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STIMULATORY EFFECT OF *NIGELLA SATIVA* ON β_2-ADRENOCEPTORS OF GUINEA PIG TRACHEAL CHAINS

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ABSTRACT

Our previous studies have demonstrated the relaxant, anticholinergic (functional antagonism), antihistaminic, and inhibitory effect of calcium channels of *Nigella sativa* on guinea pig tracheal chains. To investigate the other mechanism responsible for the relaxant effect of this plant, the stimulatory effect of *Nigella sativa* on β_2-adrenergic receptors in tracheal chains of the guinea pig was examined in this study.

The β_2-Adrenergic effects of macerated and aqueous extracts from *Nigella sativa,* 50 nM propranolol, and saline were tested by performing the cumulative Log concentration-response curves of isoprenaline induced relaxation of precontracted isolated guinea pig tracheal chains with three different conditions including: non-incubated (group 1, n=8); incubated with 1 μM chlorpheniramine, and 1 μM atropine (group 2, n=5). The effective concentration of isoprenaline, causing 50% of maximum response (EC_{50}), maximum response and the slope of isoprenaline curves obtained in the presence of extracts, and propranolol were compared with those of saline.

The results showed clear leftward shifts in isoprenaline curves obtained in the presence of both macerated and aqueous extracts compared with that of saline in group 2. The EC50 obtained in the presence of aqueous extract in group 2 was significantly lower than that of saline ($p<0.05$). The maximum response obtained in the presence of aqueous extract in group 2 was non-significantly greater than that of saline. However, in group 1 experiments there was no significant difference between EC50 and maximum responses obtained in the presence of two extracts and saline, although isoprenaline curves obtained in the presence of both macerated and aqueous extracts showed clear rightward shifts compared to that of saline. Isoprenaline curves were obtained in the presence of propranolol in both groups of experiments. The EC50 obtained in the presence of propranolol was significantly greater than that obtained in the presence of saline and extracts in both groups of experiments ($p<0.001$ for all cases).

The results of this study indicated a stimulatory effect of aqueous extract and a possible stimulatory effect of macerated extract from *Nigella sativa* on β_2-adrenoceptors.

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INTRODUCTION

Nigella sativa 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, monotropens such as p-cymene and α-pinene,[1] Nigellidine,[2] Nigellimine[3] and a saponin.[4]

Several therapeutic effects including those on digestive disorders, gynaecologic, and also anti-asthma and dyspnea have been described for the seeds of *Nigella sativa* in Iranian ancient medical books.[5] *Nigella sativa* has long been known for medical use as an antispasmodic, especially 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 volatile oil from this plant on different smooth muscle including rabbit aorta,[6] rabbit jejunum,[7] and isolated tracheal muscles of guinea pigs.[8] Mahfous 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 H_1 receptors in isolated tissues.[9] However, in an in vivo study, increasing respiratory rate and intra-tracheal pressure of guinea pigs due to i.v. administration of volatile oil from *Nigella sativa* has been demonstrated.[10] In our recent studies relaxant and anticholinergic,[11] H1 histamine receptor blocking,[12] and calcium channel blocking[13] effects of this plant on isolated guinea pig tracheal chains were demonstrated.

To elucidate the other mechanism responsible for the observed bronchodilatory effect of *Nigella sativa*, the stimulatory effect of aqueous and macerated extracts of this plant on β_2-adrenergic receptors of guinea pig tracheal chains was examined in this study.

MATERIAL 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 aqueous extract was prepared as follows: Fifty grams of the chopped, dried plant was extracted with 300 mL distilled water by suxhelat apparatus. For macerated extract, the same amount of plant was macerated with 300 mL distilled water and shaken (on a shaker) for 48 hr. The solvent of both extracts were then removed under reduced pressure until the extracts volume reached 20 mL. Plant ingredient concentration in the final extracts were 10% W/W in both extracts.

Tissue preparations

Male guinea pigs (400-700 gr) were killed by a blow on the neck and tracheae 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.[14]

Tissue was then suspended in a 10 mL organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 hr while it was washed with Krebs solution every 15 min.

Protocols

1) The stimulatory effect of *Nigella sativa* on β_2-adrenoceptors was examined by producing cumulative log concentration-response curve of isoprenaline sulphate (Sigma Chemical Ltd UK) induced relaxation of pre-contracted tracheal chains by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK) 10 min after exposing tissue to one solution (0.5 mL of propranolol hydrochloride with 1 μM concentration (Sigma Chemical Ltd UK), macerated and aqueous extracts, or saline). The consecutive concentrations of isoprenaline were added every 2 min (including 5 nM - 1000 μM); and the percentage of relaxation due to each concentration in proportion to the maximum relaxation obtained in the presence of saline was plotted against log concentration of isoprenaline.
2) The effective concentration of isoprenaline causing 50% of maximum response (EC_{50}) in each experiment was measured using the isoprenaline-response curve of the corresponding experiment. The shift of cumulative log concentration-response curves obtained in the presence of extracts and propranolol were examined by comparing the EC_{50} obtained in the presence of each solution with that of saline.
3) To examine the parallel rightward shift, the slope of the curve of each experiment was measured and the slope of the isoprenaline curves obtained in the presence of extracts and propranolol were compared with that of saline.
4) The maximum responses to isoprenaline obtained in the presence of extracts and propranolol were also compared with that of saline.

The stimulatory effect of *Nigella sativa* on β_2-adrenoceptors was tested on two different experimental conditions as follows:

a) Non-incubated tracheal chains (group 1, n=7).

b) Incubated tracheal chains 30 min prior to beginning and during obtaining the isoprenaline curve with 1 μM

atropine sulphate (Sigma Chemical Ltd UK) and 1 μM chlorpheniramine maleate (Sigma Chemical Ltd UK) , (group 2, n=5).

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

Statistical analysis

The data of EC_{50}, the slope of the curves, and maximum response to isoprenaline in different experiments were expressed as mean±SEM. The EC_{50}, the slope, and maximum response obtained in the presence of extracts and propranolol were compared with those obtained in the presence of saline using paired "t" test. The values of EC_{50}, the slope, and maximum response obtained in group 2 experiments were compared with those of group 1 using unpaired "t" test.

RESULTS

Shift in cumulative log concentration-response curves

Cumulative log concentration-response curves of isoprenaline obtained in the presence of both extracts showed clear rightward shift compared to isoprenaline curves produced in the presence of saline in group 1

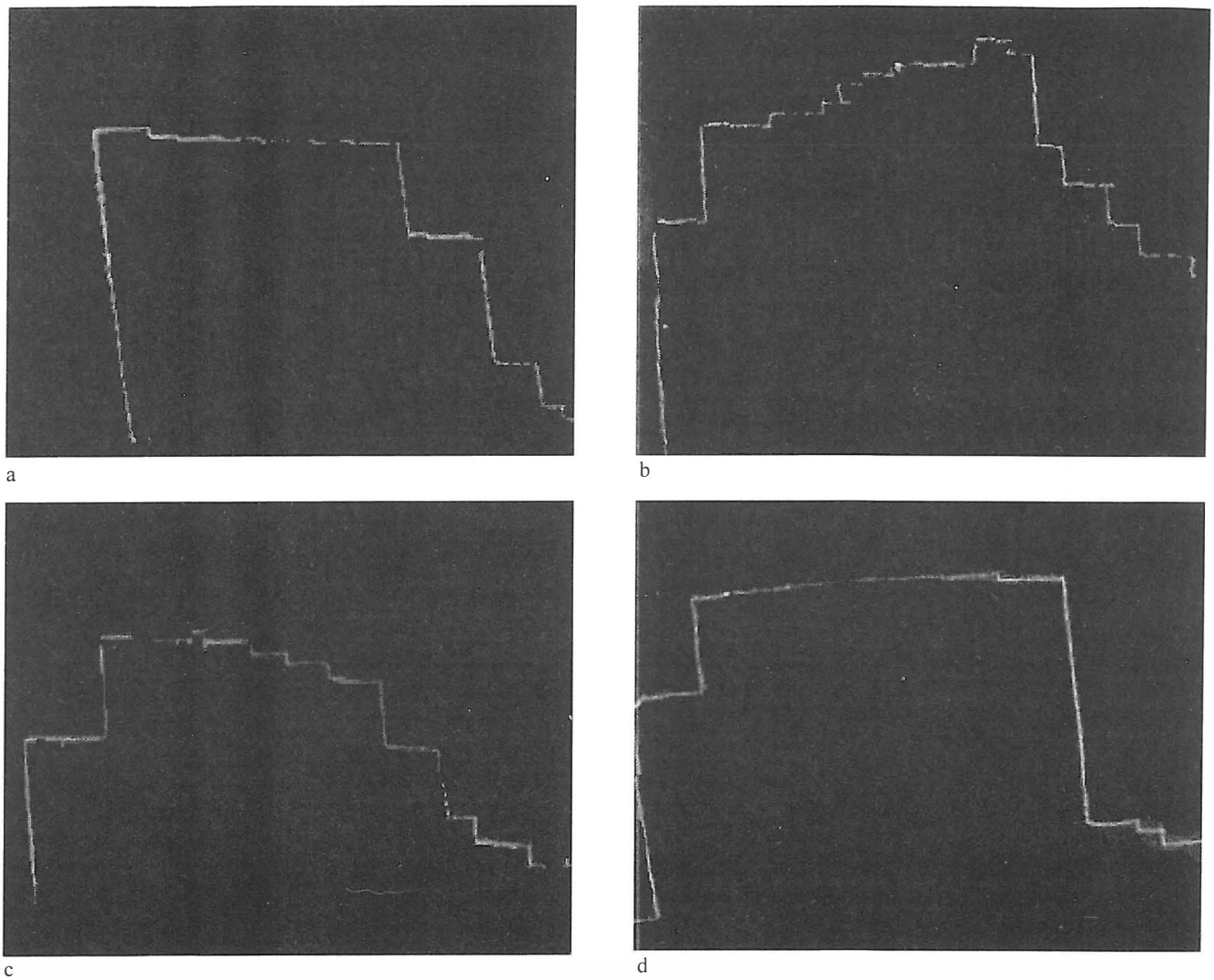

Fig. 1. Samples of kymograph records of relaxation response to isoprenaline in pre-contracted isolated guinea pig tracheal chains in the presence of saline (a), propranolol (b), aqueous extract (c), and macerated extract (d). The relaxation response of tracheal chain to isoprenaline in the presence of saline, propranolol, macerated extract and aqueous extract started at isoprenaline concentrations 5, 50, 5μM and 50 nM (in organ bath) respectively. The numbers written on kymograph indicated the concentrations of isoprenaline added to a 10 ml organ bath.

experiments. However, in group 2 experiments concentration response curves obtained in the presence of both extracts showed leftward shift compared to the curve produced in the presence of saline (Fig. 2).

EC_{50}

The EC_{50} of isoprenaline obtained in the presence of propranolol in both experimental conditions were significantly higher than those for saline ($p<0.001$). There were no significant differences between EC_{50} obtained in the presence of macerated extracts in both experimental groups and that of aqueous extract in group 1 with those for saline. However the EC_{50} obtained in the presence of aqueous extract in group 2 experiment was significantly lower than that for saline ($p<0.05$), (Table I).

Maximum response to isoprenaline

The maximum response to isoprenaline obtained in the presence of aqueous extract in group 2 experiments was non-significantly greater than those of saline and group 1. The maximum response obtained in the presence of propranolol in group 1 experiments was non-significantly lower than those of saline and group 2 (Table II).

Slope of isoprenaline-response curves

The slopes of isoprenaline-response curves obtained in the presence of extracts from *Nigella sativa* and propranolol in both experimental conditions were not significantly different from those of saline. However, the slopes of isoprenaline curve obtained in the presence of extracts in group 2 were higher than those of group 1 but only for the aqueous extract was this difference statistically significant ($p<0.05$), (Table III).

DISCUSSION

The bronchodilatory effect seen for *Nigella sativa* in our previous study might be produced due to several different mechanisms.[3,4] One possible mechanism responsible for this effect could be the stimulatory property effect of this plant on β_2-adrenoceptors.[15] The stimulatory effect of the aqueous and macerated extracts from *Nigella sativa* was therefore examined on isolated guinea pig tracheal preparations in this study.

The results on non-incubated trachea showed clear inhibitory effect of aqueous and macerated extracts from *Nigella sativa* which was very similar to that of propranolol. The reason for the difference in maximum response to isoprenaline obtained in the presence of propranolol with that of saline was perhaps inadequate concentration of isoprenaline to achieve maximum response in the presence of propranolol, because the plateu in concentration response curves in the presence of propranolol was not achieved.

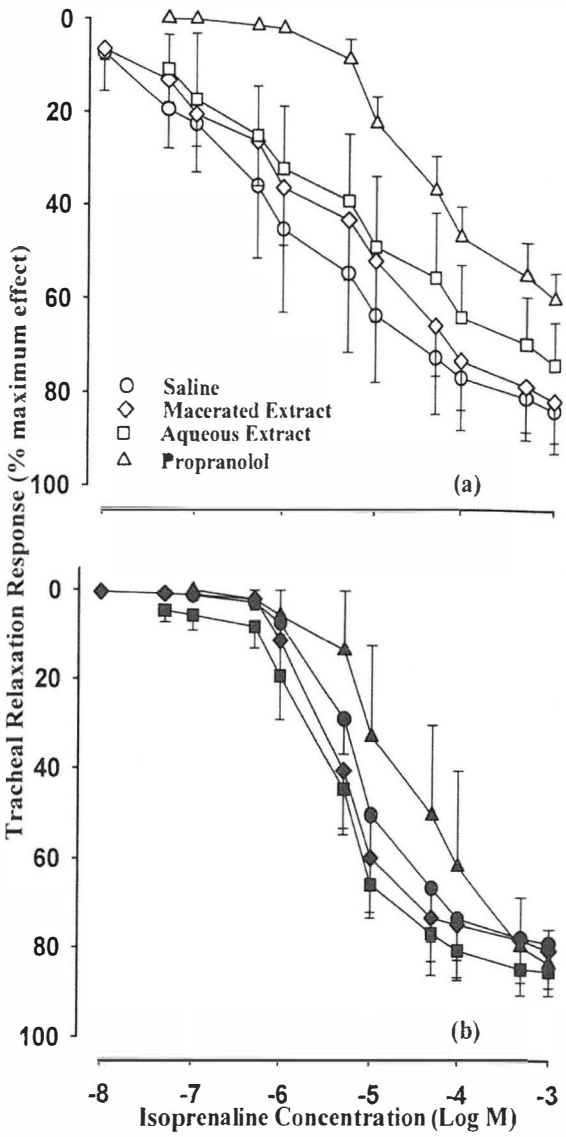

Fig. 2. Cumulative log concentration-response curves of isoprenaline induced relaxation of pre-contracted isolated guinea pig tracheal chains, in the presence of saline, macerated extract, aqueous extract, and propranolol on (a) non incubated preparation (n=7), and (b) incubated tissues atropine and chlorpheniramine (n= 5).

To evaluate the stimulatory effect of extracts on β_2-adrenoceptors more precisely, this effect of *Nigella sativa* was also examined on incubated tracheal preparation with chlorpheniramine and atropine to block both histamine H_1 and muscarinic receptors, (group 2 experiments). The results of group 2 experiments indicated a stimulatory effect of aqueous extract and a possible stimulatory effect of macerated extract on β_2-adrenoceptors (parallel leftward shift in isoprenaline response curves obtained in the presence of extracts and

Table I. EC50 (μM) of isoprenaline in the presence of extracts from *Nigella sativa*, 50 nM propranolol, and saline in two sets of experiments.

Different Solutions	Group 1		Group 2	
Saline	3.91±1.81		5.10±0.68	nS
Macerated Extract	7.15±2.63	NS	6.65±3.57	nS
	+++	NS	+++	nS
Aqueous Extract	6.24±3.47	NS	2.60±0.47	*
	+++		+++	i
Propranolol	30±6.52	***	35.00±13.79	***
				nS

Values are presented as mean±SEM. group 1 : experiments on non-incubated tracheal chains (n=7); group 2: experiments on tracheal chains incubated with 1 μM atropine and 1 μM chlorpheniramine (n=5). Statistical difference between extracts and saline; NS: non-significant difference, *: $p<0.05$, ***: $p<0.001$ .Statistical difference between the effects of extracts and propranolol; Ns: non-significant difference,+++: $p<0.001$. Statistical difference between two groups of experiments; nS: non-significant difference; i : $p<0.01$.

Table II. Maximum response to isoprenaline obtained in the presence of extracts from *Nigella sativa*, 50 nM propranolol, and saline in the two sets of experiments.

Different Solutions	Group 1		Group 2	
Saline	83.71±7.62		79.00±7.23	
Macerated Extract	81.57±8.85	NS	80.60±8.32	NS
		Ns		Ns
Aqueous Extract	73.85±6.26	NS	85.20±4.14	NS
		Ns		Ns
	nS			
Propranolol	59.85±3.91	NS	82.40±6.04	NS
				nS

For abbreviations see Table I.

Table III. Slope of isoprenaline Log concentration-response curves in the presence of extracts from *Nigella sativa*, 50 nM propranolol, and saline in two sets of experiments.

Different Solutions	Group 1		Group 2	
Saline	-0.89±0.21		-1.58 ±0.41	
Macerated Extract	-0.96± 0.21	NS	-1.24± 0.50	NS
	Ns		Ns	nS
Aqueous Extract	-0.78± 0.17	NS	-1.23± 0.13	NS
	Ns		Ns	i
Propranolol	-1.13± 0.17	NS	-1.71±0.66	NS
				nS

For abbreviations see Table I.

achievement of the maximum response to isoprenaline).[15-17] The difference in slope of response curves between two groups of experiments is unclear to us but it could be due to some interference of atropine and/or

chlorpheniramine on β_2-adrenoceptors.

In previous studies, a clear blocking effect of *Nigella sativa* on histamine H_1 receptors and a functional antagonism effect at muscarinic receptors of guinea pig tracheal chains has been demonstrated.[11,12] In addition, the existence of α-pinene in essential oil of this plant was demonstrated[1] which showed anticholinergic activity.[18] However, from the results of the present study a muscarinic and/or histamine H_1 receptor blocking effect cannot be concluded.

The different β_2-adrenoceptor stimulatory effect of aqueous and macerated extracts from *Nigella sativa* is presumably due to the variation of methods of extraction. However, the differences in ingredients of two extracts and the effect of each fraction (substance) from each extract should be clarified in further studies.

In conclusion, the results of this study showed a clear stimulatory effect of aqueous extract and a possible stimulatory effect of macerated extract from *Nigella sativa* on β_2-adrenoceptors.

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