Original Article

ANTITUSSIVE EFFECT OF *NIGELLA SATIVA* IN GUINEA PIGS

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ABSTRACT

Objective: Several therapeutic effects including anti-asthma and dyspnea have been described for the seeds of *Nigella sativa*. In previous studies the relaxant and anticholinergic (functional antagonism) effects, histamine H_1 inhibitory effect, and calcium channel blocking effect of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. In the present study the antitussive effect of this plant was evaluated.

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

Results: The results showed significant reduction of cough number observed in the presence of both concentrations of aqueous and macerated extracts, boiled extract and codeine ($p<0.05$ to $p<0.001$). The cough number observed over a period of five minutes in the presence of higher concentrations of aqueous and macerated extracts were also significantly less than those of lower concentrations ($p<0.05$ for aqueous and $p<0.01$ for macerated extracts). In addition there was not any significant difference between cough numbers observed in the presence of all extracts with that of codeine.

Conclusion: These results indicated an antitussive effect of *Nigella sativa*, which was comparable to that of codeine.

KEY WORDS: *Nigella sativa*, antitussive effect ,guinea pig, citric acid, codeine

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INTRODUCTION

Nigella sativa L. 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, monoterpens such as *p*-cymene and α-pinene[1], nigellidine[2], nigellimine[3] and a saponin[4].

Several therapeutic effects including: anti-asthma and dyspnea have been described for the seeds of *Nigella sativa* in Iranian ancient medical books[5]. In Arabian folk medicine, the whole black seeds alone or in combination with honey are used for 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 aorta[6], rabbit jejunum[7], and guinea pig isolated tracheal muscle[8]. Mahfouz and EL-Dakhakhnsy[9] reported that the volatile oil from *Nigella sativa* protected guinea pigs against histamine induced bronchospasm, but it did

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not affect histamine H_1 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], an inhibitory effect on histamine (H_1) receptors[12], and calcium channel blocking effect[13]. In the present study the antitussive effects of different extracts from this plant were evaluated.

MATERIALS AND METHODS

Plant and extracts

Nigella sativa was identified by botanists in the herbarium of Ferdowsi University of Mashhad,the specimen number of the plant is 293-0303-1. The plant extracts were prepared as follows: For macerated extract: 50 g of the chopped, dried plant was macerated with 300 ml distilled water and shaken (on a shaker) for 48 h. For aqueous extract the same amount of plant was extracted with 300 ml distilled water by suxhelt apparatus. For boiled extract 100 g of the chopped, dried plant was added to 500 ml boiled water for 10 min and then filtered. The solvent of all three extracts were then removed under reduced pressure until the extracts volume reached 10 ml. The plant ingredient concentration in the final extracts 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 by Forsberg et al[14]. Unanaesthetized unrestrained animals were placed individually in a transparent perspex chamber, dimensions 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 mass median aerodynamic diameter of 0.9 µm as determined by laser light scattering (Malvern Instruments 2600 HSD analyzer, Malvern ,UK). 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, a trained observer continuously watched the animals, and the numbers of coughs were determined. Coughs could easily be distinguished from sneeze, since there is a clear difference in sound as well as in 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):

a) Normal saline (baseline measurements)
b) Codeine solution (0.03 g/ml, positive control)
c) Macerated extract (3.3% w/w)
d) Macerated extract (5% w/w)
e) Aqueous extract (3.3% w/w)
f) Aqueous extract (5% w/w)
g) Boiled extract (5% w/w)

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

Statistical analysis

Data were expressed as mean ± 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 paired "t" test. Significance was accepted at $p<0.05$.

RESULTS

All concentrations of aqueous and macerated extracts, boiled extract, and codeine caused significant reduction in cough numbers compared to baseline value ($p<0.01$ to $p<0.001$; Table-I, Figure-I). However, the antitussive effect of both concentrations of aqueous and

Table-I Comparison of number of coughs observed in the presence of different extracts (aqueous, macerated, and boiled) from *Nigella sativa* with those obtained in the presence of saline (baseline) and codeine (for each experimental design, n=7)

Experimental design	*Number of coughs in 5 minutes*	*St. Dif. vs Baseline*	*St. Dif. vs Codeine*
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
Macerate extract 3.3 W / W	10.00±1.07	p<0.01	NS
Macerate extract 5.0 W / W	5.71±0.92	p<0.001	NS
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: nonsignificant difference.

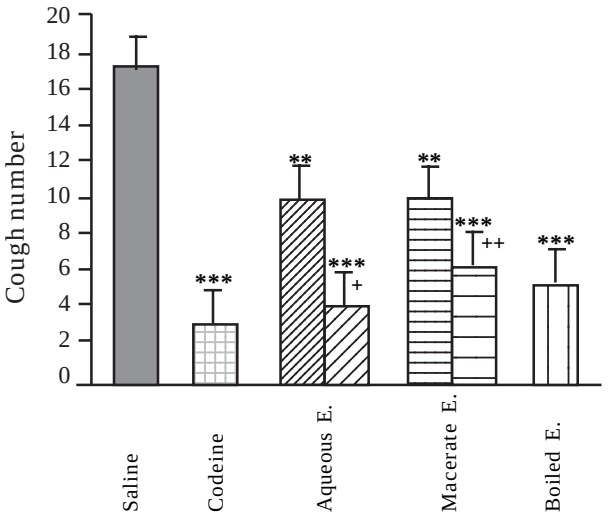

Figure-I: Cough numbers observed over a period of five minutes in the presence of lower concentration (3.3 W / W, fine filled bars) and higher concentration (5.0 W / W, medium filled bars) of aqueous, macerated, and boiled extracts from *Nigella sativa* and those obtained in the presence of saline (baseline) and codeine. Statistical differences between the effects of plant extracts and baseline value; **: p<0.01, ***: p<0.001. Statistical differences in cough numbers between two concentrations of aqueous and macerated extracts; +: p<0.05, ++: p<0.01

macerated extracts and the effect of boiled extract was not significantly different with that of codeine (Table-I, Figure-I).

Although, the antitussive effects of the higher concentration of aqueous extract was greater than those of boiled and macerated extract, the differences between the effects of three extracts (macerated, aqueous and boiled extracts) were not statistically significant. In addition the antitussive effects of higher concentrations 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

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]. The result of the present study demonstrated 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 the effect of codeine.

Misawa and Kizawa[15] 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 than that of codeine. Therefore the *Nigella sativa* has a potent antitussive effect that required further studies. 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 cannot be concluded from the results of the present study.

In a previous study[11], 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.[16].

Opioids, such as morphine and codeine, are generally considered to be the most potent and effective antitussive drugs available and are believed to inhibit coughs through suppression of a cough canter in the central nervous system[17,18]. Morphine was recently shown to reduce a vagally mediated bronchoconstriction produced by inhaled distilled water in asthmatics[19], and in healthy human subjects. The bronchoconstriction to inhaled capsaicin was attenuated by nebulized codeine and morphine[20]. 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[21], inducing vagal sensory neurons[22,23]. Some experimental data indicate that opioids may interact with the peripheral nervous system of the tracheobronchial tree. A partial antagonism of a noncholinergic neurogenic bronchoconstriction in the guinea pig by opioid agonists has been reported[24-26]. Karlsson et al.[16] also showed that nabulized codeine and morphine could inhibit bronchoconstriction and coughs induced by citric acid using a method similar to that of the present study. Therefore, the similar antitussive effect of extracts from *Nigella sativa* and codeine may indicate that the antitussive effect of this plant is due to its bronchodilator property.

In addition, coughs can be induced by irritation of sensory receptors located within and immediately below the epithelial lining. Sites of airway branching may be particulary sensitive to tussive stimuli[27]. Sensory receptors mediating reflex bronchoconstriction seem, however, to be distributed all along the tracheobronchial tree[28]. Advenier et al[29] showed the tachykinin receptor antagonists have also antitussive effect. In addition one possible mechanism responsible for bronchodilatory effect of this plant is inhibition of stimulatory non adrenergic non cholinergic nervous system (NANC)[30]. Therefore, the antitusive effect of *Nigella sativa* might be due to its possible tachykinin inhibitor substance(s) content mediating both bronchodilatory and antitussive effect.

With regard to inflammatory effect of tachykinin and because *Nigella sativa* has anti-inflammatory effect[31], the antitussive effect of this plant may be due to its anti-inflammatory effect. However, the inflammatory effect of *Nigella sativa* does not seems to occurs in a short period of time and is not effective in time period used in the present study. Therefore, the mechanism(s) of antitussive effect of *Nigella sativa* should be investigated in further studies.

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

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