



Crocini modulates IL-4/IL-13 signaling and ameliorates experimentally induced allergic airway asthma in a murine model



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ABSTRACT

Allergic asthma is a chronic respiratory disease with a prevalent T helper (Th2)-mediated immune reaction. Crocin, the major bioactive constituent of saffron, has been reported in multiple studies to have numerous pharmacological activities, including prominent anti-oxidant activities. In the current study, the anti-asthmatic potential of crocin was evaluated. Adult male Swiss Albino mice were administered 10 mg of ovalbumin (OVA) mixed with 1 mg of aluminum hydroxide intraperitoneally on days 0 and 7 and were administered crocin (25 mg/kg) orally daily for 16 days. Asthma progression was associated with significant increase in the lung/body weight index, inflammatory cell counts in bronchoalveolar lavage fluid (BALF), lung total protein content, and serious index of lung permeability, indicating pulmonary edema with accumulation of serous fluids within the lungs. Serum lactate dehydrogenase (LDH) activity and lung malondialdehyde (MDA) content were significantly increased, while lung superoxide dismutase (SOD) activity, reduced glutathione (GSH) levels, and serum and lung catalase activities were significantly decreased. These changes reflect significant pulmonary inflammation with concomitant disturbance of oxidant/antioxidant homeostasis. Moreover, tumor necrosis factor (TNF)- α , interleukin (IL)-4, and IL-13 contents in the lung were also significantly high after OVA sensitization. Crocin treatment significantly alleviated the OVA-induced allergic asthma-associated alterations in inflammatory and oxidative stress biomarkers. Crocin enhanced anti-oxidant defenses, reduced the incidence of oxidative stress, and restored pro-inflammatory cytokines to normal levels. Histopathological analysis showed significant lung improvement in crocin-treated mice. In conclusion, crocin showed a significant protective effect against allergic asthma progression, which was associated with down-regulation of inflammatory cytokine expression and restoration of oxidant/antioxidant homeostasis.

1. Introduction

The lungs are a pair of spongy, air-filled structures located on both sides of the chest. Inhaled air is conducted through the trachea down to the lungs and through its tubular branches, the bronchi and bronchioles, finally to microscopic air sacs called alveoli. Within the lungs, pulmonary macrophages, neutrophils, and eosinophils are the first line of defense against any form of tissue injury [1].

Allergic asthma is a progressive, chronic, pulmonary inflammatory disorder with a prevalent T helper (Th) 2-mediated immune reaction to inhaled allergens. It is usually characterized by bronchial hyper-reactivity and recruitment of various types of inflammatory cells, particularly mast cells, eosinophils, and lymphocytes, with hyperplasia of both smooth muscle and goblet cells and elevated serum IgE levels [2]. Several cytokines, including IL-4, IL-5, and IL-13 have been reported to contribute to both the initiation and challenge phases of an asthmatic

attack [3].

Understanding the mechanisms by which allergen challenge modifies immune and tissue responses can contribute to the development of new effective therapies with high safety profiles and wide therapeutic indices, which may offer an attractive treatment approach for the millions of asthmatic patients worldwide [4].

Crocini is a hydrophilic carotenoid that constitutes 6–16% of the total dry matter of saffron. It is a diester of the disaccharide gentiobiose and the dicarboxylic acid crocetin. Crocin 1 is the most abundant water soluble crocin derivative, due to the presence of sugar moieties [5]. Historically, saffron was widely used to promote health and fight disease [6]. In traditional medicine, saffron has been used as diaphoretic, eupeptic, expectorant, tranquilizer, aphrodisiac, as emmenagogue, and abortifacient. It has also been reported to be effective for the treatment of hepatic disorders, vomiting, spasms, flatulence, gingival and dental pain, insomnia, cognitive disorders, seizures, depression, lumbago,

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cold, asthma, cough, bronchitis, fever, cancer, and cardiovascular disorders [7].

This study was conducted to evaluate the potential beneficial effects of crocin against progression of allergic asthma and its associated pathological and biochemical consequences in an experimental model of ovalbumin (OVA)-induced allergic asthma in mice.

2. Materials and methods

2.1. Animals

Forty adult male Swiss Albino mice (obtained from the Egyptian Organization for Biological Products and Vaccines [VACSERA], Giza, Egypt), weighing 20–25 g, were used in this study. The animals were housed under standard environmental conditions and fed a standard diet during the entire study. All animal handling and experimentation were conducted in compliance with the ethical guidelines for laboratory animal experimentation adopted by the Research Ethics Committee of the Faculty of Pharmacy, Mansoura University, Egypt.

2.2. Drugs and chemicals

Crocin, as an orange fine powder, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was dissolved in 0.5% carboxy methyl cellulose (CMC) for oral administration and was prepared immediately before use. OVA, as a cream-colored white powder, was obtained from Loba Chemie PVT. Ltd. (Bombay, India).

2.3. Experimental design and treatment

Allergic airway asthma was induced as described by Hsu et al. [8]. Briefly, mice were sensitized by an intraperitoneal (IP) injection of 10 mg of OVA mixed with 1 mg of aluminum hydroxide on days 0 and 7 of the experiment, and the mixture was vortexed prior to injection to ensure uniform sampling. Animals were randomly allocated into four experimental groups (10 mice/group): the normal control, which was administered 0.2 mL of (0.9% w/v) NaCl IP on days 0 and 7 and 0.5% CMC (5 mL/kg, orally) daily for 16 days (days 0–16); the crocin control group, which was administered 0.2 mL of (0.9% w/v) NaCl IP on days 0 and 7 and crocin (25 mg/kg, orally) daily for 16 days; the allergic airway control group, which was sensitized with OVA as described above and was administered 0.5% CMC (5 mL/kg, orally) daily for 16 days; and the crocin-treated group, which was sensitized with OVA as described above and was administered crocin (25 mg/kg, orally) daily for 16 days, starting from day 0 [9].

Mice in all experimental groups, except the normal and crocin control groups, were challenged by exposure to 1% OVA in normal saline using an ultrasonic nebulizer. A large exposure box system was used, in which all the mice in the same experimental group were exposed to nebulized OVA (1%), with an adjusted flow rate for 1 h per day on days 14–16 [10,11], whereas the normal and crocin control groups were exposed to a normal saline aerosol.

The crocin control group showed no significant differences compared to the normal control group; thus, the data from the crocin control group were omitted to simplify the presentation of the results.

2.4. Specimen collection

Mice were sacrificed 24 h after the last OVA challenge, and blood samples were collected under deep sodium thiopental (40 mg/kg) anesthesia via the retro-orbital plexus. Collected blood samples were allowed to coagulate for 20 min, and then sera were separated by centrifugation (765 g for 15 min). Prepared serum samples were used immediately for biochemical assessments.

2.5. Bronchoalveolar lavage fluid (BALF) preparation and quantification of total and differential inflammatory cells

After blood samples were collected, the thoracic cavity was opened, and the upper segments of the trachea were exposed and cannulated. Ice-cold saline (0.5 mL) was infused and then extracted twice to collect BALF. The mean total retrieval volume was approximately 0.70 ± 0.30 mL per mouse. The BALF was centrifuged (340 g, 4 °C, 10 min) [12], and the cell pellets were resuspended in 200 μ L of fresh saline. Total and differential inflammatory cells were quantified using a hemocytometer by the trypan blue exclusion method as described by Said et al. [13]. Lymphocytes, neutrophils, eosinophils, and monocytes were quantified using cytospin smears stained with DiffQuick [14]. After BALF collection, the lungs were harvested, rinsed in ice-cold saline, and weighed to calculate the lung/body weight index.

2.6. Lung homogenate preparation (10% w/v) and biochemical assessment of lung malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, reduced glutathione (GSH) content, catalase activity, and total protein content

Tissue samples (500 mg) from the isolated right lobes of the lungs were homogenized by sonication in 5 mL of ice-cold KCl [15]. Malondialdehyde (MDA) content [16], superoxide dismutase (SOD) activity [17], reduced glutathione (GSH) concentration [18], and catalase activity [19] in lung homogenates were determined using Biodiagnostic assay kits (Giza, Egypt) according to the manufacturer's instructions. The BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA) was used for quantitative assessment of total lung protein content.

2.7. Biochemical assessment of serum LDH and catalase activities

Serum LDH and catalase activities were determined using commercially available Biodiagnostic assay kits (Giza, Egypt) according to the manufacturer's instructions and as described previously [20,19], respectively.

2.8. Biochemical assessment of tumor necrosis factor- α (TNF- α), interleukin-4 (IL-4), and IL-13 levels in the lungs

TNF- α , IL-13, and IL-4 levels in the lung were determined using commercially available ELISA assay kits (Bender MedSystems GmnH, Vienna, Austria) according to the manufacturer's instructions. The detection limits for mouse IL-4, IL-13, and TNF α were 2.0 pg/mL, 2.8 pg/mL, and 3.7 pg/mL, respectively.

2.9. Histopathology

The left lobes of isolated lungs were fixed with 10% buffered formalin, and tissue slides were prepared. The slides were stained with hematoxylin and eosin (H & E) to evaluate the histopathological alterations due to allergic asthma and the effects of crocin administration. Slides were examined randomly, and the pathologist was blinded to the experimental group.

Inflammation was scored semi-quantitatively as described by Zeldin et al. [21] and Elekes et al. [22] and based on the evaluation of perivascular edema, perivascular/peribronchial acute inflammation, and the numbers of macrophages/mononuclear cells in the alveolar spaces. The scores of the individual parameters were summed to yield a composite inflammatory score of 0–10.

2.10. Statistical analysis

Data are expressed as mean $\pm$ SEM. ANOVA followed by Tukey-Kramer's *post-hoc* test and Kruskal-Wallis followed by Dunn's *post-hoc* test were used for statistical comparisons between parametric and non-

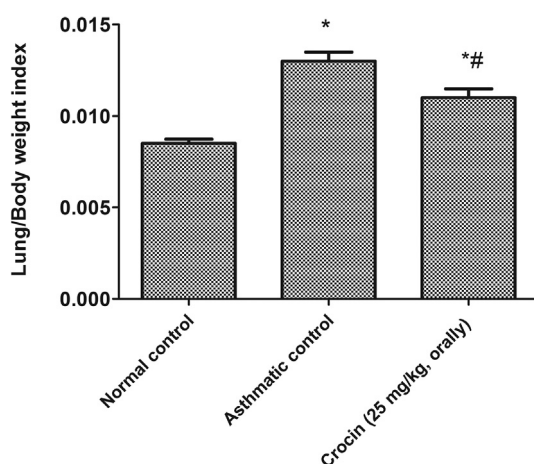


Fig. 1. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on the lung/body weight index in asthmatic mice.

Data are expressed as mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide, on days 0 and 7, and crocin was administered orally once daily from days 0 to 16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

*Significantly different vs. the normal control, $p < 0.05$.

#Significantly different vs. the asthmatic control, $p < 0.05$.

parametric data, respectively, using InStat-3 software (V2. 04; Graph Pad Software Inc., San Diego, CA, USA). P values < 0.05 were considered statistically significant.

3. Results

3.1. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on lung/body weight index in asthmatic mice

As shown in Fig. 1, OVA sensitization and repeated challenge induced a significant increase in the lung/body weight index when compared to that in normal control mice (by ~ 1.5 fold). Administration of oral crocin (25 mg/kg) once daily for 16 days significantly reduced the lung/body weight index by approximately 16% compared to that of the diseased asthmatic control.

3.2. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on the total and differential cell counts in BALF from asthmatic mice

As shown in Fig. 2, the total inflammatory cell count in asthmatic mice was significantly (~ 5 -fold) higher than that in normal control mice, and the numbers of eosinophils and neutrophils were ~ 12 -fold and ~ 6 -fold higher, respectively, in asthmatic mice. Administration of crocin (25 mg/kg, orally) once daily for 16 days led to significant decreases in the differential cell counts (monocytes, 59%; neutrophils, 63%; and eosinophils, 58%) compared to the numbers in asthmatic control mice (Fig. 2).

3.3. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on total protein content in the lung and serum LDH activity in asthmatic mice

As shown in Fig. 3, OVA sensitization induced a significant increase (~ 1.5 fold) in total protein content in the lung compared to that in normal control mice. Administration of crocin (25 mg/kg, orally) once daily for 16 days failed to significantly reduce total protein content compared to that in diseased asthmatic control mice. In contrast, OVA sensitization and repeated challenge induced a significant increase (~ 3 fold) in serum LDH activity when compared to the LDH activity in normal control mice (Fig. 4). Administration of crocin (25 mg/kg,

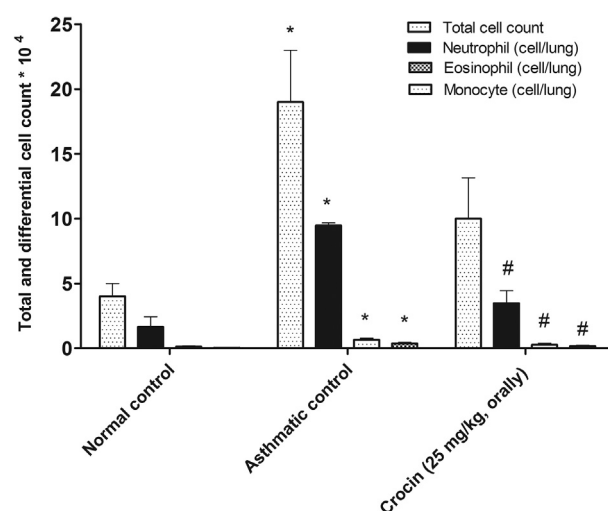


Fig. 2. Effect of daily administration of oral crocin (25 mg/kg) for 16 days on total and differential cell counts in bronchoalveolar lavage fluid (BALF) from asthmatic mice. Data are expressed as mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily from days 0 to 16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

*Significantly different vs. the normal control, $p < 0.05$.

#Significantly different vs. the asthmatic control, $p < 0.05$.

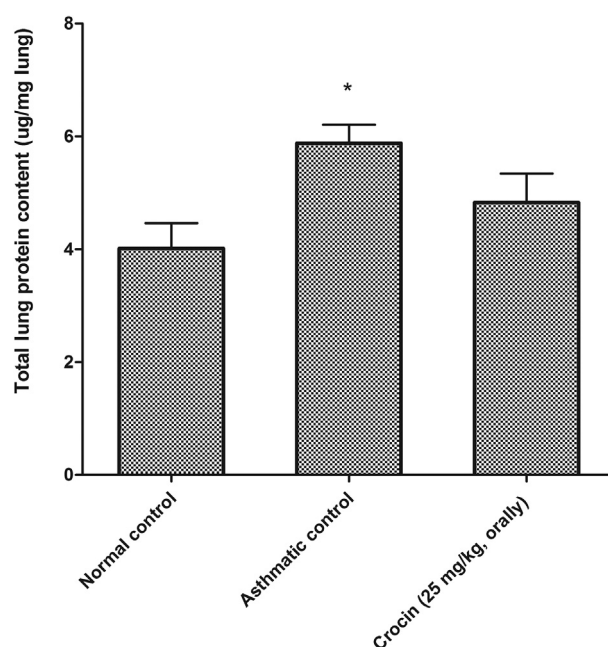


Fig. 3. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on total lung protein content in asthmatic mice.

Data are expressed as the mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily from days 0 to 16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

*Significantly different vs. the normal control, $p < 0.05$.

#Significantly different vs. the asthmatic control, $p < 0.05$.

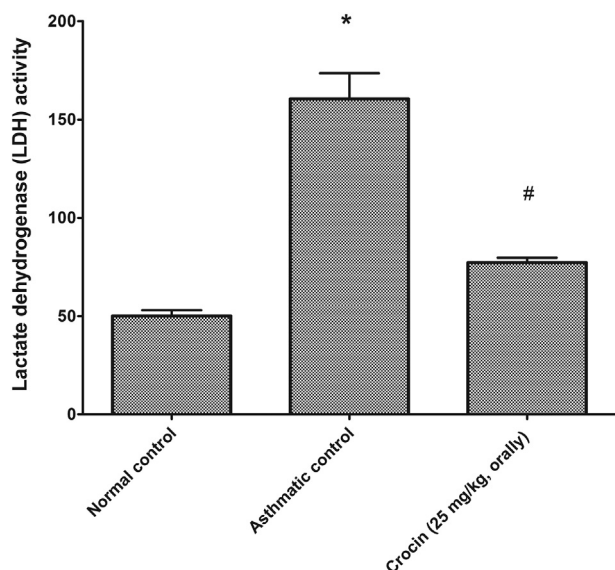


Fig. 4. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on serum LDH activity in asthmatic mice

Data are expressed as the mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily starting from day 0 to day 16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

*Significantly different vs. the normal control, $p < 0.05$.

#Significantly different vs. the asthmatic control, $p < 0.05$.

orally) once daily significantly decreased serum LDH activity by $\sim 52\%$ compared to the activity in diseased asthmatic control mice.

3.4. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on oxidative stress biomarkers: lung MDA content, SOD activity, and GSH concentration in asthmatic mice

As shown in Table 1, OVA sensitization and repeated challenge significantly increased lung MDA content by (~ 2.5 fold), with concomitant reductions in lung SOD activity and GSH concentration (by 3- and 2-fold, respectively) when compared to the levels in the normal control.

Administration of crocin (25 mg/kg, orally) once daily significantly reduced lung MDA content (by $\sim 59\%$) and significantly increased lung SOD activity and GSH levels (by $\sim 76.5\%$) and 28%, respectively, compared to the levels in the asthmatic control group. Moreover, MDA content in the lung was comparable to that in the normal control.

Table 1

Effect of daily oral administration of crocin (25 mg/kg) for 16 days on malondialdehyde (MDA) content and the oxidative stress biomarkers superoxide dismutase (SOD) and reduced glutathione (GSH) in the lungs of asthmatic mice.

Experimental group	MDA (nmol/mg)	SOD (U/g)	GSH (mg/mg)
Normal control	0.48 $\pm$ 0.031	44.230 $\pm$ 2.282	0.869 $\pm$ 0.074
Asthmatic control	1.15 $\pm$ 1.106*	12.019 $\pm$ 1.500*	0.543 $\pm$ 0.013*
Crocin (25 mg/kg, orally) + OVA (IP)	0.47 $\pm$ 0.046#	61.380 $\pm$ 1.978#	0.759 $\pm$ 0.016#

Data are expressed as mean $\pm$ SEM of 10 mice/group.

Allergic airway inflammation was induced by IP injection of 10 mg of OVA + 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily from day 0 to day 16.

Statistical analyses were performed using ANOVA followed by Tukey-Kramer multiple comparisons test.

* Significantly different vs. the normal control, $p < 0.05$.

Significantly different vs. the asthmatic control, $p < 0.05$.

Table 2

Effect of daily oral administration of crocin (25 mg/kg) for 16 days on serum and lung catalase activities in asthmatic mice.

Experimental group	Serum catalase (U/L)	Lung catalase (U/g)
Normal control	580.920 $\pm$ 18.268	567.237 $\pm$ 33.883
Asthmatic control	351.206 $\pm$ 37.384*	344.817 $\pm$ 48.279*
Crocin (25 mg/kg, orally) + OVA (IP)	676.394 $\pm$ 30.133#	860.684 $\pm$ 54.364#

Data are expressed as mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by I-P injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide, on days 0 and 7, and crocin was administered orally once daily starting from days 0 to 16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

* Significantly different vs. the normal control, $p < 0.05$.

Significantly different vs. the asthmatic control, $p < 0.05$.

Administration of crocin restored MDA and GSH levels in the lung to near normal levels, and lung SOD activity was increased beyond the level in the normal control (Table 1).

3.5. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on serum and lung catalase activities in asthmatic mice

As shown in Table 2, OVA sensitization significantly decreased serum and lung catalase activities when compared to those in normal control (by ~ 2 fold). Administration of crocin (25 mg/kg, orally) once daily significantly increased serum catalase activity (by about 48%) compared to the activity in the diseased control group. Moreover, administration of crocin (25 mg/kg, orally) once daily significantly increased lung catalase activity (by $\sim 60\%$) compared to the activity in the asthmatic control; crocin administration also elicited a significant increase in lung catalase activity (by $\sim 50\%$) compared to the activity in the normal control group (Table 2).

3.6. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on TNF- α levels in asthmatic mice

As shown in Table 3, TNF- α levels in the lungs of mice in the asthmatic control group were significantly higher (~ 4 fold) than those in mice of the normal control group. Administration of crocin (25 mg/kg, orally) significantly reduced lung TNF- α levels (by $\sim 67\%$) when compared to those in asthmatic control mice, and the TNF- α levels in the lungs of treated mice were comparable to those in the normal control group.

Table 3

Effect of daily oral administration of crocin (25 mg/kg) for 16 days on lung tumor necrosis factor- α (TNF- α) content, interleukin-13 (IL-13) and IL-4 levels in asthmatic mice.

Experimental group	TNF- α (pg/mL)	IL-13 (pg/mL)	IL-4 (pg/mL)
Normal control	15.76 $\pm$ 1.257	17.115 $\pm$ 0.662	2.868 $\pm$ 0.324
Asthmatic control	60.66 $\pm$ 4.078*	68.629 $\pm$ 5.479*	8.066 $\pm$ 0.739*
Crocin (25 mg/kg, orally) + OVA (IP)	20.28 $\pm$ 1.978#	21.831 $\pm$ 2.568#	3.634 $\pm$ 0.565#

Data are expressed as mean $\pm$ SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily on days 0–16.

Statistical analysis was performed using ANOVA followed by Tukey-Kramer's multiple comparisons test.

* Significantly different vs. the normal control, $p < 0.05$.

Significantly different vs. the asthmatic control, $p < 0.05$.

3.7. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on lung IL-4 levels in asthmatic mice

OVA sensitization and repeated challenge significantly increased lung IL-4 levels (~3 fold) when compared to those in normal control mice (Table 3). Administration of crocin (25 mg/kg, orally) once daily for 16 days led to a significant reduction in lung IL-4 levels (by ~55%) compared to the levels in the asthmatic control, and IL-4 levels in the lungs of crocin-treated mice were comparable to those in normal control mice.

3.8. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on lung IL-13 levels in asthmatic mice

IL-13 levels in the lungs of mice in the asthmatic control group were significantly higher than those in the lungs of mice in the normal control group (by ~4 fold). Administration of crocin (25 mg/kg) for 16 days significantly reduced lung IL-13 levels (by ~68%) compared to those in the asthmatic control group, and IL-13 levels in the lungs of crocin-treated mice were comparable to those observed in the normal control group (Table 3).

3.9. Effect of daily oral administration of crocin (25 mg/kg) for 16 days on lung histopathology

Histopathological examination of hematoxylin and eosin (H&E)-stained lung sections from the normal control group revealed normal lung architecture, without any evidence of perivascular edema or peribronchial inflammation (Fig. 5A), whereas sections from asthmatic control mice showed a significant increase (by 100%) in perivascular edema, peribronchial infiltration, and macrophages in the alveolar space when compared to that of the normal control (Table 4; Fig. 5B). Examination of lung specimens in the asthmatic control group revealed a severe inflammatory reaction, with perivascular edema affecting > 75% of the perivascular circumference, and peribronchial mononuclear infiltration. Moreover, the alveolar spaces were congested with alveolar macrophages and inflammatory cells, which filled > 25% of the alveolar space (Fig. 5B). In contrast, daily oral crocin administration induced a significant decrease in perivascular edema (by 30%), decrease in peribronchial inflammation (50%) and macrophage infiltration in the alveolar space (43%) compared to the levels in the asthmatic control (Table 4; Fig. 5C, D, E).

4. Discussion

Allergic asthma is considered one of the most serious respiratory disorders. Given its increased prevalence worldwide, asthma management is of serious concern. The results of the current study highlight the protective and immunomodulatory effect of crocin in the pathogenesis of asthma.

OVA sensitization and subsequent challenge significantly impaired pulmonary function as shown by the biochemical and histopathological parameters evaluated in the current study. The production of active metabolites and the generation of reactive free radicals are thought to contribute to OVA-induced pulmonary dysfunction. This was reflected in the current study as seen with the marked negative shift in several biomarkers and parameters under investigation.

OVA exposure has been reported to induce the production of highly reactive hydroxyl radicals, stimulate lipid peroxidation, and cause cellular damage. This effect may be mediated in part by increased membrane permeability to several ions, such as Ca^{+2} . MDA is a stable metabolite produced by lipid peroxidation. In the current study, MDA contents in the lung were significantly increased after OVA sensitization and challenge. In agreement with this result, lung MDA levels have been reported to increase significantly following OVA sensitization [23].

In the current study, the lung/body weight index, total lung protein content, and BALF cell counts significantly increased after OVA challenge, indicating the onset of pulmonary edema and increased leakage of serous fluids into the lung tissue. Lung histopathological examination supported these conclusions. These observations are in agreement with the observations by Klinger et al. and Zhang et al. [24,25].

Serum LDH activity and lung TNF- α , IL-4, and IL-13 levels also significantly increased, along with significant decrease in lung SOD activity, GSH concentration, and serum and lung catalase activity, reflecting significant pulmonary inflammation with a disturbance in oxidant/antioxidant homeostasis.

The antioxidant enzymes SOD and catalase and the free radical scavenger GSH play major roles in maintaining cellular redox homeostasis [26]. A decrease in SOD and catalase activities reduces cellular removal of hydrogen peroxide (H_2O_2) and superoxide ion (O_2^-) radicals [27].

Saffron and its ingredients have been reported to be useful remedies against several disorders, such as neurodegenerative disorders and related memory impairment, blood pressure abnormalities, coronary artery diseases, age-related macular degeneration and/or ischemic retinopathy, acute and/or chronic inflammatory diseases, Parkinsonism, and seizures and depression. Furthermore, saffron and its ingredients have also been shown to have tumoricidal, anti-genotoxic, anti-mutagenic, and antioxidant activities [28].

Toxicological studies have confirmed the wide safety margin and high therapeutic index of crocin [29]. Both acute and sub-acute toxicities have been evaluated in rodents. At pharmacological doses, no marked damage to major organs of the body was reported. Even following administration of high doses, up to 3 g/kg (either IP or orally), no mortality was reported after 24 and 48 h [9]. According to Loomis and Hayes [51], chemicals with LD50 in the range of 1–5 g/kg are considered to have low toxicity.

In the current study, administration of crocin (25 mg/kg) orally for 16 days significantly reduced lung MDA content, indicating decreased lipid peroxidation secondary to decrease ROS generation. This reduction in MDA highlights the role of crocin as an antioxidant that modulates the lung damage associated with the progression of allergic asthma. Stabilization of bio-membranes, ROS scavenging, and decreased peroxidation of unsaturated lipids have been reported to be among the mechanisms underlying the antioxidant properties of saffron and its components [30,31].

In the present study, administration of crocin also induced a significant increase in lung GSH content and SOD activity, along with recovery of lung and serum catalase activities, suggesting that crocin plays an important role in the modulation of anti-oxidative response. Such observations are in agreement with those by Asdag and Inamdar [40]. In two independent studies, Ochiai et al. [41] and Saleem et al. [42] reported the protective effects of saffron and its carotenoid constituents against *in vivo* and *in vitro* neuronal cell injuries, respectively. Hosseinzadeh et al. [43] reported that saffron extract and crocin have renoprotective effects against ischemia reperfusion-induced oxidative stress.

Parallel to the improvement in oxidant/antioxidant balance, there was significant attenuation of serum LDH activity, suggesting the cytoprotective effect of crocin, which is in agreement with the findings of Dianat et al. [44], who observed that administration of oral crocin significantly decreased LDH activity in heart tissue, and conferred a protective effect on myocardium cell membrane integrity.

Inhibition of inflammatory cell recruitment within the airways of OVA-challenged mice after crocin treatment was clearly observed in the current study, and these observations are validated by earlier studies reporting that saffron extract prevents allergic inflammation [32].

The significant reduction in lung TNF- α content observed in the current study may account for the anti-inflammatory and immune-modulating effects of crocin. The protective effect of crocin against OVA-induced asthmatic changes was confirmed by the concomitant

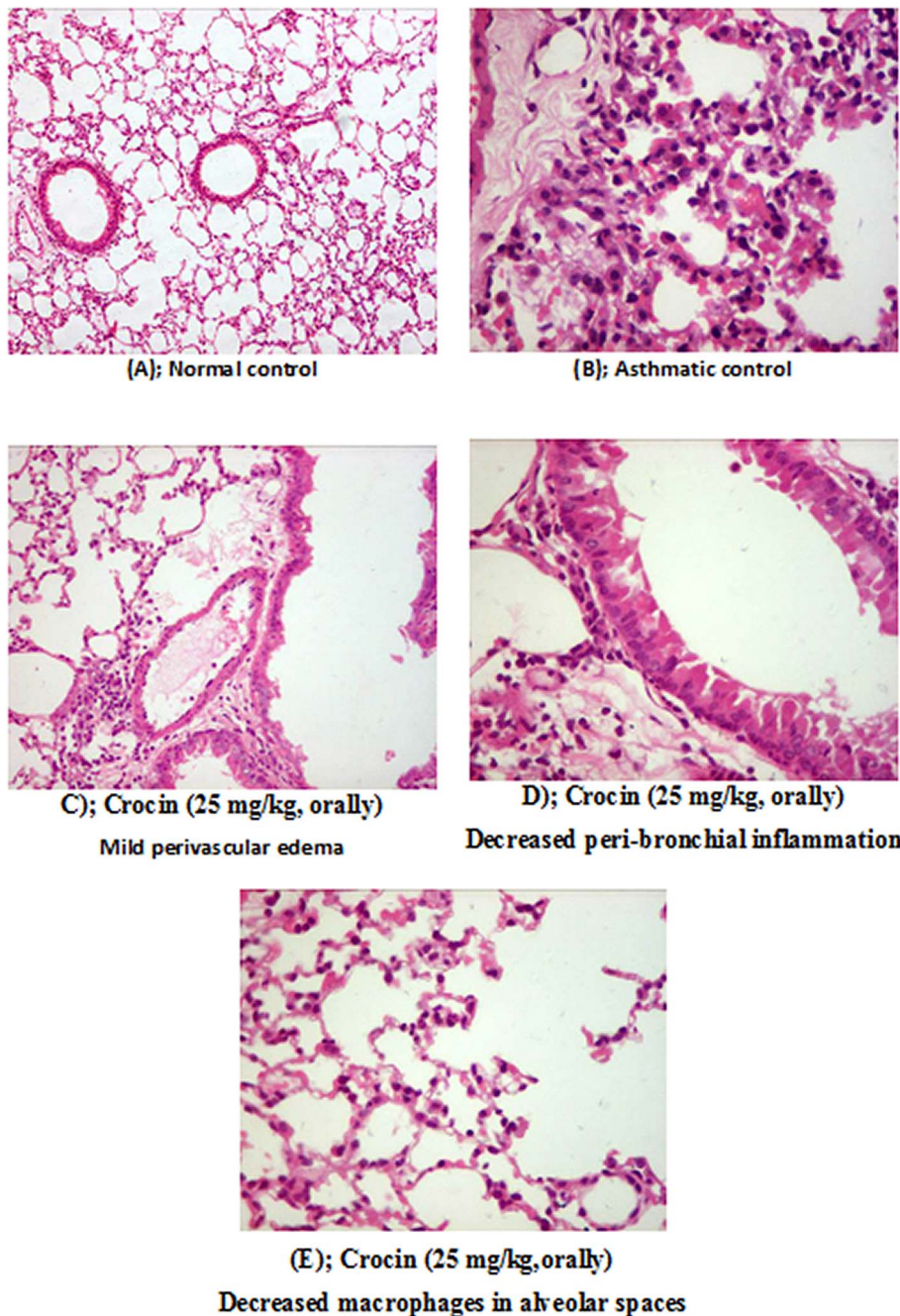


Fig. 5. H & E-stained lung specimens isolated from (A) the normal control group (100 ×); (B) diseased asthmatic control, showing perivascular edema in 75% of the perivascular circumference, peribronchial inflammation (> 5/hpf), and alveolar macrophages and inflammatory cells filling > 25% of the alveolar spaces; (C) crocin-treated group, showing mild perivascular edema in < 25% of perivascular space (100 ×); (D) crocin-treated group, showing a significant decrease in the number of perivascular and peribronchial inflammatory cells (< 5/hpf; 400 ×); (E) crocin-treated group, showing a significant reduction in the numbers of macrophages and inflammatory cells in < 25% of the alveolar spaces (400 ×).

observation of attenuated inflammatory cell recruitment within the lung and BALF. The lung histopathology showed significant improvement following administration of crocin, as shown by the significant decrease in perivascular edema, peribronchial inflammation, and macrophage infiltration in the alveolar space.

The role of TNF- α in the initiation of inflammation and its contribution to the extent of tissue injury have been described by Giebelen et al. [45]. TNF- α induces neutrophil adherence to the pulmonary capillaries and extravasation into the alveolar space [46]. Moreover, TNF- α -induced sensitization of infiltrating leukocytes has been reported to increase oxidant production, thus augmenting oxidative stress [47]. Neutrophils and macrophages convert molecular oxygen to ROS [48], contributing to local lung damage and the buildup of edematous fluid in the airspaces [49,50], which may explain the observed increase in lung/body weight index in asthmatic control mice in the present study.

Increased production of IL-4 and IL-13 is known to be essential in

pathogenesis of allergic asthma [33]. IL-4 and IL-13 are released by mast cells, lymphocytes, and eosinophils, along with a wide array of other cytokines [34], and are reported to be crucial for switching B lymphocytes to IgE production [35]. In addition, IL-4R α subunit signaling has been proven to be essential for enhanced bronchial reactivity in murine models of asthma [36,37].

IL-13 has also been reported to stimulate the activation of signal transducer and activator of transcription 6 (STAT6) through IL-4-mediated signal transduction, leading to airway hyper-responsiveness (AHR) in the allergic lung. A temporal relationship appears to exist between either IL-4 or IL-13 and the development of AHR. Blockade of IL-13 signaling, but not IL-4 signaling before aeroallergen exposure has been reported to be sufficient to attenuate AHR [37,38]. As IL-4 is a key regulator of Th2 phenotype development during sensitization [39], AHR during allergen inhalation appears to be primarily dependent on IL-13 [37,38].

Table 4

Effect of daily oral administration of crocin (25 mg/kg) for 16 days on perivascular edema, peribronchial inflammation, the numbers of macrophages and mononuclear cells in the alveolar space of asthmatic mice.

Experimental groups	Perivascular edema	Peribronchial inflammation	Macrophages and mononuclear cells in the alveolar space
Normal control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Asthmatic control	1.7 ± 0.30*	1.5 ± 0.10*	2.0 ± 0.00*
Crocine (25 mg/kg, orally) + OVA (IP)	0.5 ± 0.129 [#]	0.75 ± 0.095 [#]	0.85 ± 0.095 [#]

Data are expressed as mean ± SEM of 10 mice per group.

Allergic airway inflammation was induced by IP injection of 10 mg of ovalbumin and 1 mg of aluminum hydroxide on days 0 and 7, and crocin was administered orally once daily from days 0 to 16.

Statistical analysis was performed using Kruskal-Wallis test followed by Dunn's *post-hoc* test.

* Significantly different vs. the normal control, $p < 0.05$.

[#] Significantly different vs. the asthmatic control, $p < 0.05$.

Indeed, in the current study, allergic airway induction was associated with significant increase in lung IL-4 and IL-13 levels, mediating immune response stimulation and airway hyperactivity, respectively. Administration of crocin significantly reduced lung IL-4 and IL-13 levels and improved markers of oxidative stress and inflammatory as well as the histopathological changes.

In conclusion, crocin treatment conferred significant pulmonary and lung protective effects against OVA-induced allergic asthmatic damage. These results implicate the free radical scavenging impact of crocin, and its ability to decrease in their harmful effects on pulmonary and lung tissues, to boost antioxidant defenses, to attenuate TNF- α activity, and to modulate IL-4 and IL-13 signaling in the observed therapeutic and protective effects of crocin.

Conflicts of interest

None.

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