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# Catalpol alleviates ovalbumin-induced asthma in mice: Reduced eosinophil infiltration in the lung

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ABSTRACT

*Background: Radix Rehmanniae Preparata* is a traditional Chinese herbal medicine used to treat asthma, and catalpol is one of the main active ingredients in this herb. In the present study, the effects of catalpol on asthma and the underlying mechanism were explored.

*Methods:* Mice with ovalbumin (OVA)-induced asthma were given 5 or 10 mg/kg catalpol from Day 15 to Day 28 (intraperitoneal injection). Histopathologic changes were detected by Hematoxylin and Eosin staining and Periodic Acid Schiff staining. The levels of IgE, interleukin (IL)-4, IL-5 and eotaxin were measured by ELISA. The numbers of lymphocytes, monocytes, basophils and eosinophils in the bronchoalveolar lavage fluid were determined by Wright-Giemsa staining. The expression and distribution of eotaxin and C-C chemokine receptor 3 (CCR3) were detected by immunohistochemistry and immunofluorescence. The expression of interleukin-5 receptor α (IL-5Rα) was detected by Western blot assay.

*Results:* Catalpol inhibited OVA-induced inflammation and IgE secretion in the lung. OVA-induced type 2 inflammation was suppressed by catalpol as evidenced by decreased levels of IL-4 and IL-5. Moreover, catalpol inhibited the aberrant eosinophil infiltration in the lungs, and also suppressed OVA-induced elevation of eosinophil chemokine eotaxin and its receptor CCR3. In addition, IL-5Rα expression in the bone marrow cells derived from catalpol-treated asthmatic mice was lower than that from the untreated asthmatic mice.

*Conclusion:* Our study demonstrated that catalpol attenuated OVA-induced asthma and inhibit the infiltration of inflammatory cells, especially eosinophils, into the lung. This study suggests that catalpol may become a promising drug for the treatment of asthma.



## 1. Introduction

Asthma is a chronic lung disease that is characterized by wheezing, coughing, bronchial contraction, chest tightness and shortness of breath. It is estimated that over 300 million people are suffering from this respiratory disease [1,2]. The typical asthma symptoms result from allergen-induced airway remodeling and pulmonary inflammatory reactions [3,4]. In addition, disrupted homeostasis of various immune cells, including helper T cells, eosinophils, mast cells and neutrophils, is commonly observed in asthma [5–8].

Excessive activation of eosinophils is one of the prominent features of asthma, and eosinophils are found to be increased in the circulation and sputum [9]. Eosinophils are recruited into tissues by chemokines and release eosinophil toxic products at inflammatory sites. These events lead to epithelial damages and airway remodeling, and contribute to the airway hyperresponsiveness [5,8,10].

*Radix Rehmanniae Preparata* (Shudihuang), which has been used as a traditional health food in East Asia, is the prepared root of *Rehmannia Glutinosa Libosch* (Dihuang). *Radix Rehmanniae Preparata* is regarded as a medicine nourishing the Yin and tonifying the kidney according to the traditional Chinese medicine theory [11].

More than 70 monomeric compounds have been separated from *Radix Rehmanniae Preparata*, including catalpol. Catalpol has been reported to exhibit hypoglycemic [12], neuroprotective [12–14], and anti-tumor [15,16] properties. Catalpol also shows prominent anti-inflammatory properties inhibiting inflammatory responses induced by LPS and IFN-γ [17], high-fat diet [18] and advanced glycation end-products [19], reducing inflammatory cytokines production [20,21], and protecting against renal ischemia/reperfusion injury [22]. Hence, this anti-inflammatory function of catalpol may be associated with the protective effects of *Radix Rehmanniae Preparata* against asthma.

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*Radix Rehmanniae Preparata* is one of the common and important medicines in the treatment of asthma in traditional Chinese medicine. However, how *Radix Rehmanniae Preparata* and catalpol perform their anti-asthmatic roles is still unclear. In the present study, we explored the therapeutic effects of catalpol on asthma as well as the underlying mechanism in a mouse model of OVA-induced asthma.

## 2. Materials and methods

### 2.1. Animals

Six week-old male BALB/c mice were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The mice were housed in a controlled environment (temperature at 23 ± 2 °C and relative humidity at 50 ± 5%) with 12 h-light/12 h-dark cycles. Food and water were accessed ad libitum. The care and treatment of the animals were approved by the Animal Care and Use Committees of Heilongjiang University of Chinese Medicine.

### 2.2. Establishment of asthma model and catalpol treatment

The mice were randomly divided into four groups (Control, Model, Catalpol-L, and Catalpol-H) with 6 mice in each group. The asthma model was established by sensitization and challenge with ovalbumin (OVA). Briefly, the mice were sensitized with OVA [100 μg OVA (Sigma, St. Louis, MO, USA) and 4 mg aluminum hydroxide (Sinopharm, Shanghai, China) in 0.5 ml of normal saline, intraperitoneal injection] on Day 1 and Day 8. From Day 15 to Day 28, the mice were challenged with 1% OVA for 30 min every day using an ultrasonic nebulizer. The mice in the Control group received an equal amount of normal saline. From Day 15 to Day 28, the mice in the Catalpol-L group received a low-dose (5 mg/kg in normal saline) of catalpol (Melonepharma, Dalian, China) daily through intraperitoneal injection 0.5 h prior to each OVA challenge, and the mice in the Catalpol-H group received a high-dose (10 mg/kg in normal saline) of catalpol daily via intraperitoneal injection 0.5 h prior to each OVA challenge. The mice in the Control group and Model group received equal amounts of normal saline. On Day 29, the peripheral blood and bronchoalveolar lavage fluid (BALF) in each group were harvested, and the lung tissues were also collected. Meanwhile, bone marrow cells were obtained by perfusing each femur cavity with 3 ml heparinized Hanks solution.

### 2.3. Histopathological assay

The lung tissues of mice in each group were collected, fixed in 4% paraformaldehyde, dehydrated in increasing concentrations of ethanol, embedded in paraffin, and cut into 5-μm sections. Then the sections were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, and subjected to routine Hematoxylin and Eosin (H&E) staining or Periodic Acid Schiff (PAS) staining. The images were captured with an optical microscope under a 200× magnification.

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

The level of IgE in the BALF was measured with an IgE ELISA kit (USCN, Wuhan, China). The levels of interleukin (IL)-4 and IL-5 in the BALF and peripheral blood were measured with the corresponding ELISA kits (Boster, Wuhan, China) according to the manufacturer's instructions. The level of eotaxin in the peripheral blood of mice from each group was measured using an eotaxin ELISA kit (Boster). The optical density at 450 nm was measured using a microplate reader, and the levels of IgE, IL-4, IL-5 and eotaxin were calculated.

### 2.5. Wright-Giemsa staining

The numbers of total leukocytes, lymphocytes, monocytes, basophils and eosinophils in the BALF were detected by Wright-Giemsa staining. Briefly, the cells in BALF were resuspended in 0.5 ml phosphate buffered saline (PBS) and made into cell smears. After fixed with methanol, the cells were stained with a Wright-Giemsa staining kit (JianchengBio, Nanjing, China). Then the cells were observed under an optical microscope with a 400× magnification, and the numbers of total leukocytes, lymphocytes, monocytes, basophils and eosinophils were counted.

### 2.6. Immunohistochemistry

The expression and distribution of eotaxin and C-C chemokine receptor 3 (CCR3) in the lung tissues were detected by immunohistochemistry. The lung tissues were harvested, fixed in 4% paraformaldehyde, dehydrated in increasing concentrations of ethanol, embedded in paraffin, and cut into 5-μm sections. Then the sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Antigen retrieval was carried out in sodium citrate buffer and endogenous peroxidase was quenched by incubating the sections with 3% hydrogen peroxide for 15 min at room temperature. Subsequently, the sections were blocked with goat serum for 15 min, and then incubated with primary antibodies against eotaxin (1: 50 in PBS, Santa Cruz, Dallas, TX, USA) or CCR3 (1: 50 in PBS, Sangon Biotech, Shanghai, China) at 4 °C overnight. Thereafter, the sections were washed with PBS for three times and incubated with the corresponding biotin-labeled secondary antibody (1: 200 in PBS, Beyotime, Haimen, China) at 37 °C for 30 min, and then horseradish peroxidase (HRP)-labeled avidin (Beyotime) at 37 °C for 30 min. After washing with PBS for three times, the sections were developed with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. The images were captured with an optical microscopy under a 400× magnification.

### 2.7. Immunofluorescence

Bone marrow cells were made into cell smears. After fixed in methanol, the cell smears were blocked with goat serum at room temperature for 15 min. Then the cell smears were incubated with primary antibody against CCR3 (1: 50 in PBS) at 4 °C overnight. After washing with PBS, the cell smears were incubated with the corresponding Cy3-labeled secondary antibody (1: 200 in PBS, Beyotime) in the dark at room temperature for 60 min. After washing with PBS, the cell nucleus were stained with 4′,6-diamidino-2-phenylindole (DAPI). The cell smears were observed with a fluorescence microscope, and the images were captured under a 200× magnification. The percentage of CCR3-positive cells was calculated as follows: the percentage of CCR3-positive cells = the number of CCR3-positive cells in each group/the total cell number in each group × 100%.

### 2.8. Western blot assay

Proteins in bone marrow cells were extracted using radio immunoprecipitation assay lysis buffer with 1% phenylmethanesulfonyl fluoride. The protein concentrations were measured using a BCA protein assay kit (Wanleibio, Shenyang, China). Then equal amount of proteins from each group were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Thereafter, the separated proteins were transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). After blocking with 5% skim milk, the membranes were incubated with primary antibodies against interleukin-5 receptor α (IL-5Rα) (1: 500, Bioss, Beijing, China) or β-actin (1: 1000, Wanleibio) at 4 °C overnight. After washing, the membranes were incubated with the corresponding HRP-labeled secondary antibodies (1: 5000, Wanleibio). The target proteins were visualized using ECL detection system.