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Original article

The Effect of *Melissa officinalis* L. Extract on Ovalbumin- Induced Lung Inflammation in Rats

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Abstract

Background and objectives: Asthma is an inflammatory chronic disease that has become prevalent internationally. *Melissa officinalis* L. as a medicinal plant has long been used in the European and the Iranian traditional medicine for the treatment of several diseases. The biological activities such as antioxidant, anti-tumour, antiviral, antimicrobial, and anti-inflammatory effects of *M. officinalis* have been reported. Therefore, the effect of *Melissa officinalis* L. extract on tracheal smooth muscle responsiveness, white blood cell (WBC) counts, and lung pathological changes of ovalbumin (OVA) induced asthma model rat was examined in the current study. **Methods:** The hydroalcoholic extract of *M. officinalis* was prepared using 300 g of powdered leaves. Tracheal smooth muscle responsiveness, lung pathology, and WBC counts were evaluated in control, sensitized to OVA, and sensitized rats treated with dexamethasone and three doses of *M. officinalis* extract (50, 150 and 200 mg/kg). **Results:** Tracheal smooth muscle responsiveness to methacholine hydrochloride in all sensitized groups was greater than that of the control group ($p<0.001$). The treatment of asthma-induced rats with dexamethasone and *M. officinalis* extracts (50, 100 and 200 mg/kg) remarkably reduced pathological alterations, including; inflammation, muscle hypertrophy and mucus plaques in the lung compared to the sensitized group ($p<0.05$ to $p<0.001$). Additionally, *M. officinalis* extract significantly improved total and differential WBC counts in broncho-alveolar lavage fluid (BALF) ($p<0.001$ for all groups). **Conclusion:** Results of the current study showed a preventive effect of *M. officinalis* extracts on the responsiveness of tracheal smooth muscle and lung inflammation in OVA-sensitized rats.

Keywords: experimental lung inflammations; *Melissa officinalis*; ovalbumin alum, tracheal stenoses

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Introduction

Asthma as an inflammatory disease which is characterized by airway and eosinophilic inflammation, hyper-secretion of mucus and airway hyper-responsiveness [1]. This disease is triggered by interaction between immunoglobulin E (IgE) and the release of inflammatory cytokines such as interleukin (IL)-4, 5 , and 13 by T-helper 2 cells (Th2) [2].

Asthma is described by airway hyper-responsiveness (AHR), hypertrophy and/or hyperplasia in airway smooth muscle, mucous production and infiltration of inflammatory cells [3]. Increase in airway responsiveness could trigger the inflammatory process in the lung [4]. The control of asthma symptoms and a drop of airflow limitation as well as the risk of

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exacerbations, are the aims for asthma control [5]. The earliest recorded treatments for asthma, such as tobacco, opium, coffee and tea, express pharmacological effects and direct smooth muscle relaxation [3].

The use of medicinal herbs in complementary medicine due to their availability, cultural acceptability, efficacy, and safety claims have been increased in different countries [6]. However, there is unsatisfactory data on the effectiveness of complementary medicine in asthma. The medicinal herbs with pharmacological properties including anti-inflammatory, immune-regulatory, and smooth-muscle relaxant effects, may be suitable for controlling asthma [7,8]. The possible therapeutic effects of medicinal herbs for the relief of respiratory symptoms had been reported previously [9].

Melissa officinalis L., commonly known as lemon balm belongs to the Lamiaceae family. The fragrant leaves of this medicinal herb have been widely used as a spice in cooking to add flavor and have been used for the treatment of cardiovascular and mental diseases as well as respiratory problems in traditional medicine [10]. *Melissa officinalis* contains triterpenoids, volatile compounds, flavonoids, and phenolic acids. Crude extracts of *M. officinalis* showed several pharmacological properties including anxiolytic, antioxidant, anti-inflammatory, antispasmodic and antiviral activities in phytochemical investigations [11]. Therefore, the effect of *M. officinalis* extract on pathological changes in the lung and physiological properties of the airways on sensitization of rats was investigated in the present study.

Material and Methods

Ethical consideration

The male Wistar rats with an approximate weight range of 200 ± 250 g were sourced from the Animal Breeding Center at Birjand University of Medical Sciences. The standard conditions (22±1°C, 12-hr light and dark cycle) were maintained for the animals. All laboratory procedures conducted in this study received ethical approval from Birjand University of Medical Sciences Ethics Committee under protocol number IR.BUMS.REC.1400.272.

Chemicals

The chemicals including aluminum hydroxide $Al(OH)_3$, Ovalbumin (OVA) (98% pure), and methacholine hydrochloride, were purchased from Sigma Company (United States). Formalin solution 10% was provided from Merck, Germany. Methanol was purchased from the Razi Yeast Company, in Iran. Wright-Giemsa Stain and Turk solution were provided by Betagen Company, Iran. Ketamine injectable solution (Alfasan, Netherlands) and dexamethasone powder were purchased from Abidi Pharmaceutical Co. (Tehran, Iran).

Plant material

Melissa officinalis was collected from Tabas, located in South Khorasan province, 2021. A botanical expert from Payam Noor University, Birjand Center confirmed the plant (voucher number: L-110). The hydro-alcoholic extract was prepared using 300 g of powdered leaves dissolved in a solution containing 80% methanol. The extract was filtered with the filter paper after 24 h and evaporated using a Heidolph rotary evaporator. The extract was analysed by the Iranian Forestry and Pasture Research Institute using high-performance liquid chromatography (HPLC) to identify rosmarinic acid.

Determination of rosmarinic acid

The quality of the extract of *M. officinalis* was characterized by high- performance liquid chromatography (HPLC), KNAUER Company (HPLC PUMP K-1001). Separations were carried out using a C_{18} column (250 mm × 4.6 mm, 5 µm) at 30 °C.

A powdered sample (0.5 g) of *M. officinalis* was accurately weighed and dissolved in 20 mL solvent (methanol: water, 50:50) for 30 min at room temperature and filtered through 0.45 µm membrane filter. An aliquot sample was injected into the HPLC. The mobile phase consisted of (A) acetonitrile and (B) acetic acid/water 1% (15:85v/v). The concentrations of the standard for plotting the calibration curve were 100 ppm, 200 ppm and 500 ppm. The flow rate of the mobile phase was 1 mL/min, the volume of the injected sample and standard solutions was 10 µL, and the absorbance was monitored at 330 nm.

Sensitization

Sensitization of rats to Ovalbumin (OVA) was done using the method previously described [12,13]. In brief, rats received 1 mg/kg OVA and 100 mg $Al(OH)_3$ as adjuvant in sterile saline (0.9%) as intraperitoneal (i.p.) injection at three

times on days 1, 2 and 3 of the study. The animals were then exposed to aerosols of (2%) OVA with an airflow nebulizer (403B Air-compressing nebulizer, YunYang Industrial Park, China), on days (6, 9, 12, 15, 18 and 21) for 20 min/day. The aerosol was conducted in a closed chamber, dimensions 30 × 20 × 20 cm, while the animals breathed normally. Control animals were treated similarly with saline instead of OVA solution.

Animal grouping

Forty-eight male Wistar rats (200 ± 20 g) were maintained in an animal house at the School of Medicine, Birjand University of Medical Sciences, Birjand, Iran, under 22 ± 2 °C with 12/12 hours off light cycle. Animals were kept at standard conditions (free access to food and water ad libitum) during the study. Rats were randomly divided into six groups (n = 8 for all groups) as follows: Control group: received normal saline as (i.p.) and inhalation (exposed); Sensitized group (asthma): received OVA as (i.p.) and inhalation; Dexamethasone treated group (Dex): sensitized rats treated with dexamethasone (1 mg/kg); Three groups of *M. officinalis* extract (Ext 1, Ext2 and Ext3): sensitized rats treated with *M. officinalis* extract (50, 100 and 200 mg/kg, respectively).

Animals were treated with dexamethasone or *M. officinalis* extracts by gavage during the sensitization period.

Tissue preparations

After anesthetizing rats by injection (i.p.) of ketamine hydrochloride (50 mg/kg) 24 h after the last challenge, the animals' tracheal was dissected. The trachea ring containing almost four cartilages was obtained. The tracheal ring was suspended in a 10 mL organ bath containing Krebs-Henseleit solution: composed of NaCl (120), KCl (4.72), KH2PO4 (1.2), MgSO4 (0.5), CaCl2· (2.5), NaHCO3 (25) and dextrose (11 mM). The Krebs solution was gassed with 95% O_2 and 5% CO_2 maintained at 37±0.5 °C [14].

Tissue preparation was done using the previously described method [15]. In brief, the tissues were suspended in an organ bath containing a Krebs solution under an isotonic tension of 1 g. The isotonic transducer (MLT0420, AD Instruments, Australia) connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia) recorded the contraction responses of the trachea smooth muscle.

Tracheal response to methacholine (Met) assessment

The cumulative log concentration-response curves of Met induced contraction of tracheal smooth muscle. The consecutive Met doses (including 10^{-7} to 10^{-2} M) were added to the organ bath every 2 min, and the contraction due to each dose was recorded at the end of 2 minutes [15]. The effective concentration of Met causing 50% of maximum response (EC_{50}) using the Met response curve was also measured.

Broncho alveolar lavage fluid (BALF) and inflammatory cells

After anesthetizing animals, lungs were removed, and the left lung was lavage five times with 1 mL of saline. One milliliter of BALF was stained with Turk solution and counted with a hemocytometer (in a Burker chamber). Total white blood cells (WBC) count was performed using a Neubauer lam in the light microscope.

Then, the remained BAL fluid was centrifuged at 2000 g at ٤C for 10 min. The smear was set from the cells and stained with Wright-Giemsa. The differential cell investigation by morphological criteria was carried out under a light microscope.

Pathological evaluation

The right lung was removed and placed into the formalin solution 10%. The tissue was dried by passage through ethanol 70 - 100% and cleared by passage through xylol. The paraffin-embedded tissue blocks were stained with hematoxylin and eosin (H&E). The slice samples of the tissues were assessed under a light microscope.

The pathologic changes in the lung of animals were scored as follows: no pathologic changes = 0; patchy changes=1, local changes= 2 and scattered changes = 3 [16].

Statistical analysis

All values presented in this study are expressed as mean±standard deviation (SD). The Kolmogorov-Smirnov test was used to confirm the normal distribution of the data. The comparison of data between the sensitized group, sensitized animals treated with dexamethasone,

and plant extracts with control animals were done using One-way analysis of variance (ANOVA) with a post-hoc Tukey test. The statistical analysis was performed by SPSS for Windows version 19, and $p<0.05$ was accepted as a significant difference.

Results and Discussion

Rosmarinic acid content of the extract from *M. officinalis* was identified 1.037% using the HPLC method in the Iranian Forestry and Pasture Research Institute.

Dose- response curves to Met in all sensitized groups showed a leftward shift compared to the curve of the control group. However, the curves of treated sensitized animals with dexamethasone and three doses of plant extracts were shifted to the right compared to the sensitized group (Figure 1).

The mean values of EC_{50} in tracheal smooth muscle in the sensitized group (asthma) and sensitized animals treated with dexamethasone and three doses of *M. officinalis* extracts were lower than control group ($p<0.001$ for all groups), (Figure 2). The mean values of EC_{50} in the tracheal smooth muscle of treated animals with dexamethasone and higher dose of extracts (200 mg/kg) were significantly greater than asthma group ($p<0.001$ and $p<0.01$, respectively). However, the mean values of EC_{50} in tracheal smooth muscle of all treatment groups extracts of *M. officinalis* (all doses) were still significantly lower than dexamethasone group ($p<0.001$, $p<0.01$ and $p<0.05$, respectively), (Figure 2).

The pathologic changes in the lungs of control and sensitized groups included lung inflammation, muscle hypertrophy, and mucin. Scoring of the pathological changes in all OVA-induced sensitization were significantly higher than the control group (Figure 3). Treatment with dexamethasone and *M. officinalis* extracts, especially at higher doses (100 and 200 mg/kg), improved these changes.

The pathological changes including inflammation, muscle hypertrophy and mucus plaques were greater in sensitized groups without treatment and treatment with lower doses of extract (50 mg/kg) than control group ($p<0.001$ and $p<0.05$, respectively), (Figure 4).

Treatment of sensitized animals with dexamethasone and *M. officinalis* extracts (all doses) reduced pathological changes as dose-dependently ($p<0.05$ to $p<0.001$), Table 1 and Figure 4. In addition, the higher dose of the extract (200 mg/kg), significantly reduced inflammation compared to the dexamethasone group ($p<0.01$), (Figure 4a).

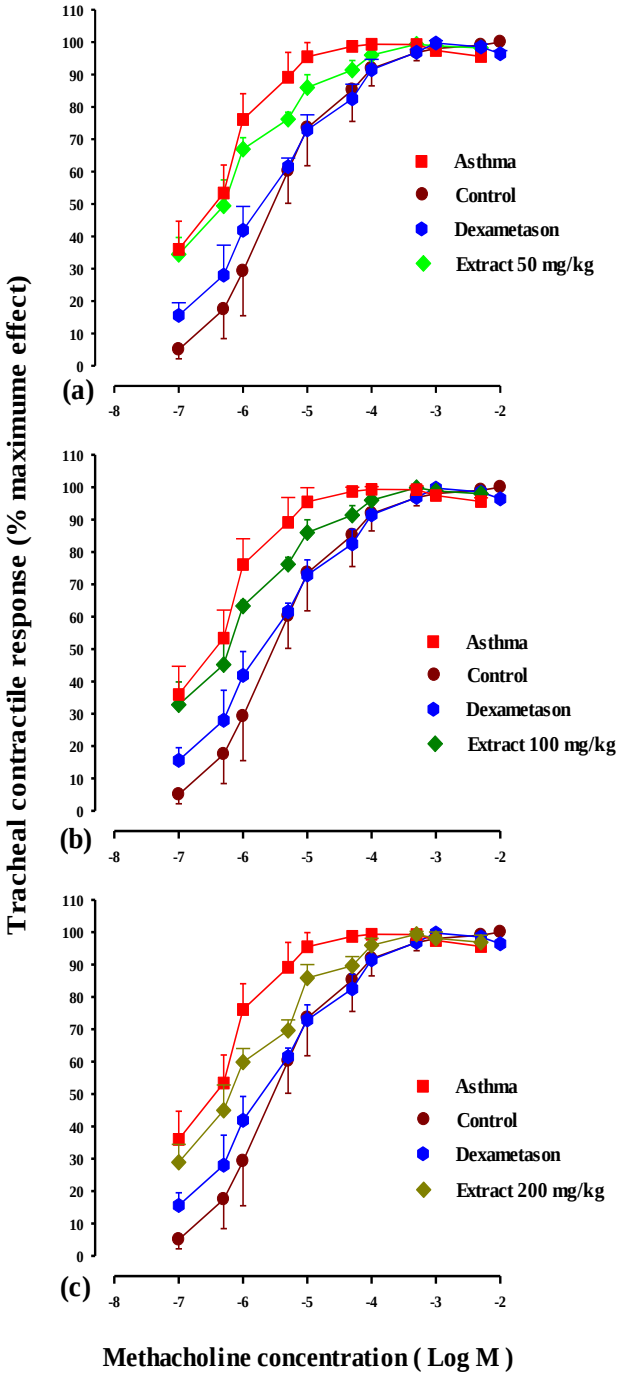

Figure 1. Cumulative log concentration-response curves of Methacholine (Met) induced contraction of smooth muscle in isolated trachea in control and sensitized groups treated with dexamethasone and *Melissa officinalis* extracts 50 mg/kg (a), 100 mg/kg (b) and 200 mg/kg (c)

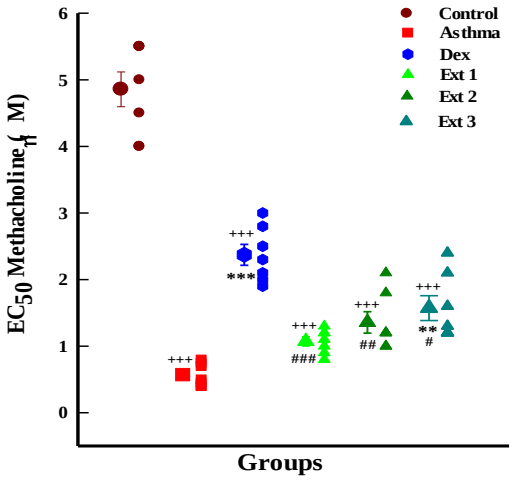

Figure 2. The mean ± SD of tracheal response to Methacholine (Met) (EC_{50}) in the control and sensitized animals treated with dexamethasone (Dex) and *Melissa officinalis* extracts (Ext 1, 2 and 3), (50, 100 and 200 mg/kg, respectively). Comparison of the data between groups was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc test; +++: p<0.001 compared between control and asthma groups; **: p<0.01 and ***: p<0.001 compared between asthma and treated groups; #: p<0.05, ##: p< 0.01, ###: p<0.001, compared between dexamethasone and other treated groups.

The mean values of total WBC in BALF of sensitized groups without treatment (asthma) and treatment with dexamethasone and *M. officinalis* extracts (50, 100 and 200 mg/kg) were remarkably greater than control group (p<0.001 in all cases), (Figure 5). The mean values of WBC were significantly reduced in treated animals with dexamethasone and *M. officinalis* extracts (all doses) (p<0.001 for all groups), Table 2 and Figure 5. Treatment with higher doses of extracts (100 and 200 mg/kg) showed significant improvement compared to the lower dose of extract (50 mg/kg).

The percentage of eosinophils, monocytes, and neutrophils remarkably increased; however, the percentage of lymphocytes decreased in BALF of asthma animals compared to the control group (p<0.001 for all cases) (Figure 6). Treatment with dexamethasone and all plant extract (all doses) remarkably decreased the percentage of neutrophils (p<0.001 for all cases), (Figure 6d) and caused decrease in the percentage of eosinophils by higher doses of extracts (100 and 200 mg/kg), (Figure 6a). Treatment of sensitized animals with dexamethasone and plant extract (50, 100 and 200 mg/kg) significantly changed the percentage of monocytes in BAL fluid (p<0.05 and p<0.001), (Figure 6b).

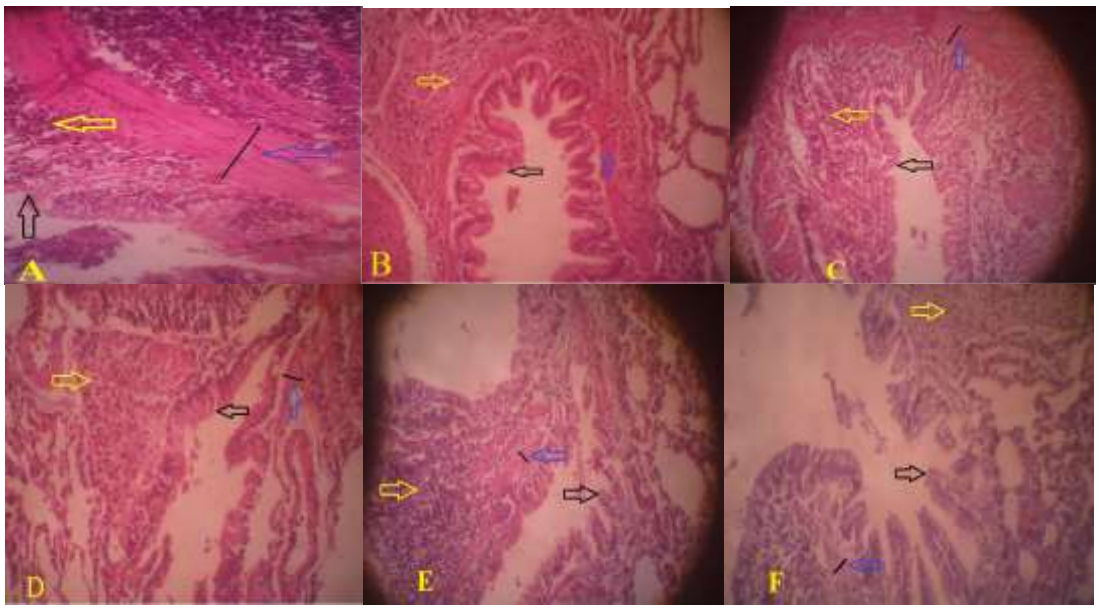

Figure 3. The lung specimens in asthma (A), control (B) and sensitized animals treated with dexamethasone (C) and *Melissa officinalis* extracts (50, 100 and 200 mg/kg), (D, E and F, respectively); inflammation (yellow arrow), muscle hypertrophy (blue arrow) and mucin (black arrow); (magnification 10x40).

Table 1. The pathological changes in the control, sensitized and treated groups

Parameters	Control	Asthma	Dex	Ext 1	Ext 2	Ext 3
Lung inflammation	0.37±0.41	$2.50\pm0.5^{+++}$	$0.75\pm0.43^{**}$	1.75±0.43	$1.00\pm0.35^{**}$	$0.25\pm0.43^{***\&\&}$
Muscle hypertrophy	0.50±0.35	$2.62\pm0.41^{+++}$	$1.00\pm0.35^{***}$	$1.25\pm0.43^{**}$	$1.00\pm0.35^{***}$	$0.87\pm0.21^{***}$
Mucus plaques	0.25±0.43	$2.25\pm0.43^{+++}$	$0.62\pm0.41^{***}$	1.34±0.41	$1.12\pm0.21^{*}$	$0.87\pm0.21^{**}$

The mean±SD values of the lung inflammation, muscle hypertrophy and mucus plaques, in control and sensitized groups treated with dexamethasone (Dex) and *Melissa officinalis* extracts (Ext 1, Ext 2 and Ext 3), (50, 100 and 200 mg/kg, respectively); comparison of the data between groups was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post-test; +++: $p<0.001$ compared between control and asthma groups; *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ compared between asthma and treated groups; &&: $p<0.01$ compared between extract (Ext 50) and (Ext 200)

The percentage of lymphocytes was remarkably decreased in sensitized without and treated animals compared to the control group ($p < 0.001$ for all cases) (Figure 6c). Treatment with Dex and higher doses of extracts (100 and 200 mg/kg) increase the percentage of lymphocytes compared to the asthma group ($p < 0.001$ for all cases).

The improvement in eosinophils, monocytes, lymphocytes and neutrophils percentage in the BALF of treated animals with higher doses of extracts (100 and 200 mg/kg) were remarkably greater than that of the lower plant extract (50 mg/kg), Figure 6 and Table 2.

Melissa officinalis as a natural food additive has been used traditionally in Mediterranean cuisine and medicine for different purposes including sedative, spasmolytic, carminative, diaphoretic properties and also as medicine, for treating anxiety, depression, gastrointestinal disorders and for the relief of stress and strengthening the memory [17].

In the current study, the effect of *M. officinalis* extracts on tracheal smooth muscle responsiveness to the Met, lung pathology and total and differential WBC counts in OVA-induced sensitization rats were examined.

The results revealed that treatment of sensitized rats with different doses of *M. officinalis* extracts improved the increased tracheal smooth muscle response to the Met and the EC_{50} using Met response curve.

In a previous study the extract of *Zataria multiflora* as a well-known medicinal plant also reduced tracheal smooth muscle response to the Met on in the guinea pig model of chronic obstructive pulmonary disease (COPD) [18]. This is consistent with the present study. It has been reported that concentration-response curves to Met were shifted to the right and EC_{50} Met were higher in the presence of atropine (an anti-muscarinic agent) and *Portulaca oleracea* extract similar to the current results study [15]. The antispasmodic effects of *M. officinails* on smooth muscles in primary dysmenorrhea in a randomized clinical trial has been shown [19]. It has been reported that *Melissa officinalis* shows affinity to acetylcholine receptor in central nervous system and also has binding properties with nicotinic and muscarinic receptors that help in modulating the cognitive performance in Alzheimer's disease patients [20]. Furthermore, the relaxant effect of *M. officinalis* oils on rat-isolated ileum contractions by KCl (80 mM) and acetylcholine (320 nM) was evaluated [21]. The relaxant effects of volatile oils of different plants such as *M. officinalis* on smooth muscles in tracheal and ileal were also reported [22]. All these results support the relaxant effects of the *M. officinalis* extract on tracheal smooth muscle in the present study.

Treatment of OVA-sensitized animals with the *M. officinalis* extracts led to improvement of almost all lung pathological changes. Although treatment with dexamethasone showed more effective on lung pathological changes, its effects were lower than the high dose of plant extract especially on lung inflammation.

Administration of *M. officinalis* extracts also reduced total and differential WBC counts except lymphocyte in treated groups. Airway inflammation and airway remodeling are the main characteristic features of asthma [23]. Therefore, treatment of allergic asthma should be focused on reducing airway inflammation and airway remodeling.

A previous study indicated that an increase in the inflammatory cell numbers in the BALF including eosinophils, lymphocytes, neutrophils as well as lung epithelial cell proliferation, mucus secretion and hyper-reactivity to methacholine increased in the lung tissues after sensitization in murine models of asthma [24]. These results confirmed the sensitization of an experimental model of asthma in animals.

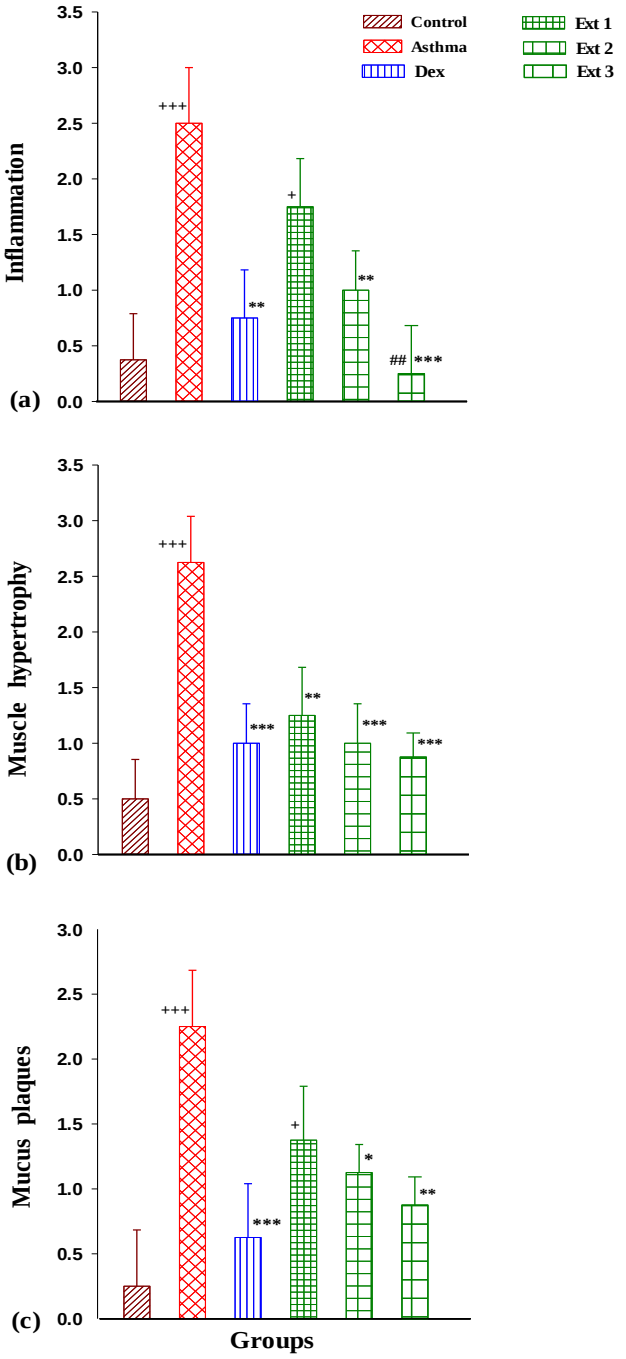

Figure 4. Mean ± SD of score of the lung inflammation (a), muscle hypertrophy (b) and mucus plaques (c) in control and sensitized animals treated with dexamethasone (Dex) and *Melissa officinalis* extracts (Ext 1, 2 and 3), (50, 100 and 200 mg/kg, respectively); Comparison of the data between groups was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc test. +++: p<0.001 compared between control and asthma groups; *: p<0.05, **: p<0.01 and ***: p<0.001 compared between asthma and treated groups; ##: p<0.01 compared between Dex and other treated groups

Previous studies also showed that the total WBC count, neutrophils, eosinophils and monocytes count increased, while lymphocytes reduced in lung lavage of sensitized animals compared to control group which confirm the findings of our study [13,25]. In addition, the cells in BALF of asthmatic cats contained a significantly higher percentage of eosinophils and significantly lower percentages of macrophages and lymphocyte cats compared to the control group [26].

The regulation of apoptosis could be a factor influencing lymphocyte numbers. In asthmatics patients, but not in healthy subjects, the physiological ligand of CD95, Fas ligand (CD95L), increased on T cells after segmental allergen challenge [34],which suggests increased apoptosis of lymphocytes in the BAL in asthmatics. The improvement in lung pathological changes, total and differential WBC count in BALF of OVA-induced sensitization in animals treated with *M. officinalis* extracts, might be due to its suppressing effect on inflammation.

Histamine is an effective inflammatory mediator that is produced during an allergic reaction and promotes vascular permeability [27].

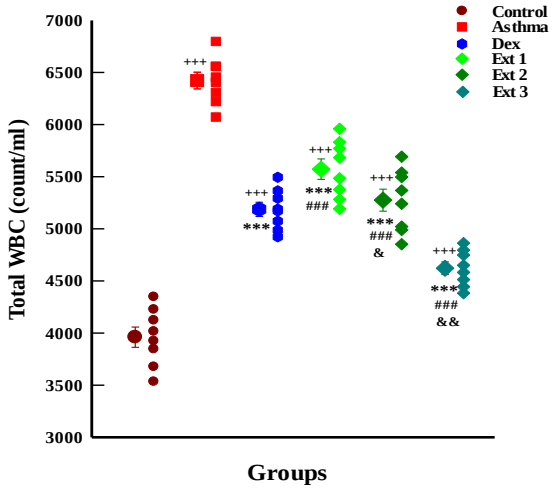

Figure 5. The mean ± SD of total WBC count in control and sensitized animals treated with dexamethasone (Dex) and *Melissa officinalis* extracts (50, 100 and 200 mg/kg) Ext 1, Ext 2 and Ext 3, respectively); Comparison of the data between control, asthma and treated sensitized rats was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc test; +++: p < 0.001, compared between control and asthma groups; ***: p < 0.001, compared between asthma and treated groups; ###: p < 0.001, compared between Dex and other treated groups; &: p<0.05 and &&: p<0.01, compared between extract Ext 50 vs Ext 100 and 200.

Table 2. Total and differential WBC count in the BALF of control, sensitized and treated rats

	Control	Asthma	Dex	Ext1	Ext2	Ext3
Total WBC	3960.50±256.7	6422.60±210.9^{+++}	5187.20±180.5***	5572.20±260.7$^{***\#}$	5275.10±280.0***	4622.10±160.9***
Lymphocyte	50.51±0.72	25.25±0.77^{+++}	43.13±0.78***	25.75±0.73$^{\#\#\#}$	34.05±0.90$^{***\#}$	46.02±1.01***
Neutrophile	15.00±0.54	26.40±0.75^{+++}	17.54±0.36***	24.90±0.54$^{\#\#\#}$	22.10±0.63**	15.25±0.36***
Eosinophils	12.95±0.64	24.59±0.46^{+++}	16.58±0.40***	23.59±0.53$^{***\#\#\#}$	19.08±0.97***	15.11±1.32***
Monocyte	21.52±0.48	23.75±0.53^{+++}	22.73±0.82***	25.75±0.74$^{\#\#\#}$	24.75±0.69***	23.61±0.66***

The mean±SD values of total WBC are its count in one mL and values of different WBC cell types are presented as percentage of each cell type in control and sensitized animals treated with dexamethasone (Dex) and *Melissa officinalis* extracts (Ext 1, Ext 2 and Ext 3), (50, 100 and 200 mg/kg, respectively). Comparison of the data between groups was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc test; +++: p<0.001 compared between control and asthma groups; *: p<0.05, **: p<0.01 and ***: p < 0.001, compared between asthma and treated groups; #: p<0.05, ###: p<0.001, compared between Dex and other treated groups

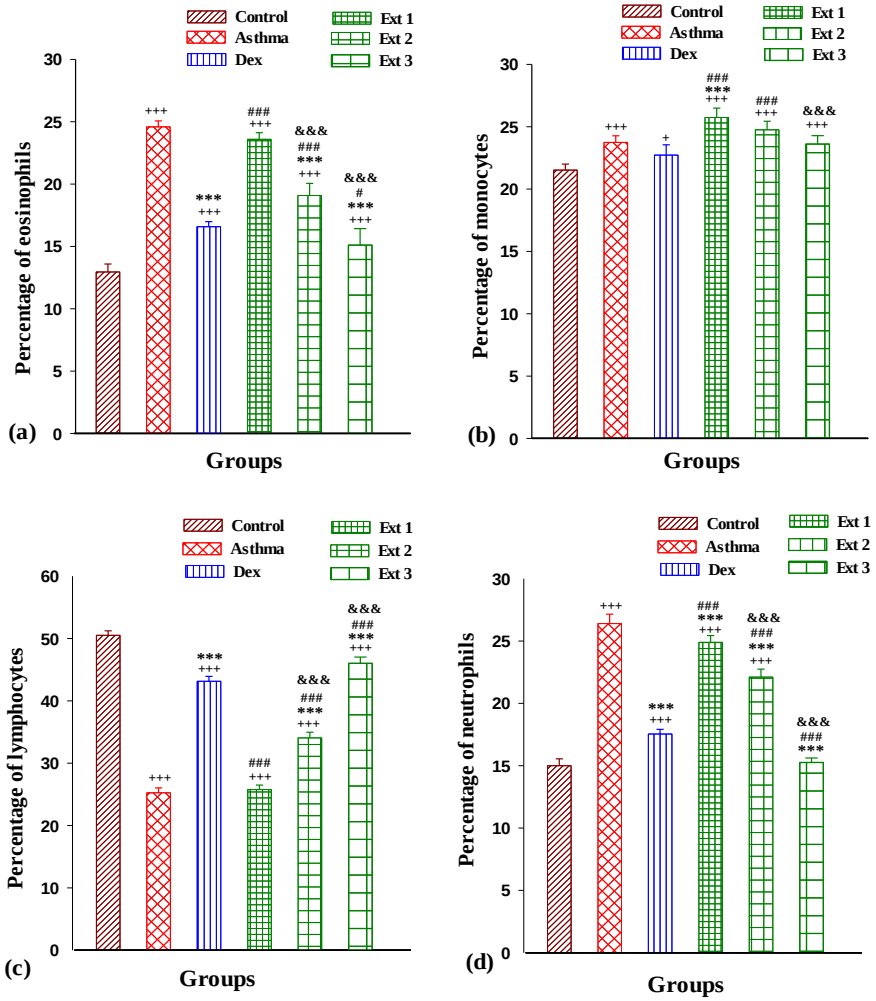

Figure 6. Mean ± SD of eosinophils (a), monocytes (b), lymphocytes (c), and neutrophils (d) percentages in BALF of control and sensitized animals treated with dexamethasone (Dex) and *Melissa officinalis* extracts (50, 100 and 200 mg/kg), (Ext 1, Ext 2 and Ext 3, respectively). Comparison of the data between control, asthma and treated sensitized rats was done using One-way analysis of variance (ANOVA) with Tukey-Kramer post hoc test; +++: p<0.001 compared between control and asthma groups; ***: p<0.001 compared between asthma and treated groups; #: p<0.05 and ###: p<0.001 compared between Dex and other treated groups; &&&: p<0.001 compared between extract Ext 50 vs Ext 100 and 200.

Administration of the aqueous extract *M. officinalis* (50-400 mg/kg) by gavage remarkably inhibited the edema produced by histamine at 3 h in rats by decreasing the inflammatory response

[28]. Oral administration of *M. officinalis* essential oil (200, 400 mg/kg) remarkably inhibited edema induced by carrageenan by 61.76% and 70.58%, respectively, compared with control and indomethacin as a standard drug [29]. In a similar study, treatment of OVA sensitized rats with *Crocus sativus* extract (50-200 mg/kg) remarkably reduced total WBC count as well as neutrophil and eosinophil percentages compared with the sensitized group [30].

In other studies, administration of *Nigella sativa* in drinking water significantly improved total and differential WBC in the BALF of OVA and sulfur mustard-induced lung injury in guinea pigs [31,32]. Based on the current study and those of previous studies, *M. officinalis* may have therapeutic effects on OVA induce asthma by reducing of lung inflammation, which supports the traditional uses of this plant for treating various diseases associated with inflammation.

The relaxant effect of *M. officinalis* extract on tracheal smooth muscle (possible broncho-dilatory effects) and effect on inflammatory cells in BALF are the strengths of this study, while not investigating effects of *M. officinalis* on inflammatory mediators as well as oxidative stress biomarkers are the limitations of the current study.

Further studies, including basic ones with different plant extracts and animal models, different constituents of the plant on animal models as well as clinical trials, are needed to examine the possible therapeutic effect of *M. officinalis* on asthma.

Conclusions

The results of the current study show that the hydroalcoholic extract of *M. officinalis*, can effectively limit tracheal smooth muscle responsiveness to methacholine and increased EC_{50}. The plant extracts also improved total and differential WBC cells in BAL fluid as well as lung pathological changes such as inflammation, muscle hypertrophy and mucus plaques in sensitized rats.

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Author contributions

Mohammad Reza Khazdair, designed and supervised the study; Vahideh Sadat Abbasnia, contributed to designing the study and collecting the data; Delaram Eslimi Esfahani, Mohsen Foadoddini and Shahrbanoo Oryan contributed to the conceptualization, methodology; Fatemeh Geramian, assisted in analyzing the data; all authors approved the final draft of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

AHR: airway hyper-responsiveness; ANOVA: one-way analysis of variance; BALF: broncho-alveolar lavage fluid; COPD: chronic obstructive pulmonary disease; Dex: dexamethasone; $EC_{50:}$ 50% of maximum response; HPLC: high-performance liquid chromatography; IgE: immunoglobulin E; IL: interleukin; IP: intraperitoneal injection; Met: methacholine; OVA: ovalbumin; Th2: T-helper 2 cells; WBC: white blood cell