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The Effects of *Allium Cepa* Extract on Tracheal Responsiveness, Lung Inflammatory Cells and Phospholipase A2 Level in Asthmatic Rats

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ABSTRACT

Antioxidant, antimicrobial, anti-hyperglycaemic, anti-diabetic and anti-inflammatory effects of *Allium cepa* (*A. cepa*) have been previously shown. In this study, the effects of *A. cepa* aqueous-alcoholic extract on tracheal responsiveness, lung inflammatory cells and phospholipase A2 (PLA2) level in bronchoalveolar fluid (BALF) of asthmatic rats were examined.

Wistar rats were randomly divided into control group (C), asthmatic group (A), asthmatic group (A) treated with *A. cepa* extract (AC, 0.175, 0.35, and 0.7 mg/mL) and dexamethasone (D, 1.25 µg/mL). The extract of *A. cepa* and dexamethasone were added to animal's drinking water during sensitization period. Tracheal responsiveness to methacholine and ovalbumin, lung inflammatory cells and PLA2 level in BALF were assessed.

Tracheal responsiveness to methacholine and ovalbumin, PLA2 level, total and most differential WBC count were increased but lymphocytes was decreased in asthmatic animals compared to group C ($p<0.05$ to $p < 0.001$). Treatment of sensitized rats with dexamethasone and all concentrations of *A. cepa* lead to a significant decrease in total WBC and PLA2 level compared to asthmatic group ($p<0.001$). The two higher concentrations of *A. cepa* also significantly decreased tracheal responsiveness, neutrophil and eosinophil counts but led to a significant increase in lymphocytes count compared to asthmatic group ($p<0.05$ to $p<0.001$). Treatment of sensitized group with the highest concentration of *A. cepa* also significantly reduced monocyte count compared to asthmatic group ($p< 0.001$).

Anti-inflammatory and preventive effects of *A. cepa* on tracheal responsiveness and lung inflammation in asthmatic animals may suggest its potential therapeutic effect on airway diseases such as asthma.

Keywords: Allium cepa; Animal asthma model; Inflammatory cells; Phospholipase A2; Tracheal responsiveness

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INTRODUCTION

Asthma is a widespread chronic inflammatory disease characterized by reversible airway obstruction, airway inflammation, infiltration of airway inflammatory cells, bronchial hyperresponsiveness and increased mucus production.[1-3]

Various cells such as T cells, mast cells, basophils, macrophages, and eosinophils, are involved in the inflammatory processes of asthma.[4] Among these cells, eosinophils are predominantly the characteristic feature of asthma.[5,6] Exposure to allergens causes migration of inflammatory cells and production of inflammatory mediators. Interactions between these factors initiate and continue the inflammatory response.[1, 7] Several studies indicated that total WBC and eosinophil counts were enhanced in sensitized animals and asthmatic patients.[8,9] Therefore, attenuation of the inflammation is essential for the treatment of asthma.

Allium cepa (*A. cepa*), commonly known as onion, is a biennial plant belonging to genus *Allium* and the family Alliaceae that on the basis of its color is divided into three types (red, yellow and white).[10,11] A large number of onion types and cultivars grows all around the world and has been consumed as a vegetable.[12] This plant massively produces by China, India, United States, Russia,Turkey, and Iran.[13] Additionally, this plant is considered as an herbal medicine for the treatment of some diseases such as hypercholesterolemia, cardiovascular diseases, cancer, hypertension, asthma, and diabetes mellitus.[10,12]

A. cepa contains several bioactive phytomolecules such as phenolic components, sulfurous components and flavonoids especially quercetin,[14,15] components and activities of onion is different on the basis of cultivars.[16] Sunpower cultivar had the most phenolic content, total flavonoids and antioxidant activity among 18 Korean onion cultivars[16] and quercetin 4′glucoside and quercetin 3,4′-diglucoside were most abundant components among southern Italian red onion.[17] In adition, Tropea ecotype onion has a higher content of

flavonols than the Montoro type onion[18,19] and content of health-promoting phenols and antioxidant in *Rossa di Toscana* is more than other two *A. cepa* cultivars in Italy.[20] Red onion variety of nigerian cultivars has also highest antioxidant potential and phenolic contents.[21] Higher antioxidant activity, total flavonols, and specific quercetin glucosides were demonestrated in two onion varieties (Hyskin and Red Baron) grown under organic conditions.[11] The major flavonoids in five onion cultivars from Tenerife were quercetin monoglucoside 1 and quercetin diglucoside for 80% of the total quercetin content.[22]

There is evidence for *A. cepa* pharmacological activities like antioxidant, antimicrobial, anti-hyperglycaemic, anti-diabetic and anti-inflammatory effects which have been attributed to the presence of these biologically active components.[23] immunomodulatory, bronchodilatory,[14] and anti-inflammatory properties for this plant hae been shown.[24]

Therefore, the present study aimed to examine the effects of *A. cepa* aqueous-alcoholic extract on tracheal responsiveness to methacholine and ovalbumin (OVA), lung inflammatory cells and phospholipase A2 (PLA2) level in asthmatic rats.

MATERIALS AND METHODS

Preparation of the Onion Extract

A. cepa was purchased from a local herbal shop in Mashhad, Iran. To prepare the extract, *A. cepa* was cut and peeled and the juice was obtained. Then, using a rotary evaporator, a dry powder of the juice was prepared.

Animals

Experiments were performed using Wistar rats (200±22 g) prepared from Animal house, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. The animals were kept in cages receiving clean filtered air (Maximiser, Thorens Caging System Inc., Hazleton, PA, USA.) under standard condition at 22±2^{o}C and regular 12 hr/ 12 hr light/dark cycle. They also had free access to food and water *ad libitum* during experimental period. Study protocol was approved by ethical committee of Mashhad University of Medical Sciences (No. 910898) and experiments were performed in compliance with the regulations of the Institute of Laboratory Animals Resources Commission on Life Sciences.

Experimental Groups

Animals were randomly divided into six groups (n=7 for each group) including: 1) Control group (group C) which was given intra-peritoneal (i.p.) and inhaled normal saline; 2) Asthmatic group (group A)

which was sensitized with ovalbumin (OVA); 3) Asthmatic group treated with dexamethasone 1.25 µg/mL added to animal's drinking water during sensitization period (group A+D); 4-6) Asthmatic groups treated with *A. cepa* extract at 3 concentrations of 0.175, 0.35, and 0.7 mg/mL added to the animal's drinking water during sensitization period (groups A+AC 0.175, A+AC 0.35, and A+AC 0.70) which were equal to 35, 70 and 140 mg/kg-bw/day, respectively. Each animal consumed almost 40 mL water per day and this volume of consumption was not significantly different among different groups.

Animal Sensitization

Experimental groups of rats were sensitized with three i.p. injections of 1 mg/kg OVA, along with 0.9% sterile saline containing 100 mg $Al(OH)_3$ as an adjuvant on days 1-3 of the experiment, on a daily basis. On days 6, 9, 12, 15, 18 and 21 of the experiment, animals were challenged with 1% OVA aerosol produced by a DeVilbiss PulmoSonic nebulizer (DeVilbiss Health Care Ltd., Feltham, UK) in a whole-body inhalation exposure chamber of 0.8 m^3 for 20 min/day. All animal were sacrificed 24 h after the last OVA challenge (on day 22).[25]

White Blood cell (WBC) Count in Bronchoalveolar Lavage Fluid (BALF)

After scarification of rats on day 22, the lungs were completely removed from the chest. The right lung was clamped and left lung was lavaged with 1 mL normal saline for five times at room temperature through tracheal cannula. To measure total leukocyte count, 1 mL of lung lavage fluid was stained by Turk solution (1 mL glacial acetic acid, 1 mL gentian violet solution 1% and 100 mL pure water) and total WBC was determined in duplicate using a hemocytometer (in a Burker chamber). The rest of BAL fluid was centrifuged (2500 g at 4°C for 10 min). For differential WBC count, a smear was prepared from the cell pellet in BALF and stained with Wright-Giemsa. After staining, differential counts were measured in accordance with standard morphologic protocol under the light microscope by counting a total of 100 cells/slide and the percentage of each leukocyte was calculated. Supernatants were collected into the test tube and stored at -80°C for measurement of PLA2.[26, 27] PLA2 level in BALF was quantified by ELISA kit, according to the manufacturer's protocol (MyBioSource, San Diego, CA, USA).

Tracheal Tissue Preparations

After sacrificing the animals' chest was opened and tracheal was dissected. The trachea was divided into rings of 3 to 4 mm length containing almost four cartilages for the formation of tracheal ring. Each tracheal ring was suspended in a 10 mL organ bath containing Krebs-Henseleit solution (KHS) (composed of NaCl 120 mM, KCl 4.72 mM, KH_2PO_4 1.2 mM, $MgSO_4 \cdot 7H_2O$ 0.5 mM, $CaCl_2 \cdot 2H_2O$ 2.5 mM, $NaHCO_3$ 25 mM and Dextrose 11 mM). This solution was maintained at 37±0.5°C and gassed with 95% O_2 and 5% CO_2. Tissues were suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 hr while they were being washed with KHS solution every 15 min. In all experiments, contraction responses were measured using an isotonic transducer (MLT0202, AD Instruments, Australia) connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia).

Assessment of Tracheal Response (TR) to Methacholine

Cumulative concentrations (log) -response curves to methacholine hydrochloride (Sigma Chemical Ltd, UK) were plotted for each experiment. Increasing concentrations of methacholine (10^{-8} to 10^{-3} M) were added every 2 min and the contraction caused by each concentration was recorded at the end of the intervals.[28] 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 concentration (log) of methacholine. It should be also noted that the effect reached a plateau in all experiments. Additionally, the effective concentration of methacholine causing 50% of maximum response (EC_{50}) was calculated using methacholine response curve in each experiment.

Measurement of Tracheal Response (TR) to Ovalbumin

The tracheal response to 0.1% solution of OVA was measured. For this purpose, 0.5 mL of 2% OVA solution was added to the 20 mL organ bath and tracheal smooth muscle contraction was recorded after 15 min.[28]

Statistical Analysis

In this study, data was represented as mean±SEM. Normality quantitative variables were checked by Kolmogorov Smirnov test. Statistical comparisons

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among and within groups were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer *post hoc* test. The results were considered statistically significant if the p value was less than 0.05. InStat (GraphPad Software, Inc, La Jolla, USA) was used for data analysis.

RESULTS

Total and Differential WBC Count

Total WBC count, percentages of eosinophils, neutrophils and monocytes in the BALF of sensitized animals were significantly higher than those of the control group but lymphocytes percentage was lower ($p<0.05$ to $p<0.001$), (Figures 1 and 2). Treatment of sensitized groups with all concentrations of the extract led to significant and concentration-dependent decrease in total WBC, the percentages of eosinophils and neutrophils. Treatment with AC 0.35 and 0.7 mg/mL resulted in significant reduction in the percentages of monocytes but significant increase in the percentage of lymphocytes compared to untreated sensitized group ($p<0.05$ to $p<0.001$), (Figures 1 and 2). In addition, the percentages of eosinophils, neutrophils and monocytes in sensitized animals treated with AC 0.175 mg/mL and the percentages of lymphocytes in groups treated with AC 0.175 and 0.35 mg/mL were significantly different from those of control group ($p<0.001$ for all cases), (Figures 1 and 2).

Treatment of sensitized animals with dexamethasone caused significant reduction in total WBC, percentages of eosinophils, neutrophils and monocytes but increased lymphocytes percentage compared to sensitized group ($p<0.01$ to $p<0.001$), (Figures 1 and 2). Total WBC count and the percentages of neutrophils in the group treated with dexamethasone were significantly higher but the percentage of lymphocytes was lower than those of the control group ($p<0.05$ to $p<0.001$), (Figures 1 and 2).

PLA2 Level

In sensitized group, PLA2 levels were significantly increased compared to control animals ($p<0.001$), (Figure 3). In groups treated with all concentrations of the extract and dexamethasone, PLA2 levels were significantly decreased compared to sensitized group ($p<0.001$ for all cases), (Figure 3). PLA2 values in groups treated with AC 0.35 and 0.7 mg/mL were still significantly higher compared to control group ($p<0.001$ for both cases), (Figure 3).

Tracheal Response to Methacholine

Concentration-response curves to methacholine in asthmatic group showed leftward shifts compared to the control group. However, in comparison to group A, the curves in all treated groups shifted to the right (Figure 4a).

The mean value of EC_{50} in group A was significantly lower than that of control group ($p<0.001$), (Figure 4b). Compared with sensitized group, the mean values of EC_{50} was significantly increased in groups treated with AC 0.35 and 0.7 mg/mL and dexamethasone ($p<0.01$ to $p<0.001$), (Figure 4b). However, in asthmatic rats treated with all concentration of extract, EC_{50} value was significantly lower than that of group C ($p<0.05$ to $p<0.001$), (Figure 4b).

In untreated asthmatic group and group treated with the lowest concentration of the extract, maximum response to methacholine was significantly higher than that of the control group ($p<0.01$ for both cases), (Figure 5a). However, groups treated with AC 0.35 and 0.7 mg/mL and dexamethasone led to a significant decrease in maximum response to methacholine compared to group A ($p<0.05$ to $p<0.01$), (Figure 5a).

Tracheal Response to Ovalbumin

Tracheal responsiveness to OVA in untreated asthmatic groups and those treated with all concentrations of the extract was significantly higher than that of the control group ($p< 0.05$ to $p<0.001$), (Figure 5b). In groups treated with dexamethasone or AC 0.35 and 0.7 mg/mL, tracheal response to OVA was significantly decreased as compared to group A ($p<0.05$ to $p<0.001$), (Figure 5b).

Comparison between the Effects of the Extract and the Effects of Dexamethasone

The effect of treatment with the lowest concentration of the extract on eosinophils, neutrophils, and monocytes counts, PLA2 level, EC_{50}, and maximum response to methacholine was significantly lower than those observed following dexamethasone treatment ($p<0.01$ to $p<0.001$), (Figures 1, 2, 3, 4b and 5a). In addition, the effects of AC 0.175 and 0.35 mg/mL on reduction of the tracheal response to ovalbumin were significantly lower than dexamethasone effect ($p<0.001$), (Figure 5b). However, the effect of AC 0.7 mg/mL on neutrophils count was significantly higher than that of dexamethasone ($p<0.01$), (Figures 1 and 2).

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Comparison of the Effects of Three Concentrations of the Extract

There was no significant difference in total WBC among different concentrations of the extract. The effects of the medium and high concentrations of extract (0.35 and 0.7 mg/mL) on eosinophils, neutrophils, lymphocytes, monocytes percentages and PLA2 level were significantly higher than those of the lowest concentration (0.175 mg/mL), (p<0.01 to p<0.001), (Table 1). The effect of the highest concentration of the extract on neutrophils and lymphocytes percentages and PLA2 value were also significantly higher than the medium concentrations (p<0.01 and p<0.001 for neutrophils and lymphocytes counts respectively), (Table 1).

The two higher concentrations of extract (0.35 and 0.7 mg/mL) caused significantly higher effects on EC_{50} and maximum response to methacholine compared to its lowest concentration, while only the effect of high concentrations of the extract on OVA response was significantly higher than that of its lowest concentration (p<0.05 to p<0.001), (Table 1).

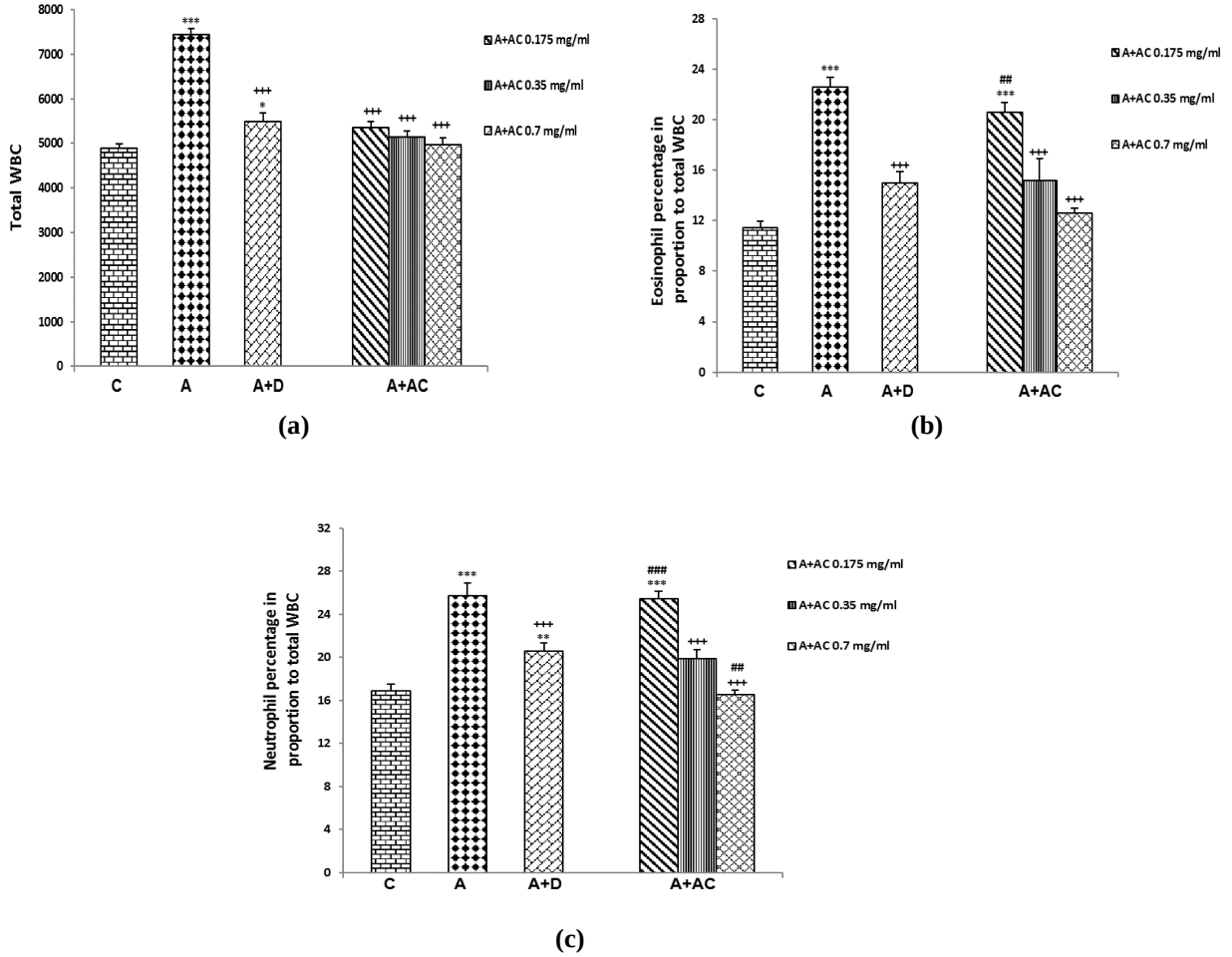

Figure 1. The effect of *A. cepa* extract on total WBC number (count/mL of bronchoalveolar lavage), (a), percentage of eosinophils (b) and neutrophils (c) in control group (C), asthmatic group (A), A treated with dexamethasone (D) and *A. cepa* (AC), (n=7 in each group). Data are presented as mean ± SEM values. *p<0.05, **p<0.01 and ***p<0.001 as compared to group C. +++p<0.001 as compared to group A. ## p<0.01 and ### p<0.001 as compared to group D. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test.

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Table 1. Comparisons of total and differential WBC count, methacholine EC_{50}, maximum response and OVA response among the three concentrations of onion extract

Parameters	A+AC 0.175	A+AC 0.35	A+AC 0.7
Total WBC (Count/mL)	5355±130.16	5150±129.56	4964.29±166.8
Eosinophils (Percentage)	20.57±0.78	15.14±1.77**	12.57±0.43***
Neutrophils (Percentage)	25.43±0.72	19.86±0.83***	16.57±0.37*** ++
Lymphocytes (Percentage)	27±0.53	43±1.23***	50.29±1.36*** +++
Monocytes (Percentage)	26.86±1.12	21.86±0.83**	20.57±0.78***
PLA2 (pg/mL)	8.15±0.12	6.89±0.09***	5.99±0.03***++
EC_{50} (μM)	0.09±0.02	0.33±0.07*	0.42±0.06***
Max Response (g)	1.16±0.17	0.72±0.04*	0.68±0.06*
OVA Response (g)	0.63±0.08	0.53±0.06	0.356±0.03**

Data were presented as mean±SEM

Comparison of A+AC 0.7 and A+AC 0.35 vs A+AC 0.175: *p<0.05, **p<0.01, and ***p<0.001 using ANOVA test.

Comparison of A+AC 0.7 and A+AC 0.35: +p<0.05, ++p<0.01, and +++p<0.001 using ANOVA test.

A+AC: asthmatic animals treated with *Allium cepa*, WBC: white blood cells, PLA2: phospholipase A2, EC50: effective concentration causing 50% maximum response, Max: maximum, OVA: ovalbumin.

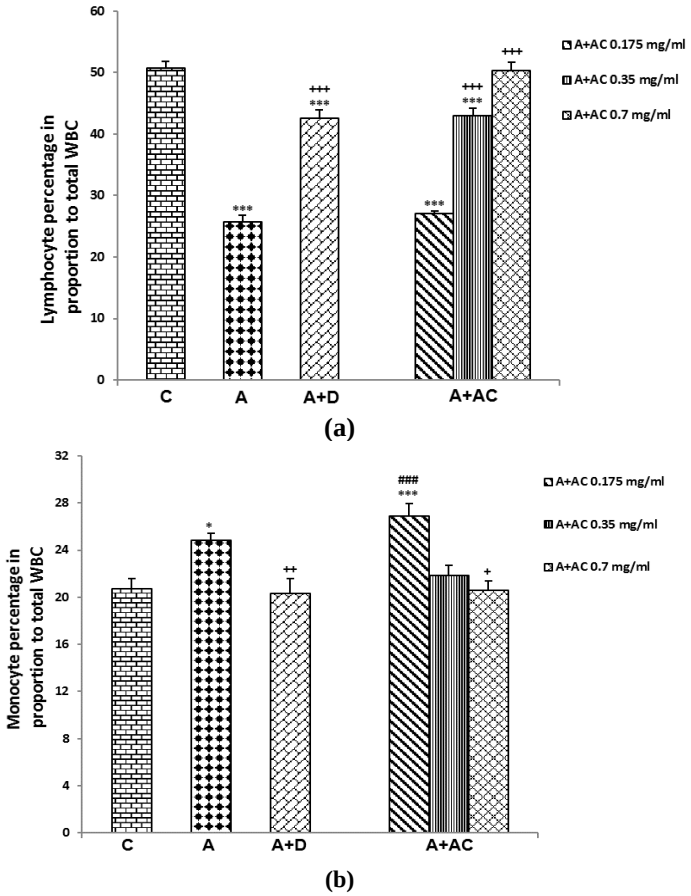


Figure 2. The effect of *A. cepa* extract on the percentage of lymphocytes (a) and monocytes (b) in control group (C), asthmatic group (A) , A treated with dexamethasone (D) and *A. cepa* (AC), (n=7 in each group). Data are presented as mean ± SEM values. *p<0.05 and ***p<0.001 as compared to group C. +p<0.05, ++p<0.01 and +++p<0.001 as compared to group A. ### p<0.001 as compared to group D. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test.

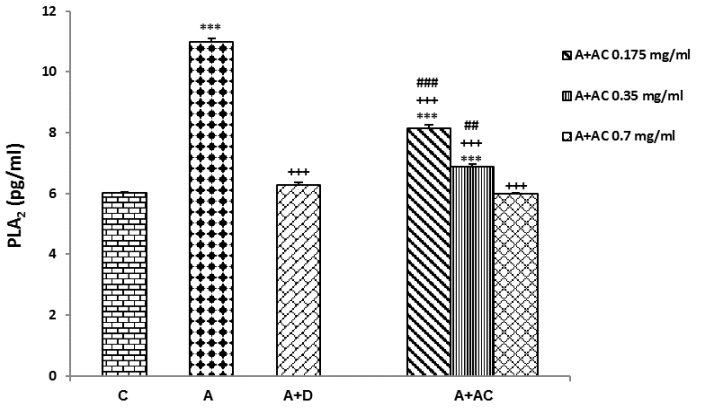
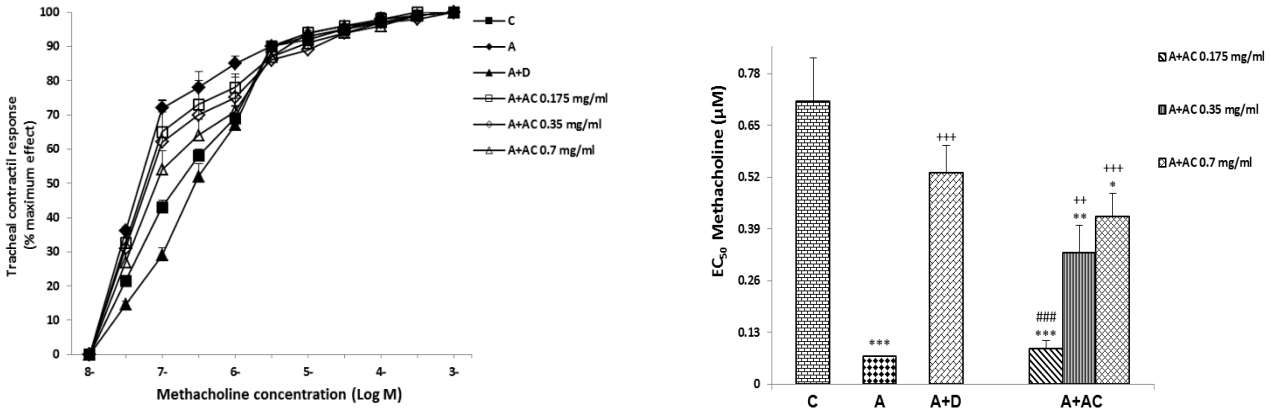


Figure 3. The effect of *A. cepa* extract on PLA2 level (pg/mL) in broncho-alveolar lavage of control group (C), asthmatic group (A), A treated with dexamethasone (D) and *A. cepa* (AC), (n=7 in each group). Data are presented as mean ± SEM values. ***p<0.001 as compared to group C. +++p<0.001 as compared to group A. ## p<0.01 and ### p<0.001 as compared to group D. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test.

Comparison of the Effects of Three Concentrations of the Extract

There was no significant difference in total WBC among different concentrations of the extract. The effects of the medium and high concentrations of extract (0.35 and 0.7 mg/mL) on eosinophils, neutrophils, lymphocytes, monocytes percentages and PLA2 level were significantly higher than those of the lowest concentration (0.175 mg/mL), (p<0.01 to p<0.001), (Table 1). The effect of the highest concentration of the extract on neutrophils and lymphocytes percentages and PLA2 value were also significantly higher than the medium concentrations (p<0.01 and p<0.001 for neutrophils and lymphocytes counts respectively), (Table 1).

The two higher concentrations of extract (0.35 and 0.7 mg/mL) caused significantly higher effects on EC_{50} and maximum response to methacholine compared to its lowest concentration, while only the effect of high concentrations of the extract on OVA response was significantly higher than that of its lowest concentration (p<0.05 to p<0.001), (Table 1).



(a) **(b)**

Figure 4. The effect of *A. cepa* extract on cumulative concentration (log) -response curves of methacholine-induced contraction of isolated trachea (a) and methacholine EC_{50} (b) in control group (C), asthmatic group (A), A treated with dexamethasone (D) and *A. cepa* (AC), (n=7 in each group). Data are presented as mean ± SEM values. *p<0.05, **p<0.01, and ***p<0.001 as compared to group C. ++p<0.01 and +++p<0.001 as compared to group A. ### p<0.001 as compared to group D. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test.

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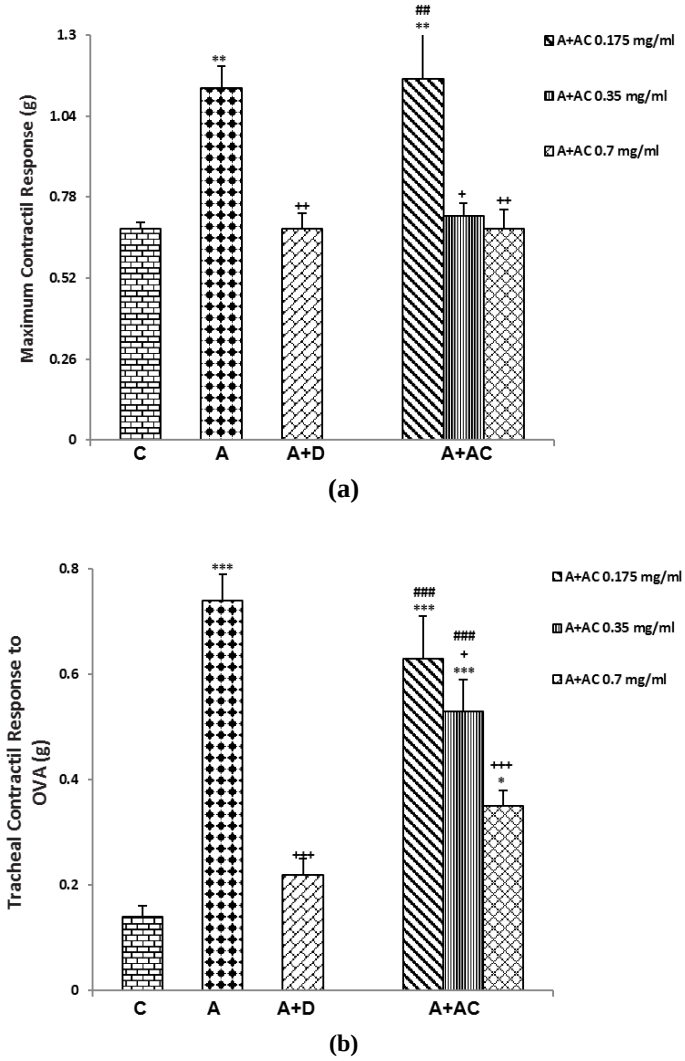

Figure 5. The effect of *A. cepa* extract on maximum contractile response of methacholine (a) and tracheal contractile response to OVA (b) in control group (C), asthmatic group (A), A treated with dexamethasone (D) and *A. cepa* (AC), (n=7 in each group). Data are presented as mean ± SEM values. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ as compared to group C. +$p<0.05$, ++$p<0.01$, and +++$p<0.001$ as compared to group A. ## $p<0.01$ and ### $p<0.001$ as compared to group D. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test.

DISCUSSION

The effects of *A. cepa* extract on non-specific and specific tracheal responsivenss (responsiveness to methacholine and ovalbumin respectively), lung inflammatory cells and PLA2 level in BALF of sensitized rats were investigated in this study.

In this experiment, increase total WBC, eosinophils, neutrophils, monocytes count and reduction of lymphocytes percentage in lung lavage were observed in sensitized rats. These results confirmed sensitization or induction of an experimental model of asthma in animals. Previous studies also indicated similar results which confirm the findings of our study. These studies have shown that inflammatory cells including total WBC, neutrophils and eosinophils were increased in lung lavage of sensitized animals compared to control group.[29,30] In addition, increase in inflammatory cells especially eosinophils in subjects with asthma supported the results of the present research.[4] The role of eosinophils in pathogenesis of a number of diseases such as asthma have been reported.[31] In this regard, a

correlation between total cell counts and eosinophils and airway responsiveness in a small group of individuals with atopic asthma, have been shown.[32] Immune reinforcement effect of onion was also reported through increasing some immunological cells.[33]

Moreover, a significant increase in PLA2 level in BALF of sensitized rats was observed in the present study. PLA2 in inflammatory diseases such as asthma, increases the release of arachidonic acid, production of lysophospholipids, sPLA 2-mediated activation of cPLA2 with increased leukotriene production, surfactant degradation, release of cytokines, and the impact on immune and inflammatory cells.[34,35]

The results of the current study showed that treatment of sensitized animals with the extract of *A. cepa* resulted in a significant reduction in total WBC, neutrophils, eosinophils and monocytes counts. These findings are consistent with those of previous studies indicating a decrease in inflammatory cells including total WBC and eosinophils in bronchoalveolar lavage in asthmatic mice treated with *A. cepa* extract.[14,36] Dawud et al., 2016 also reported significant decrease in number of eosinophil in the blood and BALF of asthmatic rats after treatment with *A. cepa*.[34]

In asthmatic rats treated with three concentrations of *A. cepa* extract, PLA2 level in BALF was significantly reduced. It was reported that *A. cepa* extract in animal models inhibits the production of thromboxane A2 (a potent bronchoconstrictor) via PLA2.[37,38] PLA2 also leads to conversion of membrane phospholipids into arachidonic acid (AA) from which TXA2 is produced. Possibly, *A. cepa* extract inhibits PLA2, reduced the production of arachidonic acid and eventually, decreases TXA2.[38] These findings support the results of the present study. Altogether, the results of the present and previous studies showed anti-asthmatic effect of *A. cepa* extract by suppression of lung inflammation via decreasing BALF inflammatory cells and PLA2 level.

Increased tracheal responsiveness to methacholine and OVA in sensitized group compared to control rats was also observed in the present study. Previous studies done using the same model of asthmatic animals, also showed similar findings[31,39,40] which confirms the induction of rat model of asthma.

Treatment of sensitized animal with all concentrations of *A. cepa* extract and dexamethasone resulted in a concentration-dependent reduction of enhanced tracheal responsiveness to methacholine and OVA in asthmatic rats. But, methacholine EC_{50} in groups treated with dexamethasone and the two higher concentrations of the extract significantly increased. Therefore, consistent with previous studies, our results suggested a preventive effect for *A. cepa* on tracheal responsiveness which is the main characteristic feature of asthma. Anti-spasmodic activity of *A. cepa* saponins was reported in the ileum isolated from guinea pig.[41] Relaxing effect of quercetin, one of the active compounds of onion, on airway smooth muscle was also reported.[42] In addition, *in vitro* inhibitory effect of quercetin on rat trachea contractility was indicated.[43] Furthermore, *A. cepa* 10, 100 or 1000 µg/mL had relaxing effects on tracheal rings of asthmatic mice.[14]

In fact, the results of the present study regarding the effect of the extract of *A. cepa* on total and differential WBC counts, PLA2 level and tracheal responsiveness were very similar to the results of the study of Oliveira et al., 2015.[14] However, the study of Oliveira et al., 2015 was performed in mice sensitized with *Blomia tropicalis* treated with methanolic extract 100 and 1000 mg/kg from the 8th to the 14th day of the experiment; while the current study was done on rats sensitized with OVA and treated with the juice of peeled onion at the doses of 35, 70 and 140 mg/kg/day for 21 days of the experimental period.

Therefore, the results of the present study along with the above-mentioned studies suggest a potential therapeutic effect for *A. cepa* against asthma by decreasing lung inflammation and tracheal responsiveness. However, some studies were reported the allergic sensitization and bronchial asthma induced by onion.[44,45] Almogren et al., 2013 indicated onion allergic sensitization in a large number of studied patients by detection of onion specific IgE antibodies.[46] Thus, this plant may show dual effects of both aggravations of allergic disorders such as asthma in conditions and prevention of asthma in other cases which should be clarified in further studies.

In the present study total and differential WBC were calculated using standard method and according to previous studies as well as PLA2 level in BALF was quantified by ELISA kit. However, accurate evaluations such as flow cytometry method could be used for more precious assessment of WBCs lung. In addition, qPCR data of PLA2 could be evaluated in further studies.

Based on the results of this study, *A. cepa* extract

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reduced tracheal responsiveness, lung inflammatory cells and PLA2 level in BALF of asthmatic rats and these effects were comparable with those of dexamethasone. Hence, it can be concluded that this plant may possess a therapeutic potential in the treatment of allergic disorders and airway diseases such as asthma.

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