Effect of Zataria multiflora Bois L. on histamine (H₁) receptor of guinea pig tracheal chains

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Received 29 November 2010; revised 2 May 2011

The effects of three concentrations (2.5, 5 and 10 µg/ml) of aqueous-ethanolic extract of Z. multiflora bois, 10 nM chlorpheniramine, and saline on histamine (H₁) receptors were tested on two groups of guinea pig tracheal chains [trachea incubated with indomethacin (Gr. 1), and indomethacin and propranolol (Gr. 2)]. The effective concentration of histamine causing 50% of maximum response (EC₅₀) obtained in presence of chlorpheniramine in both groups, all concentrations of the extract in group 1 and its two higher concentrations in group 2 were significantly greater than those of saline. The values of concentration ratio minus one (CR-1) obtained in presence of all the three concentrations of the extract in group 1 and 10 µg/ml concentration in group 2 were significantly greater than those of chlorpheniramine. The values of EC₅₀ obtained in presence of all the three concentrations of extract and CR-1 obtained in the presence of 2.5 and 5 µg/ml concentrations in group 2 were lower than group 1. There was not significant difference in maximum response obtained in presence of different concentrations of extract between two groups. There were parallel right ward shift in concentration response curves obtained in presence of all concentrations of the extract in both the groups. These results indicated an inhibitory effect of Z. multiflora at histamine H₁ receptors.

Keywords: Guinea pig, Histamine receptor, Inhibitory effect, Trachea, Zataria multiflora Bois

Zataria multiflora Bois L (Labiatae) contains terpenes, phenols, aliphatic alcohols, flavonoids, saponins and tannins. Some of the constituents particularly terpenes such as thymol and carvacrol have been identified as bioactive chemicals. Z. multiflora also contains apigenin, luteolin, 6-hydroxyluteolin glycosides and di, tri, and tetramethoxylated1,2.

In Iranian traditional medicine, the extract of this plant have different therapeuetic effect on coughts due to common cold, bronchitis and pertussis, laryngitis and tonsillitis (as a gargle) disorders of the oral cavity, and as an antibacterial agent in oral hygiene3-5. It is also used to treat pertussis, stomatitis, and halitosis2.

The relaxant effect of Z. multiflora in ileum6-8 and uterus9 and effect of another plant of this family (Tymus volgaris) in tracheal smooth muscle have been reported7,8,10. The therapeutic effect of Zataria in respiratory disorders of chemical war victims11, antitussive effect9 and relaxant effect of its constituent have been shown. A potent brochodilatory effect for carvacrol has been reported13 while thymol showed no bronchodilatory effect14.

To examine one possible mechanism of the observed relaxant effect for the plant on smooth muscle, in the present study, the effect of aqueous-ethanolic extracts of Z. multiflora on histamine (H₁) receptors was examined on tracheal chains of guinea pigs.

Materials and Methods

Plant and extraction—Zataria multiflora was collected form mountains in the region between Tabas and Yazd, (centre east region of Iran), Fleurine mine and identified by MR Joharghi. A voucher specimen (Herbarium No: 35314, FUMH) was preserved in the Herbarium of the School of Agriculture, Ferdowsi University. For preparing the aqueous-ethanolic extract of the isolated stigmata, 50 g Z. multiflora seeds were grounded, added to 700 ml of 50% ethanol using the Soxhlet apparatus. The solvent was then
removed under reduced pressure. The extract concentration in the final extract was adjusted to 10 mg/ml by adding distilled water to the dried extract.

**Tissue preparation**—Male Dunkin-Hartley guinea pigs (400-700 g) were sacrificed by a blow on the neck and the trachea were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form tracheal chain\(^{15}\). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent UK) having Krebs-Henseleit solution containing (mM): NaCl 120, NaHCO\(_3\) 25, MgSO\(_4\) 0.5, KH\(_2\)PO\(_4\) 1.2, KCl 4.72, CaCl\(_2\) 2.5 and dextrose 11.

Krebs solution was maintained at 37°C and gassed with 95% O\(_2\) and 5% CO\(_2\). Tissue was suspended under isotonic tension (1 g) and allowed to equilibrate for at least 1 hr while it was washed with Krebs solution every 15 min.

This study was approved by the University's Ethics Committee. The allowance number of the relevant ethical committee for the animal experiments is 85301.

**Protocols**—The inhibitory effect of *Z. multiflora* on histamine H\(_1\) receptors was examined by producing the cumulative log concentration-response curve of histamine acid phosphate (BDH Chemical Co, Ltd, UK) induced contraction of tracheal chains 10 min after the exposure of tissue to 10 nM chlorpheniramine maleate (Sigma Chemical Ltd, UK, Catalogue No. C4915), three concentrations (2.5, 5 and 10 µg/ml) of aqueous-ethanolic extract from *Z. multiflora*, or 0.2 ml saline. The consecutive concentrations of histamine (0.1-1000 µM) were added every 2 min. The percentage of contraction due to each concentration in proportion to the maximum contraction obtained in presence of saline was plotted against log concentration of histamine. The effective concentration of histamine causing 50% of maximum response (EC\(_{50}\)) in each experiment was measured using the log concentration-response curve of the corresponding experiment. The shift of cumulative log concentration-response curves obtained in presence of extracts and chlorpheniramine were examined by comparing the EC\(_{50}\) obtained in presence of each solution with that of saline. In addition the maximum responses to histamine obtained in presence of extracts and chlorpheniramine in all sets of experiments were compared with that of saline. To examine the parallel rightward shift, the slope of the histamine-response curve of each experiment was measured and was compared with that of saline. In experiments with parallel shift in histamine-response curve, the concentration-ratio minus one (CR-1) as an index of the competitive antagonism effect was calculated by the following equation:

\[
CR-1 = \frac{EC_{50} \text{ obtained in the presence of effective solutions}}{EC_{50} \text{ obtained in the presence of saline}} - 1
\]

The study was performed on incubated tracheal chains 30 min prior to the beginning and at the time of obtaining histamine-response curve with two different experimental designs as follows:

(i) 1.4 µM indomethacin in order to inhibit arachidonic acid metabolism (Sigma Chemical Ltd, UK), (Gr. 1; n=7).

(ii) 1.4 µM indomethacin and 1 µM propranolol hydrochloride in order to inhibit arachidonic acid metabolism and β-adrenoceptor (Gr. 2; n=5).

All the experiments were performed randomly with 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and measured after fixation.

**Statistical analysis**—Data were expressed as mean±SE. The EC\(_{50}\), slope, and maximum response obtained in presence of extract, and chlorpheniramine were compared with those obtained in the presence of saline using paired t test. The values of concentration ratio minus one (CR-1) obtained in presence of extract were also compared with those obtained in the presence of chlorpheniramine using paired t test. The values of EC\(_{50}\), the slope, CR-1, and maximum response obtained in 2 groups were compared using unpaired t test. Significance was accepted at \(P<0.05\).

**Results**

**Shift in cumulative log concentration-response curves**—Cumulative log concentration-response curves of histamine obtained in presence of all concentration of the extract and chlorpheniramine showed clear rightward shift compared to histamine curves produced in presence of saline in both groups of experiments (Fig. 1).

**Tracheal responsiveness (EC\(_{50}\))**—The EC\(_{50}\) histamine obtained in presence of chlorpheniramine and all concentrations of the extract in Gr 1 and its two higher concentrations (5 and 10 µg/ml) in Gr. 2
were significantly higher than those of saline (Table 1). The EC$_{50}$ histamine obtained in presence of all concentrations of the extract in Gr. 2 were significantly less than those of group 1 (Table 1).

**Shift in histamine concentration-response curves (CR-1)**—The values of CR-1 obtained in presence of all concentrations of the extract (2.5, 5 and 10 µg/ml) in Gr. 1 and high concentration of the extract (10 µg/ml) in Gr. 2 were significantly greater than those of chlorpheniramine (Table 2). The values of CR-1 obtained in presence of low and medium concentration of the extract (2.5 and 5 µg/ml) were significantly less in Gr. 2 compared to Gr. 1 (Table 2).

**Maximum response to histamine**—The maximum responses to histamine obtained in presence of high concentration of Zataria (10 µg/ml) in Gr 2 was significantly lower than those of saline (Table 3). There was not significant difference in maximum responses obtained in presence of different concentrations of the extract between two groups (Table 3).

**Slope of histamine-response curves**—There were parallel right ward shift in concentration response curves obtained in presence of all the concentrations of the extract in two experimental conditions compared to those of saline, (Table 3). There was no significant difference in the slopes of histamine-response curves between different concentrations of the extract in two experimental conditions (Table 3).

**Schild plot**—The slopes of Schild plot for the extract were -0.929±0.09 and -0.856±0.12 in groups 1 and 2 respectively.

**Discussion**

In the present study, the inhibitory effect of the aqueous-ethanolic extract of the plant was examined on histamine (H$_1$) receptors of isolated guinea pig tracheal preparations. The relaxant effect seen for another plant of this family on tracheal chains (bronchodilatory)$^{10}$, may be produced due to several different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H$_1$
receptors or an anticholinergic property of the plant, because indication of the relaxant effect of these mechanisms have been shown\textsuperscript{16-18}.

The parallel rightward shifts in histamine log concentration-response curves, obtained in presence of the aqueous-ethanolic extract, higher EC\textsubscript{50} and achievement of maximum contraction effect to histamine compared to those of saline in Gr 1 experiments (incubated trachea with only indomethacin) indicated a competitive antagonistic effect of \textit{Z. multiflora} at histamine H\textsubscript{1} receptors of guinea pig trachea\textsuperscript{16,19,20}.

Although in Gr 1, parallel rightward shift in histamine-concentration curve and maximum response to histamine were obtained in the presence of the of extract, to evaluate the contribution of \(\beta\)-adrenergic stimulatory mechanism on the effect of the extract at histamine H\textsubscript{1} receptors, the effects of the extract was also examined on incubated tracheal preparation with indomethacin, propranolol in Gr. 2.

In Gr. 2 also the parallel rightward shift in histamine-response curves and maximum responses to histamine were obtained in presence of the extract compared to that of saline. However, the values of EC\textsubscript{50} obtained in presence of different concentrations of the extract in this group were significantly lower than those of Gr. 1.

These results indicated that the changes obtained in Gr. 2 compared to Gr. 1 were mainly due to stimulatory effect of the extract on \(\beta\)-adrenergic receptors. The lower values of CR-1 obtained in presence of different concentrations of the extract in Gr 2 compared to those of Gr. 1 also supported this phenomenon of the extract. In fact, in a previous study the relaxant effect of macerated and aqueous extract of other plant species from this family almost completely abolished in incubated tracheal preparations incubated with propranolol and chlorpheniramine\textsuperscript{10} which confirms the result of the present study.

The higher values of CR-1 obtained in presence of different concentrations of the extract in both the groups compared to that of chlorpheniramine indicates higher antagonistic effect of the extract relative to chlorpheniramine at used concentrations.

The slope of Schild plot for the extract in both groups were closed to -1 which is another indication of competitive antagonistic effect of the extract.

The values of EC\textsubscript{50} and CR-1 obtained in presence of different concentration of the extract were concentration dependent. These results showed that for the extract, the inhibitory effect on histamine (H\textsubscript{1}) receptors was increased with increasing the concentration.

Table 2—Values of (CR-1) in the presence of extract from \textit{Z. multiflora} and 10 nM chlorpheniramine in two sets of experiments

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Concentration</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Gr 2 v Gr 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine</td>
<td>2.5 µg/mL</td>
<td>1.37±0.11 **</td>
<td>0.73±0.14</td>
<td>+</td>
</tr>
<tr>
<td>Extract</td>
<td>5 µg/mL</td>
<td>2.07±0.16 ***</td>
<td>1.31±0.22</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10 µg/mL</td>
<td>2.68±0.28 ***</td>
<td>2.2±0.20 **</td>
<td></td>
</tr>
</tbody>
</table>

For details see Table 1.

Table 3—Maximum response to histamine obtained and slope of histamine log concentration-response curves obtained in presence of extract from \textit{Z. multiflora}, 10 nM chlorpheniramine, and saline in the two sets of experiments

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Concentration</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Max.</td>
<td>100.00±0.00</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.96±0.01</td>
<td>0.98±0.04</td>
</tr>
<tr>
<td></td>
<td>2.5 µg/mL</td>
<td>Max.</td>
<td>83.57±4.31</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.97±0.02</td>
<td>0.99±0.03</td>
</tr>
<tr>
<td>Extract</td>
<td>5 µg/mL</td>
<td>Max.</td>
<td>90.57±3.92</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.96±0.03</td>
<td>0.99±0.02</td>
</tr>
<tr>
<td></td>
<td>10 µg/mL</td>
<td>Max.</td>
<td>89.29±4.82</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.97±0.02</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Max.</td>
<td>87.00±4.37</td>
<td>83.75±4.67</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.98±0.02</td>
<td>0.96±0.02</td>
</tr>
</tbody>
</table>

For details see Table 1. Max.; maximum response. *; \(P<0.05\) vs saline. There was no significant difference in maximum response and slope between Gr 1 and 2.
In conclusion, the results of this study suggested a competitive antagonistic effect of the extract of *Z. multiflora* at histamine H₁ receptors. In addition, the results also suggested a stimulatory effect of the extract on β-adrenergic receptors.

**Acknowledgment**

This study was financially supported by Research Department of Mashhad University of Medical Sciences.

**References**