

Alpha-linolenic acid ameliorates bronchial asthma features in ovalbumin-sensitized rats

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Keywords

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Abstract

Objectives Effect of alpha-linolenic acid (ALA) against ovalbumin (OVA)-induced inflammation, oxidant/antioxidant imbalance and pathological features was examined in rat.

Methods Total and differential WBC count and oxidant/antioxidant levels in BALF (bronchoalveolar lavage fluid) as well as lung pathological features were investigated in five groups of rats including controls (group C), rats sensitized with OVA (group S) and S treated with either ALA (0.2 and 0.4 mg/ml) or dexamethasone.

Key findings As compared to group C, in OVA-sensitized rats, increases in WBC counts, levels of oxidant biomarkers and most pathological scores were observed while lymphocyte percentage and antioxidants levels decreased. Treatment with ALA (0.2 and 0.4 mg/ml) significantly reduced total WBC, NO₂ and NO₃ levels, interstitial fibrosis and emphysema compared to sensitized group. The higher dose of ALA also significantly decreased neutrophil, eosinophil, and monocyte counts, MDA levels and interstitial inflammation but increased lymphocyte counts, as well as antioxidants levels, compared to sensitized group. Dexamethasone administration led to a significant improvement of most factors compared to group S but had no effects on total WBC count, bleeding and epithelial damage.

Conclusions Alpha-linolenic acid suppressed inflammation and oxidative stress, making it a potential therapeutic candidate for treatment of airway inflammatory diseases such as bronchial asthma.

Introduction

Asthma as a chronic condition is regarded as a major health issue worldwide. This disease is described by pulmonary eosinophilia, lymphocyte and neutrophil infiltration, mucus hypersecretion and airway hyperresponsiveness (AHR).^[1] Various inflammatory elements like eosinophils, lymphocytes, mast cells, and neutrophils contribute to asthma pathogenesis.^[2] In clinical trials and animal experiments, it was indicated that total WBC and eosinophil numbers increase in asthmatic groups compared to healthy controls.^[3,4] Eosinophils' count in peripheral blood and bronchial lavage of asthmatic patients correlated with the disease severity.^[5]

It was also shown that increased oxidative stress (OS) is a major player in chronic airway inflammation observed in asthma.^[6] In this regard, overproduction of reactive oxygen species (ROS) and enhanced levels of OS biomarkers were observed in asthma.^[7] High levels of ROS lead to OS induction in the airways, cause smooth muscle hyperplasia, increase subepithelial fibrosis, enhance smooth muscle mass, result in airway hyperreactivity and trigger mucus secretion through induction of histamine release from mast cells.^[8]

Alpha-linolenic acid (ALA, C₁₈H₃₀O₂) is a naturally occurring omega-3 fatty acid that is produced by several plants such as *Portulaca oleracea*. It has been used for human growth and development and prevention of

diseases.^[9] So far, several therapeutic activities such as antioxidant,^[10] anti-inflammatory,^[11] anticonvulsant^[12] and relaxant effects^[13] have been shown for ALA. Salvinorin A (C₂₃H₂₈O₈) also reduced airway hyperreactivity and interleukin (IL) 13 and inhibited mast cell degranulation but was not effective against pulmonary inflammation, or IL-4, IgE and leukotriene C4 levels in the lung of sensitized mice.^[14] It was also reported that palmitoylethanolamide (PEA, C₁₈H₃₇NO₂), which is coreleased with endocannabinoid anandamide, was reduced in the bronchi of OVA-sensitized animals and this observation was significantly associated with upregulation of cannabinoid (CB2) and G protein-coupled receptor 55 (GPR55) receptors. PEA treatment prevented bronchial hyperreactivity, lung inflammation, pulmonary inflammation and mast cell degranulation but did not affect IgE levels.^[15]

In this study, the effects of ALA on lung pathological features, total and differential WBC counts, as well as OS biomarkers in BALF (bronchoalveolar lavage fluid) of OVA-sensitized rats as an animal model of bronchial asthma, were investigated for the first time.

Methods

Chemicals and reagents

Alpha-linolenic acid, ovalbumin (OVA), grade V with 98% purity, alpha-linolenic acid and dexamethasone were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals including ketamine, formalin, ethanol, xylol, glacial acetic acid, gentian violet solution, Al (OH)₃, sulfanilamide (SULF), N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD), zinc sulphate, HCl, vanadium (III) chloride, thiobarbituric acid, pyrogallol, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 5, 5'-dithiobis-(2-nitrobenzoic acid (DTNB), Tris-ethylene diamine tetra acetic acid (Tris-EDTA), NaCl, KCl, KH₂PO₄, MgSO₄, CaCl₂, NaHCO₃ and dextrose were all of analytical grade and purchased from Merck Company (Merck, Darmstadt, Germany).

Animals

In this experiment, a total of 36 male Wistar rats (220 ± 50 g) were obtained from the Animal House of

Mashhad University of Medical Sciences and maintained in cages in a room with clean, filtered air (Maximiser; Thorens Caging System Inc, Hazleton, PA, USA) under standard conditions (i.e. at 22 ± 2 °C with 12 h/12 h light/dark cycle). Throughout the study period, animals had free access to water and food, *ad libitum*. Animals were randomly divided into five groups including control or saline-treated (group C), OVA-sensitized (group S), S animals treated with either alpha-linolenic acid 0.2 and 0.4 mg/ml (groups ALA 0.2 and ALA 0.4) or S animals treated with dexamethasone 1.25 µg/ml (group S+D), as shown in Table 1.^[16] On average, each animal took 40 ml drinking water/day. Therefore, ALA 0.2 and 0.4 mg/ml were equal to 40 and 80 mg/kg per day, and dexamethasone 1.25 µg/ml was equal to 0.25 mg/kg per day.

Ovalbumin sensitization

Rats were sensitized with intraperitoneal (i.p.) injections of OVA 1 mg/kg + 100 mg Al(OH)₃ as adjuvant, in 0.9% sterile saline on days 1, 2 and 3. Then, rats were exposed to 1% OVA aerosol for 20 min on days 6, 9, 12, 15, 18 and 21 with animal normal-breathing. OVA aerosol was produced by a DeVilbiss PulmoSonic nebulizer (DeVilbiss Health Care Ltd, Feltham, UK) with an air flow of 8 l/min in a 0.8-m³ chamber, and brief presentation of sensitization protocol is presented in Table 2.^[17,18] Animal handling was performed according to the institutional guidelines following approval of the ethics committee of Mashhad University of Medical Sciences (Approval No. 910968).

Determination of WBC counts in rats' BALF

To obtain the BALF, animals were sacrificed on day 22 using ketamine (50 mg/kg; i.p.). Next, chest was opened, and lungs along with trachea were dissected. The right lung was then washed with 1 ml of saline for five times (total = 5 ml) at room temperature.^[18]

For counting total WBC, 1 ml of BALF was stained with Turk's solution (consisted of 1 ml glacial acetic acid, 1 ml gentian violet solution 1% and 100 ml distilled water) and WBC were counted in duplicate using a hemocytometer (in a Bruker chamber). Differential WBC count was measured

Table 1 Experimental animal groups

Groups	Definition	Dose	Abbreviated name	n
Control	Nonsensitized rats		C	8
Sensitized	Nontreated, OVA-sensitized rats		S	8
	OVA-sensitized rats treated with alpha-linolenic acid	0.2 mg/ml/0.4 mg/ml	S+ALA 0.2 mg/ml/S+ALA 0.4 mg/ml	66
	OVA-sensitized rats treated with dexamethasone	1.25 µg/ml	D	8

Dexamethasone and alpha-linolenic acid were added to animals' drinking water during the sensitization period. Averagely, each rat consumed 40 ml drinking water/day; no significant differences were found in water consumption among different groups.

Table 2 Timeline of rat sensitization

Intraperitoneal injection of 1 mg/kg OVA + 100 mg Al(OH) ₃										
Inhalation exposure to 1% OVA aerosol, 20 min/day					Animal sacrificing and measurement performance					
Day 1	Day 2	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 22	
Animal death were not seen due to OVA sensitization procedure in this study.										

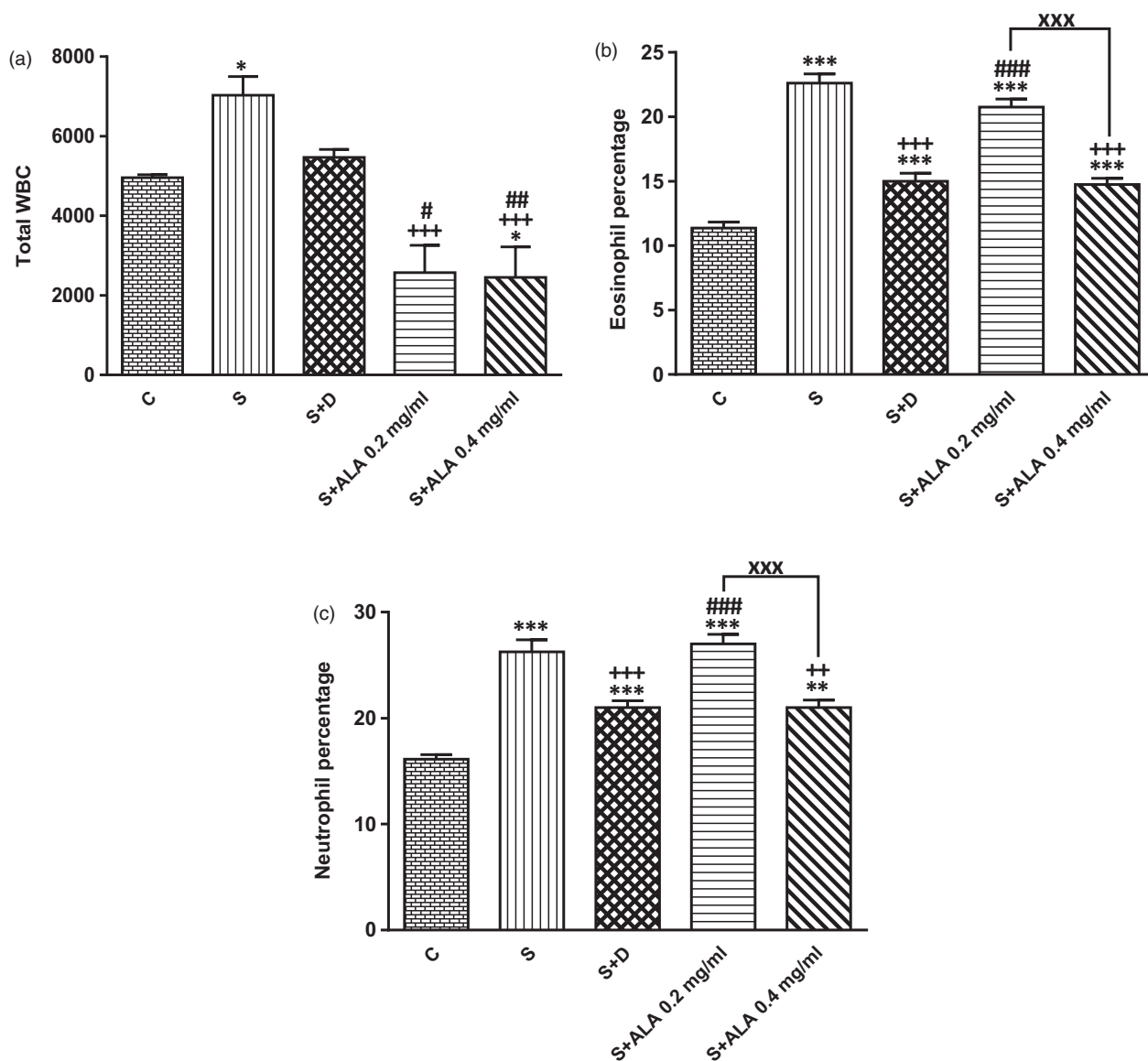


Figure 1 Total WBC (count/ml of bronchoalveolar lavage fluid) (a), percentages of eosinophils (b) and neutrophils (c) in control animals (C), sensitized group (S), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are presented as mean $\pm$ SEM values. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared to group C. ++ $P < 0.01$ and +++ $P < 0.001$ show significant differences compared to group S. # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ show significant differences compared to group S+D. XXX $P < 0.001$ shows significant differences between the two concentrations of alpha-linolenic acid. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer's post-test.

by staining and morphological criteria were evaluated, under a light microscope by counting 100 cells and calculating the percentage of each cell type.^[18]

Determination of oxidant/antioxidant biomarkers levels

In BALF supernatant, the levels of oxidant/antioxidant biomarkers including NO oxidation products ($\text{NO}_2^-/\text{NO}_3^-$), malondialdehyde (MDA) and thiol levels as well as the activity of superoxide dismutase (SOD) and catalase (CAT) were assessed as described in a previous study.^[4]

Pathological evaluations

After sacrificing the animal, left lung was dissected and fixed in formalin 10% (Merck). After 7 days, tissues were dried by an Auto Technicon apparatus; then, samples were cleared by serial dilutions of ethanol 70–100% and xylol, and the paraffin blocks were made. Afterwards, 4- μm sections were prepared from blocks and stained with haematoxylin and eosin (H&E). The tissues were examined using light microscopy. The pathological features including interstitial inflammation and fibrosis, bleeding, emphysema and insults to epithelial cells were graded from 0 to 2 based on the following criteria: (0) no pathological insults; (1) patchy changes; and (2) severe damages (throughout the lung).^[19]

Statistical analysis

In this study, data are represented as mean $\pm$ SEM. Differences among different groups were analysed using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test by InStat (GraphPad Software, Inc., La Jolla, CA, USA). *P* values <0.05 were considered statistically significant.

Results

ALA effects WBC counts in sensitized rats

Following OVA sensitization, total WBC count as well as monocytes, eosinophils and neutrophils percentages significantly increased in the BALF while lymphocyte percentage decreased as compared to the control rats (i.e. nonsensitized animals; $P < 0.05$ to <0.001, Figures 1 and 2).

Total WBC count following treatment with the two concentrations of ALA (0.2 and 0.4 mg/ml), and monocytes, eosinophils, neutrophils and lymphocyte percentages following treatment with ALA 0.4 mg/ml were markedly reduced compared to the sensitized group (group S; $P < 0.05$ to <0.001, Figures 1 and 2).

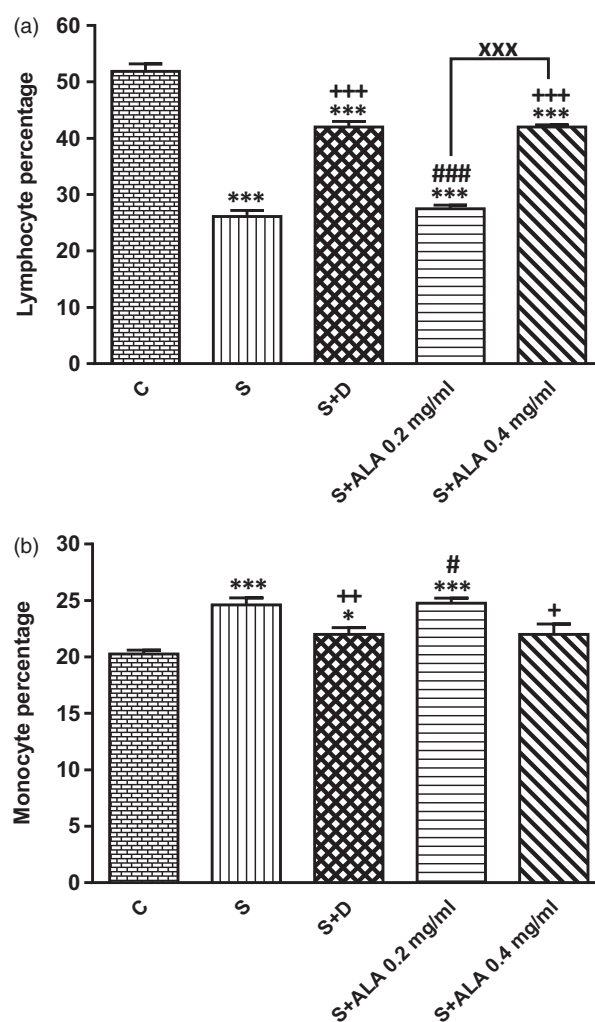


Figure 2 Percentages of lymphocyte (a) and monocyte (b) in bronchoalveolar lavage fluid of control animals (C), sensitized group (S), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are presented as mean $\pm$ SEM values. * $P < 0.05$ and *** $P < 0.001$ show significant differences compared to group C. + $P < 0.05$, ++ $P < 0.01$, and +++ $P < 0.001$ show significant differences compared to group S. # $P < 0.05$ and ### $P < 0.001$ show significant differences compared to group S+D. xxx $P < 0.001$ shows significant differences between the two concentrations of alpha-linolenic acid. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer's post-test.

Compared to group S, dexamethasone treatment significantly reduced monocyte, eosinophil and neutrophil percentages while augmented lymphocyte percentage ($P < 0.01$ to <0.001, Figures 1 and 2).

Furthermore, monocyte percentage in ALA 0.2 mg/ml-treated group, total WBC count in ALA 0.4 mg/ml-treated group, and the percentages of eosinophils, neutrophils and lymphocytes in rats treated with both concentrations of

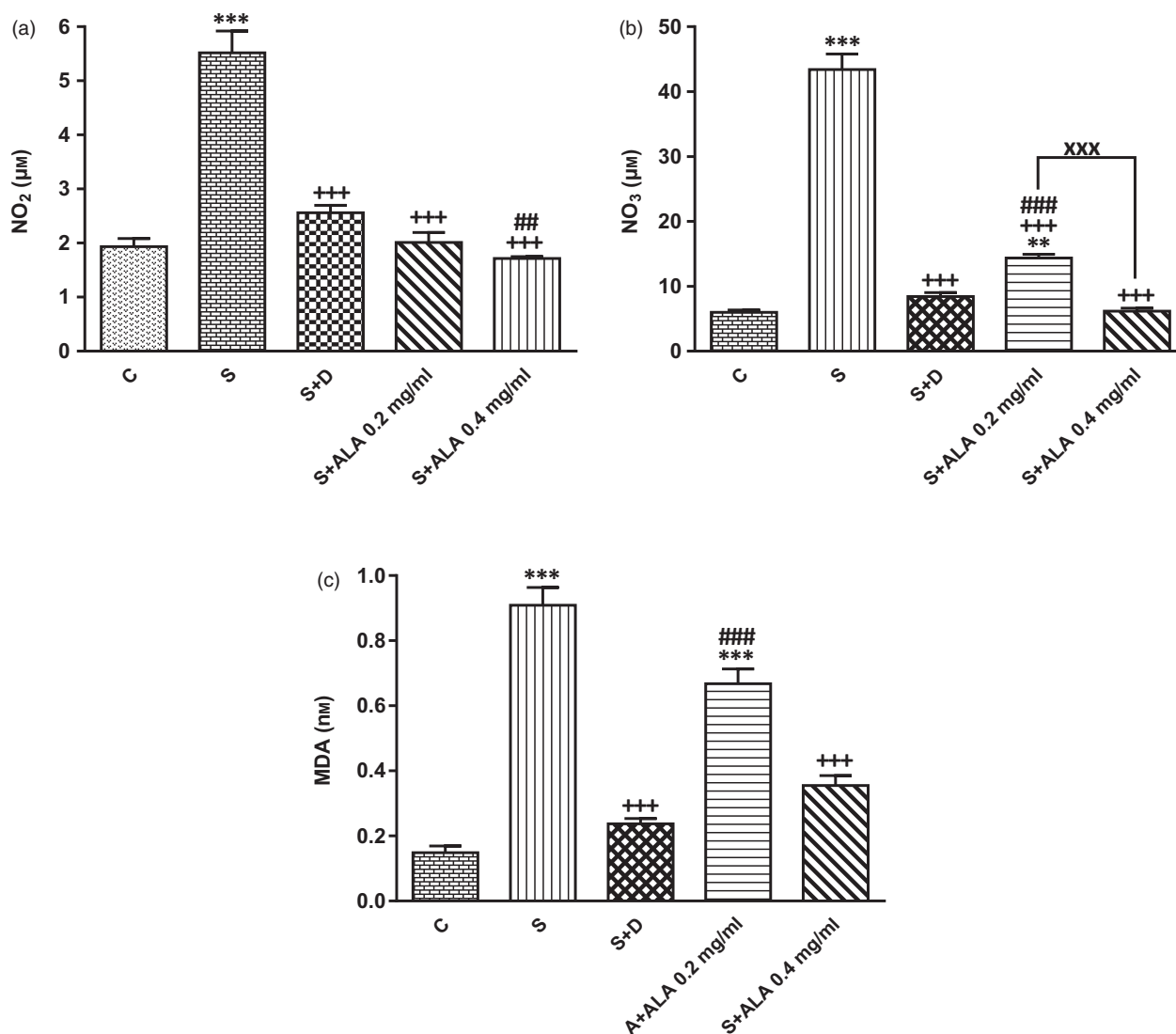


Figure 3 NO₂ (a), NO₃ (b) and malondialdehyde (c) concentrations in bronchoalveolar lavage fluid of control (C) sensitized group (S), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are expressed as mean $\pm$ SEM values. $**P < 0.01$ and $***P < 0.001$ show significant differences compared to group C. $+++P < 0.001$ shows significant differences compared to group S. $##P < 0.01$ and $###P < 0.001$ show significant differences compared to group S+D. $xxxP < 0.001$ shows significant differences between the two concentrations of ALA. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer’s post-test.

ALA were significantly different from those of group C ($P < 0.05$ to <0.001 , Figures 1 and 2).

Also, differential WBC counts in dexamethasone-treated sensitized rats were significantly different from those of group C ($P < 0.05$ to <0.001 , Figures 1 and 2).

As compared to dexamethasone, ALA 0.2 mg/ml had less significant effects on all differential WBC counts ($P < 0.05$ to <0.001 , Figures 1 and 2). However, in terms of total WBC, treatment with the two concentrations of ALA had more pronounced effects compared to those of dexamethasone ($P < 0.05$ for both cases, Figure 1).

As compared to ALA 0.2 mg/ml, ALA 0.4 mg/ml significantly showed greater effect on eosinophil, neutrophil and lymphocyte percentages ($P < 0.001$ for all cases, Figures 1 and 2).

ALA ameliorated oxidant/antioxidant balance in sensitized rats

In BALF of OVA-sensitized rats, significant increases in NO₂, NO₃ and MDA levels but significant decrements in SOD, CAT and thiol levels were observed as

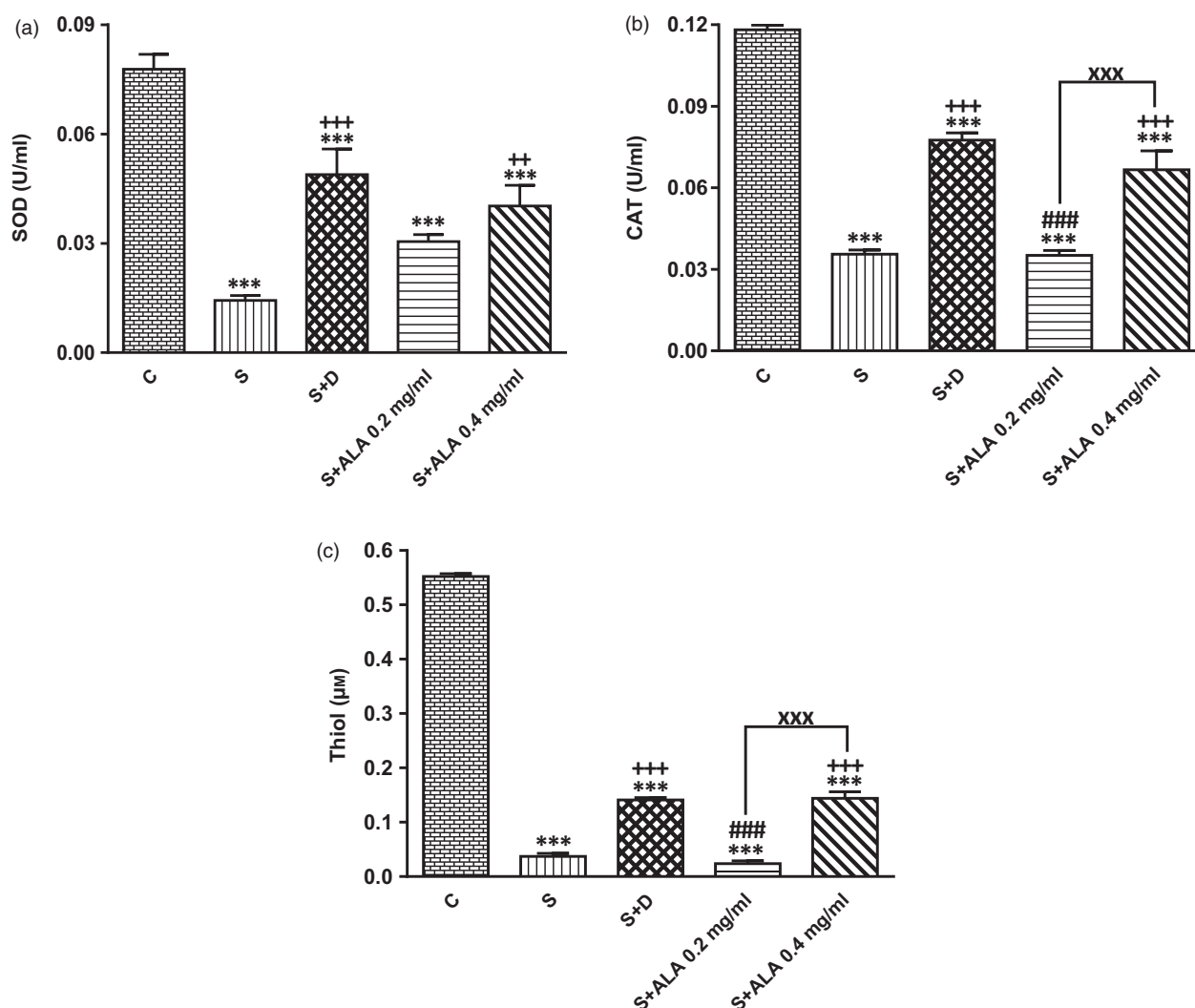


Figure 4 Superoxide dismutase (a), catalase (b) and Thiol (c) levels in bronchoalveolar lavage fluid of control (C), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are presented as mean $\pm$ SEM values. *** $P < 0.001$ shows significant differences compared to group C. ++ $P < 0.01$ and +++ $P < 0.001$ show significant differences compared to group S. ### $P < 0.001$ shows significant differences compared to group S+D. XXX $P < 0.001$ show significant differences between the two concentrations alpha-linolenic acid. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer’s post-test.

compared to control rats ($P < 0.001$ for all cases), (Figures 3 and 4).

Bronchoalveolar lavage fluid levels of NO_2 and NO_3 in ALA 0.2 and 0.4 mg/ml-treated rats, as well as BALF levels of MDA, SOD, CAT and thiol in ALA 0.4 mg/ml-treated rats, were significantly improved compared to group S ($P < 0.01$ to < 0.001 , Figures 3 and 4).

Dexamethasone also significantly reduced NO_2 , NO_3 and MDA levels but enhanced SOD, CAT and thiol levels as compared to group S ($P < 0.001$ for all cases; Figures 3 and 4).

NO_3 and MDA levels in rats treated with ALA 0.2 mg/ml and SOD, CAT and thiol levels in ALA 0.2 and 0.4

mg/ml-treated rats, were significantly different from those of group C animals ($P < 0.01$ to < 0.001 ; Figures 3 and 4).

Superoxide dismutase, CAT and thiol levels in dexamethasone-treated sensitized rats were also significantly different from those of group C animals ($P < 0.001$ for all cases; Figures 3 and 4).

Importantly, the effects of ALA 0.2 mg/ml on BALF levels of NO_3 , MDA, thiol and CAT were significantly less marked than those of dexamethasone ($P < 0.001$ for all cases; Figures 3 and 4). However, the effects of ALA 0.4 mg/ml on NO_2 level were markedly more pronounced than those of dexamethasone ($P < 0.01$, Figure 3).

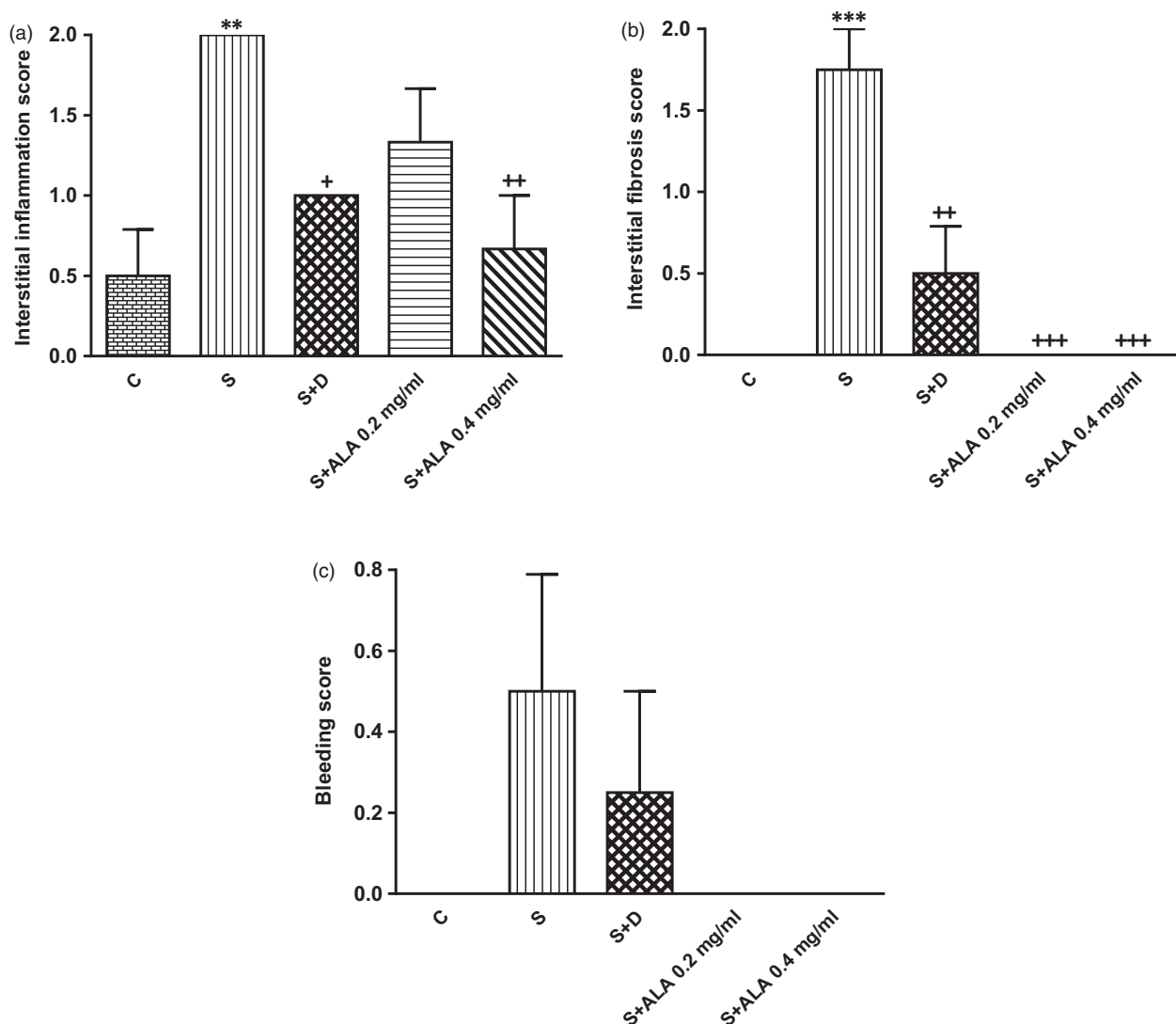


Figure 5 Interstitial inflammation (a), interstitial fibrosis (b) and bleeding (c) scores in control (C), sensitized group (S), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are presented as mean $\pm$ SEM values. ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared to group C. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared to group S. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's post-test.

As compared to ALA 0.2 mg/ml, ALA 0.4 mg/ml induced more marked effects in terms of NO_3 and CAT levels ($P < 0.001$ for both cases; Figures 3 and 4).

ALA improved pathological insults in the lung of sensitized rats

All pathological insults except for bleeding were significantly more severe in group S than group C ($P < 0.01$ to <0.001 ; Figures 5 and 6).

Interstitial fibrosis and emphysema in sensitized groups treated with ALA 0.4 and 0.2 mg/ml and interstitial

inflammation in ALA 0.4 mg/ml-treated group were significantly reduced as compared to group S animals ($P < 0.01$ to <0.001 ; Figures 5 and 6).

Treatment with dexamethasone also significantly reduced all pathological damages except for bleeding and epithelial damage, compared to group S ($P < 0.05$ to <0.01 ; Figures 5 and 6).

Epithelial damage score in the ALA 0.2 mg/ml-treated animals was significantly different from that of group C ($P < 0.05$, Figure 6).

Control and dexamethasone groups were not significantly different in terms of all pathological changes.

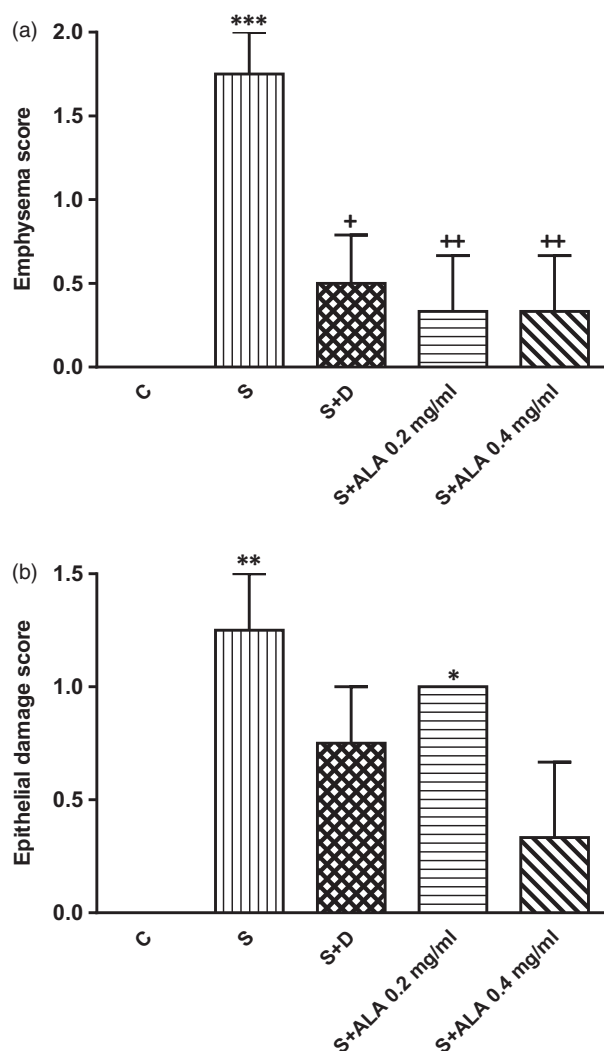


Figure 6 Emphysema (a) and epithelial damage (b) scores in control (C), sensitized group (S), sensitized rats treated with dexamethasone (S+D), ($n = 8$ in each group) and sensitized rats treated with alpha-linolenic acid (S+ALA, $n = 6$). Data are expressed as mean $\pm$ SEM values. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared to group C. + $P < 0.05$ and ++ $P < 0.01$ show significant differences compared to group S. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's post-test. Pathological changes were scored as (0) no pathological changes; (1) patchy changes; and (2) severe damages (throughout the lung).

Similarly, no significant difference was found in all pathological changes among dexamethasone and ALA-treated groups.

Treatment with ALA 0.4 or 0.2 mg/ml did not significantly differ in terms of all pathological insults. Figure 7 shows lung photographs obtained from different studied group.

Discussion

The current experiment showed increases in total WBC count, eosinophils, monocytes and neutrophils percentages, BALF levels of NO_2 , NO_3 , MDA and pathological insults scores (with respect to interstitial inflammation and fibrosis, epithelial damage and emphysema) but significant reductions in lymphocytes percentage, as well as SOD, CAT and thiol levels in the BALF of OVA-sensitized animals (group S) compared to groups C.

These results confirmed the induction of asthma in animals. Furthermore, the findings of our previous study showed increased tracheal responsiveness to methacholine and OVA,^[19] which is another proof confirming the sensitization of animals. Consistent with our findings, previous studies indicated similar results.^[4,18] In the current study, in BALF obtained from OVA-sensitized rats, lymphocyte percentage was reduced which was probably due to increased total WBC count. Consistently, a previous study reported augmented absolute lymphocyte count in OVA-sensitized animals using the same method of sensitization.^[20] In addition, another study reported increases in inflammatory cells especially neutrophils in subjects with asthma which supports the results of the present research.^[21] A positive correlation was revealed between neutrophilic inflammation of the airways and severity of asthma.^[22]

Results indicated marked increases in levels of NO_2 and NO_3 (as NO oxidation products) and MDA (as a lipid peroxidation marker) while the levels of SOD, CAT and thiol as antioxidant enzymes decreased in OVA-sensitized rats which also confirmed the induction of sensitization. Recently, it was reported that oxidant enzymes are increased and antioxidant enzymes are decreased in BALF of sensitized animals which confirm the findings of our study.^[18] There is strong evidence supporting the role of OS in the inflammatory processes in bronchial asthma.^[23] In this regard, a human study also reported increases in oxidant levels in asthmatic subjects.^[24]

Our pathological examinations showed the occurrence of interstitial inflammation and fibrosis, emphysema and epithelial damage in the lung of sensitized animals, which also confirmed the induction of sensitization and was in line with the results of a previous study.^[19]

Based on our data, ALA significantly reduced total WBC count, neutrophils, eosinophils and monocytes percentages, NO_2 , NO_3 and MDA levels, and pathological insults (in terms of interstitial inflammation and fibrosis, emphysema, and epithelial damage) but increased lymphocyte percentages as well as SOD, thiol and CAT levels compared to the sensitized group. These findings indicate preventive effects

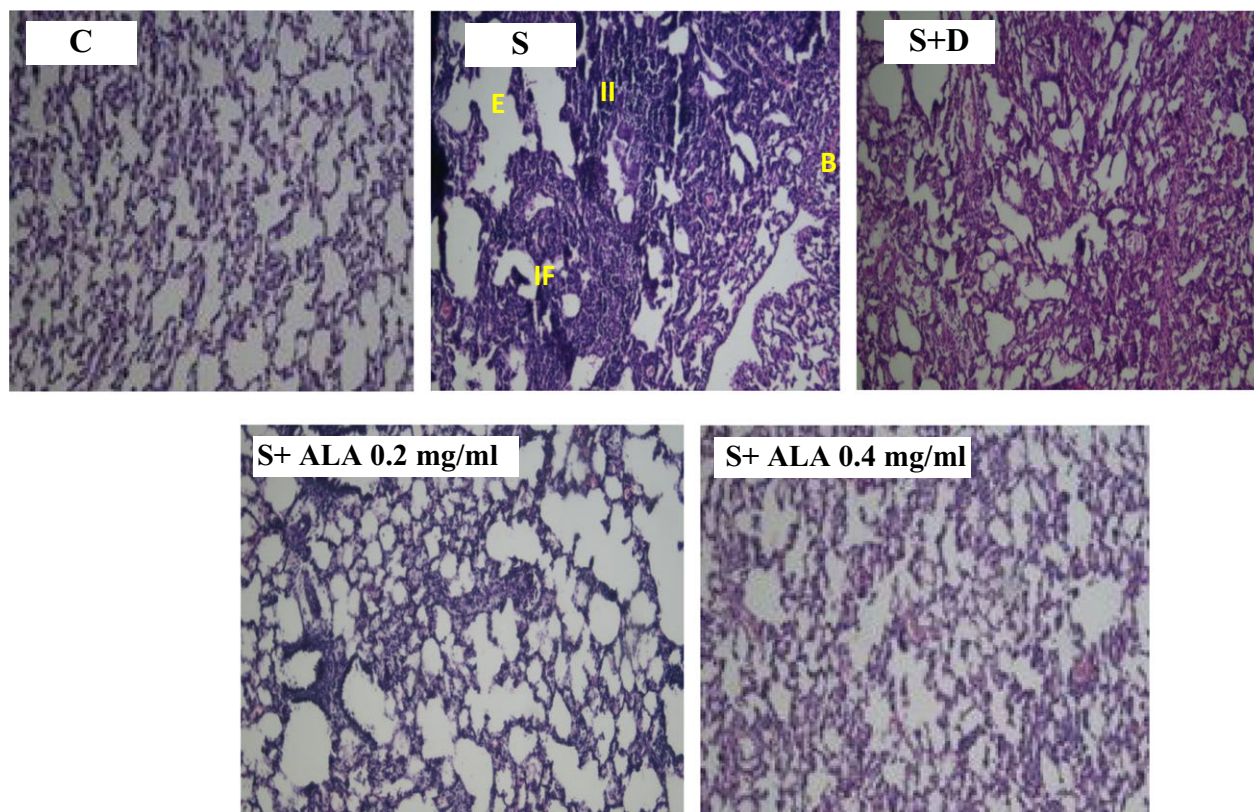


Figure 7 Photographs of lung specimens under a light microscope (X40), in control (C), sensitized (S) group with interstitial inflammation, interstitial fibrosis, bleeding and emphysema, sensitized rats treated with dexamethasone (S+D) or two concentrations of alpha-linolenic acid (S+ALA). Pathological changes were scored as (0) no pathological changes; (1) patchy changes; (2) and severe damages (throughout the lung).

of ALA on inflammatory cells, oxidant–antioxidant markers and pathological damages in OVA-sensitized animals.

Consistent with the findings of this study, decreases in the WBC count (total and differential) in the blood of OVA-sensitized rats following treatment with ALA were previously shown.^[16] Administration of ALA 600 mg/day significantly reduced total leucocytes in hypertensive patients.^[25] Treatment with ALA normalized the augmented levels of NO₂, NO₃ and MDA but reduced SOD, CAT and thiol levels in OVA-sensitized rats. Moreover, ALA enhanced the activity of SOD, CAT and GST while reduced MDA concentration following gentamicin-induced nephrotoxicity in rats.^[26] In another study, ALA (500 µg/kg, intragastric gavage) diminished MDA levels, enhanced SOD, CAT and GSH-Px activities in cardiac tissues and attenuated cardiac histopathological damages and cardiomyocytes apoptosis in a rat model of doxorubicin-induced cardiotoxicity.^[27] In addition, *Zanthoxylum bungeanum* (*Z. bungeanum*) Maxim. seed oil (0.5, 1.5 and 3.0 g/kg, i.p.) suppressed the *R_L* (lung resistance) increases and the *C_{dyn}* (dynamic lung compliance) decreases in plethysmograph test in acetylcholine/

histamine-sensitized guinea pigs. The anti-asthmatic effects of *Z. bungeanum* might be mediated via the polyunsaturated fatty acids such as α-linolenic acid, linoleic acid and oleic acid.^[28] The results of a recent study also showed increased INF-γ and INF-γ/IL4 ratio (as an index of Th1/Th2) but decreased levels of IL-4 as an immune modulatory effect, in sensitized rats due to ALA treatment. ALA treatment also showed preventive effects on inflammatory markers and tracheal responsiveness in sensitized animals.^[29] Therefore, ALA by modulating oxidant/antioxidant markers, and exerting anti-inflammatory and immune modulatory effects, can affect tracheal responsiveness as the hallmark of bronchial asthma. In a previous study, the effects of ALA on reduction of total protein, phospholipase A2, IgE, IL-4 but increment of INF-γ levels were shown.^[29] However, the effect of ALA on signalling pathways and genes expression should be examined in further studies.

All the above-described studies along with the results of the current study indicate that ALA ameliorates oxidative stress and lung inflammation in OVA-sensitized rats, supporting the asthma-preventive potential of ALA.

In the present study, dexamethasone (1.25 µg/ml) was administered in drinking water during the sensitization period (21 days). In our experiment, each rat used 40 ml water/day, and thus, the dose of dexamethasone was 0.25 mg/kg per day which was equal or even higher than that used in previous studies (0.5 mg/kg per day orally only for 3 days) during sensitization period.^[30,31] In addition, dexamethasone at the dose administered in the present study, affected almost all measured parameters suggesting that it was used at an effective dose. However, the effect of higher dose of dexamethasone may show similar or even higher effects compared to ALA which should be examined in further studies.

Together, these data indicated an asthma-preventive potential for ALA which is mediated through ALA effects on lung inflammatory cells, oxidative markers and lung pathological damages in sensitized rats. These effects were comparable to the effect of dexamethasone at used

concentrations. It should be noted that more thorough investigations are required to unveil ALA therapeutic effects against bronchial asthma in *in-vivo* and clinical studies.

Declarations

Conflict of interest

No conflict of interests to declare.

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