The role of *Smyrnium cordifolium* Boiss extract and curzerene on withdrawal syndrome in mice

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**Abstract:** Morphine is the most effective medication to relieve pain, antinociception and withdrawal syndrome, but clinical application of these compounds is greatly affected by the occurrence of addiction. The aim of this research was the influence of SE and curzerene (Cur) on withdrawal syndrome signs in mice contrasted with clonidine. Extraction of the *S. cordifolium* extract (SE) was done by the Soxhlet method. Addiction was produced using the subcutaneous injections of morphine for 7 days. To estimate the influences of SE and Cur, the 64 male mice were separated into eight of 8. Sets 1, 2 and 3 were treated SE (100,200,300 mg/kg). Sets 4, 5 and 6 were treated Cur (0.03, 0.06, 0.12mg/kg). The findings showed that curzerene was the most important effective component *S. cordifolium* extract. Curzerene and SE reduced the mean of weight loss, diarrhea, and wet dog shakes in morphine-dependent mice in comparison with clonidine. All doses of Curzerene and SE extract reduced the locomotor activity and body weight loss when compared to the control group in morphine-dependent mice but not to clonidine compared. The SE (100mg/kg) and Cur (0.03mg/kg) are reduced signs of withdrawal syndrome equally to clonidine. SE (200 mg/kg) and Cur (0.06 mg/kg), are reduced of the body weight loss significantly in relation to clonidine (P<0.05). SE (200 mg/kg) and Cur (0.06 mg/kg), are reduced of diarrhea significantly in relation to clonidine (P<0.01). This was while SE (300mg/kg) and cur (0.12mg/kg) had deadly effect. Curzerene and SE are able to decrease the signs of withdrawal syndrome, which might have a human therapeutic capacity. Curzerene may be one of the terpenoids responsible for the effect of the *S. cordifolium* extract. Nevertheless, more investigations are needed to determine the exact mechanism of the effect of SE and curzerene.

**Key words:** Curzerene; Locomotor activity; *Smyrnium cordifolium*; Withdrawal syndrome.

**Introduction**

Opioids bind to specific receptors that are located in the central nervous system (CNS) and many other organs such as cardiovascular tissue. Morphine binds to opioid receptors and can induce oxidative stress under some certain conditions. Opioid dependence is a major health and economic problem worldwide that often requires long-term treatment and care. Morphine is one opioid analgesic mainly used for alleviating pain, anxiety, withdrawal syndrome and chronic diarrhea. Its repeated use leads to physical dependence and tolerance (1).

The chemical variety of plants extracts has made them one of the main sources for the isolation of bioactive natural compounds. Many reports have shown that the use of medicinal herbs can decrease the signs of opioids discontinuation. In old medicine, many plants are used such as *Portulaca oleracea* to decrease morphine dependence (2), *Sophora alopecuroides* alleviates morphine withdrawal syndrome (3) and *Pimpinella anisum* reduces morphine tolerance and physical dependence (4). Moreover, *Teucrium chamaedrys* with anxiolytic and anti-inflammatory activity (5) is used in traditional medicine.

In traditional Iranian medicine, *Smyrnium cordifolium* is consumed to treat antinociception, anxiety and insomnia. There are incomplete reports regarding the analysis of SE hydro alcoholic extract. So that in a study of *S. cordifolium* of essential oil, identification constituents were made by GC/MS. This oil was illustrated by a high content of sesquiterpenes, most of them oxygenated sesquiterpenes with curzerene (16.9%), and curzerenone (33.8%) as major constituents (6,7).

Conforming to several studies of the mechanisms implicated in opioid tolerance and addiction, neurotransmitter systems for instance glutamate (8), dopamine (9), and receptors of stimulatory amino acids specifically the N-methyl-D-aspartate (NMDA) receptor are of greater importance. The function of glutamate receptors (NMDA) has been proven in the opioid synaptic shaping process (10). The investigation has exposed that morphine demonstrates an analgesic and phobia effect by binding to the μ-receptor (11).

The *Smyrnium cordifolium* Boiss is only species of the genus *Smyrnium* in the family apiaceae in the flora of Iran, which is a biennial plant and extensively distributed in the Zagros Mountains at a height of 1400-2000m in the west and northwest of Iran. *S. cordifolium* has many nutritional and medicinal that is used raw materials (12).

In traditional medicine of Iran, *S. cordifolium* is
used to treat pain, anxiety, and addiction withdrawal syndrome (12). Numerous medicinal characteristics, such as a diuretic, reduce urine oxalate, restorative and counteracting renal calculus has been also illustrated for all parts of the plant. Additionally, the aequous and fleshy stems of the plant are used as vegetables (13). Since prolonged use of opioids has adverse effects on the central nervous system, on the other hand, a little information on the analysis of \textit{S. cordifolium} extract and effect on symptoms withdrawal syndrome of has been published. Thus, the aim of this research was to investigate the effects of SE and curzerene on symptoms addiction withdrawal syndrome in mice compared with clonidine.

**Materials and Methods**

**Preparation of extract**

The aerial parts of \textit{S. cordifolium} used for the current research were collected during the flowering phase between February and March 2017 from of the Ghalajah Mountains of Ilam state in Southwest of Iran. A voucher sample of the collection was recognized by Dr. Attar F. (Botanist, Tehran University, Iran) and was deposited at the Department of Biology, Ilam Azad University, Iran. The aerial fragments of the plant were dried in shade for a week and exposed to air flow at room temperature before powdered with an electric mill. The powder plant extract was prepared by Soxhlet extraction method. Each 30g of powder was crushed into a thimble paper and extracted with 500ml of 70\% ethanol. The process of extraction continues for till the solvent in siphon tube of an extractor become colorless (about 48 hours). In order to evaporate the excessive solvent, the sample was then concentrated in a rotary vacuum distiller under negative pressure at 50 °C. The extract was incubated at 30-40 °C until the solvent was fully isolated from the extract and powdered. The powder was conserved in sterile bottles at -20°C until experimentations (8). In our previous studies, the amount of curzerene was determined by HPLC (15).

**Animal**

Seventy-two adult male mice (25-30g) obtained from the Pasteur Institute (Tehran, Iran). The animals were separated into nine groups, each group consisting of eight numbers. The mice were individually housed in eight per cages with temperature was maintained at 23±3 °C with a 12 hr light/dark cycle and had free access to food standard laboratory rodent’s chow) and water. All experiments were performed in accordance with the Guiding Principles for Care and Use of Laboratory Animals of the Faculty of Medicine, Ilam University. The ethical guidelines for the investigation of experimental animals were followed in all tests. All efforts were made to minimize animal suffering and to reduce the number of animals used (8).

**Drugs**

Clonidine hydrochloride tablets, Morphine and Naloxone ampoules were provided from Tolid Daru Co, Tehran-Iran, and also Curzerene of ChemFaces Co.

**Induction of morphine physical dependence**

The mice were injected subcutaneously with morphine twice daily for 7 days. The dose of morphine on days 1 & 2 was 2.5 mg/kg; this dose was doubled every day after that to reach on day 6 a total dose of 40 mg/kg. On day seven, the mice received the last injection of morphine, 50 mg/kg. Previous studies have shown that this method can well addict (15).

**Naloxone-precipitated withdrawal syndrome**

Mice of all groups received (3mg/kg i.p.) naloxone 4h after the last injection of morphine on day 7 of morphine treatment. Therefore, each mouse was placed in a transparent cylinder to observe the frequency of withdrawal manifestations (weight loss, writhing, Grooming, diarrhea, and wet dog shakes) for 30 minutes (15). The weight of the animals was determined before injection of naloxone and 3h after the injection of naloxone. Behaviors of animals were recorded by using a digital video camera.

**Effect of simultaneous of SE and morphine on withdrawal syndrome**

Groups 1(E100), 2 (E200) and 3 (E300), were treated with different doses SE (100, 200, 300mg/kg, Gavage orally) respectively and morphine.

**Effect of co-administration morphine and Cur on the withdrawal syndrome**

Groups fourth (Cur1), fifth (Cur2), and sixth (Cur3), were treated with different doses of Curzerene (0.03, 0.06, 0.12mg/kg) respectively, and morphine. These groups, morphine and Cur received intraperitoneal, respectively.

**Effect of simultaneous of clonidine hydrochloride and morphine on the development of morphine dependence of withdrawal syndrome**

Group7 clonidine hydrochloride (CLO, 0.2 mg/kg i.p.) was administered simultaneously with morphine, twice daily for 6 days; and on day 7, the animals in group 7 received the last doses of clonidine with morphine concurrently 4h before the naloxone challenge. The effect of CLO on the attenuation of morphine dependence was evaluated by comparing the frequency of the behavioral withdrawal symptoms of animals in group 7 and also the ability of SE and Cur in attenuation of morphine dependence versus clonidine was assessed by comparing groups 1, 2, 3, 4, 5 and 6. Group 8th of mice received just morphine.

**Locomotor activity**

The device, built of white timber, had a bottom of 100×100 cm, divided by red lines into 25 squared of 20×20 cm. The walls, 50 cm high, were also highlighted in white. The test room was brightened at the same intensity as the colony room. Every of mouse was located in the middle of the open field and its behavior was checked for 5 minutes. Total activity was taken as the parameter to evaluate the effect of Curzerene and plant extracts on the locomotion activity (14).

**Statistical analysis**

In this research, quantitative data (total locomotion activity, number of behaviors) were analyzed using ANOVA followed by Tukey’s test.
The role of Smyrnium cordifolium... 

Results

The result of showed that yields of SE were obtained as being 17.55% (w/w). Repeated administration of morphine produced physical dependence and addiction as assessed by a characteristic set of behavioral responses including locomotor activity following naloxone challenge. All doses of SE extract reduced the locomotor activity when compared to control group in morphine dependent mice. But only doses of E300 extract reduced the locomotor activity when compared to clonidine group in morphine dependent mice.

In Figure 1, the comparison of mean locomotor activity due to withdrawal syndrome in E100, E200, E300 mg/kg groups receiving three doses is shown. In the experimental group receiving morphine and extract at doses of E100mg/kg and E200, compared to the control group receiving morphine and carrier (p<0.05), and in the group receiving the dose of E300mg/kg extract, also decreased significantly control was observed (P<0.001).

In Figure 2, the comparison of mean locomotor activity due to withdrawal syndrome in Cur1, Cur2, Cur3 groups receiving three doses is shown (0.03, 0.06 and 0.12 mg/kg, respectively). In the experimental group receiving morphine and extract at doses of Cur1 compared to the control group receiving morphine and carrier (p<0.05), and in the group receiving the dose of Cur2, Cur3 extract, also decreased significantly control was observed (P<0.001). However, the concentration of 0.12 curzerene had a fatal effect.

Also, Figure 1 and 2, distinctly shows that chronic morphine injection not reduced the mean frequency of locomotor activity and Body weight loss in dependent mice treated during morphine withdrawal in Cur and SE groups compared to the clonidine group.

In Figure 3, the comparison of the amount of body weight loss due to withdrawal syndrome in the experimental group with three doses of curzerene was shown in comparison with the control group. Body Weight loss in the Cur1 compared to the control group was reduced at P<0.01 but decreased compared to clonidine P<0.05. Whereas in Cur2 and Cur3 groups, body weight loss was observed at P<0.05 compared to the control group. But there was no significant decrease in clonidine.

In Figure 4, the comparison of the sum of weight loss due to withdrawal syndrome in the experimental group with three doses of SE was shown in comparison with the control group. In the experimental group receiving a dose of SE100mg/kg (p<0.01), doses of SE200 and SE300 mg/kg of extract were significantly decreased compared to the control group (P<0.05).

Table 1, clearly show that in morphine dependent mice doses of the SE (200,300 mg/kg) and all three doses of Cur (0.03, 0.06 and, 0.12 mg/kg) compared to control group reduced writhing, grooming, wet dog shakes, and diarrhea. But, dose of the SE 100 mg/kg not reduced the grooming, wet dog shakes, diarrhea and writing behavior. Furthermore, clonidine as a reference drug compared to control group decreased the grooming, writhing and diarrhea (P<0.01).

Statistical analysis showed that clonidine was more effective than Cur1 and Cur2 at a dose of 0.03 and 0.06
Effect of different doses of Curzerene and *S. cordifolium* extract on the wet dog shakes, diarrhea, grooming and writhing in morphine-dependent mice manifestations during 30 min observation period. Qualitative data (wet dog shakes, diarrhea, grooming and writhing) were expressed as 25%-median-75%, and for data analysis, Mann-Whitney U test was used. *P <0.05; **P <0.01, compared to control group. N=8.

All does extracts reduced the wet dog shakes, diarrhea, grooming and writhing episodes. The maximum effects were observed at a dose of curzerene (0.12 mg/kg) and E300 (300mg/kg) hydroalcoholic extract, respectively. The curzerene reduced the wet dog shakes, diarrhea, grooming and writhing more effectively than the hydroalcoholic extract.

**Discussion**

The present results indicate that the different dose of *S. cordifolium* extracts and curzerene reduced the morphine dependence. Medicinal plants are extensively used, currently, for the preparation of different pharmaceutical forms, or as food additives. Our previous studies showed that the SE and the effective combination of the extract of this plant can be valued in reducing the symptoms of withdrawal syndrome such as mean of the jumping and teeth chattering (9). It is reported that some flavonols, terpenoids, and Sesquiterpenes decrease the dependence of morphine (16). So, it may be suggested that these constituents implicated in reducing the withdrawal syndrome.

In vegetable and plants more than 40,000 compounds have been distinguished with molecular structures and diverse biological activities. Most of them are generated by secondary metabolism (17). Terpenoids and sesquiterpenes have the most diversity among plant compounds (18). Phytochemical studies of *S. cordifolium* caused the identification of some sesquiterpins, especially Curzerenone and Curzerene, which had the highest amount in its essential oil (19). Also, studies by Molleken et al have been found in the *S. perfoliatum* Sesquiterpene Germacrone, Isopimarol, D-Germacrone and β-pinene compounds in this plant (17, 19). In another study, Maggi et al. identified the compounds of Isofuranogermacrone, Curzerene, Germacrone, D-Germacrone, Isofuranodiene, β-Phyllocladanal, α-pinene and β-pinene, from *S. olusatrum* (20). Amiri et al. studies indicate that the major part of the essential oil of *S. cordifolium* is sesquiterpene compounds, of which Curzerene (16.9%) and Curzerenone (33.8%) have the highest amount in the essential oil of this plant, and the other compounds of this plant include D-Germacrone 13% and Isopimarol with 10.9%(21), that our research findings are consistent with all of the above studies. Also,

mg/kg and there was no significant statistical difference between the mean frequency of these withdrawal signs induced by naloxone in co-administration of Cur in doses 0.06 mg/kg with morphine compared with clonidine, but in dose of 0.12mg/kg Cur3 the statistical analysis showed that Cur3 significantly was stronger than clonidine in attenuating the morphine withdrawal syndrome. One disadvantage was detected at dose of 0.12mg/kg Cur3 which increased the mortality rate of mice in co-administration with morphine.

Results showed that orally by a NG tube administration of SE at concentrations of 100, 200 and 300 mg/kg significantly, according to the dose administered, and reduced the amounts of weight loss and total motor activity episodes in morphine dependent mice. Clonidine, as a reference drug caused a significantly reduced weight loss and total motor activity episodes.

Also, statistical analysis (Table 1) showed that the effect of chronic morphine injection on the amounts of wet dog shakes, diarrhea, grooming and writhing in the SE100, SE200 and SE300 groups, was significant decrease compared to the Control group in the level (P<0.01). But, the wet dog shakes, diarrhea, grooming and writhing in these three groups, had a significant decrease in control and clonidine at the level (P<0.05).

### Table 1. The effect of intraperitoneal injection of different amounts of SE and Cur on the morphine deprivation syndrome symptoms in male mice.

| Group No. | Wet dog shakes | Diarrhea | Grooming | Writhing |
|-----------|----------------|----------|----------|----------|
| Control   | (2-1)±2.5      | (3-1)±2.5| (3-2)±2  | (3-2)±2.5|
| CLO       | (1-1)±1        | (1-0)±0.5**| (1-0)±0.5**| (1-0)±0.5**|
| SE100     | (1-1)±1        | (3-1)±2.5| (2-1)±1.75| (3-1)±2.5|
| SE200     | (1-0.5)±0      | (1-0)±1 | (2-1)±1* | (2-0)±1* |
| SE300     | (0-0)±0*       | (1-0)±0.5**| (1-0)±0.5**| (1-0)±1** |
| Cur1      | (1-1)±1        | (2-0)±1* | (2-1)±1.5| (2-0)±1 |
| Cur2      | (1-0)±1*       | (1-0)±1**| (2-1)±0.75*| (1-0)±0.5*|
| Cur3      | (0-0)±0*       | (1-0)±0.5*| (1-0)±0.25**| (1-0)±0**|

All does extracts reduced the wet dog shakes, diarrhea, grooming and writhing more effectively than the hydroalcoholic extract.
previous studies with GC-MS and GC-FID showed that S. cordifolium contains more than 16 substances that Curzerene 65.26% of plant composition and δ-Cadinene 14.3%, γ-elemene 5.15%, Curzerenone 2.43%, and Germacrone 2.18% of other S. cordifolium compounds (6).

S. cordifolium is used in traditional medicine by Iran to treat anxiety, inflammatory and insomnia. Although the anticonvulsant properties of S. cordifolium essential oil have been investigated (7). But the reducing signs of addiction withdrawal effects of the SE and Curzerene have not been completely investigated yet.

The reduction in locomotion activity could be by reason of inhibitory effects of the extracts on the CNS or the peripheral muscle relaxant activity which was reported in other studies (2). Therefore, the amounts of weight loss and total motor activity episodes in morphine dependent mice may be influenced by these effects of the SE and extracts.

Morphine is a potent opioid analgesic and anxiolytic that is extensively used for acute and chronic pain control (23). However, repeated or long term usage of opioids improves opioid tolerance and dependence which reduces the therapeutic efficacy of these drugs (24). The results of this research indicate that the major part of the SE contains Curzerene. Lately, the pharmacotherapy of opioid tolerance and addiction has drawn intense interest. The results of this study showed that chronic administration of morphine for 7 days causes dependence and tolerance to the analgesic effects of this substance. On the other hand, after withdrawal of morphine, appeared the symptoms of withdrawal syndrome. These findings are consistent with the Zarindast studies (10).

The results showed that treatment with different doses of SE during the 7 day program with morphine reduced the symptoms of addictive drug withdrawal syndrome in morphine-dependent mice. Terpenoids, sesquiterpenes and herbs containing these compounds exhibit anti-nociceptive, anti-anxiety and sedative activity (18). Therefore, curzerene and the compounds present in the SE of this plant may be part of the anti-nociceptive, anxiolytic and decreasing effects of the syndrome of withdrawal syndrome through various mechanisms such as GABAergic, opioidergic and serotonergic mechanisms.

There are opioid receptors within the CNS as well as throughout the peripheral tissues. The μ, κ and δ opioid receptors are related with the transfer of pain and anxiety. The opioid receptors of the protein group form at the molecular level and attach to the G-protein and affect the opening of the ion channels (K+ channels), intracellular transmissions and protein phosphorylation. The general agonist of these receptors (naloxone) is capable of inhibiting the analgesic and anxiolytic effects of opioid receptors and preventing analgesia. It is identified that naloxone inhibits the effectiveness of analgesic and anxiolytic drugs such as morphine. This drug tends to have the μ opioid receptor in the central nervous system, but less opioid receptors κ and δ (26). Based on studies conducted, with the activation of these receptors calcium entry into the cell increases. Obviously, increasing intracellular calcium concentrations can activate some types of calcium-dependent secondary propagandists. This leads to numerous effects, such as facilitating the activity of Ca2+/calmodulin-dependent protein kinase II (CaMkII)(24), controlling positive feedback activity of protein kinase C (25), the activation of nitric oxide synthase, and finally, the production of nitric oxide (27,28).

Nitric oxide is a nerve modulator derived from the L-arginine nitric oxide synthase enzyme. The nitric oxide synthase enzyme is activated by calcium-calmodulin, protein kinase II (CaMkII) (29). A lot of research suggests that nitric oxide is involved in inducing morphine tolerance and dependence. Also, research has shown that nitric oxide can be co-administered with other neurotransmitter systems, such as the glutamatergic system and the NMDA receptor for its role (30). The previous studies have indicated that there is a two-way relationship between the production of nitric oxide and the release of dopamine (31).

It seems that the SE with an effect on the CNS and PNS can effect of the treatment of anxiety, pain, insomnia, and reduce the symptoms of its addiction withdrawal syndrome. The present study showed that injection of Curzerene and SE had a significant decrease in the amounts of weight loss and total motor activity in morphine-dependent mice comparison to the control and clonidine groups, that this is consistent with Abbasi et al. studies of the anticonvulsant effect of S. cordifolium essential oil (7). Creating a rapid dependence restricts the usefulness of morphine in long-term treatment, but it is thought that the Curzerene and SE are thought to be good agonists for the replacement of morphine.

Central serotonergic nerves are one of the neurological systems involved in opioid dependence. On the other hand, sesquiterpenes such as Curzerene may have analgesic properties, and as an activator of the CNS, by stimulating the anxiety interventionist system, it has an effect on the surface of the GABA receptor, and has an effect that has been studied by Asadi and colleagues (32, 33). It is possible that the SE can reduce the symptoms of discontinuation when it discontinues drug use by reducing pain and relaxation.

Previous studies have shown that discontinuation of morphine leads to a significant reduction in serotonin in several CNS regions, including dorsal raphe nuclei, and also the electrical stimulation of the dorsal raphe nucleus, enhances the release of serotonin. Serotonin in the hypothalamus leads to stimulation of release Enkephalins and decreases the withdrawal symptoms. Therefore, it seems that serotonin is one of the factors involved in physiological changes and behaviors involved in opiate withdrawal (34). Research has shown that terpenoids also have anxiolytic, analgesic and sedative effects through interference with the opioid system (35). Probably effective compounds such as Curzerene and SE have the ability to stimulate the serotonergic system. Therefore, according to the above, it can be justified that the SE may reduce withdrawal symptoms by releasing serotonin.

Our data indicate that SE and curzerene suppresses naloxone-precipitated increase in locomotor activity, wet dog shakes, grooming and writhing behaviors morphine withdrawn mice. Opioids inhibit the activity of locus coeruleus neurons, and naloxone-precipitated withdrawal is associated with the disinhibition of locus coeruleus neurons through blockade of μ-opioid recep-
Noda Y, Nabeshima T. Opiate physical dependence and N-73, 941-9.

The datasets supporting the conclusions of this article

Availability of data and material
Not applicable.

The authors report no conflicts of interest.

Conflict of Interest
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Ethical considerations
All experimental procedures were conducted with the approval of the Ethics Committee of Islamic Azad University of Science and Research Branch of Tehran with an ethical code of 52734.

Consent for publication
Not applicable.

References
1. Jalili C, Sohrabi M, Jalili F, Salahshoor MR. Assessment of thymoquinone effects on apoptotic and oxidative damage induced by morphine in mice heart. Cell Mol Biol 2018; 64(9): 33-38.
2. Karimi, G., Ziaee, T., Nazari, A. Effect of Portulaca oleracea L. extracts on the Morphine Dependence in Mice. Iranian J Basic Med Sci 2008; 10(4): 229-232.
3. Kianbakht S, Hashem Dabaghiyan F. Sophora alopecuroides L. alleviates morphine withdrawal syndrome in mice: involvement of alkaloid fraction and matrine. Iranian J Basic Med Sci 2016; 19(10), 1090-1095. DOI: 10.22038/ijbms.2016.7734.
4. Shirzadi Dara, Abbasi Maleki Saeid, Zanbouri Ali. Ethanolic extract of anise (Pimpinella anisum L.) attenuates morphine physical dependence in mice. J Herbed Pharmacol. 2017; 6(2):69-73.
5. Pourmotabbed A, Farshchi A, Ghiasi G, Malek Khatabi P. Analgesic and Anti-inflammatory Activity of Teucrium chamaedrys Leaves Aqueous Extract in Male Rats. Iranian J Basic Med Sci 2010; 13(3): 119-125.
6. Abbasi N, Mohammadyari E, Asadollahi KH, Tahmasebi M, Ghebad A, Taheri Kalani M. Medicinal characteristics of Smyrnium cordifolium boiss. J Med Plant Res 2014; 8:395-400.
7. Abbasi N, Mohammadpour S, Karimi E, Aidy A, Karimi P, Azizi M, Asadollahi K. Protective effects of Smyrnium cordifolium boiss essential oil on pentylenetetrazol-induced seizures in mice: involvement of benzodiazepine and opioid antagonists. J Biologic Reg Homoes Ag 2017; 31(3): 683-689.
8. Capone F, Adriani W, Shumilina M, Izykenova G, Granstrem O, Dambinova S, et al. Autoantibodies against opioid or glutamate receptors are associated with changes in morphine reward and physical dependence in mice. Psychopharmacol (Berl). 2008;197(4):555-48. DOI:10.1007/s00213-007-1062.
9. Zarrindast MR, Bahreini T, Adl M. Effect of imipramine on the expression and acquisition of morphine-induced conditioned place preference in mice. Pharm Biochem Behav 2002; 73, 941-9.
10. Noda Y, Nabeshima T. Opiate physical dependence and N-methyl-D-aspartate receptors. Eur J Pharmacol 2004; 500(1-3):121-8.
11. Kest B, Palmese CA, Hopkins E, Adler M, Juni A. Assessment of acute and chronic morphine dependence in male and female mice. Pharm Biochem Behav 2001; 70: 149-156.
12. Zargari, A. Iranian medicinal plants. Tehran University Publications, Tehran, 1997; 1:558-565. In Persian.
13. Feily A, Abbasi N. The Inhibitory Effect of Hypericum perforatum extract on morphine withdrawal syndrome in rat and comparison with Clonidine. Phytother. Res. 2009; 23(4):119-125.
14. Pardon M, Pérez-Díaz F, Joubert C, Cohen-Salmon C. Age-dependent effects of a chronic ultramild stress procedure on open-field behavior in B6D2F1 female mice. Physiol Behav 2000; 70(1)-7-13.
15. Nazari P, Yaghmaei P, Rangin A, Abbasi, N. Effects of curzerene and Smyrnium cordifolium Boiss extract on addiction withdrawal syndrome in mice. J Herbmed Pharmacol 2018; 7(4)280-286. doi: 10.15171/jhp.2018.42
16. Molleken U, Sinnwell V, Kubeczka KH. The essential oil composition of fruits from *Smyrnium perfoliatum*. Phytochem 1998; 47:1079-83.

17. Ulubelen A, Goren N, Bohlmann F, Jakupovic J, Granz M, Tanker N. Sesquiterpene lactons from *Smyrnium cordifolium*. Phytochem 1985; 24:1305-8.

18. Roberts, S. C. Production and engineering of terpenoids in plant cell culture, Nat Chem Biol 2007; 3: 387–395.

19. Maggi F, Papa F, Giuliani C, Maleci Bini L, Venditti A, Bianco A, et al. Essential oil chemotaxification and secretory structures of the neglected vegetable *Smyrnium olsatrum* L. (Apiaceae) growing in central Italy. Flavour Fragr J 2015; 30(2):139-59, doi: 10.1002/ffj.3221.

20. Withers Sydor T, Keasling Jay D. Biosynthesis and engineering of isoprenoid small molecules. Appl Microbiol Biotechnol 2007; 73: 980–990.

21. Amiri H, Khavari-Nejad RA, Rustaiyan A. Chemical composition of essential oil and the study of secretory anatomy from *Smyrnium cordifolium* Boiss. Pajouhesh Sazanegi 2007; 20(1):11-6.

22. Ozdemir E, Bagcivan I, Durmus N, Altun A, Gursoy S. The nitric oxide-cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. Can J Physiol Pharmacol. 2011; 89(2):89-95, doi: 10.1139/y10- 10.

23. Shahnouri M, Abousohseini Tabari M, Araghi A. Neuropharmacological properties of farnesol in Murine model. Iranian J Vet Res 2016; 17(57): 259-264.

24. Lutz P-E, Ayragnci G, Chu-Sin-Chung P, Matifas A, Koebel P, Filol D, Befort K, Ouagazzal A-M, Kieffer BL. Distinct Mu, Delta, and Kappa Opioid Receptor Mechanisms Underlie Low Sociability and Depressive-Like Behaviors During Heroin Abstinence. Neuropharmacol 2014; 39(11): 2694-705.

25. Takata T, Kimura J, Tsuchiya Y, Naito Y, Watanabe Y. Calcium/calmodulin-dependent protein kinases as potential targets of nitric oxide. J Nitric Oxide. 2011; 25(2):145-52.

26. Mestek A, Hurley JH, Bye LS, Campbell AD, Chen Y, Tian M. The human μ-opioid receptor: modulation of functional desensitization by calcium/calmodulin – dependent kinase and protein kinase C. J Neurosci. 1995; 15:2396–2406.

27. Heinzen EL, Pollack EM. Pharmacodynamics of morphine-induced neuronal Nitric Oxide production and antinociceptive tolerance development. Brain Res 2004; 1023(2):175-84.

28. Hoque KE, Indorkar RP, Sammut S, Mostafaie A, Yari K. Genetic diversity study of some medicinal plant accessions belong to Apiaceae family based on seed storage proteins patterns. Mol Biol Rep. 2012; 39:10361-10365.

31. Kiss JP and Vizi ES. Nitric oxide: a novel link between synaptic and nonsynaptic transmission. Trends Neurosci 2001; 24(4): 211-5.

32. Assadi A, Zarrindast MR, Jouyban A, Samimi M. Comparing of the effects of hypericin and synthetic antidepressants on the expression of morphine-induced conditioned place preference. Services Iranian J Pharmaceut Res 2011; 10: 619-26.

33. Alavian F, Ghiasvand S. GABAB receptors within the central nucleus of amygdala may involve in the morphine-induced incentive tolerance in female rats. Iranian J Basic Med Sci 2017; 20(7): 822-828. DOI: 10.22038/jbims.2017.9018

34. Feireidoni M, Etemadi L. Involvement of Opioidergic and Serotonergic systems in the anti-nociceptive effect of Tanacetum parthenium. Physiol Pharmacol 2008; 12:115-20.

35. Koob GF, Maldonado R, Stinus L. Neural substrates of opiate withdrawal, Trends Neurosci 15 (1992) 186-191.

36. Quillian N, Lau E, Virk M, Zastrow MV, Williams J. Recovery from Mu-opioid receptor desensitization following chronic treatment with morphine and methadone. J Neurosci 2011; 23:4434-43.

37. Sbhan F, Khan M, Ibrar M, Islam N, Gilani A, Khan A. Effect of aqueous ethanol extract of *Hypericum perforatum* on naloxone-precipitated opiate withdrawal syndrome. Iranian J Pharmaceutical Res 2004; 3:42-43.

38. Spivak C.E., Beglan C.L., Seidleck B.K., Hirshbein L.D., Blaschak C.J., Uhl G.R., Surratt C.K., Naloxone activation of mu-opioid receptors mutated at a histidine residue lining the opioid binding cavity, Mol Pharmacol 52, 1997; 983-992.

39. Taylor J.R., Elsworth J.D., Garcia E.J., Grant S.J., Roth R.H., Redmond D.E., Clonidine JR., infusions into the locus coeruleus attenuate behavioral and biochemical changes associated with naloxone-precipitated withdrawal, Psychopharmacol 96 1988; 121-134.

40. Zebarjadi A., Kazem S, Kahrizi D. Cell dedifferentiation and multiplication of Burdock (Arctium Lappa) as a medicinal plant. Cell Mol Biol 2018; 64(7), 92-96.

41. Keshvare T, Najaphy A, Kahrizi D, Zebarjadi AR. Callus induction and somatic embryogenesis in Stevia rebaudiana Bertoni as a medicinal plant. Cell Mol Biol 2018; 64(2): 46-49.

42. Kazemi N, Kahrizi D, Mansouri M, Karim MH, Vaziri S, Zar-gooshi J, Kahanmadi M, Shokrinia M, Mohammad M. Induction of linalool as a pharmaceutical and medicinal metabolite via cell suspension culture of cumin (Cuminum cyminum L.). Cell Mol Biol 2016; 62(6): 65-68.

43. Esmaeili F, Kahrizi D, Mansouri M, Yari K, Kazemi N, Ghaheri M. Cell dedifferentiation in Stevia rebaudiana as a pharmaceutical and medicinal plant. J Rep Pharmacutic Sci 2016; 5(1): 12-17.

44. Kahrizi D, Rostami-Ahmadvandi H, Akbarabadi A. Feasibility Cultivation of Camelina (Camelina sativa) as Medicinal-Oil Plant in Rainfed Conditions in Kermanshah-Iran’s First Report. J Med Plants By-prod 2015; 2: 215-218.

45. Soomi J, Kahrizi D. Effect of genotype, explant type and 2,4-D on cell dedifferentiation and callus induction in cumin (*Cuminum cyminum L.*) medicinal plant. J Appl Biotechnol Rep 2015; 2(3): 265-270.

46. Kahrizi D, Molsaghi M, Faramarzi A, Yari K, Kazemi E, Farhadzadeh AM, Hemiati S, Hozhabri F, Asgari H, Chaghhamirza K, Zebarjadi A, Yousofovand N. Medicinal plants in Holy Quran. Am J Sci Res 2012; 42: 62-71.

47. Masoumi SM, Kahriz D, Rostami Ahmadvandi H, Soumi J, Kani S, Mostafaeia A, Yari K. Genetic diversity study of some medicinal plant accessions belong to Apiaceae family based on seed storage proteins patterns. Mol Biol Rep. 2012; 39:10361-10365.