Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES
October 2021, Volume 15, Number 4

Research Paper:

Analgesic Effects of the *Cressa Cretica* Extract on Induced Neuropathic Pain in Rats, and the Potential Role of Opioid Receptors

Mohsen Zabihi[1*], Ali Mohammad Ranjbar[1], Mohammad Hossein Mosaddegh[1], Nasrin Zare[1]

1. Department of Pharmacology, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Use your device to scan and read the article online

How to cite this paper Zabihi M, Ranjbar AM, Mosaddegh MH, Zare N. Analgesic Effects of the *Cressa Cretica* Extract on Induced Neuropathic Pain in Rats, and the Potential Role of Opioid Receptors. Iranian Journal of Toxicology. 2021; 15(4):241-248. http://dx.doi.org/10.32598/ijt.15.4.708.3

http://dx.doi.org/10.32598/ijt.15.4.708.3

Article info:
Received: 12 Apr 2021
Accepted: 14 Jun 2021
Online Published: 01 Oct 2021

***Corresponding author:**
Mohsen Zabihi, PhD.
Address: *Department of Pharmacology, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
E-mail: *mzabihi100@gmail.com*

ABSTRACT

Background: Damages to the peripheral fibers of sensory nerve cells and central neurons cause neuropathic pain. Manifestations of neuropathic pain occur in various conditions, including diabetes mellitus, chemotherapy, and as the side effects of some medications. *Cressa cretica* has long been used in traditional medicine for pain control. This study was conducted to determine the role of opioid receptors in the analgesic effect of the hydroalcoholic extract of *C. cretica* in an experimental model of neuropathic pain.

Methods: The hydroalcoholic extract of *C. cretica* was prepared, and its total phenolic and flavonoid contents were standardized. Painful peripheral neuropathy was induced in rats by Chronic Constriction Injury (CCI) of the sciatic nerve. To evaluate the effects of the extract, the animals were orally given *C. cretica* extract (300 mg/kg), gabapentin (70 mg/kg) or normal saline (4 mL/kg) on days 3, 7, 14, and 21 after surgery, and behavioral tests were performed 45 minutes after taking the medications. To evaluate the role of the opioid receptors, Naloxone (1 mg/kg, IP) was given to rats treated with the extract 30 minutes after the extract and then the behavioral tests were performed after 15 minutes.

Results: The hydroalcoholic extract of *C. cretica* attenuates neuropathic pain induced by CCI in rats. The extract works acutely and chronically, depending on the dosage and duration of use.

Conclusion: The hydroalcoholic extract of *C. cretica* reduces CCI-induced neuropathic pain in rats, and Naloxone, as an opioid receptor antagonist, inhibits this effect.

Keywords: *Cressa cretica,* Neuropathic pain, Naloxone, Opioid receptors

Introduction

Damage to the peripheral fibers of sensory nerve cells and central neurons can lead to neuropathic pain. Manifestations of neuropathic pain occur under various circumstances, e.g. diabetes mellitus, chemotherapy, and as the side effects of some medications, such as isoniazid, vincristine, and metronidazole [1-3]. Neuropathy is typically characterized by symmetric distal sensory loss, burning sensations, and/or muscle weakness [4].

Management of the underlying diseases along with symptomatic therapy are the main stay of treating neuropathic pain [4]. Gabapentin, pregabalin, tricyclic antidepressants, and duloxetine are among the drugs that reduce the pain associated with neuropathy and are generally well tolerated [5-9]. Simultaneous treatment with tramadol, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs),

or low-dose narcotics may be necessary in some patients for occasional flair-up of pain conditions [10, 11].

Cressa cretica (*C. cretica*) is a blooming plant species of the morning glory family. This plant grows in parts of Africa, Europe, Asia and Australia. It has a long history of application in traditional medicine, with some of its therapeutic effects studied and confirmed. It inhibits bacterial and fungal growth, is used as expectorant and antitussive drug, and improves digestion [12]. It has been effective as an anticancer drug in combination with *Tridax procumbens* and *Euphorbia thymifolia* and has been shown to improve testicular function in rats [13, 14]. The extract of *C. cretica* is used in traditional medicine in Iran to treat pain. This medicinal plant is a source of many chemical compounds, including quercetin, which is a flavonoid with analgesic effects [15]. There is little research on the effect of *C. cretica* on the management of neuropathic pain. This herb is likely to be accepted by patients in Iran as a better treatment option for neuropathic pain than synthetic drugs due to their potential side effects.

Since no research has been done on the mechanism of analgesic action of this plant, and due to the proven role of opioid receptors in pain management, this study was planned to investigate the role this plant extract as a putative analgesic agent for neuropathic pain and an inhibitor of opioid receptors.

Materials and Methods

Extract preparation: The plant, *Cressa cretica* L., was collected from farms in Yazd, Iran. The scientific name and quality of the plant was confirmed by the Department of Pharmacognosy, Faculty of Pharmacy at Shahid Sadoughi University of Medical Sciences (SSU0043). The aerial parts of the plant were dried at shade and powdered. The hydroalcoholic extract was prepared over four weeks by mixing 100g of the powder in ethanol 80%. The total hydroalcoholic extract was filtered and dried by evaporating the solvent at 40°C, and the total phenolic and flavonoid concentrations were determined. The desired concentration of the extract was prepared by dilution in distilled water.

Extract standardization: The Folin-Ciocalteu method was used to measure the phenolic compounds [15]. The desired hydroalcoholic extract samples were prepared in distilled water at the concentrations of 0.1, 0.01 or 0.001 µg/mL. The extract samples (200 µL each) were collected into separate test tubes, and distilled water (400 µL) was used as the blank. Then 3 mL and 1.5 mL of Folin-Ciocalteu solution (Merck, Germany) were added to the test tubes, containing the extract and distilled water, respectively, and allowed to incubate at 22°C. After 5 minutes of incubation, 1.5 mL and 3 mL of 0.6% sodium bicarbonate (Merck, Germany) were added to the tubes, containing the extract and distilled water, respectively, and incubated again for 5 minutes at 22°C. The optical absorption of the test tubes was read at the wavelength range of 725 nm on a spectrophotometer (Figure 1). Using the following formula, Y=0.0049 x-0.0016; Y=0.1494, X=3.0163 µg /mL, R^2=0.9979, the total phenolic content was determined to be 8.4 µg/mL.

The hydroalcoholic extract samples of the total flavonoid contents were prepared by dilution in distilled water, using quercetin method, At concentrations of 0.1, 0.01 and 0.001µg/mL. Then 300 µL of 5% sodium nitrite (Merck, Germany) was added to the blank and test tubes. After 5 minutes, 300 µL of 10% aluminum chloride (Merck, Germany) and 2 mL of 1M NaOH (Merck, Germany) was added and brought up to a final volume of 100 mL. The absorbances of the samples were read at 510 nm on a spectrophotometer (Figure 2). Using the below formula: Y=0.0025; X = - 0.1495; Y=0.01, X=6.38 µg/mL, R^2=0.9975, the total flavonoid content was determined to be 6.38 µ/mL.

Chemicals: Ketamine (Rotexmedica, Germany), xylazine (Westberg, Netherland), gabapentin (Dr. Abidi Pharmaceutical Co., Iran), and Naloxone (Kaspian Tamin Co., Iran) were purchased. The hydroalcoholic extract of *C. cretica* was prepared by dissolving it in distilled water at 100 or 300 mg/mL [16].

Animals: The study's tests were carried out on adult male Wistar rats, weighing 250±30 g. The animals were housed in cases under a 12-h alternating light-dark cycle at 20-23°C temperature and 50%-60% humidity with free access to standard food and water. All animal experiments were approved by the Institutional Animal Care and Use Committee (Certificate #: IR.SSU.MEDICINE.REC.1397.231) and were conducted according to the institutional guidelines. To minimize animal suffering, additional animal care was provided, and their number was limited to experiments. Efforts were made to minimize animal distress, and the experiments were performed in a quiet room in the morning between 8 to 11 a.m.

Induction of neuropathic pain: Painful peripheral neuropathy was induced by Chronic Constriction Injury (CCI) of the sciatic nerve as described by Bennett and Xie [17] with minimal modifications. General anesthesia was induced by intraperitoneal injection of a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg). After

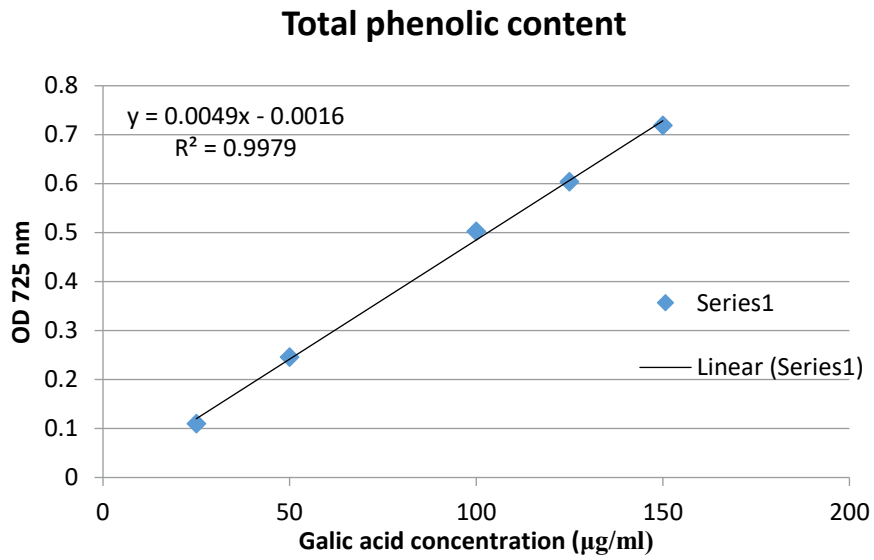

Figure 1. The Folin-Ciocalteu standard curve

shaving the area, a 2cm incision was made on the skin of the lateral surface of the left thigh, and another cut was made directly through the biceps femoris muscle to expose the sciatic nerve. Four silk ligatures (4-0) were loosely tied around the nerve, proximal part of the trifurcation with a distance of 1mm between each ligature. Double knots were used to prevent the slippage. The muscular and skin layers were then sutured with silk threads, and the animals were housed in individual cases after their recovery.

Study protocol: Thirty-six rats were rando mLy divided into six groups six rats each as follows: 1) Control, CCI rats treated with Normal Saline (NS) (4 mL/kg); 2) Sham rats with identical surgery without sciatic nerve ligature, and treated with NS; 3) rats with CCI treated orally with C. critica ethanolic extract (300 mg/kg); 4) Gabapentin, CCI rats treated orally with gabapentin (70 mg/kg); 5) Naloxone, CCI rats treated Intraperitoneally (IP) with Naloxone (1 mg/kg), and 6) Naloxone + Cressa, CCI rats treated orally with *C. cretica* extract (300 mg/kg) plus Naloxone (1 mg/kg, IP). The selection of drug Dosages was based on the pilot and previous studies [13, 18].

To evaluate the effects of treatments, the animals received the medications on days 3, 7, 14, and 21 after surgery, and the behavioral tests were conducted 45 minutes

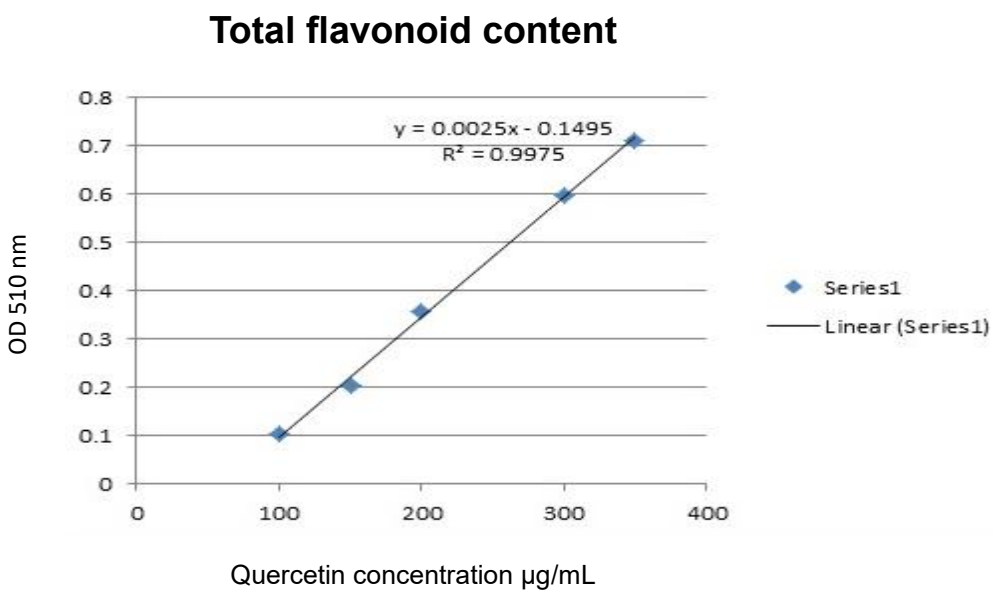

Figure 2. The Quercetin standard curve

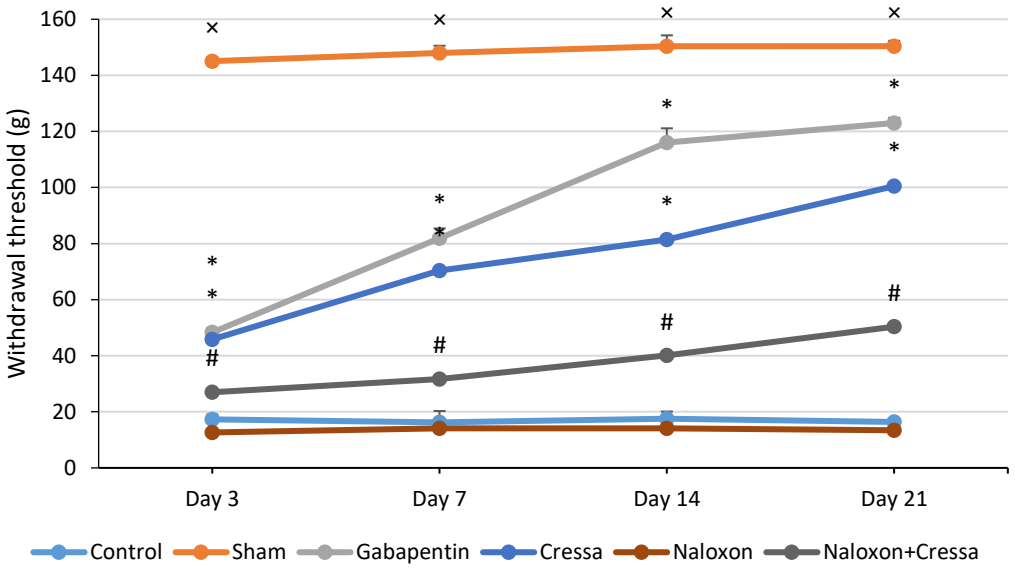

Figure 3. Mechanical allodynia test responses in rats in which neuropathic pain was induced by Chronic Constriction Injury (CCI) method

Control, CCI rats treated orally with normal saline (4 mL/kg). Sham, rats underwent similar surgery except for sciatic nerves not ligated and were treated orally with normal saline (4 mL/kg). Gabapentin, CCI rats treated orally with gabapentin (70 mg/kg), Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/kg), Naloxone, CCI rats treated orally with Naloxone (1 mg/kg, IP), Naloxone+Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/kg) and received Naloxone (1 mg/kg, IP) 45 minutes after the extract. *C. cretica* extract and gabapentin significantly increased the withdrawal threshold compared to Control and Naloxone+Cressa groups on all the evaluation days ($P<0.01$). ×$P<0.001$ in comparison with Control group; * $P<0.01$ in comparison with Control group, #$P<0.01$ in comparison with Naloxone group.

after taking the medicines in each group. In Naloxone + Cressa group, Naloxone was given to rats 30 minutes after administering the extract. This is because the extract takes time to be absorbed when given orally while the IP given Naloxon is absorbed rapidly. The behavioral tests were subsequently performed after 15 minutes.

Mechanical allodynia test: Mechanical allodynia test was performed, using von Frey test as described earlier by Chacur et al. [19]. The von Frey electronic sensor device (ELVAMED, Iran) was touched sub plantar to examine the rats' withdrawal threshold for a mechanical stimulus. The test was repeated five times at two seconds intervals. The response threshold (g) was recorded.

Thermal hyperalgesia test: A plantar test apparatus (Yo Xun 702, Iran) was used to determine paw withdrawal latency time in response to radiant heat (40°C, cut-off time: 22 seconds) [20]. The mean latency of the withdrawal response was recorded in seconds.

Cold allodynia test: Cold allodynia test was performed, using the acetone test (evaporation-evoked cooling) by pouring 1 mL acetone on the hind paws [21]. The test was done five times at 3-min intervals. The paw withdrawal frequency was determined in percentage.

Statistical analyses: Data were expressed as mean±S.E.M, which were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. To compare the variables based on repeated observations at different times, repeated measures ANOVA was used. A P-value <0.05 was considered to show significant differences for all comparisons. All statistical analyses were done using SPSS v. 24.

Results

Induction of neuropathic pain: The CCI-induced neuropathic pain did not affect the motor activity of the animals. Closure of the sciatic nerve used in the model reduced the response threshold (g) significantly upon the mechanical allodynia test (Figure 3, $P<0.001$), increased the paw withdrawal latency time in response to radiant heat (Figure 4, $P<0.001$), and increased the frequency of paw withdrawal on the cold allodynia test (Figure 5, $P<0.001$).

Mechanical allodynia response to induced neuropathic pain: The *C. cretica* extract and gabapentin significantly increased the withdrawal threshold compared to normal saline, and Naloxone mixed with *C. cretica* extract on all pain evaluations. By adding Naloxone to the *C. cretica* extract, the withdrawal threshold was reduced

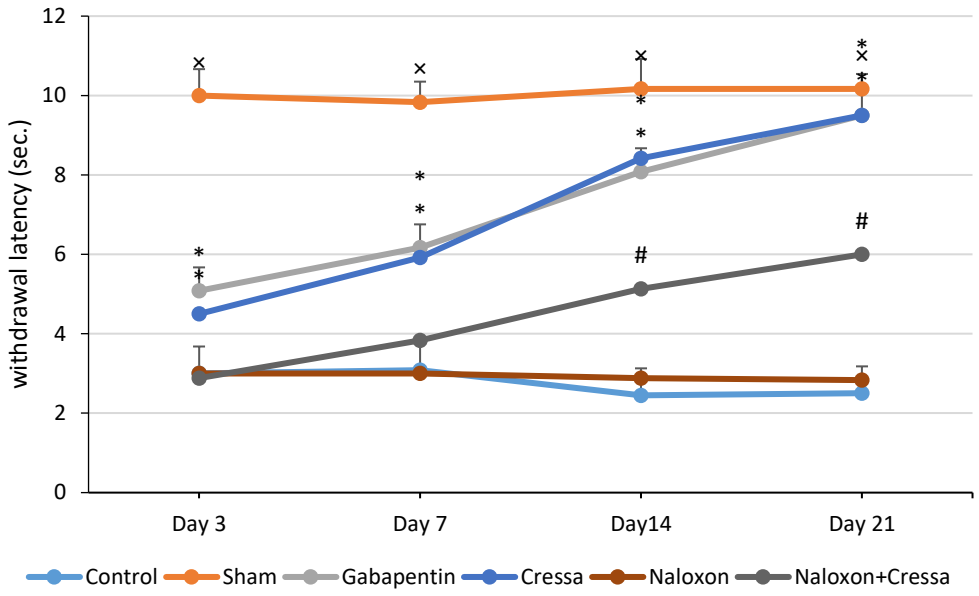

Figure 4. Thermal hyperalgesia test responses in rats in which neuropathic pain was induced by Chronic Constriction Injury (CCI) method

Control, CCI rats treated orally with normal saline (4 mL/kg). Sham, rats underwent similar surgery except for sciatic nerves not ligated and were treated orally with normal saline (4 mL/kg). Gabapentin, CCI rats treated orally with gabapentin (70 mg/kg), Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/kg), Naloxone, CCI rats treated orally with Naloxone (1 mg/kg, IP), Naloxone+Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/kg) and received naloxone (1mg/kg, IP) 45 minutes after the extract. *C. cretica* extract and gabapentin significantly increased withdrawal latency time compared to Control and Naloxone+Cressa groups on all the evaluation days ($P<0.01$). ˣ$P<0.001$ in comparison with Control group, * $P<0.01$ in comparison with Control group, #$P<0.01$ in comparison with Naloxone group.

compared to the extract alone, while Naloxone alone did not alter this effect (Figure 3, $P<0.01$). The effects of gabapentin were more significant than the *C. cretica* extract on days 7, 14, and 21 post surgery (Figure 3, $P<0.05$).

Thermal hyperalgesia response to induced neuropathic pain: The *C. cretica* extract and gabapentin significantly increased the withdrawal latency time compared to that of normal saline and Naloxone mixed with *C. cretica* extract on all pain evaluations. By adding naloxone to the *C. cretica* extract, the withdrawal latency declined compared to that of the extract alone, while Naloxone alone did not alter this effect (Figure 4, $P<0.01$). On the thermal hyperalgesia test, there was no difference between the *C. cretica* extract and gabapentin on days 3, 7, 14, and 21 after surgery.

Cold allodynia response to induced neuropathic pain: The *C. cretica* extract and gabapentin significantly reduced the paw withdrawal frequency in the rats by acetone test compared to that of the normal saline or Naloxone combined with the *C. cretica* extract on all pain evaluations. Also, there was no significant difference between the extract and gabapentin for their analgesic effect. By adding Naloxone to the *C. cretica* extract, the withdrawal frequency was significantly decreased compared to that of the *C. cretica* extract alone, while Naloxone alone did not alter this parameter (Figure 5, $P<0.01$).

Discussion

Based on the results, the technique used in this study, i.e., Chronic Constriction Injury (CCI) model induced neuropathic pain in experimental rats, as manifested by the behavioral changes in the animals. Neuropathic pain developed three days after the surgery and lasted at least over the course of the study (21 days). Most of the pain occurred on day 14 post surgery and then remained almost constant until the end of the study.

Gabapentin, as an analgesic drug improved the behavioral symptoms resulting from the induced neuropathic pain in rats, confirming the accuracy of neuropathic pain induction [22]. The data analysis shows that the effects of the *C. cretica* extract (300 mg/kg, orally) in improving the neuropathic pain in animals are very similar to those of gabapentin (70 mg/kg, orally). Further, *C. cretica* contains edible oils that may be used safely in humans [23]. Numerous studies have shown that it has anti-inflammatory, antipyretic, and analgesic effects [24, 25]. Also, the

Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

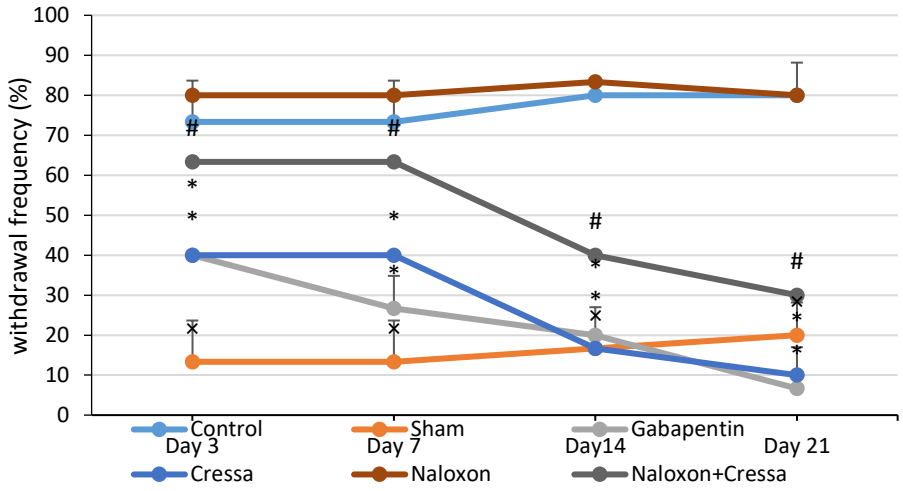

Figure 5. Cold allodynia (acetone) test responses in rats in which neuropathic pain was induced by Chronic Constriction Injury (CCI) method.

Control, CCI rats treated orally with normal saline (4 mL/kg). Sham, rats underwent similar surgery except for sciatic nerves not ligated and were treated orally with normal saline (4 mL/kg). Gabapentin, CCI rats treated orally with gabapentin (70 mg/Kg), Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/Kg), Naloxone, CCI rats treated orally with Naloxone (1 mg/Kg, IP), Naloxone+Cressa, CCI rats treated orally with *C. cretica* hydroalcoholic extract (300 mg/Kg) and received Naloxone (1 mg/Kg, IP) 45 minutes after the extract. *C. cretica* extract, and gabapentin significantly decreased paw withdrawal frequency by acetone test compared to Control and Naloxone+Cressa groups on all the evaluation days ($P<0.01$). × $P<0.001$ in comparison with Control group, * $P<0.01$ in comparison with Control group, # $P<0.01$ in comparison with Naloxone group.

antioxidant effect of the *C. cretica* extract against the oxidation process has been determined [18]. Also, *C. cretica* is a source of many chemical compounds, including quercetin, quercetin-3-Oglucoside, cressa-tetra-cosanoate, cressa-tetra-triacontanoic acid, cressa-triacontanone, cressa-naphthacenone, creticane, 3,5-dicaffeoylquinic acid, rutin, syringaresinolh-d-glucoside scopoletin, and kampferol-3-O-glucoside [26]. Although the physiological effects of most of the compounds in *C. cretica* have not yet been studied, the presence of quercetin in this plant is well known.

Quercetin is an anti-inflammatory and analgesic agent. It modulates the inflammatory and immune systems by acting on various cells, including mast cells, dendritic cells, and glial cells. It inhibits Lipopolysaccharide (LPS)-induced Tumor Necrosis Factor α (TNF-α) production in macrophages and LPS-induced IL-8 production in the lung A549 cells. Quercetin inhibits the production of inflammatory enzymes, including cyclooxygenase and lipoxygenase. It significantly inhibits neurological and inflammatory pain by acting on several systems involved, including vanilloid, D2-dopamine, alpha-2-adrenergic, 5-HT3, and glutamate receptors [27]. The antioxidant effects of the flavonoids, rutin and quercetin, inhibit oxaliplatin-induced chronic painful peripheral neuropathy [28, 29]. Quercetin has reduced pain in a diabetic neuropathic pain model by modifying opioid mechanisms [30]. In another study, however, Naloxone did not block the analgesic effects of quercetin and the hot-plate test responses, indicating that the opioid system is not involved in its analgesic property [31].

Based on the compounds found in the *C. cretica* extract as mentioned earlier, quercetin and rutin are considered as known compounds in the extract, justifying the effect of the extract on neuropathic pain. Further, there is evidence to suggest that opioid receptors are involved in the analgesic effects of rutin and quercetin [32, 33]. In this study, Naloxone alone, as an opioid receptor antagonist, did not affect the behavioral tests in the rats with induced neuropathic pain, whereas the administration of Naloxone (1 mg/kg, IP) 15 minutes after giving the *C. cretica* extract (300 mg/kg) to the rats orally, significantly reduced the neuropathic analgesic effects of the extract. Considering that opioid receptors are present in peripheral, spinal, and supraspinal areas, and the fact that Naloxone inhibits all types of opioid receptors (μ, δ, and κ), it may be suggested that opioid receptors may be involved in the neuropathic analgesic effects of the *C. cretica* extract [34-38].

Due to the lack of sufficient information about the effects of other compounds in the *C. cretica* hydroalcoholic extract, further studies are warranted to fully understand other compounds present in the *C. cretica* extracts, able to relieve neuropathic pain. Also, it is important to study the

primary mechanism of action of the *C. cretica* extract and its molecular interaction with opioid receptors.

Conclusions

The hydroalcoholic extract of *C. cretica* reduced CCI-induced neuropathic pain in experimental rats, and Naloxone, as an opioid receptor antagonist, counteracted the analgesic effects of the *C. cretica* extract in induced neuropathic pain.

Ethical Considerations

Compliance with ethical guidelines

All animal experiments were approved by the Institutional Animal Care and Use Committee (Registered #: IR.SSU.MEDICINE.REC.1397.231) and were conducted according to the institutional guidelines. To minimize animal suffering, additional animal care was provided, and their number was limited to experiments. Efforts were made to restrict animal distress, and the experiments were performed in a quiet room in the morning, 8-11 a.m.

Funding

This paper was extracted from a PhD. dissertation conducted by Nasrin Zare at the Department of Pharmacology, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd. Also, the study was supported by the School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd.

Author's contributions

Conceptualization and supervision: Mohsen Zabihi, Mohammad Hossein Mosaddegh and Ali Mohammad Ranjbar; Methodology: Mohsen Zabihi and Nasrin Zare; Investigation; Writing – original draft, and writing – review & editing: All authors; Data collection: Nasrin Zare; Data analysis: Mohsen Zabihi and Nasrin Zare.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Hughes R. Investigation of peripheral neuropathy. BMJ. 2010; 341:c6100. [DOI:10.1136/bmj.c6100] [PMID]

[2] Russell JW, Zilliox LA. Diabetic neuropathies. Continuum (Minneap Minn). 2014; 20(5):1226-40. [DOI:10.1212/01.CON.0000455884.29545.d2] [PMID] [PMCID]

[3] Staff NP, Windebank AJ. Peripheral neuropathy due to vitamin deficiency, toxins, and medications. Continuum (Minneap Minn). 2014; 20(5):1293-306. [DOI:10.1212/01.CON.0000455880.06675.5a] [PMID] [PMCID]

[4] Watson JC, Dyck PJB. Peripheral neuropathy: A practical approach to diagnosis and symptom management. Mayo Clin Proc. 2015; 90(7):940-51. [DOI:10.1016/j.mayocp.2015.05.004] [PMID]

[5] Backonja M, Glanzman RL. Gabapentin dosing for neuropathic pain: Evidence from randomized, placebo-controlled clinical trials. Clin Ther. 2003; 25(1):81-104. [DOI:10.1016/S0149-2918(03)90011-7]

[6] Sindrup SH, Otto M, Finnerup NB, Jensen TS. Antidepressants in the treatment of neuropathic pain. Basic Clin Pharmacol Toxicol. 2005; 96(6):399-409. [DOI:10.1111/j.1742-7843.2005.pto_96696601.x] [PMID]

[7] Zabihi M, Hajhashemi V, Minaiyan M, Talebi A. Evaluation of the central and peripheral effects of doxepin on carrageenan-induced inflammatory paw edema in rat. Res Pharm Sci. 2017; 12(4):337-45. [DOI:10.4103/1735-5362.212052] [PMID] [PMCID]

[8] Wiffen PJ, Derry S, Moore RA, Kalso EA. Carbamazepine for chronic neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev. 2014; (4):CD005451. [DOI:10.1002/14651858.CD005451.pub3] [PMID] [PMCID]

[9] Birse F, Derry S, Moore RA. Phenytoin for neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev. 2012; (5):CD009485. [DOI:10.1002/14651858.CD009485.pub2] [PMID] [PMCID]

[10] Moore RA, Chi CC, Wiffen PJ, Derry S, Rice AS. Oral nonsteroidal anti-inflammatory drugs for neuropathic pain. Cochrane Database Syst Rev. 2015; (10):CD010902. [DOI:10.1002/14651858.CD010902.pub2] [PMID] [PMCID]

[11] Duehmke RM, Derry S, Wiffen PJ, Bell RF, Aldington D, Moore RA. Tramadol for neuropathic pain in adults. Cochrane Database Syst Rev. 2017; 6(6):CD003726. [DOI:10.1002/14651858.CD003726.pub4] [PMID] [PMCID]

[12] Warrier PK. Indian medicinal plants: A compendium of 500 species. India: Orient Longman; 1993. https://books.google.com/books/about/Indian_Medicinal_Plants.html?id=uxrbAAAAMAAJ&source

[13] Priyashree S, Jha S, Pattanayak SP. A review on Cressa cretica Linn.: A halophytic plant. Pharmacogn Rev. 2010; 4(8):161-6. [DOI:10.4103/0973-7847.70910] [PMID] [PMCID]

[14] Austin DF. A revision of Cressa L. (Convolvulaceae). Bot J Linn Soc. 2000; 133(1):27-39. [DOI:10.1111/j.1095-8339.2000.tb01535.x]

[15] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007; 2(4):875-7. [DOI:10.1038/nprot.2007.102] [PMID]

[16] Lekar A, Borisenko S, Vetrova E, Sushkova S, Borisenko N. Extraction of quercetin from Polygonum hydropiper L. by subcritical water. Am J Agric Biol Sci. 2014; 9(1):1-5. [DOI:10.3844/ajabssp.2014.1.5]

[17] Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988; 33(1):87-107. [DOI:10.1016/0304-3959(88)90209-6]

[18] Afshari A, Sayyed-Alangi SZ. Antioxidant effect of leaf extracts from Cressa cretica against oxidation process in soybean oil. Food Sci Nutr. 2017; 5(2):324-33. [DOI:10.1002/fsn3.396] [PMID] [PMCID]

[19] Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, et al. A new model of sciatic inflammatory neuritis (SIN): Induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain. 2001; 94(3):231-44. [DOI:10.1016/S0304-3959(01)00354-2]

[20] Li X, Angst MS, Clark JD. Opioid-induced hyperalgesia and incisional pain. Anesth Analg. 2001; 93(1):204-9. [DOI:10.1097/00000539-200107000-00040] [PMID]

[21] Bridges D, Ahmad K, Rice AS. The synthetic cannabinoid WIN55, 212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br J Pharmacol. 2001; 133(4):586-94. [DOI:10.1038/sj.bjp.0704110] [PMID] [PMCID]

[22] Moore RA, Wiffen PJ, Derry S, Rice AS. Gabapentin for chronic neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev. 2014; (4):CD007938. [DOI:10.1002/14651858.CD007938.pub3]

[23] Weber D, Ansari R, Gul B, Khan MA. Potential of halophytes as source of edible oil. J Arid Environ. 2007; 68(2):315-21. [DOI:10.1016/j.jaridenv.2006.05.010]

[24] Hussain S, Ahmed E, Malik A, Jabbar A, Arshad M. Phytochemical studies on Cressa cretica. J Chem Soc Pakistan. 2005; 27(3):296-8. https://www.researchgate.net/publication/286960300_Phytochemical_studies_on_Cressa_cretica

[25] Tall JM, Raja SN. Dietary constituents as novel therapies for pain. Clin J Pain. 2004; 20(1):19-26. [DOI:10.1097/00002508-200401000-00005] [PMID]

[26] Dyson A. Discovering indigenous healing plants of the herb and fragrance gardens at Kirstenbosch National Botanical Garden. South Afric: National Botanical Institute; 1998. https://books.google.com/books/about/Discovering_Indigenous_Healing_Plants_of.ht mL?id

[27] Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. Nutrients. 2016; 8(3):167. [DOI:10.3390/nu8030167] [PMID] [PMCID]

[28] Azevedo MI, Pereira AF, Nogueira RB, Rolim FE, Brito GA, Wong DV, et al. The antioxidant effects of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. Mol Pain. 2013; 9:53. [DOI:10.1186/1744-8069-9-53] [PMID] [PMCID]

[29] Bureau G, Longpré F, Martinoli MG. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. J Neurosci Res. 2008; 86(2):403-10. [DOI:10.1002/jnr.21503] [PMID] [PMCID]

[30] Anjaneyulu M, Chopra K. Quercetin, a bioflavonoid, attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain. Prog Neuropsychopharmacol Biol Psychiatry. 2003; 27(6):1001-5. [DOI:10.1016/S0278-5846(03)00160-X]

[31] Willain Filho A, Cechinel Filho V, Olinger L, de Souza MM. Quercetin: Further investigation of its antinociceptive properties and mechanisms of action. Arch Pharm Res. 2008; 31(6):713-21. [DOI:10.1007/s12272-001-1217-2] [PMID]

[32] Selvaraj G, Kaliamurthi S, Thirungnasambandam R, Vivekanandan L, Balasubramanian T. Anti-nociceptive effect in mice of thillai flavonoid rutin. Biomed Environ Sci. 2014; 27(4):295-9. [DOI:10.3967/bes2014.052] [PMID]

[33] Ruiz-Miyazawa KW, Staurengo-Ferrari L, Mizokami SS, Domiciano TP, Vicentini FT, Camilios-Neto D, et al. Quercetin inhibits gout arthritis in mice: Induction of an opioid-dependent regulation of inflammasome. Inflammopharmacology. 2017; 25(5):555-70. [DOI:10.1007/s10787-017-0356-x] [PMID]

[34] Heyman JS, Koslo RJ, Mosberg HI, Tallarida RJ, Porreca F. Estimation of the affinity of naloxone at supraspinal and spinal opioid receptors in vivo: Studies with receptor selective agonists. Life Sci. 1986; 39(19):1795-803. [DOI:10.1016/0024-3205(86)90099-8]

[35] Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem. 2004; 73:953-90. [DOI:10.1146/annurev.biochem.73.011303.073940] [PMID]

[36] Vaught JL, Mathiasen J, Raffa RB. Examination of the involvement of supraspinal and spinal mu and delta opioid receptors in analgesia using the mu receptor deficient CXBK mouse. J Pharmacol Exp Ther. 1988; 245(1):13-6. https://jpet.aspetjournals.org/content/245/1/13.short

[37] Khalefa BI, Shaqura M, Al-Khrasani M, Fürst S, Mousa SA, Schäfer M. Relative contributions of peripheral versus supraspinal or spinal opioid receptors to the antinociception of systemic opioids. Eur J Pain. 2012; 16(5):690-705. [DOI:10.1002/j.1532-2149.2011.00070.x] [PMID]

[38] Quock RM, Best JA, Chen DC, Vaughn LK, Portoghese PS, Takemori AE. Mediation of nitrous oxide analgesia in mice by spinal and supraspinal κ-opioid receptors. Eur J Pharmacol. 1990; 175(1):97-100. [DOI:10.1016/0014-2999(90)90158-3]