Arch Iranian Med 2002; 5 (2): 103-107

ORIGINAL ARTICLE

EFFECT OF *NIGELLA SATIVA* ON ISOLATED GUINEA PIG TRACHEA

Mohammad Hossein Boskabady MD PhD•, Batool Shirmohammadi MSc

Department of Physiology, Ghaem Medical Center, Mashad University of Medical Sciences, Mashad, Iran

Background-Previous studies have demonstrated the relaxant, anticholinergic (functional antagonism) and antihistaminic effects of *Nigella sativa* on guinea pig tracheal chains. Mechanisms responsible for the relaxant effect of this plant on tracheal chains were examined in this study.

Methods- The calcium-antagonistic effects of three increment concentrations of the aqueous extracts of *Nigella sativa* and the calcium channel blocker diltiazem in comparison with saline were tested by measuring the cumulative concentration response curves of $CaCl_2$-induced contractions of isolated guinea pig tracheal chains in the presence of calcium-free Krebs-Henseleit solution and 60 mM of KCl under each experimental condition (n = 7).

Results-A rightward shift in the $CaCl_2$ response curves was obtained in the presence of two different concentrations of diltiazem (1 and 5 μM) and the aqueous extract compared to the saline control curves. The effective concentration of $CaCl_2$ causing 50% of maximum response (EC_{50}) in the presence of two concentrations of aqueous extract and diltiazem was significantly higher than that of saline ($p < 0.05$ to $p < 0.02$). However, the maximum response to $CaCl_2$ obtained in the presence of the final concentrations of aqueous extract and diltiazem was lower than of saline ($p < 0.05$ and $p < 0.005$ for aqueous extract and diltiazem, respectively).

Conclusion-The results suggest a calcium antagonistic effect of *N. sativa* in isolated tracheal chains of guinea pigs.

Keywords • calcium antagonistic effect • guinea pig • *Nigella sativa* • trachea

Introduction

Nigella sativa, commonly known as Fitch (Siah-Daneh in Persian), is a grassy plant with green- to blue-colored flowers and small black seeds that grows in temperate and cold climates. The seeds contain thymoquinone, monoterpenes such as *p*-cymene and α-pinene,[1] nigellidine,[2] nigellimine[3] and a saponin.[4]

N. sativa has long been known for its antispasmodic properties, especially in gastro-intestinal and respiratory diseases. Ancient Iranian medical books state that these seeds were used in digestive and gynecologic disorders as well as in asthma and dyspnea.[5] The whole black seeds alone or in combination with honey are used in the treatment of bronchial asthma in traditional Arabian medicine.

Studies have shown that the volatile oil of this plant has a relaxant effect on different smooth muscles including the aorta[6] and jejunum of rabbits,[7] and isolated tracheal muscles of guinea pigs.[8] Mahfouz and El-Dakhakhny reported that the volatile oil of *Nigella sativa* protected guinea pigs from histamine-induced broncho-spasm, but did not affect H_1 receptors in isolated tissues.[9] However, in an in vivo study in guinea pigs, increasing respiratory rate and intratracheal pressure were demonstrated after intravenous administration of the volatile oil of *N. sativa*.[10] In our recent study, a relaxant effect of this plant was demonstrated on isolated guinea pig tracheal

• **Correspondence:** *M.H. Boskabady MD, PhD, Department of Physiology, Ghaem Medical Center, Mashad 91735, Iran. Fax: +98-511- 8413579.*

chains.[11]

To elucidate the mechanism(s) responsible for the bronchodilatory effect of *N. sativa*, the calcium-channel antagonistic effects of the aqueous extract of this plant were examined on guinea pig tracheal chains.

Materials and Methods

Plant and extracts

N. sativa was identified by botanists in the herbarium of Ferdowsi University of Mashad, Iran (specimen number 293-0303-1).

For preparation of the aqueous extract, 50 g of the chopped dried plant was Soxhlet extracted using 300 mL of distilled water. The solvent of the extract was then removed under reduced pressure until a volume of 20 mL remained. The extract concentration in the final solution was 10% W/W.

Tissue preparations

Male guinea pigs (400–700 g) were sacrificed by a blow to the neck and the trachea were removed. Each trachea was cut into 10 rings (each ring contained 2–3 cartilaginous rings). The rings were then cut open opposite the trachealis muscle and sutured together to form a tracheal chain (n = 7).[12]

Tracheal chain tissue was then suspended in a 10 mL organ bath (Organ Bath 61300, Bio Science Palmer-Washington, Sheerness, Kent, UK) containing calcium-free Krebs-Henseleit solution with the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72 and dextrose 11 (all chemicals were from Merck, Germany).

The Krebs solution was maintained at 37˚C and aerated with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic force of 1 g and allowed to come to equilibrium for at least 1 hour while washing with Krebs solution every 15 min.

Protocols

1. The inhibitory effect of *N. sativa* on calcium channels was examined by producing a cumulative concentration-response curve of $CaCl_2$-induced contraction of tracheal chains in the presence of 60 mM KCl, 5 min after exposing tissue to each experimental solution. Seven experimental solutions were used: a) three increment concentrations of aqueous extract (0.25, 0.5 and 1 g%); b) three concentrations of diltiazem (Sigma Chemical Ltd, UK, 0.5, 1 and 5 mM), and c) 0.1 mL normal saline. Consecutive concentrations of $CaCl_2$ were added every 2 min (range, 5–65 μM). The percentage of maximum contraction obtained with each concentration in proportion to the maximum contraction obtained with saline was plotted against the concentration of $CaCl_2$.
2. The effective concentration of $CaCl_2$ causing 50% of maximum response (EC_{50}) in each experiment was determined using the concentration-response curve of the corresponding experiment. The shift of the cumulative concentration-response curves obtained in the presence of different concentrations of aqueous extract and diltiazem was examined by comparing the EC_{50} of each solution with that of saline.
3. In addition, the maximum response to $CaCl_2$ obtained in the presence of all aqueous extract concentrations and diltiazem was compared with that of saline.

The effect of different concentrations of aqueous extract of *N. sativa* and diltiazem was tested on each tracheal chains (n = 7). All experiments were performed randomly with a 1-hour resting period of the tracheal chains between each experiment. Meanwhile, the tissue was washed with Krebs solution every 15 minutes. Responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and measured after tissue fixation.

Statistical analysis

EC_{50} and maximum response to $CaCl_2$ data were expressed as the mean ± SEM. The EC_{50} and maximum response to $CaCl_2$ in the presence of different concentrations of aqueous extract were compared with those of diltiazem; results of both aqueous extract and diltiazem were compared with those obtained in the presence of saline using a paired *t*-test. Instat software version 2.01 was used for data analysis. Statistical significance was accepted at $p < 0.05$.

Results

Shift in cumulative concentration-response curves

Cumulative concentration-response curves of $CaCl_2$ obtained with three different concentrations of aqueous extract and diltiazem all showed clear rightward shifts compared to that produced with saline (Figure).

EC_{50}

The EC_{50}s of $CaCl_2$ obtained in the presence of

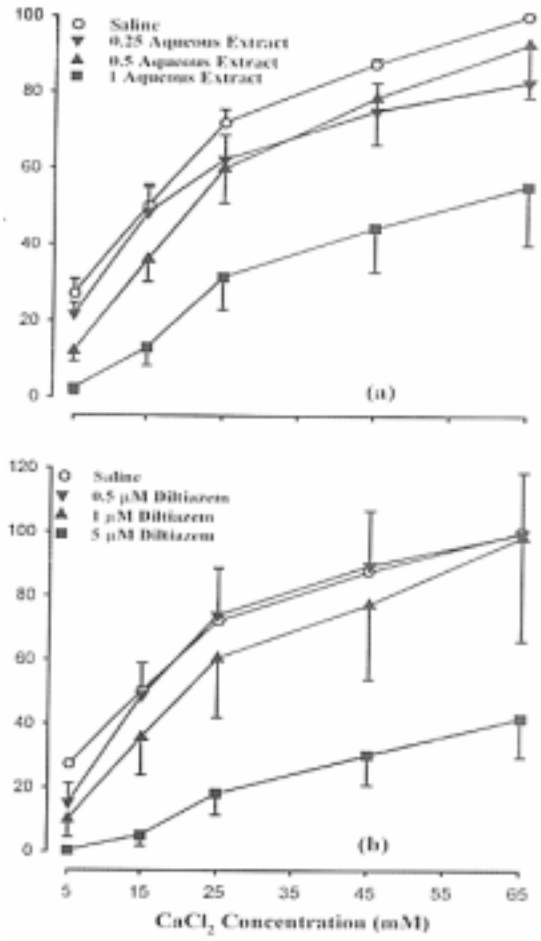

Figure. Cumulative concentration-response curves of $CaCl_2$-induced contraction of guinea pig tracheal chains in the presence of three increment concentration of aqueous extract from N. sativa (a) and diltiazem (b). Three concentrations of both aqueous extract and diltiazem caused rightward shifts in $CaCl_2$-response curves compared to the curves obtained in the presence of saline.

two concentrations (0.5 and 1 g %) of aqueous extract and diltiazem were significantly higher ($p < 0.05$ and $p < 0.02$, respectively) than those for saline (Table 1). However, there were not significant differences between the EC_{50}s obtained for different concentrations of aqueous extract and diltiazem (Table 1).

Maximum response to $CaCl_2$

The maximum responses to $CaCl_2$ obtained with 1 g% of extract alone ($p < 0.05$) and 5 μm diltiazem ($p < 0.005$) were significantly lower than that of saline (Table 2). However, the maximum response to $CaCl_2$ obtained in the presence of different concentrations of the aqueous extract were not significantly different from that of diltiazem (Table 2).

Relationship between diltiazem concentration and *N. sativa* extract with EC_{50}

There was a significant positive correlation between the EC_{50} of $CaCl_2$ (inhibitory effect on calcium channels) with different concentrations of diltiazem ($r = 0.728$, $p < 0.001$), and *N. sativa* aqueous extract ($r = 0.570$, $p < 0.02$).

Discussion

The bronchodilatory effect of *N. sativa* found in our previous study[11] might have been produced by different mechanisms, including the inhibitory effect of this plant on calcium channels.[13]

The concentration-dependent rightward shifts in $CaCl_2$ concentration-response curves, obtained in the presence of three increment extract concentrations, the concentration-dependent increase in EC_{50}, and the achievement of maximum effect of $CaCl_2$ obtained in the presence of the two lower extract concentrations indicate a concentration-dependent inhibitory effect of *N. sativa* extract on calcium channels, which provide further proof for other pharmacologic studies.[14–16]

Results obtained in the presence of increment concentrations of diltiazem, a calcium channel blocker, were similar to those of the aqueous extract, including the concentration-dependent

Table 1. EC_{50} (mM) of $CaCl_2$ obtained in the presence of three concentrations of aqueous extract (AE), diltiazem and saline (S).

Aqueous extract (%)		St. Dif. vs S	Diltiazem		St. Dif. vs S	St. Dif. vs AE
---	---	16.57 ± 0.55		---	16.57 ± 0.55	
0.25 g	14.67 ± 2.11	NS	0.5 μM	16.17 ± 0.74	NS	NS
0.5 g	21.08 ± 1.12	p<0.02	1 μM	18.83 ± 1.16	p<0.05	NS
1 g	22.20 ± 1.44	p<0.02	5 μM	32.75 ± 5.12	p<0.02	NS

Values are presented as mean ± SEM. St. Dif.= statistical difference; NS = non-significant difference (for each experimental condition n = 7).

Table 2. Maximum response to $CaCl_2$ in the presence of three concentrations of aqueous extract from *Nigella sativa* diltiazem, and saline.

Aqueous extract (%)		St. Dif. vs S	Diltiazem		St. Dif. vs S	St. Dif. vs AE
—	—	100.0 ± 0.0		—	100.0 ± 0.0	
0.25 g	82.57 ± 9.34	NS	0.5 μM	99.64 ± 19.16	NS	NS
0.5 g	92.75 ± 14.07	NS	1 μM	98.43 ± 32.54	NS	NS
1 g	55.17 ± 15.33	$p<0.05$	5 μM	41.79 ± 12.03	$p < 0.005$	NS

Values are presented as mean ± SEM. St. Dif.= statistical difference; NS = non-significant difference (for each experimental condition n = 7).

rightward shift in the concentration-response curves of $CaCl_2$, concentration-dependent increase in EC_{50} and achievement of maximum response to $CaCl_2$ in the presence of the two lower concentrations diltiazem. These similarities strongly support the idea of an inhibitory effect of *N. sativa* on calcium channels. The positive correlation between EC_{50} and concentrations of both diltiazem and aqueous extract also support the concentration-dependent inhibitory effect of *N. sativa* on calcium channels. The results of this study support those of Aqel in 1993,[17] which showed an inhibitory effect of the volatile oil of this plant on calcium channels by studying concentration-dependent relaxation of precontracting tracheal chains, while we examined the effect of aqueous extract using a more scientific method (shift in concentration response curves of $CaCl_2$). The maximum response to $CaCl_2$ was not achieved in the presence of the highest concentrations of both diltiazem and aqueous extract, which may suggest a functional antagonistic effect on calcium channels for the aqueous extract. In fact, our previous studies showed anticholinergic[11] and inhibitory effects on histamine (H_1) receptors[18] of this plant, which may interfere with calcium channels. However, the same findings for diltiazem indicate that the concentration of $CaCl_2$ is not sufficient for achievement of maximum response to $CaCl_2$ in the presence of the highest concentrations of diltiazem and extract. In the final experiment day, the 85-mM $CaCl_2$ concentration was examined in the presence of the highest concentration of diltiazem (5 μM) and maximum effect was achieved. The similar EC_{50}s of $CaCl_2$ obtained with different concentrations of aqueous extract and diltiazem indicates an equal inhibitory effect of the aqueous extract of *N. sativa* and diltiazem at the concentrations used.

The results of our previous studies[11,18] and the present study suggest that anticholinergic, histamine-H_1 inhibitory, and calcium antagonistic effects of *N. sativa* may contribute to the bronchodilatory effect of this plant.

Other possible mechanisms responsible for the bronchodilatory effect of *N. sativa* include:

1. Stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC;[19]
2. Methylxanthine activity of the plant;[20]
3. Other mechanisms such as opening of potassium channels and inhibition of phosphodiesterase;[21]

The contribution of these mechanisms and importance of those seen in our studies on the bronchodilatory effect of *N. sativa* 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 *N. sativa* might also have an anti-inflammatory effect, which would contribute to the therapeutic effect of this plant on asthma. The inhibitory effects of the essential oil of *N. sativa* and thymoquinone have been shown on both cyclooxygenase and 5-lipo-oxygenase pathways of arachidonic acid metabolism and also on membrane lipid peroxidation.[22] However, the effect of this plant on airway inflammation in asthma should be investigated in further studies.

In conclusion, the results of this study suggest an inhibitory effect of *N. sativa* very similar to that of diltiazem on calcium channels in isolated guinea pig tracheal chains.

References

1 El-Dakhakhny M. Studies on chemical constitution of Egyptian *Nigella sativa* L seeds. II. The essential oil. *Planta Medica*. 1963; **11:** 465-70.

2 Atta UR, Hasan S. Nigellidine, a new indazole alkaloid from the seeds of *Nigella sativa. Tetrahedron letters*. 1995; **36:** 1993-6.

3 Atta UR, Malik S. Zaman K. Nigellimine, a new isoquinoline alkaloid from the seeds of *Nigella sativa. J Nat Prod Lloydia.* 1992; **55:** 676-8.

4 Ansari AK, Sadiy HAS. Structural studies on a saponin isolated from the seeds of *Nigella sativa. Phyto Chem*. 1989; **27:** 377-9.

5 Sharafkhandy A. *Avicenna's Law in Medicine* [translated into Persian]. Tehran: Ministry of Guidance Publication; 1990: 314.

6 Aqel MB. The relaxing effect of volatile oil of *Nigella sativa* seed on vascular smooth muscle. *Jordan Ser B*. 1992; **1:** 91-100.

7 Aqel MB. Effects of *Nigella sativa* seeds on intestinal smooth muscle. *Int J Pharmacogn*. 1993; **31:** 55-60.

8 Reiter M, Brandt W. Relaxant effects on tracheal and ileal smooth muscles of the guinea pig. *Arzneimittelforschung.* 1985; **35:** 408-14.

9 Mahfouz M, El-Dakhakhny M. Chemical and pharmacological properties of the new anti-asthmatic drug, nigellone. *Egypt Pharm Bull*. 1960; **42:** 411-24.

10 El-Tahir KEH, Ashour MMS, Al-Harbi MM. The respiratory effect of the volatile oil of the Black seed (*Nigella sativa*) in guinea pig: elucidation of the mechanism(s) of action. *Gen Pharmacol*. 1993; **24:** 1115-22.

11 Boskabady MH, Shahabi M. Bronchodilatory and anticholinergic effect of *Nigella sativa* on isolated guinea pig a tracheal chains. *Irn J Med Sci*. 1997; **22:** 127-33.

12 Holroyde MC. The influence of epithelium on the responsiveness of guinea pig isolated trachea. *Br J Pharmacol*. 1986; **87:** 501-7.

13 Miyahara Y, Kizawa Y, Sano M, Murakami H. Effect of organic and inorganic Ca(2+)-antagonists on acetylcholine-induced contraction in molluscan (*Mytilus edulis*) smooth muscle. *Gen Pharmacol*. 1993; **24:** 1419-23.

14 Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. *Br J Pharmacol*. 1997; **120:** 148-61.

15 Ariens EJ. Pharmacology of the airway smooth muscle. In: Nadel JA, Pauwels R, Snashall PD, eds. *Bronchial Hyper responsiveness*. Boston: Blackwell Scientific Publication; 1987: 7-23.

16 Linden A, Bergendal A, Ullman A, et al. Salmetrol, formeterol, and salbutamol in the isolated guinea pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. *Thorax*. 1993; **48:** 547-53.

17 Aqel MB. The calcium antagonistic effect of the volatile oil of *Nigella sativa* seeds. *Jordan Ser B.* 1992; **1:** 119-33.

18 Boskabady MH, Shiravi N. Inhibitory effect of *Nigella sativa* on histamine (H_1) receptors of isolated guinea pig tracheal chains. *Eur Respir J*. 2000; **16:** 461.

19 Linden A, Lofdahl CG, Ullman A, Skoogh BE. Nonadrenergic, noncholinergic responses stabilize smooth muscle tone, with and without parasympathetic activation, in guinea pig isolated airways. *Eur Respir J*. 1993; **6:** 425-33.

20 Meini S, Ballati L, Evangelista S, Manzini S. Isbufylline, a xanthine derivative, inhibits bronchoconstrictor responses produced by stimulation of capsaicin-sensitive sensory nerves in guinea pig: in vitro and in vivo evidence. *Pulm Pharmacol.* 1993; **6:** 279-86.

21 Buckle DR, Arch JRS, Bowring NE, et al. Relaxant effects of potassium channel activators BRL 38227 and pinacidil on guinea pig and human airway smooth muscle, and blockade of their effects by glibenclamide and BRL 31660. *Pulm Pharmacol.* 1993; **6:** 77-86.

22 Houghton PJ, Zarka R, De las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med*. 1995; **61:** 33-6.