Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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ORIGINAL ARTICLE

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Effect of *Allium cepa* extract on total and differential WBC, TP level, oxidant and antioxidant biomarkers, and lung pathology in ovalbumin-sensitized rats

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Received 31 October 2022; Accepted 22 March 2023
Available online 1 May 2023

KEYWORDS
Allium cepa;
sensitized rat;
white blood cells;
oxidative stress;
lung pathology

Abstract
Background: Previous studies have shown that *Allium cepa* (*A. cepa*) has relaxant and anti-inflammatory effects. In this research, *A. cepa* extract was examined for its prophylactic effect on lung inflammation and oxidative stress in sensitized rats.
Methods: Total and differential white blood cell (WBC) count in the blood, serum levels of oxidant and antioxidant biomarkers, total protein (TP) in bronchoalveolar lavage fluid (BALF), and lung pathology were investigated in control group (C), sensitized group (S), and sensitized groups treated with *A. cepa* and dexamethasone.
Results: Total and most differential WBC count, TP, NO_2, NO_3, MDA (malondialdehyde), and lung pathological scores were increased while lymphocytes, superoxide dismutase (SOD), catalase (CAT), and thiol were decreased in sensitized animals compared to controls ($p < 0.01$ to $p < 0.001$). Treatment with all concentrations of extract significantly improved total WBC, TP, NO_2, NO_3, interstitial fibrosis, and emphysema compared to the S group ($p < 0.05$ to $p < 0.001$). Two higher concentrations of the extract significantly decreased neutrophil and monocyte count, malondialdehyde, bleeding and epithelial damage but increased lymphocyte, CAT, and thiol compared to the S group ($p < 0.05$ to $p < 0.001$). Dexamethasone treatment also

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https://doi.org/10.15586/aei.v51i3.532

substantially improved most measured parameters ($p < 0.05$ to $p < 0.001$), but it did not change eosinophil percentage. It was proposed that *A. cepa* extract could affect lung inflammation and oxidative stress in sensitized rats.

Introduction

Asthma is defined as inflammation of the airways, airway infiltration of eosinophils and neutrophils, swelling of the airway walls, formation of mucus plug, enhanced bronchial responsiveness to different stimuli, and variable and recurrent airflow blockage.[1] Asthma is caused by a variety of cells, including eosinophils, basophils, macrophages, lymphocytes, mast cells, and neutrophils.[2]

Oxidative stress has been identified as a mismatch between generation and removal of reactive oxygen species (ROS).[3] Airway inflammation and oxidative stress are the principal causes of recurring airflow restrictions in asthma.[4] More ROS from airspace leucocytes also cause chronic asthma inflammation in the airways,[5] along with increasing blood expression of pro-inflammatory genes.[6] It is strongly demonstrated that the inflammatory airways cells of asthma patients produce more superoxide anion radicals than those of healthy controls.[7] Analysis of expired air in asthmatic patients identified enhanced levels of nitro tyrosine and other oxidation stress markers.[8] Inflammatory intermediaries lead to the lung pathology changes such as thickening of the airway walls, inflammatory cell infiltration, increased smooth muscle mass, mucous gland hypertrophy, vascular obstruction, reduced airway diameter, airway epithelial shedding, and mucus plugs.[9]

Allium cepa (*A. cepa*), or common onion, belonging to the Liliaceae family is a monocot bulbous perennial, which is cultivated extensively all over the world, especially in Europe, Asia, North America, and Africa.[10] Various pharmacological properties have been identified for this herb, such as anti-asthmatic[11] and anti-inflammatory[12] effects. Moreover, several experimental studies have shown that *A. cepa* has substantial antioxidant activity due to its high level of flavonoids including quercetine and kaempferol.[13,14]

The effect of the extract of *A. cepa* on total and differential white blood cell (WBC) count in blood, the serum levels of nitric oxide (NO), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), thiol and total protein (TP) in bronchoalveolar lavage fluid (BALF), and lung pathological changes in sensitized rats were examined in this research.

Materials and Methods

Animal sensitization and animal groups

Male Wistar rats weighing 200–250 g were collected from Mashhad University of Medical Sciences' animal house and housed in a 22 ± 2°C temperature with a 12 h light/dark period.

The research (Ethics Allowance No. 910898) was approved by MUMS Ethics Committee and regulations were adopted by the Life Sciences Commission of Institute of Laboratory Animals Resources in animal manipulation. Animals were sensitized to ovalbumin (OVA) as previously described.[15]

Animals were randomly divided into six groups of control group (C), sensitized group with OVA (S), and S group treated with three concentrations of the extract of *A. cepa* (groups AC 0.175, AC 0.35, and AC 0.70 mg/mL) and dexamethasone (group D 1.25 µg/mL). In treated animals, the extract of *A. cepa* and dexamethasone were applied to potable water during the sensitization process in treated animals (Table 1).

Plant and extract

As already mentioned, the extract of *A. cepa* was prepared.[16]

Table 1 Studied groups and treatment with dexamethasone (D) or the extract of *A. cepa* (AC).

Group	Sensitized	Treatment	Abbreviate	n
Control	Saline	Drinking water (DW)	C	6
Sensitized	Ovalbumin	DW	S	6
S treated with D	Ovalbumin	DW + 1.25 µg/ml D	S+D	6
S treated with low AC concentration	Ovalbumin	DW + 0.175 mg/ml AC		7
S treated with medium AC concentration	Ovalbumin	DW + 0.35 mg/ml AC		7
S treated with high AC concentrations	Ovalbumin	DW + 0.70 mg/ml AC		7

The extract of *A. cepa* and dexamethasone were added in animals' drinking water during sensitization period. Each animal used almost 40 ml water per day and this volume was not significantly different among different groups.

A. cepa extract characterization

The HPLC fingerprint was carried out using Shimadzu (LC-2030) to characterize the extract of *A. cepa* (Figure 1).

Total and differential WBC count

In the method previously mentioned, total and differing WBC count were calculated.[17]

Serum oxidant and antioxidant biomarkers and TP measurements in the BALF

The products of NO metabolism (NO_2^-/NO_3^-), MDA, superoxide-dismutase (SOD), and CAT activities and total thiol in the BALF supernatant were measured using previously described methods.[17]

Lung pathological evaluation

Lung pathological changes were measured according to the previously described procedure.[18]

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey–Kramer *post-hoc* test was used for comparisons among and within groups using InStat (GraphPad Software, Inc, La Jolla, USA). The data are shown as mean ± standard error of the mean (SEM). $P < 0.05$ was used as the level of statistical significance.

Results and Discussion

The effect of A. cepa on total and differential WBC count in sensitized animals

In sensitized animals, total WBC count, and the percentages of eosinophil, neutrophil, and monocyte in the blood were significantly higher but lymphocyte percentage was lower than those of the C group ($p < 0.01$ to $p < 0.001$, Figures 2 and 3). Treatment with various concentrations of *A. cepa* significantly reduced total WBC in the blood compared to the S group ($p < 0.05$ to $p < 0.001$, Figures 2 and 3). There were significant improvements in lymphocyte, neutrophil, and monocyte percentages due to treatment of medium and high concentrations of the extract and eosinophil due to high concentration of the extract compared to the S group ($p < 0.01$ to $p < 0.001$, Figures 2 and 3). However, the percentage of neutrophil in the S group treated with low and medium concentrations of the extract and the percentage of lymphocyte in the S group treated with low concentration of extract were significantly different from those of the C group ($p < 0.05$ to $p < 0.001$), (Figures 2 and 3).

Dexamethasone treatment substantially reduced total WBC count, the percentages of monocyte and neutrophil but increased the lymphocyte percentage compared to the S group ($p < 0.01$ to $p < 0.001$, Figures 2 and 3). The percentage of neutrophil in the S group treated with dexamethasone was significantly different from those of the C group ($p < 0.01$ to $p < 0.001$, Figure 3).

The effects of low and medium concentrations of *A. cepa* (0.35, 0.70 mg/mL) on monocyte and lymphocyte percentages and its highest concentration on the percentages of neutrophil and eosinophil were significantly higher than its low concentration (0.175 mg/mL), ($p < 0.01$ to $p < 0.001$, Figures 2 and 3). In addition, there was a significant difference between high and medium concentrations of *A. cepa* on eosinophil and lymphocyte percentages ($p < 0.01$ for both cases, Figures 2 and 3).

In addition, the effects of high concentration of *A. cepa* on neutrophil, eosinophil, and lymphocyte percentages were significantly greater than the effect of dexamethasone ($p < 0.05$ to $p < 0.0$, Figures 2 and 3).

The effect of A. cepa on oxidant and antioxidant biomarkers in sensitized animals

The serum levels of NO_2, NO_3, and MDA were significantly increased but SOD, CAT, and thiol decreased in sensitized animals compared to the C group ($p < 0.001$ for all cases)

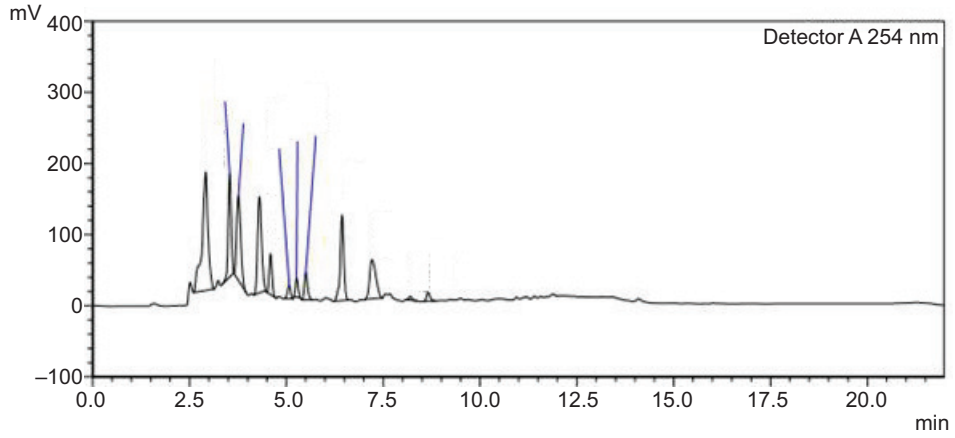

Figure 1 The extract of *A. cepa* HPLC finger print at 254 nm (40 mg/mL).

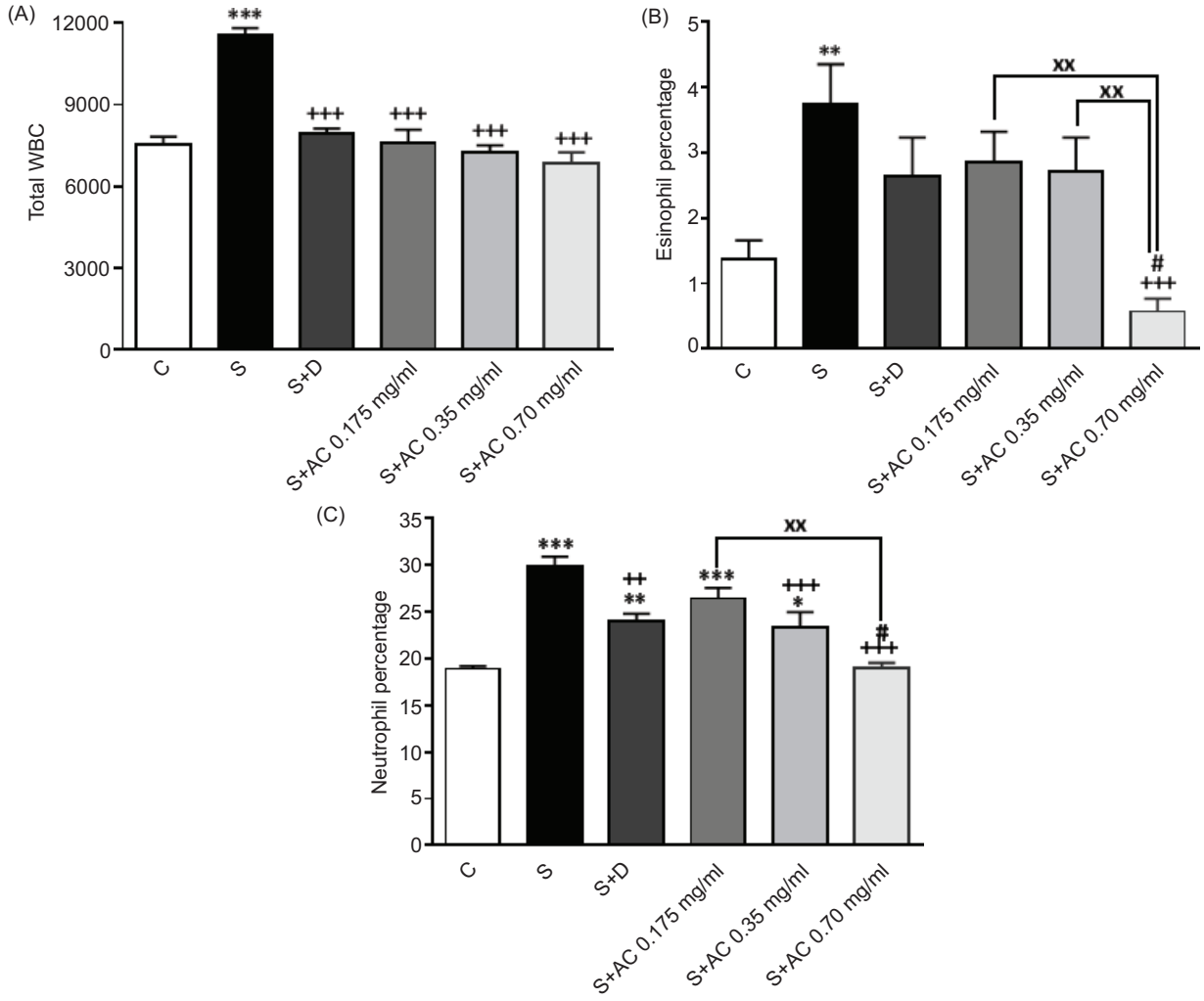

Figure 2 Total WBC number (A), percentage of eosinophil (B), and neutrophil [C] in control animals (C), sensitized group [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC] (n = 6 for C, S, and S treated with dexamethasone and n = 7 for S treated with the extract groups). *p < 0.05, **p < 0.01, ***p < 0.001 compared to group C. ++p < 0.01, +++p < 0.001 compared to S group. #p < 0.05 compared to group S + D. xx p < 0.01 comparison among three concentrations of *A. cepa*.

(Figures 4 and 5). The levels of NO_2 and NO_3 in the S groups treated with various concentrations of *A. cepa*, were significantly reduced compared to the non-treated S group. Also, the levels of MDA, CAT and thiol in the S groups treated with two higher concentrations of *A. cepa* were significantly improved compared to the S group. Treatment with high concentration of *A. cepa* significantly increased SOD level ($p < 0.01$ to $p < 0.001$, Figures 4 and 5). The levels of NO_3, MDA, SOD, CAT, and thiol in the S groups treated with various concentrations of extract did not achieve their control values and showed significant difference compared to the C group ($p < 0.05$ to $p < 0.001$, Figures 4 and 5).

Treatment with dexamethasone also significantly reduced NO_2, NO_3, and MDA levels and increased SOD, CAT, and thiol levels compared to the S group ($p < 0.001$ for all cases, Figures 4 and 5). However, the levels of NO_3, SOD, CAT, and thiol in the sensitized animals treated with dexamethasone were significantly different from those of the C group ($p < 0.01$ to $p < 0.001$, Figures 4 and 5).

The effects of medium and high concentrations of *A. cepa* (0.35, 0.70 mg/mL) on CAT, thiol, and MDA levels, and its highest concentration on the levels of NO_2, NO_3, and SOD were significantly higher than its low concentration (0.175 mg/mL) ($p < 0.05$ to $p < 0.001$, Figures 4 and 5). The effect of high concentrations of *A. cepa* on all oxidant and antioxidant markers except NO_2 level was also significantly higher than its medium concentration ($p < 0.05$ to $p < 0.01$, Figures 4 and 5).

The effect of the three extract concentrations on CAT, thiol, and MDA levels, and its two lower concentrations on the level of SOD were significantly lower than the dexamethasone treatment ($p < 0.01$ to $p < 0.001$, Figures 4 and 5).

The effect of A. cepa on TP in sensitized animals

TP level in lung lavage in the S group was significantly higher than the C group ($p < 0.001$, Figure 6). Treatment with

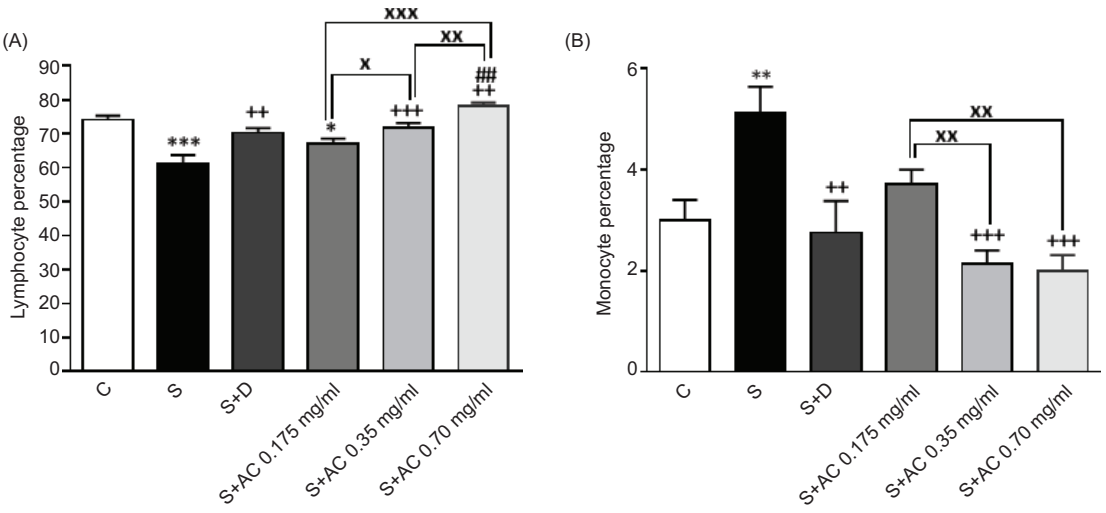

Figure 3 Percentage of lymphocyte (A) and monocyte (B) in the blood of control animals [C], sensitized group [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC] (n = 6 for C, S, and S treated with dexamethasone and n = 7 for S treated with the extract groups). *p < 0.05, **p < 0.01, ***p < 0.001 compared to group C. ++p < 0.01, +++p < 0.001 compared to S group. ##p < 0.05, ##p < 0.01 compared to group S + D group. x p < 0.05, xx p < 0.01, xxx p < 0.001 comparison among three concentrations of *A. cepa*.

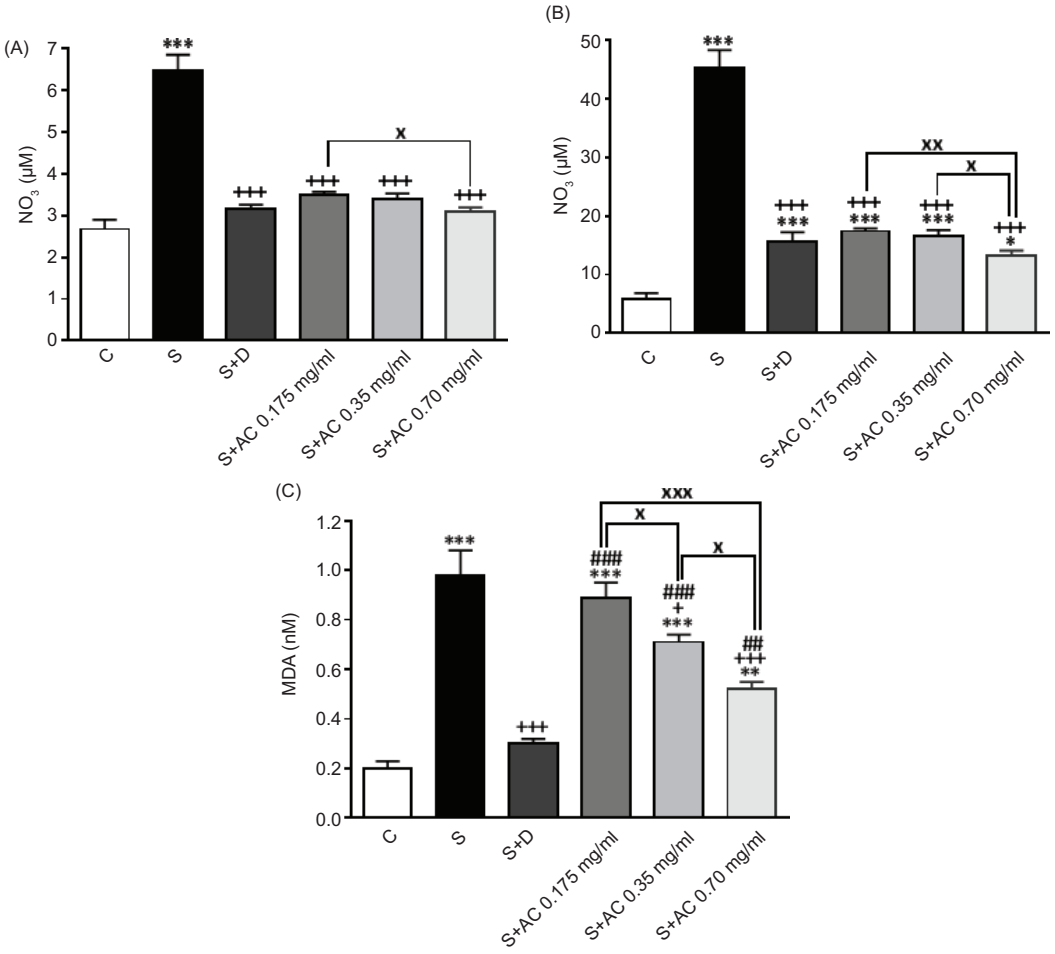

Figure 4 NO_2 (A), NO_3 (B), and MDA [C] concentrations in serum of control (C) sensitized group [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC], (n = 6 for C, S, and S treated with dexamethasone and n = 7 for S treated with the extract groups). *p < 0.05, **p < 0.01, ***p < 0.001 compared to group C. +p < 0.05, +++p < 0.001 compared to S group. ##p < 0.01, ###p < 0.001 compared to group S + D. x p < 0.05, xx p < 0.01, xxx p < 0.001 comparison among three concentrations of *A. cepa*.

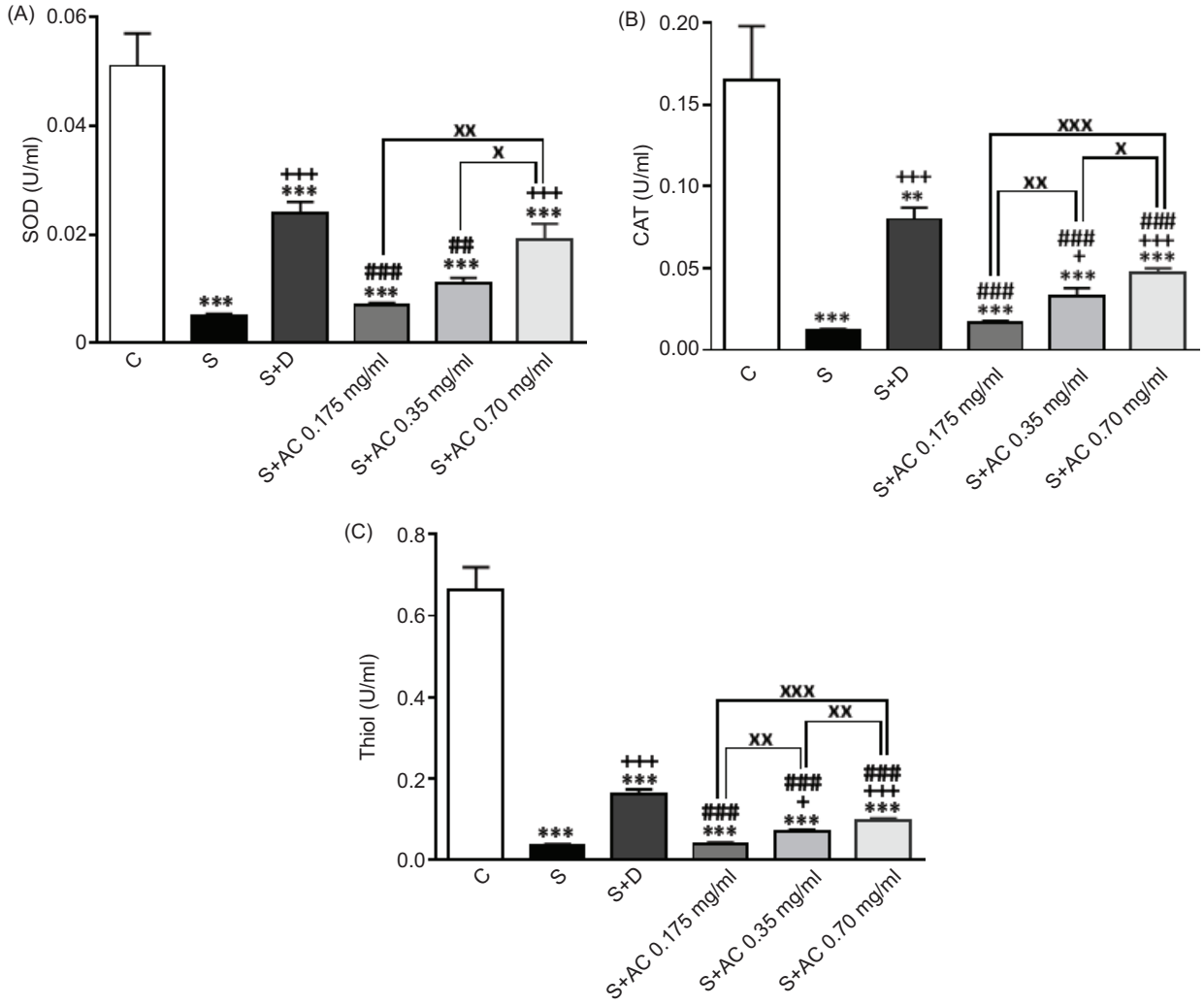

Figure 5 SOD (A), CAT (B), and thiol [C] levels in serum of control (C), sensitized [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC], (n = 6 for C, S, and S treated with dexamethasone and n = 7 for S treated with the extract groups). **p < 0.01, ***p < 0.001 compared to group C. ++p < 0.05, +++p < 0.001 compared to S group. ##p < 0.01, ###p < 0.001 compared to group S + D. x p < 0.05, xx p < 0.01, xxx p < 0.001 comparison among three concentrations of *A. cepa*.

various concentrations of *A. cepa* significantly reduced TP level in the BALF compared to the S group (p < 0.05 to p < 0.001, Figure 6).

Treatment with dexamethasone also significantly reduced TP level compared to the S group (p < 0.001, Figure 6). TP level in the S group treated with low and medium concentrations of the extract and dexamethasone did not achieve the control values and were significantly different from the C group (p < 0.001 for all cases, Figure 6).

The effects of medium and high concentrations of *A. cepa* (0.35, 0.70 mg/mL) on TP level were significantly higher than its low concentration (0.175 mg/mL), (p < 0.001 and p < 0.01 for high and medium concentrations, respectively, Figure 6).

The effect of high concentration of *A. cepa* on TP level was significantly greater than the effect of dexamethasone (p < 0.0, Figures 6).

The effect of A. cepa on lung pathology in sensitized animals

Compared with the C group, the results of all pathological changes in the S group were significantly increased (p < 0.01 to p < 0.001) (Figures 7 and 8). Interstitial fibrosis and emphysema in the S groups treated with various concentrations of *A. cepa*, were significantly reduced compared to the S group. Also, epithelial damage, and bleeding in the treated groups with two higher concentrations of *A. cepa*, were significantly reduced compared to the S group. Treatment with high concentration of *A. cepa* also significantly reduced interstitial inflammation p < 0.05 to p < 0.001, Figures 7 and 8). Epithelial damage in the S group treated with low concentration of the extract did not reach the control value and was significantly higher than in the C group (p < 0.05, Figure 8).

Dexamethasone also considerably reduced all pathological changes compared to the S group (p < 0.05 to

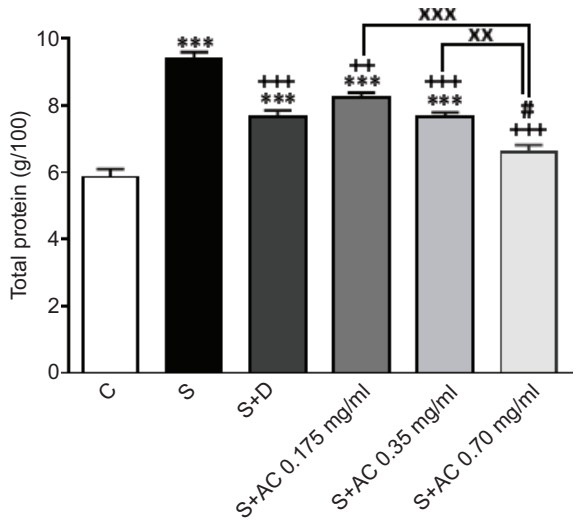

Figure 6 TP in bronchoalveolar lavage fluid (BALF) of control animals [C], sensitized group [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC], (n = 6 for C, S, ant S treated with dexamethasone and n = 7 for S treated with the extract groups). ***p < 0.001 compared to group C. ++p < 0.01, +++p < 0.001 compared to S group. #p < 0.05 compared to group S + D. xx p < 0.01, xxx p < 0.001 comparison among three concentrations of *A. cepa*.

p < 0.001, Figures 7 and 8). The pathological scores between group C and dexamethasone-treated group were not significantly different.

The effect of treatment with various concentrations of the extract on interstitial fibrosis was significantly higher than the dexamethasone treatment (p < 0.01 for all cases, Figures 7 and 8). Figure 9 showed a photograph specimen of lung in each group.

Discussion

In the present study, effects of *A. cepa* extract on total and differential WBC count in the blood, levels of oxidant (NO_2, NO_3, and MDA) and antioxidant (CAT, SOD, and thiol) biomarkers in the serum, TP level in BALF, and lung pathological changes in sensitized rats were investigated.

The results showed increased total WBC, eosinophil, neutrophil, and monocyte counts but reduced lymphocyte percentage in the blood of sensitized animals compared to control animals. These findings confirmed an asthma model for animals in rats. Previous studies have also shown increased total WBC, neutrophil and eosinophil percentages, and reduced lymphocyte percentage in BALF and blood of sensitized rats.[15,19] Findings 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 but increased lymphocyte percentages. In a previous study, the effect of the aqueous extract of *A. cepa* on total and differential WBC count in asthmatic rats was shown, which confirm the results of the present study.[11] In this study, increased TP level in BALF was observed in sensitized rats. Increased TP level was also shown in asthma, both in animal and human studies,[15,20] which supports the results of the present study. In sensitized rats treated with three concentrations of *A. cepa* extract, TP level in BALF was significantly reduced. It was reported that ethanolic extract of *A. cepa* significantly decreased the serum level of TP in gentamicin-induced nephrotoxicity in rats.[21]

Findings also showed that NO_2, NO_3, and MDA significantly increased and SOD, CAT, and thiol as antioxidant markers decreased in the asthmatic group, which also support the sensitization of animals. Recent studies has demonstrated that NO and MDA levels were increased in asthma.[4,15] Reduced SOD[19] and CAT[22] activities, as well as glutathione[23] were shown in asthmatic patients, which support the findings of the current study.

Interestingly, NO has been considered to act as a free radical, especially in an overproduced condition.[3] There was a positive relationship between oxidative stress and enhanced NO and MDA levels.[24,25] Previous studies showed that NO inhibited the Th1 and interferon gamma (IFN-γ) level but increased the interleukin 4 (IL-4) and IL-5, thereby increasing inflammatory responses.[26] Treatment with the extract of *A. cepa* significantly reduced the levels of NO_2, NO_3, and MDA, and enhanced SOD, CAT, and thiol in sensitized animals. *A. cepa* has been shown to increase SOD, CAT, and thiol, and decrease MDA in alloxan-induced diabetic rabbits.[27] Also, NO in a diabetic model induced by streptozotocin[28] was also shown, which confirms the findings of the present study.

The findings also showed a rise in pathological changes that are close to the results of some previous studies of the lung tissue in sensitized animals,[18,29] which support the sensitization of animals. Treatment of sensitized animals with different concentrations of *A. cepa* extract resulted in a significant reduction in all lung histological changes. In a previous research, it was also shown that intranasal methanolic extract of *A. cepa* attenuated inflammatory cell infiltration and airway mucus hyper activation in the lung tissue of murine model of asthma.[30] Aqueous extract of *A. cepa* decreased cell infiltration, edema, and congestion in sensitized rats with OVA.[10] These results confirm the results of this report.

Overall, the current and previous studies found that *A. cepa* extract has anti-inflammatory and antioxidant properties by lowering inflammatory cells and TP levels, inhibiting oxidative stress by lowering oxidative markers and increasing antioxidant agents, and effecting pathological changes in sensitized rats.

Respiratory reactions for *A. cepa* have been documented, such as bronchial asthma, rhinoconjunctivitis, and dermatitis.[31] In a clinical trial, the effects of garlic and onion on Saudi patients were determined by detecting specific IgE antibodies with a radioallergosorbent test (RAST), indicating that garlic and onion have sensitization and allergenic potential.[32] As a result, this plant could have paradoxical effects on asthma prevention, which should be explained in future research.

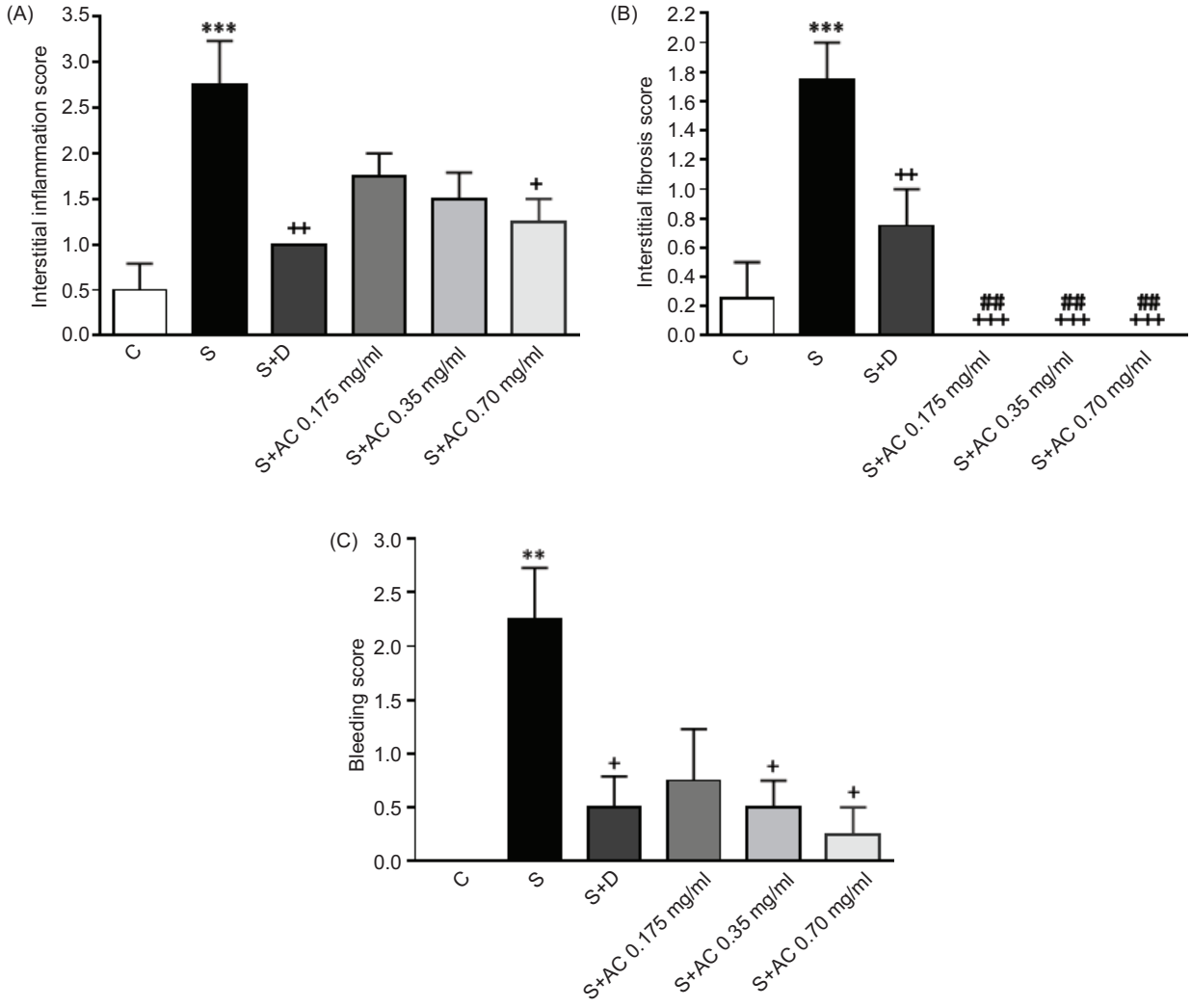

Figure 7 Interstitial inflammation (A), interstitial fibrosis (B), and bleeding [C] scores in control (C), sensitized [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC], (n = 6 for C, S, andS treated with dexamethasone and n = 7 for S treated with the extract groups). **p < 0.01, ***p < 0.001 compared to group C. ++p < 0.05, ++p < 0.01, +++p < 0.001 compared to S group. ##p < 0.01 compared to group S + D.

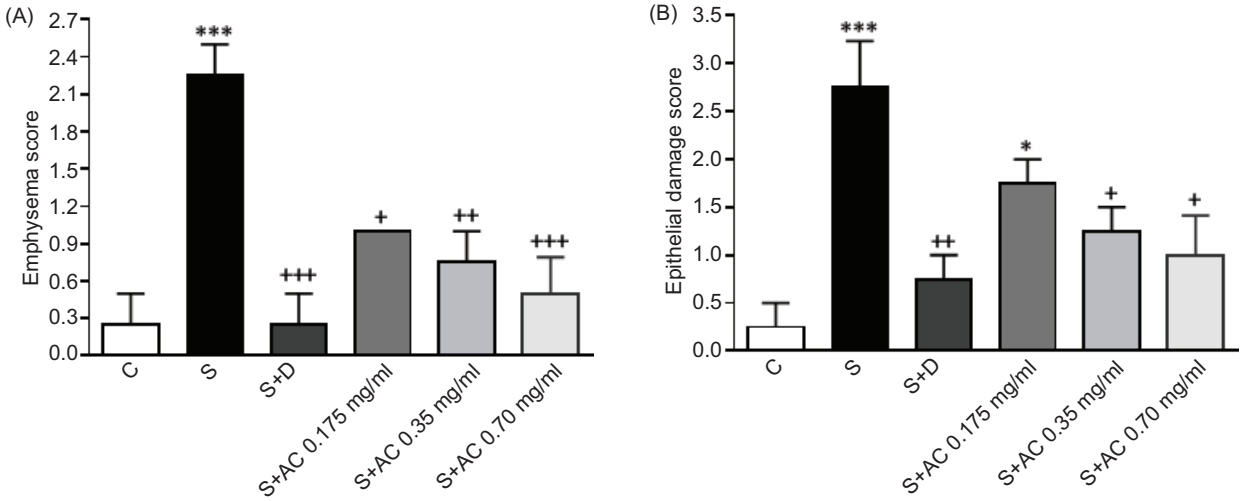

Figure 8 Emphysema (A) and epithelial damage (B) scores in control [C], sensitized [S], S groups treated with dexamethasone [S + D], and *A. cepa* [AC], (n = 6 for C, S, and S treated with dexamethasone and n = 7 for S treated with the extract groups). *p < 0.05, ***p < 0.001 compared to group C. ++p < 0.05, ++p < 0.01, +++p < 0.001 compared to S group.

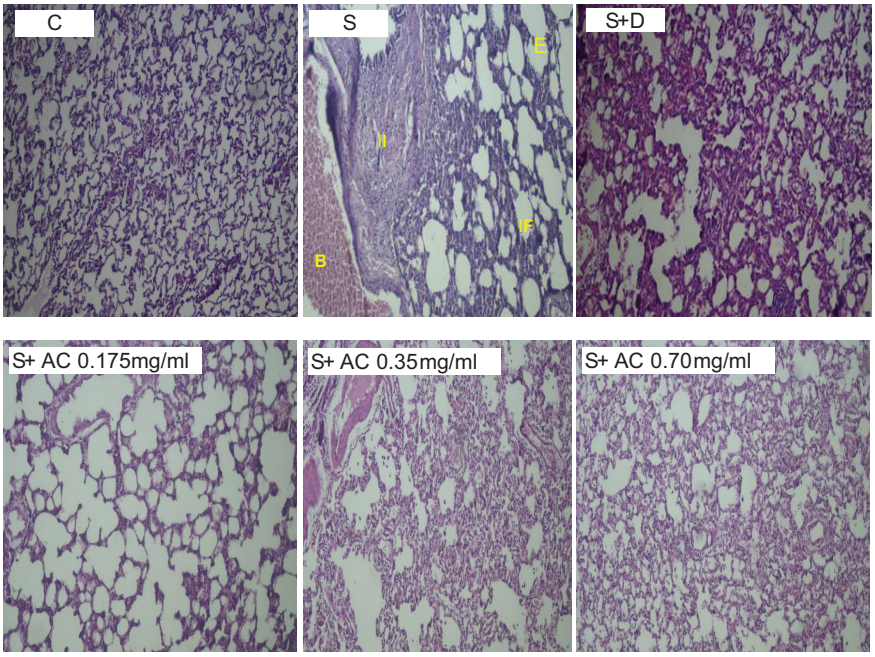

Figure 9 Pathological studies in control [C], sensitized [S] group with interstitial inflammation [II], interstitial fibrosis [IF], bleeding [B], and emphysema [E], sensitized rats treated with dexamethasone [S + D] and sensitized rats treated with *A. cepa (AC; 0.175, 0.35 and 0.70 mg/mL)*.

Conclusions

It is concluded from this study that extract of *A. cepa* has a preventive effect on total and differential WBC in blood; serum levels of NO_2, NO_3, MDA, SOD, CAT, and thiol; TP level in BALF; and lung pathological changes of sensitized rats similar to the effect of a standard anti-inflammatory drug, dexamethasone. These findings indicate that *A. cepa* in asthma has a therapeutic preventative potential.

Conflict of Interest

No conflict of interest.

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