



Intranasal curcumin ameliorates airway inflammation and obstruction by regulating MAPKinase activation (p38, Erk and JNK) and prostaglandin D2 release in murine model of asthma

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ARTICLE INFO

Article history:

Received 15 August 2015

Received in revised form 18 December 2015

Accepted 18 December 2015

Available online 4 January 2016

Keywords:

Prostaglandin D2

MAPK

Extracellular regulated kinases and c-Jun

N-terminal kinases

Eosinophils

ABSTRACT

Asthma, a multifactorial, chronic inflammatory disease encompasses multiple complex pathways releasing number of mediators by activated mast cells, eosinophils and T lymphocytes, leading to its severity. Presently available medications are associated with certain limitations, and hence, it is imperative to search for anti-inflammatory drug preferably targeting signaling cascades involved in inflammation thereby suppressing inflammatory mediators without any side effect. Curcumin, an anti-inflammatory molecule with potent anti-asthmatic potential has been found to suppress asthmatic features by inhibiting airway inflammation and bronchoconstriction if administered through nasal route. The present study provides new insight towards anti-asthmatic potential of intranasal curcumin at lower doses (2.5 and 5.0 mg/kg) in Balb/c mice sensitized and challenged with ovalbumin (OVA) which is effective in inhibiting airway inflammation. These investigations suggest that intranasal curcumin (2.5 and 5.0 mg/kg) regulates airway inflammation and airway obstruction mainly by modulating cytokine levels (IL-4, 5, IFN- γ and TNF- α) and sPLA2 activity thereby inhibiting PGD₂ release and COX-2 expression. Further, the suppression of p38 MAPK, ERK 42/44 and JNK54/56 activation elucidate the mechanism behind the inhibitory role of intranasal curcumin in asthma progression. Thus, curcumin could be better alternative for the development of nasal formulations and inhalers in near future.

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1. Introduction

Asthma, a complex, multifactorial, chronic respiratory disease associated with bronchial inflammation is generally characterized by airway inflammation, hyperresponsiveness (AHR) and remodeling in response to a variety of physical and chemical stimuli. [1]. Mast cells play a significant role by degranulating and releasing an array of inflammatory mediators including histamine, cytokines, chemokines, serotonin and eicosanoids (leukotrienes, prostaglandins, and platelets activating factor) either by IgE-antigen aggregation or by other effectors are responsible for airway obstruction leading to difficulty in breathing. Mastcell numbers are increased in the airway tissues of asthmatic-ics concomitantly with local recruitment and activation of Th2 lymphocytes. [2,3].

Clinical and experimental investigations suggest crucial role of specific Th2 cytokines (IL-4, 5, 9, 13) in orchestrating the allergic inflammatory response and have been linked with IgE production, eosinophilic inflammation and AHR [4,5,6,7]. Apart, Th1 cytokine (IFN- γ) and proinflammatory cytokines (TNF- α) also contribute to dysregulated inflammatory response by inducing iNOS synthesis leading to elevation of

exhaled NO level [8,9,10]. Also, lipid mediators including prostaglandin (PG) and leukotrienes (LT) and their associated enzymes as secretory phospholipase A2 (sPLA2) through arachidonic acid metabolism have also been implicated with the progression of asthma towards severity [11,12].

Studies suggest the compelling role of various signaling pathways within the activated cells as mitogen activated phosphorylated kinase (MAPK) cascades are responsible for the release of inflammatory mediators and as an important event in the activation of various immune cells, by a wide range of cellular stresses in response to inflammatory cytokines [13]. Kinases such as p38 and extracellular signal-regulated kinase (ERK) has been implicated in the regulation of IL-5 and other cytokines [14,15] while c-Jun, N-terminal kinase (JNK) have been reported for IgE class switching [16,17]. The inhibition of these signaling kinases involved in airway inflammations now-a-days has become an important therapeutic strategy to treat various inflammatory diseases and could be a possible treatment target.

Curcumin (diferuloylmethane), a lipophilic, polyphenol and a principal active constituent of turmeric, rhizome of *Curcuma longa* (Zingiberaceae) encompasses wide spectrum of biological and pharmacological activities [18]. Overcoming the problem of bioavailability, we have reported earlier that curcumin (5 mg/kg) through nasal

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administration is effective in ameliorating inflammation and bronchoconstriction in OVA induced murine model of asthma without any side effect in both acute and chronic asthma [19,20]. Therefore, present study unravels the mechanism of action of nasal curcumin on signaling cascades involved in inflammatory mediator release. Suppression of various kinase expressions involved in asthma inflammation by nasal curcumin was compared with standard drug di sodium cromoglycate (DSCG, 50 mg/kg, i.p.), a known mast cell stabilizer, that has been used for more than 35 years, not only for the control of bronchial asthma, but also for the management of various other allergy based diseases. In present study, nasal curcumin showed better response over standard DSCG.

2. Materials and methods

2.1. Animals

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 of the current study were approved by the Central Animal Ethical Committee, Banaras Hindu University, Varanasi, India.

2.2. Reagents

Aluminum hydroxide gel (Pierce Chemical Company; Rockford, USA), Curcumin, purity $\geq 99.5\%$, Ovalbumin grade V (OVA), Methacholine (MCh), Dimethyl Sulphoxide (DMSO) and Vanadium Chloride were purchased from Sigma-Aldrich Corporation, USA. Bovine Serum Albumin (Amresco, USA), HRP conjugated IgE (Southern biotech, India), COX-2 Antibody, Antibodies for MAPKs, p-p38, p-ERK, p-JNK (Cell Signaling, USA), HRP conjugated secondary Antibody (Cayman Chemicals, USA), and ECL kit (Thermo fisher, USA), ELISA Kits for PGD2 and sPLA2 (Cayman Chemicals, USA), Cytokines IL-4, IL-5, IFN- γ and TNF- α from Biologend, USA.

2.3. Sensitization and challenge protocol

Mice were randomly divided into six groups (5 mice/group; Table 1) and were named according to the status of sensitization/challenge/treatment. They were Sham/Sal (saline sensitized and challenged); OVA/OVA (OVA sensitized and challenged), OVA/OVA/Veh (OVA sensitized and challenged and vehicle-treated), OVA/OVA/Cur 2.5 mg/kg and OVA/OVA/Cur 5.0 mg/kg and OVA/OVA/DSCG (50 mg/kg, i.p.). Mice were sensitized by intraperitoneal (i.p.) injections of 0.2 ml saline suspension containing 50 μ g OVA emulsified in 4 mg of aluminum hydroxide on 0, 7 and 14 days. From 19 to 22 days, mice were subjected to OVA aerosol (1%) inhalation for 30 min in a plexiglass chamber by nebulizer (Omran, Japan) with air flow rate of 9 l/min (Fig 1). Control mice received i.p. injection of 0.2 ml saline and were challenged with saline alone. Curcumin was dissolved in DMSO and administered 2.5 and 5 mg/kg (in the form of nasal drops and mice were kept in a supine position to ease inhalation) an hour before every OVA challenge from days 19 to 22. Similarly, vehicle (DMSO) was given in equal volume as curcumin to study the effect of DMSO alone on asthma.

Table 1
Grouping of animal according to their sensitization and challenge.

Group	Sensitization (i.p.)	Treatment	Challenge (1% OVA aerosol)
I	OVA	–	Saline
II	OVA	–	OVA
III	OVA	DMSO (Veh.)	OVA
IV	OVA	Cur 2.5 mg/kg (i.n)	OVA
V	OVA	Cur 5.0 mg/kg (i.n)	OVA
VI	OVA	DSCG 50 mg/kg (i.p)	OVA

2.4. Airway hyperresponsiveness to methacholine (MCh)

AHR was assessed by MCh induced airflow reactivity by determining the specific airway resistance (SRaw) immediately after the final OVA challenge, using whole body plethysmography (Buxco Electronics, USA). To determine AHR, each mouse was placed, acclimatized in the whole body plethysmograph and exposed to saline to get basal SRaw level and then exposed to progressively increasing doses of MCh aerosol (3.125 mg/ml, 12.5 mg/ml and 50 mg/ml) for 3 min each.

2.5. Bronchoalveolar lavage fluid (BALF)

After 24 h of airway reactivity measurement, mice were sacrificed and BALF, serum and lungs were collected and stored at -80°C for further analysis. BALF was collected by tracheal cannulation and washing the airway lumen by chilled sterile phosphate buffered saline (PBS). The suspension was centrifuged at 3000 rpm for 10 min at 4°C . The supernatant was used to analyze cytokine levels of TNF- α , IFN- γ , IL-4 and IL-5, sPLA2 activity responsible for the release of PGD2. IgE and nitrite level was measured in serum. Lung lobes were homogenized and further used to study enzymes expression of COX-2 and phosphorylation of MAPKs (p-38, ERK and JNK) by Western blotting.

2.6. IgE level

OVA specific IgE was detected in the blood serum by indirect ELISA [21]. For serum isolation, blood was centrifuged at 3500 rpm for 30 min at 4°C . Microtitre plates were coated with 20 μ g of OVA in 100 μ l coating buffer (100 mM sodium carbonate and bicarbonate buffer, pH 9.6), incubated at 37°C for 2 h followed by overnight incubation at 4°C . The wells were washed and blocked (1% BSA) at 37°C for 2 h, followed by repeated washing with TBST (0.05% Tween 20 in Tris buffer). 100 μ l of serum (1:9 in blocking buffer) was added to the wells and incubated for 2 h at 37°C . Again after washing the wells, incubation with 100 μ l of horseradish peroxidase labeled anti-IgE antibody (1:1000) at 37°C for 2 h was followed. Finally, 100 μ l of substrate (TMB3,3',5,5' tetramethylbenzidine) was added to the wells in dark and the reaction was stopped by adding 50 μ l of 2.4 M H_2SO_4 . Plates were read at 450 nm reader (Biotek, USA).

2.7. Th1, Th2 and proinflammatory cytokines

IL-4, IL-5, IFN- γ and TNF- α levels were measured in BALF supernatant by using ELISA kits as per the manufacturer's instructions. Briefly, the plates were incubated overnight at 4°C with capture antibody. Following several washings with TBST, blocking was performed for 1 h to reduce nonspecific binding and further incubation with 100 μ l of BALF for 2 h at 37°C . Each well was incubated with individual detection antibodies for 1 h and further incubated with avidin-HRP for 30 min. After final washing, wells were incubated with TMB substrate solution in the dark till the appearance of blue color. The reaction was stopped by adding 2N H_2SO_4 and the plates were read at 450 nm. The concentrations in the test samples were determined by the standard curve and results are expressed as pg/ml.

2.8. Nitrite detection in the serum and BALF

Nitrite level, as a measure of NO was detected by Griess reagent in serum and BALF [22]. Briefly, 100 μ l of sample was mixed with 100 μ l of vanadium chloride (8 mg/ml in 1 N HCL) rapidly followed by the addition of Griess reagent (50 μ l of sulphamide which was 1% sulphanilic acid in 5% orthophosphoric acid) and 50 μ l of N-(1-naphthyl) ethylenediamine (0.1% in distilled water). Plate was incubated for 45 min at 37°C and absorbance was measured at 540 nm in microplate reader. Concentration of the sample was measured against sodium nitrate (NaNO_2) used as standard. Values are expressed in $\mu\text{mole/ml}$.

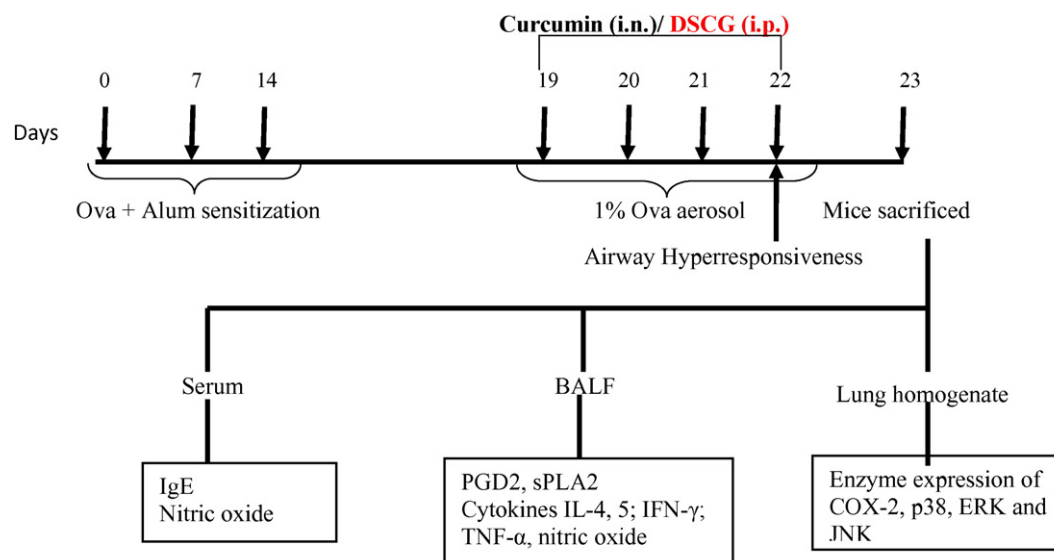


Fig. 1. Experimental protocol: BALB/c mice were sensitized by intraperitoneal injection of OVA on days 0, 7 and 14 and challenged from 19 to 22 days by 1% OVA aerosol. To evaluate the protective effect, intranasal curcumin was administered from days 19 to 22, an hour before every OVA aerosol.

2.9. sPLA2 activity

sPLA2 activity was measured in the BALF supernatant by using sPLA2 kit according to the manufacturer's instructions and expressed in $\mu\text{mol}/\text{min}/\text{ml}$. The enzyme activity was read for 16 min at 405 nm using ELISA plate reader.

2.10. PGD2 release

The level of PGD2 was measured in the BALF supernatant by ProstaglandinD2 EIA kit based on competitive enzyme immunoassay using a monoclonal antibody to PGD2 to bind in a competitive manner. Briefly, after overnight incubation of samples at 4 °C washing was followed by addition of substrate (Ellman's reagent). Plates were read at 405 nm. Using the standard curve, the PGD2 concentration for each unknown sample was determined and expressed in pg/ml.

2.11. Western blotting

The lung tissue was homogenized in lysis buffer containing Tris HCl, NaCl, Sodium orthovanadate, PMSF, EDTA and Triton X100 and centrifuged at 12,000 rpm for 30 min at 4 °C. The total protein concentration in the supernatant was determined and equal amount of protein (60 μg) was subjected to gel electrophoresis (10% SDS PAGE) at 100 V for 120 mins. Proteins were transferred onto a nitrocellulose membrane (Millipore) from the gel and membranes were blocked with 5% BSA dissolved in TBST buffer (10 mM Tris and 150 mM NaCl and 0.5% Tween-20) for 2 h and incubated with individual antibodies COX-2 (1:1000), p-ERK (1:2000), p-JNK (1:2000) and p-p38 MAPK (1:2000) overnight at 4 °C followed by incubation with HRP conjugated secondary antibody (1:10,000) for 2 h at room temperature. Subsequently, membrane was washed with TBST buffer and bands were visualized using ECL reagent.

The intensity and imaging of the bands were analyzed by the biospectrum multispectral imaging system by the vision work LS image acquisition and analysis software (UVP, Germany). The difference of the two bands (lower and upper) has been taken for analysis.

3. Data analyses

Experimental data are presented as mean $\pm$ SEM of $n = 5$. Multiple group comparisons were made using one-way analysis of variance

(ANOVA), followed by a Post hoc test analyzed by SPSS software version 16 (IBM, USA). P values <0.05 were considered as significant.

4. Results

4.1. Curcumin inhibits airway hyperreactivity (AHR) to methacholine

AHR is considered as hallmarks of asthma as it defines the lung function. Immediately after final OVA sensitization, AHR was assessed by MCh induced airflow reactivity by determining the specific airway resistance (sRaw) using whole body plethysmograph. MCh induction showed an altered lung function with increased specific resistance in mice exposed to OVA as compared to the normal mice. However, intranasal curcumin effectively improved the lung function and reduced the lung reactivity in response to the MCh-induced specific resistance. Curcumin at higher dose (5 mg/kg) was effective in improving the lung function than the lower dose (2.5 mg/kg). DMSO alone did not affect the AHR (Table. 2).

4.2. Curcumin inhibits OVA specific IgE release

IgE was measured in the serum by ELISA as discussed in the materials and methods. The OVA sensitized and challenged mice showed a significant increase in OVA specific IgE level as compared to the normal which was significantly reduced by nasal curcumin pretreatment. IgE level was suppressed more effectively at higher curcumin dose (5.0 mg/kg) as compared to lower dose (2.5 mg/kg) and DSCG (Fig 2).

Table 2

Effect of curcumin on AHR in response to increasing concentrations of methacholine. OVA exposure resulted in increased lung response with higher methacholine while nasal curcumin resulted in decreased lung response. Values are expressed as mean $\pm$ SEM; $n = 5/\text{group}$ in an experiment.

Group	Baseline Saline	sRaw measurement in $\text{cm}^2/\text{H}_2\text{O}/\text{ml}/\text{s}$ with increasing Methacholine concentrations ($\mu\text{g}/\text{ml}$)		
		3.125	12.5	50
Normal	0.33 $\pm$ 0.05	0.33 $\pm$ 0.09	0.71 $\pm$ 0.09	0.94 $\pm$ 0.08
OVA	0.52 $\pm$ 0.01	1.57 $\pm$ 0.15	2.62 $\pm$ 0.14	3.89 $\pm$ 0.12
DMSO	0.53 $\pm$ 0.03	1.50 $\pm$ 0.03	2.33 $\pm$ 0.16	3.37 $\pm$ 0.50
Cur 2.5	0.36 $\pm$ 0.35	1.17 $\pm$ 0.05	1.92 $\pm$ 0.70	2.45 $\pm$ 0.72
Cur 5.0	0.26 $\pm$ 0.16	0.98 $\pm$ 0.04	1.53 $\pm$ 0.09	1.97 $\pm$ 0.04

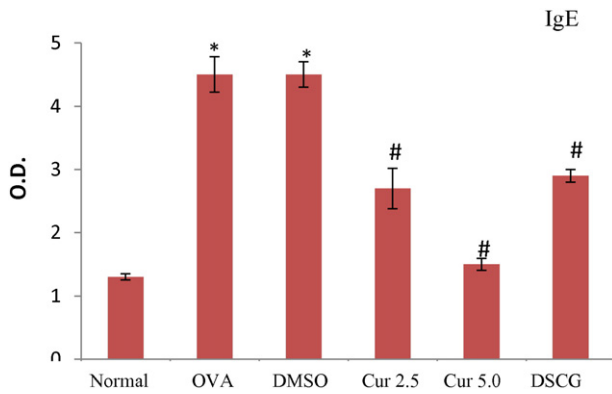


Fig. 2. Effect of nasal curcumin on OVA specific IgE in serum: Increased IgE level in the serum was significantly suppressed dose dependently by nasal curcumin. Values are expressed as mean $\pm$ SEM; n = 5/group in an experiment. (* $P < 0.05$ as compared to the normal # $P < 0.05$ as compared to the OVA).

4.3. Curcumin modulates Th1 and Th2 cytokine response

IL-4 and IL-5 (Th2) levels were enhanced whereas IFN- γ (Th1) level was lowered in asthmatic mice as compared to the normal. Intranasal curcumin has effectively downregulated IL-4 and IL-5 levels (Fig 3) and up regulated the IFN- γ level dose-dependently in the BALF. Up to 74% of inhibition was observed in the IL-4 and IL-5 release in curcumin pretreatment group (5 mg/kg) which was better than DSCG (50 mg/kg, i.p.).

4.4. Curcumin inhibits proinflammatory cytokine TNF- α in the BALF

Asthmatic inflammation is well known to be initiated by the secretion of series of proinflammatory mediators like TNF- α and IL- β . The OVA exposed mice showed a significant increase in the TNF- α level which has been remarkably attenuated by the intranasal curcumin treatment (5 mg/kg, up to 90%) and was better than DSCG (72%, Fig 5).

4.5. Curcumin inhibits nitric oxide release

Nitric oxide in the form of nitrite was measured in the BALF and serum both. The nitrate release was significantly higher in asthmatic group as compared to normal both in the BALF and serum. Intranasal curcumin suppressed nitrite level which was comparable to standard drug DSCG (Fig 6).

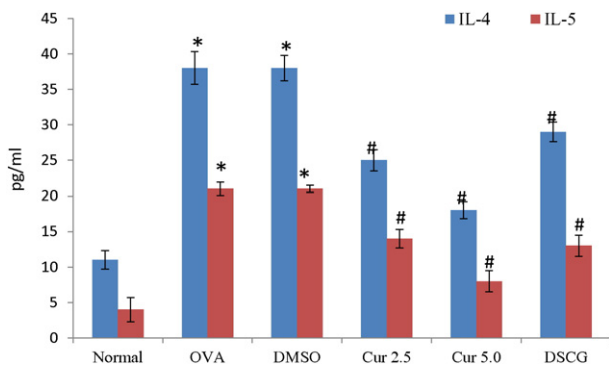


Fig. 3. Effect of intranasal curcumin on Th2 cytokines (IL-4 and IL-5): Curcumin significantly downregulated the release of both the cytokines which were upregulated in OVA sensitized and challenged mice. Values are expressed as mean $\pm$ SEM; n = 5/group in an experiment. (* $P < 0.05$ as compared to the normal # $P < 0.05$ as compared to the OVA).

4.6. Curcumin inhibits sPLA2 activities

Enhanced sPLA2 activity was noted in asthmatic mice as compared to normal. Curcumin (5 mg/kg, i.n.) treatment has significantly reduced sPLA2 activity which was equivalent to DSCG (table 3).

4.7. Curcumin inhibits PGD2 release

PGD2 level was elevated significantly in asthmatic mice as compared to the normal which was significantly and dose-dependently inhibited in curcumin treatment group (up to 30% and 61.5% at 2.5 and 5.0 mg/kg respectively, Fig 7). The release was blocked only up to 45.4% after DSCG treatment.

4.8. Curcumin ameliorates Cyclooxygenase-2 (COX-2) expression

Activation of COX-2 enzyme is responsible for arachidonic acid metabolism and PGD2 release. In asthmatic condition, COX-2 expression and PGD2 release both were enhanced and were effectively suppressed in intranasal curcumin pretreatment groups which was comparable to DSCG (Fig. 8).

4.9. Curcumin suppresses activation (phosphorylation) of MAPKinase pathway enzymes

Signaling molecules like MAPKinase pathway enzymes play a crucial role in allergic airway inflammation by activating recruitment of inflammatory cells to the lungs. Therefore, we looked for activation of three MAPkinases (p38, ERK and JNK) in OVA-induced allergic asthma model. It was observed that asthmatic mice showed enhanced expressions of all the three MAPkinases whereas intranasal curcumin was effective in inhibiting the activation of p38, ERK and JNK significantly in dose-dependent manner (Figs. 9, 10, 11).

5. Discussion

Multiple pathways regulate several pathological conditions leading to the worsening of allergic asthma. Apart from several new targets, medications available are still unlikely to have a major clinical impact as most of them are highly specific, targeting single receptor, enzyme or mediator [23]. Thus, it is imperative to find anti-inflammatory drug targeting several factors with no/minimal side effects and effective for long term use.

Curcumin being pleiotropic molecule exhibits several pharmacological effects by regulating multiple molecular targets including growth factors, transcription factors, kinases, inflammatory cytokines, adhesion molecules and apoptosis-related proteins [24]. The anti-asthmatic potential of intranasal curcumin has been studied earlier where airway inflammation and bronchoconstriction (airway obstruction) was successfully inhibited [19]. Hence, present study has focused mechanism of action of curcumin in regulating signaling cascades associated with inflammations and airway obstruction.

Following sensitization, OVA inhalation induces inflammatory changes characterized by inflammatory cell infiltration and bronchoconstriction

Table 3

sPLA2 activity in the BALF: Mice sensitized and challenged with OVA showed elevated sPLA2 level. Curcumin showed significant suppression in the sPLA2 activity in BALF as compared to OVA/OVA. Values are expressed as mean $\pm$ SEM.

Group	sPLA2 concentration (μ mol/min/ml) Mean $\pm$ SEM
Normal	0.004 $\pm$ 0.0005
OVA	0.06 $\pm$ 0.003
DMSO	0.06 $\pm$ 0.002
Cur 2.5	0.028 $\pm$ 0.002
Cur 5.0	0.01 $\pm$ 0.001
DSCG	0.02 $\pm$ 0.001

marking the development of asthma. T lymphocytes play an important role in the initiation and progression of asthma by up regulating the release of Th2 cytokines mainly IL-4, IL-5 and IL-13 and down regulating the release of Th1 cytokine, IFN- γ [4]. In present study, the enhanced release of IL-4 and IL-5 were ameliorated after intranasal curcumin treatment a dose-dependent manner (Fig 3). The enzymatic activation of mast cells lead to IL-4 production which participate in Ig class switching of activated B lymphocytes to synthesize IgG4 and IgE, promoting CD4⁺ Th2 cells and inhibiting Th1 cell development [25]. The decrease in IL-4 can be further correlated with the IgE production where intranasal curcumin has significantly declined the IgE formation (Fig 2) which is consistent with the previous findings where IL-4 inhibitor has reduced IgE level [26]. Studies have reported link between reduced IFN- γ level and increased IgE production which lead to the development of AHR in asthmatics [27]. Likewise, present finding downregulates IL-4 and IgE where a IFN- γ level was upregulated after intranasal curcumin treatment in dose-dependent manner (Figs 3 & 4).

IL-5, a potent eosinophilic inflammation marker is pivotal for growth, differentiation, recruitment and survival of eosinophils which has also been reported to cause airway hyperreactivity [28]. In the present study, IL-5 level has been significantly attenuated in curcumin administered group as compared to OVA which could be correlated with decline in eosinophilic inflammation as reported immunofluorescence study earlier [19].

Curcumin through nasal route reduces most of the OVA-induced asthmatic symptoms like bronchoconstriction and hyperreactivity to methacholine which is another interesting context of this study. Following OVA sensitization and challenge, mice developed hyperreactivity to increasing doses of aerosolized MCh. Effect of OVA induced hyperreactivity measured in terms of sRaw (specific resistance) was reduced in curcumin treatment groups (table 2) and Intranasal curcumin has restored airway function to inhaled methacholine.

Based on these results, it can be suggested that inhibition in Th-2 cytokine levels might be responsible for the immunomodulatory effects of curcumin. Th1/Th2 imbalance could be one of the mechanisms for the reduction of AHR to methacholine and other features of asthma.

Apart from the Th1 and Th2 cytokines, potent proinflammatory cytokine TNF- α , also plays a key role in elevating allergic response by acting as a chemoattractant of eosinophils [9]; thereby enhancing AHR [29] and migration them to the lungs [30]. We demonstrate an enhanced TNF- α level in OVA sensitized/challenged mice (asthmatic) while intranasal curcumin resulted in the significant inhibition (Fig 5).

TNF- α has also been reported to increase the NO production by several cells in the airways [31] where NO acts on Th1 cells to down regulate IFN- γ production thereby up regulate IL-4 and IL-5 production which in turn increases IgE level and eosinophilic inflammation [9]. The nitrite level was elevated in the asthmatic mice and significantly declined after curcumin treatment (Fig 6) both in the BALF and serum which explains the inhibition in airway inflammation. Our results are

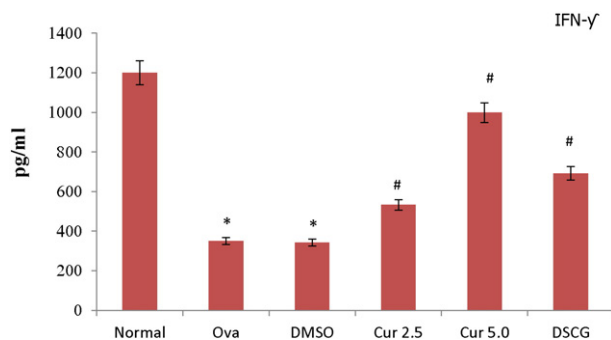


Fig 4. Effect of curcumin on Th1 cytokines (IFN- γ) level: Curcumin restored the level of IFN- γ near normal which was suppressed in asthmatic mice. Values are expressed as mean $\pm$ SEM; n = 5/group in an experiment. (* P < 0.05 as compared to the normal # P < 0.05 as compared to the OVA).

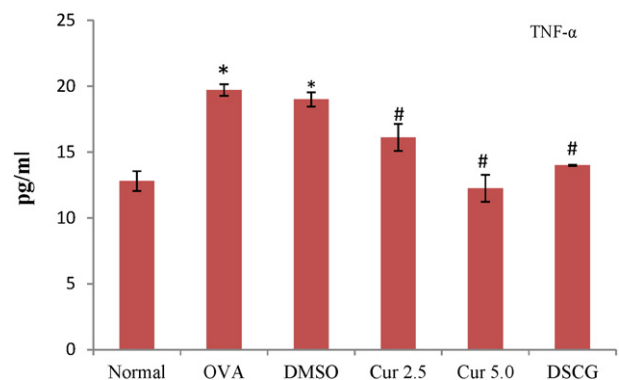


Fig 5. Effect of curcumin on proinflammatory cytokine (TNF- α): TNF- α level was elevated in asthmatic mice and was significantly inhibited by nasal curcumin. Values are expressed as mean $\pm$ SEM; n = 5/group in an experiment. (* P < 0.05 as compared to the normal # P < 0.05 as compared to the OVA).

consistent with previous report where curcumin (20 mg/kg, i.p.) attenuated OVA-induced inflammation in mice by suppressing iNOS expression and NO release by modulating Th1/Th2 balance and inflammation [32]. Thus, NO inhibition might be a factor responsible for the regulation of Th1/Th2 balance after curcumin treatment.

Lipid mediators are presently being focused for drug development against asthma as they constitute a series of mediators in inflammation progression for longer duration. The present study also emphasizes the inhibitory effect of intranasal curcumin on PGD2 release which has been reported to induce severe bronchoconstriction in allergic asthma (Fig 7) [33]. To investigate the molecular mechanism involved in its metabolic cascade, upstream factors were observed and confirmed by COX-2 expression from Western blot analysis. Activity of sPLA2, a key enzyme involved in the mobilization of membrane phospholipids were examined initially. Enhanced sPLA2 has been reported as a key factor in asthma [11,12,34] and curcumin has been found to inhibit its activity [35]. The activity of sPLA2 was elevated in OVA sensitized/challenged mice and intranasal curcumin has dose-dependently suppressed its activity (Table 3). The suppressive effect of curcumin was further correlated with PGD2 which was released after arachidonic acid metabolism catalyzed by COX-2. Interestingly, increased level of PGD2 in asthmatic mice was also suppressed (85.15%) by intranasal curcumin (5 mg/kg) which was further confirmed by significant inhibition in COX-2 expression in dose-dependent manner. Therefore, decreased sPLA2 activity could be responsible for decreased PGD2 level and COX-2 expression which is consistent with the earlier study where mepacrine inhibited the activity of COX-2 protein and decreased PGD2 level in the BALF of murine lungs [36]. It has also been reported that sPLA2 from bee venom induce IgE formation in CBA/J mice and airway inflammation in knockout mice

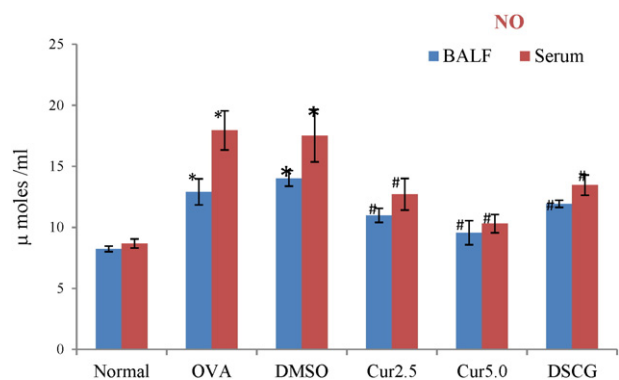


Fig 6. Release of nitric oxide in serum and BALF of mice. Nitric oxide level found elevated in OVA/OVA mice both in the serum and BALF was suppressed by curcumin treatment in dose-dependent manner. Values are expressed as mean $\pm$ SEM; n = 5/group in an experiment. (* P < 0.05 as compared to the normal # P < 0.05 as compared to the OVA).

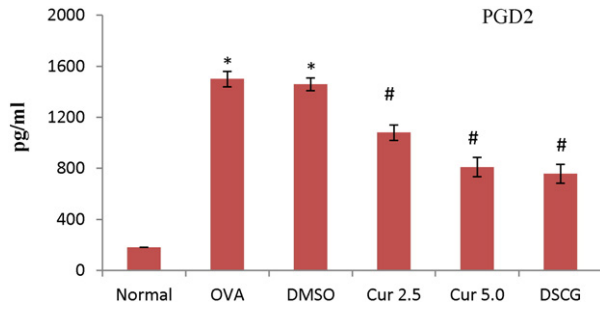


Fig. 7. Level of PGD2 in BALF: PGD2 release in the BALF of mice sensitized and challenged with OVA exhibited increased prostaglandin D2 level. Significant reduction in PGD2 level was found after curcumin treatment. Values are expressed as mean $\pm$ SEM, $n = 5$ /group in an experiment. (* $P < 0.05$ as compared to normal; # $P < 0.05$ as compared to OVA).

[37–38]. In present study inhibition in sPLA2 enzyme activity and release of PGD2 might be responsible for the inhibition in airway inflammation and IgE production.

COX-2 expression in asthmatic airways has been reported to be induced by proinflammatory cytokines IL-1 β and TNF- α and further up-regulated by NO [32]. Here, the increased production of TNF- α and NO might be responsible for the over expression of COX-2 in asthmatic mice and attenuation in TNF- α and NO level after intranasal curcumin treatment might have inhibited its activity.

MAPKs have a critical role in the expression and activation of inflammatory mediators in the airways where both resident and infiltrating cells play a crucial role [39]. MAPkinases regulate the expression of inflammatory cytokines and are activated by different upstream kinases (G proteins, Rac, cdc42 for CD40; Ras for BCR), resulting in the activities of different transcription factors [16,17,40]. Here, activation of signaling MAPkinases, including p38, ERK42/44 and JNK were ameliorated after intranasal curcumin treatment (Figs 9, 10, 11).

Present study suggests possible mechanism of action of intranasal curcumin which could significantly ameliorate phosphorylation and expression of MAPKs which is responsible for the release of proinflammatory cytokines and inflammation in allergic asthma. It is better over DSCG, which has been used as an inhalational drug for bronchial asthma for more than thirty-five years in Japan. DSCG has played an important

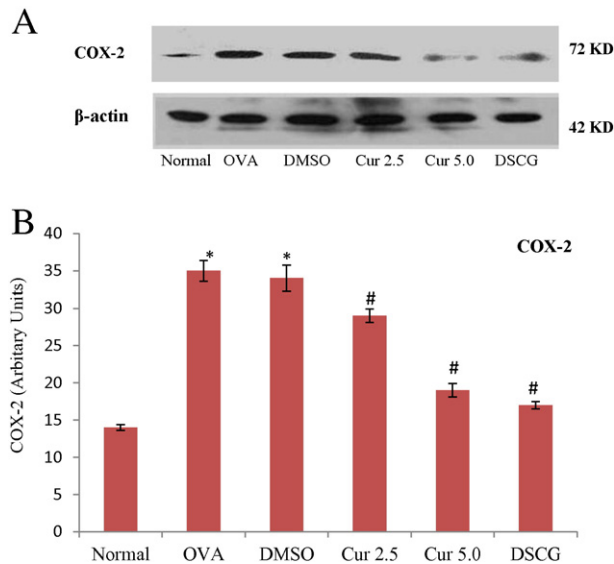


Fig. 8. A&B. COX-2 expression in the tissue homogenate (4A) and its intensity by densitometry (4B): The asthmatic mice showed increased expression of COX-2. However, mice treated with curcumin and DSCG showed reduced expression as compared to the asthmatic mice. β -actin was used as an internal control. Values are expressed as mean $\pm$ SEM $n = 5$ /group in an experiment. (* $P < 0.05$ as compared to normal; # $P < 0.05$ as compared to OVA).

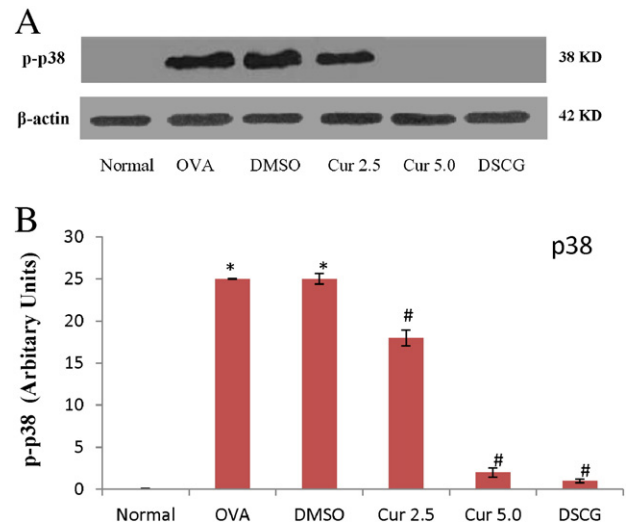


Fig. 9. A&B. Expression of p-p38 in lung tissue (A) and its intensity (B): Curcumin reduced the expression of the phosphorylated p38 which was enhanced in asthmatic mice. Values are expressed as mean $\pm$ SEM $n = 5$ /group in an experiment. (* $P < 0.05$ as compared to normal; # $P < 0.05$ as compared to OVA).

role in the treatment of children with bronchial asthma since the time when the regular use of DSCG and salbutamol inhalation solution was initially recommended [41]. DSCG and inhaled corticosteroids both are recommended as first choice preventive treatment in young children rather than DSCG being recommended as the first choice before inhaled corticosteroids. According to GINA 2015, Sodium cromoglycate and nedocromil sodium have a limited role in the long-term treatment of asthma in children. Their anti-inflammatory effect is weak and they are less effective than low-dose inhaled corticosteroids [42–43].

6. Conclusion

Intranasal curcumin can alleviate allergen induced airway inflammation in mouse model of asthma as lungs are directly targeted and curcumin might be absorbed through nasal mucosa. Besides restoring bronchoconstriction, it could inhibit AHR and the late phase markers

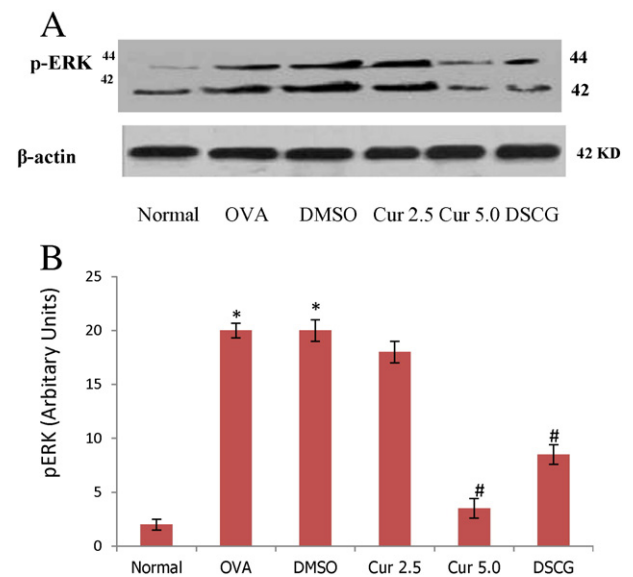


Fig. 10. A&B. Expression of p-ERK in lung tissue (A) and its intensity (B). Curcumin reduced the expression of the phosphorylated ERK in dose-dependent manner which was enhanced in asthmatic mice. Values are expressed as mean $\pm$ SEM $n = 5$ /group in an experiment. (* $P < 0.05$ as compared to normal; # $P < 0.05$ as compared to OVA).

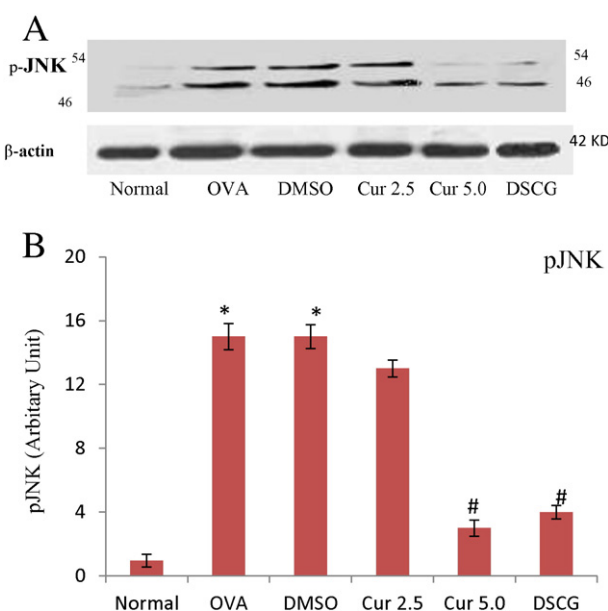


Fig. 11. A&B: Expression of p-JNK in lung tissue (A) and its intensity (B). Curcumin reduced the expression of the phosphorylated JNK which was enhanced in asthmatic mice. Values are expressed as mean $\pm$ SEM $n = 5$ /group in an experiment. (* $P < 0.05$ as compared to normal; # $P < 0.05$ as compared to OVA).

like PGD₂, eosinophils recruitment through modulating various signaling molecules. Therefore, intranasal curcumin could be useful lead molecule in the development of anti-allergic drug as it can hit multiple targets at a time and can be used safely for longer.

Acknowledgments

Authors are thankful to University Grants Commission (F.No. 39-610/2010(SR)) and Science and Engineering Research Board (SERB-DST) (grant no. SR/FT/LS-84-2011), New Delhi, India in part for financial assistance.

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