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Evaluation of analgesic and anti-inflammatory activities of *Hydrocotyle umbellata* L., Araliaceae (acariçoba) in mice

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ABSTRACT

The *Hydrocotyle umbellata* L. is a specimen of the Araliaceae family popularly known as acariçoba. Its indications in folk medicine include treatment of skin ulcers, and rheumatism. The aim of this study was to evaluate the antinociceptive and anti-inflammatory activities of the ethanolic extract from acariçoba’s underground parts (EEA). EEA reduced the nociceptive response of the animals as evaluated in the acetic acid-induced writhing test and in both phases of formalin test. EEA also presented a supraspinal analgesic activity by increasing the pain latency in the hot plate test. Moreover, EEA reduced the leukocytes migration and plasma extravasation to pleural cavity in the carrageenan-induced pleurisy, besides reducing the edema induced by carrageenan until the second hour and also the edema induced by dextran. In conclusion our results showed that EEA of *H. umbellata* L. presents analgesic and anti-inflammatory activities, and that a blockade of activity or reduction in the release of different mediators, such as histamine and serotonin, could be involved in these pharmacologic effects.

Key words: Acariçoba, anti-inflammatory, antinociceptive, Biomedical Sciences, *Hydrocotyle umbellata* L.

INTRODUCTION

The *Hydrocotyle umbellata* L., popularly known as acariçoba, is a creeping specimen that belongs to the Araliaceae family. It species is native in Argentina, Cuba, India and Brazil, where this plant can be found in MidSouth states of São Paulo, Paraná, Santa Catarina, Rio Grande do Sul and Goiás (Corrêa 1984, Fischer et al. 1994). Plant species as *Hydrocotyle umbellata* L. and *Hydrocotyle asiatica* (L.) have great interest in folk phytotherapy and in the Ayurvedic medicine (Indian) because of its potential anxiolytic, memory stimulant effects and its use in the cosmetic industry (Reis et al. 1992). Ethnobotanical studies suggest...
that these plants are used as true panaceas, being employed as healing, diuretic, anti-hypertensive and to treat skin ulcers, eczema, dermatitis, psoriasis, erysipelas, rheumatism, tuberculosis, and spleen, liver and intestinal disorders. *H. umbellata* L. leaves in high doses produce emetic effects and are potentially toxic (Corrêa 1984, Reis et al. 1992, Fischer et al. 1994). Our previous study found that the ethanolic extract from *H. umbellata* L. showed anxiolytic-like and sedative effects in mice (Rocha et al. 2011).

*Acaríçoba* is a plant with long and petiolate leaves, white small and numerous flowers and a small fruit-shaped capsule flattened. In the Brazilian folk medicine a decoction of its leaves (fresh or dried) is used as baths, and orally for the treatment of inflammatory processes (Pharmacopeia dos Estados Unidos do Brasil, 1926 – Brazilian Pharmacopeia). Inflammation is the body’s immediate response to tissue aggressions caused by pathogens, toxic substances or physical harm. The inflammatory process is characterized by the release of several mediators and the development of the classic signs of inflammation such as swelling, warmth, redness and pain (Queiroz et al. 2010).

Phytochemical studies showed the presence of flavonoids, essential oils, saponins, tannins and absence of alkaloids and anthracene derivatives (Adams et al. 1989, Fischer et al. 1994). Thus, the aim of this study was to evaluate the pharmacological activity of ethanolic extract from *Hydrocotyle umbellata* L. (acariçoba) underground parts in pain and inflammation models seeking to scientifically give support the use of this plant by the Brazilian population.

**MATERIALS AND METHODS**

**BOTANICAL MATERIAL AND EXTRACTION**

*Hydrocotyle umbellata* L. underground parts were authenticated by Prof. Heleno Dias Ferreira (Institute of Biological Sciences/Federal University of Goiás/ICB/UFG) and collected in the Medicinal Plants Garden of Faculty of Pharmacy from the Universidade Federal de Goiás (UFG). A voucher specimen has been deposited in the Herbarium of UFG under the number UFG/22.394.

To prepare the ethanolic extract from acaríçoba underground parts (EEA) the vegetal material was dried and milled and the powder was then macerated in ethanol (96° GL) for three days, followed by filtration. After that, the filtrated was concentrated to dryness under reduced pressure in rotavapor (at 40 °C). The extract was obtained by this extractive process with a yield of 3.0 %.

**ANIMALS**

Male *Swiss* albino mice weighing approximately 30 g from the Central Animal House of UFG were used in this study. The animals received food and water *ad libitum* and were maintained in a room with light and temperature regulation. All experimental protocol was developed in accordance with the principles of ethics and animal welfare designated by the SBCAL/COBEA, as well as approved by the Ethics Committee in Research of UFG (number 104/08).

**DRUGS**

Acetone (Isofar, Brazil); carrageenan (Sigma, USA); cyproheptadine (Sigma, USA); croton-oil (Sigma, USA); dexamethasone - decadron (Ache, Brazil); dextran (Sigma, USA); ethanol 95% P.A. (Synth, Brazil); formaldehyde (Synth, Brazil); glacial acetic acid (Vetec, Brazil); Heparin (Hipolabor, Brazil); indomethacin (Prodome, Brazil); morphine - Dimorf® (Cristalia, Brazil); NaCl (Belga); Türk solution (Bioshop, Brazil).

**ACUTE ORAL TOXICITY STUDY**

Acute oral toxicity assay was performed in adult male albino *Swiss* mice (30-35 g) divided into different groups following the OECD guidelines-423 (OECD, 2001). Two groups of mice
n=3 albino Swiss were treated with EEA (4,000 mg/kg), orally. The vehicle group received distilled water at the same volume and route.

**ANALGESIC ACTIVITY**

The analgesic activity of the ethanolic extract from underground parts acariçoaba was investigated using the following models.

**Acetic acid-induced abdominal writhing**

Experimental groups of mice (n = 9) were treated (p.o.) with vehicle (10 mL/kg), EEA (250, 500 or 1,000 mg/kg) or indomethacin (10 mg/kg) 60 min before the administration of 1.2% acetic acid solution (10 mL/kg, i.p.). The number of writhing produced in each group for the following 30 min was counted and the results were expressed as mean ± standard error of mean (SEM) in percentage of control group (Koster et al. 1959, Vacher et al. 1964).

**Formalin-induced pain**

Experimental groups of mice (n = 9) were treated with vehicle (10 mL/kg, p.o.), EEA (1,000 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) or morphine (5 mg/kg s.c.), 60 min after the p.o. treatments or 30 min after s.c. treatment. The animals were treated with formalin 3% (20 μL) in the right hindpaw. Following injection of the phlogistic agent, the mouse was placed into an acrylic box, with a mirror placed under the box at 45° to facilitate the observation of the formalin-injected paw for 30 min. The pain reaction time (time for licking the paw) was observed in two periods, 0-5 min (neurogenic pain) and 15-30 min (inflammatory pain) (Hunskaar and Hole 1987).

**Hot plate test**

The latency (in seconds) to reaction of the mice, expressed as licking, shaking or lifting the hind paws, on hot plate at 55.5 ± 0.5°C was analyzed according to Woolfe and MacDonald (1944). The animals were divided into five experimental groups (n = 9) consisting of animals treated with vehicle (10 mL/kg, p.o.), EEA (250, 500, or 1,000 mg/kg, p.o.) or morphine (10 mg/kg, s.c.). The latency to pain reaction was measured at -60, -30, 0, 30, 60, 90 and 120 min of the treatment.

**ANTI-INFLAMMATORY ACTIVITY**

The anti-inflammatory activity of the ethanolic extract from acariçoaba underground parts was investigated using the following models.

**Carrageenan-induced pleurisy**

Each animal received, intravenously, 200 μL of 2.5% Evan’s blue in normal saline, two hours after the animals were being treated with vehicle (10 mL/kg, p.o.), EEA (250, 500, or 1,000 mg/kg, p.o.) or dexamethasone (2 mg/kg, p.o.). One hour after the treatments, the animals received an injection of 100 μL of 1% carrageenan into pleural cavity. Four hours after phlogistic agent administration the pleural exudate was collected with 1 mL of heparinized PBS. One aliquot was used to count the number of total leukocytes, using Türk solution, in a Neubauer’s chamber. Other aliquot was used to determine the Evan’s blue concentration on a spectrophotometer at 600 nm (Saleh et al. 1999, Vinegar et al. 1973).

**Croton oil-induced ear edema**

Animals were treated (p.o.) with vehicle (10 mL/kg, p.o.), dexamethasone (2 mg/kg, p.o.), or EEA (250, 500, or 1,000 mg/kg, p.o.). One hour after the treatments, the inflammation was induced by the application of 2.5 % (v/v) croton oil solution in acetone (2.5%) on the inner surface of the right ears. The same volume of acetone was applied to the left ear (Zanini et al. 1992). After 4 hours, the mice were sacrificed and segments of both ears were removed. The inflammatory was measured by the difference between the weights of the segments.
Carrageenan-induced paw edema

Groups of mice (n = 9) were treated orally with vehicle (10 mL/kg), EEA (250, 500 or 1,000 mg/kg) or indomethacin (10 mg/kg) 1 h before the injection of 50 µL of 1% carrageenan in the right paw. The left paw was used as control and received the same volume of 0.9% NaCl solution. Then, the edema was measured by the difference in the volume between the paws using a plethysmometer (Ugo Basile Co. - Italy) at several time-points after injection of phlogistic agent (Passos 2007).

Dextran-induced paw edema

Experimental groups of mice were treated orally with vehicle (10 mL/kg), EEA (1,000 mg/kg) or cyproheptadine (5 mg/kg) one hour before the injection of 50 µL of 1% dextran in the right paw. The left paw was used as control and received the same volume of 0.9% NaCl solution. After the injections the edema was measured in time intervals of 0, 30, 60, 90 and 120 min using a plethysmometer (Ugo Basile Co.- Italy). The formation of edema was assessed by the difference between the paws volume.

STATISTICAL ANALYSIS

Results were expressed as means ± S.E.M. Differences between two means were detected using the Student’s t test. Differences between more than 2 means were detected using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls’ test. The results were considered significant when \( p < 0.05 \) (Sokal and Rohlf 1981).

RESULTS

ACUTE ORAL TOXICITY

In the acute oral toxicity test doses up to 4,000 mg/kg of EEA did not cause any death in mice during 14-days of observation. Mice did not show any signs of toxicity or change in general behavior or other physiological activities.

ACETIC ACID-INDUCED ABDOMINAL WRITHING

The EEA (250, 500 or 1,000 mg/kg) produced a significant reduction (24, 32 and 41%, respectively) in the number of acetic acid-induced writhes when compared with control values (vehicle 10 mL/kg; 113.0 ± 6.4). The indomethacin reduced the abdominal writhes by 40% (Figure 1).

![Figure 1 - Effect of the ethanolic extract from acariçoba underground parts (EEA) (250, 500 and 1,000 mg/kg p.o.) in the number of acetic acid-induced writhes in mice. Indomethacin (10 mg/kg p.o.) was used as positive control. Vertical bars represent mean ± SEM of cumulated writhings in 30 min for each experimental group. *p < 0.05; ***p < 0.001. According ANOVA followed by Student-Newman-Keuls’ test.](image)

FORMALIN-INDUCED PAIN

Treatment with EEA 1,000 mg/kg p.o. or morphine 10 mg/kg s.c. reduced by 41 and 99%, respectively, the licking time of hind paw after the intraplantar injection of formalin in the neurogenic phase (0-5 min) from control values (vehicle 10 mL/kg; 62.6 ± 4.8 s). In the inflammatory phase (15-30 min), treatment with EEA 1,000 mg/kg p.o., morphine or indomethacin reduced the licking time by 48, 99 and 43% respectively, from a control value of 131.0 ± 17.1 s (Figure 2).

HOT PLATE TEST

Treatment with EEA increased by 60% the latency to reaction only with the highest dose (1,000 mg/kg) 1 h after the treatment from a control value (vehicle...
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10 mL/kg) of 10.6 ± 0.82. The positive control morphine (10 mg/kg s.c.) increased the latency to reaction to 90 min after the treatment (Figure 3).

CARRAGEENAN-INDUCED PLEURISY

The EEA (500 and 1,000 mg/kg) or dexamethasone (2.0 mg/kg) reduced by 40, 44 and 50 % the number of migrated leukocytes/mL to the pleural cavity from a control value of 4.8 ± 0.46 × 10^6 leukocytes/mL (vehicle 10 mL/kg), respectively (Figure 4). Treatment with EEA (250, 500 and 1,000 mg/kg) reduced by 33, 38.8 and 50% the Evan’s blue concentration in the pleural exudate from a control value (vehicle 10 mL/kg) by 1.8 ± 0.17 µg/mL, respectively. Dexamethasone reduced this value by 50% (Figure 5).

CROTON OIL-INDUCED EAR EDEMA

EEA (250, 500 and 1,000 mg/kg) reduced the edema by 20, 25 and 38%, in a dose-dependent manner, from a control value (vehicle 10 mL/kg) of 17.8 ± 0.77 mg, respectively. Dexamethasone (2.0 mg/kg) reduced the edema by 78% (Figure 6).
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At the first hour, it was observed a reduction of 27, 44, 57 and 21% from control value (vehicle 10 mL/kg) of 55.0 ± 4.3, respectively. At the second hour, from control value of 57.66 ± 3.51 to 39.33 ± 2.54; EEA reduced the edema by 32, 59 and 47%, respectively.

Figure 5 - Effect of the ethanolic extract from acariçoba underground parts (EEA) (250, 500 and 1,000 mg/kg p.o.), in carrageenan-induced pleurisy in mice. Dexamethasone (2.0 mg/kg p.o.) was used as positive control. The bars represent the means ± SEM of Evan’s blue concentration (µg/mL) in the pleural exudate after carrageenan injection. ** p < 0.01 and *** p < 0.001. According ANOVA followed by Student-Newman-Keuls’ test.

The indomethacin reduced the edema by 45%. After the second hour of edema, only indomethacin was able to reduce the edema formation (Figure 7).

Figure 7 - Effect of the ethanolic extract from acariçoba underground parts (EEA) (250, 500 and 1,000 mg/kg p.o.) or indomethacin (10 mg/kg p.o.) on the carrageenan-induced paw edema. The edema was measured 1, 2, 3 and 4 h after the injection of the phlogistic agent. Vertical bars represent mean ± SEM of the difference between the volumes of the paws. *p < 0.05; **p < 0.01; ***p < 0.001. According ANOVA followed by Student-Newman-Keuls’ test.

DEXTRAN-INDUCED PAW EDEMA

In the model of dextran-induced paw edema, treatment with EEA (1,000 mg/kg) or cyproheptadine (5.0 mg/kg) reduced the edema (µL) of 36 and 37%, respectively, at 30 min after the injection of dextran, from a control value of 87.8 ± 7.4, as well as, at 60 min after the treatment, there was a reduction of 41 and 45%, from control value (60.0 ± 7.3), respectively (Figure 8).

DISCUSSION

In the present study the analgesic and anti-inflammatory effects of the ethanolic extract (EEA) from acariçoba’s underground parts was evaluated, since this plant has the popular reputation of these effects. The results obtained with this extract in the acute oral toxicity evaluation indicated no toxic effects (Santana 2001).

The acetic acid induce nociception by stimulates nociceptive fibers directly, besides promote
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as histamine, bradykinin, serotonin, prostaglandins, tachykinins and glutamate by cells activated by formalin (Fujimaki et al. 1992, Santos and Calixto 1997, Cao et al. 1998, Omote et al. 1998 and Beirith et al. 2002). The formalin also activates primary afferent sensory neurons through a specific and direct action on TRPA1, which is a member of the Transient Receptor Potential family of cation channels that is highly expressed by a subset of C-fiber nociceptors (McNamara et al. 2007).

Considering the fact that formalin-induced pain test is more specific for pain study than abdominal contortions induced by acetic acid, with the expectation to confirm the true analgesic activity of this EEA, we decided to work with the higher dose.

The EEA reduced the pain reactivity in both phases of the formalin test. This effect may be a TRPA1 inhibition, seen that TRPA1 antagonist receptor eliminated pain-related flinching in both phases of the formalin response in vivo (McNamara et al. 2007). But also, this action in the both phases of formalin test does not allow us to separate an anti-inflammatory activity independent of a central analgesic, making necessary to evaluate the extract in specific models of inflammation.

The inflammation is a complex process, thus it is necessary to use various inflammation models, involving the analysis of several parameters, to evaluate an anti-inflammatory activity of a given substance (Chiabrando et al. 1989).

The formalin test, on the other hand, allows the study of two types of pain. The first phase (0-5 min) corresponds to a neurogenic pain with the involvement of mediators such as substance P, bradykinin, histamine and serotonin, and the evidence of direct stimulation of nociceptors by formalin (Dubuisson and Dennis 1977, Hunksaar and Hole 1987, Shibata et al. 1989, Corrêa and Calixto 1993, Munron 2007). The second phase (15-30 min) is associated with the production and release of various pro-inflammatory mediators such as histamine, bradykinin, serotonin, prostaglandins, tachykinins and glutamate by cells activated by formalin (Fujimaki et al. 1992, Santos and Calixto 1997, Cao et al. 1998, Omote et al. 1998 and Beirith et al. 2002). The formalin also activates primary afferent sensory neurons through a specific and direct action on TRPA1, which is a member of the Transient Receptor Potential family of cation channels that is highly expressed by a subset of C-fiber nociceptors (McNamara et al. 2007).

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The highest dose of EEA also increased the nociceptive threshold to thermal stimulation 1 h after the treatment. This effect in the hot plate test suggests a central analgesic activity (Yaksh and Rudy 1977).

In the carrageenan-induced pleurisy test, it is possible to evaluate an important inflammation parameter, the leukocyte migration. Carrageenan is a phlogistic agent that when injected in the pleural cavity promotes a severe inflammation, in which various inflammatory mediators are released, such as...
as histamine, serotonin, cytokines and eicosanoids, resulting in an intense leukocyte migration, and an pleural exudate formation caused by protein extravasation, being this extravasation indirectly quantified by Evan’s blue concentration in the pleural exudate (Steele and Wilhelm 1966).

Treatment with EEA at the doses of 500 and 1,000 mg/kg reduced the number of leukocytes migration into the pleural cavity. These effects suggest a reduction in the level of mediators responsible for the cell migration, such as cytokines (IL-1 and TNF-α), nitric oxide, prostanoids, serotonin and histamine (Henriques et al. 1987, Hopkins 2003). A similar reduction was observed in Evan’s blue concentration in the pleural exudate, where the EEA promoted a reduction up to 50%, suggesting a reduction of protein extravasation to pleural cavity (Saleh et al. 1997, Vianna and Calixto 1998).

The croton oil causes leukocyte migration, increases the vascular permeability and the plasma exudation (Lapa 2003, Swingle et al. 1981). The anti-edematogenic effect observed in this method suggests an anti-inflammatory action. Nevertheless, this model does not clearly show in which stage of the inflammatory process EEA acts.

The carrageenan-induced paw edema is a model of acute inflammation which involves a gradual and complex response (Winter et al. 1962). The edema evolution is marked by the release of various inflammatory mediators such as histamine, bradykinin, serotonin and prostaglandins. These mediators are released at different times. In the first two hours, histamine and serotonin are the primary mediators; in the third hour, prostaglandins are predominant in the acute edema, while the complement system operates throughout the process (Di Rosa et al. 1971). In this test EEA reduced the edema in the first two hours.

Similar results were observed with extracts of leaf and stem bark of *Lafoentsia pacari*, a Cerrado’s species. These extracts showed antinociceptive activity in writhing test. The two phases of the formalin test reduced the ear edema and the cell migration, suggesting an analgesic and anti-inflammatory effect (Nascimento et al. 2011, Guimarães et al. 2010). Nascimento et al. (2011) showed that this analgesic activity may be independent of anti-inflammatory action of ellagic acid and that this effect is not blocked by naloxone.

The *Spiranthera odoratissima*, other Cerrado’s specie popularly known by manacá, has only anti-inflammatory activity by reducing the writhing test, the second phase of formalin test, the ear edema, the second and third hours of paw edema, leukocyte migration, protein extravasation and inhibition of inflammation mediators such as phospholipase A2 and TNF-α, without analgesic effect in the first phase of the formalin and hot plate tests (Barbosa et al. 2012, Nascimento et al. 2012).

The inhibition in the edema caused by EEA in the first two hours suggests that the extract interferes with the action or release of serotonin or histamine, because these mediators are predominant in the initial phase of carrageenan-induced edema (Santos and Rao 2000). The effect observed with EEA in the dextran-induced edema model corroborates with this possibility. The dextran is a phlogistic agent known to induce edema mediated by 5-hydroxytryptamine and histamine (Lo et al. 1982, Katz et al. 1984).

Histamine is a vasoactive amine, which is among the preformed mediators released during the inflammatory process (Kumar et al. 2005). This amine causes vasodilation and increases the vascular permeability through the action over specific receptors (H1 and H2), together with other inflammatory mediators (Sherwood and Toliver-Kinsky 2004).

In this regard, serotonin (5-hydroxytryptamine [5-HT]) has long been associated with pain processing and modulation (Eide and Hole 1993). Some studies have shown a spinal analgesic action of 5-HT released from brainstem structures (Yaksh
and Wilson 1979). However, the peripheral action of serotonin is different from its central actions. Serotonin is considered an inflammatory mediator in the periphery, being released from platelets and mast cells after tissue injury (Dray 1995).

Moreover, our results with cyproheptadine pretreatment in the edema induced by dextran suggest a blocker action on both histaminergic H1 and serotonergic 5HT2 receptors (Rang et al. 2007).

CONCLUSIONS

Results showed that the ethanolic extract (EEA) from acariçoba (Hydrocotyle umbellata) presents analgesic and anti-inflammatory activity in different experimental models, suggesting an anti-migration and anti-edematous action that can be due to the blockade or inhibition in the release of histamine or serotonin.

The authors declare that there are no conflicts of interest.

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RESUMO

A Hydrocotyle umbellata L., é uma espécie pertence à família Araliaceae, popularmente conhecida como acariçoba. Suas indicações na medicina popular incluem tratamento de úlceras cutâneas, eczemas e reumatismo. O objetivo deste trabalho é avaliar as atividades antinociceptiva e/ou anti-inflamatória do extrato etânólico das partes subterrâneas da acariçoba (EEA). O EEA reduziu a resposta nociceptiva do animais no teste das contorções abdominais induzidas por ácido acético e em ambas as fases do teste da formalina. O EEA também mostrou uma atividade analgésica supra-espinhal ao aumentar o limiar nociceptivo no método da placa quente. O EEA reduziu tanto o número de leucócitos migrados quanto o extravasamento plasmático para a cavidade pleural na pleurisia induzida por carragenina. Além de reduzir o edema de pata induzido por carragenina até a segunda hora e também o edema de pata induzido por dextrana. Em conclusão os nossos resultados mostram que o EEA de H. umbellata L. possui atividades analgésica e anti-inflamatória, e que um bloqueio da atividade ou redução da liberação de diferentes mediadores tais como histamina e serotoninina, podem estar envolvidos nestes efeitos farmacológicos.

Palavras chave: Acariçoba, anti-inflamatório, antinociceptivo, Ciências Biomédicas, Hydrocotyle umbellata L.

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