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Original Article

# Effect of Tong Qiao drops on the expression of eotaxin, IL-13 in the nasal mucosa of rats with allergic rhinitis

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**Abstract**

*Background*: In recent years, particularly in China, many Chinese medicines and prescriptions for treating allergic rhinitis have been evaluated for their clinical relevance. Studies have found that numerous herbs and their constituent compounds can significantly alleviate allergic symptoms and are effective treatments for allergic rhinitis. The purpose of this study was to examine the modulatory effect of Tong Qiao nose drops on allergy symptoms and the expression of cytokines in the nasal mucosa of rats with ovalbumin-induced allergic rhinitis.

*Methods*: Sixty healthy male Sprague Dawley rats were randomly divided into three groups ($n = 20$): negative, control, and experimental. Rats in the control or experimental groups were sensitized with ovalbumin to induce allergic rhinitis. The sensitized rats in the experimental group were subsequently exposed to Tong Qiao nose drops, whereas the sensitized control rats were given saline nose drops. Negative control rats were only treated with saline. Allergic symptoms and the pathologic features of the nasal mucosa were observed. The expression of eotaxin in the mucous membrane of rat nasal septums was detected by immunohistochemical staining, and the expression levels of interleukin (IL)-5 and IL-13 were measured by enzyme-linked immunosorbent assay.

*Results*: The symptom scores for the experimental group were significantly lower than those of control rats ($p < 0.01$). Histopathologic examination revealed pathologic changes of nasal mucosa edema in the experimental group was mild and the infiltration of eosinophils was insubstantial. The expression levels of eotaxin, IL-5, and IL-13 in the nasal mucosa from experimental rats were significantly lower than that of control rats ($p < 0.01$).

*Conclusion*: Tong Qiao nose drops alleviated the symptoms of allergic rhinitis in a rat model and lowered the expression levels of eotaxin, IL-5, and IL-13.



*Keywords:* allergic rhinitis; eotaxin; interleukin; rat model; Tong Qiao nose drops

## 1. Introduction

Allergic rhinitis (AR) is a type 1 immunoglobulin (Ig) E-mediated hypersensitivity disease that is increasing in incidence.[1] In recent years, particularly in China, many Chinese medicines and prescriptions for treating allergic rhinitis have been evaluated for their clinical relevance. Studies have found that numerous herbs and their constituent compounds can significantly alleviate allergic symptoms and are effective treatments for allergic rhinitis.[2,3]

Tong Qiao drops (Chengdu Huashen Zhiyao, Ltd Co., Chenddu, China), a new Chinese therapeutic formula that contains active ingredients from three species of medicinal plants: *Angelica dahurica* (Bai Zhi), *Gleditsia sinensis Lam* (Zao Jia), and *Flos Magnoliae* (Xin Yi). Each agent of the Tong Qiao drops has been demonstrated to have an anti-iflammatory effect, and they can be used effectively to minimize symptoms in many allergic diseases.[4–6] However, whether Tong Qiao drops have any demonstrable effect in the treatment of allergic rhinitis has yet to be established. This study sought to investigate the effect of Tong Qiao drops on

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experimental allergic rhinitis in rats and to explore its mechanism of action. We used Tong Qiao nasal drops in rats with ovalbumin-induced allergic rhinitis and measured its effect on cytokine levels in the nasal mucosa.

## 2. Methods

### 2.1. Reagents

Tong Qiao nasal drops (10 g) *A dahurica* Bai Zhi, 1 g *G sinensis Lam* Zao Jia, 10 g *F Magnoliae* Xin Yi, 80 mL glycerol, and sodium chloride were purchased from Chengdu Huasheng Modern Biology Science & Technology Co (Chengdu, China). Ovalbumin (OVA) was purchased from Sigma (St. Louis, MO, USA), aluminum hydroxide gel (40 mg/mL) was purchased from Gibco (Life Technologies Corp, Grand Island, NY, USA), and rat interleukin (IL)-5 and IL-13 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Yi-Li Bio-Technology Co., Ltd. (Shanghai, China). Rabbit antimouse antieotaxin polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the ready-to-use EnVision reagent (Dako Corporation, Copenhagen, Denmark) were also used.

### 2.2. Rats

Institutional Review Board approval for the study was obtained through our hospital. Eighty special pathogen free (SPF) male Sprague Dawley (SD) rats, 6−8 weeks old and weighing 180−220 g each, were housed in the First Affiliated Hospital, Zhejiang University School of Medicine Laboratory Animal Center. Using a random number table the rats were divided into experimental, control, negative, and positive control groups ($n = 20$ per group).

### 2.3. Induction of allergic rhinitis in rats

Rats were sensitized following a revised version of the protocol published by An *et al.*[7] The sensitizing solution was prepared by dissolving 0.3 mg OVA into 1 mL saline using 30 mg aluminum hydroxide as an adjuvant (negative control rats were given 1 mL saline plus 30 mg aluminum hydroxide). Rats were injected intraperitoneally every other day for 14 days (for a total of seven injections per rat). Starting on Day 14, the rats were treated with 50 mL 2% OVA-saline solution in the form of intranasal drops on each side of the nose, once a day for 7 days (negative control rats were given saline drops). After the development of allergic rhinitis (Day 21), experimental rats were given Tong Qiao intranasal drops, two or three drops (10 uL/kg/nostril) per treatment, three times per day for 7 or 15 days. Control rats were given saline nose drops. Positive control rats were given mometasone furoate nasal spray was administered topically at a volume of 10 μL into the bilateral nasal cavities by micropipette 1 hour before the nasal antigen challenge (according to previous studies with modifications[8,9]).

Adhering to previous allergic rhinitis model scoring criteria,[4] each animal was observed after nasal provocation for nose scratching, sneezing, nasal discharge, and feeding behavior. Animal behavior was observed 30 minutes after the last nasal provocation and was scored using the superposition and quantitative method,[4] in which a total score more than five points indicates successful induction of allergic rhinitis. Symptoms were scored as follows: nasal itch, 1 = scratching the nose lightly one to two times, 2 = scratching the nose and face constantly; sneeze, 1 = one to three times, 2 = four to 10 times, 3 = 11 or more times; nasal discharge, 1 = secretions flow to the anterior nostril, 2 = secretions surpass the anterior nostril, 3 = secretions cover the face.

### 2.4. Nasal mucosa tissue collection

Following 30 minutes after the last nasal provocation, all animals were anesthetized with 1% pentobarbital sodium (50 mg/kg body weight), which was administered by intraperitoneal injection. The nasal septum mucosa of each rat was harvested, and a portion of each sample was fixed in 10% formaldehyde. Additional portions of each sample were immediately stored in liquid nitrogen for future use.

### 2.5. Pathologic observation

After fixing in formaldehyde, the nasal septum mucosa specimens were embedded in paraffin, sliced into 4 mm-thick sections, and then stained by hematoxylin and eosin. Samples were observed under an optical microscope.

### 2.6. Immunohistochemical staining for eotaxin in nasal mucosa

Cryopreserved nasal mucosa tissue samples were thawed, rehydrated, embedded in paraffin, and sliced into 4 μm-thick sections. Samples were deparaffinized using a baking sheet and water, washed with phosphate-buffered saline (PBS) for 3 minutes (two times), then subjected to two rounds of submersion in 0.3% hydrogen peroxide for 10 minutes, followed by washing in PBS for 3 minutes (two times) to block endogenous peroxidase activity. Rabbit anti-mouse antieotaxin polyclonal antibody was added to the samples at a 1:100 dilution at 37°C for 2 hours, followed by the addition of the ready-to-use EnVision reagent. After incubation at 37°C for 30 minutes, samples were washed in PBS for 3 minutes (three times) before staining with diaminobenzidine for 1−3 minutes, as well as hematoxylin. Sections were dried, mounted with neutral resin, and observed under an optical microscope. The nuclei stained blue, while eotaxin staining appeared as yellow or brown granules in the cytoplasm. Positive staining was defined visually, using the semi-quantitative staining intensity method: negative (−), not stained or appeared as background; weakly positive (+), few small granules; strongly positive (+++), numerous coarse granules; positive staining ranged between (+) and (+++).

### 2.7. Measurement of IL-5 and IL-13 in nasal mucosa tissue

Approximately 200 mg of nasal mucosa tissue was homogenized in saline solution on ice. Samples were centrifuged at