The effect of hydro-ethanolic extract of *Curcuma longa* rhizome and curcumin on total and differential WBC and serum oxidant, antioxidant biomarkers in rat model of asthma

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ARTICLE INFO	ABSTRACT
	Objective(s): The effects of *Curcuma longa* (*C. longa*) and curcumin on total and differential WBC count and oxidant, antioxidant biomarkers, in rat model of asthma were evaluated. ***Materials and Methods:*** Total and differential WBC count in the blood, NO_2, NO_3, MDA, SOD, CAT and thiol levels in serum were examined in control, asthma, Asthmatic rats treated with *C. longa* (0.75, 1.50, and 3.00 mg/ml), curcumin (0.15, 0.30, and 0.60 mg/ml), and dexamethasone (1.25 µg/ml) rats. ***Results:*** Total and most differential WBC count, NO_2, NO_3 and MDA were increased but lymphocytes, SOD, CAT and thiol were decreased in asthmatic animals compared to controls ($P<0.001$). Total WBC, NO_2 and NO_3 were significantly reduced in treated groups with dexamethasone and all concentrations of *C. longa* and curcumin compared to asthmatic group ($P<0.001$ for all cases). MDA was significantly decreased, but SOD, CAT and thiol increased in treated asthma animals with dexamethasone and two higher concentrations of *C. longa* and curcumin ($P<0.01$ to $P<0.001$). There were significant improvement in eosinophil percentage due to treatment of highest concentration of the extract and curcumin, neutrophil and monocyte due to highest concentration of curcumin and lymphocyte due to highest concentration of the extract and two higher concentrations of curcumin compared to asthmatic group ($P<0.01$ to $P<0.001$). Dexamethasone treatment improved monocyte ($P<0.001$) and lymphocyte ($P<0.01$) percentages. ***Conclusion:*** Antioxidant and anti-inflammatory effects of *C. longa* extract and its constituent curcumin in animal model of asthma was observed which suggest a therapeutic potential for the plant and its constituent on asthma.

Introduction

Asthma is a chronic disease and is one of the major global health problems (1) which is characterized by inflammation of the airways, variable and recurring airflow obstruction, the airway walls swelling, mucus plug formation, and bronchial hyper responsiveness to physical and pharmacological stimuli (2).

Different inflammatory cells, including eosinophils, lymphocytes, mast cells, and neutrophils involved in airway inflammation of asthma (3).

Airway inflammation is the most prime cause of the recurrent episodes of airflow restriction in asthma and is related with incremented oxidative stress (4). Increased oxidative stress (oxidant antioxidant imbalance) of the airways also contribute in chronic airway inflammation of asthma (5) which is accompanied with increased oxidant activity in the lung and blood (6). The lung is the main exposable organ to environmental oxidants such as air pollutants and cigarette smoke as well as endogenous oxidant produced by mitochondrial electron transport during respiration released from phagocytes (7). There are strong evidences that inflammatory cells from peripheral blood and bronchoalveolar lavage (BAL) fluid of asthmatic subjects generate more superoxide anion radicals than those from healthy controls (8). Measurement of exhaled air in asthmatic patients showed enhanced levels of nitro tyrosine and other markers of oxidative stress (9). The normal lung contains high levels of antioxidant resources to prevent oxidant-induced lung injury. This is supported by the studies demonstrating an oxidant–antioxidant imbalance in asthmatic airways, reduced total antioxidant capacity and individual antioxidants in plasma and BAL fluid of asthmatic subjects (10). Studies indicated that the use of antioxidants may be effective in the treatment of asthma by restoring the oxidant-antioxidant balance (11).

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In traditional medicines, plant-based drugs as well as natural antioxidant have been used for either treatment or prevention of lung disease. *Curcuma longa* (*C. longa*), or turmeric belonging to Zingiberaceae family is a perennial herb with pointed leaves and bears funnel-shaped yellow flowers which grows to a height of three to five feet (12), and is cultivated extensively in Asia mostly in India, China and other countries with a tropical climate. *C. longa* has also mentioned in Ayurvedic medicine for its anti-asthmatic and anti-dyspnea effects. Different pharmacological effects such as anti-asthmatic (13), antioxidant (14) and anti-inflammatory (15) effects for this plant have been reported.

Vogel (1842), primarily isolated curcumin, (a bright yellow spice, extracted from the rhizome of *C. longa*), it was structurally characterized by Milobedeska and colleagues (1910) and synthesized and confirmed by Lampe and colleagues in 1913 (16, 17). Several studies have reported considerable biological properties for curcumin including; anti-inflammatory (18), anti-microbial (19), and hepatoprotective (20). Curcumin has been demonstrated to inhibit nitric oxide and reactive oxygen species (ROS) production in macrophage (21) and to enhance the activity of many antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) (22) and heme oxygenase-1 (OH-1) (23).

In the present study, the effect of the hydro-ethanolic extract of *C. longa* compared to the effect of its constituent, curcumin on total and differential white blood cell (WBC) count as an indicator of inflammation, and the levels of nitric oxide (NO), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and thiol, in serum of rat model of asthma were investigated.

Materials and Methods

Plant, extract and constituent

C. longa rhizomes were purchased from a local herbal shop in Mashhad, Razavi Khorasan Province, Iran and identified by botanists in the herbarium of Ferdowsi University of Mashhad.

The rhizomes (100 g) was cleaned, grounded, weighed, and homogenized in 96% ethanol at a ratio of 1:10 of plant to ethanol and left to soak for 3 days at 37 °C with occasional shaking and stirring. The mixture was then filtered and the resulting liquid was concentrated under reduced pressure at 45 °C in an Eyela (Heidolph, Germany) rotary evaporator to yield a dark gummy-yellow extract (24). The yield extract was 14%.

Experimental animals and induction of animal model of asthma

The study was performed in sixty nine male Wistar rats weighing approximately 200–250 g in animal house, School of Medicine, Mashhad University of Medical Sciences, Iran. The animals were kept in a 22±2 °C temperature with a 12 hr light/dark cycle and fed with standard diet and tap drinking water *ad libitum*.

For induction of animal model of asthma, the rats were sensitized by three IP injections of 1 mg/kg chicken egg albumin (Ovalbumin=OVA, grade V, 98% pure; Sigma, St. Louis, MO, USA) 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 an aerosolized of 1% OVA concentration for 20 min/day, on days 6, 9, 12, 15, 18 and 21 (25). A solution of 1% OVA in normal saline was aerosolized by delivery of compressed air to a jet nebulizer (26). Exposures were carried out in a whole body inhalation exposure chamber dimensioned 30×20×20 cm. The rat in the control group received IP and inhaled normal saline instead of OVA using the same procedure as OVA administration in sensitized animals.

The study was carried out in the following groups:
A) Control or saline treated (group C).
B) Animal model of asthma or OVA sensitized (group A).
C) Asthma animals treated with the extract of *C. longa* (0.75, 1.5, 3 mg/ml), (groups CL 0.75, CL 1.50, and CL 3.00).
D) Asthma animals treated with curcumin (0.15, 0.3, 0.6 mg/ml), (groups Cu 0.15, Cu 0.30, and Cu 0.60).
E) Asthma animals treated with dexamethasone (1.25 μg/ ml), (group D).

The extract of *C. longa*, curcumin and dexamethasone were prescribed in animals' drinking water during sensitization period. The number of animals for each curcumin treated group was 7 and for other groups, it was 8. Averagely 40 ml drinking water was used by each rat/day and there wasn't significant difference in the drinking water used by the animals in different groups.

Total and differential WBC count

At the end of the experiment period, rats were anesthetized by IP injection of ketamine (50 mg/kg). Blood samples (4 ml) were taken by cardiac puncture immediately after anesthetizing and exposing the animals' chest. Two ml of blood sample was collected into the test tube containing anticoagulant EDTA for counting total and differential WBC. The other part of sample was centrifuged at 3500 rpm for 10 min. Supernatants were collected and stored at -80 °C for measurement of MDA, NO, SOD, CAT levels.

The blood sample was stained with Turk solution (1:10 dilution) and total WBC counted in duplicate in a hemocytometer. Differential cell counts were done on a thin slide prepared with a smearing blood sample, using Wright-Giemsa's stain. According to staining and morphological criteria, differential cell

analysis was carried out under a light microscope by counting 100 cells, and the percentage of each cell type was calculated.

Measurement of oxidant biomarkers

Using a Griess reagent, total stable oxidation products of NO metabolism (NO_2-/NO_3-) were measured. The Griess reagent contained of sulfanilamide (SULF) and N-(1-Naphthyl) ethylene diamine dihydrochloride (NEDD). The frozen serum was allowed to thaw and to reach a temperature of 25°C followed by being deproteinized by zinc sulfate solution (Sigma, America). Serum was then centrifuged at 10000 g for 10 min. One hundred twenty microlitre of the clear supernatant was mixed with Griess reagents including 50 µl SULF (2% w/v, Sigma, America) in 5% HCl and 50 µl NEDD (0.1% w/v, Sigma, America) in H_2O in a test tube and incubated at controlled room temperature for 10 min and the absorbance of samples was then measured at 540 nm for measuring NO_2. For the reduction of nitrate to nitrite, 50 µl saturated solutions of vanadium (III) chloride (VCl_3; Sigma, America) in 1 M HCl was added and incubated for 2 hr 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 concentrations linear regression were used from standard curve of $NaNO_2$ and the results were expressed as µM (27).

Malondialdehyde (MDA) levels, as an index of lipid peroxidation reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) which produce a red coloured complex with peak absorbance at 535 nm. Two ml reagent of TBA/trichloroacetic acid (TCA)/HCl was added to 1 ml of serum and the solution was heated in a water bath for 40 min and then cooled. The whole solutions were centrifuged at 1,000×g for 10 min and the absorbance was measured at 535 nm (28). The MDA concentration (C) in nM was calculated as; C= Absorbance/ (1.56×105),

Measurement of antioxidant biomarkers

Superoxide dismutase (SOD) activity was measured as previously described (29) by a colorimetric assay. This assay involved generation of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to its formazan by SOD and was measured at 570 nm. The level of SOD was expressed as unit (U)/ml. The amount of enzyme causing 50% inhibition in the MTT reduction rate was defined as one unit of SOD activity.

Catalase (CAT) activity was also estimated using the previously described method (30) based on determination of the rate constant, k, (dimension: s-1, k) of hydrogen peroxide decomposition. The constant rate of the enzyme was determined using measurement of the decrease in absorbance at 240 nm per min. Activities were expressed as k (constant rate) per litter and expressed as unit (U)/ml (31).

Total thiol concentration was measured using DTNB as the reagent which reacts with the thiols to produce a yellow coloured complex with a peak absorbance at 412 nm. One ml Trisethylene diamine tetraacetic acid (EDTA) buffer (pH 8.6) was added to 50 µl serum in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Twenty microliter DTNB reagents (10 mmol in methanol) were then added to the mixture and keep it in laboratory temperature for 15 min and the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mmol) was calculated using the following equation (32); Total thiol concentration (mmol)= (A2–A1–B)×1.07/0.05×13.6.
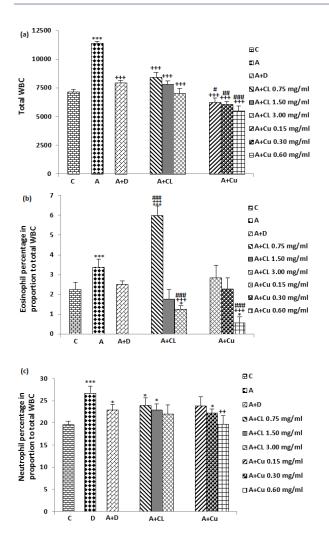
Statistical analysis

The statistical analysis was performed using InStat (GraphPad Software, Inc, La Jolla, USA). All data were presented as mean±SEM. The comparisons between the results of treated groups, un-treated asthma group and control group were performed using one way analysis of variance (ANOVA) with Tukey-Kramer's post-test. The data of three concentrations of extract and curcumin were also compared using ANOVA with Tukey-Kramer's post-test. Significance was considered at *P*<0.05.

Results

The effect of the extract of *C. longa* and its constituent, curcumin on total and differential WBC count

Total WBC count, in the blood of asthma group was significantly higher than those of the control group (*P*<0.001, Figure 1a). Significant and concentration-dependent decrease in total WBC count was seen in asthmatic animals treated with all concentrations of the extract and curcumin compared to untreated asthma group (*P*<0.001 for all cases), (Figure 1a). Dexamethasone treatment also significantly reduced total WBC count compared to asthma group (*P*<0.001, Figure 1a). Reduction of total WBC count in treated groups with all concentrations of curcumin were significantly greater compared to dexamethasone treated group (*P*<0.05 to *P*<0.001), (Figure 1a).

The percentages of eosinophil, neutrophil and monocyte in the blood of asthma group were significantly greater but the percentage of lymphocyte was less than those of the control group (*P*<0.001 for all cases), (Figures 1 and 2). The percentage of eosinophil was significantly reduced in treated asthma groups with highest concentration of the extract (3 mg/ml) and curcumin (0.6 mg/ml), (*P*<0.001 for both cases)
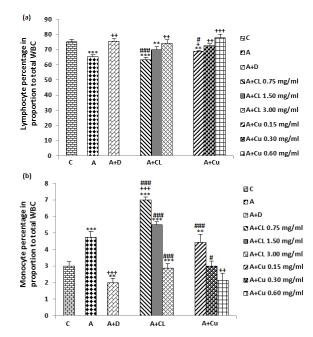
Figure 1. The effect of *C. longa* and its constituent, curcumin on total WBC number (count/ml of blood), (a), percentage of eosinophil (b) and neutrophil (c) in control animals (C), asthma (A) group, A treated with dexamethasone (D), *C. longa* (CL) (for each group, n=8) and curcumin (Cu, n=7). Data are presented as mean±SEM values. *$P<0.05$, ***$P<0.001$; compared to group C. ++ $P<0.01$, +++ $P<0.001$ compared to group A. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ compared to group D. Statistical analyses were performed using one way analysis of variance (ANOVA) with Tukey-Kramer's post-test

Figure 2. The effect of *C. longa* and its constituent, curcumin on percentage of lymphocyte (a) and monocyte (b) in control animals (C), asthma (A) group , A treated with dexamethasone (D), *C. longa* (CL), (for each group, n=8) and curcumin (Cu, n=7). Data are presented as mean±SEM values. *$P<0.05$, **$P<0.01$, ***$P<0.001$; compared to group C. +$P<0.05$, ++$P<0.01$, +++$P<0.001$ compared to group A. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ compared to group D. Statistical analyses were performed using ANOVA with Tukey-Kramer's posthoc test

in comparison with untreated asthma group (Figure 1b). Dexamethasone treatment did not significantly reduced eosinophil percentage compared to untreated asthma group. The effects of highest concentrations of the extract and curcumin on reduction of eosinophil percentage were significantly higher than dexamethasone treated group ($P<0.001$ for both cases), (Figure 1b). The percentage of eosinophil in asthma group treated with low concentration of the extract was significantly higher than control group ($P<0.001$), (Figure 1b).

The percentage of neutrophil in treated group with only highest concentration of curcumin was significantly reduced compared to untreated asthma group ($P<0.01$), (Figure 1c). However, the percentage of neutrophil were significantly higher in A treated groups

with dexamethasone, two lower concentrations of the extract and medium concentration of curcumin compared to the control group ($P<0.05$ for all cases), (Figure 1c).

Compared to asthma group, the percentage of lymphocyte in dexamethasone, highest concentration of the extract ($P<0.01$) and all three concentrations of curcumin ($P<0.05$ to $P<0.001$) were significantly increased (Figure 2a). The change in percentage of lymphocyte due to treatment of low concentration of the extract and curcumin was significantly lower compared to the effect of dexamethasone ($P<0.001$ and $P<0.05$ receptively), (Figure 2a). The percentage of lymphocyte in asthma groups treated with two lower concentrations of the extract and low concentration of curcumin were higher ($P<0.01$ to $P<0.001$) compared to control group (Figure 2a).

Significant decreases were seen in the percentage of monocyte in treated asthma groups with highest concentration of curcumin ($P<0.01$) and dexamethasone ($P<0.001$), (Figure 2b). The percentage of monocyte were significantly higher in treated groups with all concentrations of the extract ($P<0.001$ for all cases) and two lower concentrations of curcumin ($P<0.001$ for low and $P<0.05$ for medium concentration) compared to dexamethasone treated group (Figure 2b). In asthma groups treated with two higher concentrations of the extract ($P<0.001$) and low concentration of curcumin ($P<0.01$), the percentage of monocyte was significantly higher compared to control group (Figure 2b).

Table 1. Comparisons of total and differential WBC count in asthma (A) groups treated with three concentrations of *Curcuma longa* (CL, 0.75, 1.50, and 3.00 mg/ml) and curcumin (Cu, 0.15, 0.30, and 0.60 mg/ml)

Substances	Total WBC (Cunt/ml)	Eosinophil (Percentage)	Neutrophil (Percentage)	Lymphocyte (Percentage)	Monocyte (Percentage)
A+CL 0.75	8400.00±452.37	6.00±0.46	24.00±1.61	63.00±1.82	7.00±0.38
A+CL 1. 50	7806.25±303.17	1.75±0.49 +++	23.00±1.30	69.75±2.08	5.50±0.19 +++
A+CL 3.00	7025.00±412.64 +	1.26±0.16 +++	22.00±2.03	73.87±2.47 ++	2.87±0.29 +++
A+Cu 0.15	6207.14±415.68 **	2.85±0.63 ***	23.85±2.08	68.87±0.80	4.43±0.48 ***
A+Cu 0.30	6042.86±263.32 *	2.28±0.56	22.29±0.80	72.43±1.46	3.00±0.31 ***
A+Cu 0.60	5471.43±426.20	0.57±0.30 +	19.71±1.90	77.57±2.58 ++	2.15±0.40 *** ++

Data were presented as mean±SEM
Comparison of CL vs Cu:* $P<0.05$, **$P<0.01$, *** $P<0.001$ using unpaired t test
Comparison of CL 3.00 and CL 1.50 vs CL 0.75:+ $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ using ANOVA test
Comparison of Cu 0.60 and Cu 0.30 vs Cu 0.15:+ $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ using ANOVA test
Comparison of CL 3.00 vs CL 1.50:# $P<0.05$, ## $P<0.01$, ### $P<0.001$ using ANOVA test
Comparison of Cu 0.60 vs Cu 0.30:#$P<0.05$, ## $P<0.01$, ### $P<0.001$ using ANOVA test
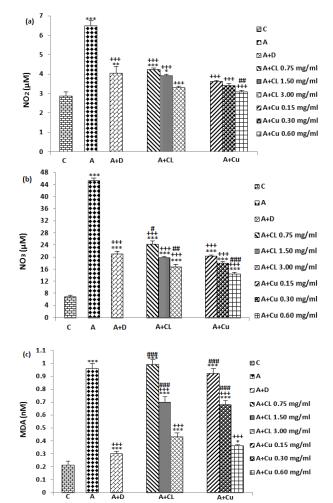
The effects of high concentration of the extract (3.00 mg/ml) on total WBC count, eosinophil, lymphocyte and monocyte as well as the effect of its medium concentration (1.50 mg/ml) on eosinophil and monocyte were significantly higher than its low concentration (0.75 mg/ml, $P<0.01$ to $P<0.001$). The effect of highest concentration of curcumin (0.60 mg/ml) on eosinophil, lymphocyte and monocyte percentage were significantly higher than its lower concentration (0.15 mg/ml, $P<0.05$ to 0.01), (Table 1).

The effects of all concentrations of curcumin on monocyte, its two lower concentrations on total WBC and its lowest concentration on eosinophil were significantly higher than those of the extract concentrations ($P<0.05$ to $P<0.001$), (Table 1).

Effect of the extract of C. longa and its constituent, curcumin on oxidant biomarkers

NO_2, NO_3 and MDA levels were significantly increased in asthma animals compared to control group ($P<0.001$ for all cases), (Figure 3). NO_2 level was significant and concentration-dependently decreased in treated asthma groups with dexamethasone, three concentrations of the extract and curcumin compared to asthma group ($P<0.001$ for all cases), (Figure 3a). The effect of treatment with highest concentration of curcumin on NO_2 level was significantly higher compared to dexamethasone treatment ($P<0.01$, Figure 3a). In treated groups with dexamethasone and two lower concentrations of extract NO_2 level was significantly higher compare to control group ($P<0.05$ to $P<0.001$), (Figure 3a).

The treatment of asthma animals with dexamethasone and all concentrations of the extract and curcumin caused significant reduction in NO_3 level compared to untreated asthma group ($P<0.001$ for all cases). Compared with dexamethasone treated group, NO_3 level was significantly lower in treated group with low concentration of the extract but was higher in treated groups with two higher concentrations of the

Figure 3. The effect of *Curcuma longa* and its constituent, curcumin on NO_2 (a), NO_3 (b) and MDA (C) concentration in control (C), and asthma (A) groups, A treated with dexamethasone (D), *C. longa* (CL) (for each group, n=8) and curcumin (Cu, n=7). Data are mean±SEM values. *$P<0.05$, ** $P<0.01$*** $P<0.001$; compared to group C. +++ $P<0.001$ compared to group A. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ compared to group D. Statistical analyses were performed using ANOVA with Tukey-Kramer's *post-hoc* test

Table 2. Comparisons of various oxidant and antioxidant variables in in asthma (A) groups treated with three concentrations of *Curcuma longa* (CL, 0.75, 1.50, and 3.00 mg/ml) and curcumin (Cu, 0.15, 0.30, and 0.60 mg/ml)

Substances	NO$_2$ (μM)	NO$_3$ (μM)	MDA (nM)	SOD (U/ml)	CAT (U/ml)	Thiol (μM)
A+CL 0.75	4.22±0.09	24.24±0.99	0.99±0.04	0.01±0.002	0.01±0.002	0.05±0.01
A+CL 1. 50	3.90±0.09 +	19.77±0.42 ++	0.70±0.04 +++	0.03±0.002 +++	0.03±0.003 +++	0.09±0.00 +
A+CL 3.00	3.30±0.08 +++ ###	16.73±0.88 +++ #	0.43±0.03 +++ ###	0.06±0.001 +++ ###	0.08±0.001 +++ ###	0.20±0.01 +++ ###
A+Cu 0.15	3.60±0.08 ***	20.25±0.42 **	0.92±0.04	0.03±0.003 **	0.02±0.002	0.07±0.00
A+Cu 0.30	3.41±0.10 **	17.99±0.50 +	0.68±0.03 +++	0.04±0.002 ++	0.04±0.002 +++	0.12±0.01 +
A+Cu 0.60	3.07±0.10 ++	14.34±0.49 +++ ###	0.36±0.01 +++ ###	0.07±0.002 *** +++ ###	0.09±0.002 +++ ###	0.25±0.01 * +++ ###

Data were presented as mean±SEM
Comparison of CL vs Cu: *P<0.05, ** *P*<0.01, *** *P*<0.001 using unpaired t test
Comparison of CL 3.00 and CL 1.50 vs CL 0.75:+ *P*<0.05, ++ *P*<0.01, +++ *P*<0.001 using ANOVA test
Comparison of Cu 0.60 and Cu 0.30 vs Cu 0.15: + *P*<0.05, ++*P*<0.01, +++ *P*<0.001 using ANOVA test
Comparison of CL 3.00 vs CL 1.50: # *P*<0.05, ##*P*<0.01, ### *P*<0.001 using ANOVA test
Comparison of Cu 0.60 vs Cu 0.30: # *P*<0.05, ## *P*<0.01, ### *P*<0.001 using ANOVA test

extract and all concentrations of curcumin (*P*<0.05 to *P*<0.001), (Figure 3b).

MDA level in treated groups with dexamethasone, two higher concentrations of the extract and curcumin was significantly decreased compared to untreated asthma group (*P*<0.001 for all cases). The effect of two lower concentrations of extract and curcumin on MDA level was significantly lower compared to dexamethasone treated group (*P*<0.001 for all cases). MDA level in treated groups with dexamethasone, all concentrations of the extract and curcumin were significantly higher compared with control group (*P*<0.001 for all cases), (Figure 3c).

The effect two higher concentrations of extract (1.50, 3.00 mg/ml) on NO$_2$, NO$_3$ and MDA level were significantly higher than its low concentration (0.75 mg/ml), (*P*<0.05 to *P*<0.001). In addition, the effect of high concentration of extract (3.00 mg/ml) on NO$_2$, NO$_3$ and MDA level was higher than its medium concentration (1.50 mg/ml), (*P*<0.001). The effect of two higher concentrations of curcumin (0.30. 0.60 mg/ml) on NO$_3$ and MDA level and its high concentration on NO$_2$ level were significantly higher than its low concentration (0.15 mg/ml), (*P*<0.05 to *P*<0.001). A significant difference was seen between high and medium concentrations of curcumin (0.30, 0.60 mg/ml) on NO$_3$ and MDA levels (*P*<0.01). In addition, The effect of low concentration of the extract on NO$_2$ and NO$_3$ levels and its medium concentration on NO$_2$ level was statistically lower compared to corresponding concentrations of extract (*P*<0.01 to *P*<0.001), (Table 2).
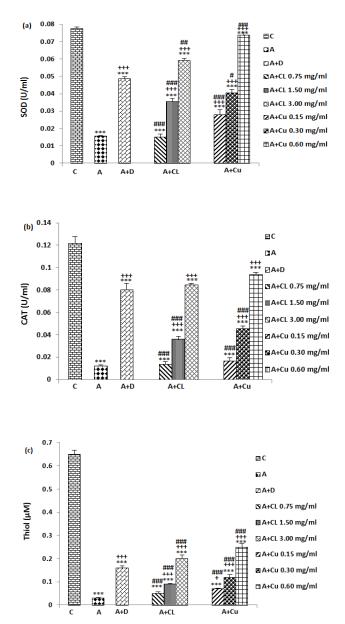
Effect of the extract of *C. longa* and its constituent, curcumin on antioxidant biomarkers

Compared with control group, SOD, CAT and thiol levels were significantly decreased in asthma group (*P*<0.001 for all cases), (Figure 4). SOD level significantly and concentration-dependently increased in asthma groups treated with all concentrations of

curcumin and the extract compared to untreated asthma group (*P*<0.001 for all cases). Dexamethasone treatment also significantly enhanced SOD level compared to asthma group (*P*<0.05 to *P*<0.001), (Figure 4a). Although the effects of treatment with two lower concentrations of curcumin and dexamethasone on SOD level were lower, the effect of treatment with highest concentration of the extract and curcumin on these markers was significantly higher compared to the effect of dexamethasone treatment (*P*<0.001 for all cases), (Figure 4a). In addition, SOD level was significantly lower in treated groups with dexamethasone, all concentrations of the extract and curcumin compared to control group (*P*<0.001 for all cases), (Figure 4a).

Treatment with dexamethasone, two higher concentrations of the extract and curcumin caused significant increase in CAT level compared to untreated asthma group (*P*<0.001 for all cases), (Figure 3b). The effect of treatment with two lower concentrations of the extract and curcumin on CAT level was significantly less than dexamethasone treatment (*P*<0.001 for all cases), (Figure 4b). However, CAT level in treated groups with dexamethasone, all concentrations of the extract and curcumin was significantly lower than control group (*P*<0.001 for all cases), (Figure 4b).

A concentration-dependent and significant increase of thiol level was observed in treated groups with two higher concentrations of the extract, all concentrations of curcumin and dexamethasone compared to untreated asthma group (*P*<0.05 for low concentration of curcumin and *P*<0.001 for other cases), (Figure 4c). Thiol level in treated groups with two lower concentrations of extract and curcumin was significantly lower (*P*<0.001 for all cases), but, in treated groups with highest concentration of the extract and curcumin was higher (*P*<0.001 for both cases) compared to dexamethasone treated group (Figure 4c). The concentration of total thiol in asthma groups, treated with dexamethasone, all concentrations of the extract and curcumin was significantly lower compared

Figure 4. The effect of *Curcuma longa* and its constituent, curcumin on serum levels of SOD (a), CAT (b) and Thiol (c) in control (C) and asthma groups (A), A treated with dexamethasone (D), *C. longa* (CL), (for each group, n=8), and curcumin (Cu, n=7). Data are mean ± SEM values. ***$P<0.001$; compared to group C. **+** $P<0.05$, +++$P<0.001$ compared to group A, #$P<0.05$, ## $P<0.01$, ### $P<0.001$ compared to group D. Statistical analyses were performed using ANOVA with Tukey-Kramer's post-test

to control group ($P<0.001$ for all cases), (Figure 4c).

The effect of two higher concentrations of extract and curcumin on SOD, CAT and thiol levels were significantly higher than their low concentration ($P<0.05$ to $P<0.001$). In addition, the effect of high concentration of the extract and curcumin on SOD, CAT and thiol levels were higher than its medium concentration ($P<0.001$ for all cases). The effects of high and low concentrations of curcumin on thiol and SOD levels were significantly higher than

corresponding concentrations of the extract ($P<0.01$ for low and $P<0.001$ for high concentration), (Table 2).

Discussion

C. longa a medicinal plant native to the Indian subcontinent is known to possess anti-inflammatory and antioxidant properties. In the present study, the effect of the hydro-ethanolic extract of *C. longa* and its constituent, curcumin on total and differential WBC count, and levels of oxidant (NO, MDA) and antioxidant (CAT, SOD and thiol) biomarkers in serum of a rat model of asthma were investigated.

Oxidative stress occurs as a result of the production of ROS in consequence of aerobic metabolism. Inflammatory cells such as activated eosinophils, neutrophils, monocytes, and macrophages can generate ROS in response to various stimuli (33). Previous evidences indicating increased oxidative stress is a major feature of many airway diseases, such as ischemia-reperfusion, cystic fibrosis, chronic obstructive pulmonary disease, and asthma (34). Studies indicated that oxidative stress is an important consequence of lung inflammation in asthma which contributes in induction of airway hyper responsiveness (9), and correlate to antioxidant capacity in the lung and blood (7).

The results of the present study showed increased total WBC, eosinophil, neutrophil and monocyte, but reduction of lymphocyte count in asthma compared to control animal. The findings of the present study indicate the induction of an asthma model (sensitization) in rat. Increased total WBC and eosinophil count was shown in asthmatics, both in animal and human studies compared to healthy controls (35, 36). The reduction of lymphocyte percentage in sensitized rat is in fact due to increased total WBC number. In fact, increased absolute number of the lymphocyte was shown in sensitized animals using similar method of sensitization (37). Increased lymphocyte percentage in treated groups is also due to reduction of total WBC count. Treatment with the extract, curcumin and dexamethasone reduced total WBC, eosinophil, neutrophil and monocyte but increased lymphocyte count. In fact, a previous study showed the effect of the extract of *C. longa* on total and differential WBC count in asthmatic rat, which support the results of the present study (13). Reduction of eosinophil, neutrophil and monocyte in asthma group treated with *C. longa* extract and curcumin observed in the present study, suggested the anti-inflammatory effect of the plant. Therefore, this plant may have preventive effect on asthma by reduction of inflammatory cells and airway inflammation. The absent of the effect of the treatment of low concentration of the extract on eosinophil may indicate that this concentration of the extract is not effective on this cell type. However, treated with two

higher concentrations resulted in reduction of eosinophil count which showed the effect of the plant on eosinophilic inflammation in asthma.

Findings showed that NO_2 and NO_3, the oxidative products of NO significantly increased in asthma group which also suggest the induction of animal model of asthma in rat. Recent studies has demonstrated that NO is present in exhaled air and that its level is increased in asthma and chronic obstructive pulmonary disease (38). NO is produced via oxidation of L-arginine by nitric oxide synthases enzyme. In the lung, NO acts as a vasodilator, bronchodilator and non-adrenergic non-cholinergic (NANC) neurotransmitter and is an important mediator which may reinforce the inflammatory response in asthma (39). NO is a by-product of airway inflammation and tissue damage and a probable index of oxidative stress in the airway diseases (40). NO has an inhibitory effect on the Th1 and interferon gamma (IFN-γ). This would lead to the increase in the number of Th2 cells and its cytokines, interleukin 4 (IL-4) and IL-5, which augment the inflammatory response (41). NO in air and in fluids of the respiratory tract could cause to a variety of more reactive and thus more toxic nitrogen oxides (42).

Treatment with dexamethasone as positive control, all concentrations of the extract and curcumin significantly decreased NO level. Previous study showed that curcumin treatment normalized the elevated nitrate in asthma rat (13). These results indicated the antioxidant and anti-inflammatory property of the plant and the mechanism of this effect may be mediated through inhibition of nitric oxide synthases enzyme.

The levels of MDA as an oxidant marker of lipid peroxidation significantly increased in asthmatic group which also support the sensitization of animals. A positive correlation was reported between ROS and MDA level (43). Oxidative stress is associated with an increase MDA and protein carbonyls in both BAL fluid and peripheral blood in asthma (4). In patients with different airway diseases including asthma, COPD, and bronchiectasis increased MDA levels in biological fluids was observed (44). MDA was significantly higher in asthmatic patients, and its level was higher during the acute attack of asthma (45).

MDA level was significantly reduced in treated groups with two higher concentrations of extract and curcumin. Therefore, treatment with the extract and curcumin can improve bronchial asthma during an acute asthma attack by reduction of oxidative biomarkers. Decreased MDA level was found in asthmatic animals after treatment with curcumin previously, which supports the findings of the present study (13).

The serum SOD level also decreased in asthma group in the present study. The main antioxidants enzymes in the lungs are SOD, CAT, and glutathione peroxidases as well as heme oxygenase-1, thio-redoxins, peroxiredoxins, and glutaredoxins (46). Since superoxide radical is the primary oxidant generated from a variety of sources, its dismutation by SOD. H_2O_2 that results from the action of SOD is reduced to water by catalase and the GSH-Pxs. SOD activity has been reported to be decreased (10), increased (47) or unchanged (48) in asthmatics as compared to controls.

In the present study, the SOD activity increased in asthma groups treated with two higher concentrations of the extract and all concentrations of curcumin in a dose-dependent manner. Increased SOD activity was seen in human hepatocyte L02 cell line after treatment with curcumin (49), which confirms the findings of the present study. The loss of SOD activity probably reflects the increased oxidative stress in asthma. Thus, this plant which enhanced SOD activity could be effective in improvement of asthma symptom.

In the present study, the CAT activity significantly reduced in untreated asthma group. CAT is a metalloprotein enzyme and the main scavenger of H_2O_2. Both animal and human studies have indicated that CAT activity in BAL fluid of asthmatics reduced compared to healthy controls (50). Decrease in CAT activity probably amplifies oxidative stress, contributing to the chronic inflammatory state of the asthmatic airway (51). Two higher concentrations of extract and curcumin reversed CAT activity in treated groups. Other study have shown that curcumin increased CAT activity and the hepatic total antioxidant capacity in liver from rats with hepatic damage and high level of oxidative stress induced by thallium acetate (52). These results showed that *C. longa* and its constituent, curcumin with antioxidant property are able to inhibit inflammatory responses in asthma.

Results of this study showed that thiol concentration significantly decreased in asthmatic rat which also confirm the induction of animal model of asthma. Thiols are potent antioxidants which protect cells against the oxidative stress. The most thiol antioxidant in the epithelial lining fluid is the tripeptide glutathione (GSH), which is a multifunc-tional intracellular antioxidant and is noticed to be the main thiol-disulphide redox buffer of the cell (53). The antioxidant capacity of thiol compounds is related to the sulphur atom, which can easily accommodate the loss of a single electron (54). GSH is also vital for airway innate immune defense and regulation alveolar macrophage apoptosis and phagocytosis of infectious particles (55). GSH depletion further leads to activation of NF-κB and enhanced pro-inflammatory gene transcription (56).

Treatment with various concentrations of curcumin and extract significantly increased thiol level. The reduced level of glutathione (GSH) in a lung carcinogenesis model induced by benzo (a) pyrene (a major carcinogenic pollutant) in mice was increased by curcumin (57). These findings also indicated antioxidant property of the plant and its constituent curcumin in asthma.

The effects of the extract and its constituent curcumin were concentration dependent. Eosinophil, lymphocyte and monocyte improvement due to high concentration of the extract and curcumin, total WBC due to high curcumin concentration and eosinophil and monocyte due to medium curcumin concentration were significantly higher than their low concentration. The effects of two higher concentrations of the extract and curcumin in almost all oxidant and antioxidant markers were significantly higher than their low concentration. The effects of the highest concentration of the extract and curcumin on most oxidant and antioxidant markers were also higher than the effects of their medium concentration. The concentration dependency effect of the extract and its constituent also support the anti-inflammatory and antioxidant effect of the extract of *C. longa* and its constituent, curcumin on rat animal model of asthma.

The results of current study also showed that the effects of all concentrations of curcumin on monocyte percentage, the effects of its two lower concentrations on total WBC and the effect of its low concentration on eosinophil percentage were significantly higher than the effects of corresponding concentrations of the extract. In addition, the effect of low concentration of curcumin on NO_2, NO_3 and SOD, the effect of its medium concentration on NO_2 and the effect of its high concentration on SOD and thiol values were higher than the effects corresponding extract concentrations. Therefore the effects of curcumin on total and differential WBC as well as oxidant and antioxidant markers were higher than the extract of the plant at studied concentrations. The curcumin concentrations used in the present study were one fifth of those of the extract of *C. longa*. However, the curcumin concentration in the pure powder is 31.9% (58), which is higher than the concentrations used in the present study. Therefore, the effect of the extract of the plant on total and differential WBC as well as on oxidant and antioxidant markers on animal model of asthma is suggested to be due to its constituent, curcumin.

The effect of the two lower concentrations of the extract and curcumin on most measured variables were lower than the effect of dexamethasone treatment or there was no significant difference between the effect of dexamethasone and the extract and curcumin treatment except for the effect of the first concentration of curcumin on total WBC count which was significantly higher than the effect of dexamethasone. However, the effects of highest concentration of the extract and curcumin were not significantly different or even higher (on most variables) than the effect of dexamethasone treatment except for the effect of highest concentration of the extract on monocyte percentage. These results also show comparable or even higher anti-inflammatory and antioxidant effect of the highest concentration of the extract of *C. longa* and its constituent, curcumin on rat animal model of asthma compared to the effect of dexamethasone.

Conclusion

The results of the present study indicated a preventive effect of *C. longa* and its constituent curcumin on total and differential WBC, serum levels of NO_2, NO_3, MDA, CAT and thiol group of animal model of asthma which is comparable to the effect of dexamethasone at used concentrations. Therefore, the results suggest a preventive therapeutic potential for *C. longa* and its constituent, curcumin on inflammatory cells and oxidative stress in asthma.

Acknowledgment

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