Vol.67: e24240230, 2024
https://doi.org/10.1590/1678-4324-2024240230
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Biological and Applied Sciences

Anti-inflammatory and Antinociceptive Activities of Aqueous and Ethanolic Extracts from *Cereus jamacaru* DC. (Cactaceae)

Alyne Almeida de Lima[1]
https://orcid.org/0000-0002-6174-7402

Max Denisson Maurício Viana[2]
https://orcid.org/0000-0002-1650-4460

Geraldo José da Silva Neto[3]
https://orcid.org/0000-0002-4872-9269

Suellen Maria Albuquerque da Silva[1]
https://orcid.org/0000-0003-1944-9513

Pedro Henrique Simões Bezerra[1]
https://orcid.org/0000-0002-1356-8557

Aline Cavalcanti de Queiroz[4]
https://orcid.org/0000-0002-6362-2726

Magna Suzana Alexandre Moreira[1]
https://orcid.org/0000-0002-9979-1994

Pedro Santana Sales Lauria[2]
https://orcid.org/0000-0002-6986-8444

Eliane Aparecida Campesatto[1]*
https://orcid.org/0000-0003-3689-888X

[1]Universidade Federal de Alagoas, Instituto de Ciências Biológicas e da Saúde, Maceió, AL, Brasil; [2]Universidade Federal da Bahia, Faculdade de Farmácia, Salvador, BA, Brasil; [3]Universidade Federal de Alagoas, Instituto de Química e Biotecnologia, Maceió, AL, Brasil; [4]Universidade Federal de Alagoas, Complexo de Ciências Médicas e Enfermagem, Arapiraca, AL, Brasil

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 11-Mar-2024; Accepted: 21-Mar-2024

*Correspondence: eliane_campesatto@hotmail.com (E.A.C.).

HIGHLIGHTS

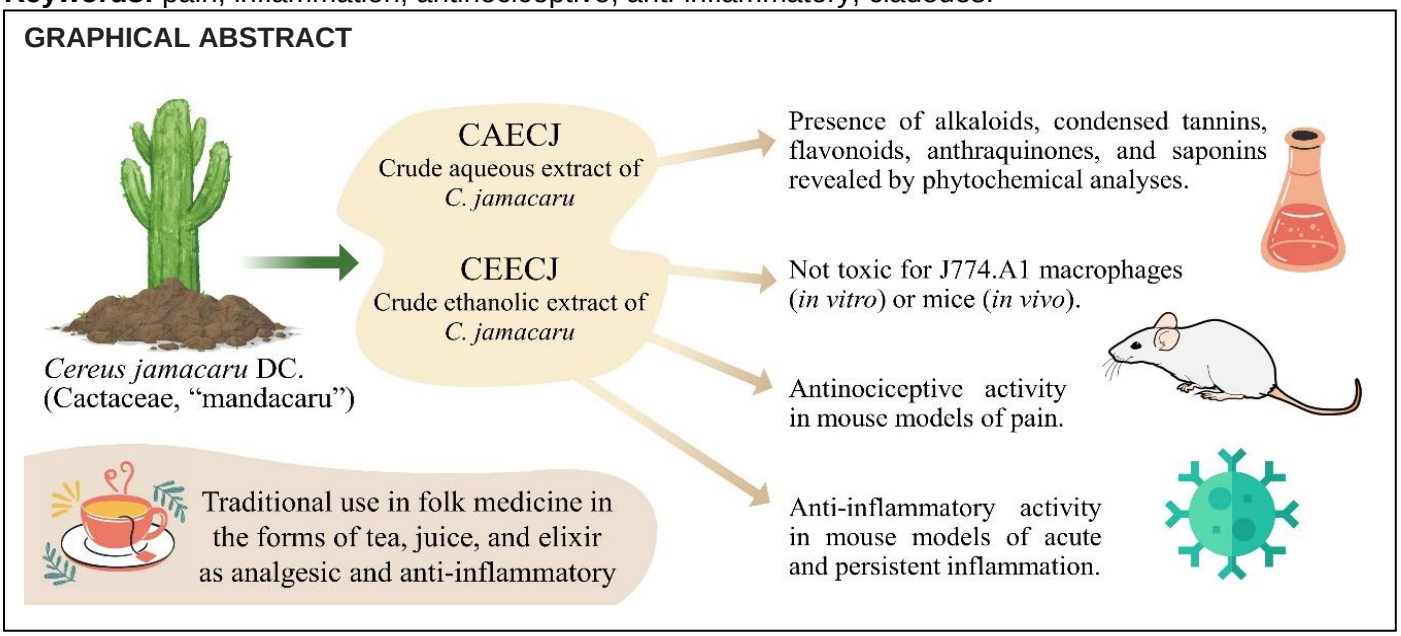
- *C. jamacaru* extracts contain alkaloids, tannins, flavonoids, anthraquinones, and saponins.
- *C. jamacaru* extracts promote antinociceptive and anti-inflammatory effects in mice.
- *C. jamacaru* extracts are not toxic *in vitro* or *in vivo*.

Abstract: *Cereus jamacaru* DC. (Cactaceae) is used in folk medicine for treating pain and inflammation. However, the therapeutic potential of *C. jamacaru* extracts has not been assessed experimentally. This study aimed to characterize the effect of both aqueous (CAECJ) and ethanolic (CEECJ) extracts of *C. jamacaru* in mouse models of pain and inflammation. CAECJ and CEECJ were chemically characterized by qualitative methods. Toxicity of extracts was evaluated *in vitro* against J774.A1 macrophages and *in vivo* by monitoring mice for 14 days following a single oral treatment at 2000 mg/kg. The antinociceptive activity of the extracts was assessed in models of pain in mice: acetic acid-induced writhing test, formalin test, hot plate test, and glutamate-induced nociception assay. The anti-inflammatory activity of the extracts was assessed following zymosan-induced peritonitis and in the model of arthritis induced by Complete Freund's Adjuvant (CFA). Phytochemical analyses revealed alkaloids, condensed tannins, flavonoids, and anthraquinones in both extracts; saponins were present in CEECJ only. Neither extract was cytotoxic *in vitro* or induced toxicity in

mice. Both extracts reduced nociception in all models of nociception without impairing motor function. Both extracts reduced leucocyte migration during experimental peritonitis and reduced paw edema during experimental arthritis. The effects of the extracts can be both due to anti-inflammatory and centrally-mediated mechanisms. This paper corroborates the traditional use of *C. jamacaru* extracts and demonstrates their therapeutic potential in the development of new analgesic and anti-inflammatory drugs with a good safety profile.

Keywords: pain; inflammation; antinociceptive; anti-inflammatory; cladodes.

GRAPHICAL ABSTRACT



INTRODUCTION

Current pharmacological strategies used in pain management include non-steroidal anti-inflammatory drugs (NSAID) and opioid analgesics. NSAID, along with steroidal anti-inflammatories, are also used in the control of inflammatory conditions. Despite their therapeutic value, these drugs can induce toxicity and well-known side effects that can limit their clinical use [1,2]. Because of these disadvantages, novel anti-inflammatory and analgesic compounds are being actively searched for. Many plant-based products are reported to have medicinal properties with few adverse effects [3]. The pharmacological study of medicinal plants greatly contributes to the dissemination of therapies based on natural products, especially those investigations that support folk medicine and the efficient use of natural resources aiming sustainable consumption [4–7].

Cereus jamacaru DC. (Cactaceae) is an herbaceous plant native to Brazil, where it is known as "mandacaru". It occurs mainly in the Northeastern region of the country and composes the vegetation of the Caatinga biome [8]. In folk medicine, *C. jamacaru* is widely used in the form of tea, juice, and elixir for treating backache, inflammatory conditions, and infections [9–11]. The major secondary metabolites described for *C. jamacaru* include tannins, alkaloids, anthraquinones, flavonoids, and polyphenols [12–14]. Some compounds that are known to promote anti-inflammatory and antinociceptive effects have been identified in *C. jamacaru*, including the polyphenols ellagic acid and caffeic acid [15–19] as well as the alkaloid hordenine [20,21]. Moreover, the fact that this cactus species is widely consumed by wild animals suggests its low toxicity and safety [12,20,22].

Despite its traditional use in the treatment of inflammatory and painful conditions and the phytochemical data suggesting its analgesic potential, no studies have explored the antinociceptive and anti-inflammatory properties of *C. jamacaru*. Hence, the goal of this study was to characterize the effect of both aqueous and ethanolic extracts of *C. jamacaru* in mouse models of pain and inflammation.

MATERIAL AND METHODS

Plant material

C. jamacaru DC. cladodes were collected in Cachoeirinha (PE, Brazil; 08° 29' 11" S, 36° 13' 59" W) in July 2018 and correctly identified by Dr. Flávia de Barros Prado Moura. A plant specimen was deposited in the Arapiraca Herbarium of the Federal University of Alagoas, under the register code ARA000077.

Preparation of the extracts

Aqueous and ethanolic extracts of *C. jamacaru* simulate traditional preparations used in folk medicine: juice and elixir, respectively. Cladode samples were weighed, cleaned, and grinded. To prepare the crude aqueous extract of *C. jamacaru* (CAECJ), half of the plant material was submitted to turbolysis following the proportion of 20 g of plant to 100 mL of distilled water. The extract was then filtered and stored in a refrigerator (2 to 8°C). To prepare the crude ethanolic extract of *C. jamacaru* (CEECJ), the other half of the plant material was dried at room temperature for 10 days. Next, it was submitted to maceration for 7 days following the proportion of 10 g of plant material to 100 mL of ethanol PA. The mixture was stirred twice a day. In the last day, the extract was filtered and the excess of solvent was removed with a rotatory evaporator. The extract was then placed in a desiccator for total removal of the solvent and stored at room temperature. For *in vivo* assays, doses of extracts are represented in milligrams of extract per kilogram of body weight. For CAECJ, doses were calculated based on the weight of raw plant material and dilutions were made in spring water. For CEECJ, doses were calculates based on the weight of the dry extract and dilutions were made in 5% Tween 80 in 0.9% saline.

Phytochemical screening of *C. jamacaru* extracts

CAECJ and CEECJ were screened for the presence of alkaloids, tannins, flavonoids, saponins, and anthraquinones as previously described [23]. The procedures described in the following subsections were performed for both extracts, independently.

Alkaloids

1 g of extract was added to 2 mL of HCl (0.1 M), heated for 10 min, and allowed to cool to room temperature. The solution was filtered and equally divided into 3 tubes. A few drops of Dragendorff reagent were added to each tube. The formation of precipitate was considered a positive result for the presence of alkaloids.

Tannins

1 g of extract was diluted in 2 mL of methanol followed by the addition of 5 mL of distilled water. The solution was filtered and a few drops of ferric chloride (10% $FeCl_3$ in water) were added. Hydrolyzed tannins are detected by the solution turning blue, while condensed tannins are detected by the solution turning green.

Flavonoids

1 g of extract was diluted in 2 mL of a methanol-water solution (1:1). Magnesium chips were added to the solution, followed by the addition of concentrated hydrochloric acid. The presence of flavonoids was detected by the presence of red or light brown hues.

Saponins

1 g of extract was diluted in 2 mL of methanol. Then, 5 mL of boiling water was added. After cooled to room temperature, the tube was vigorously shaken for 15 s and then allowed to stand for 20 min. The presence of saponins was detected by the formation of stable foam.

Anthraquinones

1 g of extract was diluted in 2 mL of methanol. The solution was filtered and 2 mL of sulfuric acid was added. The solution was kept in a warm water bath for 1 min. After cooled to room temperature, a double liquid-liquid extraction was performed with 10 mL ethyl acetate. Reduced anthraquinones were detected by the presence of a yellow hue, while oxidized anthraquinones were detected by the presence of a red hue.

Drugs and reagents

The following substances were used: distilled water (Farmace, Brazil), ethanol (Sigma-Aldrich, USA), polyoxyethylenesorbitan monoleate (Tween 80®, Sigma-Aldrich, USA), acetic acid A.R. (Vetec, Brazil), glutamate (Sigma-Aldrich, USA), formaldehyde A.R (Vetec, Brazil), zymosan (Sigma-Aldrich, USA), complete Freund's adjuvant (Sigma-Aldrich, USA), dipyrone (Sigma-Aldrich, USA), indomethacin (Merck, Brazil), morphine (Cristália, Brazil), diazepam (Cristália, Brazil), dexamethasone (Fagron, Brazil), [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium] (MTT, Sigma-Aldrich, USA), Roswell Park Memorial Institute medium (RPMI, Sigma-Aldrich, USA), fetal bovine serum (Cutilab, Brazil), non-essential pyruvate (Sigma-Aldrich, USA) and glutamine (Sigma-Aldrich, USA) amino acids, dimethylsulfoxide (DMSO, Sigma-Aldrich, USA), and Triton™ X-100 (Neon, Brazil).

Cytotoxicity assay

The cytotoxicity of the extracts was determined on murine macrophages (J774.A1 cell line) obtained from the Cell Bank of Federal University of Rio de Janeiro (RJ, Brazil). This adherent-phenotype macrophage line was cultured in RPMI medium supplemented with fetal 10% bovine serum, pyruvate, glutamine and non-essential amino acids, at 37 °C, 95% humidity, and 5% CO_2 atmosphere. Briefly, cell suspensions containing 1.0 x 105 cells/mL were placed in a 96-well plate in triplicate and incubated at 37 °C for 1 h. Then, CAECJ and CEECJ were added at concentrations of 0.1, 1, 10, and 100 µg/mL. The cells were also cultured with medium only (basal growth control) or with 0.1% DMSO (vehicle control). 1% Triton™ X-100 was used as positive control to induce cell death. After 48 h, the cytotoxicity was evaluated by mitochondrial activity of the cells via MTT reduction and the absorbance was measured in a spectrophotometer at 530 nm. Results were expressed as percentual cell viability in relation to basal growth control [24].

Animals

Swiss mice of both sexes, 6-8 weeks of age, weighing 25-30 g, were obtained from the Animal Facilities of the Federal University of Alagoas (AL, Brazil). They were housed in single-sex cages under a 12 h light/dark cycle (dark phase: 7 pm to 7 am), at constant temperature (22 ± 2°C) with water and food *ad libitum*. The experiments were approved by the Ethics Committee for Animal Handling – UFAL (Protocol # 27/2018) and performed in accordance with the guidelines established by the Guide for the Care and Use of Laboratory Animals [25].

Acute toxicity

Acute oral toxicity was evaluated in mice (n = 6 per group) according to the Guidelines for Testing Chemicals n° 420 of the Organization for Economic Co-operation and Development (OECD) [26]. After acclimatization procedure, mice were orally treated with vehicle (control), CEECJ, and CAECJ at limit dose (2000 mg/kg). Then, mice were evaluated for signs of toxicity, according to parameters described by Malone [27] every hour for the first 4 h and then daily for 14 days. Food and water intake, weight gain, and incidence of death were also verified daily.

Antinociceptive activity

Acetic acid-induced abdominal writhing test

This model was used as a screening assay to detect the antinociceptive effects of CAECJ and CEECJ. Dose-response curves were built to determine the doses to be used in the following tests. Abdominal writhing in mice was induced by intraperitoneal injection of acetic acid (0.6% solution, 0.1 mL/10 g of body weight). Mice (n = 6 per group) were orally treated with CAECJ (100, 150, and 300 mg/kg), CEECJ (100, 150, and 300 mg/kg), dipyrone (40 mg/kg), and vehicle (10 mL/kg) 40 minutes before intraperitoneal injections. Nociceptive responses were determined for 20 minutes after a latency period of 5 minutes. The observed behaviors included stretching of the hind limbs, contraction of abdominal muscles, and arching of the back [28,29].

Formalin-induced nociception

Mice (n = 6 per group) were orally treated with CAECJ (150 mg/kg), CEECJ (150 mg/kg), indomethacin (30 mg/kg), and vehicle (10 mL/kg), or intraperitoneally treated with morphine (5 mg/kg) 40 minutes before intraplantar injection of 2.5% formalin (20 µL) into the right hind paw. The nociceptive response was

considered as the time that mice spent licking the injected paw. Following the intraplantar injection, the neurogenic phase (0 – 5 min) and the inflammatory phase (15 – 30 min) were evaluated [30].

Hot plate test

The hot plate test was performed as previously described [31]. Mice (n = 6 per group) were orally treated with CAECJ (150 mg/kg), CEECJ (150 mg/kg), and vehicle (10 mL/kg), or intraperitoneally treated with morphine (5 mg/kg). Mice were placed on a hot plate at 54 ± 1°C. The reaction time until mice licked either fore paws or hind paws and/or jumped was recorded at 30, 60, 90, and 120 minutes. The cut-off time used to prevent skin damage was 15 seconds.

Glutamate-induced nociception assay

Mice (n = 6 per group) were orally treated with CAECJ (150 mg/kg), CEECJ (150 mg/kg), indomethacin (30 mg/kg), and vehicle (10 mL/kg), or intraperitoneally treated with morphine (5 mg/kg) 40 minutes before subcutaneous injection of glutamate (30 µmol/paw in 20 µL) in the dorsal surface of the hind paw. Nociceptive responses were recorded as the time mice spent licking the injected paw during the 15 minutes that followed glutamate administration [32].

Motor performance in the rotarod test

Considering that the behaviors evaluated in the nociceptive tests depend on the integrity of mice's motor function, the rotarod test was performed to exclude possible non-specific depressive or myorelaxant effects of CAECJ and CEECJ. The test consisted in placing each mouse on a rotating bar (16 rpm), 2.5 cm in diameter, raised 25 cm above ground. Mice need to keep moving forward to avoid falling from the bar. Mice were previously selected by being challenged to remain on the rotating bar for 240 seconds with a limit of up to three falls. On the following day, mice (n = 6 per group) were orally treated with vehicle (10 mL/kg), CAECJ (150 mg/kg), and CEECJ (150 mg/kg), or intraperitoneally treated with diazepam (5 mg/kg). After 40 min (for oral treatments) or 30 min (for the intraperitoneal treatment), each mouse was placed on the rotarod and the number of falls as well as fall latency time were evaluated [33].

Anti-inflammatory activity

Zymosan-induced peritonitis

The model of peritonitis [34] was used to evaluate the acute anti-inflammatory potential of CAECJ and CEECJ. Dose-response curves were built to determine the doses to be used in the next test. Mice were orally pretreated with CAECJ (100, 150, and 300 mg/kg), CEECJ (100, 150, and 300 mg/kg), indomethacin (30 mg/kg), or vehicle (10 mL/kg). After 40 minutes, leukocyte migration was induced by intraperitoneal injection of zymosan (2 mg/mL, 250 µL/mouse). Six hours after zymosan injection, mice were euthanized and the peritoneal cavity was washed with cold PBS (3 mL), from which the exudate was retrieved. Naïve mice did not receive any treatment or zymosan injection prior to peritoneal washing. The number of recruited cells to the peritoneum was counted in a Neubauer chamber and the results were obtained using Equation 1:

$$number\ of\ cells/mL = \frac{total\ cell\ count * dilution\ factor * 10000}{number\ of\ quadrants\ counted} \quad (1)$$

Arthritis induced by Complete Freund's Adjuvant (CFA)

Experimental arthritis was induced in mice as previously described [35]. CFA (20 µL/paw) was injected into the right hind paw of mice. After 13 days, mice with pronounced arthritis were orally treated with CAECJ (150 mg/kg), CEECJ (150 mg/kg), dexamethasone (1 mg/kg), or vehicle (10 mL/kg) daily from the 14th to the 21st days after CFA injection. Naïve mice did not receive any treatments or CFA injections. Paw volume was measured on the 1st day followed by a daily assessment from the 14th to the 21st day. Paw edema was expressed by the mean difference between the right (arthritic) and left (without arthritis) paws using a digital plethysmometer (LE 7500; Panlab, Harvard Apparatus).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and the statistical differences among groups were determined using one-way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test. For repeated measures, two-way ANOVA followed by Bonferroni's post-hoc test. Results were considered

statistically significant when $p < 0.05$. Statistical analysis and creation of figures were performed using the software GraphPad Prism 8.0.

RESULTS

C. jamacaru extracts contain several classes of secondary metabolites

Qualitative screening of secondary metabolites showed the presence of alkaloids, condensed tannins, flavonoids, and anthraquinones in both CAECJ and CEECJ (data not shown). On the other hand, saponins were present in CEECJ, but not in CAECJ (data not shown).

Viability of murine macrophages is not affected by *C. jamacaru* extracts *in vitro*

Cell viability of J774.A1 murine macrophages (Figure 1) was not affected by incubation with CAECJ (0.1 – 100 µg/mL), CEECJ (0.1 – 100 µg/mL), or vehicle (0.1% DMSO). Cell viability was significantly lower in positive control wells treated with 1% Triton X-100 ($p < 0.05$). Since the extracts did not induce cytotoxicity at any of the tested concentrations, the study continued with *in vivo* assays.

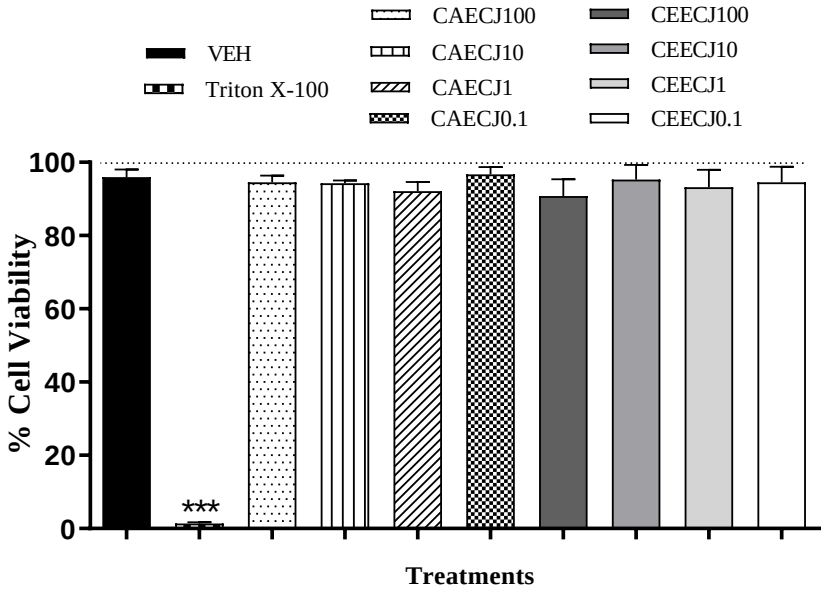


Figure 1. Influence of *C. jamacaru* extracts on murine macrophages viability. J774.A1 macrophages were incubated with vehicle (VEH; 0.1% DMSO), crude aqueous extract of *C. jamacaru* (CAECJ; 0.1, 1, 10, and 100 µg/mL), crude ethanolic extract of *C. jamacaru* (CEECJ; 0.1, 1, 10, and 100 µg/mL), or 1% Triton X-100 (positive control; CTRL) for 48 hours. Cell viability in relation to basal growth control (axis of abscissa) was evaluated using the MTT assay. Results are expressed as mean ± SEM (n = 3). *** $p < 0.001$ compared to VEH as determined by one-way ANOVA followed by Dunnett's post-hoc test.

C. jamacaru extracts do not induce acute oral toxicity in mice

Oral administration of CAECJ (2000 mg/kg) and CEECJ (2000 mg/kg) did not induce visible manifestations of acute toxicity in mice during the two-week period of daily assessments. During the evaluations, no instances of drowsiness, salivation, tremor, restlessness, convulsion, piloerection, diarrhea, or death were registered (data not shown).

Acetic acid-induced nociception is partially prevented by *C. jamacaru* extracts

Intraperitoneal injection of 0.6% acetic acid induced nociceptive behaviors marked by lower body stretching and contraction of abdominal muscles (Figure 2). Oral treatments with CAECJ significantly reduced acetic acid-induced nociception at 150 mg/kg (53.4% reduction; $p < 0.001$) and at 300 mg/kg (50.6% reduction; $p < 0.001$) compared to vehicle-treated mice (40.6 ± 4.0 writhes). Similarly, oral treatments with CEECJ reduced acetic acid-induced nociception at 100 mg/kg (36.9% reduction; $p < 0.05$) and at 150 mg/kg (57% reduction; $p < 0.001$). The efficacy of the extracts was similar to that of the standard analgesic drug dipyrone (40 mg/kg), which reduced the nociceptive behaviors by 51.5% ($p < 0.001$). Conversely, CAECJ at 100 mg/kg and CEECJ at 300 mg/kg did not influence the nociceptive effect of acetic acid. Thus, the following assays were conducted using only the dose of 150 mg/kg for both extracts.

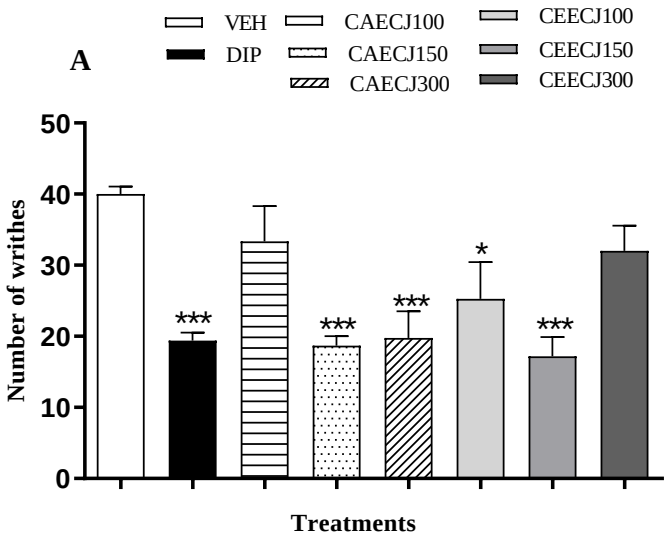


Figure 2. Influence of *C. jamacaru* extracts on acetic acid-induced nociception. Mice were orally treated with vehicle (VEH), dipyrone (DIP; 40 mg/kg), crude aqueous extract of *C. jamacaru* (CAECJ; 10, 150, and 300 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 10, 150, and 300 mg/kg). After 40 minutes, mice received intraperitoneal injections of 0.6% acetic acid and the nociceptive behaviors (axis of abscissa) were quantified for 20 minutes. Results are expressed as mean ± SEM (n = 6). * $p < 0.05$; *** $p < 0.001$ compared to VEH group as determined by one-way ANOVA followed by Dunnett's post-hoc test.

C. jamacaru extracts reduce both phases of the formalin test

Intraplantar injection of 2.5% formalin caused nociception, as shown by intense licking behavior in vehicle-treated mice in the first (85.4 ± 6.6 s) and second phases (265.0 ± 21.9 s) of the test (Figure 3). Both CAECJ (150 mg/kg) and CEECJ (150 mg/kg) significantly reduced the pain-like licking behaviors of mice in both phases of the formalin test. In the first phase, CAECJ and CEECJ reduced the licking time by 43% and 42.9%, respectively, in comparison to vehicle-treated mice ($p < 0.01$; Figure 3A). In the second phase, CAECJ and CEECJ reduced the licking time by 32.7% and 46.2%, respectively ($p < 0.01$; Figure 3B). Additionally, the reference opioid drug morphine (5 mg/kg) reduced the licking time in both phases (62.2% and 59%, respectively; $p < 0.001$). On the other hand, the reference anti-inflammatory drug indomethacin (30 mg/kg) reduced the licking time only in the second phase of the test (77.4%; $p < 0.001$).

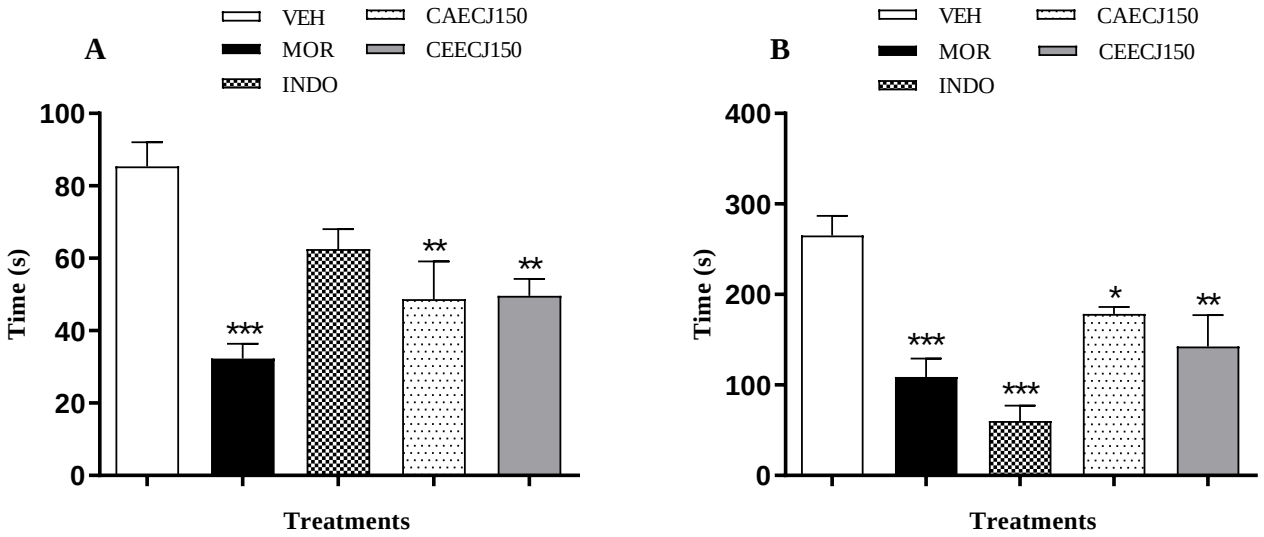


Figure 3. Effects of *C. jamacaru* extracts in the formalin test. Mice were orally treated with vehicle (VEH), indomethacin (INDO; 30 mg/kg), crude aqueous extract of *C. jamacaru* (CAECJ; 150 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 150 mg/kg), or intraperitoneally treated with morphine (MOR; 5 mg/kg) 40 minutes before intraplantar injection of 2.5% formalin into the right hind paw. Time mice spent licking the injected paw (axis of abscissa) was quantified in the neurogenic phase (Panel A; 0 – 5 min) and in the inflammatory phase (Panel B; 15 – 30 min). Results are expressed as mean ± SEM (n = 6). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to VEH group as determined by one-way ANOVA followed by Dunnett's post-hoc test.

C. jamacaru extracts promote antinociception in the hot plate test

Vehicle-treated mice showed a stable latency time until reaction to the hot plate throughout the experimental period (3.16 ± 0.2 s), with no significant differences between time points (Figure 4). CAECJ (150 mg/kg) significantly increased latency time when compared to the vehicle group at 60 ($p < 0.01$), 90 ($p < 0.01$), and 120 minutes ($p < 0.05$). On the other hand, CEECJ (150 mg/kg) increased latency time only at 90 ($p < 0.001$) and 120 minutes ($p < 0.01$). As expected, the reference opioid drug morphine (5 mg/kg) also significantly prolonged latency time to response the thermal stimulus at 60 ($p < 0.001$), 90 ($p < 0.001$), and 120 minutes ($p < 0.001$).

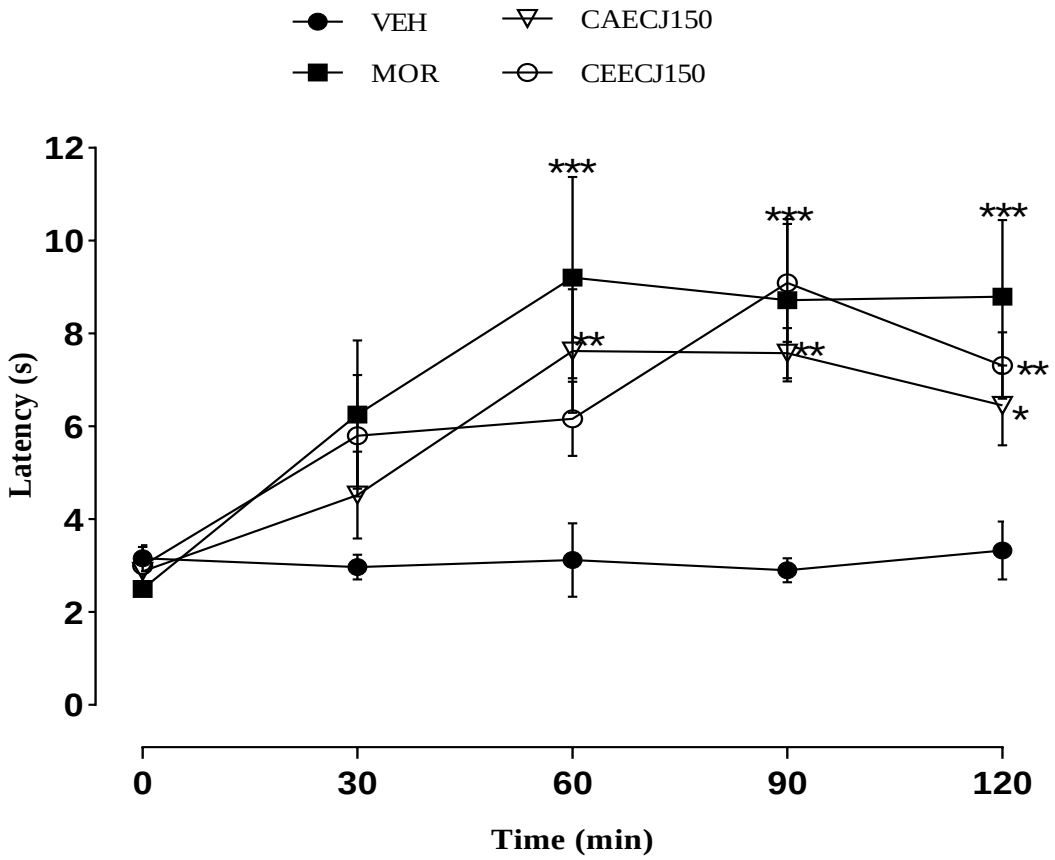


Figure 4. Effects of *C. jamacaru* extracts in the hot plate test. Mice were orally treated with vehicle (VEH), crude aqueous extract of *C. jamacaru* (CAECJ; 150 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 150 mg/kg), or intraperitoneally treated with morphine (MOR; 5 mg/kg). Mice were placed on the hot plate at 54 ± 1°C and the latency time until reaction (axis of abscissa) was recorded at 30, 60, 90, and 120 minutes following treatments. Results are expressed as mean ± SEM (n = 6). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to VEH group as determined by two-way ANOVA followed by Bonferroni's post-hoc test.

Glutamate-induced central nociception is reduced by *C. jamacaru* extracts

Because the results of the neurogenic phase of the formalin test and the hot plate test suggested that *C. jamacaru* extracts promote centrally-mediated antinociception, CAECJ (150 mg/kg) and CEECJ (150 mg/kg) were then tested against glutamate-induced nociception (Figure 5). Vehicle-treated mice showed intense pain-like paw licking behaviors (131.0 ± 12.8 s) that were significantly reduced by oral treatments with CAECJ (60.4%; $p < 0.001$) and CEECJ (47.2%; $p < 0.01$) or by intraperitoneal treatment with the reference opioid drug morphine (5 mg/kg) (93.4%; $p < 0.001$).

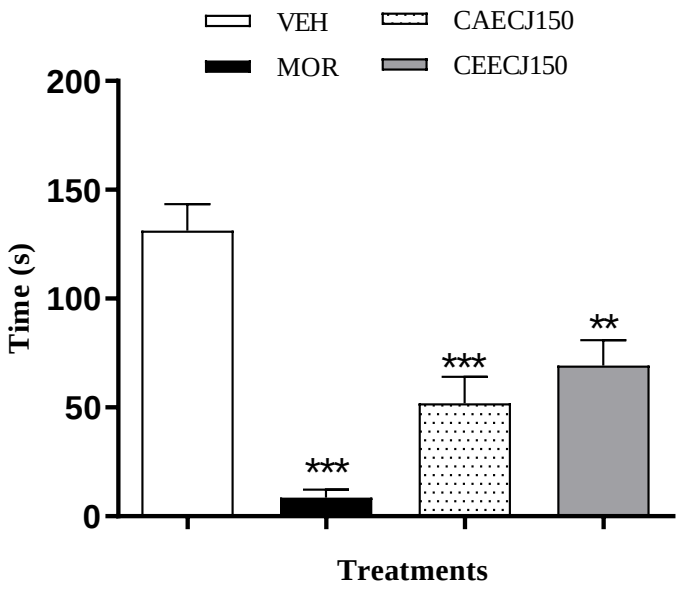


Figure 5. Influence of *C. jamacaru* extracts on glutamate-induced nociception. Mice were orally treated with vehicle (VEH), crude aqueous extract of *C. jamacaru* (CAECJ; 150 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 150 mg/kg), or intraperitoneally treated with morphine (MOR; 5 mg/kg) 40 minutes before subcutaneous injection of glutamate (30 µmol/paw in 20 µL) in the dorsal paw surface. Time mice spent licking the injected paw (axis of abscissa) was quantified for 15 minutes. Results are expressed as mean ± SEM (n = 6). ** $p < 0.01$; *** $p < 0.001$ compared to VEH group as determined by one-way ANOVA followed by Dunnett's post-hoc test.

Motor function of mice is not impaired by *C. jamacaru* extracts

Since the experiments aiming to evaluate nociception rely on the integrity of mice's motor capacities, impairment of motor function was assessed in the rotarod test. As shown in Table 1, oral treatments with CAECJ (150 mg/kg) and CEECJ (150 mg/kg) did not affect the number of falls nor fall latency in comparison with vehicle-treated mice. On the other hand, intraperitoneal treatment with the anxiolytic-sedative drug diazepam (5 mg/kg) significantly increased the number of falls ($p < 0.001$) and decreased fall latency ($p < 0.001$) when compared to vehicle-treated mice.

Table 1. Influence of *C. jamacaru* extracts on mice's motor function. Mice were orally treated with vehicle (VEH), crude aqueous extract of *C. jamacaru* (CAECJ; 150 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 150 mg/kg), or intraperitoneally treated with diazepam (DZP; 5 mg/kg) and then challenged to remain on the rotating rod. Results are expressed as mean ± SEM (n = 6). *** $p < 0.001$ compared to VEH group as determined by one-way ANOVA followed by Dunnett's post-hoc test.

Treatment	Number of falls	Fall latency (s)
VEH	0.0 ± 0.0	240 ± 0.0
CAECJ	0.0 ± 0.0	240 ± 0.0
CEECJ	0.0 ± 0.0	240 ± 0.0
DZP	11.40 ± 1.89***	23.27 ± 5.22***

C. jamacaru extracts reduce leucocyte migration induced by experimental peritonitis

Vehicle-treated mice induced with zymosan showed a significant increase in the number of cells present in peritoneal washing compared to the Naïve group ($p < 0.001$), confirming the development of experimental peritonitis (Figure 6). CAECJ significantly inhibited cell recruitment at 100 mg/kg (61.2%; $p < 0.01$), 150 mg/kg (66.6%; $p < 0.01$), and 300 mg/kg (66%; $p < 0.01$) compared to vehicle-treated mice. CEECJ also inhibited zymosan-induced leukocyte migration at 100 mg/kg (73.5%; $p < 0.001$), 150 mg/kg (59.5%; $p < 0.01$), and 300 mg/kg (47.1%; $p < 0.05$). The reference anti-inflammatory drug indomethacin (30 mg/kg) significantly decreased cell recruitment by 46% ($p < 0.05$).

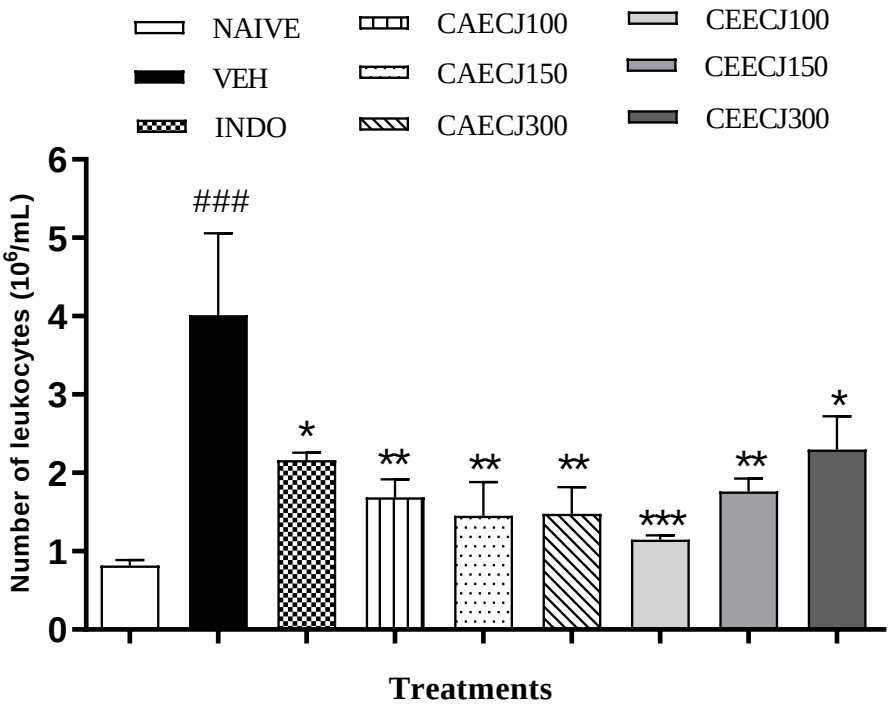


Figure 6. Influence of *C. jamacaru* extracts on leucocyte migration during zymosan-induced experimental peritonitis. Mice were orally pretreated with vehicle (VEH), indomethacin (INDO; 30 mg/kg) crude aqueous extract of *C. jamacaru* (CAECJ; 100, 150, and 300 mg/kg), and crude ethanolic extract of *C. jamacaru* (CEECJ; 100, 150, and 300 mg/kg) and then intraperitoneally injected with zymosan (500 µg) after 40 minutes. Naïve mice did not receive any pretreatments or zymosan injection. After 6 hours, the peritoneal cavity was washed with cold PBS (3 mL) and leukocyte migration was determined by counting the number of cells (axis of abscissa). Results are expressed as mean ± SEM (n = 6). ### $p < 0.001$ compared to Naïve group and * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to VEH group as determined by one-way ANOVA followed by Dunnett's post-hoc test.

C. jamacaru extracts promote anti-inflammatory effects during experimental arthritis

The results gathered so far were used for selecting the dose of *C. jamacaru* extracts to be tested in the CFA model of arthritis. Since this model has an important inflammatory component while also causing hypernociception, we selected a dose that promoted both anti-inflammatory and antinociceptive activities. Thus, the effect of oral daily treatments with CAECJ (150 mg/kg) and CEECJ (150 mg/kg) was tested in this model of chronic inflammation (Figure 7). Intraplantar injections of CFA triggered an inflammatory response marked by local edema, as shown by the significant increase in paw volume of vehicle-treated mice compared to the Naïve group ($p < 0.001$). Paw edema was persistent throughout the entire experimental period (1st and then 14th to 21st day after CFA injection). Both CAECJ (150 mg/kg) and CEECJ (150 mg/kg) as well as the reference drug dexamethasone (1 mg/kg) significantly reduced paw edema from the 15th to the 21st day following the induction of the model ($p < 0.05$).

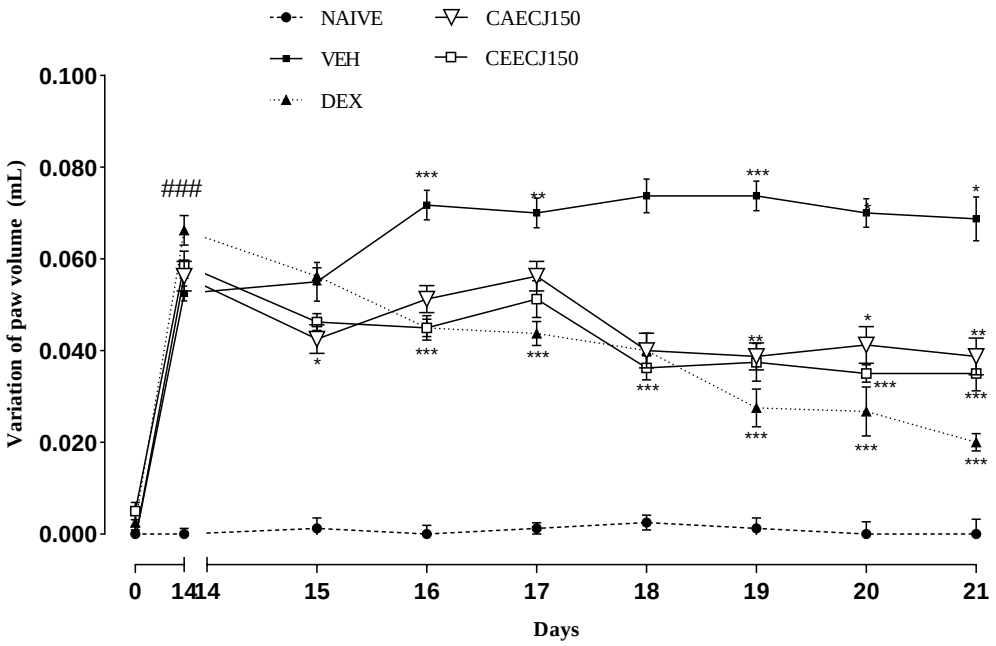


Figure 7. Antiedematogenic effect of *C. jamacaru* extracts in the CFA model of arthritis. Experimental arthritis was induced by intraplantar injection of CFA (20 µL). From the 14th to the 21st day following CFA injections, mice were orally treated with CAECJ (150 mg/kg), CEECJ (150 mg/kg), dexamethasone (1 mg/kg), or vehicle (10 mL/kg) daily. Naïve mice did not receive any treatments or CFA injections. Paw volume was measured daily and edema was expressed as variation in paw volume (axis of abscissa) between the right (arthritic) and left (without arthritis) paws. Results are expressed as mean ± SEM (n = 6). ### $p < 0.001$ compared to Naïve group and * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to VEH group as determined by two-way ANOVA followed by Bonferroni's post-hoc test.

DISCUSSION

This is the first experimental study to demonstrate the antinociceptive and anti-inflammatory activities of both aqueous and ethanolic extracts of *Cereus jamacaru* DC. in murine models of pain and inflammation. Our research corroborates the traditional use of this plant for treating painful and inflammatory conditions, a common practice in the Northeastern Brazilian folk medicine [9,10]. In addition to confirming the therapeutic potential of CAECJ and CEECJ, this paper provided evidence that supports the safety of *C. jamacaru* extracts, as neither induced noticeable toxic effects in mice nor were they cytotoxic for murine macrophages *in vitro*.

Prior to pharmacological characterization in models of pain and inflammation, CAECJ and CEECJ were screened for safety both *in vitro* and *in vivo*. The extracts were not cytotoxic for J774.A1 murine macrophages, which agrees with a previous *in vitro* study showing that the hydroalcoholic extract from *C. jamacaru* is not cytotoxic against lymphocytes [36]. We then proceeded to perform an assay for detecting acute oral toxicity in mice; neither of the extracts at the dose of 2000 mg/kg induced signs of systemic toxicity. Treatments did not impair the general behavior or mobility of mice, nor did they alter food and water intake or body weight during the 14 days of evaluation (data not shown). No deaths were registered during the 14 days following oral treatments. According to the guidelines established by OECD [26], we concluded that both CAECJ and CEECJ can be considered low toxicity xenobiotics, so their pharmacological characterization could continue.

The antinociceptive and anti-inflammatory activities of CAECJ and CEECJ were assessed by using classical animal models of pain and inflammation often employed in the process of drug discovery [37]. The maximum dose used during the pharmacological characterization was 300 mg/kg for both extracts, which corresponds to 15% of the limit dose tested for acute toxicity (2000 mg/kg). The antinociceptive effect of the extracts was initially tested in the acetic acid-induced abdominal writhing assay, which has been largely used as a screening test for detecting the analgesic potential of new compounds [29]. The intraperitoneal injection of acetic acid leads to the release of endogenous mediators that promote peripheral sensitization and nociception in mice [38]. These mediators include biogenic amines (e.g., histamine and serotonin) and prostaglandins as a result of the induction of cyclooxygenase (COX) enzymes [28,38]. Both CAECJ and CEECJ reduced the abdominal writhes induced by acetic acid; the antinociceptive effect of the extracts could be due to the inhibition of the aforementioned nociceptive mediators.

In the writhing test, the maximum effective dose for both extracts was 150 mg/kg. Therefore, following the ethical procedures established by OECD [26], this dose was selected and used in all the subsequent

nociceptive assays. Interestingly, CEECJ at 300 mg/kg did not promote antinociception in the writhing test, demonstrating that this effect was not dose-dependent. This apparent inconsistency can be explained by the fact that plant extracts are complex mixtures containing different compounds that can interfere with each other's pharmacological actions [39]. In fact, it is not uncommon that studies that characterize crude plant extracts report similar results, as demonstrated for *Allium macrostemon* (Alliaceae) [40] and *Heteropterys brachiata* (Malpighiaceae) [41].

To further characterize the antinociceptive activity of CAECJ and CEECJ, we performed other behavioral assays that can provide insights into the antinociceptive mechanisms of the extracts. Formalin-induced nociception is known to be driven by a biphasic mechanism; the neurogenic phase is caused by the direct activation of nociceptors and can be inhibited by opioid drugs, whereas the inflammatory phase results from the release of inflammatory mediators and is inhibited by anti-inflammatory drugs such as NSAID [42,43]. The extracts of *C. jamacaru* reduced the pain-like licking behavior of mice in both phases of the test, suggesting that CAECJ and CEECJ have the potential to promote both centrally-mediated antinociception and anti-inflammatory effects. Therefore, both actions were investigated in the following steps of this study.

Two assays for evaluating centrally-mediated antinociception were performed: the hot plate test, a thermal pain model that requires a complex and integrated response that involves supraspinal regions [31]; and glutamate-induced nociception, that involves the activation of NMDA and non-NMDA receptors in the central nervous system and depends on the activation of the L-arginine-nitric oxide pathway [32]. Both CAECJ and CEECJ increased the latency time of mice on the hot plate and reduced the pain-like licking behavior caused by intraplantar glutamate injections, supporting the hypothesis that the extracts promote antinociception by central mechanisms. Centrally-mediated antinociception has been reported for other species of Cactaceae, including *Opuntia dillenii* [44] and *Pereskia bleo* [45]. Importantly, neither CAECJ nor CEECJ caused motor impairment in mice in the rotarod test. These results validate the antinociceptive effect of *C. jamacaru* extracts, since they indicate that changes in behavior caused by CAECJ and CEECJ in the nociceptive tests were not due to non-specific depressant or muscle relaxing effects.

Considering that the results of the formalin test indicated that *C. jamacaru* extracts effectively reduced inflammatory nociception, we then tested the effects of CAECJ and CEECJ in murine models of inflammation. To investigate whether the extracts modulate the inflammatory response, CAECJ and CEECJ were tested against zymosan-induced leukocyte migration. Intraperitoneal injection of zymosan causes an inflammatory reaction marked by leukocyte influx and increased expression of the inducible nitric oxide synthase (iNOS) and COX-2, with consequent release of nitric oxide and prostaglandins [46]. Both *C. jamacaru* extracts reduced cell migration induced by zymosan in the peritoneal cavity, corroborating their anti-inflammatory potential. Similarly, it has been demonstrated that the juice of *Opuntia ficus-indica* (Cactaceae) reduces the release of pro-inflammatory mediators and, consequently, reduces mucosa damage during intestinal inflammation [47].

The anti-inflammatory action of CAECJ and CEECJ was also tested in the mouse model of arthritis induced by CFA. Rheumatoid arthritis is a chronic autoimmune disease characterized by persistent inflammation of the joints accompanied by leucocyte infiltration, increased levels of cytokines, chemokines, and C-reactive protein, as well as intense pain [48,49]. The pharmacological treatment of rheumatoid arthritis is based on the use of anti-inflammatory and immunosuppressive drugs that cause deleterious side effects [50], which justifies the need for new treatments that are safe and effective. Daily treatments with both CAECJ and CEECJ significantly reduced paw edema throughout the experimental period, demonstrating the potential of the extracts in the treatment of persistent joint inflammation. Accordingly, other authors have reported that extracts of *Opuntia dillenii* and *Opuntia ficus-indica* promote therapeutic effects in rodent models of arthritis [44,51].

Although investigating the mechanisms of the antinociceptive and anti-inflammatory effects of *C. jamacaru* extracts was beyond the scope of this study, the phytochemical screening of CAECJ and CEECJ provided valuable clues about the chemical classes of compounds that could contribute to these effects. The presence of alkaloids, condensed tannins, flavonoids, and anthraquinones was detected in both extracts, whereas saponins were only found in CEECJ. The qualitative chemical characterization of the extracts used in our research agrees with previous studies that explored the composition of *C. jamacaru* extracts and reported the presence of similar chemical classes [12,13,52,53]. Several studies have demonstrated the antinociceptive and/or anti-inflammatory activity of alkaloids, anthraquinones, saponins, and polyphenols (especially tannins and flavonoids) [54–58]. Therefore, it is possible that these classes of compounds contribute to the antinociceptive and anti-inflammatory effects of CAECJ and CEECJ, either by acting on their own or synergically with other compounds.

CONCLUSION

This study provides pharmacological evidence that supports the traditional use of the cladodes of *Cereus jamacaru* in the treatment of inflammatory and painful conditions. We demonstrated that both the aqueous and the ethanolic extracts of *C. jamacaru* promote antinociception in murine models of pain; our results suggest that this effect is both due to anti-inflammatory and centrally-mediated mechanisms. Additionally, both CAECJ and CEECJ reduced signs of acute and chronic inflammation in mouse models. The extracts did not induce signs of toxicity, sedation, or motor impairment in mice. Therefore, this paper demonstrates that *C. jamacaru* extracts have therapeutic potential in the development of new analgesic and anti-inflammatory drugs with a good safety profile. Future studies should aim to elucidate the mechanisms of action and fractions of the extracts responsible for the antinociceptive and anti-inflammatory effects described herein.

Funding: This research was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant numbers 479822/2013-1, 485854/2011-2, 404344/2012-7; the Instituto Nacional de Ciência e Tecnologia de Fármacos e Medicamentos (INCT-INOFAR), grant number 573.564/2008-6; the Fundação de Amparo à Pesquisa do Estado de Alagoas (FAPEAL), grant number Pronem 20110722-006-0018-0010; and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) along with the Financiadora de Estudos e Projetos – Brasil (FINEP) with the joint grant of student fellowship.
Acknowledgments: The authors thank their colleagues from Federal University of Alagoas for their constructive criticism and assistance during the development this project.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

1. Bordoni A, Danesi F, Dardevet D, Dupont D, Fernandez AS, Gille D, et al. Dairy products and inflammation: a review of the clinical evidence. Crit Rev Food Sci Nutr. 2017;57(12):2497–525.
2. Gené E, Calvet X, Morón A, Iglesias ML. [Recommendations for the prescription of anti-inflammatories and indications for gastroprotection in emergencies]. (Sant Vicenç dels Horts). 2009;21(4):295–300.
3. Uchôa VT, Dias T de LMF, Melo GM de A, Silva YKCD, Queiroz AC, Goulart HF, et al. Antinociceptive and anti-inflammatory activities of the ethanolic extract, of fractions and of epicatechin isolated from the stem bark of *Ximenia americana* L. (Oleacaceae). Rev. Virtual Quim. 2018;10(1).
4. Migacz IP, Raeski PA, Almeida VPD, Raman V, Nisgoski S, Muniz GIBD, et al. Comparative leaf morpho-anatomy of six species of *Eucalyptus* cultivated in Brazil. Rev. Bras. Farmacogn. 2018;28(3):273–81.
5. Bobek VB, Heiden G, De Oliveira CF, De Almeida VP, De Paula JP, Farago PV, et al. Comparative analytical micrographs of “vassouras” (*Baccharis*, Asteraceae). Rev. Bras. Farmacogn. 2016;26(6):665–72.
6. Budel JM, Duarte MR, Farago PV, Takeda IJM. [Anatomic characteristics of leaves and stem from *Calea uniflora* Less., Asteraceae]. Rev. Bras. Farmacogn. 2006;16(1).
7. Justus B, Almeida VPD, Gonçalves MM, Assunção DPDSFD, Borsato DM, Arana AFM, et al. Chemical composition and biological activities of the essential oil and anatomical markers of *Lavandula dentata* L. cultivated in Brazil. Braz. Arch. Biol. Techn. 2018;61:e18180111.
8. Hernández-González O, Villarreal OB. Crassulacean acid metabolism photosynthesis in columnar cactus seedlings during ontogeny: the effect of light on nocturnal acidity accumulation and chlorophyll fluorescence. Am J Bot. 2007;94(8):1344–51.
9. Andrade CTS, Marques JGW, Zappi D. [Use of cacti by Bahia country people: connective types to define utilitarian categories]. Sitientibus Série Ciências Biológicas. 2006;6:3–12.
10. Ribeiro DA, Macêdo DG, Oliveira LGS, Saraiva ME, Oliveira SF, Souza MMA, et al. [Therapeutic potential and use of medicinal plants in a Caatinga area in the state of Ceará, northeast of Brazil]. Rev Bras Plantas Med. 2014;16:912–30.
11. Silva PPP, Lima M, Silva T, Melo A, Cordeiro R. [Determination of antimicrobial activity and evaluation of toxicity of *Cereus jamacaru* DC (mandacaru) and *Opuntia ficus-indica* Mill. (forage palm)]. Rev. Eletrônica Far. 2017;14(3).
12. Davet A, Carvalho JLS, Dadalt RC, Vituoso S, Dias JFG, Miguel MD, et al. *Cereus jamacaru*: a non buffered LC quantification method to nitrogen compounds. Chroma. 2009;69(2):245–7.
13. Medeiros IU. [Identification of the active ingredients present in the ethanolic extract of *Cereus jamacaru* and evaluation in rats of the possible toxic and/or behavioral effects of prolonged exposure] [Master Thesis]. Universidade Federal do Rio Grande do Norte; 2011.
14. Zapata R, Sanabria ME, Rodríguez D. [Reduction of the development of phytopathogenic fungi with lefaria cardon extract (*Cereus deficiens* Otto & Diert)]. Interciencia. 2003;28(5):302–7.
15. Abbasi M, Taghi Mansouri SM, Naghizadeh B, Ghorbanzadeh B. Ellagic acid enhances the anti-nociceptive effects of cyclooxygenase inhibitors in a mouse visceral pain model. Jundishapur J. Physiol. 2018;1(1):22–8.

16. Campos-Buzzi F, Franzoi CL, Antonini G, Fracasso M, Cechinel Filho V, Yunes RA, et al. Antinociceptive properties of caffeic acid derivatives in mice. Eur J Med Chem. 2009;44(11):4596–602.
17. Gainok J, Daniels R, Golembiowski D, Kindred P, Post L, Strickland R, et al. Investigation of the anti-inflammatory, antinociceptive effect of ellagic acid as measured by digital paw pressure via the Randall-Selitto meter in male Sprague-Dawley rats. AANA J. 2011;79(4 Suppl):S28-34.
18. Park SH. The effect of caffeic acid on the antinociception and mechanisms in mouse. J Korean Soc Appl Biol Chem. 2011;54(2).
19. Silva GC da. [*Cereus jamacaru* D.C.: phytochemical profile and cytotoxic evaluation]. Universidade Federal de Pernambuco; 2017.
20. Meiado MV, De Albuquerque LSC, Rocha EA, Rojas-Aréchiga M, Leal IR. Seed germination responses of *Cereus jamacaru* DC. ssp. jamacaru (Cactaceae) to environmental factors. Plant Species Biology. 2010;25(2):120–8.
21. Pinto N de CC, Duque AP do N, Pacheco NR, Mendes R de F, Motta EV da S, Bellozi PMQ, et al. *Pereskia aculeata*: a plant food with antinociceptive activity. Pharm Biol. 2015;53(12):1780–5.
22. Brito AER de M. [Coastal vegetation in the semi-arid Northeast: illustrated guide]. Universidade Federal do Ceará Ed.; 2006. 275 p.
23. Farias-Silva D, Simões Bezerra PH, Lopes de Sousa Ribeiro L, Viana MDM, de Lima AA, da Silva Neto GJ, et al. *Costus spiralis* (Jacq.) Roscoe leaves fractions have potential to reduce effects of inflammatory diseases. J Ethnopharmacol. 2021;268:113607.
24. Hussain RF, Nouri AM, Oliver RT. A new approach for measurement of cytotoxicity using colorimetric assay. J Immunol Methods. 1993;160(1):89–96.
25. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US); 2011. (The National Academies Collection: Reports funded by National Institutes of Health).
26. OECD. Test no. 425: acute oral toxicity: up-and-down procedure. OECD; 2022. (OECD guidelines for the testing of chemicals, section 4).
27. Malone MH. Pharmacological approaches to natural product screening and evaluation. In: Wagner H, Wolff P, editors. New natural products and plant drugs with pharmacological, biological or therapeutical activity. Berlin, Heidelberg: Springer Berlin Heidelberg; 1977. p. 23–53. (Proceedings in Life Sciences).
28. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. 1968;32(2):295–310.
29. Koster R, Anderson W, Beer E. Acetic acid for analgesic screening. Federation Proceedings. 1959;18:412–7.
30. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30(1):103–14.
31. Kuraishi Y, Harada Y, Aratani S, Satoh M, Takagi H. Separate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal algesic tests. Brain Research. 1983;273(2):245–52.
32. Beirith A, Santos ARS, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res. 2002;924(2):219–28.
33. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc. 1957;46(3):208–9.
34. Doherty NS, Poubelle P, Borgeat P, Beaver TH, Westrich GL, Schrader NL. Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptidoleukotrienes and prostaglandin E2. Prostaglandins. 1985;30(5):769–89.
35. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Experimental Biology and Medicine. 1956;91(1):95–101.
36. Vencioneck Dutra JC, Moisés Ferreira J, Costalonga Pereira PR, Ben-Hur de Oliveira J, Vitorino Gervásio S, Bernardes Xavier M, et al. *Cereus jamacaru* D.C. hydroalcoholic extract promotes anti-cytotoxic and antitumor activity. Pharmaceuticals (Basel). 2018;11(4):130.
37. Spindola HM, Vendramini-Costa DB, Rodrigues MT, Foglio MA, Pilli RA, Carvalho JE. The antinociceptive activity of harmicine on chemical-induced neurogenic and inflammatory pain models in mice. Pharmacol Biochem Behav. 2012 Jul;102(1):133–8.
38. Duarte ID, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res. 1988;21(2):341–3.
39. Williamson EM, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant material. J Med Chem. 1997;40(10):1559.
40. Lee S, Kim DH, Lee CH, Jung JW, Seo YT, Jang YP, et al. Antidepressant-like activity of the aqueous extract of *Allium macrostemon* in mice. J Ethnopharmacol. 2010;131(2):386–95.
41. Huerta-Reyes M, Herrera-Ruiz M, González-Cortazar M, Zamilpa A, León E, Reyes-Chilpa R, et al. Neuropharmacological in vivo effects and phytochemical profile of the extract from the aerial parts of Heteropterys brachiata (L.) DC. (Malpighiaceae). J Ethnopharmacol. 2013;146(1):311–7.
42. Dickenson AH, Sullivan AF. Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. Neurosci Lett. 1987;83(1–2):207–11.

43. Yaksh TL, Ozaki G, McCumber D, Rathbun M, Svensson C, Malkmus S, et al. An automated flinch detecting system for use in the formalin nociceptive bioassay. J Appl Physiol (1985). 2001;90(6):2386–402.
44. Loro JF, del Rio I, Pérez-Santana L. Preliminary studies of analgesic and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. J Ethnopharmacol. 1999;67(2):213–8.
45. Guilhon CC, Abdul Wahab IR, Boylan F, Fernandes PD. Central antinociceptive and mechanism of action of *Pereskia bleo* Kunth leaves crude extract, fractions, and isolated compounds. Evid Based Complement Alternat Med. 2015;2015:915927.
46. Aquino PEA, Magalhães TR, Nicolau LAD, Leal LKAM, de Aquino NC, Dos Santos SM, et al. The anti-inflammatory effects of N-methyl-(2S,4R)-trans-4-hydroxy-l-proline from *Syderoxylon obtusifolium* are related to its inhibition of TNF-alpha and inflammatory enzymes. Phytomedicine. 2017;24:14–23.
47. Matias A, Nunes SL, Poejo J, Mecha E, Serra AT, Madeira PJA, et al. Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate recovered from *Opuntia ficus-indica* juice. Food Funct. 2014;5(12):3269–80.
48. Calabresi E, Petrelli F, Bonifacio AF, Puxeddu I, Alunno A. One year in review 2018: pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol. 2018;36(2):175–84.
49. Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. Nat Rev Dis Primers. 2018;4:18001.
50. Hart BA, Otten HG. Prospects of immunotherapy for rheumatoid arthritis. Pharm World Sci. 1995;17(6):178–85.
51. Park EH, Kahng JH, Lee SH, Shin KH. An anti-inflammatory principle from cactus. Fitoterapia. 2001;72(3):288–90.
52. Medeiros IU, Medeiros RA, Bortolin RH, de Queiroz FM, Silbiger VN, Pflugmacher S, et al. Genotoxicity and pharmacokinetic characterization of *Cereus jamacaru* ethanolic extract in rats. Biosci Rep. 2019;39(1):BSR20180672.
53. Ndhlala AR, Kasiyamhuru A, Mupure C, Chitindingu K, Benhura MA, Muchuweti M. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chem. 2007;103(1):82–7.
54. Choi RJ, Ngoc TM, Bae K, Cho HJ, Kim DD, Chun J, et al. Anti-inflammatory properties of anthraquinones and their relationship with the regulation of P-glycoprotein function and expression. Eur J Pharm Sci. 2013; 48(1–2):272–81.
55. Giorno TBS, Santos CHCD, Carvalho MG de, Silva VC da, Sousa PT de, Fernandes PD, et al. Study on the antinociceptive activity and mechanism of action of isolated saponins from *Siolmatra brasiliensis* (Cogn.) Baill. Molecules. 2019;24(24):4584.
56. Malik EM, Müller CE. Anthraquinones as pharmacological tools and drugs. Med Res Rev. 2016;36(4):705–48.
57. Radulovic NS, Blagojevic PD, Randjelovic PJ, Stojanovic NM. The last decade of antinociceptive alkaloids: structure, synthesis, mechanism of action and prospect. Curr Top Med Chem. 2013;13(17):2134–70.
58. Souto AL, Tavares JF, da Silva MS, Diniz M de FFM, de Athayde-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. Molecules. 2011 Oct 11;16(10):8515–34.