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Effects of hydro-ethanolic extract of *Echinophora platyloba* L. (Apiaceae) on the expression and acquisition of morphine-induced place conditioning in female mice

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ABSTRACT

Morphine is a drug that can lead to addiction because of its rewarding effects. The present study investigated the effects of a hydro-ethanolic extract of *Echinophora platyloba* L. (Apiaceae) on the rewarding effects of morphine. The rewarding effects of morphine and the plant extract were evaluated using a conditioned place preference (CPP) paradigm in mice. Additionally, the effects of administering the plant extract on the expression and acquisition of morphine CPP were investigated. Finally, the role of opioid receptors in the CPP of the plant extract was determined. Both morphine and the extract induced a significant CPP. Furthermore, naloxone could inhibit the CPP of the plant extract. The plant extract had no effect on the expression of morphine CPP, but it significantly inhibited the acquisition of morphine CPP. Consequently, the extract of *E. platyloba* L. has rewarding effects, which may be mediated through opioid receptors. The extract can also inhibit the acquisition of morphine CPP.

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1. Introduction

Opioids are a class of drugs with the ability to suppress pain and are of great importance in medicine (Rosenblum et al., 2008; Paiceet al., 2023; Varga et al., 2023). However, the risk of substance use disorder for this class of drugs is high. This is generally concluded from the increasing number of people addicted to opioids around the world (Manchikanti et al., 2012; Nolan et al., 2018). Moreover, a health problem resulting from opioid dependence is a universal public crisis (Kerr, 2019; Damiescu et al., 2021). Morphine, as a natural opioid, is the main alkaloid of the *Papaver somniferum* plant and has beneficial medical uses in healthcare facilities (Cooper et al., 2017). The drug, by binding to opioid receptors, exerts its central and peripheral effects. Although morphine is a ligand for different opioid receptors, it has a greater affinity for mu-opioid receptors (Chahl, 1996). Morphine-induced stimulation of mu-opioid receptors in the dopaminergic mesolimbic pathway of the brain increases dopamine release in the nucleus accumbens, which in turn leads to its euphorogenic or rewarding effects (Bull et al., 2017; Listos et al., 2019). The rewarding effects are the main challenge in the way of morphine use in medicine because such effects facilitate morphine abuse. In animal models, conditioned place preference (CPP) is a standard and simple method for evaluating the rewarding effects of drugs of abuse (Tzschentke, 2007). Many opioids, especially morphine, have been studied for their ability to induce CPP, and this effect has been observed in various species (Tzschentke, 2007).

Historically, medicinal plants have been used for the treatment of various diseases (Foghiset et al., 2023; Nwozoet et al., 2023; Yedjouet et al., 2023). Nowadays, the use of herbal medicine is on the rise due to its efficacy in treating various diseases (Khan and Ahmad, 2019). Even in modern medicine, many

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drugs have herbal origins (Calixto, 2019). Previous reports have demonstrated that different medicinal plants or their constituents may interfere with different pharmacological effects of morphine, such as tolerance, sensitization, dependence, and reward (Sahraei et al., 2006a; Sahraei et al., 2006b; Ghoshooni et al., 2011).

Apiaceae, also known as Umbelliferae, is a family of fragrant flowering plants named after the type genus Apium. It is commonly referred to as the celery, carrot, or parsley family, or simply as umbellifers. From a botanical point of view, the Apiaceae family is considered the 16th largest family of flowering plants, consisting of over 3,800 species spread across approximately 446 genera (Mohammadhosseini, et al., 2013; Mohammadhosseini, et al., 2019). This valuable plant family involves a large number of well-known and economically important plants such as asafoetida, sea holly ajwain, parsnip, lovage, anise, chervil, cumin, coriander, carrot, dill, celery, fennel, cow parsley, parsley, angelica, and caraway. It also encompasses silphium, a plant whose existence is uncertain and which may be on the verge of extinction. Furthermore, the Apiaceae family contains a significant number of species representing remarkable phototoxic impacts, such as giant hogweed (Nahar and Sarker, 2021; El Jabboury et al., 2023). It also includes a smaller number of highly poisonous species, including spotted cowbane, poison hemlock, fool's parsley, water hemlock, along with various species of water dropwort.

E. platyloba L. (Apiaceae) is a native medicinal plant mainly found in the western and northwestern regions of Iran (Mozaffarian, 1996). Traditionally, because of its antifungal effects, local people use this edible plant in dairy products as a flavoring agent (Hosseini, 2017). Moreover, in traditional medicine, the drug is recommended for the treatment of diarrhea and menstrual cycle disorders (Delaram et al., 2011; Anbari et al., 2019). In addition, several reports in the literature indicate other potential medicinal uses of the plant. For example, previous findings have shown that the essential oil and extract of *E. platyloba* L. may have antioxidant, antimicrobial, antimutagenic, anticancer, and analgesic effects (Avizhgan et al., 2006; Sharafati-chaleshtori et al., 2012; Shahneh et al., 2014; Heidarian et al., 2014; Asgari-Nematian and Mohammadi, 2016; Sodeifian and Sajadian, 2017).

The different medicinal properties of the plant extract or its essential oil can be ascribed to the various phytochemicals that are present in these phytochemical sources. It has been shown that the essential oil of *E. platyloba* L. contains ocimene, α-pinene, myrcene, α-phellandrene *trans*-β-ocimene, 2-furanone, myrcene, linalool, *cis*-β-ocimene, asarone, anethole, eugenol, dimethyl styrene, dimethyl styrene isomer, nuciferol, cedran, and isosafrole. In addition, different secondary metabolites have been found in the plant extract, of which saccharose, stigmasterol, sitosterol, stigmasterol-β-D-glycoside, saponins, flavonoid (quercetin), and alkaloids can be mentioned (Hosseini et al., 2017).

In previous research, the hydro-methanolic extract of this plant has been reported to have antinociceptive effects that may be mediated through opioid receptors (Asgari-Nematian and Mohammadi, 2016). Based on such a finding, we hypothesized that the hydroalcoholic extract of *E. platyloba* L. might also interfere with the rewarding effects of morphine. Therefore, the purpose of the present research was to evaluate the effects of the hydro-ethanolic extract of *E. platyloba* L. on the rewarding effects of morphine using an unbiased conditioned place preference paradigm.

2. Experimental

2.1. Subjects of experiments

Female albino mice (NMRI strain), weighing 24-28 g, were used in this research. The animals were purchased from the Pastor Institute in Karaj, Iran. After being transported to the animal house at the University of Maragheh, the mice were housed in standard polycarbonate cages and provided with ad libitum access to rodent diet and tap water. The vivarium temperature was set at 22 ± 2°C, and a 12:12-hour light-dark cycle was established. To relieve the transportation stress and acclimatize the animals to the new conditions, the mice underwent a 7-day adaptation period until the onset of the experiments. The keeping conditions and experimental protocols were approved by the Institutional Ethics Committee for Animal Care and Use at the University of Maragheh, Iran (ethical code: IR.UM.1400.001). In each experimental group, eight mice were used.

2.2. Drugs

Ampoules of naloxone HCl (0.4 mg/mL; Toliddaru Pharma. Co., Tehran, Iran) and morphine sulfate ampoules (10 mg/mL; Darou Pakhsh Pharmaceutical Mfg. Tehran, Iran) were used. Required amounts of these drugs were dissolved in normal saline to prepare different doses of the drugs. The volume of injection was 10 mL/kg. Morphine and naloxone were administered subcutaneously (s.c.) and intraperitoneally (i.p.) to the mice, respectively.

2.3. Plant extract

Aerial parts of *E. platyloba* L. were collected in the spring from Maragheh, which is located in the East Azerbaijan province of Iran. The plant was identified as *E. platyloba* L. by a plant taxonomist at the University of Maragheh, and a voucher herbarium specimen (code: UM-DB-001) was deposited. The plant material was dried in the shade and then ground into a powder. Then, 100 g of the powder was added to 100 mL of 80% ethanol and placed in a shaker-incubator for 72 hours at room temperature. After that, the plant matrix was separated from the hydro-ethanolic solution using filter paper. In the next phase, a rotary evaporator was used to separate the ethanolic part of the solvent. Finally, to acquire the dry material of the plant extract, the liquid material obtained from the rotary apparatus was placed under a hood for five days. After the solvent evaporated, the dry material was stored in a refrigerator until it was used in the experiments. The plant extract was dissolved in saline and administered intraperitoneally to the mice.

2.4. CPP apparatus

Four identical CPP devices were used, each containing two chambers. Each apparatus was similar to the device in our previous experiment with some modifications (Sahraei et al., 2006a). It consisted of two adjacent wooden cubes with identical dimensions (15 cm × 15 cm × 15 cm). The walls of both parts of the device were white but had different patterns. In addition, the floor of one part was smooth, but the other part was rough. The two parts of the device could be separated from each other by one of two types of guillotine doors. One type completely separated the two parts of the CPP apparatus. The other type had a hole (5 cm × 5 cm, which allowed the animals to freely access both parts of the apparatus. In a pilot study, it was shown that in this particular type of apparatus, the mice did not exhibit any inherent preference for one side of the apparatus over the other. Therefore, an unbiased CPP procedure was chosen for the current study.

2.5. Conditioning procedure

The CPP procedure for mice consisted of six consecutive days. The first two days were considered as preconditioning. At this time, the guillotine door with the hole was inserted into the apparatus. Then, each mouse was placed into the apparatus and allowed to explore it freely for 10 minutes. In the following three days, known as the conditioning period, the alternative type of guillotine door was installed into the apparatus, resulting in the separation of the two parts. Then, the mice received two injections each day during the conditioning process. In the first and third conditioning days, the animals received either the drug (morphine) or the plant extract in the morning conditioning session and were confined to one side for 40 minutes. The conditioning procedure was counterbalanced because half of each animal group (n = 4) was confined to one side and the other half to the other side of the apparatus. In the afternoon session, saline was injected into the mice, and they were confined to the opposite side for the same period. On day 2 of the conditioning days, the order of injections was the opposite of the first and third conditioning days, *i.e.*, the animals were given saline and the drug in the morning and afternoon sessions, respectively. The third phase of CPP was the post-conditioning phase. In this phase, the door with the hole was replaced in the apparatus. Each mouse was individually placed into the apparatus for 10 minutes, and their behaviors were recorded with a camera. An individual who was unaware of the test conditions recorded the time each mouse spent on each part of the apparatus using a chronometer. The conditioning score was calculated by subtracting the time each animal spent in the drug-treated part from the time spent in the saline-treated part.

2.6. Locomotion assessment

To ensure that changes in ambulatory activity did not affect the results of CPP experiments, the locomotor activity of mice was measured during the test session. The floor of both parts of the apparatus was divided into four quadrants using cross-shaped lines. During the test, the researchers considered the number of times each animal moved from one quadrant to another as a measure of locomotor activity (Chalabi-Yani et al., 2015).

2.7. Design of the experiments

2.7.1. Dose-response effects of morphine and *E. platyloba* L. extract on the induction of CPP

For acquiring dose-response curves of morphine and the plant extract on mouse conditioning, different doses of morphine (2.5, 5, 10, 15, and 20 mg/kg, s.c.) or *E. platyloba* L. extract (25, 50, 100, and 200 mg/kg, i.p.) were administered to 9 groups of mice (n = 8) on the conditioning days. Two control groups, one for morphine and the other for the plant extract, received saline (10 mL/kg) in all conditioning sessions. Immediately after the injections of saline or drugs, the mice were confined to one part of the apparatus for 40 minutes. On test day, the conditioning score was calculated for mice that received different doses of morphine, the plant extract, or saline.

2.7.2. Effects of *E. platyloba* L. on the expression of morphine-induced CPP

In this part of the experiment, five groups of mice were conditioned with the effective dose of morphine (15 mg/kg, s.c.). Then, on the day of the test, various doses of the plant extract (25, 50, 100, and 200 mg/kg, i.p.) or saline were administered one hour before the test. The control animals received the vehicle one hour before the test.

2.7.3. Effects of *E. platyloba* L. on the acquisition of morphine-induced CPP

To evaluate the effects of the plant extract on the acquisition of morphine conditioning, five groups of mice were administered an effective dose of morphine on conditioning days. On these days, one hour before each morphine injection, they received saline or *E. platyloba* L. extract (25, 50, 100, and 200 mg/kg, i.p.). On the day of the test, the mice were tested while being drug-free.

2.7.4. The role of the brain's opioidergic system on CPP induced by an effective dose of *E. platyloba* L.

Two groups of mice were chosen. On the conditioning days, half an hour before receiving the effective dose of the plant extract (50 mg/kg, i.p.), one group, as a control, received saline (10 mL/kg), and the other group received naloxone (0.1 mg/kg, i.p.). On the test day, the animals received no injection.

2.8. Statistical analysis

In this research, data were presented as mean ± SEM. SPSS software was used to perform the one-way analysis

of variance and the independent samples T-test to find any significant difference between groups. In the case of a significant *p*-value in the ANOVA test, the LSD post hoc test was conducted to confirm whether the differences between the means of the control group and the experimental groups were statistically significant. In our evaluations, a *p*-value of less than 0.5 was considered statistically significant.

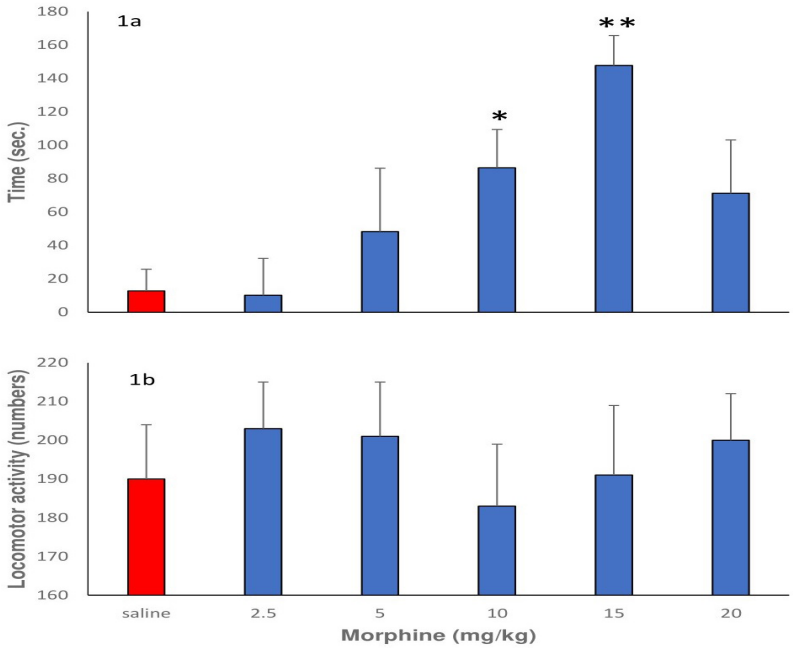

Fig. 1. Effects of morphine on the induction of CPP and locomotor activity. Six groups of mice received morphine (2.5-20 mg/kg, s.c.) or saline (10 mL/kg) on the conditioning days. Morphine could induce a significant CPP at doses of 10 and 15 mg/kg (Fig. 1a) compared with the saline group. Moreover, morphine had no effect on locomotor activity on test day (Fig. 1b). The signs * and ** indicate *p* less than 0.05 and 0.01, respectively.

3.2. Effects of *E. platyloba* L. on CPP and locomotor activity

Administration of different doses of hydro-ethanolic extract of *E. platyloba* L. (0, 25, 50, 100, and 200 mg/kg, i.p.) could induce a significant CPP in the animals [F (4, 35) = 3.27, $p < 0.05$] (Fig. 2a). Post hoc tests showed that low doses of the plant extract, *i.e.*, 25 and 50 mg/kg, could induce a significant CPP compared to the saline group. Analysis of locomotor activity on the test day showed that there was a significant effect of the plant extract on the locomotor activity of the mice on the test day [F(5, 42) = 3.18, $p < 0.05$] (Fig. 2b).

3.3. Effects of hydro-ethanolic extract of *E. platyloba* L. on the expression of morphine CPP

Administering various doses of the plant extract (0, 25, 50, 100, and 200 mg/kg, i.p.) one hour before the test in animals that received the effective dose of morphine (15 mg/kg, s.c.) on conditioning days, could induce a significant CPP [F(5, 42) = 3.04, $p < 0.05$] (Fig. 3a). Further analysis (LSD test) showed that only *E. platyloba* L. at a dose of 50 mg/kg had a significant effect on the morphine conditioning score. Moreover, the analysis of locomotor activity showed a significant decrease in extract-treated mice compared to saline-treated mice [F (5, 42) = 14.72, $p < 0.001$] (Fig. 3b).

3.4. Effects of a hydro-ethanolic extract of *E. platyloba* L. on the acquisition of morphine CPP

Treatment of animals with different doses of *E. platyloba* L. one hour before the administration of morphine (15 mg/kg, s.c.) on conditioning days had a significant inhibitory effect on morphine-induced CPP [F (5, 42) = 5.91, $p < 0.001$]. Further analysis showed that all doses of the plant extract were able to significantly reduce the conditioning effects of morphine. Interestingly, the lowest dose (25 mg/kg) exhibited the highest inhibitory effect on morphine CPP, as depicted in Fig. 4a. Additionally, the analysis of locomotor activity on the test day revealed no significant effect of the plant extract on locomotor activity [F (5, 42) = 0.86, $p > 0.05$] (Fig. 4b).

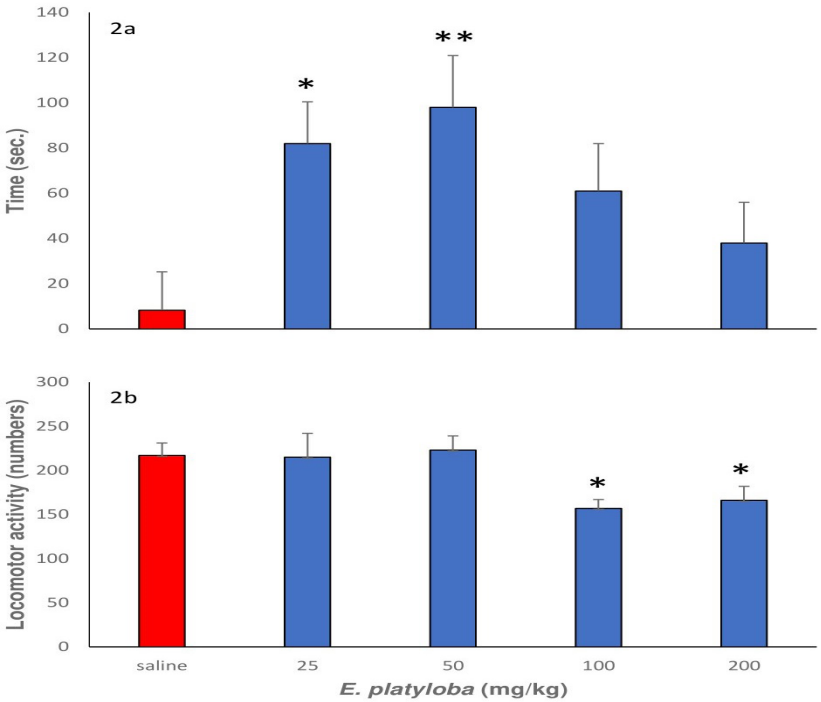

Fig. 2. Effects of a hydro-ethanolic extract of *E. platyloba* L. on the induction of CPP and locomotor activity. Administration of plant extract on conditioning days could induce a significant CPP at doses of 25 and 50 mg/kg (Fig. 2a). The plant extract at doses of 100 and 200 mg/kg decreased locomotor activity on test day (Fig. 2b). The signs * and ** indicate *p* less than 0.05 and 0.01, respectively.

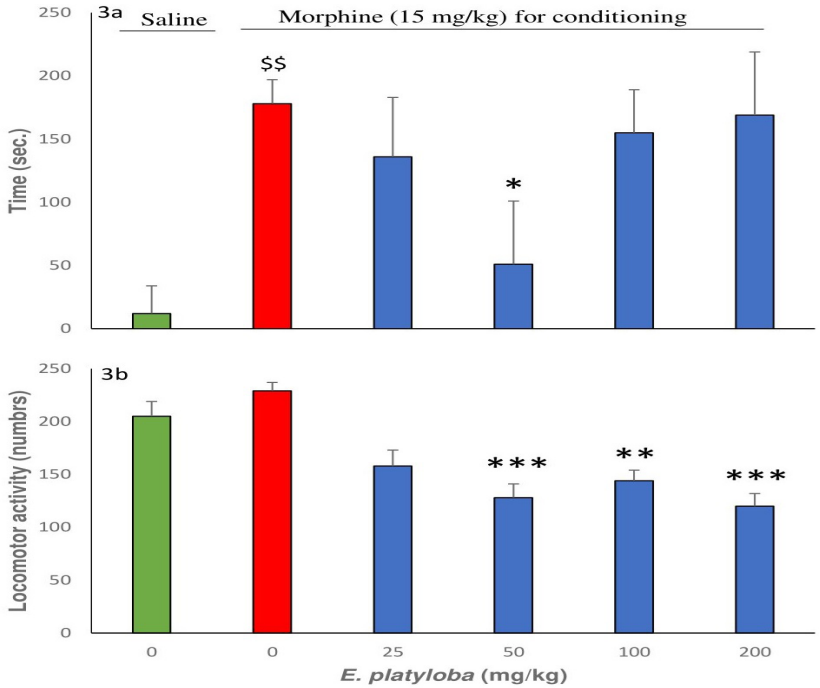

Fig. 3. Effects of a hydro-ethanolic extract of *E. platyloba* L. on the expression of morphine CPP and locomotor activity. Injection of plant extract (25-200 mg/kg, i.p.) one hour before the test significantly decreased morphine CPP compared with the positive control group (Fig. 3a). Moreover, a significant reduction of locomotor activity was observed at doses of 50, 100, and 200 mg/kg (Fig. 3b). The signs *, **, and *** show *p* less than 0.05, 0.01, and 0.001 compared with the positive control (morphine + saline) group. $$ indicates *p* less than 0.01 compared with the negative control (saline + saline) group.

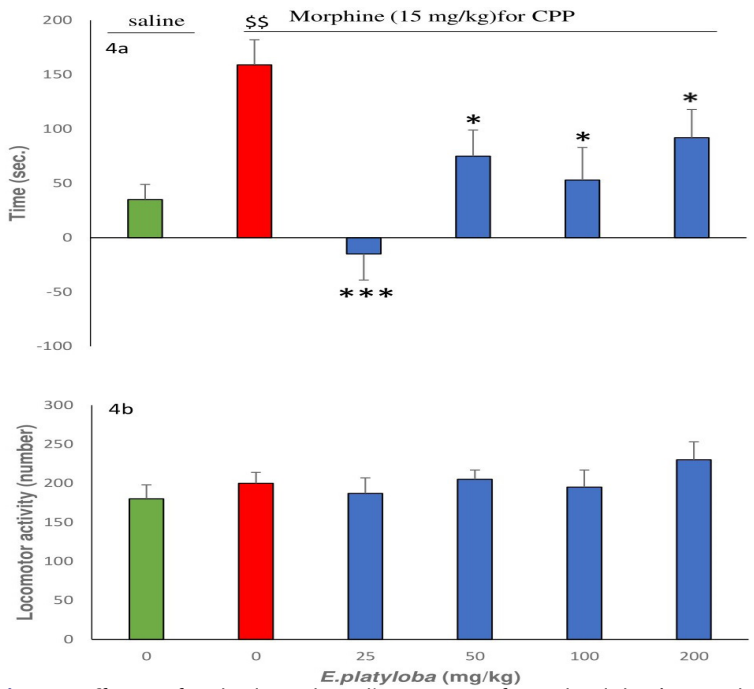

Fig. 4. Effects of a hydro-ethanolic extract of *E. platyloba* L. on the acquisition of morphine-induced CPP and locomotor activity. Injection of different doses of plant extract (25-200 mg/kg) during conditioning with an effective dose of morphine (15 mg/kg) could decrease morphine CPP compared with the positive control group (Fig. 4a). Moreover, locomotor activity did not change on test day (Fig. 4b). The signs * and *** show *p* less than 0.05 and 0.001, respectively, compared with the positive control (morphine + saline) group. $$ indicates *p* less than 0.01 compared with the negative control (saline + saline) group.

3.5. Effect of naloxone on conditioned place preference induced by an effective dose of *E. platyloba* L.

On conditioning days, two groups of mice received saline or naloxone (0.1 mg/kg, i.p.) before receiving an effective dose of the plant extract (50 mg/kg, i.p.). The CPP score was determined on the test day for the animals. The results of the independent samples T-test showed that there were statistically significant differences in CPP scores between the two groups. In other words, administering naloxone before the extract on the conditioning days significantly decreased the conditioned place preference induced by the plant extract. The mice that received saline before the plant extract had significantly higher CPP scores (124 ± 30.7) than the groups that received naloxone before the extract (-58.5 ± 49.8), t (14) = -3.1, and $p < 0.01$.

3.6. Interpretation of the results

The results showed that both morphine and the plant extract had rewarding effects. In addition, the plant extract can inhibit the rewarding effects of morphine. In the initial phase of the study, it was demonstrated that when mice were injected with morphine and exposed to neutral contextual stimuli on one side of the apparatus, it resulted in an increased amount of time spent by the animals on that side during the test day. This indicates that the neutral stimuli in our apparatus have transformed into conditioned stimuli. In other words, morphine exhibited rewarding effects, which manifested as an increase in conditioning scores in the animals that were administered morphine on conditioning days, as opposed to the mice treated with saline. The findings agree with the results of numerous studies showing the rewarding effects of morphine in the CPP paradigm (Tzschentke, 2007).

In the next phase of the current study, the mice that received low doses of the hydro-ethanolic extract of *E. platyloba* L. (25 and 50 mg/kg, i.p.) on the conditioning days showed a significant CPP. On the other hand, the locomotor activity on the test day decreased significantly only in the animal groups that received the high doses (100 and 200 mg/kg) of the plant extract. The results indicate that low doses of the plant extract may have rewarding effects by themselves. To our knowledge, this is the first time that the possible rewarding effects of the plant extract have been revealed using a CPP paradigm. Furthermore, the administration of naloxone before the plant extract on conditioning days could inhibit the CPP caused by the effective dose of the plant extract (50 mg/kg). Given that low doses of naloxone preferentially inhibit mu-opioid receptors (Karimi et al., 2011), it can be concluded that the plant

extract exerted its rewarding effects through mu-opioid receptors. In line with our results, it has been found that opioid receptors are involved in the analgesic effects of a hydro-methanolic extract of *E. platyloba* L. (Asgari-Nematian and Mohammadi, 2016). Previous research has shown that the plant extract contains alkaloids (Valizadeh et al., 2014). Alkaloids may stimulate mu-opioid receptors (Listos et al., 2019; Kaserer et al., 2020), thereby inducing CPP in laboratory animals (Yusoff et al., 2017; Listos et al., 2019). Therefore, the alkaloids of the plant extract may stimulate mu-opioid receptors in the reward pathway and induce the rewarding effects of the plant extract. The intriguing aspect of the rewarding effects of the plant extract in the CPP paradigm was its inverted U-shaped curve, similar to a dose-response curve of morphine.

In the next phase of the research, we investigated the effects of the plant extract on the expression and acquisition of morphine CPP. The acquisition and expression of opioid CPP are the results of complicated interactions between different neurotransmitter systems in different brain regions (Zarindast and Rezayof, 2007). The findings of the present research showed that the hydroalcoholic extract of the plant (50 mg/kg) could inhibit the expression of morphine CPP. In the expression of CPP, various brain regions, especially those that are related to memory, are involved (Shen et al., 2016; Jiang et al., 2018). However, the plant extract's effect on the expression of morphine CPP could not be attributed to the impairment of morphine-induced memory. This is because all doses of the extract were able to inhibit the locomotor activity of the animals on the test day. Therefore, the locomotor activity data ruled out any inhibitory effects of the plant extract on the expression of morphine CPP.

Finally, it was found that injection of the plant extract on the conditioning days could inhibit the acquisition of morphine-induced CPP. On the other hand, in the acquisition test, administration of the plant extract had no significant effect on locomotor activity. Thus, changes in the locomotor activity of the animals had no effect on the obtained results in this part of the experiment. The roles of alkaloids and flavonoids of the plant on the inhibitory effects of the plant extract on the acquisition of morphine CPP should not be neglected (Valizadeh et al., 2014). Alkaloids and flavonoids may possess rewarding properties on their own or may also influence the rewarding effects of morphine. For instance, mitragynine, the major alkaloid of the kratom plant, showed a rewarding effect and inhibited the acquisition and the expression of morphine-induced CPP in rats (Yusoff et al., 2017). Sinomenine, the natural alkaloid derived from *Caulis sinomenii*, could also inhibit the acquisition of morphine-related CPP in mice (Mo et al., 2006). Flavonoids may also have similar effects on morphine reward. For example, dihydromyricetin and baicalin had inhibitory effects on the development and expression of the rewarding effects of morphine in mice. Although the opioid system may have a central role in the effects of plant extract, the importance of other neurotransmitter systems should not be overlooked. In line with this statement, Valizadeh et al. (2014) showed that the extract of aerial parts of *E. platyloba* L. had different phytosterols (stigmasterol, sitosterol, and stigmasterol-β-D-glycoside). Previous research has shown that these compounds may have modulatory effects on the dopamine, serotonin, and GABA systems of the brain (Yin et al., 2018; Karim et al., 2021). Consequently, these secondary metabolites may also alter the rewarding effects of morphine. Considering the various compounds present in the plant extract, a limitation of the current research is the inability to accurately determine the specific compounds and their respective contributions to the observed effects of the plant extract. Therefore, further studies should be conducted to find out the separate roles of each of the phytochemicals. Moreover, future research can explore the potential roles of other brain neurotransmitter systems in the rewarding properties of the plant extract and its inhibitory effects on morphine CPP.

4. Concluding remarks

The present research indicates that *E. platyloba* L. may have rewarding effects, and its rewarding effects may be mediated, at least in part, through the opioidergic system of the brain. The role of the opioid system in morphine tolerance, dependence, and sensitization is well documented in previous research. Thus, the plant extract may also affect the other phenomena of morphine addiction, which can be investigated in the future studies. In addition, the data from this study showed impairment of the acquisition of morphine-induced CPP by the hydro-ethanolic extract of the plant. Moreover, although the plant extract could inhibit the expression of morphine CPP, this effect may be due to its inhibitory effect on locomotor activity on the test day. Therefore, the extract of *E. platyloba* L. does not affect the expression of morphine CPP. The results demonstrate that the plant extract has the ability to inhibit the rewarding effects of morphine. Since morphine and other addictive substances such as nicotine, heroin, cocaine, and alcohol exert their rewarding effects through a final common pathway (mesolimbic dopaminergic system of brain), one may conclude that *E. platyloba* L. extract may also interfere with the rewarding effects of other addictive substances. In conclusion, the plant extract may influence different aspects of addiction caused by addictive substances. However, further research is needed to investigate this hypothesis.

Author contribution statement

Conceptualization and literature search were performed by Amir Abbas Barzegari and Shiva Khezri. The first draft of the manuscript was prepared by Amir Abbas Barzegari. Shiva Khezri critically analyzed the manuscript and provided suggestions for finalization. Parisa Valatabar conducted the laboratory work in the present research. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Anbari, K., Abbaszadeh, S., Basati, G., 2019. Medicinal plants with preventive and therapeutic effect on diarrhoea: A cross-sectional epidemiologic and ethnobotanical study in traditional therapists of Shahrekord, South-West of Iran. Plant. Sci. Today 6(4), 512-517.

Asgari-Nematian, M., Mohammadi, S., 2016. The analgesic effect of *Echinophora platyloba* hydroalcoholic extract in male rats. J. Babol Univ. Med. Sci. 18(5), 31-37.

Avizhgan, M., Hafizi, M., Saadat, M., Nilforoushzadeh, M.A., 2006. Antifungal effect of *Echinophora Platyloba*'s extract against Candida albicans. I.J.P.R. 4, 285-289.

Bull, F.A., Baptista-Hon, D.T., Lambert, J.J., Walwyn, W., Hales, T.G., 2017. Morphine activation of mu opioid receptors causes disinhibition of neurons in the ventral tegmental area mediated by β-arrestin2 and c-Src. Sci. Rep. 7(1), 1-11.

Calixto, J.B., 2019. The role of natural products in modern drug discovery.An. Acad. Bras. Cienc. 91.

Chahl, L.A., 1996. Opioids-Mechanisms of action. Aust. Prescr. 19(3), 5.

Chalabi-Yani, D., Sahraei, H., Meftahi, G.H., Hosseini, S.B., Sadeghi-Gharajehdaghi, S., Beig, H.A., Bourbour, Z., Ranjabaran, M., 2015. Effect of transient inactivation of ventral tegmental area on the expression and acquisition of nicotine-induced conditioned place preference in rats. Iran.Biomed. J. 19(4), 214-219.

Cooper, T.E., Chen, J., Wiffen, P.J., Derry, S., Carr, D.B., Aldington, D, Cole, P., Moore, R.A., 2017. Morphine for chronic neuropathic pain in adults. CochraneDatabase Syst. Rev. (5).

Damiescu, R., Banerjee, M., Lee, D.Y., Paul, N.W., Efferth, T., 2021. Health (care) in the crisis: reflections in science and society on opioid addiction. Int. J. Environ. Res. Public Health 18(1), 341.

Delaram M, Kheiri S, Hodjati MR.,2011. Comparing the effects of *Echinophora-platyloba*, fennel and placebo on pre-menstrual syndrome. J. Reprod. Infertil. 12(3), 221-226.

El Jabboury, Z., Bentaib, R., Stevanovic, Z.D., Ousaaid, D., Benjelloun, M., El Ghadraoui, L., 2023. *Ammi visnaga* (L.) Lam.: An overview of phytochemistry and biological functionalities. Trends Phytochem. Res. 7(3), 141-155.

Foghis, M., Bungau, S.G., Bungau, A.F., Vesa, C.M., Purza, A.L., Tarce, A.G., Tit, D.M., Pallag, A., Behl, T., ul Hassan, S.S., Radu, A.F., 2023. Plants-based medicine implication in the evolution of chronic liver diseases. Biomed. Pharmacother. 158,1-16.

Ghoshooni, H., Daryaafzoon, M., Sadeghi-Gharjehdagi, S., Zardooz, H., Sahraei, H., Tehrani, S., Noroozzadeh, A., Bahrami-Shenasfandi, F., Kaka, G., Sadraei, S., 2011. Saffron (*Crocus sativus*) ethanolic extract and its constituent, safranal, inhibits morphine-induced place preference in mice. Pak. J. Biol. Sci. 14(20), 939-944.

Heidarian, E., Saffari, J., Jafari-Dehkordi, E., 2014. Hepatoprotective action of *Echinophora platyloba* DC leaves against acute toxicity of acetaminophen in rats. J. Diet Suppl. 11(1), 53-63.

Hosseini, Z., Lorigooini, Z., Rafieian-Kopaei, M., Shirmardi, H.A., Solati, K., 2017. A review of botany and pharmacological effect and chemical composition of *Echinophora* species growing in Iran. Pharmacogn. Res. 9(4), 305.

Jiang, J.X., Liu, H., Huang, Z.Z., Cui, Y., Zhang, X.Q., Zhang, X.L., Cui, Y., Xin, W.J., 2018. The role of CA3-LS-VTA loop in the formation of conditioned place preference induced by context-associated reward memory for morphine. Addict. Biol. 23(1), 41-54.

Karim, N., Khan, I., Abdelhalim, A., Halim, S.A., Khan, A., Al-Harrasi, A., 2021. Stigmasterol can be new steroidal drug for neurological disorders: Evidence of the GABAergic mechanism via receptor modulation. Phytomedicine 90, 153646.

Karimi, S., Karami, M., Zardooz, H., Salimi, S.H., Sahraei, H., 2011. Biphasic effects of naloxone in the rats receiving morphine overdose a place preference study. I.J.P.R. 10(3), 605.

Kaserer, T., Steinacher, T., Kainhofer, R., Erli, F., Sturm, S., Waltenberger, B., Schuster, D., Spetea, M., 2020. Identification and characterization of plant-derived alkaloids, corydine and corydaline, as novel mu opioid receptor agonists. Sci. Rep. 10(1), 13804.

Kerr, T., 2019. Public Health Responses to the Opioid Crisis in North America. BMJ Publishing Group Ltd, pp. 377-378.

Khan, M.S.A., Ahmad, I., 2019. Herbal Medicine: Current Trends and Future Prospects, New look to phytomedicine. Academic Press., Cambridge, pp. 3-13.

Listos, J., Łupina, M., Talarek, S., Mazur, A., Orzelska-Górka, J., Kotlińska, J., 2019. The mechanisms involved in morphine addiction: An overview. Int. J. Mol. Sci. 20(17), 4302.

Manchikanti, L., Helm 2nd, S., Fellows, B., Janata, J.W., Pampati, V., Grider, J.S., Boswell, M.V., 2012. Opioid epidemic in the United States. Pain Physician 15(3s), 9-38.

Mo, Z., An, S., Zhou, J., 2006. Effects of Caulis sinomenii and sinomenine on morphine-induced place preference and brain histamine level in mice. Nan Fang yi ke da xue xue bao = J.S.M.U. 26(12), 1709-1713.

Mohammadhosseini, M., Mahdavi, B., Akhlaghi, H., 2013. Characterization and chemical composition of the volatile oils from aerial parts of *Eryngium bungei* Bioss. (Apiaceae) by using traditional hydrodistillation, microwave assisted hydrodistillation and head space solid phase microextraction methods prior to GC and GC/MS analyses: A comparative approach. J. Essent. Oil-Bear. Plants 16(5), 613-623.

Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., Akbarzadeh, A., 2019. The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities – A review. Ind. Crops Prod. 129, 350-394.

Mozaffarian, V., 1996. A dictionary of Iranian Plant Names, Farhang Moaser. Tehran, Iran, pp. 542-544.

Nahar, L., Sarker, S.D., 2021. 'Thankuni' [*Centella asiatica* (L.) Urban]: When food is medicine. Trends Phytochem. Res. 5(3), 164-165.

Nolan, S., Socias, M.E., Wood, E., 2018. The threat of an

international opioid crisis. Curr. Addict. Rep. 5(4), 473-477.
Nwozo, O.S., Effiong, E.M., Aja, P.M. and Awuchi, C.G., 2023. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. Int. J. Food Prop. 26(1), 359-388.
Paice, J.A., Bohlke, K., Barton, D., Craig, D.S., El-Jawahri, A., Hershman, D.L., Kong, L.R., Kurita, G.P., LeBlanc, T.W., Mercadante, S. and Novick, K.L., 2023. Use of opioids for adults with pain from cancer or cancer treatment: ASCO guideline. J. Clin. Oncol. 41(4), 914-930.
Rosenblum, A., Marsch, L.A., Joseph, H., Portenoy, R.K., 2008. Opioids and the treatment of chronic pain: Controversies, current status, and future directions. Exp. Clin. Psychopharmacol.16(5), 405.
Sahraei, H., Barzegari, A.A., Shams, J., Zarrindast, M.R., Haeri-Rohani, A., Ghoshooni, H., Sepehri, H., Salimi, S.H., 2006a. Theophylline inhibits tolerance and sensitization induced by morphine: a conditioned place preference paradigm study in female mice. Behav. Pharmacol. 17(7), 621-628.
Sahraei, H., Fatemi, S.M., Pashaei-Rad, S., Faghih-Monzavi, Z., Salimi, S.H., Kamalinegad, M., 2006b. Effects of *Papaver rhoeas* extract on the acquisition and expression of morphine-induced conditioned place preference in mice. J. Ethnopharmacol. 103(3), 420-424.
Shahneh, F., Baradaran, B., Majidi, J., Babaloo, Z., 2014. *Echinophora platyloba* DC (*Apiaceae*) crude extract induces apoptosis in human prostate adenocarcinoma cells (PC 3). Biomed. J. 37(5), 298-304.
Sharafati-chaleshtori, R., Rafieian-kopaei, M., Mortezaei, S., Sharafati-chaleshtori, A., Amini, E., 2012. Antioxidant and antibacterial activity of the extracts of *Echinophora platyloba* DC. Afr. J. Pharm. Pharmacol. 6(37), 2692-2695.
Shen, F., Wang, X.-W., Ge, F.-F., Li, Y.-J., Cui, C.-L., 2016. Essential role of the NO signaling pathway in the hippocampal CA1 in morphine-associated memory depends on glutaminergic receptors. Neuropharmacology 102, 216-228.
Sodeifian, G., Sajadian, S.A., 2017. Investigation of essential oil extraction and antioxidant activity of *Echinophora platyloba* DC. using supercritical carbon dioxide. J. Supercrit. Fluids 121, 52-62.
Tzschentke, T.M., 2007. Review on CPP: Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. Addict. Biol. 12(4), 227-462.
Valizadeh, H., Mahmoodi, K., Alizadeh, Z., Bahadori, M., 2014. Isolation and structure elucidation of secondary metabolites from *Echinophora platyloba* DC from Iran. J. Med. Plants 13 (49), 15-21.
Varga, B.R., Streicher, J.M., Majumdar, S., 2023. Strategies towards safer opioid analgesics-A review of old and upcoming targets. Br. J. Pharmacol. 180(7), 975-993.
Yedjou, C.G., Grigsby, J., Mbemi, A., Nelson, D., Mildort, B., Latinwo, L. and Tchounwou, P.B., 2023. The management of diabetes mellitus using medicinal plants and vitamins. Int. J. Mol. Sci. 24(10) ,9085.
Yin, Y., Liu, X., Liu, J., Cai, E., Zhao, Y., Li, H., Zhang, L., Li, P., Gao, Y., 2018. The effect of beta-sitosterol and its derivatives on depression by the modification of 5-HT, DA and GABA-ergic systems in mice. RSC Adv. 8(2), 671-680.
Yusoff, N.H., Mansor, S.M., Müller, C.P., Hassan, Z., 2017. Opioid receptors mediate the acquisition, but not the expression of mitragynine-induced conditioned place preference in rats. Behav. Brain Res. 332, 1-6.
Zarindast, M., Rezayof, A., 2007. Morphine-induced place preference: interactions with neurotransmitter systems. Iran. J. Pharm. Res. 6 (1), 3-15.