

Short communication

# Relaxant effect of *Pimpinella anisum* on isolated guinea pig tracheal chains and its possible mechanism(s)

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## Abstract

We have studied the relaxant effect of *Pimpinella anisum* on isolated guinea pig tracheal chains and its possible mechanism(s). The bronchodilatory effects of aqueous and ethanol extracts and essential oil were examined on precontracted isolated tracheal chains of the guinea pig by 10  $\mu\text{M}$  methacholine in two different conditions including: non-incubated tissues (group 1) and incubated tissues with 1  $\mu\text{M}$  propranolol and 1  $\mu\text{M}$  chlorpheniramine (group 2). In addition, the anticholinergic effects of essential oil and 10 nM atropine were tested by comparing the cumulative log concentration–response curves of methacholine induced contraction of tracheal chains and the effective concentration of methacholine, causing 50% of maximum response ( $\text{EC}_{50}$ ) in the presence of essential oil or atropine. Aqueous and ethanol extracts, essential oil and theophylline (1 mM) showed significant relaxant effects compared to those of controls. Although relaxant effect of essential oil was lower than theophylline, there was no significant difference between the effect of aqueous and ethanol extracts and that of theophylline. There was also no significant difference between the relaxant effects obtained in group 1 and 2 experiments. The results also showed parallel rightward shifts of methacholine–response curves and significant increase in  $\text{EC}_{50}$  with the presence of atropine or essential oil. These results indicated bronchodilatory effects of essential oil, aqueous, and ethanol extracts from *P. anisum*. The results also showed that the relaxant effect of this plant is not due to an inhibitory effect of histamine ( $\text{H}_1$ ) or stimulatory effect of  $\beta_2$ -adrenergic receptors, but due to inhibitory effects on muscarinic receptors. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *Pimpinella anisum*; Bronchodilatory; Anticholinergic; Guinea pig

## 1. Introduction

*Pimpinella anisum* is a grassy plant with white flowers and small green to yellow seeds which grows in Egypt, Turkey, and Greece. The seeds of *P. anisum* contain anethole (Chandler and

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Hawkes, 1984), pseudoisoeugenol (Reichling et al., 1995), coumarins, scopoletin, umbelliferon, estrols (Burkhardt et al., 1986), terpene hydrocarbons (Kartnig et al., 1975), polyenes and polyactylenes (Schulte et al., 1970).

Several therapeutic effects including those on digestive disorders, gynaecologic, and also anti-convulsant, anti-asthma and dyspnea have been described for the seeds of *P. anisum* in Iranian ancient medical books (Aboabrahim, 1970).

There is evidence of relaxant effects of volatile oil from this plant on isolated tracheal muscles of guinea pigs (Reiter and Brandt, 1985). However, the contractile effect of anethole, the major constituent of this plant on ileal smooth muscle of guinea pigs has also been demonstrated (Reiter and Brandt, 1985). In contrast it has been shown that anethole has a relaxant effect on skeletal muscle (Albuquerque et al., 1995).

In the present study the relaxant effects of essential oil, aqueous and ethanol extracts of this plant in comparison with both saline and theophylline were studied by a more standard method (Martin et al., 1994). In addition the anticholinergic effect of essential oil of *P. anisum* in comparison with atropine was also studied. The  $\beta_2$  adrenergic receptor stimulatory and  $H_1$  histamine receptor blocking effects of essential oil and plant extracts were also indirectly examined.

## 2. Materials and methods

### 2.1. Plant and extracts

*P. anisum* was identified by Botanists in the herbarium of Ferdowsi University, Mashhad and the specimen number of the plant is 7701. The plant extracts were prepared as follow: For aqueous extract, 50 g of the chopped, dried plant were extracted with 300 ml distilled water by suxhelat apparatus. Ethanol extract was prepared the same as aqueous extract except the solvent was ethanol instead of distilled water. The solvent of both extracts were then removed under reduced pressure until the extract volume reached 20 ml. Plant ingredient concentration in the final

extracts were 33.3% W/V in both aqueous and ethanol extracts. From 100 g of the chopped, dried plant with 1000 ml distilled water, 1 ml essential oil was extracted by steam distilled apparatus. The concentration of plant ingredients in essential oil was 10% V/V.

### 2.2. Tissue preparations

Male guinea pigs (400–700 g) were killed by a blow on the neck and trachea 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 (Holroyde, 1986). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs–Henseliet solution of the following composition (mM): NaCl 120,  $\text{NaHCO}_3$  25,  $\text{MgSO}_4$  0.5,  $\text{KH}_2\text{PO}_4$  1.2, KCl 4.72,  $\text{CaCl}_2$  2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

### 2.3. Protocols

The relaxant effects of 0.02 ml essential oil, 0.6 ml of aqueous and 0.1 ml ethanol extracts from *P. anisum* and 0.1 ml theophylline anhydrous as positive control (Sigma, UK) at 0.1 M concentration and anticholinergic effects of the same volume of essential oil and 0.1 ml atropine sulphate (Sigma) at 1  $\mu\text{M}$  concentration were examined. Saline (0.6 ml) was used as negative control for essential oil and aqueous extract, and ethanol (0.1 ml) for ethanol extract.

#### 2.3.1. Relaxant effect

In each experiment the effect of one of the solutions on contracted tracheal smooth muscle with 10  $\mu\text{M}$  methacholine hydrochloride (Sigma) was measured after exposing tissue to the solution for 10 min.

A decrease in tone was considered as relaxant effect and expressed as positive percentage change in proportion to maximum contraction obtained due to 10  $\mu\text{M}$  of methacholine; and an increase in tone was considered as contractile effect which was expressed as negative percentage change (Martin et al., 1994).

The relaxant effect of *P. anisum* was tested with two different experimental designs as follows: On non-incubated tracheal chains (group 1 experiments), and on incubated tracheal chains 30 min prior to beginning and during the testing relaxation of different solutions with 1  $\mu\text{M}$  propranolol hydrochloride (Sigma) and 1  $\mu\text{M}$  chlorpheniramine maleate (Sigma), (group 2 experiments).

### 2.3.2. Anticholinergic effect

The anticholinergic effect of essential oil and 10 nM atropine was examined by producing cumulative log concentration–response curve of methacholine induced contraction of tracheal chain 10 min after exposing tissue to each solution. The effective concentration of methacholine causing 50% of maximum response ( $\text{EC}_{50}$ ), the slope of the curves and the maximum response to methacholine were then compared to those of saline.

In experiments with parallel or approximately parallel shift in methacholine response curve, the concentration-ratio minus one (CR-1) was calculated by the following equation:

$$\frac{\text{EC}_{50} \text{ obtained in the presence of effective solutions}}{\text{EC}_{50} \text{ obtained in the presence of saline}} - 1$$

The relaxant effect in group one and two experiments, and the anticholinergic effect of different solutions were examined in three different series of tracheal chains (for each experimental condition,  $n = 8$ ). 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 after fixation were measured.

## 2.4. Statistical analysis

The data of bronchodilatory effect,  $\text{EC}_{50}$ , maximum response to methacholine, and the slope of methacholine response curve of different experiments were expressed as mean  $\pm$  SEM. The data of bronchodilatory effects of extracts and essential oil,  $\text{EC}_{50}$ , the slope, and maximum response of the curves obtained in the presence of essential oil were compared with the results of negative and positive control using a paired *t*-test. The data of bronchodilatory effect obtained in group 2 experiments were compared with those of group 1 using unpaired *t*-test. Significance was accepted at  $P < 0.05$ .

## 3. Results

### 3.1. Bronchodilatory effect

Essential oil, aqueous, and ethanol extracts of *P. anisum* showed potent relaxant effects on tracheal chains of guinea pig. Bronchodilatory effects of ethanol extract, essential oil, and aqueous extract were statistically significant as compared to those of control (Table 1). The relaxant effects of aqueous, and ethanol extracts were not significantly different from that of theophylline but the effect of essential oil was significantly lower than theophylline. In addition, there was no significant difference between the relaxant effect of extracts and essential oil in two experimental conditions (Table 1).

### 3.2. Anticholinergic effect

Cumulative log concentration–response curves of methacholine obtained in the presence of essential oil and atropine showed a clear, parallel, rightward shift, but the shift in methacholine–response curve obtained in the presence of atropine was greater (Fig. 1). The slope of methacholine–response curves obtained in the presence of both essential oil and atropine were not significantly different from that of saline (Table 2).

The maximum response of tracheal smooth muscle to methacholine obtained in the presence

Table 1

Relaxant effect of extracts and essential oil from *Pimpinella anisum* in comparison with negative control (ethanol for ethanol extract, saline for aqueous extract and essential oil) and positive control (theophylline)<sup>a</sup>

Exp. group		Saline	Ethanol	Aqueous extract	Ethanol extract	Essential oil	Theophylline
1	Relaxant effect	$-0.13 \pm 0.3$	$23.6 \pm 4.5$	$46.9 \pm 10.3$	$74.1 \pm 5.7$	$19.9 \pm 4.7$	$56.8 \pm 7.5$
	St. sig. vs. neg. cont.			$P < 0.005$	$P < 0.001$	$P < 0.005$	
	St. sig. vs. pos. cont.	$P < 0.001$	$P = 0.005$	NS	NS	$P = 0.002$	
	cont.						
2	Relaxant effect	$-1.9 \pm 3.0$	$30.9 \pm 6.8$	$55.3 \pm 12.2$	$70.5 \pm 8.0$	$16.0 \pm 7.2$	$54.6 \pm 7.8$
	St. sig. vs. neg. cont.			$P < 0.005$	$P < 0.001$	$P < 0.05$	
	St. sig. vs. pos. cont.	$P < 0.001$	$P < 0.05$	NS	NS	$P < 0.02$	
	cont.						
	St. sig. vs. group 1	NS	NS	NS	NS	NS	NS

<sup>a</sup> Values are presented as mean  $\pm$  SEM. Exp., experimental; St. sig., statistical significance; Neg. cont., negative control; Pos. cont., positive control; NS, nonsignificant difference. For both groups of experiments,  $n = 8$ .

of essential oil and atropine were also not significantly different with that of saline (Table 2). However, the  $EC_{50}$  of methacholine obtained in the presence of essential oil and atropine were significantly higher than that for saline (Table 2).

### 3.3. Comparison between anticholinergic effect of *P. anisum* and atropine

The  $EC_{50}$  obtained in the presence of essential oil from *P. anisum* was significantly lower than that of atropine (Table 2). The (CR-1) produced due to essential oil also showed a significant difference as compared with that of atropine (Table 2).

## 4. Discussion

Essential oil, aqueous, and ethanol extracts of this plant showed relatively potent relaxant effects compared to the effect of saline in group 1 and 2 experiments, supporting the previous study which demonstrated relaxant effect of volatile oil from *P. anisum* on isolated tracheal and ileal smooth muscle of the guinea pig (Reiter and Brandt, 1985). The relaxant effects of aqueous, and ethanol extracts were comparable with that of

theophylline, but the effect of essential oil was lower at examined concentrations. Obviously, with increasing the concentration of essential oil a similar effect to theophylline could be achieved.

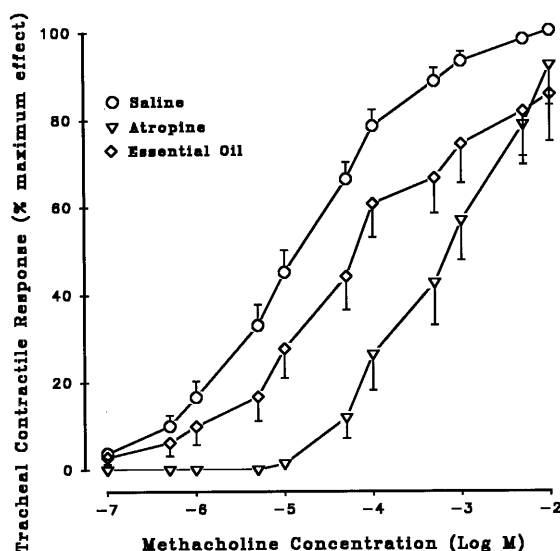


Fig. 1. Cumulative log concentration–response curves of methacholine induced contraction of isolated guinea pig tracheal chains, in the presence of saline, atropine, and essential oil of *Pimpinella anisum* ( $n = 8$ ). Both atropine and essential oil showed parallel rightward shifts in methacholine response curves compared to the curve obtained in the presence of saline.

Table 2

Values of  $EC_{50}$  of methacholine ( $\mu$ M), maximum response, and slope of methacholine–response curves obtained in the presence of essential oil from *Pimpinella anisum*, 10 nM atropine, and saline and (CR-1) produced by atropine and essential oil ( $n = 8$ )<sup>a</sup>

Solutions	$EC_{50}$	Slope	Maximum response	CR-1
Saline	$19.4 \pm 5.2$	$1.26 \pm 0.07$	100	–
Atropine st. sig. vs. saline	$590.6 \pm 128.3$ $P < 0.005$	$1.46 \pm 0.20$ NS	$92.2 \pm 9.04$ NS	$46.3 \pm 14.45$ –
Essential oil st. sig. vs. saline st. sig. vs. atropine	$59 \pm 10.5$ $P < 0.01$ $P = 0.002$	$1.20 \pm 0.15$ NS NS	$85.5 \pm 10.8$ NS NS	$2.9 \pm 1.22$ – $P < 0.02$

<sup>a</sup> For abbreviations, see Table 1.

The lower relaxant effect observed for essential oil compared to those of two extracts in both experimental conditions could be due to lower volume of essential oil, existence of greater anethole which have a contractile effect on smooth muscle (Reiter and Brandt, 1985) or lower effective substance(s) in essential oil than extracts. This is presumably due to the variation of methods of extraction between extracts and essential oil. The most probable explanation for this finding could be the existence of anethole in essential oil of this plant because the effective substances is usually greater in essential oil than other extracts of plants. Indeed, in previous studies, the greater bronchodilatory effect for essential oil than those of other extracts for some plants have been observed (Boskabady et al., 1998; Boskabady and Talebi 1999). The relaxant effect of ethanol may suggest that the effect of ethanol extract might be due to its solvent content. However, the relaxant effect of ethanol extract was significantly higher than the same volume of ethanol.

The bronchodilatory effect of *P. anisum* might be produced due to several different mechanisms including stimulation of  $\beta$  adrenergic receptors, inhibition of histamine  $H_1$  receptors or anticholinergic property of this plant. To evaluate the contribution of  $\beta$  adrenergic stimulatory and/or  $H_1$  histamine blocking effect of this plant on its bronchodilatory effects, the effects of essential oil, aqueous and ethanol extracts from *P. anisum* were re-examined on inhibited  $\beta$  adrenergic and  $H_1$  histamine receptors by propranolol and chlorpheniramine in group 2 experiments. There was no significant difference between group 1 and 2

experiments in relaxant effects of plant's solutions. These findings indicate that the bronchodilatory of this plant is not due to its adrenergic stimulatory and/or histamine  $H_1$  blocking property.

To evaluate the contribution of the inhibitory property of *P. anisum* at muscarinic receptors on its bronchodilatory finding, the anticholinergic effect of essential oil from this plant was also examined on isolated guinea pig tracheal preparations. The clear and parallel rightward shift in methacholine log concentration–response curve obtained in the presence of essential oil compared to methacholine response-curve obtained in the presence of saline and achievement of maximum response in the presence of essential oil observed in this part of the study, indicated a competitive antagonism effect of essential oil from *P. anisum* at muscarinic receptors (Arunlakshana and Schild, 1959; Ariens, 1987; Linden et al., 1993).

However, the competitive antagonism effect (CR-1) produced by essential oil was significantly lower than that of atropine indicating smaller competitive antagonism effect of essential oil compared to atropine at concentration used. Therefore, inhibitory property of the plant at muscarinic receptors may contribute in its bronchodilatory effect. The smaller antagonism effect of essential oil than atropine could be at least, in part, due to its anethole constituents.

With regard to the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, *P. anisum* might also have an anti-inflammatory effect which will contribute to the therapeutic effect of this plant on asthma. In fact, the

antioxidant effect (Rehman et al., 1991) and inhibitory effect of coumarins, a constituent of essential oil from *P. anisum* on prostaglandins biosynthesis (Lee et al., 1981) have been shown. The extracts of *P. anisum* also contain different strolls (Burkhardt et al., 1986) which could have anti-inflammatory effect. However, the effect of *P. anisum* on airway inflammation existing in asthma disease should be investigated in further studies.

In conclusion, the results of this study showed a relatively potent relaxant (bronchodilatory) effect of *P. anisum* on tracheal chains of guinea pig. The  $\beta_2$  adrenergic receptor stimulatory and histamine  $H_1$  receptor blocking property of this plant do not contribute to its relaxant effect on tracheal chains. However, inhibitory property of the plant at muscarinic receptors (anticholinergic effect) may, at least in part, be responsible for this broncodilatory effect.

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