



## Antinociceptive activity of the ethanolic extract from barks and leaves of *Cnidoscolus quercifolius* (Euphorbiaceae) in mice

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### ABSTRACT

*Cnidoscolus quercifolius* is a species native to the Brazilian Caatinga (semi-arid vegetation) popularly known as “favela” and “faveleira” and used in folk medicine to treat pain. The objective of this work was to evaluate the antinociceptive effect of the ethanolic extract from barks (Cqb-EtOH) and leaves (Cql-EtOH) of *C. quercifolius* in mice using experimental models of nociception. The antinociceptive activity was evaluated by writhing, hot plate and formalin tests. In addition, the rota-rod test was used to evaluate motor coordination. In the acetic acid-induced writhing test, the Cqb-EtOH (100, 200 and 400 mg/kg, i.p.) reduced the number of writhing by 83.70, 81.40 and 88.10%, respectively, while Cql-EtOH reduced by 71.30, 79.40, and 98.70%, respectively. In the formalin test, the extracts reduced the paw licking time in the first and second phases, but the best results were observed in the second phase (inflammatory pain), reducing by 66.08, 78.26 and 73.97%, as well as 60.11, 75.58, and 79.46% for Cqb-EtOH and Cql-EtOH, respectively. In the hot plate test, the extracts increased the reaction time when compared to control only at dose of 400 mg/kg. Using the rota-rod test, mice treated did not demonstrate any significant motor performance changes. It can be concluded that Cqb-EtOH and Cql-EtOH of *C. quercifolius* have antinociceptive activity, which supports the popular use of this plant to treat pain.

**Key words:** Caatinga biome, *Cnidoscolus quercifolius*, Euphorbiaceae, pain, medicinal plants

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### INTRODUCTION

Medicinal plants are used over the years by humanity in order to promote healing for diseases and pain relief, with several clinical procedures registered with them. Hence, while allopathic medicine has obtained a breakthrough from the second half of the XX century, there are still some

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barriers in their use by disadvantaged populations. Thus, the use of medicinal plants stood out among the poorest people in developing countries due to its easy to obtain and great tradition of using medicinal plants.<sup>1</sup>

Many ethnopharmacological studies in Brazil have shown that a large number of plant species are used by the local population to treat their diseases particularly the family Euphorbiaceae, which has great medicinal use, as well as the highly cited genus *Cnidoscopus*, popularly known as “urtiga” or “favela.” This genus has 50-75 representatives, which are predominantly concentrated in tropical America, almost exclusively in Mexico and northeastern Brazil.<sup>2,3</sup> The distinct feature of this genus is the presence of stinging trichomes that, when stimulated by contact with skin, can cause severe and localized pain.<sup>4</sup>

In the Caatinga biome, this genus is represented by four *Cnidoscopus* medicinal species (*Cnidoscopus infestus*, *Cnidoscopus pubescens*, *Cnidoscopus quercifolius* and *Cnidoscopus urens*), which are utilized for a variety of indications, including as an anti-inflammatory, an antitumor agent for the genito urinary system, an antiseptic and to treat kidney infections, dermatological and ophthalmic lesions, bruises, fractures, wounds, warts, dysentery, hemorrhage, appendicitis and rheumatism.<sup>3,5-7</sup>

*C. quercifolius* is a species known as “favela” and “faveleira,” and is a deciduous species, heliophytic, which occurs in xerophytic woods and exhibits irregular dispersion. The species has been used in reforestation of eroded areas of different soil habitats in the Caatinga. *C. quercifolius* also has high economic value to be used as a food source for local populations and livestock.<sup>8</sup> Several species of the genus *Cnidoscopus* are used in folk medicine for treatment of diseases, including pain and inflammation.<sup>7</sup>

In our continuing search of the pharmacological properties of species from the Caatinga biome, in the present paper we demonstrate the antinociceptive effects of crude ethanol extracts from barks (Cqb-EtOH) and leaves (Cql-EtOH) of *C. quercifolius* in experimental models in mice.

## MATERIALS AND METHODS

### Drugs and chemicals

Acetic acid, formaldehyde, indomethacin (INDO) and polyoxyethylenesorbitan monolated (Tween 80) were purchased from Sigma United States of America and morphine (MORPH) was obtained from União Química (Brazil). All drugs and extracts were administered intraperitoneally (i.p.) in volumes of 0.1 mL/10 g (mice).

### Plant material

The barks and leaves of *C. quercifolius* Pohl. were collected in the city of Petrolina (Coordinates: S 09°03'55"; W 40°20'06"), State of Pernambuco, Brazil, in February of 2012. The sample was identified by a biologist from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Semiárido). A voucher specimen (19202) was deposited at the Herbarium Vale do São Francisco of the Federal University of San Francisco Valley.

### Extraction

The barks and leaves were dried and pulverized (1481 g and 482 g, respectively), and macerated with ethanol 95% at room temperature for 72 h. The solution was filtered and concentrated under reduced pressure in a rotatory evaporator at 50°C, producing 0.392 g of crude Cqb-EtOH (13.07% yield on dry weight of the plant) and 0.063 g of crude Cql-EtOH (26.46% of yield on dry weight of the plant).

### Animals

Adult male albino Swiss mice (25-35 g) were used throughout this study. The animals were randomly housed in appropriate cages at 22°C ± 2°C on a 12 h light/dark cycle with free access to food and water. Mice were used in groups of six animals each, according to the requirements of individual experiments. All tests were carried out by the same visual observer. Experimental protocols and procedures were approved by Federal University of San Francisco Valley Animal Care and Use Committee by number 0030/82012.

### Pharmacological experiments

#### Acetic acid-induced writhing in mice

This test was performed as described by Collier *et al.*<sup>9</sup> with modifications. Male mice ( $n = 6$ ) were intraperitoneally pre-treated 30 min before the nociceptive agent, acetic acid 0.9% (v/v, 0.1 ml/10 kg). Vehicle (saline + drops of tween 80), Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg, i.p.), INDO (20 mg/kg, i.p.) and MORPH (10 mg/kg, i.p.) were administered before acetic acid injection. Following the injection of acetic acid, the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring between 5 and 15 min after injection of the acetic acid.

#### Formalin test

The method used was described by Hunskaar and Hole<sup>10</sup> with some modifications. 20 microliters of 2.5% formalin

was injected subcutaneously into the right hind paw of male mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 0-5 min (first phase, neurogenic phase) and 15-30 min after formalin injection (second phase, inflammatory phase). Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg, i.p.), INDO (20 mg/kg, i.p.) and MORPH (10 mg/kg, i.p.) were administered 60 min before the formalin injection. Control animals received the same volume of saline with drops of tween 80. Mice were observed in the chambers with a mirror mounted on three sides to allow view of the paws.

### Hot plate test

Mice were divided into five groups of six mice each. Mice were pre-selected on the hot plate at  $55 \pm 0.5^\circ\text{C}$ . Licks on the rear paws were the parameters of observation. Animals showing a reaction time (latency for licking the hind feet or jumping)  $>20$  s were discarded. The mice were then treated with Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg, i.p.), vehicle (saline + drops of tween 80 i.p.) and MORPH (10 mg/kg i.p.). The animals were placed individually on a hot plate with constant temperature ( $55 \pm 0.5^\circ\text{C}$ ) and the latency was measured at 30, 60, 90 and 120 min after administration of extracts and MORPH.<sup>11</sup> In order to protect the animal from potential tissue damage was chosen a cutoff time of 20 s.

### Motor coordination test (rota-rod test)

A rota-rod treadmill device (Insight, Brazil) was used for the evaluation of motor coordination.<sup>12</sup> Initially, 24 h before the test, mice capable of remaining on the rota-rod apparatus longer than 180 s (7 rpm) were selected. 30 min after administration of Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg, i.p.), vehicle (saline + drops of tween 80) or diazepam (DZP)(2.5 mg/kg, i.p.), each animal was tested in the rota-rod apparatus for 0.5, 1 and 2 h post-treatment, and time (seconds) the rats were able to remain on top of the bar was recorded for 180 s.

### Statistical analysis

The data are expressed as the means  $\pm$  standard error of the mean, and statistical significance was determined using an analysis of variance followed by Dunnett's test. All analysis was performed with the GraphPad Prism 4.0 program. Values were considered as significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

This study showed for the first time the antinociceptive effect of crude extracts obtained from bark and leaves of

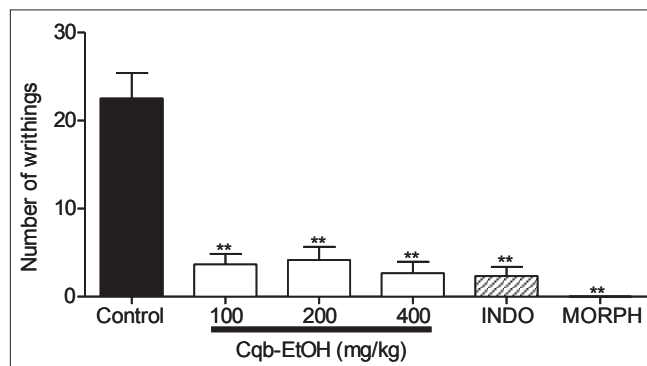
*C. quercifolius* in different nociceptive responses generated by different noxious stimuli. Three experimental models (acetic acid-induced writhing, formalin and hot plate tests) were used to assess the antinociceptive activity and a model (rota-rod test) was used to assess motor coordination.

In folk medicine in the Northeast of Brazil, the decoction, infusion and maceration of the stem bark and inner bark of *C. quercifolius* are used against pain, ovarian inflammation, and general inflammation.<sup>13</sup>

The intraperitoneal administration of Cqb-EtOH (100, 200 and 400 mg/kg) decreased significantly ( $P < 0.01$ ) the number of writhing movements induced by the administration of the acetic acid when compared with the control group (Figure 1). The inhibitions of writhes in percentage were 83.70, 81.40, and 88.10%, respectively.

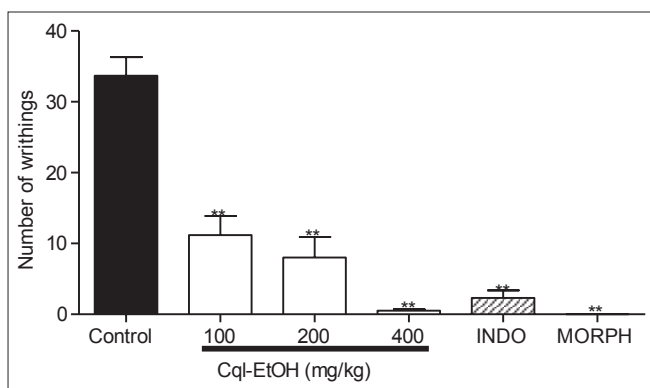
The administration of Cql-EtOH (100, 200 and 400 mg/kg) also decreased significantly ( $P < 0.01$ ) the number of writhing movements (Figure 2). The inhibitions of writhes in percentage were 71.30, 79.40 and 98.70%, respectively.

The model of the abdominal writhing test is employed for the initial screening of new molecules with potential analgesic. The constriction response of the abdomen, induced by acetic acid, is a sensitive method for peripheral analgesic agents.<sup>14</sup> Due to its low selectivity, most agents cause a change in the number of writhes, as other models should be tested. Pain is generated through the release of endogenous mediators such as bradykinin and serotonin, besides the involvement of cyclooxygenase release of arachidonic acid through the biosynthesis of prostaglandins.<sup>15</sup> Thus, the administration of Cq-EtOH from barks and leaves can cover hypothesized to act by inhibiting prostaglandin synthesis.



**Figure 1:** Effect of ethanolic extract of barks of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg), (indomethacin 20 mg/kg) and (morphine 10 mg/kg), on acetic acid induced writhing test in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control, analysis of variance followed Dunnett's test ( $n = 6$ , by group)

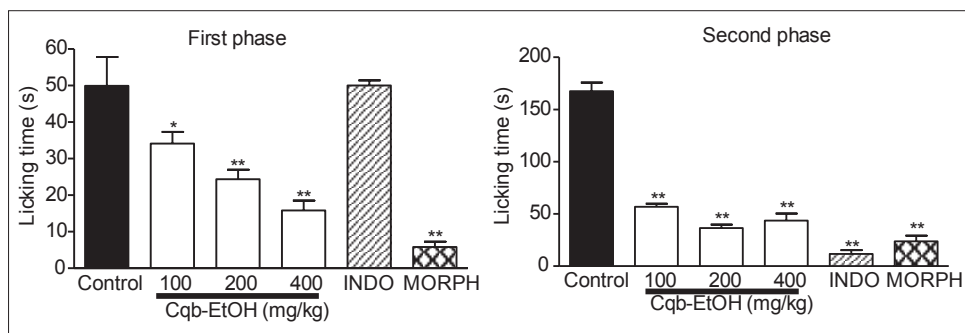
The results of the formalin test are shown in Figures 3 and 4. Cqb-EtOH caused a significant inhibition of both neurogenic and inflammatory phases in the licking induced by formalin. The result was most significant in the inflammatory phase. Cqb-EtOH (100, 200 and 400 mg/kg, i.p.) decreased by 31.62, 51.10, and 68.33%, respectively, the paw licking time in the first phase, as well as 66.08, 78.26, and 73.97%, respectively, the second phase of the formalin test.



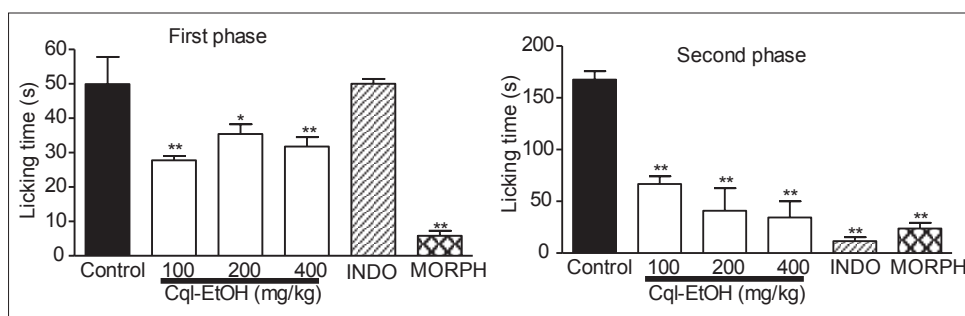
**Figure 2:** Effect of ethanolic extract of leaves of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg), (indomethacin 20 mg/kg) and (morphine 10 mg/kg), on acetic acid induced writhing test in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control, analysis of variance followed Dunnett's test ( $n = 6$ , by group)

The Cql-EtOH extract also caused a significant inhibition of both neurogenic and inflammatory phases in the formalin test. The result was most significant in the inflammatory phase. Cql-EtOH (100, 200 and 400 mg/kg, i.p.) decreased by 44.28, 29.05 and 36.27%, respectively, the paw licking time in the first phase, as well as 60.11, 75.58 and 79.46%, respectively, in the second phase of the formalin test.

To distinguish between the central and peripheral antinociceptive action of the extracts, the formalin test was performed. This model acts to promote a chemical stimulus that induces a spontaneous response to pain, being divided into two distinct phases, which reflect different types of pain.<sup>10</sup> The first phase (neurogenic pain) starts immediately after injection and lasts for approximately 5 min, this stage is followed by a quiescent period of about 5-10 min, after which develops a second phase (inflammatory pain) response, which starts 15-30 min after injection.<sup>10</sup> In this experiment, the Cqb-EtOH and Cql-EtOH decreased the licking time in both phases, but the effect was more significant for both extracts in the second phase. The decrease of licking time in both phases is characteristic of drugs acting at the central level and the possibility of interaction with opioid receptors. The first stage is more sensitive



**Figure 3:** Effect of ethanolic extract of barks of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg), (indomethacin 20 mg/kg) and (morphine 10 mg/kg), on formalin test (first and second phase) in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)

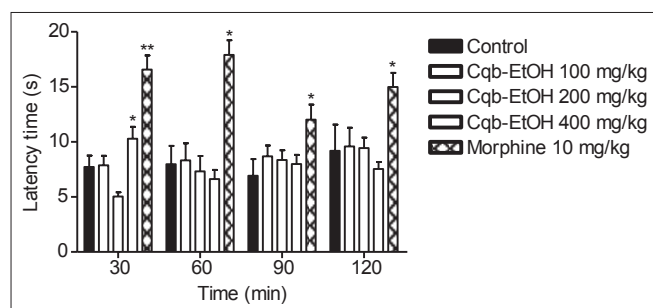


**Figure 4:** Effect of ethanolic extract of leaves of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg), (indomethacin 20 mg/kg) and (morphine 10 mg/kg), on formalin test (first and second phase) in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)

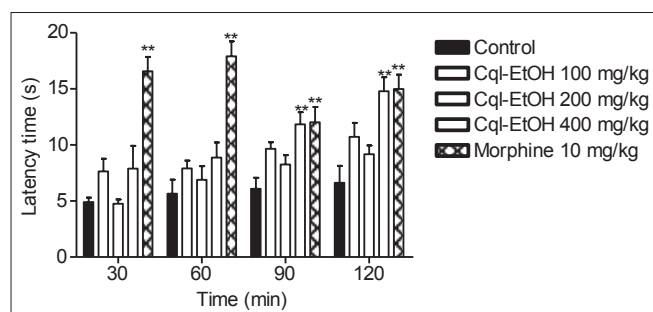
to opioid analgesics, but INDO and acetylsalicylic acid respond better in the second phase.<sup>16</sup> The extracts showed effects in the second phase, indicating a possible anti-inflammatory effect.

In the hot plate test, significant effect was observed only at dose of 400 mg/kg of Cqb-EtOH in the time of 30 min ( $P < 0.05$ ), while the administration of Cql-EtOH showed an increase in latency time for the dose of 400 mg/kg at 90 and 120 min after administration (Figures 5 and 6).

The hot plate test is one of the most common tests of nociception that is based on a phasic stimulus of high intensity. Thermally induced pain in hot plate test is specific for centrally mediated nociception.<sup>17</sup> The evaluation of Cqb-EtOH and Cql-EtOH administration with the hot plate showed that the extracts presented effect in this test. As the hot plate is a specific central antinociceptive test, it is possible that Cqb-EtOH and Cql-EtOH exerts an antinociceptive effect at least in part through central mechanisms. This result is similar to what was observed in the formalin test with Cqb-EtOH and Cql-EtOH inhibiting of both phases of nociception.



**Figure 5:** Effect of ethanolic extract of barks of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg) and (morphine 10 mg/kg), on hot plate test in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)

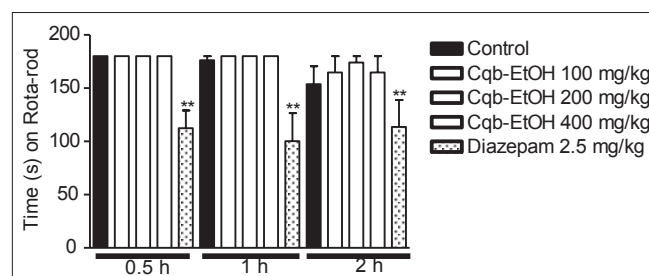


**Figure 6:** Effect of ethanolic extract of leaves of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg) and (morphine 10 mg/kg), on hot plate test in mice. Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)

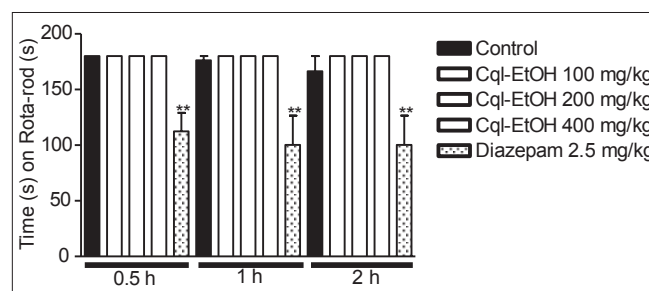
Finally, to assess whether Cqb-EtOH and Cql-EtOH produces a loss of motor coordination in animals, a rota-rod test was performed. In this test, Cqb-EtOH and Cql-EtOH did not impair motor coordination at 0.5, 1 and 2 h post-administration. The results revealed that the extracts did not produce changes in motor coordination of treated animals. DZP (2.5 mg/kg) caused a significant decrease in time that the animals remained on the rota-rod apparatus, compared to the control group (Figures 7 and 8).

## CONCLUSION

It can be concluded that the Cqb-EtOH and Cql-EtOH have antinociceptive activity, and its effect is probably mediated by inhibition of peripheral and central mediators. However, the extracts did not affect motor coordination of animals. Thus, our results confirm that *C. quercifolius* has therapeutic potential to act in combating of pain, corroborating its popular use. Other studies are underway to enable us to understand the precise mechanisms of action of Cqb-EtOH and Cql-EtOH.



**Figure 7:** Effect of ethanolic extract of barks of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg) and diazepam (2.5 mg/kg), on motor coordination test in mice (rota-rod test). Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)



**Figure 8:** Effect of ethanolic extract of leaves of *Cnidoscopus quercifolius* (100, 200 and 400 mg/kg) and diazepam (2.5 mg/kg), on motor coordination test in mice (rota-rod test). Values are mean  $\pm$  standard error of the mean. \* $P < 0.05$ , significantly different from control; analysis of variance followed Dunnett's test ( $n = 6$ , by group)

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