

## Possible Relaxant Effects of Thymoquinone on Guinea Pig Tracheal Chains

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Received 6 November 2004; revised 25 January 2005; accepted 6 March 2005

### ABSTRACT

In the previous studies the relaxant, anti-cholinergic (functional antagonism) and anti-histaminic effects of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. To elucidate the main substance responsible for the relaxant effect of this plant, the effect of thymoquinone, one of the main constituent of *Nigella sativa* was examined in this study. The bronchodilatory effects of three cumulative concentrations (40, 80, and 120  $\mu$ M) of thymoquinone were examined by their relaxant effects on precontracted tracheal chains of guinea pigs using (1) 10  $\mu$ M methacholine and (2) 60 mM KCl. The results were compared with the effects of saline, theophylline, and extracts (macrated and aqueous) of *Nigella sativa* ( $n = 5$  for each group). The results showed significant relaxant effects of theophylline and extracts from *Nigella sativa* as compared to saline in group 1 experiments ( $P < 0.05$  and  $P < 0.005$ ). There were no significant differences between the effects of two higher concentrations of extracts with those of theophylline. However, none of the three thymoquinone concentrations showed any relaxant effect in both groups. There were also significant differences between the relaxant effect of theophylline and extracts with those of thymoquinone concentrations ( $P < 0.05$  and  $P < 0.001$ ). In group 2, only theophylline showed a significant relaxant effect ( $P < 0.05$  and  $P < 0.001$ ). The effects of two higher concentrations of both extracts and thymoquinone were significantly lower than those of theophylline in group 2 ( $P < 0.05$  to  $P < 0.001$ ). These results indicated that the relaxant effect of *Nigella sativa* is not due to its constituent thymoquinone. *Iran. Biomed. J.* 9 (3): 123-128, 2005

**Keywords:** Thymoquinone, *Nigella sativa*, Relaxant effect, Tracheal chain, Guinea pig

### INTRODUCTION

*Nigella sativa* (black seed) is a grassy plant with green to blue flowers and small black seeds, which grows in temperate and cold climate areas. This plant grows in Arak, Isfahan and other city of Iran. The seeds of *Nigella sativa* contain thymoquinone, monotropens such as *p*-cymene and  $\alpha$ -pinene [1], nigellidine [2], nigellimine [3] and a saponin [4].

Several therapeutic effects including antiasthma and dyspnea have been described for the seeds of *Nigella sativa* in Iranian ancient medical books. In Arabian folk medicine, the whole black seeds alone or in combination with honey are also prompted for treatment of bronchial asthma. There is evidence of relaxant effects of the volatile oil from this plant on different smooth muscle preparations including

rabbit aorta [5], rabbit jejunum [6], and guinea pig isolated tracheal muscle [7]. Mahfouz and EL-Dakhakhnsy [8] 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. 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 [9].

The results of previous studies also showed a relaxant effect of this plant on isolated guinea pig tracheal chains. The functional antagonistic effect of this plant on muscarinic receptors [10], inhibitory effect on histamine ( $H_1$ ) receptors [11], inhibitory effect on calcium channels [12], opening effect on potassium channels [13] and stimulatory effect on  $\alpha$ -adrenergic receptors [14] of guinea pig

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tracheal chains were studied. In this report, we studied the relaxant effect of thymoquinone on guinea pig tracheal chains.

## MATERIALS AND METHODS

**Plant and extracts.** Botanists in the Herbarium Dept. of Ferdowsi University of Mashhad (Iran) identified *Nigella sativa* and the specimen number of the plant was determined as 293-0303-1.

The aqueous extract was prepared as follow: chopped dried seeds of the plant (50 g) were extracted with 300 ml distilled water by suxhelat apparatus. For macerated extract, the same amount of seeds was macerated with 300 ml distilled water and shaken on a shaker for 48 h. The solvent of both extracts was removed under reduced pressure until the extracts volume reached 20 ml. In the final extract, the concentration was 10% W/V in both extracts.

**Tissue preparations.** Male guinea pigs (400-700 g) were killed 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 [15]. Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliet solution of the following composition in mM: NaCl, 120; NaHCO<sub>3</sub>, 25; MgSO<sub>4</sub>, 0.5; KH<sub>2</sub>PO<sub>4</sub>, 1.2; KCl, 4.72; CaCl<sub>2</sub>, 2.5 and dextrose, 11. The Krebs solution was maintained at 37°C and gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

**Protocols.** The relaxant effects of three cumulative concentrations of thymoquinone (Sigma, UK), (40, 80, and 120 µM), theophylline anhydrous (Sigma, UK) (0.25, 0.5, 0.75 mM), extracts from *Nigella sativa* (0.25, 0.5, and 1 g% W/V for both aqueous and macerated extracts), and 1 ml saline as negative control were examined. To produce different concentrations of thymoquinone 0.04 ml of 1 mM and for theophylline, 0.25 ml of 10 mM concentrated solutions was added to a 10 ml organ bath three times. For producing concentrations of extracts 0.25, 0.25 and 0.5 ml of 10 W/V concentrated of each extracts were added to organ bath. The consecutive volumes were added

to organ bath at five minutes intervals.

In each experiment, the effects of three cumulative concentrations from one of the solutions (thymoquinone, theophylline, extracts or saline) on contracted tracheal smooth muscle were measured. A decrease in tone was considered as relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to maximum contraction obtained due to contractile substance; and an increase in tone was considered as contractile (bronchoconstrictory) effect, which was expressed as negative percentage change [16].

The relaxant effect of different solutions was tested with two different experimental designs as follows. Group 1: On tracheal chains contracted by 10 µM methacholine hydrochloride (Sigma, UK). Group 2: On contracted tracheal chains by 60 mM KCl.

The relaxant effects in both groups were examined in two different series of tracheal chains (for each group, n = 5). All of the experiments were performed randomly with a 1 h 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) and measured after fixation.

**Statistical analysis.** The data of relaxant effect of different experiments were expressed as mean ± SEM. The data of relaxant effects of different concentrations of thymoquinone were compared with the results of negative and positive control and extracts from *Nigella sativa* using one-way analysis of variance (ANOVA) test. The data of relaxant effect obtained in both groups were compared using unpaired student's *t*-test. The relaxant effect of thymoquinone, extracts and theophylline was related with concentrations of the each solution, using least square regression. Significance was accepted at *P*<0.05.

## RESULTS

**Relaxant effect in group 1.** In group 1, all concentrations of both extracts and two higher concentrations of theophylline showed a significant relaxant effect compared to the effect of saline. There were no significant differences between the effects of two higher concentrations of extracts with those of theophylline (*P*<0.05 and *P*<0.001, Table 1). However, thymoquinone did not show any significant relaxant effect as compared to the effect of saline. The effects of two higher concentrations

**Table 1.** Relaxant effect of thymoquinone and extracts from *Nigella sativa* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (contracted tracheal chains by 10  $\mu$ M methacholine).

Different concentrations	Saline	Thymoquinone	Aqueous extract	Macerated extract	Theophylline
1	-2.00 $\pm$ 0.95	-3.71 $\pm$ 0.96	16.21 $\pm$ 4.49	16.98 $\pm$ 3.13	1.84 $\pm$ 0.29
St. Dif. vs S.		NS	$P < 0.001$	$P < 0.001$	NS
St. Dif. vs The.		NS	$P < 0.001$	$P < 0.001$	
St. Dif. vs A.E.				NS	
2		-3.94 $\pm$ 1.12	23.62 $\pm$ 4.39	21.08 $\pm$ 5.19	11.78 $\pm$ 2.69
St. Dif. vs S.		NS	$P < 0.001$	$P < 0.001$	$P < 0.05$
St. Dif. vs The.		$P < 0.05$	NS	NS	
St. Dif. vs A.E.				NS	
3		-6.08 $\pm$ 0.83	37.01 $\pm$ 12.67	29.32 $\pm$ 6.14	34.05 $\pm$ 4.44
St. Dif. vs S.		NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
St. Dif. vs The.		$P < 0.001$	NS	NS	
St. Dif. vs A.E.				NS	

Values are presented as mean  $\pm$  SEM. Tested concentrations for thymoquinone were; 40, 80, and 120 nM and for theophylline 0.25, 0.5 and 1 mM. For assessing relaxant effect of extracts, 0.25, 0.5, and 1 g% W/V and for saline, 1 ml was used (for each group, n = 5). St. Dif. vs S., Statistical differences between the effect of saline and other solutions; St. Dif. vs The, Statistical differences between the effect of thymoquinone and extracts with those of theophylline; St. Dif. vs A.E., Statistical differences between the effect of aqueous and macerated extracts; NS, non-significant difference.

of thymoquinone were significantly lower than those of theophylline ( $P < 0.05$  to  $P < 0.001$ , Table 1). In addition there were significant differences between the effects of all concentrations of thymoquinone and extracts ( $P < 0.01$  to  $P < 0.001$ ). There was no significant difference in the effects of different concentrations between two extracts (Table 1).

**Relaxant effect in group 2.** In group 2, only 2 higher concentrations of theophylline showed a significant relaxant effect compared to that of saline ( $P < 0.05$  to  $P < 0.001$ , Table 2). The different

concentrations of extracts and thymoquinone did not show any significant relaxant effect compared to that of saline (Table 2). There were significant differences between the effects of two higher concentrations of both extracts with those of theophylline ( $P < 0.05$  to (or and)  $P < 0.001$ , Table 2). The relaxant effects of two higher concentrations of both extracts were significantly less negative than those of thymoquinone ( $P < 0.01$  for all cases). There were no significant differences in the effects of different concentrations between two extracts (Table 2).

**Table 2.** Relaxant effect of thymoquinone and extracts from *Nigella sativa* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (contracted tracheal chains by 60 mM KCl).

Different concentrations	Saline	Thymoquinone	Aqueous extract	Macerated extract	Theophylline
1	-1.40 $\pm$ 0.75	-5.95 $\pm$ 2.67	-0.20 $\pm$ 0.49	-0.40 $\pm$ 0.68	-4.35 $\pm$ 2.43
St. Dif. vs S.		NS	NS	NS	NS
St. Dif. vs The.		NS	NS	NS	
St. Dif. vs A.E.				NS	
2		-7.84 $\pm$ 2.28	-0.20 $\pm$ 0.80	-0.40 $\pm$ 0.98	14.61 $\pm$ 7.44
St. Dif. vs S.		NS	NS	NS	$P < 0.05$
St. Dif. vs The.		$P < 0.01$	$P < 0.01$	$P < 0.01$	
St. Dif. vs A.E.				NS	
3		-7.60 $\pm$ 1.53	-0.14 $\pm$ 0.66	-0.28 $\pm$ 0.72	51.00 $\pm$ 7.13
St. Dif. vs S.		NS	NS	NS	$P < 0.001$
St. Dif. vs The.		$P < 0.001$	$P < 0.001$	$P < 0.001$	
St. Dif. vs A.E.				NS	

Values are presented as mean  $\pm$  SEM. Tested concentrations for thymoquinone were; 40, 80, and 120 nM and for theophylline 0.25, 0.5 and 1 mM. For assessing relaxant effect of extracts, 0.25, 0.5, and 1 g% W/V and for saline, 1 ml was used (for each group, n = 5). St. Dif. Vs S., Statistical differences between the effect of saline and other solutions; St. Dif. Vs The, Statistical differences between the effect of thymoquinone and extracts with those of theophylline; St. Dif. Vs A.E., Statistical differences between the effect of aqueous and macerated extracts; NS, non-significant difference.

**Table 3.** Correlation between the relaxant effects of thymoquinone, extracts from *Nigella sativa* and theophylline with concentration in groups 2 experiments.

Experimental groups	Group 1		Group 2	
	r	P value	r	P value
Thymoquinone	0.286	NS	0.068	NS
Aqueous extract	0.468	$P<0.05$	0.020	NS
Macerated extract	0.450	$P<0.05$	0.033	NS
Theophylline	0.907	$P<0.001$	0.848	$P<0.001$

NS, non-significant difference.

**Comparison of the relaxant effect between two groups.** The relaxant effects of thymoquinone and theophylline were not significantly different between two groups of experiments. However, the effects of all concentrations of both extracts in group 2 were significantly lower than those of group 1.

**Correlation between the relaxant effect and concentrations.** There were significant correlations between the relaxant effect and concentrations of theophylline and both extracts in group 1 ( $P<0.05$  to  $P<0.001$ ). The correlation between the relaxant effects and concentrations of theophylline in group 2 was also statistically significant ( $P<0.001$ ). However, there were no any significant correlations between the relaxant effects and concentrations for thymoquinone in both groups of experiments and extracts in group 2 (Table 3).

## DISCUSSION

In the present study, the relaxant (bronchodilatory) effects of thymoquinone in comparison with extracts (aqueous and macerated) from *Nigella sativa*, theophylline, and saline were studied. Theophylline was used as a positive control with a non-specific relaxant property on tracheal chains. In group 1 (contracted tracheal chains by methacholine), both extracts showed a significant and concentration dependent relaxant effect. The effects of all concentrations of extracts were comparable to those of theophylline. In fact, the effects of first concentration of both extract in group 1 were significantly greater than that of theophylline. The results of group 1 confirm the findings of our previous study indicating the relaxant effect of extracts from *Nigella sativa* [10]. However, thymoquinone did not show any relaxant effect in this group. The effects of all concentrations of thymoquinone were significantly lower than those

of both extracts.

In group 2, thymoquinone and both extracts from *Nigella sativa* did not show any relaxant effect on tracheal chains of guinea pig. The effect of two higher concentrations of thymoquinone and extracts were significantly lower than those of theophylline. There were no significant differences between the effect of thymoquinone and extracts.

Since KCl affects calcium channels [17] and with regard to the bronchodilatory effect of calcium channel blockers [18, 19], these findings showed the absence of a blocking effect of the extracts on calcium channels. The absence of obvious relaxant effect of aqueous and macerated extracts from this plant in group 2 and the relatively potent relaxant effect of this extract in groups 1 and 2 may also indicate an opening effect of these fractions on potassium channels because the bronchodilatory effect of potassium channel opening has been demonstrated previously [20]. If the aqueous and macerated extracts from *Nigella sativa* had a potassium channel opening effect, they would not have relaxant effect on tracheal chains contracted by KCl, while they could show relaxant effect when the tracheal chain was contracted by metacholine. In fact, the results of groups 1 and 2 may support the effects of aqueous and macerated extracts. However, the effect of this plant on potassium channel should be examined in further studies.

The results of our previous studies [10-14] and present study suggest that anticholinergic, histamine  $H_1$  inhibitory,  $\alpha$ -adrenoceptor stimulatory and potassium channel opening effects of *Nigella Sativa* may contribute to bronchodilatory effect.

The other possible mechanisms responsible for bronchodilatory effect of *Nigella sativa* are as follows: Stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC [21], methylxanthine activity of the plant [22] and inhibition of phosphodiesterase [23]. The contribution of these mechanisms and the importance of those seen in

our studies in the bronchodilatory effect of *Nigella sativa* should be clarified in further studies.

Although the seeds of *Nigella sativa* contain different active substances, only thymoquinone is widely studied and showed to have different pharmacological effect including; antioxidant and protective effect against hepatotoxicity [24, 25], inhibitory effect on eicosanoid generation [26], anti-carcinogenic effect [27], inhibitory effect on hyperlipidemic nephropathy [28] and anti-nociceptive effect [29].

In conclusion, the results of the present study showed that the relaxant effect of *Nigella sativa* on tracheal chains of guinea pig is not due to its main constituent, thymoquinone. However the results suggested a potassium channel opening effect for this plant which may contribute in its bronchodilatory effect.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. H. Hosseinzadeh for providing thymoquinone.

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