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Stimulatory effect of *Crocus sativus* (saffron) on β_2-adrenoceptors of guinea pig tracheal chains

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Abstract

To study the mechanism(s) of the relaxant effects of *Crocus sativus* (Iridaceae), the stimulatory effect of aqueous-ethanolic extracts of this plant and one of its constituent, safranal was examined on β-adrenoceptors in tracheal chains of guinea pigs.

The β_2-adrenergic stimulatory was tested by performing the cumulative concentration–response curves of isoprenaline-induced relaxation of pre-contracted isolated guinea pig tracheal chains. The studied solutions were included two concentrations of aqueous-ethanolic extract from *Crocus sativus* (0.1 and 0.2 g%), safranal (1.25 and 2.5 μg), 10 nM propranolol, and saline. The study was done in two different conditions including: non-incubated (group 1, $n = 9$) and incubated tissues with 1 μM chlorpheniramine (group 2, $n = 6$).

The results showed clear leftward shifts in isoprenaline curves obtained in the presence of only higher concentration of the extract in group 1and its both concentrations in group 2 compared with that of saline. The EC_{50} (the effective concentration of isoprenaline, causing 50% of maximum response) obtained in the presence of both concentrations of the extract (0.17 ± 0.06 and 0.12 ± 0.02) and safranal (0.22 ± 0.05 and 0.22 ± 0.05) in group 1 and only in the presence of two concentrations of the extract (1.16 ± 0.31 and 0.68 ± 0.21) in group 2 was significantly lower compared to saline (1.00 ± 0.22 and 4.06 ± 1.04 for groups 1 and 2, respectively) ($p < 0.05$–0.001). The maximum responses obtained in the presence of both concentrations of the extract and safranal in group 1 were significantly lower than that of saline ($p < 0.005$ for all cases). All values (CR−1 = (EC_{50} obtained in the presence of active substances/EC_{50}, obtained in the presence of saline)−1) obtained in the presence of higher concentrations of extract in group 1, its both concentrations and higher concentration of safranal in group 2 were negative and there were significant differences in this value between propranolol and those obtained in the presence of extract and safranal ($p < 0.05$ to $p < 0.001$).

The results indicated a relatively potent stimulatory effect of the extract from *Crocus sativus* on β_2-adrenoceptors which is partially due to its constituent, safranal. A possible inhibitory effect of the plant on histamine (H_1) receptors was also suggested.

Keywords: *Crocus sativus*; Iridaceae; Stimulatory effect; β_2-Adrenoceptors; Guinea pig; Trachea

Introduction

Crocus sativus L, commonly known as saffron, is a small perennial plant from the iris family (Iridaceae) which is cultivated in many places, but particularly in

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Spain and Iran. It has green, hairy leaves about 1–1/2 ft long with 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 constituents of this plant are crocins, safranal, picrocrocin, ketoisophorone, isophorone, glycosidic terpenoids (Trantilis et al., 1995).

In traditional medicine, *Crocus sativus* is used as an antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac, and emmenagogue (Rios et al., 1996; Abdullaev and Espinosa-Aguirre, 2004).

Previous studies have shown different pharmacological effects for this plant including: anticonvulsant (Hosseinzadeh and Khosravan, 2002; Hosseinzadeh and Talebzadeh, 2005), antidepressant (Hosseinzadeh et al., 2004; Akhondzadeh et al., 2005, 2007), antiinflammatory (Hosseinzadeh and Younesi, 2002), radical scavenger and antioxidant properties (Abe et al., 1999; Verma and Bordia,1998; Assimopoulou et al., 2005; Papandreou et al., 2006; Kanakis et al., 2007), and antitumour effects (Rios et al., 1996; Abdullaev and Espinosa-Aguirre, 2004; Abdullaev, 1993; Escribano et al., 1996; Abdullaev and Ferenkel, 1992; Das et al., 2004; Chryssanthi et al., 2007). It has been also reported that the plant has learning and memory improving properties (Zhang et al., 1994; Abe and Saito, 2000; Pitsikas and Sakellaridis, 2006). 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, 2003, 2006). A lowering blood pressure effect (Rios et al., 1996) and relaxant effect on vascular (Fatehi et al., 2003), and tracheal smooth muscle (Boskabady and Aslani, 2006) has also been described for this plant.

To study the mechanism(s) of the relaxant effects of saffron, the stimulatory effect of aqueous-ethanolic extracts of *Crocus sativus* and one of its constituent, safranal on β-adrenoceptors was examined on tracheal chains of guinea pigs.

Material and methods

Plant and extracts

Crocus sativus was collected from Torbat Heydarieh (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 whole plant was collected and identified but only the stigma of the identified plant was isolated and used in the study. The aqueous-ethanolic extract of the isolated stigmata was prepared as follows: 10 g of chopped, dried isolated stigmata of the plant were extracted with 50 ml of ethanol 50% (25 ml distilled water and 25 ml ethanol) by soxhelt apparatus. The solvent was then removed under reduced pressure. The plant ingredient concentration in the final extract was adjusted to be 10 g% by adding distilled water to the dried extract.

Tissue preparations

Male Dunkin–Hartley guinea pigs (400–700 g) were scarified by a blow on the neck and the trachea 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 UK) containing Krebs–Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5, and dextrose 11.

The Krebs solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension (1 g) and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

The study was approved by the University's Ethic Committee for animal use. The allowance number of the relevant ethical committee for the animal experiments is 85301.

Protocols

The stimulatory effect of different solutions was examined on β_2-adrenoceptors by producing cumulative log concentration–response curves of isoprenaline sulphate (Sigma Chemical Ltd., UK)-induced relaxation of pre-contracted tracheal chains by 10 μM methacholine hydrochloride (Sigma Chemical Ltd., UK) 10 min after exposing tissue to the tested solutions. Different tested solutions were included: 10 nM propranolol (0.1 ml of propranolol hydrochloride with 0.1 μM concentration, Sigma Chemical Ltd., UK), two concentrations of aqueous-ethanolic extract from *Crocus sativus* (0.1 and 0.2 g% equivalent to 0.48 and 0.96 ml of 10 g% extract), safranal (1.25 and 2.5 μg equivalent to 1 and 2 ml of 10 g% extract), or 0.2 ml saline. The consecutive concentrations of isoprenaline were added every 2 min (including 5 nM – 1000 μM); and the percentage of relaxation due to each concentration in proportion to the maximum relaxation obtained in the presence of