

Crocin alleviates the local paw edema induced by histamine in rats

Esmaeel Tamaddonfard^{1*}, Amir Abbas Farshid², Leila Hosseini¹

Abstract

Objective: Crocin, as an active constituent of saffron, has many biological functions including antioxidant and anti-inflammatory activities. The present study was aimed to investigate the effects of crocin and chlorpheniramine on local edema induced by histamine.

Materials and Methods: Local edema was induced by subcutaneous injection of histamine (100 μ l, 0.1%) in ventral surface of right hind paw. The thickness of paw was measured at 1 h before and 1, 2, 3 h after injection of histamine, using a fine caliper. The number of neutrophils in paw tissue sections was counted 3 h after intraplantar injection of histamine.

Results: Intraperitoneal injection of crocin at doses of 100 and 200 mg/kg and chlorpheniramine at a dose of 10 mg/kg significantly ($p < 0.05$) decreased both paw thickness and infiltration of neutrophils in paw tissues. In a combined treatment, intraperitoneal injection of an effective dose of crocin (100 mg/kg) with an ineffective dose of chlorpheniramine (2.5 mg/kg) produced a more documented response in comparison with crocin (100 mg/kg) and chlorpheniramine (2.5 mg/kg) used alone.

Conclusion: The results suggested that both crocin and chlorpheniramine suppressed histamine-induced local paw edema. Moreover, histamine H₁ receptors function may be affected by crocin.

Keywords: Crocin, Chlorpheniramine, Histamine, Paw edema, Rats

1- Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, I. R. Iran

2- Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, I. R. Iran

*Corresponding author: Tel: +984412770508; Fax: +984412771926.

E-mail: etamaddonfard@yahoo.com

Introduction

Crocin is a digentiobiosyl all-*trans*-crocetin (8,8'-di-apocarotene-8,8'-dioic acid) ester that is the major yellow pigment in gardenia yellow and saffron, which are extracts of *Gardenia jasminoides* fruits and *Crocus sativus* stigmas, respectively (Aung *et al.*, 2007; Lee *et al.*, 2007). Saffron is used in folk medicine for various purposes such as an antispasmodic, nerve sedative, eupeptic, anticatarrhal, carminative, diaphoretic, stomachic, and aphrodisiac substance, and also as an emmenagogue and expectorant (Schmidt *et al.*, 2007). Saffron contains carotenoid pigments called tricrocin, bicrocin, and crocin, a bitter glycoside called picocrocin, and the volatile, aromatic substance, safranal (Ochiai *et al.*, 2007; Rios *et al.*, 1996).

In modern pharmacological studies, saffron and its constituents including crocin and safranal, have demonstrated anticonvulsant, antidepressant, antioxidant, and antitumour properties (Abdullaev, 2002; Hosseinzadeh and Khosravani 2002; Hosseinzadeh *et al.*, 2004; Zheng *et al.*, 2007). Crocin inhibited xylene-induced ear edema and carrageenan-induced paw edema in rats (Xu *et al.*, 2009). Moreover, in isoproterenol-induced cardiotoxicity, crocin suppressed edema as well as inflammatory cells infiltration in myocardium (Goyal *et al.*, 2010).

Histamine is a biogenic amine and through its specific membrane receptors, H₁, H₂, H₃, and H₄, plays an important role in physiological and pathological processes such as gastric acid secretion, smooth muscle contraction, neurotransmission, immunomodulation, angiogenesis, and allergic disorders (Parsons and Ganellin, 2006; Tripathi *et al.*, 2010). Histamine affects the local inflammatory responses by activation of vasodilation, vascular permeability, edema formation, polymorphonuclear leukocyte infiltration, and cytokine release (Bilici *et al.*, 2001; Chimona *et al.*, 2007; Ozbakis-Dengiz *et al.*, 2007; Kalokasidis *et al.*, 2009; Farshid *et al.*, 2011).

Histamine-induced inflammation has been widely used to explore the anti-inflammatory effects of some medicinal plants. The aqueous and methanol extract of the dried latex of *Calotropis procera* and the leaf extract of *Garcinia gardneriana* reduced paw edema induced by histamine, serotonin, and prostaglandin E₂ (Arya and Kumar, 2005; Castardo *et al.*, 2008).

The effects of crocin on histamine-induced paw edema, to the best of our knowledge, have not been studied before. In the present study, the effect of crocin was investigated on paw edema induced by intra-plantar injection of histamine in rats. In addition, to identify the mechanism that possibly mediates the effect of crocin on local paw edema induced by histamine, the contribution of histamine H₁ receptors was assessed using chlorpheniramine, a histamine H₁ receptor antagonist, with and without crocin.

Materials and Methods

Animals

Healthy adult male Wistar rats, weighing 210 – 230 g were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum*, in a laboratory with controlled ambient temperature (23°±0.5°C) and under a 12:12 h light-dark cycle (lights on from 07:00 h). Six rats were used in each experiment. The experimental protocol was approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University.

Drugs

The following drugs were administered: histamine, crocin, and chlorpheniramine. The drugs were purchased from Sigma-Aldrich (Chemical Co., Inc., St Louis, MO, USA). The drugs were dissolved in sterile normal saline.

Treatment groups

Rats were divided into following seven groups of six animals each. Group I was

Effect of crocin on histamine-induced paw edema

treated with intraperitoneal injection of normal saline followed by intraplantar injection of 0.1% histamine. In groups II, III and IV, intraplantar injections of histamine were performed after intraperitoneal injection of crocin at doses of 50, 100 and 200 mg/kg. Groups V and VI were treated with intraperitoneal injection of chlorpheniramine at doses of 2.5 and 10 mg/kg before intraplantar injection of histamine. Group VII received intraperitoneal injection of crocin (100 mg/kg) plus chlorpheniramine (2.5 mg/kg) before intraplantar injection of histamine. Crocin and chlorpheniramine were administered 30 and 20 min before intraplantar injection of histamine, respectively. The drug doses used here were selected according to the investigations in which the used doses of crocin and chlorpheniramine were 25-200 mg/kg and 3-10 mg/kg, respectively (Kohno et al., 1987; Shivkar and Kumar, 2003; Tamaddonfard and Hamzeh-Gooshchi, 2010). In the present study, we used chlorpheniramine as a reference drug according to the previous studies (Arya and Kumar, 2005; Boskabady et al., 2010; Boskabady et al., 2011).

Induction of paw edema

For induction of paw edema, each rat was subcutaneously injected with 100 μ l histamine (0.1%) in the ventral surface of the right hind paw using a 29-gauge injection needle and was returned to its cage (Bilici et al., 2001; Ozbakis-Dengiz et al., 2007; Farshid et al., 2011). The magnitude of paw edema was assessed by measuring dorsal-plantar paw thickness with a fine caliper (Farshid et al., 2010 and 2011), at 1 h before and 1, 2 and 3 h after histamine injection (Farshid et al., 2011). Edema was expressed as the increase in paw thickness (mm) after histamine injection relative to the pre-injection value for each animal (Farshid et al., 2010 and 2011).

Histopathological evaluation

For histopathological evaluation of paw tissues, the animals were euthanized by

decapitation 3 h after histamine injection, and their paw tissues were collected for histopathological investigation. The specimens were fixed in 10% buffer formal saline and routinely processed for paraffin embedding. For each sample, 4-5 μ m thick sections were cut and stained with hematoxylin-eosin, to evaluate the acute inflammation. Neutrophils were counted by special morphometric lens in 0.25 mm² microscopic field, from 10 different areas of the sections and the mean values were calculated. The final number of neutrophils was expressed as the mean of the number counted in six animals per group (Farshid et al., 2010 and 2011)

Statistical analysis

Reported values are the mean \pm SEM. Statistical analysis was performed by repeated measure analysis of variance (ANOVA) and Duncan's test for the data obtained from the paw edema. Data obtained from the neutrophil infiltration were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. The significant level was expressed as $p < 0.05$.

Results

Intraplantar injection of histamine evoked a local edema with maximal rate detected within 1 h after injection and thereafter declined to the end of the experiment. Intraperitoneal injections of crocin at doses of 100 and 200 mg/kg, but not at a dose of 50 mg/kg, significantly decreased 1, 2 and 3 h paw thickness induced by histamine ($F_{(3,80)} = 23.636$, $p < 0.05$) (Figure 1).

Intraperitoneal injection of chlorpheniramine at a dose of 10 mg/kg, but not at a dose of 2.5 mg/kg, significantly decreased 1, 2 and 3 h paw thickness induced by histamine ($F_{(2,60)} = 32.222$, $p < 0.05$) (Figure 2).

Intraperitoneal co-administration of an effective dose of crocin (100 mg/kg) with an ineffective dose of chlorpheniramine (2.5 mg/kg) decreased the paw thickness as

compared with crocin (100 mg/kg) and chlorpheniramine (2.5 mg/kg) used alone ($F_{(3,80)}=17.458, p<0.05$) (Figure 3).

Histopathologically, congestion, edema, haemorrhages, and leukocytic infiltration, mainly neutrophils, were observed in the inflamed area. As presented in Figure 4 and showed in Figure 5a, the number of neutrophils was highest (39.2 ± 2.7) in the intraplantar injected (control) group. Intraperitoneal injection of crocin at a dose of 50 mg/kg had no significant effect, but at doses of 100 and 200 mg/kg, crocin significantly decreased the number of neutrophils in the inflamed area ($F_{(3,20)}=11.336, p <0.05$) (Figures 4, 5b, 5c, and 5d). Intraperitoneal injection of chlorpheniramine at a dose of 10 mg/kg, but not at a dose of 2.5 mg/kg, significantly decreased the neutrophil infiltration ($F_{(2,15)}=22.104, p <0.05$) (Figures 4, 5e, and 5f). Intraperitoneal co-administration of an effective dose of crocin (100 mg/kg) with an ineffective dose of chlorpheniramine (2.5 mg/kg) decreased the number of neutrophils as compared with crocin (100 mg/kg) and chlorpheniramine (2.5 mg/kg) used alone ($F_{(3,20)}=23.729, p<0.05$) (Figures 4 and 5g).

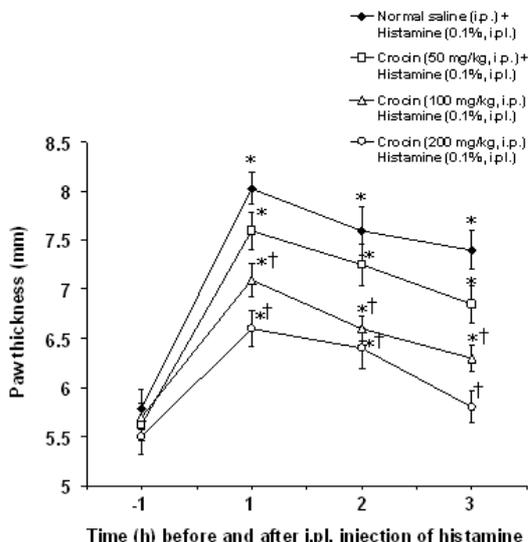


Figure 1. Effect of crocin on paw thickness induced by intraplantar injection of histamine in rats. All values are expressed as mean±SEM (n=6). Statistical comparisons among groups were carried out by factorial ANOVA followed by Duncan's tests. *p<0.05 in comparison with 1 h before histamine injection. †p<0.05 in comparison with

normal saline + histamine group. ip: intraperitoneal, ipl: intraplantar

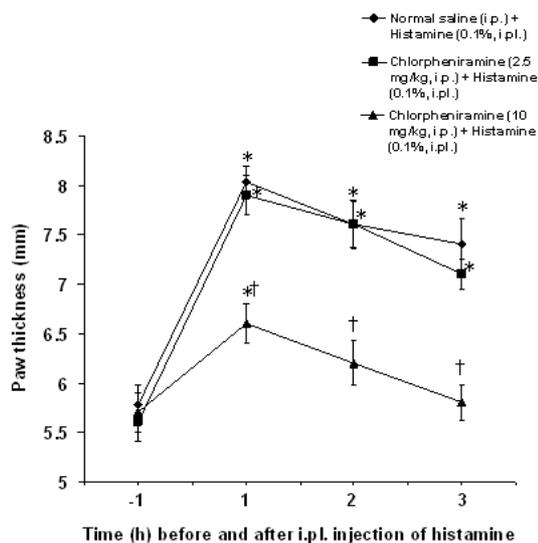


Figure 2. Effect of chlorpheniramine on paw thickness induced by intraplantar injection of histamine in rats. All values are expressed as mean ±SEM (n=6). Statistical comparisons among groups were carried out by factorial ANOVA followed by Duncan's tests. *p<0.05 in comparison with 1 h before histamine injection. †p<0.05 in comparison with normal saline+histamine and chlorpheniramine (2.5 mg/kg) + histamine groups. ip: intraperitoneal, ipl: intraplantar

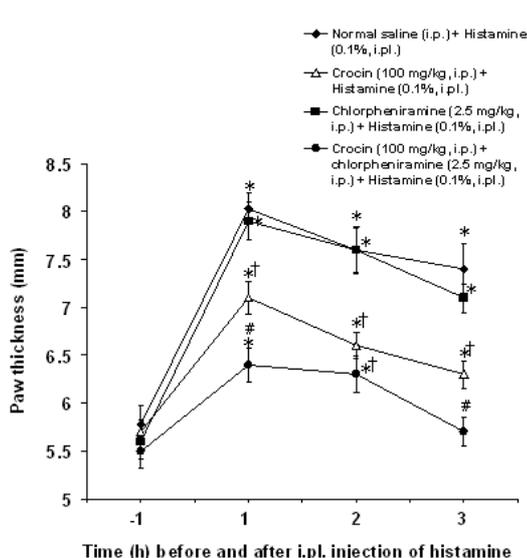


Figure 3. Effect of co-administration of crocin with chlorpheniramine on paw thickness induced by intraplantar injection of histamine in rats. All values are mean±SEM (n=6). Statistical comparisons among groups were carried out by factorial ANOVA followed by Duncan's tests. *p<0.05 in comparison with 1 h before histamine injection. †p<0.05 in

Effect of crocin on histamine-induced paw edema

comparison with normal saline+histamine and chlorpheniramine (2.5 mg/kg) + histamine groups. †p<0.05 in comparison with crocin (100 mg/kg) +histamine and chlorpheniramine (2.5 mg/kg) +histamine groups. ip: intraperitoneal, ipl: intraplantar.

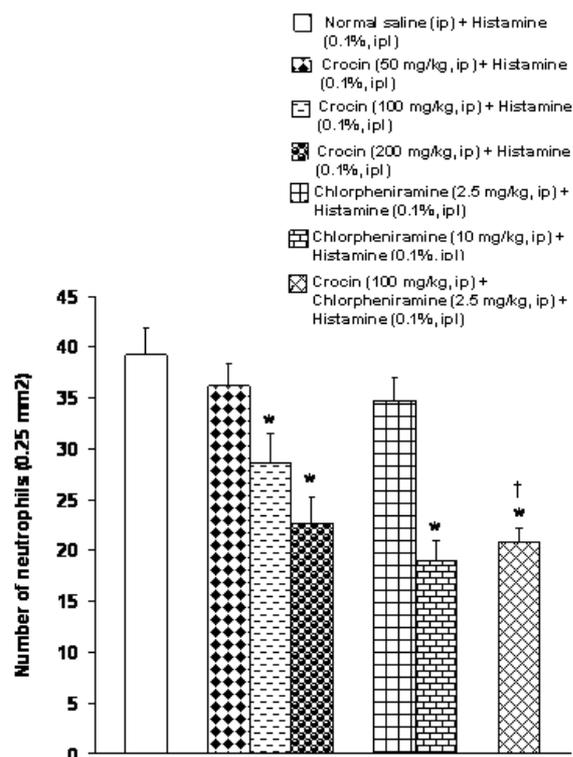


Figure 4. Effects of separate and combined treatments with crocin and chlorpheniramine on the number of neutrophils in paw tissues in rats. All values are mean±SEM (n=6). Statistical comparisons among groups were carried out by one way ANOVA followed by Duncan's tests. *p<0.05 in comparison with normal saline+histamine group. †p<0.05 in comparison with chlorpheniramine (2.5 mg/kg) +histamine and crocin (100 mg/kg) +histamine groups. ip: intraperitoneal, ipl: intraplantar.

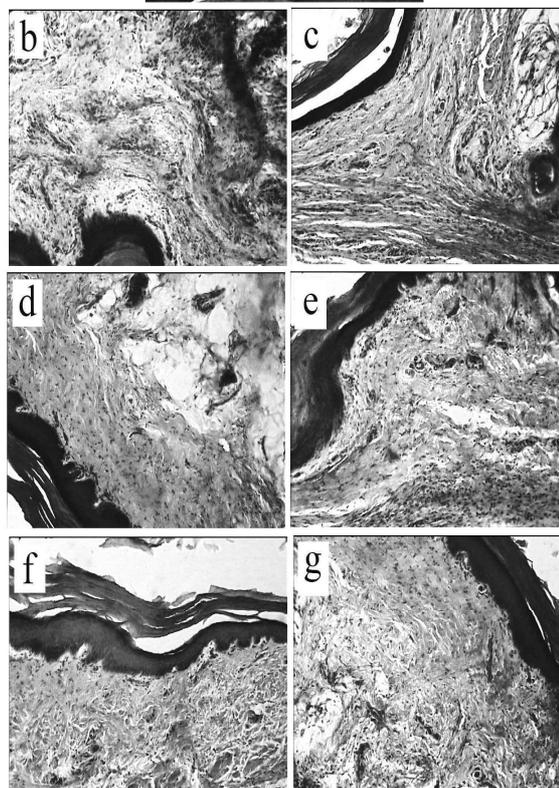
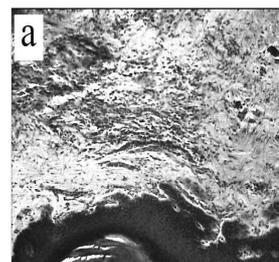


Figure 5. Effects of separate and combined treatments with crocin and chlorpheniramine on neutrophil infiltration induced by histamine in rat paw tissues. Animals were treated with: (a) normal saline + histamine, (b) crocin (50 mg/kg) +histamine, (c) crocin (100 mg/kg) + histamine, (d) crocin (200 mg/kg) +histamine, (e) chlorpheniramine (2.5 mg/kg) +histamine, (f) chlorpheniramine (10 mg/kg) + histamine, (g) crocin (100 mg/kg) + chlorpheniramine (2.5 mg/kg) +histamine. Extensive neutrophil infiltrations are seen in a, b, and e. Moderate neutrophil infiltrations are seen in c, d, f, and g (H&E × 100).

Discussion

In this study, subcutaneous injection of histamine in the ventral surface of hind paw produced paw edema as well as neutrophil infiltration in the paw tissue. In addition, pretreatment with chlorpheniramine suppressed histamine-induced paw inflammatory responses. Histamine-induced inflammation has been well established as a valid model to study paw edema and neutrophil infiltration in paw tissue after inflammatory states (Bilici *et al.*, 2001; Ozbakis-Dengiz *et al.*, 2007; Farshid *et al.*, 2011). Several reports have confirmed that histamine alone and in participation with chemoattractants such as platelet activating factor, interleukin 8, and leukotriene B₄ involve in the regulation of neutrophil recruitment (Bilici *et al.*, 2001; Chimona *et al.*, 2007; Ozbakis-Dengiz *et al.*, 2007; Kalokasidis *et al.*, 2009; Farshid *et al.*, 2011; Perretti *et al.*, 1994).

Histamine H₁ receptors are involved in mediating the inflammation induced by various inflammatory agents. Oral administration of chlorpheniramine suppressed histamine-induced paw edema in rats (Arya and Kumar, 2005). Moreover, intravenous injection of chlorpheniramine inhibited the edema response induced by subcutaneous injection of substance P, a mediator of inflammation (Gilligan *et al.*, 1994), in mouse ear (Inoue *et al.*, 1996). Paw edema induced by subplantar injection of zymogen was inhibited by intraperitoneal injections and oral administrations of clemastine, cyproheptadine, cetirizine, loratadine, and terfenadine (histamine H₁ receptor antagonists) in rats (Blazso and Gabor, 1997). In addition, oral administration of desloratadine and levocetirizine (histamine H₁ receptor antagonists) for 3 days reduced the oedema, vascular dilation and congestion induced by transtympanically injection of histamine on the right middle ear mucosa in rabbits (Chimona *et al.*, 2008).

The results of present study indicated that crocin suppressed the paw edema and

neutrophil recruitment induced by histamine. Moreover, a potentiation effect was observed between crocin and chlorpheniramine when an effective dose of crocin was used with an ineffective dose of chlorpheniramine. These indicate that crocin produces an anti-edematous effect on histamine-induced local paw edema. In addition, crocin may affect the histamine H₁ receptors function. To our knowledge, this is the first report that provided an evidence of anti-edematous effect of crocin on histamine-induced paw edema in rats. It is well known that histamine is synthesized and released by different cells including basophils, mast cells, platelets, histaminergic neurons, lymphocytes, and enterochromaffin cells (Criado *et al.*, 2010).

Crocine is a major constituent of saffron (Rios *et al.*, 1996), and the inhibitory effect of a water extract of a mixture of eight herbs including chamomiles, saffron, anise, fennel, caraway, licorice, cardamom, and black seed was reported on compound 48/80- and IgE/anti-IgE-induced histamine release from peritoneal mast cells (Haggag *et al.*, 2003). Crocin may affect the function of histamine H₁ receptors, because the inhibitory effect of aqueous-ethalonic extract of saffron has been reported on the function of histamine H₁ receptors in the guinea pig tracheal chains (Boskabady *et al.*, 2010).

Besides, recently the inhibitory effect of saffron on the function of histamine H₁ receptors has been attributed to safranal, another constituent found in saffron (Boskabady *et al.*, 2011). In other inflammatory states, crocin exerts beneficial effects. Oral administration of crocin inhibited the xylene-induced ear edema in mice and carrageenan-induced paw edema and reduced the rise in hind paw prostaglandin E₂ level in carrageenan-induced paw edema in rats (Xu *et al.*, 2009). In the isoproterenol-induced cardiotoxicity, the preventive effect of chronic oral administration of crocin on myocardial necrosis, edema, and inflammation has been attributed to the

Effect of crocin on histamine-induced paw edema

recovery effects of crocin on cardiac levels of CK-MB, LDH, MDA, catalase, SOD, and GSH in rats (Goyal et al., 2010). It seems that crocin exerts its anti-inflammatory effect through several mechanisms.

In conclusion, crocin produced an anti-edematogenic effect in histamine-induced local paw edema in rats. A potentiation effect was observed between crocin and chlorpheniramine. Histamine H₁ receptors function may be influenced by crocin.

Acknowledgments

This work was supported by Dean of Faculty of Veterinary Medicine of Urmia University. The authors declare that they have no conflict of interest.

References

- Abdullaev FI. 2002. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Exp Biol Med*, 227: 20-25.
- Arya S, Kumar VL. 2005. Anti-inflammatory efficacy of extracts of latex of *Calotropis procera* against different mediators of inflammation. *Mediators Inflamm*, 4: 228-232.
- Aung HH, Wang CZ, Ni M, Fishbein A, Mehendale SR, Xie JT, Shoyoma AY, Yuan CS. 2007. Crocin from *Crocus sativus* possesses significant anti-proliferation effects on human colorectal cancer cells. *Exp Oncol*, 29: 175-180.
- Bilici D, Akpinar E, Gursan N, Ozbakis-Dengiz G, Bilici S, Altas S. 2001. Protective effect of T-type calcium channel blocker in histamine-induced paw inflammation in rats. *Pharmacol Rep*, 44: 527-531.
- Blazso G, Gabor M. 1997. Evaluation of anti-oedematous effects of some H₁-receptor antagonists and methysergide in rats. *Pharmacol Res*, 35: 65-71.
- Boskabady MH, Ghasemzadeh Rahbardar M, Nemati H, Esmaeilzadeh M. 2010. Inhibitory effect of *Crocus sativus* (saffron) on histamine H₁ receptors of guinea pig tracheal chains. *Pharmazie*, 65: 300-305.
- Boskabady MH, Ghasemzadeh Rahbardar M, Safari Z. 2011. The effect of safranal on histamine H₁ receptors of guinea pig tracheal chains. *Fitoterapia*, 82: 162-167.
- Castardo JC, Prudente AS, Ferreira J, Guimaraes CL, Monache FD, Filho VC, Otuki MF, Cabrini DA. 2008. Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from *Garcinia gardneriniana* leaves in mouse paw oedema. *J Ethnopharmacol*, 118: 405-411.
- Chimona TS, Panayiotides JG, Papadakis CE, Helidonis ES, Velegrakis GA. 2007. Transtympanic versus intramuscular steroid administration in a histamine-induced inflammatory middle-ear model. *J Laryngol Otol*, 121: 630-634.
- Chimona TS, Panayiotides JG, Papadakis CE, Helidonis ES, Velegrakis GA. 2008. Antihistamine effects on experimental middle ear inflammatory model. *Eur Arch Otorhinolaryngol*, 265: 899-905.
- Criado PR, Criado RFJ, Maruta CW, Filho CAM. 2010. Histamine, histamine receptors and antihistamines: new concepts. *An Bras Dermatol*, 85: 195-210.
- Farshid AA, Tamaddonfard E, Morvaridi A. 2011. Effects of histidine and dexamethasone on the local inflammation induced by histamine in rats. *Vet Res Forum*, 2: 31-36.
- Farshid AA, Tamaddonfard E, Yahyae F. 2010. Effects of histidine and N-acetylcysteine on diclofenac-induced anti-inflammatory response in acute inflammation in rats. *Indian J Exp Biol*, 48: 1136-1142.
- Gilligan JP, Lovato SJ, Erion MD, Jeng AY. 1994. Modulation of carrageenan-induced hind paw edema by substance P. *Inflammation*, 18: 285-292.
- Goyal SN, Arora S, Sharma AK, Joshi S, Ray R, Bhatia J, Kumari S, Arya DS. 2010. Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultrastructural alterations in isoproterenol-induced cardiotoxicity in rats. *Phytomedicine*, 17: 227-232.
- Haggag EG, Abou-Mostafa MA, Boucher W, Theoharides TC. 2003. The effect of a herbal water-extract on histamine release from mast cells and allergic asthma. *J Herb Pharmacother*, 3: 41-54.
- Hosseinzadeh H, Karami GH, Niapoor M. 2004. Antidepressant effects of *Crocus sativus* stigma extracts and its constituents,

- crocin and safranol, in mice. *Acta Hort*, 650: 435-445.
- Hosseinzadeh H, Khosravani V. 2002. Anticonvulsant effects of aqueous and ethalonic extracts of *Crocus sativus* L. stigma in mice. *Arch Irn Med*, 5: 44-47.
- Inoue H, Nagata N, Koshihara Y. 1996. Involvement of tachykinin receptors in oedema formation and plasma extravasation induced by substance P, neurokinin A, and neurokinin B in mouse ear. *Inflamm Res*, 45: 316-323.
- Kalokasidis K, Molyva D, Mirtsou V, Kokkas B, Gaulas A. 2009. Evidence for the contribution of tumor necrosis factor in oedema formation induced by histamine in the hind paw of the rat. *Inflamm Res*, 58: 437-440.
- Kohno H, Sugahara M, Ohkubo Y, Kubodera A. 1987. Evaluation of histamine-induced acute inflammation by ⁶⁷Ga-citrate in conscious rats. *Eur J Nucl Med*, 13: 371-373.
- Lee IA, Lee JH, Baek NI, Kim DH. 2005. Antihyperlipidemic effect of crocin isolated from the fructus of *Gardenia jasminoides* and its metabolite crocetin. *Biol Pharm Bull*, 28: 2106-2110.
- Ochiai T, Shimeno H, Mishima K, Iwasaki K, Fujiwara M, Tanaka H, Shoyama Y, Toda A, Eyanagi R, Soeda S. 2007. Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. *Biochim Biophys Acta*, 1770: 578-584.
- Ozbakis-Dengiz G, Halici Z, Akpina E, Cardici E, Bilici D, Gursan N. 2007. Role of polymorphonuclear leukocyte infiltration in the mechanism of anti-inflammatory effect of amiodarone. *Pharmacol Rep*, 59: 538-544.
- Parsons ME, Ganellin CR. 2006. Histamine and its receptors. *Br J Pharmacol*, 147: 127-135.
- Perretti M, Harris JG, Flower RJ. 1994. A role for endogenous histamine in interleukin-8-induced neutrophil infiltration into mouse air-pouch: investigation of the modulatory action of systemic and local dexamethasone. *Br J Pharmacol*, 112: 801-808.
- Rios JL, Recio MC, Ginger RM, Manz S. 1996. An update review of saffron and its active constituents. *Phytother Res*, 10: 189-193.
- Schmidt M, Betti G, Hensen A. 2007. Saffron in phytotherapy: pharmacology and clinical uses. *Wien Med Wochenschr*, 157: 315-319.
- Shivkar YM, Kumar VL. 2003. Histamine mediates the pro-inflammatory effect of latex of *Calotropis procera* in rats. *Mediators Inflamm*, 12: 299-302.
- Tamaddonfard E, Hamzeh-Gooshchi N. 2010. Effect of crocin on the morphine-induced antinociception in the formalin test in rats. *Phytother Res*, 24: 410-413.
- Tripathi T, Shahid M, Khan HM, Negi MPS, Siddiqui M, Khan RA. 2010. Modulation of in vivo immunoglobulin production by endogenous histamine and H₁R and H₂R agonists and antagonists. *Pharmacol Rep*, 62: 917-925.
- Xu GL, Li G, Ma HP, Zhong H, Liu F, Ao GZ. 2009. Preventive effect of crocin in inflamed animals and in LPS-challenged RAW 264.7 cells. *J Agric Food Chem*, 57: 8325-8330.
- Zheng YQ, Liu JX, Wang JN, Xu L. 2007. Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global ischemia. *Brain Res*, 1138: 86-94.