RELAXANT EFFECTS OF *OCIMUM BASILICUM* ON GUINEA PIG TRACHEAL CHAINS AND ITS POSSIBLE MECHANISM(S)

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ABSTRACT

Therapeutic effects of *Ocimum basilicum* on respiratory diseases especially dyspnea have been reported in Iranian ancient medical books. In the present study, the relaxant effects of macerated and soxhlet extracts of this plant on tracheal chains of guinea pigs were evaluated. The relaxant effects of 4 cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 W/V) in comparison with saline as negative control and 4 cumulative concentrations of theophylline (0.25, 0.5, 0.75, and 1.0 mM) as positive control were examined on precontracted tracheal chains of two groups of 6 guinea pig by 60 mM KCl (group 1) and 10 μM methacholine (group 2). Decrease in contractile tone of tracheal chains was considered as relaxant effect. In group 1 experiments only the last two higher concentrations of theophylline showed significant relaxant effect compared to that of saline ($p<0.001$ for both concentrations), which were significantly greater than those of macerated and soxhlet extracts ($p<0.001$ for all cases) and in group 2 experiments both macerated and soxhlet extracts showed concentration-dependent relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$ for both extracts). There were significant differences between the relaxant effects of both extracts with those of theophylline in group 2 experiments ($p<0.01$ to $p<0.001$). The relaxant effects of macerated and soxhlet extracts in group 1 were significantly lower than those of groups 2. These results showed a potent relaxant effect of *Ocimum basilicum* on tracheal chains of guinea pigs which were lower than theophylline at concentrations used.

Keywords: *Ocimum basilicum* **,** Respiratory diseases Dyspnea, Relaxant

INTRODUCTION

Ocimum basilicum L. (labiatae) is a grassy and annual plant. The leaves of this plant are oval with a sharp tip and the flowers are yellow, white and pink. It is a native plant of Iran, Afghanistan and India (1,2,3). This plant is a popular culinary herb and its essential oils have been used for many years in food perfumery and dental produts (4) in Iranian traditional medicine (5,6).

According to the literature, about 45 compounds are found in volatile oil of this plant and the major compounds are linalol, eugenol, methyl-chavicol, methylcinnamat, linolen, ocimene, pinene, cineol, anethol, estragol, thymol, citral and comphor (1,7,8).

Different parts of *Ocimum basilicum* have diuretic (1,3), antipyretic (1) and antitussive effects (1,2) and have been used to treat gastritis, stomach-ache, flatulence and constipation (1-3,9-11), vomiting and hiccup (1).The seeds and the boiled extract are used for treatment of diarrhea (1). This plant also has therapeutic effects for nasal polyps (1), upper respiratory tract diseases and dyspnea (1,2), inflammation of the urinary tract (2) and it has been used as a bathing solution for treatment of ulcers (2,9) and increase in lactation (3). Inhalation of the fragrance of this plant is useful to alleviate headache (1), and also it produces a favorable impression on the mental activity (12). In the Previous studies, the anti-inflammatory (10,13,14), anti-HIV (15), anti-oxidant (5,16-18), antibacterial and antifungal (4,19-21) effects of this plant have been reported.

In the present study, the relaxant effect of macerated and soxhlet extracts of *Ocimum basilicum* on guinea pig tracheal chains was examined.

MATERIALS AND METHODS

Plant and extracts

Ocimum basilicum was Collected from the School of Pharmacy and identified by Mr. Joharchi and the specimen number of the plant is 19484. The soxhlet extract was prepared as follows: Fifty grams of the chopped, dried plant leaves were extracted with 300 ml distilled water by suxhelat apparatus. For the preparation of the macerated extract, the same amount of plant was macerated with 300 ml distilled water (on a shaker) for 48 hr. The solvent of both extracts were then removed under reduced pressure at 50 ^{o}C and distilled water were added to residues in such a way that plant ingredient concentration in the final soxhlet extracts were 10% W/W.

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Tissue preparations

Male guinea pigs (400-700 g) were killed by a blow on the neck and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). The cartilages of all rings were then cut open opposite to the trachealis muscle, and sutured together to form a tracheal chain (22). Tissue was 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, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was kept at 37^{o}C under stream of 95% O_2 and 5% CO_2 gases. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Protocols

The relaxant effects of four cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 g/100 ml), four cumulative concentrations of theophylline anhydrous (Sigma Chemical Ltd UK) (0.25, 0.5, 0.75, and 1.0 mM) as positive control, and saline as negative control were examined. For preparation of different concentrations in the case of macerated and soxhlet extracts, 0.25 ml of 10% W/V of the concentrated extracts and in the case of theophylline, 0.25 ml of 10 mM solution were added to the organ bath. The consecutive volumes were added to organ bath at 5 min intervals.

In each experiment the effect of four cumulative volumes of each extract, four cumulative volumes of theophylline, or saline on contracted tracheal smooth muscle were determined after exposure of tissue to the solution for 5 min. A decrease in tone was considered as a relaxant (broncho-dilatory) effect and expressed as positive percentage change in proportion to the maximum contraction and an increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change (23).

The relaxant effect of different solutions were tested with two different experimental designs (n=6 for each group) as follows:

1. On tracheal chains contracted by 60 mM KCl (group 1 experiments).
2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 2 experiments).

The relaxant effects in two groups of experiments, were examined in two different series of tracheal chains. All experiments were performed randomly with a 1 h resting period of tracheal chains between each consecutive 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 were measured after fixation.

Statistical analysis

All data were expressed as mean±SEM. Data of relaxant effects of different concentrations of extracts were compared with the results of negative and positive control using ANOVA with Tukey Kramer post test. The data of relaxant effect obtained in two groups of experiments were also compared using unpaired t test. The relaxant effect of two extracts and theophylline were related to the concentrations using least square regression. Significances were accepted for $p<0.05$.

RESULTS

Relaxant (bronchodilatory) effect

In group 1 experiments, only the two higher concentrations of theophylline showed significant relaxant effects compared to those of saline ($p<0.001$ for both cases). In addition, the effects of the last two higher concentrations of both extracts were significantly lower than those of theophylline ($p<0.001$ for both cases). There was no significant difference between the effects of different concentrations of the two extracts (Table 1).

In groups 2 experiments, both extracts from O*cimum basilicum* and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea pig. The relaxant effects of the most concentrated extracts and theophylline were significantly higher than those of saline ($p<0.05$ to $p<0.001$). Only the low concentration of theophylline did not show significant relaxant effect compared to that of saline (Table 2).However ,the effects of two higher concentrations of macerated extract and only the highest concentration of soxhlet extract in group 2 were significantly lower than those of theophylline ($p<0.01$ to $p<0.001$), (Table 2). In contrast, the effects of the lowest concentrations of both extracts were significantly greater than that of theophylline ($p<0.01$ and $p<0.001$ for macerated and soxhlet extract respectively), (Table 2). In addition, the effects of the different concentrations of soxhlet extract in this group were greater than those of macerated extracts but only for the lower concentration, which were statistically significant ($p<0.05$), (Table 2).

Table 1. Relaxant effect of two different extracts of *Ocimum basilicum* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (contracted tracheal chains by 60 mM KCl).

Concentration	Saline	Macerated extract	Soxhlet extract	Theophylline
0.25	-	1.67±1.31 NS b	2.83±1.83 NS bb nS	-5.20±2.16 NS
0.5	-	2.17±1.64 NS ns	4.83±3.37 NS ns nS	13.65±6.15 NS
0.75	-	3.17±2.32 NS bbb	7.00±5.00 NS bbb nS	49.00±5.77 aaa
1	0.90±0.640	4.17±2.99 NS bbb	9.50±6.70 NS bbb nS	85.83±6.40 aaa

Values are presented as mean±SEM. Statistical differences between the effect of extracts and theophylline with negative control (saline); NS: non-significant difference, aaa: $p<0.001$. Statistical differences between the effect of extracts and positive control (theophylline); ns: non-significant difference, b: $p<0.05$, bb: $p<0.01$, bbb: $p<0.001$. Statistical differences between the effect of two extracts; nS: non-significant difference. The unit of concentration for extracts was g/100 ml and for theophylline was mM.

Table 2. Relaxant effect of two different extracts of *Ocimum basilicum* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (contracted tracheal chains by 10 µM methacholine).

Concentration	Saline	Macerated extract	Soxhlet extract	Theophylline
0.25	-	7.00±2.31 a bb	13.83±2.32 aaa bbb c	-2.01±0.34 NS
0.5	-	11.33±2.89 aa ns	19.5±4.05 aaa ns nS	12.32±2.22 aa
0.75	-	15.00±3.16 aa bb	25.5±4.72 aaa ns nS	33.21±4.12 aaa
1	0.90±0.640	19.50±3.22 aa bbb	30.83±5.24 aaa bbb nS	74.88±6.09 aaa

Values are presented as mean±SEM. Statistical differences between the effect of extracts and theophylline with negative control (saline); NS: non-significant difference, a: $p<0.05$, aa: $p<0.01$, aaa: $p<0.001$. Statistical differences between the effect of extracts and positive control (theophylline); ns: non-significant difference, bb: $p<0.01$, bbb: $p<0.001$. Statistical differences between the effect of two extracts; nS: non-significant difference, c: $p<0.05$. The unit of concentration for extracts was g/100 ml and for theophylline was mM.

Table 3. Correlation (r) of the relaxant effects of two different extracts of *Ocimum basilicum* and theophylline with their concentrations in three groups of experiments.

DifferentSubstances	Group 1	Group 2
Macerated extract	0.192 NS	0.576**
Soxhlet extract	0.234 NS	0.558**
Theophylline	0.935***	0.930***

Statistical significances; NS: non-significant difference, **: $p<0.01$, ***: $p<0.001$.

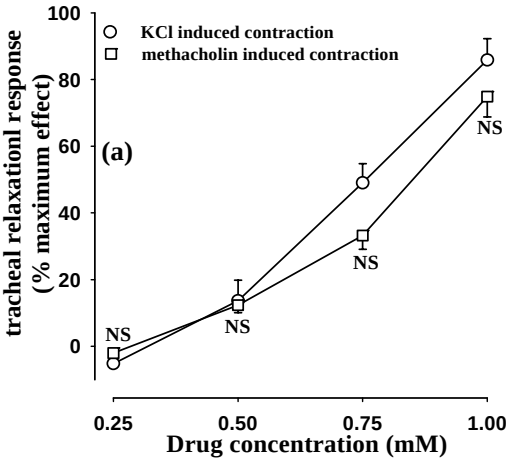

Figure 1. Concentration response curves of the relaxant effect of theophylline in two groups of experiments (group 1; KCl induced contraction (o) and group 2; methacholine induced contraction of tracheal chains (□, n=6 for each group). Statistical differences in the relaxant effect between group 1 and group 2; NS: non-significant difference.

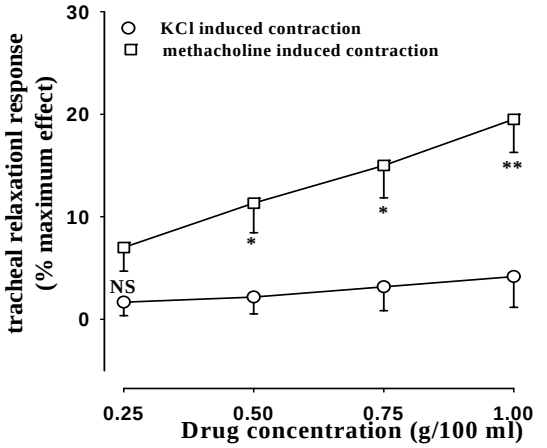

Figure 2. Concentration response curves of the relaxant effect of macerated extract of *Ocimum basilicum* in two groups of experiments (group 1; KCl induced contraction (o) and group 2; methacholine induced contraction of tracheal chains (□, n=6 for each group). Statistical differences in the relaxant effect between group 1 and group 2; NS: non-significant difference, *; p<0.05, **: p<0.01.

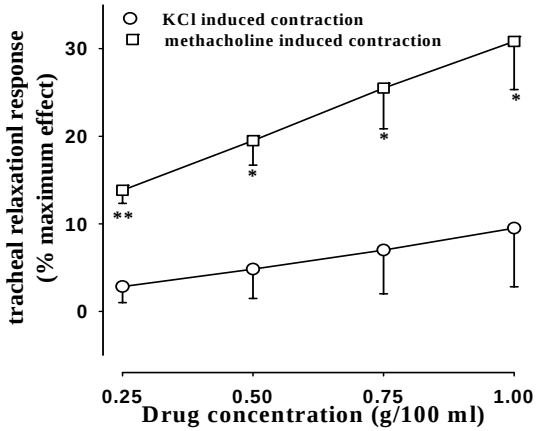

Figure 3. Concentration response curves of the relaxant effect of soxhlet extract from *Ocimum basilicum* in two groups of experiments (group 1; KCl induced contraction (o) and group 2; methacholine induced contraction of tracheal chains (□, n=6 for each group). Statistical differences in the relaxant effect between group 1 and group 2; NS: non-significant difference, *; p<0.05, **: p<0.01.

Comparison of the relaxant effect between two groups of experiments

The relaxant effects of higher concentrations of both extracts in group 2 were statistically greater than those of group 1 experiments (p<0.05 to p<0.01). However, there were no significant differences in the effects of all concentrations of theophylline between groups 1 and 2 (Fig. 1, 2, and 3)

Correlation between concentrations of solutions and their relaxant effects

There were significant positive correlations between the relaxant effects of both extracts and theophylline with concentrations of the solutions in group 2 experiments (p<0.01 to p<0.001), (Table 3). The correlation between the relaxant effect and concentration of theophylline in group 1 was also significant (p<0.001).

DISCUSSION

In this study the relaxant (bronchodilatory) effects of macerated and soxhlet extracts of O*cimum basilicum* in comparison with saline as negative control and theophylline as positive control were studied. The relaxant effect of both extracts and theophylline were concentration dependent. There were positive correlations between increase in concentrations and the relaxant effects of both extracts in group 2, and theophylline in groups 1 and 2 experiments. The relaxant effects of all concentrations of macerated extract were non-significantly smaller than those of soxhlet extract in both groups of experiments.

The absence of any obvious relaxant effect of macerated and soxhlet extract of O*cimum basilicum* in group 1 and relatively potent effect of the extracts in group 2 experiments may be due to the opening of potassium channels which has been demonstrated previously (27). If the extracts from *Ocimum basilicum* open potassium channels, they should not have any effect on tracheal chains contracted by KCl, while they should have effects on the tracheal chain which were contracted by metacholine. In fact, the results of groups 2 may support this hypothesis. Since KCl affect calcium channels (28) and calcium channel blockers are bronchodilator (29,30), another explanation for results of the experiment is the absence of blocking effects of this extract on calcium channels.

The effects of different concentrations of soxhlet extract in both groups of experiments were greater than those of macerated extract. Which may suggest that the effective substances in soxhlet extract are higher than that of macerated extract. The other possible mechanisms for broncho-dilatory effect of O*cimum basilicum* include: stimulation of β-adrenergic receptors (23,24), blocking of histamine H_1 receptors (25), anticholinergic activity (26), stimulation of inhibitory nerve other than adrenergic or cholinergic system (NANC) or inhibition of stimulatory NANC (31), methyl xanthin like activity (32), due to inhibition of phospho-diestrase (33). However, contributions of these mechanisms to bronchodilatory effect of extracts of O*cimum basilicum* on tracheal chains of guinea pigs (s) should be clarified in further studies.

In addition O*cimum basilicum* might also have an anti-inflammatory effect, which will contribute to the therapeutic effect of this plant on asthma. While anti-inflammatory (10,13,14) and anti-oxidant (5,16,17,18) effects of this plant have been reported, the effect of, O*cimum basilicum* on airway inflammation which is present in asthma should be investigated in further studies.

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