



Intranasal curcumin and its evaluation in murine model of asthma

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ABSTRACT

Curcumin, a phytochemical present in turmeric, rhizome of *Curcuma longa*, has been shown to have a wide variety of pharmacological activities including anti-inflammatory, anti-allergic and anti-asthmatic properties. Curcumin is known for its low systemic bioavailability and rapid metabolism through oral route and has limited its applications. Over the recent decades, the interest in intranasal delivery as a non-invasive route for drugs has increased as target tissue for drug delivery since nasal mucosa offers numerous benefits. In this study, we evaluated intranasal curcumin following its absorption through nasal mucosa by a sensitive and validated high-performance liquid chromatography (HPLC) method for the determination of intranasal curcumin in mouse blood plasma and lung tissue. Intranasal curcumin has been detected in plasma after 15 min to 3 h at pharmacological dose (5 mg/kg, i.n.), which has shown anti-asthmatic potential by inhibiting bronchoconstriction and inflammatory cell recruitment to the lungs. At considerably lower doses has proved better than standard drug disodium cromoglycate (DSCG 50 mg/kg, i.p.) by affecting inflammatory cell infiltration and histamine release in mouse model of asthma. HPLC detection revealed that curcumin absorption in lungs has started after 30 min following intranasal administration and retained till 3 h then declines. Present investigations suggest that intranasal curcumin (5.0 mg/kg, i.n.) has effectively being absorbed and detected in plasma and lungs both and suppressed airway inflammations at lower doses than the earlier doses used for detection (100–200 mg/kg, i.p.) for pharmacological studies (10–20 mg/kg, i.p.) in mouse model of asthma. Present study may prove the possibility of curcumin as complementary medication in the development of nasal drops to prevent airway inflammations and bronchoconstrictions in asthma without any side effect.

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1. Introduction

Asthma is one of the most common chronic inflammatory diseases. As per latest report from World Health Organization, about 300 million people worldwide have suffered from asthma [1]. It is characterized by airway inflammation, with intermittent episodes of wheezing and coughing [2,3]. Inhaled corticosteroids provide one of the most effective therapies, but this method has severe side effects after long-term use [4,5] such as hypertension, cataracts, osteoporosis in elderly patients and stunted growth in children. However, inhaled corticosteroids are still required in the systemic administration of glucocorticoids for intractable asthma [6,7].

It is well established that mast cells play a key role in the pathogenesis of allergic diseases including asthma [8,9]. Mast cells participate in airway inflammation by IgE-receptor (FcεRI) cross-linking with exposed antigen, secreting variety of pro-inflammatory mediators

including histamine, serotonin, eicosanoids and platelet activating factors as well as various cytokines and chemokines [10–12]. Among the released mediators, histamine from mast cells and peroxidases (EPO) released from eosinophils are one of the most important chemical mediators in the allergic reactions, which are responsible for airway constriction leading to difficulty in breathing. Another most striking feature is the accumulation of large number of inflammatory cells in perivascular and peribronchial region of the lung parenchyma. These inflammatory cells include lymphocytes, macrophages, neutrophils with strikingly large number of eosinophils. Local allergen challenge in patients with bronchial asthma [13], allergic conjunctivitis [14] and atopic dermatitis [15] has been shown to result in rapid elevation of prostaglandin D2 (PGD2) level in nasal and bronchial lavage fluids, tears and skin chamber fluids. PGD2 is the major cyclooxygenase metabolite produced by mast cells responding to IgE-dependent stimuli [16].

Recent years have seen the development of highly targeted biological treatment and synthetic therapies with some serious side effects. Natural medicines obtained from plants have been used in

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therapeutics from ancient times as these have the benefit to have minimal toxicity. Thus, natural treatments as complementary medicines have drawn the attention of the scientific community to ancient remedies. Curcumin, a phytochemical present in turmeric, the rhizome of *Curcuma longa*, has been shown to have a wide variety of pharmacological activities including anti-allergic and anti-inflammatory properties [17]. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione], being a phenolic substance, is used as a dietary spice and natural colouring agent for foods. Its yellow colour is imparted primarily by curcumin (diferuloyl methane) and polyphenolic pigment [18]. In recent years, it has attracted interest because of its antioxidant, anti-inflammatory and potential cancer chemopreventive activities [19]. Studies have suggested that curcumin has downregulated Th2 responses and reduced lung inflammation in latex sensitized mice suggesting possible role of curcumin in controlling allergic disorders [20,21].

In common with several other diet-derived polyphenols, curcumin has low systemic bioavailability [22]. This pharmacokinetic feature of curcumin, which has been observed across several species, is the result of poor absorption and avid metabolic conjugation and reduction [23]. Despite the evidence that curcumin is poorly available following oral administration, there are reports that curcumin at 50–200 mg/kg doses exerts biological activity on sites distant from the locus of absorption in rodents, such as breast [24–26], prostate, lung and especially the liver [27–29]. Studies on gall

bladder contractility following oral curcumin in humans suggest that curcumin may exert biological actions at doses of 20 mg [30–33].

Several studies have suggested poor bioavailability of curcumin in both rodents and humans despite the promising biological effects that have been observed (Fig. 1) [34,35]. Pan et al. [36] investigated the pharmacokinetic properties of curcumin administered either orally or intraperitoneally (i.p.) in mice. With oral administration of 1.0 g/kg of curcumin, low plasma levels of 0.13 µg/ml appeared after 15 min, while a maximum plasma level of 0.22 µg/ml was obtained at 1 h; plasma concentrations declined then below detection limit by 6 h. Entirely different plasma curcumin levels were found after i.p. administration of 0.1 g/kg. Plasma curcumin levels peaked (2.25 µg/mL) within 15 min of administration and declined rapidly within 1 h. An investigation reported that it was difficult to detect curcumin in plasma following 180 mg oral dose [35]. Poor absorption through oral route could be due to its extremely low aqueous solubility and/or extensive pre metabolism [37]. Earlier studies reported that nasal cavity may be exploited as a route of entry into systemic circulation since nasal mucosa is highly vascular and increases the absorption of the drug [38,39].

Curcumin is unstable at neutral and basic pH values and is degraded to ferulic acid ([4-hydroxy-3-methoxycinnamic acid]) and feruloylmethane (4-hydroxy-3-methoxycinnamoyl-methane) [34]. In the course of investigations, it was found that more than 90% of curcumin decomposes rapidly in buffer systems at neutral basic

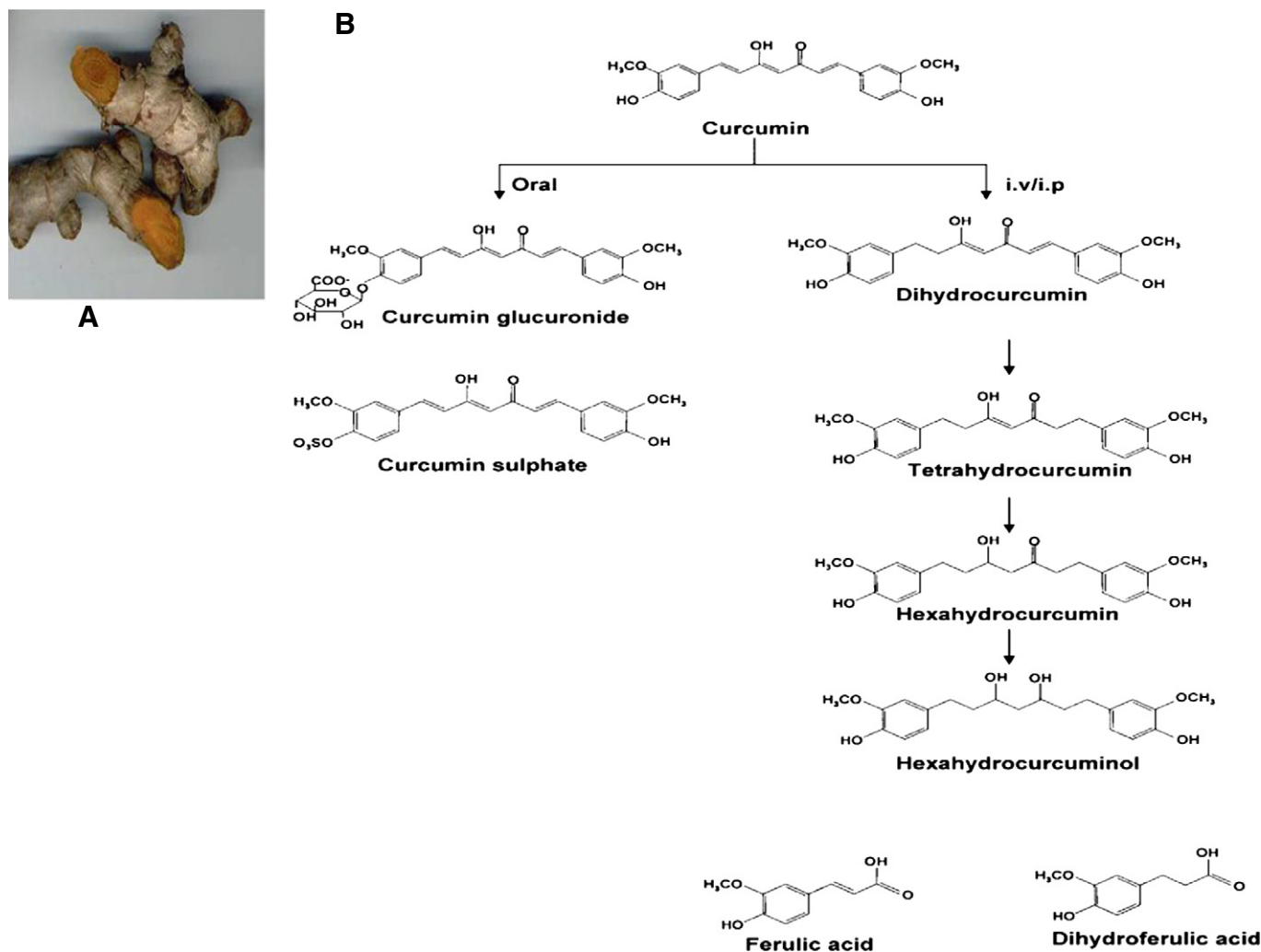


Fig. 1. (A) Rhizome of curcumin. (B) Structure and its metabolites (Anand et al. [34]).

pH conditions. Oetari et al. (1996) [40], using spectrophotometry, also found that curcumin is unstable in phosphate buffer at pH 7.4. Although the exact mechanism of degradation is not clear yet, it appears to occur through an oxidative mechanism because the presence of antioxidants such as ascorbic acid, *N*-acetylcysteine, or glutathione completely blocks the degradation of curcumin at pH 7.4. The effect of pH on metabolites of curcumin has not been investigated previously. Curcumin should be stable in the stomach and small intestines because the pH is between 1 and 6, and degradation of curcumin is extremely slow in these conditions [36]. The normal pH of nasal mucosa is 5.5 to 6.5, and therefore it can be detected in plasma and could reach to lungs without degradation.

The disposition of curcumin in blood plasma and lung tissue following intranasal administration is unknown in rodents and human both. Therefore, we aimed to determine achievable levels of curcumin in mouse plasma following nasal administration. In particular, lungs are the affected organ in airway inflammations, and due to highly vascular nature of nasal mucosa, the idea is to study the absorption of curcumin directly to the lungs for suppressing airway inflammations in asthma. Analytical methods to determine curcumin concentrations in biological samples has been reported, which include liquid chromatography (LC) with UV [41,42] and tandem mass spectrometric detection [43,44]. In the present study, we have evaluated curcumin in mouse plasma and lung tissue after intranasal administration. Earlier studies have detected curcumin in plasma after intraperitoneal injection at higher doses (100–200 mg/kg, i.p.) other than therapeutic dose (10–20 mg/kg, i.p.), but here curcumin has been detected in plasma and lungs both at same therapeutic dose (5 mg/kg, i.n.), where it has shown anti-inflammatory potential by inhibiting bronchoconstriction and airway inflammation both. Inhibition in histamine and eosinophil peroxidase (EPO) release as markers of inflammation has been observed at similar doses.

2. Methods

2.1. Reagent and Apparatus for HPLC method

Curcumin (purity $\geq 99.5\%$), ovalbumin (grade V) and acetonitrile (high-pressure liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Aluminium hydroxide gel was obtained from Pierce Chemical Company (Rockford, IL). Balb/c mice (6–8 weeks old; 20–22 g) were procured from Central Drug Research Institute, Lucknow, India, and acclimatized for a week under standard laboratory conditions. Experimental protocols were approved by the Central Animal Ethical Committee, Banaras Hindu University, Varanasi, India. HPLC was performed by Hamilton CECIL-UV detector (CECIL Ce 420) with variable w/L detector HX SII9hydrogen silicon) C18 column (25 cm $\times$ 4.6 mm, 5 μ m); mobile phase, 80% methanol with 20% water; flow rate 1.0 ml/min; sample detection was achieved at wavelength, 420 nm, and injection volume was 20 μ l. Calibration curves were prepared over the range from 10 μ g/ml to 50 μ g/ml. The amount of curcumin in the sample was determined from the peak area using a calibration curve constructed of standard curcumin.

2.2. Preparation of Stock and calibration samples

Five hundred microliters of plasma was taken with working stock of curcumin 100 μ g/ml (curcumin in solvent system methanol–water) 80:20 ratio. Then various concentrations were prepared accordingly, ranging from 0 to 50 μ g/ml in extracting reagent (ethyl acetate 95%–methanol 5%) in equal ratio with the plasma and then centrifuged for 15 min for each sample. Two hundred microliters of supernatant was collected, and organic solvent was evaporated. Then filtered (0.45 μ m) mobile phase (80:20) methanol–water was added, and

HPLC was performed. The retention time was found to be 2.57 min. All described solutions were protected from light and stored at 4 °C. The system was run isocratically at a flow rate of 1 ml/min. Sample detection was achieved at 420 nm, and injection volumes were 20 μ l. Calibration curves over the range of 0 to 50 μ g/ml were established for the quantitation of curcumin. Curcumin was given at a dose of 5 mg/kg, i.n., and at 20 mg/kg, i.p., respectively. Blood samples were collected via orbital plexus from each mice at each time point, ranging from 15 min to 3 h post dosing. Plasma was obtained from blood (treated with 0.2 mg/ml heparin) after centrifugation at 1500 rpm for 15 min. The resulting plasma was deproteinized by mixing it with a two volumes of acetonitrile. The lungs were removed from euthanized mice at the same time points and were collected, weighed and then minced with scissors and homogenized in phosphate buffer and sonicated in PBS and centrifuged for 20 min at 12000 rpm. The curcumin was extracted by adding acetonitrile: PBS (1:1 v/v). After centrifugation, the supernatants were subjected to HPLC analysis. Results were normalized to protein level. The curcumin levels were analysed by HPLC.

2.3. Development of experimental mouse model

Mice were randomly divided into different groups (5 mice/group), and each group was named according to the status of sensitization/challenge/treatment. They were Sham/Sal/Veh (saline-sensitized, saline-challenged and vehicle-treated), OVA/OVA/VEH, OVA/OVA/CUR 2.5 mg/kg and 5.0 mg/kg (nasal drops were delivered and animals were kept in supine position for a few minutes), 10 mg/kg (i.p. route) and DSCG (50 mg/kg,i.p.). Mice were sensitized with 0.2 ml saline suspension containing 10 μ g ovalbumine (OVA) and 2 mg aluminium hydroxide in saline on days 0, 7 and 14. From days 19 to 22, mice were subjected to aerosol of ova (1%) inhalation 30 min daily (Fig. 2) in a Plexiglas chamber by nebulizer (Omran, Japan) with an airflow rate 9 l/min. Control mice received i.p. injections of 0.2 ml saline containing 2 mg alum and were challenged with saline alone. Curcumin was dissolved in dimethyl sulphoxide (DMSO) and given an hour before every ova challenge (days 19 to 22). Vehicle (DMSO) was also given to OVA sensitized mice in a similar volume as curcumin has been used.

2.4. Collection of bronchoalveolar lavage (BAL) fluid and lung histology

Mice were sacrificed after 24 h of OVA-aerosol challenge. Trachea was cannulated by a small incision on ventral skin of the neck region. Lungs were washed three times with PBS in total volume of 1 ml. The BAL fluid was centrifuged for 5 min at 2000 rpm at 4 °C. Cell pellet was used to count total inflammatory cell, especially eosinophils, neutrophils, mast cells and lymphocytes. The supernatant was removed and used for analysis of histamine content released from airway mast cells. For histological analyses, mice were sacrificed 24 h after the final challenge. Lungs and trachea were washed with 10% of neutral buffer formalin (NBF) before removal and then fixed. Lung tissues were embedded in paraffin, cut into sections of 5 μ m thickness and stained with hematoxylin–eosin (H&E). The slices were evaluated via light microscopy under similar conditions. The degrees of peribronchial and perivascular lung inflammation was scored with having special reference on inflammatory cell types recruited to the lungs.

2.5. Total inflammatory cell count

Total cell numbers were counted by hemocytometer. Smears of bronchoalveolar lavage (BAL) cells prepared and were stained with giemsa for differential cell count. Two independent, blinded investigators counted the cells using a microscope. Randomly, three locations were selected for differential cell count.

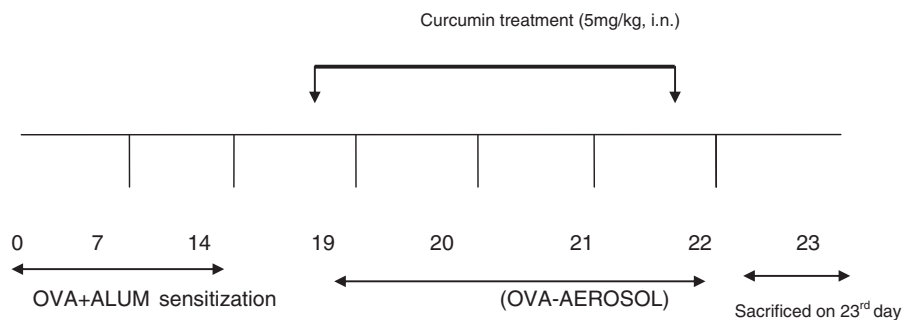


Fig. 2. Schematic diagram of protocol for sensitization and airway challenge.

2.6. Immunofluorescence (IF) study for detection of eosinophil granule major basic protein (MBP) as eosinophil recruitment marker

Pulmonary eosinophilia is one of the distinguishing features of asthma. To detect eosinophils recruitment to the lungs of asthmatic mice, immunostaining for eosinophil major basic protein was adapted from Stelts et al. [45]. Lungs were fixed in 10% neutral buffer formalin for 24 h and then processed. In order to free up any formalin fixed epitopes, the slides were incubated in 0.1% trypsin and 0.1% CaCl_2 (pH 7.8) at 37°C for 5 min. Sections were rinsed with 0.05% PBST two times. Slides were then overlaid with 10% normal goat serum for 2 h at 37 °C and incubated overnight with 1:500 rat anti-mouse major basic protein or normal rat IgG (as a negative control) for 24 h. Again, the sections were washed with PBST five times and then treated with 1% chromotrope 2R (Loba) for 30 min at room temperature to prevent non specific staining of eosinophils with fluorescein. The sections were then washed and overlaid with fluorescein-labeled goat anti-mouse IgG (Invitrogen) for 30 min. Slides were washed and mounted in 50% glycerol and covered with coverslip and observed under a fluorescent microscope (Nikon).

2.7. Measurement of histamine in BALF

Histamine was measured in the BALF supernatant as discussed previously with slight modification [46]. Briefly, 90 μl of supernatant was mixed with 90 μl of 0.1 M HCl, 36 μl of 1 M NaOH and 9 μl of OPT (O-phthalaldehyde) at room temperature. After 7 min, the reaction was stopped by adding 18 μl of 3 M HCl. Samples were transferred to a flat bottom 96 well black microplate. Fluorescence intensity was measured using 360-nm excitation filter and 460-nm emission filter. Values are expressed as percentage of net histamine release.

2.8. Measurement of EPO activity in BALF

The level of EPO in BALF supernatant was determined by taking 100 μl of substrate solution (0.1 mM O-phenylene-diamine-dihydrochloride, 0.1% Triton-X 100, 1 mM hydrogen peroxide in 0.05 M Tris HCl) to 100 μl of supernatant in microtiter plates and incubated for 30 min at 37 °C. The reaction was stopped by adding 50 μl of 4 M sulphuric acid, and optical density was read at 490 nm. This method has been shown specific for peroxidase activity of eosinophils.

2.9. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

The liver function test was performed in order to test the toxicity of intranasal curcumin. In this test, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were observed by modified Reitman and Frankel's colorimetric DNPH method by standard kits (Avecon).

2.9.2. Measurement of creatinine level

For the kidney function test, creatinine was measured by using creatinine reagent kit (Beacon). The principle was based on the formation of orange coloured complex by the reaction of picric acid with creatinine. The intensity of colour formed is directly proportional to the amount of creatinine present and measured at 490 nm.

3. Results

3.1. HPLC-UV conditions and stability

An appropriate wavelength was important for the detection of curcumin since it has special conjugation structure (Fig. 1), which leads to strong UV absorption at wavelength of 420 nm. Therefore, detection wavelength was set at 420 nm. Internal standard was used in between to compensate for extraction variation, efficiency and extraction errors (results not shown here). Methanol was selected as organic phase, and according to chemical structure of curcumin, appropriate concentrations of acetic acid were added to get good peaks. Calibration curves were constructed by plotting the peak area ratios (curcumin standards 0–50 $\mu\text{g}/\text{ml}$) of normal plasma verses nominal concentrations and five different lots of plasma were linear and in the range (Fig. 3). Bench-top stability was assessed to ensure that curcumin has not been degraded in plasma samples at room temperature for a time period to cover sample preparation and by exposing them to normal laboratory conditions. Freeze–thaw stability was also considered during analysis. All stability samples were analysed and compared with freshly prepared samples used for therapeutic studies (results not shown here).

3.2. Intranasal curcumin inhibits bronchoconstriction and inflammation (H&E staining)

Histological analyses revealed typical pathological features in OVA-challenged mice showing numerous inflammatory cells around the bronchioles. As shown in Fig. 4, lungs of mice without sensitization and challenge with OVA (only saline) showed normal bronchioles and epithelial lining as normal lung morphology without any bronchoconstriction and inflammation, whereas lungs of OVA sensitized/challenged mice showed clear cut bronchoconstriction as both the bronchiolar walls with reduced lumen volume and significant inflammation with large population of inflammatory cells including eosinophils. As curcumin was dissolved in DMSO, OVA/OVA/DMSO treated lungs were used to study where no inhibition in bronchoconstriction and anti-inflammatory effect of DMSO was observed. Curcumin administrations at 2.5 mg/kg and 5.0 mg/kg (i.n.) have shown significant reduction in bronchoconstriction with good lumen volume and decreased number of eosinophils with significant reduction in inflammatory cell infiltration in the peribronchiolar and perivascular regions.

3.3. Curcumin suppresses total leukocyte count and infiltration of inflammatory cells to the lungs

Total number of inflammatory cells including eosinophils were counted from the BALF of curcumin-treated and control mice as per

experimental protocol. As shown in Fig. 5, OVA-treated mice show a rise in the total number of leukocytes and eosinophils in BALF and were reduced in curcumin treated groups. Values are expressed as mean $\pm$ SEM, $n = 5/\text{group}$, and were reproducible in experiments ($p < 0.05$).

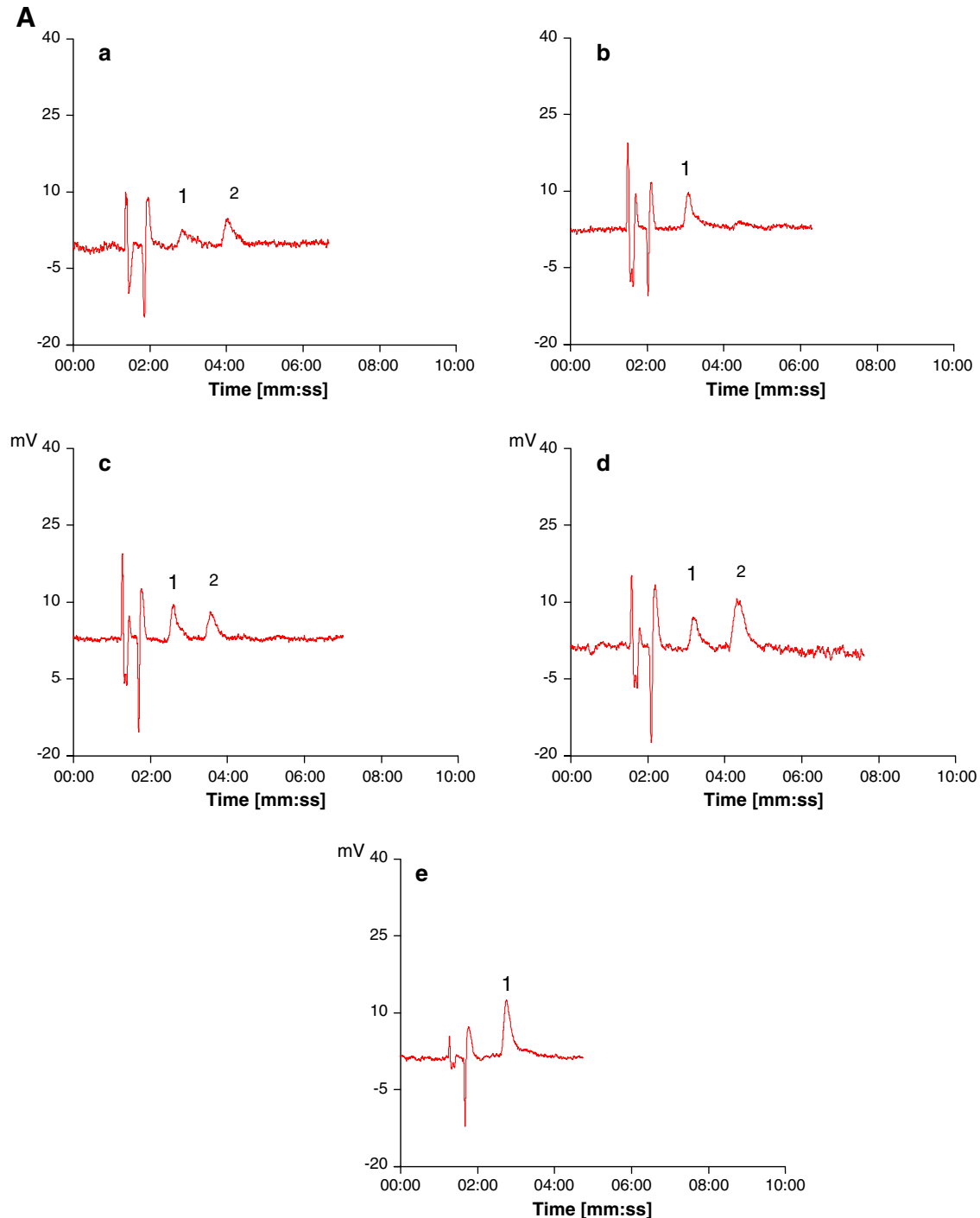


Fig. 3. A: Intranasal curcumin detection in plasma. Representative chromatograms (a–d) showing curcumin detection in plasma samples prepared post intranasal curcumin administration (5 mg/kg, i.n.) at different time points; 15 min, 30 min, 1 h and 3 h. In all four chromatograms sharp peak of curcumin has been detected. Up to 0.9 µg/ml of curcumin was detected after 15 min and increased peak up to 1.5 µg/ml after 3 h in plasma has been detected. (e) Blank plasma spiked with 10 µg/ml of curcumin; (1) curcumin peak, (2) unknown peak. B: Intranasal curcumin detection in lung homogenate. Representative chromatograms (f–j) showing curcumin peaks in lung homogenate post curcumin dosing (5 mg/kg, i.n.) at different time points; 15 min, 30 min, 1 h and 3 h; (f–i) in all four chromatograms sharp peak of curcumin has been detected. Up to 0.27 µg/ml of curcumin was detected after 15 min and up to ten fold increased peak i.e., 3.6 µg/ml was detected in lung homogenate after 3 h; (j) blank lung homogenate spiked with 10 µg/ml of curcumin. C: Intraperitoneal curcumin detection in lung homogenate. In representative chromatograms (k–n) Intraperitoneal curcumin (20 mg/kg, i.p.) has been detected in lung homogenate samples prepared post administration after 15 min, 30 min, 1 h and 3 h; up to 0.3 µg/ml of curcumin was detected after 15 min and curcumin spiked up to 5.0 µg/ml after 3 h in lung homogenate; (1) curcumin peak.

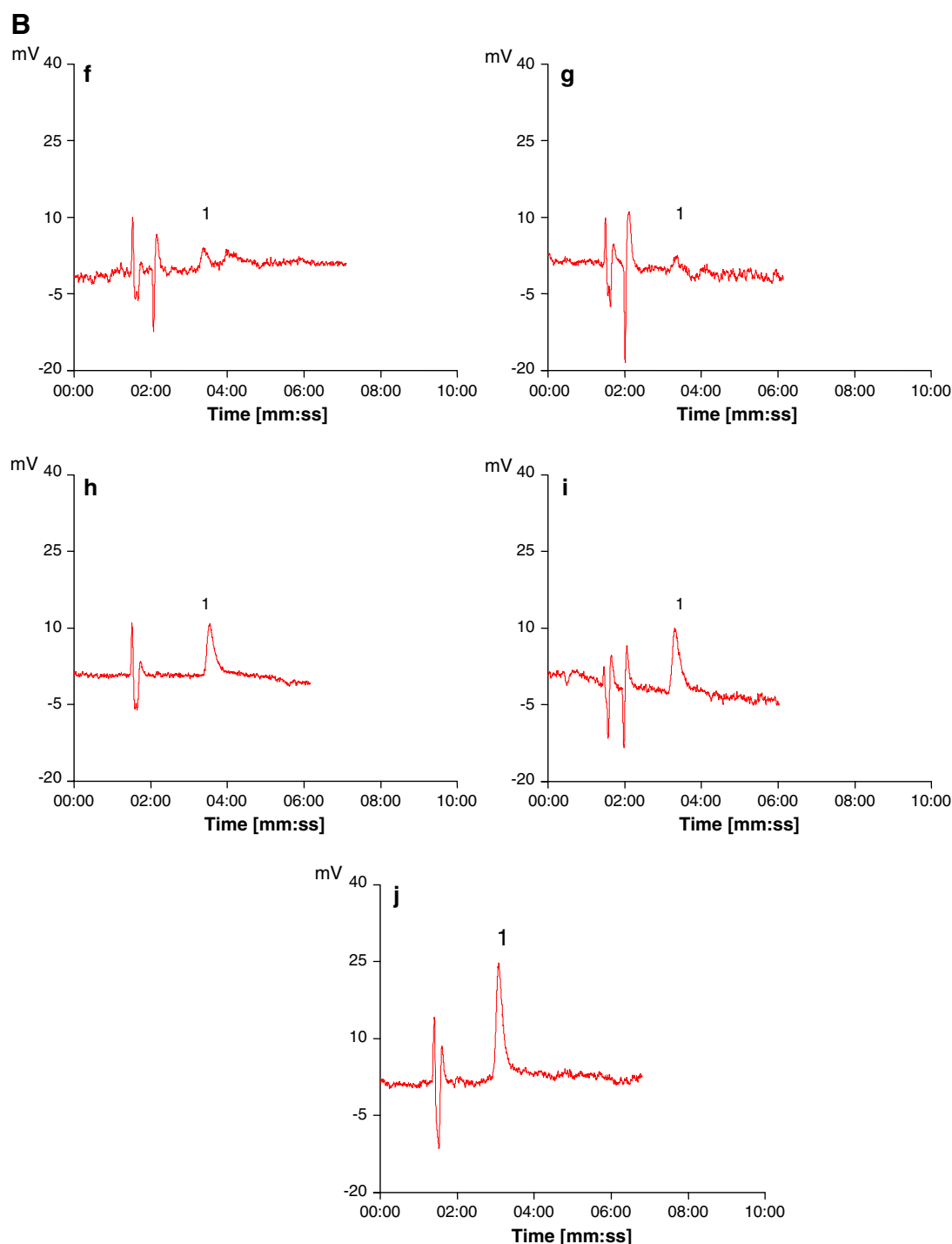


Fig. 3 (continued).

3.4. Immunofluorescence staining of lung tissues for eosinophil detection

Lung tissue sections from nonsensitized (normal) mice showed neither peribronchial nor alveolar infiltration of eosinophils as shown by immunofluorescent negative cells. In contrast, OVA sensitized/challenged mice consistently showed eosinophils in alveolar region as well as in peribronchial region as profound immunofluorescence for anti-MBP antibody used specific for eosinophil granule major basic protein (Fig. 6A–D). Moreover, when asthmatic mice were challenged with intranasal curcumin (2.5 to 5.0 mg/kg), eosinophil recruitment was inhibited in alveolar region and no immunofluorescence was detected in the peribronchiolar region. Immunofluorescent studies revealed

curcumin more potent than standard drug di sodium cromoglycate (50 mg/kg, i.p.).

3.5. Curcumin inhibits histamine release from mast cells

Histamine release in BAL fluid collected from ova-sensitized and ova-challenged mice treated with curcumin through nasal route was dose-dependently inhibited. It was found that intranasal challenge of curcumin (5.0 mg/kg) has significantly inhibited histamine release up to 80%. However, there are no reports till date regarding histamine release inhibition through nasal route of curcumin administration (Fig. 7A and B). Histamine release was compared with

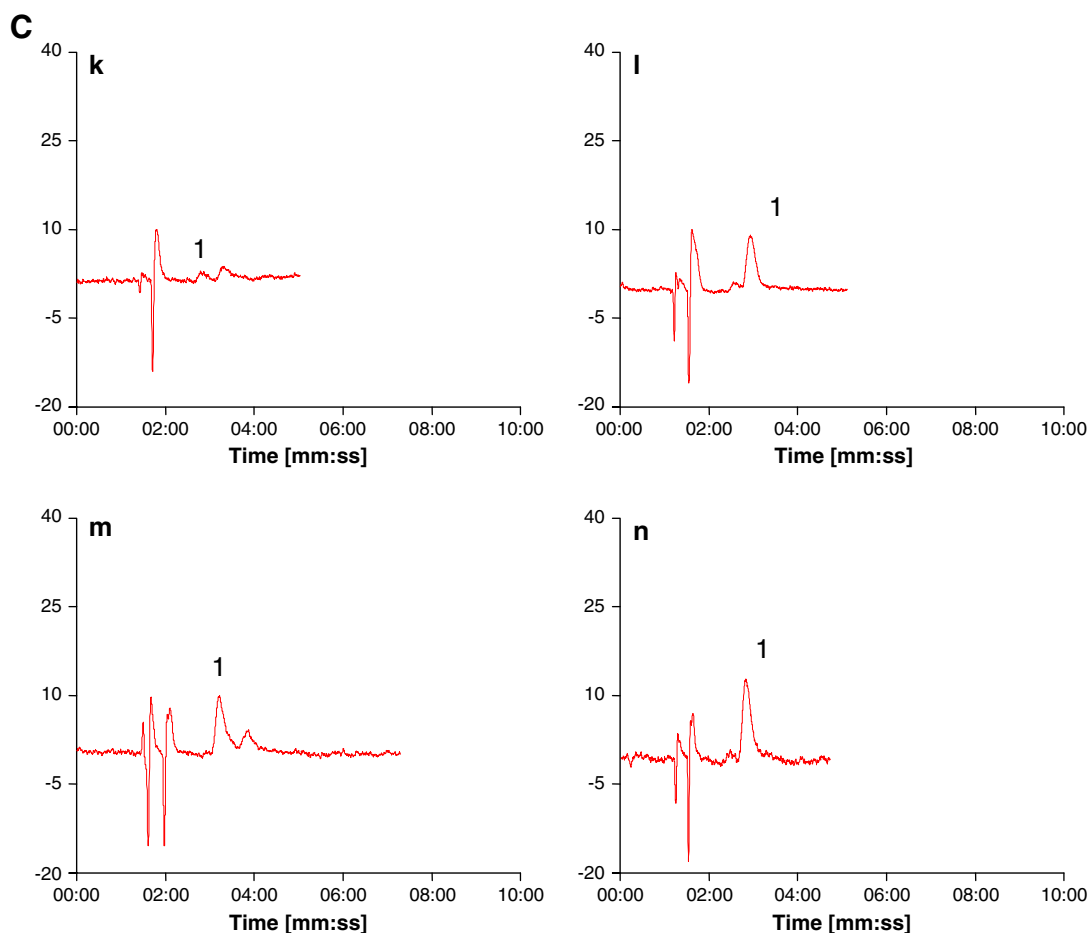


Fig. 3 (continued).

curcumin administration intraperitoneally at higher doses. Curcumin dose-dependently inhibited histamine release in BALF of asthmatic mice as described in methods. Here, the effects of curcumin by both the routes were compared, and intranasal route has shown as better parenteral route in terms of histamine release and % inhibition. Each data represent the mean $\pm$ SEM of 5 independent experiments.

3.6. Curcumin modulates level of eosinophil peroxidases (EPO) and eosinophil recruitment to the lungs

Eosinophils contain large amount of granules that induce severe airway inflammation, and increased levels of EPO in allergic patients are considered to be one of the inflammatory marker of eosinophil recruitment [47,48]. Other inflammatory cells are also recruited to the perivascular areas, but eosinophils are recruited most in airway inflammations and EPO has been reported to be significantly increased in OVA-induced asthma. In present investigations, intranasal curcumin has significantly inhibited eosinophil recruitment to the lungs (Fig. 4), and decreased EPO levels has been observed in BALF of mice where curcumin was administered through nasal route (Fig. 8).

3.7. Cytotoxicity of curcumin in a murine model of allergic asthma

AST and ALT levels, commonly known indicators of hepatotoxicity, were not changed by intranasal curcumin administration, indicating normal liver function. Creatinine levels measured as normal kidney function test was found constant with and without curcumin treatment (Figs. 9 and 10).

4. Discussion

Asthma is a chronic inflammatory airway disease in which multiple complex pathways are involved. Being a chronic disease, long-term medications are required for the therapy. Currently, available therapeutic agents have major adverse implications. Hence, it is more important to find drugs preferably non-steroidal in nature, which can be taken for longer without any adverse effects. Therefore, we have chosen a very well-known phytochemical with anti-inflammatory potential in allergic asthma model through nasal route. In the present study, we are evaluating experimental evidence of intranasal curcumin absorption in murine model of asthma.

Because of the complex nature of plasma, a pre-treatment procedure is often needed to remove protein and potential interferences prior to HPLC analysis. Sample preparation is essential and more important in high-throughput analysis than the instrumentation. Specificity can be described as the ability of the method to discriminate all the possible interfering substances. Studies has also been performed to investigate curcumin detection at higher doses (up to 100 mg/kg, i.n.), and it was found that at 30 min, it was low (0.2 μ g/ml) in plasma but has been increased up to 25 μ g/ml after 3 h, and only 3.6 μ g/ml could be reach to the lungs till 3 h (data not shown here). It is possible that higher dose of curcumin might have taken longer to get absorbed and could reach to plasma till 3 h, but it was unable to reach to the lungs at the same rate could be due to the reason that being present in large quantity in circulation, its diffusion is very slow and might take still longer to reach to the lungs (more than 3 h, we have not detected). Drugs that remain in the circulation tend to have a low volume of distribution.

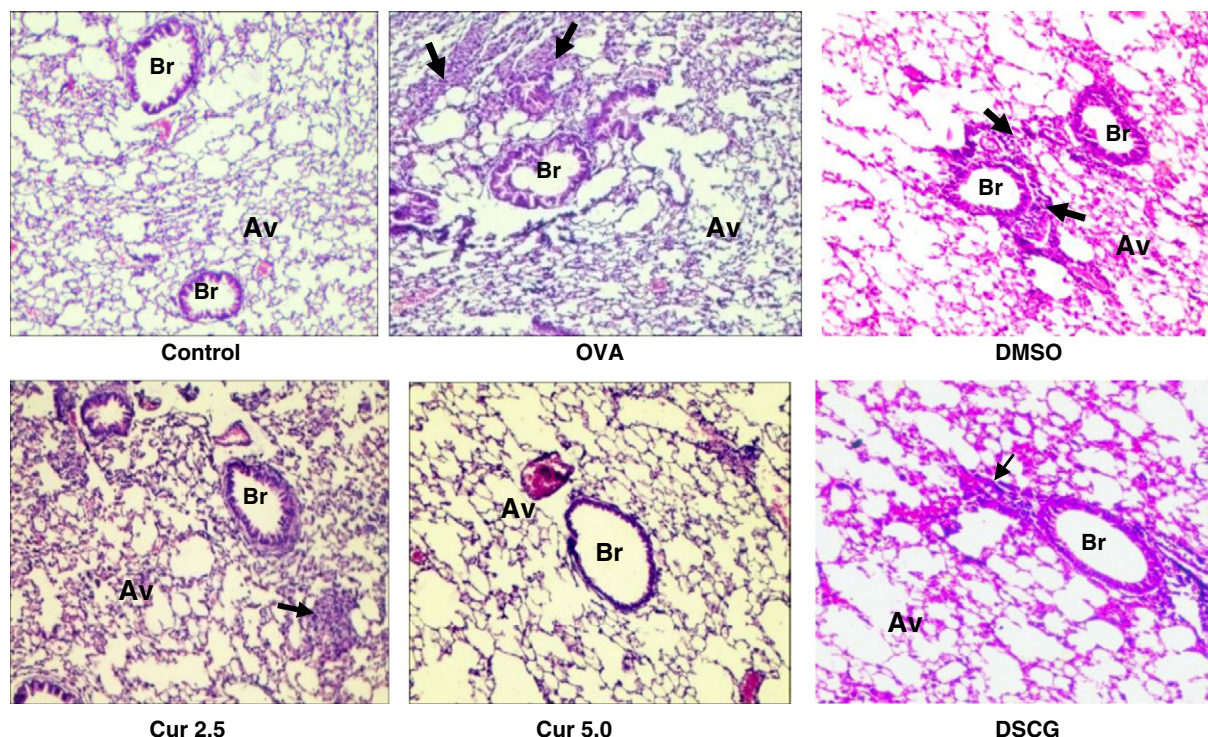


Fig. 4. Effect of intranasal curcumin on inflammation and bronchoconstriction. Mice were sensitized and challenged with OVA as described in [Methods](#). Lungs were removed after 24 h of last OVA challenge. Lung sections (5 μ m) were stained with hematoxylin and eosin. Control: without sensitization and challenge (saline only); OVA: OVA sensitized/challenged section; DMSO: OVA sensitized/challenged section with intranasal DMSO as vehicle; Cur 2.5: OVA sensitized/challenged section with intranasal curcumin 2.5 mg/kg; Cur 5.0: OVA sensitized/challenged section with intranasal curcumin 5.0 mg/kg; DSCG: OVA sensitized/challenged section with intraperitoneal DSCG 50 mg/kg (inflammation and bronchoconstriction in each is comparable; magnification 100 $\times$).

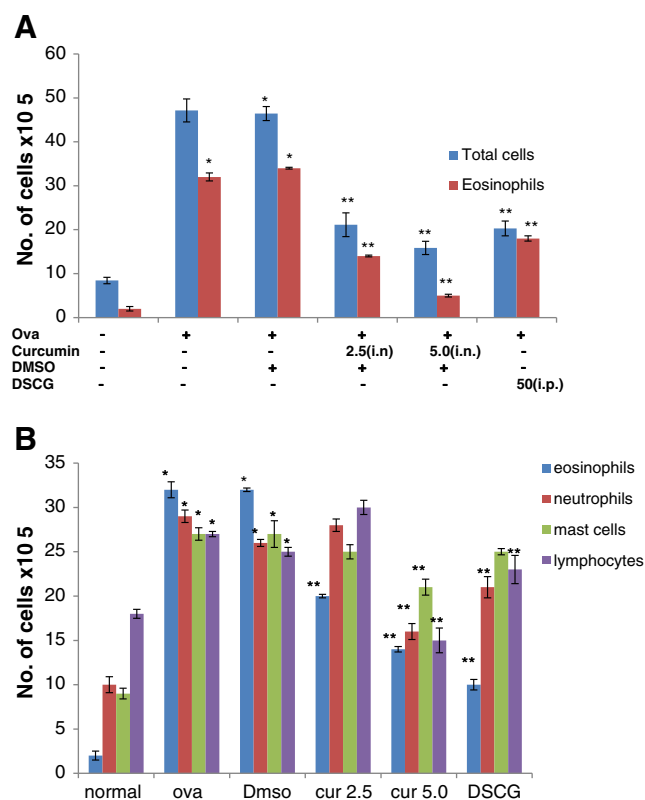


Fig. 5. (A and B) Effect of curcumin on eosinophils and total inflammatory cells [A] and other inflammatory cells [B] in BALF of mice 24 h after the final OVA challenge. Cells were isolated by cytocentrifuge and stained with Giemsa for differential cell count. (* $p < 0.05$ versus normal, ** $p < 0.05$ versus OVA challenged mice.)

In the present study, 0.9 μ g/ml of curcumin was detected after 15 min which spiked up to 3 h till 1.5 μ g/ml in plasma after intranasal administration of 5 mg/kg curcumin. Lung homogenate retained up to 3.6 μ g/ml of curcumin till 3 h after same dose administration (5 mg/kg, i.n.), which was detectable from 15 min (0.27 μ g/ml) and was on higher side as compared to earlier studies. Since nasal respiratory mucosa is constituted by the epithelium, basement membrane, lamina propria and microvilli play important role in enhancing respiratory surface area. Highly vascular mucosa might have enhanced the absorption and bioavailability of curcumin in both plasma and lung homogenates; therefore, its detection was possible till 3 h. Therefore, we could see a very good inhibition in bronchoconstriction and inflammation in lung tissues without degradation at lower doses after intranasal administration. According to previous studies, a maximum of 1.35 μ g/ml curcumin was detected in serum after 2 g/kg oral dose in rats. Plasma curcumin level peaked up to 2.25 μ g/ml after 15 min of intraperitoneal administration and declined rapidly after 1 h ([Fig 3, A-C](#)).

It was reported earlier that curcumin attenuate allergen induced airway hyperresponsiveness in sensitized guinea pigs [49]. In the present study, curcumin have shown effectiveness in attenuating bronchoconstriction and lung inflammation in sensitized and Ovalbumin exposed Balb/c mice at lower doses through intranasal route as compared to the intraperitoneal route. Activated mast cells and eosinophils contribute to pulmonary inflammation by producing a variety of chemical mediators and cytokines. The recruitment of inflammatory cells from blood vessels to the airway need various selectins and blocking of these selectins has been demonstrated to inhibit antigen induced airway inflammations.

Present investigations are providing evidence of intranasal curcumin absorption through nasal mucosa and showing better effect on airway inflammations for the first time. OVA sensitized and challenged mice showed certain characteristic features of asthma along

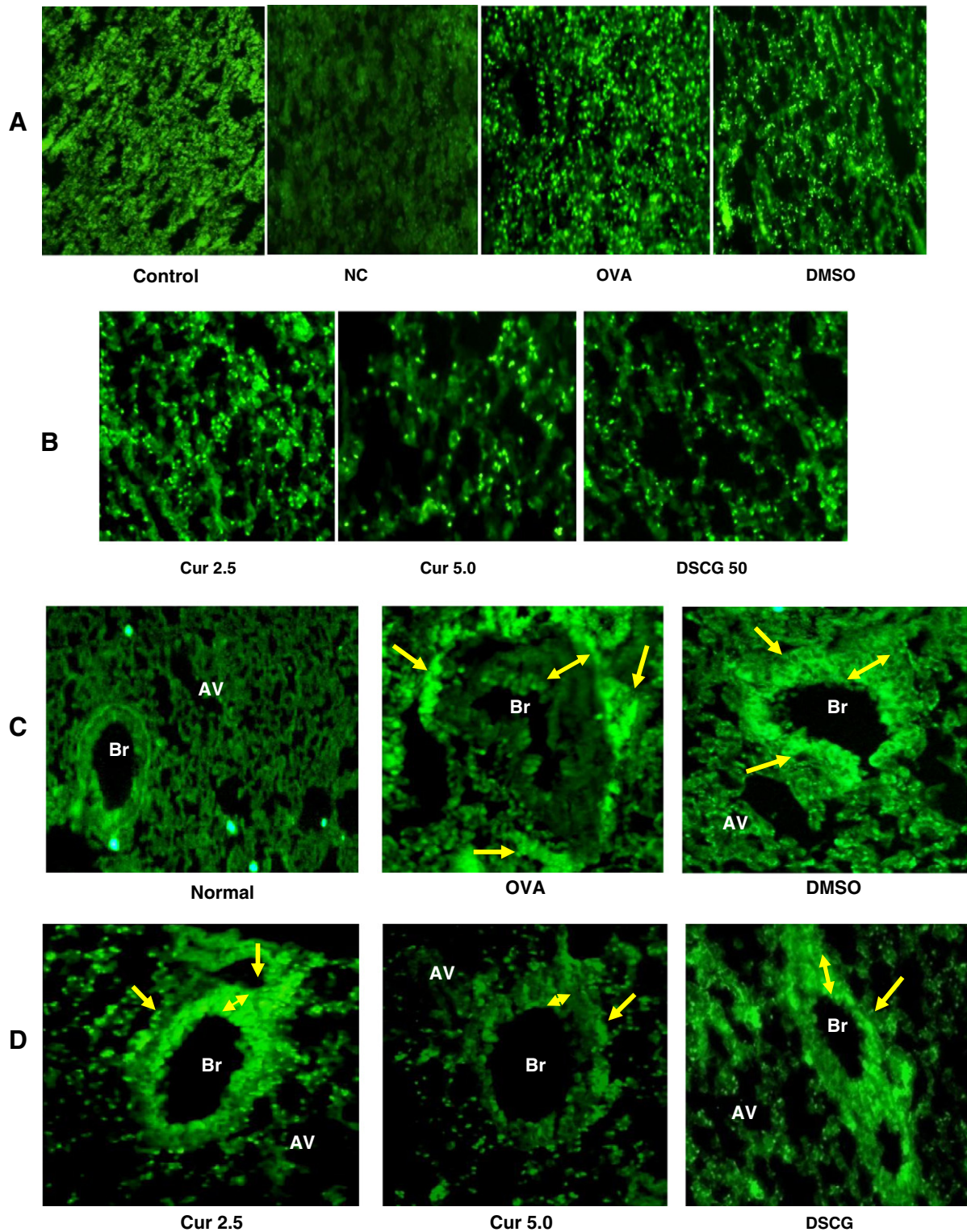


Fig. 6. (A–D) Recruitment of eosinophils in the lung parenchyma by immunofluorescence staining; representative alveolar region (A, B) and peribronchial region (C, D) of the lung sections (5 μ m) incubated with rat anti-mouse MBP antibody or normal IgG cross-linked with fluorescein-labeled goat anti-mouse IgG control: without sensitization; NC: negative control stained with normal IgG, no fluorescence; OVA: OVA-sensitized/challenged mice, maximum fluorescence for recruited eosinophils positive for mMBP in alveolar and peribronchial regions; DMSO: OVA-sensitized/challenged mice treated with vehicle, no effect of vehicle on fluorescence in alveolar and peribronchial regions; Cur 2.5: OVA-sensitized/challenged mice treated with intranasal curcumin 2.5 mg/kg, less fluorescence related to inhibition in infiltration; Cur 5.0, OVA-sensitized/challenged mice treated with intranasal curcumin 5 mg/kg, very few eosinophils in alveolar region and almost suppressed fluorescence in bronchial regions; DSCG: OVA-sensitized/challenged mice treated with intraperitoneal DSCG 50 mg/kg with countable numbers in alveolar region and little fluorescence in bronchial region. Double headed arrows show thickness in epithelial foldings during bronchoconstriction which has been reduced in curcumin treated groups (original magnification, $\times 200$.)

with bronchoconstriction and inflammation in lungs (Fig. 4). The BALF of the asthmatic animals showed increased number of total inflammatory cells, including neutrophils, lymphocytes and mast cells

along with large number of eosinophils. The anti-inflammatory effect of vehicle, DMSO, was not seen in any observations including histology (Fig. 5A and B).

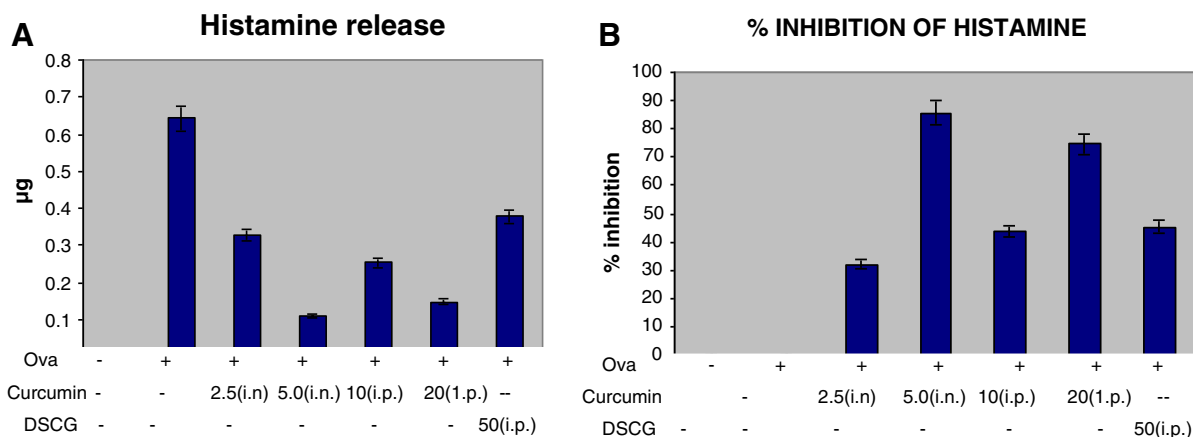


Fig. 7. Effect of curcumin on histamine release in BALF of asthmatic mice. BAL fluid supernatant was assessed for histamine release spectrofluorometrically, and it was found that 5.0 mg/kg curcumin (i.n.) has significantly decreased histamine release from mast cells and intranasal route is more effective than intraperitoneal route at lower doses. Effectiveness of curcumin is comparable to standard drug disodium cromoglycate (i.p.), a known mast cell stabilizer. [A] showing histamine release in µg in BALF of Ovalbumine and curcumin/DSCG treated mice. [B] % inhibition in histamine release ($p < 0.05$ OVA challenged mice to curcumin).

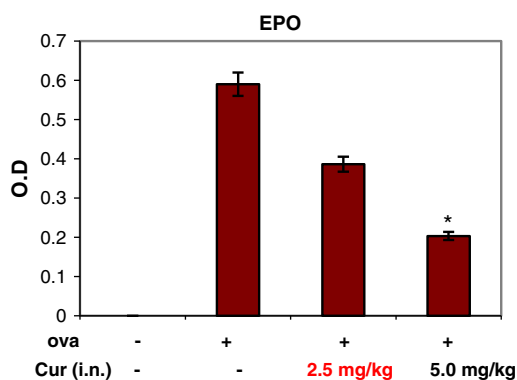


Fig. 8. Effect of curcumin on EPO activity. Mice were sensitized and challenged with OVA as described in materials and methods. EPO activity was measured in BALF after 24 h of last airway challenge (as described in materials and methods). Data present the mean $\pm$ SEM from 5 independent experiments ($p < 0.05$ Vs OVA challenged mice).

Immunofluorescence studies revealed maximum recruitment of eosinophils in alveolar spaces and accumulation of fluorescence in peribronchial region of asthmatic lungs, whereas consistent reduction in the recruitment of eosinophils, as presence of MBP, an eosinophil marker in the curcumin-treated lung sections with very few numbers in alveolar spaces and almost complete inhibition to the peribronchial region, was found (Fig. 6A–B). These findings thus indicate that intranasal curcumin reduces the asthmatic features by inhibiting eosinophil

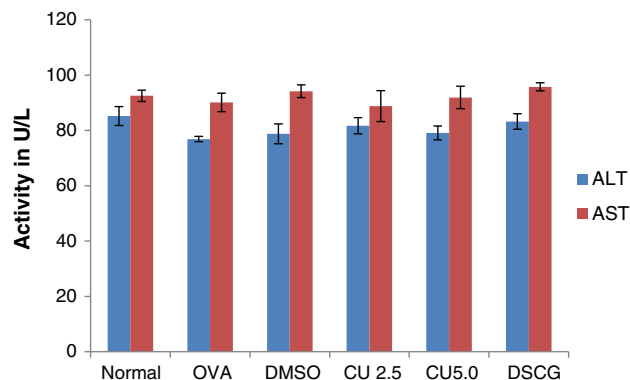


Fig. 9. Levels of AST and ALT in serum as liver function test. Serum was collected after the final OVA challenge. Samples ($n = 5$) were analyzed using commercial kit as described in Materials and Methods.

recruitment and release of histamine at the tissue site. Curcumin has not shown any toxic effects as investigated by liver and kidney function tests. In recent years many drugs have shown to achieve better systemic bioavailability through nasal route than by oral administration or peritoneal route. Intranasal absorption and therefore detection of curcumin revealed impacts on the feasibility of its role as modulator of allergic airway inflammation in directly targeting lungs at considerably lower doses. Nasal drops and sprays of curcumin could prove to be better medication without any side effect. As most of the drugs are even inconvenient for long-term therapy by parenteral route, intranasal drug delivery may be a better choice. Nasal drops of curcumin has shown better absorption than intraperitoneal route, and it can have better effects if used through nebulizer in human subjects. Curcumin is effective in suppressing asthmatic features at lower doses with better efficacy than DSCG. Further investigations like its pharmacokinetic and pharmacodynamic study is in progress to prove its enhanced bioavailability through intranasal route at lower doses.

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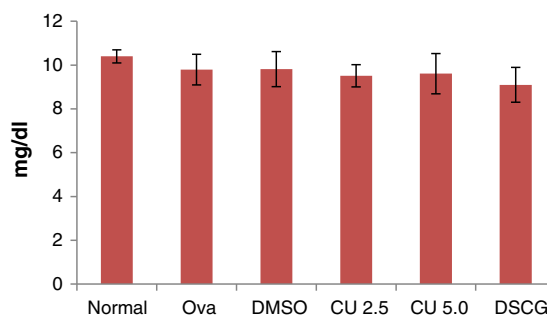


Fig. 10. Levels of creatinine as kidney function test in serum. Serum was collected after the final OVA challenge. Samples ($n = 5$) were analyzed using commercial kit as described in Materials and Methods.

References

- [1] Yin LM, Jiang GH, Wang Y, Wang Y, Liu YY, Jin WR, et al. Use of serial analysis of gene expression to reveal the specific regulation of gene expression profile in asthmatic rats treated by acupuncture. *J Biomed Sci* 2009;16(46):1–13.
- [2] Ray Cohn L. Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation. *J Clin Invest* 1999;104(8):985–93.
- [3] Yin LM, Jiang GH, Wang Y, Wang Y, Liu YY, Jin WR, et al. Serial analysis of gene expression in rat lung model of asthma. *Respirology* 2008;13(7):972–82.
- [4] Edwards MR, Bartlett NW, Clark D, Birrell M, Belvisi M, Johnston SL. Targeting the NF- κ B pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol Ther* 2009;121(1):1–13.
- [5] Inapagolla R, Guru BR, Kurtoglu YE, Gao X, Lih-Lai M, Bassett DJ, et al. In vivo efficacy of dendrimer-methylprednisolone conjugate formulation for the treatment of lung inflammation. *Int J Pharmacol* 2010;39991–2:140–7.
- [6] Strehle EM. Long-term management of children with neuromuscular disorders. *J Pediatr (Rio J)* 2009;85(5):379–84.
- [7] Matsuo Y, Ishihara T, Ishijaki J, Miyamoto K, Higaki M, Yamashita N. Effect of betamethasone phosphate loaded polymeric nanoparticle on murine asthma model. *Cell Immunol* 2009;260(1):33–8.
- [8] Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;50:515–96.
- [9] Murray JJ, Tonnel AB, Brash AR, Roberts II LJ, Gosset P, Workman R, et al. Release of prostaglandin D2 into human airways during acute antigen challenge. *N Engl J Med* 1986;315:800–4.
- [10] Proud D, Sweet J, Stein P, Settipane RA, Kagey-Sobotka A, Friedlaender MH, et al. Inflammatory mediator release on conjunctival provocation of allergic subjects with allergen. *J Allergy Clin Immunol* 1990;85:896–905.
- [11] Charlesworth EN, Kagey-Sobotka A, Scheilmer RP, Norman PS, Lichtenstein LM. Prednisolone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late phase response to allergen. *J Immunol* 1991;146:671–6.
- [12] Lewis RA, Sorter NA, Diamond PT, Austen KF, Oates JA, Roberts II LJ. Prostaglandin D2 generation after the activation of rat and human mast cells with anti-IgE. *J Immunol* 1982;129:1627–31.
- [13] Boyce JA. The role of mast cells in asthma. *Prostaglandins Leukot Essent Fatty Acids* 69; 2003 195–205 [Aug-Sep(2-3)].
- [14] Sur R, Cavender D, Malviya R. Different approaches to study mast cell functions. *Int Immunopharmacol* 2007;7:555–67.
- [15] Hart PH. Regulation of inflammatory response in asthma by mast cell products. *Immunol Cell Biol* 2001;79:149–53.
- [16] Kalesnikoff J, Galli SJ. New developments in mast cell biology. *Nat Immunol* 2008;9:1215–23.
- [17] Gilani AH, Shah AJ, Ghayur MN, Majeed KK. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. *Life Sci* 2005;76:3089–105.
- [18] Cooper TH, Clark G and Guzinski J in *Food Phytochemicals II: Teas, Spices, and Herbs* (Ho CT ed) 994; pp 231–236, American Chemical Society, Washington, DC.
- [19] Ireson CR, Orr S, Jones DL, Verschöyle R, Lim CK, Luo JL, et al. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in rat plasma and evaluation of their ability to inhibit cyclooxygenase-2 expression. *Cancer Res* 2001;61:1058–64.
- [20] Kurup V, Barrios CS. Immunomodulatory effects of curcumin in allergy. *Mol Nutr Food Res* 2008;52:1031–9.
- [21] Kurup V, Barrios CS, Barrios R, Raju BD, BL Michael Johnson, Fink JN. Immune response modulation by curcumin in a latex allergy model. *Clin mol allergy* 2007;5.
- [22] Ireson CR, Jones DJL, Orr S, Coughtrie MWH, Boocock D, Williams ML, et al. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomarkers Prev* 2002;11:97–104.
- [23] Ireson CR. Disposition and metabolism of the cancer chemopreventive agent curcumin in rodents and humans. University of Leicester; 2002 [PhD thesis].
- [24] Inano H, Onoda M. Prevention of radiation-induced mammary tumours. *Int J Radiat Oncol* 2002;52:212–23.
- [25] Frank N, Knauff J, Amelung F, Nair J, Wesch H, Bartsch H. No prevention of liver and kidney tumours in Long-Evans cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. *Mutat Res* 2003;127–135:523–4.
- [26] Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16F10 melanoma by polyphenols. *Cancer Lett* 1995;95:221–5.
- [27] Busquets S, Carbo N, Almendro V, Quiles MT, Lopez-Soriano FJ, Argiles JM. Curcumin, a natural product present in turmeric, decreases tumor growth but does not behave as an anticachectic compound in a rat model. *Cancer Lett* 2001;167:33–8.
- [28] Park EJ, Jeon CH, Ko G, Kim J, Sohn DH. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J Pharm Pharmacol* 2001;52:437–40.
- [29] Nanji AA, Jokelainen K, Tipoe GL, Rahemtulla A, Thomas P, Dannenberg AJ. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF- κ B-dependent genes. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G321–7.
- [30] Rasyid A, Lelo A. The effect of curcumin and placebo on human gallbladder function: an ultrasound study. *Aliment Pharmacol Ther* 1999;13:245–59.
- [31] Rasyid A, Rahman AR, Jaalam K, Lelo A. Effect of different curcumin dosages on human gall bladder. *Asia Pacific J Clin Nutr* 2002;11:314–8.
- [32] Marone G, Triggiani M, Genovese AA, De Paulis. Role of human mast cells and basophils in bronchial asthma. *Adv Immunol* 2005;88:97–160.
- [33] Gilani AH, Shah AJ, Ghayur MN, Majeed KK. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. *Life Sci* 2005;76:3089–105.
- [34] Anand Preetha, Kunnumakkara Ajaikumar B, Newman Robert A, Aggarwal Bharat B. Bioavailability of curcumin: problems and promises. *Mol Pharm* 2007;4(6):807–18.
- [35] Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, et al. Pharmacodynamic and pharmacokinetic study of oral curcuma extract in patients with colorectal cancer. *Clin Cancer Res* 2001;7(7):1894–900.
- [36] Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab Dispos* 1999;27(4):486–94.
- [37] Pak Y, Patek R, Mayersohn M. Sensitive and rapid isocratic liquid chromatography method for the quantitation of curcumin in plasma. *J Chromatogr B* 2003;796:339–46.
- [38] Pires Anaísa, Fortuna Ana, Alves Gilberto. Intranasal drug delivery: how, why and what for? *J Pharm Pharm Sci* 2009;12(3):288–311.
- [39] Türker S, Onur E, Ozer Y. Nasal route and drug delivery systems. *Pharm World Sci* 2004;26(3):137–42 [Jun].
- [40] Oetari S, Sudibyo M, Commandeur JN, Samhoedi R, Vermeulen NP. Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver. *Biochem Pharmacol* 1996;51:39–45.
- [41] Ma Z, Shayeganpour A, Brocks DR, Lavasanifan A, Samuel J. High-performance liquid chromatography analysis of curcumin in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. *Biomed Chromatogr* 2007;21(5):546–52.
- [42] Liu A, Lou H, Zhao L, Fan P. Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. *J Pharm Biomed Anal* 2006;40:720–7.
- [43] Wang YY, Pan MH, Cheng AL, Lin LI, Ho YS, Ho YS, et al. Stability of curcumin in buffer solutions and characterization of its degradation products. *J Pharm Biomed Anal* 1997;15:1867–76.
- [44] Yang KY, Lin LC, Tseng TY, Wang SC, Tsai TH. Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007;853:183–9.
- [45] Stelts D, Egan RW, Falcone A, Garlisi CG, Gleich GJ, kreutner W, et al. Eosinophils retain their granule major basic protein in a murine model of allergic pulmonary inflammation. *Am J Respir Cell Mol Biol* 1998;18:463–70.
- [46] Shore P, Purkhalter A, Cohn Jr V. A method for the fluorometric assay of histamine in tissues. *J Pharmacol Exp Ther* 1959;127:182–6.
- [47] Erjefalt JS, Greiff LM, Anderson E, Mattson H, Petersen M, Linden PK, et al. Allergen-induced eosinophil cytotoxicity is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999;160:304–12.
- [48] Metso T, VengeP Haahtela T, Perterson CG, Seveus L. Cell specific markers for eosinophils and neutrophils in sputum and bronchoalveolar lavage fluid of patients with respiratory conditions and healthy subjects. *Thorax* May 2002;57(5):449–51.
- [49] Das M, Ram A, Ghosh B. Curcumin attenuates allergen induced airway hyperresponsiveness in sensitized guinea pig. *Biol Pharm Bull* 2003;26:1021–4.