

# The Prophylactic and Therapeutic Effects of Saffron Extract and Crocin on Ethanol Withdrawal Syndrome in Mice

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## Key Words

alcohol withdrawal, pentylenetetrazol, *Crocus sativus*, crocin, open field test

## Abstract

**Objectives:** Ethanol withdrawal following its chronic use is a serious outcome and challenging to treatment. The chronic use of ethanol induces a progressive neuroplasticity in different regions of brain. In this study we evaluated the effects of aqueous extract of *Crocus sativus* L. (saffron) and its active compound, crocin, on the withdrawal behavior induced after repeated administration of ethanol, in two regimens of prophylactic (administration of drugs concomitant with the induction of dependence) and treatment (administration of drugs during the period of ethanol withdrawal) in mice which received ethanol.

**Methods:** Ethanol dependence was induced by oral administration of 10% v/v ethanol (2 g/kg) for 7 days. The aqueous extracts of saffron (40, 80 and 160) and crocin (10, 20 and 40 mg/kg) were administered to mice in two regimens of prophylactic (along with ethanol) and treat-

ment (during withdrawal period). Diazepam (1 mg/kg) was used as a positive control. Six hours after discontinuation of the ethanol, seizure was evaluated by the sub-convulsive dose of pentylenetetrazole (PTZ) (30 mg/kg). The open field test and Rota rod test were used for evaluation of locomotor activity and motor incoordination, respectively.

**Results:** Both extracts and crocin increased the number of crossed lines in the open field test. PTZ kindling seizure was inhibited in animals received extract (80 and 160 mg/kg) in both regimens. Motor incoordination was only improved following administration of crocin.

**Conclusion:** The aqueous extract of saffron and crocin can be considered as safe agents and reliable alternative to diazepam in management of ethanol withdrawal syndrome.

## 1. Introduction

Addiction is a chronic and relapsing disturbance which is reflected in an individual pathologically pursuing reward and relief by substance use. This disturbance leads to dysfunction in the biological, psycho-

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logical and social manifestations and also contributes to loss of control over drug intake and dysfunctional emotional responses such as dysphoria, anxiety and irritability that explain a motivational withdrawal syndrome subsequent to drug barricade [1].

Ethanol and drug dependence are important public health problems worldwide. The outbreak of ethanol and drug dependence in the United States and other parts of the world is really significant [2]. One of the problems of chronic ethanol consumption is physical dependence which resulting in physical withdrawal responses after stop taking of ethanol. The mechanisms which are involved in tolerance, dependence and withdrawal syndrome following chronic ethanol consumption are complex. Ethanol has depressant effects on the central nervous system (CNS) and when large amounts of ethanol are used over time the brain adapts to this depressant effect, in this way, stimulate a level of tolerance [3]. In proportion to the duration of a recent drinking habit and the amount of ethanol consumed, clinical symptoms of alcohol withdrawal syndrome (AWS) occur with varying intensity [4]. Different studies have shown that benzodiazepines, such as diazepam, are the perfect choice for the treatment of withdrawal syndrome, because it can modulate symptoms of seizures and anxiety. Although, dependence to these drugs can be mentioned as important problem in treatment of ethanol dependence [5].

*Crocus sativus* L., universally known as saffron, is a plant cultivated in different parts of the world and has traditional uses [6, 7] and multiple pharmacological properties [8, 9]. Saffron contains several constituents such as safranal (accountable for saffron odor and aroma), picrocrocin (responsible for saffron bitter flavor) and crocin (the main saffron antioxidant as a dye agent) [10]. Saffron or its main component crocin have been exhibited valuable effects including cardioprotective [11, 12] hepatoprotective [13-15], neuroprotective [16, 17] antidote against different toxins [18], antinociceptive [19], anti-inflammatory [20] and antitumor [21] properties. Additionally effect of saffron in digestive disorder [22], metabolic syndrome [18] and erectile dysfunction [23] has been reported. Regarding to safety of saffron and crocin [24, 25] different clinical applications of them have been mentioned in various disorders [26].

Interestingly, saffron or crocin could change the chemical neurotransmitters such as dopamine in the brain, [27] and showed several effects on nervous system [28] including, antidepressant [29], anticonvulsant [30], memory enhancer [31, 32], hypnotic [33] and attenuation of morphine withdrawal syndrome [34].

The aim of this study was to determine the prophylactic and therapeutic effect of saffron extract and crocin on ethanol-induced withdrawal syndrome and dependence in mice. It should be noted that the study focused on the effects of saffron extract and crocin on the important symptoms of ethanol withdrawal syndrome including decrease the seizure threshold and motor impairment. Anticonvulsant, antidepressant and anxiolytic effects of saffron extract and crocin will help us to evaluate their efficiency in treatment of ethanol withdrawal as these compounds does not induce any dependence in comparison to benzodiazepines.

## 2. Material and Methods

### 2.1. Chemicals

Diazepam ampul (10 mg/2 ml, Caspian Tamin, Iran), Pentylentetrazol (Sigma-Aldrich), ethanol 96% (Taghtir Khorasan, Iran) were used in our study.

### 2.2. Crocin preparation

Stigmas of *Crocus sativus* L. were purchased from Novin Saffron (collected from Ghaen, Khorasan province, North-east of Iran). Crocin was extracted and purified according to the method which previously described [35].

### 2.3. Preparation of extract

30 g of stigma powder was soaked in 1500 ml water for 72 hours. The mixture of plant and water was subsequently centrifuged (5 minutes, 3000 RPM) and was placed overnight in the freezer (-20 °C). Then, the extract was dried in Freeze dryer [33].

### 2.4. Animals

Male mice weighing (25 - 35 g) used in the study. The animals were housed in colony rooms with (21 ± 2°C) under a 12/12 hours light/dark cycle. Animals had free access to food and water. The experiments were approved by with Mashhad University of Medical Sciences, Ethical Committee Acts (No=922237).

### 2.5. Experimental Design

The mice (n=7 per group) were divided into 18 groups which described as follows:

Group 1- Control group treated with water by gavage (negative control)

Group 2- Ethanol alone received animals (2 g/kg ethanol as the 10% (v/v) solution by gavage) [36].

#### (Prophylactic groups)

Group 3- Animals received ethanol + diazepam (1 mg/kg, IP) [36].

Group 4, 5, 6- Animals received ethanol + aqueous saffron extract (40, 80, 160 mg/kg in groups 4, 5, 6 respectively) [37].

Group 7, 8, 9- Animals received ethanol + crocin (10, 20, 40 mg/kg in groups 7, 8, 9 respectively) [11].

Group 10- Aqueous saffron extract alone administered animals at the highest dose (160 mg/kg) Group 11- Crocin alone administered animals at the highest dose (40 mg/kg)

In mentioned groups, for induction dependence, ethanol was administrated once daily for 7 days. Withdrawal symptoms for ethanol were evaluated 6 hours after the last administration of ethanol. Additionally, diazepam, aqueous saffron extract or crocin were administrated IP once daily for 7 days and 6 hours after the last administration

of ethanol.

### (Treatment groups)

Group 12- Animals received ethanol + diazepam (1 mg/kg, IP) [36].

Group 13, 14, 15- Animals received ethanol + aqueous saffron extract (40, 80, 160 mg/kg. Group 16, 17, 18- Animals received ethanol + crocin (10, 20, 40 mg/kg).

In treatment groups a single dose of diazepam, aqueous saffron extract or crocin was administrated 6 hours after the last administration of ethanol. In both (prophylactic and treatment groups) ethanol withdrawal symptoms were evaluated using open field test, Rota rod test and PTZ Kindling.

## 2.6. Open field test (OFT)

The apparatus for this test, made of white wood, had a floor of 45 × 45 cm divided by lines into 25 squares of 9 × 9 cm. The walls, 40-cm high, were also painted in white. Each mouse was placed in the middle of the apparatus and its behavior was observed for 10 minutes. The total number of squares crossed was evaluated [38].

## 2.7. Rota rod test

Rota rod test includes a rotating bar that the animal is placed on it. In this test total time each animal was able to maintain its balance walking on top of the rod was recorded. The speed of the Rota rod accelerated from 10 to 20 rpm over a 20 seconds period. The maximum time the animal remains on the device, 300 seconds was considered. In this method, 24 hours before the test, mice were trained on the apparatus and only the mice that have the ability to maintain on the rotating rod at least 30 seconds were used [39].

## 2.8. PTZ Kindling

To induce kindling, a subconvulsant dose of pentylenetetrazole (30 mg/kg, IP) was injected 6 hours after the last dose of ethanol. After the PTZ injection, the animals were observed for the occurrence of convulsive behavior for 30 minutes. Animals entering into convulsion have shown convulsive state which include ear and facial jerks, myoclonic body jerks without upright position, myoclonic jerks, upright position with bilateral forelimb clonus, clonic-tonic seizures and generalised clonic-tonic seizures and loss of postural control [36].

## 2.9. Statistical Analysis

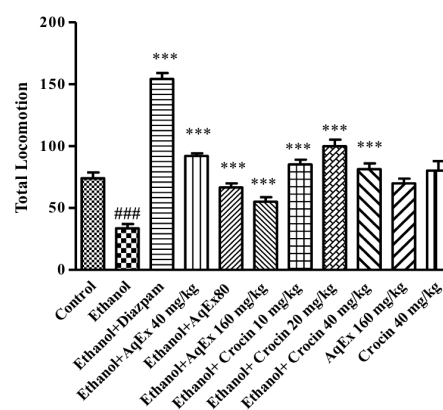
The results were expressed as mean ± SD (n=7). The statistical analysis was performed by One-way ANOVA followed by Tukey test in the Open field and Rota rod test and Fisher exact test were used in the PTZ. In all three tests p value at < 0.05 was considered as statistically significant.

## 3. Results

### 3.1. Open field test

#### 3.1.1. Effect of aqueous saffron extract and crocin on locomotor activity in ethanol withdrawal mice model in prophylactic groups

Administration of ethanol decreased the number of lines crossed during the open field test as compared to negative control ( $P < 0.001$ ). Administration of diazepam, the extracts (40, 80 and 160 mg/kg) or crocin (10, 20 and 40 mg/kg) once a day during induction of ethanol dependence significantly increased animal total locomotion in comparison to ethanol-treated group (figure 1).



**Figure 1** Prophylactic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocin (10, 20, 40 mg/kg) during open field test in ethanol withdrawal mice model. Bars represent mean ± SD (n=7). ###P < 0.001 vs control, \*\*\*P < 0.001 vs ethanol group. The statistical analysis was performed by One-way ANOVA followed by Tukey test. AqEx = aqueous saffron extract.

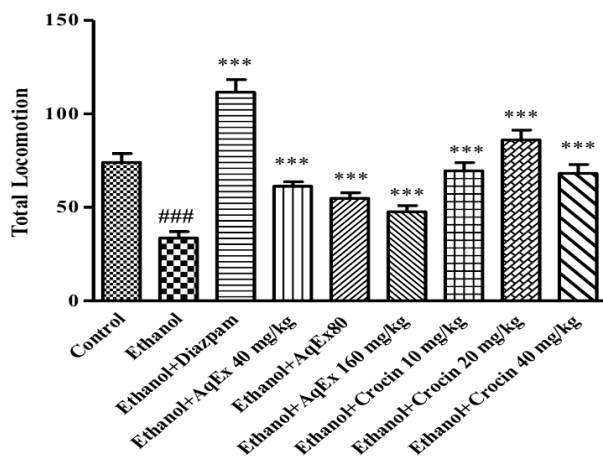
#### 3.1.2. Effect of aqueous saffron extract and crocin on locomotor activity in ethanol withdrawal mice model in treatment groups

As shown in figure 2, in ethanol-treatment animals the number of lines crossed during the open field test markedly reduced as compared to negative control ( $P < 0.001$ ), while single dose administration of diazepam, the extracts (40, 80 and 160 mg/kg) or crocin (10, 20 and 40 mg/kg) could improve locomotor activity in comparison to ethanol group ( $P < 0.001$ ).

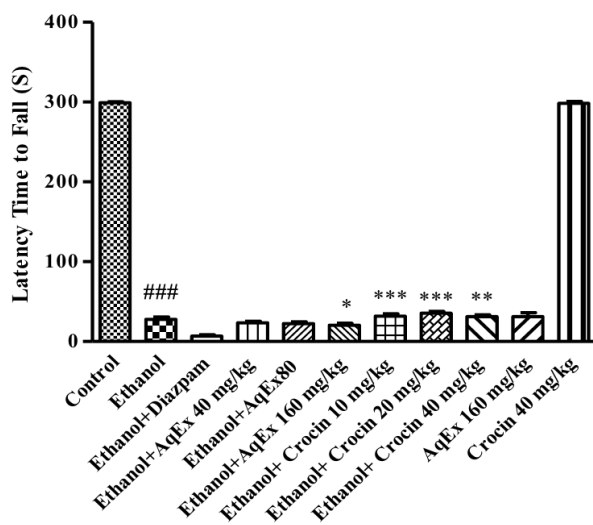
### 3.2. Rota rod test

#### 3.2.1. Effect of aqueous saffron extract and crocin on motor coordination in ethanol withdrawal mice model in prophylactic groups

The findings from the Rota rod test demonstrated that following ethanol withdrawal, motor coordination signifi-



**Figure 2** Therapeutic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocinn (10, 20, 40 mg/kg) during open field test in ethanol withdrawal mice model. Bars represent mean  $\pm$  SD (n=7). ###P < 0.001 vs control, \*\*\*P < 0.001 vs ethanol group. The statistical analysis was performed by One-way ANOVA followed by Tukey test. AqEx = aqueous saffron extract.



**Figure 3** Prophylactic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocinn (10, 20, 40 mg/kg) during Rotarod test in ethanol withdrawal mice model. Bars represent mean  $\pm$  SD (n=7). ###P < 0.001 vs control, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs ethanol group. The statistical analysis was performed by One-way ANOVA followed by Tukey test. AqEx = aqueous saffron extract.

cantly diminished (P < 0.001 vs control group). Treatment of animals with crocinn (10, 20 and 40 mg/kg) during induction of ethanol dependence improved the performance of mice in rota rod test. Administration of aqueous saffron extract in doses of 40 and 80 mg/kg could not change motion balance when compared to ethanol group (figure 3).

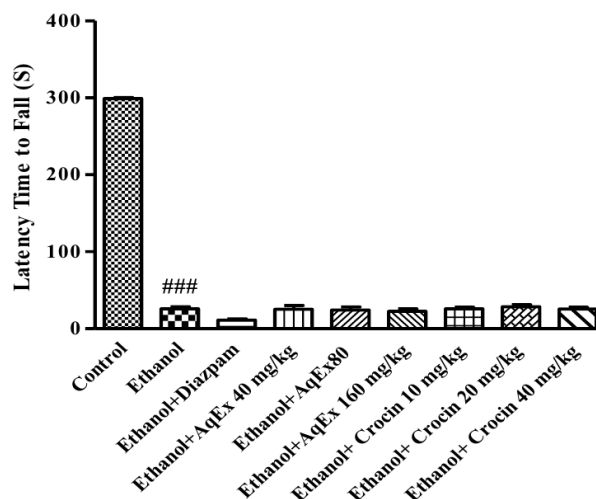
### 3.2.2. Effect of aqueous saffron extract and crocinn on motor coordination in ethanol withdrawal mice model in treatment groups

As shown in figure 4, single dose administration of diazepam, the extracts (40, 80 and 160 mg/kg) or crocinn (10, 20 and 40 mg/kg) didn't recover mice performance in Rota rod test.

### 3.3. PTZ Kindling

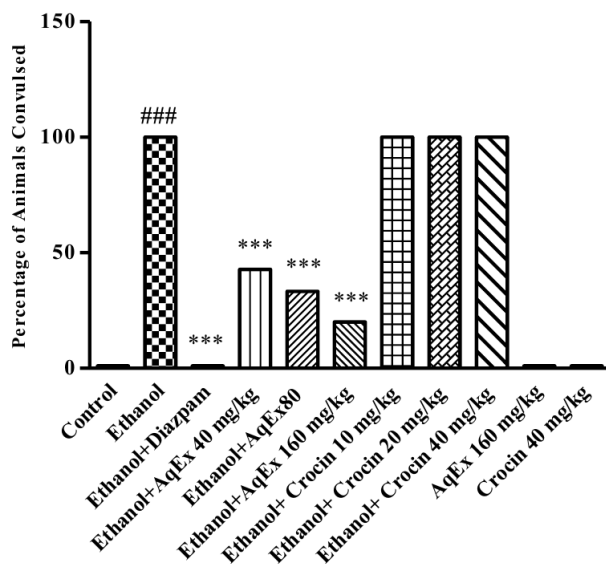
#### 3.3.1. Effect of aqueous saffron extract and crocinn on PTZ Kindling in ethanol withdrawal mice model in prophylactic groups

Administration of sub-convulsive dose of PTZ (30 mg/kg i.p) in negative control group has not induced any convulsions, whereas in ethanol withdrawal mice, 100% convulsion was observed following PTZ administration. As shown in figure 5, treatment of animals with diazepam (1 mg/kg) or aqueous saffron extract (40, 80, 160 mg/kg) markedly attenuated the percentage of convulsion (P < 0.001 vs ethanol group), while crocinn in all doses could not decrease seizure threshold.

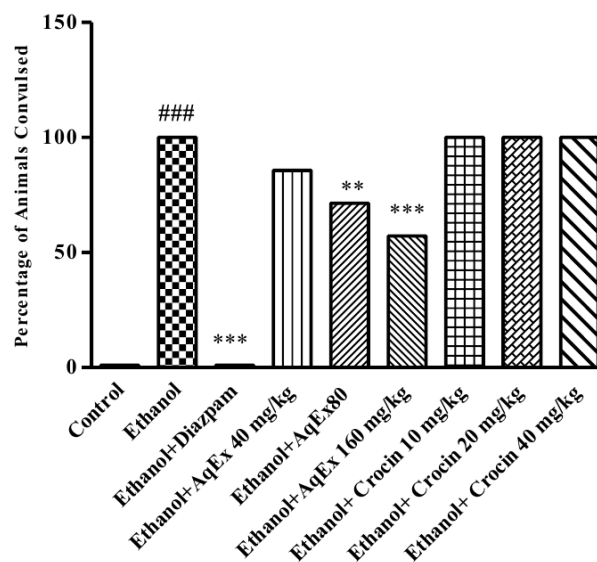


**Figure 4** Therapeutic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocinn (10, 20, 40 mg/kg) during rotarod test in ethanol withdrawal mice model in treatment groups. Bars represent mean  $\pm$  SD (n=7).

###P < 0.001 vs control. The statistical analysis was performed by One-way ANOVA followed by Tukey test. AqEx = aqueous saffron extract.



**Figure 5** Prophylactic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocin (10, 20, 40 mg/kg) during PTZ test in ethanol withdrawal mice model. Bars represent mean  $\pm$  SD (n=7). ###P < 0.001 vs control, \*\*\*P < 0.001 vs ethanol group. The statistical analysis was performed by Fisher exact test. AqEx = aqueous saffron extract.



**Figure 6** Therapeutic effect of aqueous saffron extracts (40, 80, 160 mg/kg) and crocin (10, 20, 40 mg/kg) during PTZ test in ethanol withdrawal mice model. ###P < 0.001 vs control, \*\*P < 0.01, \*\*\*P < 0.001 vs ethanol group. The statistical analysis was performed by Fisher exact test. AqEx = aqueous saffron extract.

### 3.3.2. Effect of aqueous saffron extract and crocin on PTZ Kindling in ethanol withdrawal mice model in treatment groups

According to results which have been shown in figure 6, single dose administration of diazepam (1 mg/kg), or aqueous saffron extracts (80 mg/kg P < 0.01, 160 mg/kg P < 0.001) significantly increased seizure threshold in comparison to ethanol-received animals. However, crocin in all doses was not effective in control of convulsion during ethanol withdrawal period.

## 4. Discussion

In this study, the prophylactic and therapeutic effect of saffron extract and crocin on ethanol-induced withdrawal syndrome and dependence in mice were examined. Both extract and crocin increased the number of crossed lined of withdrawn animals in the open field test. PTZ kindling seizure was inhibited in animals treated with aqueous extract (40, 80 and 160 mg/kg). Administration of crocin but not extract improved motor incoordination in animals.

Ethanol withdrawal syndrome occurs when a heavy drinker suddenly stops or significantly reduces his ethanol use. This syndrome includes psychological symptoms and physical signs. The results of animal studies have been shown, administration of ethanol with multiple periodic

pattern lead to more consecutive signs of withdrawal [40].

In previous studies it was found that saffron extracts and their constituents, crocin and safranal have serotonergic, noradrenergic and dopaminergic activity [29]. In withdrawal process the level of dopamine and serotonin neurotransmitters significantly change. [41]. Therefore, it can be concluded saffron extracts and its constituent crocin based on effects on these mediators may have advantages in the treatment of ethanol withdrawal.

Ethanol withdrawal leads to reduction of animal exploratory behaviors in open field test. This reduction often resulted from withdrawal anxiety, which can be explained by decrease in number of lines crossed by animals. The results of this test in the current study exhibited the number of lines crossing activity by mice significantly increased following treatment with aqueous saffron extract (40, 80, 160 mg/kg) and crocin (10, 20, 40 mg/kg) in both prophylactic and treatment groups similar to diazepam. These data can be due to anxiolytic activity of aqueous saffron extract and crocin. In the previous research Hosseinzadeh et al (2009) [33] showed that the aqueous saffron extract had an anxiolytic effect in mice which was comparable to diazepam. This finding was obtained in the elevated plus-maze test. Since GABA agonist has anti-anxiety properties [42], the beneficial effects of saffron extract and crocin in this study may be mediated in part through GABA-mimetic effects. Although more mechanistic studies should be done.

The NMDA receptors and glutamatergic system are partly responsible for the anxiety-like behavior seen during ethanol withdrawal [43]. Based on Berger et al. study (2011)

[44], effect on NMDA receptor may be involved in the antagonistic effect of saffron extract on ethanol withdrawal syndrome. Also, previous results suggested that crocin antagonized the inhibitory effect of ethanol on NMDA receptor-mediated responses in hippocampal neurons [45].

According to the results of the Rota rod test, administration of crocin (10, 20 and 40 mg/kg) significantly increased motion balance in prophylactic models. In the previous study [33], saffron showed muscle relaxation and motor imbalance effects while these effects were not observed during administration of crocin.

In the treatment groups, there were no significant differences between the aqueous saffron extract (40, 80 and 160 mg/kg) and crocin (10, 20 and 40 mg/kg) groups for motion balance as compared to ethanol group. To achieve this different mechanism and effect, further studies are needed.

In this study, administration of diazepam (1 mg/kg) or aqueous saffron extracts (80 mg/kg and 160 mg/kg) significantly increased seizure threshold in comparison to ethanol-received animals. It can be concluded that aqueous saffron extract and diazepam have similar antiepileptic effects in PTZ model of epilepsy. It seems safranal is responsible for the antiepileptic effects of saffron extract. According to previous studies, safranal exhibited an anticonvulsant activity in PTZ-induced seizures while crocin did not show anticonvulsant activity [30, 46]. Also, antiabuse properties of safranal in acute experimental seizure models have been reported [47].

Our data suggested that crocin was not involved in the anticonvulsant effects of saffron stigma in current study.

## 5. Conclusion

This study exhibited that the aqueous extract of saffron and crocin may have possible benefit in the treatment of ethanol withdrawal and can be used as a safe agent and a reliable alternative to benzodiazepines.

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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