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RESEARCH ARTICLE

Ocimum basilicum affects tracheal responsiveness, lung inflammatory cells and oxidant–antioxidant biomarkers in sensitized rats

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ABSTRACT

The anti-inflammatory and antioxidant effects of *Ocimum basilicum* (*O. basilicum*) was shown previously. In the present study, the effect of *O. basilicum* on tracheal responsiveness (TR) to methacholine and ovalbumin (OVA), bronchoalveolar lavage fluid (BALF) levels of oxidant–antioxidant biomarkers as well as total and differential white blood cell (WBC) in sensitized rats was examined. Six groups of rats including control (group C), sensitized rats to OVA (group S), S groups treated with three concentrations of *O. basilicum* (0.75, 1.50, and 3.00 mg/ml) and one concentration of dexamethasone (1.25 μg/ml) ($n = 8$ for all groups) were studied. TR to methacholine and OVA, total WBC count, percentages of eosinophils, monocytes, neutrophils, and levels of oxidant biomarkers were significantly increased but other measured parameters were significantly decreased in group S compared to group C. TR to methacholine and OVA, percentages of eosinophils, monocytes, neutrophils, and levels of oxidant biomarkers were significantly decreased but lymphocytes and antioxidant biomarkers were significantly increased in S groups treated with dexamethasone and at least two higher concentrations of the extract compared to group S. Total WBC count was also decreased in treated S groups with dexamethasone and high extract concentration. The effect of extract on most measured parameters was significantly lower than dexamethasone treatment. The effects of two higher concentrations of the extract on most variables were significantly higher than the effect of low extract concentration. These results showed the concentration-dependent effect of *O. basilicum* on tracheal responses, lung inflammatory cells, and oxidant–antioxidant parameters in sensitized rats.

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KEYWORDS
Ocimum basilicum; tracheal responsiveness; inflammatory cells; oxidant–antioxidant markers; sensitized rats

Introduction

Asthma is a chronic inflammatory airway disorder which is associated with airway hyperresponsiveness (AHR) (Yin *et al*. 2008). Respiratory tract inflammation and AHR are main characteristic features of asthma (Meurs *et al*. 2008, Dodig *et al*. 2011). It has been demonstrated that increased oxidative stress occurs in the airways of asthmatic patients and causes inflammation (Dworski 2000). Increased nitrite, nitrate, and nitrotyrosine concentrations and loss of antioxidant defenses, such as superoxide dismutase, catalase, and glutathione were shown in asthma which could be contribute in physiopathology of the airways inflammation (Ricciardolo *et al*. 2004, Kaleli *et al*. 2006, Robroeks *et al*. 2007, Comhair and Erzurum 2010). In sensitized animals (Hogan *et al*. 2003) and asthmatic patients (Luksza and Jones 1982), increased total WBC and eosinophil count were also shown. Increased airway responsiveness has been shown in animal model of asthma and in asthmatic patients (Boskabady and Snashall 2000, Boskabady *et al*. 2011).

Ocimum basilicum (*O. basilicum*) known as Basil is a yearly herb of the *Lamiaceae* family. It is consumed as a seasoning in dry and fresh form. It is native to tropical Asia, but is now cultivated all over the world. *O. basilicum* derived compounds include mainly triterpenoids, polyphenols, steroids, and phenylpropanoids and rosmarinic acid is one of its main phenolic compound (Siddiqui *et al*. 2007, Strazzer *et al*. 2011).

The safety of *O. basilicum* in animal and human models has been shown previously (Katalinic *et al*. 2006). Anti-HIV (Yamasaki *et al*. 1998), anti-aging, anti-cancer (Manosroi *et al*. 2006, Almeida *et al*. 2007), and anti-inflammatory (Singh 1998, 1999a, 1999b) effects of *O. basilicum* have been reported. The relaxant effect of this plant on tracheal smooth muscle was observed in our previous study (Boskabady *et al*. 2005). Spasmolytic, bronchodilator, and vasodilator activities of aqueous methanolic extract of *O. basilicum* were also reported (Janbaz *et al*. 2014). Fathiazad and colleagues (2012) showed that cardioprotective effects of this plant are correlated with its antioxidant compounds. Antioxidant properties of Ocimum genus have been also documented previously (Lee and Shibamoti 2002, Jayasinghe *et al*. 2003, Dasgupta *et al*. 2004, Khaki *et al*. 2011).

In the present study, the effect of hydroalcoholic extract of *O. basilicum* on tracheal responsiveness (TR),

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bronchoalveolar lavage fluid (BALF) levels of NO_2, NO_3, MDA, thiol, SOD, and CAT as well as total and differential WBC in sensitized rats was examined.

Materials and methods

Animals and experimental groups

Wistar rats weighing 200 ± 220 g were kept in a stainless steel cage with clean filtered air (Maximiser, Thorens Caging System Inc., Hazleton, PA). Water and food were available *ad libitum* during experimental period and the temperature was maintained at 22 ± 2 °C on a 12 h light/dark cycle (Salmon *et al*. 1999).

Animals were randomly divided into six groups ($n = 8$ for each group) including: (1) non-sensitized control animals (group C), (2) untreated sensitized animals, (group S), (3) sensitized group treated with 1.25 μg/ml dexamethasone (group D), (4–6) sensitized groups treated with three concentrations of 0.75, 1.50, and 3.00 mg/ml *O. basilicum* extract (groups OB 0.75, OB 1.50, and OB 3.00).

Dexamethasone and *O. basilicum* extract were added to animal's drinking water during sensitization period. Each rat drank in average 40 ml/day drinking water which was not significantly different between the animals of different groups. The study was approved by Ethical Committee of Mashhad University of Medical Sciences and also all experiments were done according to 'FUM animal ethics committee rules'.

Method of animal sensitization

Animals were sensitized on days 1, 2, and 3 by intraperitoneal (IP) injection of 1 mg/kg of ovalbumin (OVA) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in 0.9% sterile saline containing 100 mg $Al(OH)_3$ (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as adjuvant. Rats were then exposed to 1% OVA aerosol on days 6, 9, 12, 15, 18, and 21, produced by a DeVilbiss PulmoSonic nebulizer (DeVilbiss Health Care Ltd., Feltham, UK) for 20 min with air flow of 8 L/min. Challenges were took place in a 0.8 m^3 chamber, with animal normal-breathing. In control, non-sensitized group, saline was used instead of OVA for IP injection and inhalation (Salmon *et al*. 1999).

Preparation of plant extract

The leaves of *O. basilicum* (identified by Research Center for Plant Sciences with specimen number 12937061, preserved in the herbarium of school of agriculture, Ferdowsi University of Mashhad) were dried in shadow and grinded. The macerated hydro-ethanolic extract was prepared by dissolving 100 g of *O. basilicum* powder in 1000 ml ethanol 70% in laboratory temperature for 72 h. The solution was removed by rotary evaporator to prepare dried extract. The yield extract was 19%.

Preparation of BALF

As previously mentioned, animals were sacrificed on day 22 of experiment. After opening the chest, trachea and lungs were dissected and external surfaces were washed with normal saline. The left lung was lavaged with 1 ml saline 5 times (total = 5 ml) at room temperature. The BAL fluid was centrifuged at 2500 *g* at 4 °C for 10 min. Supernatants were collected and stored at −80 °C for measurement of NO_2, NO_3, MDA, thiol, SOD, and CAT levels (Dong *et al*. 2014).

Total and differential WBC in lung lavage

One milliliter of BALF was stained with Turk solution. Leukocyte count was determined in a Neubauer counting chamber. The smear of cells after centrifuge was prepared from the cells and stained with Wright–Giemsa. Differential cell analysis was carried out according to staining and morphological criteria, under a light microscope by counting 100 cells and the percentage of each cell type was calculated (Dong *et al*. 2014).

Measurement of oxidant and antioxidant biomarkers in BALF

Measurement of oxidant biomarkers

The total stable oxidation products of NO metabolism (NO_2^-/NO_3^-) of BALF supernatant were assessed using a Griess reagent. The Griess reagent consists of sulfanilamide (SULF) and N-(1-Naphthyl) ethylenediaminedihydrochloride (NEDD). The frozen BALF was allowed to thaw and to reach a temperature of 25 °C that was followed by being deproteinized by zinc sulfate solution (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). The liquefied BALF was then centrifuged at 12 000 *g* for 10 min. Aliquots (300 μl) of the clear supernatant was mixed with Griess reagents including 300 μl SULF (2% w/v, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in 5% HCl and 300 μl NEDD (0.1% w/v, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in H_2O in a test tube. For reduction of nitrate to nitrite, 300 μl saturated solutions of vanadium (III) chloride (VCl_3; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in 1 M HCl was added and incubated for 2 h at 30 °C in the dark. Then, the absorbance of samples was measured at 540 nm against a blank containing the same concentrations of ingredients but no biological sample. Linear regression was used to determine NO concentration from standard curve of $NaNO_2$. The final results were expressed as μmol (Yousefniapasha *et al*. 2015).

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as a thiobarbituric acid reactive substance (TBARS) to produce a red-colored complex which has peak absorbance at 535 nm. Two milliliters from reagent of TBA/trichloroacetic acid (TCA)/HCl was added to 1 ml of BALF supernatant and the solution was heated in a water bath for 40 min. After cooling, the whole solutions were centrifuged within 1000 × *g* for 10 min. The absorbance was measured at 535 nm (Janero 1990, Hall and Andrus 2009).

Measurement of antioxidant biomarkers

Using DTNB (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as the reagent, total thiol concentration was measured. This

reagent reacts with thiols to produce a yellow-colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml Tris-ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) buffer (pH 8.6) was added to 50 µl BALF supernatant in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then 20 µl DTNB reagents (10 mmol/L in methanol) was added to the mixture and after 15 min (stored in laboratory temperature, the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mmol/l) was calculated using the following equation (Hosseinzadeh and Sadeghnia 2005).

$$\text{Total thiol concentration } (\text{mmol/L}) = (\text{A2}-\text{A1}-\text{B}) \times 1.07/0.05 \times 13.6$$

SOD activity was measured by the procedure described by Madesh and Balasubramanian (1998). A colorimetric assay involving generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) to its formazan by SOD was measured at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

CAT activity was estimated using the method of Aebi (Beers and Sizer 1952). The principle of the assay is based on determination of the rate constant, k, (dimension: $s-1$, k) of hydrogen peroxide decomposition. By measuring the decrease in absorbance at 240 nm per minute, the rate constant of the enzyme was determined. Activities were expressed as k (rate of constant) per liter.

Tissue preparations

Animals were sacrificed at day 22 of experiment by ketamine (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). After opening the chest, trachea was removed. The trachea was cut into two parts, each containing 5–6 cartilaginous rings.

One part of the trachea was hung between two Nichrome hooks inserted into the lumen, and placed in a 10 ml organ bath containing Krebs–Henseliet solution (KHS), with the following composition (mM): NaCl 120, KCl 4.72, KH_2PO_4 1.2, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2{\cdot}2H_2O$ 2.5, $NaHCO_3$ 25, and Dextrose 11. KHS solution was maintained at $37 \pm 0.5\,^{\circ}C$ and bubbled constantly with 5% CO_2–95% O_2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for 1 h, while it was washed with KHS solution every 15 min. In all experiments contraction responses were measured using an isotonic transducer (MLT0202, AD Instruments, Australia) which was connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia).

Assessment of tracheal response (TR) to methacholine

In each experiment a cumulative log concentration–response curves of methacholine hydrochloride (Sigma Chemical Ltd., UK) induced contraction of tracheal smooth muscle was obtained as previously described (Keyhanmanesh *et al*. 2010). Consecutive concentrations (10^{-8} to 10^{-3} M) were added every 2 min, the contraction due to each concentration was recorded at the end of 2 min, and the effect reached to a plateau in all experiments. 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. The effective concentration of methacholine, causing 50% of maximum response (EC_{50}) 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 the X axis.

Measurement of tracheal response (TR) to OVA

Tracheal response to 0.2% solution of OVA (adding 1 ml of 2% OVA solution to the 10 ml organ bath) was measured according to the previously described method (Keyhanmanesh *et al*. 2010). After 10 min, tracheal smooth muscle contraction was measured and expressed as gram contraction force.

Statistical analysis

The data were expressed as means ± SEM. The comparison of the results between treated, untreated and control groups as well as between three concentrations of extract were performed using one way analysis of variance (ANOVA) with Tukey–Kramer's post-test. Significance was considered at $p < 0.05$. InStat (GraphPad Software, Inc., La Jolla, CA) was used for statistical analysis.

Results

Total and differential WBC counts in the BALF of untreated ant treated sensitized rats

Total WBC count, percentages of eosinophils, monocytes, and neutrophils in BALF of S group were significantly increased but percentage of lymphocytes decreased compared to control group ($p < 0.05$ to $p < 0.001$, Figures 1 and 2).

Treatment of sensitized animals with dexamethasone leads to significant decrease in percentages of eosinophils, monocytes, and neutrophils but increased lymphocytes percentage ($p < 0.01$ to $p < 0.001$). Treatment of sensitized animals with two higher concentrations of the extract (1.5 and 3.0 mg/ml) led to significant reduction in eosinophils and neutrophils but increased lymphocytes percentage ($p < 0.05$ to $p < 0.001$). Treatment with high concentration of the extract (3.0 mg/ml) also decreased total WBC count and percentage of monocytes ($p < 0.01$ for all cases, Figures 1 and 2).

However, percentages of eosinophils, neutrophils, and lymphocytes in treated groups with dexamethasone and all concentrations of the extract and percentage of monocytes in treated group with low extract concentration were significantly different compared to control group ($p < 0.05$ to $p < 0.001$, Figures 1 and 2).

The effects of two higher concentrations of the extract (1.5 and 3.0 mg/ml) on percentages of all types of WBC were

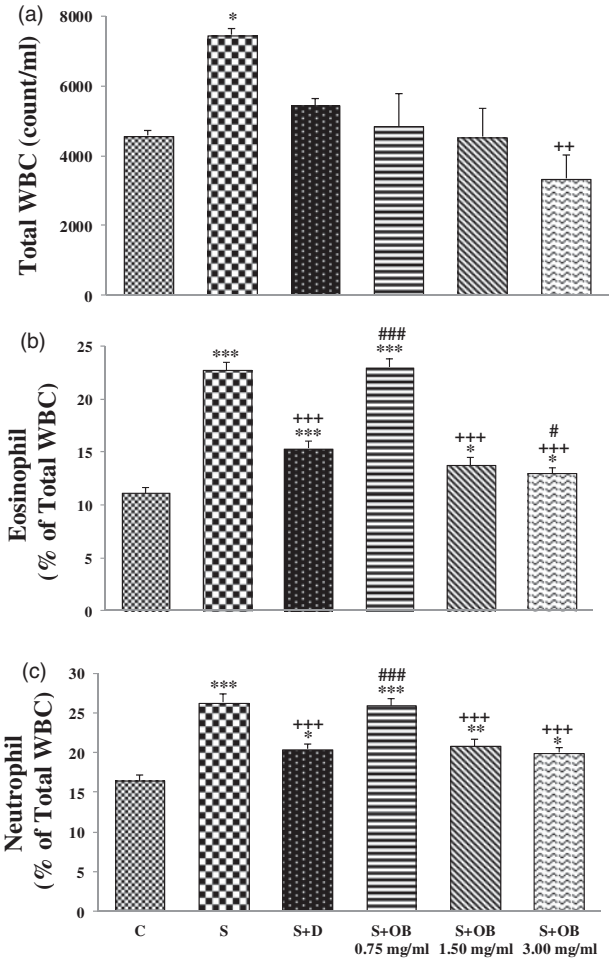

Figure 1. Total WBC number (mean ± SEM) in 1 ml bronchalveolar lavage fluid (BALF) (a), the percentages of eosinophil (b) and neutrophil (c) of control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$, for all groups). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, comparison of groups S, S + D, and S + OB *versus* group C. $^{++}p < 0.01$, $^{+++}p < 0.001$, comparison of groups S + D and S + OB *versus* group S. $\#p < 0.05$, $\#\#\#p < 0.001$, comparison of groups S + OB *versus* group S + D. The statistical comparisons were made using Tukey–Kramer's multiple post-test.

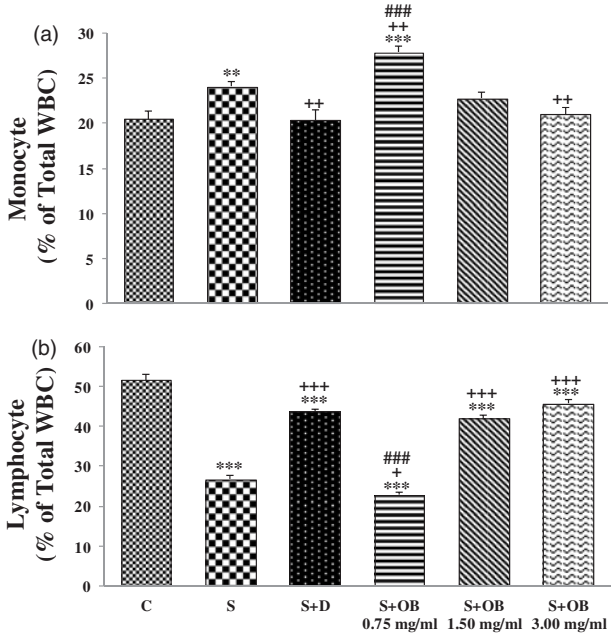

Figure 2. Percentages (mean ± SEM) of monocyte (a) and lymphocyte (b) in the BALF of control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups). $^{***}p < 0.001$, comparison of groups S, S + D, and S + OB *versus* group C. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, comparison of groups S + D and S + OB *versus* group S. $\#\#\#p < 0.001$, comparison of groups S + OB *versus* group S + D. The statistical comparisons were made using Tukey–Kramer's multiple post-test.

significantly higher than the effect of its low (0.75 mg/ml) concentration ($p < 0.001$ for all cases). The effect of high concentration of the extract (3.0 mg/ml) on percentage of lymphocytes was also significantly higher than the effect of its medium concentration ($p < 0.05$, Table 1).

The levels of oxidant biomarkers in BALF of untreated and treated sensitized rats

The levels of NO_2, NO_3, and MDA in BALF of S group were significantly increased compared to control group ($p < 0.001$ for all cases, Figure 3).

Treatment of sensitized animals with dexamethasone and all concentrations of the extract significantly decreased BALF levels of NO_2 and NO_3. Treatment with two higher concentrations of the extract (1.5 and 3.0 mg/ml) also caused significant reduction in MDA value compared to untreated sensitized group ($p < 0.001$ for both cases, Figure 3). However, there were significant differences between control group and sensitized animals treated with all concentrations of the extract on MDA and NO_3, and low concentration of the extract on NO_2 values ($p < 0.05$ to $p < 0.001$, Figure 3).

The effects of two higher concentrations of the extract (1.5 and 3.0 mg/ml) on BALF concentrations of all oxidant biomarkers were significantly higher than the effect of treatment with low extract (0.75 mg/ml) concentration ($p < 0.001$ for all cases). The effect of high concentration of the extract (3.0 mg/ml) on all oxidant biomarkers was also significantly higher than the effect their medium concentration ($p < 0.05$ to $p < 0.001$, Table 2).

The levels of antioxidant biomarkers in BALF of untreated and treated sensitized rats

The BALF levels of thiol, SOD, and CAT were significantly decreased in sensitized compared to control group ($p < 0.001$ for all cases, Figure 4). Treatment of sensitized animals with dexamethasone and two higher concentrations of the extract lead to significant increase in SOD and CAT values and treatment with its highest concentration (3.0 mg/ml) enhanced thiol value compared to untreated sensitized group ($p < 0.01$ to $p < 0.001$, Figure 4). However, there were significant differences between control group and sensitized animals treated with all concentrations of the extract on thiol, SOD, and CAT values ($p < 0.001$ for all cases, Figure 4).

The effects of two higher concentrations of the extract (1.5 and 3.0 mg/ml) on BALF concentrations of all antioxidant biomarkers were significantly higher than the effect of its low (0.75 mg/ml) concentration ($p < 0.05$ for medium extract concentration on thiol and $p < 0.001$ for other cases). The effect of high concentration of the extract (3.0 mg/ml) on thiol and

Table 1. Values of total and differential WBC count in bronchoalveolar fluid (BALF) of control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups).

Groups	Total WBC	Eosinophil	Monocyte	Neutrophil	Lymphocyte
S + OB 0.75	4835.62 ± 953.2	23 ± 0.77	27.87 ± 0.74	26 ± 0.86	22.87 ± 0.76
S + OB 1.5	4550 ± 824.1	13.75 ± 0.75+++	22.75 ± 0.7+++	20.87 ± 0.78+++	42 ± 0.96+++
S + OB 3.00	3343.75 ± 692.2	13 ± 0.56+++	21 ± 0.7+++	20 ± 0.65+++	45.75 ± 0.95+++,#

The data of WBC are their count in 1 ml BALF and those of each type of WBC is the percentage of total WBC. Three concentrations of *O. basilicum* were 0.75, 1.50, and 3.00 mg/ml.
+++$p < 0.001$, comparison of groups S + OB 1.5 and S + OB 3.0 versus group S + OB 0.75.
#$p < 0.05$, comparison of group S + OB 3.0 versus S + OB 1.5 group.
Values are presented as mean ± SEM. The statistical comparisons were made using Tukey–Kramer's multiple post-test.

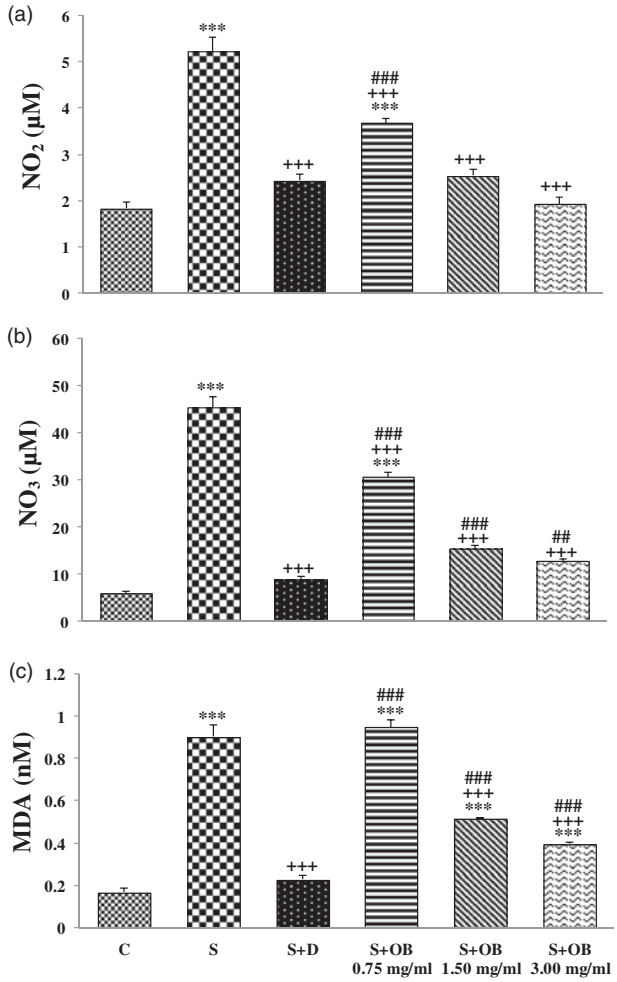

Figure 3. Bronchoalveolar lavage fluid levels of NO_2 (a), NO_3 (b) and malondialdehyde (MDA), (c) in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups). **$p < 0.01$, ***$p < 0.001$, comparison of groups S, S + D, and S + OB *versus* group C. +++$p < 0.001$, comparison of groups S + D and S + OB *versus* group S. ##$p < 0.01$, ###$p < 0.001$, comparison of groups S + OB *versus* group S + D. The statistical comparisons were made using Tukey–Kramer's multiple post-test.

CAT levels was also significantly higher than the effects of its medium concentration ($p < 0.05$ to $p < 0.001$, Table 2).

Tracheal responses to methacholine and OVA

Concentration response curves to methacholine showed leftward shift in group S compared to group C but the curves of treated groups with dexamethasone and all concentrations of the extract were shifted to right compared to group S (Figure 5).

The value of EC_{50} in group S was significantly lower than group C ($p < 0.001$). The values of EC_{50} in treatment groups with dexamethasone and two higher concentrations of the extract were significantly improved compared to the group S ($p < 0.05$ to $p < 0.001$, Figure 5).

Maximum response to methacholine in group S was significantly higher than group C ($p < 0.01$). Treatment with dexamethasone and two higher concentrations of extract led to significant decrease in maximum response compared to group S ($p < 0.01$ to $p < 0.001$). Maximum response to methacholine in treated group with low extract concentration was significantly higher than that of dexamethasone treatment ($p < 0.05$, Figure 5).

TR to OVA in group S was significantly higher than group C ($p < 0.001$). TR to OVA in treated groups with dexamethasone and two higher concentrations of the extract were significantly improved compared to group S ($p < 0.001$ for all cases). However, TR to OVA in treated groups with low concentration of the extract were still significantly higher than group C ($p < 0.001$, Figure 6).

The effects of treatment with two higher concentrations of the extract (1.5 and 3.0 mg/ml) on TR to OVA and the effect of high concentration of the extract (3.0 mg/ml) on EC_{50} value were significantly higher than the effect of treatment with its low (0.75 mg/ml) concentration ($p < 0.05$ to $p < 0.001$, Table 3).

Comparison of the effect of dexamethasone with the extract treatment

The effects of treated groups with all concentrations of extract on BALF concentrations of NO_3, MDA, and thiol, the effects of treated groups with two lower extract concentrations on CAT and the effects of treated groups with low extract concentration on EC_{50} value, maximum response to metacholine, TR to OVA, percentages of eosinophils, neutrophils, lymphocytes, levels of NO_2 and SOD were significantly lower but the effect of treated group with high extract concentration on percentage of eosinophils was significantly higher than dexamethasone treatment ($p < 0.05$ to $p < 0.001$, Figures 1–6).

Discussion

In the present study, the effect of *O. basilicum* on total and differential WBC counts, NO_2, NO_3, MDA, thiol, SOD, and CAT levels of BALF as well as TR to methacholine and OVA in sensitized rats were examined. Total WBC count, percentages of

Table 2. Bronchoalveolar lavage fluid levels of NO_2, NO_3, MDA, thiol, SOD, and CAT in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups).

Mediators	NO_2 (μM)	NO_3 (μM)	MDA (nM)	Thiol (μM)	SOD (U/ml)	CAT (U/ml)
S + OB 0.75	3.67 ± 0.09	30.66 ± 0.88	0.95 ± 0.04	0.014 ± 0.001	0.018 ± 0.002	0.036 ± 0.0003
S + OB 1.5	2.53 ± 0.15+++	15.44 ± 0.60+++	0.52 ± 0.002+++	0.036 ± 0.002+	0.038 ± 0.0001+++	0.060 ± 0.004+++
S + OB 3.00	1.91 ± 0.16+++,#	12.82 ± 0.50+++,#	0.39 ± 0.007+++,##	0.069 ± 0.009+++,##	0.044 ± 0.003+++	0.075 ± 0.004+++,#

Values are presented as mean ± SEM. Three concentrations of *O. basilicum* were 0.75, 1.50, and 3.00 mg/ml.
$+p < 0.05$, $+++p < 0.001$, comparison of groups S + OB 1.5 and S + OB 3.0 versus group S + OB 0.75.
$\#p < 0.05$, $\#\#p < 0.01$, comparison of groups S + OB 3.0 versus S + OB 1.5 group.
The statistical comparisons were made using Tukey–Kramer's multiple post-test.

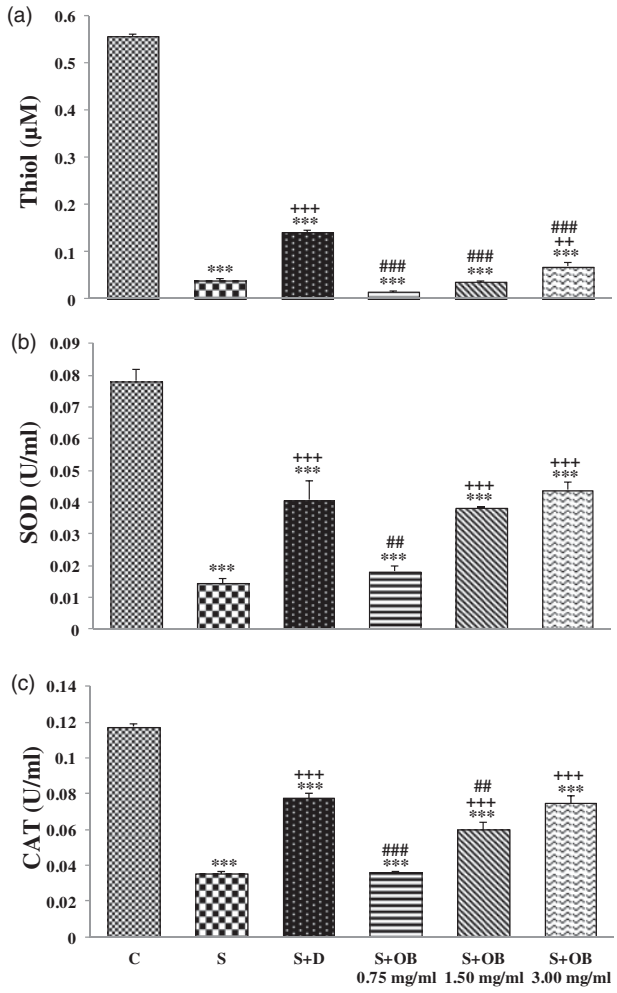

Figure 4. Bronchoalveolar lavage fluid levels of thiol (a), superoxide dismutase (SOD), (b), and catalase (CAT), (c) in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups). $***p < 0.001$, comparison of groups S, S + D, and S + OB *versus* group C. $++p < 0.01$, $+++p < 0.001$, comparison of groups S + D and S + OB *versus* group S. $\#\#p < 0.01$, $\#\#\#p < 0.001$, comparison of groups S + OB *versus* group S + D. The statistical comparisons were made using Tukey–Kramer's multiple post-test.

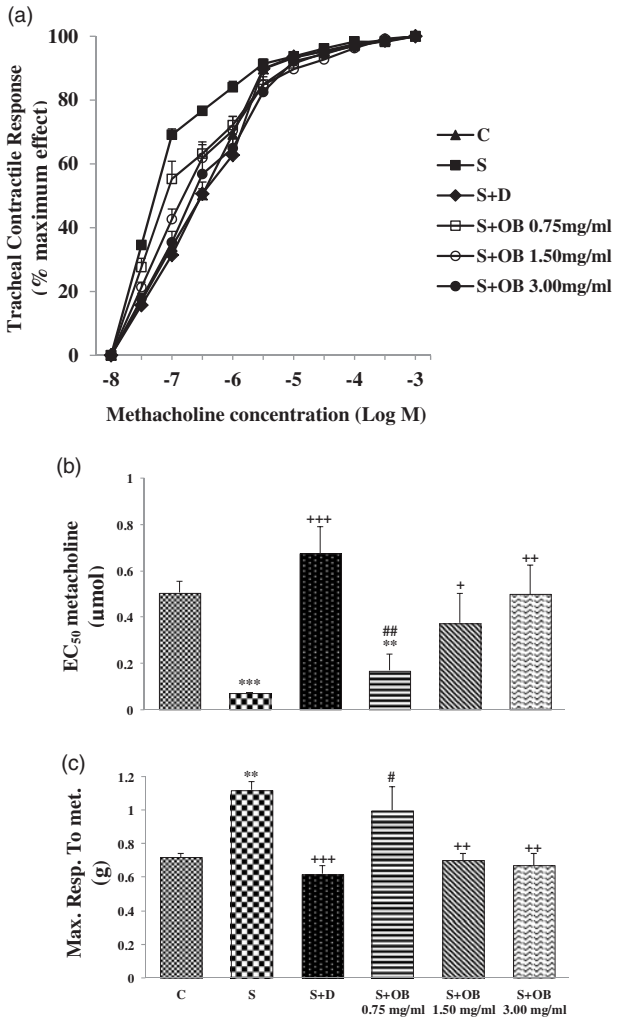

Figure 5. Cumulative log concentration-response curves of methacholine induced contraction of isolated tracheal smooth muscle (a), tracheal response to methacholine (EC_{50}, the effective concentration of methacholine, causing 50% of maximum response), (b) and maximum response to methacholine in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups). $**p < 0.01$, $***p < 0.001$, comparison of groups S, S + D, and S + OB *versus* group C. $+p < 0.05$, $++p < 0.01$, $+++p < 0.001$, comparison of groups S + D and S + OB *versus* group S. $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.01$, comparison of groups S + OB *versus* group S + D.

eosinophils, monocytes, neutrophils, levels of oxidant biomarkers in BALF and TR to methacholine and OVA were significantly increased but percentage of lymphocytes and antioxidant biomarkers levels were significantly decreased in group S compared to group C which indicated the sensitization of animals. Previous studies showed that asthma is characterized by AHR, multicellular inflammation (such as mast cells, neutrophils, and eosinophils), increased oxidant markers (NO and MDA) and decreased antioxidant factors (thiol groups, SOD, and CAT) (Busse 1998, Sahiner *et al*. 2011). Regarding the reduction of lymphocyte percentage, a previous study, using similar method of animal sensitization showed increased absolute number of the lymphocyte (Keyhanmanesh *et al*. 2010). Therefore, the reduction of percentage of lymphocytes in sensitized rats observed in the present study is due to increased total WBC count.

The results of the present study showed that treatment of sensitized animals with dexamethasone and at least two higher concentrations of the extract caused significant

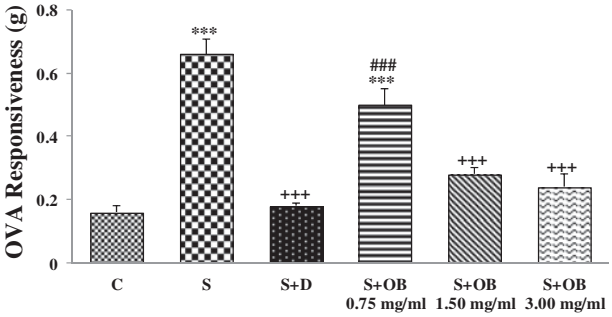

Figure 6. Values of tracheal response to ovalbumin in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups). $^{**}p < 0.01$, $^{***}p < 0.001$, comparison of groups S, S + D, and S + OB versus group C. $+p < 0.05$, $++p < 0.01$, $+++p < 0.001$, comparison of groups S + D and S + OB versus group S. $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.01$, comparison of groups S + OB versus group S + D.

Table 3. Values of tracheal responsiveness to methacholine (EC_{50}), maximum response to methacholine (Max. Resp.) and tracheal responsiveness to ovalbumin (OVA) in control rats (C), sensitized animals (S), S treated with dexamethasone (S + D) and three concentrations of *O. basilicum* (S + OB) ($n = 8$ for all groups).

Parameters	EC_{50} (μmol)	Max. Resp. (g)	OVA (g)
S + OB 0.75	0.16 ± 0.07	1 ± 0.14	0.5 ± 0.05
S + OB 1.5	0.37 ± 0.12	0.7 ± 0.04	0.28 ± 0.02++
S + OB 3.00	0.498 ± 0.12+	0.67 ± 0.07	0.24 ± 0.04+++

Values are presented as mean ± SEM. Three concentrations of *O. basilicum* were 0.75, 1.50, and 3.00 mg/ml.
$+p < 0.05, ++p < 0.01, +++p < 0.001$, comparison of groups S + OB 1.5 and S + OB 3.0 versus group S + OB 0.75.
The statistical comparisons were made using Tukey–Kramer's multiple post-test.

reduction in the percentages of eosinophils, monocytes, neutrophils, levels of oxidant biomarkers in BALF, and TR to methacholine and OVA but caused increase percentage of lymphocytes and antioxidant biomarkers levels compared to untreated S group. These results suggest that the extract has anti-inflammatory, antioxidant, and also preventive effect on TR which is the main characteristic feature of asthma. Increased lymphocyte percentage in treated groups seen in the present study is perhaps due to reduction of total WBC count in these groups.

All prophylactic drugs used in the treatment of asthma should focus on reduction of airway inflammation and AHR. The preventive effect of long-term administration of the extract of *O. basilicum* on TR (which is mainly due to airway inflammation) of sensitized animals is perhaps due to its suppressing effect on airway inflammation. In fact, anti-inflammatory, antioxidant, and bronchodilatory effects of this plant have been shown previously which support the results of current study. In our previous study, the relaxant effect of this plant on tracheal smooth muscle was observed (Boskabady *et al*. 2005) that may be due to the opening of potassium channels (Buckle *et al*. 1993). The spasmolytic, bronchodilator and vasodilator activities of aqueous methanolic extract of *O. basilicum* were also shown which suggested to be mediated through Ca 2+ channel blocking activities (Janbaz *et al*. 2014). The most important characteristic features of asthma are airway responsiveness and TR (Yin *et al*. 2008, Dodig *et al*. 2011). It is also well known that airway responsiveness is mainly due to airway inflammation (Meurs *et al*. 2008, Dodig *et al*. 2011). Therefore, in the present study as well as several previous studies to examine the possible therapeutic effect of hydroalcoholic extract of *O. basilicum* on asthma, its effect on TR to methacholine as nonspecific airway responsiveness and to OVA as specific airway responsiveness was evaluated. The reduction effect of the extract on specific and nonspecific TR indicated its therapeutic potential on asthma.

In addition, the alcoholic extract of *O. basilicum* showed anti-inflammatory activity in human peripheral blood mononuclear cells (PBMC). The extract can inhibit proinflammatory cytokines and mediators (Selvakkumar *et al*. 2007). Decreased paw edema induced by carrageenan in male Wistar rats was also shown for the ethanolic extract of the plant which could be related to its antioxidant effect (Rameshrad *et al*. 2015). Petroleum ether fraction and ethanolic fraction of the seeds of *O. basilicum* also reduced inflammation induced by histamine and prostaglandins in rats by significant inhibition of the paw edema produced by histamine and PGF2a (Rakha *et al*. 2010). All these studies showed anti-inflammatory effect for the seeds of *O. basilicum* which support the results of the present study.

In addition, antioxidant activity of *O. basilicum* was shown by isolation of potential antioxidant compounds of this plant (Durga *et al*. 2009). The extract of the plant is able to reduce the reproductive alterations induced by an organophosphorus insecticide (diazinon) with serious oxidative effects, in albino rats which may be due to the potent antioxidant effects of extract (Sakr and Abdel Samie 2015). Basil extract can also decrease the oxidative effects of electromagnetic field on neural cells of rats (Khaki 2016). These studies showed the effect of the plant on oxidant stress which supports the results of the present study indicating antioxidant property of the plant.

The results of the present study showed concentration-dependent preventive effect of the extract on almost all measured values. The effects of two higher concentrations of the extract on the percentages of all types of WBC, concentrations of all oxidant and antioxidant biomarkers in BALF and TR to OVA and the effect of highest concentration of the extract on the mean value of EC_{50} were significantly higher than the effect of treatment with low extract concentration. The effect of highest concentration of the extract on percentage of lymphocytes and concentrations of all oxidant and antioxidant biomarkers except SOD was also significantly greater than medium extract concentration. The concentration dependency effect of the extract also could be another evidence for its antioxidant and anti-inflammatory effect of the plant.

The comparable effect of extract of the plant with that of dexamethasone is also another indicator for anti-inflammatory and antioxidant properties of the plant. The inhibitory effect of dexamethasone on respiratory tract inflammation and its effect on lymphocytes in asthmatic mice have been shown, which support the results of the current study (Tang *et al*. 2011). The effects of treated groups with all concentrations of extract on BALF concentrations of NO_3, MDA and thiol, the effects of treated groups with two lower extract concentrations on CAT and the effects of treated groups with low extract concentration on EC_{50} value, maximum response to metacholine, TR to OVA, percentages of eosinophils,

neutrophils, lymphocytes, levels of NO_2 and SOD were significantly lower than but the effect of treated group with high extract concentration on percentage of eosinophils was significantly higher than dexamethasone treatment.

Regarding the extract doses used in the present study, considering the weight of animals as 200 g and used drinking water by each animal as 40 ml, the administered dose of the extract in the present study were 0.15, 0.3, and 0.6 g/kg. However, according to pharmacological principle the administered dose in human is one tenth of animal. In this regard, the maximum dose in human would be 0.06 g/kg or 60 mg/kg which is not a high dose. In addition, even low dose of the extract (0.15 g/kg in this study equal to 15 mg/kg in human) was significantly affected oxidant, antioxidant markers and TR and the medium dose o the extract (0.3 g/kg) affected most measured parameters.

Our recent study (Eftekhar *et al*. 2018) showed similar effect of rosmarinic acid on sensitized animals. The results of this study suggest that the effect of the plant is mainly due to its constituent, rosmarinic acid.

The results of the present study showed the improvement in BALF total and defferential WBC count, levels of oxidant and antioxidant biomarkers as well as TR in sensitized rats treated with *O. basilicum*. These results together with other studies indicate anti-inflammatory, antioxidant of the plant and its preventive effect on TR. These results suggest a therapeutic effect of *O. basilicum* on asthma by both bronchodilation and preventive effect on lung inflammation and oxidant–antioxidant imbalance. However, further studies needed to evaluate the effect of the plant on asthmatic patients.

Conclusions

The results of this study showed a concentration-dependent preventive effect of the extract of *O. basilicum* on BALF inflammatory cells, oxidant biomarkers and TR of sensitized rats which was comparable to the effect of dexamethasone at used concentrations. These results may suggest the potential therapeutic effect of this plant on asthma disease.

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No potential conflict of interest was reported by the authors.

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