A study on the anti-inflammatory effect of aromatic rhinitis spray

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The aim of this study was to study the anti-inflammatory effect of a compound containing volatile oils of Flos magnoliae, Centipeda minima and Mentha haplocalyx. The anti-inflammatory effect of the compound was investigated using xylene-, egg white- and cotton pellet-induced inflammation method. The medium- and high-doses of the compound (0.04 and 0.08 ml/kg) significantly inhibited the xylene-induced ear swelling in mice; low-, medium- and high-doses of the compound (0.02, 0.04, and 0.08 ml/kg) all had significant inhibitory effects on degree of paw swelling 30 min after the induction of inflammation; high-dose of the compound (0.08 ml/kg) significantly lowered the weight of cotton pellet granuloma in rats. The compound has a preferable therapeutic effect on both the acute and chronic inflammations, which can be developed as an anti-inflammatory traditional Chinese medicine.

Key words: Anti-inflammation, Flos magnoliae, Centipeda minima, Mentha haplocalyx, volatile oil.

INTRODUCTION

The formula of the aromatic rhinitis spray consists of three traditional Chinese herbs, namely Flos magnoliae, Centipeda minima and Mentha haplocalyx. The nasal spray is mainly used in the treatment of acute and chronic rhinitis. F. magnoliae is the dry bud of the Magnoliaceae plants such as Magnolia biondii Pamp., Magnolia denudate Desr., or Magnolia sprengeri Pamp. (The State Pharmacopoeia Committee of China, 2010). Through the analysis and identification, it was found out from the study of Qin et al. (2001) that the main composition of volatile oil from F. magnoliae were aromatic alcohol compounds and unsaturated ester compounds. Modern pharmacological studies have shown that F. magnoliae has an astringent effect, which can improve local blood circulation and promote the absorption of secretions, thereby diminishing inflammation, clearing nasal passage, and relieving or eliminating symptoms (Tao, 1996); it also has an anti-allergic effect, which can effectively fight against the nasal itching, sneezing, runny nose, and other symptoms caused by allergic rhinitis (Wen-bin et al., 2002). C. minima is the whole dry plant of the C. minima (L.) A. Br. et Aschers., which belongs to the Compositae plant family, it has the effects of dispersing wind-cold, clearing nasal passage, and relieving cough. Its volatile oil has an anti-inflammatory effect (Ren-an et al., 2001, 2006; Wen-kui et al., 2000). M. haplocalyx is the dry aerial part of M. haplocalyx Briq., which belongs to the Lamiaceae plant family. Its main composition is volatile oil, and the main active ingredient in the volatile oil is menthol. In this study, the anti-inflammatory effect of a compound containing these three herbs was studied, thus providing the basis for the development of the compound.

MATERIALS AND METHODS

Reagents and apparatus

F. magnoliae (Anhui Yiyuan Bio-Engineering Co., Ltd); C. minima (Anhui Shuzhong Pharmaceutical Co., Ltd); M. haplocalyx (Anhui Shuzhong Pharmaceutical Co., Ltd); prednisone (Hubei Xianhe...
other reagents were all of analytical grade; DHG-101-3A electro thermostatic blast oven (Gongyi Yuhua Instrument CO., Ltd); volatile oil extractor (Beijing Midwest Technology Co., Ltd); electronic balance (Beijing Sartorius Instrument System Co., Ltd).

Animals

National Institutes of Health (NIH) strain mice (50) of either sex, weighing 18 to 22 g were provided by the Laboratory Animal Center of the China Medical University. Sprague Dawley (SD) rats (80), half male and half female, weighing 130 to 150 g were provided by the Laboratory Animal Center of the China Medical University.

Extraction of volatile oil

F. magnoliae, C. minima and M. haplocalyx herbs were crushed, and a total of 200 g of three kinds of powders were weighed at a certain proportion (2:1:1). Volatile oil was extracted using the determination method A as prescribed in the appendix XD of the Volume I of the “Chinese Pharmacopoeia” 2010 edition. After continuous reflux extraction for 8 h, 2.6 ml of oily matter was obtained, which was prepared in certain concentrations later when administering (Wei et al., 2010).

Xylene-induced ear inflammation experiment in mice

Healthy male mice (50) were randomly divided into blank control, positive control (prednisone, 10 mg/kg), compound low-dose, compound medium-dose, and compound high-dose groups. Animals in each group were continuously administered the compound once daily for 5 days. 0.5 h after the last administration on the fifth day, 30 µl of (15 µl for each the outer and inner ears) xylene was applied to the right ear of mice to induce inflammation; xylene was not applied in the left ears, which served as the controls. 1 h after the induction of inflammation, mice were sacrificed, ear pieces at the same site of the left and right ears were removed with a 8 mm-diameter puncher, and the swelling inhibition rate was calculated taking the weight difference between the left and right ear pieces as the degree of swelling (Liu-ying et al., 2005; Maria et al., 2010).

Egg white-induced paw swelling experiment in rats

Healthy SD rats (40) weighing 130 to 150 g were randomly divided into 5 groups, namely blank control, positive control (prednisone), compound low-dose, compound medium-dose, and compound high-dose groups according to their body weights. Animals in each group were intragastrically administered the compound twice per day for 5 consecutive days. Before the experiment, paw volume of each rat was determined three times using the paw volume determination method, and the average value was taken as the normal paw volume of every rat before administration. 30 min after the last administration, right rear paw of each rat was subcutaneously injected with 0.1 ml of 10% fresh egg white saline solution to induce inflammation. 5, 30, 60, 120, and 180 min after the induction of inflammation, the volume of right rear paw of each rat was determined, respectively using the same method, change in value of the right rear paw volume of each rat before and after the induction of inflammation was calculated, and the anti-inflammatory effect of the drug was estimated by the degree of swelling (Shu-yun et al., 2002; Shen et al., 2008).

Cotton pellet granuloma experiment in rats

Healthy SD rats (40) were randomly divided into 5 groups according to their body weights. On the first day, rats in each group after administration of ether anesthesia and routine disinfection were anesthetized, under sterile conditions, the left and right armpits of these rats were separately implanted with a sterilized cotton pellet (weight 50±1 mg, high-pressure sterilized, each added with 1 mg/0.1 ml of ampicillin, and dried in the 50°C oven). The compound was administered once daily for 7 consecutive days, on the 8th day, the rats were sacrificed by cervical dislocation, and the cotton pellets were removed, and placed in a 60°C oven for 12 h, and were weighed by subtracting the weights of the original cotton pellets, and the net weights of granulomas were obtained (Shu-yun et al., 2002; Alfreda and Takayuki, 2010; Ji-Yan et al., 2012).

Statistical methods

Experimental results were analyzed using Statistical Package for Social Sciences (SPSS) software version 13.0. T-test was used for the mean comparison between the two groups. Multiple pairwise comparisons were done using analysis of variance.

RESULTS AND DISCUSSION

Results of xylene-induced ear inflammation experiment in mice

The results of the xylene-induced ear inflammation experiment in mice are shown in Table 1. The results revealed that the compound medium- and high-dose groups significantly inhibited xylene-induced ear swelling in mice (P<0.05).

Results of egg white-induced paw swelling experiment in rats

The results of the egg white-induced paw swelling experiment are shown in Table 2. The results revealed that: compared with the blank control group, compound low-, medium- and high-dose groups significantly inhibited paw swelling 30 min after the induction of inflammation (P<0.01); compound medium- and high-dose groups had inhibitory effects on degree of paw swelling 60 min after the induction of inflammation.

Results of cotton pellet granuloma experiment in rats

The results of the cotton pellet granuloma experiment are shown in Table 3. The experimental results revealed that: the granuloma weights were significantly lowered in the high-dose compound group (P<0.01), granuloma weights were also markedly lowered in the medium-dose compound group (P<0.05).
Table 1. Effect of the compound on xylene-induced ear swelling in mice (±S).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ml/kg)</th>
<th>Number of animals</th>
<th>Degree of ear swelling (mg)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>-</td>
<td>10</td>
<td>21.4±3.87</td>
<td>-</td>
</tr>
<tr>
<td>Prednisone</td>
<td>10mg/kg</td>
<td>10</td>
<td>8.9±3.73**</td>
<td>58.14</td>
</tr>
<tr>
<td>Low-dose</td>
<td>0.02</td>
<td>10</td>
<td>20.8±4.13</td>
<td>2.80</td>
</tr>
<tr>
<td>Medium-dose</td>
<td>0.04</td>
<td>10</td>
<td>18.2±3.96*</td>
<td>14.92</td>
</tr>
<tr>
<td>High-dose</td>
<td>0.08</td>
<td>10</td>
<td>16.3±4.51*</td>
<td>23.82</td>
</tr>
</tbody>
</table>

Comparison with the blank control group. *P<0.05; **P<0.01

Table 2. Effect of the Compound on Egg White-Induced Paw Swelling in Rats (±S).

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal paw volume (ml)</th>
<th>Degree of swelling at different time periods (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Blank control</td>
<td>1.78±0.12</td>
<td>0.25±0.12</td>
</tr>
<tr>
<td>Prednisone</td>
<td>1.87±0.21</td>
<td>0.14±0.11</td>
</tr>
<tr>
<td>low-dose</td>
<td>1.85±0.13</td>
<td>0.17±0.23</td>
</tr>
<tr>
<td>medium-dose</td>
<td>1.79±0.15</td>
<td>0.15±0.24</td>
</tr>
<tr>
<td>high-dose</td>
<td>1.82±0.17</td>
<td>0.14±0.16</td>
</tr>
</tbody>
</table>

Comparison with the blank control group. *P<0.05; **P<0.01

Table 3. Effect of the Compound on Cotton Pellet Granuloma in Rats (±S).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Dose (ml/kg)</th>
<th>Weight of granuloma (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>8</td>
<td>-</td>
<td>67.8±4.60</td>
</tr>
<tr>
<td>Prednisone</td>
<td>8</td>
<td>10mg/kg</td>
<td>35.6±3.50**</td>
</tr>
<tr>
<td>low-dose</td>
<td>8</td>
<td>0.02</td>
<td>51.3±5.20</td>
</tr>
<tr>
<td>medium-dose</td>
<td>8</td>
<td>0.04</td>
<td>44.7±2.50*</td>
</tr>
<tr>
<td>high-dose</td>
<td>8</td>
<td>0.08</td>
<td>40.3±3.10**</td>
</tr>
</tbody>
</table>

Comparison with the blank control group. *P<0.05; **P<0.01

DISCUSSION

At present, volatile oils of single herb medicines have been widely studied (Liu-ying et al., 2005; Maria et al., 2010; Shu-yun et al., 2002; Tian-qin et., 2006; Zhi-gang et., 2005; Guang-liang et., 2001), but studies on the compound of the three herbs are scanty. In this paper, the volatile oil from the three herbs was studied in general. *F. magnoliae* is pungent in flavor, warm in nature, and acts on the lung and stomach channels, which has a favorable nasal passage clearing effect, a good therapeautic effect on nasal diseases, and can help stop headaches. *C. minima* is pungent in flavor, and warm in nature, it acts on the lung channel, clears the nasal passage, facilitates nine orifices, and expels wind phlegm. Suplemented by the *M. haplocalyx* aroma, it can also relieve stuffiness. Volatile oil of the three traditional Chinese medicines was extracted to make the nasal spray, the spray is easy to use, with smaller droplets and large functioning area, can evenly dispersed in the nasal cavity, difficult to drain, and highly bioavailable. The method used in this study is simple and practical, which includes acute and chronic inflammatory animal models, experimental results also showed the inhibitory effect of the compound on both the acute and chronic inflammations, and can significantly reduce mouse xylene-induced ear swelling and rat carrageenan induced paw swelling and a significant reduction in granuloma weight in rats. This is consistent with the effect of single herb medicine reported in other literature, thus providing the reference for further study of the compound. However, its mechanism to play anti-inflammatory effects remains to be further studied.

REFERENCES


