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Original Article

The potential effect of the extract of *Crocus sativus* and safranal on the total and differential white blood cells of ovalbumin-sensitized guinea pigs

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Abstract

Previous studies have indicated relaxant, inhibitory effect on histamine (H1) and muscarinic receptors, and stimulatory effect on β-drenoceptor of *Crocus sativus* on guinea pig tracheal chains. In the present study, the effect of the extract of *C. sativus* and one of its constituents, safranal, on the inflammatory changes of sensitized guinea pigs was examined. Eight groups of sensitized guinea pigs to ovalbumin were studied. One group was given drinking water alone (group S), while other 7 groups were received drinking water containing; three concentrations of safranal (4, 8 and 16 μg/ml), three concentrations of extract (0.1, 0.2 and 0.4 mg/ml) and one concentration of dexamethasone (S+D group), (six animal in each group). Total and differential white blood cell (WBC) counts in blood were evaluated. Total blood WBC number, eosinophyl and lymphocyte percentage in blood were increased, but neutrophil decreased in sensitized animals compared to those of control groups (*P*<0.05 to *P*<0.001). Treatment of animals with dexamethasone, all concentrations of the extract and safranal significantly improved most types of WBCs but total WBC number was only decreased in treated groups with dexamethasone and high concentration of the extract compared to group S (*P*<0.05 to *P*<0.001). Safranal was more effective in the improvement of eosinophil and lymphocyte compared to the extracts (*P*<0.001 for both cases). However, the preventive effect of the extract of *C. sativus* on total WBC count was more prominent than that of the safranal (*P*<0.01). These results showed a preventive effect of the extract of *C. sativus* and its constituent safranal on total and differential count of WBC in blood of sensitized guinea pigs. The results also suggest that the effect of the plant is perhaps due to its constituent of safranal.

Keywords: *Crocus sativus;* Safranal; Asthma; Sensitization; Inflammation; WBC

INTRODUCTION

The main pathological characteristic feature of asthma is airway inflammation (1). Many inflammatory cells are involved in the pathogenesis of airway inflammation in asthma (2). These cells overproduce reactive oxygen species (superoxides, hydrogen peroxide and hypohalites, etc.) which lead to airway inflammation (2). Increased total WBC and eosinophil count in sensitized animals (3) and asthmatic patients (4) were also shown. Mainly two types of drugs including bronchodilators and anti-inflammatory or preventive drugs are used for the treatment of asthma. However, there is no definite treatment for this common disease.

Crocus sativus L, known as saffron, is a small perennial plant from the iris family (Iridaceae) which is cultivated in many regions of the globe mainly in Iran. Crocins, safranal, picrocrocin, ketoisophorone, isophorone, glycosidic terpenoids and crocetin are constituents of the stigma of the plant (5). The central part of the flower (female sexual organ) or stigma of *C. sativus* (Saffron) is used as antispasmodic and expectorant in the traditional medicine (6,7).

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Anti-inflammatory (8), radical scavenging, antioxidant properties (9,10) and anti-tumour effects (11) have been previously shown for *C. sativus*. Apoptotic and mutagenic effects have also been demonstrated for saffron and crocin (12). Chemopreventive and genoprotective effects of the plant have been also described which could protect the mice from genotoxin-induced oxidative stress (13). The anti-tumor activity of saffron and its constituents were also shown in various studies (14-17).

Several pharmacological effects including anticonvulsant (17-19) anxiolytic and hypnotic activities (20), antioxidant and chemo-pereventive properties (21,22) and anticancer effect (23) have been shown for safranal. The antitussive effect of *C. sativus* stigma and its components, safranal and crocin have also been demonstrated (24).

The relaxant effect of saffron on tracheal smooth muscle (25), inhibitory effect of the plant on histamine (H_1) receptor (26) and its stimulatory effect on β-adrenoceptors (27) have been also shown in our previous studies. The results of these studies may indicate the bronchodilatory property of the plant. The effect of saffron on Th1/Th2 balance was also documented (28). With regard to the results of this study and other studies indicating antioxidant effect (9,10), this plant may have anti-inflammatory or preventive effect on some inflammatory diseases such as asthma.

Therefore, in the present study, the effect of the extract of *C. sativus* and its constituent, safranal, on total and differential WBC count in blood as an indicator of inflammation, was examined in sensitized guinea pigs .

MATERIALS AND METHODS

Plant and extract

C. sativus was collected from Torbat Heydarieh (northeast Iran) in November 2010, and its stigma was dried at room temperature in the absence of sunlight. The plant was identified by botanists in the herbarium of Ferdowsi University of Mashhad; the specimen number of the plant is 293-0303-1. The hydro-ethanolic extract was prepared as follows: three grams of chopped *C. sativus* stigma were mixed with 50 ml ethanol 70% for 72 h at room temperature and the solution was separated by maceration method. This process was repeated for three times. The solutions were dried in room temperature, stored in -4°C and away from light.

Animal sensitization and animal groups

Sensitization of animals to ovalbumin (OA) was performed using the method described previously (29). Briefly, adult Dunkin-Hartley guinea pigs (400-700 g, both sexes) were sensitized to OA (Sigma Chemical Ltd, UK) by injecting 100 mg i.p. and 100 mg s.c. on the day one and a further 10 mg i.p. on the day 8. From day 14 sensitized animals were exposed to an aerosol of 4% OA for 18 ± 1 days, 4 min daily. The aerosol was administered in a closed chamber in the dimensions of 30 × 20 × 20 cm using a nebulizer (CX3, Omron Healthcare Europe B.V., Netherlands). Control animals were treated similarly but saline was used instead of OA solution. The study was approved by the ethical committee of the Mashhad University of Medical Sciences. Animals were housed in individual cages with access to food and water *ad libitum* and were maintained at 22 ± 2ºC on a 12 h light/dark cycle (light period 0700 and 1900 h).

The study was performed on control groups (group C, treated the same as sensitized group but normal saline was used instead of OA, and the animals were given drinking water alone) and eight different groups of sensitized animals (6 animal in each group) which were given drinking water alone or drinking water containing dexamethasone, saffron extract, safranal (dissolved in water using few drops of ethanol) during sensitization period as follows:

1) Drinking water alone (group S, an animal model of asthma)
2) 5 µg/ml dexamethasone (group S+D)
3) 0.1 mg/ml extract
4) 0.2 mg/ml extract
5) 0.4 mg/ml extract
6) 4 µg/ml safranal
7) 8 µg/ml safranal
8) 16 µg/ml safranal

White blood cells count

A two ml blood sample was taken by cardiac puncture immediately after sacrificing

and exposing the animals' chest, and was collected into the test tube containing anticoagulant EDTA. The blood sample was stained with Turk solution (1:10 dilution) and total white blood cell (WBC) counted in duplicate in a hemocytometer (in a Burker chamber). The Turk solution consisted of 1 ml of glacial acetic acid, 1 ml of gentian violet solution 1 % and 100 ml distilled water.

Differential cell counts were done on a thin slide, prepared with a smearing blood sample, using Wright-Giemsa's stain. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 100 cells, and the percentage of each cell type was calculated.

Statistical analysis

The data of total and differential WBC count were presented as mean ± SEM. According to the Kolmogorov Smirnov test these data showed normal distribution. The data of sensitized group were compared with those of control guinea pigs using unpaired *t* test. Comparison of the data between sensitized groups and each treated group was also done using unpaired *t* test. The data of three groups of animals treated with the extract or safranal were compared using unpaired one way ANOVA with Tukey Kramer post hoc test. In addition, the comparison between the data of animals treated with each concentration of the extract and that of safranal was performed using unpaired *t* test. Significance was accepted at $P<0.05$.

RESULTS

Total and differential WBC count

Total WBC count, the percentage of eosinophil and lymphocyte in blood of group S were significantly higher, but the percentage of neutrophil was less than those of group C ($P<0.05$ to $P<0.001$) (Fig. 1 and 2). The treatment of sensitized animals with dexamethasone and all concentrations of the extract and safranal caused a significant reduction in eosinophil and monocyte but it led to a significant increase in the percentage of neutrophil ($P<0.05$ to $P<0.001$), (Fig. 2). In sensitized animals treated with dexamethasone and high concentration of the extract, total WBC and lymphocyte count were significantly decreased compared to sensitized group ($P<0.05$ for all cases), (Fig. 1 and 2). The treatment of sensitized animals with middle concentration of safranal (8 μg/ml) also led to a significant reduction in the percentage of lymphocyte ($P<0.05$, Fig. 2 B).

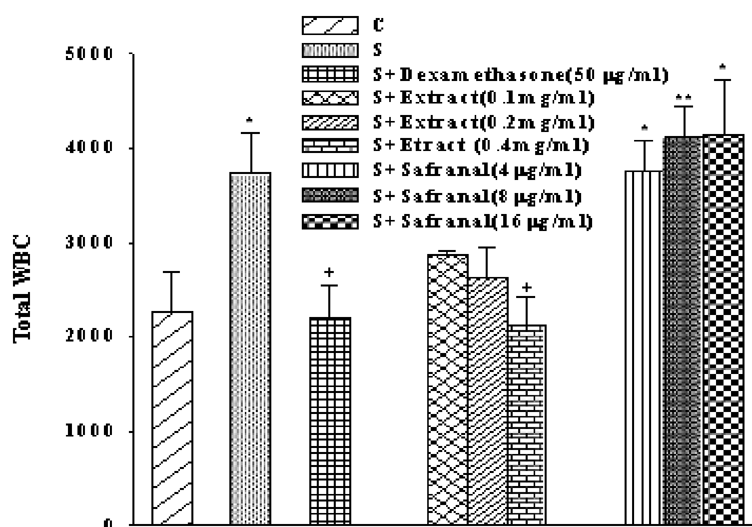

Fig. 1. Mean ± SEM values of total blood WBC number in control (C), sensitized (S), S treated with dexamethasone (S+D), S treated with three concentrations of the extract (S+Extract), and three concentrations of safranal (S+Safranal), (for each group, n=6). Statistical differences between control *and* sensitized group: *: $P<0.05$, **: $P<0.01$. Statistical differences between sensitized and treated groups: +; $P<0.05$.

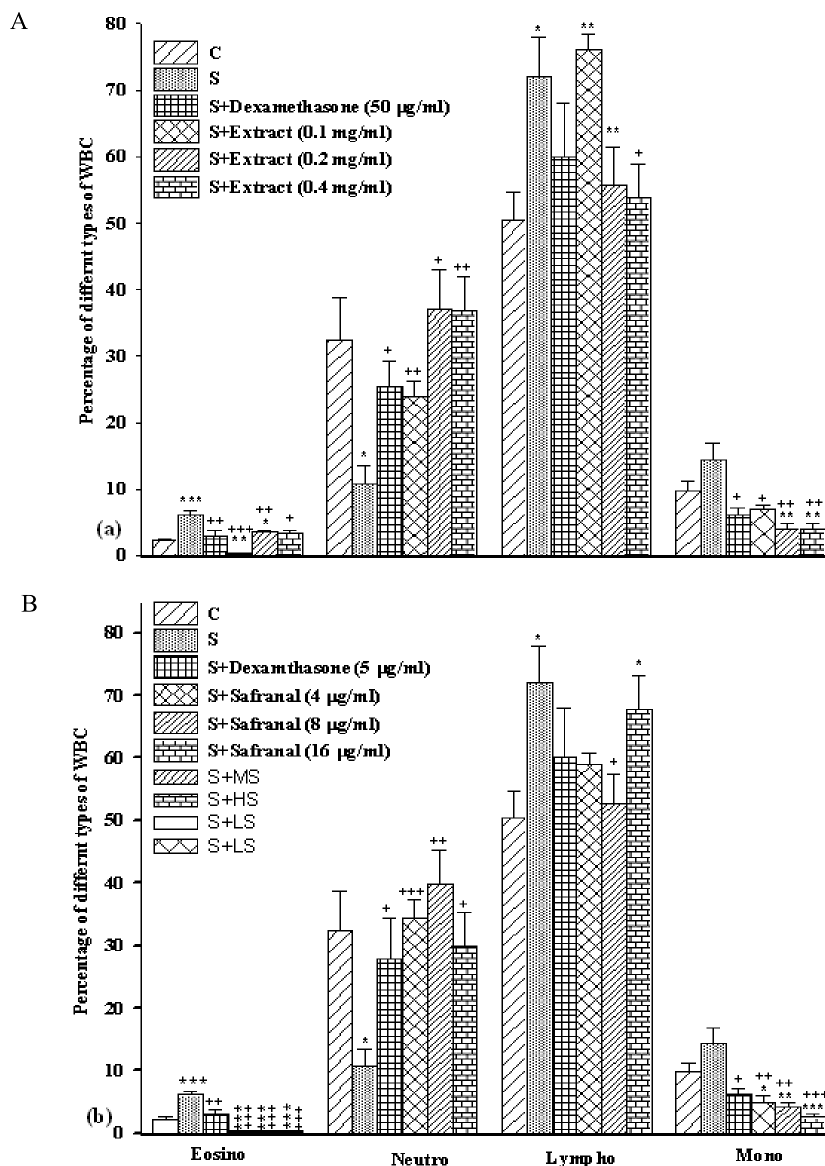

Fig. 2. Precentage (mean ± SEM values) of differential WBC count in control (C), sensitized (S), S treated with dexamethasone (S+D), S treated with three concentrations of the extract (S+Extract), (A) and three concentrations of safranal (S+Safranal), (B), (for each group, n=6). Statistical differences between control *vs* sensitized group: *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. Statistical differences between sensitized and treated groups: +; $P<0.05$, ++; $P<0.01$, +++; $P<0.001$. The percent of eosinophil in the treatment groups with low concentration of the extract and all concentrations of safranal was 0.00±0.00.

Differences in total and differential WBC count between three concentrations of the extract and safranal

The effects of two higher concentrations of the extract (0.2 and 0.4 mg/ml) on lymphocyte and monocyte were significantly greater than its low concentration (0.1 mg/ml), ($P<0.05$ for all cases). However, the effect of the low concentration of the extract on eosinophil was greater than the effect of two higher concentrations ($P<0.001$). There was no significant difference amongst the effects of three concentrations of safranal.

Differences in different parameters among the extract, safranal and dexamethasone

The effect of two higher concentrations of extract on total WBC number was significantly higher than that of the safranal ($P<0.01$ for middle and $P<0.05$ for high concentration). However, the effect of two higher concentrations of safranal on eosinophil and its

low concentration on lymphocyte count were significantly higher than that of the extract ($P<0.001$).

The effect of dexamethasone treatment on total WBC number was significantly higher than all concentrations of safranal, and its effect on lymphocyte count was significantly higher than low concentration of the extract ($P<0.01$ for all cases). However, the effect of dexamethasone treatment was lower than the effect of all concentrations of safranal and low concentration of the extract on eosinophil, and also than the effect of high concentration of safranal on monocyte count ($P<0.01$ for all cases).

DISCUSSION

The preventive effect of long term administration of the extract of *C. sativus* and one of its constituents, safranal, on total and differential WBC count in blood of sensitized guinea pigs was examined in the present study. The results showed increased total WBC, eosinophil and lymphocyte, but reduction of neutrophil count in sensitized compared to control animal. The increased total WBC and eosinophil count in sensitized animals (3) and asthmatic patients (4) are documented well enough to support the sensitization of animals. Our previous studies also showed increased total WBC and eosinophil but decreased neutrophil count in sensitized guinea pigs (30,31).

The treatment of sensitized animals with both the extract and safranal prevented increased eosinophil and lymphocyte but increased neutrophil count of sensitized guinea pigs. However, total WBC number was only decreased in the groups treated with the extract and dexamethasone.

Airway inflammation is the most characteristic feature of asthma (1). Increased eosinophil can release a variety of preformed mediators including Eosinophil Cationic Protein, Major Basic Protein, (which are both cytotoxic to the respiratory epithelium and could therefore account for the denudation of epithelium seen in asthma (32)), Eosinophil Derived Neurotoxin, Eosinophil Peroxidase, superoxide ion and lipoxin A. Eosinophils have the capacity to produce newly generated mediators such as leukotriene C_4/D_4 (33), PAF (34), PGE_2 and PGI_2. All the above mediators can in turn cause airway inflammation. Increased lymphocytes can also recognize antigen through specific receptors and thereby initiate an inflammatory response by releasing cytokines (35). Increased numbers of activated lymphocytes were found in bronchial biopsies from subjects with mild asthma in an ultrastructural morphologic study (35) which supports the findings of the present studies. Comparable effects of the extract of the plant and its constituent, safranal, with that of dexamethasone is another important evidence indicating the anti-inflammatory effect of *C. sativus* and its constituent. In fact the inhibitory effect of dexamethasone on airway inflammation and its effect on lymphocytes in asthmatic mice have been shown, which support the results of our study (36).

The reduction of eosinophil and lymphocyte in sensitized animals, treated with the extract of *C. sativus* and its constituent safranal seen in the present study, suggest their anti-inflammatory property. Therefore, the extract of this plant may have preventive effects on asthma disease by reduction of inflammatory cells and airway inflammation. In fact, the reduction of eosinophil and lymphocyte due to anti-inflammatory drug has been shown in our previous study (30) which support the anti-inflammatory action of the extract of the plant and it's constituent. Antioxidant properties of safranal (13,33,34) and anti-inflammatory and anti-oxidant effects of *C. Sativus* (8,10,12) have been shown previously. The inhibitory effect of safranal on histamine (H_1) receptor (26) and its antitussive effect (24) can contribute to its anti-inflammatory effect and could support the results of the present study.

The concentrations of the safranl used in the present study were 0.4 times of those of the extract (0.1, 0.2 and 0.4 mg/ml for the extract 4, 8 and 16 μg/ml for safranal). The HPLC results indicate that the extract of saffron contains only 0.26% safranal (37) which is lower than concentration of safranal used in the present study. These findings suggest that the preventive effect seen for the extract of

saffron on the total and specially on differential WBC count of sensitized guinea pigs is perhaps due to its constituent, safranal.

The anti inflammatory effect of several other plants such as *Rosmarinus officinalis, Platanus orientalis, Cydonia oblonga* was reported in previous studies (38-40). Therefore, the observed effect should exist in other plants and the effect of these plants on sensitized animals should be studied in future studies.

CONCLUSION

In conclusion, these results indicated the preventive effect of the extract of *C. sativus* and its constituent, safranal on WBC count in blood of sensitized guinea pigs which could indicate a prophylactic effect on inflammatory disorders such as asthma. The results also suggest that the effect of the plant on total and differential WBC count is at least, in part, due to its constituent, safranal.

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REFERENCES

1. Zhang WX, Li CC. Airway remodeling: a potential therapeutic target in asthma. World J Pediatr. 2011;7:124-128.
2. Kelly CA, Ward C, Stenton SC, Bird G, Hendrick DJ, Walters EH. Numbers and activity of cells obtained at bronchoalveolar lavage in asthma, and their relationship to airway responsiveness. Thorax. 1998;43:684-692.
3. Hogan MB, Weissman DN, Hubbs AF, Gibson LF, Piktel D, Landreth KS. Regulation of eosinophilopoiesis in a murine model of asthma. J Immunol. 2003;171;2644-2651.
4. Luksza AR, Jones DK. Comparison of whole-blood eosinophil counts in extrinsic asthmatics with acute and chronic asthma. British Med J. 1982;285:1229-1231.
5. Tarantilis PA, Tsoupras G, Polissiou M. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatograghy-UV-visible photodiode-array detection-mass spectrometry. J Chromato. 1995;99:107-118.
6. Rios JL, Recio MC, Giner RM, Manez S. An update review of saffron and its active constituents. Phytother Res. 1996;10:189-193.
7. Abdullaev FI, Espinosa-Aguirre JJ. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detec Prev. 2004;28:426-432.
8. Hosseinzadeh H, Younesi H M. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol. 2002;2:1-8.
9. Assimopoulou AN, Sinakos Z, Papageorgiou VP. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. Phytother Res. 2005;19:997-1000.
10. Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P, Margarity M, *et al*. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. J Agric Food Chem. 2006;54:8762-8768.
11. Chryssanthi DG, Lamari FN, Iatrou G, Pylara A, Karamanos NK, Cordopatis P. Inhibition of breast cancer cell proliferation by style constituents of different *Crocus* species. Anticancer Res. 2007;27:357-362.
12. Salomi MJ, Nair SC, Panikkar KR. Cytotoxicty and non- mutagenicity of Nigela sativa and saffron (*Crocus sativus*) *in vitro*. Proc Ker Sci Congr. 1991;5:244.
13. Premkumar K, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A. Inhibition of genotoxicity by saffron (*Crocus sativus* L.) in mice. Drug Chem Toxicol. 2001;24:421-428.
14. Nair SC, Varghese CD, Pannikar KR, Kurumboor SK, Parathod RK. Effects of saffron in vitamin A levels and its antiumor activity on the growth of solid tumors in mice. Int J Pharmacog. 1994;32:105-114.
15. Martin G, Goh E, Neff A W. Evaluation of the developmental toxicity of crocetin on Xenopus. Food Chem. Toxicol. 2002;40:959-964.
16. Tavakkol-Afshari J, Brook A, Mousavi SH. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol. 2008;46:3443-3447.
17. Hosseinzadeh H, Sadeghnia HR. Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. Phytomedicine. 2007:14:256-262.
18. Hosseinzadeh H, Talebzadeh F. Anticonvulsant evaluation of safranal and crocin from Hosseinzadeh H, Sadeghnia HR, Rahimi A. Effect of safranal on extracellular hippocampal levels of glutamate and aspartate during kainic Acid treatment in anesthetized rats. Planta Med. 2008;74:1441-1445.
19. Sadeghnia HR, Cortez MA, Liu D, Hosseinzadeh H. Antiabsence effects of safranal in acute experimental seizure models: EEG and autoradiography. J Pharm Pharm Sci. 2008;11:1-14.
20. Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract

and its constituents, crocin and safranal, in mice. Phytother Res. 2009;23:768-774.
21. Hosseinzadeh H, Mohaghegh MH, Saffari Z: *Crocus Sativus* L. (saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. Evid Based Complement Alternat Med. 2009;6:343-350.
22. Masutani H, Otsuki R, Yamaguchi Y, Takenaka M, Kanoh N, Takatera K, Kunimoto Y, Yodoi J. Fragrant unsaturated aldehydes elicit activation of the Keap1/ Nrf2 system leading to the up-regulation of thioredoxin expression and protection against oxidative stress. Antioxid Redox Signal. 2009;11:949-962.
23. Hosseinzadeh H, Sadeghnia HR. Effect of safranal, a constituent of *Crocus sativus* (saffron), on methyl methanesulfonate (MMS)-induced DNA damage in mouse organs: an alkaline single-cell gel electrophoresis (comet) assay. DNA Cell Biol. 2007;26:841-846.
24. Hosseinzadeh H, Ghenaati J. Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in guinea pigs. Fitoterapia. 2006;77:446-448.
25. Boskabady MH, Aslani MR. Relaxant effect of *Crocus sativus* on guinea pig tracheal chains and its possible mechanisms. J Pharm Pharmacol. 2006;58:1385-1390.
26. Boskabady M H, Ghasemzadeh M, Nemati H, Esmaeilzadeh M. Inhibitory effect of *Crocus sativus* (saffron) on histamine (H_1) receptors of guinea pig tracheal chains. Pharmezie 2010;65:300-305.
27. Nemati H, Boskabady MH, Ahmadzade Vostakolaei H. Stimulatory effect of *Crocus sativus* (saffron) on $ß_2$-adrnoceptors of guinea pig tracheal chains. Phytomed. 2008;15:1038-1045.
28. Boskabady MH, Seyedhosseini Tamijani SM, Rafatpanah H, Rezaei AR. The effect of *Crocus sativus* on human lymphocyte's cytokines and Th_2/Th_1 balance. In Press: J Med Food 2011;14:1538-1545.
29. Boskabady MH, Kiani S, Aslani MR. Tracheal responsiveness to both isoprenaline and beta-adrenoreceptor blockade by propranolol in cigarette smoke exposed and sensitized guinea pigs. Respirology. 2006;11:572-578.
30. Keyhanmanesh R, Boskabady MH, Khamneh S, Ebrahimi MA. The effect of thymoquinone, the main constituent of *Nigella sativa* on tracheal responsiveness and WBC count in lung lavage of sensitized guinea-pigs. Planta Med. 2009;75:1-5.
31. Neamati A, Boskabady MH, Tavakol Afshari J, Mohaghegh Hazrati S, Haeri Rohani A. The effect of the natural adjuvants on tracheal responsiveness and cell count in BAL and blood of sensitized guinea-pig. Respirology. 2009;14:877-884.
32. Filley WV, Kephart GM, Holley KE, Gleich GJ. Identification by immunofluorescence of eosinophil granule MBP in lung tissues of patients with bronchial asthma. Lancet. 1982;3:11-15.
33. Weller PF, Lee CW, Corey EJ, Austen KF, Lewis RA. Generation and metabolism of 4-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C4. Proceeding National Academy of Science of USA. 1983;80;7626-7630.
34. Lee TC, Lenihan DJ, Malone B, Roddy LL, Wasserman SI. Increased biosynthesis of platelet activating factor in activated human eosinophils. J Biol Chem. 1984;259:5526-5530.
35. Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. Am Rev Respir Dis. 1990;141:970-977.
36. Tang XY, Yu HP, Deng HJ, Chen X, Fan HZ, Gong YX, *et al*. Pathogenic mechanism of CD8(+)CD28(-)T cell and the effect of dexamethasone in asthmatic mouse. Zhonghua Yi Xue Za Zhi. 2011;91:1861-1865.
37. Hadizadeh F, Mahdavi M, Emami SA, Khashayarmanesh Z, Hassanzadeh M, Asili J, *et al*. Evaluation of IS0 Method in Saffron Qualification. Acta Hort (ISHS). 2007;739:405-409.
38. MinaiyanM, Ghannadi AR, Afsharipour M and Mahzouni P. Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats. Res Pharm Sci. 2011;61:13-21.
39. Hajhashemi1 V, Ghannadi A and Mousavi S. Antinociceptive study of extracts of *Platanus orientalis* leaves in mice. Res Pharm Sci. 2011;6:123-128.
40. Minaiyan M, Ghannadi A, Etemad M and Mahzouni P. A study of the effects of *Cydonia oblonga* Miller (Quince) on TNBS-induced ulcerative colitis in rats. Res Pharmac Sci 2012;7:103-110.