

Comparison of Antitussive Effect of *Nigella Sativa* with Codeine in Guinea Pig

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Abstract

Background: The relaxant and anticholinergic (functional antagonism) effects, histamine H₁ inhibitory effect, and calcium channel blocking effect of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. Several therapeutic effects including anti-asthma and dyspnea have also been ascribed to the seeds of *Nigella sativa*.

Objective: To evaluate the antitussive effect of this plant.

Methods: The antitussive effects of aerosols of two different concentrations of aqueous and macerated extracts as well as an extract of concentrated boiled seeds, codeine, and saline were tested by counting the number of coughs produced 10 min after exposing animal to aerosols of different solutions of citric acid (n=7 for each solution).

Results: The results showed significant reduction in the number of coughs obtained in the presence of both concentrations of aqueous and macerated extracts, boiled seeds extract and codeine (p<0.05 to p<0.001). The cough number induced in the presence of higher concentrations of aqueous and macerated extracts were also significantly less than those with lower concentrations (p<0.05 for aqueous and p<0.01 for macerated extracts). There was no significant difference between the number of coughs obtained in the presence of all extracts with that of codeine.

Conclusion: These results indicate an antitussive effect of *Nigella sativa* comparable to that of codeine.

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Keywords • *Nigella sativa* • guinea pigs • citric acid • codeine

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Introduction

N*igella sativa* L. is a grassy plant with green to blue flowers and small black seeds which grows in temperate and cold climates. The seeds of *Nigella sativa* contain thymoquinone, monoterpenes such as *p*-cymene and α -pinene¹, nigellidine², nigellimine³ and a saponin.⁴ Several therapeutic effects including anti-asthma and dyspnea have been ascribed to the seeds of *Nigella sativa* in Iranian traditional medical books.⁵ In Arabian folk medicine, the black seeds alone or in combination with honey are used for

Table 1: Comparison of number of cough in the presence of different extracts (aqueous, macerated, and boiled) from <i>Nigella sativa</i> with those in the presence of saline (baseline) and codeine (for each experimental design, n=7)					
Experimental design		Number of cough	St. Dif. vs Baseline	St. Dif. vs Codeine	St. Dif. vs 3.3 W/W
Baseline		17.43±1.77			
Aqueous extract	3.3 W/W	10.00±1.34	p<0.01	NS	
Aqueous extract	5.0 W/W	4.14±1.58	p<0.001	NS	p<0.05
Macerated extract	3.3 W/W	10.00±1.07	p<0.01	NS	
Macerated extract	5.0 W/W	5.71±0.92	p<0.001	NS	p<0.01
Boiled extract	5.0 W/W	4.86±1.62	p<0.001	NS	
Codeine	0.03 g/ml	3.17±2.01	p<0.001		

Values are presented as mean±SEM. St. Dif.: statistical difference; NS: not significant difference.

treatment of bronchial asthma.

There is evidence of the relaxant effects of the volatile oil from this plant on different smooth muscle preparations including rabbit aorta⁶, rabbit jejunum,⁷ and isolated tracheal muscle of guinea pig.⁸ Mahfouz and EL-Dakhakhnsy⁹ reported that the volatile oil from *Nigella sativa* protected guinea pigs against histamine induced bronchospasm, but 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.¹⁰

The results of our studies also show a relaxant effect of this plant on isolated guinea pig tracheal chains and functional antagonistic effect of this plant on muscarinic receptors,¹¹ inhibitory effect on histamine (H₁) receptors,¹² and calcium channel blocking effect.¹³ In the present study, antitussive effects of different extracts from this plant were evaluated.

Material and Methods

Plant and extracts

Nigella sativa was identified by botanists in the Herbarium of Ferdowsi University of Mashhad, under specimen number of 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 Soxhlet apparatus. C) Boiled extract: 100 g of the chopped, dried plant was added to 500 ml boiled water for 10 min and filtered. The solvent of all three extracts were then removed under reduced pressure until the extract volume reached 10

ml. The plant ingredient concentration in the final preparations was 10% W/W in all extracts.

Protocols

Dunkin-Hartley guinea pigs of both sexes were used in the study (body weight 500-600g). The method used has been described previously.¹⁴ Unanaesthetized unrestrained animals were placed individually in a transparent perspex chamber, measuring 30 x 20 x 20 cm and exposed to a nebulized aqueous solution of 0.1 g/ml citric acid for 7 min. The aerosol was produced by an air flow of 8 l/min through a Wright nebulizer. The aerosol particles had a median aerodynamic diameter of 0.9 µm. The output of nebulizer was 0.65±0.04 ml solution per minute. The same nebulizer was used throughout the experiment. During the last 5 min of the exposure, the animals were continuously watched by a trained observer, and the number of coughs was determined. Coughs could easily be distinguished from sneeze, since there was a clear difference in sound as well as the behaviour of the animals.¹⁴

The above protocol was performed 10 min after exposing animals to aerosols of the following solutions for a period of 7 min (n=7 for each solution):

- Normal saline (baseline measurements)
- Codeine solution (0.03 g/ml, positive control)
- Macerated extract (3.3% w/w)
- Macerated extract (5% w/w)
- Aqueous extract (3.3% w/w)
- Aqueous extract (5% w/w)
- Boiled extract (5% w/w)

All of the experiments were performed randomly with 2h resting period between each two experiments.

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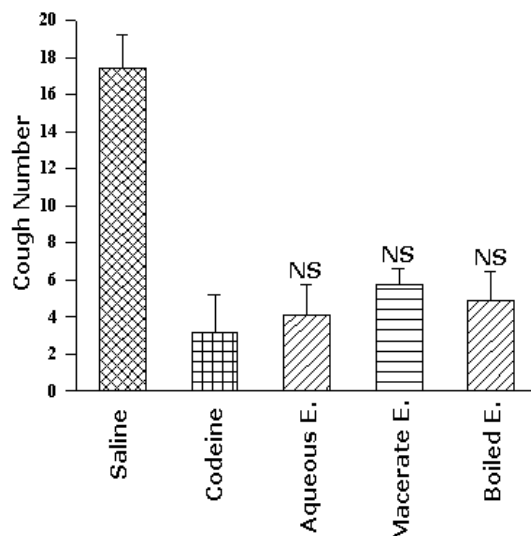


Fig 1: Cough number in the presence of 5.0 W/W of aqueous, macerated, and boiled extracts from *Nigella sativa* compared to those in the presence of saline and codeine. $p < 0.001$ NS: non significant

Statistical analysis

The data are expressed as mean \pm SEM. Comparison of baseline data with number of coughs obtained in the presence of plant extracts and codeine were made using ANOVA. Comparison of data obtained in the presence of two different concentrations of aqueous and macerated extracts were made using the paired "t" test. P value < 0.05 was considered significant.

Results

All concentrations of aqueous and macerated extracts, boiled extract, and codeine caused significant reduction in cough number compared to baseline value ($p < 0.01$ to $p < 0.001$; Table 1, Fig. 1). However, the antitussive effect of both concentrations of aqueous and macerated extracts and the effect of boiled extract was not significantly different from that of codeine (Table 1, Fig.1).

Although, the antitussive effect of the higher concentrations of aqueous extract was greater than those of boiled and macerated extract, the difference between the effect of three extracts (macerated, aqueous and boiled) was not statistically significant. In addition, the antitussive effects of higher concentration of aqueous and macerated extract were significantly greater than those of lower concentrations ($P < 0.05$ for aqueous and $p < 0.01$ for macerated extracts).

Discussion

The present study demonstrates a relatively potent antitussive effect for all three extracts from *Nigella sativa*. The antitussive effects of both aqueous and macerated extracts were concentration dependent and the effect of the higher concentration of each extract was significantly greater than those of the lower concentrations. The antitussive effects of all three extracts from *Nigella sativa* were comparable with that of codeine.

In the present study, the antitussive effects of extracts from *Nigella sativa* were evaluated using a standard method used previously by several investigators.^{14,15}

Although the antitussive effects of different extracts from *Nigella sativa* were similar to that of codeine, the mechanism(s) of antitussive effect of this plant can not be concluded from the results of the present study.

In a previous study¹¹ we demonstrated a relative potent relaxant effect of aqueous and macerated extracts from *Nigella sativa*. Therefore, the bronchodilatory effect of extracts of this plant may be responsible for its antitussive property as stated by Karlsson et al.¹⁵

Opioids, such as morphine and codeine, are generally considered to be the most potent and effective antitussive drugs available and are believed to inhibit cough through suppression of the cough center in the CNS.^{16,17} Morphine was recently shown to reduce a vagally mediated bronchoconstriction produced by inhaled distilled water in asthmatics,¹⁸ and in healthy human subjects. The bronchoconstriction due to inhaled capsaicin was attenuated by nebulized codeine and morphine.¹⁹ The mechanism behind this inhibitory effect is unknown, but suppression of neurotransmitter release has been suggested. Inhibitory opioid receptors have been demonstrated on peripheral nerves,²⁰ inducing vagal sensory neurons.^{21,22} Some experimental data indicate that opioids may interact with the peripheral nervous system of the tracheobronchial tree. A partial antagonism of a non-cholinergic neurogenic bronchoconstriction in the guinea pig by opioid agonists has been reported.²³⁻²⁵ Kerlson et al.¹⁵ also showed that nebulized codeine and morphine can inhibit bronchoconstriction and cough induced by citric acid using a method similar to that of the present study. Therefore, similar antitussive effect of extracts from *Nigella sativa* and codeine may indicate that the antitussive effect of this plant is due to its bronchodilatory property.

However, cough can be induced by irritation of sensory receptors located within and immediately below the epithelial lining. Sites of airway branching may be particularly sensitive to tussive stimuli.²⁶

Sensory receptors mediating reflex bronchoconstriction seem, however, to be distributed all along the tracheobronchial tree.²⁷ Cumulative data suggest that the two airway reflexes of cough and bronchoconstriction have separate afferent neural pathways and may have a differing sensitivity to inhibitory drugs.²⁷ Therefore, the antitussive effect of *Nigella sativa* seems not to be due to its bronchodilatory property.

Misawa and Kizawa²⁸ also showed the antitussive effect of several volatile oils by inhalation and i.p. injection. The antitussive effect of volatile oils in their study was smaller to that of codeine. Advenier et al²⁹ has shown that the tachykinin receptor antagonists have also an antitussive effect. In regard to inflammatory effect of tachykinin and anti-inflammatory effect of *Nigella sativa*,³⁰ the antitussive effect of this plant may be due to its anti-inflammatory effect. However, the inflammatory effect of *Nigella sativa* does not seem to be operative in a short period of time and is not effective in the time period used in the present study.

In conclusion, the results of the present study show antitussive effect of *Nigella sativa* which is comparable to that of codeine but the exact mechanism of this effect needs to be clarified in further studies.

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