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Effects of aqueous saffron extract *(Crocus sativus* L.*)* on the acquisition and reinstatement of morphine-induced conditioned place preference in mice

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Abstract

Objective: In the present study, the effects of aqueous saffron extract *(Crocus sativus L.)* on the acquisition and reinstatement of morphine-induced conditioned place preference (CPP) in mice were investigated.

Materials and Methods: Subcutaneous administration of morphine (40 mg/kg for four days) produced place preference. Intraperitoneal administration of aqueous extract (40 and 80 mg/kg for four days) 30 min before the morphine administration decreased the acquisition of morphine CPP. In other groups of animal, following extinction of a place preference induced by morphine (40 mg/kg), single administration of morphine (10 mg/kg) reinstated the place reference. The aqueous extract (80 mg/kg) 30 min before this priming dose of morphine blocked morphine-induced reinstatement of place preference.

Results: These results show that aqueous saffron extract can reduce the acquisition and reinstatement of morphine-induced conditioned place preference.

Keywords: Aqueous saffron extract, *Crocus sativus* L., CPP (conditioned place preference), Morphine, Mice

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Introduction

The rate of relapse to opioid use following periods of abstinence is very high in detoxified opioid addicts and remains as a major clinical problem in treatment of drug abuse. Drug craving is a subjective feeling which motivates human drug addicts to drug seeking and can produce relapse to drug abuse even long-time after withdrawal (O'Brien, 1997).

The mesolimbic dopamine (DA) system is the principal pathway involved in psychological dependence to opioids (Wise, 2000). Activation of this system is associated with the feeling of euphoria, which causes continuing drug abuse (Popik and Kolasiewicz, 1999). A large body of evidence indicates that the mesocorticolimbic DA system contributes to the acute reinforcing effects of opioids (Manzanedo et al., 2001; Olmstead and Franklin, 1997; Wise, 1996). Opiates activate DA neurons in the ventral tegmental area (VTA) through the inhibition of the GABAergic inhibitory interneurons, which subsequently increase the DA transmission to the nucleus accumbens (NAcc) (Do Couto et al., 2005). Dopamine antagonists (haloperidol, clozapine, risperidone and SCH 23390) have reversed Morphine-induced conditioned place preference (CPP) in mice (Manzanedo, 2001).

However, it appears that this DA pathway may not be the only one responsible for opioid reward. The VTA and NAcc received glutamatergic projections from the prefrontal cortex (PFC) and limbic areas. Biochemical studies have demonstrated the regulation of DA release by glutamateand NMDA receptors (Do Couto et al., 2005). It has been shown that memantine as a NMDA receptor antagonists, is capable of preventing the acquisition of morphine-induced CPP (Do Couto et al., 2004).

Crocus sativus L., commonly known as saffron, is a perennial stemless herb of the Iridaceae family. In modern pharmacological studies, saffron has demonstrated several pharmacological effects in central nervous system including anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Hosseinzadeh et al., 2004), anti-inflammatory and antinociceptive (Hosseinzadeh and Younesi, 2002), learning and memory-improving properties (Zhang et al., 1994; Abe et al., 1999) and reducing physical signs of morphine withdrawal (Hosseinzadeh and Jahanian, 2010). We showed that crocin, a constituent of saffron, can reduce the acquisition and reinstatement of morphine-induced conditioned place preference (Imenshahidi, et al., 2011). In a recent study it has been showed that Saffron extract can reduce the acquisition of morphine-induced CPP in mice (Sahraei, et al., 2007) but there is not any study about the effect of Saffron extract on reinstatement of morphine-induced conditioned place preference. In this study we evaluate the effect of aqueous saffron extract on acquisition and reinstatement of morphine-induced CPP.

Materials and Methods

Animals

Male NMRI mice (25–30 g) were housed in plastic cages in an animal room maintained at 21°±2° C on a 12-h dark cycle. Animals had free access to water and food except for the time of behavioral tests. The experimental protocol was approved by the "Animal Studies Ethics Committee" of the School of Pharmacy, Mashhad University of Medical Sciences and all procedures were carried out in accordance with institutional guidelines for laboratory animal care and use. Each mouse was used only once and each treatment group consisted of 7 animals.

Drugs

Animals were injected IP with crocin (Sigma), or morphine sulphate (Daru

Pakhsh, Iran), dissolved in physiological saline (NaCl 0.9%), in a volume of 0.1 ml/10 g. Control group were injected IP with physiological saline. The used doses of aqueous saffron extract had been shown to be active in previous studies (Hosseinzadeh, 2007).

Apparatus

Identical plexiglas boxes with two equal size compartments (30 length×30 width×35 height) separated by a grey central area (15 length×30 width×35 height) were used. The compartments were connected by guillotine doors. The compartments had different colored walls (black vs. white) and also distinct floor textures (fine and wide grid in the black and white compartment respectively). A drop of banana extract was placed at the corner of the black compartment floor and a drop of acetic acid at the corner of the white compartment floor, to provide the olfactory difference between the compartments. After each behavioral test or place conditioning, the whole box was cleaned to prevent interference from the smell of feces and urine.

Experimental procedure

Acquisition of place preference

Pre-conditioning phase: The experiment consists of three phases. During the first phase (pre-conditioning) mice had free access to both compartments of the apparatus for 15 min each day for 2 days. On day 3, the time spent by the animal in each compartment was recorded for 15 min. The animals showing a strong unconditioned aversion or preference (less than 33% or more than 66% of the session time) for any compartment were discarded.

Conditioning phase: In the second phase (conditioning), which had a duration of 4 days, animals received an injection of normal saline immediately before being confined to the black compartment for 1 h, and after an interval of 4 h, received the drugs immediately before confinement in the white compartment for 1 h. Confinement was carried out by closing the guillotine door that separated the two compartments. According to the treatment received during this phase (conditioning), animals were divided into 8 groups (n=7): saline+saline (SAL); saline+40 mg/kg of morphine (MOR); 40 mg/kg of morphine+10, 40, 80 mg/kg of extract (MOR+EXT); saline+10, 40, 80 mg/kg of extract (EXT); as we mentioned above, drugs were administrated immediately before confinement in the white compartment for 1 h.

Post-conditioning: During the third phase (post-conditioning), on day 8 the guillotine door was removed and the time spent by the mice in each compartment was recorded for 15 min. The time spent in the central area was proportionally divided between both conditioning compartments. The difference between the time spent in the white compartment in the post and pre-conditioning test is a measure of the degree of conditioning induced by the drug. If this difference is positive, it means that the drug has induced a preference for the drug-paired (white) compartment, while the opposite indicates the induction of an aversion. (Do Couto, 2004)

Extinction of place preference

In some other groups of animal, after performing three phases of CPP acquisition according to the protocol described above for MOR group experiment continued to evaluate the effect of extract on the reinstatement of morphine-induced CPP. For this purpose, animals underwent a 15 min daily extinction session schedule, which consisted of placement of animals in the apparatus (without guillotine doors separating the compartments) for 8 days so that the time spent in the white compartment for each group of animals became similar to those of pre-conditioning sessions.

Reinstatement of place preference

On the day following the last extinction session, a priming dose of morphine (10 mg/kg) was injected to induce a reinstatement of CPP. Thirty minutes before the priming dose of morphine, extract (10, 40, 80 mg/kg) or normal saline had been injected. After administration of the priming dose of morphine, the time spent by the mice in each compartment was recorded for 15 min similar to Post-conditioning phase.

Statistical analysis

Data of the time spent in white compartment were analyzed with analysis of variance (ANOVA). For post-hoc comparisons Tukey-Kramer test was used.

Results

Effects of aqueous saffron extract on Acquisition of place preference

As shown in Figure1, in MOR group the time spent in the white compartment was longer in post-conditioning (day 8), in comparison to Saline group suggesting that animals in this group acquired CPP after repeated administration of morphine**.** In group received the dose of 10 mg/kg of aqueous saffron extract (MOR+EXT10), there was also significant difference between the time spent in pre- and post-conditioning days. It means that the administration of saffron extract in dose 10 mg/kg during conditioning phase could not prevent acquisition of place preference. But in doses 40 and 80 mg/kg of aqueous saffron extract (MOR+EXT40; MOR+EXT80), there was no significant difference between the time spent in pre- and post-conditioning days. It means that the administration of saffron extract in doses 40 and 80 mg/kg during conditioning phase could prevent acquisition of place preference. In groups of EXT (aqueous saffron extract 10, 20 and 80 mg/kg) there was no significant difference between the time spent in pre- and post- conditioning days that means saffron extract in doses 10, 40 and 80 mg/kg could not induces place preference or aversion by itself.

Effects of aqueous saffron extract on reinstatement of place preference

As showed in Figure 2, lack of differences between the last extinction sessions (extinction) and Pre-conditioning suggests that after daily extinction sessions the conditioning has been disappeared. Administration of the priming dose of morphine could reinstate CPP. Saffron extract in dose 80 mg/kg blocked the reinstatement of place preference due to priming dose of morphine.

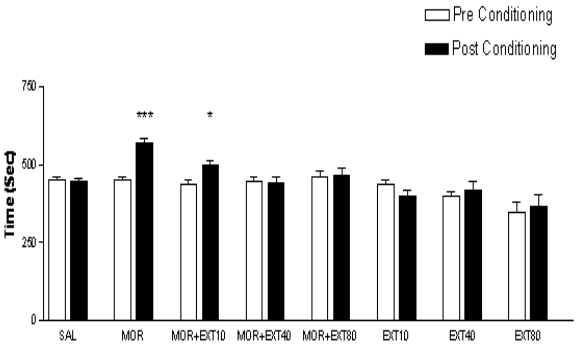

Figure 1. Effects of aqueous saffron extract on morphine-induced CPP.

During the phase of conditioning, animals received the following treatments in the drug-paired compartment: SAL, saline plus saline; MOR, saline plus 40 mg/kg of morphine; MOR+EXT10, 10 mg/kg of extract plus 40 mg/kg of morphine; MOR+EXT40, 40 mg/kg of extract plus 40 mg/kg of morphine; MOR+EXT80, 80 mg/kg of extract plus 40 mg/kg of morphine;EXT10, 10 mg/kg of extract plus saline; EXT40, 40 mg/kg of extract plus saline; EXT 80, 80 mg/kg of extract plus saline. The bars represent the time spent in the drug-paired compartment before conditioning sessions in preconditioning test (white bars) and after conditioning sessions in post-conditioning test (black bars). ***$p<0.001$, *$p<0.05$ significant difference in the time spent in the drug-paired compartment in pre-conditioning vs. post-conditioning sessions tests.

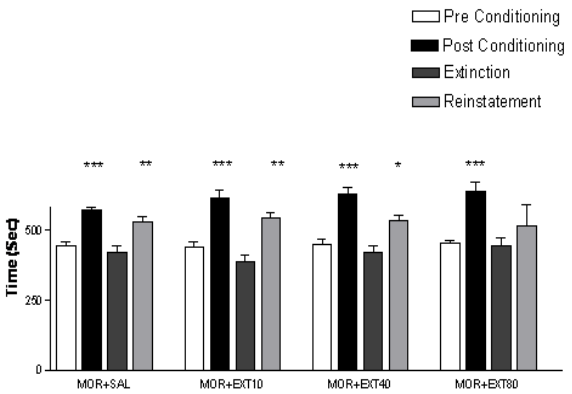

Figure 2. Effects of aqueous saffron extract on the reinstatement of morphine induced CPP. After acquisition and extinction of morphine-induced CPP, during reinstatement phase, animals received priming dose of morphine as following treatments as : MOR+SAL, morphine (10 mg/kg) plus saline; MOR+EXT10, morphine (10 mg/kg) plus extract (10mg/kg); MOR+EXT40, morphine (10 mg/kg) plus extract (40mg/kg); MOR+EXT80, morphine (10 mg/kg) plus extract (80mg/kg). The bars represent the mean (±S.E.M.) time spent in the drug-paired (white) compartment before conditioning sessions (white bars), after conditioning sessions (black bars), in the last extinction session (dark grey bars) and in the reinstatement test (light grey bars). For extinction, animals underwent a 15 min daily extinction session schedule, which consisted of the placement of animals in the apparatus (without guillotine doors separating the compartments) for 8 days so that the time spent in the white compartment for each group of animals became similar to those of pre-conditioning sessions. In reinstatement phase, a priming dose of morphine (10 mg/kg) was injected to induce a reinstatement of CPP. ***$p<0.001$, **$p<0.01$, *$p<0.05$ significant difference in the time spent in pre-conditioning vs. post-conditioning sessions or reinstatement tests.

Discussion

In this study we showed that aqueous saffron extract inhibits morphine-induced CPP. Aqueous saffron extract by itself produced neither CPP nor CPA. Moreover, using a morphine prime to induce reinstatement, we observed that aqueous saffron extract can block the morphine-induced re-approach to the morphine-paired compartment.

Saffron has demonstrated several pharmacological effects in central nervous system including anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Hosseinzadeh et al., 2004), anti-inflammatory and antinociceptive (Hosseinzadeh and Younesi, 2002), learning and memory-improving properties (Zhang et al., 1994; Abe et al., 1999) and reducing physical signs of morphine withdrawal (Hosseinzadeh and Jahanian, 2010). It has been showed that crocin, a constituent of saffron, can reduce the acquisition and reinstatement of morphine-induced conditioned place preference (Imenshahidi, et al., 2011). Also in another study it has been showed that Saffron extract can reduce the acquisition of morphine-induced CPP in mice (Sahraei, et al., 2007) but there is no study about the effect of Saffron extract on reinstatement of morphine-induced conditioned place preference.

By comparing our result to the result of previous work, we can conclude that in both studies saffron reduces the acquisition of morphine-induced CPP in mice but the effective dose of aqueous saffron extract in our study was 40 mg/kg while in the work of Sahraei and et al., they reported 100 mg/kg as the effective dose. This difference could be related to the yield of extraction in our source plant. In the present study we also showed for the first time that aqueous saffron extract could block the reinstatement of place preference.

It has been shown that aqueous saffron extract could interact with dopaminergic system in CNS (Shafique et al., 2005) and dopaminergic pathways in the ventral tegmental area had an important role in morphine induced CPP (Manzanedo et al., 2001).

Antagonists of NMDA receptor can also prevent morphine induced CPP (Do Couto et al., 2004). Saffron extract has interaction to NMDA receptors that may have role in the effects of saffron on CPP. (Lechtenberg

et al., 2008). Imipramine as a tricyclic antidepressant can reverse morphine-induced CPP in mice (Zarrindast et al., 2002) and Saffron extract has also shown antidepressant activity (Akhondzadeh et al., 2004; Hosseinzadeh, 2004). It is possible that saffron extract also act via a similar mechanism to prevent morphine-induced CPP. Saffron possesses a sedative effect in mice that may be induced via Gabaergic system. (Zhang et al., 1994; Pitsikas et al., 2008). It has been shown that administration of the GABA(A) receptor agonist, muscimol and GABA(B) receptor agonist, baclofen significantly inhibit the morphine-induced CPP and administration of the GABA(A) receptor antagonist, bicuculline in combination with an ineffective dose of morphine elicits a significant CPP Effect (Rezayof et al., 2006; Sahraei et al., 2009). Therefore, Gabaergic system may be involved in this effect of saffron.

In the present study we also showed for the first time that aqueous saffron extract in dose of 80 mg/kg blocks the reinstatement of place preference due to priming dose of morphine. As we mentioned above, NMDA receptor antagonists can show similar effect and saffron extract has interaction to NMDA receptors that may have role in the effects of saffron on reinstatement of CPP (Lechtenberg et al., 2008).

In summary, we have observed that aqueous saffron extract can block morphine-induced CPP and also the reinstatement of place preference due to priming dose of morphine. On the other hand aqueous saffron extract produced neither CPP nor CPA. These results suggest that saffron could be clinically evaluated as a treatment for addiction to opiates in humans.

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