

Effects of catalpol on bronchial asthma and its relationship with cytokines

Yanlin Li¹ | Hai Wang²  | Xu Yang²

¹Heilongjiang University of Traditional Chinese Medicine, Harbin, Heilongjiang, China

²Department of Pediatrics, The First Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, Harbin, China

Correspondence

Hai Wang, Department of Pediatrics, the First Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, No.26 Heping Road, 150040 Harbin, China.
Email: hai_wang_hljucm@163.com

Abstract

An animal (BALB/c mice) model of catalpol associated with bronchial asthma in vivo was established, and the effects of catalpol and its relationship with cytokines were investigated. A total of 30 adult BALB/c mice were randomly divided into a positive control group, a model group, and a catalpol group, with 10 mice in each group. The lung function of mice, the cell count, and the cytokine concentrations in bronchoalveolar lavage fluid (BALF) were detected. The levels of cytokines [interleukin 4 (IL-4), interleukin 5 (IL5), and interferon gamma (IFN- γ)] in BALF were measured with enzyme-linked immunosorbent assay methods. The total number of cells in the BALF of the group treated with catalpol was significantly lower than the model group. After treatment with catalpol, the eosinophils and neutrophils of the mice were remarkably reduced compared with the model group. The malondialdehyde content in the lung tissue homogenate of the mice was also decreased in the catalpol group. The cytokines IL-5 and IL-4 exhibited a similar tendency: the concentrations of IL-4 and IL-5 for the catalpol group were dramatically decreased compared with the model group. However, the IFN- γ concentration for the catalpol group was higher than the model group. The results indicated that IL-5 may involve in the pathologic process of asthma-like IL-4, and an inflammatory reaction may still exist in the airway during the remission stage of asthma. The imbalances of the cytokine network might be an important molecular basis in the asthma pathogenesis. It is suggested that catalpol may be a potential drug for the clinical treatment of asthma.

KEYWORDS

bronchial asthma, catalpol, IFN- γ , IL-4, IL-5

1 | INTRODUCTION

Bronchial asthma is a common respiratory disease. Statistics have shown that about 100 million people suffer from asthma worldwide, and there are at least 20 million patients in China. Thus, asthma has become a serious threat to people's health.¹⁻³ The prevention and control of asthma has always been a concern in medical research and treatment. Previous studies have demonstrated that asthma

is an airway inflammatory disease characterized by recurrent reversible airway obstruction and development of airway hyperreactivity, which includes the participation of kinds of immunity inflammation cells such as adipose cells, acidophilia granular cells, lymphocyte, etc. and the cell components.⁴⁻⁷ The mucus suppository formed by airway inflammation, edema, and mucus secretion is the main reason why asthma is difficult to reverse or is, sometimes, completely irreversible.^{4,5} Therefore, the control

of airway inflammation, the elimination of airway edema, the reduction of mucus secretion, and the improvement of the immune function of the body are the key approaches for the treatment of asthma.

Currently, the exact pathogenesis of asthma is not clear. It has been considered that Th1/Th2 dysfunction is an important factor in the pathogenesis of asthma.⁸⁻¹² Interleukins (IL-4, IL-5)—produced from excess growth, proliferation, and activation of Th2 cell—might cause asthma, and interferon (IFN- γ)—secreted by Th1 cells—can inhibit the development of Th2 cells and the immune response.¹³⁻¹⁶ Inflammation is the basis of bronchial asthma. Oxygen-free radicals are important inflammatory mediators.^{14,16} Asthma attacks affect the bronchi and bronchioles in the lungs, the enhancement of the inflammatory reaction increases the formation of free radicals and increases the lipid peroxidation, and the content of the intermediate product, malondialdehyde (MDA), increases.^{8,9,12-16} Therefore, the changes in MDA can indirectly reflect the level of oxygen-free radicals in the body.

Radix Rehmanniae Preparata (Shudihuang) is a common traditional Chinese herbal medicine for nourishing the kidney.¹⁷⁻²⁵ The pharmacological effects of this herb have been widely investigated, including its anti-aging effect, antitumor function, and its ability to improve immunity. According to the traditional Chinese medicine theory, asthma is a kidney deficiency. In clinical practice, *Radix Rehmanniae Preparata* is commonly used to treat asthma. Catalpol is one of the main active ingredients in *Radix Rehmanniae Preparata*. The chemical structure of catalpol is shown in Figure 1. Catalpol can help determine the quality of the herb. It is well known that catalpol exhibits functions of diuresis, hypoglycemia, and slow diarrhea.^{17,18,20,21,23,25,26} However, there are few pharmacological studies about catalpol on the regulation and improvement of the immune function of the body and the anti-asthmatic airway allergic inflammation.²⁷⁻²⁹ In this study, the effect of catalpol on airway inflammation was investigated using an ovalbumin (OVA)-induced asthma model in BALB/c mice to observe the changes in MDA and cytokines in the asthmatic mice to probe a new approach for the prevention and control of asthma.

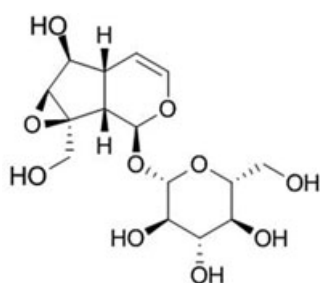


FIGURE 1 Chemical structure of catalpol

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Thirty SPF grade BALB/c mice (age: 5-6 weeks; weight: 180-210 g) were purchased from Vital River Laboratory Animal Technology (Beijing, China). The BALB/c mice were divided into the experimental group and the control group with 30 rats in each group. The temperature and humidity were $25 \pm 1^\circ\text{C}$ and 55%-60%, respectively. Both the illumination time and the dark time were 12 hours. There were no restrictions on drinking water and feeding. The care and treatment of the animals were approved by the Animal Care and Use Committees of the Heilongjiang University of Chinese Medicine.

2.2 | Establishment of asthma model and catalpol treatment

The mice were randomly divided into three groups: a positive control group, a model group, and a catalpol group, with 10 mice in each group. The asthma model was established by sensitization and challenge with OVA.

For the control group, the mice were treated by intraperitoneal injection using 0.5 mL of normal saline on day 1, day 7, and day 14. From day 15 to day 28, the mice were challenged with normal saline for 30 minutes every day using an ultrasonic nebulizer. For the model group, the mice were sensitized with OVA, which contains 100 μg OVA (Sigma, St. Louis, MO) and 2 mg aluminum hydroxide (Sinopharm, Shanghai, China) through intraperitoneal injection on day 1, day 7 and day 14. From day 15 to day 28, the mice were challenged with 50 g/L OVA in an equal amount of normal saline used for the control group for 30 minutes every day using an ultrasonic nebulizer. For the catalpol group, the sensitization and OVA challenge were the same as that for the model group. One hour before OVA challenge, the mice received a dose (5 mg/kg in normal saline) of catalpol (Melonepharma, Dalian, China) daily through intraperitoneal injection from day 15 to day 28.

2.3 | Bronchoalveolar lavage fluid and lung tissue homogenate

On day 29, the mice were executed. Normal saline was injected into the right ventricle two times to completely remove the residual blood in the lung. The lung was taken from the chest cavity after washing. A tube was inserted into the syringe to inject 1 mL of PBS solution for bronchoalveolar lavage three times. Bronchoalveolar lavage fluid (BALF) was obtained, and the total number of cells was counted with 10 μL . BALF was centrifuged for 10 minutes at 1500 rpm.

The supernatant was extracted for cytokine detection, and the cells were classified and counted after precipitation and suspension to determine the percentages of neutrophils, eosinophils, lymphocytes, and macrophages. The lung tissue of mice was loaded in normal saline according in a weight ratio of 1:9 to prepare 10% lung tissue homogenate, which was centrifuged for 10 minutes at 3500 rpm. The supernatant was extracted to measure the MDA content.

2.4 | Enzyme-linked immunosorbent assay

The level of IgE in the BALF was determined using an IgE enzyme-linked immunosorbent assay (ELISA) kit (USCN, Wuhan, China). The levels of IL-4, IL-5, and IFN- γ in the BALF were determined using the corresponding ELISA kits (Boster, Wuhan, China) according to the manufacturer's instructions. In the measurement, the optical density (OD) values were recorded using an enzyme standard instrument (Biorad450) at the wavelength of 450 nm. OD values more than twice of the negative control were defined as positive.

2.5 | Statistical analysis

SPSS 13.0 software was used for statistical analysis. Single-factor Analysis of Variance (ANOVA) was used to compare the difference between the groups. The least significant difference method was used for the comparison of two sets of data with the homogeneity of variance. Dunnett's *t*-test was used for heterogeneity of variance. Spearman's rank correlation analysis was used for correlation analysis. $P < 0.05$ indicated that there were no significant differences between the data.

3 | RESULTS

3.1 | Determination of IgE in BALF

The level of IgE in BALF was determined using the ELISA method. As shown in Figure 2, the level of IgE in BALF was increased in the model group, and it was significantly higher than that in the control group with $P < 0.01$. After catalpol treatment, the IgE level was dramatically decreased, and it was significantly lower than that in the model group with $P < 0.01$.

3.2 | Cell counts in BALF

After the sensitization and OVA-challenge of the mice, the total number of cells in the model group and the catalpol group was significantly increased compared with the control group, with $P < 0.01$, as shown in Figure 3. The

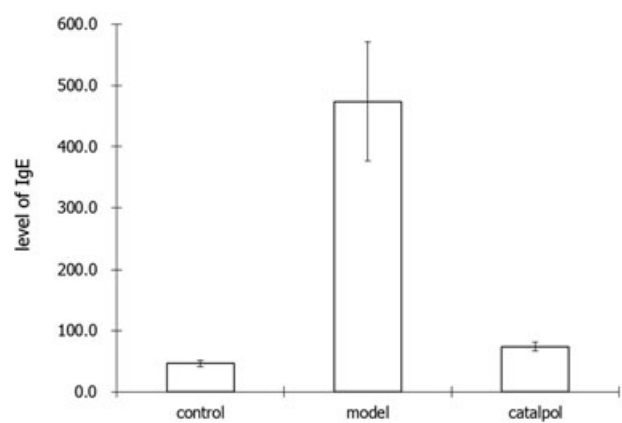


FIGURE 2 The level of IgE in BALF of mice from each group

percentages of eosinophils and neutrophils in the model group and the catalpol group were also increased significantly compared with the control group, with $P < 0.01$, as shown in Figure 4. Furthermore, the total number of cells and the percentages of eosinophils and neutrophils in the catalpol group were significantly decreased compared with the model group with $P < 0.05$ (Figures 3 and 4).

3.3 | Effect of catalpol on MDA

The result for MDA in lung tissue homogenate is shown in Figure 5. The MDA content in the model group was higher than that in the control group, with $P < 0.05$, and the MDA content in the catalpol group was lower than that in the model group, with $P < 0.05$.

3.4 | Concentration of cytokines

The concentrations of cytokine in the BALF of mice from each group are shown in Figure 6. The cytokines IL-4 and

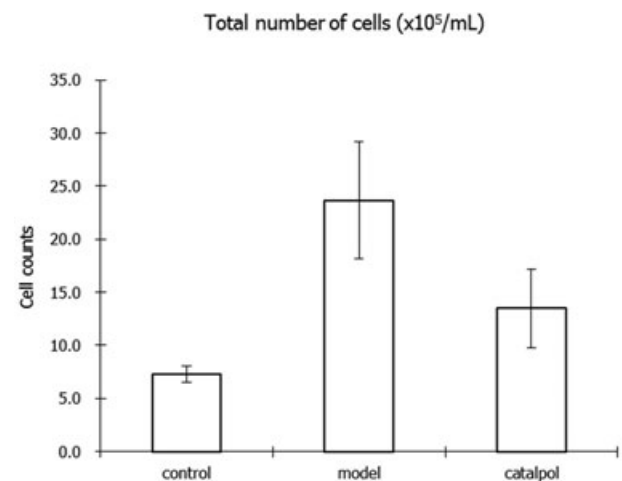


FIGURE 3 Total number of cells in BALF of mice from each group

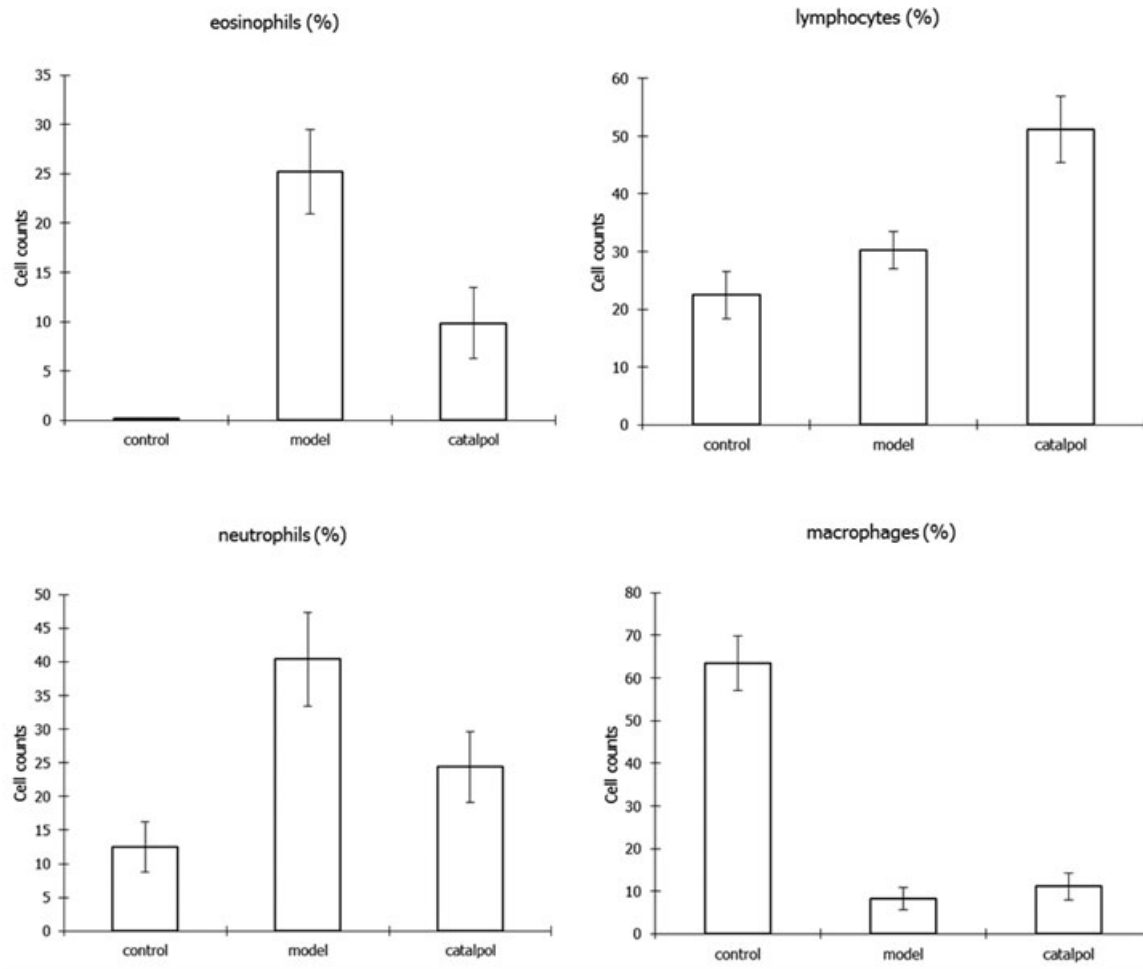


FIGURE 4 Percentages of neutrophils, eosinophils, lymphocytes, and macrophages in BALF of mice from each group

IL-5 in the model group were significantly increased compared with the control group with $P < 0.05$, and the cytokines IL-4 and IL-5 in the catalpol group were significantly decreased compared with the model group with $P < 0.05$. However, the IFN- γ in the model group was decreased compared with the control group with $P < 0.05$, and IFN- γ in the catalpol group was increased compared with the model group with $P < 0.05$.

4 | DISCUSSION

Radix Rehmanniae Preparata is a traditional Chinese medicine that has been successfully applied in the clinical treatment of asthma. However, there have been very few studies about the clinical effects of catalpol, the most important component of *Radix Rehmanniae Preparata*, in the treatment of asthma. In this study, a BALB/c mice model on asthma was established by repeated sensitization and OVA-challenge. A large number of inflammatory cells, mainly eosinophils and lymphocytes, infiltrated around bronchioles and capillaries. Moreover, the airway mucosa

and submucosa of experimental mice in the catalpol-treated group still exhibited partial congestion and edema, a small number of inflammatory cells infiltrated around bronchioles and capillaries, and a small amount of secretion accumulated in bronchioles. The level of IgE in the model group was higher than that of the control group. These

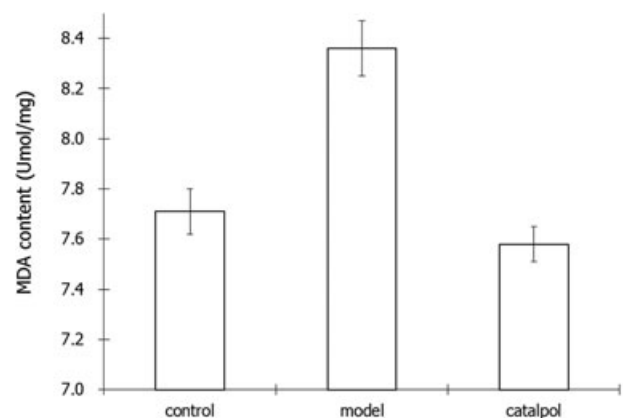


FIGURE 5 MDA content in lung tissue homogenate of mice from each group

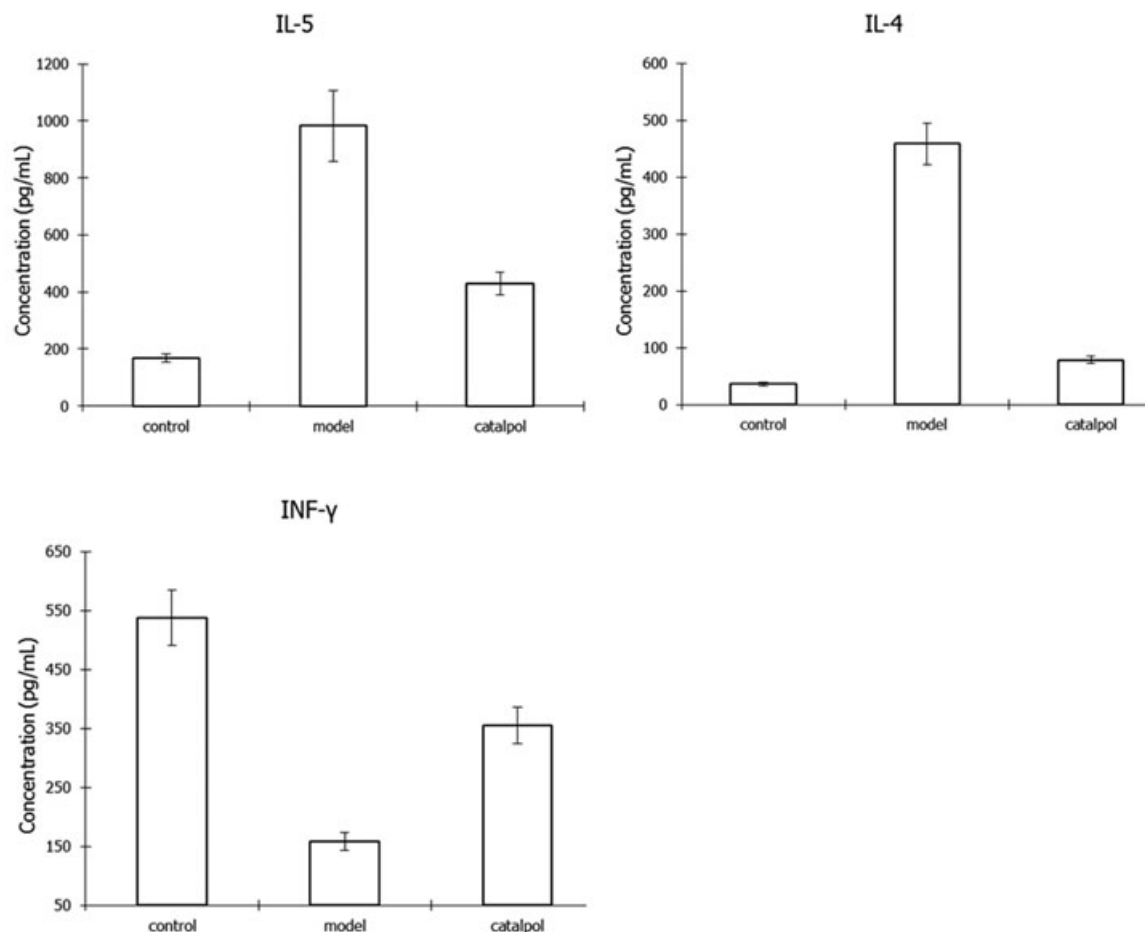


FIGURE 6 Cytokine concentrations in BALF of mice from each group

results indicate that the model was successfully established and replicated. The airway inflammation in mice was significantly alleviated after catalpol treatment, and the level of IgE in the BALF of experimental mice for the catalpol groups was significantly lower than that in the model group.

Previous studies have demonstrated that asthma is actually a chronic inflammation involving a variety of inflammatory cells, inflammatory mediators, and cytokines.⁸⁻¹⁶ Oxygen-free radicals are also one of the important inflammatory mediators. They can directly affect airway smooth muscle and airway tissue cells, resulting in contraction of airway smooth muscle, destruction of the cell membrane, and cell death. These free radicals directly target pulmonary microvasculature to increase the permeability of the blood vessel wall and promote plasma exudation and interstitial edema; thus pulmonary gas exchange is affected. They also target inflammatory cells to promote the release of inflammatory mediators, damage airway epithelium, and expose peripheral nerve fibers, resulting in enhanced airway response to various stimuli to form a sustained airway hyperresponsiveness. During asthma attacks, the enhance-

ment of inflammation leads to the increase of free radical production and lipid peroxidation, and consequently, the content of MDA increases. Therefore, the change in MDA can indirectly reflect the level of oxygen-free radicals in the body. The content of MDA in the lung tissue homogenate of BALB/c mice was measured in this study. It was found that the content of MDA in the model group initially increased but decreased after catalpol treatment. This result was inconsistent with previous studies, probably because fewer cases were investigated in the current research.

The pathogenesis of asthma is still unclear. Th1/Th2 dysfunction and hyperactivation of Th2 cells have been considered as the key factors in the pathogenesis of asthma.^{8,9,13,14,16} Th1 cells mainly secrete IL-5 and IFN-γ, thereby inducing macrophage activation and delayed hypersensitivity. Th2 cells mainly secrete IL-4 and IL-5, which induce the growth and differentiation of mast cells and EOS, as well as the production of immunoglobulins such as IgE. IL-4 is the primary cytokine that promotes the synthesis of IgE in vivo. IL-4 can promote the proliferation and growth of B lymphocytes by inducing the transcription of immunoglobulin and heavy chain and the expression of

CD23, thus facilitating the synthesis of IgE. It can also induce the proliferation of T lymphocytes, which is the differentiation of unshaped T cells into Th2. IL-4 can also promote the expression of vascular endothelial cell adhesion molecules, and enhance the binding of inflammatory cells and endothelial cells to participate in the infiltration of asthmatic airway inflammatory cells. IFN- γ is an antagonist of IL-4; it can inhibit the production of IgE in vivo by inhibiting the transcription of IL-4 mRNA and the expression of low-affinity IgE receptors in B cells induced by IL-4. IFN- γ can also inhibit the differentiation of Th0 cells into Th2 cells and the proliferation and activation of Th2 cells to reduce the infiltration and activity of eosinophils. IFN- γ and IL-4 are the characteristic cytokines of Th1 and Th2 cells, respectively. Therefore, some studies have concluded that IL-4/IFN- γ can reflect the Th1/Th2 cell immune response pattern. Furthermore, the imbalance of IL-4 and IFN- γ is the main reason for excessive IgE synthesis in asthmatic patients. The results of the present study indicated that the level of IL-4 in the serum of BALB/c mice in the model group increased, and IFN- γ decreased, and IL-4 and IFN- γ were unbalanced, which was consistent with the theory of asthma pathogenesis. After catalpol treatment, the level of IFN- γ increased, which was significantly different from the model group, indicating that the clinical effects of asthma treatment using catalpol could be improved by increasing the IFN- γ level. After catalpol treatment, the IL-4 level of experimental mice decreased and the level of IFN- γ increased and the balance IL-4/IFN- γ was improved.

In summary, catalpol can significantly increase the level of IFN- γ and balance the ratio IL-4/IFN- γ in BALF and improve chronic airway inflammation for asthmatic mice. This study suggests that catalpol might become an effective drug for the treatment of asthma.

5 | CONCLUSIONS

Catalpol can significantly alleviate airway inflammation for experimental mice, indicating that catalpol has an anti-inflammatory effect in the treatment of asthma. After sensitization, the levels of IL-4 and IL-5 in BALF of asthmatic mice in the model group increased; however, the level of IFN- γ decreased in the model group, indicating that there was an imbalance of IL-4 to IFN- γ . After catalpol treatment, the level of IFN- γ in the serum of mice significantly increased, but the level of IL-4 decreased, suggesting that catalpol can improve the balance of the ratio IL-4/IFN- γ in asthma. Therefore, catalpol has potentially anti-inflammatory and immunomodulatory effects in the treatment of asthma.

ORCID

Hai Wang  <http://orcid.org/0000-0003-4225-1580>

REFERENCES

1. Asher I, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis*. 2014;18:1269-1278.
2. Braman SS. The global burden of asthma. *Chest*. 2006;130:4S-12S.
3. Desai D, Brightling C. Cytokine and anti-cytokine therapy in asthma: ready for the clinic? *Clin Exp Immunol*. 2009;158:10-19.
4. Humbles AA, Lloyd CM, McMillan SJ, et al. A critical role for eosinophils in allergic airways remodeling. *Science*. 2004;305:1776-1779.
5. Venarske D, deShazo RD. Molecular mechanisms of allergic disease. *South Med J*. 2003;96:1049-1054.
6. Wardlaw AJ, Brightling C, Green R, Woltmann G, Pavord I. Eosinophils in asthma and other allergic diseases. *Br Med Bull*. 2000;56:985-1003.
7. Brightling CE, Bradding P, Pavord ID, Wardlaw AJ. New insights into the role of the mast cell in asthma. *Clin Exp Allergy*. 2003;33:550-556.
8. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res*. 2001;2:64-65.
9. Shi H, Qin S, Huang G, et al. Infiltration of eosinophils into the asthmatic airways caused by interleukin 5. *Am J Respir Cell Mol Biol*. 1997;16:220-224.
10. Wei Y, Luo QL, Sun J, Chen MX, Liu F, Dong JC. Bu-Shen-Yi-Qi formulae suppress chronic airway inflammation and regulate Th17/Treg imbalance in the murine ovalbumin asthma model. *J Ethnopharmacol*. 2015;164:368-377.
11. Zhang X, Jiang B, Li Z, Hao S, An L. Catalpol ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. *Pharmacol Biochem Behav*. 2007;88:64-72.
12. Durrant DM, Metzger DW. Emerging roles of T helper subsets in the pathogenesis of asthma. *Immunol Investig*. 2010;39:526-549.
13. Garcia G, Taille C, Laveneziana P, Bourdin A, Chanez P, Humbert M. Anti-interleukin-5 therapy in severe asthma. *Eur Respir Rev*. 2013;22:251-257.
14. Huang WC, Liou CJ. Dietary acacetin reduces airway hyperresponsiveness and eosinophil infiltration by modulating eotaxin-1 and th2 cytokines in a mouse model of asthma. *Evid. Based Complement. Alternat. Med*. 2012;2012:910520.
15. Ok IS, Kim SH, Kim BK, Lee JC, Lee YC. *Pinellia ternata*, *Citrus reticulata*, and their combinational prescription inhibit eosinophil infiltration and airway hyperresponsiveness by suppressing CCR3 + and Th2 cytokines production in the ovalbumin-induced asthma model. *Mediat Inflamm*. 2009;2009:413270.
16. Wegmann M. Targeting eosinophil biology in asthma therapy. *Am J Respir Cell Mol Biol*. 2011;45:667-674.
17. Jiang B, Du J, Liu J, Bao YM, An LJ. Catalpol attenuates the neurotoxicity induced by beta-amyloid(1-42) in cortical neuron-glia cultures. *Brain Res*. 2008;1188:139-147.
18. Jin D, Cao M, Mu X, et al. Catalpol inhibited the proliferation of T24 human bladder cancer cells by inducing apoptosis through the blockade of Akt-mediated anti-apoptotic signaling. *Cell Biochem Biophys*. 2015;71:1349-1356.

19. Park KS. Catalpol reduces the production of inflammatory mediators via PPARgamma activation in human intestinal Caco-2 cells. *J Nat Med*. 2016;70:620-626.
20. Tian YY, An LJ, Jiang L, Duan YL, Chen J, Jiang B. Catalpol protects dopaminergic neurons from LPS-induced neurotoxicity in mesencephalic neuron-glia cultures. *Life Sci*. 2006;80:193-199.
21. Zhang RX, Li MX, Jia ZP. *Rehmannia glutinosa*: a review of botany, chemistry, and pharmacology. *J Ethnopharmacol*. 2008;117:199-214.
22. Zhang X, Jin C, Li Y, Guan S, Han F, Zhang S. Catalpol improves cholinergic function and reduces inflammatory cytokines in the senescent mice induced by D-galactose. *Food Chem Toxicol*. 2013;58:50-55.
23. Zhou J, Xu G, Ma S, et al. Catalpol ameliorates high-fat-diet-induced insulin resistance and adipose tissue inflammation by suppressing the JNK and NF-kappaB pathways. *Biochem Biophys Res Commun*. 2015;467:853-858.
24. Choi HJ, Jang HJ, Chung TW, et al. Catalpol suppresses advanced glycation end-products-induced inflammatory responses through inhibition of reactive oxygen species in human monocytic THP-1 cells. *Fitoterapia*. 2013;86:19-28.
25. Gao N, Tian JX, Shang YH, Zhao DY, Wu T. Catalpol suppresses proliferation and facilitates apoptosis of OVCAR-3 ovarian cancer cells through upregulating microRNA-200 and downregulating MMP-2 expression. *Int J Mol Sci*. 2014;15:19394-19405.
26. Jiang B, Shen RF, Bi J, Tian XS, Hinchliffe T, Xia Y. Catalpol: a potential therapeutic for neurodegenerative diseases. *Curr Med Chem*. 2015;22:1278-1291.
27. Lee HK; Oh SR; Ahn KS; et al. Pharmaceutical composition comprising an extract of pseudotsugomachion longifolium and the catalpol derivatives isolated therefrom having antiinflammatory, antiallergic and antiasthmatic activity, US8455541B2 (2013).
28. Park EJ, Lee HS, Oh SR, Lee HK, Lee HS. Pharmacokinetics of verproside after intravenous and oral administration in rats. *Arch Pharm Res*. 2009;32:559-564.
29. Chen Y, Zhang Y, Xu M, et al. Catalpol alleviates ovalbumin-induced asthma in mice: reduced eosinophil infiltration in the lung. *Int Immunopharmacol*. 2017;43:140-146.
30. Zhu J, Chen X, Wang H, Yan Q. Catalpol protects mice against renal ischemia/reperfusion injury via suppressing PI3K/Akt-eNOS signaling and inflammation. *Int J Clin Exp Med*. 2015;8:2038-2044.

How to cite this article: Li Y, Wang H, Yang X. Effects of catalpol on bronchial asthma and its relationship with cytokines. *J Cell Biochem*. 2018;1-7. <https://doi.org/10.1002/jcb.28170>