Saudi Journal of Biological Sciences 25 (2018) 1806–1811

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Effect of *Fructus schisandrae* syrup on bronchial asthma mice model

Mengfan Peng, Mingsan Miao *, Tan Wang, Dandan Liu

College of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, China

ARTICLE INFO

Article history:
Received 8 May 2018
Revised 4 July 2018
Accepted 8 July 2018
Available online 09 July 2018

Keywords:
Fructus schisandrae syrup
Bronchial asthma
Morphological changes

ABSTRACT

Purpose: To investigate the effect of *Fructus schisandrae* syrup on bronchial asthma mice model.
Methods: Sixty Kunming mice were randomly divided into normal control group, bronchial asthma model group, low-, middle-, and high-dose *Fructus schisandrae* syrup groups. Bronchial asthma was induced by injection of ovalbumin combined smoking. Two hours after the last administration, the change of lung function were observed, the contents of NO, IL-6 in serum were detected, the morphological changes of lung and bronchial were also observed, so as to explore the effect of *Fructus schisandrae* syrup on bronchial asthma mice.
Results: Compared with model group, the Schisandrae Fructus syrup groups can significantly increase the tidal volume of mice and decrease the respiratory frequency and the degree of bronchial stenosis ($P < 0.01$); The Schisandrae Fructus syrup groups can decrease the levels of NO and IL-6 in serum and improve the pathological changes of lung and bronchus in different degrees.
Conclusion: *Fructus schisandrae* syrup can significantly improve the biochemical indexes and pathological status of mice with bronchial asthma.

1. Introduction

Bronchial asthma is a common chronic inflammatory disease of airway, which has the characteristics of long duration and recurrence. The clinical manifestations of bronchial asthma include shortness of breath, cough, chest tightness and wheezing, and often happens in the morning and at night (Yang, 2017). Modern medicine believes that allergic reaction, airway inflammation, airway hyperresponsiveness, airway remodeling, nerve regulation mechanism and psychological factors are the pathogenesis of bronchial asthma. According to traditional Chinese medicine, the pathogenesis of bronchial asthma is the result of "wind". Phlegm blocked in the lungs, sputum stasis causes wheezing, zang-fu disharmony are the pathogenesis of bronchial asthma (Zhao, 2016). *Chinese pharmacopoeia* records that Guilongkechuanning capsule has the effect of relieving cough and reducing sputum, lowering the adverse flow of qi and relieving asthma. And when used for the treatment of acute and chronic bronchitis, has curative effect (National Pharmacopoeia Committee, 2015). *Fructus schisandrae* syrup is recorded in *Standards for pharmaceutical preparations for medical units of the people's liberation army of China*, which is a traditional Chinese medicine preparation made by processing *Fructus schisandrae* (The general logistics department of the people's liberation army., 1991). *Fructus schisandrae* syrup has the effects of supplementing qi and promoting the production of body fluid, tonifying the kidney and stabilizing the mind, it is used to treat chronic cough, shortness of breath, palpitation, insomnia etc. But has not seen *Fructus schisandrae* syrup for the treatment of bronchial asthma, therefore, select Guilongkechuanning capsule as a positive control drug, *Fructus schisandrae* syrup for the reagent, to observe the effect of *Fructus schisandrae* syrup on bronchial asthma mice model.

2. Materials

2.1. Animals and methods

Kunming mice, half male and half female, weighing 18–22 g, provided by the experimental animal center of Shandong Province. The certificate number: No. 37009200004567; Production license number of experimental animals, SCXK (Lu) 20140007. Breeding conditions: temperature 20–25 °C, relative humidity 40–60%.

* Corresponding author.
E-mail address: miaomingsan@163.com (M. Miao).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.07.003
1319-562X/

2.2. Drugs and reagents

Fructus schisandrae syrup provided by Shanghai haihong industrial chaohu industrial co. LTD, batch number:160630; Guilongkechuanning capsule, provided by Guilong pharmaceutical (anhui) co. LTD, batch number:160404; Ovalbumin (OVA) provided by Sigma-Aldrich LLC, batch number:A-5253; Aluminum hydroxide, Tianjin zhiyuan chemical reagent co. LTD, batch number:20131116; Mouse nitric oxide (NO) Elisa test kit, and IL-6 Elisa test kit, Biocalvin (Suzhou Calvin biology technology co. LTD), batch number: E20161201A.

2.3. Experimental instruments

FA(N)/JA(N) series electronic scales, Shanghai minqiao precision instruments co. LTD; KDC-160HR high-speed freezing centrifuge, Science and technology innovation co. LTD; 680-type enzyme marker, BIO-RAD co. LTD; Animal lung function detector, Beijing amuka biotechnology co. LTD.

3. Method

3.1. Model replicating methods of bronchial asthma mice (Li et al., 2014; Sa et al., 2014; Liu et al., 2013)

Sixty Kunming mice were randomly divided into normal control group, bronchial asthma model group, low-, middle-, and high-dose *Fructus schisandrae* syrup groups after adaptive feeding three days. In addition to the blank group, 0.1 ml of sensitizing fluid was administered to each group in the abdominal cavity on day 1, 4, 8 [10% ovalbumin and 10% aluminum hydroxide]. After sensitization, the mice were induced to have asthma attacks by smoking for 20 min at a time, lasting for 7 days.

3.2. Drug delivery

Guilongkechuanning capsule group (1.125 g/kg), low-, middle-, and high-dose *Fructus schisandrae* syrup groups (3 g/kg, 1.5 g/kg, 0.75 g/kg) were orally administrated after sensitization, once time a day for 7 days. The model group and blank group give the same volume of physiological saline.

3.3. Indexs

After two hours of the last administration, tidal volume, tracheal stenosis degree and respiratory inhalation frequency were measured by lung function detection instrument. The blood was taken from the eye socket and the upper serum is taken to be frozen for the contents detection of NO and IL-6 in the serum. Remove lungs and bronchi with surgical scissors for HE staining to observe the morphological changes.

3.4. Statistics processing method

The data were analyzed by SPSS 17.0 for windows statistical software, measurement data are expressed by mean ± standard ($x \pm s$) deviation, single factor variance analysis was used among the groups, the least significant difference (LSD) method was used to test the variance homogeneity and the Games-Howell method was used to test the heterogeneity of variance, ranked data using Ridit test.

4. Results

4.1. Effects on lung function of bronchial asthma mice

As we can see from Table 1: Compared with the blank group, the respiratory inhalation frequency and degree of bronchial stenosis increased significantly ($P < 0.01$), tidal volume decreased significantly ($P < 0.01$). Compared with model group, the respiratory inhalation frequency and degree of bronchial stenosis decreased significantly ($P < 0.01$), tidal volume increased significantly in low-, middle-, and high-dose *Fructus schisandrae* syrup groups ($P < 0.01$).

4.2. Effect of biochemical indexes in the serum of bronchial asthma mice

As we can see from Table 2: compared with the blank group, the contents of NO and IL-6 in the serum of the model group increased significantly ($P < 0.01$). Compared with model group, the levels of NO and IL-6 in the serum of the middle-, high-dose *Fructus schisandrae* syrup group decreased significantly ($P < 0.01$), the contents of NO in the serum of the low-dose *Fructus schisandrae* syrup group decreased significantly ($P < 0.01$).

Table 2
Effects on the contents of NO and IL-6 in serum of bronchial asthma mice ($\bar{x} \pm s$,N = 10).

Group	Dose (g/kg)	NO(u/l)	IL-6(pg/ml)
Blank	–	22.05 ± 2.11	83.03 ± 11.09
Model	–	34.04 ± 3.25△△	133.16 ± 12.74△△
Guilongkechuanning capsule	1.125	32.27 ± 2.98	98.8 ± 15.5**
Fructus schisandrae syrup	3	28.96 ± 3.03**	94.91 ± 10.76**
	1.5	28.18 ± 3.88**	107.09 ± 19.81**
	0.75	29.97 ± 3.09**	127.14 ± 11.01

*Compared with model group $P < 0.05$.
△Compared with blank group $P < 0.05$.
△△ Compared with blank group $P < 0.01$.
** Compared with model group $P < 0.01$.

Table 1
Effects on respiratory rate, tidal volume and the degree of bronchial stenosis of bronchial asthma mice ($\bar{x} \pm s$,N = 10).

Group	Dose (g/kg)	respiratory inhalation frequency(bpm)	tracheal stenosis degree	tidal volume(ml)
Blank	–	304.1 ± 29.6	0.36 ± 0.05	0.224 ± 0.015
Model	–	481.4 ± 25.8△△	0.48 ± 0.04△△	0.183 ± 0.017△△
Guilongkechuanning capsule	1.125	379.7 ± 28.6**	0.4 ± 0.04**	0.209 ± 0.012**
Fructus schisandrae syrup	3	280.2 ± 47.8**	0.36 ± 0.04**	0.225 ± 0.02**
	1.5	379.1 ± 38**	0.36 ± 0.04**	0.217 ± 0.019**
	0.75	431.2 ± 34.5**	0.36 ± 0.03**	0.204 ± 0.019**

*Compared with model group $P < 0.05$.
△Compared with blank group $P < 0.05$.
△△ Compared with blank group $P < 0.01$.
** Compared with model group $P < 0.01$.

4.3. Effect of morphological changes in the bronchial asthma mice'lungs and bronchi

4.3.1. Effect of morphological changes in the bronchial asthma mice'lungs

Blank group: The pulmonary lobule structure was normal and the alveolar epithelial cells showed no degeneration, necrosis and abscission. There was no hyperemia and edema in the alveolar wall, no exudate in the alveolar cavity and bronchial cavity, no inflammatory cell infiltration in the interstitium; the bronchial wall is intact, the smooth muscle thickness is normal, and the cells are arranged in a regular way. No exudate or epithelial cells were observed in the lumen, and no inflammatory cell infiltration was observed in the stroma (see Fig. 1). Model group: The pulmonary lobule structure was damaged, and the alveolar wall was significantly thickened, hyperemia, edema and inflammatory cell infiltration. Airway wall thickening, lumen stenosis, some vascular wall smooth muscle hyperplasia, glassy degeneration. The blood vessels and bronchi are surrounded by a large number of inflammatory cells (see Fig. 2). Guilongkechuanning capsule group: the lobule structure is close to normal. Erythrocytes, exudate and inflammatory cell infiltration are occasionally seen in the alveolar cavity. The capillary dilatation and hyperemia of the alveolar wall were improved obviously, the inflammatory cell infiltration around the wall of the alveolar wall was decreased significantly, and the bronchial mucosa was intact (see Fig. 3). High-dose *Fructus schisandrae* syrup group: The alveolar cavity gradually widens, the alveolar septum becomes narrow, and the alveolar wall can see lung macrophages and dust cells. The bronchial mucosa is intact and there is little inflammatory cell infiltration (see Fig. 4). Middle- and low-dose *Fructus schisandrae* syrup group: The mucosa of the bronchial tube was partially detached, and the alveolar cavity gradually became wider and the alveolar septum became narrower. A small amount of inflammatory cell infiltration was observed in lung stroma and blood vessels, and bleeding was observed in stroma (see Figs. 5 and 6).

As we can see from Table 3: By Ridit test, compared with the blank group, the lung tissue of the model group showed obvious inflammatory morphological changes ($P < 0.01$). Compared with the model group, High-dose *Fructus schisandrae* syrup group could significantly improve the morphological changes of the lung ($P < 0.05$). Although other groups could improve the morphological

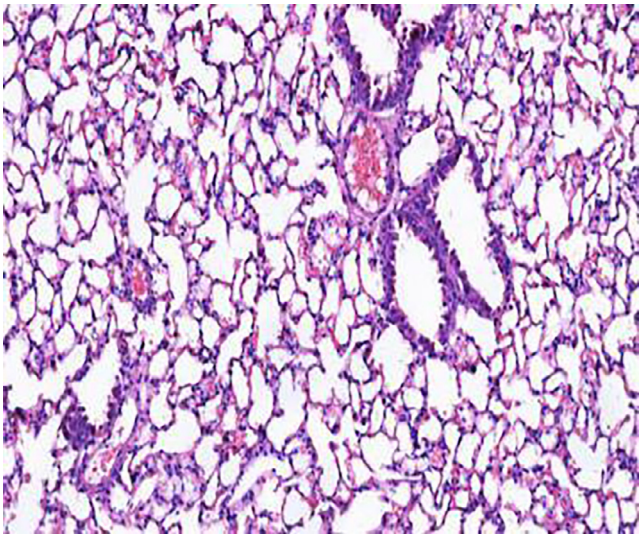

Fig. 1. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). Blank.

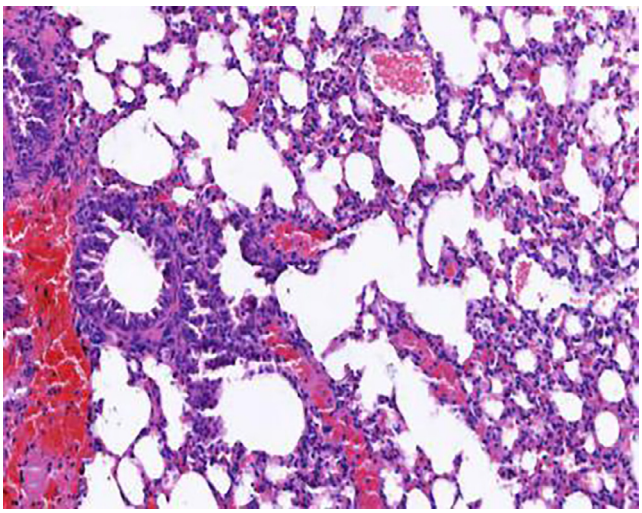

Fig. 2. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). Model.

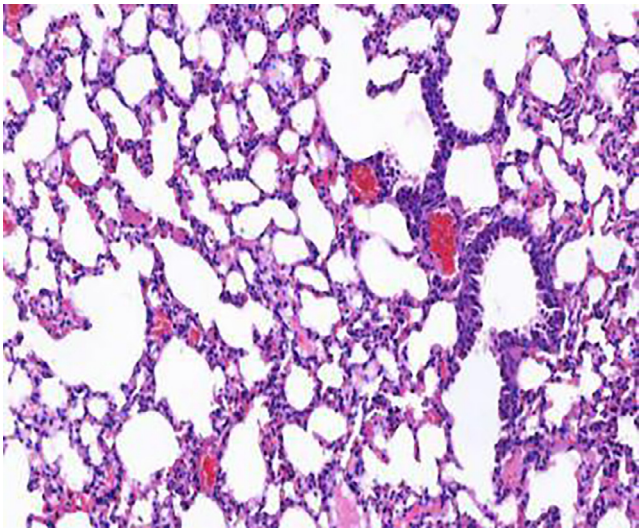

Fig. 3. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). Guilongkechuanning capsule.

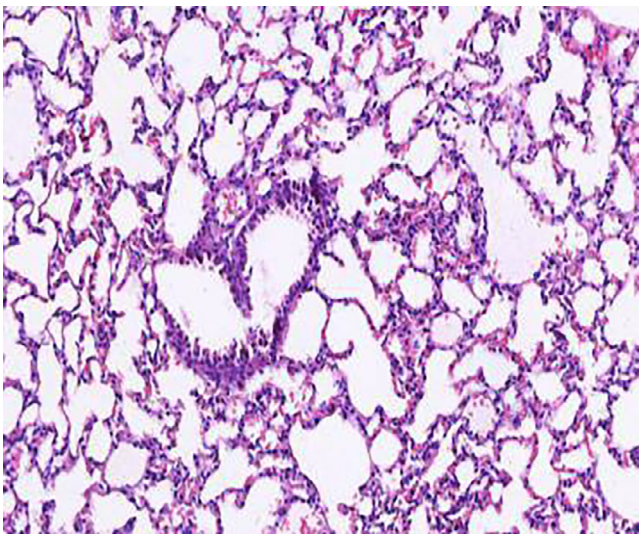

Fig. 4. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). High-dose *Fructus schisandrae* syrup.

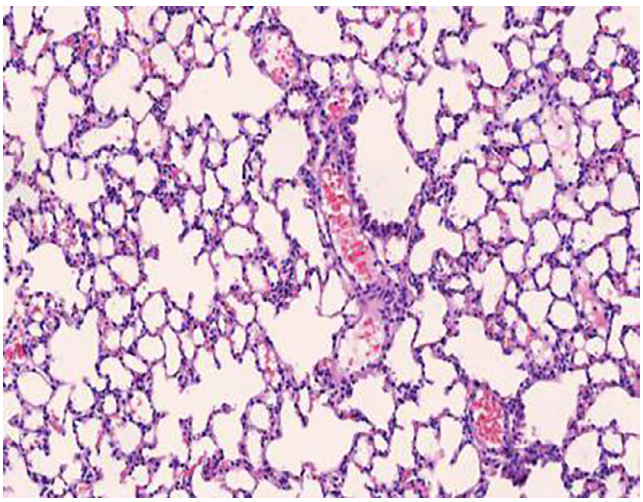

Fig. 5. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). Middle-dose *Fructus schisandrae* syrup.

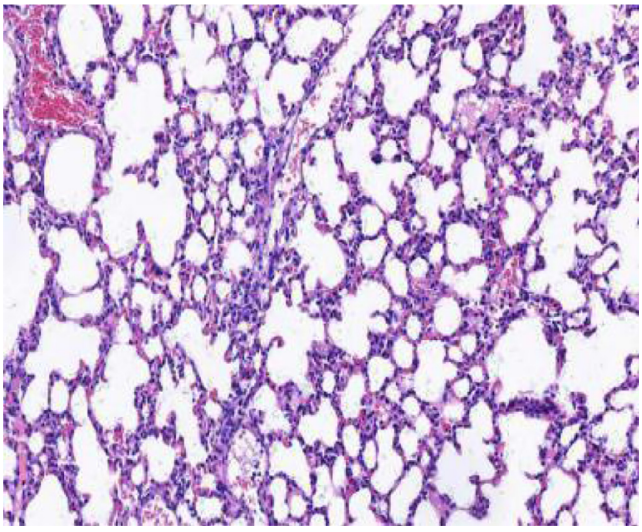

Fig. 6. Effect of pathological changes in the bronchial asthma mice' lungs (HE × 200). Low-dose *Fructus schisandrae* syrup.

changes of the lung tissue, there is no statistical significance ($P > 0.05$).

4.3.2. Effect of morphological changes in the bronchial asthma mice'bronchi

Blank group: the tracheal wall is intact, the wall and smooth muscle thickness are normal, without hyperemia and inflammatory cell infiltration (see Fig. 7). Model group: the mucosal epithelium

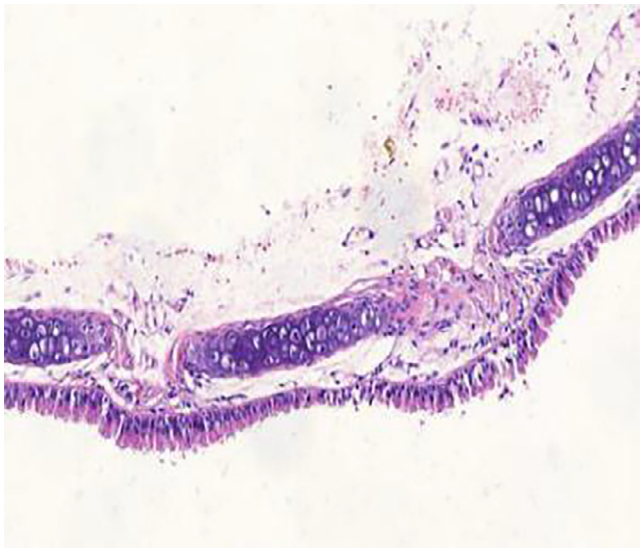

Fig. 7. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). Blank.

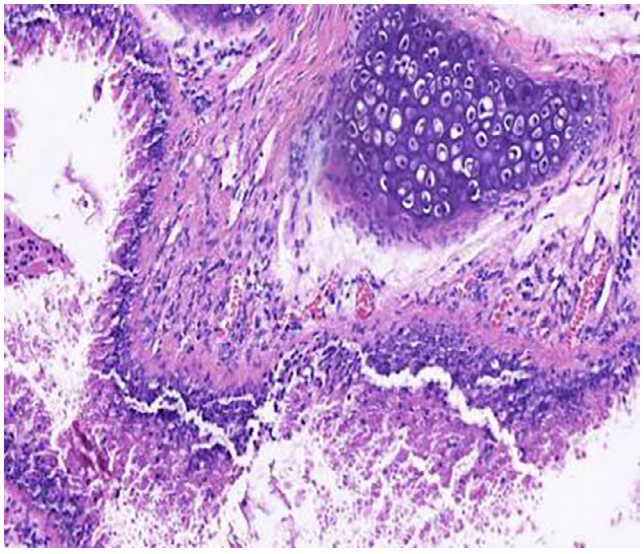

Fig. 8. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). Model.

was locally detached, the basement membrane was significantly thickened and glassy degenerated, the bronchial wall was dilated and filled with blood, and the surrounding inflammatory cells were infiltrated. Bronchial mucosal wrinkled wall increased and prolonged, goblet cells increased, wall smooth muscle hyperplasia hypertrophy (see Fig. 8). Guilongkechuanning capsule group:

Table 3
Effect of morphological changes in the bronchial a ($\bar{x} \pm s$, N = 10).

Group	Dose (g/kg)	–	+	++	+++	P
Blank	–	10	0	0	0	
Model	–	0	1	6	3	<0.01
Guilongkechuanning capsule	1.125	2	4	4	0	>0.05
Fructus schisandrae syrup	3	2	6	2	0	<0.05
	1.5	1	5	3	1	>0.05
	0.75	1	4	3	2	>0.05

"–" The alveolar cavity is well dilated, and the capillary endothelium in the alveolar septum is smooth, showing a very small number of inflammatory cells. "+" Bronchial mucosa is intact and there are very few inflammatory cells around the blood vessels and airway. "++" Inflammatory cells infiltrate the lung interstitium, epithelial cells shed, and a small number of inflammatory cells are seen around the blood vessels and airway. "+++" EOS and lymphocyte infiltration were observed in the interstitial lung, and epithelial cells were partially ablated. Small amounts of inflammatory cells were seen around the vessels and airway, and bleeding was seen.

tracheal tube cavity is narrow, mucosa part slightly thickens, rarely inflammatory cell infiltration, part epithelium necrosis falls off (see Fig. 9). High-dose *Fructus schisandrae* syrup group: the mucosa is partially detached, the epithelium is slightly thickened, the hyperemia and expansion are not obvious. Small amounts of inflammatory cells were infiltrated (see Fig. 10). Middle-, and low-dose *Fructus schisandrae* syrup group: the mucosal epithelium was locally ablated, the cells were moderately proliferated, and the basement membrane was slightly thickened. Small amounts of inflammatory cell infiltration can be seen in peripheral blood vessels (see Figs. 11 and 12).

As we can see from Table 4: By Ridit test, compared with the blank group, the tracheal tissue of the model group showed obvious inflammatory morphological changes ($P < 0.01$). Compared with the model group, the high-dose *Fructus schisandrae* syrup group could significantly improve the morphological changes of the bronchi tissue ($P < 0.05$). Although other groups could improve the morphological changes of the bronchi tissue, there is no statistical significance ($P > 0.05$).

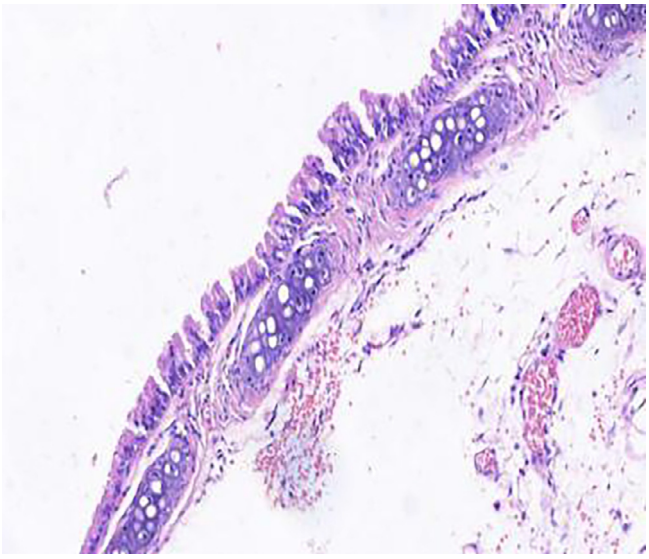

Fig. 9. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). Guilongkechuanning capsule.

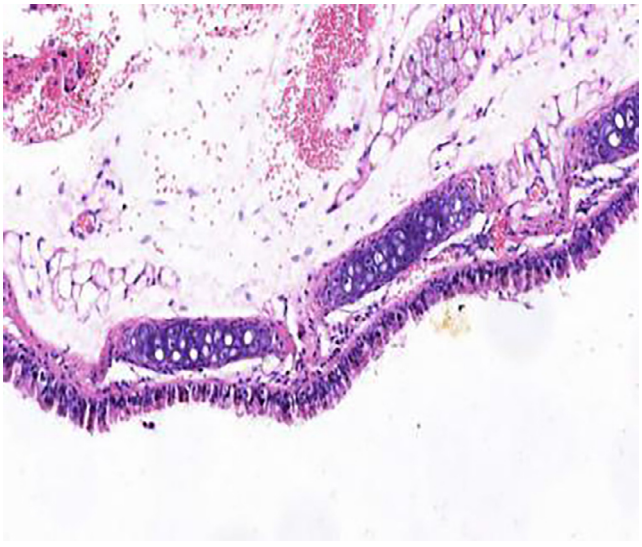

Fig. 10. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). High-dose *Fructus schisandrae* syrup.

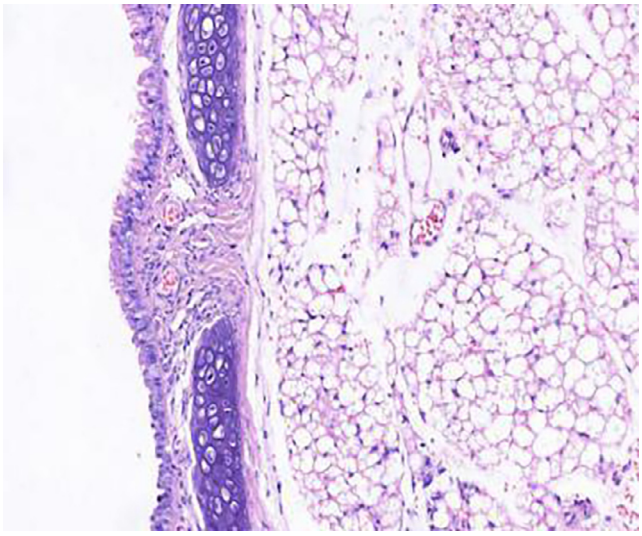

Fig. 11. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). Middle-dose *Fructus schisandrae* syrup.

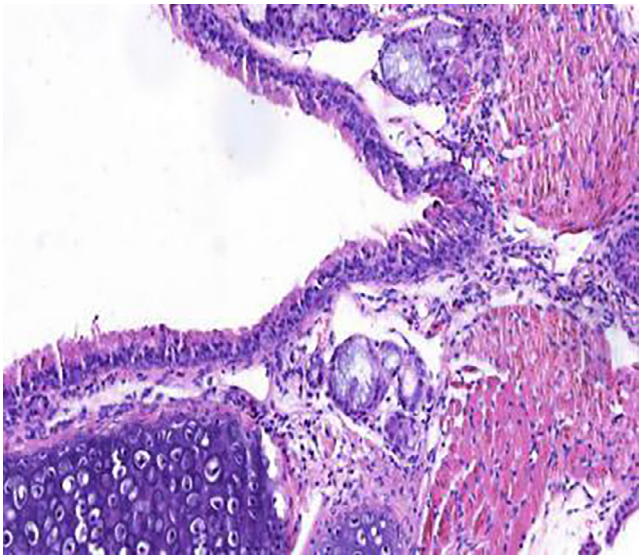

Fig. 12. Effect of pathological changes in the bronchial asthma mice' bronchi (HE × 200). Low-dose *Fructus schisandrae* syrup.

5. Discuss

Asthma is a recognized medical problem in the world, and the world health organization ranks it as one of the four major intractable diseases. The main pathological basis of bronchial asthma is airway allergic inflammation, which is mainly infiltrated by mast cells, eosinophils and T lymphocytes. Other studies have shown that the immune disorders are one of the key factors in the development of the disease (Kleinjan et al., 2014). Clinical treatment of bronchial asthma is mainly anti-airway allergic inflammation, but the clinical effect is not obvious. Traditional Chinese medicine believers bronchial asthma belong to the category of "asthma syndrome" and "gasp syndrome", Lung and kidney impaired, exogenous pathogenic factors, airway obstructed, qi obstructed due to phlegm and so on are the etiology and pathogenesis. Therefore, the convalescence of lung and kidney function is critical for the treatment of bronchial asthma (Xu, 2017; Chen, 2017). *Fructus schisandrae* price is low, can play a balanced role among the viscera

Table 4
Effect of morphological changes in the bronchial asthma mice' bronchi ($\bar{x} \pm s$, N = 10).

Group	Dose(g/kg)	–	+	++	+++	P
Blank	–	10	0	0	0	
Model	–	0	1	5	4	<0.01
Guilongkechuanning capsule	1.125	2	5	3	0	<0.05
Fructus schisandrae syrup	3	3	5	2	0	<0.05
	1.5	1	4	3	2	>0.05
	0.75	1	5	2	2	>0.05

"–"Tracheal wall is intact, the tracheal wall and smooth muscle thickness are normal. "+"Tracheal tube cavity is narrow, mucosa has slight thickening, the surrounding blood vessel individual inflammatory cell infiltration. "++"Tracheal stenosis, a small amount of mucosa shed and thickened, the subtracheal mucosa. There is infiltration of inflammatory cells scattered around the vessels. "+++"Tracheal stenosis, a small amount of mucosa shed and thickened, the subtracheal mucosa. There is a small infiltration of inflammatory cells around the vessels.

of heart, liver, spleen, lungs and kidneys. It has the function of invigorating lung and kidney, promoting the secretion of body fluid (Ma et al., 2014). *Fructus schisandrae* syrup is processed by *Fructus schisandrae*, containing lignin components and volatile components such as schisandra chinensis a, schisandra chinensis b, schisandra chinensis. And it has certain influence on kidney, lung and inflammation, immune function and so on. Therefore, this study selected fructus schisandra chinensis syrup as the test drug to observe its therapeutic effect on bronchial asthma mice model.

Bronchial asthma is one of the most common airway inflammatory diseases in human beings. It is of great significance to study the changes of the content of inflammatory factors in the pathogenesis. Nitric oxide (NO) is the main non-adrenal no-cholinergic nerve that causes bronchial smooth muscle relaxation and can dilate bronchi. It can also increase capillary exudation, leading to airway mucosal edema and aggravate airway obstruction. NO reacts with oxygen to form superoxide nitrite anions, releasing free radicals, damaging airway tissue, aggravating inflammation, causing high airway reactivity, leading to asthma attack or aggravation. Excessive amount of NO can expand blood vessels and promote the exudation of inflammatory cells and the release of inflammatory mediators (Guilford and Hope, 2014; Zuo et al., 2014). IL-6 is a cytokine with a variety of biological activities, mainly produced by monocytes and macrophages, which are closely related to local inflammatory responses. It can participate in inflammatory response injury and immune response of the body, stimulate and accelerate cell growth and the synthesis of inflammatory cytokines. High IL-6 level indicates the aggravation of vascular endothelial cell injury (Gan et al., 2016). Many literatures have shown that the change of il-6 level in serum is closely related to asthma, and the increase of il-6 level will aggravate airway inflammation (Li et al., 2014). Traditional Chinese medicine believes that the qi obstructed in lungs is the main factor that causes the recurrent symptoms such as asthma and cough (Fu et al., 2014). Therefore, the study selected NO, IL-6, as well as the changes in the morphology of lung tissues and bronchial tissues as the evaluation indexes for the therapeutic effect.

Experimental results showed that the mice model of bronchial asthma was successfully replicated. Compared with the blank group, the respiratory inhalation frequency, degree of bronchial stenosis, the levels of NO and IL-6 in the serum increased significantly, tidal volume decreased significantly in the model group. Compared with model group, *Fructus schisandrae* syrup could decrease the respiratory inhalation frequency, degree of bronchial stenosis, the levels of NO and IL-6 in the serum and increase tidal volume. The pathological changes of bronchial and pulmonary tissues were also improved.

To sum up, the following conclusions are drawn. *Fructus schisandrae* syrup can achieve the therapeutic effect on bronchial asthma mice model by decrease the levels of NO and IL-6 in the serum, improve the morphological changes of bronchus and lung tissue lesions, the effect of High-dose *Fructus schisandrae* syrup group is optimal. At present, the experiment is limited to laboratory studies, and the clinical effects need to be further studied.

Acknowledgement

The work was financially supported by the Foundation item of National and international cooperation bases (2016-151) and Henan province results transformation project (142201610011).

References

Chen, F.G., 2017. Pulmonary function improvement of traditional Chinese medicine on patients with bronchial asthma. Clin. Med. Res. Pract. 2 (12), 127–128.
Fu, Y., Liu, Z.H., Wang, B.K., Cheng, S., Tan, C., Zhu, Q., Zhao, J.P., 2014. Effect of acupuncture with lung and large intestine Theory on the Pulmonary Function of Patients with Bronchial Asthma Basic medical journal of traditional. Chin. Med. 20 (3), 364–367.
Gan, X.J., Wang, X.H., Hu, G., 2016. Changes of serum levels of TNF-α and IL-6 in acute exacerbation patients with chronic bronchitis and their clinical significance. J. Pract. Cardiocerebral Pulm. Vasc. Dis. 24 (05), 127–128.
Guilford, F.T., Hope, J., 2014. Deficient glutathione in the pathophysiology of mycotoxin-related illness. Toxins (Basel) 6 (2), 608–623.
Kleinjan, A., Klein Wolterink, R.G., Levani, Y., de Bruijn, M.J., Hoogsteden, H.C., van Nimwegen, M., Hendriks, R.W., 2014. Enforced expres-sion of Gata3 in T cells and group 2 innate lymphoid cells increases susceptibility to allergic airway inflammation in mice. J. Immu-nol. 192 (4), 1385–1394.
Li, Y.N., Ma, Y.C., Liu, L., Yin, J.N., Cheng, H.J., 2014. Cigarette smoke exposure of immature mice increase asthmatic airway inflammation in adult mice. Chin. J. Immunol. 30 (09), 1165–1168. +1173.
Li, Q., Tian, F.L., Cui, J.M., Liu, G.R., Zheng, D.S., Chen, J.M., Ma, S.X., Wang, H.B., Li, X. Q., 2014. Effect of massage on IL-17, IL-33 and IL-6 of pediatric asthma in different time. Health Care for Chin. Women Children 29 (04), 530–532.
Liu, P., Yu, J.N., Bai, L., 2013. Role of Pingchuan Recipe on microRNA126 and GATA-3 expression in lung tissue of mice with asthma. Proprietary Chin. Med. 35 (11), 2324–2328.
Ma, Y.X., Huang, Y.X., Zhou, H.C., Wang, Y.L., Li, Y., 2014. Advances in modern pharmacology and clinical research of *Fructus schisandrae*. Chin. Med. Inform. 31 (01), 125–126.
National Pharmacopoeia Committee, 2015. Pharmacopoeia of People's Republic of China Beijing: China medical. Science and Technology Press.
Sa, R.G., Hong, M., Na, S.S., 2014. The effects of Mongolian medicine compound sandthorn granules on chronic tracheobronchitis in mice. Chin. J Nat. Med. 20 (01), 39–40.
The general logistics department of the people's liberation army, 1991. Regulations on the preparation of medical units of the people's liberation army. Beijing: People's military medical publishing house. 103.
Xu, X.M., 2017. Observation on the therapeutic effect of TCM syndrome differentiation in the treatment of bronchial asthma. Light Traditional Chin. Med. 32 (01), 51–52.
Yang, B.H., 2017. Clinical observation on treating bronchial asthma with doxophylline. Clin. Studies Traditional Chin. Med. 9 (22), 116–117.
Zhao, C.X., 2016. The development of pathogenesis of bronchial asthma. Med. Theory Pract. 29 (07), 870–872.
Zuo, L., Koozechian, M.S., Chen, L.L., 2014. Characterization of reactive nitogen species in allergic asthma. Ann. Allergy Asthma Immunol. 112 (1), 18–22.