

THE EFFECT OF *ZATARIA MULTIFLORA BOISS* ON β_2 -ADRENOCEPTORS OF GUINEA PIG TRACHEAL CHAINS

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Summary

The effect of aqueous-ethanolic extract of *Zataria multiflora Boiss* (Labiatae), on β -adrenoceptors was examined on tracheal chains of guinea pigs. The effects of three concentrations of aqueous-ethanolic extract (0.05, 0.1 and 0.2 mg/mL), 10 nM propranolol, and saline on β -adrenoceptors were tested (n=8). The results showed clear leftward shifts in isoprenaline curves obtained in the presence of all concentration of the extract compared with that of saline. The EC₅₀ (the effective concentration of isoprenaline, causing 50% of maximum response) obtained in the presence of all concentrations of the extract was significantly lower compared to saline (p<0.05 to p<0.001). The maximum responses obtained in the presence of all three concentrations of the extract were not significantly different compared with that of saline. All values of (CR-1 = (EC₅₀ obtained in the presence of active substances/EC₅₀, obtained in the presence of saline) -1) obtained in the presence of concentrations of extract were negative and there were significant differences in this value between propranolol and those obtained in the presence of extract (p<0.01 for all three cases). The results indicated a relatively potent stimulatory effect of the extract from *Zataria multiflora Boiss* on β_2 -adrenoceptors.

Key words: *Zataria multiflora Boiss*, Stimulatory effect, β_2 -adrenoceptors, trachea, guinea pig

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Effect of *Zataria multiflora Boiss* on β_2 -Adrenergic receptors

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Introduction

Zataria multiflora Boiss L is a grassy and annual plant which grows in many areas of the world. Main active constituents of this plant are: terpenes, phenols, thymol, carvacrol, terpenoids, glycosides of phenolic monoterpenoids, eugenol and aliphatic alcohols, the flavonoids thymonin, cirsilineol, and 8-methoxycirsilineol, biphenyl compounds of monoterpenoid origin, caffeic and rosmarinic acids, [1], tannins, labiatic acid, ursolic acid, and oleanolic acid. *Zataria multiflora* also contains apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di, tri, and tetramethoxylated flavones [2].

The extract of this plant has been used for treatment of coughs due to colds, bronchitis and pertussis; and laryngitis and tonsillitis. Topical applications of the extract have been used in the treatment, the common cold, disorders of the oral cavity, and as an antibacterial agent in oral hygiene. Both the essential oil and thymol are ingredients of a number of proprietary drugs including antiseptic and healing ointments, syrups for the treatment of respiratory disorders, and preparations for inhalation [1]. It has also been used to treat pertussis, stomatitis, and halitosis [2].

The relaxant effect of this plant in ileum [3-5] and uterus [6] smooth muscle was shown. The relaxant effect of the plant on tracheal smooth muscle also has been demonstrated [4, 5, 7]. The therapeutic effect of *Zataria* in respiratory disorders of chemical war victims is also demonstrated [8]. An anti-tussive effect for the plant was also documented [9]. Several other effects have been also shown for the plant including; anti-fungal, anti-candida and effect on different parasites [10-14]. The antibacterial activity of the plant has been demonstrated [15, 16]. It has been also shown that *Zataria multiflora* has anti-inflammatory and analgesic effects [17-20].

In the present study, the stimulatory effect of aqueous-ethanolic extracts of *Zataria multiflora* Boiss on β -adrenoceptors was examined on tracheal chains of guinea pigs to examine one possible mechanism for the relaxant effect of the plant on smooth muscle,

Material and Methods

Plant and extracts

Zataria multiflora Boiss was Collected from form mountain in the region between Tabas and Yazd, (centre east region of Iran), Fleurine mine and identified by MR Joharghi. A voucher specimen was preserved in the Herbarium of the School of Agriculture, Ferdowsi University (Herbarium No: 35314, FUMH).

The aqueous-ethanolic extract of the isolated stigmata was prepared as follows: fifty grams of *Zataria multiflora* seeds were grinded, added to 700 mL of ethanol 50% (350 mL distilled water and 350 mL ethanol) using the Soxhlet apparatus. The solvent was then removed under reduced pressure. The plant ingredient concentration in the final extract was adjusted to 0.1 g/mL by adding distilled water to the dried extract.

Tissue preparations

Male Dunkin-Hartley guinea pigs (400-700 g) were sacrificed by a blow on the neck and the trachea were 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 [21].

Tissue was then suspended in a 10 mL organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseleit solution with the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under isotonic tension (1 g) and allowed to equilibrate for at least 1 hr while it was washed with Krebs solution every 15 min.

The study was approved by the University's Ethics Committee. The allowance number of the relevant ethical committee for the animal experiments is 85301.

Protocols

The stimulatory effect of of *Zataria multiflora* Boiss was examined by producing the cumulative log concentration-response curve of isoprenaline sulphate (Sigma Chemical Ltd UK) induced relaxation of pre-contracted tracheal chains by 10 µM methacholine hydrochloride (Sigma Chemical Ltd UK) 10 min after the exposure of tissue to one solution (n = 8). Different tested solutions were included: 10 nM propranolol (0.1 ml of propranolol hydrochloride with 0.1 µM concentration, Sigma Chemical Ltd UK), three concentrations of aqueous-ethanolic extract from *Zataria multiflora* Boiss (0.05, 0.1 and 0.2 mg/ml) or 0.2 ml saline. The consecutive concentrations of isoprenaline were added every 2 min (including 1 nM - 1000 µM); and the percentage of relaxation due to each concentration in proportion to the maximum relaxation obtained in the presence of saline was plotted against log concentration of isoprenaline. The effective concentration of isoprenaline causing 50% of maximum response (EC₅₀) in each experiment was measured using the log concentration-response curve of the corresponding experiment.

The shift of cumulative log concentration-response curves obtained in the presence of different concentrations of extract and propranolol were examined by comparing the EC₅₀ obtained in the presence of each solution with that of saline. In addition the maximum responses to isoprenaline obtained in the presence of different concentrations of extract and propranolol in all sets of experiments were compared with that of saline. To examine the parallel rightward shift, the slope of the isoprenaline -response curve of each experiment was measured and was compared with that of saline. In experiments with parallel shift in isoprenaline -response curve, the concentration-ratio minus one (CR-1) as an index of the competitive antagonism effect was calculated by the following equation:

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

All of the experiments were performed randomly with 1 hour resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments contractions were measured using an isotonic transducer (Harvard APP LTD, 50-6360 SINO . 0210) and measured using a software by computer(Acer model NO.: G781) recording.

Statistical analysis

All data were expressed as mean±SEM. The EC₅₀, the slope, and the maximum response obtained in the presence of extract and propranolol were compared with those obtained in the presence of saline and (CR-1) obtained in the presence of extract with those obtained in the presence of propranolol using the paired t test. The comparison of the data of different concentrations of extract were performed using One-way Analysis of Variance (ANOVA) with Tukey- Kramer multiple pot test. Significance was accepted at p<0.05.

Results

Shift in cumulative log concentration-response curves

Cumulative log concentration-response curves to isoprenaline obtained in the presence of all concentration of the extract and propranolol showed clear rightward shift compared to isoprenaline curves produced in the presence of saline (Figure 1).

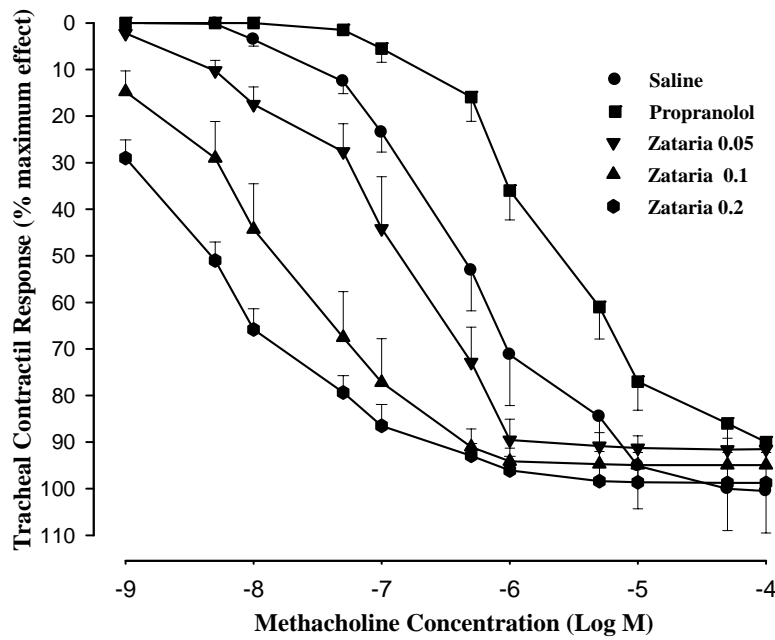


Fig. 1 Cumulative log concentration-response curves of isoprenaline induced relaxation of guinea pig tracheal chains, in the presence of saline, three concentrations of aqueous-ethanolic extract and 10 nM propranolol (n=8).

Tracheal response to isoprenaline (EC₅₀)

The EC₅₀ isoprenaline obtained in the presence of propranolol was significantly higher than that of saline (p<0.001). However, the EC₅₀ obtained in the presence of all concentrations of the extract were significantly lower than those of saline (P<0.05 to p<0.001), (Figure 2).

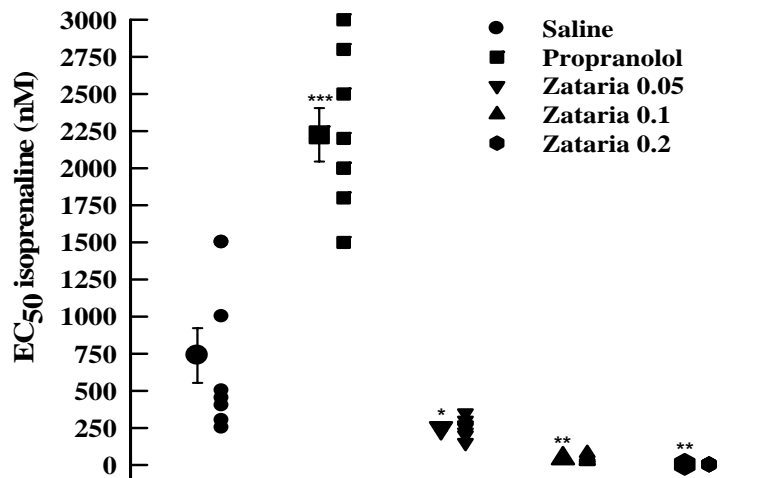


Fig. 2 EC₅₀ metacholin obtained in the presence of three concentrations of aqueous-ethanolic extract from *Zataria* (0.05 ▼, 0.1 ▼, and 0.2 mg/ml, ◆) and 10 nM propranolol (■) (n=8). Statistical comparison in EC₅₀ between saline and other solutions *: p<0.05, **: p<0.01, ***: p<0.001.

Maximum response to isoprenaline and the slope of isoprenaline concentration response curves

The maximum responses to methacholine obtained in the presence of all concentrations of the extract were not significantly different compared to that of saline (Table 1).

The slopes of isoprenaline -response curves obtained in the presence of all three concentrations of the extract also were significantly different with those of saline (Table 1).

Table 1. Differences in maximum response and slope obtained in the presence of propranolol, different concentration of zataria and carvacrol, with those of saline.

Solutions	Concentration	Maximum Response		Slope	
		Values	Stat. Dif.	Values	Stat. Dif.
Saline		100.00±0.00		-0.99±0.002	
Propranolol		89.42±5.20	NS	-0.98±0.003	NS
Extract	0.05 mg/ml	97.30±5.01	NS	-0.97±0.015	NS
	0.1 mg/ml	94.93±2.70	NS	-0.96±0.011	NS
	0.2 mg/ml	95.92±3.40	NS	-0.97±0.007	NS

Values are presented as mean±SEM (n=8). Stat. Dif.: statistical difference, NS: non-significant difference

Shift in methacholine concentration-response cuves (CR-1)

The values of (CR-1) obtained in the presence of all concentrations of the extract were negative and significantly different with that of atropine (p<0.01 for all cases), (Figure 3).

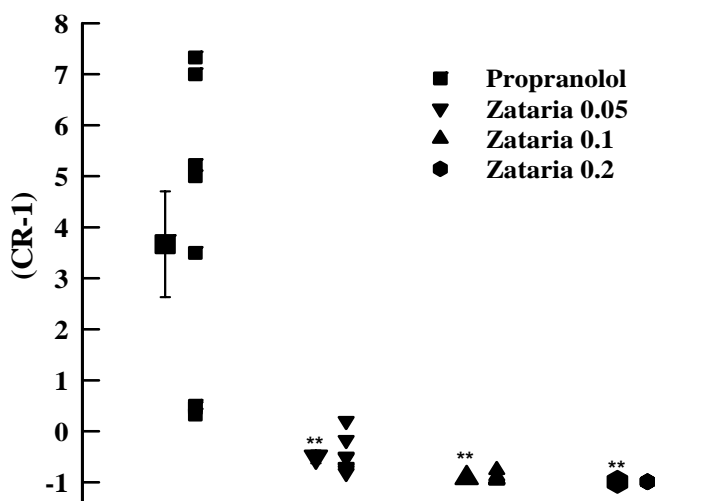


Fig. 3 The values of (CR-1) obtained in the presence of three concentrations of aqueous-ethanolic extract from *Zataria* (0.05 ▼, 0.1 ▼, and 0.2 mg/ml, ●) and 10 nM propranolol (■) (n=8). Statistical comparison in (CR-1) between propranolol and other solutions **: p<0.01.

Correlations between values of EC₅₀ and (CR-1) with concentrations of the extract

There was significant negative correlations between the concentrations of the extract and the values of EC₅₀ and (CR-1), ($r = -0.804$ and $r = -0.625$, respectively, $p < 0.001$ for both cases).

Discussion

The present study was performed in order to examine one possible mechanism responsible for the observed relaxant effect seen for the extract of *Zataria multiflora Boiss* on tracheal and other smooth muscle [3- 7]. Therefore, the stimulatory effect of the aqueous-ethanolic extract of the plant on β -adrenoceptors of tracheal chains was examined because relaxant effect of inhibition of this receptors has been shown [22]. The parallel rightward shifts in isoprenaline log concentration-response curves obtained in the presence of the different concentrations of aqueous-ethanolic extract, lower EC₅₀ and achievement of maximum relaxation effect to isoprenaline compared to that of saline showed possible competitive antagonistic effects of the hydro-ethanolic extract of the plant suggested a competitive antagonistic effect of the extract on β -adrenoceptors of guinea pig trachea [22-24]. The values of (CR-1) obtained in the presence of the concentrations of the extract were negative but that of propranolol was positive. These findings also supported competitive antagonistic effect of the extract on β -adrenoceptors of guinea pig trachea. The significant negative correlations between both values of EC₅₀ and (CR-1) and the concentrations of the extract indicated concentration-dependent effect of the extract. In conclusion, the results of this study suggested a competitive antagonistic effect of *Zataria multiflora Boiss* on β -adrenoceptors of guinea pig trachea.

Acknowledgments

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