Phytochemical screening and anti-inflammatory activity of *Cnidoscolus quercifolius* (Euphorbiaceae) in mice

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ABSTRACT

Background: *Cnidoscolus quercifolius* is a species popularly known as "favela" and "faveleira", and belonging to the Caatinga biome (semi-arid vegetation, Brazil), where is used in folk medicine as an anti-inflammatory. **Objective:** The aim was to evaluate the anti-inflammatory effect of the ethanolic extract from barks (Cqb-EtOH) and leaves (Cql-EtOH) of *C. quercifolius* in mice using experimental models of inflammation. **Materials and Methods:** The preliminary phytochemical analysis of the ethanolic extract was performed. The activity was evaluated by paw edema induced by carrageenan and leukocytes migration to the peritoneal cavity induced by carrageenan methods. **Results:** A preliminary analysis of Cqb-EtOH revealed that it contained coumarins, flavonoids, monoterpenes/diterpenes and naphthoquinones, while the Cql-EtOH showed positive reaction to coumarins, anthracene derivatives, flavonoids, lignans and triterpenes/steroids. Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg) inhibited significantly ($P < 0.01$) the increase in the edema volume after administration of carrageenan. In the peritonitis test, acute pretreatment with Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg) inhibited the leukocyte migration. **Conclusions:** It can be concluded that extracts from the barks and leaves of *C. quercifolius* have anti-inflammatory activity, which supports the popular use of this plant to treat inflammation. Thus, extracts has significant anti-inflammatory properties, which are related probably to inhibition of release of mediators of the inflammatory process.

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INTRODUCTION

The Euphorbiaceae family is considered of big economic importance among the Angiosperms.[1] The chemistry of Euphorbiaceae is one of the most diverse and interesting one of the flowering plant families and is comparable to the biological diversity of the family.[2] It is characteristic of this family the occurrence of various secondary metabolites, among which stand out alkaloids, flavonoids, tannins and coumarins.[3] Another important class of secondary metabolites present in species of this family are the diterpenes, as they are considered the most characteristic group of substances in this complex family.[4]

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Inside the family Euphorbiaceae is the genus *Cnidoscolus*, which is well represented in Brazil (about 18 species), especially in the northeastern (about 10 species), and this region is one of the probable centers of diversity of *Cnidoscolus*.[5] In relation to the phytochemistry, there are reports of the presence of alkaloids and terpenoids in the genus *Cnidoscolus*.[6,7] Chemical investigation of *Cnidoscolus phyllacanthus* had led to isolation of three new terpenoids from the barks: A bis-nor-diterpene, phyllacantone and two triterpenes, 3 β-*O*-cinnamoyl-lupeol and 3 β-*O*-dihidrocinnamoyl-lupeol.[8] Regarding to biological activity, the species *Cnidoscolus aconitifolius* has showed hepatoprotective, nephroprotective, anti-inflammatory, and analgesic properties.[9]

Cnidoscolus quercifolius Pohl. is a species popularly known as "favela" and "faveleira", the tree is known to possess stinging trichomes distributed throughout the plant. This species produces latex, which is widely used for medicinal purposes. It's roots are tuberous and store nutrients used

during the dry season, a period when flowering and fruiting of this species occurs.[10] Regarding use in folk medicine, *C. quercifolius* is used to fight inflammation.[11]

The inflammation is the earliest organic response before tissue damage or infection. Before a tissue injury, the local accumulation of prostaglandins, thromboxane's, and other chemical mediators cause a change in the threshold nociceptors, resulting in hyperalgesia.[12,13] The inflammatory process is part of a mechanism of host defense against stimuli that cause injuries, but when this process is not controlled, can damage the health of the individual.[14] The cardinal signs that identify the inflammation are heat, flushing (redness), tumor (swelling), pain and loss of function, of which the first four were described by Cornelius Celsus.[15]

A number of natural products are used in various traditional medical systems to treat relief of symptoms from inflammation.[16] Considering the large consumption in our region and scarcity of chemical and pharmacological studies about *C. quercifolius*, in our continuing search of the pharmacological properties of species from the Caatinga biome, in this paper we demonstrate the anti-inflammatory effects of crude ethanol extracts from barks and leaves of this species in experimental models in mice.

MATERIALS AND METHODS

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 EMBRAPA Semiárido. A voucher specimen (19202) was deposited at the Herbarium Vale do São Francisco of the Federal University of San Francisco Valley.

Preparation of the extracts

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 ethanol extract of barks (Cqb-EtOH, 13.07% yield on dry weight of the plant) and 0.063 g of crude ethanol extract of leaves (Cql-EtOH, 26.46% of yield on dry weight of the plant).

Animals

Adult male albino Swiss mice (30-40 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.

Preliminary phytochemical screening

Preliminary phytochemical analyses of the extracts were performed as described by Wagner and Bladt,[17] seeking to highlight the major groups of secondary metabolites. It was evaluated the presence of alkaloids, anthocyanin's, coumarins, anthracene derivatives, flavonoids, lignans, mono, and diterpenes, naphthoquinones, saponins, steroids and terpenoids.

Pharmacological experiments

Carrageenan-induced hind paw edema

The test of paw edema induced by carrageenan 2% consists of injecting a volume of 20 µl/animal in the subplantar region, the right paw of the mouse.[18] Subsequently, to assess the extent of the volume of the paws immersed itself right paw to the lateral malleolus in an electric plethysmometer. The mice were divided into five groups of six animals each. The animals were treated with Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg, i.p.), vehicle (saline) and indomethacin (20 mg/kg, i.p.) 30 min before the injection of carrageenan. The mice pedal volume up to the ankle joint was measured using plethysmometer (PanLab LE 7500, Spain) before (VA, baseline) the intraplantar administration of carrageenan and 1, 2, 3, 4 and 5 h after (VB), as described previously.[19] The inhibition of the edema paw was calculated by (VB - VA)/VA, where VA is the volume of the right hind paw before carrageenan injection, and VB is the volume of the right hind paw after carrageenan injection.

Leukocyte migration to the peritoneal cavity

The animals were divided into five groups of six animals each. Leukocyte migration was induced by injection of carrageenan (1%, i.p., 0.25 ml) into the peritoneal cavity 1 h after administration of Cqb-EtOH and Cql-EtOH (100, 200 and 400 mg/kg i.p.), vehicle (saline, i.p.) and dexamethasone (2 mg/kg, i.p.), the solution of carrageenan was administered 30 min later of pre-treatments. The migration of leukocytes was measured 4 h after stimulation, and the animals euthanized and cells were harvested from the peritoneal cavity with 3 ml of saline containing 1 mM ethylenediaminetetraacetic acid (EDTA). Immediately, a short massage was performed and then the peritoneal fluid was collected, it was centrifuged (3000 rpm for 6 min) at room temperature. The supernatant was discarded and 300 µl of EDTA was added to the precipitate, which was removed at a rate of 10 µl. Two hundred microliter solution Turk at the rate and total cells were counted in a Neubauer

chamber, in an optical microscope was added. The results were expressed as the number of leukocytes/ml.[20]

Statistical analysis

The data are expressed as the means ± 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 significant at $P < 0.05$.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis indicated a positive reaction for the presence of coumarins, flavonoids, monoterpenes/diterpenes and naphthoquinones in Cqb-EtOH, while the Cql-EtOH showed positive reaction to coumarins, anthracene derivatives, flavonoids, lignans, and triterpenes/steroids. However, Cqb-EtOH showed negative for the presence of alkaloids, anthocyanin's, anthracene derivatives, lignans, saponins and triterpenes/steroids and Cql-EtOH negative for alkaloids, anthocyanin's, monoterpenes/diterpene quinones, saponins and triterpenes/steroids. The results are presented in the Table 1.

Carrageenan-induced hind paw edema

The paw edema induced by carrageenan test revealed an anti-inflammatory effect of the extracts. Pretreatment with Cqb-EtOH (i.p.) significantly inhibited at all times only at the dose of 200 mg/kg ($P < 0.01$), whereas a dose of 100 mg/kg inhibited only from 3 h to 5 h ($P < 0.01$) after using the carrageenan induced edema [Figure 1].

The paw edema induced by carrageenan in mice was reduced after pre-treatment with the Cql-EtOH (100, 200 and 400 mg/kg, i.p.). The edema was significantly inhibited only at a dose of 100 mg from 4 h ($P < 0.01$) to 5 h ($P < 0.01$) [Figure 2].

Table 1: Phytochemical characterization of crude ethanol extracts of the bark and leaves of *Cnidoscolus quercifolius*

Chemical class	Cqb-EtOH	Cql-EtOH
Alkaloids	-	-
Anthocyanins	-	-
Coumarins	++	+
Anthracene derivatives	-	+
Flavonoids	++	+
Lignans	-	+
Monoterpenes/diterpenes	+	-
Naphthoquinones	+	-
Saponins	-	-
Triterpenes/steroids	-	+++

-=Not detected; +=Low presence; ++=Moderate presence; +++=Strong presence

Leukocyte migration to the peritoneal cavity

The i.p. administration of Cqb-EtOH (100, 200 and 400 mg/kg), 1 h before injection of carrageenan (1%, i.p., 0.25 ml) inhibited leukocyte migration ($P < 0.01$) when compared to the control group [Figure 3].

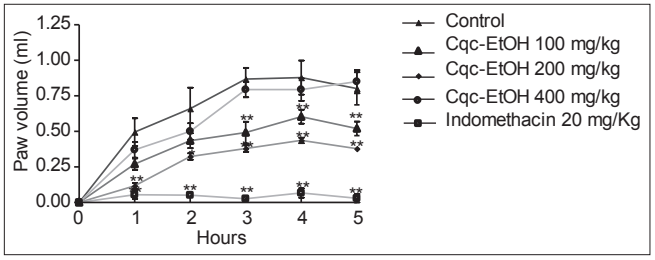

Figure 1: Effect of ethanolic extract of barks of *Cnidoscolus quercifolius* (Cqb-EtOH 100, 200 and 400 mg/kg) and indomethacin (20 mg/kg), on paw edema induced by carrageenan test in mice. Values are mean ± standard error of the mean. *$P < 0.05$, significantly different from control; analysis of variance followed Dunnett's test ($n = 6$, by group)

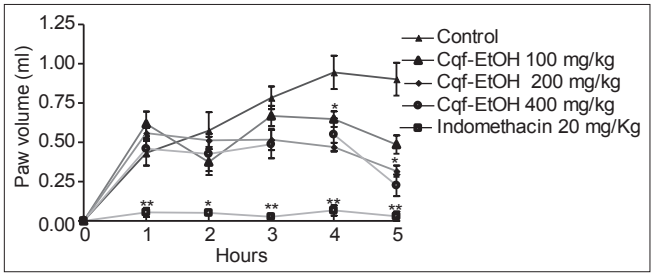

Figure 2: Effect of ethanolic extract of leaves of *Cnidoscolus quercifolius* (Cql-EtOH 100, 200 and 400 mg/kg) and indomethacin (20 mg/kg), on paw edema induced by carrageenan test in mice. Values are mean ± standard error of the mean. *$P < 0.05$, significantly different from control; analysis of variance followed Dunnett's test ($n = 6$, by group)

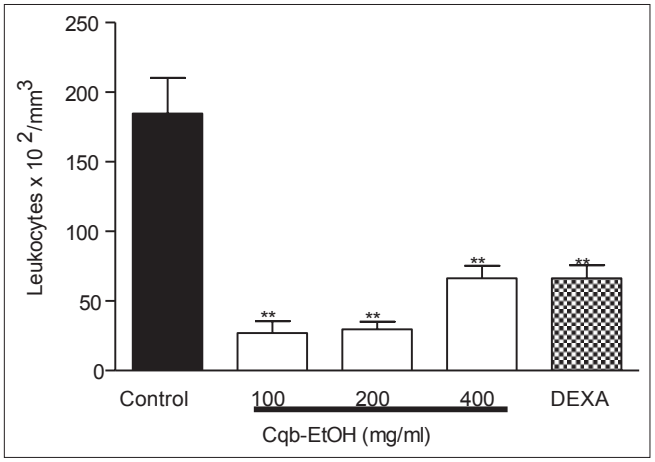

Figure 3: Effect of ethanolic extract of barks of *Cnidoscolus quercifolius* (Cqb-EtOH 100, 200 and 400 mg/kg) and dexametasone (2 mg/kg), on migration of leukocytes into the peritoneal cavity induced by carrageenan test in mice. Values are mean ± standard error of the mean. *$P < 0.05$, significantly different from control; analysis of variance followed Dunnett's test ($n = 6$, by group)

Pre-treatment with crude ethanolic extract of leaves (Cql-EtOH 100, 200 and 400 mg/kg, i.p.) 1 h before carrageenan injection also inhibited leukocyte migration ($P < 0.01$) when compared to the control group [Figure 4].

DISCUSSION

Many synthetic drugs reported to be used for the treatment of inflammatory disorders are of least interest now a days due to their potential side effects and serious adverse effects and as they are found to be highly unsafe for human assistance. Since the last few decades, herbal drugs have regained their popularity in treatment against several human ailments.[21]

In the present study, we demonstrate for the first time, the anti-inflammatory effects of crude ethanol extract of the barks and leaves of *C. quercifolius* through various inflammatory responses. This anti-inflammatory effects presented by Cqb-EtOH and Cql-EtOH, are probably associated with a variety of chemical constituents that were identified in the preliminary phytochemical analysis of the extracts [Table 1].

In the evaluation of anti-inflammatory activity, the paw edema test was performed. Carrageenan-induced paw edema as an *in vivo* model of inflammation has been frequently used to assess the antiedematous effect of natural products.[22] This model consists of two stages, the first being evaluated between 1 and 1.5 h after carrageenan injection is characterized by the formation of a nonphagocytic edema, whereas the second stage 2-5 h after injection, is characterized by increased formation of edema, which remains to 5 h.[23] Inflammation induced by carrageenan involves three distinct phases of release of the mediators. In the first phase occurs liberation of serotonin and histamine (0–2 h), in the second phase kinins are liberated (3 h), and prostaglandin in the third phase (after 4 h).[24] Thus, the Cqb-EtOH inhibited paw edema significantly at all times only at the dose of 200 mg/kg, while the dose of 100 mg/kg inhibited only from 3 h to 5 h, while the Cql-EtOH inhibited paw edema at a dose of only 100 mg from 4 h to 5 h.

The inflammation produced by injection of carrageenan into the peritoneal cavity is slow and prolonged, making it possible to evaluate the leakage of liquid, as well as cell migration, besides the participation of cytokines, enzymes, and chemical mediators, such as nitric oxide, prostaglandin E_2, interleukin (IL)-1 β, IL-6 and tumor necrosis factor-alpha, by utilizing different phlogistic agents.[25] In the test of carrageenan-induced peritonitis,

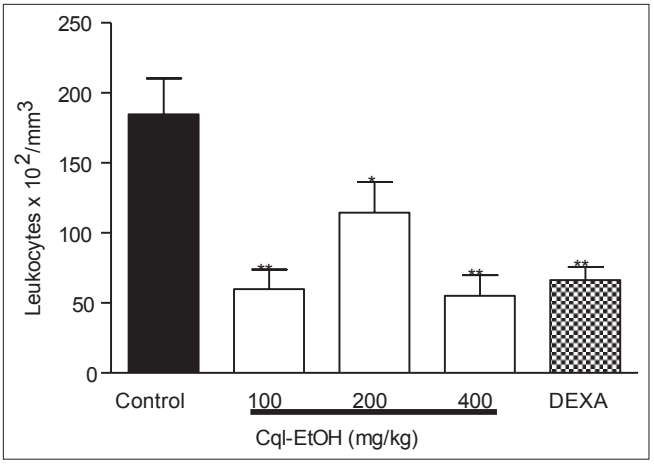

Figure 4: Effect of ethanolic extract of leaves of *Cnidoscolus quercifolius* (Cql-EtOH 100, 200 and 400 mg/kg) and dexametasone (2 mg/kg), on migration of leukocytes into the peritoneal cavity induced by carrageenan test in mice. Values are mean ± standard error of the mean. *$P < 0.05$, significantly different from control; analysis of variance followed Dunnett's test ($n = 6$, by group)

Cqb-EtOH and Cql-EtOH inhibited the leukocyte migration at all tested doses. Probably the extracts contain substances that act in blocking the synthesis of mediators responsible for leukocyte chemotaxis, and it is not possible to establish the mechanism involved.

The present study has demonstrated that crude ethanol extracts of the barks and leaves of *C. quercifolius* has significant anti-inflammatory properties, which are related probably with inhibition of release of mediators of the inflammatory process, such as prostaglandins, histamine and neutrophils.

CONCLUSION

It can be concluded that extracts from the barks and leaves of *C. quercifolius* have anti-inflammatory activity, which supports the popular use of this plant to treat inflammation.

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