http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (20): 939-944, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.939.944

Saffron (*Crocus sativus*) Ethanolic Extract and its Constituent, Safranal, Inhibits Morphine-induced Place Preference in Mice

[1]H. Ghoshooni, [2]M. Daryaafzoon, [1]S. Sadeghi-Gharjehdagi, [3]H. Zardooz, [1]H. Sahraei, [2]S.P. Tehrani, [1]A. Noroozzadeh, [1]F. Bahrami-Shenasfandi, [1]G.H. Kaka and [1]S.H. Sadraei
[1]Neuroscience Research Center, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran
[2]Department of Psychology, Tehran University, Tehran, Iran
[3]Neuroscience Research Center, Department of Physiology,
Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract: The effects of saffron ethanolic extract and its constituent, safranal, on the acquisition and expression of morphine-induced place preference (CPP) in male Swiss Webster mice (20-25 g) were investigated in the present study. An unbiased place conditioning method was applied for assessment of morphine reward properties. The saffron extract and safranal were administered intraperitoneally (i.p.) during (acquisition) or after induction (expression) of morphine CPP. In a pilot study, the extract and safranal were alone administered to the animals to assess if they have any reward properties. Subcutaneous (s.c.) of morphine (4 and 8 mg kg^{-1}) and extract (50 mg kg^{-1}; i.p.) induced CPP. Extract (10, 50 and 100 mg kg^{-1}; i.p.) reduced the acquisition and expression of morphine CPP. The same results were obtained when safranal (1, 5 and 10 mg kg^{-1}, i.p.) was used. It may be concluded that both ethanolic saffron extract and safranal can inhibit the acquisition and expression of morphine-induced CPP in the mice.

Key words: Morphine, conditioned place preference, safranal, mice, saffron

INTRODUCTION

Opioid dependence has become one of the major problems worldwide. Humans use opioids seeking pleasure or avoiding stress (Cami and Farre, 2003). The dopaminergic projections originating from the ventral tegmental area and projecting to the nucleus accumbens are considered as the main biological substrate of the reinforcing and stimulant effects of opioids (Di Chiara, 2002). Opioids produce their effects by reducing tonic inhibition of the dopaminergic neurons through actions at μ-opioid receptors on GABAergic interneurons (Johnson and North, 1992). Data also confirmed that morphine elevates the extra cellular concentration of dopamine in the nucleus accumbens (Pontieri *et al*., 1995). Unfortunately, despite of developments occurred in our understanding about how opioids function in the central nervous system, the problem of opioid addiction remains unresolved. Several therapeutic strategies have developed for opioid dependence (Cami and Farre, 2003). In this regard, studies have shown that natural products such as the extracts of medicinal herbs which have good efficacy and low toxicity can be use for opioid abuse (Pourmotabbed *et al*., 2004; Sahraei *et al*, 2006).

Saffron, *Crocus sativus* L. (Iridaceae), is used in folk medicine for aphrodisiac, antispasmodic, expectorant and antidepressant (Sarris, 2007). Recent studies have demonstrated that saffron extract and its constituent crocin, shows interactions with morphine reward properties (Imenshahidi *et al*., 2011; Khakpour *et al*., 2008; Mobasher *et al*., 2006; Mojabi *et al*., 2008a, b; Sahraei *et al*., 2007, 2008). Interestingly, these investigators have shown that the extract may interact with the neural elements located in the shell part of the nucleus accumbens (Mojabi *et al*., 2008b). On the other hand, Hosseinzadeh and Younesi, (2002) have shown that the extract and safranal and crocin can reduce the signs of morphine withdrawal syndrome in mice (Hosseinzadeh and Jahanian, 2010).

Chemical studies on *Crocus sativus* have shown the presence of constituents such as crocin, crocetin, safranal and picrocrocin. Among the constituents of saffron extract, safranal is considered as one of the main element responsible for some of these pharmacological activities (Schmidt *et al*., 2007). However, there is no

Corresponding Author: H. Zardooz, Nurosciance Research Center, Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, P.O. Box 19395-6558, Tehran, Iran
Tel/Fax: (+9821) 22281561

study considering the effects of the ethanolic extract of *Crocus sativus* and safranal on rewarding effects of morphine. In the present study, the effects of peripheral administrations of ethanolic extract of *Crocus sativus* stigma and safranal on the acquisition and expression of morphine-induced CPP in male mice were investigated.

MATERIALS AND METHODS

Animals: Male Swiss-Webster mice (20-25 g, Pasture Institute, Tehran, IRAN) were used throughout the study (6-8 mice for each experiment). Animals were housed in groups of 10 per cage in a 12/12 h light-cycle (lights on at 07.00 a.m), with *adlib* food and water available. The animals were randomly allocated to different groups of the experiment. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 81/021, July 10, 2002).

Drugs: The following drugs were used in these experiments: morphine sulfate (TEMAD, Iran), safranal (Fluka, Germany). The drugs were dissolved in sterile saline. Morphine was injected subcutaneously (s.c) to the animals in a volume of 10 mL kg^{-1}. The extract and safranal were given intraperitoneally in a volume of 10 mL kg^{-1} and was prepared before use. The control groups received saline.

Plant material: The saffron used in this study was dedicated by Talakaran-E- Mazraeh agricultural Co. (Torbat Heydarieh, Khorasan-e-Razavi, Iran). The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran) and a voucher specimen coded P-408 has been deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The part of *Crocus sativus* that were being used as additive and also herbal medicine was stigma. The stigma's extract was prepared as follow: 100 g of dried and milled stigmas were extracted with 1000 mL ethanol 100% by maceration procedure. The extract was dried by evaporation in temperature between 35 and 40°C. The yield of extraction was 12 mg of freeze-dried powder for 100 mg of the dry stigma. The extract was dissolved in normal saline and was immediately administered to the animals.

Quantification of safranal in saffron extract: The quantifying method for safranal in saffron extract was used elsewhere (Hosseinzadeh and Jahanian, 2010), with modifications. The extract doses were used in these experiments was standardized according to its safranal content.

Apparatus: A two compartment CPP apparatus (15×15×30 cm) was used in these experiments. The apparatus was made of wood. Both compartments were identical in size (the apparatus was divided into two equal-sized compartments by means of a removable white guillotine door) and shading (both were white) but distinguishable by texture, olfactory and visual cues. To provide the tactile difference between the compartments, one of the compartments had a smooth floor, while the other compartment had a nylon white mesh floor. A drop of menthol was placed at the right center of the compartment with a textured (nylon mesh) floor, to provide the olfactory difference between the compartments. For visual differences, the compartments were differently striped black on their sides. In this apparatus, mice showed no consistent preference for either compartment, which supports our un-biased CPP paradigm.

Behavioral testing: Each conditioning session consists of 5 days. On the first day of the experiments, each mouse was placed separately into the apparatus for 10 min, with free access to all compartments and the time spent by mice in each compartment was measured. In the second phase which consisted of a 3-day schedule animals received three trials in which they experienced the effects of the morphine while confined in one compartment for 45 min and three trials in which they experienced the effects of saline while confined in the other compartment for 45 min. Access to the other compartments was blocked on these days. On the 5th day (the preference test day) the partition was removed and the mice could access the entire apparatus. The mean time for each mouse spent in either compartment during a 10 min period was determined as the preference criterion. No injection was given during the acquisition tests.

Experimental design

Experiment 1: Dose-response effects of place conditioning produced by morphine, saffron extract and safranal: In these experiments, we established a dose-response function for morphine, saffron extract and safranal on place conditioning paradigm. Morphine (1, 2, 4 and 8 mg kg^{-1}, s.c.), saffron extract (10, 50 and 100 mg kg^{-1}, i.p.) and safranal (1, 5 and 10 mg kg^{-1}, i.p.) were tested for producing place preference. In order to confirm that the injection and conditioning schedules did

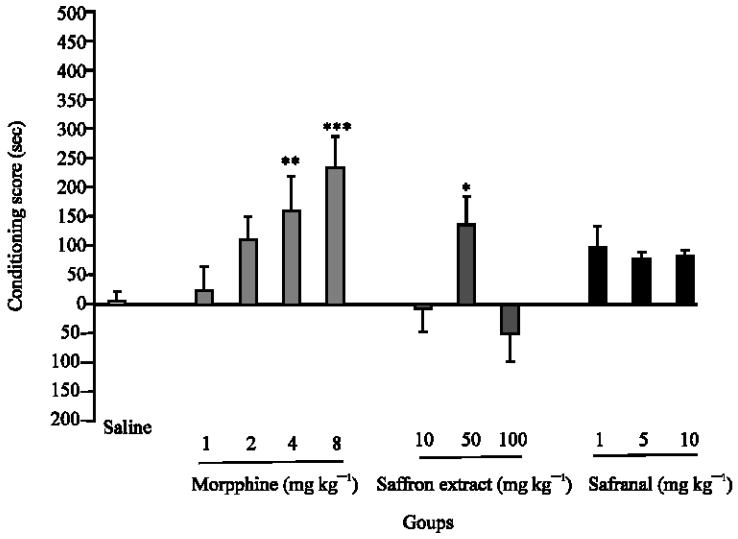

Fig. 1: Conditioned place preferences induced by morphine (a) and *Crocus sativus* L. extract (b). Each point shows the Mean±SEM of conditioning score for 6-8 rats, *p<0.05 *p<0.05, **p<0.01, ***p<0.001 different from the saline control groups

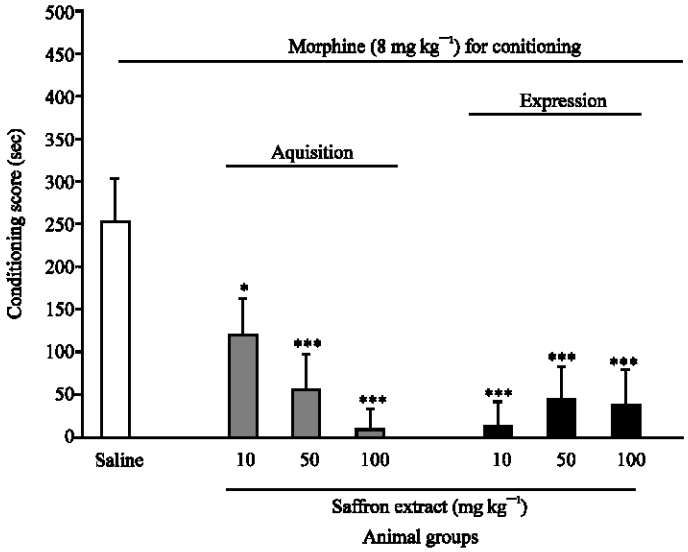

Fig. 2: Effects of different doses of the extract of *Crocus sativus* on the acquisition (a) and expression (b) of morphine-induced CPP. Each point is the Mean±SEM for 6-8 rats, *p<0.05, **p<0.01, ***p<0.001 different from the saline control groups

not affect the time spent in the compartments, three separate groups of animals received saline (10 mg kg^{-1}, s.c. or i.p.) in two compartments. These groups were used as control (Fig. 1).

Experiment 2: Effects of the saffron extract and safranal on the acquisition of conditioned place preference induced by morphine: To test the effects of saffron extract and safranal on the acquisition of place preference induced by morphine, seven groups of animals received either saline (10 mg kg^{-1}, i.p.), the extract (10, 50 and 100 mg kg^{-1}, i.p.), or safranal (1, 5 and 10 mg kg^{-1}, i.p.) and 30 min later was injected with morphine (8 mg kg^{-1}, s.c.) during the conditioning session (Fig. 2).

Experiment 3: Effects of the saffron extract and safranal on the expression morphine-induced conditioned place preference: In order to examine the possible influence of

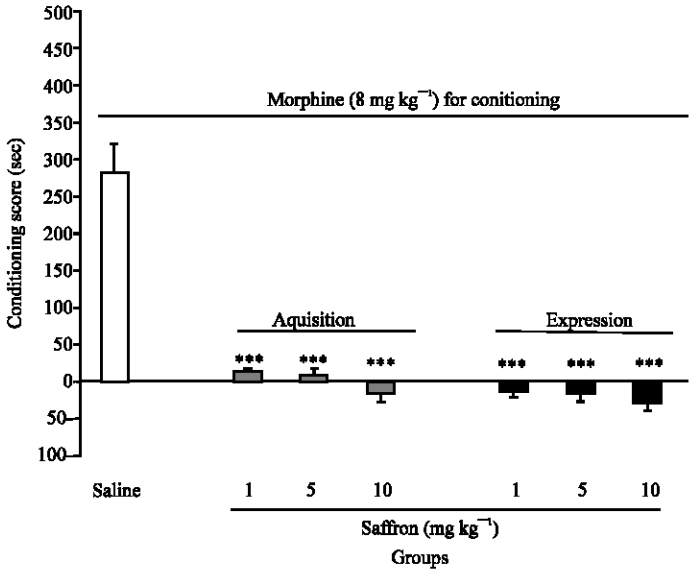

Fig. 3: Effects of intra-nucleus accumbens administration of the *Crocus sativus* methanolic extract (1, 5 and 10 μg rat) on the acquisition (a) and expression (b) of morphine-induced CPP. Each point is the Mean±SEM for 6-8 rats, **p<0.01, ***p<0.001 different from the control groups

saffron extract and safranal on the expression of morphine-induced place preference, seven groups of the animals were conditioned with morphine (8 mg kg^{-1}, s.c.) and tested 24 h later. They received either saline (10 mg kg^{-1}, i.p.) as control or different doses of saffron extract (10, 50 and 100 mg kg^{-1}, i.p.), or safranal (1, 5 and 10 mg kg^{-1}, i.p.) 30 min before the test session (Fig. 3).

Data analysis: Conditioning score represents the time (in seconds) spent in drug-paired compartment minus the time spent in the saline-paired compartment was calculated for each animal and expressed as Mean±SEM. Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Newman-Keuls. Differences with $p<0.05$ were considered significant.

RESULTS

Morphine, saffron extract and safranal dose-response on CPP paradigm: The effects of morphine, saffron extract and safranal are shown in Fig. 1. Injection of morphine (4 and 8 mg kg^{-1}, s.c.) to mice caused a significant CPP [$F(4, 32) = 3.25$, $p<0.01$]. Subcutaneous injection of saline to the animals (saline control group) did not produce any preference or aversion for either place. I addition, administration of different doses of the extract (10, 50 and 100 mg kg^{-1}; i.p.) also induced place preference [$F(3, 20) = 2.11$, $p<0.05$], while safranal had no effect [$F(3, 20) = 0.723$, $p>0.05$] (Fig. 1).

Effects of systemic injections of saffron extract on the acquisition and expression of morphine CPP: Different doses of the extract (10, 50 and 100 mg kg^{-1}) was administered either 30 min before the morphine (8 mg kg^{-1}, s.c.) injection in the conditioning period of the experiments (acquisition) or 30 min before the test in post conditioning phase (expression) (Fig. 2). The control animal groups received sterile saline (1 mL kg^{-1}) instead of the extract. Present results indicated that both the acquisition and expression of morphine CPP were reduced significantly [$F(3, 25) = 28.54$, $p<0.0001$] and [$F(3, 27) = 20.357$, $p<0.0001$], respectively.

Effects of systemic injections of safranal on the acquisition and expression of morphine CPP: Different doses of safranal (1, 5 and 10 mg kg^{-1}, i.p.) was administered either 30 min before the morphine (8 mg kg^{-1}, s.c.) injection in the conditioning period of the experiments (acquisition) or 30 min before the test in post conditioning phase (expression) (Fig. 3). The control animal groups received sterile saline (1 mL kg^{-1}) instead of safranal. Our results indicated that both the acquisition and expression of morphine CPP were reduced by safranal pre-administration [$F(3, 20) = 66.2$, $p<0.0001$ and $F(3, 24) = 48.24$, $p<0.0001$], respectively.

DISCUSSION

The results obtained from these experiments indicate that both saffron ethanolic extract and safranal may

interact with the positive reinforcement properties of morphine and suppress both the acquisition and expression of morphine-induced place preference and provide the animals' response to place aversion. Furthermore, the ethanolic extract of *Crocus sativus* but not safranal per se enables to induce place preference.

The present study revealed that animals exhibit a marked preference for the environment associated with administration of morphine. Previous studies revealed that morphine can activate μ-opioid receptors located on the GABAergic interneurons plasma membrane in the Ventral Tegmental Area (VTA) and inhibit their tonic inhibition on dopaminergic neurons and increase dopamine release in the nucleus accumbens and induce reward (Johnson and North, 1992; Pontieri *et al*., 1995). In addition, previous studies have indicated that saffron water extract may have rewarding properties as shown by place conditioning paradigm and locomotor activity (Mobasher *et al*., 2006; Sahraei *et al*., 2007; Sahraei *et al*., 2008; Khakpour *et al*., 2008; Mojabi *et al*., 2008a, b). More recently Shams *et al*., (2010) have shown that saffron extract can increase dopamine concentration in the rat brain. Since as mentioned above the abused drugs such as morphine induce their reward properties by increasing dopamine release in the brain, it is likely that saffron extract can induce place preference by similar mechanism. However, safranal did not induce place preference or place aversion in our study. The drug cannot induce dopamine release in the rat brain in the previous study (Shams *et al*., 2010). In addition, there is no study concerning the effects of the safranal on brain reward system.

In the next part of the experiments, peripheral administration of the extract has reduced the acquisition and expression of morphine CPP. One may conclude that the extract interacts with reward properties of morphine in the way that inhibits the acquisition of morphine CPP and as a result the animals do not feel or receive reward properties of morphine when the opioid injection is matched with the extract.

The extract may induce dopamine release in the brain (Shams *et al*., 2010). Considering the role of dopamine in morphine reward (Pontieri *et al*., 1995), it is anticipated that the extract could enhance the acquisition and expression of morphine CPP. It must be noted that increase in brain dopamine activity can reduce morphine reward properties in rat (Karami, *et al*., 2002). However, the opposite effect has been obtained in the present study is in agreement with our previous data about the effects of the saffron water extract on morphine CPP in mice (Mobasher *et al*., 2006; Sahraei *et al*., 2007, 2008; Khakpour *et al*., 2008) and rat (Mojabi *et al*., 2008a, b). In addition Imenshahidi and co-workers have shown that another saffron extract constituent, crocin, also can inhibit morphine CPP (Imenshahidi *et al*., 2011). Moreover, several data indicated that the extract may improve learning and memory by activation of N-Methyle-D-Aspartat (NMDA) glutamate receptors (Abe and Saito, 2000). Because it has been shown that NMDA receptor agonists and antagonists can impair the acquisition and expression of morphine CPP in the rat (Tzschentke, 2007), the same mechanism may be involved in the present study. Further researches should be focused on these contradicted results. We suggest that applying different methods including morphine self-administration may provide more clear results. Unknown pharmacokinetic interactions between morphine and the extract also may be the cause of the results obtained from the extract to reduce the acquisition of morphine CPP.

In the last part of the experiments, the results show that administration of safranal as one of the extract constituents can also reduce the acquisition and expression of morphine CPP. It is important finding which indicates the interaction between safranal and morphine reward through the mechanisms which are not fully understand. As mentioned above, safranal can increase dopamine release in the rat brain (Shams *et al*., 2010) which may safranal may interact with morphine by such mechanism. However, safranal cannot induce CPP by itself, which may indicate that above mentioned mechanism is not true for the effect observed from safranal.

CONCLUSION

It seems that the extract and safranal are effective for inhibition of the reward and/or memory mechanisms, which are activated by morphine under chronic and acute administration. Since the extract and safranal may improve the memory (Abe and Saito, 2000), it is surprising that they inhibit the acquisition and expression of morphine CPP. However, several mechanisms including NMDA, dopamine and serotonine receptor and/or systems in the brain may be activated after the extract administration (Ahmad *et al*., 2005; Akhondzadeh *et al*., 2004; Abe and Saito, 2000). Investigators have shown that all of these mechanisms are involved in morphine CPP (Tzschentke, 2007). It is possible that the extract and safranal may inhibit the acquisition and expression of morphine CPP by one or more of these mechanisms.

ACKNOWLEDGMENTS

The authors would like to thank Mr Jafari, head of the Talakaran-E- Mazraeh agricultural Co. (Torbat Heydarieh, Khorasan-e-Razavi, Iran) for kindly offering the saffron. This study was supported by grant from Neuroscience Research Center, Baqyiatallah (a.s.) University of Medical Sciences.

REFERENCES

Abe, K. and H. Saito, 2000. Effects of saffron extract and its constituent crocin on learning behavior and long-term potentiation. Phytother. Res., 14: 149-152.

Ahmad, A.S., M.A. Ansari, M. Ahmad, S. Saleem, S. Yousuf, M.N. Hoda and F. Islam, 2005. Neuroprotection by crocetin in a hemi-parkinsonian rat model. Pharmacol. Biochem. Behav., 81: 805-813.

Akhondzadeh, S., H. Fallah-Pour, K. Afkham, A.H. Jamshidi and F. Khalighi-Cigaroudi, 2004. Comparison of *Crocus sativus* L. and imipramine in the treatment of mild to moderate depression: a pilot double-blind randomized trial [ISRCTN45683816]. BMC Complement Altern. Med., 4: 12-16.

Cami, J. and M. Farre, 2003. Drug addiction. New Engl. J. Med., 4: 975-986.

Di Chiara, G., 2002. Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. Behav. Brain Res., 137: 75-114.

Hosseinzadeh, H and H.M. Younesi, 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol., 1: 7-15.

Hosseinzadeh, H. and Z. Jahanian, 2010. Effect of *Crocus sativus* L. (saffron) stigma and its constituents, crocin and safranal, on morphine withdrawal syndrome in mice. Phytother. Res., 24: 726-730.

Imenshahidi, M., H. Zafari and H. Hosseinzadeh, 2011. Effects of crocin on the acquisition and reinstatement of morphine-induced conditioned place preference in mice. Pharmacologyonnline, 1: 1007-1013.

Johnson, S.W. and R.A. North, 1992. Opioids excite dopamine neurons by hyperpolarization o local interneurons. Neuroscience, 12: 483-488.

Karami, M., M.R. Zarrindast, H. Sepehri and H. Sahraei, 2002. Role of nitric oxide in the rat hippocampal CA1 area on morphine-induced conditioned place preference. Eur. J. Pharmacol., 449: 113-119.

Khakpour, B., M. Rostampour, H. Sahraei, M. Kamalinejad and J. shams, 2008. Effects of the aqueous extract of *Crocus sativus* flower stigmata on the acquisition and expression of morphine induced motor sensitivity in male mice. Kowsar Med. J., 12: 313-321.

Mobasher, M., H. Sahraei, B. Sadeghi-Rad, M. Kamalinejad and J. Shams, 2006. The effects of the *Crocus sativus* extract on the acquisition and expression of morphine-induced conditioned place preference in mice. J. Rafsanjan Univ. Med. Sci. Health Serv., 5: 143-150.

Mojabi, N., A. Eidi, H. Sahraee, M. Kamalnejad, F. Khamseh and A. Khoshbaten, 2008a. Study of the effects of alcoholic extract of *Crocus sativus* on the acquisition and expression of morphine-induced conditioned place preference in rats. Kowsar Med. J., 13: 197-210.

Mojabi, N., A. Eidi, M. Kamalinejad, A. Khoshbaten and A. Noroozzadeh *et al*., 2008b. Study of the effects of intra-nucleus accumbens shell injections of alcoholic extract of *Crocus sativus* on the acquisition and expression of morphine-induced conditioned place preference in rats. Physiol. Pharmacol., 12: 121-128.

Pontieri, F.E., G. Tanda and G. Di Chiara, 1995. Intravenous cocaine, morphine and amphetamine preferentially increase extracellular dopamine in the shell as compared with the core of the rat nucleus accumbens. Proc. Natl. Acad. Sci. USA., 92: 12304-12308.

Pourmotabbed, A., B. Rostamian, G. Manouchehri, G. Pirzadeh-Jahromi and H. Sahraei *et al*., 2004. Effects of papaver rhoeas extract on the expression and development of morphine-dependence in mice. J. Ethnopharmacol., 95: 431-435.

Sahraei, H., S.M. Fatemi, S. Pashei-Rad, Z. Faghih-Monzavi, S.H. Salimi and M. Kamalinegad, 2006. Effects of Papaver rhoeas extract on the acquisition and expression of morphine induced conditioned place preference in mice. J. Ethnopharmacol., 103: 420-424.

Sahraei, H., J. Shams, S. Marjani, S. Molavi and M. Kamalinejad, 2007. Effects of the *Crocus sativus* L. extract on the acquisition and expression of morphine-induced behavioral sensitization in female mice. J. Med. Plants, 6: 26-35.

Sahraei, H., M. Mohammadi, M. Kamalinejad, J. Shams, H. Ghoshooni and A. Noroozzadeh, 2008. Effects of the *Crocus sativus* L. extract on the acquisition and expression of morphine-induced conditioned place preference in female mice. J. Med. Plants., 7: 39-48.

Sarris, J., 2007. Herbal medicines in the treatment of psychiatric disorders: A systemic review. Phytother. Res., 21: 703-716.

Schmidt, M., G. Betti and A. Hensel, 2007. Saffron in phytotherapy: Pharmacology and clinical uses. Wien. Med. Wochenschr., 157: 315-319.

Shams, J., M. Hedayati, F. Asefi and H. Sahraei, 2010. Water extract of saffron (*Crocus sativus*) increases brain dopamine and glutamate concentration in the rat. Eur. Neuropsychopharmacol., 20: S353-S353.

Tzschentke, T.M., 2007. Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. Addiction Biol., 12: 227-462.