

Original Article

Petiveria alliacea Suppresses Airway Inflammation and Allergen-Specific Th2 Responses in Ovalbumin-Sensitized Murine Model of Asthma

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ABSTRACT **Objective:** To examine the effect of metanol extract of *Petiveria alliacea* (PM) on airway inflammation in a murine model of chronic asthma. **Methods:** Two-month-old male BALB/c mice ($n=6-8$ /group) were sensitized on days 0 and 14 by intraperitoneal injection of 20 μ g ovalbumin (OVA). On day 25, the mice received an airway challenge with OVA (3%, w/v, in phosphate buffered saline). PM was administered orally by oral gavage to mice at doses of 100, 200 and 400 mg/kg body weight once daily from days 18 to 23. Control mice were orally administered phosphate buffered saline (PBS) to induce a model of asthma. At the end of the test, respiratory reactivity was assayed, the total cell number, interleukin-4 (IL-4), IL-5, IL-13, tumor necrosis factor- α (TNF- α) and reactive oxygen species (ROS) in the bronchoalveolar lavage fluid (BALF) were determined and the levels of serum IgE, intercellular cell adhesion molecule 1 (ICAM-1) and eotaxin were measured. In addition, lung tissue was used to qualify the IL-4, IL-5, IL-13, TNF- α and transforming growth factor beta 1 (TGF- β 1). Histologic examination was performed to observe inflammatory cellular infiltration. **Results:** The administration of PM in comparison with the OVA-only treated group significantly attenuated the infiltration of eosinophils and other inflammatory cells ($P<0.01$). Airway resistance (RI) in the OVA-only induced group was significantly higher than that of the PBS control group ($P<0.01$) when methacholine was added. TNF- α , IgE, TGF- β 1 and cytokine levels IL-4, IL-5, IL-13 in the BALF decreased compared to control mice ($P<0.01$ or $P<0.05$). PM treatment also inhibited the production of chemokines, eotaxin and ICAM-1 in BALF ($P<0.01$), which improved lung function. Histopathological examination revealed that the sensitized treated PM groups had significant lower inflammatory scores similar to dexamethasone treatments and the untreated group. **Conclusion:** Administration of PM could inhibit airway inflammation, regulate cytokines, chemokines and enhance pulmonary conditions in allergic murine model of asthma.

KEYWORDS *Petiveria alliacea*, allergy, antiinflammation, asthma

Asthma is a chronic inflammatory disease, of the respiratory system, in which the airways gets narrow and are covered with excessive amounts of mucus, often in response to one or more triggers. This airway narrowing causes shortness of breath, wheezing, coughing, chest tightness, bronchospasm, reversible airflow obstruction induced by an inflammatory state that lead to a decreased of lung function. Allergic asthma is characterized by an overexpression of allergen-reactive reactions including IgE mediated inflammation of the nasal mucosa, which involves the infiltration of inflammatory cells. Eosinophils are the major inflammatory cell type that contributes to asthma, they release proinflammatory mediators, such as platelet activating factor and leukotriene C4 that increase the permeability of mucosal cells and stimulate further eosinophil infiltrate. T helper type 2

(Th2) cells release cytokines like [interleukin-4 (IL-4), IL-5, and IL-13] that promote IgE production by B cells and mucus deposit in the airways. In addition, eotaxin promotes the selective recruitment of eosinophils into the inflamed area.⁽¹⁾

Pharmacological treatments for asthma are often incapable to control the symptoms of the disease and

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do not halt the subsequent process.⁽²⁾ Consequently, there is a need to connect the ethno-pharmacological knowledge with the efforts to discover new active natural metabolites that could be a better treatment to diseases like this.⁽³⁾ Asthmatic mice induced by ovalbumin (OVA) have high concentrations of proinflammatory cytokine tumor necrosis factor α (TNF- α) and Th2 cytokines in the lung, serum and bronchoalveolar lavage fluid (BALF).⁽⁴⁾ In this study, we use this model to evaluate *Petiveria alliacea* (PA) for its pharmacological activity to treat the symptoms.

Petiveria alliacea L. (Phytolaccaceae) is used in folk medicine in the treatment of cancer, inflammatory process, as asthma, to enhance memory to treat flu and other viral or bacterial infections etc. Recently, a study reported the isolation and characterization of a large number of sulfur-containing amino acids.⁽⁵⁻⁹⁾ Considering that *Petiveria alliacea* possess antiinflammatory function⁽¹⁰⁾ and its use is described in traditional medicine to treat asthma. The aim of this study was to explore if *P. alliacea* could have a therapeutic effect in a murine model of allergic asthma.

Chemicals

OVA (grade II), aluminum hydroxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), α -tocopherol, dexamethasone, 3, 3', 5, 5'-tetramethylbenzidine (TMB), pentobarbital sodium, methacholine and 2,7-dichlorofluorescein diacetate were purchased from Sigma-Aldrich (Sto. Louis, MI, USA). IL-4, IL-5, IL-13, TNF- α , transforming growth factor β 1 (TGF- β 1), eotaxin, intercellular adhesion molecule-1 (ICAM-1) and gene IgE were measured using immunosorbent assay (ELISA) kits according to the manufacturer's instructions (eBioscience, San Diego, CA, USA).

Plant Extracts

Leaves of *Petiveria alliacea* L. were collected from Bahia de Bandera about 70 km of Puerto Vallarta. The plant was authenticated by Biology Aurora Chemal, and a voucher specimen (No. 5943) was deposited at the Herbarium of Universidad Autonoma Metropolitana-Xochimilco, (Mexico) for future reference. The plant material was air-dried in shade at room temperature and ground into powder (1 kg). Extraction was performed in Soxhlet apparatus with hexane (PH), chloroform (PC) and methanol (PM) consecutively for 3 h and then the solvent was removed at 40 °C under reduced pressure, with a

yield of 48, 29, and 65 g respectively. The extracts were kept in a desiccator.

Animals

BALB/c mice were chosen for the study because they are highly susceptible to asthma-like airway hyperresponsiveness. Male mice 2 months old with body weights between 20 and 25 g, were acclimatized for 1 week before use. Mice were housed under specific pathogen-free conditions, maintained at constant temperature and humidity, with food and *ad libitum* water. The experimental protocol and animal handling throughout the study were performed following the guidelines stated in the Principles of Laboratory Animal Care (National Institute of Health publication (NIH) 85-23), in Mexican Official Normativity (NOM-062-Z00-1999) and with the recommendations for the use of laboratory animals (No. 756/lab/ENCB).

Experimental Asthma Model Induction

To induce asthma, OVA sensitization and airway challenge were performed as described previously.⁽¹¹⁾ The mice were sensitized on days 0 and 14 by intraperitoneal injection of 20 μ g OVA emulsified in 2 mg aluminum hydroxide in 200 μ L phosphate buffered saline (PBS) buffer (pH 7.4). The OVA-challenged mice were exposed to 3%, OVA (W/v) in PBS for 20 min (over period of 5 min) by inhalation using an ultrasonic nebulizer (Just nebulizers equipment, Toronto, Canada). PM was completely dissolved in PBS and was prepared fresh daily before each administration. PM was administered by oral gavage to mice at doses of 100, 200 and 400 mg/kg body weight once daily (Figure 1). Control mice were orally administered with PBS. At the end of OVA challenge, BALF samples were obtained from the trachea of mice and processed. Serum samples were collected from the retro-orbital sinus of mouse. The airway responsiveness to aerosolized methacholine (MCh) was measured one day after the last OVA challenge. The OVA sensitized group developed pronounced increased airway responsiveness to MCh inhalation compared to the control group. Other groups of OVA-sensitized mice were administered with 3 different doses of PM extract respectively, with 50 mg/mL aerosolized OVA challenge to further induce airway hyperresponsiveness (AHR). Inflammatory cells were counted at least 5 squares of a hemocytometer.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed by instilling

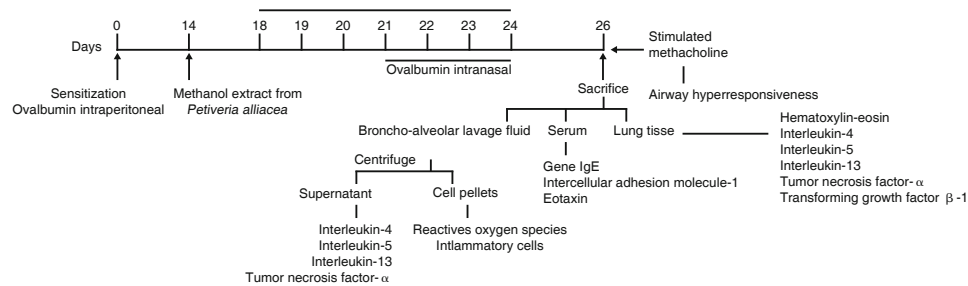


Figure 1. Effect of Treatment of Extracts from *P. alliacea* on Mouse Model of Airway Inflammation Induced by OVA

1 mL saline solution into the lungs through a tracheal cannula and then gently aspirating the fluid. This lavage was repeated 3 times. The recovered fluid was filtered through a double layer of sterile gauze to remove mucus. The resulting BALF was centrifuged (1,000 r/min, 4 °C, 10 min) and supernatants were collected; cells were fixed in 1% formalin with the addition of 2% sucrose and collected on cytocentrifugation glass slides. The supernatant was stored at −40 °C until the cytokine assays were performed. The cell pellets were used for the measurement of intracellular reactive oxygen species (ROS) and cell numbers.

Inflammatory Cell Counts in BALF

The total inflammatory cell number was assessed according to the previously described method.⁽¹²⁾ BALF was centrifuged, the pellet was washed 3 times with PBS, and finally resuspended in 100 µL of PBS. The total cell number was estimated using a hemocytometer. A cytospin preparation of BALF cells was fixed and stained using a Diff-Quik Staining reagent (Dade Behring Inc., Deerfield, IL, USA). The different cell types were enumerated based on their morphology and staining profile.

Evaluation of Mucus Secretion

For evaluation of mucus secretion, a semi-quantitative scoring system on periodic acid-Schiff (PAS) stained sections was used to identify goblet cells. Mucus was stained with 1% Alcian blue in 3% acetic acid (pH 2.5) for 15 min, followed by 2 washes in still tap water. Sections were treated for 5 min with 0.5% periodic acid followed by 2 washes in still tap water. Neutral mucus were stained with Schiff's reagent for 10 min. Tissue sections were washed thoroughly in still tap water. The number of goblet cells per crypt was calculated from an average of 10 crypts per tissue section, for 6 to 8 mice. The increased production of mucus by lung goblet cells is used as a parameter of allergic airways disease. To determine PM influence

on the secretion of mucus, lung sections were stained with PAS.

Measurement of DPPH Radical Scavenging Activities and Intracellular ROS in BALF

In order to evaluate the antioxidant activity of PM, a DPPH radical scavenging assay was performed as described previously.⁽¹³⁾ Several α-tocopherol concentrations (1, 5, 10, 25 and 50 µmol/L) were mixed with 120 µL of DPPH radical solution (1.5×10^{-4} mol/L), and the decrease in absorbance at 517 nm was monitored. DPPH solution with metanol was used as control, and α-tocopherol was used as a standard antioxidant and positive control.

The intracellular ROS was measured by the 2,7-dichlorofluorescein diacetate (DCF-DA) method.⁽⁹⁾ After the incubation of the BALF cells with 50 µmol/L DCF-DA for 45 min and the removal of PBS with the DCF-DA, cells were washed twice with 1 × PBS (pH 7.4), and DCF fluorescence was measured using a fluorometric microplate reader (BioTek, Winooski, VT, USA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

Measurements of Cytokines, Chemokines and OVA-Specific IgE in BALF and Lung Tissue

The amount and presence of cytokines, including IL-4, IL-5, IL-13, and chemokines, eotaxin, and ICAM-1 in BALF fluid and lung tissues, were measured using sandwich ELISA kits according to the manufacturer's instructions.

The levels of OVA-specific IgE in serum were measured by an ELISA as described previously.⁽⁴⁾ Briefly, microtitre plates were coated with 1% OVA in coating buffer (0.05 mol/L sodium carbonate-bicarbonate, pH 9.6) overnight at 4 °C. After blocking and washing, diluted serum samples (1/20) were added at room temperature and left for 2 h and

then washed and incubated with a biotinylated anti-mouse IgE antibody, followed by multiple washings and incubation for 30 min with extravidin-peroxidase conjugated reagent. For colour development at room temperature, 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate was added, and the reaction was stopped by 2 mol/L sulphuric acid (H₂SO₄). Optical density (OD) values were measured at 450 nm using a Thermo Fisher Scientific microplate reader (Waltham, MA, USA). In addition, Dex (2 mg/kg) was used as a positive control.

Effect of PM on Airway Inflammation

Semi-quantitative analysis of inflammatory cell infiltration and mucus production in lung sections were performed as previously described.⁽¹⁴⁾ The scoring data of inflammatory cell infiltration and mucus production were assessed at least 3 different fields for each lung section.

Determination of AHR

Airway function was measured by the whole-body plethysmography as described previously.⁽¹⁵⁾ Twenty-four hours after the secondary challenge, AHR was measured when mice were stimulated with Mch using a whole-body plethysmography (Buxco, Wilmington, NC). Mice were placed in the main chamber of a whole-body plethysmography and challenged with aerosolized Mch, at increasing concentration from 12.5 to 50 mg/mL in PBS buffer. After the end of aerosolization, the enhanced pause (Penh) values were measured during each 3 min period.

Lung Histology Study

After the lavage, the lungs were immediately removed and fixed in 10% v/v buffered formalin (in 0.01 mol/L PBS; pH 7.2). Pulmonary tissues were subsequently sliced, embedded in paraffin, and cut into 5- μ m-thick sections. These frozen sections were stained with hematoxylin-eosin (HE) to evaluate inflammatory cell infiltration. Light microscopy was used for the histopathological assessment.

Statistical Analysis

The data from the 3 independent experiments were expressed as the mean $\pm$ standard deviation ($\bar{x} \pm s$). The data were analyzed using the SPSS package (Version 10.0, SPSS, SPSS Inc., Chicago, IL, USA), and one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests was used to compare the results from different

treatments. The data were considered to have statistical significances at $P < 0.05$.

RESULTS

PM Decreased Cells Infiltration in Airway Allergic Inflammatory of OVA-Sensitized Mice

The groups with the induced-allergic airway disease by sensitization with OVA exhibited an increase in eosinophils and other inflammatory cells in the BALF compared with the control group (Table 1). In contrast mice treated with PM extract showed a lower number of eosinophils and total inflammatory cells in the BALF. Extracts of PH and PC were tested as well but no significant activity was present (data not shown).

Table 1. Effect of PM on Inflammatory Cells in BALF of Mice (1×10^4 cells/mL, $\bar{x} \pm s$)

Group	n	Eosinophil	Other inflammatory cells	Total cells
Control	6	—	59.17 $\pm$ 6.12	59.17 $\pm$ 4.29
OVA	6	210.0 $\pm$ 9.6	489.00 $\pm$ 12.52	699.00 $\pm$ 14.76
OVA+PM-100	8	121.0 $\pm$ 7.3*	193.00 $\pm$ 8.76*	314.00 $\pm$ 10.37*
OVA+PM-200	8	85.0 $\pm$ 7.8**	142.00 $\pm$ 12.69**	227.00 $\pm$ 9.56**
OVA+PM-400	8	65.0 $\pm$ 10.6**	123.00 $\pm$ 8.90**	188.00 $\pm$ 9.56**

Notes: * $P < 0.05$, ** $P < 0.01$, compared with OVA group

PM Decreased ROS Production of OVA-Sensitized Mice

The DPPH radical scavenging activity of PM was found to be near 100% at concentrations of 10, 25 and 50 μ g. α -Tocopherol was used as a positive control. In this study, α -tocopherol and PM performed a similar way on the DPPH. The effect of PM on ROS production is shown in Figure 2. In the OVA group, the levels of ROS in the BALF increased significantly compared with the PBS-control group ($P < 0.05$). These results suggested that PM exerted an inhibitory effect on ROS production.⁽¹⁶⁾

PM Suppressed Chemokines and Th2 Responses of OVA-Sensitized Mice

OVA groups have a remarkable elevation of serum-specific IgE chemokines levels ICAM-1 and eotaxin (Table 2). Despite the effects of OVA sensitization, when PM was administered there was a notable attenuation of the characteristic inflammatory pattern ($P < 0.05$). The protection of PM at a dose of 400 mg/kg was comparable to the PBS-challenged control group and greater than that produced by Dex at dose of 2 mg/kg ($P < 0.01$ vs. the OVA+ Dex group).

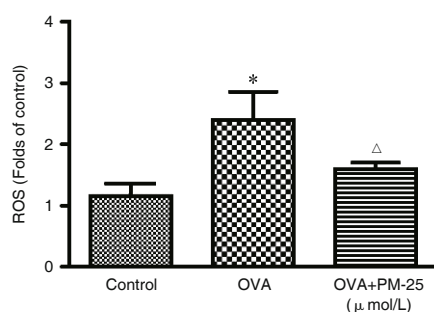


Figure 2. Effect of PM on ROS in BALF of Mice ($n=8$, $\bar{x} \pm s$)

Notes: * $P<0.05$, compared with the control group; $\Delta P<0.05$, compared with the OVA group

Table 2. Effect of PM on OVA-Specific IgE Level in Serum, ICAM-1 and Eotaxin in BALF of Mice by Sandwich ELISA (pg/mL, $\bar{x} \pm s$)

Group	<i>n</i>	IgE level	ICAM-1	Eotaxin
Control	6	0.074 ± 0.004	2.9 ± 0.8	16.0 ± 4.1
OVA	8	0.130 ± 0.009	28.0 ± 1.9	126.0 ± 7.4
OVA+PM-100	8	0.110 ± 0.006	20.0 ± 3.2	99.0 ± 6.2
OVA+PM-200	8	0.082 ± 0.008**	14.0 ± 2.4*	70.0 ± 5.5**
OVA+PM-400	8	0.075 ± 0.008** ^Δ	12.0 ± 2.6* ^Δ	65.0 ± 5.5** ^Δ
OVA+Dex	8	0.084 ± 0.008**	15.0 ± 3.1*	73.0 ± 5.5**

Notes: * $P<0.05$, ** $P<0.01$, compared with OVA group; $\Delta P<0.01$, compared with OVA+Dex group

BALF from OVA group have an increase in the typical Th2 cytokines profile, including IL-4, IL-5, IL-13 ($P<0.01$). The administration of PM (100, 200 and 400 mg/kg) significantly reduced all of these cytokines in the BALF compared with those in the OVA group ($P<0.01$). However, Dex (2 mg/kg) also reduced the levels of IL-4, IL-5, and IL-13 in BALF ($P<0.01$). As expected, the TNF- α levels in the BALF were also significantly decreased in the group treated with PM compared with the OVA group ($P<0.05$, Table 3).

To further determine the effect of PM on inflammatory protein like IL-4, IL-5, IL-13, TNF- α

Table 3. Effect of PM on Cytokines IL-4, IL-5, IL-13, and TNF- α Protein in BALF of Mice by Sandwich ELISA (pg/mL, $\bar{x} \pm s$)

Group	<i>n</i>	IL-4	IL-5	IL-13	TNF- α protein
Control	6	19.0 ± 2.4	22.0 ± 2.0	26.0 ± 5.9	9.0 ± 1.2
OVA	8	84.0 ± 4.2	118.0 ± 5.4	128.0 ± 7.4	27.0 ± 3.5
OVA+PM-100	8	71.0 ± 5.4	99.0 ± 4.9	93.0 ± 4.8	22.0 ± 4.2
OVA+PM-200	8	58.0 ± 3.7** ^Δ	68.0 ± 6.2** ^Δ	85.0 ± 6.7** ^Δ	19.0 ± 3.5*
OVA+PM-400	8	31.0 ± 4.6**	57.0 ± 2.9**	62.0 ± 5.4**	15.0 ± 3.1*
OVA+Dex	8	29.0 ± 3.3**	59.0 ± 6.1**	64.0 ± 3.3**	13.0 ± 2.5*

Notes: * $P<0.05$, ** $P<0.01$, compared with OVA group; $\Delta P<0.01$, compared with OVA+Dex group

and TGF- β 1 in lung tissue, in the OVA group, there was a significantly increased expression of these inflammatory proteins compared with the control group (Table 4). All these changes were reversed with treatment with PM or Dex, resulting in a marked reduction in the production of inflammatory proteins in BALF and lung tissue ($P<0.01$).

The administration of PM (400 mg/kg) or Dex (2 mg/kg) attenuated the magnitude of inflammation and alleviated mucus secretion in the lung tissue compared with OVA group ($P<0.01$). What is more similar efficacy was observed between PM and Dex treated group ($P<0.01$, Table 5).

Table 5. Effect of PM on Airway Inflammation and Mucus Hypersecretion of Mice ($\bar{x} \pm s$)

Group	<i>n</i>	Inflammation score	Mucus score (grade)
NC	6	1.1 ± 0.2	No mucus detected (0)
OVA	8	3.2 ± 0.6	3.5 ± 0.4
OVA+PM-100	8	2.9 ± 0.2	3.0 ± 0.3 (1)
OVA+PM-200	8	2.0 ± 0.6*	2.8 ± 0.1 (1)
OVA+PM-400	8	1.5 ± 0.4*	1.7 ± 0.2* (3)
OVA+Dex	8	1.9 ± 0.2*	2.1 ± 0.3* (2)

Notes: * $P<0.05$ vs. OVA group. 0=no mucus detected, 1=0–20%, 2=21%–40%, 3=41%–60%, of cells in the airway epithelium were positive for mucus.

Table 4. Effect of PM on IL-4, IL-5, IL-13, TNF- α Protein and TGF- β 1 in Lung Tissue of Mice by Sandwich ELISA (pg/mL, $\bar{x} \pm s$)

Group	<i>n</i>	IL-4	IL-5	IL-13	TNF- α protein (pg/mg protein)	TGF- β 1
Control	6	6.0 ± 1.7	18.0 ± 3.8	5.0 ± 1.2	73.0 ± 2.6	132.0 ± 4.2
OVA	8	24.0 ± 5.5	52.0 ± 6.0	11.0 ± 1.0	113.0 ± 5.7	421.0 ± 9.7
OVA+PM-100	8	21.0 ± 3.4	31.0 ± 3.0	10.0 ± 2.4	106.0 ± 3.9	268.0 ± 7.2
OVA+PM-200	8	18.0 ± 4.3* ^Δ	26.0 ± 4.8** ^Δ	8.0 ± 3.1**	89.0 ± 6.2** ^Δ	242.0 ± 8.4** ^Δ
OVA+PM-400	8	10.0 ± 2.6*	19.0 ± 5.3**	7.0 ± 1.7**	77.0 ± 5.2**	210.0 ± 6.4**
OVA+Dex	8	9.0 ± 2.2*	18.0 ± 3.8**	7.0 ± 0.9**	79.0 ± 4.3**	200.0 ± 5.7**

Notes: * $P<0.05$, ** $P<0.01$, compared with OVA group; $\Delta P<0.01$, compared with OVA+Dex group

PM Decreased AHR of OVA-Sensitized Mice

PM is capable of decrease AHR in OVA-sensitized mice. OVA group had significantly higher AHR, measured as Penh value and the RI level was significantly higher than that of the PBS control group (Figure 3).

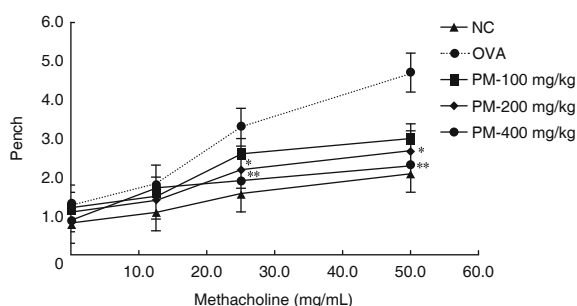


Figure 3. Inhibitory Effects of PM on AHR ($n=6-8$, $\bar{x} \pm s$)

Notes: * $P<0.05$, ** $P<0.01$, compared with OVA group

Effect of PM on Lung Histology Study of OVA-Sensitized Mice

The results of the histological analysis of lung tissues are consistent with those obtained in the previous experiment. PM treatment reduced lung inflammation, goblet cell hyperplasia, and improved the infiltration of inflammatory cells in peribronchiolar regions (Figure 4).

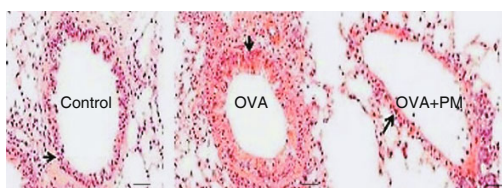


Figure 4. Effects of PM on Airway Inflammation in Lung Tissue of Mice by HE Staining ($\times 200$)

Notes: Arrows indicate the infiltrated inflammatory cells in the lung tissue.

DISCUSSION

Mouse models of asthma have become increasingly popular as they provide a valuable for studies relevant to the process and its treatment. Therefore, it provided an ideal model to directly mimics the usual route of human exposure.

Oxydative stress can stimulate inflammatory responses that can lead to allergic asthma.⁽¹⁷⁾ In this study our results showed that ROS are increased in bronchial inflammatory cells in the OVA-sensitized murine model of asthma and that the administration of PM can significantly reduce the generation of

ROS. We also found a high antioxidant activity of PM against DPPH radical scavenging. The antioxidant effect of *P. alliacea* found in this study is matched with that reported by Okada, et al.⁽¹⁸⁾ Our results present that PM is a potential source of natural antioxidants, which improve oxidative stress. Inhibiting the generation of ROS is an important therapeutic target in the inflammatory allergic asthma, since ROS are implicated in the mechanism of chronic inflammation of airways.⁽¹⁹⁾

Cytokines IL-4, IL-5, and IL-13 produced by Th2 cells are responsible for initiating and maintaining Th2-associated asthma.⁽¹⁸⁻²⁰⁾ These cytokines are responsible for the excessive recruitment of eosinophils and mast cells, IgE production, mucus hypersecretion, and development of AHR, which are directly linked to the major clinical manifestations of allergic asthma.⁽²¹⁾

In response to allergic stimuli, eosinophils are recruited from the circulation to inflammatory tissues contributing to the pathogenesis of allergic asthma.⁽²²⁾ Smooth muscle cells, endothelial cells, epithelial cells, eosinophils and alveolar macrophages in the lungs can produce eotaxin. In the groups treated with PM, we observed eotaxin levels probably due to the decreased recruitment of eosinophils, which are the source of eotaxin production. IL-4 receptor is associated with the risk of asthma,⁽²³⁾ and plays a critical role in the production of IgE. This leads to assume that PM may have an antiallergic effect by suppressing IL-4 secretion and consistently reducing IgE production by B cells. IL-5 is best characterized in the growth and differentiation of eosinophils in bone marrow but in allergic asthma it causes airway inflammation,⁽²⁴⁾ by amplifying allergic responses through Th2 cell immunity and as terminal effector cells.⁽²⁵⁾ In this study, after administration of PM, IL-5 levels in the BALF were significantly reduced. This suggest that the reduction in eosinophils in part may be due to inhibition of IL-5 secretion by Th2 cells. PM-treated mice exhibit less eosinophil infiltration and activation in lungs resulting in reduced IL-5 levels in the BALF. IL-13 cytokine induces the eotaxin release by airway epithelial cells,⁽²⁶⁾ causing AHR through direct effects on resident airway cells. PM caused attenuation of AHR induced by an allergen in mice.

Inflammation and asthma are associated with an

increase of production of TNF- α , which is abnormally expressed in the OVA-sensitized/challenged mouse model. In the lung and BALF, the levels of TNF- α were significantly elevated by airway challenge with OVA, however, after PM administration the levels of TNF- α were significantly reduced compared with those in the control group (asthma group). Reduction of TNF- α may be due to inhibition of IgE level.⁽²⁷⁾

TGF- β 1 increases levels of the Th2 cytokines IL-4, IL-5, and IL-13, resulting in an increased inflammatory response in the lung.⁽²⁸⁾ PM treatment leads to a reduction of 50% of the TGF- β 1 levels in mice, similar to the reduction caused by Dex treatment which is of 52%. This reduction in the TGF- β 1 levels after treatment with PM correlates with the reduction of eosinophil infiltrations, shown by histopathology observed in the lung and the improvement of lung inflammation. Based on these data we can deduce that part of the PM effect act novice airway inflammation is through the inhibition of the expression of TGF- β 1. Other way that PM seems to improve the symptoms in the asthma model by modulating the immunity response which is caused by inflammation activating cytokines produced by the Th2 type cells.

Mucus hypersecretion is a major cause of airway obstruction leading to wheezing and coughing. Mucus is produced by goblet cells in the airways. Administration of PM to OVA-induced asthmatic mice markedly reduced mucus hypersecretion and goblet cell hyperplasia.⁽²⁹⁾ These results further demonstrate that PM has favorable effects on the development of airway inflammation in this OVA-sensitized murine model of asthma.

Our results indicate that PM shows improving effects on AHR, which may be due to lower inflammation in lung, bronchiole, and cell infiltration of eosinophils suggesting that PM attenuates inflammation and AHR by inhibiting proallergic mediators and eosinophils.

In conclusion, PM extract inhibited the activation of IgE, TGF- β 1 levels, and ICAM-1 and reduced mucus hypersecretion. In addition, PM reduced inflammatory cytokine levels. These results suggest that *Petiveria alliacea* leaves are capable of reversing the features of asthma and has proven to be a potential candidate for the treatment of asthma.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution

Rosa Martha PG was responsible for conception and design, drafted the manuscript and revised it critically for important intellectual content and contributed to the data analysis and interpretation of the findings. Jose Maria MF conducted the experiments, organized the data analysis, and interpretation of data. All authors contributed to the drafting of the manuscript. In addition, all authors read and approved the final manuscript.

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