Inhibitory Effect of *Nigella sativa* on Histamine (H₁) Receptors of Isolated Guinea Pig Tracheal Chains

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Abstract

In a previous study, the relaxant and anticholinergic (functional antagonism) effects of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. To elucidate the other mechanisms responsible for this relaxant effect, the inhibitory effect of this plant on histamine H₁ receptors was examined in this study. The antihistaminic effects of aqueous and macerated extracts, 5 nM chlorpheniramine, and saline were tested by performing the cumulative log concentration-response curves of histamine-induced contraction of isolated guinea pig tracheal chains incubated with three different conditions including: 1) 1.4 μM indomethacin, 2) indomethacin, 1 μM propranolol, and 10 nM atropine, and 3) indomethacin and propranolol (for each group n = 8). The results showed clear parallel rightward shifts in histamine-response curves obtained in the presence of macerated extract in group 1 and aqueous extract in group 2 experiments compared with the curves obtained in the presence of saline. The EC₅₀ (effective concentration of histamine causing 50% of maximum response) obtained in the presence of extracts, and chlorpheniramine in all three sets of experiments were significantly higher than that of saline (P < 0.05 to p = 0.002), but maximum response to histamine obtained in the presence of extracts were lower (P < 0.01 to P < 0.001). However, the maximum response obtained in the presence of aqueous extract in group 2 experiments compared to the other two sets of experiments was improved. These results indicated a competitive antagonistic effect of *Nigella sativa* at histamine H₁ receptors.

Keywords: *Nigella sativa*, antihistaminic effect, trachea, guinea pig.

Introduction

*Nigella sativa* L. (Ranunculaceae) is a grassy plant with green to blue flowers and small black seeds which grows in temperate and cold climate areas. The seeds of *Nigella sativa* contain thymoquinone, monotropenes such as p-cymene and α-pinene (El-Dakhakhny, 1963), nigellidine (Atta & Malik, 1995), nigellimine (Atta & Malik, 1985) and a saponin (Ansari & Sadiy, 1989).

Several therapeutic effects including those on digestive disorders, gynaecologic, and anti-asthma and dyspnea have been described for the seeds of *Nigella sativa* in Iranian ancient medical books (Avesina, 1990). *Nigella sativa* has long been known for medical use as an antispasmodic medicine specially against gastrointestinal disorders or respiratory ailments in many countries. In Arabian folk medicine also, the whole black seeds alone or in combination with honey are prompted for treatment of bronchial asthma.

There is evidence of relaxant effects of the volatile oil from this plant on different smooth muscle preparations including rabbit aorta (Aqel, 1992a), rabbit jejunum (Aqel, 1993), and guinea pig isolated tracheal muscle (Reiter & Brandt, 1985). Mahfouz and El-Dakhakhny (1960) reported that the volatile oil from *Nigella sativa* protected guinea pigs against histamine-induced bronchospasm, but it did not affect histamine H₁ receptors in isolated tissues. However, in an in vivo study, increasing respiratory rate and intratracheal pressure of guinea pigs due to i.v. administration of volatile oil from *Nigella sativa* has been demonstrated (El-Tahir et al., 1993). In our recent study, a relaxant effect of this plant on isolated guinea pig tracheal chains was demonstrated (Boskabady & Shahabi, 1997). The results of our study also showed a functional antagonistic effect of this plant on muscarinic receptors.
To elucidate the other mechanisms responsible for the observed bronchodilatory effect of *Nigella sativa*, the inhibitory effect of aqueous and macerated extracts of this plant on histamine H\textsubscript{1} receptors of guinea pig tracheal chains in comparison with both saline and chlorpheniramine were examined in this study.

Materials and methods

Plant and extracts

*Nigella sativa* was identified by botanists in the herbarium of Ferdowsi University of Mashhad and the specimen number of the plant is 293-0303-1. The plant extracts were prepared as follows: A) Macerated extract: 50 g of the chopped, dried plant was macerated with 300 ml distilled water and shaken (on a shaker) for 48 h. B) Aqueous extract: The same amount of plant was extracted with 300 ml distilled water by a suxhelet apparatus. The solvent of both extracts was then removed under reduced pressure until the extract volume reached 20 ml. The plant ingredient concentrations in the final extracts were 18 and 24% W/W in macerated and aqueous extracts, respectively.

Tissue preparations

Male guinea pigs (400–700 g) were killed by a blow on the neck and the trachea 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 (Holroyde, 1986).

Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, NaHCO\textsubscript{3} 25, MgSO\textsubscript{4} 0.5, KH\textsubscript{2}PO\textsubscript{4} 1.2, KCl 4.72, CaCl\textsubscript{2} 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Protocols

1) The inhibitory effect of *Nigella sativa* on histamine H\textsubscript{1} receptors was examined by producing a cumulative log concentration-response curve of histamine acid phosphate (BDH Chemical Co, Ltd UK) induced contraction of tracheal chains 10 min after exposing tissue to one solution [macerated and aqueous extracts 0.3 ml, 0.05 ml of 1 μM chlorpheniramine maleate (Sigma Chemical Ltd UK), or 0.3 ml saline]. Consecutive concentrations of histamine were added every 2 min (range 0.1–1000 μM), and the percentage of contraction due to each concentration in proportion to the maximum contraction obtained in the presence of saline was plotted against log concentration of histamine.

2) The effective concentration of histamine causing 50% of maximum response (EC\textsubscript{50}) in each experiment was measured using the log concentration-response curve of the corresponding experiment. The shift of the cumulative log concentration-response curves obtained in the presence of extracts and chlorpheniramine was examined by comparing the EC\textsubscript{50} obtained in the presence of each solution with that of saline.

3) To examine the parallel rightward shift, the slope of the histamine-response curve of each experiment was measured and the slope of the histamine curves obtained in the presence of extracts and chlorpheniramine was compared with that of saline.

4) In addition, the maximum responses to histamine obtained in the presence of extracts and chlorpheniramine in all three sets of experiments were compared with that of saline.

5) In experiments with parallel shift in the histamine-response curve, the concentration-ratio minus one (CR – 1) as competitive antagonism effect was calculated by the following equation:

\[
\left( \frac{[EC_{50} \text{ obtained in the presence of effective solutions}]}{EC_{50} \text{ obtained in the presence of saline}} \right) - 1
\]

The inhibitory effect of *Nigella sativa* on histamine H\textsubscript{1} receptors was tested on incubated tracheal chains 30 min prior to beginning and while obtaining the histamine-response curve with three different experimental conditions (for each condition, n = 8) as follows:

a) 1.4 μM indomethacin (Sigma Chemical Ltd UK) (group 1 experiments).

b) 1.4 μM indomethacin, 1 μM propranolol hydrochloride (Sigma Chemical Ltd UK), and 10 nM atropine sulphate (Sigma Chemical Ltd UK) (group 2 experiments).

c) 1.4 μM indomethacin and 1 μM propranolol hydrochloride (group 3 experiments).

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

Statistical analysis

The EC\textsubscript{50} data, the slope of the histamine-response curves, the maximum response to histamine, and the values of (CR – 1) of different experiments were expressed as mean ± SEM. The EC\textsubscript{50}, the slope of the histamine-response curves, the maximum response to histamine obtained in the presence of extracts and chlorpheniramine were compared with those obtained in the presence of saline and
values of \((CR - 1)\) obtained in the presence of extracts with those of chlorpheniramine using a paired "t" test. The values of \(EC_{50}\), the slope of the histamine-response curves, and the maximum response to histamine between three groups of experiments were compared using a one-way analysis of variance (ANOVA) 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 the presence of extracts and chlorpheniramine in all three experimental conditions showed a clear rightward shift compared to histamine-response curves produced in the presence of saline (Fig. 1).

**\(EC_{50}\)**

The \(EC_{50}\) of histamine obtained in the presence of extracts and chlorpheniramine in all three experimental conditions was significantly higher than those for saline \((p < 0.05\) to \(p = 0.002)\). However, there were no significant differences between \(EC_{50}\) values obtained in the three experimental conditions (Table 1).

**Slope of log concentration-response curves**

The slope of the histamine-response curves obtained in the presence of aqueous extract in group 1, that of macerated extract in group 2 and those of both extracts from *Nigella sativa* in group 3 experiments were significantly lower than those obtained in the presence of saline \((p < 0.02\) to \(p < 0.001)\). However, there was no significant difference between the slope of histamine-response curves obtained in the presence of extracts from *Nigella sativa* in the three groups of experiments except that of aqueous extract which was significantly higher in group 2 than those of group 1 and 2 experiments \((p < 0.01\) vs. groups 1 and 3) (Table 2).

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**Figure 1.** Cumulative log concentration-response curves of histamine induced contraction of guinea pig tracheal chains, in the presence of saline, extracts, and chlorpheniramine on incubated preparations with three different conditions; a) indomethacin, b) indomethacin, propranolol and atropine, and c) indomethacin and propranolol (for each condition, \(n = 8\)). Macerated extract in group 1 and aqueous extract in group 2 experiments caused parallel rightward shifts in histamine-response curves compared to the curves obtained in the presence of saline. The shifts of histamine-response curves obtained in the presence of chlorpheniramine in all three sets of experiments were also parallel.
Table 1. EC50 (μM) of histamine in the presence of aqueous extract (AE), macerated extract (ME), 5 nM chlorpheniramine (C), and saline (S) in three sets of experiments.

<table>
<thead>
<tr>
<th>Different Solutions</th>
<th>group 1</th>
<th>St. Diff. vs. S</th>
<th>group 2</th>
<th>St. Diff. vs. S</th>
<th>group 3</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>13.06 ± 2.50</td>
<td>–</td>
<td>23.13 ± 3.99</td>
<td>–</td>
<td>NS</td>
<td>19.88 ± 3.09</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>AE</td>
<td>59.75 ± 8.47</td>
<td>p = 0.002</td>
<td>52.13 ± 9.90</td>
<td>p = 0.01</td>
<td>NS</td>
<td>45.25 ± 9.32</td>
<td>p &lt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>ME</td>
<td>36.38 ± 6.25</td>
<td>p &lt; 0.01</td>
<td>68.63 ± 18.4</td>
<td>p &lt; 0.05</td>
<td>NS</td>
<td>32.00 ± 5.67</td>
<td>p = 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>25.94 ± 5.40</td>
<td>p &lt; 0.01</td>
<td>42.38 ± 8.9</td>
<td>p &lt; 0.02</td>
<td>NS</td>
<td>37.13 ± 4.62</td>
<td>p &lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. Group 1: experiments on tracheal chains incubated with 1.4 μM indomethacin; group 2: experiments on tracheal chains incubated with 1.4 μM indomethacin, 1 μM propranolol and 10 nM atropine; group 3: experiments on tracheal chains incubated with 1.4 μM indomethacin and 1 μM propranolol (for each group, n = 8); St. Diff.: statistical difference; NS: non-significant difference.

Table 2. Slope of histamine log concentration-response curves in the presence of aqueous extract (AE), macerated extract (ME), 5 nM chlorpheniramine (C), and saline (S) in three sets of experiments.

<table>
<thead>
<tr>
<th>Different Solutions</th>
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<th>group 2</th>
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<th>group 3</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2.46 ± 0.19</td>
<td>–</td>
<td>2.76 ± 0.18</td>
<td>–</td>
<td>NS</td>
<td>2.58 ± 0.11</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>AE</td>
<td>0.95 ± 0.20</td>
<td>p &lt; 0.001</td>
<td>2.08 ± 0.3</td>
<td>NS</td>
<td>p &lt; 0.01</td>
<td>0.91 ± 0.21</td>
<td>p &lt; 0.001</td>
<td>NS</td>
</tr>
<tr>
<td>ME</td>
<td>2.12 ± 0.13</td>
<td>NS</td>
<td>1.49 ± 0.24</td>
<td>p = 0.001</td>
<td>NS</td>
<td>1.43 ± 0.24</td>
<td>p &lt; 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>2.82 ± 0.21</td>
<td>NS</td>
<td>2.83 ± 0.24</td>
<td>NS</td>
<td>NS</td>
<td>2.46 ± 0.06</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

For abbreviations see Table 1.

Table 3. Maximum response to histamine obtained in the presence of aqueous extract (AE), macerated extract (ME), 5 nM chlorpheniramine (C), and saline (S) in three sets of experiments.

<table>
<thead>
<tr>
<th>Different Solutions</th>
<th>group 1</th>
<th>St. Diff. vs. S</th>
<th>group 2</th>
<th>St. Diff. vs. S</th>
<th>group 3</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 1</th>
<th>St. Diff. vs. group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>100.0 ± 0.0</td>
<td>–</td>
<td>100.0 ± 0.0</td>
<td>–</td>
<td>NS</td>
<td>100.0 ± 0.0</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>AE</td>
<td>36.48 ± 6.88</td>
<td>p &lt; 0.001</td>
<td>73.76 ± 8.38</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.005</td>
<td>30.91 ± 5.36</td>
<td>p &lt; 0.001</td>
<td>NS</td>
</tr>
<tr>
<td>ME</td>
<td>71.05 ± 5.71</td>
<td>p = 0.001</td>
<td>59.89 ± 7.56</td>
<td>p = 0.001</td>
<td>NS</td>
<td>49.54 ± 8.10</td>
<td>p &lt; 0.001</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>92.14 ± 4.72</td>
<td>NS</td>
<td>97.79 ± 4.74</td>
<td>NS</td>
<td>NS</td>
<td>88.33 ± 3.15</td>
<td>p &lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

For abbreviations see Table 1.

**Maximum response to histamine**

The maximum response to histamine obtained in the presence of extracts from *Nigella sativa* was significantly lower than those of saline in all three sets of experiments (p = 0.02 to p < 0.001). However, the maximum response to histamine obtained in the presence of aqueous extract in group 2 experiments was significantly improved compared to the other two sets of experiments (p = 0.01 vs. group 1 and p < 0.001 vs. group 3). The maximum response to histamine obtained in the presence of macerated extract in group 2 and 3 experiments was also not significantly lower than that of group 1 (Table 3).

**Comparison between the antihistaminic effect of *Nigella sativa* and chlorpheniramine**

The values of (CR – 1) obtained only in the presence of macerated extract in group 1 (2.28 ± 0.65) was significantly higher (p < 0.05) and that of aqueous extract in group 2 experiments (1.55 ± 0.57) showed a non-significant difference with that of chlorpheniramine (1.11 ± 0.30 and 0.94 ± 0.32 for group 1 and 2 experiments, respectively).

**Discussion**

The bronchodilatory effect seen for *Nigella sativa* in our previous study (Boskabady & Shahabi, 1997) might be
produced due to several different mechanisms. One possible mechanism responsible for this effect could be the inhibitory effect of this plant on histamine H₁ receptors (Popa, 1977). The inhibitory effect of the extracts from this plant was therefore examined on isolated guinea pig tracheal preparations in this study.

The non-parallel rightward shifts in histamine log concentration-response curves, obtained in the presence of aqueous extract and the parallel shift obtained in the presence of macerated extract, greater EC₅₀, but lower maximum contraction effect of histamine compared to those of saline in group 1 experiments (incubated trachea with only indomethacin) indicated a functional antagonistic effect of Nigella sativa at histamine H₁ receptors of guinea pig trachea (Arunlakshana & Schild, 1959; Ariens, 1987; Linden et al., 1993a). However, for macerated extract with a parallel shift in histamine-response curves a component of competitive antagonism and a functional antagonistic effect could be postulated. The (CR – 1) produced by the macerated extract was significantly higher than that of chlorpheniramine, indicating a greater antagonistic effect of this extract than that of chlorpheniramine on histamine H₁ receptors at the concentrations used.

To evaluate the contribution of β-adrenergic stimulatory and/or muscarinic blocking effect on the functional antagonism of Nigella sativa at histamine H₁ receptors, the antihistaminic effects of extracts from this plant were also examined on incubated tracheal preparations with indomethacin, propranolol, and atropine. The parallel rightward shift in histamine-response curves obtained in the presence of aqueous extract compared to that of saline and the significant improvement of the maximum response to histamine obtained in this part of the study, relative to that of group 1 experiments, showed a possible competitive antagonistic effect of this extract on histamine H₁ receptors. Improvement of the maximum response to histamine in this part of the study without a significant change in EC₅₀ obtained in the presence of aqueous extract indicates anticholinergic and/or adrenergic stimulatory effects of this solution. The values of (CR – 1) obtained in the presence of aqueous extract in this part of the study was not significantly different from that of chlorpheniramine, indicating a comparable antagonistic effect relative to chlorpheniramine at the concentrations used. Although there was an improvement in the maximum response obtained in the presence of aqueous extract in group 2 experiments compared to that of group 1, there was still significant difference between the maximum response obtained in the presence of aqueous extract and that of saline, indicating a small functional antagonistic effect of this solution at histamine H₁ receptors other than β-adrenergic stimulatory and/or a muscarinic blocking effect.

In order to investigate whether the functional antagonistic effect of aqueous extract from Nigella sativa at histamine H₁ receptors seen in group 1 experiments and that of macerated extract in group 2 experiments is due to β-adrenergic stimulatory or muscarinic blocking effects, the antihistaminic effect of the plant was also examined on tracheal chains incubated with indomethacin and propranolol. The results of this part of the study obtained in the presence of aqueous extract were fairly similar to those of group 1 and those obtained in the presence of macerated extract were fairly similar to those of the group 2 study. These results indicate that the functional antagonism of aqueous extract at histamine H₁ receptors is mainly due to the blocking effect of this extract on muscarinic receptors. In fact, the existence of α-pinene in essential oil of this plant was demonstrated (El-Dakakhny, 1963), which showed anticholinergic activity (Bogats & Epshtein, 1959). Therefore, the non-parallel shift in histamine-response curves obtained in the presence of aqueous extract in group 1 and 3 experiments could be due to the presence of α-pinene or other substances with a muscarinic receptor blocking effect and also substances with a competitive antagonistic effect at histamine H₁ receptors in this extract from Nigella sativa. In a previous study, we have also demonstrated a competitive antagonistic effect of aqueous extract and a non-competitive antagonistic effect of macerated extract from Nigella sativa at muscarinic receptors (Boskabady & Shahabi, 1997) which confirm the results of the present study.

There were non-significant decreases in maximum response to histamine and in slope of the curves obtained in the presence of macerated extract in group 2 and 3 experiments (both on incubated tissue with propranolol) compared to those of group 1. These results may indicate an inhibitory effect of this extract on β-adrenergic receptors. The postulated β-adrenergic receptor inhibitory effect of this plant may support the results of El-Tahir et al. (1993) which showed an increase in tracheal pressure due to volatile oil from Nigella sativa. The cause of the significant reduction in maximum response to histamine obtained in the presence of chlorpheniramine compared to that of saline in group 3 experiments is uncertain to us.

The results of our previous study (Boskabady & Shahabi, 1997) and of the present study, indicating bronchodilatory and histamine H₁ inhibitory effects of Nigella sativa, respectively, may seem at variance with those of El-Tahir et al. (1993). However, El-Tahir et al. were also unable to show a contractile effect of volatile oil from this plant on the isolated trachea. Therefore, Nigella sativa may contain substances contractile on tracheal smooth muscle including thymoquinone and substances inhibitory on β-adrenergic receptors and also substances relaxant on smooth muscle including inhibitory agents on histamine H₁ and muscarinic receptors seen in our studies.

In order to inhibit the arachidonic acid metabolism, in all three parts of the study, tissues were incubated with indomethacin. The different blocking effect of extracts on histamine H₁ receptors is presumably due to the variation in the methods of extraction.

The other possible mechanisms responsible for the bronchodilatory effect of Nigella sativa and the functional antagonism of macerated and aqueous extracts at histamine H₁ receptors are as follows:
1) Stimulation of the inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC (Linden et al., 1993b).
2) Methylxanthine activity of the plant (Meini et al., 1993).
3) Other possible mechanisms including opening of potassium channels and inhibition of phosphodiesterase (Bucke et al., 1993; Van Amsterdam et al., 1989) and especially calcium antagonism (Miyahara et al., 1993), because the calcium antagonistic effect of volatile oil from *Nigella sativa* on the tracheal smooth muscle of rabbit has been shown (Aqel et al., 1992b). The contribution of these mechanisms in the bronchodilatory effect of *Nigella sativa* and the functional antagonism of macerated and aqueous extracts at histamine H1 receptors should be clarified in further studies.

With regard to the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, the antihistaminic effect of *Nigella sativa* might also have an anti-inflammatory effect which will contribute to the therapeutic effect of this plant on asthma. In fact, the inhibitory effects of essential oil from *Nigella sativa* and thymoquinone on both the cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism and also on membrane lipid peroxidation have been shown (Houghton et al., 1995). However, the effect of this plant on airway inflammation existing in asthma disease should be investigated in further studies.

In conclusion, the results of this study suggest a competitive antagonistic effect of *Nigella sativa* at histamine H1 receptors. In addition, the results indicate a blocking effect of aqueous extract from this plant at muscarinic receptors.

**Acknowledgment**

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**References**


