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Article

Evaluation of the antinociceptive and anti-inflammatory effects of the acetone extract from *Anacardium occidentale* L.

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The stem bark of *Anacardium occidentale* L. (Anacardiaceae), commonly called cashew, is used in Brazilian traditional medicine for the treatment of gastric and inflammatory disorders. The present study was carried out to investigate the in vivo anti-inflammatory activities of the acetone extract (AE) of the stem bark of *A. occidentale*. We evaluated the pharmacological activities of this plant material through the analgesic, antiedematogenic and chemotaxic inhibitory effects produced by the AE. The oral administration (p.o.) of mice with the AE (0.1, 0.3 and 1.0 g/kg) or positive control indomethacin (10 mg/kg) inhibited acetic acid-induced writhing by 18.9, 35.9, 62.9 and 68.9%, respectively ($ID_{50\%}$ = 530 mg/kg). The highest dose of the AE was able to inhibit croton oil-induced ear edema formation by 56.8% (indomethacin at 10 mg/kg, p.o. - 57.6% inhibition). When submitted to the carrageenan-induced peritonitis test, the AE (0.1, 0.3 and 1.0 g/kg, p.o.) impaired leukocyte migration into the peritoneal cavity by 24.8, 40.5 and 49.6%, respectively. The positive control, dexamethasone (2 mg/kg, s.c.), inhibited leukocyte migration by 66.9%. These results indicate the presence of anti-inflammatory and antinociceptive principles in the acetone extract of *Anacardium occidentale*, and reinforce the plant's potential therapeutic use against pain and inflammatory diseases.

Uniterms: *Anacardium occidentale* L./analgesic activity. *Anacardium occidentale* L./anti-inflammatory activity. *Anacardium occidentale* L./antiedematogenic activity. Anacardiaceae. Cashew.

As cascas do caule do *Anacardium occidentale* L. (Anacardiaceae), conhecido como cajueiro, são popularmente utilizadas no Brasil para o tratamento de doenças gástricas e inflamatórias. Este estudo teve como objetivo a avaliação farmacológica in vivo da atividade antiinflamatória do extrato acetônico (AE) obtido das cascas do *A. occidentale*, investigando os efeitos analgésico, antiedematogênico e inibitório sobre a quimiotaxia deste material botânico. A administração oral (p.o.) em camundongos com o AE (0,1; 0,3 e 1 g/kg) ou o controle positivo indometacina (10 mg/kg) inibiu as contorções abdominais induzidas pelo ácido acético em 18,9; 35,9; 62,9 e 68,9% respectivamente ($ID_{50\%}$ = 530 mg/kg). Esta maior dose do AE também inibiu o edema de orelha produzido pelo óleo de cróton em 56,8% (indometacina, 10 mg/kg, p.o. - 57,6% de inibição). No teste da peritonite induzido pela carragenina, o AE (0,1; 0,3; e 1,0 mg/kg, p.o.) reduziu a migração de leucócitos para a cavidade peritoneal em 24,8; 40,5; e 49,6% respectivamente, enquanto que o controle positivo dexametasona (2 mg/kg, s.c.) inibiu a migração de leucócitos em 66,9%. Estes resultados indicam a presença de princípios ativos antiinflamatórios e antinociceptivos no extrato acetônico de *Anacardium occidentale* e reforçam o potencial terapêutico da planta em doenças que envolvem dor e inflamação.

Unitermos: *Anacardium occidentale* L./atividade analgésica. *Anacardium occidentale* L./atividade antiinflamatória. *Anacardium occidentale* L./atividade antiedematogenica. Anacardiaceae. Cajueiro.

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INTRODUCTION

Anacardium occidentale L. (Anacardiaceae) is a tropical tree native to Brazil that is now extensively cultivated in India and East Africa, popularly known as cashew. The fruits, stem bark and leaves are used in folk medicine for the treatment of several diseases such as hypertension, inflammatory diseases, asthma, bronchitis, and gastric (peptic ulcers) and intestinal disturbances (Corrêa, 1984). The stem bark is astringent and rich in tannins, which possibly supports its popular use in healing. In fact, previous studies conducted by Mota *et al.* (1985) demonstrated that tannins isolated from the stem bark of *A. occidentale* possess analgesic and anti-inflammatory effects. The anti-inflammatory activity of the plant's stem bark has been recently described in two different models of inflammation, having reduced paw edema induced by fresh egg albumin in rats (Ojewole, 2004) and inhibited skin dye leakage in mice after subcutaneous injection of LPS (Olajide *et al.*, 2004). More specifically, the anti-inflammatory and cicatrizant effects of the aqueous extract of *Anacardium occidentale*, rich in tannin constituents, was shown by the clinical evaluation of patients with skin injury or mucosa membrane lesions (oral or vulva erosion, uterus inflammation and body ulceration). The aqueous extract reduced the characteristic lesions up to a total cure, without any evidence of side effects or adverse reactions (Lopes *et al.*, 2003). Recently, the antioxidant activities of the methanol extract obtained from the stem bark (Gomes *et al.,* 2006) and the antiulcerogenic effect of a hydroethanolic extract of *Anacardium occidentale* L. leaves (Konan, Bacchi, 2007) were characterized. Although the anti-inflammatory activity of the *A. occidentale* extracts has been established in different models of inflammation, its analgesic effect has been poorly studied, and the mechanisms of both activities remain unclear. The present work studied the analgesic and antiedematogenic effects of *Anacardium occidentale* L. stem bark acetone extract (AE) and investigated the effect of the plant on leukocyte migration, an important component of the inflammatory process.

MATERIAL AND METHODS

Plant material

The stem bark samples of *Anacardium occidentale* were collected in September, 2005, in Natal (RN) Brazil, and identified by Maria Iracema Bezerra Loiola. A voucher specimen (1782) was deposited in the Herbarium of Botanic Ecology and Zoology Department (UFRN, Brazil).

Extraction

To obtain the acetone extract (AE), 1.3 kg of powdered bark of *A. occidentale* was extracted with acetone in a Soxhlet apparatus to yield 58 g of the AE.

Animals

Adult male Swiss mice (25–35 g) were housed in plastic cages, with food and tap water available *ad libitum* in the colony room. Mice were acclimatized in the laboratory for at least 60 min prior to the test procedure and left without food for 12–18 h before the gavages. All experiments were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines on the use of animals in pain research (CIOMS 1985; Zimmermann, 1986). Experimental protocols were approved by the local Animal Care and Use Committee (007/2006/CEPEB/UFRRJ), and the minimum number of animals and duration of observation required to obtain consistent data were employed.

Drugs and reagents

Acetic acid, acetone (Merck AG, Darmstadt, Germany), croton oil, indomethacin, carrageenan (Sigma Chemical Co., St. Louis, MO, USA), fentanyl (Janssen Pharmaceutical), dexamethasone (Prodome, Brazil), the AE and other drugs were diluted in water (*p.o.*) or saline solution (*s.c*. or *i.p.*).

Acetic acid-induced writhing test

We orally treated groups of six mice with water (10 mL/kg), the AE (0.1 to 1.0 g/kg) or indomethacin (10 mg/kg) 60 min before acetic acid injection (1.2%, 0.1 mL/10 g), and counted the number of times the mice writhed during the following 30 min (Koster *et al.*, 1959).

Hot-plate test

We measured the latency (seconds) of heat stimulus every 30 min, starting 30 min before and up to 2 h after treatment of the mice ($n = 8$) with the AE (1 g/kg, *p.o.*), fentanyl (100 µg/kg, *s.c.*) or water (10 mL/kg, *p.o.*) (D'Amour, Smith, 1941).

Croton oil induced ear oedema

One hour after the oral administration of water, the AE (1 g/kg) or indomethacin (10 mg/kg), we treated

each animal (n = 8) with 20 µL of freshly prepared croton oil (2.5% in acetone) on the inner surface of the right ear. The left ear was treated with the same volume of acetone (control). Four hours after the treatment, the mice were killed by cervical dislocation and a plug (6 mm in diameter) was taken from both the treated and untreated ears with a punch. We monitored the inflammatory response (edema) by weighing (mg) both plugs (Δ) and testing the differences. The test was employed as described by Tubaro *et al.* (1986) and Zanini *et al.* (1992).

Carrageenan-induced peritonitis

We orally treated groups of mice (n = 10) with a vehicle (water) or the AE (0.1 to 1.0 g/kg) 60 min prior to an injection of carrageenan (1% in saline solution; 250 µL/mouse) into the right peritoneal cavity. The positive control group was pre-treated (30 min) with dexamethasone (2 mg/kg, *s.c.*). The animals were anesthetized with ether and sacrificed 4 h later. We washed the peritoneal cavity with 2 mL of PBS-heparin (10 UI/mL) and collected the peritoneal exudates, which were then diluted (Türk solution 1:20). We counted the number of leukocytes that had migrated to the peritoneum in a Neubaüer chamber, and the results were expressed as cells x 10^6/mL, or as the percentage of inhibition of leukocyte migration compared to control groups as described by Ferrándiz and Alcaraz (1991).

Statistical analysis

Data were analyzed statistically by one-way ANOVA. The differences between the means were detected using the unpaired Student's *t*-test. The values are reported as mean ± standard error of the mean (SEM). Probability values of less than 0.05 ($p < 0.05$) were considered significant.

RESULTS

To evaluate the antinociceptive effect of the AE, we assessed its performance in the acetic acid-induced abdominal writhing model of analgesia. As can be seen in Table I, pre-treatment with the AE (0.1, 0.3 and 1.0 g/kg, *p.o.*) produced a dose-related inhibition of acetic acid-induced abdominal writhing (18.9, 35.9, and 62.9%, respectively) compared to the control group (43.7 ± 3.4 number of writhings). The inhibitory dose calculated ($ID_{50\%}$) was 530.8 mg/kg, and pre-treatment with indomethacin also showed a decrease in accumulated abdominal writhing (15.3 ± 3.4 number of writhings).

In the hot-plate test, Figure 1 shows that the basal latency of the control group was 8.3 ± 0.8 sec, and the AE (1 g/kg, *p.o.*) did not alter the pain latency of the animals, even at a dose level high enough to induce maximal response in the acetic acid test. The positive control fentanyl increased the latency of heat stimulus 2.7-fold (30 min) and 1.9-fold (60 min).

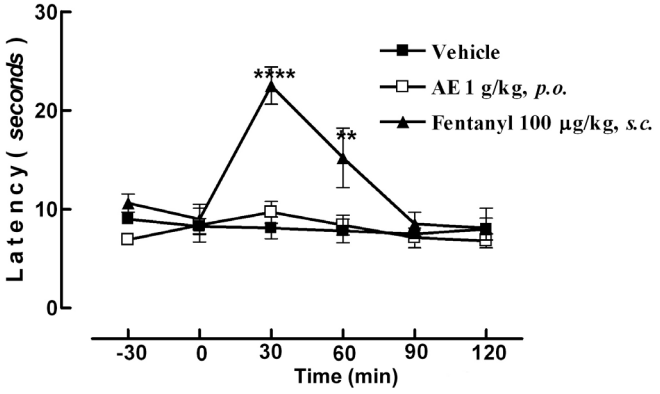

FIGURE 1- Effect of the acetone extract (AE) of *Anacardium occidentale* or fentanyl on the nociceptive response of mice in the hot-plate test. The symbols and vertical bars express means ± S.E.M. of the latency for the nociceptive behavior. $^{**}p < 0.01$, $^{****}p < 0.0001$ when compared with the vehicle control group (Two tail unpaired Student's *t* test). n = 8.

TABLE I - Effect of AE from *Anacardium occidentale* on acetic acid-induced abdominal writhing in mice

Treatment (*p.o.*)	**Dose (mg/kg, *p.o.*)**	Number of writhing (30 min)	**Inhibition (%)**
vehicle	-	43.7 ± 3.4	-
AE	100	35.4 ± 3.3	18.9
AE	300	28.0 ± 4.5**	35.9
AE	1000	16.2 ± 4.3***	62.9
indomethacin	10	15.3 ± 3.4****	65.0

Values represent the mean ± S.E.M. of 6 mice. Significantly different from control group: $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

To assess the antiedematogenic effect of the AE, the croton oil-induced mice ear oedema method was used. Figure 2 shows that the difference of the right and left ear weight (**Δ**) obtained in the control group was 7.4 ± 1.2 mg, and that AE (1 g/kg) or indomethacin oral pre-treatment inhibited the edematogenic response after topical application of croton oil by 56.8 (**Δ** = 3.1 ± 0.9 mg) and 57.6% (**Δ** = 3.1 ± 0.8 mg), respectively.

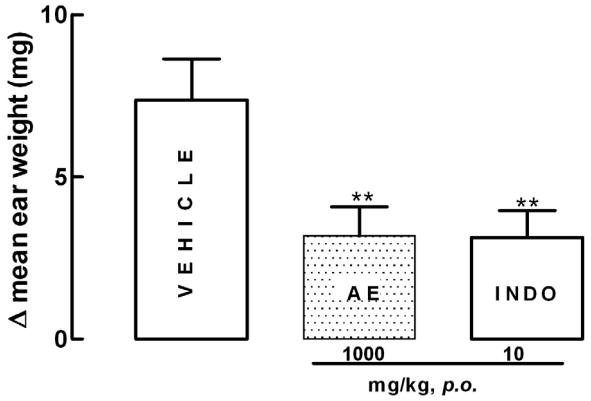

FIGURE 2 - Oral administration effect of vehicle (water), acetone extract (AE - 1 g/kg) from *Anacardium occidentale* and the positive control indomethacin (INDO - 10 mg/kg) 240 minutes after croton oil-induced ear oedema in mice. (Δ) represents differences between right ear (croton oil) and left ear (acetone) weights. The columns and vertical bars represent the mean ± S.E.M. of 8 mice. Significantly different from control group: $^{**}p < 0.01$.

To evaluate the influence of the oral administration of the AE on leukocyte migration, we assessed its performance in the inflammatory model of carrageenan-induced peritonitis. As can be seen in Table II, compared to the group administered only with the vehicle (12.1 ± 1.0 leukocytes x 10^6/mL), the AE administered group (0.1, 0.3 and 1 g/kg) exhibited a dose-related reduction of leukocyte migration by 24.8, 40.5 and 49.6%, respectively. The positive control, dexamethasone (2 mg/kg, *s.c.*), was also effective, inhibiting leukocyte migration by 66.9%.

DISCUSSION

The antinociceptive effect of the AE was tested in two models of analgesia: the acetic acid-induced abdominal writhing and the hot-plate test. The constrictions induced by acetic acid in mice result from an acute inflammatory reaction related to the increase in the peritoneal fluid levels of PGE_2 and $PGF_{2\alpha}$ (Vinegar *et al.*, 1976; Deraedt *et al.*, 1976, 1980). Treatment with AE promoted a dose-related antinociceptive effect in the acetic acid-induced abdominal writhing model. Although this model could be commonly used as a screening method to identify drugs with antinociceptive activity potential, several groups of drugs with different mechanisms of action can inhibit abdominal writhing (Koster *et al.*, 1959; Hendershot, Forsaith, 1959). Moreover, the involvement of different mediators like prostaglandins (Deraedt *et al.*, 1980), neurokinin$_A$ (Julia, Bueno, 1997) and CGRP (Friese *et al.*, 1997), for example, has been described in acid-induced abdominal writhing, which means that it is not possible to suggest any mechanism of the antinociceptive effect of the AE based only on this model. The fact that the AE was able to inhibit constrictions showed that this extract has a peripheral antinociceptive effect, interfering with the acute phase of the inflammatory process. This hypothesis was reinforced when the present results showed that the AE has no central action in the hot-plate test, commonly used to assess narcotic analgesics or other centrally acting drugs (Beirith *et al.*, 1998). Also, antinociception is not related to nonspecific central effects, since no detectable effect was observed in the rota-rod test (results not shown). Later, we tested this anti-inflammatory hypothesis in two other models that evaluate different aspects of the inflammatory process: edema formation and leukocyte migration. The AE significantly reduced croton oil-induced ear edema by 56.8%, and also reduced the total leukocyte migration to the peritoneum after carrageenan stimulation in a dose-related manner with a highest inhibition value of 49.6%.

The production of exudates in these models is related

TABLE II - Effect of AE from *Anacardium occidentale* on carrageenan-induced peritonitis in mice

Treatment	Dose (mg/kg)	Number of leukocytes (x 10^6 / mL)	Inhibition (%)
Vehicle (*p.o.*)	-	12.1 ± 1.0	-
AE (*p.o.*)	100	9.1 ± 1.3	24.8
AE (*p.o.*)	300	7.2 ± 0.3**	40.5
AE (*p.o.*)	1000	6.1 ± 0.2****	49.6
Dexamethasone (*s.c.*)	2	4.0 ± 0.3****	66.9

Values represent the mean ± S.E.M. of 10 mice. Significantly different from control group: $^{**}p < 0.005$, $^{****}p < 0.0001$.

to a local release of vasoactive substances (histamine and kinins) and the synthesis of prostaglandins. The migration of leukocytes would not be directly related to cyclooxygenase products, but the process could be inhibited by some non-steroidal anti-inflammatory compounds, indicating that many mechanisms could be implicated in its control (Higgs *et al.*, 1980; Mikami, Miyasaka, 1983; Brooks, Day, 1991). The *A. occidentale* stem bark acetone extract produced potent antinociceptive activity against the acetic acid-induced pain response without interfering with the hot-plate response, and also inhibited croton oil-induced ear edema formation and reduced leukocyte migration in the peritoneum after carrageenan stimulation. Our main hypothesis to explain these results is that compounds present in the AE can inhibit the cyclo-oxygenase and/or the lipoxygenase pathways of arachidonate metabolism, blockading the leukotriene mechanism, which is important to chemotaxis, and/or also blockading the prostaglandin mechanism, which is important to the genesis of the edema and pain (Morrow and Roberts II, 2001).

In conclusion, we herein report that the acetone extract from *Anacardium occidentale* produced antinociceptive, antiedematogenic and chemotaxis inhibitory activities, reinforcing the therapeutic potential of this traditional medicinal herb against pain and inflammatory diseases. A bioassay-guided fractioning of this extract is now in progress to identify the bioactive substance(s).

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