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Original Research

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

Nazari Pari[1], Yaghmaei Parichehreh[1*], Rangin Alireza[2], Abbasi Naser[3]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Biology, Ilam Branch, Islamic Azad University, Ilam, Iran
[3] Department of Pharmacology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

[*]**Correspondence to:** Yaghmaei_p@yahoo.com

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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 oleraceae* 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-Daspartate (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 aqueous 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 *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 *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 locomo-

tor activity and body weight loss) were expressed as mean±SD and qualitative data (wet dog shakes and diarrhea) in the median. For analysis of quantitative data, one-way ANOVA and Tukey test were used and the Mann-Whitney test was used for qualitative data analysis. Significant level was considered in $P<0.05$ test.

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

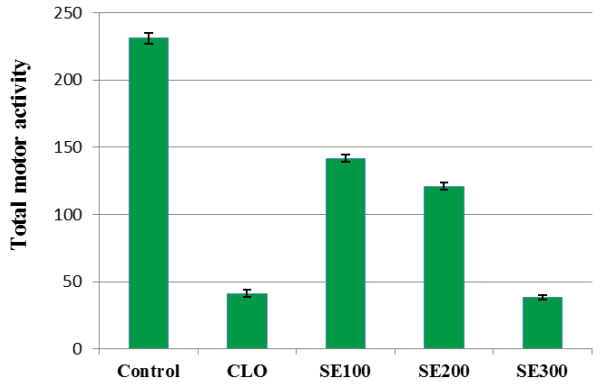

Figure 1. Mean of the locomotor activity SE100 (The group received SE at a concentration of 100mg/kg and morphine), SE200 (The group received SE at a concentration of 200mg/kg and morphine), SE300 (The group received SE at a concentration of 300mg/kg and morphine), CLO (the group received clonidine with 0.2 mg/kg and morphine), Control (the recipient just morphine group).

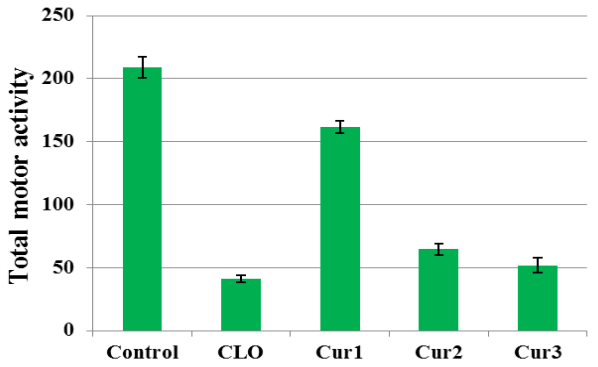

Figure 2. Mean of the total motor activity Cur1 (The group received Cur (0.03 mg/kg) and morphine), Cur2 (The group received Cur (0.06 mg/kg) and morphine), Cur3 (The group received Cur (0.12 mg/kg) and morphine), CLO (the group received clonidine (0.2 mg/kg) and morphine), Control (the recipient just morphine group).

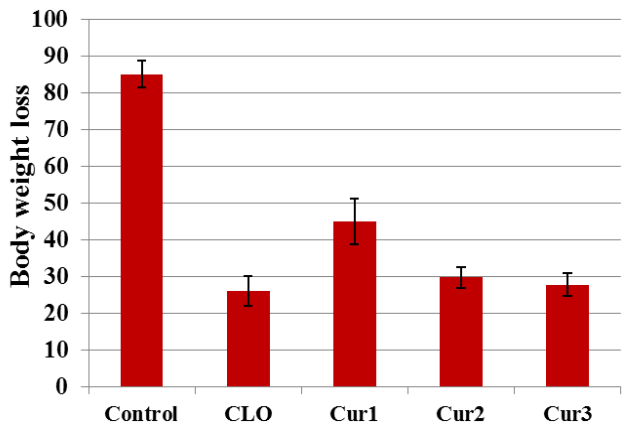

Figure 3. Mean of Body weight loss Cur1 (The group received Cur (0.03 mg/kg) and morphine), Cur2 (The group received Cur (0.06 mg/kg) and morphine), Cur3 (The group received Cur (0.12 mg/kg) and morphine), CLO (the group received clonidine (0.2 mg/kg) and morphine), Control (the recipient just morphine group).

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)±1.25	(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**	(1-0)±0.5**	(1-0)±1**
Cur1	(1-1)±1	(2-0)±1*	(2-1)±1.5	(2-0)±1
Cur2	(1-0)±0*	(1-0)±1**	(2-1)±0.75*	(1-0)±0.5*
Cur3	(0-0)±0*	(1-0)±0**	(1-0)±0.25**	(1-0)±0**

Effect of different doses of Curzerene and *S. cordifollum* 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.

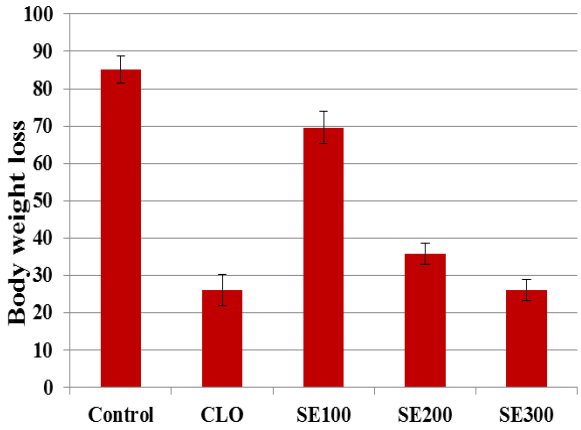

Figure 4. Mean of the Body weight loss SE100 (The group received SE at a concentration of 100mg/kg and morphine), SE200 (The group received SE at a concentration of 200mg/kg and morphine), SE300 (The group received SE at a concentration of 300mg/kg and morphine), CLO (the group received clonidine with 0.2 mg/kg and morphine), Control (the recipient just morphine group).

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).

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 Germacrene, Isopimarol, D-Germacrone and β-pinene compounds in this plant (17, 19). In another study, Maggi *et* al. identified the compounds of Isofuranogermacrene, Curzerene, Germacrone, D-Germacrene, 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,

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 of 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 antagonist 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 Ca^{2+}/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 kinaseII (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-

tors (38). An increase in the activity of locus coeruleus neurons is associated with the expression of withdrawal signs (39). These results suggest that SE and curzerene suppresses withdrawal symptoms by inhibiting central sympathetic outflow, likely by acting on GABAergic interneurons.

The administration of naloxone as an opioid receptor antagonist has reduced the analgesic effect of the SE and it is stated that the analgesic effect of the SE is probably related to the effect of the opioid system. Therefore, with chronic morphine administration, the possibility of endogenous opioids, including Met-enkephalin, is reduced, and this reduction is apparent when discontinued with naloxone injection, the SE has been able to prevent these symptoms. It can be argued that some of the SE compounds such as Curzerene, with opioid-like properties, and thus reduces morphine withdrawal symptoms, which is consistent with studies (36, 37).

Several reports have highlighted the importance of medicinal plants (40-47).

Our findings provide the first evidence that SE and curzerene suppresses morphine induced behavioral sensitization and ameliorates naloxone-precipitated withdrawal in morphine treated mice. The pattern of results supports our view that the curerene and SE is potential target for preventing morphine abuse and dependence. In overall, considering the results of this study, it can be concluded that the compounds present in the SE of *S. cordifolium* increase the antinociceptive and anxiolytic response of morphine and consequently, decrease in dependence and tolerance. So, curzerene and SE may hold promise as a non-addicting alternative to standard opiate replacement therapies to transition patients to opiate abstinence.

Curzerene was found to be more efficient than *S. cordifolium* extract in controlling the opioid withdrawal and craving for the abused substance. It appears that the compounds present in S. cordifolium extract such as Curzerene are capable of reducing the symptoms of withdrawal syndrome and their effectiveness may be more than clonidine. This may have human therapeutic potential.

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Conflict of Interest
The authors report no conflicts of interest.

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.

Availability of data and material
The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AA and NA conceived the project. PY and AA did all the experiments and analysis, overall research was supervised by NA. The manuscript was written by PN, PY and AA. All authors read and approved the final manuscript.

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