Relaxant effects of different fractions from *Nigella sativa* L. on guinea pig tracheal chains and its possible mechanism(s)

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The relaxant effects of four cumulative concentrations of n-hexane, dichloromethane, methanol and aqueous fractions of *N. sativa* (0.8, 1.2, 1.6 and 2.0 g%) in comparison with saline as negative control and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mM) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 60 mM KCl (group 1) and 10 µM methacholine (group 2). In group 1, all concentrations of only theophylline showed significant relaxant effects. However, in group 2, all concentrations of theophylline and methanol fraction, 1.2, 1.6 and 2.0 g% concentrations of dichloromethane and 1.2 and 2.0 g% concentrations of n-hexane fractions showed significant relaxant effects compared to that of saline. In addition, in group 1, the relaxant effect of most concentrations of all fractions except the low concentration (0.8 g%) of dichloromethane and methanol fraction were significantly less than those of theophylline. The relaxant effect of different concentrations of methanol and dichloromethane fraction and 1.2, 1.6 and 2.0 g% concentrations of n-hexane fraction were significantly greater in group 2 compared to group 1 experiments. There were significant positive correlations between the relaxant effects and concentrations for all fractions (except aqueous fraction) in group 2 and for theophylline in both groups but a negative correlation for n-hexane, dichloromethane and methanol fractions in group 1. The results showed relaxant effect of most fractions from *N. sativa* on tracheal chains of guinea pigs which was more potent for methanol and dichloromethane fractions.

**Keywords**: Bronchodilatory, Fractions, Guinea pig, *Nigella sativa*, Trachea

*Nigella sativa* L. (Ranunculaceae) is a grassy plant that grows in temperate and cold climate areas and has green to blue flowers and small black seeds. The seeds contain thymoquinone, monotropens such as *p*-cymene and *α*-pinene¹, nigellidine², nigellimine³ and a saponin⁴. The chemical composition of the plant was summarized in a recent review⁵.

The therapeutic use of the seeds of *N. sativa* for the treatment of asthma and dyspnea have been described in ancient Iranian literature⁶. The relaxant effects of the volatile oil of seeds on different isolated smooth muscle preparations like rabbit aorta⁷, rabbit jejunum⁸, and guinea pig tracheal muscle⁹ have been reported. Mahfouz and El-Dakhakhnsy¹⁰ reported that the volatile oil from *N. sativa* protected guinea pigs against histamine-induced bronchospasm, but it did not block histamine H₃ receptors in isolated tissues. Both systemic and local administrations of essential oil from this plant have been shown to have anti-inflammatory activity¹¹. The therapeutic effect of *N. sativa* oil on patients with allergic diseases (allergic rhinitis, bronchial asthma, atopic eczema) was also demonstrated¹². In addition, Labib Salem⁵ reported the potent immunomodulatory effects of seeds of the plant.

Different pharmacological effects of *N. sativa* on guinea pig tracheal chains have been reported¹³-¹⁷. The antitussive effect of this plant on guinea pig¹⁸ was also demonstrated.

The objective of present study is to evaluate, the effects of n-hexane, dichloromethane, methanol and aqueous fractions of *N. sativa* on isolated tracheal chains of guinea pigs.

**Materials and Methods**

**Plant and fractions**— *Nigella sativa* was collected from Torbat Heydarieh (north east Iran) during spring of 2002, and dried in shade at room temperature. The plant was identified by botanists of Ferdowsi
University of Mashhad, and the voucher specimen (293-0303-1) preserved. For separating fractions 300 ml n-hexane was added to 500 g of the chopped, dried seeds and the solution was kept at the room temperature for 48 hr. The solution was then decanted and the solvent was evaporated. The dichloromethane was added to the remaining powder and the mixture was allowed to remain at the room temperature for 48 hr. The solution was decanted and the solvent was evaporated. Methanol was added to the remaining powder and the mixture was allowed to remain at the room temperature for 48 hr. The solution was decanted and evaporated. Finally, distilled water was added to the remaining powder and the mixture was allowed to remain at the room temperature for 48 hr and the solution was decanted and solvent was evaporated (Fig. 1). In these manners all of solvents were added until the solutions became clear. Then the final solution contained 40 g/100 ml extract (40 g%).

Tissue preparation — Adult Dunkin-Hartley guinea pigs (400–700 g, both sexes) were used. Animals were housed in big cages (4–5 per cage) with free access to food and water ad libitum, maintained at 22º ± 2ºC on a 12 hr light/dark cycle (light period 0700 and 1900 hrs). Guinea pigs were sacrificed by a blow on the neck and tracheas 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 a tracheal chain. Tissue was then suspended in a 20 ml organ bath (schuler organ bath type 809, March- Hugstetten, Germany) containing Krebs-Henseliet solution containing (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11. The Krebs solution was maintained at 37ºC and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 hr and washed with Krebs solution every 15 min.

Protocol of experiments — The tissue was contracted with KCl (60 mM) or methacholine (10 μM). The relaxant effects of four cumulative concentrations (0.8, 1.2, 1.6 and 2.0 g/100 ml) of n-hexane, dichloromethane, methanol and aqueous fractions of N. sativa and theophylline anhydrous (Sigma Chemical Ltd UK) (0.2, 0.4, 0.6 and 0.8 mM) as positive control, and saline (1 ml) as negative control were examined. To produce the first concentration of each fraction, 0.4 ml of 40 g% was added to a 20 ml organ bath. This will produce a 0.8 g% concentration of fraction in organ bath as follows: 0.4 × 40 g% = 16 g%, 16 g%/20 ml = 0.8 g% (0.8 g/ 100 ml). For other three concentrations, 0.2 ml of 40 g% was added to organ bath respectively, three times. For theophylline, 0.2 ml of 20 mM concentrated solution was added to organ bath 4 times. The consecutive volumes were added to organ bath at 5 min intervals.

In each experiment the effect of four cumulative concentrations from each fraction, theophylline or saline on KCl or methacholine contracted tracheal smooth muscle was measured after exposing tissue to each concentration of the solution for 5 min. A decrease in tone was considered to be a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction. An increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change.

The relaxant effect of different solutions was tested with following two different experimental designs, (n= 6 for each group):

1. On tracheal chains contracted by 60 mM KCl (group 1 experiments).
2. On tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 2 experiments).

The relaxant effects in two groups of experiments were examined in two different sets of tracheal chains. All the experiments were performed randomly with a 1 hr resting period of tracheal chains between two experiments while washing the tissues every
15 min with Krebs solution. In all experiments responses were amplified with amplifier (ML/118 quadribridge amp, March-Hugstetten, Germany) and recorded on powerlab (ML-750, 4 channel recorder, March-Hugstetten, Germany). The research protocol was approved by ethical committee of Mashhad University of Medical Sciences and the norms of international animal ethics were followed.

**Statistical analysis** — The data were expressed as mean±SE. Data of relaxant effects of different concentrations of each fraction were compared with the results of negative and positive control using paired \( t \) test. The data of relaxant effects obtained in two groups of experiments were compared using unpaired \( t \) test. The relaxant effects of different concentrations of four different fractions were compared with each other using One-way Analysis of Variance (ANOVA) with Tukey-Kramer post test. The relaxant effect of four fractions and theophylline were related to the log concentrations using least square regression. Significance was accepted at \( P<0.05 \).

**Results**

**Relaxant effect** — In group 1 experiments all concentrations of only theophylline showed significant relaxant effects compared to that of saline \( (P<0.001 \text{ for all concentrations}) \). Two last concentrations \( (1.6 \text{ and } 2.0 \text{ g%}) \) of n-hexane fraction and three last concentrations \( (1.2, \ 1.6 \text{ and } 2.0 \text{ g%}) \) of dichloromethane and methanol fractions showed small but significant contractile effects compared to that of saline in this group (Table 1).

In group 2 experiments all concentrations of theophylline and methanol fraction, three last concentrations \( (1.2, \ 1.6 \text{ and } 2.0 \text{ g%}) \) of dichloromethane and two higher concentrations \( (1.6 \text{ and } 2.0 \text{ g%}) \) of n-hexane fractions showed significant relaxant effects compared to that of saline. However, aqueous fraction in both group 1 and group 2 did not show any significant relaxant effect on tracheal chains (Table 2).

**Comparison of the relaxant effect of theophylline with different fractions** — In group 1 the relaxant effect of all concentrations and in group 2, the relaxant effect of all concentrations of different fractions except the first concentration of dichloromethane and methanol fraction were significantly less than those of theophylline (Tables 1 and 2).

**Comparison of the relaxant effect of different fractions** — In group 1, the effect of the higher concentration of methanol and dichloromethane

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### Table 1

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Saline</th>
<th>N-hexane Fraction</th>
<th>Dichlorom fraction</th>
<th>Methanol fraction</th>
<th>Aqueous fraction</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-2.67±1.6^c</td>
<td>-2±1.6^c</td>
<td>-3.34±1.7^c</td>
<td>-0.84±0.84</td>
<td>17.22±2.34</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-4.75±1.92^c</td>
<td>-8.28±2.91^d,c</td>
<td>-9.33±3.28^c</td>
<td>-2.91±2.08^c</td>
<td>48.50±4.30</td>
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<tr>
<td>3</td>
<td>-</td>
<td>-8.12±2.53^d,c</td>
<td>-14.33±3.4^e</td>
<td>-16.17±3.47^b,c</td>
<td>-4.59±2.91^c</td>
<td>66.69±3.43</td>
</tr>
<tr>
<td>4</td>
<td>0.42±0.27</td>
<td>-12.41±3.03^de</td>
<td>-22±3.31^b</td>
<td>-21.34±3.78^b,c</td>
<td>-1.25±4.27^e</td>
<td>81.32±2.51</td>
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</table>

Table 1.—Relaxant effect of four different fractions (n-hexane, dichloromethane, methanol and aqueous) from *N. sativa* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (contracted tracheal chains with 60 mM KCl)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Saline</th>
<th>N-hexane Fraction</th>
<th>Dichlorom fraction</th>
<th>Methanol fraction</th>
<th>Aqueous fraction</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>0.83±0.83^a,f</td>
<td>5.84±2.71^b,g</td>
<td>8.34±2.47^d,g</td>
<td>0.00±0.00</td>
<td>15.42±3.56</td>
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<tr>
<td>2</td>
<td>-</td>
<td>4.17±2.00^a,f</td>
<td>11.67±3.57^a,c</td>
<td>18.04±3.28^b,e</td>
<td>1.87±1.2^a,c</td>
<td>43.75±4.99</td>
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<tr>
<td>3</td>
<td>-</td>
<td>8.34±2.79^d,e</td>
<td>17.5±4.95^c,e</td>
<td>27.48±4.73^b,e</td>
<td>3.75±2.4^c</td>
<td>62.50±5.28</td>
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<tr>
<td>4</td>
<td>0.55±0.37</td>
<td>11.67±3.86^d,e</td>
<td>25±7.07^b,c</td>
<td>40.33±6.86^b</td>
<td>10±4.65^c</td>
<td>82.50±6.55</td>
</tr>
</tbody>
</table>

Table 2.—Relaxant effect of four different fractions (n-hexane, dichloromethane, methanol and aqueous) from *N. sativa* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (contracted tracheal chains by 10 μM methacholine)

For other details Table 1.
fractions were significantly lower than that of aqueous fraction \( (P<0.01 \text{ for both fraction}, \) (Fig. 2).

In group 2 the relaxant effect of three last concentrations of methanol fraction were significantly greater than those of n-hexane fraction and aqueous fractions \( (P<0.05 \text{ to } <0.01), \) (Fig. 2).

**Comparison of the relaxant effect between two groups of experiments** — The relaxant effect of different concentrations of methanol and dichloromethane fractions were significantly greater in group 2 compared to group 1 experiments. The relaxant effect of three last concentrations of n-hexane fraction were also significantly greater in group 2 compared to group 1 experiments. However, there was no significant difference in the relaxant effect of different concentrations of theophylline and aqueous fraction between two groups (Fig. 2a and b).

**Correlation between concentrations of solutions and their relaxant effect** — There were significant positive correlations between the relaxant effects and concentrations for all fractions (except aqueous fraction) in group 2 and for theophylline in both groups. However, there were significant negative correlations between the relaxant effects and concentrations for all fractions (except aqueous fraction) in group 1 (Table 3).

**Discussion**

In the present study the relaxant (bronchodilatory) effects of four different fractions (n-hexan, dichloromethane, methanol and aqueous) from *N. sativa* were compared with saline as negative control and theophylline as positive control. In group 1 experiments (contracted tracheal chains by KCl) while theophylline showed significant relaxant effect, different fraction of *N. sativa* did not show any relaxant effect. However, in group 2 experiments (contracted tracheal chains by methacholine) all concentrations of theophylline and methanol fraction, three last concentrations (1.2, 1.6 and 2.0 g%) of dichloromethane and two higher concentrations (1.6 and 2.0 g%) of n-hexane fractions showed significant relaxant effects on tracheal smooth muscle.

The absence of relaxant effect of different fractions from *N. sativa* in group 1 may indicate an opening effect for fractions of this plant on potassium channels because the bronchodilatory effect of potassium channel opening has been demonstrated previously\(^{21}\). If the fractions from *N. sativa* had a potassium channel opening effect, they would not have a relaxant effect on tracheal chains contracted by KCl, while they could show a relaxant effect when the tracheal chain was contracted by methacholine.

However, with regard to bronchodilatory effect of calcium channel blockers\(^{21,22}\) and because KCl affect

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Table 3—Correlation \((r)\) between the relaxant effects of four different fractions (n-hexane, dichloromethane, methanol and aqueous) from *N. sativa* and theophylline with their log concentrations in two groups of experiments

<table>
<thead>
<tr>
<th>Different solutions</th>
<th>N-hexane fraction</th>
<th>Dichloromethane fraction</th>
<th>Methanol fraction</th>
<th>Aqueous fraction</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R)</td>
<td>(P) value</td>
<td>(r)</td>
<td>(P) value</td>
<td>(r)</td>
</tr>
<tr>
<td>Group 1</td>
<td>-0.553</td>
<td>&lt;0.005</td>
<td>-0.739</td>
<td>&lt;0.001</td>
<td>-0.688</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.571</td>
<td>&lt;0.005</td>
<td>0.536</td>
<td>&lt;0.005</td>
<td>0.739</td>
</tr>
</tbody>
</table>
concentrations used. The most potent relaxant effect was shown by methanol and dichloromethane fractions.

**Acknowledgement**

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**References**

6. Ave-Sina, Law in Medicine, Sharafkhandy A; Interpreter, Ministry of Culture publication, Teheran (1990) 314.


