

Departement of Physiology and Pharmacological Research Centre of Medicinal Plants, Medical School Mashhad University of Medical Sciences, Mashhad, Iran

## Inhibitory effect of *Crocus sativus* (saffron) on histamine (H<sub>1</sub>) receptors of guinea pig tracheal chains

M. H. BOSKABADY, M. GHASEMZADEH RAHBARDAR, H. NEMATI, M. ESMAEILZADEH

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M. H. Boskabady, M.D., Ph.D., Department of Physiology, Medical School Mashhad, Post Code 9177948564, University of Medical Sciences, Mashhad, Iran  
boskabady@ums.ac.ir, mhboskabady@hotmail.com

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The inhibitory effects of aqueous-ethanolic extracts of *Crocus sativus* (Iridaceae), on histamine (H<sub>1</sub>) receptors was examined on tracheal chains of guinea pigs. The effects of three concentrations of aqueous-ethanolic extract, 10 nM chlorpheniramine, and saline on histamine (H<sub>1</sub>) receptors were tested on three groups of guinea pig tracheal chains as follows; incubated trachea with: 1) indomethacin, 2) indomethacin, propranolol, and atropine and 3) indomethacin and propranolol. The EC<sub>50</sub> (effective concentration of histamine causing 50% of maximum response) obtained in the presence of chlorpheniramine and all concentrations of the extract in all three groups were significantly greater than those of saline ( $p < 0.05$  to  $p < 0.001$ ) except low concentration of the extract in groups 1 and 3. The EC<sub>50</sub> obtained in the presence of two higher concentrations of extract in group 2 were greater than group 1 and 3 ( $p < 0.05$  to  $p < 0.001$ ). Maximum response obtained in the presence of two higher concentrations of extract in group 2 were greater than those of group 1 and group 3 ( $p < 0.001$  for all cases). There were parallel right ward shift in concentration response curves obtained in the presence of only low and medium concentrations of the extract in group 2 compared to the those of saline. These results indicated an inhibitory effect of *Crocus sativus* at histamine H<sub>1</sub> receptors.

### 1. Introduction

*Crocus sativus* L, commonly known as saffron, is a small perennial plant from the Iridaceae family which is cultivated in many places, but particularly in France, Spain, Sicily, and Iran. It has green, hairy leaves about 1-1/2 feet long and a funnel-shaped, reddish-purple flower. The medicinally used part of the plant is its stigma, also called the style (central part of the flower, female sexual organ). The main active constituents of this plant are picrocrocin and its derivatives including safranal, flavonoid derivatives and crocin (Trantilis et al. 1995).

*Crocus sativus* is used in folk medicine as an antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue (Rios et al. 1996).

Previous studies have shown different pharmacological effects for this plant including: anticonvulsant (Hosseinzadeh and Khosravan 2002), antidepressant (Hosseinzadeh et al. 2004), anti-inflammatory (Hosseinzadeh and Younesi 2002), antioxidant effect (Abe et al. 1999), antitumour and radical scavenger effects (Rios et al. 1996; Abdullaev 1993; Escribano et al. 1996; Abdullaev and Ferenkel 1992) learning and memory improving properties (Zhang et al. 1994). Saffron extract also has chemopreventive and genoprotective effects and protects from genotoxins-induced oxidative stress in mice (Abdullaev and Ferenkel 1992; Nair et al. 1995; Premkumar et al. 2001; Premkumar et al. 2003). A blood pressure lowering effect (Rios et al. 1996) and relaxant effect on vascular smooth muscle (Fatehi et al. 2003) has also been described for this plant. The

relaxant effect of the plant on tracheal smooth muscle is also demonstrated (Boskabady and Aslani 2006).

There is evidence of usage of the plant as an antispasmodic and expectorant in folk medicine. The relaxant effect on vascular smooth muscle (Fatehi et al. 2003) and tracheal smooth muscle (Boskabady and Aslani 2006) has been described for the plant. Therefore to examine one possible mechanism responsible for the relaxant effect of the plant on tracheal smooth muscle and also the contribution of its constituent, safranal in this effect, the inhibitory effect of aqueous-ethanolic extracts of *Crocus sativus* on histamine (H<sub>1</sub>) receptors was examined on tracheal chains of guinea pigs in the present study.

### 2. Investigations, results and discussion

Cumulative log concentration-response curves of histamine obtained in the presence of all concentrations of the extract and chlorpheniramine showed a clear rightward shift compared to histamine curves produced in the presence of saline in all three groups of experiments (Fig. 1).

The EC<sub>50</sub> histamine obtained in the presence of chlorpheniramine under all experimental conditions was significantly higher than that of saline ( $p < 0.05$  to  $p < 0.001$ ). The EC<sub>50</sub> obtained in the presence of two higher concentrations of the extract in groups 1 and 3 and its all concentrations in group 2 were significantly higher than that of saline ( $p < 0.05$  to  $p < 0.001$ ), (Fig. 2). The EC<sub>50</sub> histamine obtained in the presence of saline, two higher concentrations of the extract and

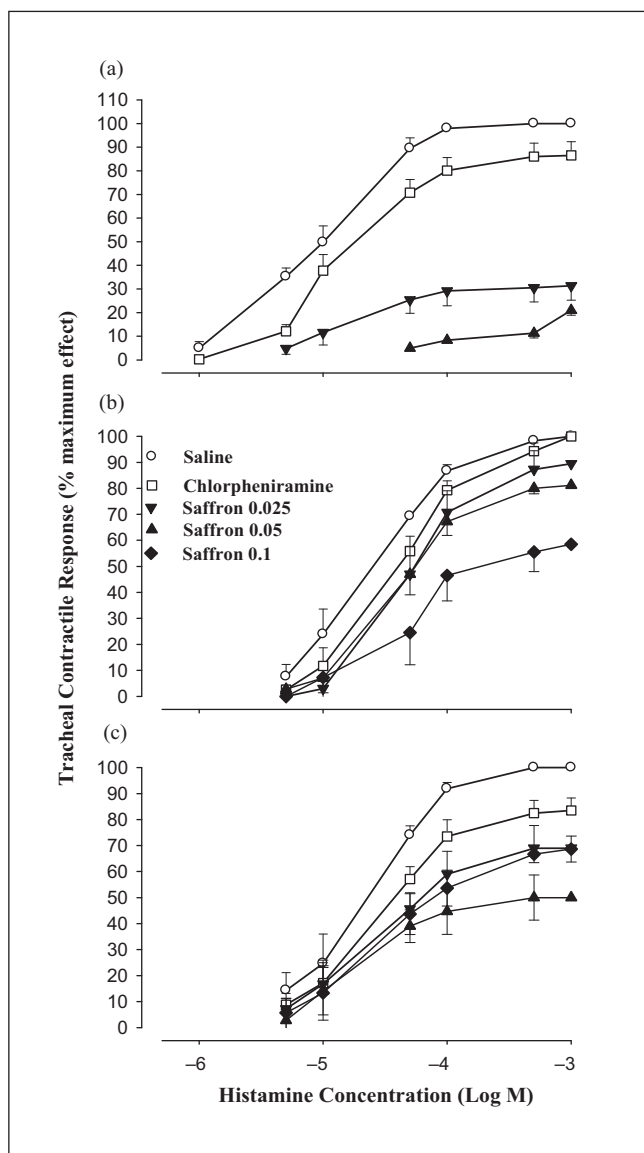


Fig. 1: Cumulative log concentration-response curves of histamine induced contraction of guinea pig tracheal chains, in the presence of saline, three concentrations of aqueous-ethanolic extract, and 10 nM chlorpheniramine on (a) incubated trachea with 1.4  $\mu$ M indomethacin (group 1, n=8), (b) incubated trachea with 1.4  $\mu$ M indomethacin, 1  $\mu$ M propranolol and 10 nM atropine (group 2, n=7) and (c) incubated trachea with 1.4  $\mu$ M indomethacin and 1  $\mu$ M propranolol (group 3, n=6)

chlorpheniramine in group 2 were significantly higher than those of group 1 ( $p < 0.05$  to  $p < 0.001$ , Table 1). In addition, the  $EC_{50}$  histamine obtained in the presence of two higher concentrations of the extract in group 3 were significantly greater than those of group 1 ( $p < 0.01$  to  $p < 0.001$ ). The  $EC_{50}$  histamine obtained in the presence of all concentrations of extract and chlorpheniramine in group 3 were less than those of group 2 but these differences were only significant for low concentration of the extract ( $p < 0.05$ , Table 1).

The maximum responses to histamine obtained in the presence of two higher concentrations of the extract in group 1 were significantly lower than that of saline ( $p < 0.01$  to  $p < 0.001$ , Table 2). The maximum responses obtained in the presence of the high concentrations of the extract in groups 2 were also significantly lower than those of saline ( $p < 0.001$ ). The maximum responses obtained in the presence of all concentration of extract in group 3 were lower than saline ( $p < 0.01$  to  $p < 0.001$ , Table 2). The maximum responses obtained in the presence of two higher

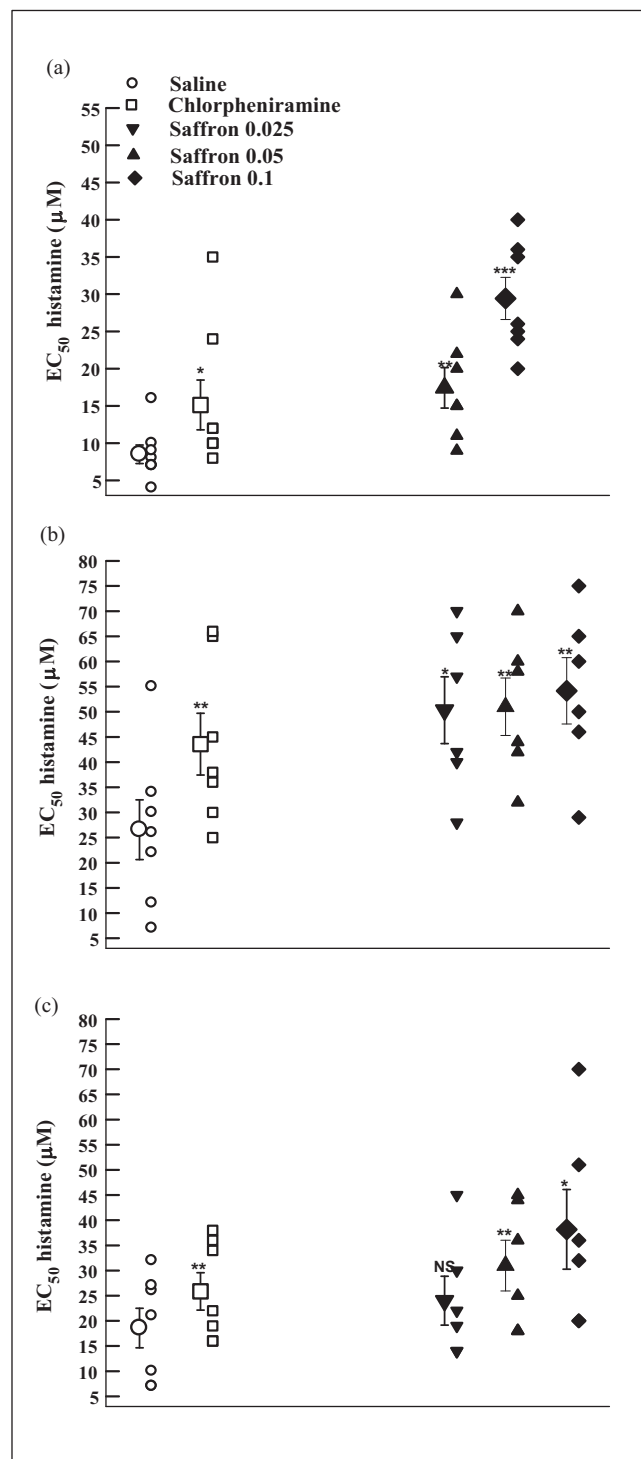


Fig. 2:  $EC_{50}$  histamine obtained in the presence of three concentrations of aqueous-ethanolic extract from saffron (0.025  $\mu$ g/mL  $\nabla$ , 0.05  $\mu$ g/mL  $\Delta$ , and 0.1 mg/mL  $\diamond$ ), 10 nM chlorpheniramine ( $\square$ ), and saline ( $\circ$ ) in incubated trachea with (a) 1.4  $\mu$ M indomethacin (group 1, n=8), (b) incubated trachea with 1.4  $\mu$ M indomethacin, 1  $\mu$ M propranolol and 10 nM atropine (group 2, n=7) and (c) incubated trachea with 1.4  $\mu$ M indomethacin and 1  $\mu$ M propranolol (group 3, n=6). Statistical comparison in  $EC_{50}$  between saline and other solutions NS: non-significant difference, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$

concentrations of the extract in groups 2 and 3 were significantly higher than those of group 1 ( $p < 0.001$  for all cases, Table 3). However, the maximum responses obtained in the presence of only two low concentrations of the extract in group 2 were significantly higher than those of group 3 ( $p < 0.05$  to  $p < 0.01$ , Table 3).

**Table 1: EC<sub>50</sub> (μM) of histamine obtained in the presence of aqueous-ethanolic extract from *Crocus sativus*, 10 nM chlorpheniramine and saline in three groups of experiments**

Solutions	Concentration	Group 1	Group 2	Stat. dif. G1 vs G2	Group 3	Stat. dif. G1 vs G3	Stat. dif. G2 vs G3
Saline		8.50 ± 1.24	26.57 ± 5.96	p < 0.05	20.50 ± 4.07	NS	NS
Extract	0.025 g%	–	50.33 ± 6.64	–	26.00 ± 5.41	–	p < 0.05
	0.05 g%	17.43 ± 2.72	51.00 ± 5.72	p < 0.001	33.60 ± 5.30	p < 0.05	NS
	0.1 g%	29.43 ± 2.83	54.17 ± 6.59	p < 0.05	41.8 ± 8.62	p < 0.05	NS
Chlorpheniramine		15.13 ± 3.34	43.57 ± 6.14	p < 0.001	27.50 ± 3.91	NS	NS

Values are presented as mean ± SEM. G1: group 1, experiments on incubated tracheal chains with 1.4 μM indomethacin (n = 8); G2: group 2, experiments on tracheal chains incubated with 1.4 μM indomethacin, 1 μM propranolol and 10 nM atropine (n = 7) and G3: group 3, experiments on incubated tracheal chains with 1.4 μM indomethacin and 1 μM propranolol (n = 6). Stat. Dif.: statistical difference, NS: non-significant difference

**Table 2: Differences in maximum response and slope obtained in the presence of chlorpheniramine, different concentrations of the extract of *Crocus sativus* with those of saline**

Solutions	Concentration	Maximum Response			Slope		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Chlorpheniramine		NS	NS	NS	NS	NS	NS
Extract	0.025 g%	–	NS	p < 0.01	–	NS	p < 0.05
	0.05 g%	p < 0.001	NS	p < 0.001	p < 0.05	NS	p < 0.001
	0.1 g%	p < 0.01	p < 0.001	p < 0.001	p < 0.001	p < 0.05	p < 0.01

**Table 3: Maximum response to histamine obtained in the presence of extract from *Crocus sativus*, 10 nM chlorpheniramine, and saline in the three groups of experiments**

Solutions	Concentration	Group 1	Group 2	Stat. dif. G1 vs G2	Group 3	Stat. dif. G1 vs G3	Stat. dif. G2 vs G3
Saline		100.00 ± 0.00	100.00 ± 0.00	NS	100.00 ± 0.00	NS	NS
Extract	0.025 g%	–	89.83 ± 4.90	–	69.00 ± 8.67	–	p < 0.05
	0.05 g%	31.57 ± 4.25	81.33 ± 1.50	p < 0.001	66.2 ± 4.02	p < 0.001	p < 0.01
	0.1 g%	20.86 ± 1.30	58.67 ± 5.43	p < 0.001	65.20 ± 3.80	p < 0.001	NS
Chlorpheniramine		86.50 ± 5.84	100.00 ± 0.00	NS	83.50 ± 4.79	NS	NS

For abbreviations see Table 1

The slopes of histamine-response curves obtained in the presence of two higher concentrations of the extract in group 1, the last concentrations of the extract in group 2 and in the presence of all concentrations of the extract in group 3 were significantly lower than those of saline (p < 0.05 to p < 0.001, Table 2). The slopes of histamine-response curves obtained in the presence of medium and high concentrations of the extract in group 1 were significantly lower than those of groups 2 and 3 (p < 0.05 to p < 0.001). However, there was no significant difference in the slope of the curves between group 2 and 3 (Table 4). The values of (CR-1) obtained in the presence of high concentrations of the extract in group 1 was significantly greater than those of chlorpheniramine (p < 0.01, Fig. 3). There was not significant difference in the value of (CR-1) between three groups (Table 5).

The bronchodilatory effect seen for *Crocus sativus* in our previous study might be produced due to several different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H<sub>1</sub> receptors or an anticholinergic property of the plant, because the relaxant effect of β<sub>2</sub>-stimulatory (Martin et al. 1994; Linden et al. 1993), histamine H<sub>1</sub> receptor inhibitory (Popa et al. 1984), and anticholinergic drugs (Loenders et al. 1992) have been shown in previous studies. Therefore, the inhibitory effect of the aqueous-ethanolic extract of *Crocus sativus* was examined on isolated guinea pig tracheal preparations in this study. In order to inhibit arachidonic acid metabolism, in all three parts of the study, tissues were incubated with indomethacin.

The non-parallel rightward shifts in histamine log concentration-response curves, obtained in the presence of the aqueous-

**Table 4: Slope of histamine Log concentration-response curves in the presence of extract from *Crocus sativus*, 10 nM chlorpheniramine, and saline in three groups of experiments**

Solutions	Concentration	Group 1	Group 2	Stat. dif. G1 vs G2	Group 3	Stat. dif. G1 vs G3	Stat. dif. G2 vs G3
Saline		1.14 ± 0.14	1.54 ± 0.11	NS	1.54 ± 0.17	NS	NS
Extract	0.025 g%	–	1.26 ± 0.14	–	1.00 ± 0.12	–	NS
	0.05 g%	0.49 ± 0.06	1.08 ± 0.25	p < 0.05	0.80 ± 0.09	p < 0.05	NS
	0.1 g%	0.17 ± 0.01	0.81 ± 0.14	p < 0.001	0.94 ± 0.04	p < 0.001	NS
Chlorpheniramine		1.27 ± 0.12	1.49 ± 0.07	NS	1.28 ± 0.04	NS	NS

For abbreviations see Table 1

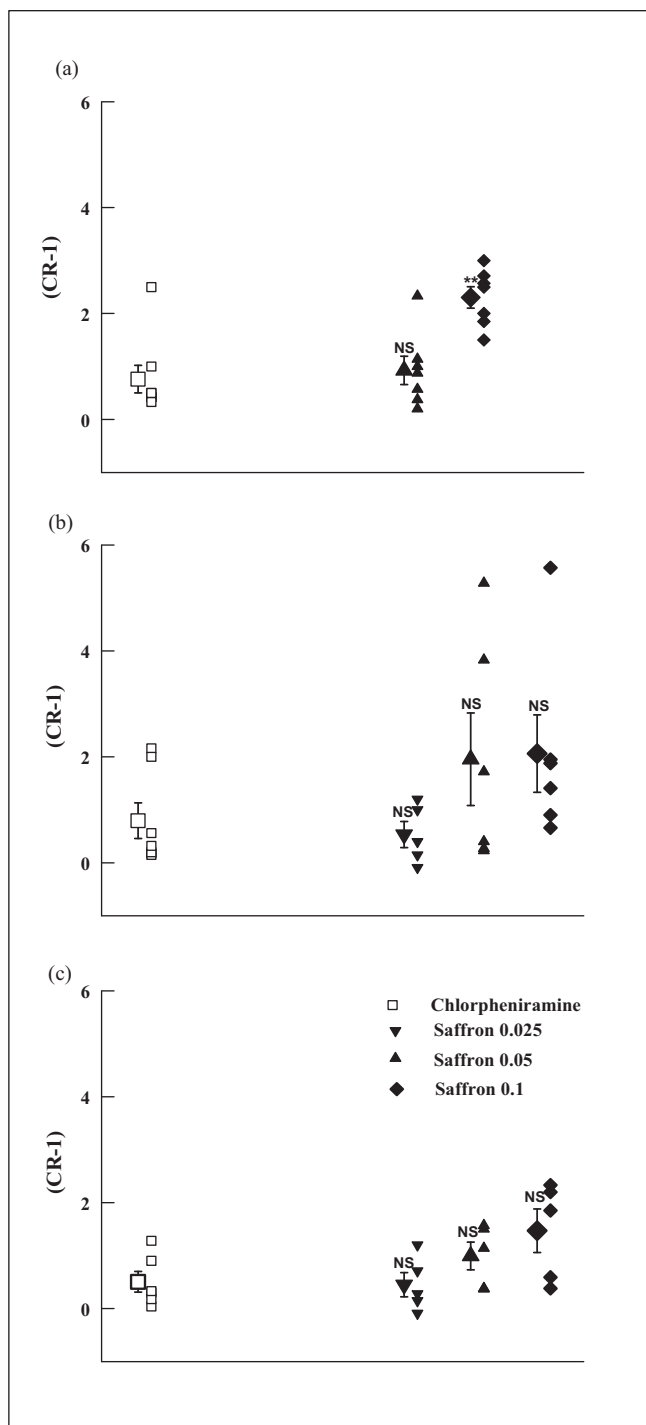


Fig. 3: The values of (CR-1) obtained in the presence of three concentrations of aqueous-ethanolic extract from saffron (0.025  $\nabla$ , 0.05  $\Delta$ , and 0.1 mg/mL  $\diamond$ ), and 10 nM chlorpheniramine ( $\square$ ) in incubated trachea with (a) 1.4  $\mu$ M indomethacin (group 1, n = 8), (b) incubated trachea with 1.4  $\mu$ M indomethacin, 1  $\mu$ M propranolol and 10 nM atropine (group 2, n = 7) and (c) incubated trachea with 1.4  $\mu$ M indomethacin and 1  $\mu$ M propranolol (group 3, n = 6). Statistical comparison in  $EC_{50}$  between chlorpheniramine and other solutions NS: non-significant difference, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . The unite of the (CR-1) is the proportion of  $EC_{50}$  obtained in the presence of tested solutions/ $EC_{50}$  obtained in the presence of saline - 1

ethanolic extract greater  $EC_{50}$  but lower maximum contraction effect to histamine compared to those of saline in group 1 experiments (incubated trachea with only indomethacin) indicated a functional antagonistic effect of *Crocus sativus* at histamine  $H_1$  receptors of guinea pig trachea (Linden et al. 1993; Arunlakshana and Schild 1959; Ariens 1987).

To evaluate the contribution of  $\beta$ -adrenergic stimulatory and/or muscarinic blocking effect on functional antagonism of *Crocus sativus* at histamine  $H_1$  receptors, the antihistaminic effects of the extract from this plant were also examined on an incubated tracheal preparation with indomethacin, propranolol, and atropine. The parallel rightward shift in histamine-response curves obtained in the presence of the extract compared to that of saline and significant improvement of maximum responses to histamine obtained in the presence of the extract in this group of the experiment, relative to those of group 1 showed possible competitive antagonistic effects of the hydro-ethanolic extract of *Crocus sativus* on histamine  $H_1$  receptors. Parallel shift in concentration-response curves and improvement of maximum response to histamine and significant increase in  $EC_{50}$  obtained in the presence of the extract in this group of the study indicates anticholinergic and/or adrenergic stimulatory effects of this plant. The non-significant difference between the values of (CR-1) obtained in the presence of all concentrations of the extract in group 2 of the study with that of chlorpheniramine indicates comparable antagonistic effect of the solutions relative to chlorpheniramine at concentrations used. Although improvement in maximum responses was obtained in the presence of different concentrations of the extract in group 2 experiments compared to those of group 1, there were still significant differences between maximum responses and slopes obtained in the presence of the final concentrations of the extract and that of saline. These results indicate small functional antagonistic effects of the extract at histamine  $H_1$  receptors other than  $\beta$ -adrenergic stimulatory and/or muscarinic blocking effect.

In order to investigate whether changes of concentration response curves observed in group 2 experiments are due to  $\beta$ -adrenergic stimulatory or muscarinic blocking effects, the antihistaminic effect of the plant was also examined on tracheal chains incubated with indomethacin and propranolol. The results of this part of the study showed reduction in maximum response and  $EC_{50}$  compared to group 2 although these values were higher than those of group 1. These results indicate that functional antagonism of the extract at histamine  $H_1$  receptors is due to both the blocking effect on muscarinic receptors and  $\beta$ -adrenergic stimulatory although the results suggest that the  $\beta$ -adrenergic stimulatory of the extract is prominent. Non-parallel shift in histamine-response curves obtained in presence of the extract in group 1 and 3 experiments could be due to the muscarinic receptor blocking effect. The results obtained in the presence of the extract in this part of the study showed that the functional antagonism of this solution observed in group 1 experiments is mainly due to stimulatory effect on  $\beta$ -adrenergic receptors. The values of (CR-1) obtained in the presence of the extract in this part of the study were not significantly different from that of chlorpheniramine indicating a comparable antagonistic effect relative to chlorpheniramine at concentrations used. In fact our previous study which examined the stimulatory effect of the plant on  $\beta$ -adrenergic receptors of tracheal chains using standard method of performing concentration-response curves to isoprenaline on non-incubated and incubated tissues with chlorpheniramine, suggested an inhibitory effect of the extract on histamine  $H_1$  receptors in addition to a stimulatory effect on  $\beta$ -adrenergic receptors which confirms the findings of the present study (Nemati et al. 2008).

The results of the present study confirm those of our previous study indicating a potent relaxant effect for aqueous-ethanolic extracts of *Crocus sativus* which is almost entirely absent in tracheal chains incubated with propranolol, chlorpheniramine and atropine (Boskabady et al. 2006). In addition, the results of that study also showed that the relaxant effect of the extract of *Crocus sativus* was significantly greater than that

**Table 5: Values of (CR-1) in the presence of extract from *Crocus sativus* and 10 nM chlorpheniramine in three groups of experiments**

Solutions	Concentration	Group 1	Group 2	Stat. dif. G1 vs G2	Group 3	Stat. dif. G1 vs G3	Stat. dif. G2 vs G3
Extract	0.025 g%	–	1.90 ± 0.85	–	0.53 ± 0.25	–	NS
	0.05 g%	0.92 ± 0.20	1.96 ± 0.87	NS	0.99 ± 0.26	NS	NS
	0.1 g%	2.30 ± 0.20	2.06 ± 0.73	NS	1.47 ± 0.41	NS	NS
Chlorpheniramine		0.76 ± 0.26	0.80 ± 0.34	NS	0.51 ± 0.20	NS	NS

For abbreviations see Table 1

of safranal at the concentration used indicating that the relaxant effect of the plant is partially due to its main constituent, safranal.

In conclusion, the results of this study suggest a competitive antagonistic effect of *Crocus sativus* at histamine H<sub>1</sub> receptors. In addition, the results also suggest a blocking effect of safranal at muscarinic receptors and a stimulatory effect of the extract on β-adrenergic receptors.

### 3. Experimental

#### 3.1. Plant and extracts

*Crocus sativus* was collected from Torbat Heydariyeh (east of Iran) and identified by Mrs. Molaei. A voucher specimen was preserved in the Herbarium of the School of Agriculture, Mashhad University of Ferdowsi (Herbarium No: 143-0319-1). The aqueous-ethanolic extract was prepared as follows: 10 grams of chopped, dried plant was extracted with 25 ml distilled water and 25 ml ethanol by soxhlet apparatus. The solvent was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final extract was 10 g%.

#### 3.2. Tissue preparations

Male guinea pigs (400–700 g) were killed by a blow on the neck and the tracheae were removed. Each trachea was cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form tracheal chain (Holroyde 1986).

Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, NaHCO<sub>3</sub> 25, MgSO<sub>4</sub> 0.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, KCl 4.72, CaCl<sub>2</sub> 2.5 and dextrose 11.

The Krebs solution was maintained at 37 °C and gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

#### 3.3. Protocols

The inhibitory effect of *Crocus sativus* on histamine H<sub>1</sub> receptors was examined by producing cumulative log concentration-response curve of histamine acid phosphate (BDH Chemical Co., Ltd., UK) induced contraction of tracheal chains 10 min after exposing tissue to one solution (10 nM chlorpheniramine maleate (Sigma Chemical Ltd., UK), three concentrations of aqueous-ethanolic extract from *Crocus sativus* (0.025, 0.05 and 0.1 g%), or 0.2 ml saline). The consecutive concentrations of histamine were added every 2 min (range 0.1–1000 μM); and the percentage of contraction due to each concentration in proportion to the maximum contraction obtained in the presence of saline was plotted against log concentration of histamine. The effective concentration of histamine causing 50% of maximum response (EC<sub>50</sub>) in each experiment was measured using the log concentration-response curve of corresponding experiment. The shift of cumulative log concentration-response curves obtained in the presence of extracts and chlorpheniramine were examined by comparing the EC<sub>50</sub> obtained in the presence of each solution with that of saline. In addition the maximum responses to histamine obtained in the presence of extracts and chlorpheniramine in all sets of experiments were compared with that of saline. To examine the parallel rightward shift, the slope of the histamine-response curve of each experiment was measured and was compared with that of saline. In experiments with parallel shift in histamine-response curve, the concentration-ratio minus one (CR-1) as competitive antagonism effect

was calculated by the following equation:

$$\frac{EC_{50} \text{ obtained in the presence of effective solutions}}{EC_{50} \text{ obtained in the presence of saline}} - 1$$

The inhibitory effect of *Crocus sativus* on histamine H<sub>1</sub> receptors was tested on incubated tracheal chains 30 min prior to the beginning and while obtaining histamine-response curve with three different experimental designs as follows:

1. 1.4 μM indomethacin (Sigma Chemical Ltd., UK), (group 1 experiments), (n = 8).
2. 1.4 μM indomethacin, 1 μM propranolol hydrochloride (Sigma Chemical Ltd., UK), and 10 nM atropine sulphate (Sigma Chemical Ltd., UK), (group 2 experiments), (n = 7).
3. 1.4 μM indomethacin and 1 μM propranolol hydrochloride (group 3 experiments), (n = 6).

All of the experiments were performed randomly with 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boullitt, Paris) and was measured after fixation.

#### 3.4. Statistical analysis

The data of EC<sub>50</sub>, the slope of the curves, the values of (CR-1) and maximum response to histamine in different experiments were expressed as mean ± SEM. The EC<sub>50</sub>, the slope, and maximum response obtained in the presence of extract and chlorpheniramine were compared with those obtained in the presence of saline using paired t test. The values of (CR-1) obtained in the presence of extract were also compared with those obtained in the presence of chlorpheniramine using paired t test. The comparison of the data of different concentrations of extract were performed using ANOVA with Tukey-Kramer multiple post test. The values of EC<sub>50</sub>, the slope, (CR-1), and maximum response obtained in three groups were compared using ANOVA. Significance was accepted at p < 0.05.

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