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

*Boskabady MH., Aslani MR., Mansuri F., Amery S.

Department of Physiology, Ghaem Medical Centre, Mashhad University of Medical Sciences, Mashhad, Iran.

Received: 17 Feb 2007; Revised: 15 Apr 2007; Accepted: 24 May 2007

ABSTRACT

Background: In Iranian ancient medical books, the therapeutic effects of *Satureja hortensis* on respiratory diseases have been reported. In order to verify these reports, the relaxant effect of aqueous-ethanolic extract of *Satureja hortensis* on guinea pig trachea was examined.

Methods: The relaxant effects of 6 cumulative concentrations of extract (0.15, 0.3, 0.45, 0.6, 0.75 and 0.9 g %) in comparison with saline and 4 cumulative concentrations of theophylline (0.15, 0.3, 0.45, and 0.6 mM) by their effects on precontracted tracheal chains of guinea pig by 10 µM methacholine (group 1) or 60 mM KCl in two different conditions including: non-incubated tissues (group 2) and incubated tissues with 1 µM propranolol, 1 µM chlorpheniramine and 1 µM atropine (group 3, for each group n=6) were examined.

Results: In group 1, the extract and theophylline showed concentration dependent relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$). In group 2, three last concentrations of theophylline and four higher concentrations of extract showed significant relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$). The effects of four lower concentrations of extracts in groups 1 and 2 were significantly lower than those of theophylline ($p<0.05$ $p<0.001$). In group 3, the extract did not show any significant relaxant effect. There were significant correlations between the relaxant effects and concentrations of extract and theophylline in groups 1 and 2 ($p<0.001$ for all cases).

Conclusion: These results showed a potent relaxant effect of *Satureja hortensis* on guinea pigs trachea which was comparable to that of theophylline.

Keywords: Satureja hortensis · Bronchodilatory · Guinea pig. Trachea

INTRODUCTION

Satureja hortensis L. is a grassy and annual plant which grows in south Europe, central and south-west of Asia countries such as Iran and Siberia. The leaves are small, narrow and elliptical, greenish-grey in colors. The flowers terminate the branches in whorls with white to pale red color. The leaves and flowers of *S. horte* have medicinal properties (1). Main active constituents of this plant are: thymol, carvacrol, γ-terpinene and burniol (1-4).

It has been reported in Iranian ancient medical books that *Satureja hortensis* has therapeutic effect on respiratory diseases and has been used orally to treat exacerbation of asthma and coughs. In addition, this plant has also been used for treatment of gastrointestinal disorders. A tonic property has also been described for this plant (1). Previous studies have shown antibacterial, antifungal, and antioxidant activities of the essential oil and methanolic extracts of herbal parts and callus cultures of *Satureja hortensis* (5-7), and antioxidant effect of phenolic (8) and flavonoid compounds of the plant (9). Antispasmodic (10,11) and anti-diarrhoeal effects (11), and anti-HIV activity of the plant (12), and the benefits of the isolated flavonoids from *Satureja hortensis* L. on hypercholesterolemic rabbits have also been shown (13).

The therapeutic effect of the plant on asthma could be due to relaxant effect on airway smooth muscles leading to dilation of the airways. In the present study, the relaxant effect of aqueous-ethanolic extract of the leaves of *Satureja hortensis* and its possible mechanism(s) on guinea pig tracheal chains were examined.

MATERIALS AND METHODS

Plant and extracts

Satureja hortensis 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

Correspondence: M-boskabady@mums.ac.ir

University of Medical Sciences (Herbarium No: 240-1615-12). The aqueous-ethanolic extract was prepared as follows: Fifty grams of the chopped, dried leaves of the plant was extracted with 150 ml distilled water and 150 ml ethanol by soxhlet apparatus. The solvent of the extract was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final aqueous extract was 10 g % in the extract.

Tissue preparations

Male Dunkin-Hartly 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). All rings were then cut open opposite the tracheal muscle, and sutured together to form a tracheal chain (14). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bioscience 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 maintained at 37^{o}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 while it was washed with Krebs solution every 15 min.

Protocols

The relaxant effects of six cumulative concentrations of aqueous-ethanolic extract (0.15, 0.3, 0.45, 0.6, 0.75 and 0.9 g %)), four cumulative concentrations of anhydrous theophylline (Sigma Chemical Ltd UK) (0.15, 0.3, 0.45, and 0.6 mM) as positive control, and saline as negative control (0.1 ml) were examined. An amount of 0.15 ml of 10 g % concentrated aqueous extracts was added to a 10 ml organ bath respectively six times. For theophylline, 0.15 ml of 10 mM concentrated solution was added to organ bath four times. The consecutive volumes were then added to organ bath at five minutes intervals.

In each experiment the effect of six cumulative volumes from the extract, four cumulative volumes from theophylline, or saline on contracted tracheal smooth muscle were measured after exposure of tissue to the solution for 5 min. 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 (bronchoconstrictory) effect, which was expressed as negative percentage change (15).

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

1. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 1).
2. On tracheal chains contracted by 60 mM KCl (group 2).
3. On incubated tracheal chains with 1 μM propranolol hydrochloride (Sigma Chemical Ltd UK) and 1 μM chlorpheniramine maleate (Sigma Chemical Ltd UK) and 1 μM atropine sulfate (Sigma Chemical Ltd UK) 30 min prior to beginning and during testing of relaxation of different solutions. In this series of experiments, tracheal chains were also contracted by 60 mM KCl (group 3).

The relaxant effect of theophylline was examined only on groups1 and 2. The relaxant effects in three groups of experiments were examined in three different series of tracheal chains (n=6 for each group). Experiments were performed randomly within a 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 were measured after fixation.

Statistical analysis

All data were expressed as mean±SEM. Data of relaxant effects of different concentrations of extract were compared with the results of negative and positive control using ANOVA. The data of relaxant effect obtained in three groups of experiments were also compared using ANOVA. The relaxant effect of extract and theophylline were related to the concentrations using least square regression. Significance was accepted at $p<0.05$.

RESULTS

Relaxant (bronchodilatory) effects

In groups 1 extract from *Satureja hortensis* and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea pig. The relaxant effects of all concentrations of extract and theophylline were significantly higher than those of saline ($p<0.01$ to $p<0.001$), (Table 1). The effects of four lower concentrations of extract in the group 1 were significantly lower than those of theophylline ($p<0.05$ o $p<0.01$), (Table 1).

In group 2 experiments, 3 last concentrations of theophylline and 4 higher concentrations of extract showed significant relaxant effects compared to those of saline ($p<0.05$ to $p<0.001$). The effects of four lower concentrations of aqueous extract were significantly lower than those of theophylline ($p<0.05$ to $p<0.001$). (Table 2).

In group 3, the extract of *Satureja hortensis* did not show any significant relaxant effect in comparison with saline (Table 3).

Comparison of the relaxant effects between three groups of experiments

The relaxant effects of most concentrations of extract in groups 1 and 2 were statistically greater than those of group 3 experiments ($p<0.01$ to $p<0.001$). The relaxant effects of most concentrations of extract in group 1 were also significantly higher than those of group 2 ($p<0.05$ to $p<0.001$), (Fig. 1b). In addition, there were no significant differences in the effect of all concentrations of theophylline between groups 1 and 2 (Fig. 1a).

Correlation between concentrations of solutions and their relaxant effects

There were significant positive correlations between the relaxant effects of extract and theophylline with solutions of different concentrations in groups 1 and 2 of experiments ($p<0.001$ for all cases), (Table 4).

Table 1. Relaxant effects of aqueous-ethanolic extract of *Satureja Hortensis* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (non incubated preparation contracted by 10 µM methacholine, n=6).

Conc.	Saline	Extract	Stat. Dif. *vs* Saline	Stat. Dif. *vs* Theo.	Theo.	Stat. Dif. *vs* Saline
0.15	-	13.54±3.38	P<0.01	P<0.01	32.59±6.35	P<0.01
0.30	-	30.16±7.05	P<0.01	P<0.01	57.56±6.85	P<0.001
0.45	-	46.11±7.49	P<0.001	P<0.01	73.59±7.10	P<0.001
0.60	-	58.97±6.92	P<0.001	P<0.01	87.39±6.22	P<0.001
0.75	-	72.25±5.38	P<0.001	-	-	-
0.90	1.37±0.62	89.90±3.62	P<0.001	-	-	-

Values are presented as mean±SEM. Stat. Dif. Stat. Dif.: Statistical differences, Theo.: theophylline NS: non-significant difference. The unit of concentration for extracts was g % and for theophylline was mM.

Table 2. Relaxant effects of aqueous-ethanolic extract of *Satureja Hortensis* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (non incubated tracheal chains contracted with 60 mM KCl, n=6).

Conc.	Saline	Extract	Stat. Dif. *vs* Saline	Stat. Dif. *vs* Theo.	Theo.	Stat. Dif. *vs* Saline
0.15	-	3.06±0.48	NS	P<0.05	18.91±1.86	P<0.01
0.30	-	14.84±6.30	NS	P<0.001	52.36±2.20	P<0.001
0.45	-	26.21±5.93	P<0.05	P<0.001	70.13±0.78	P<0.001
0.60	-	36.21±5.93	P<0.01	P<0.001	83.69±1.10	P<0.001
0.75	-	45.52±8.17	P<0.001	-	-	-
0.90	1.57±0.72	54.70±10.68	P<0.001	-	-	-

For abbreviations see Table 1.

Table 3. Relaxant effects of aqueous-ethanolic extract of *Satureja Hortensis* in comparison with negative control (saline) in group 3 experiments (incubated preparations with 1 µM propranolol, 1 µM cholorpheniramine and 1 µM atropine contracted by 60 mM KCl, n=6).

Concentration	Saline	Etract	Stat. Dif. *vs* Saline
0.15	-	0.32±0.32	NS
0.30	-	2.15±1.40	NS
0.45	-	6.19±4.47	NS
0.60	-	10.60±7.02	NS
0.75	-	3.05±1.79	NS
0.90	1.46±0.69	6.85±4.04	NS

For abbreviations see Table 1.

Table 4. Correlations (r) between the relaxant effects of aqueous-ethanolic extract of *Satureja Hortensis* and theophylline and concentration in three groups of experiments.

Different Substances	Group 1		Group 2		Group 3	
	r	P value	r	P value	r	P value
Aqueous-ethanolic extract	0.783	p<0.001	0.886	p<0.001	0.234	NS
Theophylline	0.800	p<0.001	0.967	p<0.001	--	--

NS: non-significant difference

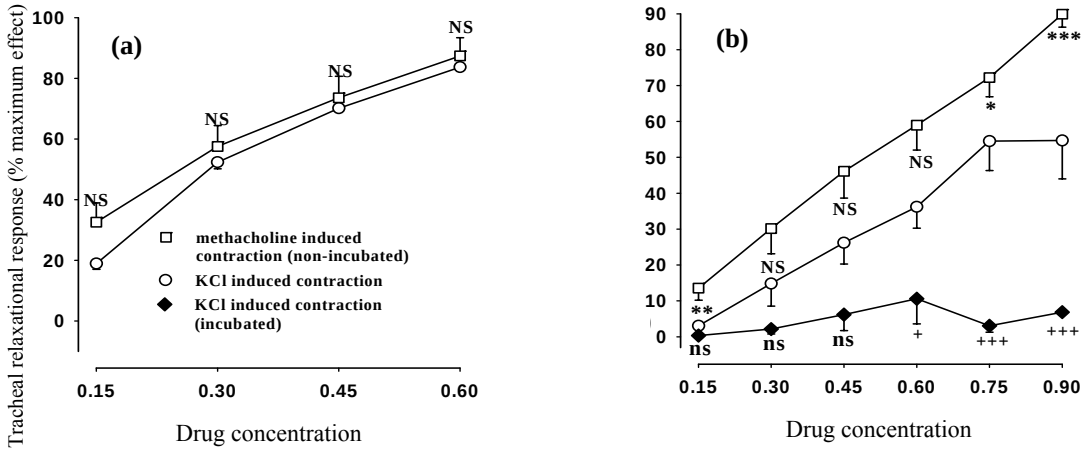

Figure 1. Concentration response curves of the relaxant effects for theophylline (a) and aqueous-ethanolic extract (b) of *Satureja Hortensis* in three groups of experiments (group 1; methacholine induced contraction on non - incubated tracheal chains (□), group 2; KCl induced contraction on non - incubated tracheal chains (o), and group 3 experiments; KCl induced contraction on incubated tracheal chains of guinea pig with atropine, propranolol and chlorpheniramine (♦, n=6 for each group) Statistical differences in the relaxant effects of different substances between group 1 compared with those of group 2; NS: non-significant difference, *; $p<0.05$, **; $p<0.01$, ***; $p<0.002$. Statistical differences in the relaxant effect of different substances between group 3 with those of groups 1 and 2; ns: non-significant difference, +; $p<0.05$, ++; $p<0.01$.

DISCUSSION

In this study the relaxant (bronchodilatory) effects of aqueous-ethanolic extract from *Satureja hortensis* in comparison with saline as negative control and theophylline as positive control were studied. In group 1 experiment (contracted tracheal chains by methacholine) extract from *Satureja hortensis* and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea pig. In group 2 experiments (contracted tracheal chains by KCl), 3 last concentrations of theophylline and 4 higher concentrations of extract showed significant relaxant effects compared to those of saline. However, the effects four lower concentrations of extract were significantly lower than those of theophylline. In-group 3, the extract of *Satureja hortensis* did not show any significant relaxant effects compared to that of saline. The effect of theophylline was not examined in group 3 experiments.

The relaxant effects of extract and theophylline was concentration dependent. There were positive correlations between increasing concentrations and the relaxant effects of extract in groups 1 and 2 experiments. The relaxant effects of 3 last concentrations of extract in group 2 were lower than those of groups 1. In addition, the effects of extract in group 1 experiments were comparable to that of theophylline.

The relaxant effects of the extract from *Satureja hortensis* on tracheal chains of guinea pigs might be produced by different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H_1 receptors or an anticholinergic property of this plant, because the relaxant effect of β_2-stimulatory (15,16), histamine H_1 receptor inhibitory (17), and anticholinergic drugs (18) have been shown in previous studies. To evaluate the contribution of β-adrenergic stimulatory, H_1 histamine and muscarinc blocking effect of extract, the effects of these extracts on tracheal chains inhibited β-adrenergic, muscarinuc and histamine H_1 receptors by propranolol, atropine and chlorpheniramine, were re-examined in group 3 experiments. The extract of the plant did not show any relaxant effect in group 3 experiments. The relaxant effects of all concentrations of the extract from *Satureja hortensis* obtained in the group 3 experiments were significantly lower than those of group 2. These findings suggest probable ß-adrenergic stimulatory, muscarinic and/or histamine H_1 blocking properties of the plant extract that may contribute to their relaxant effect on tracheal chins of guinea pig.

While KCl affects calcium channels (19) and becuse calcium channel blockers have bronchodilatory effect (20, 21), the relaxant effect of extract from *Satureja hortensis* in group 2 experiments may be due to blocking effects of the calcium channels. Another explanation for these findings is an opening effect of the extract on potassium channels (22). The weak relaxant effects of extract on tracheal chains contracted by KCl and high relaxant effect on tracheal chain contracted by metacholine may support opening

of potassium channels.

Since the plant showed a potent relaxant effect on methacholine induced contraction which was completely blocked in tissues incubated with atropine and chorpheniramine, the most possible mechanisms of the relaxant effect of *Satureja hortensis* might be due to its inhibitory effects on muscarinct receptors. Howevere, the inhibitory effect of the plant on histamine (H_1) receptors and its stimulatory effect on β-adrenoceptors can not ruled out with these results and shoud be re-examined in further studies.

The results of this study confirmed those of Shipochliev (10) and Hajhashemi *et al* (11) indicating relaxant effects of this plant on smooth muscles. Our previous study (23) showed a potent relaxant effect on tracheal chains for carvacol, which is one of the constituents of *Satureja hortensis.* Therefore, the carvacol content of the plant may be responsible for its relaxant effects on tracheal chains. The relaxant effect of the plant is not due to thymol as the plant main constituent, because another study failed to show any relaxant effect for thymol on tracheal chains of guinea pig (24).

ACKNOWLEDGMENTS

This study was financially supported by Vice Chancellor of Research of Mashhad University of Medical Sciences.

REFERENCES

1. Zargari A, *Medicinal plants*.Vol.2. 5th ed. Tehran University publications. Tehran (1992) 281-284.
2. Paskov D, Drianovska-Noninska L. Chemical and pharmacological studies on *Satureia hortensis* L. *Farmatsiia* 1954; 4: 31-37
3. Radonic A, Milos M. Chemical composition and antioxidant test of free and glycosidically bound volatile compounds of savory (*Satureja montana* L. subsp. montana) from Croatia. *Nahrung* 2003; 47: 236-7.
4. Baser K H C, Özek T, Kirimer N, Tümen G. A Comparative Study of the Essential Oils of Wild and Cultivated *Satureja hortensis* L. *J Essential Oil Res* 2004; 9: 1-4.
5. Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A, Sahin F. In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J Agric Food Chem* 2003; 51: 958-65.
6. Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A, Ozturk S, Kotan R. Evaluation of antimicrobial activities of *Satureja hortensis* L. *J Ethnopharmacol* 2003; 87: 61-65.
7. Lindberg HM, Andersen L, Christiansen L, Brockhoff P and Bertelsen G. Antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A* 1996; 1431-4630.
8. Kuchukashvili Z, Davitaia I, Gorozia G, Kvesitadze E, Lomidze G, Khatisashvili. Antioxidant characteristics of phenolic compounds of *Satureia hortensis*. *J Biol Phys Chem* 2003; 3: 5–88.
9. Kuchukashvili Z, A valiani N, Davitaia, G. Influence of Flavonoid fraction from *satureja hortensis* on microsomal oxidation. *Proceeding od of the Georgian Academy of Sceinces* 2003; 1-18.
10. Shipochliev T. Pharmacological study of several essential oils. I. Effect on the smooth muscle. *Vet-Med Nauki* 1968; 5: 63-9.
11. Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M. Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. *J Ethnopharmacol* 2000; 71: 187–192.
12. Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N, Oishi I, Inami R, Yamane M, Nakamura M, Murata H, Nakanishi T. Anti-HIV-1 activity of herbs in Labiatae. *Biol Pharm Bull* 1998; 21: 829-33.
13. Mchedlishvili D, Kuchukashvili Z, Tabatadze T, Davitaia G. Influence of flavonoids isolated from *Satureja hortensis* L. on hypercholesterolemic rabbits. *Ind J Pharmacol* 2005; 37: 259-260.
14. Holroyde MC. The influence of epithelium on the responsiveness of guinea pig isolated trachea. *Br J Pharmacol* 1986; 87:501-507.
15. Martin CAE, Naline E, Bakdach H, Advenier C. $Beta_3$ adrenoceptor agonists, BRL 37344 and SR 58611A do not induce relaxation of human, sheep and guinea pig airway smooth muscle in vitro. *Eur Respir J* 1994; 7: 1610-1615.
16. Linden A, Bergendal A, Ullman A, Skoogh BE, Lofdahl CG. Salmetrol, formetrol, and salbutamol in the isolated guinea-pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. *Thorax* 1993; 48: 547-553.
17. Popa VT, Somani P, Simon P, Simon V. The effect of inhaled verapamil on resting bronchial tone and airway constriction by histamine and acetylcholine in normal and asthmatic subjects. *Am Rev Respir Dis* 1984; 130: 106-113.

18. Lronards B, Rampart M, Herman AG. Selective M_3 muscarinic receptor inhibit smooth muscle contraction in rabit trachea without increasing the release of acetylcholine. *J Pharmacol Exp Ther* 1992; 263: 773-770.
19. Perez-Guerrero C, Suarez J, Herrera MD, Marhuenda E. Spasmolytic effect of tetrazepam on rat duodenum and guinea pig ileum. *Pharmacol Res* 1997; 35: 493-494.
20. Miyahara Y, Kizawa Y, Sano M, Murakami H. Effect of organic and inorganic Ca^{2+} antagonists on acetylcholine induced contraction in molluscan (Mytilus edulis) smooth muscle. *Gen Pharmacol* 1993; 24: 1419-1423.
21. McCaig D, DeJonckeere S. Effect of two Ca^{2+} modulator in normal and albumin sensitised guinea pig trachea. *Eur J Pharmacol* 1993; 249: 53-63.
22. Buckle DR, Arch JRS, Boering NE,Foster KA, Taylor JF, Taylor SG, Shaw DJ. Relaxation effect of potassium channel activators BRL 38227 and Pinacidil on guinea pig and human airway smooth muscle, and blockade of their effects by Glibenclamide and BRL 31660. *Pulmon Pharmacol* 1993; 6: 77-86.
23. Boskabady MH, Jandaghi P. Relaxant effect of carvacrol on guinea pig tracheal chainsand its possible mechanisms. *Pharmazie* 2003; 58: 661-663.
24. Boskabady MH, Rakhshandah H, Moetamedshariati V. Bronchodilatory and anticholinergic effects of *Carum copticum* on isolated guinea pig tracheal chains. *Med J I R Iran* 1998; 11: 329-334.