

## *Basic Science In Medicine*

### BRONCHODILATORY AND ANTICHOLINERGIC EFFECTS OF *CARUM CARVI* ON ISOLATED GUINEA PIG TRACHEAL CHAINS

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#### ABSTRACT

*Carum carvi* (CC) is a grassy plant which is believed to have several therapeutic effects, including anti-asthmatic properties. We have therefore studied the bronchodilatory and anticholinergic effects of this plant.

The bronchodilatory effects of the aqueous extract (AE), macerated extract (ME), essential oil (EO), and 4  $\mu$ M theophylline (T) in comparison with saline (S) were examined by their relaxant effects on precontracted [by 10  $\mu$ M methacholine (M)] isolated tracheal chains of guinea pigs (n=10). The anticholinergic effects of AE, ME, EO, and 5 nM atropine (A) were tested by comparing the cumulative Log concentration-response curves (LCRCs) of M-induced contraction of tracheal chains and the effective concentration of M causing 50% of maximum response ( $EC_{50}$ ) in the presence of AE, ME, EO, and A with that of S.

Although the bronchodilatory effects of AE, ME, and EO were lower than that of T ( $p < 0.001$  for all cases), they were significantly higher than the effect of S ( $p < 0.05$  for AE,  $p < 0.01$  for ME, and  $p < 0.005$  for EO). There were also clear non-parallel right-ward shifts in LCRCs obtained in the presence of AE, ME, and EO, but a parallel shift in the presence of A compared to the curve obtained in the presence of S. The slope of LCRCs obtained in the presence of AE, ME, and EO were significantly lower than that of S ( $p < 0.01$  for AE,  $p < 0.05$  for ME, and  $p < 0.001$  for EO). The  $EC_{50}$  obtained in the presence of AE ( $p < 0.005$ ), ME ( $p = 0.005$ ), EO ( $p < 0.02$ ), and A ( $p < 0.001$ ) were significantly higher than that of S. However, the  $EC_{50}$  values obtained in the presence of AE, ME, EO and dose ratio (DR-1) produced by ME were not significantly different from that of A. The LCRCs obtained in the presence of EO on incubated tracheal chains with 1  $\mu$ M propranolol and 1  $\mu$ M chlorpheniramine also showed a clear non-parallel right-ward shift comparing with that of S. However, the slope of this curve was

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significantly higher than that of non-incubated experiments ( $p < 0.05$ ).

The results of this study indicated that the bronchodilatory effect of *Carum carvi* is mainly due to the non-competitive antagonistic property of this plant at muscarinic receptors. The  $\beta$  stimulatory effect and/or anti-histaminic effect of EO might be contributed to its non-competitive property. The variation between anticholinergic behaviours of different extracts is probably due to the variation of methods used, leading to extraction of different substances or destruction of some substances due to high temperature.

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**Keywords:** *Carum carvi*, bronchodilatory, anticholinergic, guinea pig.

## INTRODUCTION

*Carum carvi* is a grassy plant with white or pink flowers and small brownish seeds which grows in warm climate areas. The seeds of CC contain carvone, limonene,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene and other substances.<sup>1,2</sup>

Several therapeutic effects including those on digestive disorders, urinary tract disorders, and diuretic, gynecologic, anti-convulsive, anti-helminthic and also anti-asthmatic and dyspneic effects have been described for the seeds of CC in Iranian ancient medical books.<sup>3</sup> *Carum carvi* is therefore used in folk medicine as an antispasmodic, especially against gastrointestinal disorders or respiratory ailments in many countries including Iran and Germany.<sup>4</sup> According to ancient medical books, the volatile oil of CC acts predominantly as a relaxant on smooth muscles, mostly of the alimentary tract at the site of absorption after oral ingestion and of the respiratory system where this effect is exerted to some extent.<sup>5</sup>

In a previous study the relaxant effect of the alcoholic extract and volatile oil of this plant has been shown on isolated ileal smooth muscles of the guinea pig by performing dose-response curves to acetylcholine and histamine in the absence and presence of the plant extract.<sup>4</sup> In another study the relaxant effect of volatile oil from this plant has been demonstrated on isolated ileal and tracheal smooth muscles of guinea pigs by performing relaxation dose-response curves to increasing amounts of volatile oil on stretched muscle preparations.<sup>5</sup>

Therefore, in the present study the relaxant effect of AE, ME, and EO of this plant in comparison with both S and T were examined by a more standard method.<sup>6</sup> In addition, the anticholinergic effects of different extracts (AE, ME, and EO) of CC in comparison with atropine were also studied.

## MATERIAL AND METHODS

### Plant extracts

#### A-Aqueous extract

Fifty grams of the chopped, dried plant was extracted with 300 mL distilled water by suxhelat apparatus, and the solvent was removed under reduced pressure until the volume of extract reached 20 mL. The final extract contained 24% W/W of plant ingredients.

#### B-Macerated extract

Fifty grams of the chopped, dried plant was macerated with 300 mL distilled water and shaken (on a shaker) for 48 h; then the solvent was removed under reduced pressure until the volume of extract reached 20 mL. The concentration of plant ingredients in the final extract was 18% W/W.

#### C-Essential oil

From 100 g of the chopped, dried plant, 1.5 mL EO was extracted with 1000 mL distilled water by steam distilled apparatus. The concentration of plant ingredients in EO was 1.5% W/V.

### Tissue preparations

Male guinea pigs (400-700 g) were sacrificed by a blow on the neck and their tracheas 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>7</sup>

Tissues were 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 (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.

**Table I.** Slope of cumulative log concentration-response curves of methacholine-induced contraction of isolated guinea pig tracheal chains (mean  $\pm$  SEM), in the presence of AE, ME, and EO (both on non-incubated and incubated tracheal chains with 1  $\mu$ M propranolol and 1  $\mu$ M chlorpheniramine), compared to the slope of the LCRC obtained in the presence of S.

| Solution               | Slope Mean $\pm$ SEM | n  | p-value     |
|------------------------|----------------------|----|-------------|
| Saline (S)             | 1.60 $\pm$ 0.08      | 10 |             |
| Aqueous Extract (AE)   | 1.09 $\pm$ 0.12      | 10 | $p < 0.01$  |
| Saline (S)             | 1.29 $\pm$ 0.08      | 10 |             |
| Macerated Extract (ME) | 1.09 $\pm$ 0.09      | 10 | $p < 0.05$  |
| Essential Oil (EO)     | 0.34 $\pm$ 0.07      | 9  | $p < 0.001$ |
| Incubated S            | 1.40 $\pm$ 0.13      | 8  |             |
| Incubated EO           | 0.68 $\pm$ 0.13      | 8  | $p < 0.01$  |

The Krebs solution was maintained at 37°C and gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissues were suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while they were washed with Krebs solution every 10 min.

#### Protocols

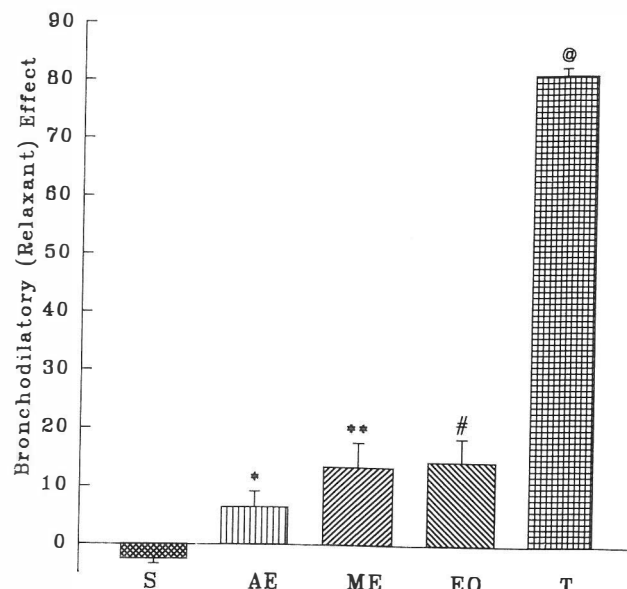
The bronchodilatory effects of 0.3 mL AE, 0.3 mL ME, 0.005 mL EO of *Carum carvi*, and 0.04 mL theophylline anhydrous (MW = 180.2, Sigma Chemical Co. Ltd., UK) with 1 mM concentration (4  $\mu$ M in organ bath), (for each solution, n=10) and the anticholinergic effects of the same volume of AE (n=10), ME (n=10), EO (n=9), and 0.05 mL atropine sulphate (MW = 695, Sigma Chemical Co. Ltd., UK) with 1  $\mu$ M concentration (5 nM in organ bath, n=10), in comparison with 0.3 mL S were then examined as follows:

#### A- Bronchodilatory effect

1) In each experiment the tracheal smooth muscle was contracted with 10  $\mu$ M methacholine hydrochloride (MW = 196, Sigma Chemical Co. Ltd., UK) and the muscle tone was measured.

2) One of the solutions (AE, ME, EO, T, or S) was added to the bath while exposing the tissue to the solution for 10 min and the effect of the solution on the tone of precontracted tracheal muscle was then measured.

3) Decrease in tone was considered as a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to maximum contraction



**Fig. 1.** Relaxant (bronchodilatory) effects of saline (S), aqueous extract (AE), macerated extract (ME), essential oil (EO), and theophylline (T) on 10 isolated guinea pig tracheal chains. AE, ME and EO all showed significant bronchodilatory effects compared to S. However, the bronchodilatory effects of plant solutions were significantly lower than that of T ( $p < 0.001$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , # $p < 0.005$ , @  $p < 0.001$  compared to the effect of S.

obtained due to 10  $\mu$ M of methacholine; and the increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change.<sup>6</sup>

#### B- Anticholinergic effect

1) In each experiment one solution (AE, ME, EO, A, or S) was added to the bath, and the tissue was exposed to the solution for 10 min.

2) Cumulative log concentration-response curves of methacholine induced contraction of tracheal chains were obtained, with addition of consecutive concentrations every 2 min (including concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000, and 10000  $\mu$ M). To obtain the curves, 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 (10mM) was calculated and plotted against the log concentration of M.

3) The effective concentration of methacholine causing 50% of the maximum response ( $EC_{50}$ ) in each experiment was measured using the LCRC of the corresponding experiment.

4) The shift of the cumulative log concentration-response curves obtained in the presence of AE, ME, EO, and A were examined by comparing the  $EC_{50}$  obtained in the presence of each solution with that of S.

5) The slope of the LCRC of each experiment was also measured and the slope of the methacholine curve obtained in the presence of AE, ME, and EO was compared with that of S.

6) The anticholinergic effect of EO and S on incubated tracheal chains with 1  $\mu$ M propranolol (MW = 296, Sigma Chemical Co. Ltd., UK) and 1  $\mu$ M chlorpheniramine (MW = 391, Sigma Chemical Co. Ltd., UK), 1 h prior to beginning and during obtaining the LCRC, were also examined (n=8).

7) In experiments with parallel or approximately parallel shifts in LCRC, the dose-ratio minus one (DR-1) was calculated by the following equation:

$$\frac{EC_{50} \text{ obtained in the presence of effective solution}}{EC_{50} \text{ obtained in the presence of S}} - 1$$

The bronchodilatory effect of different solutions, the anticholinergic effect of AE, the anticholinergic effect of A, and the anticholinergic effect of EO on incubated tissue were examined on 5 different series of tracheal chains. All of the experiments were performed randomly with 1 h resting periods of tracheal chains between each two experiments while washing the tissues every 10 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and after fixation were measured.

**Statistical analysis**

The data of bronchodilatory effects,  $EC_{50}$ , and the slope of LCRCs of different experiments were expressed as mean  $\pm$  SEM, but in values with large SEMs geometric means were also quoted. The bronchodilatory effects,  $EC_{50}$ , and the slope of LCRCs obtained in the presence of AE, ME, EO, and A experiments were compared with the results of S experiments using the paired "t" test. The slope of LCRC and  $EC_{50}$  values obtained in the presence of EO on incubated tracheas were compared with those of non-incubated tracheas using the unpaired "t" test. The  $EC_{50}$  values obtained in the presence of AE, ME, EO, and (DR-1) produced by ME were also compared with those of A using the unpaired "t" test. In comparison, for values with large SEMs and unequal mean and medians obtained in the presence of plant solutions with those of S, the Wilcoxon test, and in comparing these values with those of A, the Mann-Whitney "U" test was applied.

**RESULTS**

**Bronchodilatory effect**

The aqueous extract, macerated extract and essential oil of *Carum carvi* all showed potent bronchodilatory effects compared to saline. The differences between the bronchodilatory effect of AE ( $6.40 \pm 2.70$ ,  $p < 0.05$ ), ME

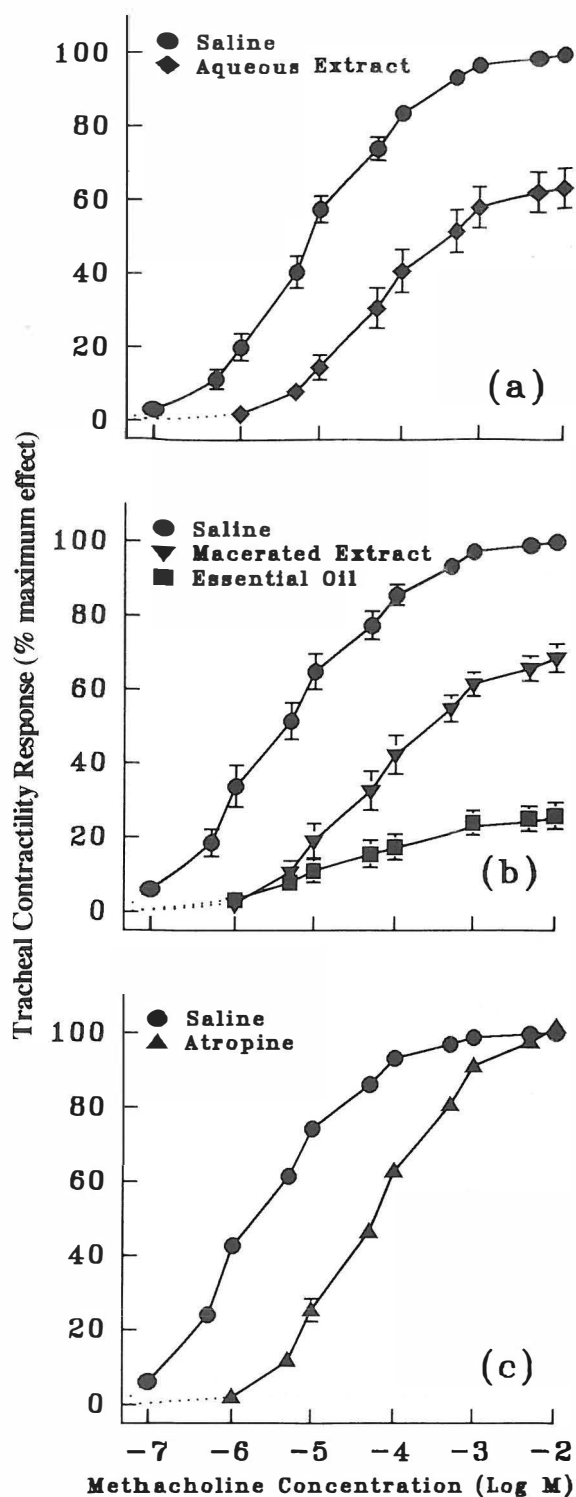


Fig. 2. Cumulative log concentration-response curves of methacholine-induced contraction of isolated guinea pig tracheal chains in the presence of S and AE (n=10)(a); S and ME (n=10) and EO (n=9) (b); and S and A (n=10) (c). AE, ME, and EO all showed a non-parallel, but A a parallel rightward shift in their LCRCs compared to the LCRC obtained in the presence of S.

**Table II.**  $EC_{50}$  ( $\mu$ M) of methacholine (mean $\pm$ SEM), obtained in the presence of aqueous extract (AE), macerated extract (ME), and essential oil (EO) both on non-incubated and incubated tracheal chains with 1  $\mu$ M propranolol and 1  $\mu$ M chlorpheniramine, atropine (A), and saline (S) and the statistical differences between the  $EC_{50}$  obtained in the presence of plant solutions with that of S and A. In comparison of the  $EC_{50}$  obtained in the presence of ME and EO with that of S, the Wilcoxon test was applied. G.X.: geometric mean.

| Solution | $EC_{50}$<br>Mean $\pm$ SEM     | n  | Statistical Difference |          |
|----------|---------------------------------|----|------------------------|----------|
|          |                                 |    | S                      | A        |
| S        | 8.69 $\pm$ 1.98                 | 10 |                        |          |
| AE       | 53.5 $\pm$ 10.10                | 10 | $p<0.005$              | $p=0.61$ |
| S        | 6.36 $\pm$ 2.20                 | 10 |                        |          |
| ME       | 83.9 $\pm$ 37.43<br>G.X.= 40.35 | 10 | $p=0.005$              | $p=0.53$ |
| EO       | 56.97 $\pm$ 31.6<br>G.X.=18.50  | 9  | $p<0.02$               | $p=0.93$ |
| S (inc)  | 3.53 $\pm$ 1.57                 | 8  |                        |          |
| EO (inc) | 5.14 $\pm$ 1.33                 | 8  | $p<0.05$               |          |
| S        | 1.97 $\pm$ 0.84                 | 10 |                        |          |
| A        | 59.7 $\pm$ 6.43                 | 10 | $p<0.001$              |          |

(13.37 $\pm$ 4.28,  $p<0.01$ ), and EO (14.45 $\pm$ 4.02,  $p<0.005$ ), were statistically significant compared to saline (-2.60 $\pm$ 0.79) (Fig. 1). However, the bronchodilatory effects of AE, ME, and EO were significantly lower than T (81.50 $\pm$ 1.28) ( $p<0.001$  for all three cases) (Fig. 1).

#### Anticholinergic effect

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

Cumulative log concentration-response curves of methacholine obtained in the presence of AE, ME, and EO (both in non-incubated and incubated experiments) showed clear, non-parallel, right-ward shifts but LCRCs obtained in the presence of A showed clear parallel right-ward shifts compared to the LCRCs produced in the presence of S. The degree of contractions of tracheal smooth muscle in all concentrations of M in the presence of A (except two final concentrations) were significantly lower than those of S ( $p<0.05$  to  $p<0.001$  in all cases) (Figs. 2 and 3). Note that obtaining the LCRCs in the presence of EO on incubated tissues was stopped at an M concentration of 1 mM, because at higher concentrations of M, the degree of contractions was reduced (Fig. 3).

The slope of LCRCs obtained in the presence of AE ( $p<0.01$ ), ME ( $p<0.05$ ), and EO ( $p<0.001$ ) were all

significantly lower than the slope of LCRCs obtained in the presence of S (Table I). The slope of LCRCs obtained in the presence of EO on incubated preparations was also significantly lower than that of S ( $p<0.01$ ), but showed a significant rise compared to that of non-incubated experiments (0.68 $\pm$ 0.13 vs. 0.34 $\pm$ 0.07,  $p<0.05$ ) (Table I, Fig. 3).

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

The  $EC_{50}$  of methacholine obtained in the presence of AE ( $p<0.005$ ), ME ( $p=0.005$ ), EO ( $p<0.02$ ), and A ( $p<0.001$ ) were all significantly higher than that for S. The  $EC_{50}$  obtained in the presence of EO on incubated tracheal chains was also significantly higher than that for S ( $p<0.05$ ) (Table II).

##### C- Comparison between the anticholinergic effect of *Carum carvi* and atropine

The  $EC_{50}$  values obtained in the presence of AE, ME, and EO were not significantly different from that of atropine using both the unpaired "t" test and the Mann-Whitney "U" test (Table II). The (DR-1) produced due to ME (with the almost parallel shift in LCRC), also did not show a significant difference with that of A (19.5 $\pm$ 9.87 vs. 34.07 $\pm$ 6.31,  $p>0.05$ ).

## DISCUSSION

In this study the bronchodilatory effect of AE, ME, and EO from *Carum carvi* in comparison with saline and theophylline were studied. Both extracts and EO showed relatively potent bronchodilatory effects, supporting the previous study which demonstrated the relaxant effect of the volatile oil of this plant on isolated tracheal and ileal smooth muscle of the guinea pig.<sup>5</sup> Although the bronchodilatory effects of the plant solutions were lower than that of T, obviously by increasing the concentration of extracts a bronchodilatory effect similar to T could be achieved.

This bronchodilatory effect might be produced by several different mechanisms. One possible mechanism responsible for the bronchodilatory effect of *Carum carvi* could be the anticholinergic property of this plant. The anticholinergic effect of the extracts and EO of this plant were therefore also examined on isolated guinea pig tracheal preparations. This part of the study showed clear and noticeable but non-parallel right-ward shifts in methacholine log concentration-response curves obtained in the presence of AE, ME, and EO compared to the LCRC obtained in the presence of S. The  $EC_{50}$  of M obtained in the presence of AE, ME, and EO was significantly larger than that for S, confirming the antagonistic effect of these solutions on muscarinic receptor activation. The  $EC_{50}$  values obtained in the presence of both extracts and EO were not significantly different than that for A, indicating comparable antagonistic effects of the extracts and EO of the plant with that of A. However, the slope of the LCRC obtained in the presence of AE, ME, and EO was significantly lower than the slope of the methacholine response curve obtained in the presence of S. The maximum contraction effect of M in the presence of AE, ME, and EO also was not achieved. These results indicated a functional antagonistic effect of *Carum carvi* at muscarinic receptors of the guinea pig trachea.<sup>8-10</sup> Although the existence of  $\alpha$ -pinene in the essential oil of CC was demonstrated<sup>11</sup> which showed anticholinergic activity,<sup>12</sup> this plant perhaps contains abundant amounts of substances with functional antagonistic effects at muscarinic receptors. Therefore, a non-parallel shift in the LCRC could be produced in the presence of a small amount of  $\alpha$ -pinene and large amounts of substances with functional antagonistic effects on muscarinic receptors. In fact, the right-ward shift in LCRC produced by ME was nearly parallel, indicating a component of competitive antagonistic effect of this extract at muscarinic receptors which was very similar to the results of the effect of ethanol extract on guinea pig ileum, which is perhaps due to the existence of  $\alpha$ -pinene in these two extracts.<sup>4</sup> The different muscarinic blocking effects of both extracts and EO are presumably due to variation of methods of extraction, leading to destruction of substances with

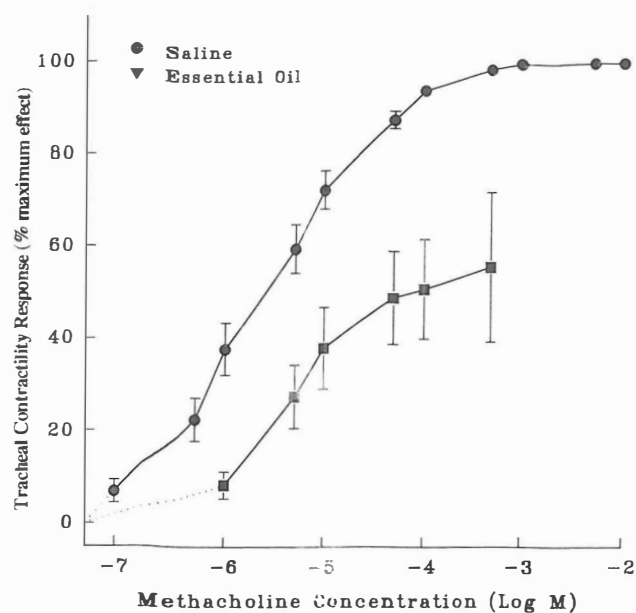


Fig. 3. Cumulative log concentration-response curves of methacholine-induced contraction of isolated guinea pig tracheal chains in the presence of S and EO on incubated tissues with  $1 \mu\text{M}$  propranolol and  $1 \mu\text{M}$  chlorpheniramine ( $n=8$ ).

competitive antagonism or extraction of larger amounts of substances with functional antagonistic effects at muscarinic receptors in AE and EO due to high temperature.

To evaluate the contribution of  $\beta$  adrenergic stimulatory and/or  $H_1$  histamine blocking effects on functional antagonism of EO at muscarinic receptors, which caused the greatest non-parallel shift in LCRC, the anticholinergic effect of EO was also examined on incubated tracheal preparations with  $1 \mu\text{M}$  propranolol and  $1 \mu\text{M}$  chlorpheniramine. The LCRC obtained in the presence of EO on incubated tracheas also showed a non-parallel shift compared to the LCRC obtained in the presence of S. However, the slope of the curve obtained in the presence of EO on incubated trachea was significantly higher than that of non-incubated experiments (Table I), indicating a  $\beta$  receptor stimulatory and/or  $H_1$  blocking effect of EO. The reason for reduction of the degree of contraction at high M concentrations during LCRC obtaining in these experiments is uncertain to us.

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

- 1) Stimulation of the inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC.<sup>13</sup>
- 2) Methyl-xanthine activity of the plant.<sup>6,14</sup>
- 3) Other possible mechanisms including calcium antagonism, opening of potassium channels and inhibition of phospho-diesterase.<sup>15-17</sup>

The contribution of these mechanisms in the

bronchodilatory effect of *Carum carvi* should be clarified in further studies.

With regard to the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, *Carum carvi* might also have an anti-inflammatory effect which will contribute to the therapeutic effect of this plant on asthma. In fact, in a previous study an anti-oxidant effect of the essential oil of this plant has been demonstrated.<sup>18</sup> Another study has demonstrated a suppressing effect of the essential oil of CC on the initial stage of an inflammatory process.<sup>19</sup> However, the effect of CC on the airway inflammation existing in asthma should be investigated in further studies.

In conclusion, the results of this study showed a relatively potent relaxant (bronchodilatory) effect of *Carum carvi* on the tracheal chain of the guinea pig, but this effect was not due to a pure competitive antagonist substance at cholinergic, muscarinic receptors. The  $\beta_2$  adrenergic receptor stimulatory and/or  $H_1$  histamine receptor blocking effect might be contributed to functional antagonism of the EO of CC at tracheal muscarinic receptors.

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