Antihistaminic Effect of *Bunium persicum* on Guinea Pig Tracheal Chains

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ABSTRACT

In a previous study, the relaxant and anticholinergic (functional antagonism) effects of *Bunium persicum* (*B. persicum*) have been demonstrated on guinea pig tracheal chains. To elucidate the other mechanisms responsible for this relaxant effect, the inhibitory effect of this plant on histamine H_1 receptors was examined in this study. The antihistaminic effects of aqueous and macerated extracts, essential oil, 20 nM chlorpheniramine, and saline were tested by performing the cumulative log concentration-response curves of histamine induced contraction of isolated guinea pig tracheal chains incubated with three different conditions including: 1) 1.4 µM indomethacin, 2) 1.4 µM indomethacin, 1 µM propranolol, and 10 nM atropine, and 3) 1.4 µM indomethacin and 1 µM propranolol (for each group n = 8). The results showed clear parallel rightward shifts in histamine-response curves obtained in the presence of macerated extract in group 2, aqueous extract in group 3, and essential oil in group 2 and 3 experiments compared with the curves obtained in the presence of saline. The EC_{50} (effective concentration of histamine causing 50% of maximum response) obtained in the presence of essential oil, extracts, and chlorpheniramine in all three sets of experiments were significantly higher than that of saline ($p<0.05$ to $p<0.001$), but maximum response to histamine obtained in the presence of essential oil and extracts were lower ($p<0.01$ to $p<0.001$). However, the maximum response obtained in the presence of aqueous extract in group 3 compared to group 1 and that of macerated extract in group 2 compared to the other two sets of experiments were improved. These results indicated a competitive antagonistic effect of *B. persicum* at histamine H_1 receptors. *Iran. Biomed. J. 8 (3): 149-155, 2004*

Keywords: *Bunium persicum*, Antihistaminic effect, Trachea, Guinea pig

INTRODUCTION

. *persicum* (Boiss.) B. Fedtsch. (or *Carum persicum*) is a grassy plant with white or pink flowers and small brownish seeds which grows in warm climate areas of Iran. The seeds of *B. persicum* contain p-Mentha-1, 4-dien-7-al, gamma-terpinene, beta-pinene and cuminaldehyde [1].

Several therapeutic effects including those on digestive disorders, urinary tract disorders, diuretic, gynaecologic, anti-convulsion, anti-helmetic and also anti-asthma and dyspnea have been described for the seeds of *B. persicum* in Iranian ancient medical books. A similar plant (*Carum carvi*) is used in folk medicine as antispasmodic especially against gastrointestinal disorders or respiratory ailments in many countries including Germany and Iran [2]. In addition, there is no report regarding any side effect of this plant or its extracts.

In the previous studies antimicrobial [3] and antifungal [4] effects of this plant have been demonstrated. In our recent study, relaxant effects of this plant was demonstrated on isolated guinea pig tracheal chains [5]. We showed a functional antagonistic effect of this plant on muscarinic receptors.

To elucidate the other mechanisms responsible for the observed bronchodilatory effect of *B. persicum*, the inhibitory effect of essential oil, aqueous and macerated extracts of this plant on histamine H_1 receptors of

guinea pig tracheal chains in comparison with both saline and chlorpheniramine were examined in this study.

MATERIALS AND METHODS

Plant and extracts. *B. persicum* was purchased from local market and identified by botanists in the herbarium of the Ferdowsi University of Mashhad. The specimen number of the plant is 17612. The plant extracts were prepared as follows: For macerated extract, the chopped and dried plant (50 g) was macerated with 300 ml distilled water and shaken (on a shaker) for 48 h. For aqueous extract, the same amount of plant was extracted with 300 ml distilled water by suxhelat apparatus. The solvent of both extracts were then removed under reduced pressure until the extracts volume reached 20 ml. The plant ingredient concentration in the final extracts were 45.8 and 36.9% W/W in macerated and aqueous extracts, respectively. From 100 g of the chopped and dried plant in 1,000 ml distilled water, one millilitre essential oil was extracted by steam distilled apparatus. The concentration of plant ingredients in the essential oil was 1.5% V/V.

Tissue preparations. Male guinea pigs (400-700 g) were killed by a blow on the neck and then trachea was 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 tracheal chain [6].

Tissue was suspended in 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent UK.) containing Krebs-Henseleit 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 maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The 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.

Protodols. The inhibitory effect of *B. persicum* on histamine H_1 receptors was examined by producing cumulative log concentration-response curve of histamine acid phosphate (BDH Chemical Co, Ltd UK) induced contraction of tracheal chains 10 min after exposing tissue to one solution (essential oil 3 µl, macerated and aqueous extracts 0.08 ml, 0.2 ml of 1 µM chlorpheniramine maleate (Sigma Chemical Ltd UK), or 0.08 ml saline). The consecutive concentrations of histamine were added every 2 min (range 0.1-1,000 µM); and the percentage of contraction due to each concentration in proportion to the maximum contraction obtained in the presence of saline was plotted against log concentration of histamine. The effective concentration of histamine causing 50% of maximum response (EC_{50}) in each experiment was measured using the log concentration-response curve of corresponding experiment. The shift of cumulative log concentration-response curves obtained in the presence of extracts and chlorpheniramine were examined by comparing the EC_{50} obtained in the presence of each solution with that of saline. In addition the maximum responses to histamine obtained in the presence of extracts and chlorpheniramine in all sets of experiments were compared with that of saline. To examine the parallel rightward shift, the slope of the histamine-response curve of each experiment was measured and were compared with that of saline. In experiments with parallel shift in histamine-response curve, the concentration-ratio minus one (CR-1) as competitive antagonism effect was calculated by the following equation:

$$\left(\frac{EC_{50} \text{ obtained in the presence of effective solutions}}{EC_{50} \text{ obtained in the presence of saline}}\right) - 1$$

The inhibitory effect of *B. persicum* on histamine H_1 receptors was tested on incubated tracheal chains 30 min prior to beginning and during obtaining histamine-response curve with three different experimental designs (for each design, n = 8) as follows:

1. 1.4 µM indomethacin (Sigma Chemical Ltd UK), (group 1 experiments).
2. 1.4 µM indomethacin, 1 µM propranolol hydrochloride (Sigma Chemical Ltd UK), and 10 nM atropine sulphate (Sigma Chemical Ltd UK), (group 2 experiments).
3. 1.4 µM indomethacin and 1 µM propranolol hydrochloride (group 3 experiments).

All of the experiments were performed randomly with 1 h 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 was measured after fixation.

Statistical analysis. The data of EC_{50}, the slope of histamine-response curves, maximum response to histamine, and values of (CR-1) of different experiments were expressed as mean ± SEM. The EC_{50}, the slope of histamine-response curves, and maximum response to histamine obtained in the presence of essential oil, extracts, and chlorpheniramine were compared with those obtained in the presence of saline and values of (CR-1) obtained in the presence of extracts and essential oil with those of cholorpheniramin using paired *t*-test. The values of EC_{50}, the slope of histamine-response curves, and maximum response to histamine between three groups of experiments were compared using one-way analysis of variance (ANOVA) test. Significance was accepted at $p<0.05$.

RESULTS

Shift in cumulative log concentration-response curves. Cumulative log concentration-response curves of histamine obtained in the presence of essential oil, extracts, and chlorpheniramine in all three experimental groups showed clear rightward shift compared to histamine-response curves produced in the presence of saline (Fig. 1).

EC_{50}. The EC_{50} of histamine obtained in the presence of essential oil, extracts, and chlorpheniramine in all three experimental conditions were significantly higher than those for saline ($p<0.05$ to $p<0.001$). Comparison of EC_{50} between 3 groups of experiments showed, only EC_{50} obtained the presence of essential oil and macerated extract in group 3 were significant higher than those of group 1 experiments ($p<0.05$ for both cases), (Table 1).

Slope of log concentration-response curves. The slope of histamine-response curves obtained in the presence of essential oil and macerated extract in group 2 and those of essential oil and aqueous extract from *B. persicum* in group 3 experiments were not significantly different from those obtained in the presence of saline. However, the slope of histamine-response curves obtained in the presence of essential oil obtained in groups 2 and 3 ($p<0.05$ for both cases) and that of aqueous extract in group 3 was significantly higher than group 1 ($p<0.001$). The slope of histamine-response curve obtained in the presence of macerated extract obtained in group 2 was also significantly higher than those of another two groups ($p<0.001$ for both cases), (Table 2).

Maximum response to histamine. The maximum response to histamine obtained in the presence of essential oil and extracts from *B. persicum* were significantly lower than those of saline in all three sets of experiments ($p<0.01$ to $p<0.001$). However, the maximum response to histamine obtained in the presence of aqueous extract in group 3 experiments was significantly improved compared to group 1 ($p<0.05$) and that of macerated extract in group 2 was improved compared to the other two sets of experiments ($p<0.001$ vs group 1 and $p<0.05$ *vs* group 3), (Table 3).

Fig. 1. Cumulative log concentration-response curves of histamine induced contraction of guinea pig tracheal chains, in the presence of saline, extracts from *B. persicum*, and chlorpheniramine on incubated preparations with three different conditions; a) indomethacin, b) indomethacin, propranolol and atropine, and c) indomethacin and propranolol (for each condition, n = 8). Essential oil in group 2 and 3, macerated extract in group 2 and aqueous extract in group 3 experiments caused parallel rightward shifts in histamine-response curves compared to the curves obtained in the presence of saline. The shifts of histamine-response curves obtained in the presence of chlorpheniramine in all three sets of experiments were also parallel.

Table 1. EC_{50} (μM) of histamine in the presence of aqueous extract, macerated extract, essential oil, 20nM chlorpheniramine, and saline in three groups of experiments.

Solutions	Group 1	Group 2	Group 3
Saline	17.00 ± 3.88	22.80 ± 2.72 ns	27.75 ± 2.48 ns nS
Aqueous extract	37.25 ± 4.94 *	49.88 ± 3.63 **** ns	45.63 ± 3.85 **** ns nS
Macerated extract	45.13 ± 5.80 ***	50.50 ± 2.60 **** ns	60.88 ± 2.79 **** # nS
Essential oil	35.75 ± 5.36 *	45.50 ± 2.35 **** ns	53.63 ± 4.84 **** # nS
Chlorpheniramine	39.13 ± 3.92 ***	45.00 ± 3.93 **** ns	53.63 ± 4.61 **** ns nS

Values are presented as mean ± SEM. Group 1, experiments on tracheal chains incubated with 1.4 µM indomethacin; Group 2, experiments on tracheal chains incubated with 1.4 µM indomethacin, 1 µM propranolol and 10nM atropine; Group 3, experiments on tracheal chains incubated with 1.4 µM indomethacin and 1 µM propranolol (for each group, n = 8); Significance of differences: (I), plant solutions vs saline; NS, non-significant difference; *, $p<0.05$; ***, $p<0.005$; ****, $p<0.001$; (II), the results of group 2 and 3 vs group 1; ns, non-significant difference; #, $p<0.05$; (III), the results of group 3 vs group 2; nS, non-significant difference.

Comparison between antihistaminic effect of B. persicum and chlorpheniramine. The values of (CR-1) obtained in the presence of essential oil and macerated extract in group 2 and those of essential oil and aqueous extract in group 3 experiments showed non-significant difference with that of chlorpheniramine (Table 4).

DISCUSSION

The bronchodilatory effect seen for *B. persicum* in our previous study [5] might be produced due to several different mechanisms. One possible mechanism responsible for this effect could be the inhibitory effect of this plant on histamine H_1 receptors [7]. The inhibitory effect of the essential oil and extracts from this plant was therefore examined on isolated guinea pig tracheal preparations in this study.

The non-parallel rightward shifts in histamine log concentration-response curves, obtained in the presence of essential oil and extracts, greater EC_{50} but lower maximum contraction effect to histamine compared to those of saline in group 1 experiments (incubated trachea with only indomethacin) indicated a functional antagonistic effect of *B. persicum* at histamine H_1 receptors of guinea pig trachea [8-10].

To evaluate the contribution of β-adrenergic stimulatory and/or muscarinic blocking effect on functional antagonism of *B. persicum* at histamine H_1 receptors, the antihistaminic effects of extracts and essential oil from this plant were also examined on incubated tracheal preparation with indomethacin, propranolol, and atropine. The

Table 2. Slope of histamine Log concentration-response curves in the presence of aqueous extract, macerated extract, essential oil, 20nM chlorpheniramine, and saline in three groups of experiments .

Solutions	Group 1	Group 2	Group 3
Saline	1.3 ± 0.13	1.57 ± 0.09 ns	1.57 ± 0.08 ns nS
Aqueous extract	0.76 ± 0.06 ***	1.00 ± 0.14 * ns	1.40 ± 0.09 NS # # nS
Macerated extract	0.62 ± 0.08 ***	1.34 ± 0.11 NS # #	0.81 ± 0.10 **** ns $$
Essential oil	0.80 ± 0.16 *	1.37 ± 0.09 NS #	1.40 ± 0.15 NS # nS
Chlorpheniramine	1.26 ± 0.12 NS	1.56 ± 0.15 NS ns	1.46 ± 0.07 NS ns nS

Values are presented as mean ± SEM. Group 1, experiments on tracheal chains incubated with 1.4 µM indomethacin; Group 2, experiments on tracheal chains incubated with 1.4 µM indomethacin, 1 µM propranolol and 10nM atropine; Group 3, experiments on tracheal chains incubated with 1.4 µM indomethacin and 1 µM propranolol (for each group, n = 8); Significance of differences: (I), plant solutions vs saline; NS, non-significant difference; *, $p<0.05$; **, $p< 0.01$; ***, $p<0.005$; ****, $p<0.001$; (II), the results of group 2 and 3 vs group 1; ns, non-significant difference; #, $p<0.05$; # #, $p<0.001$; (III), the results of group 3 vs group 2; nS, non-significant difference; $$, $p<0.001$.

Table 3. Maximum response to histamine obtained in the presence of aqueous extract, macerated extract, essential oil, 20nM chlorpheniramine, and saline in three groups of experiments .

Solutions	Group 1	Group 2	Group 3
Saline	100.0 ± 0.00	100.0 ± 0.0 ns	100.0 ± 0.0 ns nS
Aqueous extract	53.75 ± 5.36 ****	59.00 ± 6.19 **** ns	74.38 ± 3.87 *** # nS
Macerated extract	43.06 ± 6.20 ****	72.38 ± 7.16 ** # #	50.00 ± 3.74 **** ns $
Essential oil	59.06 ± 10.5 **	64.75 ± 5.40 **** ns	74.88 ± 5.36 *** ns nS
Chlorpheniramine	91.50 ± 3.02 NS	87.75 ± 3.60 NS ns	91.75 ± 2.63 * ns nS

Values are presented as mean ± SEM. Group 1, experiments on tracheal chains incubated with 1.4 µM indomethacin; Group 2, experiments on tracheal chains incubated with 1.4 µM indomethacin, 1 µM propranolol and 10nM atropine; Group 3, experiments on tracheal chains incubated with 1.4 µM indomethacin and 1 µM propranolol (for each group, n = 8); Significance of differences: (I), plant solutions vs saline; NS, non-significant difference; *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$, ****, $p<0.001$; (II), the results of group 2 and 3 vs group 1; ns, non-significant difference; #, $p<0.05$; # #, $p<0.001$; (III), the results of group 3 vs group 2; nS, non-significant difference; $, $p<0.05$.

parallel rightward shift in histamine-response curves obtained in the presence of macerated extract and essential oil compared to that of saline and significant improvement of maximum responses to histamine obtained in the presence of these two solutions in this part of the study, relative to those of group 1 experiments showed possiblecompetitive antagonistic effects of these two solutions on histamine H_1 receptors. Parallel shift in concentration-response curves and improvement of maximum response to histamine without significant change in EC_{50} obtained in the presence of macereted extract and essential oil in this part of the study indicates anticholinergic and/or adrenergic stimulatory effects of these solutions. The nonsignificant difference between the values of (CR-1) obtained in the presence of both solutions in this part of the study with that of chlorpheniramine indicates comparable antagonistic effect of the solutions relative to chlorpheniramine at concentrations used. Although improvement in maximum responses was obtained in the presence of macerated extract and essential oil in group 2 experiments compared to those of group 1, there were still significant differences between maximum responses obtained in the presence of both solutions and that of saline, indicating small functional antagonistic effects of these solutions at histamine H_1 receptors other than β-adrenergic stimulatory and/or muscarinic blocking effect.

In order to investigate whether changes of concentration response curves observed in group 2 experiments are due to β-adrenergic stimulatory or muscarinic blocking effects, the antihistaminic effect of the plant was also examined on tracheal chains incubated with indomethacin and propranolol. The results of this part of the study obtained in the presence of macerated extract were fairly similar to those of group 1 and those obtained in the presence of essential oil were fairly similar to those of group 2 study. These results indicate that functional antagonism of macerated extract at histamine H_1 receptors is mainly due to the blocking effect of this extract on muscarinic receptors. Non-parallel shift in histamine-response curves obtained in presence of macerated extract in group 1 and 3 experiments could be due to the presence of substances with muscarinic receptor blocking effect

Table 4. Values of (CR-1) obtained in the presence of macerated extract, aqueous extract, essential oil, and 20 nM chlorpheniramine in three groups of experiments.

Solutions	Group 1	Group 2	Group 3
Aqueous extract	--	--	0.68 ± 0.14 NS
Macerated extract	--	1.40 ± 0.28 NS	--
Essential oil	--	1.23 ± 0.31 NS	1.00 ± 0.21 NS
Chlorpheniramine	1.80 ± 0.35	1.06 ± 0.16	1.06 ± 0.28

NS, non-significant difference of (CR-1) values between chlorpheniramine and plant solutions.

and also substances with competitive antagonistic effect at histamine H_1 receptors in this extract from *B. persicum*. The results obtained in the presence of essential oil and aqueous extract in this part of the study showed that the functional antagonism of these two solutions observed in group 1 experiments is mainly due to stimulatory effect on β-adrenergic receptors. In a previous study, we have also demonstrated a competitive antagonistic effect of macerated extract and a non-competitive antagonistic effect of aqueous extract and essential oil from *B. persicum* at muscarinic receptors [5] which confirm the results of the present study. The values of (CR-1) obtained in the presence of both solutions in this part of the study were not significantly different from that of chlor-pheniramine indicating comparable antagonistic effect relative to chlorpheniramine at concentrations used.

In order to inhibit arachidonic acid metabolism, in all three parts of the study, tissues were incubated with

indomethacin. The different histamine H_1 receptors blocking effect of extracts is presumably due to the variation of methods of extraction.

The other possible mechanisms responsible for bronchodilatory effect of *B. persicum* and functional antagonism of its extracts and essential oil at histamine H_1 receptors are: stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC [11], Methylxanthine activity of the plant [12], opening of potassium channels [13], inhibition of phosphodiesterase [14], and calcium antagonism [15]. The contribution of these mechanisms in the bronchodilatory effect of *B. persicum* and functional antagonism of its extracts and essential oil at histamine H_1 receptors should be clarified in further studies.

With regard of the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, the *B. persicum* 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 essential oil of some species of Umblliferate family (*Carum carvi*) has been demonstrated [16]. Another study has demonstrated a suppressing effect of the essential oil of *Bunium carvi* on initial stage of inflammatory proses [17]. However, the effect of *B. persicum* on airway inflammation existing in asthma disease, especially on experimental model of asthma should be investigated in further studies.

In conclusion the results of this study suggested a competitive antagonistic effect of *B. persicum* at histamine H_1 receptors. In addition, the results indicated a blocking effect of macerated extract from this plant at muscarinic receptors, a stimualtory effect of essential oil and aqueous extract on β-adrenergic receptors.

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