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Effects of hydroalcoholic extract of *Borago officinalis* on naloxone-precipitated withdrawal syndrome in morphine-dependent mice

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Abstract

The aim of the present study was to investigate the effect of hydroalcoholic extract of *Borago officinalis* on morphine withdrawal syndrome in mice. Morphine-dependent group received morphine for nine days and then received naloxone via intraperitoneal injection. Control group received saline for nine days. Post-treated group received *B. officinalis* extract intraperitoneally (100 mg/kg) on the day 10 before naloxone injection. Co-treated group received *B. officinalis* extract intraperitoneally (100 mg/kg) and morphine for nine days and then received naloxone. Extract-treated group received extract for nine days and then received naloxone. Naloxone injection significantly increased the frequency of jumping, blinking, ptosis, defecation, paw trembling, and two-legged standing in comparison to the control group. Co-treatment and post-treatment with *B. officinalis* extract significantly decreased the withdrawal symptoms. In conclusion, hydroalcoholic extract of *B. officinalis* significantly attenuated the symptoms of morphine withdrawal syndrome.

Introduction

Withdrawal symptoms occur in substance-dependent people when the drug or its equivalent is not available for use. These symptoms include anxiety, restlessness, insomnia, nausea and vomiting, diarrhea, muscle cramp (leg and abdominal muscle), sweating, rhinorrea or epiphora, involuntary muscle contraction or muscle twitching, and sometimes convulsion. If dependence is severe, the withdrawal symptoms last for 6-10 days. The severity of symptoms depends on the degree of dependence. Psychological dependence is the desire for drug use and the psychological symptoms of withdrawal takes much more time than physical dependence (Ferriola et al., 1989).

The chemical compounds in some plants affect drug withdrawal through different cerebral systems, such as gabaergic and dopaminergic systems, and therefore cause sedation and relief in substance-dependent people.

Seed oil, leaves, flowers and flowering shoots of *Borago officinalis* are used in traditional medicine. The compounds of *B. officinalis* seed oil include β-caryophyllene, p-simen-8-L, monoterpenes, sesquiterpenes, gamma-linoleic acid, linoleic acid and alpha-linoleic acid. The main compounds identified in *B. officinalis* leaves include phenolic acids, flavonoids and organic acids (Asadi Samani et al., 2014).

Hydroalcoholic extract of *B. officinalis* flower is able to relieve the severe and acute pain (Shahraki et al., 2015).

B. officinalis is rich in unsaturated fatty acids, especially linoleic acid. The use of *B. officinalis* causes neurons' membrane to repair more rapidly and hence the process of receiving and transmitting neural messages is improved. Clinical studies have indicated that the use of *B. officinalis* seed oil reduces cerebral arteriosclerosis by up to 30%. In addition, this oil contributes to improving memory and reducing affective arousal (Pieszak et al., 2012).

Materials and Methods

Extracting

B. officinalis flowers were provided from the reliable groceries of Shahrekord. After the flowers were identified and ground, they were mixed with alcohol 70%. The solution was kept at room temperature for one week. Then, the extract filtered and the filtered solution was left at 37°C temperature for evaporation of alcohol and water in it (Ghahremanitamadon et al., 2014).

Determining phenolic compounds

Briefly, 0.5 mL Folin-Ciocalteu reagent was introduced into 0.1 mL of the diluted extract (0.01 g/10 mL methanol at 60°C temperature). After 3-4 min, 0.4 mL sodium carbonate 7.5% was added to the solution. After 30 min incubation at room temperature, the absorbance of the samples was read against distilled water used as blank. During the experiment, different dilutions of gallic acid were prepared and standard curve was derived. The samples' absorbance was compared with the standard curve and the extract's total phenol was calculated and expressed in mg/g dried extract (Rabiei et al., 2014).

Determining flavonolid compounds

Briefly, 0.5 mL of the extract solution (0.01 g/10 mL methanol at 60°C temperature) was dissolved with 0.5 mL aluminum chloride 2%. Then, 3 mL potassium acetate was introduced into the solution. After 40 min, the absorbance of samples was read against distilled water at 440 nm wavelength. During the experiment, different dilutions at routine concentrations were prepared and tested according to the above method and the standard curve was derived. The samples' absorbance was compared with the standard curve and the extract's flavonol amount was measured and expressed in mg/g dried extract. The amounts of phenolic, flavonolic, and flavonoid compounds were measured and expressed as mean ± standard deviation (Rabiei et al., 2014).

Laboratory animals

Adult male Balb/C mice weighing 25-30 g were kept at suitable (21 ± 2°C) temperature and 12/12 hours darkness: Light cycle with food and water freely available.

Inducing physical dependence

On the first three days, 10 mg/kg body weight (BW) morphine solution per day was intraperitoneally injected. On the following three days, 20 mg/kg BW morphine solution per day was intraperitoneally injected and on the last three days, 40 mg/kg BW morphine solution per day was intraperitoneally injected (Kerachian et al., 2007).

Group 1 (morphine-dependent group) (n=8) received intraperitoneally morphine for nine days and 1 mg/kg BW on the day 10, Group 2 (control group) (n=8) received intraperitoneally 2 mL/kg BW normal saline, Group 3 (post-treatment group) (n=8) received intraperitoneally morphine for nine days and 100 mg *B. officinalis* extract one min after naloxone injection, Group 4 (co-treatment group) (n=8) received simultaneously morphine and 100 mg *B. officinalis* extract for nine days and 1 mg/kg BW naloxone on the day 10, Group 5 (extract group) (n=8) received *B. officinalis* extract for nine days and 1 mg/kg BW naloxone on the day 10.

Inducing morphine withdrawal syndrome

On the day 10 after inducing physical dependence, naloxone (1 mg/kg BW) was injected intraperitoneally to induce the symptoms of morphine withdrawal syndrome (Kerachian et al., 2007).

Recording morphine withdrawal symptoms

After naloxone injection, the symptoms of morphine withdrawal, frequency of body stretching, jumping, paw trembling, blinking and ptosis were recorded for 30 min (Kerachian et al., 2007). (**Video Clip**)

Statistical analysis

The data were analyzed by one-way ANOVA in SPSS 16. Tukey's post-hoc test was used to investigate the significance of difference among the groups. $p<0.05$ was considered the level of significance and results were expressed in mean ± SEM.

Results

The amounts of phenolic, flavonoid and flavonolid compounds of the B. *officinalis* extract are 44.9, 9.12 and 4.24 mg/kg dried extract respectively.

Figure 1 illustrates the effect of *B. officinalis* extract, as co -treatment and post-treatment, on the frequency of naloxone-induced jumping in the morphine-dependent mice. The frequency of jumping was significantly higher in the morphine-dependent group than the control group after naloxone injection ($p<0.01$). The administration of 100 mg/kg hydroalcoholic *B. officinalis* extract, as co-treatment and post-treatment, caused a significant decrease in the frequency of jumping ($p<0.01$). The frequency of jumping was significantly different between the control group and the group treated with *B. officinalis* extract alone ($p<0.01$).

Figure 1 illustrates that the frequency of paw trembling was significantly higher in the morphine-dependent group than the control group after naloxone injection ($p<0.01$). Co-treatment and post-treatment with hydro-alcoholic *B. officinalis* extract caused a significant

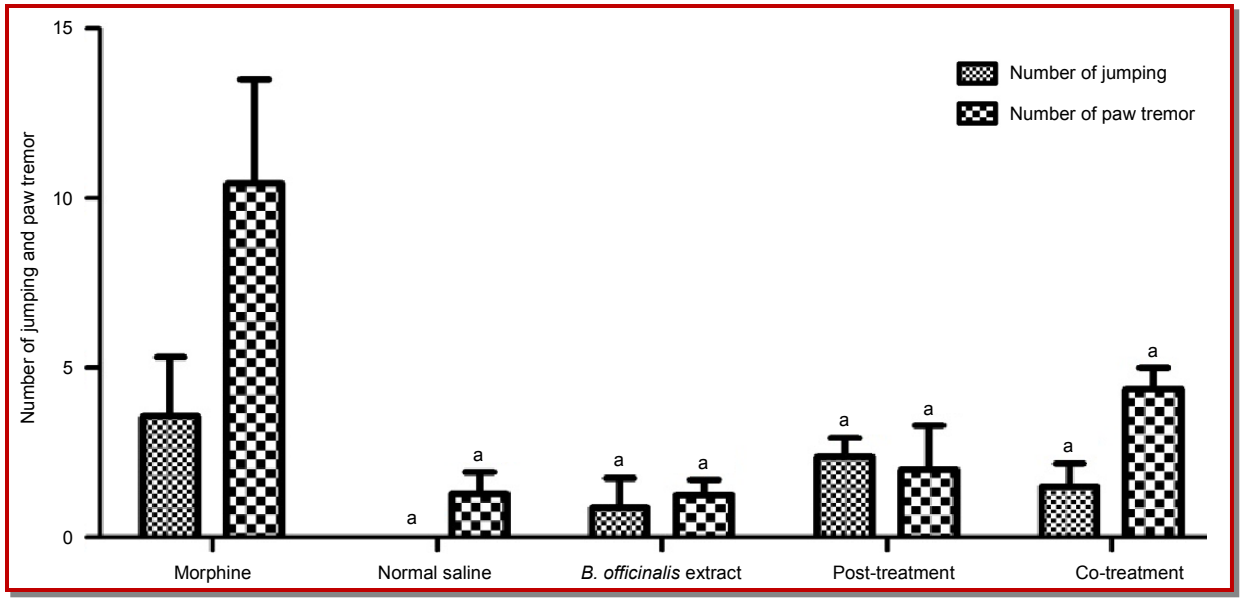

Figure 1: The effect of *Borago officinalis* extract injection, as post-treatment and co-treatment, on the frequency of naloxone-induced jumping and paw tremor in morphine-dependent mice; [a]p<0.01

decrease in the frequency of paw trembling compared to the morphine-dependent group (p<0.01). In addition, the frequency of paw trembling was not significantly different between the group treated with *B. officinalis* extract alone and the control group after naloxone injection (p<0.01).

Figure 2 illustrates that the frequency of blinking was significantly higher in the morphine-dependent group than the control group after naloxone injection (p<0.05). The administration of 100 mg/kg hydroalcoholic *B. officinalis* extract on the day 10 before naloxane injection caused a significant decrease in the frequency of blinking compared to the morphine-dependent group (p<0.05). Simultaneous injection of *B. officinalis* extract and morphine for nine days caused a significant decrease in the frequency of blinking after naloxane injection compared to the morphine-dependent group (p<0.01). The frequency of blinking was significantly different between the control group and the group treated with *B. officinalis* extract alone (p<0.05).

Figure 2 illustrates the effect of co-treatment and post-treatment with *B. officinalis* extract on the frequency of ptosis in the morphine-dependent group. The frequency of ptosis was significantly higher in the morphine-dependent group than the control group after naloxone injection (p<0.01). Co-treatment and post

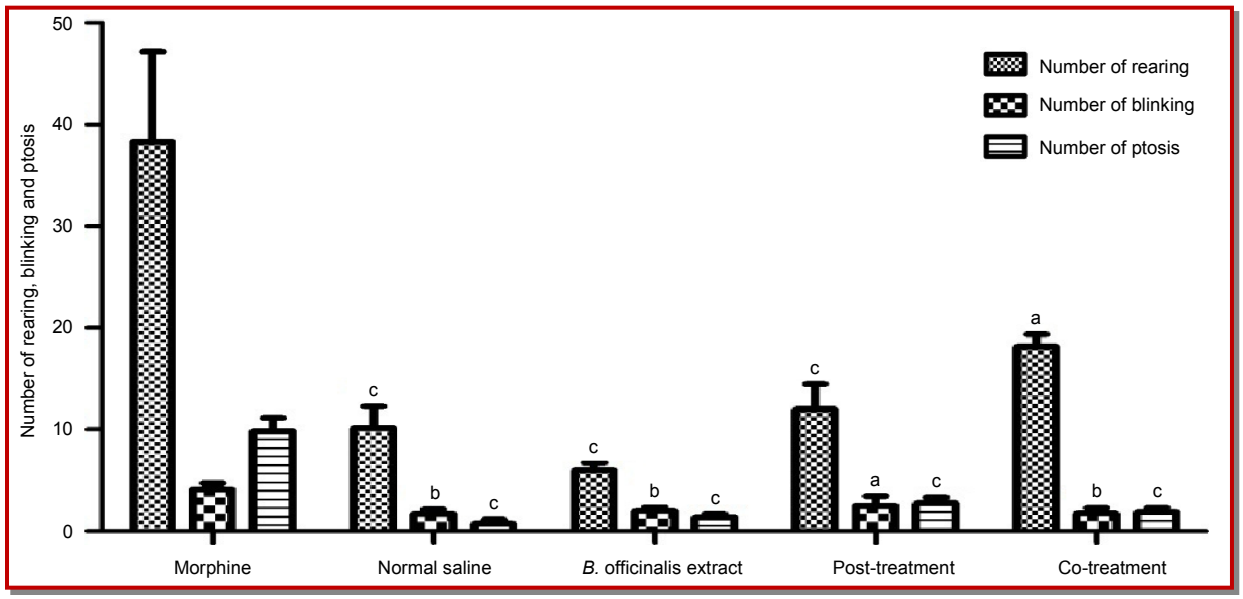

Figure 2: The effect of *Borago officinalis* extract injection, as post-treatment and co-treatment, on the frequency of naloxone-induced blinking, rearing and ptosis in morphine-dependent mice; [a]p<0.05; [b]p=0.01-0.05; [c]p<0.01

-treatment with *B. officinalis* extract caused a significant decrease in the frequency of ptosis compared to the morphine-dependent group ($p<0.01$). The frequency of ptosis was significantly different between the control group and the group treated with *B. officinalis* extract alone ($p<0.01$).

Figure 2 illustrates the effect of 100 mg/kg *B. officinalis*, as treatment and post-treatment, on the frequency of two-legged standing in the mice with morphine withdrawal syndrome. The frequency of two-legged standing was significantly higher in the morphine-dependent group than the control group ($p<0.01$). Hydroalcoholic *B. officinalis* extract, as treatment and post-treatment, caused a significant decrease in the frequency of two-legged standing compared to the morphine-dependent group ($p<0.05$). The frequency of two-legged standing was significantly different between the group treated with *B. officinalis* alone after naloxone injection and the morphine-dependent group ($p<0.01$).

As Figure 3 illustrates, the amount of diarrhea was significantly higher in the morphine-dependent group than the control group after naloxone injection ($p<0.01$). Intraperitoneal administration of 100 mg/kg hydro-alcoholic *B. officinalis* extract, as treatment and after treatment, caused a significant decrease in the diarrhea amount in the morphine-dependent group compared to the control group ($p<0.01$). Diarrhea amount was significantly lower in the group treated with the extract alone than the morphine-dependent group after nalo-xone injection ($p<0.05$).

Figure 4 illustrates the effect of co-treatment and post-treatment with *B. officinalis* on the frequency of scrat-ching in the mice with morphine withdrawal syn-drome. The frequency of scratching was not signifi-cantly different between the morphine-dependent

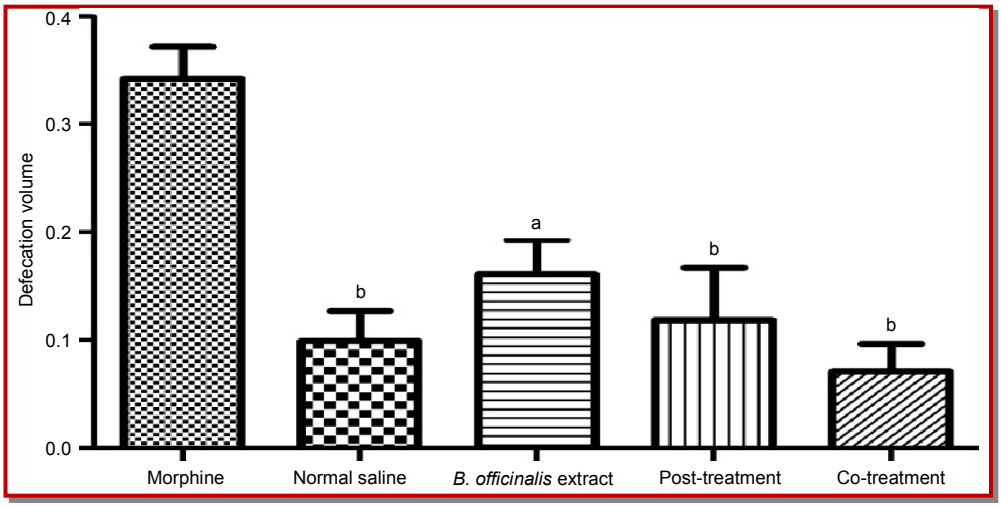

Figure 3: The effect of *Borago officinalis* extract injection, as post-treatment and co-treatment, on the amount of diarrhea after nalox-one injection in morphine-dependent mice; [a]p=0.01-0.05; [b]p<0.05

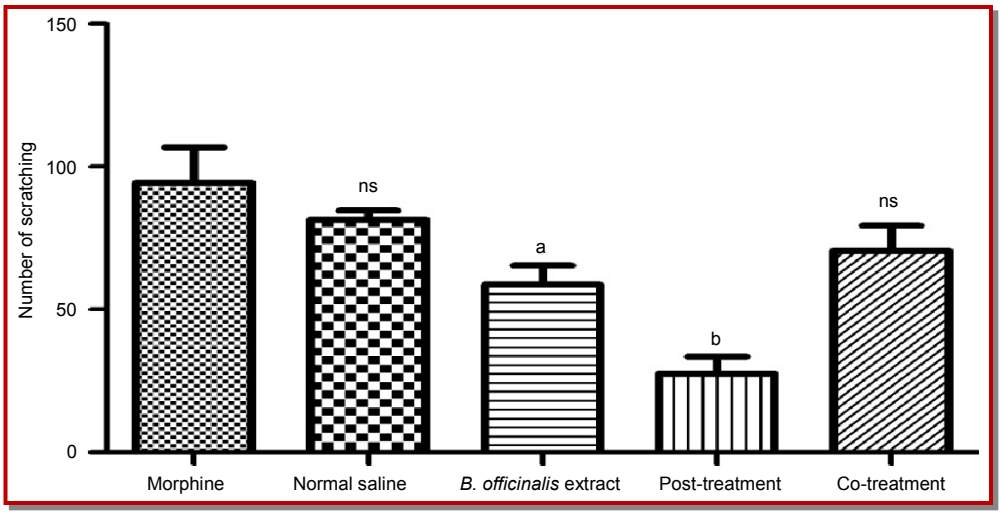

Figure 4: The effect of *Borago officinalis* extract, as post-treatment and co-treatment, on the frequency of scratching in the morphine-dependent mice after naloxone injection in morphine-dependent mice; [a]p<0.05; [b]p<0.01; ns: Not significant

group and the control group after naloxone injection ($p>0.05$). Injecting hydroalcoholic *B. officinalis* extract on the day 10, 10 min before naloxone injection caused a significant decrease in the frequency of scratching compared to the morphine-dependent group ($p<0.01$). *B. officinalis* and morphine co-treatment caused no significant effect on the frequency of scratching after naloxone injection ($p>0.05$). The frequency of scratching was significantly different between the group administered with the extract alone and the morphine-dependent group after naloxone injection ($p<0.05$).

Discussion

Injecting naloxone caused increase in the frequency of jumping, blinking, ptosis, bowel movement, and paw trembling in the morphine-administered group compared to the control group, but no significant effect was derived on scratching and abdominal twitching. Other studies reported that naloxone injection after morphine administration caused the symptoms of morphine withdrawal syndrome, including increased diarrhea, ptosis and snarling (Vafaei et al., 2011; Khajeh et al., 2015) .

Naloxone, as an opioid receptors antagonist, prevents opioid agonists from acting and prevents opioid receptors from accessing agonist molecules. This drug in administered intravenously and intensifies the symptoms of addiction withdrawal in opioid-dependent people (Rafieian Kopaei et al., 1995) .

In this study, co-treatment and post-treatment with *B. officinalis* extract caused a significant decrease in the frequency of morphine withdrawal syndrome, jumping, blinking, ptosis, bowel movement, scratching, and paw trembling.

According to some studies on the symptoms of morphine withdrawal syndrome, diarrhea may occur because of morphine effect on cholinergic system (Williams et al., 2001). Cholinergic, serotoninergic and gabaergic systems and possibly calcium ion are likely to involve in jumping, such that serotoninergic and cholinergic systems cause increase in jumping and GABAergic system cause decrease in jumping (Eriator 1998).

Flavonoid and phenolic compounds of the medicinal plants can inhibit cholinergic system function through inhibiting cholinesterase and therefore cause reduction in the symptoms of withdrawal syndrome (Baradaran et al., 2012). Given great amounts of flavonoid and phenolic compounds of B. *officinalis* flowers, the function could be attributed to the inhibitory effect on cholinergic system. However, further investigation is needed to examine this.

Protein kinase inhibitors, calcium channel blockers and Rp-cAMPS, specific and strong inhibitor of protein kinase activation by cAMPS, can reduce the withdrawal symptoms in rats (Ouyang et al., 2012). Gilani et al. reported that *B. officinalis* extract exerted antispasmodic and bronchodilating effects through inhibiting calcium channels (Gilani et al., 2007). Therefore, inhibiting calcium channels may be considered a potential mechanism and investigated.

Studies have indicated that baclofen, $GABA_B$ agonist, can reduce naloxone-induced withdrawal symptoms. In addition, certain drugs, such as benzodiazepine, can reduce naloxone-induced symptoms of morphine-dependent rats (Maldonado et al., 1991). Given that the analgesic effect of *B. officinalis* has already been demonstrated (Shahraki et al., 2015), this plant is likely to reduce the symptoms of withdrawal syndrome through affecting gabaergic system. This deserves further investigation.

In addition, opioid receptors are present in different sites, such as limbic system, spinal network pathways, intermediate nuclei, spinal gelatinous substance, spinal nucleus of trigeminal nerve, and the vagus nerve. These receptors are composed of various hairs, kappa, delta, epsilon, and sigma, any one of which has a distinct function (Maldonado et al., 1991)

Any opioid receptors are likely to be activated during phythotherapy with *B. officinalis*. It is necessary to determine the role of opioid receptors in phythotherapy with *B. officinalis* using opioid receptor agonists and antagonists. Given that the therapeutic effects of hydroalcoholic *B. officinalis* extract on the symptoms of morphine withdrawal syndrome have been investigated in this study for the first time, none of the compounds of this extract can be considered responsible for reducing the symptoms of withdrawal syndrome.

In this study, nine-day *B. officinalis* treatment followed by naloxone administration caused no significant change in the frequency of jumping, blinking, ptosis, bowel movements, and paw trembling compared to the control treatment. This confirms no induction of dependence on *B. officinalis* extract.

Several studies have shown that *B. officinalis* contains secondary metabolites such as pyerolisidine alkaloids and should not be used in the long-term (Maldonado et al., 1991). In the present study, nine-day treatment followed by one post-treatment with *B. officinalis* extract caused a significant decrease in the symptoms of morphine withdrawal syndrome. Therefore, there is no need for the repeated use of this plant.

Conclusion

B. officinalis administration for nine days followed by naloxone administration caused no significant decrease

in the frequency of jumping, blinking, ptosis, frequency of bowel movements, and paw trembling compared to the control group. This confirms no induction of dependence on *B*. *officinalis* extract.

References

Asadi Samani M, Bahmani M, Rafieian Kopaei M. The chemical composition, botanical characteristic and biological activities of *Borago officinalis*: A review. Asian Pac J Trop Med. 2014; 7: 22-28.

Baradaran A, Rabiei Z, Rafieian M, Shirzad H. A review study on medicinal plants affecting amnesia through cholinergic system. J Herbmed Plarmacol. 2012; 1: 3-9.

Eriato L. Naloxone: Acute opioid withdrawal syndrome or side effects. Anesth Analg. 1998; 87: 1214-30.

Ghahremanitamadon F, Shahidi S, Zargooshnia S, Nikkhah A, Ranjbar A, Soleimani AS. Protective effects of *Borago officinalis* extract on amyloid β-peptide (25–35)-induced memory impairment in male rats: A behavioral study. Bio Med Res Int. 2014; 2014.

Gilani AH, Bashir S, Khan AU. Pharmacological basis for the use of *Borago officinalis* in gastrointestinal, respiratory and cardiovascular disorders. J Ethnopharmacol. 2007; 114: 393-99.

Kerachian N, Alaee H, Gharavi-Naini M, Pilevarian A, Moghimi A. Effects of alcoholic extract of *Avena sativa, Hypericum perforatum, Passiflora incarnata* and *Lavandula officinalis* on symptoms of morphine withdrawal syndrome in rats. Physiol Pharmacol. 2007; 10: 313-21.

Khajeh N, Eidi A, Zarringhalam J. The effect of ethanol extract of aerial parts of the *Mentha piperita* in the cquisition, tolerance expression and dependence to morphine in adult male mice. Armaghane-danesh. 2015; 2: 53-65.

Maldonado R, Mico J, Valverde O, Saavedra M, Leonsegui I, Gibert Rahola J. Influence of different benzodiazepines on the experimental morphine abstinence syndrome. Psychopharmacology 1991; 105: 197–203.

Nasri H, Shabnam Hajian Sh, Ahmadi A, Baradaran A, Kohi G, Nasri P, Rafieian Kopaei M. Ameliorative effect of green tea against contrast-induced renal tubular cell injury. Iran J Kid Dis. 2015a; 9: 421-26.

Ouyang H, Liu S, Zeng W, Levitt RC, Candiotti KA, Hao S. An emerging new paradigmin opioid withdrawal: A Critical role for glia-neuron signaling in the periaqueductal gray. Scientific World J. 2012; 6: 2-9.

Pieszak M, Mikołajczak PŁ, Manikowska K. Borage (*Borago officinalis* L.): A valuable medicinal plant used in herbal medicine. J Herbal Drugs. 2012; 58: 96-103.

Rabiei Z, Rafieian Kopaei M, Heidarian E, Saghaei E, Mokhtari S. Effects of *Zizyphus jujube* extract on memory and learning impairment induced by bilateral electric lesions of the nucleus basalis of meynert in rat. Neurochemical Res. 2014; 39: 353-60.

Rafieian Kopaei M, Gray AM, Spencer PS, Sewell RD. Contrasting actions of acute or chronic paroxetine and fluvoxamine on morphine withdrawal-induced place conditioning. Eur J Pharmacol. 1995; 275: 185-89.

Shahraki MR, Ahmadimoghadm M, Shahraki AR. The anti-nociceptive effects of hydroalcoholic extract of *Borago officinalis* flower in male rats using formalin test. Basic Clin Neurosci. 2015; 6: 285-90.

Vafaei A, Miladi GH, Taherian A, Bagerian M. Effects of *Valeriane officinalis, Satureja hortensis*, and *Mentha piperita* extracts on the withdrawal syndrome signs in mice. J Semnan Uni Med Sci. 2011; 12: 342-47.

Williams J, Christie M, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev. 2001; 81: 299-343.

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