

# Effect of Centipeda Herba Volatile Oil on Allergic Rhinitis by Nasal Sniffing

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**Abstract** [Objectives] To explore the efficacy of different concentrations of Centipeda Herba volatile oil in the treatment of allergic rhinitis (AR) by nasal sniffing and the optimal drug concentration. [Methods] Forty-eight mice were randomly divided into control group, blank group, alcohol treatment group, low concentration group, medium concentration group and high concentration group, with 8 mice in each group. Except for the blank group, each mouse in the other groups was intraperitoneally injected with 1 mL of a mixture of 50 µgOVA + 5 mg [Al(OH)<sub>3</sub>] + 1 mL of normal saline for 14 d. The allergic rhinitis mouse model was successfully established by intranasal instillation of 5% OVA solution on both sides (20 µL per side, once a day) from the 15<sup>th</sup> day after stimulation for 7 d. The blank group was treated with the same amount of saline as above. The volatile oil of Centipeda Herba was obtained by steam distillation and petroleum ether extraction, and then was made into 1.25%, 2.5% and 5% volatile oil of Centipeda Herba with 75% alcohol. The control group was stimulated once every other day with reagent 2 after 7 d of stimulation (maintenance) until the end of treatment. The blank group was treated with 5 mL saline by nasal sniffing for 30 d, twice in the morning and evening, 30 min each time. The alcohol treatment group was treated with 5 mL of 75% alcohol, and the low concentration group, the medium concentration group and the high concentration group were treated with 5 mL of 1.25%, 2.5% and 5% Centipeda Herba volatile oil, respectively. The treatment time was the same as that of the blank group, and the treatment process was carried out in their respective closed contamination boxes. Before and after the treatment, the frequency of sneezing, the frequency of scratching nose, the amount of nasal discharge, activity and other general characteristics of the mice were observed, and the allergic behavior score was carried out. Besides, the IgE content in the serum of the mice was determined, and the eosinophils in the nasal discharge were counted. [Results] The scores of mice before and after treatment showed that there was no significant difference in the alcohol treatment group before and after treatment ( $P > 0.05$ ), and there was significant difference in the low, medium and high concentration groups before and after treatment ( $P < 0.05$ ), except that there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), and there was significant difference among the other groups ( $P < 0.05$ ). The levels of IgE and the number of eosinophils in peripheral serum of mice in the control group, alcohol treatment group, low concentration group, medium concentration group and high concentration group were higher than those in the blank group ( $P < 0.05$ ), and there was no significant difference between the two groups ( $P > 0.05$ ). [Conclusions] Volatile oil of Centipeda Herba can be used to treat allergic rhinitis by nasal sniffing, and 5% volatile oil of Centipeda Herba has the best effect. During the treatment, sneezing and runny nose in mice were reduced. The results showed that nasal sniffing was less irritating to the nasal cavity and not easy to produce discomfort, the utilization rate of drugs was higher than that of traditional therapy, and the volatile oil could be preserved longer than that of traditional Chinese medicine.

**Key words** Allergic rhinitis, Volatile oil of Centipeda Herba, Nasal sniffing therapy, IgE, Eosinophil

## 1 Introduction

As a global respiratory disease, the incidence of allergic rhinitis (AR) is increasing year by year, which has attracted wide medical attention<sup>[1]</sup>. The complexity of the disease is due to multiple factors, including environmental, genetic and immune factors, which makes its treatment full of challenges. At present, the treatment of AR is mainly divided into traditional Chinese medicine and Western medicine. Western medicine uses anti-allergic drugs and steroid nasal sprays to suppress symptoms by suppressing immune responses. However, these drugs not only have the problem of recurrence and tolerance after a period of use, but also may cause a series of adverse reactions after long-term use. Traditional Chinese medicine treatment strengthens conditioning as a whole,

using natural ingredients such as Chinese herbal medicine, and the effect varies from person to person<sup>[2]</sup>. The traditional treatment method mainly comprises the following steps: (i) using the liquid medicine obtained after the traditional Chinese medicine is boiled in water to wash the nose or drop the nose<sup>[3]</sup>; (ii) putting the dried traditional Chinese medicine into the nose after being powdered<sup>[4]</sup>; (iii) mixing the dried traditional Chinese medicine with ointment such as vaseline<sup>[5]</sup> and the like after being powdered, and then smearing the mixture into the nose. However, in practice, many people can not persist in these three ways for a long time. Each of the three methods has disadvantages, the first method of washing or dropping the nose with the liquid medicine obtained after the traditional Chinese medicine is boiled in water takes more time and energy, and the liquid medicine boiled on the same day may deteriorate if it is stored for use after the next day. The second method of putting the powdered dried traditional Chinese medicine into the nose has great irritation to the nasal cavity, thus causing disgusting and uncomfortable behaviors such

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as sneezing and runny nose, and the traditional Chinese medicine powder flows out with the nasal mucus, thus reducing the curative effect. For the third method, mixing the powder of the dried traditional Chinese medicine with the paste and then smearing it into the nose can also cause sneezing and runny nose. Besides, the mixed ointment will not only affect the exudation of the active ingredients in the traditional Chinese medicine and reduce the efficacy, but also make people feel uncomfortable when the ointment is applied in the nasal cavity for a long time, so how to make people with AR adhere to long-term treatment and find an effective and simple method is the key.

Therefore, in this study, we introduced a new treatment method, inhaling Centipeda Herba volatile oil through nasal sniffing, and explored its efficacy and the best drug concentration, in order to provide a reference for improving the traditional treatment drugs and methods.

2 Materials and methods

**2.1 Experimental animals** 48 ICR grade mice (12 – 15 g) of 3 – 4 weeks old, half male and half female, purchased from Changsha Tianqin Biotechnology Co., Ltd., with the production license number of SCXK (Xiang) 2022-0011, were raised in the Animal Experimental Center of Youjiang Medical University for Nationalities, with good indoor ventilation, indoor temperature kept at 19 – 22 °C, and fed with standard experimental mouse feed. Sterile pure water was replaced once every two days, and padding was replaced three times a week.

**2.2 Main instruments** Temperature-adjusting magnetic stirrer (TWCL-T, Guangzhou Yuhua Instrument Co., Ltd.), electronic balance (FA1204B, Shanghai Tianmei Balance Instrument Co., Ltd.), volatile oil extractor (Nanning Ditian Laboratory Supplies Business Company), multifunctional microplate reader (Mithras LB 943, Berthold, Germany), desktop high-speed centrifuge (TG16-WS, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.), intelligent constant temperature water tank (HH-Zk600, Gongyi Yingyu Hi-Tech Instrument Factory), inverted microscope (Leica DMI8, Leica, Germany), closed dosing box (IVC, Suzhou Fengshi Laboratory Animal Equipment Co., Ltd.

**2.3 Drugs and reagents** Ovalbumin (OVA), 4% aluminum hydroxide gel [Al(OH)<sub>3</sub>], 75% alcohol, Wright’s stain, mouse immunoglobulin E (IgE) ELISA research kit were purchased from Guangxi Nanning Baishi Innovation Biotechnology Co., Ltd.

2.4 Preparation of drugs

**2.4.1** Preparation of molding reagent. Reagent 1: 2 mg OVA + 200 mg Al(OH)<sub>3</sub> + 40 mL Normal saline; reagent 2: 5% OVA solution.

**2.4.2** Preparation of treatment drugs. Centipeda Herba is the dried whole herb of *Centipeda minima* (L.) A. Br. et Aschers. Centipeda Herba was purchased from Zhengyang Drugstore in Baise City, cleaned, dried and crushed, and the powder was sieved with a 50-mesh sieve for later use. It was identified by the Department of Pharmacology, School of Pharmacy, Youjiang Medical University for Nationalities that it met the standards stipulated in the *Chinese Pharmacopoeia* (2020 edition). The volatile oil of Centipeda Herba was extracted by volatile oil extractor, steam distillation and petroleum ether extraction. The purity of the volatile oil met the standard of *Chinese Pharmacopoeia* (2020 edition). The volatile oil of Centipeda Herba was prepared with 75% alcohol at low, medium and high concentrations of 1.25%, 2.5% and 5%, respectively. Took 5 mL of each of the three concentrations of Centipeda Herba volatile oil for preliminary test, and placed in an evaporating dish in closed contamination boxes. The volatile oil of each concentration of Centipeda Herba volatilizes within 15 min at room temperature.

**2.5 Establishment of allergic rhinitis animal model** The mice were randomly divided into control group, blank group, alcohol treatment group, low concentration group, medium concentration group and high concentration group, with 8 mice in each group, half male and half female. Except for the blank group, the other groups were intraperitoneally injected with reagent 1 at 14 d before modeling, and each mouse was injected with 1 mL, once every other day. On the 15<sup>th</sup> day, reagent 2 was administered by nasal drip on both sides, 20 μL on each side, once a day, for 7 consecutive days. At the same time period, the blank group was treated with the same amount of saline as above<sup>[6]</sup>.

**2.6 Treatment experiment** The control group was challenged with reagent 2 once every other day after the 7<sup>th</sup> day of stimulation (maintenance), while the blank group was treated with 5 mL saline through nasal sniffing. Alcohol treatment group was treated with 5 mL 75% alcohol by nasal sniffing. The low concentration group, the medium concentration group and the high concentration group were treated with 1.25%, 2.5% and 5% 5 mL Centipeda Herba volatile oil by nasal sniffing, and the treatment process was carried out in their own closed contamination boxes. All groups were treated for 30 d, twice in the morning and evening, 30 min each time.

2.7 Indicator detection

**2.7.1** Model evaluation. Nasal symptoms and signs were observed. The severity, frequency and duration of nasal itching, sneezing and runny nose 15 min after bilateral nasal drip of self-administered drugs were taken as the scoring criteria and recorded<sup>[7]</sup>. The scoring criteria were listed in Table 1.

Table 1 Behavioral score of anaphylaxis

Item	Score (15 min after stimulation) // points		
	1	2	3
Nose itching	≤2	Between the two	Scratching nose violently
Sneeze	1 –	3 –	11 –
Nose running	Running to anterior nostril	Beyond the anterior nostril	Streaming down the face

**2.7.2 Nasal secretion smear and serum IgE detection.** Took the nasal secretion of mice in each group with a small cotton swab, smeared, dried naturally, added Wright's A staining solution for 1 min, add 2–3 times of Wright's B solution phosphate buffer solution of a staining solution, gently shook the slide, evenly stood for 5 min, washed with water, absorbed, and observed the morphological changes and number of eosinophils under the light microscope. Five high power fields were randomly selected from each slice for counting. Serum IgE was measured by double antibody sandwich ELISA method according to ELAISA kit<sup>[8]</sup>, and serum IgE level was detected.

**2.8 Statistical methods** SPSS 26 software was used to establish a database for statistical analysis. The data were expressed as ( $\bar{x} \pm s$ ). One-way ANOVA was used to analyze the variance. The test level was  $\alpha = 0.05$  (two-sided), and  $P < 0.05$  means statistically significant.

3 Results and analysis

3.1 Scoring results of nasal provocation symptoms in mice

The scores of mice before and after treatment showed that there was no significant difference in the alcohol treatment group before and after treatment ( $P > 0.05$ ), but there was significant difference in the low, medium and high concentration groups before and after treatment ( $P < 0.05$ ). The score results among the treatment groups showed that there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), and there was significant difference among the other groups ( $P < 0.05$ ), as shown in Table 2.

Table 2 Results of nasal provocation symptom scores in mice (n = 8)

Group	Symptom score		<i>t</i>	<i>P</i>
	Before treatment	After treatment		
Blank	1.13 ± 0.35	0.75 ± 0.71	1.342	0.201
Control	7.38 ± 1.06 <sup>a</sup>	6.88 ± 1.13 <sup>a</sup>	0.914	0.376
Alcohol treatment	6.88 ± 0.84 <sup>a</sup>	6.50 ± 0.93 <sup>a</sup>	0.851	0.409
Low concentration	7.13 ± 1.13 <sup>a</sup>	4.88 ± 0.83 <sup>abc</sup>	4.541	<0.001
Medium concentration	6.75 ± 1.04 <sup>a</sup>	3.50 ± 0.93 <sup>abcd</sup>	6.619	<0.001
High concentration	6.50 ± 1.07 <sup>a</sup>	2.00 ± 0.76 <sup>abcde</sup>	9.721	<0.001
<i>F</i>	50.382	60.737	–	–
<i>P</i>	<0.001	<0.001	–	–

**NOTE** a means compared with the blank group, b means compared with the control group, c means compared with the alcohol treatment group, d means compared with the low concentration group, e means compared with the medium concentration group,  $P < 0.05$ . The same below.

**3.2 Number of secretory eosinophils** The eosinophil count in the nasal secretion of the experimental mice showed that the eosinophil count in the control group, the alcohol treatment group, the low concentration group, the medium concentration group and the high concentration group was higher than that in the blank group ( $P < 0.05$ ). Except that there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), the difference between the other groups was statistically significant ( $P < 0.05$ ), as shown in Table 3.

Table 3 Number of eosinophils in nasal secretion of mice (n = 8)

Group	Number of eosinophils	<i>F</i>	<i>P</i>
Blank	1.3 ± 0.5	166.491	<0.001
Control	14.6 ± 1.4 <sup>a</sup>		
Alcohol treatment	13.9 ± 1.7 <sup>a</sup>		
Low concentration	8.5 ± 0.9 <sup>abc</sup>		
Medium concentration	5.8 ± 1.3 <sup>abcd</sup>		
High concentration	3.3 ± 1.0 <sup>abcde</sup>		

**3.3 Serum IgE level** The serum IgE level of mice in each group was higher in the control group, alcohol treatment group, low concentration group, medium concentration group and high concentration group than in the blank group ( $P < 0.05$ ), and there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), while there was significant difference between the other groups ( $P < 0.05$ ), as shown in Table 4.

Table 4 Detection results of IgE in peripheral serum of mice (n = 8)

Group	IgE//μg/mL	<i>F</i>	<i>P</i>
Blank	0.612 ± 0.052	312.308	<0.001
Control	2.147 ± 0.101 <sup>a</sup>		
Alcohol treatment	2.125 ± 0.087 <sup>a</sup>		
Low concentration	1.769 ± 0.097 <sup>abc</sup>		
Medium concentration	1.256 ± 0.084 <sup>abcd</sup>		
High concentration	0.989 ± 0.156 <sup>abcde</sup>		

4 Discussion

Centipeda Herba is the dried whole herb of *Centipeda minima* (L.) A. Br. et Aschers. It grows in paddy fields or dark and damp places, mainly in Heilongjiang, Jilin and Liaoning provinces. It has the functions of dispelling wind, cold, dampness, nebula and nasal obstruction<sup>[9]</sup>. According to relevant experimental studies, the volatile oil of Centipeda Herba contains seven chemical components, including chlorogenic acid, cryptochlorogenic acid, caffeic acid, rutin, isochlorogenic acid B, isochlorogenic acid A and isochlorogenic acid C, with the highest content of isochlorogenic acid A, ranging from 0.032% to 0.263%<sup>[10]</sup>. Centipeda Herba has anti-tumor<sup>[11]</sup>, anti-allergic rhinitis<sup>[12]</sup>, anti-inflammatory<sup>[13]</sup>, antiasthmatic<sup>[14]</sup> and other effects. In clinical studies, Centipeda Herba has also been found to have the effect of treating nasopharyngeal carcinoma<sup>[15]</sup> and cholelithiasis<sup>[16]</sup>. In this study, we used the essential oil extracted from Centipeda Herba to establish allergic rhinitis animal model, nasal sniffing treatment, and used the detection of serum IgE and number of eosinophils to study. In this study, the volatile oil of Centipeda Herba was extracted by distillation extraction method, and the purity of the volatile oil was detected by gas chromatography-mass spectrometry (GC-MS). The purity of the volatile oil met the standard of *Chinese Pharmacopoeia* (2020 edition). The establishment of AR mouse model includes two stages: basic sensitization and local challenge. The commonly used methods include nasal drip, intraperitoneal injection, atomization inhalation, *etc.* In this experiment, the model was established by intraperitoneal injection-nasal drip<sup>[17]</sup>. In this

experiment, all the mice were successfully modeled, and the mice in each group were put into closed contamination boxes at the same time for nasal sniffing treatment. After 30 d of treatment, the results showed that there was no significant difference in the scores of mice before and after treatment in the alcohol treatment group ( $P > 0.05$ ), but there was significant difference in the scores of mice before and after treatment in the low, medium and high concentration groups ( $P < 0.05$ ). There were no significant differences between the control group and the alcohol treatment group ( $P > 0.05$ ), but there were significant differences between the other groups ( $P < 0.05$ ). In the course of treatment, the phenomena of scratching nose, runny nose and sneezing in mice were reduced, which proved that the method of nasal sniffing in this study had little irritation to the nose. Nasal secretion of mice was smeared and the number of eosinophils in the secretion was observed by Wright's staining. Eosinophils are reactive cells that are rapidly mobilized in AR. Their cytoplasmic granules are mainly composed of basic protein (MBP) and eosinophil cationic protein (ECP), as well as other neurotoxins, which can cause epithelial damage and enhance the release of leukotriene C4 (LTC4) and platelet aggregation factor (PAF)<sup>[18]</sup>. The eosinophil count in the nasal secretion of the mice showed that the eosinophil count in the control group, the alcohol treatment group, the low concentration group, the medium concentration group and the high concentration group was higher than that in the blank group ( $P < 0.05$ ), and there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), but there was significant difference between the other groups ( $P < 0.05$ ). The mice were killed, blood was taken, centrifuged for 20 min (2 500 rpm), the upper serum was taken, and the serum IgE concentration was measured by double-antibody sandwich ELISA method. Allergic rhinitis belongs to type I allergy, and secretory immunoglobulin IgE is an important antibody to induce type I allergy. Most of them originate from plasma cells in the lamina propria of nasopharynx, gastrointestinal mucosa and bronchus<sup>[19]</sup>. The level of serum IgE in the control group, alcohol treatment group, low concentration group, medium concentration group and high concentration group was higher than that in the blank group ( $P < 0.05$ ). Except that there was no significant difference between the control group and the alcohol treatment group ( $P > 0.05$ ), there was significant difference between the other groups ( $P < 0.05$ ), which proved that *Centipeda Herba* volatile oil had a certain effect on AR through nasal sniffing, and alcohol had no significant effect on AR ( $P > 0.05$ ).

In summary, the volatile oil of *Centipeda Herba* is effective in the treatment of AR by nasal sniffing, and 5% of the volatile oil of *Centipeda Herba* is the most effective. In the treatment process, the phenomena of scratching nose, sneezing and runny nose of the mice are reduced, which indicates that the nasal sniffing administration mode has small irritation to the nasal cavity and high suitability, and the patient can insist on taking the medicine for a long time, thereby improving the utilization rate of the medicine, improving the curative effect and reducing the recurrence rate.

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