

## Basic Science In Medicine

### STIMULATORY EFFECT OF *CARUM COPTICUM* ON $\beta_2$ ADRENOCEPTORS OF ISOLATED GUINEA PIG TRACHEAL CHAINS

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#### ABSTRACT

The  $\beta_2$ -adrenergic effects of macerated extract, aqueous extract, ethanol extract, and essential oil of *Carum copticum*, 5 nM propranolol, and saline were tested by performing cumulative Log concentration-response curves of isoprenaline-induced relaxation of precontracted isolated guinea pig tracheal chains in three different conditions including: non-incubated (group 1, n=9); incubated with 1  $\mu$ M chlorpheniramine and 1  $\mu$ M atropine (group 2, n=8); and incubated with 1  $\mu$ M chlorpheniramine (group 3, n=6). The effective concentration of isoprenaline, causing 50% of maximum response ( $EC_{50}$ ), the maximum response, and the slope of isoprenaline curves obtained in the presence of extracts, essential oil, and propranolol were compared with those of saline.

The results showed clear leftward shifts in isoprenaline curves obtained in the presence of only the ethanol extract compared with those of saline in group 1. In groups 2 and 3 the same finding was observed, although the effect of ethanol extract was tested in the presence of propranolol. The  $EC_{50}$  and maximum response obtained in the presence of ethanol extract were similar to those of saline in all three sets of experiments. However, the maximum response obtained in the presence of other extracts, essential oil, and even propranolol were lower than those for saline in all sets of experiments ( $p < 0.05$  to  $p < 0.001$ ). The  $EC_{50}$ s obtained in the presence of essential oil, macerated and aqueous extracts, and even propranolol were greater than those obtained in the presence of saline in the two last sets of experiments ( $p < 0.05$  to  $p < 0.001$ ). The maximum response obtained in the presence of only ethanol extract in group 3 compared to group 2 was reduced ( $p < 0.05$ ).

The results of this study indicate a stimulatory effect of only the ethanol extract of *Carum copticum* on  $\beta_2$ -adrenoceptors.

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**Keywords:** *Carum copticum*, antihistaminic effect, trachea, guinea pig.

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## INTRODUCTION

*Carum copticum* is a grassy, annual plant which grows in the east of India, Iran and Egypt with white flowers and small brownish seeds. The seeds of this plant have an odor similar to thymol; and its essential oil contains  $\gamma$ -terpinene, p. cymene,  $\alpha$ -pinene,  $\beta$ -pinene and other substances such as thymol and carvacol.<sup>1</sup>

The seeds of *Carum copticum* have several therapeutic effects including diuretic, anti-emetic, analgesic, anti-asthmatic and anti-dyspneic properties.<sup>2</sup> They also have therapeutic effects on some cutaneous, neural, and urinary tract disorders. *Carum copticum* is also used in household remedies. A watery extract of this plant is widely used to relieve gripe in children. In diarrhea either roasted seeds are taken, or a watery extract is made from them and given as a draught.

Previously, a relatively potent relaxant (bronchodilatory) effect of *Carum copticum* on isolated guinea pig tracheal chains has been reported.<sup>3,4</sup> Another study demonstrated that *Carum copticum* has a muscarinic effect on guinea pig ileum, rabbit duodenum and rat jejunum. This study also showed a depressant effect of extract from roasted *Carum copticum* seed on rat and cat blood pressure similar to acetylcholine. All of these effects were blocked by atropine and cholinesterase. The existence of choline and acetylcholine in the extract from roasted *Carum copticum* seed was also shown.<sup>5</sup> The results of our studies<sup>3,4</sup> also showed non-parallel rightward shifts of methacholine Log concentration-response curves in the presence of essential oil, ethanol, and aqueous extracts from this plant which indicated a functional antagonistic effect of *Carum copticum* at muscarinic receptors of tracheal chains.

To elucidate the other mechanisms responsible for this relaxant effect, the stimulatory effect of essential oil, macerated, aqueous, and ethanol extracts from *Carum copticum* on adrenoceptors in comparison with both saline and propranolol was examined in this study.

## MATERIALS AND METHODS

## Plant extracts

A) Macerated extract: Fifty grams of the chopped, dried plant was macerated with 300 mL distilled water and shaken (on a shaker) for 48 hr.

B) Aqueous extract: The same amount of plant was extracted with 300 mL distilled water by suxhelat apparatus.

C) Ethanol extract was prepared similar to aqueous extract except the solvent was ethanol instead of distilled water.

The solvent of all three extracts were then removed under reduced pressure until the extract volume reached 20 mL. Plant ingredient concentrations in the final extracts were 25, 27, and 23% W/W in macerated, aqueous, and

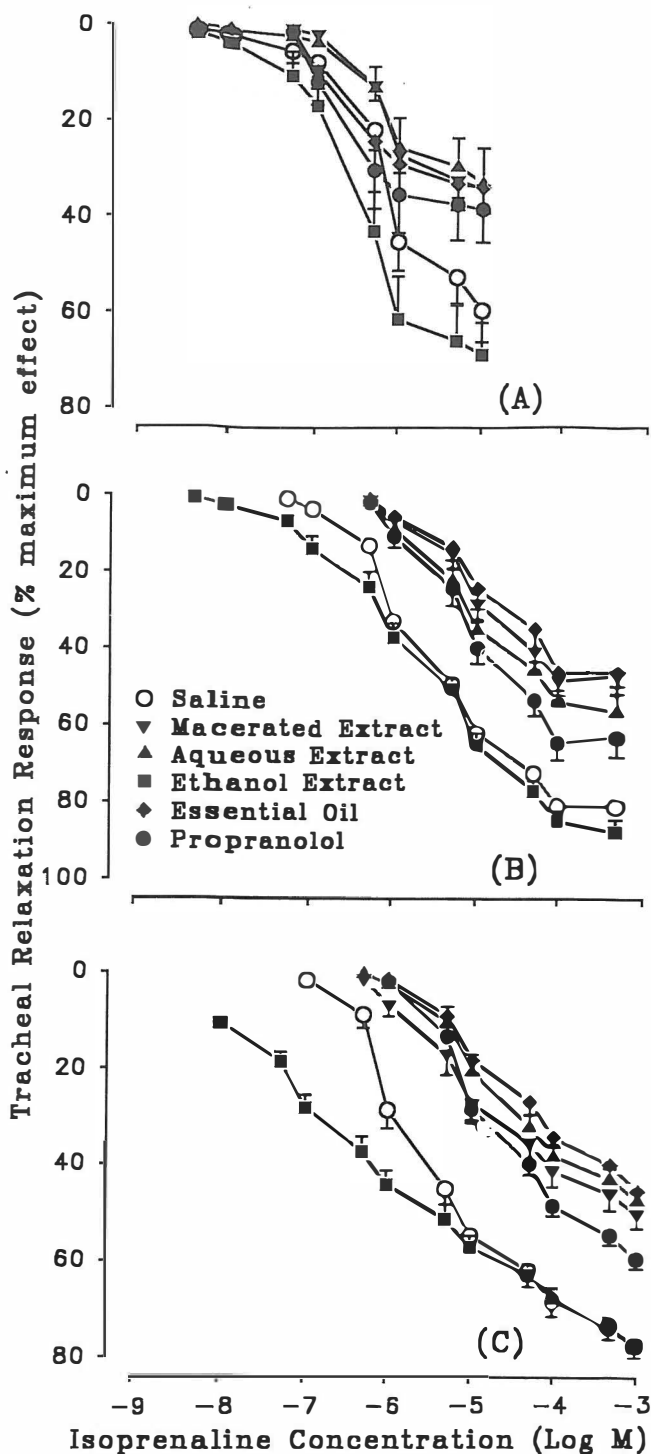


Fig. 1. Cumulative log concentration-response curves of isoprenaline-induced relaxation of precontracted isolated guinea pig tracheal chains in the presence of saline, essential oil, macerated, aqueous, and ethanol extracts and propranolol on A) non-incubated preparation (n=9), and incubated tissues with two different conditions, including B) atropine and chlorpheniramine (n=8), and C) chlorpheniramine (n=6).

Table I. EC<sub>50</sub> (μM) of isoprenaline in the presence of macerated extract (ME), aqueous extract (AE), ethanol extract (EE), essential oil (EO), 5 nM propranolol (P), and saline (S) in three sets of experiments.

Different Solutions	Group 1	St. Dif. vs S	Group 2	St. Dif. vs S	Group 3	St. Dif. vs S	St. Dif. vs group 2
S	0.78 ± 0.16	-	2.31 ± 0.71	-	2.92 ± 0.66	-	NS
ME	1.38 ± 0.53	NS	10.38 ± 1.59	p < 0.005	14.83 ± 2.60	p < 0.005	NS
AE	0.73 ± 0.12	NS	7.63 ± 0.80	p < 0.001	13.17 ± 4.09	p < 0.05	NS
EE	0.92 ± 0.52	NS	3.13 ± 0.86	NS	2.60 ± 0.71	NS	NS
EO	2.01 ± 0.71	NS	14.00 ± 2.93	p < 0.01	16.67 ± 3.07	p < 0.01	NS
P	1.39 ± 0.72	NS	7.13 ± 0.77	p < 0.001	9.83 ± 0.65	p < 0.001	p < 0.05

Values are presented as mean ± SEM. Group 1: experiments on non-incubated tracheal chains (n=9); group 2: experiments on tracheal chains incubated with 1 μM atropine and 1 μM chlorpheniramine (n=8); group 3: experiments on tracheal chains incubated with 1 μM chlorpheniramine (n=6); St. Dif. : statistical difference; NS: nonsignificant.

ethanol extracts respectively.

D) Essential oil (1.5 mL) was extracted from 100 g of the chopped, dried plant in 1000 mL distilled water by steam distilled apparatus. The concentration of plant ingredients in essential oil was 1.5% V/W.

#### Tissue preparations

Male guinea pigs (400-700 g) were killed by a blow on the neck and the 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.<sup>6</sup>

Tissues were 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<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 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 *Carum copticum* on β<sub>2</sub>-adrenoceptors was examined by producing cumulative log concentration-response curves of isoprenaline sulphate (Sigma Chemical Ltd, UK)-induced relaxation of precontracted tracheal chains 10 min after exposing tissue to one solution (macerated and aqueous extracts, 0.3 mL; ethanol extract, 0.05 mL; essential oil, 0.05 mL (100 times

diluted); 0.05 mL propranolol hydrochloride (Sigma Chemical Ltd, UK) of 1 μM concentration, or 0.3 mL saline). The consecutive concentrations were added every 2 min (including 5 nM-1000 μM); and the percentage of relaxation due to each concentration of isoprenaline in proportion to the maximum relaxation obtained in the presence of saline was plotted against the log concentration of isoprenaline.

2) The effective concentration of isoprenaline causing 50% of maximum response (EC<sub>50</sub>) 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, essential oil, and propranolol were examined by comparing the EC<sub>50</sub> 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, essential oil, and propranolol were compared with that of saline.

4) The maximum responses to isoprenaline obtained in the presence of extracts, essential oil, and propranolol were also compared with that of saline.

The stimulatory effect of *Carum copticum* on β<sub>2</sub>-adrenoceptors was tested on three different experimental conditions as follows:

- Non-incubated tracheal chains (group 1, n=9).
- Incubated tracheal chains 30 min prior to beginning and during obtaining the isoprenaline curve with two different experimental conditions:

**Table II.** Slope of isoprenaline Log concentration-response curves in the presence of macerated extract (ME), aqueous extract (AE), ethanol extract (EE), essential oil (EO), 5 nM propranolol (P), and saline (S) in three sets of experiments.

Different Solutions	Group 1	St. Dif. vs S	Group 2	St. Dif. vs S	Group 3	St. Dif. vs S	St. Dif. vs group 2
S	-1.32±0.13	-	-0.90±0.04	-	-0.64±0.02	-	p<0.001
ME	-0.88±0.15	NS	-0.71±0.05	NS	-0.51±0.01	NS	p<0.01
AE	-0.81±0.11	NS	-0.76±0.04	NS	-0.50±0.04	NS	p<0.001
EE	-1.51±0.17	NS	-0.85±0.08	NS	-0.40±0.03	NS	p<0.001
EO	-1.58±0.22	NS	-0.61±0.08	NS	-0.51±0.01	NS	NS
P	-1.89±0.24	NS	-0.88±0.05	NS	-0.65±0.03	NS	p<0.01

Values are presented as mean ± SEM. Group 1: experiments on non-incubated tracheal chains (n=9); group 2: experiments on tracheal chains incubated with 1 μM atropine and 1 μM chlorpheniramine (n=8); group 3: experiments on tracheal chains incubated with 1 μM chlorpheniramine (n=6); St. Dif. : statistical difference; NS: nonsignificant.

I) Incubated tracheal chains with 1 μM atropine sulphate (Sigma Chemical Ltd, UK) and 1 μM chlorpheniramine maleate (Sigma Chemical Ltd, UK) (group 2, n=8).

II) Incubated tracheal chains with 1 μM chlorpheniramine (group 3, n=6).

In group 1 experiments, precontraction of tracheal chains was induced by 10 μM methacholine hydrochloride (Sigma Chemical Ltd, UK) and in the other two groups by 60 mM KCl. In the last two experimental conditions the stimulatory effect of extracts and essential oil on  $\beta_2$ -adrenoceptors was examined in the presence of 5 nM propranolol.

All the experiments were performed randomly with a 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) and measured after fixation.

#### Statistical analysis

The data of EC<sub>50</sub>, the slope of the curves, and maximum response to isoprenaline in different experiments were expressed as mean ± SEM. The EC<sub>50</sub>, the slope, and maximum response obtained in the presence of extracts, essential oil, and propranolol were compared with those obtained in the presence of saline using paired "t" test. The values of EC<sub>50</sub>, the slope, and maximum response obtained in group 3 experiments were compared with those of group 2 using unpaired "t" test.

## RESULTS

### A-Shift in cumulative log concentration-response curves

Cumulative log concentration-response curves of

isoprenaline obtained in the presence of only ethanol extract showed clear leftward shift compared to isoprenaline curves produced in the presence of saline in all three experimental conditions and even in the last two sets of experiments which the isoprenaline-response curves were performed in the presence of propranolol (Fig. 1).

### B-EC<sub>50</sub>

The EC<sub>50</sub> of isoprenaline obtained in the presence of extracts (except for ethanol extract), essential oil, and propranolol in the last two experimental conditions was significantly higher than that for saline (p<0.05 to p<0.001). However, the EC<sub>50</sub>s obtained in the presence of extracts and essential oil in group 2 experiments were not significantly different from those of group 3 (Table I).

### C-Slope of isoprenaline-response curves

The slope of isoprenaline-response curves obtained in the presence of extracts and essential oil from *Carum copticum* in all three experimental conditions was not significantly different from that of saline. However, the slope of the isoprenaline curve obtained in the presence of extracts in group 2 was significantly higher than that of group 3 (p<0.01 to p<0.001) (Table II).

### D-Maximum response to isoprenaline

The maximum response to isoprenaline obtained in the presence of extracts (except for ethanol extract), essential oil, and propranolol were significantly lower than those of saline in all three sets of experiments (p=0.05 to p<0.001). In addition, the maximum response obtained in the presence of ethanol extract in group 2 was significantly higher than that of group 3 (p<0.05) (Table III).

Table III. Maximum response of isoprenaline obtained in the presence of macerated extract (ME), aqueous extract (AE), ethanol extract (EE), essential oil (EO), 5 nM chlorpheniramine (C), and saline (S) in three sets of experiments.

Different Solutions	Group 1	St. Dif. vs S	Group 2	St. Dif. vs S	Group 3	St. Dif. vs S	St. Dif. vs group 2
S	56.62±6.53	-	81.0±2.73	-	77.33±2.50	-	NS
ME	32.94±7.36	$p<0.05$	48.57±4.77	$p<0.001$	47.33±1.93	$p<0.001$	NS
AE	34.12±4.61	$p<0.05$	54.14±3.13	$p<0.001$	50.17±2.93	$p<0.005$	NS
EE	68.79±7.75	NS	84.86±2.50	NS	77.50±1.84	NS	$p<0.05$
EO	33.84±5.32	$p<0.01$	46.43±5.89	$p<0.001$	45.50±1.36	$p<0.001$	NS
C	38.37±6.95	$p<0.05$	64.86±4.39	$p<0.001$	59.50±1.93	$p<0.001$	NS

Values are presented as mean ± SEM. Group 1: experiments on non-incubated tracheal chains (n=9); group 2: experiments on tracheal chains incubated with 1 μM atropine and 1 μM chlorpheniramine (n=8); group 3: experiments on tracheal chains incubated with 1 μM chlorpheniramine (n=6); St. Dif. : statistical difference; NS: nonsignificant.

## DISCUSSION

The bronchodilatory effect seen for *Carum copticum* in our previous study might be due to several different mechanisms.<sup>3,4</sup> One possible mechanism responsible for this effect could be the stimulatory effect of this plant on β<sub>2</sub>-adrenoceptors.<sup>7</sup> The stimulatory effect of the extracts and essential oil from *Carum copticum* was therefore examined on isolated guinea pig tracheal preparations in this study.

The results on non-incubated tracheae showed clear and parallel leftward shifts in isoprenaline log concentration-response curves obtained in the presence of only ethanol extract compared to the curves obtained in the presence of saline (Fig. 1a). The slope of the isoprenaline response curves and the EC<sub>50</sub> of isoprenaline obtained in the presence of extracts, essential oil and propranolol were not statistically different from those obtained in the presence of saline (Table I and II). However, the maximum relaxation effect to isoprenaline was achieved only in the presence of ethanol extract (Table III). The cause of significant differences between the maximum response obtained in the presence of essential oil, macerated, and aqueous extracts is not clear to us because the same finding was also seen in the presence of propranolol (Table III). The parallel leftward shift of the isoprenaline curve and achievement of the maximum response seen in the presence of ethanol extract from *Carum copticum*, indicated a stimulatory effect of this extract on β<sub>2</sub>-adrenoceptors of guinea pig trachea.<sup>7-9</sup>

To evaluate the stimulatory effect of extracts and essential oil on β<sub>2</sub>-adrenoceptors more precisely, this effect of *Carum copticum* was also examined on incubated tracheal

preparations with two different conditions: one with chlorpheniramine and atropine to block both histamine H<sub>1</sub> and muscarinic receptors, and on another occasion with only chlorpheniramine to block histamine H<sub>1</sub> receptors (group 2 and 3 experiments). In addition, the effect of all extracts and essential oil was tested in the presence of propranolol in these two sets of experiments. The isoprenaline-response curves obtained in the presence of ethanol extract in these experimental conditions also showed a parallel shift compared to the curves obtained in the presence of saline and propranolol, while the effect of ethanol extract was tested in the presence of the same concentration of propranolol as testing the effect of propranolol alone. The maximum response to isoprenaline was also achieved in the presence of ethanol extract. These results confirmed the results of group 1, which indicate a β<sub>2</sub>-adrenoceptor stimulatory effect of this extract.

However, the maximum response to isoprenaline obtained in the presence of essential oil, macerated, and aqueous extracts from *Carum copticum* in the last two experimental conditions, similar to group 1, were significantly lower and values of EC<sub>50</sub> were higher than those for saline (Table I and III). These results also confirmed those obtained in group 1 experiments. The nonsignificant difference between the EC<sub>50</sub> obtained in the presence of extracts and essential oil with that of saline in group 1 is perhaps due to muscarinic and/or histamine H<sub>1</sub> receptor blockade of the plant.

Comparison between the slope, EC<sub>50</sub>, and maximum response obtained in group 2 and 3 with that of group 1 was not possible, because precontraction of tracheal chains in

group 1 was induced by methacholine, while in group 2 and 3 by KCl. However, significant reduction in the slope of the curves obtained in incubated tissues with chlorpheniramine (group 3) compared to those of incubated tissue with atropine and chlorpheniramine (group 2) is perhaps due to the depressant effect of atropine on contracted muscle. In addition, in group 3, in the presence of all extracts and essential oil, the plateau of isoprenaline-response curves could not be achieved. The depressant effect of atropine could account for this phenomenon because similar results were also seen in the presence of saline and propranolol. Atropine may potentiate the relaxant effect of isoprenaline and lead to occurrence of a plateau at lower concentrations of isoprenaline. In previous studies, a clear blocking effect of *Carum copticum* on histamine  $H_1$  receptors and a functional antagonistic effect at muscarinic receptors of guinea pig tracheal chains have been demonstrated.<sup>3,4,10</sup> In addition, the existence of  $\alpha$ -pinene in the essential oil of this plant was demonstrated<sup>1</sup> which showed anticholinergic activity.<sup>11</sup> However, from the results of the present study, muscarinic and/or histamine  $H_1$  receptor blockade cannot be concluded.

The different  $\beta_2$ -adrenoceptor stimulatory effect of extracts and essential oil is presumably due to the variation of methods of extraction.

Other possible mechanisms responsible for the bronchodilatory effect of *Carum copticum* are stimulation of the inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC,<sup>12</sup> methyl-xanthine activity,<sup>13</sup> calcium antagonism,<sup>14</sup> opening of potassium channels,<sup>15</sup> and inhibition of phosphodiesterase.<sup>16</sup> The contribution of these mechanisms in the bronchodilatory effect of this plant, especially the effect of essential oil, macerated, and aqueous extracts should be clarified in further studies.

In conclusion, the results of this study showed a clear stimulatory effect of only the ethanol extract of *Carum copticum* on  $\beta_2$ -adrenoceptors.

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