

Antidepressant Effect of *Crocus sativus* L. Stigma Extracts and their Constituents, Crocin and Safranal, in Mice

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Abstract

The effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigma and their constituents safranal and crocin were studied for the antidepressant activity using forced swimming test in mice. The extracts and constituents were injected intraperitoneally to mice. The aqueous and ethanolic extracts of stigma (0.2-0.8 g/kg) decreased immobility time in comparison to normal saline. Safranal (0.15-0.5 ml/kg) and crocin (50-600 mg/kg) also reduced immobility time. Swimming time was increased by fluoxetine and both extracts. Safranal increased swimming time. Climbing time was increased by imipramine and both extracts. Safranal with a higher dose (0.5 mg/kg) and crocin at doses 50 and 600 increased climbing time. In the open field activity test, the ethanolic extract and safranal increased stereotypic activities. On the basis of these results, the antidepressant effect of *C. sativus* stigma extracts may be mediated via safranal and crocin. Crocin may act via the uptake inhibition of dopamine and norepinephrine, and safranal via serotonin.

INTRODUCTION

Depression is a potentially life-threatening disorder that affects hundreds of millions of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as this disorder causes severe distress and disruption of life and, if left untreated, can be fatal (Bondy, 2002).

The area of pharmacotherapy of depression started in the 1950s, with landmark publications and discoveries that still govern the manner in which we treat depression. There are currently between 10 and 20 different drugs marketed as antidepressants, depending on the country. Tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs) were called first generation antidepressants, and selective serotonin reuptake inhibitors (SSRI) and reversible and selective inhibitors of monoamine oxidase A (RIMA) second generation antidepressants. Third-generation antidepressants include more recent molecules, such as mirtazapine, nefazodone, milnacipran, and reboxetine (Schulz and Macher, 2002).

Some plants like *Hypericum perforatum* (Hippius, 1998; Franklin and Cowen, 2001, Schulz 2002), *Curcuma longa* (Ya et al., 2002) and *Valeriana fauriei* (Oshima et al., 1995) also showed antidepressant effects.

Crocus sativus L., commonly known as saffron, is a perennial stemless herb of the Iridaceae family, widely cultivated in Iran and other countries such as India and Greece. Commercial saffron comprises the dried red stigma with a small portion of the yellowish style attached. Compounds considered pharmacologically active and important are volatile agents (e.g. safranal), bitter principles (e.g. picrocrocin) and dye materials (e.g. crocetin and its glycosidic, crocin) (Ríos et al., 1996). Saffron extract or its active constituents, crocetin and crocin, could be useful as a treatment for neurodegenerative disorders accompanying memory impairment (Abe and Saito, 2002). Saffron is a protective agent against chromosomal damage (Premkumar et al., 2003), a potential cancer chemopreventive agent (Nair et al., 1991; Abdullaev, 2002), a modulator of lipid

peroxidation, and an antioxidant and detoxifying spice (Premkumar et al., 2003). Antinociceptive and anti-inflammatory (Hosseinzadeh and Younesi, 2002) as well as antiseizure (Hosseinzadeh and Khosravan, 2002) effects have also been reported in animals. Recently, we showed the antidepressant effect of *C. sativus* petal and stigma aqueous and ethanolic extracts in mice (Karimi et al, 2001). In this work, we studied the antidepressant effect and preliminary mechanism of *C. sativus* stigma and its two constituents, crocin and safranal in mice using modified forced swimming test.

MATERIALS AND METHODS

Animals

Male BALB/c mice, weighing 22-25 g, were kept in the animal house of Mashhad University of Medical Sciences, in colony rooms with 12/12 h light/dark cycle at $21 \pm 2^\circ \text{C}$. The animals had free access to food and water.

Preparation of Extracts

In the maceration method, 4 g of stigma was macerated in 500 mL ethanol (80%, v/v) or distilled water for 24 h. The mixture was subsequently filtered and concentrated under reduced pressure at 50°C .

Forced Swimming Test

The modified forced swim test was used for the evaluation of antidepressant activity in mice. Mice were placed individually in Pyrex cylinders ($10 \times 25 \text{ cm}$) that were filled with water at $24\text{-}25^\circ \text{C}$ to a 20-cm depth. They were removed 15 min later, dried and placed in their home cage. Twenty-four hours after their first exposure, the animals were replaced in the swim apparatus for 6 min and behaviours were monitored. Injections were administered intraperitoneally 30 min prior to the test session. After two min swimming, behavioural activities were evaluated during four min. Climbing behaviour consisted of upward directed movements of the forepaws along the side of the swim chamber. Swimming behaviour was defined as movement usually horizontal throughout the swim chamber, which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the mice's head above the water (Cryan and Lucki, 2000)

Open Field Activity

The apparatus, made of white wood, had a floor of $100 \times 100 \text{ cm}$ divided by red lines into 25 squares of $20 \times 20 \text{ cm}$. The walls, 50-cm high, were also painted in white. The test room was illuminated at the same intensity as the colony room. Each mouse was placed in the centre of the open field, and its behaviour was observed for 10 min. The parameters evaluated were the total number of squares crossed. The numbers of leanings (one or two paws in contact with the wall), rearings (the mouse standing on its two hind paws without touching the walls), groomings (face cleaning, paw licking, fur licking, head scraping, and rubbing) were also recorded. At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel. Injections were administered intraperitoneally 30 min prior to the test session (Pardon et al., 2000).

Statistical Analysis

The data were expressed as mean \pm SEM and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer.

RESULTS

Forced Swimming Test

Fluoxetine (10 mg/kg) and imipramine (15 mg/kg) significantly reduced immobility time. The ethanolic (200-800 mg/kg) and aqueous (160-320 mg/kg) extracts

also diminished immobility time. Safranal with a higher dose (0.5 mg/kg) and crocin at doses 50 and 600 mg/kg reduced immobility time (Figures 1 and 2). Swimming time was increased by fluoxetine and both extracts. Only one constituent of saffron, i.e., safranal increased swimming time (Figures 3 and 4). Climbing time was increased by imipramine and both extracts. Safranal with a higher dose (0.5 mg/kg) and crocin at doses 50 and 600 mg/kg increased climbing time (Figures 5 and 6).

Open Field Activity

The aqueous extract and safranal (a high dose) decreased the total locomotion and the ethanolic extract and crocin with higher doses increased leaning and grooming activities (Tables 1 and 2).

DISCUSSION

In the forced swimming test (FST), the aqueous and ethanolic extracts of saffron and its constituents, crocin and safranal showed antidepressant activities as they decreased the immobility time.

Agents which decrease the immobility time in FST have antidepressant effects (Porsolt et al., 1978). The selective serotonin reuptake inhibitors such as fluoxetine (Lucki, 1997; Cryan and Lucki, 2000) and selective noradrenaline reuptake inhibitors such as reboxetine (Cryan et al., 2002) increase swimming and immobility times, respectively.

The aqueous and ethanolic extracts as well as safranal increased swimming time. This indicates that the extracts and safranal amplified the synaptic serotonin (Lucki, 1997; Cryan and Lucki, 2000).

Climbing time was increased by crocin and the high dose of safranal which implies these agents augmented the synaptic noreadrenaline (Cryan et al., 2002).

In the open field activity test, the ethanolic extract and safranal increased stereotypic activities. This may be related to the augmentation of dopaminergic system (Rodenburg et al., 2003). Some antidepressant agents such as bupropion act partially via the dopamine reuptake inhibition (Cooper et al., 1980).

It is reported that the antidepressant activity of *Hypericum perforatum* is also mediated via serotonergic, noradrenergic and dopaminergic system activation (Calapai et al., 2001).

The results indicate that *C. sativus* extracts and their constituents, crocin and safranal showed antidepressant activities. This antidepressant action may be mediated by serotonergic, noradrenergic and dopaminergic system activation.

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Tables

Table 1. Effect of *C. sativus* stigma aqueous and ethanolic extracts on locomotor activities in the open field test.

	Total locomotion	Rearing	Leaning	Grooming
Saline 10 ml/Kg	151.1 ± 5.8	2.6 ± 0.4	3.25 ± 0.5	3.5 ± 0.5
Imipramine 15 mg/Kg	181 ± 9.7	12 ± 1.3 ***	25.25 ± 0.8 ***	7.3 ± 0.6
Eth. Ext. 200 mg/Kg	143 ± 17.6	9.5 ± 1.6 ***	11.4 ± 2.8	5.9 ± 0.7
Eth. Ext. 400 mg/Kg	165.4 ± 15.9	1.1 ± 0.3	25.3 ± 2.3 ***	4.8 ± 0.7
Eth Ext. 800 mg/Kg	177.2 ± 2.9	1.9 ± 0.5	20.5 ± 10 ***	7.9 ± 1.4 *
Aqu. Ext. 80 mg/Kg	141.4 ± 14.7	0 ± 0	0.4 ± 0.2	1.3 ± 0.5
Aqu. Ext. 160 mg/Kg	34.5 ± 15.6 ***	1.7 ± 0.6	2.1 ± 1.1	2.9 ± 0.7
Aqu. Ext. 320 mg/Kg	21.8 ± 9.2 ***	4.9 ± 0.8	12.3 ± 1.8 *	3.6 ± 0.9

Agents were administered to mice intraperitoneally 30 min prior to the test. Mice behaviors were observed for 10 min. Values are the mean ± S.E.M. for 10 mice; *p<0.05, ***p<0.001, as compared to saline, Tukey-Kramer test.

Table 2. Effect of safranal and crocin on locomotor activities in the open field test.

	Total locomotion	Rearing	Leaning	Grooming
Saline 10 ml/Kg	151.1 ± 5.8	2.6 ± 0.4	3.25 ± 0.5	3.5 ± 0.5
Imipramine 15 mg/Kg	181 ± 9.7	12 ± 1.3 ***	25.25 ± 0.8 ***	7.3 ± 0.6
Crocin 50 mg/Kg	199.7 ± 63.7	7.2 ± 7.1	18 ± 3.7	6.5 ± 3.3
Crocin 200 mg/Kg	189.6 ± 72.9	6.3 ± 5.3	21 ± 14.8 ***	7.4 ± 4.3
Crocin 800 mg/Kg	203.5 ± 37.2	8.2 ± 3.9	19.5 ± 2.7 ***	12.1 ± 3.3***
Safranal 0.15 mg/Kg	129.5 ± 13.3	0.5 ± 0.5	4.5 ± 3.8	6 ± 2.3
Safranal 0.35 mg/Kg	110.5 ± 24.5	3 ± 2	4.8 ± 3.4	4.2 ± 1.2
Safranal 0.5 mg/Kg	65.1 ± 5.2 *	0 ± 0	7.8 ± 3	7.8 ± 3

Agents were administered to mice intraperitoneally 30 min prior to the test. Mice behaviors were observed for 10 min. Values are the mean ± S.E.M. for 10 mice; *p<0.05, ***p<0.001, as compared to saline, Tukey-Kramer test.

Figures

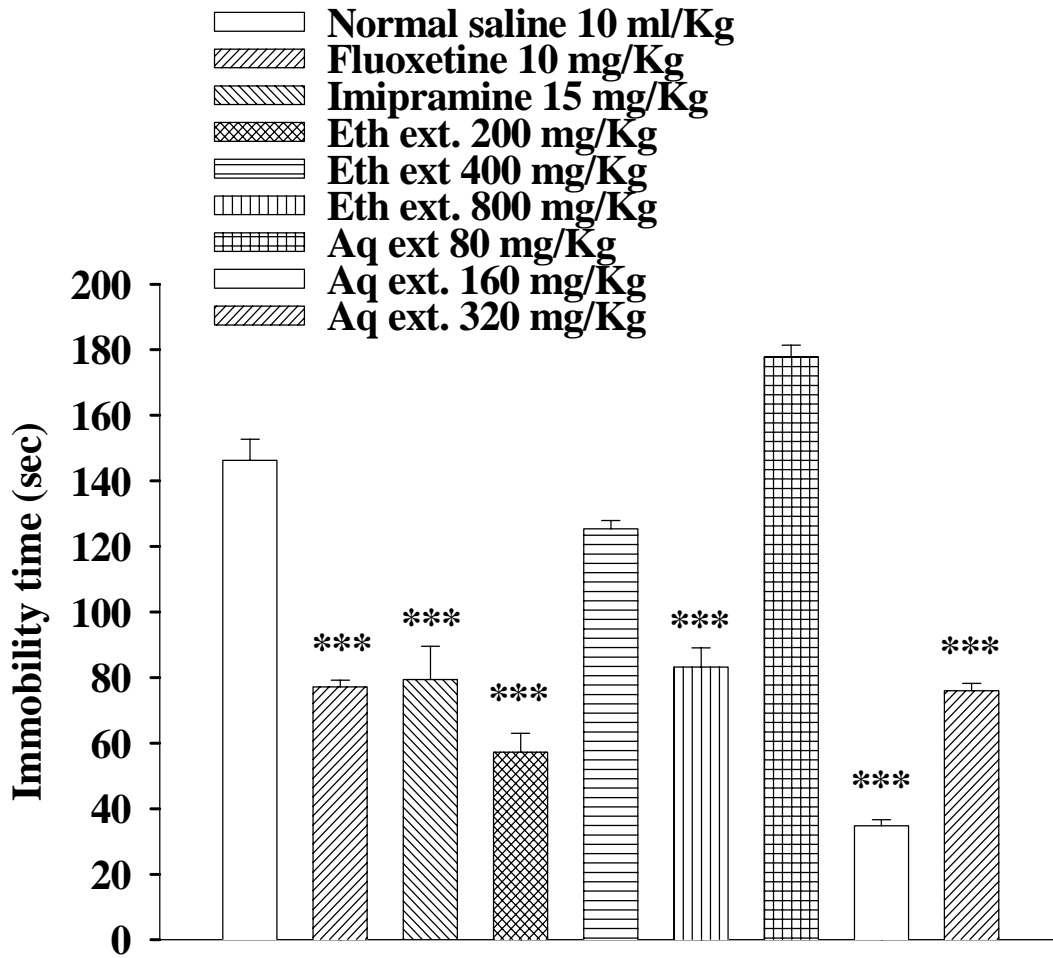


Fig. 1. Effect of *C. sativus* stigma aqueous and ethanolic extracts on immobility time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; *** $p < 0.001$, as compared to saline, Tukey-Kramer test.

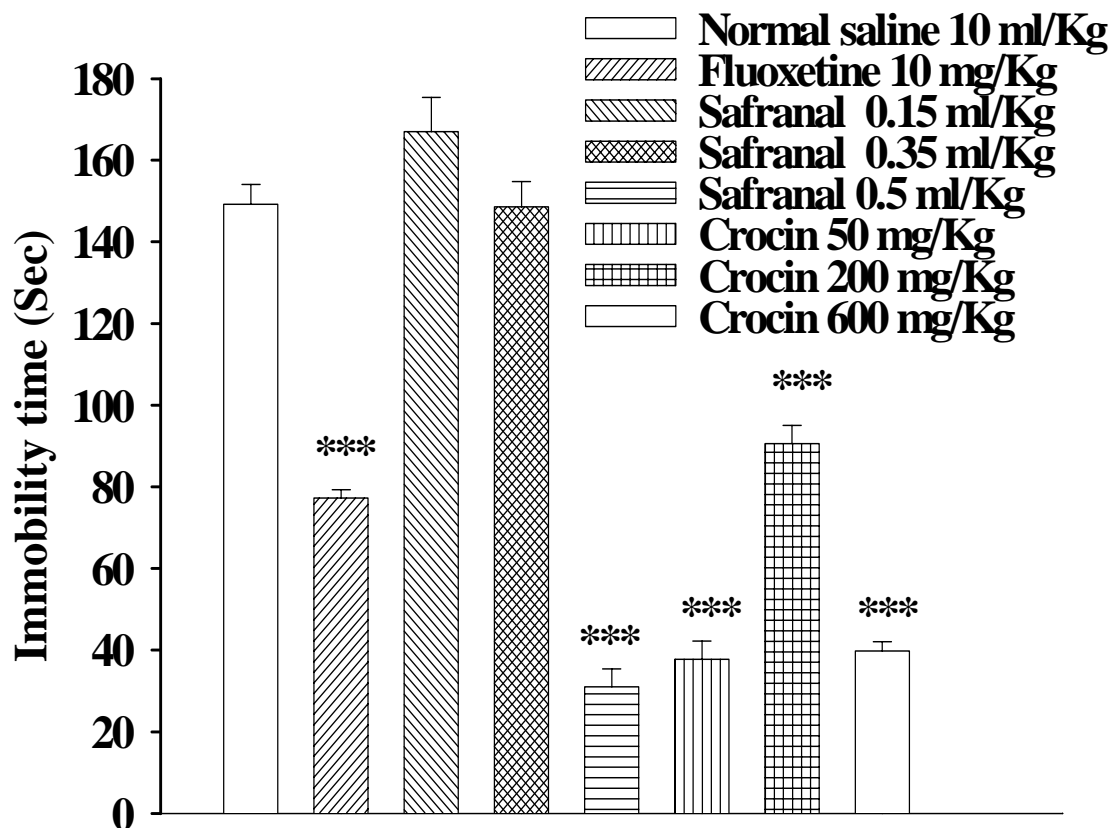


Fig. 2. Effect of crocin and safranal on immobility time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; *** p <0.001, as compared to saline, Tukey-Kramer test.

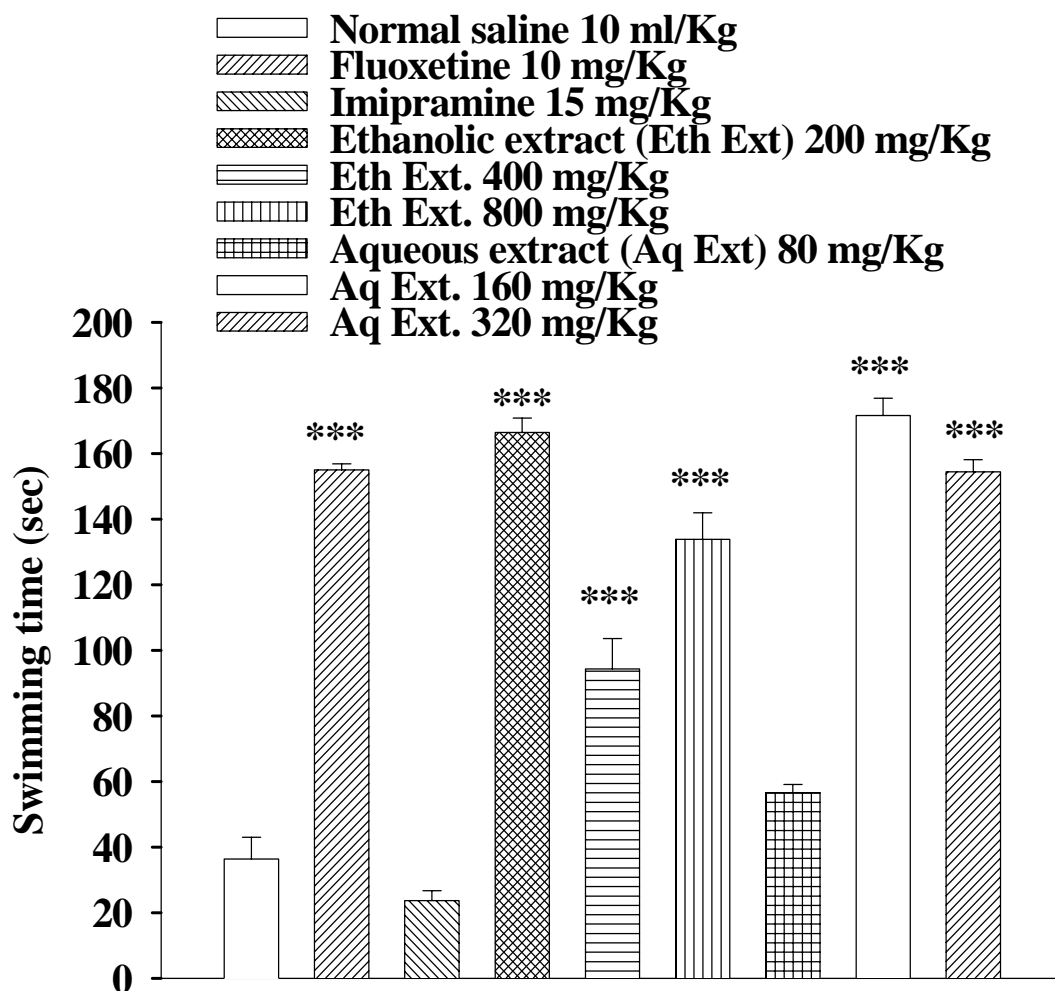


Fig. 3. Effect of *C. sativus* stigma aqueous and ethanolic extracts on swimming time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; *** p <0.001, as compared to saline, Tukey-Kramer test.

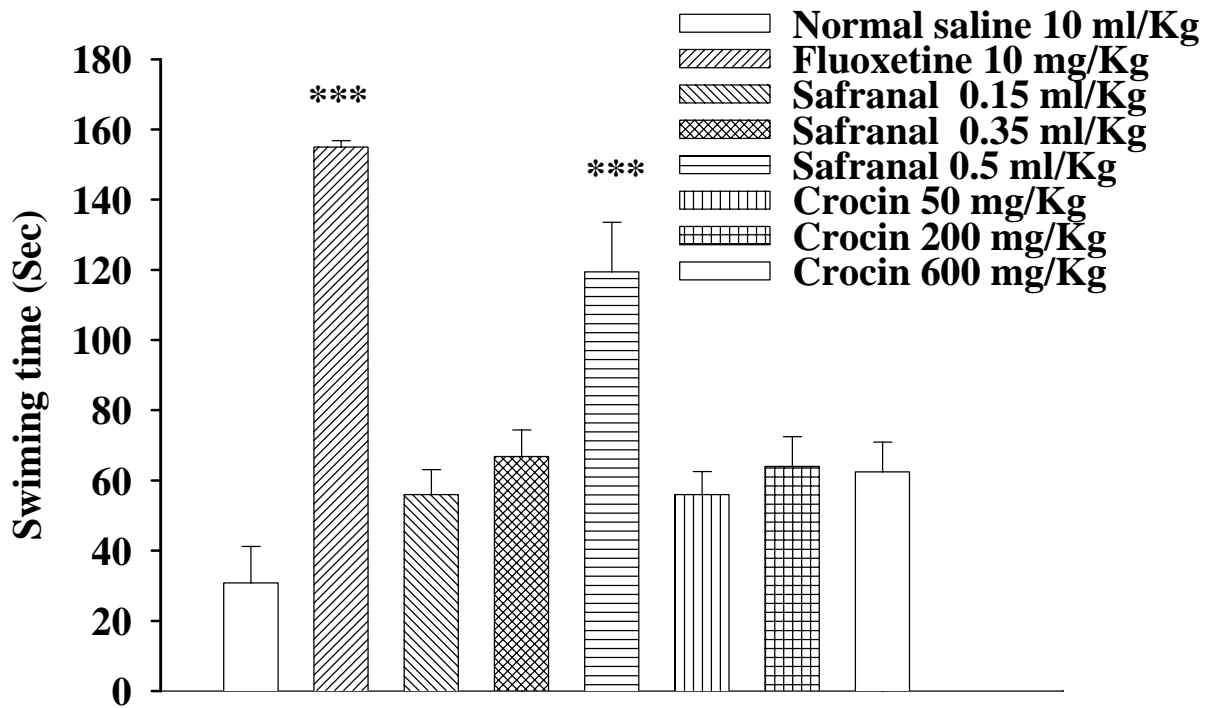


Fig. 4. Effect of crocin and safranal on swimming time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; *** p <0.001, as compared to saline, Tukey-Kramer test.

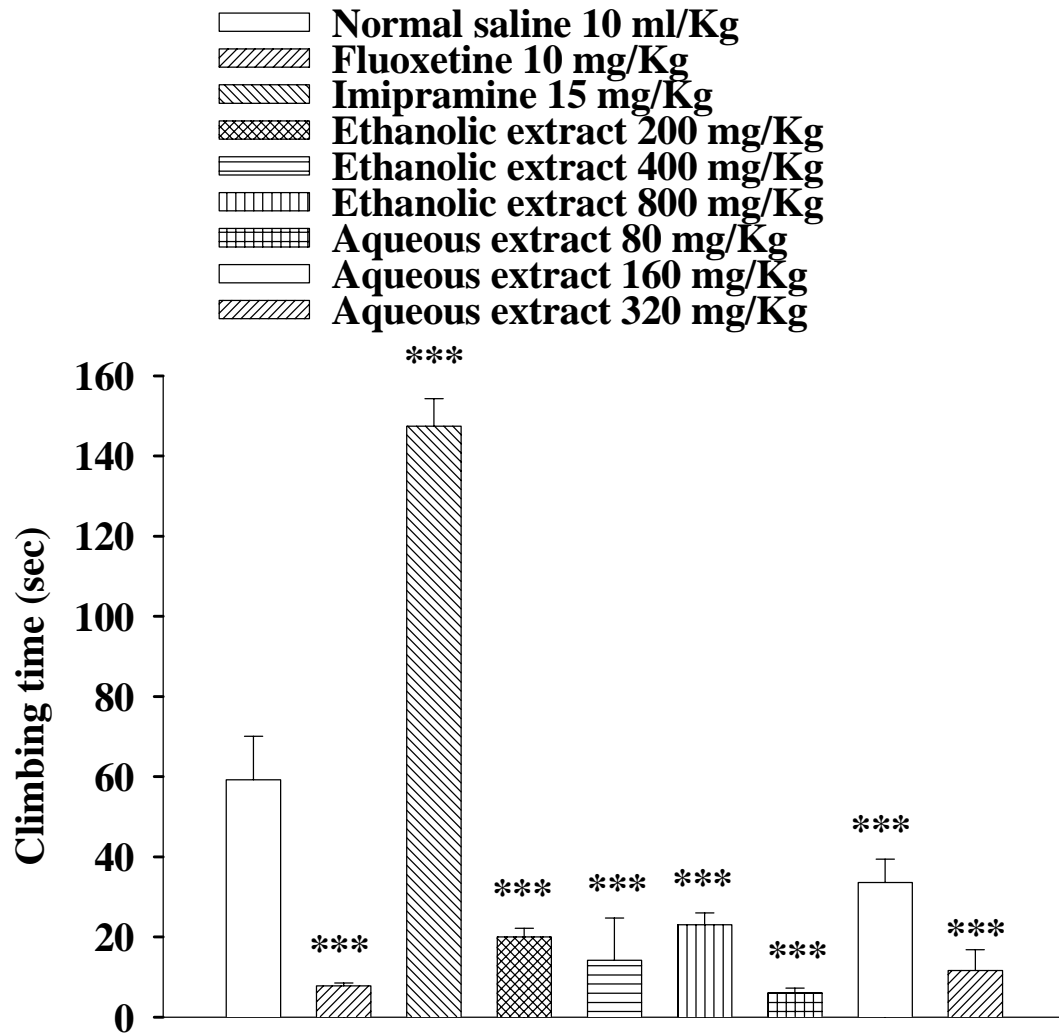


Fig. 5. Effect of *Crocus sativus* stigma aqueous and ethanolic extracts on climbing time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; *** p <0.001, as compared to saline, Tukey-Kramer test.

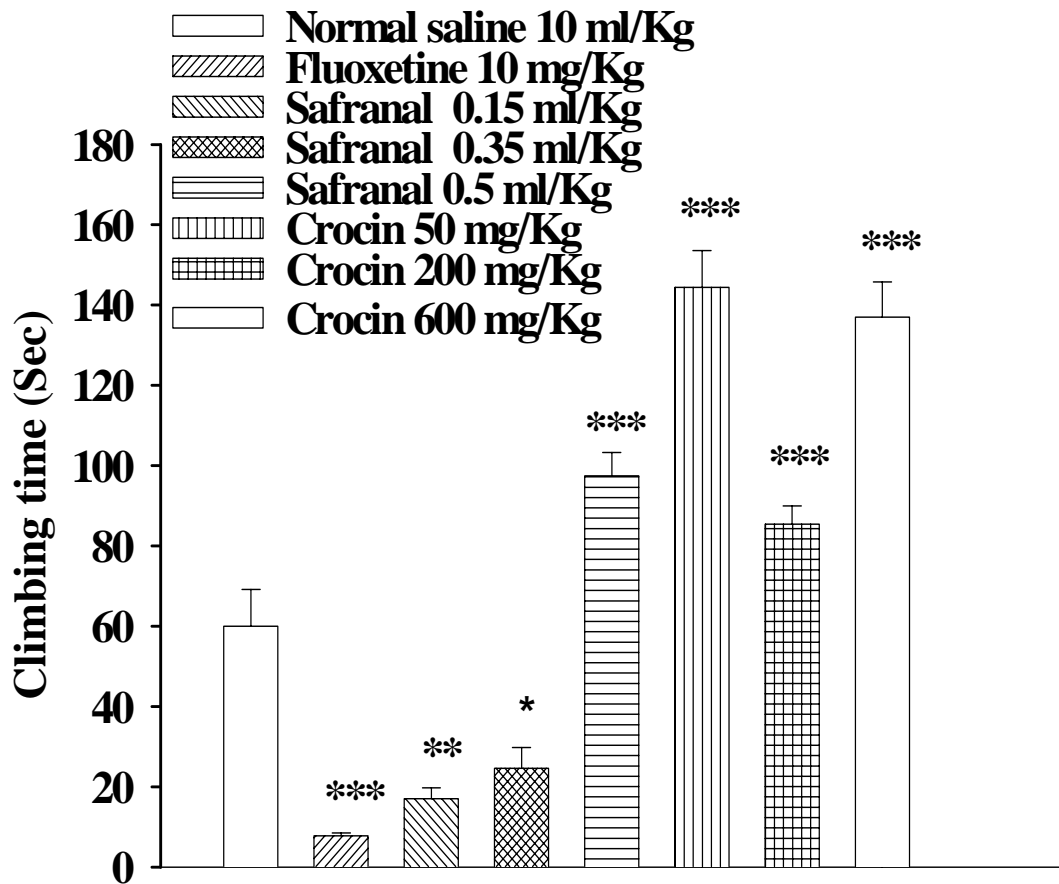


Fig. 6. Effect of crocin and safranal on climbing time in the forced swimming test. Agents were administered to mice intraperitoneally 30 min prior to the test. Values are the mean \pm S.E.M. for 5 mice; * $P < 0.05$, ** $P < 0.01$, *** $p < 0.001$, as compared to saline, Tukey-Kramer test.