



Therapeutic potential of curcumin in experimentally induced allergic rhinitis in guinea pigs

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ABSTRACT

In the present experiments, the possible role of curcumin in ovalbumin induced allergic rhinitis in guinea pig model was investigated. Various allergic rhinitis symptoms viz sneezing, rubbing frequencies, lacrimation and nasal congestion at various humidity conditions as well as on repeated sensitization were studied. The biochemical changes like serum IgE, IL-4 and nitric oxide (NO) in nasal lavage and eosinophil peroxidase activity in nasal homogenates were determined in allergic rhinitis. Curcumin treatment significantly reduced the symptoms (sneezing, rubbing frequencies, lacrimation and nasal congestion) and improved the histopathological alterations (reduction in inflammatory cells infiltration) of nasal mucosa in allergic rhinitis. Furthermore, curcumin treatment prevented significantly elevation of serum IgE, IL-4, NO in nasal lavage and eosinophil peroxidase in nasal homogenate. In the present experimental findings, we suggest that curcumin is a promising anti-allergic agent that may be useful in the clinical management of allergic rhinitis.

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1. Introduction

Allergic rhinitis is predominantly an IgE-mediated inflammation of the membranes of nasal mucosa and/or cavity, characterized by sneezing, itching, nasal congestion, rhinorrhea, post nasal drainage, lacrimation of eyes, and associated loss of smell, taste and induction of cough [1,2]. It occurs due to the interactions of resident and infiltrating inflammatory cells, in associated with inflammatory mediators viz cytokines, and neurotransmitters causing sensory nerve activation, plasma leakage, and congestion of venous sinusoids [3]. Allergic rhinitis is either seasonal or perennial depending upon the symptoms observed in response to seasonal allergen exposure or throughout the year, respectively. Common seasonal allergens are mainly pollens, grasses and weeds, while perennial allergens are derived from dust mites, molds, animal danders and other sources of occupational origin [4]. Exposure to allergens in sensitized individuals, elicits cascade of biochemical and cellular events leading to synthesis and release of IgE and subsequent allergen exposure triggers mast cell degranulation causing release of histamine, tryptase, prostaglandins, cysteinyl-leukotrienes (Cys-LT), cytokines (IL-4, IL-5, and TNF- α , etc.), and platelet-activating factor (PAF) [5].

Depending upon the symptoms of allergic rhinitis respective drug(s) has been prescribed to reduce its severity. Currently, antihistamines (Chlorpheniramine maleate, fexofenadine hydrochloride, cetirizine hydrochloride etc.), corticosteroids (betamethasone, beclomethasone, and

fluticasone dipropionate), mast cell stabilizers (sodium cromoglycate), decongestants (phenylephrine, oxymetazoline, and xylometazoline), Cys-LT-receptor antagonists (Montelukast sodium, and Zafirlukast) and anticholinergics are being successfully used clinically in the management of allergic rhinitis.

Various animal models have been developed, validated and widely employed for studying the etiopathology of allergic rhinitis [6–8]. Guinea pigs are usually preferred for studying rhinitis because they mimic human rhinitis, although, rats also exhibit rhinitis symptoms on topical sensitization with known allergen [6]. This model on systemic ovalbumin administration induced symptoms which resemble human allergic rhinitis and respond to clinical pharmacotherapy. The quantification of the individual symptoms is possible in this model due to characteristic manifestation of symptoms [9].

Curcumin an active constituents *Curcuma longa*, and chemically it is [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadine-3,5-dione (diferuloylmethane)]. Various studies are documented on therapeutic benefits of curcumin in the management of diseases/disorders because of its diverse pharmacological activities, anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive, hypercholesterolemia, antioxidant, antiallergic and cardioprotection [10–17]. Curcumin elicits inhibitory effects on cytokines viz IL-1, IL-4, IL-6, and TNF- α along with activation of nuclear factor kappa B (NF- κ B) [18,19]. The antiallergic activity of curcumin has been correlated with the inhibition of IgE-mediated degranulation of mast cells in Brown Norway rats [17]. Although, curcumin is known to exert its effects on IgE and IL-4, its effect on allergic rhinitis is scientifically unknown. Therefore, the present study was undertaken to investigate

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the beneficial effects of curcumin in experimentally induced allergic rhinitis in guinea pig model along with the probable mechanism(s) of action.

2. Materials and methods

2.1. Animals

Dunkin Hartley guinea pigs (250–300 g) of either sex were procured from Haffkine Institute of Biopharmaceutical Sciences, Mumbai, India. Animals were maintained in our animal house at desired conditions: temperature 23 ± 1 °C, and relative humidity (RH) $50 \pm 5\%$. The animals were provided with standard laboratory diet (Amrut Laboratory Animal Feed, Nava Maharashtra Chakan Oil Mills, and Pune, India) and water ad libitum. The animals were shifted from animal house to laboratory 2 h prior to experiments.

2.2. Institutional Animal Ethics committee approval

Experimental procedures and protocol for the present study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), constituted under the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. SIPS/IAEC/App/2011-12/14 and conformed to the Indian National Science Academy guidelines for the use and care of experimental animals in research. Animal house was registered with the Govt. of India (962/c/06/CPCSEA) dated 27 July 2006.

2.3. Drugs and chemicals

2.3.1. Ovalbumin

Chicken egg Grade V (Sigma, St. Louis, USA), Montelukast sodium (Cipla Ltd, Mumbai, India), aluminum hydroxide and carboxy methyl cellulose from local suppliers were procured. Purified curcumin powder was obtained as gift sample from Amsar Private Ltd. Indore, India.

2.3.2. Curcumin

The purified curcumin powder was dissolved in ethanol and quantitatively analyzed by high performance thin layer chromatography and compared with standard curcumin with scanning at 420 nm. Curcumin consisted of 3 fractions curcumin (56.99%, Rf 0.57), demethoxycurcumin (28.57%, Rf 0.39), and bisdemethoxycurcumin (14.45%, Rf 0.28). The separation of all 3 fractions was performed by preparative thin layer chromatography and analyzed for physicochemical properties and reported in our earlier experiments [16].

2.4. Oral acute toxicity study

The oral acute toxicity study of curcumin was carried out in rats as per the OECD 423 guideline. In this, animals were administered with curcumin up to 2000 mg/kg by oral route and they were observed for a period of 96 h for any clinical and behavioral symptoms. Curcumin (2000 mg/kg) did not produce clinical, toxic symptoms and no mortality; hence the doses 100 and 200 mg/kg were selected for the present studies.

2.5. Sensitization procedure

Guinea pigs of either sex in equal numbers were sensitized as per the procedure described by Underwood et al. [20] with minor modification by Bahekar et al. [9]. Briefly, Guinea pigs were randomized and divided into different groups ($n = 6$). They were sensitized on days 1, 7, 14 and 21 by intraperitoneal injection of ovalbumin (100 µg/animal) and aluminum hydroxide (5 mg/animal) dissolved and suspended, respectively in 0.9% saline solution. Nonsensitized animals received

suspension of aluminum hydroxide in saline. After 7 days of systemic sensitization, animals were topically instilled with 60 mg/ml, 20 µl/nostril ovalbumin solutions. A week after the last sensitization, the dorsal area of animals was shaved and challenged with intradermal injection of ovalbumin solution (25 µl of 200 µg/ml). Successful sensitization was confirmed by edema and redness at the site of injection. Ovalbumin dose 60 mg/ml (20 µl/nostril) for induction of allergic rhinitis was selected which elicited the marked rhinitis symptoms [9].

2.6. Induction of allergic rhinitis

The room where experiments were performed was free of noise and strong odors and maintained at a temperature of 23 ± 1 °C and RH $50 \pm 5\%$. Animals were allowed to acclimatize in clean perspex observation cages ($26 \times 26 \times 26$ cm) for 30 min. The curcumin (100 and 200 mg/kg) or vehicles (10 ml/kg), Montelukast (10 mg/kg), and sodium chromoglycate (100 mg/kg) were administered orally to overnight fasted animals or intranasally to non-fasted animals, for 3 days prior to intranasal ovalbumin challenge. The local ovalbumin challenge was performed by instilling ovalbumin solution into both the nostrils by using micropipette (Biosystems, India). Symptoms of rhinitis; number of sneezing, nose rubbing, eye lacrimation and difficulty in breathing were observed and scored for a period of 2 h. Sneezing was characterized by an explosive expiration just after a deep inspiration, and nose rubbing was characterized by the external perinasal scratching with either one or both forelimbs of animal [21]. Lacrimation was scored on a 3-point scale as follows: (+)—hazy eyes; (++)—lacrimation; and (+++)—lacrimation with the onset of conjunctivitis. Nasal acoustic phenomenon reflecting nasal congestion/obstruction was evaluated as (+)—impaired inspiration; (++)—nasal inflammation; and (+++)—severe breathing impairment [22]. The nasal secretion (watery fluid) discharged through nostrils or trickling on the walls of observation cages during sneezing was considered as the sign of rhinorrhea [9].

2.7. Effects of curcumin, Montelukast on ovalbumin induced rhinitis symptoms

The sensitized guinea pigs were divided into various groups ($n = 6$) and administered daily for 3 days with saline (20 µl/nostril), curcumin (100 and 200 mg/kg, p.o.), and Montelukast (10 mg/kg, p.o.), and 2 h after the last treatment, animals were challenged with ovalbumin (20 µl/nostril containing 1.2 mg of ovalbumin). Sneezing and rubbing frequency, lacrimation and nasal congestion were counted for 2 h and recorded. Animals were sacrificed 8 h after challenge for histopathological studies.

2.8. Histopathological studies of nasal cavity

Histopathological studies were performed as per the method described by Bahekar et al. and Yamasaki earlier [9,25]. The head was removed and lower jaw was discarded. The nasal cavity was separated from skin, muscle and soft tissues which were immersed in the freshly prepared 10% neutral buffered formalin for a period of 48 h. Thereafter, tissues were rinsed in running tap water and decalcified in 10% nitric acid solution for 5 days. After rinsing in tap water, the tissue was processed for dehydration with alcohol and embedded in paraffin. Sections (4 µm) were cut at the level of incisive papillae and first and second palatal ridges and stained with hematoxylin-eosin. Sections were examined under light microscope to observe alterations in histopathologic structure of specimen and infiltration of cells in epithelium and subepithelium. All slides were coded before the analysis and read in blind fashion to avoid observer's bias.

2.9. Effect of topical application of curcumin and sodium chromoglycate on repeated ovalbumin induced sneezing and rubbing frequency

The sensitized guinea pigs were divided into various groups ($n = 6$) and received topically daily for 3 days with normal saline (20 μ l/nostril), curcumin [100 and 200 mg/ml (20 μ l/nostril)], and sodium chromoglycate [100 mg/ml (20 μ l/nostril)], and 2 h after the last treatment animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin) 2 times a week (1st and 4th days) for 2 weeks.

2.10. Effect of curcumin on ovalbumin induced allergic rhinitis symptoms at varied humidity conditions

The experiment was performed in two different environmental conditions having temperature 23 ± 1 °C with RH 50–60% in one room and 80–90% in another room. The sensitized guinea pigs were divided into various groups ($n = 6$) and administered daily for 3 days with normal saline (20 μ l/nostril), curcumin (100 and 200 mg/kg, p.o.), Montelukast (10 mg/kg, p.o.) and sodium chromoglycate [100 mg/ml (20 μ l/nostril)], and 2 h after the last treatment, animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin). The number of sneezing and rubbing frequency was counted for 2 h and recorded.

2.11. Measurement of ovalbumin-specific serum IgE

The sensitized guinea pigs were divided into various groups ($n = 6$) in two sets of experiments; in the first set of experiment, animals were challenged on the 7th day and the second set of experiment animals were challenged on the 14th day. Both the experimental groups received saline (20 μ l/nostril), curcumin (100 and 200 mg/kg, p.o.), Montelukast (10 mg/kg, p.o.) and sodium chromoglycate [100 mg/ml (20 μ l/nostril)] daily for 3 days, and 2 h after the last treatment, animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin). The blood was collected from retro-orbital plexus under ether anesthesia from each guinea pig 2 h after the ovalbumin challenge. The serum was separated by centrifugation at 7000 rpm for 10 min at 4 °C (Remi, India) and assayed for ovalbumin specific IgE on 7th and 14th days using ELISA kit (Woburn, MA, USA) following the method described by the manufacturer.

2.12. Measurement of IL-4 concentration in nasal lavage

Sensitized guinea pigs were challenged intranasally with ovalbumin. Four hours later, animals were anesthetized by urethane (100 mg/kg, i.p.). All drugs were administered daily for 3 days, and 2 h after the last treatment animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin). Nasopharynx was cannulated and connected to a hypodermic syringe, perfused with Hank's balanced salt solution (500 μ l, pH 7.4). Nasal lavage was collected and filtered and IL-4 level was determined using ELISA kit (Woburn, MA, USA), as per the manufacturer's procedure.

2.13. Determination of eosinophil peroxidase activity

The sensitized guinea pigs were divided into various groups ($n = 6$) and administered daily for 3 days with normal saline (20 μ l/nostril), curcumin (100 and 200 mg/kg, p.o.), Montelukast (10 mg/kg, p.o.) and sodium chromoglycate [100 mg/ml (20 μ l/nostril)], and 2 h after the last treatment, animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin). Animals were sacrificed with excess of ether anesthesia, and nasal cavity was opened, removed and separated and used for eosinophil peroxidase (EPO) activity determination. The EPO activity was determined in homogenate of nasal cavity as per the method of Tsunematsu et al. [23]. Briefly, the nasal cavity was homogenized with 4 ml of 0.5% hexadecyltrimethylammonium bromide (HTAB) in phosphate buffer solution (PBS). The homogenate was freeze-thawed 3 times and centrifuged. To the supernatant (50 μ l), 16 mM of O-phenylenediamine dihydrochloride and 0.01% of hydrogen

peroxide in 50 mM of tris-hydrochloride were added and thoroughly mixed, and incubated for 15 min at 30 °C. The reaction was stopped by adding 4 mol/l sulfuric acid (50 μ l) and optical density of resultant solution was read at 490 nm on spectrophotometer (Shimadzu 1700, Japan). Results were expressed as units of EPO activity/2 ml of nasal lavage fluid (units of EPO activity corresponds to 1×10^3 eosinophil).

2.14. Measurement of nitric oxide (NO) infiltration in nasal lavage

The sensitized guinea pigs were divided into various groups ($n = 6$) and administered daily for 3 days with normal saline (20 μ l/nostril), curcumin (100 and 200 mg/kg, p.o.), Montelukast (10 mg/kg, p.o.) and sodium chromoglycate [100 mg/ml (20 μ l/nostril)], and 2 h after the last treatment, animals were challenged with ovalbumin (20 μ l/nostril containing 1.2 mg of ovalbumin). One hour after the challenge, the nasal lavage was collected [24] and centrifuged at 1000 g for 10 min at 4 °C. Equal quantity of supernatant and Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamide in 5% phosphoric acid) was mixed and incubated for 10 min in dark. The absorbance was read at 540 nm on spectrophotometer. Results were expressed as the NO activity as μ M/2 ml of nasal lavage using standard graph of sodium nitrate.

2.15. Statistical analysis

The data of symptom scores, IL-4, and IgE were analyzed for statistical significance by one way ANOVA and compared with vehicle control or treatment group by Dunnett's and SNK (Student–Newman–Keuls) tests, respectively. The effect of humidity on rhinitis symptoms was analyzed by Student's 't' test. Significance was considered at $p < 0.05$.

3. Results

3.1. Effects of curcumin, Montelukast on ovalbumin induced allergic rhinitis symptoms.

Ovalbumin sensitized animals showed manifestation of allergic rhinitis symptoms ($p < 0.001$) as compared to non-sensitized animals. The treatment with curcumin at 200 mg/kg, inhibits sneezing, rubbing frequencies significantly ($p < 0.05$), and reduction in eye lacrimation and nasal congestions (Table 1). However, Montelukast (10 mg/kg, p.o.) failed to prevent the allergic rhinitis symptoms.

3.2. Effect of topical application of curcumin, Montelukast and sodium chromoglycate on repeated ovalbumin induced sneezing and rubbing frequency

There was a significant ($p < 0.001$) increase in sneezing and rubbing frequencies on 4th sensitization as compared to 1st, 2nd and 3rd sensitizations. Administration of curcumin (100 and 200 mg/ml; 20 μ l/nostril), and sodium chromoglycate [100 mg/ml; (20 μ l/nostril)]

Table 1

Effects of curcumin, Montelukast on ovalbumin induced allergic rhinitis symptoms in guinea pigs.

Treatment and dose (mg/kg, p.o.)	Frequency/severity of symptoms in 2 h Mean $\pm$ SEM ($n = 6$)			
	Sneezing	Rubbing	Lacrimation	Congestion
Non-sensitized ^a	2.4 $\pm$ 1.1	8.8 $\pm$ 1.6	+	0
Ovalbumin sensitized ^b	40.3 $\pm$ 5.9 ^{##}	55.9 $\pm$ 8.7 ^{##}	+++	+++
Montelukast 10	38.4 $\pm$ 6.4	47.6 $\pm$ 9.1	++	++
Curcumin 100	31.9 $\pm$ 5.5	36.8 $\pm$ 6.2	++	++
Curcumin 200	21.8 $\pm$ 4.5*	25.3 $\pm$ 3.9	+	+

Experiments were repeated twice ($n = 6$, mean $\pm$ SEM).

^a Nonsensitized ovalbumin challenged.

^b Sensitized and challenged with ovalbumin (60 mg/ml, 20 μ l/nostril).

^{##} $p < 0.001$ when compared to nonsensitized animals.

* $p < 0.05$ compared to ovalbumin sensitized animals.

prevented significantly sneezing and rubbing frequencies on 4th sensitization compared to 1st, 2nd and 3rd sensitizations (Fig. 1A and B).

3.3. Effect of curcumin on ovalbumin induced allergic rhinitis symptoms at varied humidity conditions

When sensitized guinea pigs were exposed to RH (50–60%), sneezing and rubbing frequencies were higher, however, these symptoms were ameliorated significantly with the treatment of curcumin ($p < 0.001$), Montelukast and sodium chromoglycate ($p < 0.05$). At higher RH (80–90%) sneezing frequencies were reduced even in ovalbumin sensitized animals and were further reduced significantly ($p < 0.05$) with curcumin, sodium chromoglycate and Montelukast treatments, compared to RH 50–60%. However, it was observed that, increased RH (30% increase RH) during rhinitis reduced sneezing significantly with no effect on rubbing frequencies even with employed drug treatments at 80–90% RH (Fig. 2A and B).

3.4. Effects of curcumin, Montelukast on ovalbumin-specific serum IgE

There was a significant ($p < 0.001$) elevation of serum IgE antibody concentration in guinea pigs on day 7 and day 14 after the last sensitization, compared to non-sensitized guinea pigs. Treatment with curcumin

200 mg/kg prevented the elevation of serum IgE level significantly ($p < 0.001$) (Table 2).

3.5. Effects of curcumin, Montelukast on IL-4 and NO (nitric oxide) in nasal lavage

There was a significant increase in IL-4 and NO concentration of nasal lavage in ovalbumin-sensitized guinea pigs compared to non-sensitized guinea pigs. Intranasal administration of curcumin [100 and 200 mg/ml (20 μ l/nostril)], Montelukast (10 mg/ml; 20 μ l/nostril), or sodium chromoglycate (100 mg/ml; 20 μ l/nostril), or dexamethasone (5 mg/kg, 20 μ l/nostril) reduced the IL-4 concentration in nasal lavage significantly ($p < 0.001$) compared to ovalbumin sensitized guinea pigs. The NO concentration was also reduced with all employed drug treatments (curcumin, Montelukast and sodium chromoglycate) significantly (Fig. 3 and 4).

3.6. Effects of curcumin, Montelukast and sodium chromoglycate on eosinophil peroxidase (EPO) activity

The EPO activity was increased significantly ($p < 0.001$) in ovalbumin-sensitized animals. Significant inhibition of EPO activity was observed with treatment of curcumin (100 and 200 mg/kg), sodium

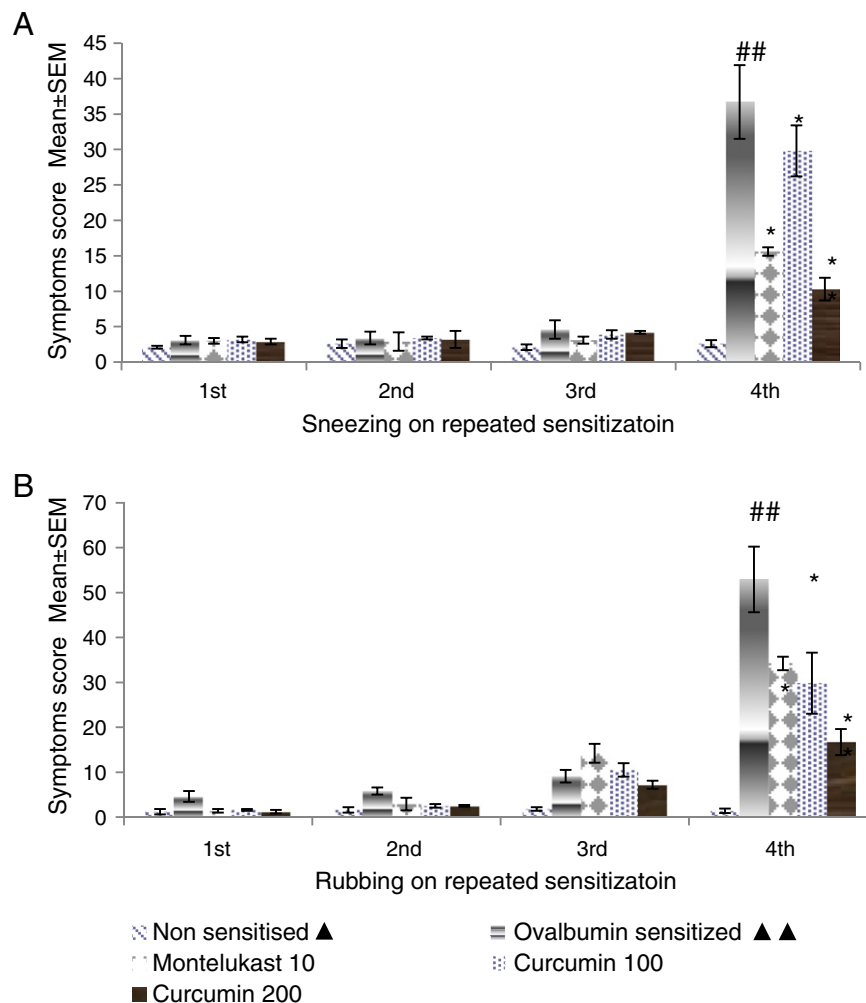


Fig. 1. Effect of topical application of curcumin, and sodium chromoglycate on repeated ovalbumin induced sneezing (A) and rubbing frequencies (B). $\blacktriangle$ Non sensitized ovalbumin challenged, $\blacktriangle\blacktriangle$ sensitized and challenged with ovalbumin (60 mg/ml, 20 μ l/nostril), ## $p < 0.001$ when compared to 1st, 2nd, and 3rd sensitizations. * $p < 0.05$, ** $p < 0.001$ when compared to ovalbumin sensitized group at 4th sensitization. Experiments were repeated twice ($n = 6$, mean $\pm$ SEM).

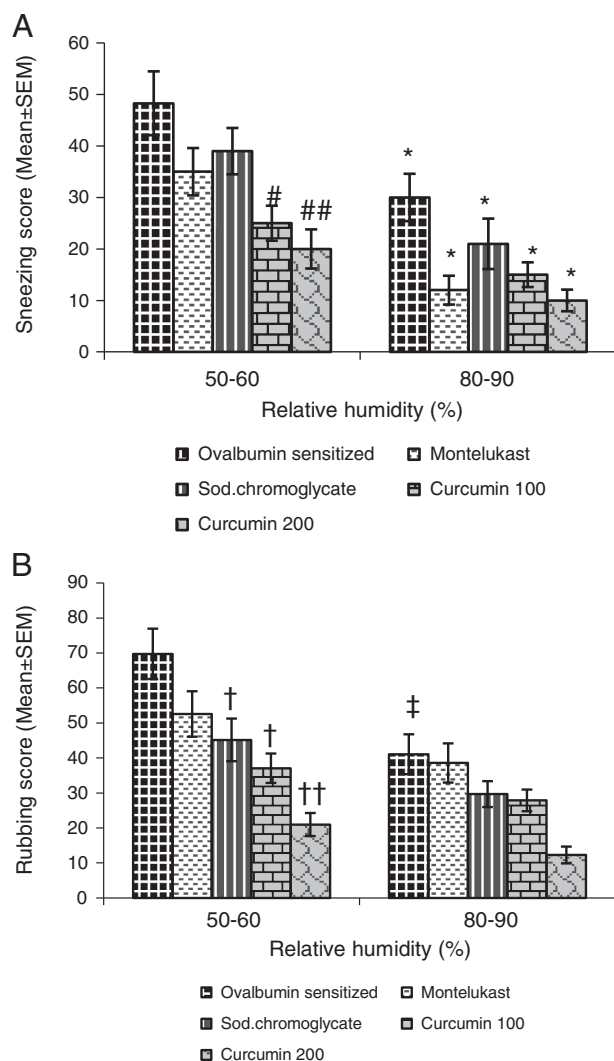


Fig. 2. Effect of curcumin, Montelukast and sodium chromoglycate on ovalbumin-induced sneezing and rubbing frequencies at varied humidities 50–60% and 80–90%. Sensitized animals were challenged with ovalbumin (60 mg/ml, 20 µl/nostril) and number of sneezing (A) and rubbing (B) were noted for a period of 2 h. ^{*}*p* < 0.05, ^{##}*p* < 0.001 when compared to ovalbumin sensitized sneezing (A), ^{*}*p* < 0.05 when compared to relative humidity 50–60% and [†]*p* < 0.05, ^{††}*p* < 0.001 when compared to ovalbumin sensitized rubbing (B), [‡]*p* < 0.05 when compared to relative humidity 50–60%. Experiments were repeated twice (*n* = 6, mean ± SEM).

Table 2

Effects of curcumin, Montelukast and sodium chromoglycate on ovalbumin specific serum IgE concentration during allergic rhinitis.

Treatment and dose (mg/kg, p.o.)	Serum IgE (ng/ml) on different days	
	Mean ± SEM (n = 6)	
	7	14
Non-sensitized ^a	2.4 ± 0.9	2.9 ± 0.4
Ovalbumin sensitized ^b	210.2 ± 24.5 ^{##}	398.4 ± 32.2 ^{##}
Montelukast 10	194 ± 19.8	264.3 ± 24.6 [*]
Sodium chromoglycate 100	202.2 ± 27.3	167.9 ± 26.3 [*]
Curcumin 100	157.3 ± 16.7 [*]	124.3 ± 15.5 [*]
Curcumin 200	102.1 ± 9.8 ^{**}	50.3 ± 6.8 ^{**}

Experiment was performed once (*n* = 6, mean ± SEM).

^a Non-sensitized ovalbumin challenged.

^b Sensitized and challenged with ovalbumin (60 mg/ml, 20 µl/nostril).

^{##} *p* < 0.001 when compared to nonsensitized animals.

^{*} *p* < 0.05.

^{**} *p* < 0.001 compared to ovalbumin sensitized animals.

chromoglycate and Montelukast when compared to ovalbumin sensitized animals (Fig. 5).

3.7. Histopathological studies

Ovalbumin sensitized groups of guinea pigs elicit marked infiltration of inflammatory cells in the nasal mucosa at 8 h of ovalbumin challenge. Inflammatory cells viz eosinophils, neutrophils and disruption of epithelium were observed in ovalbumin challenged animals. Treatment with curcumin and Montelukast showed marked prevention of inflammatory cells into the nasal mucosa (Fig. 6).

4. Discussion

The clinical symptoms of allergic rhinitis include sneezing, itchy nose, rhinorrhea, lacrimation and nasal congestion, which mainly originate from mast cells and other inflammatory cells [1]. These symptoms are presumed to be triggered by various mediators such as cytokines (IL-4, IL-6, IL-13), prostaglandins, NO from mast cells and other inflammatory cells [26]. Presently, H₁-receptor antagonist, corticosteroids, mast cell stabilizers, nasal decongestants, leukotriene receptor antagonist and anticholinergics are being used clinically in the treatment of allergic rhinitis. However, most of these drugs are known to induce wide variety of adverse effects [1].

In the present experiments, we could able to demonstrate with our findings the possible role of curcumin in prevention of allergic rhinitis symptoms in guinea pigs. Repeated intranasal administration of curcumin for 3 days prior to 4th sensitization prevented the major symptoms like sneezing and rubbing frequencies. Environmental variables (temperature, humidity and pollutants) influence rhinitis considerably at 50–60%, or dry air that irritates nasal mucosa resulting in the aggravation of allergic rhinitis [27]. Humid air (95% RH) inhibits allergen induced sneezing, congestion and nasal secretion in patients with rhinitis [28]. In present study too; ovalbumin sensitized guinea pigs when exposed to relative humidity 50–60% showed a marked enhancement in sneezing and rubbing frequencies. Treatment with curcumin at varied humidity conditions was found to inhibit significantly sneezing and rubbing frequencies. It was also observed, at higher humidity, that there was a reduction in sneezing and rubbing frequencies in sensitized as well as curcumin and Montelukast pretreated animals.

Mast cells express a high-affinity receptor (FcεRI) for the Fc region of IgE, and thus, IgE binds irreversibly to the mast cells. Re-exposure to the same antigen causes its binding on the site situated on the variable region of the IgE molecule on mast cell membrane. Cross-linking of two or more IgE molecules on mast cell causing sequence of reactions, triggers degranulation of mast cells and finally leading to an increased release of inflammatory mediators in nasal mucosa, manifested in sneezing, itching with watery discharge [29]. The released IgE is responsible for increased production of leukotrienes and prostaglandins which in turn attract eosinophils, increase microvascular leakage, edema, mucous secretion and augment the kinin action [30]. In our study, the 7th and 14th days of ovalbumin challenge induced a significant elevation of serum IgE concentration on the 14th day of ovalbumin challenged guinea pigs.

However, the increased IgE concentration was more prominent on the 14th day post challenge compared to the 7th day which might be due to continued longer (up to 14 days) exposure of antigens to immune system. The higher IgE level in systemic circulation manifested into activation of cellular events which are responsible for symptoms of allergic rhinitis. Following ovalbumin sensitization, processing of antigen via antigen-presenting cells such as dendritic cells results in T-cell activation leading to production of specific cytokines [9]. Isotype switching and specific IgE production from B cells mediated by IL-4 are a critical step in the allergic reactions [9], reflected in clinical symptoms viz sneezing, itching and rhinorrhea accompanied by infiltration

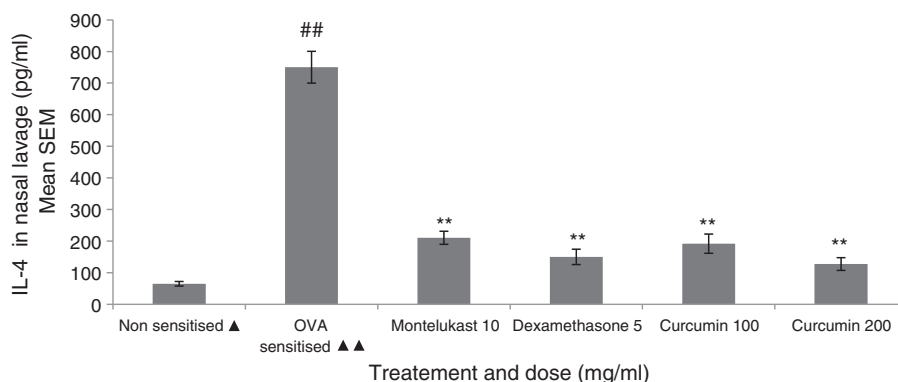


Fig. 3. Effects of curcumin, Montelukast and sodium chromoglycate on nasal IL-4 concentration in ovalbumin sensitized allergic rhinitis in guinea pig. ▲Nonsensitized ovalbumin challenged, ▲▲sensitized and challenged with ovalbumin (60 mg/ml, 20 μ l/nostril), ## $p < 0.001$ when compared to non sensitized group, ** $p < 0.001$ when compared to ovalbumin sensitized group. Experiment was performed once ($n = 6$, mean $\pm$ SEM).

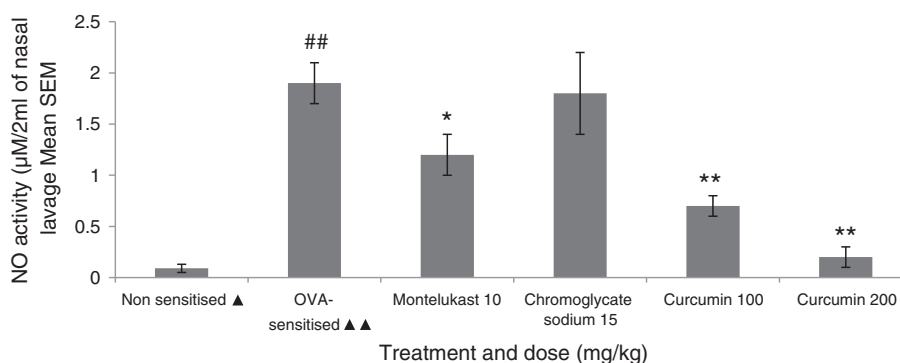


Fig. 4. Effects of curcumin, Montelukast and sodium chromoglycate on NO infiltration in ovalbumin sensitized allergic rhinitis in guinea pig. ▲Nonsensitized ovalbumin challenged, ▲▲sensitized and challenged with ovalbumin (60 mg/ml, 20 μ l/nostril), ## $p < 0.001$ when compared to nonsensitized group, * $p < 0.005$, ** $p < 0.001$ when compared to ovalbumin sensitized group. Experiment was performed twice ($n = 6$, mean $\pm$ SEM).

of inflammatory cells into the nasal mucosa. Pretreatment curcumin for 3 days prior to ovalbumin challenge prevented the elevation of serum IgE concentration; thereby affecting cellular cascade reactions due to the products released by mast cells which may be responsible for the symptomatic relief (absence of sneezing, rubbing, lacrimation and congestion of nasal mucosa) during allergic rhinitis. These results are in agreement with findings of Kobyashi et al. [31], who demonstrated, inhibition of cytokine production by curcumin, consequently affecting eosinophil function and IgE synthesis, finally preventing allergic symptoms. The greater IgE inhibition with curcumin treatment in the present experiment is not fully known, however, its effects on mast cell stabilization as well as other cellular cascade reaction might be responsible for such

inhibition. Similarly, IL-4, an important and major pro-inflammatory mediator was found to be increased in nasal lavage during allergic rhinitis [9]. Pretreatment with curcumin at both the doses 100 and 200 mg showed a significant reduction of IL-4 concentration in nasal lavage, thereby suggesting that curcumin is an inhibitor of IL-4 during rhinitis thus further symptoms of allergic rhinitis were prevented. Moreover, Montelukast, a leukotriene receptor antagonist also showed an inhibitory effect on IL-4 concentration.

In another set of experiment, we measured EPO activity, which is an index of eosinophil infiltration. The increased eosinophil infiltration into the nasal mucosa in allergic rhinitis was also reported by Tsunematsu et al. [23]. In our experiments too, we observed, a significant increase of

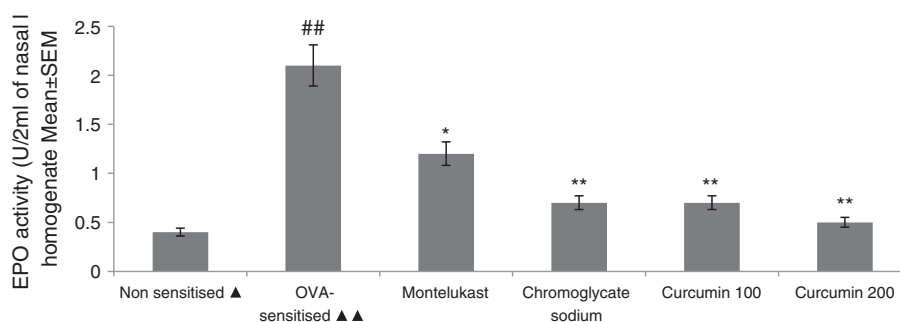


Fig. 5. Effects of curcumin, Montelukast and sodium chromoglycate on serum EPO (Eosinophil peroxidase) activity in ovalbumin sensitized allergic rhinitis in guinea pig. ▲Nonsensitized ovalbumin challenged, ▲▲sensitized and challenged with ovalbumin (60 mg/ml, 20 μ l/nostril), ## $p < 0.001$ when compared to nonsensitized group, * $p < 0.005$, ** $p < 0.001$ when compared to ovalbumin sensitized group. Experiments were performed twice ($n = 6$, mean $\pm$ SEM).

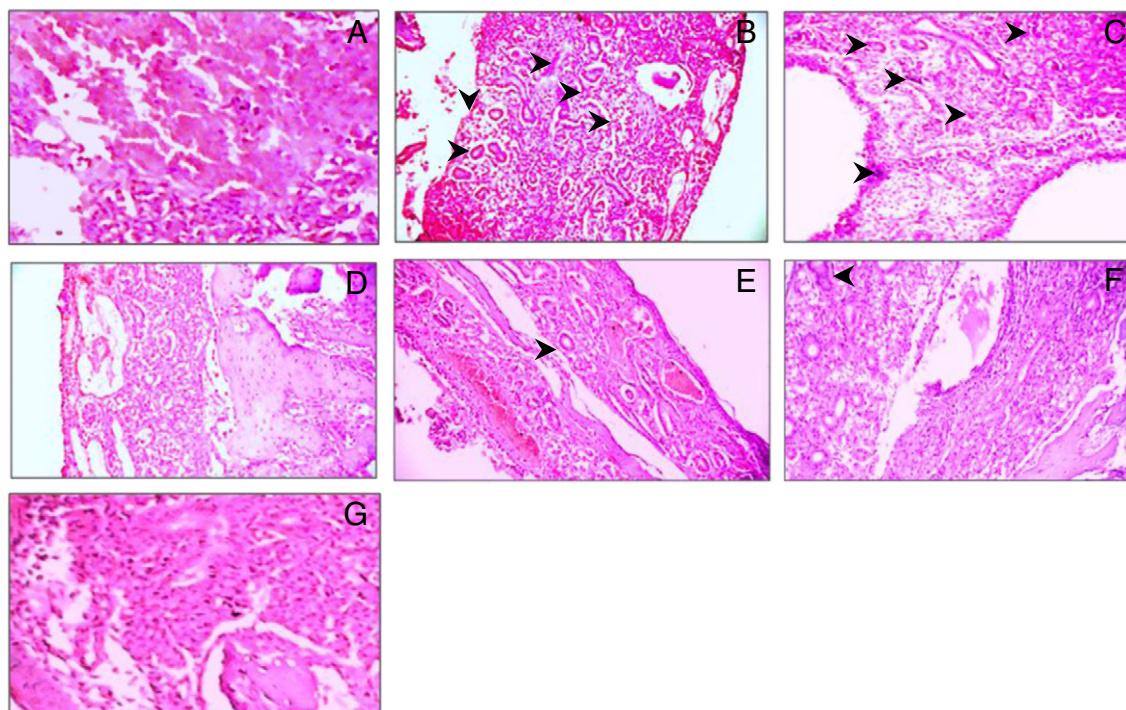


Fig. 6. Hematoxylin–eosin stained sections of nasal mucosa of the nonsensitized (A), ovalbumin sensitized (B), ovalbumin challenged (C), Montelukast sodium (D), Sodium chromoglycate (E), curcumin 100 (F) and curcumin 200 (G) at 8 h (D) after intranasal ovalbumin challenge. Black arrows indicate inflammatory cell infiltrations viz eosinophils and neutrophils on nasal mucosa in respective group. Original magnification $\times 100$.

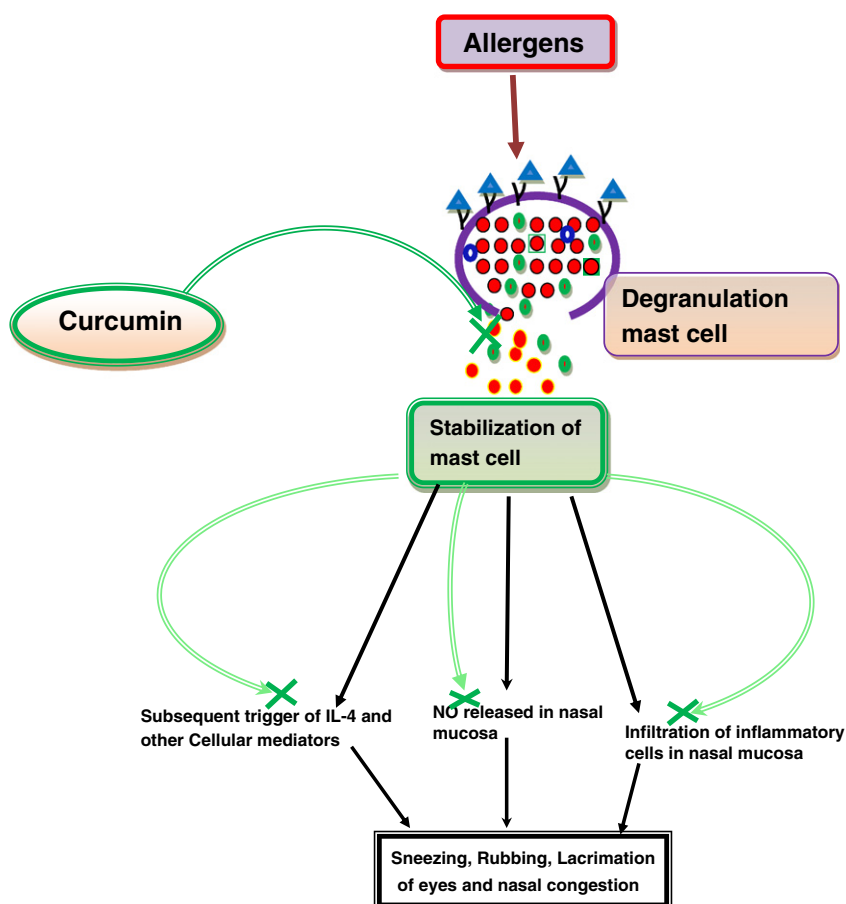


Fig. 7. Multiple effects of curcumin at various targets during allergic rhinitis.

EPO activity in nasal homogenate in ovalbumin sensitized guinea pigs. Curcumin as well as sodium chromoglycate treatment inhibited EPO activity in allergic rhinitis. Thus, our findings suggest that, curcumin is able to prevent the cellular infiltration of eosinophils during allergic rhinitis. The NO involvement has been documented during allergic rhinitis; the distribution of NOs (nitric oxide synthase) in nasal mucosa may have a specific role in the production of cytokines (IL-4) and may be required for eosinophil survival [32]. Curcumin and Montelukast treatment inhibited the NO activity in nasal lavage in ovalbumin challenged group, and may be responsible for amelioration of symptoms as well as reduction of eosinophil infiltration. In histopathological studies, it was observed that a marked infiltration of inflammatory cells, lymphocytes in nasal mucosa of ovalbumin challenged guinea pigs. Treatment of curcumin and Montelukast reduced the infiltration of inflammatory cells in nasal cavity in allergic rhinitis. Thus, the histopathological findings are in agreement with biochemical effects (IgE, IL-4, NO, EPO) of curcumin in experimental allergic rhinitis.

5. Conclusion

Based on our experimental findings, it is presumed that curcumin might act mainly; through mast cell stabilization which can subsequently prevent the other associated cellular/molecular events (IgE, IL-4, NO, EPO) leading to amelioration of the majority of allergic rhinitis symptoms (Fig. 7). Thus, we are attempted to propose that curcumin can be a promising anti-allergic agent and may be beneficial in the clinical management of allergic rhinitis.

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