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The Analgesic Effect of Echinophora Platyloba Hydroalcoholic Extract in Male Rats

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Abstract

BACKGROUND AND OBJECTIVES: In traditional Iranian medicine, use of medicinal herbs is prevalent for the treatment and alleviation of pain and inflammation. *Echinophora Platyloba* is one of the major medicinal herbs with confirmed anti-cancer, anti-microbial, and anti-fungal effects. Therefore, in this study, we aimed to evaluate the analgesic effects of *E. platyloba* hydroalcoholic extracts in male rats.

METHODS: In this experimental study, 42 rats were divided into six groups, i.e., control group, extract groups (80, 100, and 300 mg/kg via intraperitoneal injection), morphine group (1 mg/kg via intraperitoneal injection), and naloxone group (1 mg/kg of naloxone along with 300 mg/kg of the extract). To evaluate the analgesic effects of the extracts, rating, tail flick, and formalin tests were performed.

FINDINGS: In the writhing test, doses of 100 and 300 mg/kg of the extracts could decrease the rating score from 43.3±22.61 to 27.31±2.34 ($p<0.05$) and 29.0±67.91 ($p<0.01$) in the control group, respectively. In addition, administration of 300 mg/kg of the extract in tail flick test decreased the latency time from 2.84±0.76 to 5.52±1.88 sec in the control group ($p<0.05$). Moreover, in formalin test, 300 mg/kg of the extract reduced the pain score in both acute and chronic phases from 2.18±0.22 to 0.74±0.13 in the control group ($p<0.01$).

CONCLUSION: According to the present results, *E. platyloba* hydroalcoholic extracts have central and peripheral analgesic effects.

KEY WORDS: *Hydroalcoholic Extract, Analgesic, Echinophora Platyloba, Medicinal Herbs.*

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Introduction

Pain is one of the major problems in modern societies. In addition to being a sign of tissue damage, pain is an unpleasant feeling which motivates an individual to apply various therapeutic methods (1). Evidence suggests that in the United States, nearly 50 million people at different ages suffer from pain, the management of which requires over 25 million dollars. Today, non-steroidal, anti-inflammatory medications or opiates are utilized for pain management. However, these medications have various side-effects and are accompanied with gastrointestinal tract disorders, kidney damage, and drug dependence. Accordingly, researchers seek new medications, which not only lead to fewer side-effects, but are also cost-effective and accessible at all times (2). Medicinal herbs are an important source of chemical compounds, with strong therapeutic effects (3).

In traditional Iranian medicine, use of medicinal herbs is prevalent for the treatment and alleviation of pain and inflammation. Nevertheless, the origin and mechanism of action mostly remain undetermined in these plants. Therefore, evaluation of the pharmacological effects of plant extracts seems logical for discovering new medications (4,5). The genus *Echinophora* is represented in the flora of Iran (6), and four species including *E. orientalis*, *E. cinerea*, *E. sibthorpiana*, and *E. platyloba* are endemic to this country (7). *E. platyloba* DC belongs to the *Umbelliferae* family and can be found in Mediterranean areas, as well as central and western provinces of Iran (8).

The underground rhizome of this plant can extensively grow, and its vertical stems are full of branches. The leaf edges are thorny, and the plant blossoms from June to September. In Iran, fresh and dried aerial parts of some *Echinophora* species are added to cheese and yogurt as seasoning. Moreover, *Echinophora* species, given their anti-fungal and anti-flatulence characteristics, have been used in traditional medicine for wound repair and treatment of peptic ulcer (as gastric stimulators) (9).

In addition, the anti-microbial and anti-cancer effects of these plants have been confirmed in the literature (10). Furthermore, this herb has been shown to protect the liver against the toxic effects of acetaminophen (11). Flavonoids and alkaloids are among the major compounds of *E. platyloba* (6, 12). In recent years, the analgesic effects of various medicinal herbs, e.g., *Tribulus terrestris* (13), *Allium hirtifolium* (14), *Pimpinella anisum* (15), and *Bryonia dioica* (16), have been confirmed, using tail flick, rating, and formalin tests. According to our literature review, no research has been conducted on the analgesic effects of *E. platyloba* hydroalcoholic extracts via novel experimental methods. Common analgesic compounds such as morphine are derived from medicinal herbs (17) and *E. platyloba* is widely applied in traditional medicine for the treatment of various conditions; therefore, this plant might have analgesic effects (9). In the present study, we aimed to evaluate the analgesic effects of *E. platyloba* hydroalcoholic extracts, using formalin, rating, and tail flick tests.

Methods

Extract preparation: In this experimental study, 2 kg of fresh E. platyloba leaves was used in July 2015 after being confirmed by a botanist at Bu-Ali Sina University of Hamedan, Iran.

After the removal of petioles, Echinophora leaves were dried in shade at room temperature (25°C). Afterwards, the leaves were powdered, using a mechanical mill. In the next stage, 200 g of the dried powder was added to 1 L of 80% methanol and kept for 72 h in order for the effective compounds to be extracted.

The obtained mixture was placed in a rotary device after filtration, and the solvent was separated. Finally, the mixture was kept under a hood in a petri dish for another week to dry. After one week, the residues (herbal extract) were dissolved in a proper amount of the physiological serum (0.9% sodium chloride) in order to treat rats with different concentrations of the extract.

Animals: In total, 42 male Wistar rats (weight: 220-250 g) were purchased from Pasteur Institute of Iran. The animals were maintained under standard conditions in an animal room at the mean temperature of 22±1°C and relative humidity of 50-55% with 12:12 h light-dark cycle (onset: 7 a.m.). The animals had access to food and water ad libitum in their metal cages. They were accustomed to laboratory conditions for at least 2 h before the examination. Tests were carried out from 8:00 am to 12:00 pm. The examinations were in accordance with the ethical considerations of the International Association for the Study of Pain in Laboratory Animals (18). The rats were divided into six groups (seven rats per group) including: control group (normal saline), morphine

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group (1 mg/kg), aspirin group (1 mg/kg), extract groups receiving low, medium, and high doses of E. platyloba (80, 100, and 300 mg/kg, respectively), and naloxone group (1 mg/kg along with 100 mg/kg of the extract).

Pain assessment tests: Rating test: This test is mainly used to evaluate compounds with peripheral analgesic activities. Rating test is mostly utilized to distinguish central pain from peripheral pain (19). In order for the animals to be accustomed to the environment, they were maintained in standard glass boxes for 30 min before the start of the test.

Initially, 18, 38, and 80 mg/kg of the hydroalcoholic extracts were dissolved in sterile physiological serum and intraperitoneally injected in rats. After 15 min, 0.1 ml/kg of acetic acid with a concentration of 1% was injected intraperitoneally. At 5 min following the injection, abdominal contractions were counted (both paws of the rats were fully extended); it should be noted that each animal was used only once (20).

In the control group, the test was performed after the intraperitoneal injection of saline. In case abdominal contractions were less than or equal to half of the medium amount in the control group, it could be concluded that the extracts led to reduced pain in animals. Tail flick test: This test is generally used to evaluate the central analgesic effects of medications and chemical compounds. In fact, this test is specific to medications which affect the central nervous system (21). This test was conducted, using a tail-flick device (model TF-5380, Borj Sanat Co., Iran) and was carried out according to a previously proposed model (22). The light intensity was determined to be seven, and the cut-off time was estimated at 10 sec, i.e., in case the animal did not move its tail after 10 sec of exposure to burning light, it was removed to prevent tissue damage. The animals were horizontally kept in a special animal cage, while their tails were free to move. The latency time of tail flick was measured three times within 2 min intervals before the injection of medications or the extracts.

The mean of the calculated values was recorded as the latency time before drug consumption. Animals with more than 6 sec latency time in two of the three mentioned tests were removed from the experiment. Moreover, at 20 min following drug injection, the test was repeated (three times), and the mean value was recorded as the latency time after drug consumption. In the next stage, morphine was intraperitoneally injected and time of tail flick was recorded. Formalin test: The model recommended by Dubuisson and Dennis (23) was used in this test to evaluate chronic pain. For this purpose, animals were transferred to special boxes one hour before the test in order for them to be adapted to the environment.

This box was made of Plexiglas (30×30×30 dimension), and a mirror with a 45° angle was placed underneath it (in front of the observant) to observe animal movements. At 30 min following the intraperitoneal injection of drugs, 50 µl of 2.5% formaldehyde was injected to the animal's right paw. The animals were returned to the box and their behaviors were studied and scored for 60 min. The strategy was to record the motor response to pain (i.e., scores 0, 1, 2, and 3) every 15 sec.

The scoring system was as follows in this test: score 0, the animal moved with complete balance and its weight was equally distributed on both feet; score 1, the animal could not tolerate its body weight on the injected foot or take care of it; score 2, the animal raised the painful paw and had no contact with the box floor; and score 3, the animal licked, bit, or vigorously shook the painful paw. The mean score over the first 5 min was considered as the first phase of formalin test (acute phase), while the mean score recorded at 15-60 min was determined as the second phase of the test (chronic phase).

Agents: Morphine sulfate and naloxone were obtained from Darou Pakhsh Co. (Iran), while acetic acid and formalin were purchased from Merck Co. (Germany).

Data analysis: Data analysis was performed, using one-way analysis of variance (ANOVA) and Tukey's test. P-value less than 0.05 was considered statistically significant.

Results

The results of the rating test indicated that the injection of 100 mg/kg of the extract led to reduced rating score (abdominal contractions of rats) by 17 units, compared to the control group ($p<0.05$). Moreover, the highest dose of the extract (i.e., 300 mg/kg) reduced the rating by 29 units, compared to the control group ($p<0.01$). In this laboratory model, it was demonstrated that naloxone, coupled with a high dose of the extract, could recover the analgesic effects of the extract. In addition, morphine caused a 39-unit decline in the number of abdominal contractions, compared to the control group ($p<0.001$) (fig 1). In the tail flick

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test, use of 300 mg/kg of the extract resulted in a significant analgesic effect, compared to the control group and could reduce tail-flick latency from 2.84±0.76 to 5.52±1.88 sec ($p<0.05$). Nonetheless, 80 and 100 mg/kg doses showed no significant analgesic effects in this test, compared to the control group. On the other hand, simultaneous use of naloxone and the highest dose of the extract recovered the analgesic effects of the extract. Furthermore, morphine increased tail-flick latency in rats from 2.81±0.34 to 5.52±1.88 sec in the control group ($p<0.01$) (fig 2).

Based on the results of formalin test, 100 mg/kg of the extract showed significant analgesic effects in the chronic phase, compared to the control group ($p<0.05$); the pain score reduced from 2 to 0.9; however, no such effect was observed in the acute phase. Meanwhile, injection of 300 mg/kg of the extract resulted in significant analgesic effects in both chronic and acute phases, compared to the control group; the pain score reduced by 1.5 units, compared to the control group ($p<0.01$). Concomitant use of a high dose of the extract and naloxone reversed the analgesic effects in formalin test. Additionally, use of morphine, similar to 300 mg/kg of the extract, had significant analgesic effects in both chronic and acute phases, compared to the control group ($p<0.01$). Based on the findings, the analgesic effects of *E. platyloba* extracts were mainly observed in the chronic phase of formalin test (fig 3).

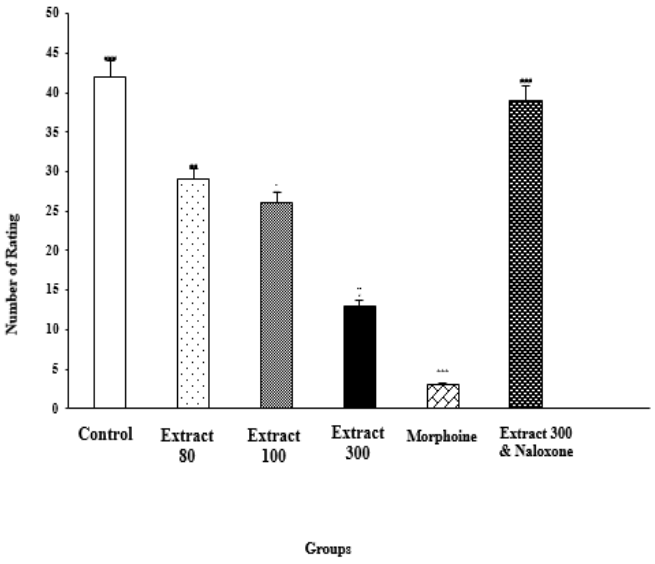

Figure 1. Comparison of the mean rating of rats receiving different concentrations of *E. platyloba* extracts in the acetic acid test

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, difference with the control group

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, difference with the morphine group

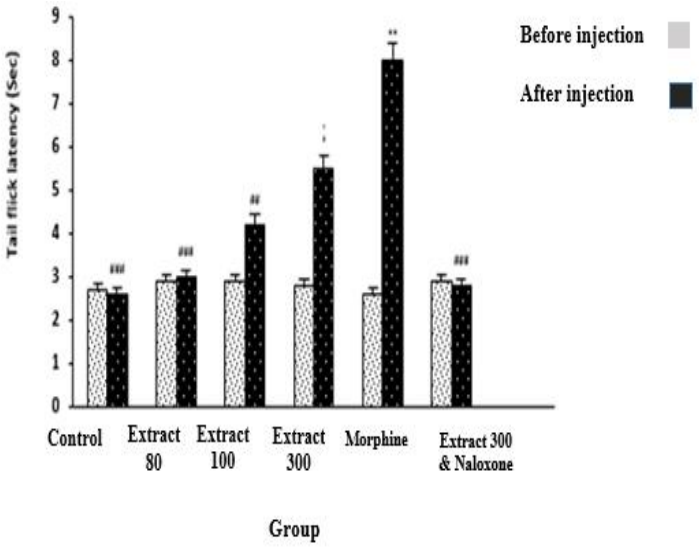

Figure 2. Comparison of the mean concentrations of the extract in tail flick test and the significant difference with the control group

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, significant difference with the morphine group

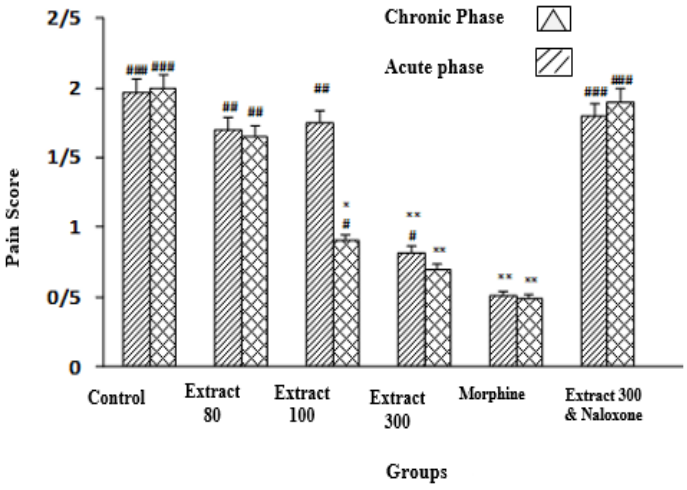

Figure 3. Comparison of the mean pain score in male rats receiving different concentrations of *E. platyloba* extracts in formalin test

$^{*}p<0.05$, $^{**}p<0.01$, compared to the control group

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, significant difference with the morphine group.

Discussion

According to the results of the present study, *E. platyloba* exhibited significant analgesic effects. In this regard, in a study by Sayyah et al., it was determined that *Cuminum cyminum*, which belongs to the *Umbelliferae* family, could exert peripheral analgesic effects (24). In addition, the analgesic effects of *Pluchea quitoc* were confirmed in a study by Barros et

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al., using acetic acid test (25). Similar to previous studies, the hydroalcoholic extract of *E. platyloba* prevented abdominal cramps induced by acetic acid in the current study. Therefore, it seems that the analgesic effects of this plant are supported by peripheral mechanisms.

Intraperitoneal injection of acetic acid could cause acute inflammation in the peritoneum. It is hypothesized that the peripheral analgesic effects of *E. platyloba* are indirectly induced by inflammatory mediators, including bradykinin, serotonin, histamine, substance P, and prostaglandins; this direct influence of mediators is due to the stimulation of peripheral nerves (26).Various studies have been performed to evaluate the analgesic effects of different herbs, using tail flick test. In this regard, in a study by Arambewla et al., it was revealed that a medium dose of *Alpinia calcarata* could reduce pain in the samples (27). Similarly, the results of the present study indicated that the injection of medium and high doses of the studied extract could decrease pain, caused by the thermal stimulator on tail flick test. Considering the application of tail flick test for the evaluation of spinal reflexes and identification of central analgesic pathways (28), it can be concluded that *E. platyloba* extracts could exert central analgesic effects.

Subcutaneous injection of formalin leads to two different pain-inducing phases. The first phase (acute phase) refers to the neurogenic phase, which is directly induced by formalin around active nerves involved in pain induction. On the other hand, the second phase (chronic phase) is known as the inflammatory phase, which is caused by the activation of ventral horn neurons in the spinal cord (29).

Furthermore, the analgesic effect of *Polygonatum verticillatum* was confirmed in a study by Haroon Khan et al., using formalin test. It was suggested that *Polygonatum verticillatum* extracts reduced pain in the chronic phase of formalin test, which was mainly induced by flavonoids and alkaloids in the extract (30). In the present study, the gathered findings were indicative of the inhibitory effect of *E. platyloba* extract on pain; however, this effect was more significant in the chronic phase, compared to the acute phase. Inhibition of the chronic phase by this extract might be due to inflammations, resulting in the release of some compounds such as E_2 and $F_{2\alpha}$ prostaglandins. This release could be responsible for the stimulation of some neurons involved in central pain induction (31). Naloxone (an opioid antagonist) was utilized to evaluate the interference of opioid system, caused by the analgesic effect of the extract; this substance could in fact prevent the activation of opioid receptors (32). The results of this study showed that naloxone caused a significant decline in the analgesic effect of the extract; therefore, it is possible that the analgesic effect of this herb be associated with opioid receptors. As mentioned earlier, *E. platyloba* contains significant phytochemical compounds, such as alkaloids and flavonoids. According to the literature, alkaloid plays a partial role in the palliative opioid system (33). Moreover, different reports have confirmed the analgesic effects of alkaloids (34). Also, different flavonoids have been shown to have various anti-inflammatory and analgesic effects (35).

Alkaloids and flavonoids could lead to reduced intercellular calcium through the inhibition of N-methyl-D-aspartate receptors, followed by the activation of nitric oxide synthases and calcium-dependent phospholipase A_2. Therefore, these compounds apply their analgesic effects through reducing nitric oxides and prostaglandins, especially $F_{2\alpha}$ and E_2 prostaglandins (36-38). Furthermore, various reports have confirmed the analgesic effects of saponins, indicating that they could inhibit the synthesis of inducible nitric oxide synthase and cyclooxygenase-2 (39).

In conclusion, we can state that the use of *E. platyloba* hydroalcoholic extracts could lead to the inhibition of chronic and acute pain in male rats. Despite the fact that the analgesic mechanism of this herb is still unknown, the extracts could both peripherally and centrally reduce pain and lead to increased resistance to pain and decreased response to chronic and acute pain. The gathered findings were substantiated by the available literature on the analgesic effects of herbal medicines, the presence of flavonoids in these herbs, and the induced effects on the central and peripheral systems. It is recommended that further studies be performed to evaluate the mechanisms involved in the analgesic effects of these extracts and focus on other medicinal herbs.

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