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Research Communication

Anti-Inflammatory, Antioxidant, and Immunomodulatory effects of curcumin in ovalbumin-sensitized rat

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Abstract

Background: Anti-inflammatory and antioxidant properties of curcumin have been shown. In this study, anti-inflammatory, antioxidant, and immunomodulatory effects of curcumin in sensitized rat were evaluated. **Methods:** Six groups of rats including control (C), ovalbumin-sensitized (as a rat model of asthma, S), S groups treated with curcumin (Cu 0.15, 0.30, and 0.60 mg/mL), and 1.25 μ g/mL dexamethasone (S + D) were studied. Curcumin and dexamethasone were given in animals' drinking water during sensitization period. Total and differential WBC count, PLA2, TP, IFN- γ , IL-4, IgE, oxidant, and antioxidant biomarker levels in bronchoalveolar lavage fluid (BALF) were examined. **Results:** Total WBC, neutrophil and eosinophil counts, levels of PLA2, TP, IgE, IL-4, NO₂, NO₃, and MDA in BALF were increased but lymphocyte percentage, SOD, CAT, thiol, and IFN- γ levels and IFN- γ /IL-4 ratio decreased in S

animals compared to controls ($P < 0.001$ for all cases). Treatment with all concentrations of curcumin significantly improved total WBC, PLA2, TP, IgE, IL-4, IFN- γ , IFN- γ /IL-4 ratio, SOD, thiol, NO₂, and NO₃ compared to S group ($P < 0.01$ to $P < 0.001$). Two higher concentrations of curcumin significantly decreased neutrophil and eosinophil counts and MDA level but increased IFN- γ , CAT and lymphocyte values compared to S group ($P < 0.001$ for all cases). Dexamethasone treatment also significantly improved most of the measured parameters ($P < 0.05$ to $P < 0.001$) but it did not change IL-4 and IFN- γ levels and IFN- γ /IL-4 ratio. **Conclusion:** Anti-inflammatory, antioxidant, and immunomodulatory effects of curcumin with more specific immunomodulatory effect on Th1/Th2 balance compared to dexamethasone in sensitized rats was shown. © 2017 BioFactors, 43(4):567–576, 2017

Keywords: curcumin; sensitized rat; lung inflammation; oxidative stress; immunomodulatory effect

1. Introduction

Curcumin (diferuloylmethane) is a bioactive component derived from the rhizomes of *Curcuma longa*. Various

pharmacological effects such as anti-inflammatory [1], antioxidant [2], and hepatoprotective [3] were reported for curcumin. Moreover, inhibitory effects of curcumin on various inflammatory mediators, cytokines, and oxidant markers have been mentioned in the literature [4].

TNF- α , MIP-2, IL-6, and MPO levels and the wet/dry weight ratio of lung and neutrophil accumulation in BALF were reduced by curcumin in LPS-induced acute lung injury in mice [5]. It has also been reported that curcumin is able to scavenge free radicals (peroxides), phenolic oxidants, and nitric acid [6] and to inhibit lipid peroxidation induced by chemical agents [7].

Asthma is an airway inflammatory disease, characterized by infiltration of eosinophils, lymphocytes, and neutrophils in the lung, mucus hypersecretion, and airway hyper-responsiveness (AHR) to inhaled allergens [8]. An association between increased eosinophil counts in the blood of asthmatic

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patients and severity of the disease was reported [9]. PLA2 has an important role in production of eicosanoids and the development of inflammation and AHR in murine models of asthma [10]. Mechanisms of action of PLA2 in inflammatory disorders are release of arachidonic acid, production of lysophospholipids, interplay between enzymes belonging to the A_2 superfamily, surfactant degradation, release of cytokines, and the impact on immune and inflammatory cells [10].

Increase in lung oxidative stress also contributes to chronic airway inflammation in asthma [11]. Increased levels of direct and indirect oxidant markers in urine, plasma, sputum, and lung tissues of asthmatic patients were also documented [12,13]. Oxidative stress has an important role in asthmatic airway inflammation, and reduction of both enzymatic and nonenzymatic antioxidant defenses have been observed in asthma [14] and it was shown to be associated with increased airway responsiveness in mice [15]. Increased production of oxidants has been reported in asthmatic patients compared to healthy subjects [13], resulting in increased airway inflammation by activating diverse proinflammatory cells including macrophages, neutrophils, and eosinophil [16]. Production of nitric oxide (NO) is generally enhanced during inflammatory diseases including bronchial asthma.

Inflammatory cells such as T lymphocytes, eosinophils, and mast cells play a vital role in the pathogenesis of asthma, in which the most important element is the activation of $CD4^+$ T cells which are divided to Th1 and Th2 subtypes [12]. Th1 lymphocytes produce IL-2 and IFN- γ that organize cell-mediated immunity against intracellular pathogens and tumors. Th2 cells secrete IL-4, IL-5, and IL-13, which are involved in humoral immune response against extracellular infections [12]. Th1 and Th2 responses have reverse effect on each other [13]. It was shown that Th2 cells start and precede allergic inflammatory diseases [17]. Thus, changes in IFN- γ /IL-4 cytokines pattern (Th1/Th2 balance) toward IL-4 (Th2) lead to allergic and atopic disease including asthma, atopic dermatitis, anaphylactic shock, and allergic rhinitis. However, IFN- γ is able to suppress both eosinophil recruitment and IgE synthesis [18]. Therefore, Th1 is able to regulate anti-inflammatory responses [19]. IgE production is the main factor required for induction of allergic asthma. IgE initiates the allergic response by activating mast cells to release inflammatory mediators and recruitment of eosinophils. Therefore, blocking the effects of IgE could be a therapeutic strategy for inhibiting allergic disorders [20].

Corticosteroid drugs play an important role in the treatment of asthma. Dexamethasone is a corticosteroid drug with well-known anti-inflammatory effects and was used as positive control in animal models of asthma [21,22].

In this study, the potential anti-inflammatory, antioxidant, and immunomodulatory effects of curcumin on sensitized rats were evaluated by measurement of total and differential WBC count, PLA2, TP, IgE, IFN- γ , IL-4, and oxidant and antioxidant biomarker levels in BALF.

2. Materials and methods

2.1. Chemicals and reagents

Ovalbumin, ketamine, dexamethasone, glacial acetic acid, gentian violet solution, $Al(OH)_3$, sulfanilamide (SULF), N-(1-naphthyl) ethylene diamine dihydrochloride (NEDD), zinc sulfate, 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), and Tris ethylene diamine tetra acetic acid (Tris-EDTA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Curcumin was purchased from Sami Labs LTD (Bangalore, India). The purity of curcumin was 96% and the content of demethoxy-curcumin and bis-demethoxy-curcumin were 12.7% and 0.71%, respectively. ELISA kits for detection of IFN- γ , IL-4, and IgE were purchased from Abcam (Cambridge, MA, USA), for measurement of phospholipase A2 (PLA2) from MyBioSource (San Diego, CA, USA), and for evaluation of total protein (TP) from Pars Azmon (Tehran, Iran).

2.2. Animals and experimental groups

Wistar rats weighing 200–250 g were kept at $22 \pm 2^\circ C$, with 12 h light/dark cycle and humidity of $55 \pm 5\%$. Animals were fed with standard diet and tap drinking water *ad libitum* in animal house, School of Medicine, Mashhad University of Medical Sciences, Iran.

Forty-five rats were randomly allocated to the following groups:

- Control group (C): saline was used instead of ovalbumin (OVA) and rats were given drinking water alone
- Sensitized group (S) which were sensitized with OVA and given drinking water alone
- S group treated with 1.25 $\mu g/mL$ dexamethasone (S+D)
- S group treated with 0.15 mg/mL curcumin (group S+Cu 0.15)
- S group treated with 0.30 mg/mL curcumin (group S+Cu 0.30)
- S group treated with 0.60 mg/mL curcumin (group S+Cu 0.60)

Curcumin and dexamethasone were given in drinking water of animals during sensitization period. Curcumin was dispersed in drinking water and the drinking water container was shaken several times a day. In average, 40 mL drinking water was used by each rat/day and there was no significant difference in the volume of the drinking water consumed by the animals in different groups ($n = 7$ for curcumin-treated groups and $n = 8$ for other groups).

2.3. Animals sensitization

Rats were sensitized by three i.p. injections of 1 mg/kg chicken egg albumin (Ovalbumin = OVA, grade V with 98% purity) in 0.9% sterile saline containing 100 mg $Al(OH)_3$ as adjuvant on days 1, 2, and 3 of the experiment. The animals were then exposed to OVA aerosol 1% for 20 min/day, on days 6, 9, 12, 15, 18, and 21 [17]. Aerosol of OVA was produced by delivery

of compressed air (8 L/min) through a jet nebulizer that contained a solution of 1% OVA in normal saline [17]. Exposures of animals to OVA aerosol were carried out in a whole body

exposure chamber (30 × 20 × 20 cm). The rats in the control group received i.p. and inhaled normal saline instead of OVA using the same route as OVA administration in sensitized animals. The study was approved by Ethical Committee of Mashhad University of Medical Sciences (Ethical allowance No. 921249) and animal procedures were done in accordance with National Laws and National Institutes of Health guidelines for the use and care of laboratory animals.

2.4. Preparation of BALF and measurement of total and differential WBC count, and inflammatory and immunological mediators

Rats were anesthetized by i.p. injection of ketamine (50 mg/kg) at the end of the experimental period and the chest was opened. Trachea and lungs were then dissected and washed with distilled water. The trachea was cannulated and the lungs were lavaged with 1 mL of saline 5 times at room temperature.

For determination of total WBC, 1 mL of BALF was stained with Turk solution (consisted of 1 mL glacial acetic acid, 1 mL of gentian violet solution 1%, and 100 mL distilled water) and counted using a Neubauer chamber in duplicate.

The remaining BALF was centrifuged at 2,500g at 4°C for 10 min. The supernatant was removed and stored at -80°C for measurement of PLA2, TP, IgE, IFN- γ , IL-4, NO₂, NO₃, MDA, SOD, CAT, and thiol levels.

For differential WBC count, the smear was prepared from the cells and stained with Wright-Giemsa. Differential cell analysis was performed according to staining and morphological criteria, using a light microscope by counting 100 cells and calculation of the percentage of each cell type.

Levels of PLA2, TP, IgE, IL-4, and IFN- γ in BALF were measured using enzyme-linked immune sorbent assay (ELISA) sandwich method according to the manufacturer's instructions with reliable sensitivity, specificity, and reproducibility to detect normal ranges of cytokines in rat's BALF. The minimum detectable level of PLA2, IgE, and cytokines was 1.0 ng/mL, 1.4192 ng/mL, and 1.5 pg/mL, respectively. The levels of IL-4 and IFN- γ in BALF were adjusted as pg/total protein.

2.5. Measurement of oxidant and antioxidant biomarkers in BALF

Using a Griess reagent (sulphanilamide (SULF) and N-(1-Naphthyl) ethylene diamine dihydrochloride (NEDD)), total stable

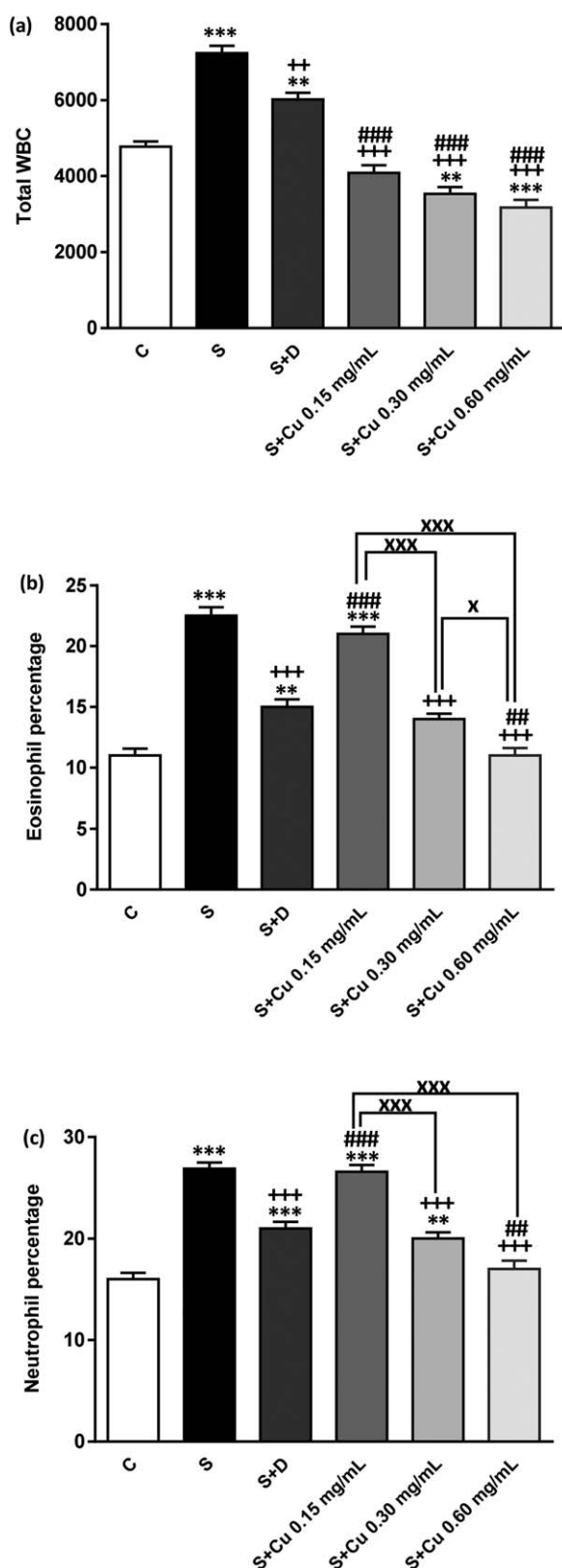


FIG 1

Total WBC number (count/mL of BALF) (a), percentage of eosinophil (b) and neutrophil (c) in bronchoalveolar lavage fluid (BALF) of control animals (C), sensitized group (S), S treated with dexamethasone (S+D) (for each group, n = 8), and curcumin (Cu, n = 7). Data are presented as mean $\pm$ SEM values. **P < 0.01, ***P < 0.001 compared to group C. ++P < 0.01, +++P < 0.001 compared to group S. ##P < 0.01, ###P < 0.001 compared to group S+D. xP < 0.05, xxxP < 0.001 comparison of three concentrations of curcumin. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's post-test.

oxidation products of NO metabolism ($\text{NO}_2^-/\text{NO}_3^-$) was measured. The frozen BALF was allowed to thaw and reach a temperature of 25°C, followed by a deproteinization process using zinc sulfate solution. BALF was then centrifuged at 10,000g for 10 min. Then, 120 μL of the clear supernatant was mixed with Griess reagents including 50 μL SULF (2% w/v) in 5% HCl and 50 μL NEDD (0.1% w/v) in H_2O , in a test tube and incubated at controlled room temperature for 10 min. Next, the absorbance of samples was measured at 540 nm for evaluation of NO_2 level. For the reduction of nitrate to nitrite, 50 μL saturated solutions of vanadium (III) chloride (VCl_3) in 1 M HCl were added and the tubes were incubated for 2 h at 30°C in the dark. The absorbance of samples was then measured at 540 nm against a blank containing the same concentrations of ingredients without biological sample. To determine NO_2 and NO_3 concentration, linear regression was used from a standard curve of NaNO_2 and the results were expressed as μM [23].

Two mL of thiobarbituric acid (TBA)/trichloroacetic acid (TCA)/HCl reagent was added to 1 mL of BALF and the solution was heated in a water bath for 40 min and then cooled; this process resulted in producing thiobarbituric acid reactive substance (TBARS) which produces a red coloured complex with peak absorbance at 535 nm. The whole solutions were centrifuged at 1000g for 10 min and the absorbance was measured at 535 nm [24]. The MDA concentration was calculated as follows:

Absorbance/(1.56×10^5) and expressed as nM.

Superoxide dismutase (SOD) activity was measured as previously described [25]. In this method, production of superoxide by pyrogallol auto-oxidation and inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to formazan by SOD, was measured at 570 nm and the level of SOD was expressed as unit (U)/mL.

Catalase (CAT) activity was measured based on determination of the rate constant k (dimension: s^{-1} , k) of hydrogen peroxide decomposition. The rate constant of the enzyme was determined using measurement of the decrease in absorbance at 240 nm/min and CAT activity was expressed as unit (U)/mL [26]. For measurement of SOD and CAT, sterile, warmed phosphate-buffered saline (pH 7.4) was used for the BALF to keep the pH at 7.4.

For measurement of total thiol concentration, 1 mL Tris-EDTA buffer (pH 8.6) was added to 50 μL BALF in 1 mL cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Twenty 20 μL of DTNB reagents (10 mmol in methanol) was added to the mixture and the samples were kept at laboratory temperature for 15 min and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mmol) was calculated using this equation [27]: Total thiol concentration (mmol) = $(A2 - A1 - B) \times 1.07/0.05 \times 13.6$.

2.6. Statistical Analysis

Data were expressed as means $\pm$ SEM. The comparisons between the data of treated groups, untreated sensitized group and

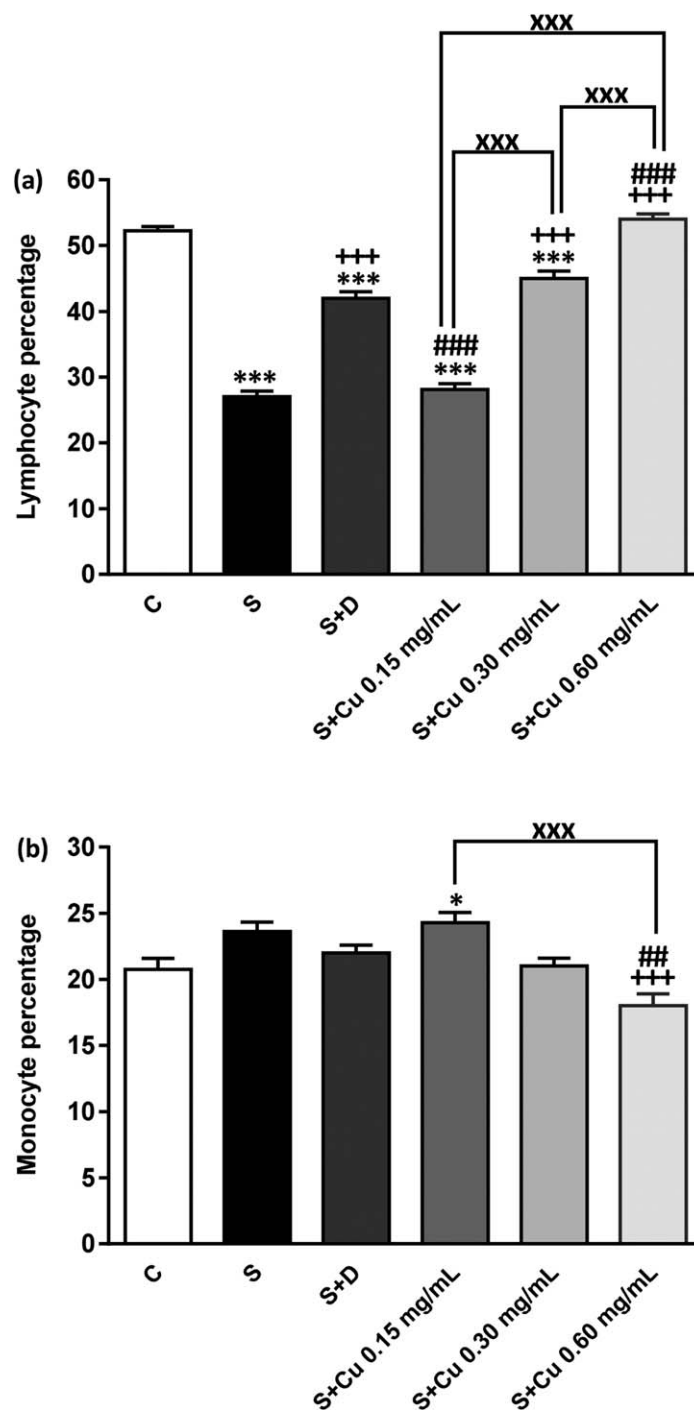


FIG 2

Percentage of lymphocyte (a) and monocyte (b) in bronchoalveolar lavage fluid (BALF) of control animals (C), sensitized group (S), S treated with dexamethasone (S+D) (for each group, $n=8$), and curcumin (Cu, $n=7$). Data are presented as mean $\pm$ SEM values. * $P < 0.05$, *** $P < 0.001$ compared to group C. + $P < 0.05$, +++ $P < 0.001$ compared to group S. ## $P < 0.01$, ### $P < 0.001$ compared to group S+D. xxx $P < 0.001$ comparison of three concentrations of curcumin. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's post-test.

control group and among treated groups with three concentrations of curcumin were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer’s post-test. InStat software

(GraphPad Software, Inc, La Jolla, USA) was used for statistical analysis. P values <0.05 were considered as statistically significant.

3. Results

3.1. The effect of animal sensitization on different variables

Total WBC count, the percentages of eosinophil and neutrophil, and the levels of IgE, IL-4, NO₂, NO₃, and MDA in the BALF of sensitized group were significantly higher than group C ($P < 0.001$ for all cases). However, lymphocyte percentage, values of IFN- γ , IFN- γ /IL-4 ratio, SOD, CAT, and thiol in sensitized animals were lower than those of the control group ($P < 0.001$ for all cases, Figs. 1–6).

3.2. The effect of curcumin treatment on different variables in sensitized rats

Total WBC count, the levels of PLA2, TP, IgE, IFN- γ , IL-4, and IFN- γ /IL-4 ratio, NO₂, NO₃, thiol, and SOD in groups treated with all concentrations of curcumin were improved compared to untreated S group ($P < 0.01$ to $P < 0.001$). The percentages of eosinophil, neutrophil, and lymphocyte, the levels of MDA and CAT with two higher concentrations of curcumin, and the monocyte percentage in group treated with the high concentration were significantly improved compared to those of untreated S group ($P < 0.001$ for all cases, Figs. 1–6).

3.3. The effect of dexamethasone treatment on different variables in sensitized rats

Dexamethasone treatment significantly reduced total WBC count, the percentages of eosinophil, neutrophil, the levels of IgE, NO₂, NO₃, and MDA but increased lymphocyte percentage, SOD, CAT, and thiol levels compared to S group ($P < 0.05$ to $P < 0.01$, Figs. 1–6). However, dexamethasone treatment did not affect IL-4, IFN- γ , and IFN- γ /IL-4 ratio (Fig. 4c).

3.4. Comparison of different variables between treated and control groups

MDA, CAT, and thiol levels in groups treated with all concentrations of curcumin and neutrophil and lymphocyte percentage, the levels of PLA2 and TP, IgE, IFN- γ , IFN- γ /IL-4 ratio, and SOD in groups treated with the two lower concentrations were not comparable with those of the control and were significantly different ($P < 0.05$ to $P < 0.001$). The percentages of eosinophil and monocyte, and the levels of IL-4 and NO₂ in the

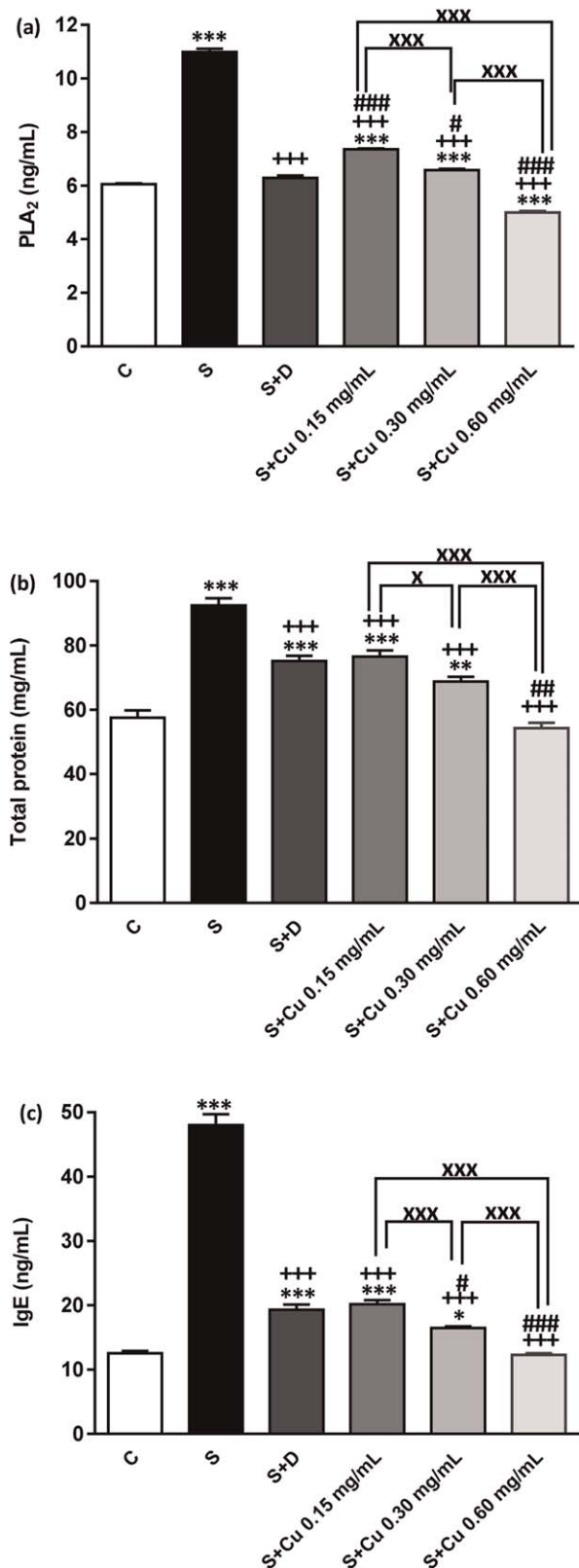
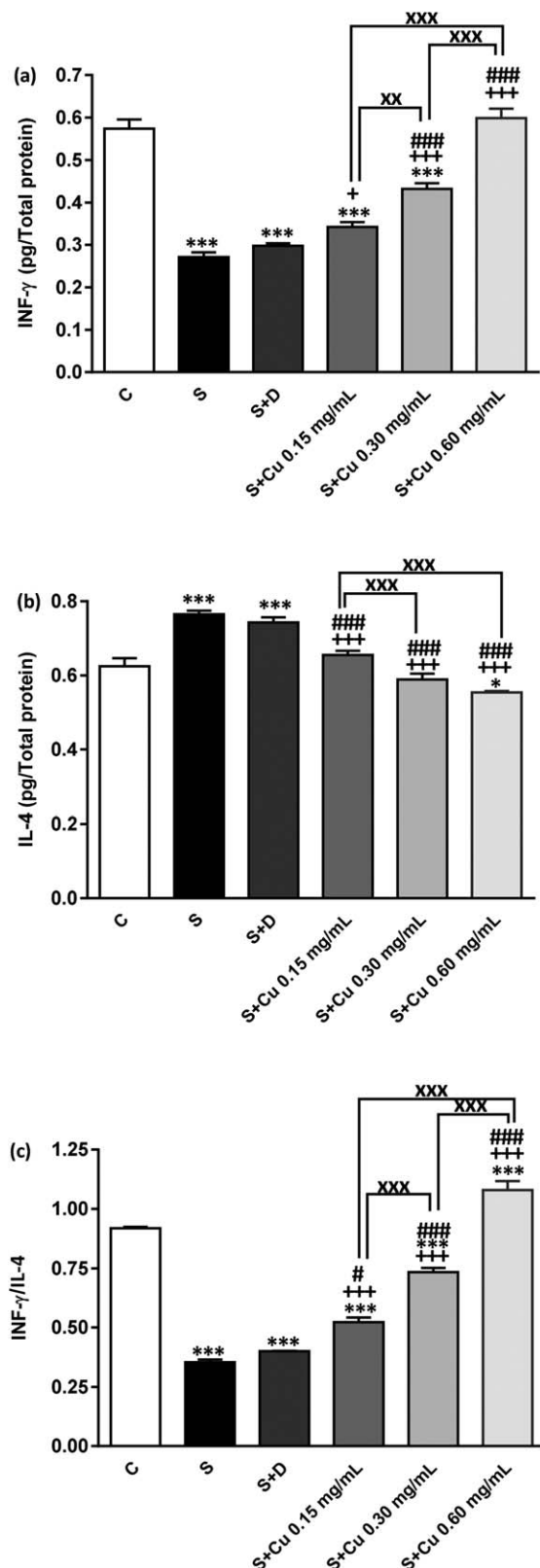


FIG 3

PLA₂ (a), TP (b), and IgE (c) in bronchoalveolar lavage fluid (BALF) of control animals (C), sensitized group (S), S treated with dexamethasone (S + D) (for each group, $n = 8$), and curcumin (Cu, $n = 7$). Data are presented as mean $\pm$ SEM values. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to group C. ++ $P < 0.01$, +++ $P < 0.001$ compared to group S. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ compared to group S + D. x $P < 0.05$, xxx $P < 0.001$ comparison of three concentrations of curcumin. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer’s post-test.

group treated with low concentration of curcumin were also significantly different as compared to control group ($P < 0.001$ for all cases, Figs. 1–6).



Total and all differential WBC count (except for monocyte percentage) and the values of TP, IgE, IL-4, IFN- γ and IFN- γ /IL-4 ratio, SOD, CAT, and thiol in S group treated with dexamethasone were also significantly different from those of control group ($P < 0.01$ to $P < 0.001$, Figs. 1–6).

3.5. Comparison between the effects of three curcumin concentrations

The effects of the two higher concentrations of curcumin (0.30 and 0.60 mg/mL) on eosinophil, neutrophil, and lymphocyte percentages, the levels of PLA₂, TP, IgE, IFN- γ , IL-4, IFN- γ /IL-4 ratio, and all oxidant and antioxidant biomarkers (except the effect of its medium concentration on NO₂) were significantly higher than its low concentration ($P < 0.01$ to $P < 0.001$). Significant differences were also seen between the effect of high and medium concentrations of curcumin on monocyte percentage and all measured inflammatory and immunologic mediators ($P < 0.01$ to $P < 0.001$). The effects of the high concentration of curcumin on monocyte percentage were significantly higher than its low concentration (0.15 mg/mL) ($P < 0.001$). The effect of high concentrations of curcumin on all oxidant and antioxidant markers was also significantly higher than its medium concentration ($P < 0.05$ to $P < 0.001$, Figs. 1–6).

3.5. Comparison between the effects of curcumin and dexamethasone

The effect of the low concentration of curcumin on eosinophil, neutrophil, and lymphocytes percentages and the levels of NO₂, SOD and CAT, and the effect of its two lower concentrations on PLA₂, NO₂, and MDA were significantly lower than the effect of dexamethasone ($P < 0.05$ to $P < 0.001$).

However, the effect of treatment with all concentrations of curcumin on total WBC, IFN- γ , IL-4, and IFN- γ /IL-4 values, the effect of its two higher concentrations on IgE, and the effect of its high concentration on eosinophils, lymphocytes, neutrophils, monocytes, PLA₂, TP, SOD, CAT, and thiol levels were significantly higher than dexamethasone treatment ($P < 0.05$ to $P < 0.001$, Figs. 1–6).

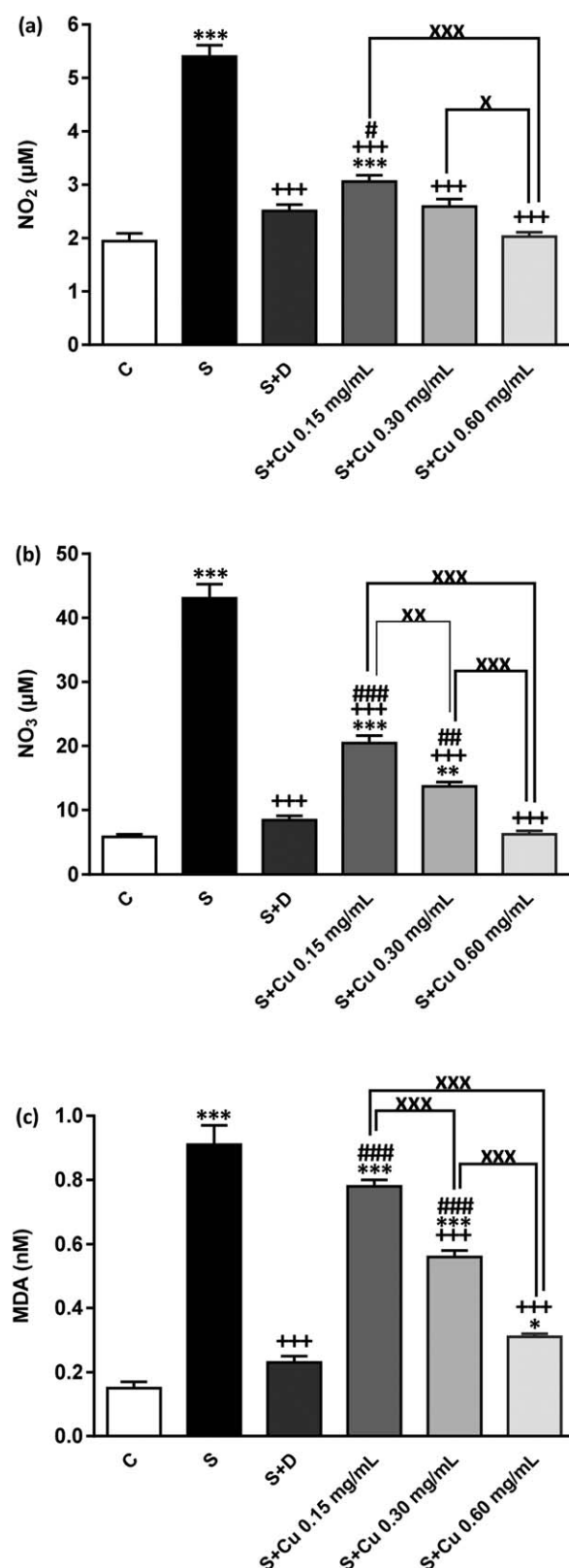
4. Discussion

The results of this study showed increased total WBC, eosinophil, neutrophil, PLA₂, TP, IgE, IL-4, NO₃, NO₂, and MDA levels but decreased lymphocyte percentage, IFN- γ , IFN- γ /IL-4 ratio,

FIG 4

INF- γ (a), IL-4 (b), and IFN- γ /IL-4 ratio (c) in bronchoalveolar lavage fluid (BALF) of control animals (C), sensitized group (S), S treated with dexamethasone (S+D) (for each group, $n = 8$), and curcumin (Cu, $n = 7$). Data are presented as mean $\pm$ SEM values. ** $P < 0.01$, *** $P < 0.001$ compared to group C. + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ compared to group S. ## $P < 0.01$, ### $P < 0.001$ compared to group S+D. xxx $P < 0.001$ comparison of three concentrations of curcumin. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey–Kramer's post-test.

SOD, CAT, and thiol levels in BALF of sensitized compared to control animal. All these changes support animal sensitization and induction of an animal model of asthma in rats.



Increased total WBC, neutrophil, and particularly eosinophil count in lung lavage of sensitized animals compared to control group are shown previously [28,29]. The reduction in lymphocyte percentage in BALF of sensitized animals in this study is mainly due to increased total WBC in sensitized rats. In fact, the increased absolute lymphocyte count in sensitized animals was previously shown using similar method of sensitization [29]. Increased PLA2 level in serum and bronchoalveolar lavage fluid from asthmatic patients was also reported [30].

Increased IgE and IL-4 levels and reduction of IFN- γ and IFN- γ /IL-4 ratio in BALF of sensitized animals were seen in the previous studies using the similar method of induction of animal model of asthma [31]. The number of CD4⁺ Th cells, especially the Th2 subtype, is increased [32] the release of Th2 cytokines (mainly IL-4, IL-5, and IL-13) upregulated and the release of Th1 cytokine such as IFN- γ downregulated [33] in asthmatic patients.

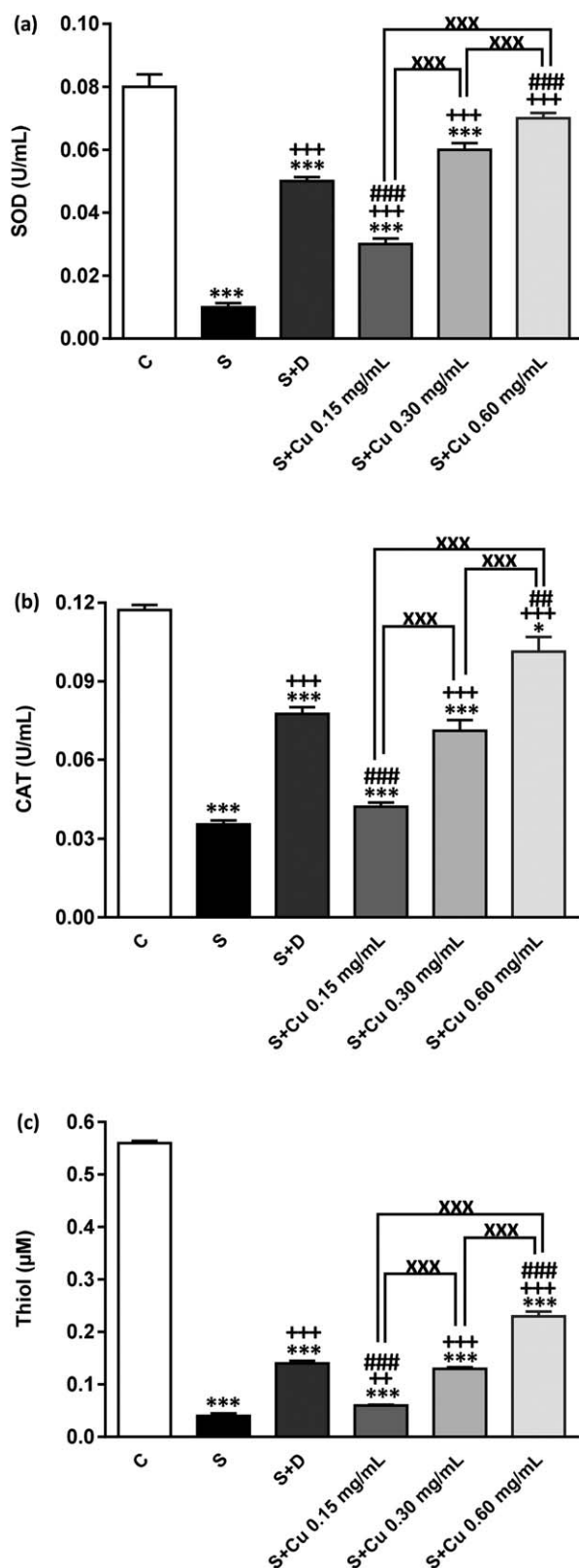
Increased NO₂ and NO₃ levels in exhaled breath condensates [34] and enhanced plasma level of MDA and protein carbonyls in BALF and peripheral blood samples in patients with stable asthma [35] were reported. Decreased airway antioxidants, such as SOD level [36], CAT activity [37], ascorbate, alpha-tocopherol, and glutathione were shown in asthmatic patients [38]. All described studies regarding total and differential WBC changes, Th1/Th2 balance, and oxidant and antioxidant biomarkers in sensitized animals and asthmatic patients support the results of this study and confirm the sensitization of animals in this study.

Treatment of sensitized animals with curcumin lead to significant reduction in total WBC, eosinophil, neutrophil, and monocyte but increased lymphocyte percentage. Increased lymphocyte percentage in BALF of treated S groups was also reported by a previous study [28] which was due to reduction of total WBC count. PLA2 and TP levels were also significantly reduced in BALF of groups treated with all concentrations of curcumin. Reduction of total and most differential WBC count and TP in BALF of OVA-sensitized rat [39] and PLA2 in a murine model of asthma [20] treated with curcumin were reported. In LPS-induced acute lung injury, administration of curcumin also significantly reduced BALF level of TP [40]. Antioxidant and anti-inflammatory properties of curcumin have been previously shown [41]. Therefore, the results of the

FIG 5

NO₂ (a), NO₃ (b), and MDA (c) concentration in bronchoalveolar lavage fluid (BALF) of control (C) sensitized group (S), S treated with dexamethasone (S+D) (for each group, n=8), and curcumin (Cu, n=7). Data are mean $\pm$ SEM values. *P<0.05, **P<0.01, ***P<0.001 compared to group C. +++P<0.001 compared to group S. #P<0.05, ###P<0.001 compared to group S+D. xP<0.05, xP<0.01, xxxP<0.001 comparison of three concentrations of curcumin. Statistical analyses were performed using ANOVA with Tukey-Kramer's post hoc test.

present and previous studies suggest the therapeutic effect of curcumin on asthma by suppressing lung inflammation changes.



Treatment of sensitized animals with all three curcumin concentrations resulted in reduction of IgE and IL-4 levels in BALF and increased IFN- γ level and IFN- γ /IL-4 ratio. Increase in IFN- γ and reduction of IL-4 in BALF of sensitized animals treated with curcumin may indicate stimulatory effect of curcumin on Th1 and an its inhibitory effect on Th2 cells. Reduction of allergic airway inflammation by regulating Treg/Th17 balance in OVA-sensitized mice by curcumin was shown previously [42]. However, intranasal curcumin dose-dependently downregulated IL-4, IL-5, and IFN- γ levels in the BALF of a murine model of asthma [43]. Decreased serum IgE levels in animals exposed to latex antigens [44], IgE signaling processes [45], and IgE level in OVA-challenged mice [40] by curcumin were also documented which supports the results of our study. The use of ELISA kits for measurements of PLA2, TP, IgE, IL-4, and IFN- γ in BALF may seem to be a limitation of this study. However, measurements of the aforementioned variables using ELISA method are not unreliable and provide their quantitative values. In fact, almost all similar studies in this field used this method for measurement of cytokines [31,39,42,43,45]. Although ELISA kits used in this study (abcam®, USA and MyBioSource®, USA) do not supply any quality control sample, as the results of different groups were compared, quality controls sample would not affect the overall results. The variation in the obtained results was also very low as indicated in Fig. 4.

Treatment of sensitized animals with various concentrations of curcumin resulted in reduction of NO₃, NO₂, and MDA and increase in SOD and CAT. Normalization of the elevated nitrate and MDA levels in asthmatic rats [39], increased SOD [46] and CAT activity following curcumin treatment were also reported [47]. It was shown that curcumin has additive effects on thiol concentration in benzo(a)pyrene-induced lung carcinogenesis in mice [48]. All these studies are in agreement with the findings of this study, which suggest that curcumin as an effective antioxidant, may have a protective effect on asthma disease by its effect on oxidant-antioxidant balance.

The effects of two higher concentrations of curcumin on inflammatory cells, inflammatory mediators, immune and oxidant and antioxidant markers were higher than its low concentration and the effect of its high concentration was also higher than the medium concentration. Concentration dependency effects of curcumin on measured parameters are another

FIG 6

SOD (a), CAT (b), and Thiol (c) levels in bronchoalveolar lavage fluid (BALF) of control (C), sensitized (S), S treated with dexamethasone (S + D) (for each group, n = 8), and curcumin (Cu, n = 7). Data are mean $\pm$ SEM values. *P < 0.05, ***P < 0.001 compared to group C. ++P < 0.05, +++P < 0.01, ###P < 0.01, ###P < 0.001 compared to group S + D. xxxP < 0.001 comparison of three concentrations of curcumin. Statistical analyses were performed using ANOVA with Tukey-Kramer's post-test.

evidence for its anti-inflammatory, immunomodulatory, and antioxidant effects in sensitized rat.

Daily uptake of curcumin in this study were 30, 60, and 120 mg/kg/day during sensitization period (0.15, 0.30, and 0.6 mg/mL in drinking water of rat, using 40 mL water/day, with weight of 200 g) which were comparable to the curcumin dose of similar previous studies. In fact, similar curcumin doses to this study (100 and 200 mg/kg) caused reduction of NO level, total and differential WBC count in BALF, and TP and MDA in lung tissue homogenates in sensitized rats [39] and also resulted in reduction of Th17 but increased Treg cell and Treg/Th17 balance in sensitized mice (50, 100, and 200 mg/kg) [42]. Treatment of OVA-sensitized mice with lower curcumin doses lead to similar results of this study, including the reduction of eosinophil and IgE in conjunctival inflammation and Th2 type cytokines (IL-4 and IL-5) in conjunctiva, spleen, and cervical lymph nodes (10 and 20 mg/kg) [49] and reduction of eosinophil and IgE levels and shifted Th2 response toward Th1 in a latex allergy model (12.5 mg/kg) [45]. In the BALF of mice sensitized with OVA and treated with 2.5 and 5.0 mg/mL, the reduction of IL-4, IFN γ , and PLA2 was reported [43] which was different with the results of this study (increases IFN γ concentration in the BALF) perhaps due to lower higher dose of curcumin in this study.

The effects of all concentrations of curcumin on total WBC, IL-4, and IFN- γ /IL-4 ratio, its two higher concentrations on IgE and IFN- γ , and its high concentration on eosinophils, lymphocytes, neutrophils, monocytes, PLA2, TP, SOD, CAT, and thiol were higher than the effect of dexamethasone. In addition, curcumin treatment caused reduction of IL-4 but increased IFN- γ level and IFN- γ /IL-4 ratio which indicate enhanced Th1/Th2 balance in sensitized rats which are the most novel finding of this study. However, dexamethasone treatment did not change IFN- γ , IL-4, and IFN- γ /IL-4 ratio (Th1/Th2 balance). These results indicate comparable or even higher anti-inflammatory and antioxidant effects of curcumin on asthmatic rats compared to the effect of dexamethasone along with more specific immunomodulatory effect which is another important evidence of the possible preventive effect of curcumin in asthma. In this study, the dose of dexamethasone was 0.25 mg/kg/day during 21 days sensitization period (1.25 μ g/mL in drinking water of animals) which was equal or even higher than previous studies which used 0.5 mg/kg/day orally only for 3 days during sensitization period [21,50] or 1 mg/kg/day intraperitoneally for 7 days in a 77-day sensitization period in mice [51]. In addition, dexamethasone at the dose administered in this study affected almost all measured parameters which suggest to be an effective dose for this substance. However, the effect of higher dose of dexamethasone may show similar or even higher effect compared to curcumin which should be examined in further studies.

The results of this study together with previous studies may suggest that the effect of curcumin treatment on oxidant-antioxidant balance may reduce airway inflammation. In addition, immunomodulatory effects of curcumin may also cause

reduction of lung inflammation. Therefore, these results may suggest complex interactions between antioxidant, anti-inflammatory, and immunoregulatory effect of curcumin in the treatment of asthma. However, further investigations are needed to evaluate the effect of curcumin on asthmatic patients.

Conclusion

In conclusion, these results indicated the preventive effect of curcumin on lung inflammatory cells, inflammatory mediators, oxidative stress, and immunomodulatory effects in sensitized animals. The anti-inflammatory and antioxidant effects of curcumin were comparable or even higher than dexamethasone at studied concentrations but its immunomodulatory effect was more specific which caused increased Th1/Th2 balance. These results suggest a promising therapeutic potential of curcumin for treatment of asthma disease.

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Conflict of interest

The authors declare no conflict of interest.

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