Relaxant effect of *Cuminum cyminum* on guinea pig tracheal chains and its possible mechanism(s)

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**ABSTRACT**

Objective: To examine the relaxant effects of the macerated and aqueous extracts of *Cuminum cyminum* on the tracheal chains of guinea pig.

Materials and methods: The relaxant effects of cumulative concentrations of macerated and aqueous extracts (0.25, 0.5, 0.75 and 1.0 g%) in comparison with saline and theophylline (0.25, 0.5, 0.75, and 1.0 mM) were examined on pre-contracted tracheal chains of guinea pigs under different conditions.

Results: In Group 1 experiments (contracted by KCl) only the last two concentrations of theophylline and the highest concentration of macerated extract showed significant relaxant effect compared to that of saline (P<0.001 and P<0.05 for theophylline and macerated extract respectively). The effects of the last two concentrations of theophylline in this group were significantly greater than those of the macerated and aqueous extracts (P<0.001). However, in Group 2 experiments (contracted by methacholine) both the extracts and theophylline showed concentration-dependent relaxant effect compared to that of saline (P<0.05 to P<0.001). The effects of the two last concentrations of both extracts were significantly lower than those of theophylline in Group 2 experiments (P<0.05 to P<0.001). In Group 3 (non-incubated, contracted by methacholine) the extracts of *Cuminum cyminum* did not show any relaxant effect of tracheal chains. The relaxant effects of macerated and aqueous extracts in Groups 1 and 3 were significantly lower than those of Group 2 (P<0.05 to P<0.001). However, the effects of different concentrations of theophylline obtained in Group 1 and 2 were not significantly different. There was a significant correlation between the effects and concentrations of theophylline in Groups 1 and 2, macerated extract in Groups 2 and 3 and aqueous extract in Group 1 (P<0.05 to P<0.001).

Conclusion: These results show a potent relaxant effect of *Cuminum cyminum* on guinea pig tracheal chains which may be due to a stimulatory effect of the plant on ß-adrenoceptors and/or an inhibitory effect on histamine H₁ receptors.

KEY WORD: Bronchodilator, smooth muscle relaxant, propranolol, chlorpheniramine

Introduction

*Cuminum cyminum* L. is a grassy plant with white or pink flowers and small green seeds, which grows in Egypt, Saudi Arabia, Iran, Mediterranean areas and some other parts of the world. The seeds contain cuminol, cymine, hellandren, carvone, cuminique alcohol.¹-³

Several therapeutic indications involving the disorders of gastrointestinal, gynecological and respiratory (asthma and dyspnea) systems have been described for the seeds of *Cuminum cyminum* in ancient Iranian medical books.⁴

The extracts and essential oil from *Cuminum cyminum* has been reported to possess, antimicrobial,⁵-⁷ antidiabetic,⁶-⁸ antihyperlipidemic,⁹ anti-inflammatory¹⁰ and anticarcinogenic¹⁰,¹¹ properties. In addition, there is evidence of the relaxant effects of the volatile oil from this plant on smooth muscle preparation.¹²

In the present study, the effect of the macerated and aqueous extracts from *Cuminum cyminum* on guinea pig tracheal chain was examined.

Materials and methods

Plant and extracts

*Cuminum cyminum* was collected from the School of Pharmacy and identified by Mr. Ahei. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Two types of extracts were prepared. Extract A with 50 g dried plant in 300 ml distilled water by soxhlet method and Extract B with the same amount of macerated plant in 300 ml distilled water.
water on a shaker for 48 h. The solvent was removed under reduced pressure and distilled water was added so that the final plant ingredient concentration was 10 w/v in both extracts.

**Tissue preparations**

Male Dunkin-Hartley guinea pigs (400-700 g) were killed by a blow on the neck and tracheas were removed. Each trachea was cut into rings, 2-3 mm in length. All the rings were then cut open opposite to the trachealis muscle, and sutured together to form a tracheal chain. The tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliot solution (composition in mM: NaCl 120, NaHCO$_3$ 25, MgSO$_4$ 0.5, KH$_2$PO$_4$ 1.2, KCl 4.72, CaCl$_2$ 2.5 and dextrose 11).

The Krebs solution was maintained at 37 °C and gassed with 95% O$_2$ and 5% CO$_2$. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h with change of fresh Krebs solution every 15 min.

The relaxant effects of four cumulative concentrations of extracts (0.25, 0.5, 0.75, and 1.0 g%), theophylline anhydrous (Sigma Chemical Ltd., UK) (0.25, 0.5, 0.75, and 1.0 mM), and 1 ml saline as control were examined. To produce different concentrations of macerated and aqueous extracts, 0.25 ml of 10% extracts were added to a 10 ml organ bath four times. For theophylline, 0.25 ml of 10 mM solution was added to an organ bath four times. The consecutive volumes were added to the organ bath at five minute intervals.

In each experiment the effect of four cumulative concentrations from each extract, theophylline, or 1 ml saline on contracted tracheal smooth muscle was measured. A decrease in tone was considered as a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction, and an increase in tone was considered as a contractile (bronchoconstrictive) effect, which was expressed as negative percentage change. $^{[14]}$

The relaxant effect of the different solutions was tested with three different experimental designs as follows:

1. On tracheal chains contracted by 60 mM KCl (Group 1, n=6 experiments).
2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd., UK), (Group 2, n=6 experiments).
3. On tracheal chains incubated with 1 μM propranolol hydrochloride (Sigma Chemical Ltd UK) and 1 μM chlorpheniramine maleate (Sigma Chemical Ltd., UK) 30 min prior to and during the relaxation test with different solutions. In this series of experiments, tracheal chains were also contracted by 10 μM methacholine hydrochloride (Group 3, n=4 experiments).

The relaxant effect of theophylline was examined only in Groups 1 and 2. The relaxant effects of the three groups of experiments were examined in three different series of tracheal chains. All the experiments were performed randomly with a 1 h resting period for the tissue between each two experiments and a washing with Krebs solution every 15 min. The responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation.

**Statistical analysis**

All data were expressed as mean±SEM. Data of the relaxant effects of different concentrations of the extracts were compared with the results of negative and positive control using one-way ANOVA with Tukey-Kramer post-test. The data of the relaxant effect obtained in three groups of experiments were also compared using the same statistical method. The relaxant effect of the two extracts and theophylline were related to the concentrations, using least square regression. Significance was accepted at P<0.05.

**Figure 1.** Concentration response curves of the relaxant effect of theophylline (a), macerated extract (b), and aqueous extract (c) from Cuminum cyminum in three groups of experiments (Group 1; KCl-induced contraction on non-incubated tracheal chains (O, n=6), Group 2; methacholine-induced contraction on non-incubated tracheal chains (□, n=6), and Group 3 experiments, methacholine-induced contraction on incubated tracheal chains of guinea pig with propranolol and chlorpheniramine (●, n=4). Statistical differences in the relaxant effect of different substances between Group 1 and those of Groups 2 and 3; *P<0.05, ***P<0.002. Statistical differences in the relaxant effect of different substances between Groups 2 and 3; *P<0.05, **P<0.01, P<0.001.
Results

Relaxant (bronchodilatory) effect

In Group 1 experiments 0.75 and 1 mM concentrations of theophylline and 1g% concentration of macerated extract showed significant relaxant effect compared to that of saline (P<0.05 to P<0.001). The effects of 0.75 and 1g% concentrations of both extracts were significantly lower than those of theophylline (P<0.001 for all cases). There was no difference between the two extracts in this group (Table 1).

In Group 2 both extracts from Cuminum cyminum and theophylline showed concentration-dependent relaxant effects on tracheal chains of guinea pig. The relaxant effects of 0.75 and 1g% concentrations of extracts and 0.75 and 1 mM concentrations of theophylline were significantly higher than those of saline (P<0.01 to P<0.001), (Table 2). In addition, the effects of the two last concentrations of both extracts in the Group 2 were significantly lower than those of theophylline (P<0.05 to P<0.001, Table 2). The effect of 1g% concentration of the macerated extract was also significantly greater than that of the aqueous extract (P<0.001, Table 2).

In Group 3 the extracts of Cuminum cyminum did not show any significant relaxant effect on guinea pig tracheal chains.

Table 1

<table>
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<th>Aqueous extract</th>
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<td>0.25</td>
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Values are presented as mean±SEM. Statistical differences between the effect of extracts and negative control (saline); *P<0.05, ***P<0.001. When compared to positive control (theophylline); +++P<0.001. The unit of concentration for the extracts is w/v (g/100 ml) and for theophylline mM.

Comparison of the relaxant effect between the three groups of experiments

The relaxant effects of most concentrations of both extracts in Group 1 and 3 were statistically lower than those of Group 2 experiments (P<0.05 to P<0.001). In addition, there were no significant differences in the effect of all concentrations of theophylline between Groups 1 and 2 (Figure 1).

Correlation between the concentrations of solutions and their relaxant effect

There was significant correlation between the effects and concentrations of theophylline in Group 1 (r=0.930, p<0.001), theophylline (r=0.953), macerated (r=0.895) and aqueous (r=0.737) extracts in Group 2 (p<0.001 for all cases) and macerated extract in Group 3 (r=0.537, P<0.05).

Discussion

In this study the relaxant (bronchodilatory) effects of the macerated and aqueous extracts from Cuminum cyminum in

Only 0.75 and 1g% concentrations of macerated extracts showed non-significant relaxant effect on tracheal chains (5.00±3.00 and 10.00±5.833 respectively).

Table 2

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<th>Aqueous extract</th>
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Values are presented as mean±SEM. Statistical differences between the effect of extracts and negative control (saline); *P<0.05, ***P<0.001. When compared to positive control (theophylline); **P<0.01. When compared made between two extracts; *P<0.001. The unit of concentration for the extracts is w/v (g/100 ml) and for theophylline mM.
comparison with saline as negative control and theophylline as positive control were studied. In Group 1 experiments (KCl contracted tracheal chains) only the two higher concentrations of theophylline and the last concentration of macerated extract showed relaxant effect. However, both extracts from Cuminum cyminum showed relatively potent relaxant effects compared with the effect of saline in Group 2 experiments.

The relaxant effect of both extracts and theophylline were concentration-dependent. There was a significant relationship between the concentrations and response of theophylline in Groups 1 and 2, macerated extract in Groups 2 and 3 and aqueous extract in Group 2 (P<0.05 to P<0.001). The relaxant effects of all concentrations of the macerated extract were greater than those of the aqueous extract in all groups of experiments. The effects of the macerated and aqueous extract at many concentration levels in Groups 1 and 2 of experiments were lower than those of theophylline.

The relaxant effect of different extracts from Cuminum cyminum on tracheal chains of guinea pigs might be due to several different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H1 receptors or an anticholinergic property of this plant, because the relaxant effect of β-stimulatory,[14,15] histamine H1 receptor inhibitory,[16] and anticholinergic drugs[17] have been shown in previous studies. To evaluate the contribution of the β-adrenergic stimulatory and/or H1 histamine blocking effect of the macerated and aqueous extracts from this plant to their bronchodilatory effects, the effects of these extracts on tracheal chains were examined in presence of propranolol and chlorpheniramine respectively. The relaxant effects of most concentrations of both extracts from Cuminum cyminum obtained in Group 3 experiments were significantly lower than those of Group 2. These findings suggest probable β-adrenergic stimulatory and/or histamine H1 blocking properties of the plant extracts that may contribute to their relaxant effect on tracheal chains.

The absence of the obvious relaxant effect of the aqueous extract from Cuminum cyminum in Group 1 and the its relatively potent relaxant effect in Group 2 experiments may be due to potassium channels opening which has been demonstrated to produce bronchodilation.[18] If this extract from Cuminum cyminum had a potassium channel opening effect, it would not have a relaxant effect on tracheal chains contracted by KCl, while it could show relaxant effect when the tracheal chain was contracted by methacholine. It has been shown that KCl affects calcium channels[19] and calcium channel blockers have bronchodilatory effect.[20,21] Therefore, the relaxant effect of macerated extract in Group 1 may indicate an inhibitory effect of this extract on calcium channels.

The effects of different concentrations of the macerated extract in all groups of experiments were greater than those of the aqueous extract. These results may suggest that the effective substances causing a relaxant effect on guinea pig tracheal chains are higher in the macerated extract than the aqueous extract. Although both the macerated and aqueous extracts are aqueous in nature, the differences in the effect of these two extracts could be due to the variation in their constituents due to the method of extraction.

With regard to the airway inflammation of asthmatic and COPD patients, Cuminum cyminum might also have an antiinflammatory effect, which will contribute to the therapeutic effect of this plant on asthma and COPD. In fact, the antiinflammatory effect of this plant has been reported.[9] However, the effect of Cuminum cyminum on airway inflammation needs further investigation.

In conclusion the results of this study indicate a relatively potent relaxant (bronchodilatory) effect of Cuminum cyminum on the tracheal chains of guinea pigs. A stimulatory effect of the plant on β-adrenoceptors and/or an inhibitory effect on histamine H1 receptors are suggested as the possible mechanisms, apart from the opening of potassium channels and inhibition of calcium channels.

Acknowledgment

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Reference

9. Sivastava KC. Extracts from two frequently consumed spices-cumin (Cuminum cyminum) and turmeric (Curcuma longa)-inhibit platelet aggregation and alter eicosanoid biosynthesis in human blood platelets. Prostaglandins Leukot Essent Fatty Acids 1989;37:57-64.
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