

## *Basic Science In Medicine*

### BRONCHODILATORY AND ANTICHOLINERGIC EFFECTS OF *Carum copticum* ON ISOLATED GUINEA PIG TRACHEAL CHAINS

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#### ABSTRACT

*Carum copticum* is a grassy and annual plant which is believed to have several therapeutic effects, including anti-asthmatic properties.

We have therefore studied the bronchodilatory and anticholinergic effects of 4 mL aqueous extract (AE), 0.05 mL ethanol extract (EE), and 0.005 mL essential oil (EO) of *Carum copticum* and 1 mL of a 5 mM solution of its main ingredient, thymol (T), in comparison with 4 mL of saline (S) on isolated tracheal chains of guinea-pigs ( $w=500-800$  g) in a 50 mL organ bath. The bronchodilatory effects of different solutions were examined by their relaxant effect on isometrically (1 g) precontracted tracheal chains by 10  $\mu$ M methacholine (M). The anticholinergic effects of different solutions were tested by comparing the cumulative log concentration-response curves of tracheal chains to cumulative concentrations of M in the presence of AE, EE, EO, and T with that of S.

The bronchodilatory effects of AE, EE, and EO were significantly higher than S in all cases ( $p<0.001$ ), but T did not show any significant bronchodilatory effect. There were also significant right-ward shifts in cumulative log concentration-response curves obtained in the presence of AE, EE, and EO in comparison with the curve obtained in the presence of S. Thymol also caused a smaller right-ward shift in the M cumulative log concentration-response curve. However, the slopes of methacholine response curves obtained in the presence of AE, EE, EO ( $p<0.001$ ), and T ( $p<0.05$ ) were significantly lower than that of S, indicating the functional antagonistic effects of these solutions.

These data showed the presence of bronchodilatory effects of AE, EE, and EO of *Carum copticum* which was not due to the presence of T or the existence of a competitive antagonist at the muscarinic receptors in this plant.

*MJIRI, Vol. 11, No. 4, 329-334, 1998.*

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## INTRODUCTION

*Carum copticum* is an annual plant which grows in the east of India, Iran and Egypt with white flowers and small brownish seeds. Its seeds have an odor similar to thymol and contain an essential oil which contains 35-50% cymenem, 30-40% alpha-pinene, gamma-terpinen and other substances such as carvacol.

The seeds of *Carum copticum* have several therapeutic effects including diuretic, anti-emetic, analgesic, anti-asthmatic and anti-dyspneic effects.<sup>1,2</sup> It also has a therapeutic effect on some cutaneous neural and urinary tract disorders. *Carum copticum* is therefore used in household remedies. A watery extract of this plant is widely used to relieve colds in children. In diarrhea, either roasted seeds are taken, or a watery extract made from them is given as a draught.

There is evidence of anti-spasmodic properties of the ethanol extract on isolated guinea pig ileum.<sup>3</sup> Another study paradoxically 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 the extract of roasted seeds of *Carum copticum* on rat and cat blood pressure similar to acetylcholine. All of these effects were blocked by atropine and cholinesterase but potentiated by physostigmine. The presence of choline and acetylcholine in the extract of roasted seeds of *Carum copticum* was also proven in this study.<sup>4</sup>

To elucidate this controversy and in order to demonstrate the therapeutic effect of this plant on asthma, the bronchodilatory and anticholinergic effects of the aqueous extract (AE), ethanol extract (EE) and essential oil (EO) of *Carum copticum* and its main ingredient thymol (T), in comparison with saline (S), were examined on isolated guinea pig tracheal preparations.

## MATERIALS AND METHODS

## Tissue preparations

Male guinea pigs (500-800 g) were killed by cervical dislocation and the trachea were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain.<sup>5</sup>

Tissues were then suspended in a 50 mL organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, U.K.) containing modified Krebs solution<sup>6</sup> with the following composition: NaCl 120mM, NaHCO<sub>3</sub> 25 mM, MgSO<sub>4</sub> 0.5 mM, KH<sub>2</sub>PO<sub>4</sub> 1.2 mM, KCl 4.72 mM, CaCl<sub>2</sub> 2.5 mM and dextrose 11 mM.

Saline was maintained at 37°C and gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissues were suspended isometrically under a tension of 1 g and allowed to equilibrate for at least 1 h while they were washed with Krebs solution every 15 min.

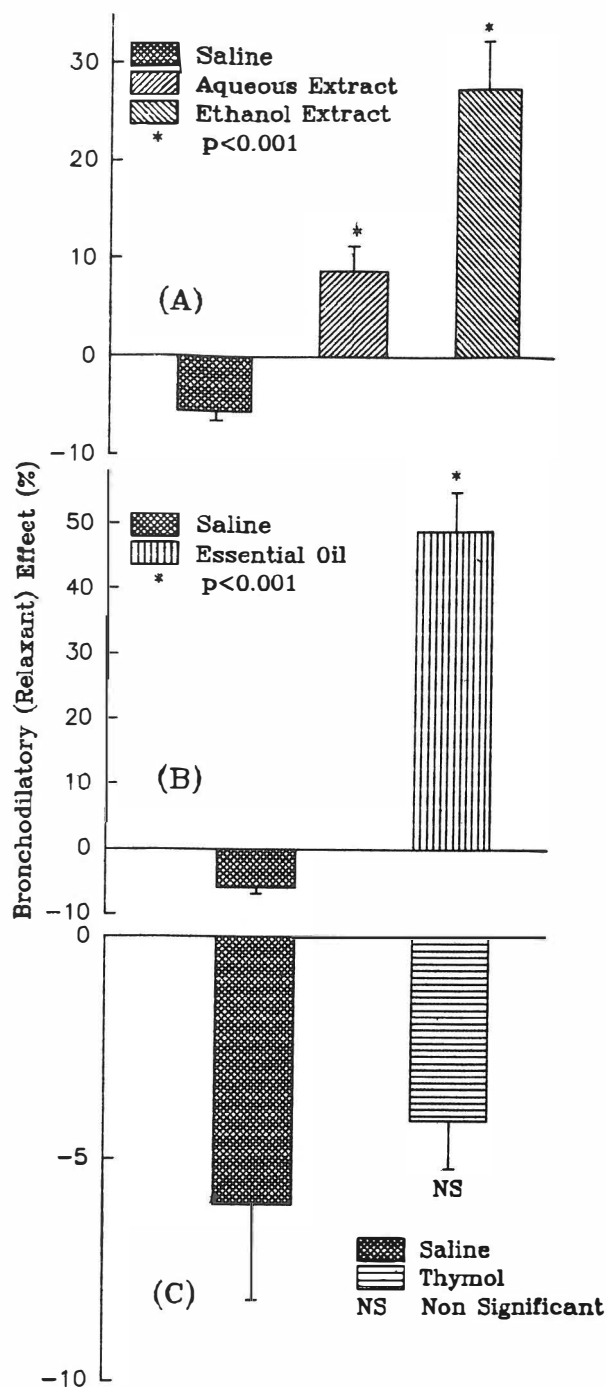
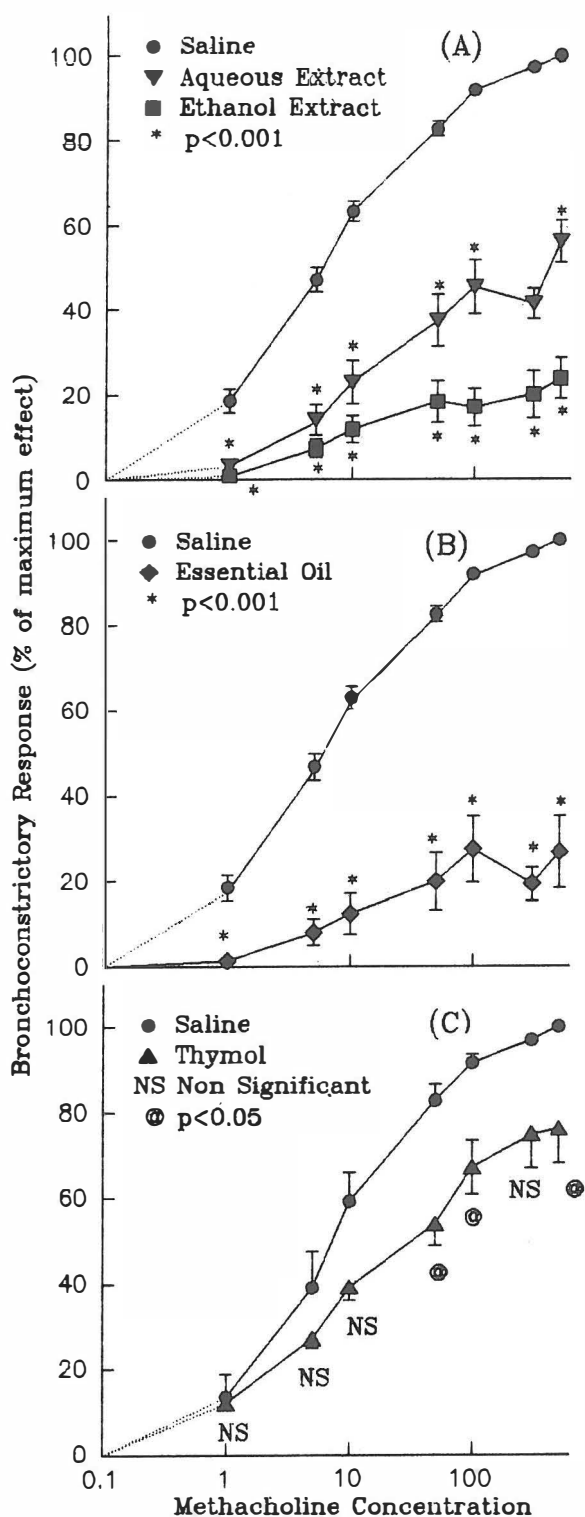


Fig. 1. Bronchodilatory (relaxant) effect of S, AE, and EE on 14 isolated guinea pig tracheal chains (A), S and EO on 12 tracheal chains (B), and S and T on 5 tracheal chains (C). AE, EE, and EO show significant bronchodilatory effects, but T does not have any significant bronchodilatory effect compared to S.

## Protocols

The bronchodilatory and anticholinergic effect of 4 mL AE, 0.05 mL EE (each on 14 tracheal chains), and 0.005 mL



**Fig. 2.** Cumulative log concentration-response curves of isolated guineapig tracheal preparations to increasing concentrations of methacholine in the presence of S, AE, and EE on 14 tracheal chains (A), S and EO on 12 tracheal chains (B), and S and T on 5 tracheal chains (C). AE, EE, EO, and T caused right-ward shifts in M-response curves compared to the M-response curve obtained in the presence of S.

**Table I.** Bronchodilatory effect (mean $\pm$ SE) of AE, EE, EO and T compared to S and the statistical differences between the bronchodilatory effects of S and other solutions

Different solutions	Relaxant effect		n	p-value
	mean (%)	SE		
Saline (S)	-5.56	0.98	14	
Aqueous extract (AE)	8.81	2.50	14	$p < 0.001$
Ethanol extract (EE)	27.48	4.83	14	$p < 0.001$
Saline (S)	-6.04	1.08	12	
Essential oil (EO)	48.92	6.08	12	$p < 0.001$
Saline (S)	-6.02	2.16	5	
Thymol (T)	-4.12	1.09	5	$p > 0.05$

EO (on 12 tracheal chains) of *Carum copticum* and 1 mL T with 5 mM concentration (on 5 tracheal chains) in comparison with the same volume of S was examined as follows.

#### A- Bronchodilatory effect

1) In each experiment tracheal smooth muscle was contracted with 10  $\mu$ M methacholine and the muscle tone was measured.

2) One of the solutions (AE, EE, EO, T or S) was added to the bath and the tissue was exposed to the solution for 5 min. The effect of the solution on the tone of precontracted tracheal muscle was then measured.

3) Decrease in tone was considered as a bronchodilatory (relaxant) effect and expressed as a positive percentage change of maximum contraction obtained due to 10  $\mu$ M of methacholine. An increase in tone was considered as a bronchoconstrictory effect which was expressed as a negative percentage change.

#### B- Anticholinergic effect

1) In each experiment one solution (AE, EE, EO, T or S) was added to the bath and the tissue was exposed to the solution for 5 min.

2) Cumulative log concentration-response curves to increasing concentrations of methacholine (1, 5, 10, 50, 100, 300, and 500  $\mu$ M) were obtained with addition of these concentrations every 2 min. To obtain the curve the percentage of contraction of the tracheal smooth muscle due to each concentration of M to the maximum contraction obtained in the presence of S due to the final concentration of M (500  $\mu$ M) was calculated and plotted against the log concentration of M.

**Table II.** EC<sub>50</sub> of methacholine (mean±SE) in the presence of AE, EE, EO and T compared to EC<sub>50</sub> in the presence of S.

Different solutions	EC <sub>50</sub>		n	p-value
	mean (μM)	SE		
Saline	6.04	0.81	14	
Aqueous extract	35.09	6.72	14	p<0.001
Ethanol extract	44.24	17.26	14	p<0.05
Saline	6.48	0.99	12	
Essential oil	22.52	7.29	12	p<0.05
Saline	14.26	4.86	5	
Thymol	9.52	1.16	5	p>0.05

3) The shift of the cumulative log concentration-response curves obtained in the presence of AE, EE, EO, or T with that of S was examined.

4) The slope of the M-response curve of each experiment was also measured and the slope of the methacholine curve obtained in the presence of AE, EE, EO, or T was compared with that of S.

5) In addition, the effective concentration of methacholine producing 50% of the maximum response (EC<sub>50</sub>) in each experiment was measured using the methacholine curve of the corresponding experiment.

All of the experiments were performed randomly with 1 hr resting periods of tracheal chains between each two experiments while washing the tissues every 15 min. In all experiments responses were recorded on a kymograph (ET 8 G-Boulitt, Paris) and were measured after fixation.

**Statistical analysis**

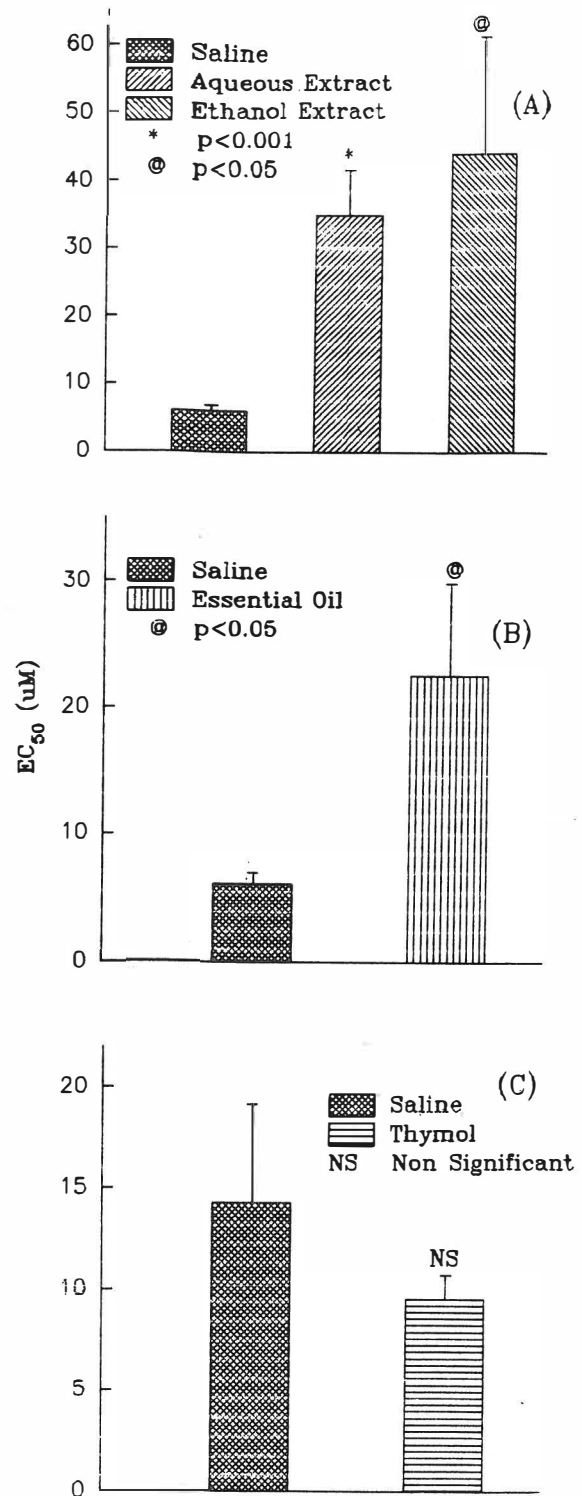
The data of bronchodilatory effects, EC<sub>50</sub>, and the slope of different experiments were expressed as mean±SEM. The bronchodilatory effect, EC<sub>50</sub> and the slope of methacholine curves of AE, EE, EO, and T experiments were compared with the results of S experiments using the paired "t" test.

**RESULTS**

**Bronchodilatory effect**

The aqueous extract, ethanol extract and essential oil of *Carum copticum* all showed potent bronchodilatory effects compared to saline.

The differences between the bronchodilatory effects of



**Fig 3.** The EC<sub>50</sub> of methacholine-response in the presence of S, AE, and EE on 14 tracheal chains (A), S and EO on 12 tracheal chains (B), and S and T on 5 tracheal chains (C). The EC<sub>50</sub> of methacholine in the presence of AE, EE, and EO are significantly higher than that of S, but there is no significant difference between the EC<sub>50</sub> obtained in the presence of T and S.

**Table III.** Slope of methacholine-response curves (mean±SE) in the presence of AE, EE, EO and T compared to the slope obtained in the presence of S.

Different solutions	Slope		n	p-value
	mean	SE		
Saline	1.00	0.03	14	
Aqueous extract	0.58	0.08	14	$p=0.001$
Ethanol extract	0.29	0.08	14	$p<0.001$
Saline	1.01	0.04	12	
Essential oil	0.22	0.10	12	$p<0.001$
Saline	1.12	0.08	5	
Thymol	0.68	0.08	5	$p<0.05$

AE, EE, and EO with saline, were highly significant for all three cases ( $p<0.001$ ), (Table I, Fig. 1). However, T did not show any significant bronchodilatory effect compared to S ( $p>0.05$ ). In fact, both S and T showed a bronchoconstrictory effect (Table I, Fig. 1).

#### Anticholinergic effect

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

Cumulative log concentration-response curves obtained in the presence of AE, EE, and EO showed clear right-ward shifts compared to the methacholine response curve produced in the presence of S. The degree of contractions of tracheal smooth muscle in all concentrations of M in the presence of AE, EE, and EO were significantly lower than those of S ( $p<0.001$  in all cases) (Fig. 2).

##### B- $EC_{50}$

The  $EC_{50}$  of methacholine in the presence of AE ( $p<0.001$ ), EE ( $p<0.05$ ), and EO ( $p<0.05$ ) were all significantly higher than that for S. However, the  $EC_{50}$  of methacholine in the presence of T was not significantly different with the  $EC_{50}$  in the presence of S ( $p>0.05$ ) (Table II, Fig. 3).

##### C- Slope of methacholine cumulative log concentration-response curves

The slope of methacholine cumulative log concentration-response curves in the presence of AE, EE, EO (for all three cases,  $p<0.001$ ), and T ( $p<0.05$ ) were all significantly lower than the slope of the methacholine-response curve in the presence of S (Table III).

## DISCUSSION

In this study the bronchodilatory and anticholinergic effects of AE, EE, EO, and T in comparison with saline were studied. All plant solutions (AE, EE and EO) showed relatively potent bronchodilatory effects. This bronchodilatory effect might be produced by several different mechanisms.

Based on a study demonstrating similar effects of the ethanol extract of *Carum copticum* and atropine on isolated guinea pig ileum,<sup>3</sup> one possible mechanism responsible for the bronchodilatory effect of *Carum copticum* could be the anticholinergic property of this plant. The anticholinergic effect of the extracts and EO of this plant was therefore examined on isolated guinea pig tracheal preparations. This part of the study showed a clear and significant right-ward shift of the methacholine log concentration-response curve in the presence of AE, EE, and EO compared to the methacholine response curve in the presence of S. The  $EC_{50}$  of M in the presence of AE, EE, and EO were also significantly higher than the  $EC_{50}$  in the presence of S. However, the slope of the curves obtained in the presence of AE, EE, and EO were significantly lower than the slope of the methacholine response curve in the presence of S, indicating a functional antagonistic effect of *Carum copticum*.<sup>7,8</sup>

The results of this study supported the existence of a relaxant effect of *Carum copticum* on smooth muscle demonstrated by a previous study.<sup>3</sup> But this effect was not due to a competitive antagonistic effect of this plant and therefore could be produced by the presence of small amounts of acetylcholine in *Carum copticum*, as demonstrated in another study.<sup>4</sup>

The bronchodilatory effect of *Carum copticum* could be due to its main ingredient, T. Thus the bronchodilatory and anticholinergic effect of T was also investigated. T caused a smaller right-ward shift in the methacholine response curve, but the  $EC_{50}$  of M in the presence of T was not significantly different with that of S, and T did not show any bronchodilatory effect. Therefore the bronchodilatory effect of *Carum copticum* is not due to the presence of T in this plant. In fact, S and T both showed bronchoconstrictory effects. This effect is perhaps due to the persistent slight bronchoconstrictory effect of M as observed in some experiments during the study. Thus the extracts and EO of *Carum copticum*, in addition to the bronchodilatory effects demonstrated by the data of this study, also antagonized the remaining bronchoconstrictory effect of M.

The other possible mechanisms responsible for the bronchodilatory effect of *Carum copticum* are as follows:

1) Beta-2 adrenergic receptor stimulation.<sup>9</sup>

2) Stimulation of the inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC.<sup>10</sup>

3) Inhibition of  $H_1$  histamine receptors.<sup>11</sup>

4) Methylxanthine activity of the plant.<sup>12</sup>

5) Other possible mechanisms including calcium antagonism, opening of potassium channels and inhibition of phosphodiesterase.<sup>13-15</sup>

The contribution of these mechanisms in the bronchodilatory effect of *Carum copticum* should be clarified in further studies.

With regard to the existence of airway inflammation in asthmatic patients, *Carum copticum* may also have an anti-inflammatory effect which contributes to the therapeutic effect of this plant on asthma. This effect should also be investigated in a further study.

In conclusion, the results of this study showed a relatively potent bronchodilatory effect for *Carum copticum*, but this effect was not due to the existence of either a competitive antagonistic substance at the cholinergic muscarinic receptors, or the thymol present in this plant.

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