Avicenna Journal of Phytomedicine
Received: Feb 11, 2011; Accepted: Mar 15, 2011
Vol. 1, No. 1, Summer 2011, 7-13

# *Zataria multiflora* Boiss inhibits muscarinic receptors of incubated tracheal smooth muscle with propranolol

Zahra Jafari[2], Mohammad Hossein Boskabady[1,*], Iran Pouraboli[2], Beheshteh Babazade[1]

## Abstract

**Objective(s):** In the present study, the effect of tissue incubation with propranolol on functional antagonism of *Zataria multiflora* Boiss (*Z. multiflora*) at muscarinic receptors of tracheal smooth muscle was examined.
**Materials and Methods:** The effects of three concentrations of aqueous-ethanolic extract, 10 nM atropine, and saline on muscarinic receptors were tested on incubated tracheal smooth muscle with propranolol (n=5).
**Results:** The $EC_{50}$ of all concentration of the extract and atropine was significantly higher than that of saline. There was parallel right ward shift in concentration response curves obtained in the presence of all concentrations of the extract. There was not any significant difference in the maximum response and slope obtained in the presence of different concentrations of extract compared to saline. There was significant positive correlation between the concentrations and the values of $EC_{50}$ ($p<0.001$). The value of (CR-1) obtained in the presence of highest concentration of the extract was significantly higher than that of atropine ($p<0.05$).
**Conclusion:** These results indicated that functional antagonism of *Z. multiflora* at muscarinic receptors of tracheal smooth muscle was mainly due to β-adrenoceptor stimulatory effect of plant.

**Keywords:** *Zataria multiflora* Boiss, Labiatae, Methacholine, Anti cholinergic effect, Trachea, Guinea pig

*1- Department of Physiology and Pharmaceutical Research Centre, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I. R. Iran*
*2- Department of Biology, School of Sciences, Shahid Bahonar University, Kerman, I. R. Iran*
***Corresponding Author:** Tel: +985118002221; Fax +985118828564
E-mail: Boskabadymh@mums.ac.ir

## Introduction

*Zataria multiflora* Boiss L is a grassy and annual plant which grows in many areas of the world. *Z. multiflora* is a perennial with a woody, fibrous root. The leaves are small, narrow and elliptical, greenish-grey in colors. The flowers terminate the branches in whorls with pale purple color. The seeds are roundish and very small.

The constituents of the 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, and saponins, tannins, labiatic acid, ursolic acid, and oleanolic acid (ESCOP, 1997; Mossa et al., 1987).

The therapeutic effects of *Z. multiflora* on common colds, bronchitis and pertussis, laryngitis, antibacterial agent in oral hygiene (ESCOP, 1997) and stomatitis have been described (Mossa et al., 1987).

This plant has relaxant effect on ileum (Gharib Naseri, 2003; Stecher, 1968; Reiter and Brandt, 1985), uterus (Gharib Naseri et al., 2006) and tracheal smooth muscle (Stecher, 1968; Reiter and Brandt, 1985; Boskabady et al., 2006). The therapeutic effects of *Z. multiflora* in respiratory disorders of chemical war victims (Mostafavi and Shasavari, 2005) and its anti-tussive effect (Afzali et al., 2003) were also documented. The anti-fungal, anti-Candida and effect on different parasites (Jafari et al., 2003; Khosravi et al., 2008; Mahmoudabadi et al., 2006), antibacterial activity (Javidnia et al., 1999; Janssen et al., 1987), analgesic, anti-inflammatory and analgesic effects (Amanlou et al., 2006; Ashtaral Nakhai et al., 2007; Hosseinzadeah and Salmani, 2003) for *Z. multiflora* were also documented.

In the present study, the effect of tissue incubation with propranolol on functional antagonism of *Z. multiflora* at muscarinic receptors of tracheal smooth muscle of guinea pigs was examined.

## Materials and Methods

### Plant and extracts

*Z. multiflora* Boiss was collected from the mountains between Tabas and Yazd, Fleurine mine (centre region of Iran) and identified by MR Joharchi. A voucher specimen was preserved in the Herbarium of the School of Agriculture, Ferdowsi University, Iran (Herbarium No: 35314, FUMH). The aqueous-ethanolic extract of the plant was prepared using the soxhlet apparatus as follows: fifty grams of *Z. multiflora* seeds were ground and added to 700 mL of ethanol 50% (350 mL distilled water and 350 mL ethanol) and extracted for 24 hours. 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 to the neck and the tracheae 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 (Boskabady et al., 2006).

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, $NaHCO_3$ 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$^{o}$C and gassed with 95% $O_2$ and 5% $CO_2$. 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 Ethical Committee of Mashhad University of Medical Sciences.

**Protocols**

The inhibitory effect of *Z. multiflora* was examined by producing the cumulative log concentration-response curve of methacholine hydrochloride (Sigma Chemical Ltd, Ltd UK) induced contraction of tracheal chains. Cumulative log concentration-response curve of methacholine was produced 10 min after the exposure of tissue to 10 nM atropine maleate (Sigma Chemical Ltd UK, Catalogue No. C4915) and three concentrations of aqueous-ethanolic extract from *Z. multiflora* (0. 5, 1.0 and 2.0 µg/mL). The consecutive concentrations of methacholine were added every 2 min (range 0.1 - 1000 µM). 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 methacholine. The effective concentration of methacholine causing 50% of maximum response ($EC_{50}$) 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 atropine was examined. For this purpose, the $EC_{50}$ obtained in the presence of each solution was compared with that of saline. In addition, the maximum responses to methacholine obtained in the presence of different concentrations of extract and atropine were compared with that of saline. To examine the parallel rightward shift, the slope of the methacholine-response curve of each experiment was compared with that of saline. In experiments with parallel shift in methacholine-response curve, the concentration-ratio minus one (CR-1) as an index of the competitive antagonism effect was calculated by the following equation:

($EC_{50}$ obtained in the presence of effective solutions/$EC_{50}$ obtained in the presence of saline) – 1

The study was performed on incubated tracheal chains 30 min prior to the beginning and while obtaining methacholine-response curve with 1 µM propranolol hydrochloride (n=5).

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 a computer (Acer model NO.: G781) recording.

**Statistical analysis**

All data were expressed as mean±SEM. The $EC_{50}$, the slope, and the maximum response obtained in the presence of extract and atropine 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 atropine using the paired t- test. Comparison of the data of different concentrations of extract was performed using One-way Analysis of Variance (ANOVA) with Tukey-Kramer multiple pot hoc test. Significance was set at $p<0.05$.

## Results

**Shift in cumulative log concentration-response curves**

Cumulative log concentration-response curves of methacholine obtained in the presence of different concentrations of the extract and atropine showed clear rightward shift compared to methacholine curves in the presence of saline (Figure 1).

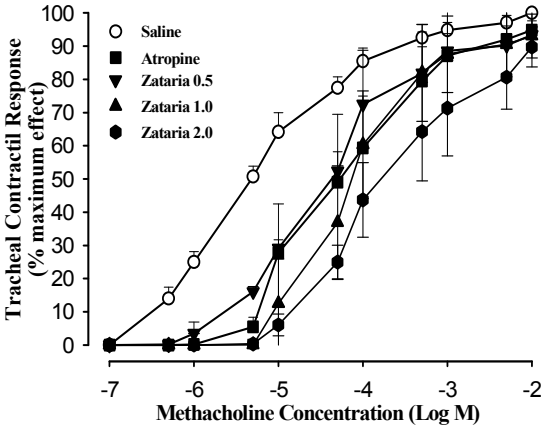


Figure 1. Cumulative log concentration-response curves of methacholine induced contraction of guinea pig tracheal chains in the presence of saline, three concentrations of aqueous-ethanolic extract and 10 nM atropine in incubated tracheal smooth muscle with 1 µM propranolol (n=5).

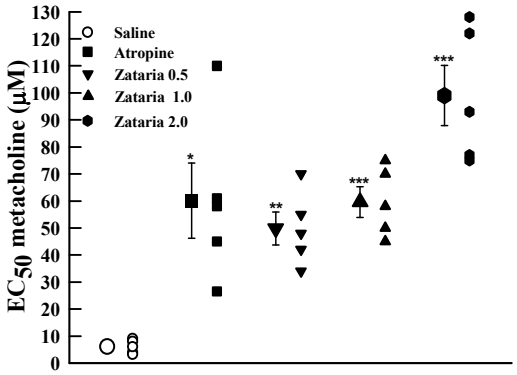


Figure 2. $EC_{50}$ of methacholine obtained in the presence of three concentrations of aqueous-ethanolic extract from *Z. multiflora* (0.5 ▼, 1.0 ▲, and 2.0 µg/mL, ⬣) 10 nM atropine (■), and saline (O) in incubated trachea with 1 µM propranolol (n=5). Statistical comparison of $EC_{50}$ between saline and other solutions NS: non-significant difference, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

**Tracheal responsiveness ($EC_{50}$)**

The $EC_{50}$ methacholine obtained in the presence of atropine was significantly higher than that of saline ($p<0.05$). The $EC_{50}$ obtained in the presence of all concentrations of the extract was also significantly higher than that of saline ($p<0.01$ for low and $p<0.001$ for two higher concentrations, Figure 2).

**aximum response to methacholine and slope of methacholine-response curves**

There was not any statistical significant difference in maximum responses to methacholine and the slopes of methacholine-response curves obtained in the presence of different concentrations of the extract with those of saline (Table 1).

**Shift in methacholine concentration response curves (CR-1)**

The values of (CR-1) obtained in the presence of high concentrations (2.0 µg/mL) of the extract was significantly higher than that of atropine ($p<0.05$, Figure 3).

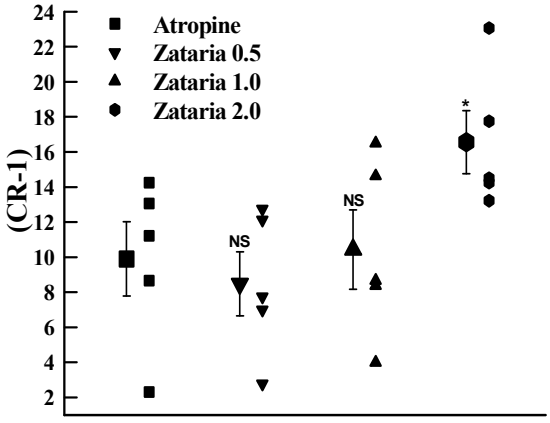


Figure 3. The values of (CR-1) obtained in the presence of three concentrations of aqueous-ethanolic extract from *Z. multiflora* (0.5 ▼, 1.0 ▲, and 2.0 µg/mL, ⬣) and 10 nM atropine on (■) incubated trachea with 1 µM propranolol (n=5). Statistical comparison of $EC_{50}$ between atropine and other solutions NS: non-significant difference, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Table 1. Maximum response to methacholine and the slope of methacholine log concentration-response curves obtained in the presence of aqueous-ethanolic extract from *Z. multiflora* on incubated tracheal chains with 1 µM propranolol (n=5).

| Solutions | Concentration | Maximum response | Difference *vs* saline | Slope | Difference *vs* saline |
|---|---|---|---|---|---|
| Saline | | 100.00±0.00 | | 0.86±0.03 | |
| | 0.5 µg/mL | 91.62±3.8 | NS | 1.26±0.31 | NS |
| Extract | 1.0 µg/mL | 90.80±4.14 | NS | 1.22±0.14 | NS |
| | 2.0 µg/mL | 90.80±3.57 | NS | 0. 90±0.21 | NS |
| Atropine | | 95.48±1.67 | NS | 95.48±1.67 | NS |

Values are presented as mean±SEM. NS: non-significant difference.

### Correlations between values of $EC_{50}$ and concentrations of the extract and Schild plot

There was statistically significant positive correlation between the concentrations of the extract and the values of $EC_{50}$ ($r=0.790$, $p<0.001$).

The slope of Schild plot [log (CR-1)] against log extract concentrations) for the extract was -0.743 (Figure 4).

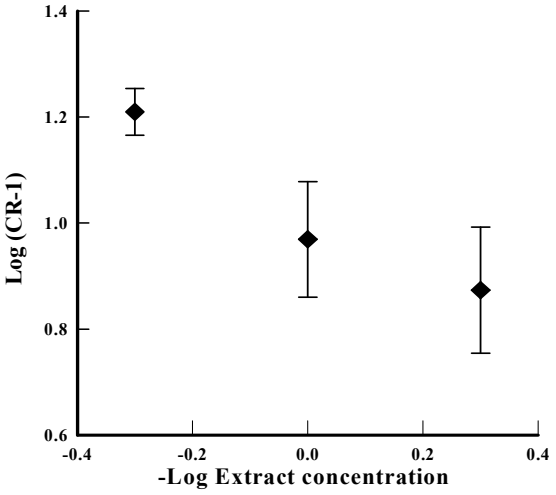


Figure 4. The Schild plot, log (CR-1) against log extract concentrations in incubated trachea with 1 µM propranolol. The slope of Schild plot for the extract was -0.743.

## Discussion

The relaxant effect on smooth muscle seen for the extract of *Z. multiflora* and other plants from this family in the previous studies (Gharib Naseri et al., 2006; Stecher, 1968; Reiter and Brandt, 1985; Boskabady et al., 2006) might be produced due to several different mechanisms including inhibitory effect on muscarinic receptors because relaxant effect of inhibition of this receptors has been shown (Loenders et al., 1992).

Previous study (In press in: Natural Product Research) showed non-parallel rightward shifts in methacholine log concentration-response curves in the presence of the of the extract and lower maximum contraction effect to methacholine compared to that of saline, indicated a functional antagonistic effect of the extract at muscarinic receptors of guinea pig trachea (Arunlakshana and Schild, 1959). Incubation of tracheal smooth muscle with propranolol and chlorpheniramine led to parallel rightward shift in methacholine-response curves and significant improvement in maximum responses to methacholine and increase in $EC_{50}$ in the presence of the extract. These results showed possible competitive antagonistic effects of the hydro-ethanolic extract on muscarinic receptors as well as inhibitory effect on histamine ($H_1$) receptors and/or adrenergic stimulatory effects for the

extract. To investigate whether changes of the results observed in incubated tissues with propranolol and chlorpheniramine are due to β-adrenergic stimulatory or histamine ($H_1$) receptor blocking effect, the effect of the extract was examined on tissues incubated with propranolol in the present study. The results of this study showed non-significant difference in slopes obtained in the presence of different concentrations of the extract with that of saline. The maximum responses obtained in the presence of concentrations of the extract also were not significant different with that of saline. The results of this study indicate a β-adrenergic stimulatory effect for the extract. In addition, $EC_{50}$, maximum response, slope and values of (CR-1) obtained in this study were not different with those of incubated tissues with chlorpheniramine and propranolol in our previous study (In press in: Natural Product Research). These results indicated that the changes in the data observed in this study and incubated tissues with chlorpheniramine and propranolol compared to non incubated tissues in our previous study (Submitted to Natural Product Research) are due to stimulatory effect of the extract on β-adrenergic receptors. The results of the present study were also supported by those of our previous study indicating the absence of the relaxant effect of the extract of another species of this plant family in tracheal chains incubated with propranolol, cholorpheniramine and contracted with methacholine (Boskabady et al., 2006).

The significant positive correlations between the effects of the extract and concentration indicated concentration-dependent effect of the extract. The similar values of (CR-1) obtained in the presence of two lower concentrations of the extract and even higher value of (CR-1) in the presence of last concentration of the extract compared to that of atropine indicate comparable or even higher antagonistic effect of the extract relative to atropine at used concentrations.

These results confirmed the competitive antagonistic effect of *Z. multiflora* at muscarinic receptors and also indicated a stimulatory effect at β-adrenergic receptors.

**Acknowledgments**

This study was financially supported by Research Department of Mashhad University of Medical sciences and Dept of Biology, Shahid Bahonar University of Kerman.

## References

Afzali H, Darabi M, Kashanian M. 2003. The effect of *Zataria multiflora* and *Foeniculum vulgare* in acute cough. 16th Iranian Congresss of Physiology and Pharmacology, p 192.

Amanlou M, Momen Beitollahi J, Abdollahzadeh S, Tohidast-Ekrad Z. 2006. Miconazole gel compared withf *Zataria multiflora* Boiss gel in the treatment of denture stomatitis. Phytother Res, 20**:** 966-969**.**

Arunlakshana O, Schild HO. 1959. Some quantitive uses of drug antagonist. Br J Pharmacol 14: 48-58.

Ashtaral Nakhai L, Mohammadirad A, Yasa N, Minaie B, Nikfar S, Ghazanfari G, Zamani MJ, Dehghan G, Jamshidi H, Shetab Boushehri V, Khorasani R, Abdollahi M. 2007. Benefits of *Zataria multiflora* Boiss in experimental model of mouse inflammatory bowel disease. Evid Based Complement Alternat Med**,** 4: 43-50.

Boskabady MH, Aslani MR, Kiani S. 2006. Relaxant effects of *Tymus volgari* on guinea pig tracheal chains and its possible mechanism(s). Phytother Res, 20: 28-33.

Boskabady MH, Jafari Z, Pouraboli I. 2010. The effect of carvacrol on muscarinic receptors of guinea pig tracheal chains. Phytother Res, [Epub ahead of print].

Boskabady MH, Jandaghi P. 2003. Relaxant effect of carvacrol on guinea pig tracheal

chains and its possible mechanisms. Pharmazie. 58: 661-663.

Boskabady MH, Kiani S, Rakhshandah H. 2006. Relaxant effects of *Rosa damascena* on guinea pig tracheal chains and its possible mechanism(s). J Ethnopharmacol, 106: 377-382.

ESCOP. 1997. "*Thymi herba*." Monographs on the medicinal uses of plant drugs**.** Exeter, U.K.: European Scientific Cooperative on Phytotherapy.

Gharib Naseri MK, Mazlomi H, Goshaiesh M, Vakilzadeh G and Heidari A. 2006. Antispasmodic effect of *Zataria multiflora* Boiss Leaf extract on the rat uterus. Iranian J Pharm Res, 2: 131-136..

Gharib Naseri MK. 2003. Effect of *Zataria multiflora* Boiss leaf hydroalchoholic extract on rat ileum. Behbood (J Kermanshah University Med Sci), 7: 18-26.

Hosseinzadeah H, Ramezani M, Salmani G. 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. J Ethnopharmacol, 73: 379-385.

Jafari S, Amanlou M, Borhan-Mojabi K, Farsam H. 2003. Comparative study of *Zataria multiflora* and *Anthemis nobelis* extracts with *Myrthus communis* preparation in the treatment of recurrent aphthous stomatitis. Daru, 11: 23-27.

Janssen AM, Scheffer JJC, Baerheim-Svendsen A. 1987. Antimicrobial activity of essential oils: A 1976–1986 literature review, Aspects of the test methods. Planta Med, 53: 395-398.

Javidnia K, Tabatabai M, Shafiee A. 1999. Volatile constituents and antimicrobial activity of *Zataria multiflora*, population Iran. Iran J Chem Chemical Egin, 18: 1-5.

Khosravi A, Eslami AR, Shokri H, Kashanian M. 2008. *Zataria multifloa* cream for the treatment of acute vaginal candidiasis. Inter J Gynaecol Obstet**,** 101: 201-202.

Loenders B, Rampart M, Herman AG. 1992. Selective $M_3$ muscarinic receptor antagonists inhibit smooth muscle contraction in rabbit trachea without increasing the release of acetylcholine. J Pharmacol Exp Ther. 263: 773-779.

Mahmoudabadi AZ, Dabbagh MA, Fouladi Z. 2007. In-vitro anti-candida activity of *Zataria multiflora* Boiss. Evid Based Complement Alternat Med**,** 4: 351-353.

Mossa JS, Al-Yahya MA, Al-Meshal IA. 1987. Medicinal plants of Saudi Arabia, Vol. 1. Riyadh, Saudi Arabia, King Saud University Libraries.

Mostafavi B, Shasavari S. 2005. The effect of *Zataria* on respiratory disorders of chemical war victims. Behbood, 7: 293-297.

Reiter M, Brandt W. 1985. Relaxant effects on tracheal and ileal smooth muscles of the guinea pig. Arzneimittelforschung, 35: 408-414.

Stecher PG, ed. 1968.The Merck Index: An encyclopedia of chemicals and drugs, 8th ed. Merck & Co., Inc: Rahway, NJ.