



Original article

Curcumin affects tracheal responsiveness and lung pathology in asthmatic rats



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ABSTRACT

Background: Curcumin has shown various pharmacological effects such as anti-inflammatory activities. In this study, the effects of curcumin on tracheal responsiveness and lung pathological features were evaluated in a rat model of asthma.

Methods: Tracheal responsiveness and lung pathological features were evaluated in control rats (C), ovalbumin (OVA)-sensitized rats (as an animal model of asthma; A), A rats treated with curcumin (Cu, 0.15, 0.30, and 0.60 mg/ml) and dexamethasone (D, 1.25 µg/ml), (n = 8 in curcumin-treated groups and n = 6 in other groups). Curcumin and dexamethasone were added to animals' drinking water during the sensitization period.

Results: Asthmatic group showed increased lung pathological score and tracheal responsiveness to methacholine and OVA compared to control group ($p < 0.01$ to $p < 0.001$). Pathological features including interstitial inflammation, interstitial fibrosis, bleeding, and emphysema as well as tracheal responsiveness to methacholine and OVA, were significantly decreased in treated groups with dexamethasone and all concentrations of curcumin compared to group A ($p < 0.05$ to $p < 0.001$). Epithelial damage was also significantly decreased in treated groups with the two higher concentrations of curcumin ($p < 0.05$ to $p < 0.001$).

Conclusion: Curcumin showed preventive effects on tracheal responsiveness and lung pathological features in asthmatic rats.

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Introduction

Asthma is a chronic inflammatory disease of the airways that is characterized by thickening of the airway walls, infiltration of inflammatory cells, enhanced smooth muscle mass and mucus secretion, epithelial shedding, hypertrophy and hyperplasia of airway smooth muscle, and inflammation of the airways [1]. In asthma, various inflammatory cells including mast cells, eosinophils, neutrophils, T- lymphocytes, epithelial cells, and macrophages contribute to airway inflammation and these cells produce

reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical [2]. ROS induce oxidative stress in the airways and induce smooth muscle contraction, airway hyper-reactivity and mucus secretion by releasing histamine from mast cells [3]. Airway hyper-responsiveness (AHR) to physical and pharmacological stimuli, is the main characteristic feature of asthma which is due to airway inflammation [4]. A correlation was found between lung inflammation and the severity of airway hyper-responsiveness in asthma [5].

Curcumin is a phenolic compound obtained from the plant *Curcuma longa* which has been used in Indian traditional medicine for treatment of ailments including diarrhea and asthma. The anti-inflammatory effect of both systemic and local administration of curcumin has been shown [6,7]. In allergic rhinitis, curcumin modulated the activation of mast cells compared to healthy controls [8,9]. The effects of curcumin on ovalbumin (OVA)-sensitized rats, were previously shown with respect to antioxidant,

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anti-inflammatory (total and differential white blood cells) and immunomodulatory effects [10,11]. Curcumin also showed relaxant effects on isolated caprine detrusor muscle and porcine coronary arterial ring segments [12,13]. Therefore, the present study aimed to evaluate the effect of curcumin on lung pathological features as well as tracheal responsiveness to methacholine and OVA, in asthmatic (i.e. OVA-sensitized) rats.

Materials and methods

Animal groups and sensitization protocol

Male Wistar rats (200–250 g) were kept in an animal house receiving clean filtered air (Maximiser, Thorens Caging System Inc., Hazleton, PA, USA) under standard conditions at $22 \pm 2^\circ\text{C}$ temperature with 12 h/12 h light/dark cycles. They also had free access to food and water ad libitum during the experimental period. Animal handling was performed in compliance with the regulations of the Institute of Laboratory Animals Resources Commission on Life Sciences.

Forty two rats were randomly divided into six groups ($n = 8$ for curcumin treated groups and $n = 6$ for other groups) as follows:

1) Control group, which was given intraperitoneal (*ip*) and inhaled normal saline (group C).

2) OVA-sensitized group, which was given *ip* and inhaled OVA (group A).

3–5) OVA-sensitized rats treated with three concentrations of curcumin (0.15, 0.30 and 0.60 mg/ml), during sensitization

period (group A + Cu 0.15 mg/ml, A + Cu 0.30 mg/ml and A + Cu 0.60 mg/ml, respectively).

6) OVA-sensitized rats treated with dexamethasone ($1.25 \mu\text{g/ml}$) during the sensitization period (group A + D).

Curcumin (Sami Labs LTD, Bangalore, India) and dexamethasone (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were stable in water at room temperature for 24 h and were added to animals' drinking water during the 21-day sensitization period. Curcumin was dispersed in drinking water and the drinking water container was shaken several times a day. Each animal used almost 40 ml water per day and water consumption volume was not significantly different among different groups. Curcumin and dexamethasone solutions were freshly prepared each day. The doses of curcumin and dexamethasone were chosen according to previous studies [10,11]. There was no significant difference in body weight between control, dexamethasone-treated and curcumin-treated groups during the experimental period.

Sensitization of rats to OVA was performed using a previously described method [14]. Briefly, 1 mg/kg chicken egg albumin (OVA, grade V, 98% pure; Sigma, St. Louis, MO, USA) dissolved in 0.9% sterile saline containing 100 mg $\text{Al}(\text{OH})_3$ as adjuvant was injected (*ip*) on days 1, 2 and 3 of the experiment. Rats were exposed to an aerosolized 1% OVA solution with an air flow of 8 lit/min produced by a DeVilbiss PulmoSonic nebulizer (DeVilbiss Health Care Ltd., Feltham, UK) for 20 min/day on days 6, 9, 12, 15, 18 and 21. Challenges took place in a 0.8 m^3 chamber, while animals were breathing normally. Control animals were treated similarly but saline was used instead of OVA solution.

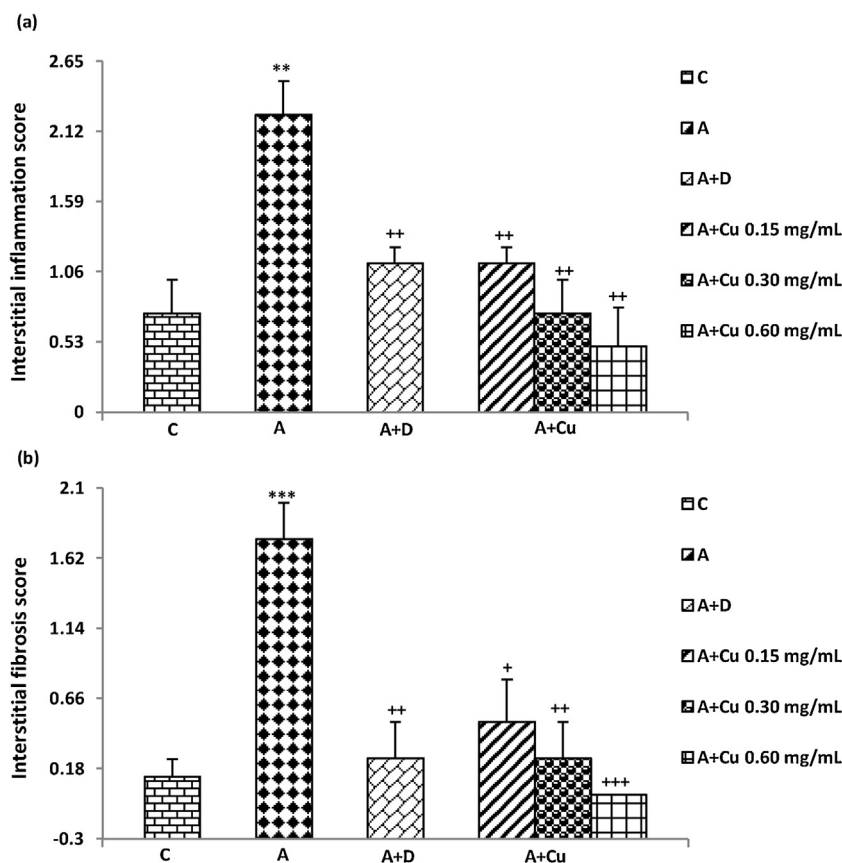


Fig. 1. Interstitial inflammation (a) and interstitial fibrosis (b) scores in control animals (C), asthmatic group (A), A groups treated with dexamethasone (A + D) and curcumin (A + Cu), (for curcumin treated groups, $n = 8$ and for other groups, $n = 6$). Data are presented as mean $\pm$ SEM values. ** $p < 0.01$, *** $p < 0.001$; compared to group C. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ compared to group A. Statistical analyses were performed using one way analysis of variance (ANOVA) with Tukey-Kramer's post-test.

Pathological evaluation

After sacrificing the animals using ketamine (50 mg/kg, *ip*), lungs were removed and placed into buffered formalin 10% (Merck, Darmstadt, Germany). Seven days later, tissues were dried using Auto Technicon apparatus and cleared by a series of ethanol 70–100% and xylol; then, paraffin blocks were prepared. The specimens were cut into 4- μ m slices and stained with hematoxylin and eosin (H&E). The tissues were then evaluated under a light microscope. The pathologic features that were studied in the lungs of sensitized and treated rats included interstitial inflammation, interstitial fibrosis, bleeding, emphysema and epithelial damage. Based on previous studies, pathological insults were scored as follows: 0) No pathologic changes; 1) Patchy changes; and 2) severe changes (in most parts of the lung) [15].

Measurement of tracheal response to methacholine and ovalbumin

After animal scarification, the chest of rats was opened and trachea was removed and cut into 2 parts, each containing 5–6 cartilaginous rings. One part of the trachea was hung between two Nichrome hooks inserted into the lumen under isotonic tension of 1 g and placed in a 10-ml organ bath containing Krebs-Henseleit solution (KHS) which was maintained at $37 \pm 0.5^\circ\text{C}$ and bubbled constantly with 5% CO_2 –95% O_2 . The KHS was consisted of (mM): NaCl 120, KCl 4.72, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5, NaHCO_3 25 and dextrose 11. Tissue was allowed to equilibrate for at least 1 h while it was being washed with KHS solution every 15 min. In all experiments, contraction responses were measured using an isometric transducer (MLT0202, AD Instruments, Australia) which was connected to a power lab system (Power Lab 8/30, ML870, AD Instruments, Australia).

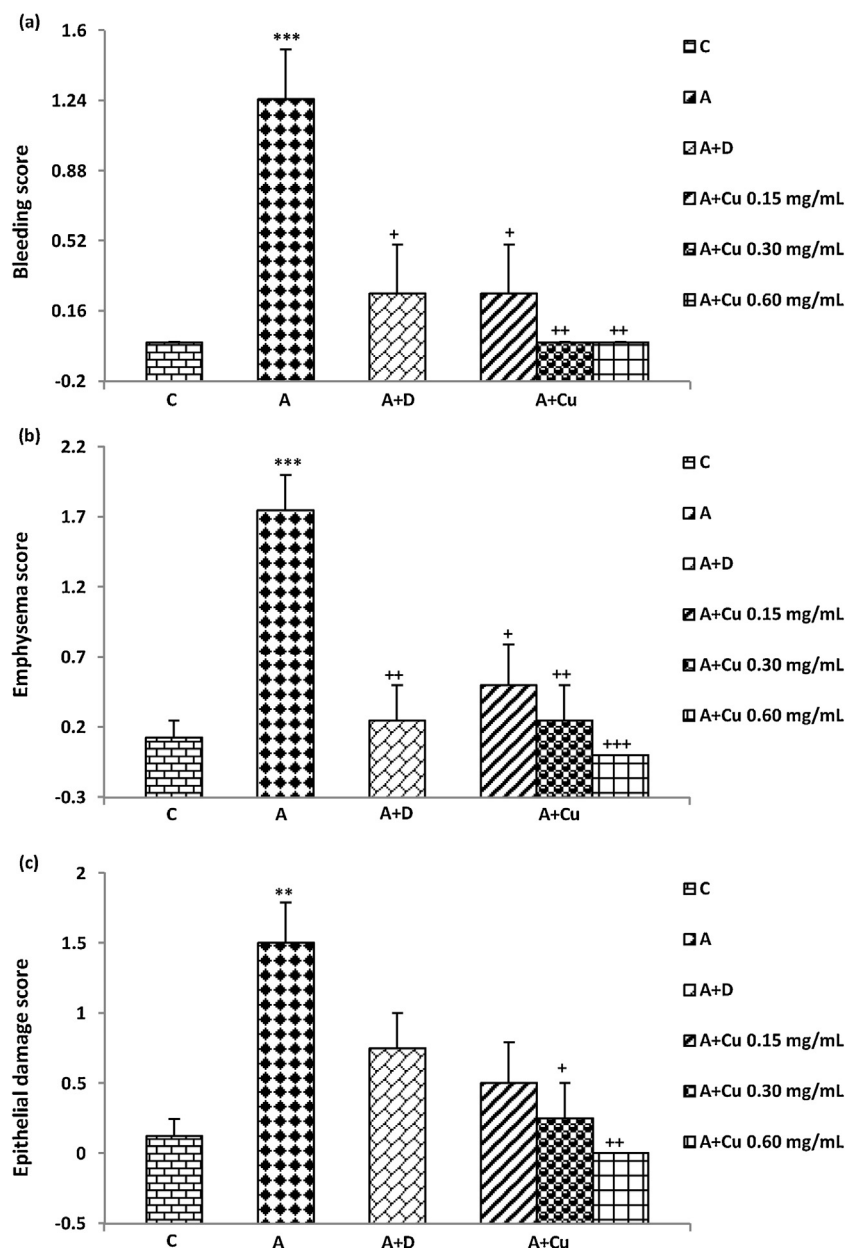


Fig. 2. Bleeding (a) emphysema (b) and epithelial damage (c) scores in control animals (C), asthmatic group (A), A groups treated with dexamethasone (A + D) and curcumin (A + Cu), (for curcumin treated groups, $n = 8$ and for other groups, $n = 6$). Data are presented as mean $\pm$ SEM values. ** $p < 0.01$, *** $p < 0.001$; compared to group C. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ compared to group A. Statistical analyses were performed using ANOVA with Tukey-Kramer's *post-test*.

For measurement of tracheal responsiveness to methacholine hydrochloride, its cumulative concentrations of 10^{-8} to 10^{-3} M (Sigma Chemical Ltd, U.K.) were added every 2 min, and the contraction due to each concentration was recorded at the end of 2 min until the contrail response reached a plateau. The percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction by its final concentration was plotted against log concentration of methacholine to obtain cumulative log concentration-response curves for methacholine in each experiment. The effective concentration of methacholine, causing 50% of maximum response (EC_{50}) was measured using methacholine response curve.

For measurement of tracheal responsiveness to OVA, 1 ml of 2% OVA solution was added to the 10-ml organ bath and the degree of tracheal smooth muscle contraction was recorded after 15 min and expressed as gram [16].

Statistical analysis

Data were presented as mean $\pm$ SEM. Data analysis was done by an investigator who was blind to experimental groups. The normality of data was checked by Kolmogorov-Smirnov test. The sample size was calculated according the previous studies [10,11]. Statistical comparisons 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 $p < 0.05$. InStat (GraphPad Software, Inc, La Jolla, USA) was used for data analysis.

Results

Lung pathological features

The scores of all pathological features in group A were significantly increased compared to the control group ($p < 0.01$ to $p < 0.001$), (Figs. 1 and 2).

There were significant and concentration-dependent decreases in interstitial inflammation, interstitial fibrosis, bleeding, and emphysema in treated groups with all concentrations of curcumin compared to asthma group ($p < 0.05$ to $p < 0.001$), (Figs. 1 and 2). Two higher concentrations of curcumin also significantly reduced epithelial damage compared to A group ($p < 0.05$ and $p < 0.01$ for medium and high concentrations of curcumin, respectively), (Fig. 2). Dexamethasone treatment also significantly decreased all pathological features compared to group A ($p < 0.01$ to $p < 0.001$), (Figs. 1 and 2).

No significant difference was seen between dexamethasone-treated group and curcumin-treated groups in terms of pathological scores. Fig. 3 shows a photograph of lung specimen from each studied group.

Tracheal responsiveness to methacholine

A leftward shift in methacholine concentration-response curves was observed in asthmatic group compared to group C, but the curves of treated groups with dexamethasone and all concentrations of curcumin were shifted to right compared to group A (Fig. 4a). A significantly lower EC_{50} value was seen in group A compared to group C ($p < 0.001$, Fig. 4b). The EC_{50} values in treated groups with all concentrations of curcumin and dexamethasone were significantly higher than that of group A ($p < 0.001$ for all cases, Fig. 4b).

Maximum response to methacholine in group A was significantly higher than that of group C ($p < 0.001$, Fig. 5a). Treatment with all concentrations of curcumin and dexamethasone led to significant reductions in maximum response compared to group A ($p < 0.05$ to $p < 0.001$, Fig. 5a).

The effect of the highest concentration of curcumin (0.60 mg/ml) on the EC_{50} for methacholine and maximum response, was significantly higher than that of the lowest concentration (0.15 mg/ml), ($p < 0.05$ for EC_{50} for methacholine and $p < 0.001$ for maximum response, Table 1).

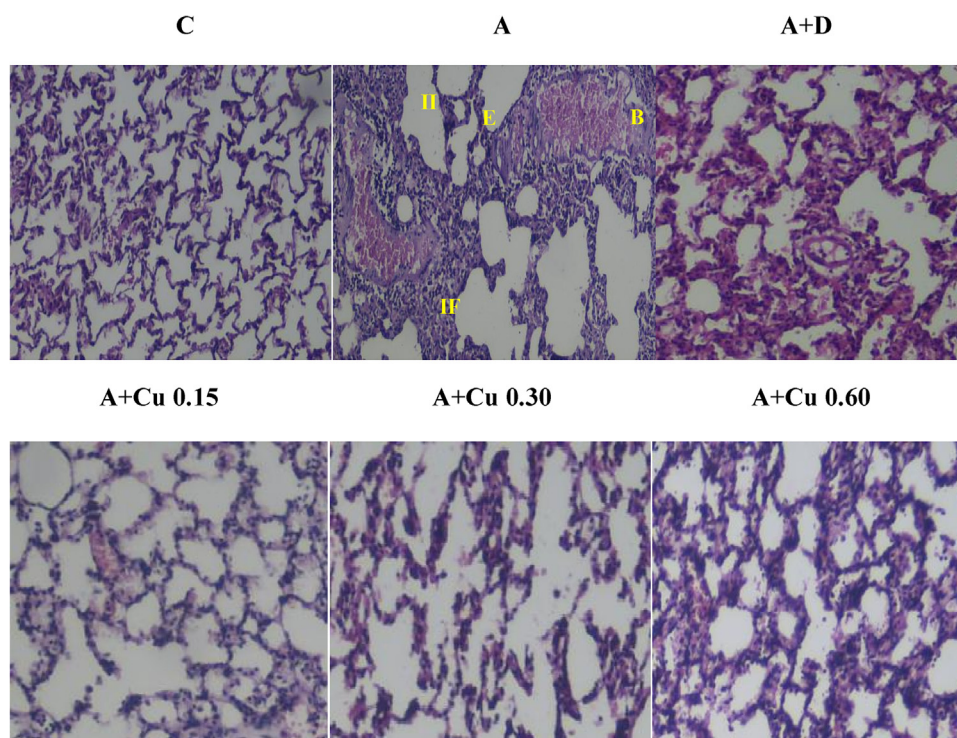


Fig. 3. Photographs of lung specimens under a light microscope with a 40 magnification, in control group (C), asthmatic (A) group with interstitial inflammation (II), interstitial fibrosis (IF), bleeding (B), emphysema (E) and epithelial damage (ED), A groups treated with dexamethasone (A + D) and three concentrations of curcumin (A + Cu).

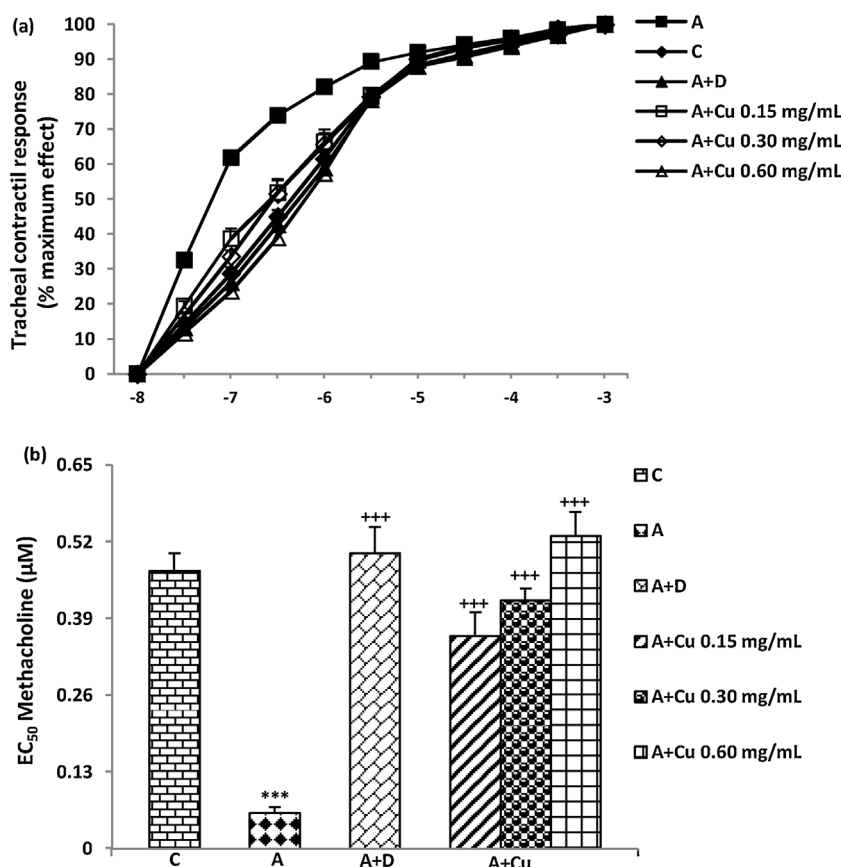


Fig. 4. Cumulative log concentration-response curves of methacholine induced contraction of isolated trachea (a) and EC₅₀ methacholine (b) in control group (C), asthmatic group (A), A groups treated with dexamethasone (A + D) and curcumin (A + Cu), (for curcumin treated groups, n = 8 and for other groups, n = 6). Data are mean $\pm$ SEM values. *** p < 0.001; compared to group C. +++ p < 0.001 compared to group A. Statistical analyses were performed using ANOVA with Tukey-Kramer's *post-test*.

Tracheal responsiveness to ovalbumin

Tracheal responsiveness to OVA in group A and treated group the with the lowest concentration of curcumin was significantly higher than that of group C (p < 0.001 for both cases, Fig. 5b). In dexamethasone-treated group and groups treated with all concentrations of curcumin, tracheal responsiveness to OVA were significantly decreased compared to group A (p < 0.001 for all cases, Fig. 5b).

The effects of two higher concentrations of curcumin (0.30 and 0.60 mg/ml) on tracheal responsiveness to OVA were significantly higher than that of the lowest concentration (0.15 mg/ml), (p < 0.01 to p < 0.001, Table 1).

The effect of the lowest concentration of curcumin on OVA responsiveness was significantly lower than dexamethasone's effect (p < 0.01, Fig. 5b).

Discussion

In this study, asthmatic rats were treated with three concentrations of curcumin to examine the possible preventive effect of this natural compound on lung pathological features and tracheal responsiveness to methacholine and OVA. The effect of dexamethasone as the positive control was also evaluated.

The results showed increased pathological changes of the lung tissues in asthmatic animals including interstitial inflammation, interstitial fibrosis, bleeding, emphysema and epithelial damage. The pathological changes observed in asthmatic rats in this experiment were similar to those reported by previous studies [15,16]. Pathological findings also confirmed the induction of an

animal model of asthma (i.e. animal sensitization) in the present study.

Treatment of asthmatic rats with curcumin led to improvement of almost all lung histological features. The effect of curcumin on neutrophil infiltration in the lung in a mouse model of acute inflammation induced by intranasal instillation of *Klebsiella pneumoniae* B5055, was previously shown [17]. In another study, oral administration of curcumin ameliorated inflammatory responses and pathological features following induction of lung injury by different aspiration materials in rats [18]. Curcumin was found effective against acute respiratory distress syndrome in female rats by alteration of inflammatory response and inhibition of subsequent fibrosis [19]; also, methanolic extract of *Curcuma longa* ameliorated inflammation with respect to peri-bronchial and alveolar congestion and intraluminal hemorrhage in the bronchus of OVA sensitized rats [20]. These evidences confirm the results of the present study. Both *in vitro* and *in vivo* studies have indicated that curcumin significantly decreased the thickness of the airway wall, the airway smooth muscle layer, the number of ASMCs and the expression of extracellular signal-regulated kinase (ERK) compared to the asthmatic animals [21].

The results also showed significant reductions in the EC₅₀ for methacholine in asthma group compared to the control animals which is a marker of increased tracheal responsiveness. The findings of this study showed increased tracheal responsiveness to methacholine and OVA which confirm sensitization of animals. The induction of an animal model of asthma by injected and inhaled OVA was shown in several studies using the similar method of the present study previously [22,23].

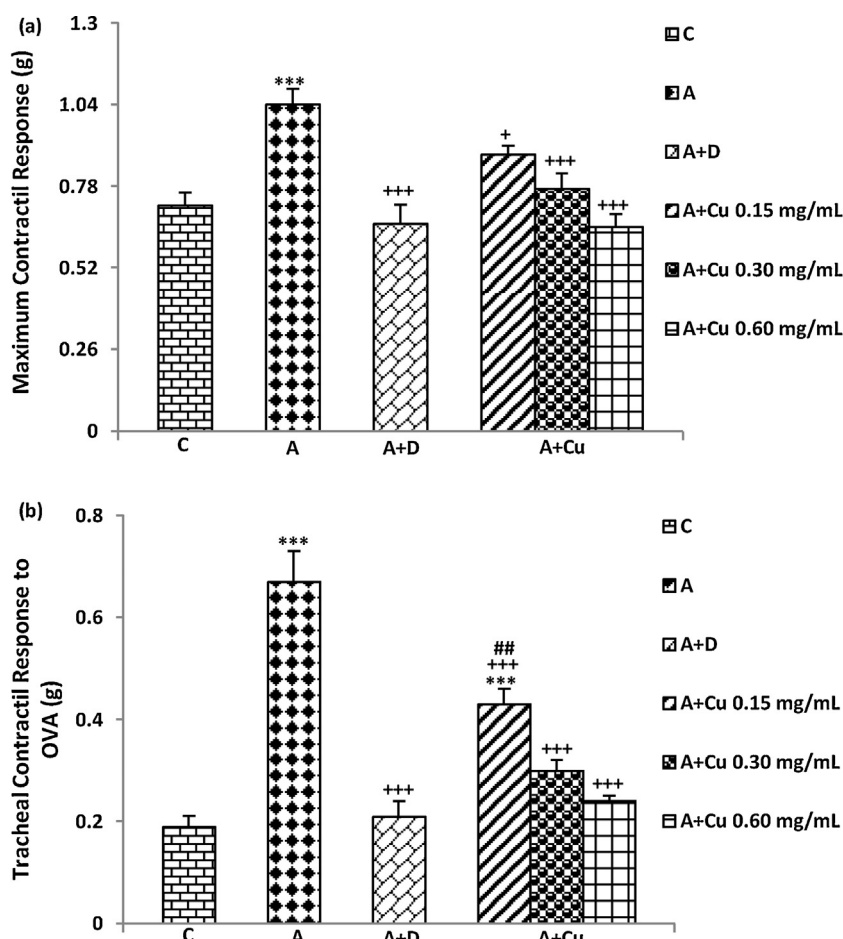


Fig. 5. Maximum contractile response to methacholine (a) and tracheal contractile response to OVA (b) in control group (C), asthmatic group (A), A treated with dexamethasone (A + D) and curcumin (A + Cu), (for curcumin treated groups, $n = 8$ and for other groups, $n = 6$). Data are mean $\pm$ SEM values. *** $p < 0.001$; compared to group C. + $p < 0.05$, +++ $p < 0.001$ compared to group A, ### $p < 0.01$ compared to group A + D. Statistical analyses were performed using ANOVA with Tukey-Kramer's *post-test*.

Table 1

Comparisons of EC_{50} methacholine, maximum response, OVA responsiveness and lung pathological changes in asthmatic animal (A) treated with three concentrations of curcumin.

Parameters	A + Cu 0.15	A + Cu 0.30	A + Cu 0.60
EC_{50} (μ M)	0.36 ± 0.04	0.42 ± 0.02	0.53 ± 0.04 +
Max Response (g)	0.88 ± 0.03	0.77 ± 0.05	0.65 ± 0.04 +++
OVA Response (g)	0.43 ± 0.03	0.30 ± 0.02 ++	0.24 ± 0.01 +++
Interstitial inflammation	1.12 ± 0.12	0.75 ± 0.25	0.50 ± 0.29
Interstitial fibrosis	0.50 ± 0.29	0.25 ± 0.25	0.00 ± 0.00
Bleeding	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
Emphysema	0.50 ± 0.29	0.25 ± 0.25	0.00 ± 0.00
Epithelial damage	0.50 ± 0.29	0.25 ± 0.25	0.00 ± 0.00

Data were presented as mean $\pm$ SEM. + $p < 0.05$ compared to Cu 0.15. Statistical analyses were performed using ANOVA with Tukey-Kramer's *post-test*.

Concentration-dependent protective effect of curcumin on enhanced tracheal responsiveness to methacholine and OVA as well as reduced EC_{50} methacholine in asthmatic rats were also observed in the present study which was similar to dexamethasone's effect. The main pathophysiological feature of asthma is airway responsiveness which is mainly due to airway inflammation. The preventive effect of curcumin on the tracheal responsiveness of asthmatic animals is perhaps due to its suppressing effect on airway inflammation. In fact, previous studies have shown anti-inflammatory and bronchodilatory effects for curcumin, which support the results of the current study. In a study, animals sensitized with OVA showed development of certain

characteristics of asthma including allergen-induced airway constriction and airway hyper-reactivity to histamine. After treatment with curcumin (20 mg/kg body weight, orally), airway constriction and hyper-reactivity were significantly inhibited [24]. In another study, curcumin (300 μ g, orally) inhibited mast cell activation and intestinal anaphylaxis in a murine model of ovalbumin (OVA)-induced food allergy [25]. In addition, inhaled curcumin (5.0 mg/kg) suppressed airway inflammations and bronchoconstriction in OVA-sensitized mice with a significant reduction in inflammatory cell infiltration in the peribronchiolar and perivascular regions [26]. The results of the above-described studies demonstrated that curcumin is able to reduce airway responsiveness by modulating the inflammatory processes, which also support the results of the present research.

The preventive effect of curcumin on tracheal responsiveness and pathological changes were concentration-dependent which is another reason indicating the potential preventive activity of curcumin in airway responsiveness in asthmatic rats. The effect of the highest concentration of curcumin on EC_{50} for methacholine and maximum response and the effects of two higher concentrations of curcumin on OVA response, were significantly higher than those of the lowest concentration of curcumin.

Dexamethasone, as a known steroid, anti-inflammatory drug which was used as the positive control drug, also reduced lung pathological features and tracheal responsiveness, comparable to the results of curcumin. The effect of curcumin on lung pathological features and tracheal responsiveness in asthmatic rats were comparable or even higher to the effect of

dexamethasone (especially on tracheal responsiveness). These finding also suggest the potential preventive effect of curcumin on asthma. In the present study, the preventive effect of curcumin in an animal model of asthma was studied with respect to lung pathological features and tracheal responsiveness and the obtained results indicated novel findings. However, the effect of curcumin and *Curcuma longa* on other aspects of asthma as well as their effects in asthmatic patients should be evaluated in further studies.

Conclusions

In conclusion, the results of this study showed the preventive effect of curcumin on asthma-induced lung pathological changes and tracheal responsiveness, which was comparable or even more potent than the effect of dexamethasone at used concentrations.

Conflict of interest

The authors declare that they have no conflict of interest.

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