Phytomedicine Plus 3 (2023) 100413

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Comparative evaluation of two intranasal forms of Curcumin: Quantitation and impact on mouse model of asthma

Payal Singh [a], Ruchi Chawla [b], Ajai Kumar Pandey [c], J.K. Mishra [d], Rashmi Singh [a,*]

[a] Department of Zoology, MMV, Banaras Hindu University
[b] Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University
[c] Department of Kaychikitsa, Institute of Medical Sciences, Banaras Hindu University
[d] Department of TB and Respiratory diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, India

ARTICLE INFO

Keywords:
Airway inflammation
Anti-inflammatory
Curcumin
Internal standard
HPLC

ABSTRACT

Aim of the study: Present study aimed to investigate impact of two different intranasal forms of curcumin on mouse model of asthma and detection of its absorption after different time points of administration. Since the systemic bioavailability of curcumin is very low, therefore, intranasal route of administration has been explored here where systemic absorption of two intranasal forms of curcumin was evaluated.

Materials and methods: To explore the possibilities of its absorption and role in amelioration of asthma, two intranasal forms of curcumin, nasal drop and aerosol were administered to control and asthmatic mice. Blood plasma and lungs were collected at different time points of curcumin administration. Separation was done by using isocratic reversed phase high performance liquid chromatography (HPLC). Airway inflammation and oxidative stress parameters like SOD, Catalase, ROS, lipid peroxidation, and total inflammatory cell recruitments were analyzed to study efficacy of two intranasal forms of curcumin.

Results: Maximum absorption was noted at 3 hrs of different intranasal forms of curcumin, administration, i.e., nasal drop (1.93 ± 0.050 plasma, 2.87 ± 0.26 in lungs) and aerosol form of curcumin where better absorption was noted in aerosol form as compared to nasal drop (Lungs 3.08 ± 0.12 aerosol, 2.05 ± 0.020 nasal drop) at 1 h. Both, curcumin aerosol and nasal drops were effective in ameliorating oxidative stress ($p < 0.05$) associated with asthma in mice model. The present study may pave the way towards the development of intranasal curcumin as complementary medication because of its better absorption in plasma and lungs.

1. Introduction

Curcumin is a major polyphenolic component of spice turmeric (*Curcumin longa*), derived from the member of the ginger family (Zingiberaceae). Three different curcuminoids are found in turmeric in which curcumin is the most active constituent. Curcumin (diferuloylmethane) provides turmeric its yellow color which is used as a food and flavor in south Asian countries mostly in India and Indonesia. In Indian medicine, curcumin has been recognized for its medicinal properties like anti-oxidative, anti-inflammatory, and antimicrobial potentials (Kolodziejczyket al., 2011; Yu, 2016; Menon and Sudhir 2007)which has also been studied to be effective in allergic asthma (Kurup and Barrios, 2008; Subhashini et al., 2013; Shahid et al., 2019), arthritis, anxiety, and metabolic syndromes (Fang and Holmgren 2005). Several studies have reported its potential to suppress tumor formation and have anticancer properties (Fang and Holmgren, 2005; Wu et al, 2015). Simultaneously, even many different curcumin analogs 2, 6-Bis-(4-hydroxyyl -3methoxybenzylidine) cyclohexanone and (B2BrBC, C66) were also reported efficient in treatment of allergic asthma and airways hyperactivity (Tham et al., 2021; Stamenkovska et al., 2020). Despite all the merits, curcumin did not get approval for clinical trials due to its poor bioavailability. The first report published in 1978, states that after oral administration of curcumin (1 g/kg), a negligible amount of curcumin was detected in the blood plasma of Sprague Dawley rats. It was reported that a major portion of curcumin was metabolized and it was poorly absorbed in the gut (Azhdari 2019; Wahlström and Blennow,

Abbreviations: EPO, eosinophil peroxidase; HPLC, High perfoprmance liquid chromatography; MDA, **malondialdehyde**; MPO, myeloperoxidase; OVA, Ovalbumine; ROS, Reactive oxygen species; SOD, superoxide dismutase.

* Corresponding author.
E-mail addresses: rashmirs98@rediffmail.com, singhras@bhu.ac.in (R. Singh).

https://doi.org/10.1016/j.phyplu.2023.100413

Available online 19 January 2023

1978). It was not detected in heart blood after oral administration (400 mg of curcumin) in rats, only traces were detected in portal blood from 15 min up to 24 h after administration (Ravindranath and Chandrasekhara, 1980). It was also reported (Pan, 1999) that higher curcumin level (2.25 μg/ml) appeared in the plasma in the first 15 min and then declined rapidly within 1h after intraperitoneal administration in mice (0.1 g/kg, *ip*), which was totally different from oral administration (1.0 g/kg., *po*.) where a very low level of curcumin (0.13 μg/mL) appeared in plasma after 15 min, while a maximum level was noted in plasma (0.22 μg/mL) within 1 h. The pharmacokinetic studies have shown its detection after *i.p.* injection [14C] and dietary uptake for a week, irrespective of the dose, in the plasma at levels near the limit of detection (5 pmol/ml) (Perkins et al., 2002). The poor absorption of oral curcumin might be due to its high metabolism and rapid elimination. However, the metabolic product of curcumin, tetrahydrocurcumin was also able to reduce airways symptoms and lungs inflammation (Wu et al., 2020). Some studies have reported that intranasal administration of curcumin avoids the gastrointestinal and hepatic presystemic metabolism, enhanced drug bioavailability in comparison to that obtained after gastrointestinal absorption (Anand, 2007; Leonard, 2007). Curcumin level was quantified for the first time in water extract of turmeric by using HPLC (Asakawa 1981). Further improved HPLC methods were developed and validated for quantification of curcumin (Jayaprakasha, 2002 Fig. 5 **Experiment II (A-J)**). Poor bioavailability of curcumin has prompted to search for better effective routes of delivery. We have shown earlier that route of curcumin administration affects its distribution and absorption as higher concentration was detected 3 h post dosing via intranasal route (5 mg/kg, *i.n.*) as compared to intraperitoneal then declined (Subhashini et al., 2013). It has been reported that aerosolized curcumin inhalation showed effective delivery to pathologically relevant regions of the brain (McClure, 2017). Here, we demonstrate a unique approach that utilizes curcumin aerosol delivery to recapitulate its bioavailability and absorption in mice. Many methods were developed for quantification of curcumin in turmeric powder and nutraceutical products. Most of the HPLC methods had certain limitations such as long run time and complicated gradient mobile phase, where these methods are restricted to the turmeric products (Li et al., 2011; Osorio Tobon., 2016). Therefore, in this method methanol and ethyl acetate was used as extraction solvent which was an appropriate solvent for extraction from biological samples. To overcome the limitations regarding peak tailing and broadening, this method is found efficient as both gradient and isocratic elution was evaluated with simple mobile phase composition. This method is reproducible also for quantitation of curcumin and gives better separation at C18 column.

In present study, absorption of two intranasal forms of curcumin, nasal drop and aerosol were quantified in blood plasma and lungs after different durations of its administration using HPLC. Their effects on inflammatory cell recruitment, lipid peroxidation and oxidative stress were evaluated in OVA-induced asthma. Exposure of antigen (OVA) results in the infiltration and activation of inflammatory cells, which further produce more ROS (Nita and Grzybowski, 2016). The pathophysiology of asthmatic airway inflammation and tissue damage, which include damaged epithelium, shedding off epithelial layer, and hyperresponsive airways, is significantly influenced by ROS (Kudo et al., 2013).Thus, therapeutic strategy has traditionally focused on antioxidant treatment of allergic asthmatic inflammation. Oxidative inactivation of SOD enhances apoptosis and degrades the epithelial barrier in conducting airways. These are believed to be potential contributors to airway hyperresponsiveness and may promote airway remodeling (Janssen-Heininger, 2005). Enzymatic and non-enzymatic mechanisms can be found in the extensive lung antioxidant defenses like catalases, Cu, Zn superoxide dismutase (SOD), and glutathione peroxidases which are the main extracellular enzymatic antioxidants (Irato and Santovito, 2021; Birben et al., 2012). We recently demonstrated intranasal curcumin pretreatment plays a protective role in OVA-induced asthma where airway inflammation and oxidative stress were regulated (Islam et al., 2022). In accordance with this discovery, we also have reported significant lowering in oxidative stress induced by LPS where marked increase in antioxidant enzymes SOD and catalase was found in acute lung injury model by intranasal curcumin pretreatment (10 mg/kg, i.n). Significantly reduced nitric oxide, MPO, MDA and MMP-9 was also reported in curcumin pretreatment group (Kumari et al., 2015). We also have explored its potential impact on silica induced lung damage, where intranasal curcumin pretreatment (5 mg/kg, i.n.) has reduced expression of fibrosis markers MMP-9, α-SMA and Hydroxyproline, which ultimately helps in reducing the structural changes thereby lung damage (Kumari and Singh, 2022). Since we have extensively explored curcumin nasal drops in OVA-induced asthma, curcumin aerosol is being investigated here for its therapeutic efficacy. Therefore, aim of the present study is to investigate whether curcumin aerosol can alleviate OVA-induced inflammation, histopathological changes in lungs and oxidative stress with same efficacy like nasal drop.

2. Material and methods

2.1. Chemicals and reagents

Curcumin, emodin (internal standard, IS) and Ovalbumin(Ova, grade V,) were obtained from Sigma Chemical Co. (St Louis, MO, USA). The purities of curcumin and emodin were >99.5%. HPLC grade acetonitrile, methanol, and water were purchased from Merck India Ltd. All other chemicals and reagents were of analytical grade and used without any further purification.

2.1.1. Experimental animals

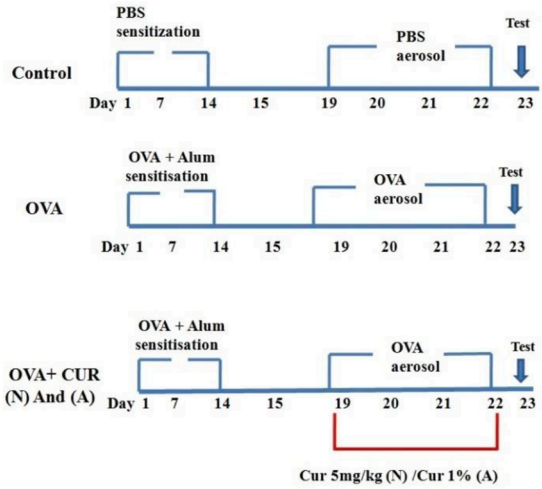
BALB/c mice (6–8weeks old; 24–26 g) were procured from Central Drug Research Institute, Lucknow, India, and acclimatized for a week under standard laboratory conditions (like 20–25 °C temperature with 40–60% humidity and 12 h light /dark cycle). The asthmatic mouse model was made by inducing with Ovalbumine (OVA + alum sensitized/ OVA challenge). Control mice were given alum-containing saline and challenged with saline alone (Fig. 1 upper panel). Experimental protocols were approved by the Central Animal Ethical Committee, Banaras Hindu University, Varanasi, India.

2.2. HPLC-UV instrumentation and chromatography parameters

Analysis was carried out on a Shimadzu SPD-M20A consisting of Photodiode array (PDA) detector, Refractive index detector, UV detector and Auto sampler available at the Central instrumentation laboratory, MMV, Banaras Hindu University, Varanasi, India.

2.2.1. Chromatographic condition for experiment I

The chromatographic separation was performed on C18 column LC, Shim-pack GIST (25 cm × 4.6 mm, 5 μm particle size); mobile phase consists 80% methanol with 20% water; flow rate 1.0 ml/min. Curcumin shows strong absorption at 420 nm due to its conjugated structure, so detection was achieved at wavelength, 420 nm, and the injection volume was kept 20 μl. HPLC was performed isocratically at 1 ml/min flow rate and the retention time of curcumin was observed at 4.43 min. Emodin, a natural anthraquinone, was used as an internal standard due to its structural similarities with curcumin (Fig 1). Behavioral characteristics and properties of emodin have confirmed chemical requirements of internal standard (IS) in HPLC. The retention time was observed for emodin, which was 7.8 min (Fig. 2). In addition, emodin is commercially available with high purity, and it is stable and non-reactive with sample or mobile phase. Meanwhile, it also has good response at the detection wavelength (420 nm). Calibration curves were prepared using curcumin standards ranging from 1 to 100 μg/ml. The amount of curcumin in the sample was determined from the peak area using a calibration curve constructed from standard curcumin. Three quality control samples of curcumin and emodin was analyzed, the



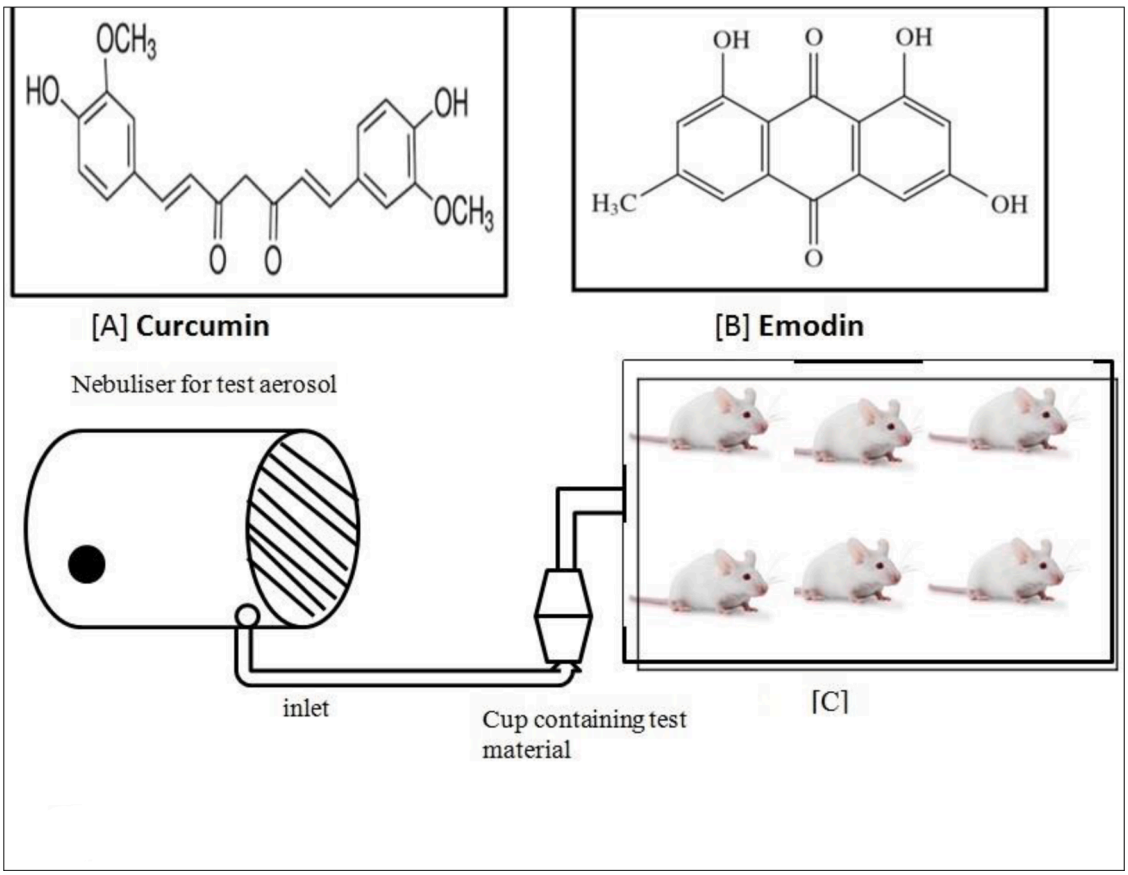


Fig. 1. Experimental plan for the generation of OVA-induced asthma model and protocol of curcumin administration (upper panel); Chemical Structure of curcumin and emodin [A] and [B] while [C] represents OVA/Curcumin aerosol exposure method using aerosol generation device (nebulizer, omron) and chamber.

absolute recovery was calculated by comparing the peak areas of the prepared QC samples with those of the standard solutions. For the extraction recovery of curcumin, three quality control samples were prepared LQC (low) MQC (medium) and HQC (high) (5 μg, 2.5 μg and 1 μg) the recovery percent were 112%, 92% and 95.8%, respectively. Emodin (internal standard) was added equally in all three quality control samples (Table 1).

2.2.2. *Chromatographic condition for experiment II*

A binary-gradient mobile phase containing 70% methanol and 30% water was employed in the optimized procedure. The mobile phase was filtered through 0.2 μm syringe filter (Axiva Sichem), vacuum-degassed, and sonicated for 30 min (ICON ISO 9001-2008). The injection volume was 20 μl, with a flow rate of 1 ml/min. Instrument control and data analysis were done with the LC solution software programme (Shimadzu, Japan) running on a Dell PC. Samples were stored in deep freezer (-80°C). In experiment II, external standard method is used for sample validation. The retention time for cucumin was observed at 8.3 min. Curcumin (10 μg/ml and 20 μg/ml) spiked samples were prepared in mice plasma where blank plasma was used as a control.

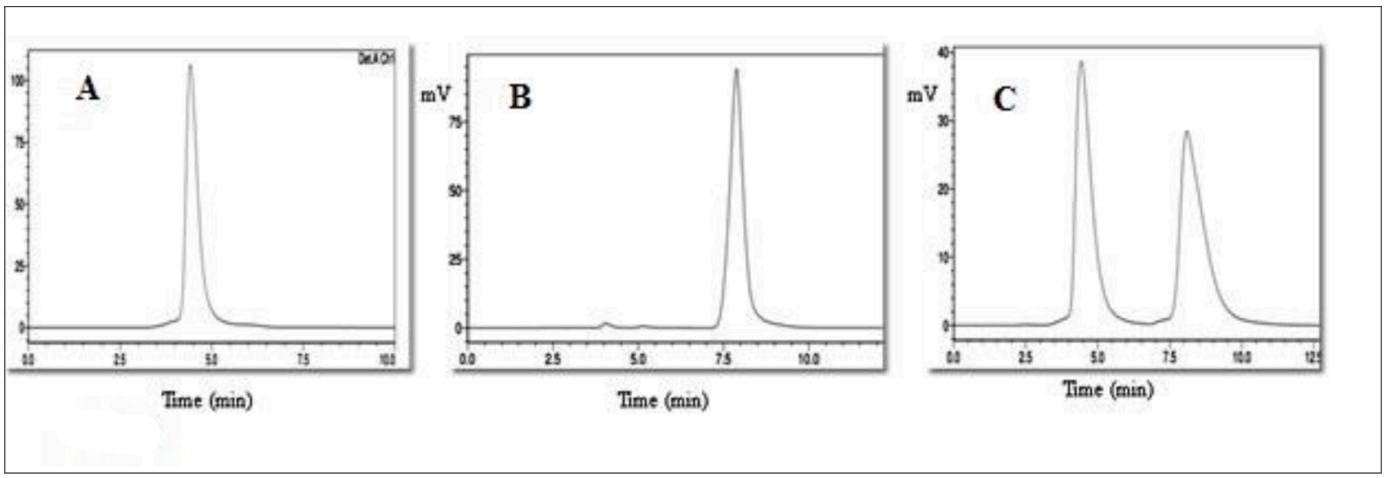


Fig. 2. Represents the peak of methanolic stock of curcumin (RT-4.7) (A), emodin (B) (IS, RT-7.5) and (C) both curcumin and emodin (C). Chromatographic conditions, mobile phase 80:20, Flow rate 1 ml/min, injection volume 20 µl, and Absorbance 420 nm. Abbreviation: RT = Retention time, IS-Internal Standard.

Table 1
Concentration of curcumin and internal standard emodin for calibration in mice plasma of three quality control samples (n = 3), expressed as recovery% (observed concentration/Nominal concentration)*100.of calibration sample.

Nominal concentration (µg/ml)		Recovery%		Observed concentration (µg/ml)	
Curcumin	Emodin	Curcumin	Emodin	Curcumin	Emodin
5	2.5	95.8	111.6	4.79	2.79
2.5	2.5	92	92	2.34	2.34
1	2.5	112	105	1.12	2.63

2.3. Preparation of stock solutions and calibration samples

Stock solutions of curcumin and emodin were prepared in methanol at 1 mg/ml (concentration). Further, both curcumin and emodin stock solutions were diluted with methanol to working solutions ranging from 1 to 100 µg /ml. Blank Plasma was used to prepare different calibration samples (1–100 µg/ml) from the working stock of curcumin (methanolic stock). All solutions were protected from light and stored at 4 °C. Curcumin was administered to mice groups as a nasal drop (5 mg/kg, *in*) and aerosol (1%). Blood samples were collected from the orbital plexus of mice at different time points (0.5, 1, 3, 6 h) post dosing (curcumin nasal drops at 5 mg/kg) or 1 h after curcumin aerosol (1% for 30 min). Plasma was separated from blood (heparinized tubes) after centrifugation at 1500 rpm for 15 min. The resulting plasma was deproteinized by mixing it with equal volumes of methanol and ethyl acetate (1:1), then it (100 µl) was mixed with 20 µl of fixed concentration of IS. After mixing, sample was vortexed for 1 min and centrifuged at 12000 rpm for 15 min. Supernatants were transferred to a fresh tube and dried at 37 °C, methanol: water (80:20) was added and injected directly into the column. Plasma was stored (at −20 °C) before analysis. To collect lung samples, mice were sacrificed and only absorbed curcumin was determined which was extracted from the samples. The lungs were removed from euthanized mice at the same time points post dosing, weighed, minced, and homogenized in phosphate buffer (sonicated in PBS), and centrifuged for 20 min at 12000 rpm. Curcumin was extracted by adding acetonitrile: PBS (1:1 v/v). After centrifugation, supernatants were subjected to HPLC analysis. Results were normalized to the protein level. Then curcumin levels were detected and quantified by HPLC.

2.4. Calibration and linearity

The linearity of the method was evaluated by plotting a curve between peak area and concentration. The curve was constructed by plotting the peak area ratios of plasma standards versus nominal concentration (curcumin/IS). Calibration curve was highly linear ($r2 > 0.999$) within the range of 1–100 µg/mL for curcumin (Table 2).

2.5. Groups for Experiment I (for quantification of curcumin in control mice)

Mice were randomly divided into two groups, curcumin **nasal drop** and **aerosol group** (5 mice at each time point in both groups). Mice were subjected to curcumin **nasal drop** (5mg/kg) whereas **aerosol** inhalation (1%) was given for 30 min in a Plexiglass chamber using a nebulizer (Omran, Japan) with an airflow rate of 9l/min. After different time points (0.5 h, 1 h, 3 h, 6 h) of intranasal curcumin administration (nasal drop), blood and lungs were collected from mice of both the groups; however, from aerosolized curcumin group (Fig 1b), blood was collected at a single time point (1h post aerosol exposure).

2.6. Groups for Experiment II (for quantification of curcumin in asthmatic mice)

Mice were randomly divided into four groups, (n = 5; either sex) (i) Wild-type (**Control**) (ii) asthmatic mice model (**OVA)** (OVA + alum sensitized/OVA challenge), Treatment groups (iii) (**OVA+N**) (OVA-+alum sensitized/OVA challenge /CUR treated (5mg/kg, *i.n.*) (iv) (**OVA+A)** (OVA + alum sensitized /OVA challenge/CUR treated (1%

Table 2
Inter and intraday precision and accuracy of standard curcumin samples. RSD is calculated as (SD/Mean)*100, (Data are represents as Mean ± SD where SD = Standard deviation).

	Curcumin standard					
	Inter day (n = 5)			Intraday (n = 5)		
Nominal Conc. (µg/ml)	Recovery %	Observed Conc. Mean ± SD	RSD %	Bias %	Recovery %	RSD %
100	100.84	100.84 ± 6.90	6.84	0.84	98.89	1.51
50	98.00	49 ± 1.58	3.22	-2	99.90	0.44
20	98.30	19.66 ± 2.11	10.73	-1.7	99.45	0.27
10	100.00	10 ± 0.070	0.7	0	99.98	2.48
5	100.2	5.01 ± 0.004	0.07	0.2	99.87	0.27
2.5	93.63	2.34 ± 0.181	7.735	-6.4	106.23	5.96
1	106.00	1.002 ± 0.070	6.98	-0.9	99.97	12.65

(w/v) aerosol). **Control** mice were given alum-containing saline (i.p.) and then challenged with saline alone. On days 0, 7, and 14, the remaining three groups received saline suspension (0.2 ml) containing ovalbumin (50g) emulsified in 4mg of aluminium hydroxide. Mice were given 1% OVA aerosol inhalation for 30 min everyday from days19 to 22. Curcumin (5 mg/kg, *i.n.*) and aerosol were delivered to mice using the same technique as in experiment I (Fig 1).

2.7. Collection of bronchoalveolar lavage fluid (BALF), serum and lungs

Mice were euthanized 24 h after the last OVA aerosol challenge, and BALF was collected and washed. Lung washings were centrifuged for 10 min (at 3000 rpm, 4 °C). Inflammatory cells from BALF cell pellet were stained with giemsa and evaluated. Lung lobes were fixed in 10% neutral buffer formalin and stored (at -80 °C), whereas BALF supernatants were used for further investigations.

2.8. Determination of Reactive oxygen species (ROS) in BALF

In BALF, ROS was measured in BALF cell pellet (Eruslanov and Kusmartsev, 2010). Briefly, BALF cells (1×10^6) were isolated from each group, washed in PBS, and plated in a 96-well black plate. DCFDA (10 M) in DMSO was incubated for 30 min at 37 °C in the dark. Microplate fluorescence reader (Bio-Tek instruments Inc., 9 Winooski, VT, USA) was used to measure fluorescence intensity at excitation (485 nm) and emission (530 nm) wavelengths and ROS levels were expressed in arbitrary units.

2.9. Total cell count in BALF

BALF cells were counted by using trypan blue dye exclusiom test where cell pellet was mixed (1:1) with trypan blue dye (0.2%)..

2.10. Determination of Eosinophil peroxidase (EPO) activity

Lungs were tested for EPO activity (Negrao-Corrêa,2003). Homogenized lungs (10% PBS (w/v) were centrifuged (6000 rpm at 4°C for 15 min) and cell pellet was re-suspended in PBS containing 0.5% HTAC and homogenized followed by three freeze thaw cycles. OPD (1.5mM) and hydrogen peroxide H_2O_2 (6.6 mM)in 75 mM Tris–HCl (pH-8.0) were combined with samples, then incubated for 30 min at 20 °C in the dark. H_2SO_4 (4 M) was added to stop the reaction, and results were displayed in absorbance units.

2.11. Myeloperoxidase (MPO) activity determination

MPO activity was measured in lung tissue homogenate to assess neutrophil infiltration and activation (Islam, 2022). Lungs were homogenized in potassium phosphate buffer (50 mM, pH 6.0) containing 0.5% CTAB (cetyltrimethyl ammonium bromide) and frozen/thawed three times at 80 degree celsius. Lung homogenate (300 ml) was added with a reaction mixture comprising 0.002% hydrogen peroxide (H_2O_2) and 0.167 mg/ml O-dianisidinedihydro-chloride. Absorbance was measured at 460 nm for 20 min.

2.12. Malondialdehyde assay (MDA)

MDA was measured using a previously established method with slight modifications (Kumari, 2015). Thiobarbituric acid (TBARS) active ingredients were used to quantify the amount of malondialdehyde (MDA) in lung tissue homogenate. In brief, 50 µl of 8.1% SDS, 375 µl of 20% acetic acid, and 375 µl of 8.1% thiobarbituric acid were added to 10% lung tissue homogenate in potassium phosphate buffer (pH = 7.4). The solution was cooled at room temperature after one hour of boiling in order to turn it pink. Then 250 µl of distilled water, pyridine, and n-butanol were added (1.25 ml of 1:1 solution),. centrifuged (2000 rpm for 10 min), absorbance of upper layer was measured at 532 nm and MDA concentration was expressed in Moles/mg.

2.13. Catalase activity

Lung homogenate was used to determine catalase activity using a technique described earlier (Kumari, 2015). In brief, lung homogenate was diluted in phosphate buffer (pH-7.4) where H_2O_2 was used as substrate. Catalase activity of each sample was measured at 240 nm for 3 min and expressed in katals per second per mg of protein.

2.14. SOD level

Superoxide dismutase (SOD) levels in lung tissue were evaluated as previously described method (Kumari, 2015). In phosphate buffer (pH 7.4), lung homogenate (50 µl) was mixed with reaction mixture containing 75 µl of 20 mM-methionine, 40 µl of TritonX-100, 75 µl of 100 mM hydroxylamine hydrochloride, and 100 µl 50 µM EDTA. After 5 min at 37 °C, 80 µl of 50 M riboflavin was added, and the reaction mixture was exposed to white light for 10 min. Griess reagent (1 ml) was added to the reaction mixture, which contained a 1:1 solution of 0.1% N-(1-naphthyl) ethylenediamine and 1.0% sulphanilic acid in 5% orthophosphoric acid. The absorbance was measured at 543 nm. The activity of SOD was measured in per mg of protein.

2.15. Lung histology

Lungs were washed with 10% neutral buffer formalin (NBF) before removal and then fixed. Lung tissues were embedded in paraffin, thin sections (5 µm) were cut and stained with hematoxylin–eosin (H&E) and observed under light microscope (Zeiss Axiocam 208 monocolor / 202).

2.16. Statistical analysis

Data are represented as mean ± SE. Statistical analysis was performed using GraphPad Prism 5 software (San Diego, CA, USA). Drug absorption analysis was performed by using a one-way analysis of variance and Student's t-test. Statistical significance reported at $*p < 0.05$.

3. Results

3.1. Evaluation of intranasal curcumin (nasal drop) absorption at different time points using control mice (Experiment I)

Intranasal curcumin (5mg/kg, n=5) absorption was evaluated at different time points and maximum absorption was observed at 3 h post curcumin administration (5 mg/kg, n = 5, *i.n*) in both plasma and lungs of control mice. However, absorption in lungs was significantly higher as compared to plasma at 3 hrs (Plasma 1.93 ± 0.090, 2.50 ± 0.26 µg in lungs) (Fig 3a and b).

3.2. Curcumin aerosol absorption and its detection after 1 hr in control mice

Curcumin aerosol (1%) showed better absorption (3.08 µg) than nasal drop (5 mg/kg; 2.05 µg) after 1 hr in lung homogenate (Fig 4), whereas significant increase was reported in lungs as compared to plasma after 1 hr of curcumin aerosol exposure (plasma 2.34 ± 0.27 and 3.08 ± 0.12 µg in **O+A group** lungs) (Mean ± SE) (Fig 4E).

3.3. Evaluation of intranasal curcumin (nasal drop and aerosol) in OVA induced asthmatic model (Experiment II)

Intranasal Curcumin was detected in plasma and lungs of mice of different groups at day 23rd after four consecutive days of

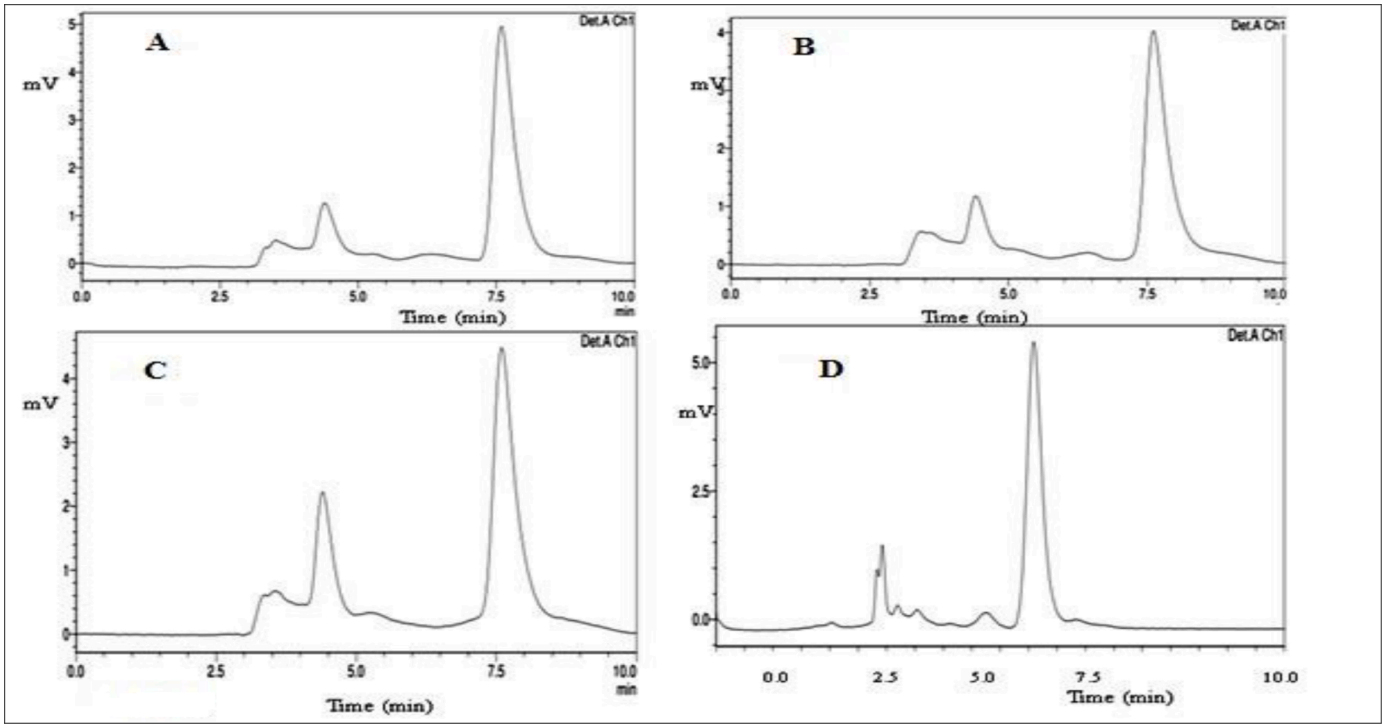


Fig. 3. (a) Intranasal curcumin detection (nasal drop) in mice lung samples at different time points.: Exhibit absorption of intranasal curcumin (5 mg/kg, n = 5) at different time points: (A) 0.5 h, (B) 1 h, (C) 3 h, (D) 6 h in lungs. Maximum absorption was found at 3 hrs in lungs at 3hrs whereas very less least absorption was noted at 6 hrs; 3b (AE) Absorption of intranasal curcumin (5mg/kg, n = 5) at different time points in plasma: (A) 0.5 h, (B) 1 h, (C)3h, (D) 6 h (E) graph represented the maximum absorption was observed at 3 h post curcumin administrationdosing (5 mg/kg, n = 5, *i.n.*) in plasma of control mice. Maximum absorption was noted in both lungs and plasma samples at 3 h, however, better absorption (2.87 μg) was found in lung homogenate as compared to plasma (1.93 μg) (*p < 0.05 Lung vs blood plasma).

administration. Similar trend of curcumin absorption was noted in control and **O+N** groups (Fig 5 A–E). No significant difference was seen between control and **O+N** groups. However, **O+A** group showed significantly higher amount of curcumin absorption in both lungs and plasma as compared to **O+N** and control groups (#p < 0.05 **O+A** vs **O+N** and Control). Instantaneously, higher absorption in lungs of **O+A** group mice was noted as compared to plasma on 23rd day (*p < 0.05 O+A lungs vs **O+A** Plasma) (Fig 5). Spiked curcumin samples (10 μg and 20 μg) were prepared in blank plasma to assure the retention time of curcumin through previously described method in experiment II (Fig 5 G–I).

3.4. Intranasal curcumin (nasal drop and aerosol) suppressed eosinophil and neutrophil recruitment in lungs

Eosinophil peroxidase activity (EPO) in lung homogenate was quantitated to assess recruitment of eosinophils to lungs which was found elevated (75%) in OVA group as compared to control. Significantly reduced (p<0.05) level was observed in both the treatment groups (Fig 6A). Myeloperoxidase activity as a measure of neutrophil recruitment was elevated in OVA group. Significant decline was noted in both, intranasal curcumin treatment groups (Fig 6A). Both **O+N** and **O+A** groups were efficient in reducing the neutrophil recruitment in asthmatic lungs.

3.5. Intranasal curcumin supressed the lipid peroxidation in OVA-induced asthmatic lungs

Malondialdehyde is the product of lipid peroxidation which was measured in lung homogenate. Increased MDA level was reported in OVA group, which were further reduced significantly in curcumin treatment groups (Fig 6A). Both the intranasal forms of curcumin O+N and O+A was found to be effective in reducing the lipid peroxidation in OVA-induced asthmatic mice.

3.6. Oxidative stress was ameliorated in both intranasal forms of curcumin treatment groups

Enzymes activities of catalase and superoxide dismutase (SOD) were measured to evaluate oxidative stress in lungs. Significant decrease (*p < 0.05 control vs OVA) in enzyme activities in OVA group was improved in both the curcumin treatment groups (Fig 6B).

3.7. Reactive oxygen species level was ameliorated in curcumin nasal drop treatment study

Increased ROS was detected in OVA group which was reduced significantly after curcumin nasal drop treatment. Both, nasal drop and aerosol treatment strategies were effective in reducing the reactive oxygen species significantly as compared to OVA group. However, **O+N** was found better in reducing the ROS (upto 42%) as compared to OVA group (Fig. 6C).In contrary to **O+N** group, **O+A** group was not much effective in reducing ROS level but it has efficiently reduced ROS level up to 32%.

3.8. Total cell count was reduced in both intranasal curcumin treatment groups

Total inflammatory cell recruitment was significantly (p < 0.05) higher in **OVA group** as compared to **control**. Further, reduced recruitment was observed in both the treatment groups. Both **O+ A** and **O+N** was efficient in reducing inflammatory cell recruitments (p <

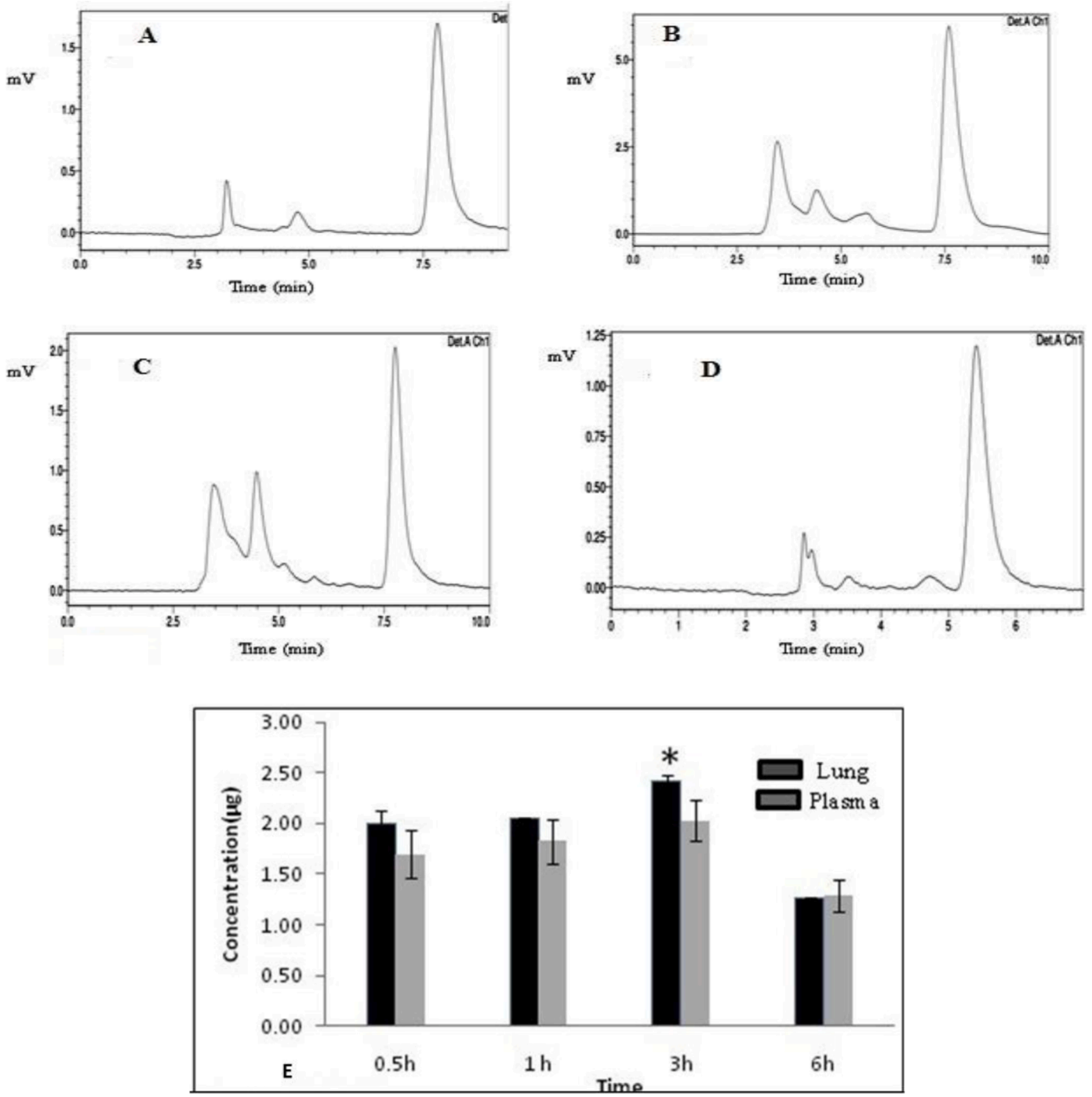


Fig. 3. (*continued*).

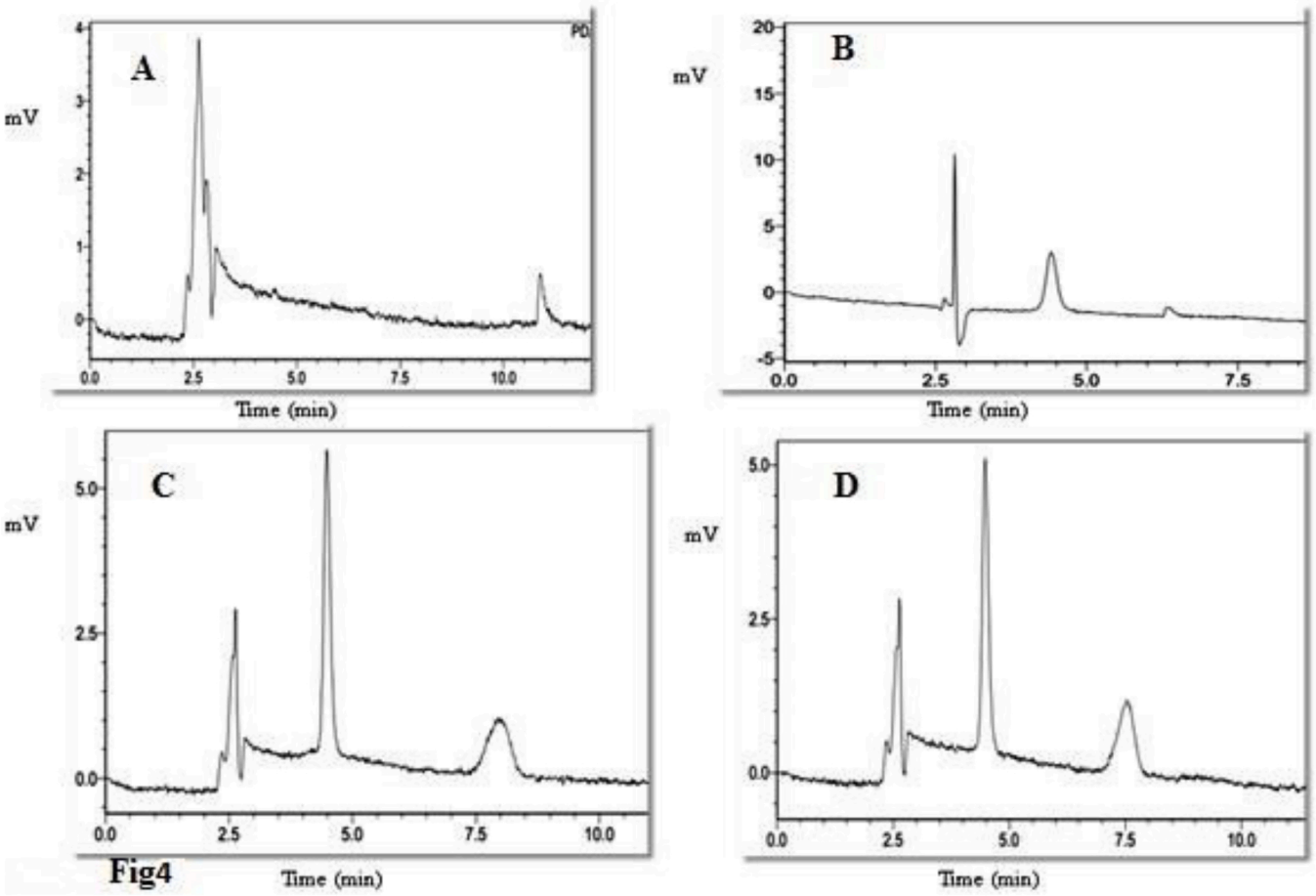
0.05) (Fig 6D).

3.9. *Effect of intranasal curcumin on histopathology of lungs*

After microscopic analysis, enhanced inflammatory cells were observed in giemsa stained cytospun preparations of BALF cells which was reduced in curcumin treatment groups. Similar trend was noted in H&E stained lung sections where large number of inflammatory cells were observed in alveolar spaces of asthmatic lungs which were ameliorated in curcumin group. Remarkable bronchoconstriction was seen in asthmatic lungs whereas relaxed bronchioles with larger lumen was seen in both the intranasal forms of curcumin treated groups (Fig 7).

4. Discussion

Being anti-inflammatory and anti-oxidant, curcumin is known for its poor bioavailability due to less aqueous solubility. These limitations should be addressed at basic level using animal model. Previous studies have reported that after oral administration of curcumin in rats (2g/kg), a maximum serum concentration was observed (1.35 µg/mL) at 0.83 h (Anand et al., 2007), whereas, Pan et al., reported maximum absorption of curcumin (0.22 µg/ml) after 1 h of oral administration (1.0 g/kg) (Pan et al., 1999). However, after 15 min of intraperitoneal administration, maximum plasma curcumin level was observed (2.25 µg/mL) and it declined rapidly within 1 h. The rapid metabolism and poor absorption of curcumin administered either oral or intraperitoneal (i.p.)



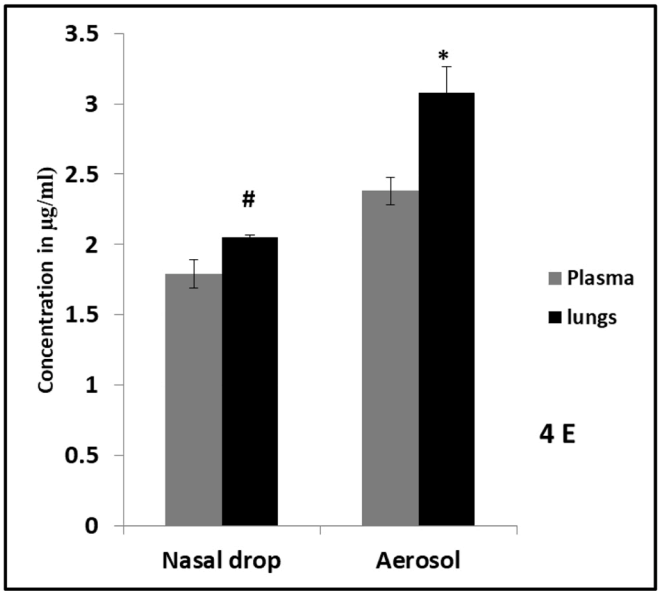
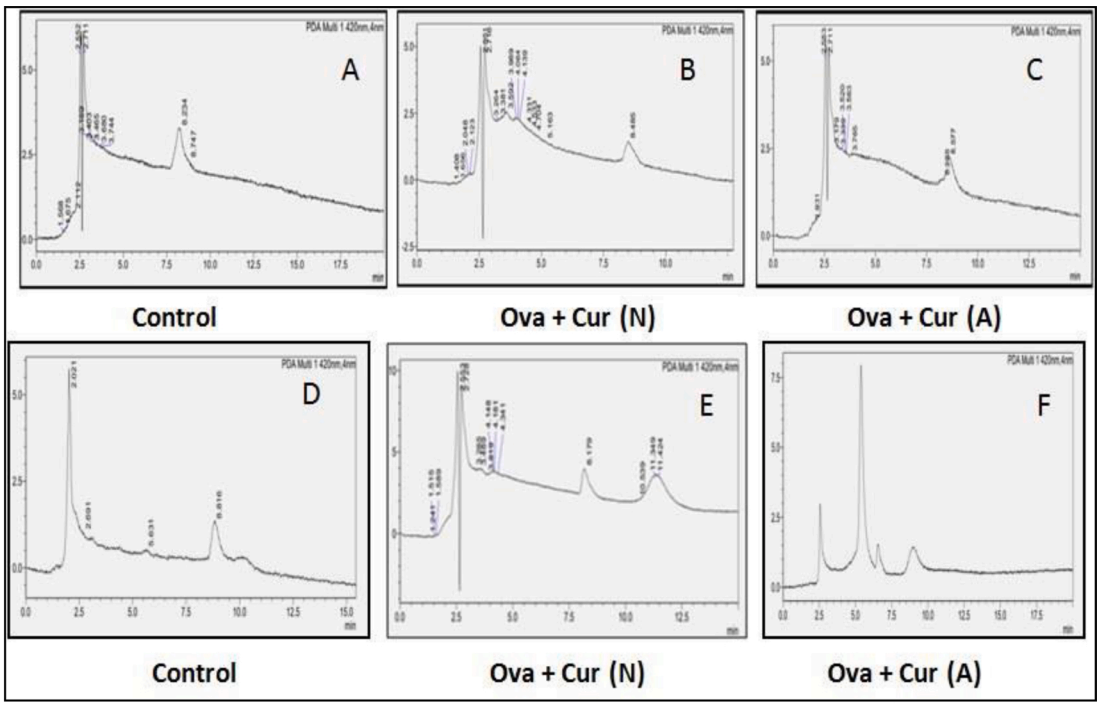
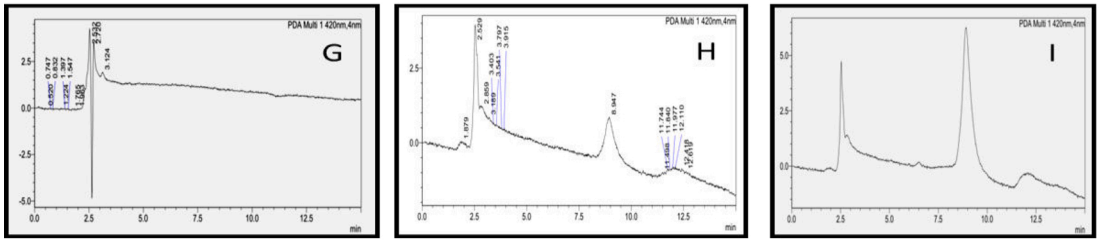


Fig. 4. (A–E) Detection of curcumin after 1 hr of aerosol inhalation: (A) blank plasma containing internal standard emodin (B) absorption in plasma, (C & D) shows absorption in lung homogenates, and absorption difference between nasal drop and aerosol after 1 hrs of post treatment (E). Aerosolised curcumin (1%) showed better absorption (3.08 µg) than nasal drop (5 mg/kg; 2.05 µg) in lung homogenate after 1 hr, (#$p < 0.05$ nasal drop vs aerosol).Significant difference was also observed between lungs and plasma of aerosol group (*$p < 0.05$ aerosol lungs vs plasma).

routes in mice prompted the search for more effective routes of systemic delivery. On the other hand, a major portion (~90%) of oral curcumin is excreted as per previous reports (Metzler, 2013). To overcome this problem, numerous methods were tried to enhance its bioavailability, like use of adjuvants such as piperine, liposomal curcumin formulations, curcumin nanoparticles, curcumin phospholipid complexes, and the use of structural analogs of curcumin such as turmeric oil (Anand, 2007; Siviero,2015). Intranasal drug delivery is now recognized as an effective and reliable alternative to oral and peritoneal routes. Undoubtedly, intranasal drug delivery may emerge as better therapeutic route for





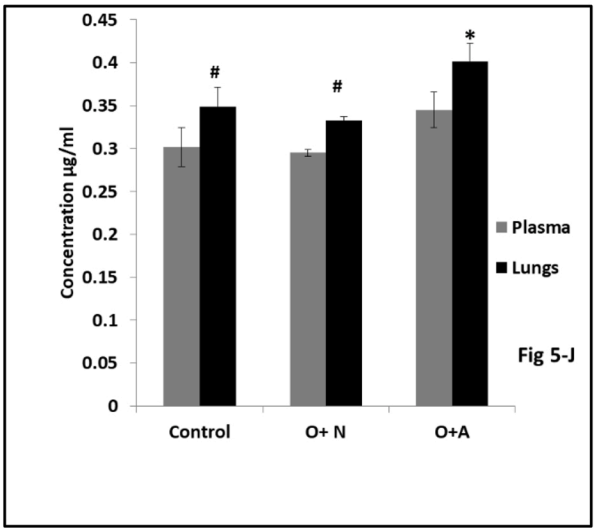


Fig. 5. (A–J) Experiment II showing detection of curcumin on 23rd day after four consecutive days of curcumin treatment in plasma and lungs of mice of different groups A, B, C represents chromatograms of plasma samples and D, E, F represents lung homogenate, (G, H & I) Chromatogram of blank and samples where (G) Blank plasma sample (H) Spiked Cur (10 µg) and (I) Cur (20 µg), (J) Graph represented the comparison between absorption of control, curcumin nasal drop and aerosol. There was no significant difference in control and **O**+**N** group. Aerosol group showed increased absorption as compared to both control and **O**+**N** groups (*$p < 0.05$ Control, **O**+**N** vs **O**+**A**comparing plasma and lungs), (#$p < 0.05$ comparing **O**+**A** to other groups).

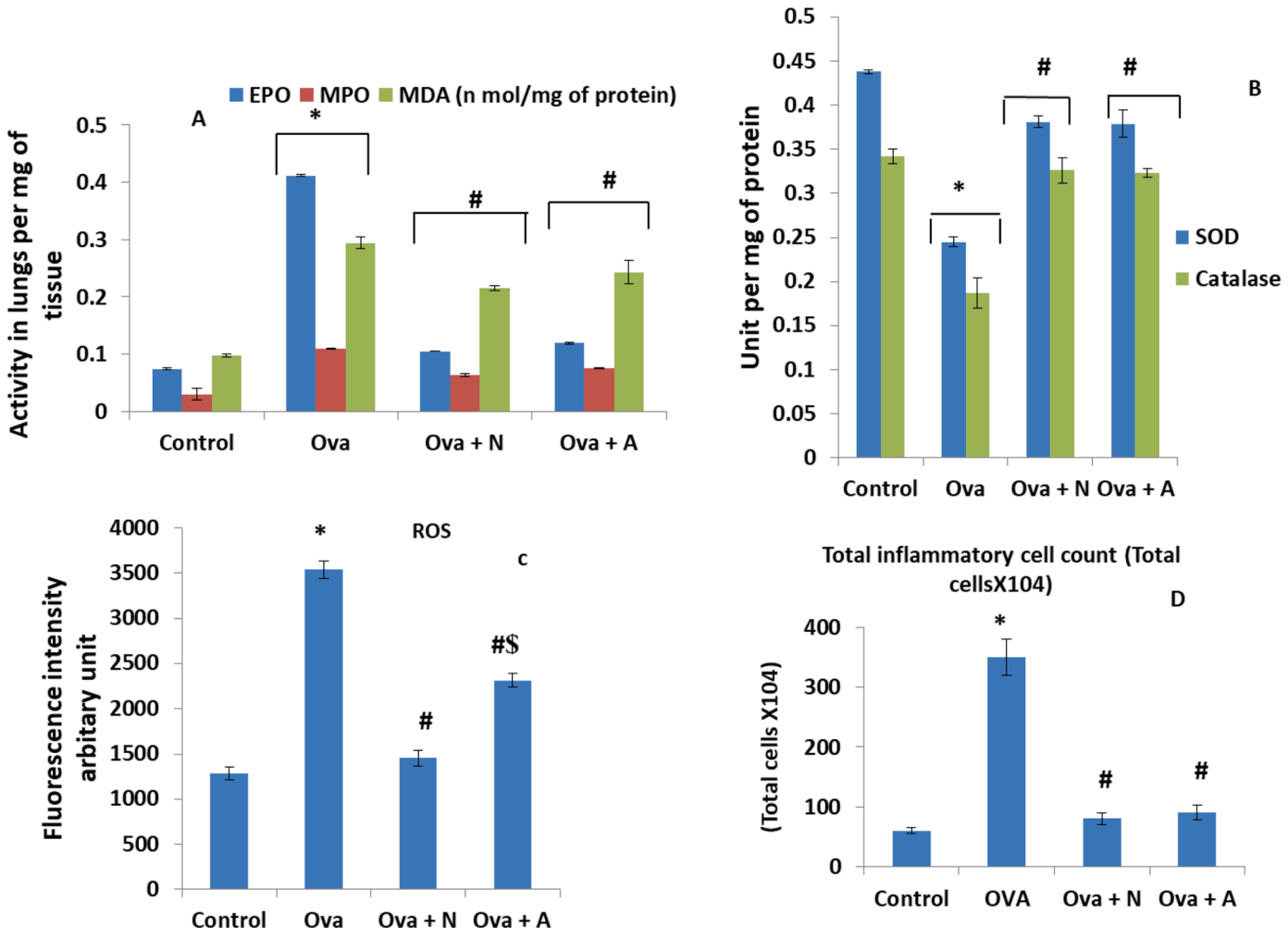


Fig. 6. Oxidative stress and inflammation in asthmatic mice after intranasal curcumin administration. Increased ROS and lipid peroxidation (MDA) levels were detected in OVA group which were significantly reduced after curcumin treatments. Antioxidant enzyme activities of catalase and superoxide dismutase were decreased in OVA group which was further improved in curcumin treatment groups. Elevated EPO and MPO levels were also reduced in curcumin treated groups. Similar trend was noted in Total cell count was reduced in treatment groups. The results are shown in means ± SE (n = 5) (*$p < 0.05$ Con verses OVA group) and (#$p < 0.05$ OVA vs O+N and O+A group), (#p<0.05 O+N vs O+A). OVA–asthmatic + Cur (N) - curcumin nasal drop treated asthmatic mice, OVA + Cur (A) curcumin aerosol treated asthmatic mice.

pharmacologically active compounds with poor absorption in the gastrointestinal tract. Therefore, in present study, the intranasal route of curcumin administration has been selected as nasal mucosa offers numerous benefits of drug delivery such as high vascularity, large surface area, avoidance of first-pass metabolism, and low enzymatic environment. For the treatment of respiratory disorders, if a drug of interest is given through the nasal route, then it can directly reach to lungs with minimal degradation. It has already been reported that aerosol-based drug delivery is capable of delivering large amount of compounds to different parts of the body especially, brain (McClure, 2015). Aerosol-based drug delivery is cheaper, effective, and has minimal side effects as compared to oral administration. Long-time delivery systems like metered dose inhalers (MDI), dry powder inhalers (DPI), and nebulizers have been widely used for the treatment of respiratory diseases. Recently, Adaptive Aerosol Delivery (AAD) System has been developed which can deliver drugs according to the tidal volume and breathing pattern of individuals with improved efficacy (Daily, 2016; Denyer and Dyche, 2010; Hess, 2008; McClur, 2017). Previous study demonstrated that intranasal administration of curcumin micro emulsion-based *in situ* ion sensitive gelling system was more effective and safe (Wang, 2012). Intranasal administration of curcumin hydrogel showed significantly increased distribution of curcumin into the rat brain tissue (Chen, 2013). Due to the lipophilic nature of curcumin, it is relatively insoluble in water, so developing aerosol based curcumin is a tedious task. Here, organic solvent like DMSO was used as curcumin solvent where toxicity was minimal as less than 0.1% DMSO was used. In this study, significantly improved absorption of curcumin was noted in aerosol form as compared to nasal drop. Additionally, in curcumin aerosol form, better absorption was noted in lungs than plasma, while in previous study, absorption of only curcumin nasal drop and its efficacy was evaluated (Subhashini et al., 2013). Besides, both asthmatic and control mice were used to investigate whether curcumin absorption has any difference. No difference in absorption of curcumin at 23rdday was seen in nasal drop and control groups**.** However, aerosol group showed significantly higher absorption in lungs and plasma both . Delivery methods, intranasal and curcumin aerosol, both, were found effective in asthma treatment where slight advantage in the aerosol mode of delivery was noted. Reactive oxygen species (ROS) is responsible for significant damage in asthmatic condition. So, the damage caused by oxygen radicals to different biomolecules, such lipids, proteins, or deoxyribonucleic acid, is frequently used to determine the level of oxidative stress. Antioxidant defense systems are frequently explored as indirect indicator of oxidative stress (Wood et al., 2000). We already have reported effectiveness of nasal drop in reducing EPO, MPO and MDA activities in allergic asthma as

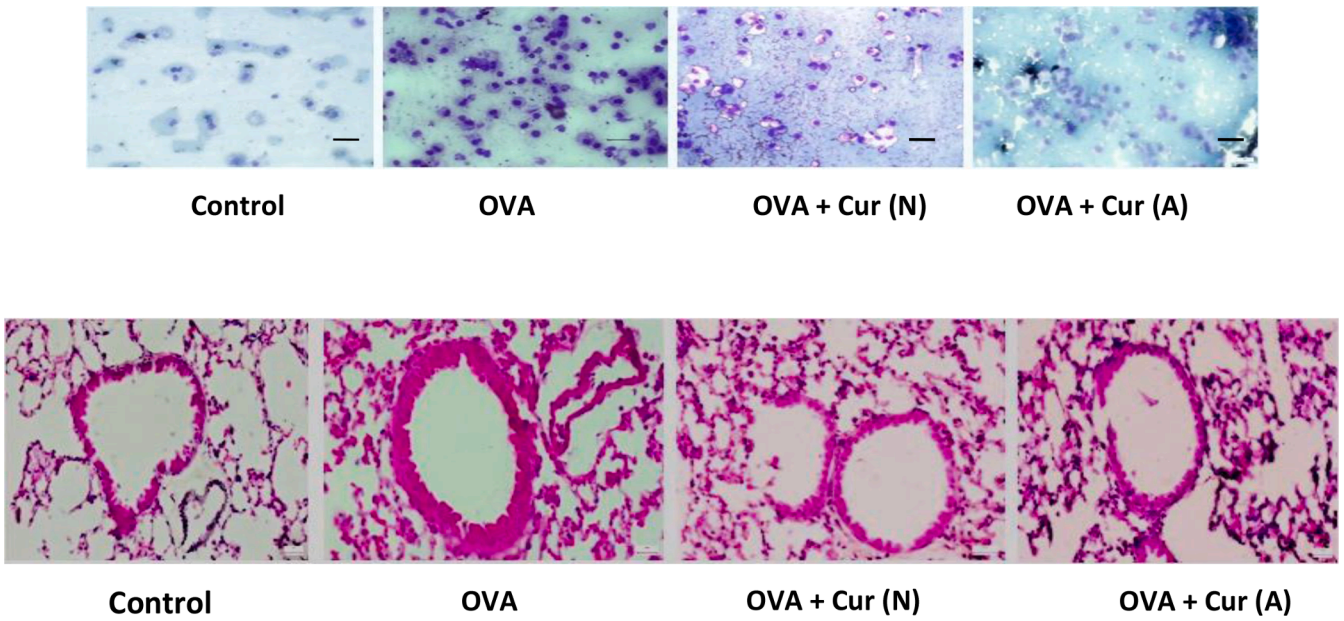


Fig. 7. Cytospun and giemsa stained BALF cells and Histopathology of lungs after H&E staining. Enhanced inflammatory cells were seen around bronchioles and alveolar spaces, constricted and thickened airway lumen was seen in OVA group in contrast to control and curcumin treatment groups. Lung sections were examined under light microscope (× 400 images) and the bar represented 20 μm.

well as in lung injury (Kumari et al., 2015; Jaiswal et al., 2022). Lipid peroxidation is particularly seen in asthma due to inflammation which can be detected in blood, sputum, bronchoalveolar lavage (BAL), and bronchial tissues of asthmatic subjects, these factors are used to gauge the severity of the disease, the presence of inflammation, and how well the disease responds to treatment (Sanz et al., 1997; Andreadis et al., 2003). Enhanced lipid peroxidation (MDA) levels were detected in OVA group which were reduced significantly after curcumin treatment in both nasal drop and aerosol treatment groups. Eosinophil and neutrophil recruitements to the affected site were considerably reduced in both forms of nasal curcumin treatments which were measured by EPO and MPO . It is a well-known fact that antioxidant enzymes play pivotal role in the homeostasis of the cell. Any disturbance results in an initiation and progression of the disease including asthma. Curcumin exhibits significant antioxidant activities by modulating the activities of the antioxidant enzymes as shown by earlier reports. Antioxidant enzyme activities of catalase and superoxide dismutase were decreased in OVA group which was further improved in both the curcumin treatment groups. Curcumin aerosol treatment improves activities of both the enzymes, SOD and catalase up to 40% in asthmatic mice. For the first time, curcumin aerosol has been used which is similar to nasal drop in reducing the OVA-induced inflammation in mice. According to some studies, oxidative stress is crucial in asthma pathogenesis. Activated inflammatory cells produce ROS and discharge them into neighboring cells. Oxidative stress has several negative impacts on airway functioning, including airway smooth muscle contraction, induction of AHR, mucus hypersecretion, epithelium shedding, and vascular exudation, when ROS levels surpass host antioxidant defenses. Here, in mice model, we found interestingly better decline in ROS parameters after curcumin nasal drop treatment as compared to aerosol where decline in ROS level was seen but not like nasal drop. On the other hand, similar trend in both forms of nasal curcumin when total cell count was compared. Additional studies are needed to confirm the finding where aerosol dose in mice can be calculated.

We have supplemented this study by comparing aerosol based curcumin with nasal drop. Aerosol based curcumin shows significant and better efficiency as compared to nasal drop with respect to absorption. Curcumin aerosol has shown almost equal efficacy like nasal drop. Present investigations may suggest that intranasal curcumin is effective in modulating bronchoconstriction and airway inflammation as it is absorbed in blood thereby reaches to the lungs. Present study has also shown equal efficacies of curcumin aerosol as possible delivery method and effective treatment strategy in human asthmatic subjects where direct inhalation may have better impact than mice model.

CRediT authorship contribution statement

Payal Singh: Data curation, Writing – original draft, Software. **Ruchi Chawla:** Visualization, Investigation, Software, Validation. **Ajai Kumar Pandey:** Writing – review & editing. **J.K. Mishra:** Writing – review & editing. **Rashmi Singh:** Conceptualization, Methodology, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors are thankful to Technology Development Program (TDT division), Department of Science and Technology, New Delhi, India (DST-SERB) for financial support (Grant number TDP/BDTD/41/2019/). Authors are thankful to the DST-PURSE scheme (50:50 grant) for providing HPLC facility at Central Instrumentation Laboratory (CIL), Mahila Mahavidyalya (MMV), Banaras Hindu University, Varanasi, India where study was performed.

References

Anand, P, Kunnumakkara, A.B, Newman, R.A., Aggarwal, B.B, 2007. Bioavailability of curcumin: problems and promises. Mol. Pharm. 4 (6), 807–818.
Andreadis, A.A, Hazen, S.L, Comhair, S.A, Erzurum, S.C, 2003. Oxidative and nitrosative events in asthma. Free Radic. Biol. Med. 35 (3), 213–225.
Asakawa, N, Tsuno, M, Hattori, T, Ueyama, M, Shinoda, A, Miyake, Y, Yakugakuzasshi, Kagei K, 1981. J. Pharm. Soc. Japan 101 (4), 374–377.
Azhdari, M., Karandish, M., Mansoori, A., 2019. Metabolic benefits of curcumin supplementation in patients with metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. Phytotherapy Res. 3 (5), 1289–1301.

Birben, E, Sahiner, U.M, Sackesen, C, Erzurum, S, Kalayci, O, 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5 (1), 9–19.
Chen, X., Zhi, F., Jia, X., Zhang, X., Ambardekar, R., Meng, Z., Yang, Y., 2013. Enhanced brain targeting of curcumin by intranasal administration of a thermo sensitive poloxamerhydrogel. J. Pharm. Pharmacol. 5 (6), 807–816.
Negrao, D., Corrêa, D, Corrêa, N., Silveira, M.R., Borges, C.M., Souza, D.G., Teixeira, M. M., 2003. Changes in pulmonary function and parasite burden in rats infected with Strongyloidesvenezuelensis concomitant with induction of allergic airway inflammation. Infect. Immun. 71 (5), 2607–2614.
Daily, J, Daily, W., Yang, M., Park, S., 2016. Efficacy of Turmeric Extracts and Curcumin for Alleviating the Symptoms of Joint Arthritis: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J. Med. Food 19 (8), 717–729.
Denyer, J, Dyche, T, 2010. The Adaptive Aerosol Delivery (AAD) technology: Past, present, and future. J. Aerosol. Med. Pulm. Drug Deliv. 23 (1), S1–S10.
Eruslanov, E., Kusmartsev, S., 2010. Identification of ROS using oxidized DCFDA and flow-cytometry. In: In: Advanced Protocols in Oxidative Stress, II, pp. 57–72.
Fang, Lu, J., Holmgren, A., 2005. Thioredoxinreductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. J. Biol. Chem. 280 (26), 25284–25290.
Hess, D, Hess, R., 2008. Aerosol delivery devices in the treatment of asthma. Respir. Care 53 (6), 699–725.
Irato, P, G, Santovito, 2021. Enzymatic and non-enzymatic molecules with antioxidant function. Antioxidants 10 (4), 579.
Islam, R, Dash, D., Singh, R., 2022. Intranasal curcumin and sodium butyrate modulates airway inflammation and fibrosis via HDAC inhibition in allergic asthma. Cytokine 149, 155720.
Janssen Heininger, Y, Ckless, K, Reynaert, N, van der Vliet, A, 2005. SOD inactivation in asthma: Bad news or NO news? Am. J. Pathol. 166 (3), 649–652.
Jaiswal, A, Dash, D, Singh, R, 2022. Intranasal curcumin and dexamethasone combination ameliorates inflammasome (NLRP3) activation in lipopolysachharide exposed asthma exacerbations. Toxicol. Appl. Pharmacol. 436, 115861.
Jayaprakasha, G.K, Jagan Mohan Rao, L., Sakariah, K.K., 2002. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. J. Agric. Food Chem. 50 (13), 3668–3672.
Kolodziejczyk, J, Olas, B., Saluk-Juszczak, J, B, Wachowicz, 2011. Antioxidative properties of curcumin in the protection of blood platelets against oxidative stress *in vitro*. Platelets 22 (4), 270–276.
Kudo, M, Ishigatsubo, Y, Aoki, I, 2013. Pathology of asthma. Front. Microbiol. (4), 263.
Kumari, A, Dash, D, Singh, R, 2015. Lipopolysaccharide (LPS) exposure differently affects allergic asthma exacerbations and its amelioration by intranasal curcumin in mice. Cytokine 76 (2), 334–342.
Kumari, S, Singh, R, 2022. Protective effects of intranasal curcumin on silica-induced lung damage. Cytokine (157), 155949.
Kumari, A, Tyagi, N., Dash, D., Singh, R., 2015. Intranasal curcumin ameliorates lipopolysaccharide-induced acute lung injury in mice. Inflammation 38 (3), 1103–1112.
Kurup, V.P, Barrios, C.S., 2008. Immunomodulatory effects of curcumin in allergy. Mol. Nutr. Food Res 52 (9), 1031–1039.
Leonard, A.K, Sileno, A.P., Brandt, G.C., Foerder, C.A., Quay, S.C., Costantino, H.R., 2007. In vitro formulation optimization of intranasal galantamine leading to enhanced bioavailability and reduced emetic response *in vivo*. Int. J. Pharm. 335 (1-2), 138–146.
Li, R, Xiang, C, Ye, M, Li, H.F, Zhang, X, Guo, D.A, 2011. Qualitative and quantitative analysis of curcuminoids in herbal medicines derived from Curcuma species. Food Chem. 126 (4), 1890–1895.
McClure, R, Yanagisawa, D., Stec, D., Abdollahian, D., Koktysh, D., Xhillari, D., Jaeger, R., tanwood, S.G., Chekmenev, E., Tooyama, I., Gore, J.C., Pham, W., 2015. Inhalable curcumin: offering the potential for translation to imaging and treatment of Alzheimer's disease. J. Alzheimer...s Dis. 44 (1), 283–295.
McClure, R, Ong, H., Janve, V., Barton, S., Zhu, M., Li, B., Dawes, M., Jerome, W.G., Anderson, A., Massion, P., Gore, J.C., Pham, W., 2017. Aerosol Delivery of Curcumin Reduced Amyloid-β Deposition and Improved Cognitive Performance in a Transgenic Model of Alzheimer's disease. J. Alzheimer...s Dis. 55 (2), 797–811.
Menon, V.P, Sudheer, A.R., 2007. Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Biol. 595, 105–125.
Metzler, M., Pfeiffer, E., Schulz, S.I., Dempe, J.S., 2013. Curcumin uptake and metabolism. BioFactors 39 (1), 14–20.
Nita, M, Grzybowski, A, 2016. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid. Med. Cell Longev. 2016.
Osorio Tobón, J.F, Carvalho, P.I, Barbero, G.F., Nogueira, G.C, Rostagno, M.A, de Almeida Meireles, M.A, 2016. Fast analysis of curcuminoids from turmeric (Curcuma longa L.) by high-performance liquid chromatography using a fused-core column. Food Chem. 200, 167–174.
Pan M.H, T.M. Huang, & Lin. J.K, 1999. **Biotransformation of curcumin through reduction and glucuronidation in mice. Drug metabolism and disposition: the biological fate of chemicals**, 27(4), 486–494.
Perkins, S, Verschoyle, R.D., Hill, K., Parveen, I., Threadgill, M.D, Sharma, R.A, Williams, M., Steward, L.W.P., Gescher, A.J., 2002. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research. cosponsored by the American Society of Preventive Oncology 11 (6), 535–540.
Ravindranath, V, Chandrasekhara, N., 1980. Absorption and tissue distribution of curcumin in rats. Toxicology 16 (3), 259–265.
Sanz, M.L, Parra, A, Prieto, I, Dieguez, I, Oehling, A.K, 1997. Serum eosinophil peroxidase (EPO) levels in asthmatic patients. Allergy 52 (4), 417–422.
Shahid, H, Shahzad, M, Shabbir, A, Saghir, G, 2019. Immunomodulatory and anti-inflammatory potential of curcumin for the treatment of allergic asthma: effects on expression levels of pro-inflammatory cytokines and aquaporins. Inflammation 42 (6), 2037–2047.
Siviero, A., Gallo, E., Maggini, V., Gori, L., Mugelli, A., Firenzuoli, F., Vannacci, A., 2015. Curcumin, a golden spice with a low bioavailability. J. Herb. Med. 5 (2), 57–70.
Stamenkovska, M, Thaçi, Q, Hadzi-Petrushev, N, Angelovski, M, Bogdanov, J, Reçica, S, Sopi, R.B, 2020. Curcumin analogs (B2BrBC and C66) supplementation attenuates airway hyperreactivity and promote airway relaxation in neonatal rats exposed to hyperoxia. Physiol. Rep. 8 (16), 14555.
Subhashini, Chauhan, P.S., Kumari, S., Kumar, J.P, Chawla, R., Dash, D., Singh, M., Singh, R, 2013. Intranasal curcumin and its evaluation in murine model of asthma. Int. Immunopharmacol. 17 (3), 733–743.
Tham, C.L, Yeoh, S.Y, Ong, C.H, Harith, H.H, Israf, D.A, 2021. A Synthetic Curcuminoid Analogue, 2, 6-Bis-4-(Hydroxyl-3-Methoxybenzylidine)-Cyclohexanone (BHMC) Ameliorates Acute Airway Inflammation of Allergic Asthma in Ovalbumin-Sensitized Mice. Mediators Inflamm.
Wahlström, B, Blennow, G., 1978. A study on the fate of curcumin in the rat. Acta Pharmacol.
Wang, S., Chen, P., Zhang, L., Yang, C., Zhai, G., 2012. Formulation and evaluation of microemulsion-based in situ ion-sensitive gelling systems for intranasal administration of curcumin. J. Drug Target. 20 (10), 831–840.
Wood, L.G, Fitzgerald, D.A, Gibson, P.C, Cooper, D.M, Garg, M.L, 2000. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. Lipids 35 (9), 967–974.
Wu, A., Noble, E.E, Tyagi, E., Ying, Z., Zhuang, Y., Gomez-Pinilla, F., 2015. Curcumin boosts DHA in the brain: Implications for the prevention of anxiety disorders. Biochim. et Biophys. Acta 1852 (5), 951–961.
Wu, Y.F., Chen, Y.Q, Li, Q, Ye, X.Y, Zuo, X, Shi, Y.L, Yang, Y, 2020. Supplementation with Tetrahydrocurcumin Enhances the Therapeutic Effects of Dexamethasone in a Murine Model of Allergic Asthma. Int. Arch. Allergy Immunol. 181 (11), 822–830.
Yu, S, Wang, X., He, X., Wang, Y., Gao, S., Ren, L., Shi, Y., 2016. Curcumin exerts anti-inflammatory and antioxidative properties in 1-methyl-4-phenylpyridinium ion (MPP(+))-stimulated mesencephalic astrocytes by interference with TLR4 and downstream signaling pathway. Cell Stress Chaperones 21 (4), 697–705.