lung tissues revealed a significant increase in p-NF-κB expression in the positive control group compared to the negative control group. Treatment with various doses of PJP decreased p-NF-κB expression compared to the positive control group. Notably, PJP at 200 mg/kg reduced p-NF-κB expression by approximately 35.3%. In addition, a significant difference was observed between the PJP-treated group and the dexamethasone-treated group (Fig. 5A and B).

PJP ameliorates lung and nasal histology in PM_{10}/OVA-induced respiratory disease in mice. Effects of PJP on the morphology and histology of lung and nasal tissues were investigated. H&E staining revealed that exposure to PM_{10} and OVA throughout the experimental period significantly increased nasal mucosal thickness in the positive control group compared to the negative control group. Treatment with various doses of PJP resulted in a reduction in epithelial thickness. Mice administered PJP exhibited a significant decrease in epithelial thickness compared to the positive control group. Notably, treatment with PJP at 100 mg/kg reduced epithelial thickness by approximately 32.9%, and this effect was not significantly different from that

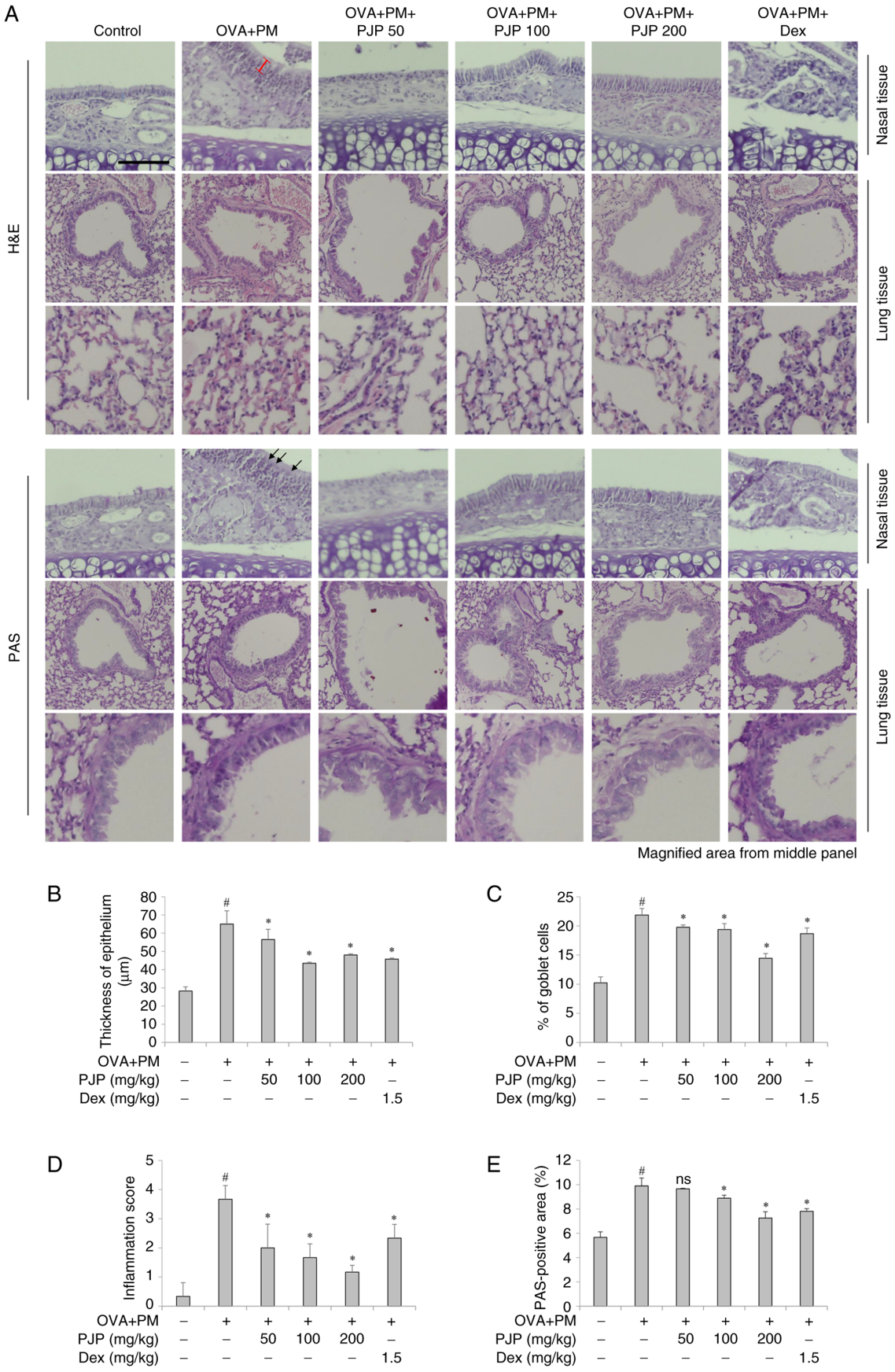

Figure 6. Histological changes in OVA + PM-induced respiratory disease mice treated with PJP. (A) H&E and PAS staining of nasal and lung tissues observed at a magnification of x100 (top and middle panels, respectively). The red bar indicates epithelial thickness, and black arrows indicate PAS-positive areas. The bottom row shows a magnified image of the lung tissue from the middle row. (B) Thickness of the epithelium and (C) goblet cell count in nasal tissue (expressed as % of total epithelial cells). (D) Inflammation score and (E) PAS-positive area in lung tissue. Scale bar, 200 μm. Each bar represents the mean ± SEM (n=6). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. OVA + PM group. ns, not significantly different from the OVA + PM group; OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; PAS, periodic acid-schiff.

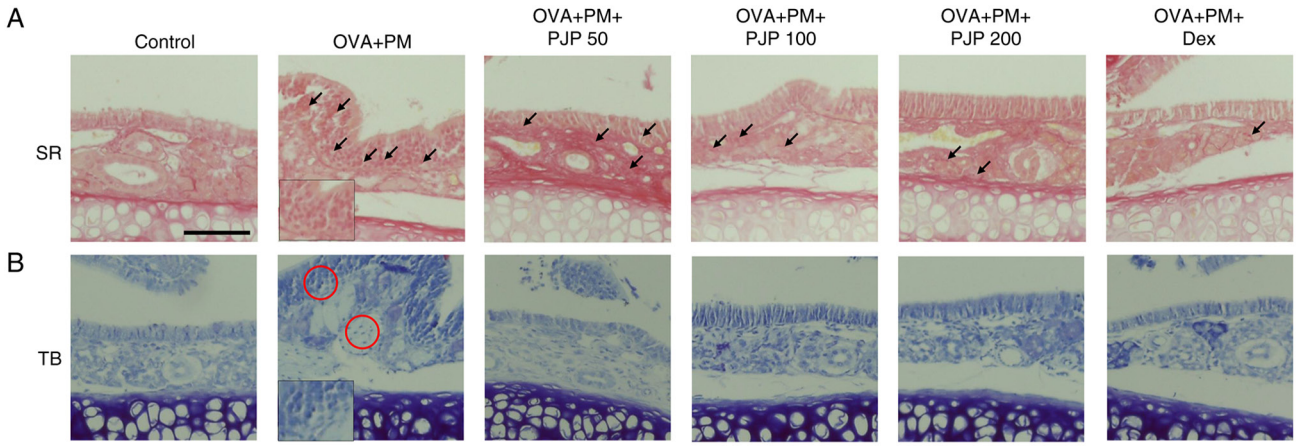

Figure 7. Histological evaluation of nasal tissue observed at a magnification of x100. (A) SR staining revealed eosinophil infiltration. Arrows indicate eosinophils. (B) TB staining highlighted mast cell infiltration. Circles indicate mast cells. Magnified areas show higher-resolution views. Scale bar, 200 μm. OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; SR, sirius red; TB, toluidine blue.

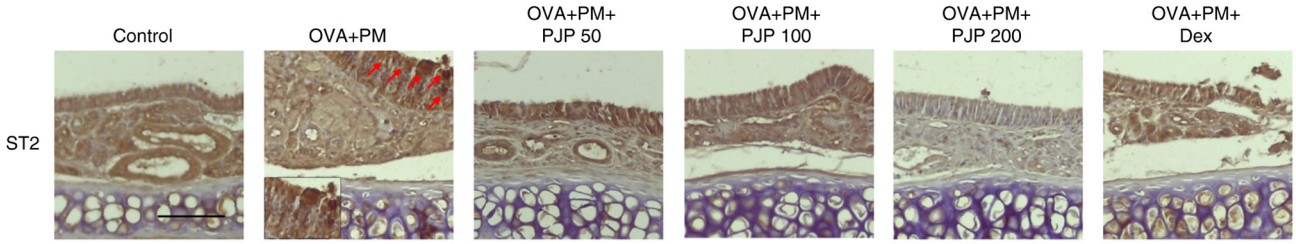

Figure 8. Immunohistochemical analysis of ST2 expression in nasal tissue observed at a magnification of x100. ST2 in nasal tissue was evaluated using immunohistochemical staining. Positive staining indicated the localization and expression levels of ST2 in the tissue sections. Arrows indicate ST2-positive staining in the nasal epithelium, and the magnified panel displays an enlarged view of these stained areas. Scale bar, 200 μm. OVA, ovalbumin; PM, particulate matter; PJP, *Petasites japonicus* propolis extract; Dex, dexamethasone; ST2, suppression of tumorigenicity 2.

observed in the dexamethasone-treated group (Fig. 6A and B). Additionally, goblet cell hyperplasia was more pronounced in mice exposed to PM_{10} and OVA compared to the negative control group. However, the number of goblet cells was significantly reduced in mice treated with PJP or dexamethasone compared to the positive control group. In particular, PJP 200 mg/kg resulted in an approximate 34.2% reduction in goblet cell numbers (Fig. 6C). Inflammatory cell infiltration in lung tissues was also evaluated using H&E and PAS staining. The inflammatory score was significantly higher in the PM_{10}/OVA-induced respiratory disease groups compared to the control group. PJP supplementation significantly reduced the inflammatory score compared to the positive control group. Treatment with PJP at all doses significantly decreased the inflammatory scores compared to the positive control group, with the PJP 200 mg/kg showing an approximately 67.6% reduction in inflammatory scores compared to the positive control group. Notably, the anti-inflammatory effect observed in the PJP 200 mg/kg group was greater than that observed in the dexamethasone-treated group (Fig. 6D). Similarly, the PAS-positive area was significantly higher in the PM_{10}/OVA-induced respiratory disease groups compared to the negative control groups. PJP administration reduced the PAS-positive area, indicating a decrease in mucus accumulation in the airways. In particular, PJP at 200 mg/kg reduced the PAS-positive area by approximately 26.3% compared to the positive control group, demonstrating a comparable effect to dexamethasone at 1.5 mg/kg (Fig. 6E).

PJP reduces eosinophil and mast cell infiltration in nasal tissues of PM_{10}/OVA-induced respiratory disease in mice. Effects of PJP on eosinophil and mast cell infiltration in nasal tissues were investigated. Exposure to PM_{10} and OVA throughout the experimental period significantly increased eosinophil and mast cell infiltration in nasal tissues of the positive control group compared to the negative control group. This was demonstrated by Toluidine blue staining of mast cells and Sirius red staining of eosinophils. Treatment with PJP at various doses resulted in decreased eosinophil and mast cell infiltration, with results similar to those of the dexamethasone-treated group (Fig. 7A and B).

PJP reduces ST2 in nasal tissues of PM_{10}/OVA-induced respiratory disease in mice. ST2 expression in nasal tissues was evaluated using IHC staining. Exposure to PM_{10} and OVA significantly increased ST2 expression in nasal tissues of the positive control group compared to the negative control group. Treatment with various doses of PJP reduced ST2 expression, such, effects of PJP were comparable to or more pronounced than those of dexamethasone (Fig. 8).

Discussion

This study explored the effects of PM_{10} and OVA exposure on airway inflammation in a murine model of allergic rhinitis and asthma, and evaluated the therapeutic potential of PJP. Our findings demonstrated that PJP effectively alleviated nasal symptoms, reduced immunoglobulin levels, suppressed inflammatory mediators, and modulated key signaling pathways involved in airway inflammation.

PM is a well-known exacerbating factor in respiratory diseases, including asthma and allergic rhinitis (3,25). PM exposure leads to increased production of pro-inflammatory cytokines and recruitment of immune cells such as mast cells, eosinophils, and macrophages, which contribute to airway hyperresponsiveness and remodeling (26). Our PM_{10}/OVA mouse model successfully recapitulated these features, including enhanced nasal rubbing, sneezing, epithelial thickening, goblet cell hyperplasia, and elevated serum IgE and IgG1 levels, consistent with previous reports using similar models (27,28).

The observed reduction of nasal symptoms and immunoglobulin levels by PJP aligns with studies showing that natural plant extracts and propolis components can modulate allergic inflammation by regulating IgE-mediated responses and immune cell activation (29,30). Notably, PJP administration significantly decreased serum OVA-specific IgG1 and IgE, which are critical in mast cell sensitization and histamine release, thereby directly linking the observed symptom relief to underlying immunomodulatory effects.

Furthermore, our results showed that PJP treatment reduced levels of IL-4 and histamine in both BALF and NLF. IL-4 is a central cytokine in Th2-mediated allergic responses that promotes IgE class switching and mast cell recruitment (31,32). The suppression of IL-4 and histamine release suggests that PJP modulates the Th2 immune axis, a mechanism corroborated by previous studies where propolis extracts inhibited Th2 cytokines and associated inflammation (33). Importantly, PJP's inhibitory effect on histamine release was more pronounced than that of dexamethasone, highlighting its potent anti-allergic properties.

In addition to Th2 cytokines, TNF-α and the IL-33/ST2 axis are critical mediators of airway inflammation. TNF-α amplifies allergic inflammation by enhancing Th2 cell migration and cytokine production (34,35). The IL-33/ST2 pathway activates downstream ERK1/2 signaling, leading to inflammatory gene expression and recruitment of innate lymphoid cells (19,36). PJP's ability to significantly downregulate TNF-α, IL-33, and ST2 expression underscores its broad-spectrum anti-inflammatory potential, targeting multiple pathways implicated in allergic airway disease pathogenesis.

The suppression of NF-κB phosphorylation in lung tissues by PJP provides further insight into its mechanism of action. NF-κB is a master regulator of inflammatory gene transcription, and its activation is a hallmark of PM-induced airway inflammation (37). By inhibiting NF-κB activation, PJP may prevent the transcription of pro-inflammatory cytokines and chemokines, thereby reducing immune cell recruitment and inflammation. This molecular mechanism is consistent with reports that *P. japonicus* and propolis components can modulate NF-κB signaling in various inflammatory models (38,39).

Histologically, PJP improved airway remodeling, as evidenced by reduced epithelial thickness, goblet cell hyperplasia, and inflammatory cell infiltration. These morphological improvements are critical because chronic airway remodeling contributes to disease severity and resistance to conventional therapies (40). The notable reduction in eosinophil and mast cell infiltration in nasal tissues further validates PJP's anti-inflammatory efficacy.

In addition, to clarify whether the observed cytokine-inhibitory effects are attributable solely to *Petasites japonicus*, propolis, or their combination, our previous *in vitro* studies using NCI-H292 lung epithelial cells and RAW264.7 macrophages demonstrated that the PJP mixture significantly suppressed inflammatory cytokines (IL-6, IL-1β, TNF-α), restored antioxidant enzyme levels (SOD, catalase, glutathione), and inhibited NF-κB and MAPK signaling pathways under PM stimulation (20,21). These findings suggest that both Petasites japonicus and propolis individually exert anti-inflammatory and antioxidant effects, but their combination enhances these actions through potential synergistic mechanisms. Although the current *in vivo* study did not include groups treated with individual extracts, our prior *in vitro* data support the hypothesis that each component contributes to the overall efficacy of PJP. Future studies should include single extract control groups to further delineate the individual and combined effects of each component *in vivo*.

Despite these promising results, there are limitations to consider. The study used an acute animal model, and the long-term effects and safety profile of PJP remain to be established. Moreover, the exact bioactive compounds responsible for these therapeutic effects require further identification and characterization. Future studies should also explore the pharmacokinetics and potential synergistic effects of PJP constituents, as well as validate these findings in clinical trials. Additionally, the PJP used in this study lacked standardization and quantification of key active compounds such as petasin, flavonoids, and CAPE, which may affect reproducibility. Although our previous *in vitro* studies confirmed anti-inflammatory effects of each component, the present *in vivo* study did not include propolis-only or *P. japonicus*-only groups, limiting the ability to distinguish their individual effects *in vivo*. Furthermore, while we focused on NF-κB signaling, additional analysis of pathways such as MAPK and ERK1/2 is needed to fully elucidate the therapeutic mechanisms. Future studies will address these limitations.

In summary, our study demonstrates that PJP mitigates PM_{10}/OVA-induced airway inflammation through modulation of immunoglobulin production, suppression of Th2 cytokines, inhibition of NF-κB signaling, and reduction of inflammatory cell infiltration and airway remodeling. These findings support the potential of PJP as a natural therapeutic agent for allergic respiratory diseases exacerbated by air pollution. Further research is warranted to fully elucidate its mechanisms and clinical applicability.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JHP and JYS made major contributions to the study design and manuscript writing, and made substantial contributions to the acquisition, analysis and interpretation of the data. DNC contributed to data interpretation, manuscript review, editing, drafting and validation. MYK contributed to methodology development and data visualization, and assisted with data analysis. YKH provided essential resources, performed formal data analysis and contributed to data interpretation. GSS contributed to data visualization, assisted in data interpretation and performed validation. BOC contributed to supervision, project administration and study conceptualization. SIJ contributed to supervision, project administration, study conceptualization and funding acquisition. JHP and SIJ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by Jeonju University Institutional Animal Care and Use Committee (approval no. jjIACUC-20230602-2022-0505-A1; Jeonju, South Korea).

Patient consent for publication

Not applicable.

Competing interests

JHP, MYK and YKH are affiliated with Unique Biotech Co., Ltd., which provided the propolis powder used in the present study. The other authors declare that they have no competing interests.

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