

## Original Research Article

# Effects of t*Ma-Xin-Di-Tan* decoction on ovalbumin-induced allergic asthma in mice

Yazun Liu<sup>1,2</sup>, Wanchao Xu<sup>1,2</sup>, Xi Ming<sup>1,2</sup>, Xinguang Zhan<sup>1</sup>, Li Bai<sup>1</sup>, Zheng Xue<sup>1\*</sup>, Jianer Yu<sup>1</sup>

<sup>1</sup>Department of Pediatrics, The Affiliated Shanghai Traditional Chinese Medicine Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai 200071, <sup>2</sup>Shanghai University of Traditional Chinese Medicine, Shanghai 201203, PR China

\*For correspondence: **Email:** lyz1041857257@126.com; **Tel:** +86-021-56639828-2303

Sent for review: 8 November 2017

Revised accepted: 18 April 2018

### Abstract

**Purpose:** To investigate the effect of the *Ma-Xin-Di-Tan* (MXDT) decoction on ovalbumin-induced allergic asthma (AA) in mice.

**Methods:** Asthma was induced in mice by ovalbumin (OVA) injection, and different doses of MXDT (150, 300, and 600 mg/kg/day) were administered orally for 28 days. Pathological changes in lung tissues were examined, while levels of cytokines, including interleukin (IL)-4, IL-6, IL-17, interferon (IFN)- $\gamma$ , and transforming growth factor (TGF)- $\beta$ , were determined using enzyme-linked immunosorbent assays (ELISAs) of the bronchoalveolar lavage fluid. Toll-like receptor (TLR)-4, GATA-binding protein (GATA)-3, Ox40 ligand (OX40L), indoleamine 2,3-dioxygenase (IDO), forkhead box P3 (Foxp3), and T box expressed in T cells (T-bet) levels were determined in lung tissues by western blot analysis.

**Results:** MXDT inhibited the inflammatory reaction of lung tissues in OVA-challenged mice. After treatment with MXDT, levels of IL-4, IL-6, IL-17, and TGF- $\beta$  were downregulated, whereas IFN- $\gamma$  levels were upregulated. In addition, MXDT decreased TLR-4, GATA-3, and OX40L levels in lung tissues but increased the expression of Foxp3, T-bet, and IDO.

**Conclusion:** MXDT has anti-allergic effects on OVA-induced AA in mice; the possible molecular mechanisms might involve the inhibition of inflammatory reactions and modulation of Th1/Th2 cytokine balance.

**Keywords:** *Ma-Xin-Di-Tan* decoction, Allergic asthma, Inflammatory reactions, Th1/Th2

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Allergic asthma (AA), characterized by serious airway inflammation, airway hyperreactivity, shortness of breath, and excessive mucous production in pulmonary airways, is a common and intractable allergic disorder that affects the life quality for millions of individuals [1-3]. It is

estimated that AA affects more than 300 million patients worldwide, with an increasing prevalence in recent years [4,5]. Currently available drugs, such as glucocorticoids, can only temporary control or relieve asthma symptoms, and long-term usage of such drugs can result in serious adverse reactions [2,5]. Thus, more effective therapies for AA are

urgently required.

It has been reported that traditional herbal medicines are a potential source of effective agents for the treatment of allergic diseases, including AA, allergic rhinitis, and eczema [2,6,7]. The *Ma-Xin-Di-Tan* (MXDT) decoction is derived from the clinical experiences of Professor Jianer Yu in the treatment of AA (Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai, China). MXDT is mainly composed of the whole *Ephedra sinica* Stapf herb, the seeds of *Amygdalus Communis* Vas, rainworm, and the seeds of *Prunus persica* (L.) Batsch. In the present study, the antiallergic effects of the *Ma-Xin-Di-Tan* decoction on ovalbumin (OVA)-induced AA was evaluated in mice, and its molecular mechanisms were also explored.

## EXPERIMENTAL

### Chemicals and reagents

All herbal medicines were purchased from the Shanghai traditional Chinese medicine company (Shanghai, China). Dexamethasone (Dex) and aluminum hydroxide were obtained from Sigma-Aldrich (Shanghai, China). Commercial enzyme-linked immunosorbent assay (ELISA) kits for interleukin (IL)-4, IL-6, IL-17, interferon (IFN)- $\gamma$ , and transforming growth factor (TGF)- $\beta$  were purchased from Bioswamp Co. (Shanghai, China). PVDF membranes were purchased from Millipore Biotech (MA, USA). All antibodies used in this research, including those against Toll-like receptor (TLR)-4, GATA-binding protein (GATA)-3, Ox40 ligand (OX40L), indoleamine 2,3-dioxygenase (IDO), forkhead box P3 (Foxp3), and T box expressed in T cells (T-bet), were purchased from the Abcam Co. (Cambridge, UK).

### Animal studies

A total of 60 mice (18 – 22 g) were obtained from the Shanghai Laboratory Animal Research Center (Shanghai, China). All animals received humane care according to the Declaration of Helsinki, which was promulgated in 1964 and amended in 1996 [8]. All experimental protocols were approved by the Animal Care and Use Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai, China (approval no. a201611-27#). Mice were randomly divided into six groups (n = 10): normal group, control group (AA mice), positive drug treatment group (Dex), and three MXDT treatment groups (150, 300, and 600 mg/kg/day). The experimental protocol was carried out according to previous reports with some modifications [2,9].

The mice in the control, Dex, and MXDT groups were immunized intraperitoneally with a mixture of 50 mg OVA and 2 mg aluminum hydroxide on days 1 and 14. Subsequently, on days 21 through 28, the mice were challenged with a 5 % OVA solution for 40 min using an ultrasonic nebulizer (OMRON Co, Tokyo, Japan). The Dex (1.5 mg/kg/day) and MXDT (150, 300, and 600 mg/kg/day) groups were administered agents orally on days 28 through 56. Twenty-four hours after the final drug treatment, the mice were sacrificed by cervical dislocation, and the bronchoalveolar lavage fluid (BALF) and lung tissues were collected and stored at  $-70^{\circ}\text{C}$  for further analysis.

### Histochemical analysis

Lungs tissues were fixed in 10 % paraformaldehyde for 24 h, then embedded in paraffin and subsequently sectioned (5- $\mu\text{m}$ -thick slices). The lung tissue sections were subsequently stained with hematoxylin and eosin for routine histochemical analysis. Finally, pathological changes in the lung tissues were examined using an optical microscope (Olympus 2H12003, Tokyo, Japan).

### ELISA

The levels of IL-4, IL-6, IL-17, IFN- $\gamma$ , and TGF- $\beta$  were quantified in the supernatant of the BALF with commercial ELISA kits following the manufacturer's instructions.

### Western blot analyses

Lung tissues were homogenized in lysis buffer. Total protein was then extracted from the lung tissues. Subsequently, 35  $\mu\text{g}$  total lung tissue protein were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to a PVDF membrane, which was then probed with primary antibodies against TLR-4, GATA-3, OX40L, IDO, Foxp3, and T-bet. The PVDF membrane was incubated with horseradish peroxidase-conjugated secondary antibodies. The target protein bands were then visualized by chemiluminescence, and GAPDH was used as an internal reference.

### Statistical analysis

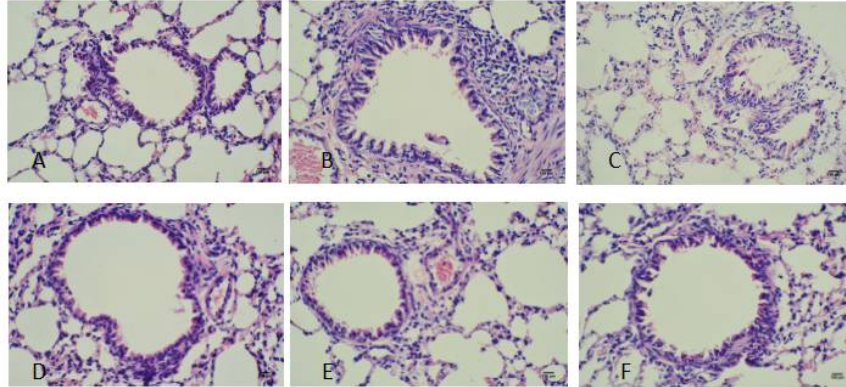
Data are presented as the mean  $\pm$  standard deviation (SD). Statistical comparisons were made by one-way analysis of variance using SPSS software (version 18.0, USA), followed by Dunnett's multiple comparison test. A *P* value less than 0.05 was considered statistically significant.

## RESULTS

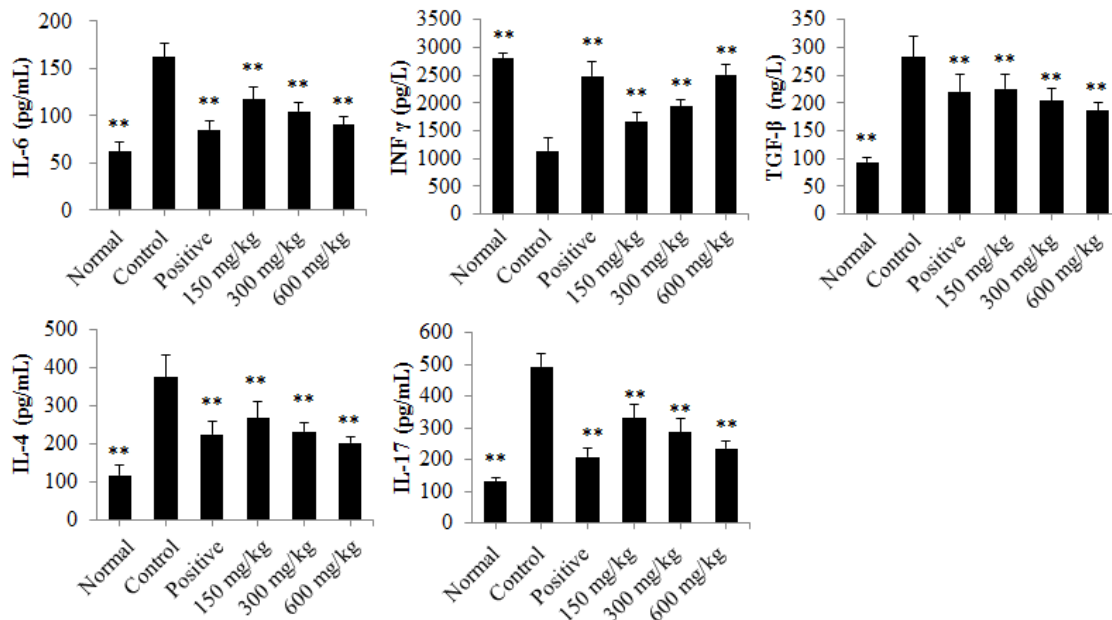
### MXDT treatments alleviate inflammatory reactions in lung tissues of OVA-challenged mice

Results of the pathological examinations of the lung tissues are shown in Figure 1. In normal mice, bronchial wall tissues were normal and the structural integrity was intact; no obvious pathological changes were observed (Figure 1A).

In contrast, in the control mice, the bronchial walls appeared thick and damaged, and the airway lumen was obviously narrower; inflammatory cell infiltration could be observed in the airway walls and other lung tissues (Figure 1B). Interestingly, for Dex- and MXDT-treated mice, the inflammatory and pathological changes mentioned above had been significantly alleviated; no obvious bronchial wall thickening was observed, but moderate inflammatory cell infiltration could be seen in the lung tissues (Figure 1C–F).



**Figure 1:** Pathological examination of lung tissues (magnification: x200). A – F represents the normal, control, Dex, and three MXDT treatment groups (150, 300, and 600 mg/kg/day). All drugs were administered orally for 28 days

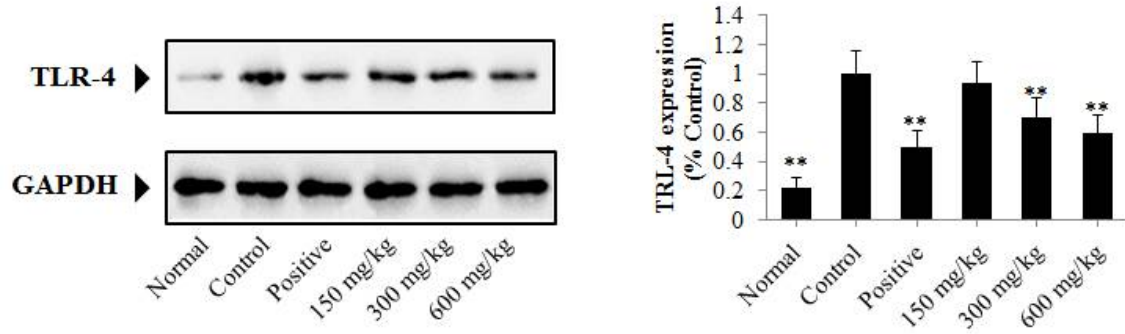


**Figure 2:** Cytokines levels in BALF. All drugs were administered orally for 28 days. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ); \*\* $p < 0.01$  compared to controls

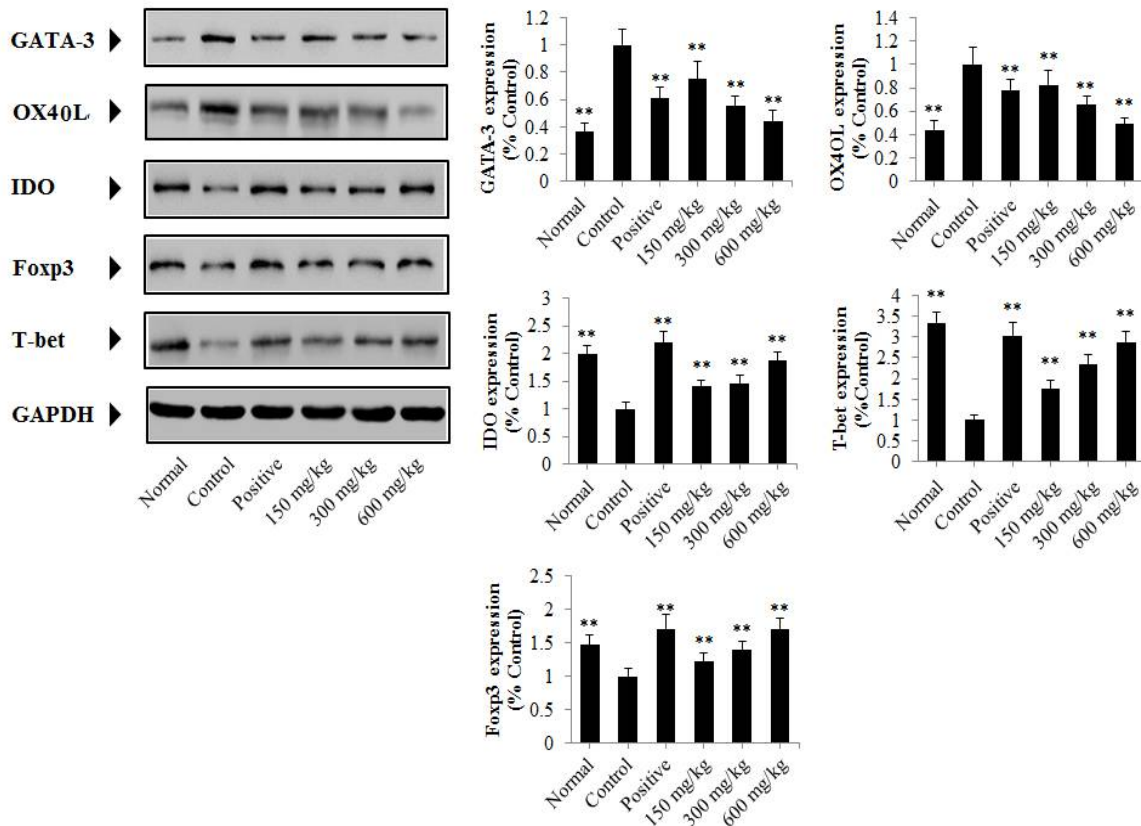
### MXDT treatments regulate cytokine levels in the BALF of OVA-challenged mice

Results of the ELISAs are shown in Figure 2. The cytokine levels of IL-4 ( $P < 0.01$ ), IL-6 ( $P <$

$0.01$ ), IL-17 ( $P < 0.01$ ), and TGF- $\beta$  ( $P < 0.01$ ) were significantly higher in the BALF of control mice than in that of normal mice; however, the cytokine levels of IFN- $\gamma$  ( $P < 0.01$ ) were significantly decreased in the BALF of control mice



**Figure 3:** TLR-4 expression in the lung tissues of OVA-challenged mice. The protein expression levels were determined using western blot assays. All drugs were administered orally for 28 days. Data are expressed as the mean  $\pm$  SD (n = 10); \*\* $p$  < 0.01 compared to controls



**Figure 4:** GATA-3, OX40L, IDO, Foxp3, and T-bet expression levels in the lung tissues of OVA-challenged mice. Protein expression was determined using western blot assays. All drugs were administered orally for 28 days. Data are expressed as the mean  $\pm$  SD (n = 10); \*\* $p$  < 0.01, compared to controls

compared to in that of normal mice. Interestingly, after treatment with MXDT (150, 300, and 600 mg/kg/day) for 28 days, the increased levels of IL-4 ( $p$  < 0.01), IL-6 ( $p$  < 0.01), IL-17 ( $p$  < 0.01), and TGF- $\beta$  ( $p$  < 0.01) were downregulated, whereas the decreased levels of IFN- $\gamma$  ( $p$  < 0.01) were upregulated, compared to control mice.

**Effect of MXDT treatments on protein expression**

Results of western blot assays are shown in

Figure 3 and Figure 4. The results showed that after stimulation with OVA, the protein expression levels of TLR-4 (Figure 3) ( $p$  < 0.01), GATA-3 ( $p$  < 0.01), and OX40L ( $p$  < 0.01) (Figure 4) in lung tissues were significantly upregulated compared with those of normal mice, whereas Foxp3 ( $p$  < 0.01), T-bet ( $p$  < 0.01), and IDO ( $P$  < 0.01) (Figure 4) expression was downregulated ( $P$  < 0.01). However, compared with control mice, treatment of OVA-challenged mice with MXDT decreased the upregulated expression levels of TLR-4 ( $p$  < 0.01) (Figure 3), GATA-3 ( $p$

< 0.01), and OX40L ( $p < 0.01$ ) (Figure 4) in lung tissues and increased the downregulated expression levels of Foxp3 ( $p < 0.01$ ), T-bet ( $p < 0.01$ ), and IDO ( $p < 0.01$ ) (Figure 4).

## DISCUSSION

To the best of our knowledge, the present report is the first animal research study to describe the anti-allergic effects of the MXDT decoction on ovalbumin-induced AA in mice. Animal models are a very important tool to explore human disease pathology and identify more useful and effective drugs to treat human diseases.

The mouse model of OVA-challenged AA is a broadly used tool for the investigation of asthma and for the screening of candidate antiasthma drugs [10,11]. Thus, the present study successfully induced AA in mice after treatment with OVA. Inflammatory reactions play important roles in the development of AA, and various studies have demonstrated that the alleviation of inflammatory reactions is a useful way to treat asthma [12,13]. The present results demonstrate that MXDT inhibits inflammatory reactions in lung tissues of mice with OVA-induced AA.

Th1/Th2 balance is recognized as an important mediator of asthma; modulation of Th1/Th2 balance is thus considered beneficial for the treatment of asthma [2,14,15]. The present study revealed that after treatment with MXDT, increased levels of Th2 cytokines (IL-4 and IL-6) were downregulated, whereas decreased levels of the Th1 cytokine IFN- $\gamma$  was upregulated, suggesting that MXDT can modulate Th1/Th2 balance. Clinical research has reported high serum/BALF levels of IL-7 and TGF- $\beta$  in asthma patients [16,17].

IL-17 is an early proinflammatory cytokine that can activate TGF- $\beta$ ; these two cytokines can then cause proinflammatory cytokine release, airway inflammation, goblet cell proliferation, and airway remodeling [16,17]. The present results show that MXDT is able to decrease the levels of IL-7 and TGF- $\beta$  in the BALF of mice with OVA-induced AA. TLR4, an important pattern recognition receptor, plays a crucial role in the reactions and secretion of inflammatory cytokines. The ratio of GATA-3, OX40L, and T-bet levels was reported to be important for Th1/Th2 balance. GATA-3 and OX40L may activate the release of Th2 cytokines, whereas T-bet commonly activates the release of Th1 cytokines [18-20]. The relative increase in GATA-3 levels may also moderate Th1/Th2 balance [19,20]. In addition, IDO and Foxp3 are

reportedly able to alleviate the allergic symptoms of AA patients [21,22]. The results of the present study suggest that MXDT can moderate Th1/Th2 balance by regulating TLR-4, GATA-3, OX40L, IDO, Foxp3, and T-bet levels.

## CONCLUSION

The present results suggest that MXDT has potential anti-allergic effects on OVA-induced AA in mice; possible molecular mechanisms may involve the inhibition of inflammatory reactions and the modulation of Th1/Th2 balance. The present work implies that MXDT is a promising candidate for the treatment of asthma.

## DECLARATIONS

### Acknowledgement

This work was funded by National Natural Science Foundation of China (General Program, no. 8157150535).

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Jianer Yu and Zheng Xue contributed equally to this manuscript.

## REFERENCES

1. Szeffler SJ. *Advances in pediatric asthma in 2013: coordinating asthma care. J Allergy Clin Immunol* 2014; 133: 654-661.
2. Xue Z, Zhang XG, Wu J, Xu WC, Li LQ, Liu F, Yu JE. *Effect of treatment with geraniol on ovalbumin-induced allergic asthma in mice. Ann Allergy Asthma Immunol* 2016; 116: 506-513.
3. Rana S, Shahzad M, Shabbir A. *Pistacia integerrima ameliorates airway inflammation by attenuation of TNF- $\alpha$ , IL-4, and IL-5 expression levels, and pulmonary edema by elevation of AQP1 and AQP5 expression levels in mouse model of ovalbumin-induced allergic asthma. Phytomed* 2016; 23: 838-845.
4. Olin JT, Wechsler ME. *Asthma: pathogenesis and novel drugs for treatment. BMJ* 2014; 349: g5517.
5. Li Q, Ding W, Gao YZ, Li YL, Jiang LB, Jiang Y. *Imperatorin inhibits allergic airway inflammatory reaction*

- and mucin secretion in ovalbumin-induced asthmatic rats. *Trop J Pharm Res* 2016; 15: 2415-2420.
6. Peng W, Ming QL, Han P, Zhang QY, Jiang YP, Zheng CJ, Han T, Qin LP. Anti-allergic rhinitis effect of caffeoylxanthiazonoside isolated from fruits of *Xanthium strumarium* L. in rodent animals. *Phytomedicine* 2014; 21: 824-829.
  7. Mao WA, Sun YY, Mao JY, Wang L, Zhang J, Zhou J, Rahman K, Ye Y. Inhibitory effects of angelica polysaccharide on activation of mast cells. *Evid Based Complement Alternat Med* 2016; 2016: 6063475.
  8. World Medical Organization. Declaration of Helsinki. *BMJ* 1996; 313: 1448-1449.
  9. Lee EJ, Song MJ, Kwon HS, Ji GE, Sung MK. Oral administration of fermented red ginseng suppressed ovalbumin-induced allergic responses in female BALB/c mice. *Phytomedicine* 2012; 19:896-903.
  10. Brüggemann TR, Fernandes P, Oliveira LM, Sato MN, Martins MA, Arantes-Costa FM. Cigarette smoke increases CD8 $\alpha$ <sup>+</sup> dendritic cells in an ovalbumin-induced airway inflammation. *Front Immunol* 2017; 8: 718.
  11. He J, Lv L, Wang Z, Huo C, Zheng Z, Yin B, Jiang P, Yang Y, Li J, Gao Y, Xue J. Pulvis *Fellis Suis* extract attenuates ovalbumin-induced airway inflammation in murine model of asthma. *J Ethnopharmacol* 2017; 207: 34-41.
  12. Dai R, Liu J, Cai S, Zheng C, Zhou X. Delivery of adipose-derived mesenchymal stem cells attenuates airway responsiveness and inflammation in a mouse model of ovalbumin-induced asthma. *Am J Transl Res* 2017; 9: 2421-2428.
  13. Antwi AO, Obiri DD, Osafo N. Stigmasterol modulates allergic airway inflammation in guinea pig model of ovalbumin-induced asthma. *Mediators Inflamm* 2017; 2017:2953930.
  14. Yeh WI, Harrington LE. Regulation of effector CD4<sup>+</sup> T cell functions by T-bet. *J Immunol* 2009; 182: 48.
  15. Yang SH, Kao TI, Chiang BL, Chen HY, Chen KH, Chen JL. Immune-modulatory effects of bu-zhong-yi-qi-tang in ovalbumin-induced murine model of allergic asthma. *PLoS One* 2015; 10: e0127636.
  16. Lajoie S, Lewkowich IP, Suzuki Y. Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol* 2010; 11: 928-935.
  17. Kim SH, Hong JH, Lee YC. Ursolic acid, a potential PPAR $\gamma$  agonist, suppresses ovalbumin-induced airway inflammation and Penh by down-regulating IL-5, IL-13, and IL-17 in a mouse model of allergic asthma. *Eur J Pharmacol* 2013; 701: 131-143.
  18. Sugimoto A, Kawakami R, Mikami N. Transcription Factors Downstream of IL-4 and TGF- $\beta$  Signals: Analysis by Quantitative PCR, Western Blot, and Flow Cytometry. *Methods Mol Biol* 2017; 1585:141-153.
  19. Kim SH, Hong JH, Lee JE, Lee YC. 18  $\beta$ -Glycyrrhetic acid, the major bioactive component of *Glycyrrhizae Radix*, attenuates airway inflammation by modulating Th2 cytokines, GATA-3, STAT6, and Foxp3 transcription factors in an asthmatic mouse model. *Environ Toxicol Pharmacol* 2017; 52: 99-113.
  20. Delayre-Orthez C, Becker J, Auwerx J, Frossard N, Pons F. Suppression of allergen-induced airway inflammation and immune response by the peroxisome proliferator-activated receptor-alpha agonist fenofibrate. *Eur J Pharmacol* 2008; 581:177-184.
  21. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells. *Nat Immunol* 2003; 4: 330-336.
  22. Miwa N, Ilayakawa S, Miyazaki S. IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-gamma increase in normal pregnancy but decrease in spontaneous abortion. *Mol Hum Reprod* 2005; 11: 865-870.