

Effects of Crocin on the Acquisition and Reinstatement of Morphine-induced Conditioned Place Preference in Mice

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Summary

In the present study, the effects of crocin, an active component of *Crocus sativus*, on the acquisition and reinstatement of morphine-induced conditioned place preference (CPP) in mice were investigated. Subcutaneous administration of morphine (40 mg/kg for four days) produced place preference. Intraperitoneal administration of crocin (600 mg/kg for four days) 30 min before the morphine administration decreased the acquisition of morphine CPP. In other groups of animals, following extinction of a place preference induced by morphine (40mg/kg), single administration of morphine (10mg/kg) reinstated the place preference. Crocin (400 and 600 mg/kg) 30 min before this priming dose of morphine blocked morphine-induced reinstatement of place preference. These results showed that crocin can reduce the acquisition and reinstatement of morphine-induced conditioned place preference.

Keywords: Crocin, Saffron, *Crocus sativus*, CPP (conditioned place preference), Morphine

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 (1).

The mesolimbic dopamine (DA) system is the principal pathway involved in psychological dependence to opioids (2). The activation of this system is associated with the feeling of euphoria, which causes continuing drug abuse (3). A large body of evidence indicates that the mesocorticolimbic DA system contributes to the acute reinforcing effects of opioids (4, 5, 6). 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) (7)..

Dopamine antagonists (haloperidol, clozapine, risperidone and SCH 23390) have reversed Morphine-induced conditioned place preference (CPP) in mice (4).

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 glutamate and NMDA receptors (7). It has been shown that memantine as a NMDA receptor antagonists, is capable of preventing the acquisition of morphine-induced CPP (8).

Crocus sativus L., commonly known as saffron, is a perennial stem less herb of the Iridaceae family. In modern pharmacological studies, saffron has demonstrated several pharmacological effects in central nervous system including anticonvulsant (9), antidepressant (10), anti-inflammatory and antinociceptive (11), learning and memory-improving properties (12, 13) and reducing physical signs of morphine withdrawal (14). In a recent study it has been showed that Saffron extract can reduce the acquisition of morphine-induced CPP in mice (15).

Crocin is a constitute of saffron that is responsible for some CNS effects of this plants including anxiolytic-like effect (16), antidepressant activity (10), aphrodisiac activity (17), learning and memory-improving properties (18) and reducing physical signs of morphine withdrawal (14). In this study we evaluate the effect of crocin on morphine-induced CPP. We also study the effect of crocin in the reinstatement of morphine-induced CPP with morphine primes.

Materials and Methods

Animals

Male NMRI mice (25–30 g) were housed in plastic cages in an animal room maintained at $21^{\circ} \pm 2^{\circ} \text{C}$ on a 12-h dark cycle. Animals had free access to water and food except during 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 with physiological saline. The doses of crocin used had been shown to be active in previous studies (14).

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 have 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 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+200, 400, 600 mg/kg of crocin (MOR+CRS); saline+200, 400, 600 mg/kg of crocin (CRS); as we mentioned above, drugs were administered 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. (8)

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 (saline+40 mg/kg of morphine)) experiment continued to evaluate the effect of crocin on the reinstatement of morphine-induced CPP. For this purpose, 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 session.

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 min before the priming dose of morphine, crocin (200, 400, and 600) or normal saline had been injected. After the 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 were presented as mean \pm SEM. Data of the time spent in white compartment were analyzed with analysis of variance (ANOVA). For post-hoc comparisons Tukey Kramer tests were used. Statistical significance was defined as $p < 0.05$.

Results

Effects of crocin on Acquisition of place preference

As can be seen in figure 1, in MOR group the time spent in the white compartment is higher in post-conditioning (day 8), in comparison to saline group suggesting that animals in this group acquired CPP after repeated administration of morphine.

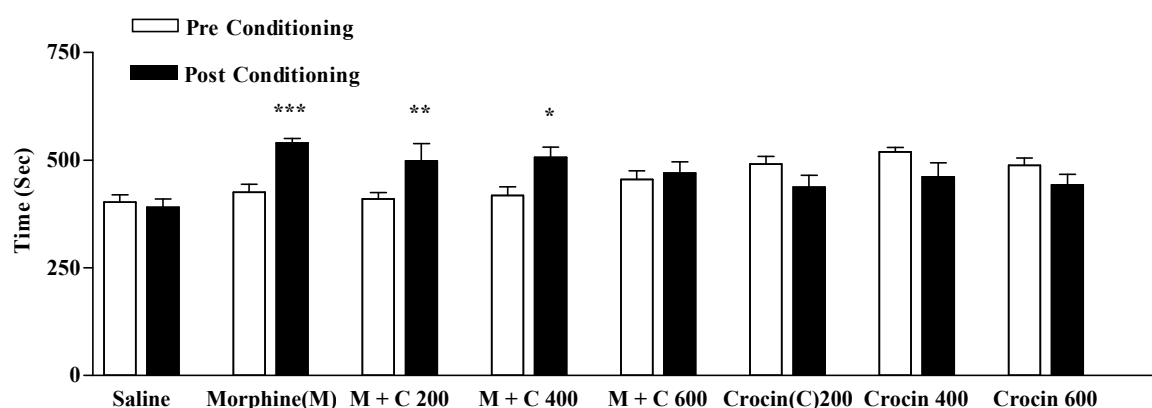


Figure 1. Effects of crocin on morphine-induced CPP. During the phase of conditioning, animals received the following treatments in the drug-paired compartment: SAL, saline plus saline; MOR, saline plus 40 mg/kg of morphine; MOR+CRC200, 200 mg/kg of Crocin plus 40 mg/kg of morphine; MOR+CRC400, 400 mg/kg of crocin plus 40 mg/kg of morphine; MOR+CRC600, 600 mg/kg of crocin plus 40 mg/kg of morphine; CRC200, 200 mg/kg of crocin plus saline; CRC400, 400 mg/kg of crocin plus saline; CRC600, 600 mg/kg of crocin plus saline. The bars represent the time spent in the drug-paired compartment before conditioning sessions in pre-conditioning test (white bars) and after conditioning sessions in post-conditioning test (black bars). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ significant difference in the time spent in the drug-paired compartment in pre-conditioning vs. post-conditioning sessions tests.

In groups received the doses of 200 and 400 mg/kg of crocin (MOR+CRS200; MOR+CRS400), there are also significant difference between the time spent in pre- and post-conditioning days. It means that the administration of crocin in doses 200 and 400 mg/kg during conditioning phase cannot prevent acquisition of place preference. But in dose 600 mg/kg of crocin (group MOR+CRS600), there is no significant difference between the time spent in pre- and post-conditioning days. It means that the administration of crocin in dose 600 mg/kg during conditioning phase can prevents acquisition of

place preference. In groups of CRS (crocin 200, 400 and 600 mg/kg) there is no significant difference between the time spent in pre- and post- conditioning days that means crocin in doses 200, 400 and 600 mg/kg cannot induces place preference or aversion by itself.

Effects of crocin on Reinstatement of place preference

As showed in figure 2 the lack of differences between the last extinction session (extinction) and Pre-conditioning suggests that after daily extinction sessions the conditioning has been disappeared. Administration of the priming dose of morphine can reinstate CPP. Crocin in doses 400 and 600 mg/kg blocks the Reinstatement of place preference due to priming dose of morphine.

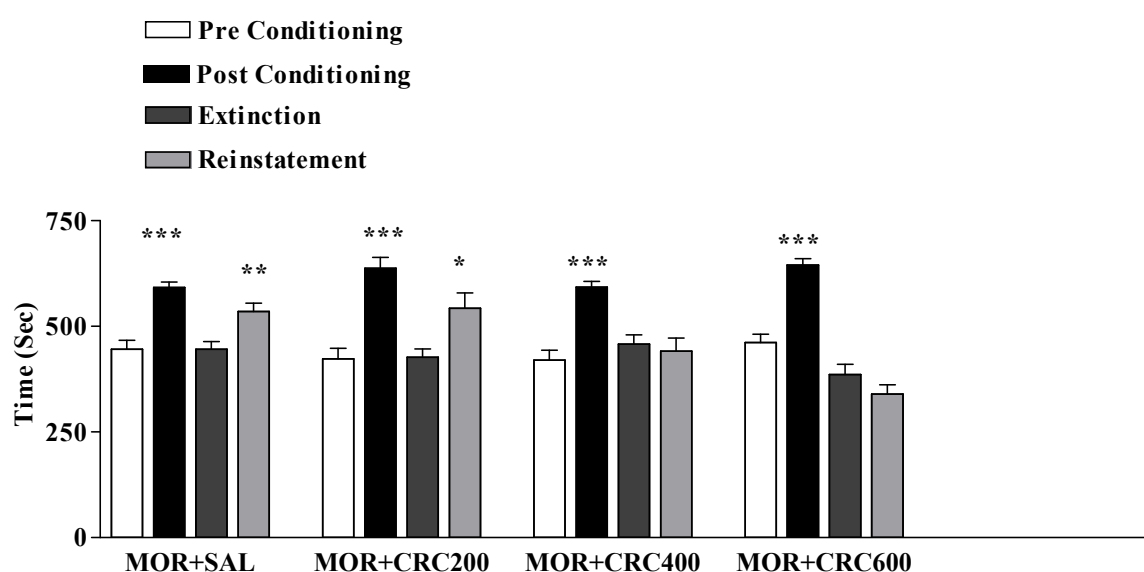


Figure 2. Effects of crocin on the reinstatement of morphine induced CPP. After acquisition and extinction of morphine- induced CPP, during reinstatement phase, animals received priming dose of morphine as following treatments as : MOR+SAL, morphine (10 mg/kg) plus saline; MOR+CR200, morphine (10 mg/kg) plus crocin (200mg/kg); MOR+CR400, morphine (10 mg/kg) plus crocin (400mg/kg); MOR+CR600, morphine (10 mg/kg) plus crocin (600mg/kg). The bars represent the mean (\pm 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). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ significant difference in the time spent in pre-conditioning vs. post-conditioning sessions or reinstatement tests.

Discussion

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

Crocin is a constituent of saffron that is responsible for some CNS effects of this plant including anxiolytic-like effect (16), antidepressant (10), aphrodisiac activity (17), learning and memory-improving properties (18) and reducing physical signs of morphine withdrawal (14). Although there is

not any study about the effect of crocin on morphine induced CPP, in a recent study, it has been showed that Saffron extract can reduce the acquisition of morphine-induced CPP in mice (15). Regarding the results of our study we can suggest that this effect of saffron may be at least partially related to crocin of the extract.

It has been showed that crocin can interact with dopaminergic system in CNS (19) and dopaminergic pathways in the ventral tegmental area have an important role in morphine induced CPP. Dopamine antagonists can reverse morphine-induced CPP (4). Antagonists of NMDA receptor can also prevent morphine induced CPP (4). Saffron extract has interaction to NMDA receptors that may have role in the effects of saffron on CPP.(20), but it seems that this mechanism could not be considered for the effect of crocin in preventing morphine induced CPP because it has been showed that crocin doesn't have any interaction with NMDA receptors (20). Imipramine as a tricyclic antidepressant can reverse morphine-induced CPP in mice (21). Saffron extract and crocin have showed antidepressant activity (10, 22). It is possible that crocin also act via a similar mechanism to prevent morphine-induced CPP. Saffron and crocin possess a sedative effect in mice that may be induced via GABAergic system. (16, 23). It has been showed that Administration of the GABA(A) receptor agonist, muscimol and of 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 (24, 25). Therefore, GABAergic system may be involved in this effect of saffron and crocin.

In the present study we also showed that crocin in doses 400 and 600 mg/kg blocks the reinstatement of place preference due to priming dose of morphine. Although it has been showed that NMDA antagonist can show similar effect, but as we mentioned above, crocin hasn't showed NMDA antagonist activity and therefore it seems that other mechanism(s) are responsible for this effect of crocin which need more study to find them.

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

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