



Research Paper

The extract of *Zataria multiflora* affect tracheal responsiveness, serum levels of NO, nitrite, PLA2, TP and histamine in sensitized Guinea pigs

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ABSTRACT

Ethnopharmacological relevance: Different therapeutic effects for *Zataria multiflora* have been described in Iranian traditional medicine. In addition anti-inflammatory and anti-oxidant effects of this plant were previously described. The effect of the extract of *Zataria multiflora* on tracheal responsiveness (TR) and inflammatory mediators of sensitized guinea pigs was examined.

Materials and methods: Five groups of sensitized guinea pigs to ovalbumin (OA) were given drinking water alone, drinking water containing three concentrations of the extract and dexamethasone. TR to methacholine and OA, serum levels of NO, nitrite, PLA2, TP and histamine were measured ($n=6$, for each groups).

Results: TR to methacholine and OA, serum levels of NO, nitrite, PLA2, TP and histamine were increased in sensitized animals compared to control group ($p < 0.05$ to $p < 0.001$). Treatment of sensitized animals with dexamethasone and most concentration of the extract lead to significantly decrease in all measured parameters compared to sensitized group ($p < 0.05$ to $p < 0.001$) except TP and histamine levels in treated group with dexamethasone.

Conclusion: These results showed a preventive effect of the extract of *Zataria multiflora* on tracheal responsiveness, serum level of NO, nitrite, total protein, PLA2 and histamine in sensitized guinea pigs which was equal or even more potent than dexamethasone at used concentrations.

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1. Introduction

Asthma is an inflammatory disorder of the airways which different inflammatory cells are involved in the pathogenesis of the airway inflammation in this disease (Kelly et al., 1998). The inflammatory cells involved in asthma produce more inflammatory mediators and reactive oxygen species than those of normal subjects (Cluzel et al., 1987).

Inflammatory cells, such as neutrophils and alveolar macrophages, and tracheal epithelial cells release PLA2 (Vadas and Pruzanski, 1986). PLA2 have an important role in the synthesis of eicosanoids by releasing arachidonic acid metabolites from the membrane phospholipids of various inflammatory cells (Chang et al., 1987) which are involved in the allergic inflammation of bronchial asthma (Kashima et al., 1993). Increased PLA2 activity was shown in serum and bronchoalveolar lavage fluid from asthmatics (Kashima et al., 1993).

It was shown that NO can induce plasma extravasation and lung edema. NO can contribute in asthma physiopathology by increasing vascular permeability, mucus hypersecretion, inflammatory cell infiltration, epithelial cell damage and perpetuation of the Th2-mediated inflammatory response in the airways (Ricciardolo et al., 2004). Increased nitrite, nitrate, and nitrotyrosine concentrations in the exhaled breath condensate of asthmatic patients were observed (Robroeks et al., 2007). Glucocorticoids and selective inhibitors can inhibit inducible NOS and may have therapeutic potential in asthma (Saleh et al., 1998). In asthmatic patients and animal model of asthma, increased serum histamine level (Busse and Swenson, 1989) was also documented.

Zataria multiflora Boiss L is a grassy and annual plant 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, cirsiolineol, and 8-methoxycirsiolineol, biphenyl compounds of monoterpenoid origin, caffeic and rosmarinic acids, and saponins, tanins, labiatic acid, ursolic acid, and oleanolic acid (ESCOP, 1997;

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Shariffar et al., 2007). *Zataria multiflora* is an edible plant. It is a well known condiment or spice and used as food additive regularly. For more information please refer to a recent published review article (Sajed et al., 2013).

Different therapeutic effects including the effect on common colds, bronchitis and pertussis, laryngitis, antibacterial in oral hygiene (ESCOP, 1997) have been described for *Zataria multiflora*.

In addition, different pharmacological effects such as relaxant effect in ileum (Reiter and Brandt, 1985), uterus (Gharib Naseri et al., 2006) and tracheal smooth muscle (Boskabady et al., 2006a) have been shown for this plant. There are reports regarding the therapeutic effect of *Zataria* in respiratory disorders of chemical war victims (Mostafavi and Shasavari, 2005) and its antitussive effect (Afzali et al., 2003). Previous studies also showed anti-fungal, anti-Candida and effect on different parasites (Mahmoudabadi et al., 2006), antibacterial activity (Shariffar et al., 2007), analgesic and anti-inflammatory effects (Hosseinzadeh and Salmani, 2003) for *Zataria multiflora*.

Zataria multiflora is widely used as a traditional medicinal plant for treatment of various diseases. The clinical uses of this plant were well documented (ESCOP, 1997). In addition, the effects of this plant on treatment of recurrent aphthous stomatitis (Jafari et al., 2003) and primary dysmenorrhea (Irvani, 2009) were shown. Different pharmacological effects such as anti-inflammatory, anti-oxidant and relaxant effect on smooth muscle were shown for this plant in *in vitro* setting previously. The immunomodulatory effect of *Zataria multiflora* was also shown recently (Boskabady et al., 2013). Therefore, in the present study the protective effect of oral administration of the extract of *Zataria multiflora* on tracheal responsiveness and serum inflammatory mediators of sensitized guinea pigs was examined *in vivo*.

2. Materials and methods

2.1. Animal sensitization and animal Groups

Animals were sensitized to OA according to the method described previously (Boskabady and Ziaei, 2003; Boskabady et al., 2006b). Briefly, guinea pigs were sensitized to 10 mg OA (Sigma Chemical Ltd, UK) and 100 mg Al(OH)₃ dissolved in 1 ml saline *i.p.* One week later they were given 2 mg OA and 100 mg Al(OH)₃ dissolved in 1 ml saline *i.p.* as a booster dose. From day 14 sensitized animals were exposed to an aerosol of 4% OA for 18 ± 1 days, 5 min daily (Fig. 1). The aerosol was administered in a closed chamber, dimensions 30 × 20 × 20 cm³. The study was approved by the ethical committee of the Mashhad University of Medical Sciences.

The study was performed in control animals (group C, treated as the same as sensitized group but normal saline was used instead of OA and animals were given drinking water alone) and five different groups of sensitized animals which were given drinking water alone (group S, an animal model of asthma) or drinking water containing treatment agents during sensitization period as follows (*n* = 6 for each group);

- 1) 50 µg/ml dexamethasone (group S+D).
- 2) 0.2 mg/ml of the extract (group S+Z1).
- 3) 0.4 mg/ml of the extract (group S+Z2).
- 4) 0.8 mg/ml of the extract (group S+Z3).

2.2. Plant and extracts

Zataria multiflora was collected from the region between Tabas and Yazd, (center east part of Iran), Fleurine mine. The plant was identified by MR Joharchi and a voucher specimen was preserved in the Herbarium of the School of Agriculture, Ferdowsi University (Herbarium no: 35314, FUMH). The aqueous-ethanolic extract was prepared by adding 50 g of grounded *Zataria multiflora* seeds to 700 ml of ethanol 50% (350 ml distilled water and 350 ml ethanol) using the Soxhlet apparatus. The solvent was removed under reduced pressure and the extract concentration in the final extract was adjusted to 10 mg/ml by adding distilled water to the dried extract. The extract was solved in distilled water by adding few drop of tween 80. The characteristic of the extract of the same plant was identified in our previous study with the same plant which showed that one of the main constituent of the plant is carvacrol (Boskabady et al., 2011a).

2.3. Tissue preparation

Trachea was removed after animal scarification by a blow on the neck. One tracheal chain was prepared in each animal by cutting trachea 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 a tracheal chain (Boskabady et al., 2004).

Tissue was suspended in a 20 ml organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing Krebs–Henseliet solution of 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 of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Responses were measured using vernier control type 850N sensor with sensitivity range: 0–20 g and resolution: 0.2 mm/turn (Hugo-Sachs Elektronik, Germany) and amplified with amplifier (ML/118 quadbridge amp, March-Hugstetten, Germany) and recorded on powerlab (ML-750, 4 channel recorder, March-Hugstetten, Germany).

2.4. Assessment of tracheal response to methacholine and OA

A cumulative log concentration-response curves of methacholine hydrochloride (Sigma Chemical Ltd., U.K.) induced contraction of tracheal chain were obtained in each experiment. Consecutive concentrations (including 10^{−7}–10^{−5} mM, dissolved in saline) were added every 3 min, the contraction due to each concentration was recorded at the end of 3 min, and the effect reached a plateau in all experiments. To obtain the curve the percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction obtained by its final concentration was plotted against log concentration of methacholine. A concentration-response curve of methacholine was performed in tracheal chain of each studied animal.

The effective concentration of methacholine, causing 50% of maximum response (EC₅₀) was measured from methacholine response curve in each experiment using 50% of maximum response in the Y axis and measuring the dose of methacholine causing this response in

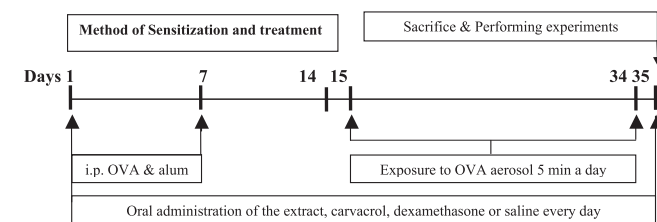


Fig. 1. Method of sensitization and administration of the extract, carvacrol, dexamethasone or saline during sensitization period.

the X axis. Contractility response to 10 μ M methacholine as the magnitude of contraction was also measured.

The tracheal response of all animals to 0.1% solution of OA was measured in each studied animal as follows: 0.5 ml of 4% OA solution (dissolved in saline) was added to the 20 ml organ bath and the degree of tracheal chain contraction was recorded after 15 min and was expressed as proportion (in percentage) to contraction obtained by 100 μ M methacholine. The measurement of tracheal response to methacholine and OA was performed in random order.

2.5. Measurement of serum nitric oxide (NO)

A total of 5 ml peripheral blood was obtained immediately after sacrificing the animals and placed at room temperature for 1 h. The samples were then centrifuged at 3500g at 4 °C for 10 min. The supernatant was collected and immediately stored at –70 °C until analysis. Serum NO levels were determined by Nitric Oxide Calorimetric Assay kit (nitric oxide colorimetric assay kit k262-200, BioVision Research Products, USA) according to the manufacturer's instructions. Nitrate in the samples and standards were converted to nitrite by nitrate reductase and Griess reagent was added in the each well plate. Absorbance was determined at 540 nm. The concentration of total NO (nitrite + nitrate) was calculated according to a standard curve of known nitrite concentrations. For measuring nitrite, a nitrite standard curve was prepared in the absence of nitrate reductase in the standard and assay samples.

2.6. Measurement of serum histamine, total protein and PLA2

Serum histamine, and PLA2 levels were measured in supernatant described above, using the enzyme-linked immunosorbent assay (ELISA) sandwich method according to the manufacturer's instructions, (LDN's histamine assay kit, BAE-1100, Co., LDN, England, PLA₂: E10217: Invitrogen, England).

The serum protein level was determined using quantitative protein assay kit (Pars Azmon, Iran) according to the manufacturer protocol with photometric method.

Serum levels of all chemicals were measured in control, sensitized and S groups treated with dexamethasone and three concentrations of the extract.

2.7. Statistical analysis

The comparisons of the data between different groups were performed using one way ANOVA with Tukey Kermar post-hoc test. Significance was accepted at $p < 0.05$.

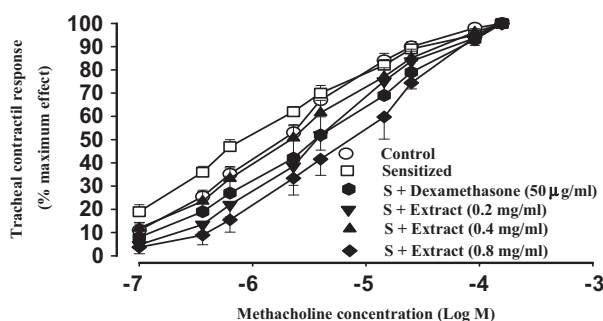


Fig. 2. Cumulative log concentration-response curves of methacholine induced contraction of isolated trachea in control guinea pigs (C), sensitized group (S), S treated with dexamethasone and three concentrations of the extract (S+Extract) (for each group, $n=6$).

3. Results

3.1. Tracheal response to methacholine

While concentration response curves to methacholine showed left ward shift in the group S compared to those in the group C, the curves of treated groups with dexamethasone and all concentrations of *Zataria multiflora* were shifted to right compared to group S (Fig. 2).

The mean value of EC₅₀ in tracheal chains of group S was significantly lower than group C ($p < 0.01$, Fig. 3a). The mean value of EC₅₀ in tracheal chains in treated groups with all three concentrations of *Zataria multiflora* and dexamethasone were

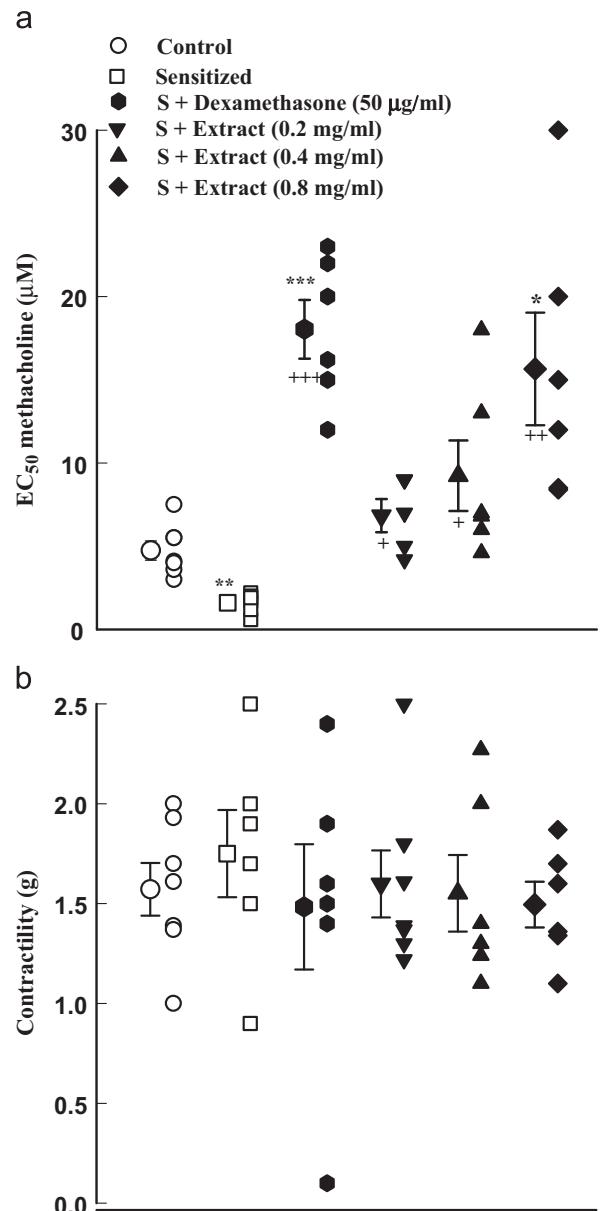


Fig. 3. Individual values and mean $\pm$ SEM (big symbols with bars) of (a) tracheal response to methacholine (EC₅₀) and (b) tracheal contractility to methacholine in control guinea pigs (C), sensitized animals (S), S treated with dexamethasone and three concentrations of the extract (S+Extract), (for each group, $n=6$). Statistical differences between control and different groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between treated and sensitized group: + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

significantly different compared to the group S ($p < 0.05$ to $p < 0.001$, Fig. 3a).

Contractility response to methacholine in tracheal chains of group S was also non-significantly lower than group C. In addition, contractility response to methacholine in tracheal chains of treated groups with all three concentrations of *Zataria multiflora* and dexamethasone was not significantly different compared to the group S (Fig. 3b).

3.2. Tracheal response to ovalbumin

Tracheal response to OA in group S was significantly higher than group C ($p < 0.001$, Fig. 4a). Tracheal response to OA in treated groups with dexamethasone and all concentrations of the extract were significantly decreased compared to group S ($p < 0.05$ for dexamethasone and $p < 0.01$ for all three concentrations of the extract, Fig. 4a).

Maximum contractility response to OA in tracheal chains of group S was also significantly higher than group C ($p < 0.01$, Fig. 4b).

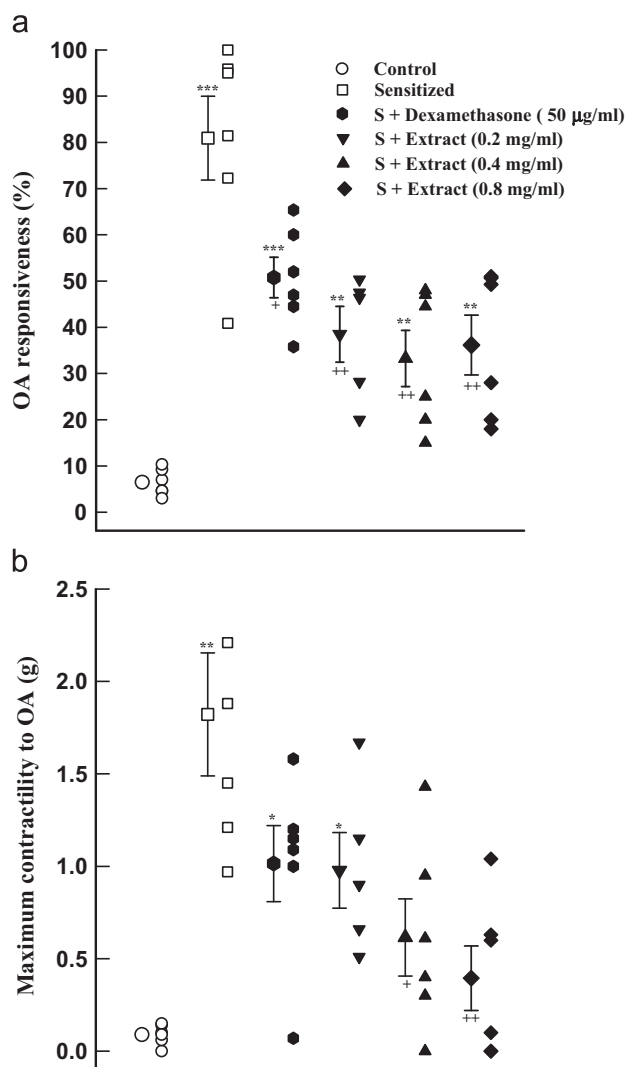


Fig. 4. Individual values and mean $\pm$ SEM (big symbols with bars) of (a) tracheal response to ovalbumin 0.1% (percent concentration in proportion to contraction obtained by 100 μ M methacholine) and (b) tracheal contractility to OA in control guinea pigs (C), sensitized animals (S), S treated with dexamethasone and three concentrations of the extract (S+Extract), (for each group, $n=6$). Statistical differences between control and different groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between treated and sensitized group: + $p < 0.05$, ++ $p < 0.005$. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

Maximum contractility response to OA in treated groups with dexamethasone and all concentrations of the extract was decreased compared to group S which was only statistically significant for two higher concentration of the extract ($p < 0.05$ and $p < 0.01$ for medium and high concentrations respectively, Fig. 4b).

3.3. Serum levels of NO, nitrite, PLA2, total protein and histamine

Serum levels of NO, nitrite, PLA2, total protein and histamine in group S were significantly higher than those of group C ($p < 0.05$ to $p < 0.001$, Figs. 5–7). Treatment of S animals with dexamethasone and all concentrations of the extract, lead to significant reduction in serum levels of NO and nitrite ($p < 0.05$ to $p < 0.001$, Fig. 5). However the level of total PLA2, protein and histamine levels in only treated groups with two higher concentrations of the extract were significant decreased compared to group S ($p < 0.05$ to $p < 0.001$, Figs. 6 and 7).

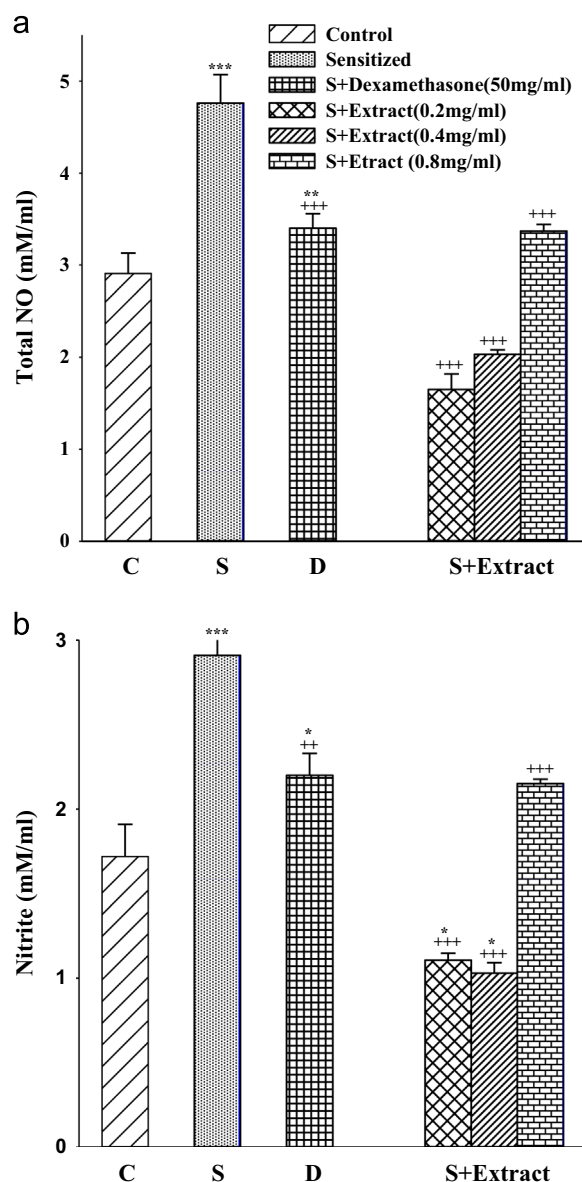


Fig. 5. The levels of serum NO₂+NO₃ and NO₃ in control guinea pigs (C), sensitized animals (S), S treated with dexamethasone and three concentrations of the extract (S+Extract), (for each group, $n=6$). Statistical differences between control and different groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between treated and sensitized group: + $p < 0.05$, ++ $p < 0.005$, +++ $p < 0.001$. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

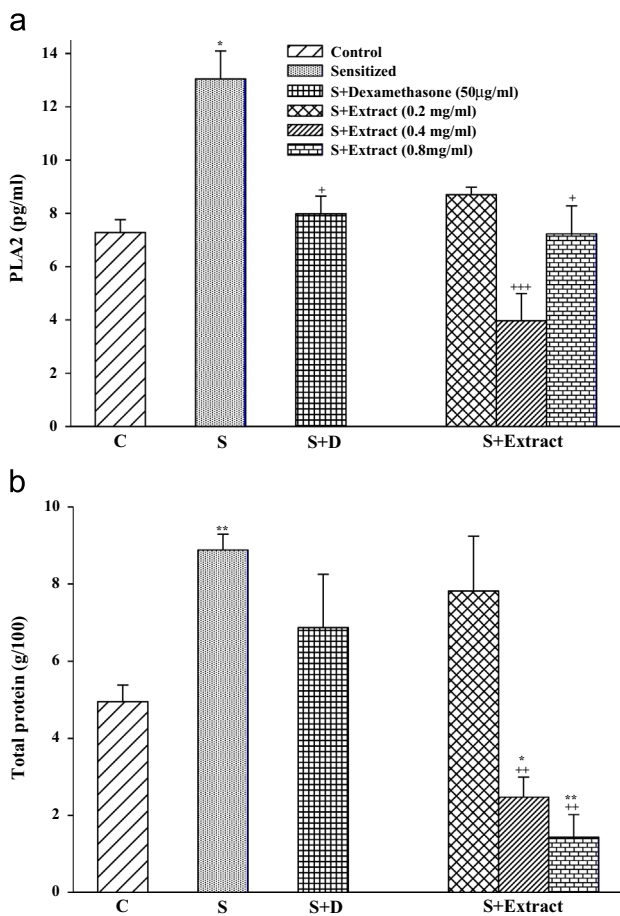


Fig. 6. The levels of serum PLA2 and total protein in control guinea pigs (C), sensitized animals (S), S treated with dexamethasone and three concentrations of the extract (S+Extract), (for each group, $n=6$). Statistical differences between control and different groups: * $p < 0.05$, ** $p < 0.01$. Statistical differences between treated and sensitized group: +++ $p < 0.001$. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

3.4. Differences between the effect of dexamethasone and *Zataria multiflora*

The effect of lower concentrations of the extract on most measures (except TR to OA) was significantly lower than the effect of dexamethasone ($p < 0.05$ to $p < 0.01$, Tables 1 and 2). However, there was not statistical difference between the effect of higher concentration of the extract with that of dexamethasone (Tables 1 and 2). In addition the effects of two higher concentrations of the extract on TR to OA were significantly higher than the effect of dexamethasone ($p < 0.05$ for both concentrations, Table 2).

3.5. Differences in between three concentrations of *Zataria multiflora*

The effect of high concentration of the extract on nitrite and nitrate was significantly higher than the effect of two lower concentrations ($p < 0.001$ for all cases, Table 2). In addition, the effects of two higher concentrations of the extract on total protein and histamine levels were significantly higher than the effect of its lower concentration ($p < 0.01$ for TP and $p < 0.001$ for histamine, Table 2). The effect of high concentration of the extract on histamine was also significantly higher than the effect of its medium concentration ($p < 0.001$, Table 2).

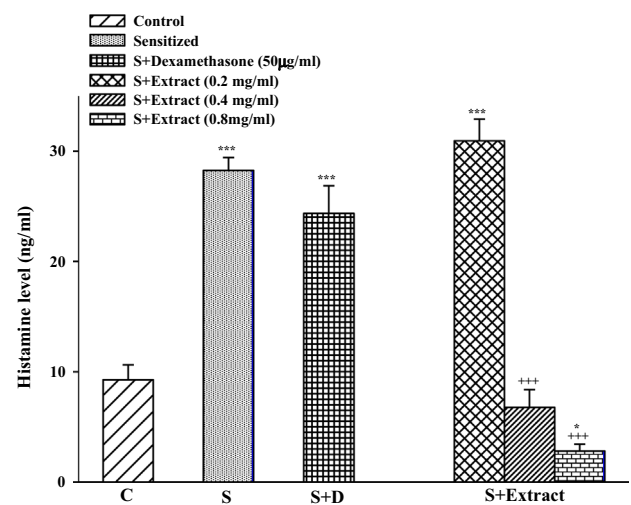


Fig. 7. The levels of serum histamine in control guinea pigs (C), sensitized animals (S), S treated with dexamethasone and three concentrations of the extract (S+Extract), (for each group, $n=6$). Statistical differences between control and different groups: * $p < 0.05$, *** $p < 0.001$. Statistical differences between treated and sensitized group: +++ $p < 0.001$. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

4. Discussion

The results showed increased TR to methacholine, OA, serum levels of NO nitrate PLA2, total protein and histamine which confirm sensitization (induction of an animal model of asthma). In fact, the results of the present study were very similar to our previous studies using the similar method of sensitization (Neamati et al., 2009, Keyhanmanesh et al., 2010) which support the sensitization of animals. Although, Tibério et al. (1997) and some others used only inhaled OA to induce sensitization which is more physiological and close to human asthma, but several studies used both injected and inhaled OA to insure sensitization in guinea pigs (Zhu et al., 2000; Li et al., 2001; Abd El Aziz et al., 2011; Boskabady and Ziaei, 2003; Boskabady and Kiani, 2007; Neamati et al., 2009, Keyhanmanesh et al., 2010, Boskabady et al., 2013).

The present study also showed that dexamethasone and all concentrations of the extract of *Zataria multiflora* caused protective effect on increased tracheal responsiveness to methacholine and OA, as well as serum levels of NO nitrate PLA2, total protein and histamine. Treatment of sensitized animals with dexamethasone also caused significant improvements in TR to methacholine, NO, nitrate and PLA2.

The preventive effect of the extract of *Zataria multiflora*, on tracheal responsiveness is perhaps due to its suppressing effect on airway inflammation in sensitized animals. The effect of the extract on serum levels of PLA2, TP and histamine support the effect of the plant on lung inflammation of sensitized animals. The preventive effects of anti-inflammatory drugs on different inflammatory mediators including histamine level (Gelfand, 2002) of sensitized and asthmatic patient were shown previously which support the findings of the present study.

In fact, the main characteristic feature of asthma is airway inflammation and prophylactic drugs used in the treatment of this disease should reduce airway inflammation. Therefore the results of the present study suggested that the extract of *Zataria Multiflora* could be used as preventive treatment of asthma disease leading to reduction in lung inflammation and airway responsiveness exists in this disease.

In addition, reduction in serum levels of total NO and nitrite in sensitized animals treated with the extract of *Zataria Multiflora* may suggest the antioxidant effect of the plant. The role of nitrite and

Table 1

Values of tracheal response to methacholine (EC₅₀) and response to ovalbumin in control guinea pigs (C), sensitized animals (S), S treated with three concentrations of the extract of *Zataria Multiflora* (S+Z1, S+Z2 And S+Z3) and dexamethasone (S+D), (for each group, n=6)

Parameters	C	S	S+D	S+Z1	S+Z2	S+Z3
EC ₅₀ (μmol)	4.74 ± 0.58	1.60 ± 0.24	18.03 ± 1.75	6.84 ± 0.99***	9.23 ± 2.11**	15.65 ± 3.37
OA (%)	6.45 ± 1.17	80.90 ± 9.0798	50.77 ± 4.39	38.49 ± 6.0*	36.78 ± 6.2*	36.15 ± 6.4*
Contractility to OA(g)	0.09 ± 0.02	1.82 ± 0.33	1.02 ± 0.21	0.98 ± 0.20*	0.61 ± 0.21	0.40 ± 0.18 ⁺⁺
Max. res. to met. (g)	1.57 ± 0.13	1.75 ± 0.22	1.48 ± 0.31	1.60 ± 0.17	1.55 ± 0.19	1.50 ± 0.11

Values are presented as mean ± SEM. Three concentrations of the extract were 0.2, 0.4 and 0.8 mg/ml and that of dexamethasone was 50 μg/ml. Max. res. to met.: maximum response to methacholine.

Statistical significance for the difference between the data of S+D and S+Z1, S+Z2 and S+Z3: **p* < 0.05, ***p* < 0.01, ****p* < 0.001. The statistical comparisons were made using unpaired *t* test. Statistical significance for the difference between the data of S+Z3 and S+Z2 and S+Z1: ⁺⁺*p* < 0.01. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

Table 2

Values of serum NO₂ + NO₃, NO₃, PLA₂, total protein and histamine levels in control guinea pigs (c), sensitized animals (S), S treated with three concentrations of the extract of *Zataria Multiflora* (S+Z1, S+Z2 and S+Z3) and dexamethasone (S+D), (for each group, N=6)

Mediators	Control	S	S+D	S+Z1	S+Z2	S+Z3
NO ₂ + NO ₃	2.91 ± 0.22	4.76 ± 0.31	3.40 ± 0.16	1.65 ± 0.167***	2.03 ± 0.05***	3.37 ± 0.07 ⁺⁺⁺ †††
NO ₃	1.72 ± 0.19	2.91 ± 0.15	2.20 ± 0.13	1.107 ± 0.04***	1.03 ± 0.062***	2.152 ± 0.026 ⁺⁺⁺ †††
PLA ₂	7.28 ± 0.48	13.05 ± 1.04	7.98 ± 0.67	8.7 ± 0.28	3.97 ± 1.02* ⁺	7.23 ± 1.05
Total protein	4.95 ± 0.43	8.88 ± 0.41	6.87 ± 1.38	7.82 ± 1.42	2.47 ± 0.52* ⁺⁺	1.43 ± 0.59* ⁺⁺
Histamine	9.28 ± 1.35	28.25 ± 1.16	24.35 ± 2.51	30.94 ± 1.95	6.77 ± 1.617*** ⁺⁺⁺	2.815 ± 0.61*** ⁺⁺⁺ †††

Values are presented as mean ± SEM. Three concentrations of the extract were 0.2, 0.4 and 0.8 mg/ml and that of dexamethasone was 50 μg/ml. The units of PLA₂, total protein and histamine are pg/ml, g/100 ml and ng/ml respectively.

Statistical significance for the difference between the data of S+D and S+Z1, S+Z2 and S+Z3: **p* < 0.05, ****p* < 0.001. The statistical comparisons were made using unpaired *t* test.

Statistical significance for the difference between the data of S+Z3 and S+Z2 and S+Z1: ⁺*p* < 0.05, ⁺⁺*p* < 0.01, ⁺⁺⁺*p* < 0.001. Statistical significance for the difference between the data of S+Z3 and S+Z2: †††*p* < 0.001. The statistical comparisons were made using ANOVA with the Tukey–Kramer multiple post test.

nitrate in pathophysiology of asthma was showed by increased nitrite and nitrate concentrations in the exhaled breath condensate of asthmatic patients (Robroeks et al., 2007). The Inhibitory effect of glucocorticoids, and selective inhibitors on inducible NOS in asthma (Saleh et al., 1998) support the antioxidant effect of the extract of *Zataria Multiflora* observed in the present study.

The anti-inflammatory, analgesic and anti-oxidant effects for *Zataria Multiflora* (Hosseinizadeh and Salmani, 2003; Sharififar et al., 2007; Karimian et al., 2011; Fatemi et al., 2012; Kavooosi et al., 2012; Kavooosi and Teixeira da Silva, 2012) and anti-inflammatory and anti-oxidant effects for its constituent carvacrol (Landa et al., 2009) have been shown which support the observed effect of the extract on sensitized guinea pigs in the present study. However, in previous studies, anti-inflammatory and anti-oxidant effect of *Zataria multiflora* extracts was studied in in vitro but in this study the anti-inflammatory effect of the plant was studied on in vivo setting which is more advanced compared to previous investigations. In addition, the effect of *Zataria multiflora* on inflammatory parameter of animal model of asthma, specially the effect of the plant on specific and non specific tracheal responsiveness, the most important characteristic feature of asthma was evaluated in the present study for first time.

The effects of lower concentration of the extract of *Zataria Multiflora* on almost all measured mediators were lower than its two higher concentrations. These results showed a concentration dependent preventive effect of the extract on inflammatory mediators of sensitized animals which could be a further evidence of specific anti-inflammatory effect of the plant.

Although the preventive effect of low concentration of the extract on most parameter in treated sensitized animals was lower, the effects of its two higher concentrations were similar or even higher than dexamethasone. These findings may also confirm the anti-inflammatory effect of the plant in sensitized guinea pigs which was comparable or even greater than the effect of dexamethasone.

The relaxant effect of the extract of other plant of this family (*Tymus vulgaris*) (Boskabady et al., 2006a), and one of the main constituent of the plant, carvacrol (Boskabady and Jandaghi, 2003) on tracheal smooth muscle, stimulatory effect of the plant on β₂-adrenoceptors (Boskabady et al., 2011a) and the inhibitory effect of the plant and carvacrol on histamine (H₁) receptors (Boskabady et al., 2012b) were shown in our previous studies. In addition the inhibitory effect of carvacrol (Boskabady et al., 2011d) and the extract of *Zataria multiflora* (Boskabady et al., 2012a; Jafari et al., 2011) on muscarinic receptors of tracheal smooth muscle were also documented. The preventive effects of carvacrol on tracheal responsiveness, total and differential WBC count in blood (Boskabady and Jalali, 2013), serum cytokines and endothelin levels (Jalali et al., 2013) in ovalbumin sensitized guinea-pigs were also observed. In addition, the effect of *Zataria multiflora* on inflammatory cytokines was previously demonstrated in both sensitized guinea pigs and human mononuclear cells which showed reduction of IL-4 but increased IFN-γ indicating enhanced Th1/Th2 balance in both conditions (Boskabady et al., 2013). The preventive effect of the extract of *Zataria multiflora* and its constituent, carvacrol, on lung pathology, total and differential WBC in lung lavage as well as serum levels of IgE, and eosinophil peroxidase (EPO) in sensitized guinea pigs was also demonstrated (Boskabady et al., 2014). The possible mechanisms of the effect of the plant and its constituents including carvacrol such as contribution of NF-κB should be examined in further studies.

The findings of this study showed improvement in tracheal responsiveness, serum levels of total NO, nitrite, total protein, PLA₂ and histamine in sensitized guinea pigs treated with the extract of *Zataria multiflora*. Therefore, these results together with other studies indicating anti-inflammatory effect for the plant and its relaxant effect on tracheal smooth muscle, indicate that this plant could have a therapeutic effect on inflammatory diseases such as asthma by causing both bronchodilation and reduction effect on lung inflammation. However further studies needed to

evaluate the effect of *Zataria multiflora* on asthmatic patients. In addition the effect of other constituents of the plant on sensitized animals also should be examined in further studies.

The results of the study clearly indicated significant differences between control and sensitized groups supporting the induction of sensitization of the animal. The findings also showed significant improvement effect of the extract which was comparable to the effect of dexamethasone. These results clearly suggest a preventive effect of the extract on sensitized group which may suggest a therapeutic value for the plant in treatment of asthma. Although, evaluating of the effect of the extract on control animal is not a major limitation of the present study, it could be examined in further studies.

In conclusion, the results of this study showed a preventive effect of the extract of *Zataria multiflora* on tracheal responsiveness, serum level of NO, nitrite, total protein, PLA2 and histamine in sensitized guinea pigs which was equal or even more potent than dexamethasone at used concentrations. These results suggest that the extract of *Zataria multiflora* could be valuable in treatment of asthma disease.

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