

RESEARCH ARTICLE

Anti-asthmatic potential of chrysin on ovalbumin-induced bronchoalveolar hyperresponsiveness in rats

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Abstract

Context: Chrysin, a flavonoid obtained from various natural sources, has been reported to act as an anti-inflammatory and antioxidant agent. However, its anti-allergic action is not fully understood.

Objective: In this study, we investigated the *in vivo* anti-asthmatic activity of chrysin.

Materials and methods: The effects of chrysin were evaluated using ovalbumin (OVA) (two subcutaneous 1 mL injections of 20 µg) to induce bronchoalveolar hyperresponsiveness in rats. Chrysin, when administered at 3, 10, and 30 mg/kg, p.o., respectively, before OVA challenge, reduced inflammatory cell (total and differential cell count) infiltration into the lungs measured from bronchoalveolar lavage fluid as supported by lung histology.

Results: The total lung injury score was reduced in a dose-dependent manner, evaluated in six different categories (infiltration of leucocytes, type of inflammatory exudates, status of bronchi, perivascular status of lung blood vessels, integrity of alveoli and activation of alveolar macrophages). Various cellular injury parameters such as alkaline phosphatase, lactate dehydrogenase, and total protein were estimated and found to be reduced by chrysin pretreatment. Further, chrysin was found to reduce nitrite concentration (NO) and lipid peroxidation, suggesting its antioxidant activity.

Discussion and conclusion: Chrysin showed anti-asthmatic potential, probably due to the alteration of Th1/Th2 polarization via the suppression of inducible nitric oxide synthase, nuclear factor-κB, and activation protein.

Keywords: Chrysin, antioxidant, asthma, flavonoid, ovalbumin hyperresponsiveness

Introduction

Asthma is a chronic inflammatory airways disease in which multiple complex pathways are involved, and is associated with genetic, allergic, environmental, infectious, emotional, and nutritional factors (Greene, 1995). When the individual is re-exposed to the allergen it stimulates secretion of various inflammatory cells which are responsible for bronchoconstriction, increased airway hyperresponsiveness, and mucus production. The pathological mechanisms involved in type-I allergy has been explained as the degranulation of mast cells, followed by the release of proinflammatory mediators from these cells (Mathew et al., 2009). Type-I hypersensitivity or allergy, is the clinical manifestation of an immune

response against foreign protein molecules, commonly known as allergens, which are potent inducers of immunoglobulin E (IgE) synthesis (Shakib et al., 2008).

Flavonoids are ubiquitous plant secondary metabolites that contains large groups of low molecular weight polyphenolic compounds, which are important to the health and maintenance of human beings. Flavonoids are reported to possess a variety of biological effects, including antioxidant, antitumor, antimicrobial, anti-allergic, and anti-angiogenic properties (Middleton et al., 2000; Chen et al., 2007).

Chrysin, a flavonoid obtained from various natural sources such as *Passiflora caerulea* L. (Passifloraceae) (Medina et al., 1990), *Lonicera japonica* Thunb.

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(Caprifoliaceae) (Rahman Kang, 2009), *Cyclanthera pedata* Scrabs (Caigua) (Montoro et al., 2005), and bee propolis (Burdock, 1998), was reported to possess inhibition of IgE and Th2 cytokine (Yano et al., 2007), inhibition of cyclooxygenase (COX), inducible nitric oxide synthase (iNOS) (Liang et al., 2001; Cho et al., 2004; Hougee et al., 2005; Woo et al., 2005; Comalada et al., 2006; Harris et al., 2006), and hypoxia-inducible factor-1 expression (Fu et al., 2007).

However, *in vivo* support for anti-asthmatic potential of chrysin is lacking. The objective of the present study is to evaluate anti-asthmatic activity of chrysin using ovalbumin (OVA)-induced bronchoalveolar hyperresponsiveness in rats.

Methods

Animals

Wistar rats (180–200 g) were used for the study and were maintained under regulated environmental conditions (temperature $22 \pm 2^\circ\text{C}$; humidity 45–55%; 12 h light/dark cycle) and provided with standard diet and water *ad libitum*. The Institution Animal Ethics Committee approved the experimental protocol (198/99/CPCSEA).

Chemicals

Chrysin was procured from Sigma (St. Louis, MO). OVA was procured from the Central Drug House, New Delhi, India. All other chemicals were of high purity reagent grade. Carboxymethyl cellulose (CMC, 1% w/v in saline) was used as vehicle for oral administration of chrysin in animals.

Sensitization and drug administration

Wistar albino rats were immunized with two subcutaneous 1 mL injections of 20 μg OVA mixed with 8 mg/mL aluminum hydroxide in 0.9% NaCl, given 7 days apart (on days 1 and 8) as previously reported (Chapman et al., 2007). Rats were kept segregated in six different groups ($n=6$). Non-sensitized (NS) animals were injected with alum only (group NS). After 1 week (on day 15) 5 h after drug administration to each group, animals were challenged (via both nostrils) by aerosolized OVA (1%) for 30 min by placing the rats into a closed Plexiglas chamber and filling the chamber with aerosolized OVA which was generated by an nebulizer (YCMO-202B, Gimmy Industrial, Taiwan).

Animals in group “NS” and in group “S” (OVA-sensitized and challenged) received vehicle (1% CMC, 10 mL/kg, p.o.). Rats in group CHR3, CHR10, and CHR30 received chrysin 3, 10, and 30 mg/kg, p.o., respectively, whereas group Dexa was treated with the standard drug dexamethasone (1 mg/kg, p.o.).

Bronchoalveolar lavage and lung histology

Twenty-four hours after challenge, bronchoalveolar lavage fluid (BALF) was collected via tracheal cannulation (Portela Cde et al., 2001). The rats were sacrificed

and a tracheal catheter was inserted. BALF was collected by lavaging the lungs with 2 aliquots of 5 mL of 0.9% sodium chloride solution. Total recovery volume per rat was approximately 8 mL. BALF was centrifuged (Remi cooling centrifuge, 3000 rpm, 10 min) to obtain the pellet. The total cell count in the BALF was performed by determining microscopically the total cell number using a Neubauer chamber. For the differential white cell count 200 μL of BALF was stained with Leishman stain. Cells were identified as eosinophils, neutrophils, lymphocytes, monocytes, and alveolar macrophages by standard morphology and 100 cells counted under (400 $\times$) magnifications. For the histological evaluation of lung tissue, the tissues were dehydrated in various concentrations of ethanol and embedded in paraffin. A series of micro sections (5 μm) were cut on a microtome and stained with H&E using standard histological techniques to assess cellular deformities due to OVA exposure (400 $\times$). The scores of lung injury for each animal were histopathologically evaluated as described previously (Verstraelen et al., 2008). Briefly, lung injury was graded from 0 (normal) to 4 (severe) in six different categories: infiltration of leucocytes, type of inflammatory exudates, status of bronchi, perivascular status of lung blood vessels, integrity of alveoli and activation of alveolar macrophages. The total lung injury score was calculated by adding the individual scores for each category.

Wet-to-dry lung ratio

Four to five animals from each group were used to determine the wet-to-dry lung ratio as an indicator of pulmonary edema. The left lung was excised and immediately weighed using a precision balance, then re-weighed after being dried for 24 h in an oven at 90°C . The left lung of the remaining animals (four to five animals) of each group was used for histological evaluation.

Biochemical parameters from BALF and serum

Concentration of cellular damage biomarkers such as alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total protein (TP) were determined from serum and BALF collected from these animals using biochemical estimation kits. The units were as follows: KA units/dL, U/L and g/dL, respectively.

Nitric oxide content from BALF

Nitric oxide production in BALF was quantified spectrophotometrically using Griess reagent (1% sulfanilamide, 2.5% H_3PO_4 , 0.1% *N*-1-naphthylethylenediamine dihydrochloride) by the reported method (Park et al., 2009). The absorbance was measured at 540 nm and the nitrite concentration was determined using a calibration curve prepared with sodium nitrite as the standard.

MDA content from lung homogenate

The assay for membrane lipid peroxidation (LPO) was done by reported method (Wright et al., 1981). The

reaction mixture in a total volume of 3 mL contained 1 mL of lung homogenate, 1 mL of trichloroacetic acid (10%) and 1 mL of thiobarbituric acid (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to an ice bath for 10 min. and then centrifuged at 2500g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm and was expressed in nm of MDA/g of tissue (Draper et al., 1993).

Statistical analysis

Results are presented as mean $\pm$ SEM. Experimental differences were tested for statistical significance using ANOVA followed by Dunnett's test. Non-parametric Kruskal Wallis followed by Dunn's tests was used to compare group medians for histopathological scores, using GraphPad Prism version 5. $p < 0.05$ was considered to be significant.

Results

Effect of chrysin on lung histopathology

Histological study reveals that sensitization and subsequent challenge to rats with OVA damages lung architecture, there was increase in hemorrhage, hyperplasia, exudation of mucus (catarrhal and mucoid material), cell infiltration (eosinophils, neutrophils, etc.), constriction of the secondary bronchus and tertiary bronchi, infiltration of mononuclear cells around the lung blood vessels (both artery and venules) and alveolar emphysema (Table 1 and Figure 1A). The NS group had normal histology with minimum infiltration of leukocytes and exudates, hyperplasia. Total score in the sensitized group was 18 which significantly increased as compared to NS group. This increase in infiltration of inflammatory cells along with exudates was significantly reduced in the dexamethasone- and chrysin-treated groups. The effect of drug treatment 5 h before also decreases activation of macrophages and perivascular bleeding. The total score in the drug-treated group was reduced to 6.6 for Dexa, 11.6 for CHR3, 9.8 for CHR10 and 7.2 for CHR30, respectively.

Effect of chrysin on total and differential cell count in BALF

The cell count in the sensitized group was significantly increased as compared to the NS group. This effect of OVA was significantly reduced in the drug-treated groups. Chrysin (3 mg/kg) had minimal effect on inflammatory cell infiltration (eosinophils, neutrophils, macrophages, lymphocytes, and monocytes) which were increased in a dose-dependent manner for 10 and 30 mg/kg chrysin-treated groups. Dexamethasone, a reference anti-inflammatory drug, also reduced the total number of inflammatory cells in the BALF as compared to the sensitized group (Figure 2A and 2B).

Effect of chrysin on lung weight ratio

To determine OVA-induced airway hypertrophy in the lung tissue, wet/dry weight ratio was performed (Table 2). Lung weight ratio in the sensitized group was raised to 4.48 compared to that in the NS group. Total lung weight was dose-dependently inhibited by chrysin administration compared to the sensitized group. These results suggest that chrysin inhibited the OVA-induced total lung weight gain in a murine model of asthma.

Effect of chrysin on biochemical parameters in serum and BALF

The sensitized group had the highest level of ALP, LDH and TP, which represents the most cellular death and lung injury in rats. Chrysin administration before OVA challenge had inhibitory action on cellular damage due to allergic reaction, when compared with the sensitized group. This inhibitory action of chrysin was found to be dose-dependent, as 3 mg/kg had minimal effect, whereas for higher doses the activity was found to be improved. It was also clear that, chrysin and dexamethasone-treated groups showed decreased levels of these parameters in both serum and BALF, as compared to the sensitized group (Figure 3A, 3B and 3C).

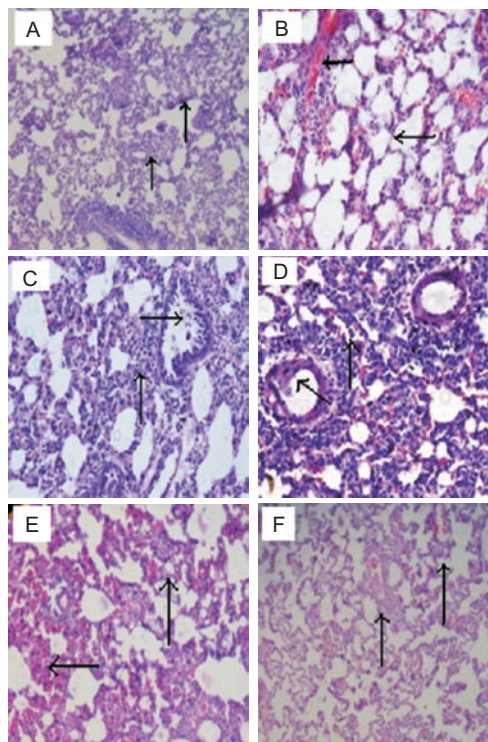


Figure 1. Effects of chrysin on pulmonary morphological changes. The lung sections from (A) NS, non-sensitized = OVA-challenged, treated with vehicle, (B) S, sensitized = OVA-sensitized and challenged, treated with vehicle), (C) Dexa, OVA-sensitized and challenged, treated with dexamethasone 1 mg/kg, (D) CHR3, (E) CHR10 and (F) CHR30 = OVA-sensitized and challenged, treated with chrysin 3, 10, and 30 mg/kg, p.o., respectively. Arrows showing hemorrhage, hyperplasia of epithelial cells.

Table 1. Effects of chrysin on lung histology.

Lung injury	Groups					
	NS	S	Dexa	CHR3	CHR10	CHR30
Infiltration of leucocytes	0.0±0.0	3.8±0.2 ^{##}	0.8±0.2 ^{**}	2.2±0.94	2.1±0.29	1.2±0.24 [*]
Type of inflammatory exudates	0.0±0.0	3.4±0.4 ^{##}	1.2±0.2 ^{**}	2.1±0.44	1.6±0.2	1.2±0.24 [*]
Status of bronchi	0.0±0.0	2.6±0.24 ^{##}	1.2±0.24 ^{**}	2.3±0.6	1.8±0.47	1.3±0.24 [*]
Perivascular status of lung blood vessels	0.2±0.2	2.4±0.24 ^{##}	0.8±0.2 ^{**}	1.9±0.87	1.6±0.91	1.0±0.2 [*]
Integrity of alveoli	0.2±0.2	2.8±0.2 ^{##}	1.4±0.24 ^{**}	1.4±0.19	1.2±0.59 [*]	1.3±0.24 [*]
Activation of alveolar macrophages	0.0±0.0	3.0±0.31 ^{##}	1.2±0.2 ^{**}	1.7±0.2	1.5±0.6 [*]	1.2±0.24 [*]
TLIS	0.4±0	18.0±1.59 ^{##}	6.6±1.28 ^{**}	11.6±3.24	9.8±3.06	7.2±1.4 [*]

TLIS, total lung injury score; NS, non-sensitized (OVA-challenged, treated with vehicle); S, Sensitized; Dexa, dexamethasone (1 mg/kg, p.o.) treated; CHR3, CHR10 and CHR30, Chrysin at 3, 10, and 30 mg/kg, p.o., respectively. Results are presented as mean ± SEM. Data of each parameter was separately analyzed by non-parametric Kruskal Wallis followed by Dunn's test. ^{##}*p*<0.01 when sensitized group compared with non-sensitized group, ^{*}*p*<0.05, ^{**}*p*<0.01 when Dexa, CHR3, CHR10, CHR30 groups compared with sensitized group.

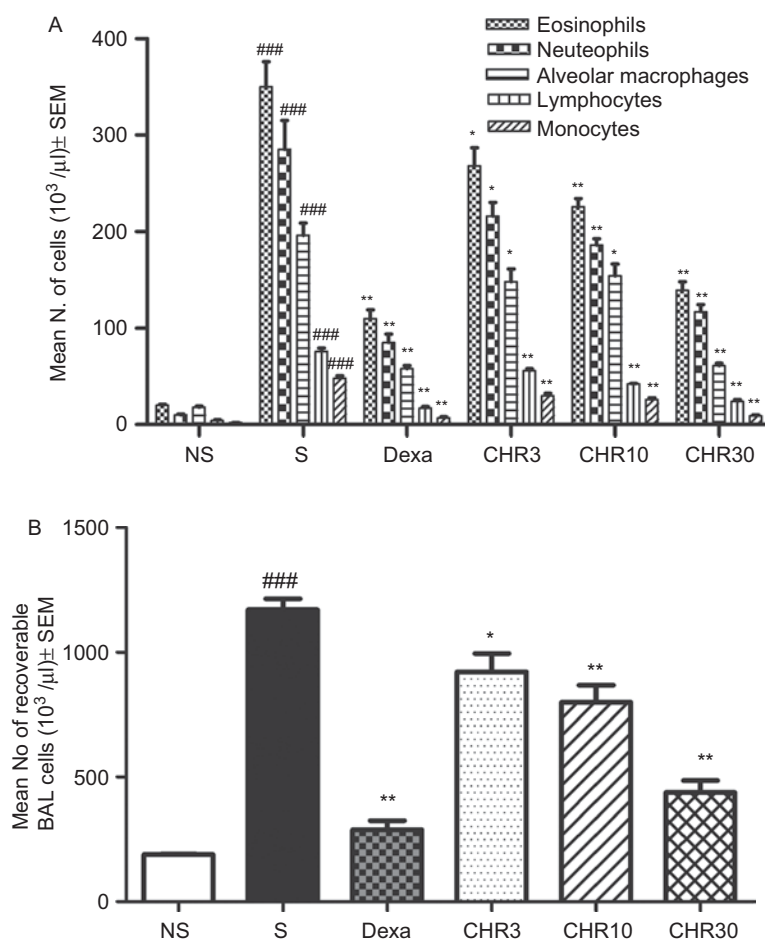


Figure 2. Effect of chrysin on (A) differential cell count (B) total cell count from BALF. NS, non-sensitized (OVA-challenged, treated with vehicle), S, sensitized (OVA-sensitized and challenged, treated with vehicle), Dexa, OVA-sensitized and challenged treated with dexamethasone 1 mg/kg, CHR3, CHR10, CHR30, OVA-sensitized and challenged, treated with chrysin 3, 10, and 30 mg/kg, p.o., respectively. Results are presented as mean ± SEM. ANOVA followed by Dunnett's test. ^{###}*p*<0.001 when sensitized group versus non-sensitized group, ^{*}*p*<0.05, ^{**}*p*<0.01 when Dexa, CHR3, CHR10, CHR30 groups versus sensitized group.

Effect of chrysin on oxidative stress

Nitric oxide level is reported to be increased in breath condensate of asthmatic patients, which further leads to increased oxidative stress in lungs and ultimately results in severe lung inflammation. Chrysin-treated groups showed decreased levels of nitrite content from BALF of OVA-sensitized and challenged rats as compared to sensitized group. Chrysin at 30 mg/kg possess

comparable results as that of dexamethasone. Another marker of oxidative stress measured in this study is LPO from lung homogenate. Here also, a similar result was obtained as that of nitrite content. Increased levels of MDA were observed in the sensitized group. Chrysin-treated groups decreased formation of peroxide of lipids (MDA content) in a dose-dependent manner (Figure 4A and 4B).

Discussion

Laboratory animals such as guinea pigs, rats, and mice, when exposed to parenteral administration of antigen (OVA) adsorbed on adjuvant leads to sustained delivery of antigen and more consistent and rapid response (Sanjar et al., 1990). Eosinophils play an important role in exacerbation of allergen-induced asthma. Increased infiltration of activated phagocytic cells (neutrophils,

eosinophils, monocytes, and macrophages) in lungs after OVA challenge, secretes cytokines, chemokines and produce large amounts of reactive oxygen species (ROS) (Henricks Nijkamp, 2001). These oxidized biomolecules may also provoke a variety of cellular responses through the generation of secondary metabolic reactive species (Rahman et al., 2006).

Exposure to allergens in susceptible individuals leads to the generation of CD4⁺ Th2 cells. Subsequent allergen exposure activates previously sensitized Th2 cells resulting in an allergic immune response that is pathologically manifested in the eyes (conjunctivitis), nose (rhinitis), lungs (asthma), skin (atopic dermatitis, eczema), or circulatory system (anaphylaxis) (Epstein, 2006). In asthma, the activation of allergen-induced Th2 cells in the lungs leads to eosinophilic lung inflammation, edema, increased secretion of mucus and recurring bronchospasm.

Type-I hypersensitivity or allergy, is the clinical manifestation of an immune response against foreign protein molecules, commonly known as allergens, which are potent inducers of IgE synthesis (Shakib et al., 2008). The resulting allergen-specific IgE (produced by the cooperation between Th2 cells and B-cells) binds to both allergen and high-affinity FcεRI receptors on the surface of mast cells and transduces a signal that causes the secretion of interleukin-4 (IL-4), IL-5, tumor necrosis factor (TNF-α),

Table 2. Effect of Chrysin on lung weight ratio.

Group no.	Group	Lung weight ratio (wet/dry) mean ± SEM
I	NS	2.96 ± 0.073
II	S	4.48 ± 0.092 ^{###}
III	D	3.2 ± 0.086 ^{**}
IV	CHR3	4.09 ± 0.037 [*]
V	CHR10	3.66 ± 0.077 ^{**}
VI	CHR30	3.21 ± 0.101 ^{**}

NS, non-sensitized (OVA-challenged, treated with vehicle); S, sensitized (OVA-sensitized and challenged treated with vehicle); Dexa, OVA-sensitized and challenged treated with dexamethasone 1 mg/kg, CHR3, CHR10, CHR30, OVA-sensitized and challenged treated with Chrysin 3, 10, and 30 mg/kg, p.o., respectively. Results are presented as mean ± SEM. ANOVA followed by Dunnett test.

^{###}*p* < 0.001 when sensitized group versus non-sensitized group, ^{*}*p* < 0.05, ^{**}*p* < 0.01 when Dexa, CHR3, CHR10, CHR30 groups versus sensitized group.

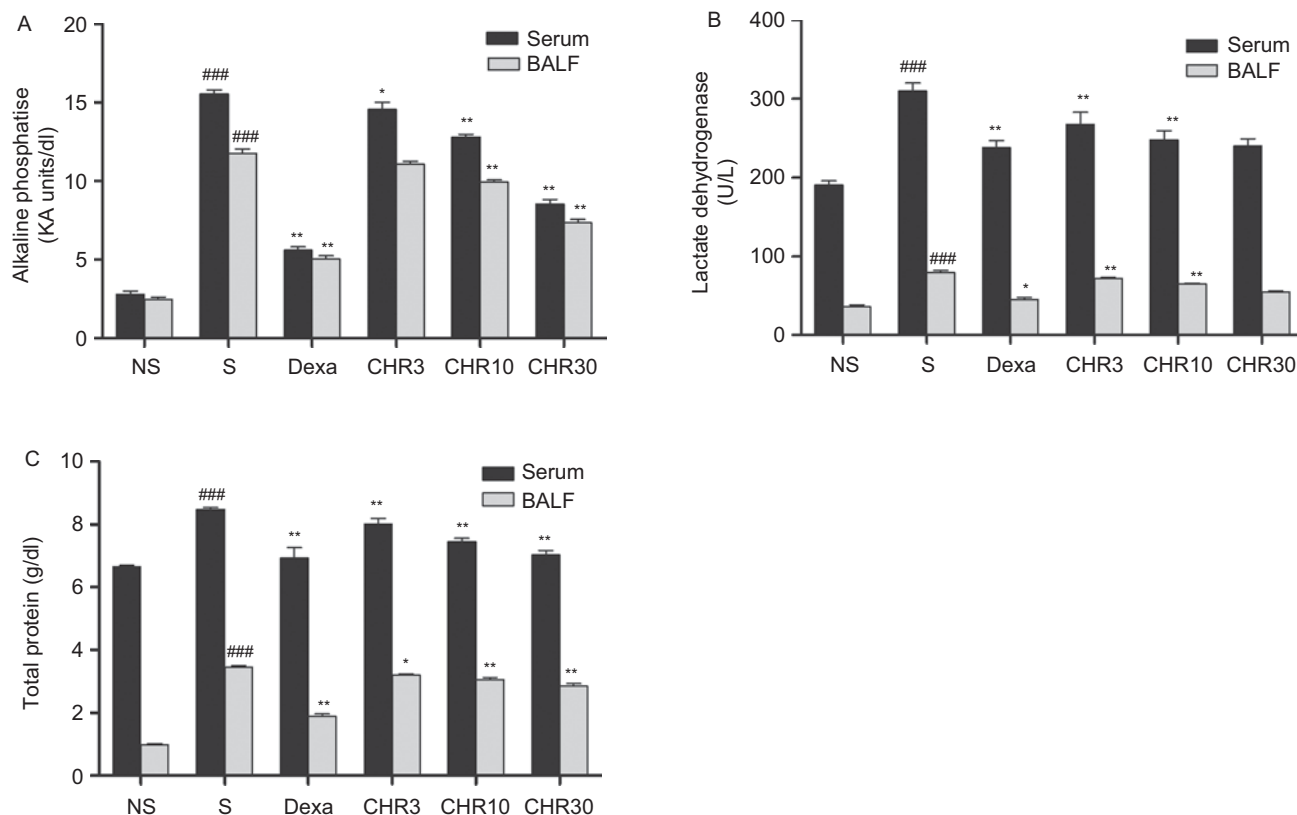


Figure 3. Effect of chrysin on (A) alkaline phosphatase (B) lactate dehydrogenase (C) total protein (D) from BALF. NS, non-sensitized (OVA-challenged, treated with vehicle), S, sensitized (OVA-sensitized and challenged, treated with vehicle), Dexa, OVA-sensitized and challenged, treated with dexamethasone 1 mg/kg, CHR3, CHR10, CHR30, OVA-sensitized and challenged, treated with chrysin 3, 10, and 30 mg/kg, p.o., respectively. Results are presented as mean ± SEM. ANOVA followed by Dunnett's test. ^{###}*p* < 0.001 when sensitized group versus non-sensitized group, ^{*}*p* < 0.05, ^{**}*p* < 0.01 when Dexa, CHR3, CHR10, CHR30 groups versus sensitized group.

leukotrienes, histamine, tryptase, prostaglandins, and chymase. These mediators are responsible for inducing the immediate type of hypersensitivity reaction or early phase response (Epstein, 2006). Eosinophils, lymphocytes, alveolar macrophages and to the lesser extent neutrophils are infiltrated due to OVA challenge, which are responsible for more sustained inflammatory or late-phase response together with alveolar epithelial cells, bronchial epithelial cells and endothelial cells (Rogerio et al., 2007). An imbalance in the Th1/Th2 ratio is reported to be responsible for allergic asthma and there is an increase in the number of Th2 cells and, while Th1 cells are found to be decreased (Packard Khan, 2003). GATA-3 and c-Maf are selective transcription factors (Epstein, 2006) involved in Th2 differentiation, and cytokine production was reported to increase during asthmatic exacerbation (Choi et al., 2009).

In this study, there was an increase in lymphocyte count as well as induced inflammatory response in rats exposed to OVA. Chrysin treatment in rats decreased infiltration cells into the lungs, probably due to alteration in Th1/Th2 cells. Histological study reveals that there was an increase in hemorrhage, hyperplasia, exudation of mucus (catarrhal and mucoid material), cell infiltration (eosinophils, neutrophils), constriction of the secondary bronchus and tertiary bronchi, infiltration of

mononuclear cells around the lung blood vessels (both artery and venules) and alveolar emphysema. This may be the reason for the reduction in the lung wet/dry ratio. The number of inflammatory cells and histology measured in this study was not solely responsible for the exacerbation of asthmatic condition, so we expanded this study to observe the effect of OVA on oxidative stress and cellular injury.

Because of its location, anatomy and vital action, the lung has continuous exposure to oxidants generated internally as well as produced by external agents (Rahman et al., 2006). *In situ* lung injury due to ROS is linked to oxidation of proteins, DNA, and lipids. At the molecular level, increased ROS/RNS levels have been implicated in initiating inflammatory responses in the lungs through the activation of transcription factors (Eynott et al., 2002). Nitric oxide and $O_2^{\cdot-}$ react to form highly reactive peroxynitrite molecule (Rahman et al., 2006). Studies demonstrated that, NO levels in exhaled air were increased 30 min after OVA challenge to sensitized animals, with a concomitant increase in airway constriction (Smith Broadley, 2007). This suggests that iNOS-derived NO promotes bronchial hyperactivity and eosinophil infiltration in the airways as a consequence of Th1/Th2 imbalance (Nevin Broadley, 2002). The decreased concentration of NO and MDA in chrysin-

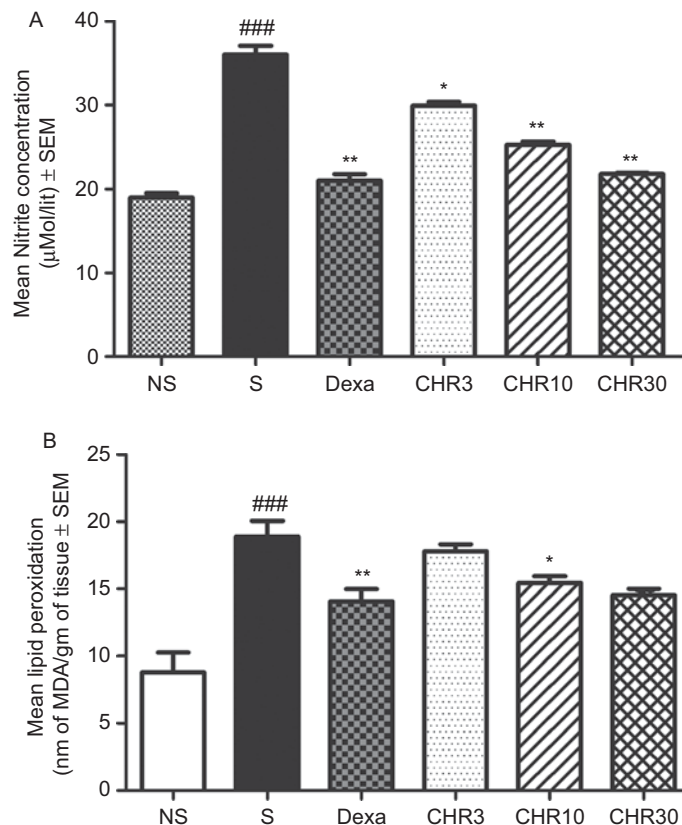


Figure 4. Effect of chrysin on (A) nitrite content from BALF (B) lipid peroxidation from lung homogenate. NS, non-sensitized (OVA-challenged, treated with vehicle), S, sensitized (OVA-sensitized and challenged, treated with vehicle), Dexa, OVA-sensitized and challenged treated with dexamethasone 1 mg/kg, CHR3, CHR10, CHR30, OVA-sensitized and challenged, treated with chrysin 3, 10, and 30 mg/kg, p.o., respectively. Results are presented as mean $\pm$ SEM. ANOVA followed by Dunnett's test. ^{###} $p < 0.001$ when sensitized group versus non-sensitized group, ^{*} $p < 0.05$, ^{**} $p < 0.01$ when Dexa, CHR3, CHR10, CHR30 groups versus sensitized group.

treated groups suggests its antioxidant activity is due to inhibition of iNOS.

Previous reports have indicated that generation of ROS is involved in regulating the activation status of a variety of transcription factors, including nuclear factor- κ B (NF- κ B) and activation protein (AP-1) through the phosphorylation and degradation of inhibitor of κ B (I κ B) by increasing I κ B kinase or Akt kinase activity (Comalada et al., 2006). Some of the pathways implicated comprise those involving the Src-family tyrosine kinases, the serine/threonine kinases protein kinase A and C, mitogen-activated protein kinase and protein kinase B/Akt. Certain flavonoids including chrysin, luteolin, apigenin, quercetin, kaempferol have been reported previously to inhibit protein kinase B/Akt, COX/LOX, NF- κ B, iNOS, protein kinase C, tyrosine kinase and phospholipases A2 and C (Cho et al., 2004; Comalada et al., 2006; Chen et al., 2007). The structural analogues of chrysin such as apigenin (Choi et al., 2009) and luteolin (Das et al., 2003) demonstrated an ability to reduce OVA-induced asthmatic conditions in experimental animals.

Apart from this action, inhibition of OVA-induced lung injury was due to binding abilities of this flavonoid to peroxisome proliferator-activated receptors (PPAR γ) (Liang et al., 2001; Ward et al., 2006). PPAR γ expression is increased in asthmatic airways (O'Leary et al., 2004). Liang et al., (2001) demonstrated that chrysin and apigenin possess PPAR γ agonistic action and stimulate its anti-inflammatory action, which down-regulates the gene expression in a ligand-dependent manner by antagonizing the activities of signal-dependent transcription factor NF- κ B. This suggestion was supported by a study conducted by Yano et al., (2007), which broadens the scope of these flavones to suppress IgE and Th2 cytokine production by activation of PPAR γ receptors. Several structure activity relationship studies of chrysin demonstrate that the double bond at 2-3 position and OH-groups at R5 and R7 are responsible for reduction in generation of proinflammatory cytokines such as TNF- α and iNOS/NO generation from macrophages and monocytes, and stimulation of IL-10 and transforming growth factor- β (Comalada et al., 2006).

Cellular enzymes when present in the extracellular space do not have any metabolic function but are still beneficial as they provide an important tool for measurement of disturbances of the cellular integrity when subjected to pathological conditions. The present study demonstrated the effect of chrysin on ALP (toxic damage or proliferation of pneumocytes type II), LDH (increased membrane permeability and cell lysis) and also checked the TP level (altered respiratory membrane and vascular permeability) (Maden et al., 2001). Tissue concentration of LDH enzyme activity is about 500 times higher than those normally found in serum (Drent et al., 1996). The results presented in this work, demonstrated that chrysin had an inhibitory action on cellular injury and tissue damage due to OVA-induced lung inflammation when administered before challenge. The concentration of

above-mentioned biochemical parameters was found to be increased in serum as compared to BALF. Chrysin treatment had a suppressive effect on all the measured parameters, suggesting an anti-inflammatory, anti-allergic action on OVA-induced type-I hypersensitivity.

In conclusion, our results strongly indicate that chrysin reduces allergic airway inflammation by degranulation of certain types of cells. Antioxidant potential of chrysin may be attributed to alteration of Th1/Th2 polarization via the suppression of iNOS, NF- κ B, and AP-1. However, pharmacokinetics studies will be required to implement chrysin as a new therapeutic approach to allergic airway diseases.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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