



Pulvis Fellis Suis extract attenuates ovalbumin-induced airway inflammation in murine model of asthma



Jiao He^{a,b,1,*}, Le Lv^{a,b,1}, Ziyi Wang^{a,b}, Chuanchuan Huo^{a,b}, Zongwei Zheng^a, Bing Yin^{a,b}, Peizhi Jiang^a, Yang Yang^{a,b}, Jing Li^{a,b}, Yuan Gao^a, Jing Xue^a

^a Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, Xi'an 710069, China

^b Biomedicine Key Laboratory of Shaanxi Province, College of Life Science, Northwest University, Xi'an 710069, China

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ABSTRACT

Ethnopharmacological relevance: Pulvis Fellis Suis (PFS), named with “Zhu Danfen” in China, has been extensively used for the therapy of enteritis, acute pharyngitis, whooping cough and asthma in folk medicine. Although PFS shows anti-inflammatory activities, its effect on airway inflammation in asthma has not been studied.

Aim of the study: To explore the protective effect of PFS ethanol extract against airway inflammation in asthmatic mice.

Materials and methods: Allergic asthma in mice was sensitized and challenged by OVA. Mice were administered in oral with PFS daily at doses of 100, 200 and 400 mg/kg on days 21–27. Inflammatory cell counts and classification in bronchoalveolar lavage fluid (BALF) were analyzed. Histopathological evaluation of the lung tissue was performed by hematoxylin and eosin (H & E) and periodic acid-schiff (PAS) staining. The IgE level in serum was measured by using enzyme-linked immunoassay (ELISA). ELISA was also used to detect the levels of Th1/Th2 cytokine and eotaxin in BALF.

Results: Histological results revealed that PFS could ameliorate OVA-induced histological changes by attenuating inflammatory cell infiltration, mucus hypersecretion and goblet cell hyperplasia in the lung. Treatment with different doses of PFS significantly decreased the elevated inflammatory cell numbers in BALF and IgE production in serum. PFS treatment reduced the production of Th2 cytokine IL-4, IL-5, IL-13, and promoted Th1 cytokine IFN- γ production in BALF. In addition, PFS also decreased the levels of eotaxin and TNF- α in BALF.

Conclusions: These findings suggest that PFS has a markedly anti-inflammatory effect on OVA-induced allergic asthma in mice, and could be a promising protective agent recommended for allergic asthma patients.

1. Introduction

Asthma is a common chronic inflammatory respiratory disease, which characterized by airway hyperresponsiveness (AHR) and airway inflammation (Busse and Lemanske, 2001; Tiwari et al., 2014). Asthma usually has varied reversible airflow limitation, which causes recurrent episodes of wheezing, shortness of breath, chest tightness and cough, often attack at night and in the early morning (Holgate et al., 2008; Shin et al., 2014). Due to the climate, environmental and some other changes, the incidence of asthma in the world is on the rise resulting in

increased mortality rates in recent years. The prevalence of allergic asthma has been particularly increased among children and has become an important problem (Mirabelli et al., 2016).

Asthma is caused by a combination of immunological, genetic and environmental interactions. In asthma, the inflammatory responses are linked to the activation of T-helper 2 (Th2) cells, which can induce AHR and airway inflammation by releasing cytokines (include interleukin (IL)-4, IL-5 and IL-13). Furthermore, Th2 cells can help B cells to activate mast cells releasing more allergic and inflammatory mediators, and produce IgE (Bosnjak et al., 2011; Larché et al.,

Abbreviations: AHR, airway hyperresponsiveness; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; IL, interleukin; OVA, ovalbumin; PBS, phosphate buffered saline; PAS, periodic acid-schiff; PFS, Pulvis Fellis Suis; SEM, standard error of mean; Th2, T-helper 2; TNF- α , tumor necrosis factor- α

* Corresponding author.

E-mail address: hejiao@nwu.edu.cn (J. He).

¹ These authors contributed equally to this work.

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2003; Liu et al., 2013). In the last decades, the medications for asthma therapy mainly include corticosteroids, antileukotrienes, anti-IgE monoclonal antibody, beta2 agonists, and anticholinergic (Chini et al., 2014). Corticosteroids are the most common therapeutical drugs in the clinics (Tritar-Cherif et al., 2002). However, the symptoms could not be prevented by these drugs in a proportion of patients, such as smokers and obese asthma patients. On the other hand, unacceptable side effects limited the use of these drugs (Manson et al., 2009). Therefore, the development of new drugs which can directly reduce or alleviate the symptoms of asthma is urgently needed.

Traditional Chinese medicine is widely used for the treatment of allergic diseases, especially in asthma (Cheng et al., 2014; Wei et al., 2015). Pulvis Fellis Suis (PFS), named with “Zhu Danfen” in China, is the dried bile of *Sus scrofa domestica* Brisson. It has been used extensively in folk medicine in the therapy of respiratory diseases, mainly used for treating acute pharyngitis, bronchitis, cough, asthma and other diseases (Liu and Geng, 1992; Pharmacopoeia of the People's Republic of China, 2015). Bile acids, especially conjugated cholic acids, are the main chemical components in PFS. The recent pharmacological research revealed that bile acids have anti-inflammatory, analgesic, and antibacterial effects. Bile acids from PFS could inhibit cough central excitatory and relieve bronchial spasm (He et al., 2011; Li et al., 2008; Song, 2001; Wu, 1993). Although PFS has been used in the therapy of respiratory disease, its inhibition on airway inflammation in asthma has not been studied yet. Therefore, the present study was designed to investigate the protective effects of ethanol extracts of PFS against allergic asthma induced by ovalbumin (OVA) in mice.

2. Materials and methods

2.1. Chemicals and drugs

Ovalbumin (OVA) was purchased from Sigma-Aldrich Trading Co., Ltd., (Shanghai, China). IL-4, IL-5, IL-13, tumor necrosis factor- α (TNF- α), IFN- γ , eotaxin and IgE enzyme-linked immunosorbent assay (ELISA) kits were purchased from R & D Systems (Minneapolis, MN, USA). Dexamethasone (DEX, No. 140806) was purchased from Xianju Pharmaceutical Co. Ltd. (Zhejiang, China). Other chemical reagents used in the experiments were analytical grade.

2.2. Preparation of the extract

Pulvis Fellis Suis was purchased from Wanshou Road Chinese Herbal Medicine Market (Xi'an, China), and was authenticated by Prof. W.J. Sun of Northwest University, Xi'an, China. The voucher specimen (ZDF-150301) was deposited at Life Science College of Northwest University, Xi'an, China. PFS (50 g) was added into a chromatographic column containing 1500 g aluminum oxide active neutral (8 cm $\times$ 50 cm), and sequentially eluted with 95% (*v/v*) ethanol. The effluents were then mixed and concentrated in vacuum at 50–60 °C by using a rotary evaporator. The dried residue obtained with a yield of 4.75 g was suspended in saline and subjected to *in vivo* experiments. The mice received the drugs with a range of doses which are equivalent to 100, 200, and 400 mg of the dried extract per kg body weight respectively.

2.3. Animals

Female ICR mice (18–22 g) were purchased from Experimental Animal Center in Xi'an Jiaotong University (Shaanxi, China). Mice were maintained in the Specific Pathogen Free (SPF) Animal Lab, fed with standard laboratory chow and water *ad libitum*. The temperature of SPF Lab was controlled at 23 ± 2 °C, and the relative humidity was set at 40–60% with a 12 h light/dark cycle. All animal experimental procedures were conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Publications, 85-23, revised 1985), and approved by ethical committee for Animal Care and Use of the Northwest University (No. 20150519).

2.4. Establishment of allergic asthma model and treatment

Mice were sensitized and challenged with OVA. Briefly, on day 0, mice were sensitized with 0.2 ml phosphate buffered saline (PBS, pH 7.4) containing 20 μ g OVA and 2 mg aluminum hydroxide gel by intraperitoneal injection. The sensitization was repeated on day 7, 14 and 21. On day 24–27, 1 h after the drug treatment, each group mice were placed into an individual chamber, challenged through inhalation atomization with 1% OVA (*w/v*) in PBS for 30 min by using an ultrasonic nebulizer (JLN -2304AS, Bang Lijian corp., Shenzhen, China). Normal control mice were sensitized and challenged with PBS buffer alone using the same technique.

Mice were divided into six groups (*n* = 10). Group 1 was normal mice without any treatment (normal control group). Group 2–6 were sensitized and challenged with OVA as described above. Group 2 (OVA control group) was not received any treatment. Group 3 was treated with DEX (2 mg/kg). Group 4, 5 and 6 were given with PFS at 100, 200, 400 mg/kg, respectively. All drugs were given in oral from day 21–27 consecutively. A schematic diagram of the treatment is shown in Fig. 1.

2.5. Preparation of bronchoalveolar lavage fluid (BALF) and inflammatory cell counting

Mice were euthanized under anesthesia 24 h after the last OVA challenge. Following by three times instillation of ice-cold PBS (0.5 ml) into the lung, BALF was aspirated via tracheal catheter (total volume 1.5 ml). BALF from each mouse was recovered and centrifuged, and the supernatants were collected and stored at -80 °C for cytokine assays. Cell pellet in each tube was re-suspended in PBS. The hemacytometer was used for total cell counts, and the differential cell counts were performed by using Wright's and Giemsa stain.

2.6. Histological assessment of lung

Neutral formalin (10%, *v/v*) was used to fix lung tissue, then the tissue was dehydrated with increasing concentrations of ethanol, embedded in paraffin, cut into 5 μ m thickness sections and stained with hematoxylin and eosin (H & E) solution. Using a treatment-blind observer, the score of lung inflammation was assessed as the following scale: 0, no inflammation; 1, mild inflammation; 2, moderate inflammation; 3, marked inflammation; and 4, severe inflammation. When

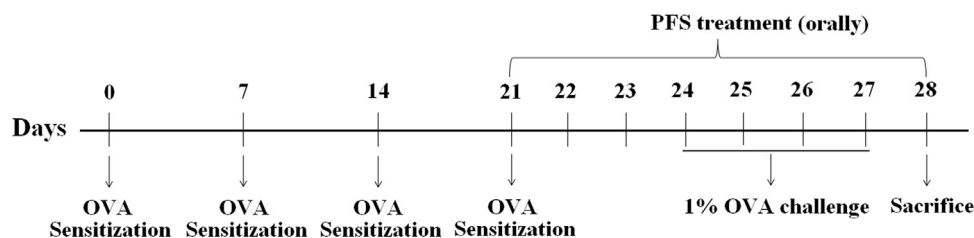


Fig. 1. Mouse model of airway inflammation and treatment with PFS ethanol extract.

the inflammation degree is between two scoring criteria, a point of 0.5 can be used (McKay et al., 2004). The identification of goblet cells in the epithelium of lung tissue was performed by using periodic acid-schiff (PAS) stain. The degree of mucus production in airway was assessed and scored as previously described (Tanaka et al., 2001; Reber et al., 2012). All stained sections were observed and photographed by using a microscope with a camera system (Olympus DP-71, MN, USA). Motic Images 2000 software was used to analyze the histopathological changes.

2.7. Measurement of IgE in serum, cytokines and chemokine in BALF

The level of total IgE in serum, and the levels of Th1/Th2 cytokines (IFN- γ , IL-4, IL-5 and IL-13), TNF- α , and eotaxin in BALF were measured by using ELISA assay. All ELISA experiments were strictly performed according to the manufacture's instruction. Absorbance was measured in an ELISA reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm.

2.8. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

To assess liver function, the levels of ALT and AST in serum were determined by using commercial kits according to the manufacture's instruction, after the OVA-induced asthmatic mice were treatment with PFS for seven days.

2.9. Statistic analysis

All results were expressed as means $\pm$ standard error of mean (SEM). Statistical analysis was performed by using GraphPad Prism 5 statistical software. Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of PFS on OVA-induced inflammatory cells in BALF

As shown in Fig. 2, the number of total inflammatory cells and eosinophils in BALF was significantly increased in asthmatic mice compared to normal mice ($P < 0.001$). However, they were significantly reduced in asthmatic mice treated with PFS at 100, 200 and 400 mg/kg, which is in a dose-dependent manner ($P < 0.05$, $P < 0.01$ or $P < 0.001$). In addition, DEX treatment also markedly decreased the number of total cells and eosinophils in BALF ($P < 0.001$).

3.2. Effects of PFS on OVA-induced histopathological change in lung tissue

Histopathologic results revealed that lung tissue exposed to OVA showed epithelium loss, airway mucosal edema, and a marked increase in the infiltration of inflammatory cells into the lung (H & E staining). Meanwhile, mucus hypersecretion and goblet cell hyperplasia were observed in the bronchial airways by PAS staining. However, pulmonary histology was normal in the normal mice (Figs. 3 and 4). Treatment with different doses of PFS or DEX markedly decreased infiltration of inflammatory cell, mucus hypersecretion and goblet cell hyperplasia in airway compared with that observed in the OVA control group (Figs. 3 and 4). These results suggest that PFS and DEX inhibited the OVA-induced inflammatory infiltration in asthmatic mice.

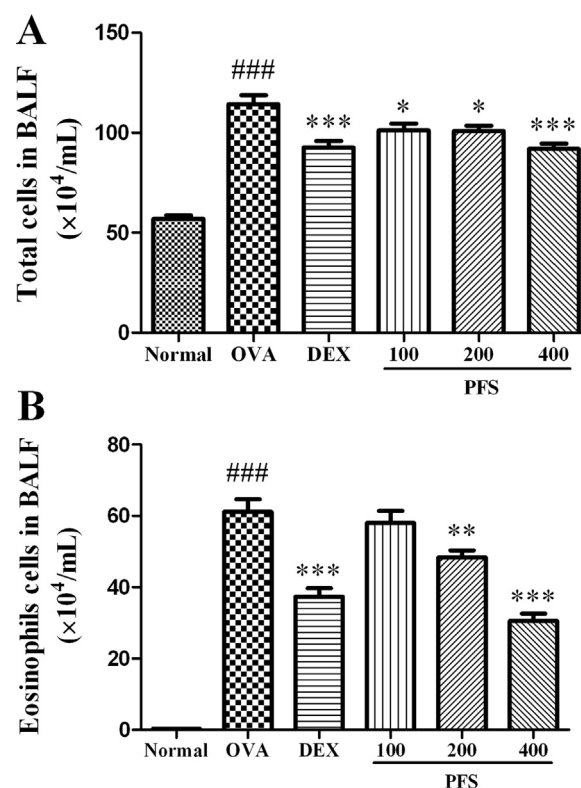


Fig. 2. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on the number of total cells and eosinophils in BALF of OVA-induced allergic asthmatic mice. A, Total cells in BALF; B, Eosinophils in BALF. Data are expressed as the means $\pm$ S.E.M. ### $P < 0.001$ vs. normal control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. OVA control.

3.3. Effects of PFS on IgE production in serum

As shown in Fig. 5, the OVA-induced asthmatic mice (OVA control group) presented significantly increase in IgE levels compared with that of normal control group (8.57 ± 0.10 vs. 8.11 ± 0.03 IU/ml, $P < 0.001$). PFS at doses of 100, 200 and 400 mg/kg significantly attenuated the IgE serum levels to 8.30 ± 0.05 , 8.26 ± 0.05 and 8.14 ± 0.08 IU/ml in asthmatic mice ($P < 0.05$ or $P < 0.001$ compare with OVA control group). Treatment with DEX could also decrease the serum levels of IgE in asthmatic mice (8.15 ± 0.04 IU/ml), while no significant differences were observed between PFS and DEX treatment group.

3.4. Effects of PFS on OVA-induced cytokine and chemokine levels in BALF

An imbalance of Th1/Th2 cytokines is the characteristics of airway inflammation in asthma. Thus, the levels of Th1/Th2 cytokines in BALF were measured to determine this imbalance modulated by PFS. As shown in Fig. 6A–D, the levels of IL-4, IL-5, IL-13 and TNF- α were significantly increased to 306.3 ± 7.29 , 29.28 ± 0.89 , 47.99 ± 1.18 , 724.00 ± 10.06 pg/ml in BALF in OVA control group ($P < 0.001$). After treated with different doses of PFS or DEX, mice showed markedly decreased levels of Th2 cytokines (IL-4, IL-5 and IL-13) and TNF- α compared with those in the OVA control group ($P < 0.001$ or $P < 0.01$ or $P < 0.05$). However, no significant differences in the levels of IL-5 and TNF- α were observed in mice treated with PFS at a small dose (100 mg/kg) (Fig. 6B and D).

IFN- γ , an important Th1 cytokine, was also measured with the

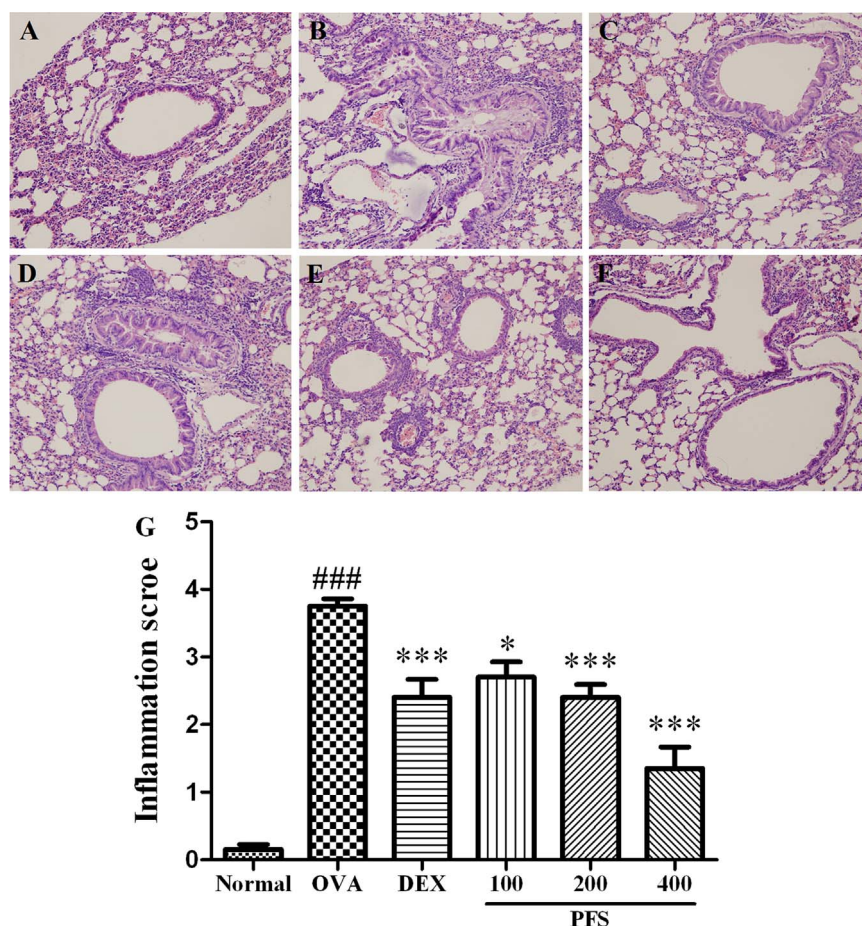


Fig. 3. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on airway inflammation in the lung tissue in OVA-induced allergic asthmatic mice (H & E staining, original magnification 200 $\times$). A, normal control group; B, OVA control group; C, dexamethasone treatment group; D-F, PFS extract treatment group, 100 mg/kg, 200 mg/kg and 400 mg/kg; G, Graphs represent inflammation scores. Data are expressed as the means $\pm$ S.E.M. *** P < 0.001 vs. normal control; * P < 0.05, *** P < 0.001 vs. OVA control.

levels by using ELISA. As shown in Fig. 6E, OVA-exposed mice showed reduction in the level of this cytokine compared with normal mice (1697 ± 73.08 vs. 2039 ± 47.60 pg/ml, P < 0.01). Asthmatic mice treated with PFS at 200 and 400 mg/kg showed increase in IFN- γ levels (1969 ± 54.17 , 1990 ± 76.39 pg/ml) compared with the OVA control mice (P < 0.05). However, no significant difference was observed in mice treated with small dose of PFS (1890 ± 50.81 pg/ml, P > 0.05). DEX treated mice also showed significant increase in IFN- γ level (2014 ± 43.02 pg/ml). Therefore, the results suggest a protective role of PFS in maintaining balance of Th1/Th2 cytokines and related anti-inflammatory effect.

Eotaxin is one of the most potent chemokines for recruiting eosinophils and neutrophils. As shown in Fig. 6F, PFS at doses of 100, 200 and 400 mg/kg significantly decreased eotaxin levels to 179.90 ± 5.39 , 169.60 ± 5.61 and 158.20 ± 2.77 pg/ml, which is in a dose-dependent manner (P < 0.001 or P < 0.01). DEX also caused significant reduction in eotaxin level compared with OVA control values (161.00 ± 2.87 vs. 202.10 ± 5.88 pg/ml, P < 0.001). This result indicated that PFS could reduce the secretions of eotaxin, and subsequently decrease the recruitment of eosinophils and neutrophils.

3.5. Effects of PFS on OVA-induced ALT and AST levels in serum

To confirm the toxicity induced by PFS in liver, the OVA-induced

asthmatic mice treated with PFS were detected with the levels of ALT and AST in serum. Mice in the PFS treatment groups did not exhibit altered ALT and AST levels (Fig. 7), demonstrating an undetectable liver toxicity from the PFS treatment.

4. Discussion

Asthma is one of the most common respiratory diseases, the characteristics of which are airway eosinophilia, high concentration of Th2 cytokine, increased chemokine levels, and elevated IgE (Chung et al., 2012, 2015). Pulvis Fellis Suis is a folk medicine used for the therapy of respiratory disease including acute pharyngitis, whooping cough and bronchitis in China (Pharmacopoeia of the People's Republic of China, 2015). Based on the traditional uses of PFS, the anti-asthmatic effects of its ethanol extract were investigated. OVA induced model is considered to be an appropriate method for experimental allergic asthma. This model has similar clinical symptoms to human asthma, which are characterized by airway mucosal edema, bronchial wall thicknesses, mucus hypersecretion and increased infiltration of inflammatory cells into the lung. In this study, PFS was administered orally to OVA-induced asthmatic mice and evaluated with the therapeutic effects. The data showed that PFS significantly reduced the level of IgE in serum, inhibited chemokine production and regulating Th1/Th2 cytokine imbalance in BALF in asthmatic mice. Our findings demonstrate for the first time that PFS is protective in the murine

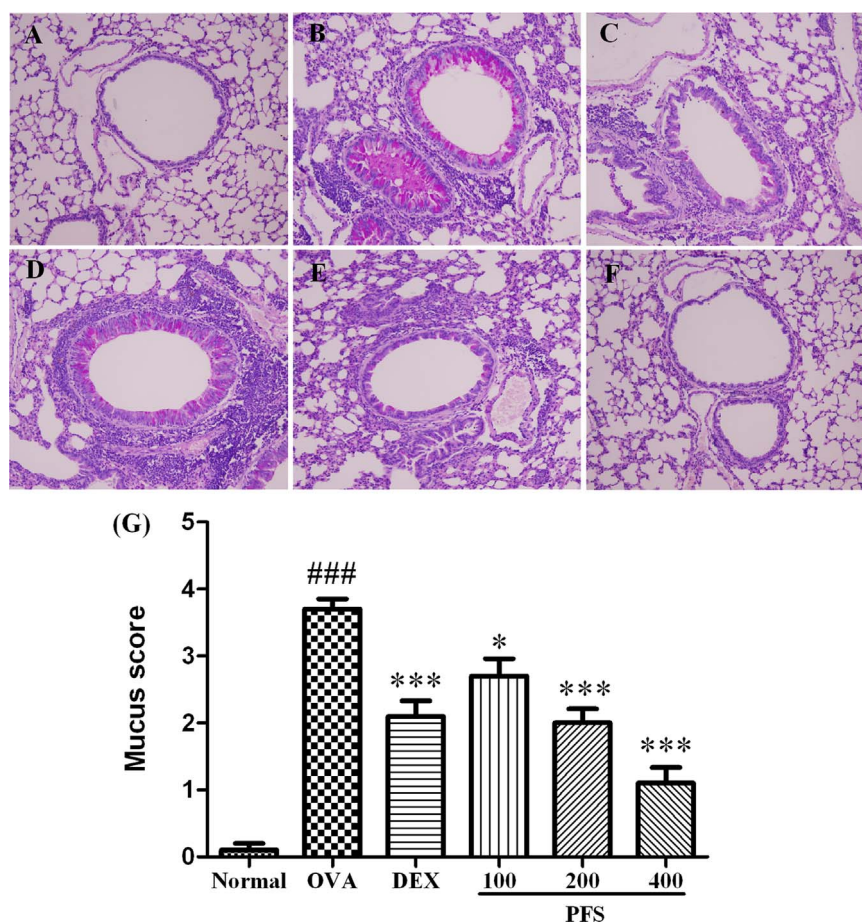


Fig. 4. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on lung mucus production in OVA-induced allergic asthmatic mice (PAS staining, original magnification 200×). A, normal control group; B, OVA control group; C, dexamethasone treatment group; D-F, PFS extract treatment group, 100 mg/kg, 200 mg/kg and 400 mg/kg; G, Graphs represent mucus scores. Data are expressed as the means $\pm$ S.E.M. *** P < 0.001 vs. normal control; * P < 0.05, *** P < 0.001 vs. OVA control.

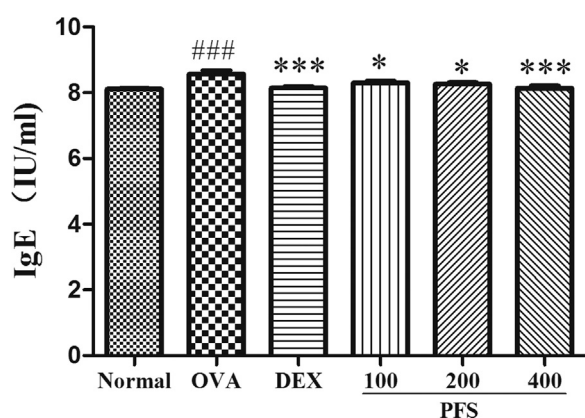


Fig. 5. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on OVA-induced IgE production in serum. Data are expressed as the means $\pm$ S.E.M. *** P < 0.001 vs. normal control; * P < 0.05, *** P < 0.001 vs. OVA control.

model of OVA-induced asthma.

Airway inflammation is the main feature of asthma, the mechanism of which is mainly associated with increasing in a variety of immune cells and inflammatory mediators, including eosinophils and lymphocyte (Schuijs et al., 2013). It has been reported that the degree of eosinophils infiltration were related to the severity of asthma, and

eosinophils contributed to the deposition of collagen and proliferation of airway smooth muscle in the chronic asthma (Humbles et al., 2004). In this study, mice sensitized and challenged with OVA showed an increased number of total cells and eosinophils in BALF. Consistent with these findings, histopathologic analysis results showed a marked increase in the infiltration of inflammatory cells in the lung in asthmatic mice. In addition, goblet cell hyperplasia and mucus overproduction in the bronchial airways were observed in asthmatic mice. On the contrary, PFS treatment could effectively inhibit the number of total cells and eosinophils in BALF, reduce the infiltration of inflammatory cells and eosinophils, as well as decrease mucus hypersecretion and goblet cell hyperplasia in the lung. These findings indicated that PFS has an anti-inflammatory effect on OVA-induced allergic asthma.

Chemotactic factor and cytokines play key roles in the pathogenesis of asthma. Eotaxin, which belongs to the chemotactic factor family, is a chemical activator of eosinophils. Eotaxin can be produced by epithelial cells, fibroblasts, smooth muscle cells, and eosinophils. In asthma, eotaxin bind to CCR3 which is highly up-regulated by eosinophils, and induce eosinophils adhesion, recruitment and degranulation in the lung (Conroy et al., 2001; Shieh et al., 2015; Sun et al., 2015). TNF- α is an anti-inflammatory cytokine secreted by mononuclear macrophages and NK cells, which is an important mediator in the inflammatory process of asthma. TNF- α can stimulate airway smooth muscle, increase the expression of adhesion molecules, and promote the adhesion of inflammatory cells (such as eosinophils and neutrophils) on the airway surface. TNF- α can also induce eosinophils release leukotrienes, platelet activating factor, prostaglandin and other inflammatory mediators (Barnes et al., 2003; Holgate et al., 2004). In our

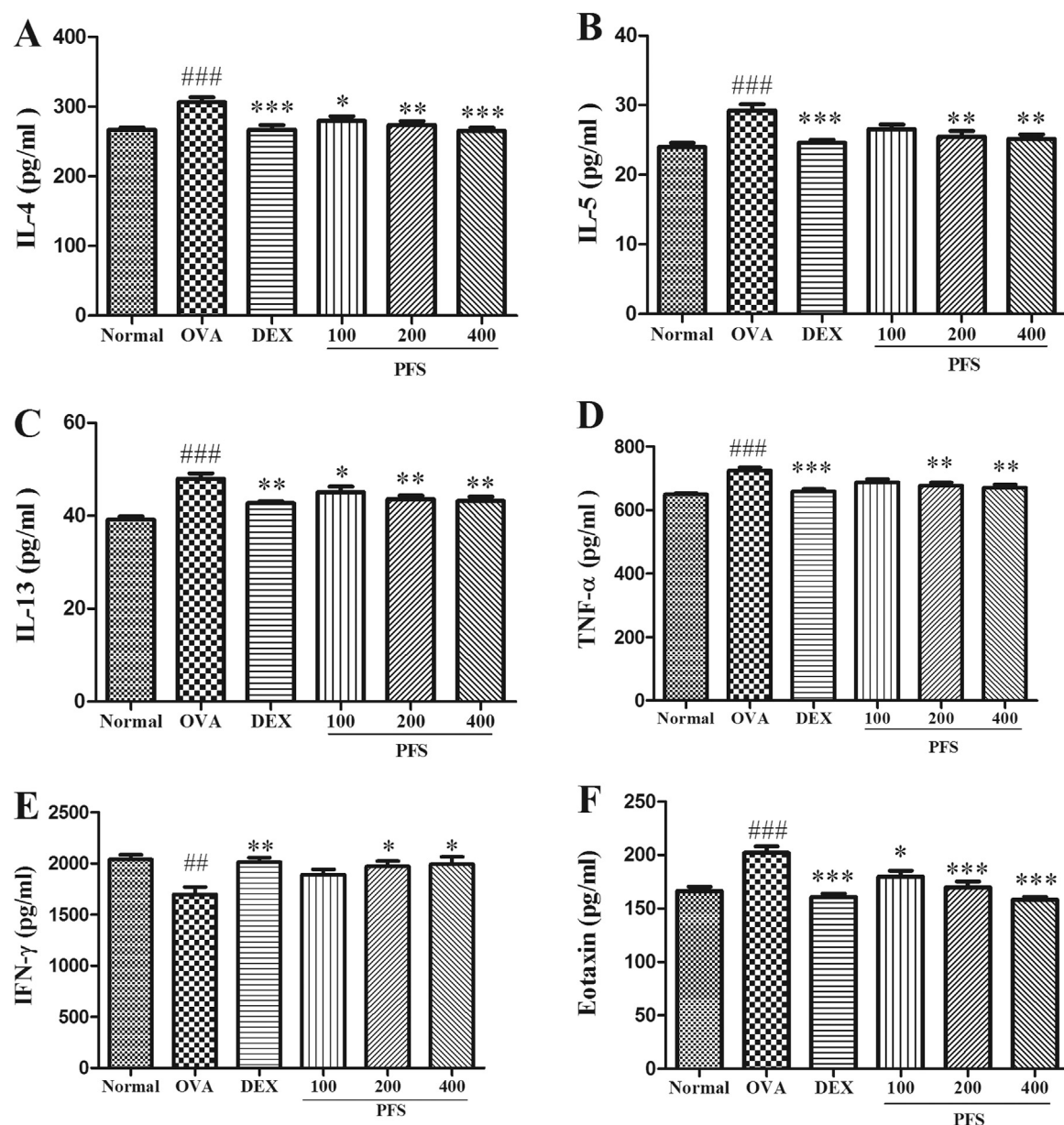


Fig. 6. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on OVA-induced cytokine and chemokine levels in BALF. Data are expressed as the means $\pm$ S.E.M. ^{***} P < 0.01, ^{###} P < 0.001 vs. normal control; ^{*} P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001 vs. OVA control.

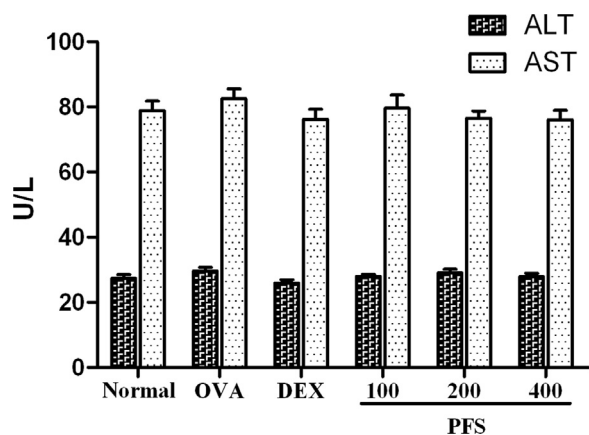


Fig. 7. Effects of different doses of PFS extract (100, 200, 400 mg/kg) and dexamethasone (2 mg/kg) on OVA-induced ALT and AST levels in serum. Data are expressed as the means $\pm$ S.E.M.

study, the levels of eotaxin and TNF- α in BALF were remarkably increased in OVA-induced asthmatic mice. However, PFS treatment could significantly decrease their levels in asthmatic mice. These result suggested that PFS could relieve allergic asthma by down-regulating eotaxin and TNF- α levels.

It is well known that T lymphocytes play a key role in regulation of immune and inflammatory reactions through the release of cytokines (Mosmann et al., 1986; Mosmann et al., 1996). The imbalance and dysfunction of helper T lymphocyte subsets Th1/Th2 cells were consider as one of the important mechanisms of allergic asthma. Th1 cell secrete cytokines including IFN- γ , which can promote Th2 cell and mucous cell apoptosis, and reduce eosinophil infiltration (Gajewski et al., 1988; Luttmann et al., 2000; Shi et al., 2002). It has been reported that IgE synthesis in asthmatic patients was related to the balance between IFN- γ and IL-4 (Stokes et al., 2004). Th2 cell can induced various pathological changes of asthma. Th2 cytokines, including IL-4, IL-5 and IL-13, can regulate the production and recruitment of eosinophil, induce transformation of immunoglobulin

subtype and produce Ig E (Lee et al., 2010; Liu et al., 2013). It has been reported that IL-4, IL-5, IL-13 are abnormally expressed in a mouse model of allergic asthma (Chung et al., 2012). IL-4 promotes the development of mast cells, and stimulates the production of IgE and vascular cell adhesion factor 1 (Fish et al., 2005). IL-5 is a key factor in eosinophils growth and differentiation, which can make bone marrow release eosinophils and induce the accumulation of eosinophils in airway (Simon et al., 2004; Farahi et al., 2007). Anti-IL-5 treatment could effectively reduce the number of eosinophils in blood and relieve the clinical symptoms of asthma (Corren et al., 2012). IL-13 plays a significant role in IgE synthesis, eosinophilic infiltration, mucus hypersecretion, goblet cell hyperplasia and AHR (Kibe et al., 2003; Wynn et al., 2003). In this study, Th1 and Th2 cytokines were analyzed in BALF. We found that the levels of IL-4, IL-5 and IL-13 were significantly elevated and IFN- γ was markedly decreased in OVA-induced mice. In contrast, PFS decreased the levels of IL-4, IL-5 and IL-13 and also prevented OVA-induced reduction of IFN- γ in asthmatic mice. These findings were consistent with the results obtained from the analysis of cell numbers in BALF and histological analysis in lung tissue. These results suggested that PFS attenuated airway inflammation via regulating the imbalance between Th1 and Th2 cytokines, where there is a decrease in Th2 cytokines production and an increase in Th1 response.

Allergic diseases are mainly mediated by T cells, and IgE is an important promoter. The allergen binds with IgE, and act on the mast cells and IgE receptor with high affinity. The interactions of allergen-specific IgE and receptors induce mast cell degranulation, release membrane lipid mediators, cytokines and chemokines, finally lead to tissue inflammation. IL-4 and IL-13 are important inducers of IgE. IgE level is considered as an indicator of Th2 cell secretion in the pathogenesis of asthma (Lee et al., 2008; Sun et al., 2015). In our experiments, IgE levels were significantly increased in OVA control group compared with that in normal control group. However, PFS treatment induced a significant reduction of IgE levels in asthmatic mice. These findings confirmed that the protective effect of PFS on OVA-induced asthma was related to the decreasing of IgE levels in serum.

In conclusion, all data in this experiment indicated for the first time that PFS has a markedly protective effect against OVA-induced allergic asthma in mice. PFS treatment can attenuate airway inflammation, decreased the levels of IgE in serum, and reduce the levels of eotaxin and TNF- α in BALF. In addition, PFS can regulate Th1/Th2 imbalance by inhibiting the release of Th2 cytokines. Moreover, further investigations are currently in progress to determine the precise underlying protective mechanisms of PFS on OVA-induced allergic asthma. Therefore, PFS could be a promising protective agent recommended for allergic asthma patients.

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