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Artigo

Antinociceptive effect of the ethanolic extract of *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, in rodents

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RESUMO: : "Efeito antinociceptivo do extrato etanólico de *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, em roedores". O extrato etanólico da entrecasca de *A. cearensis* (EEA) foi avaliado em modelos experimentais de nocicepção. No teste das contorções abdominais induzidas pelo ácido acético o EEA (200 e 400 mg/kg, *v.o.*) foi significativamente efetivo em inibir o estímulo álgico (33,4% e 40,7%), respectivamente, em relação aos animais do grupo controle. O EEA, em todas as doses, promoveu uma redução significativa do tempo de lambidas das patas na segunda fase do teste da formalina (77,5%; 79,7 e 91,3%). Os resultados sugerem uma ação antinociceptiva do EEA.

Unitermos: *Amburana cearensis,* Fabaceae, atividade antinociceptiva, plantas medicinais.

ABSTRACT: The ethanolic extract of the trunk bark of *Amburana cearensis* (EEA) was examined for its oral (*p.o.*) analgesic activity at the doses of 100, 200 and 400 mg/kg body weight. In the acetic acid-induced writhing test, the EEA (200 and 400 mg/kg, *p.o.*) reduced the number of writhing by 33.4% and 40.7%, respectively. Additionally, EEA (100, 200 and 400 mg/kg, *p.o.*) decreased by 77.5%, 79.7 and 91.3%, respectively, the paw liking time in the second phase of the formalin test. Therefore, EEA showed a dose-dependent analgesic effect in formalin test and was effective in reducing writhing in mice.

Keywords: *Amburana cearensis,* Fabaceae, antinociceptive activity, medicinal plant.

INTRODUCTION

Pain is a sensorial modality which in many cases represents the only symptom for the diagnosis of several diseases. It often has a protective function. Throughout history man has used many different forms of therapy for the relief of pain, among them, medicinal herbs are highlighted due to their wide popular use. An example is *Papaver somniferum* from which morphine was isolated (Almeida et al., 2001). In the other hand, inflammatory diseases (include inflammatory pain) including different types of rheumatic diseases are very common throughout the world (Srinivasan et al., 2001). Therefore, the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from folk medicine, especially to natural products (Falcão et al., 2005; Barbosa-Filho et al., Rocha et al., 2008; 2006; Jesus et al., 2009).

Amburana cearensis (Allemão) A.C. Sm. (Fabaceae) is a tree common to the Brazilian Northeastern "caatinga" (savannah) and trunk bark or seeds are extensively used in folk medicine to treatment of respiratory disease, including asthma (Braga, 1976; Agra et al., 2007; 2008). From the trunk bark of *A. cearensis* several compounds were isolated (Bravo et al., 1999), including protocatechuic acid, coumarin, flavonoids (isokaempferide, kaempferol, afrormosin and 4-(methoxyfisetin) and the phenol glucosides, amburosides A and B. Many pharmacological activities have been ascribed to plants rich in coumarins (as *A. cearensis*) as antiinflammatory (Paya et al., 1992) and antinociceptive (Leal et al., 2000).

This study has initiated since to best of our knowledge there has been no report on antinociceptive and anti-inflammatory activity effect of the ethanolic extract of the trunk bark of *A. cearensis*.

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MATERIAL AND METHODS

Plant material and extract preparation

Trunk bark of *A. cearensis* were collected from Petrolina-PE, Brazil. A voucher specimen (5545) is deposited at the Herbário do Vale do São Francisco (HVASF) of the Universidde Federal do Vale do São Francisco. The trunk bark were dried in an oven at 40 oC and pulverized an extracted at room temperature with 95% ethanol in water for 72 h. The extract was dried at 60 oC using rotavapor and the yield was approximately of 20% obtaining the ethanolic extract of the trunk bark of *A. cearensis* (EEA). The lyophilized extract was suspended in distilled water with one drop of tween 80 0.2% for experiments.

Animals

Male Swiss mice (25-30 g) and male Wistar rats, with 2-3 months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 22±1 °C on a 12 h light/dark cycle (lights on 06h00-18h00) with free access to food (Purina) and water. They were used in groups of ten animals each. Experimental protocols and procedures were approved by the Federal University of Vale do São Francisco Animal Care and Use Committee (CEPA/UNIVASF nº 05/2006).

Behavioural screening

The behavioral screening of the mice was performed following parameters described by Almeida et al. (1999) and animals were observed at 0.5, 1, 1.5 and 2 h after administration of AEE (100, 200, 400 and 800 mg/kg, *p.o.*).

Analgesic activity

Acetic acid-induced abdominal writhing

This was carried out according to the method described previously (Koster et al., 1959). The AEE (100, 200 and 400 mg/kg, *p.o.*) or distilled water with one drop of tween 80 0.2% (vehicle) were administered to mice before intraperitoneal (*i.p.*) injection of acetic acid (0.85% v/v in normal saline, 10 mL/kg). Acetylsalicylic acid (ASA) (200 mg/kg, *s.c.*) was used as the reference drug. The number of writhes was counted for 15 min.

Formalin test

The method used was similar to that described previously (Shibata et al., 1989; Vianna et al., 1998). Twenty microlitres of 1% formalin was injected subcutaneously into the right hind paw of 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 5 min after formalin injection (first phase) and 15-30 min after formalin injection (second phase). AEE (100, 200 and 400 mg/kg, *p.o.*) and ASA (200 mg/kg, *s.c.*) were administered 30 min before formalin injection. Control animals received the same volume of distilled water orally.

Tail flick test

The tail flick was evoked by a source of radiant heat, which was focused on the dorsal surface of the tail. Rats were examined for latency (s) to withdraw their tails from a noxious thermal stimulus using a tail flick meter (Ugo Basile, Model 7360) (n-8 for each group). Measurement of threshold was made 30 min after administration of AEE (100, 200 and 400 mg/kg, *p.o.*) or morphine (MOR) (3 mg/kg, *i.p.*). Control mice received distilled water with one drop of the tween 80 0.2%. To avoid tissue damage following treatment with plant extract trials were terminated if the animals did not respond within 30 s.

Statistical analysis

All data were expressed as mean±S.E.M. and the statistical significance was determined using an analysis of variance followed by Dunnett's test. Values were considered significantly different at $p < 0.05$. The percent of inhibition by an antinociceptive agent was determined for each experimental group using the following formula (Reanmongkol et al., 1994):

inhibition % = 100 . (control-experiment)/control

RESULTS

Behavioural screening

EEA at doses of 400 or 800 mg/kg (*p.o.*) showed behavioral alterations in animals after 1 and 1.5 h after treatment: decrease of the spontaneous activity, palpebral ptosis and sedation.

Acetic acid-induced abdominal writhing

As shown Figure 1, EEA was effective in significant reducing ($p < 0.05$, Dunnett´test) abdominal writhing at doses of 200 and 400 mg/kg (*p.o.*) by 33.4% and 40.7%, respectively, when compared to control group (vehicle).

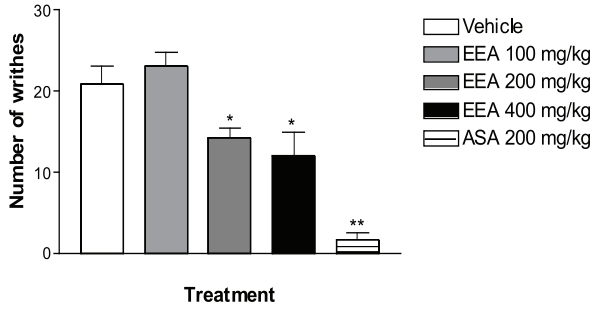

Figure 1. Effect of ethanolic extract of the trunk bark of *Amburana cearensis* (EEA) on acetic acid induced writhing test. Values are mean±S.E.M. *$p < 0.05$, **$p < 0.01$, significantly different from control; ANOVA followed Dunnet's test ($n = 8$, per group).

Formalin test

The oral administration of the EEA, all doses, was infective in alters first phase of formalin test. Moreover, EEA in doses 100, 200 and 400 mg/kg, (*p.o.*) exhibited greater effects on the second phase of the nociceptive response. Therefore, significant decreased ($p < 0.01$) by 77.5%, 79.7 and 91.3%, respectively. ASA (200 mg/kg, p.o.) significant reduced the licking time in both phases (Figure 2 and 3).

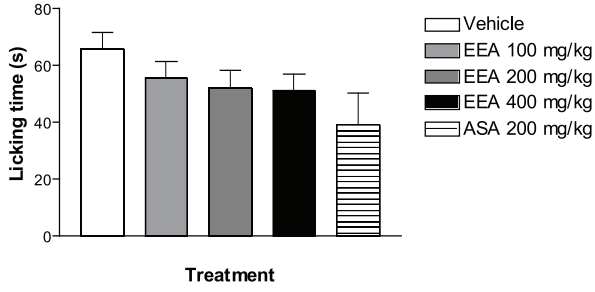

Figure 2. Effect of ethanolic extract of the trunk bark of *A. cearensis* (EEA) on formalin test (first phase). Values are mean±S.E.M.; ANOVA followed Dunnet's test ($n = 8$, per group).

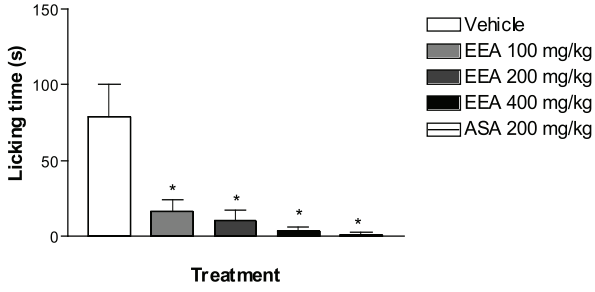

Figure 3. Effect of ethanolic extract of the trunk bark of *A. cearensis* (EEA) on formalin test (second phase). Values are mean±S.E.M. *p <0.01; **$p < 0.001$ significantly different from control; ANOVA followed Dunnet's test ($n = 8$, per group).

Tail flick test

Results for the EEA (100, 200 and 400 mg/kg, *p.o.*) are presented in Figure 4 (reaction time in s, mean+S.E.M.). However, the extract showed no significant results. Morphine sulphate at 3 mg/kg (*i.p.*) manifested its maximum protective effect of 96.6% ($p < 0.01$).

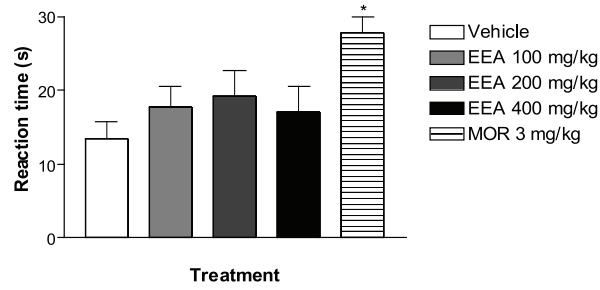

Figure 4. Effect of ethanolic extract of the trunk bark of *Amburana cearensis* (EEA) on tail flick test. Values are mean±S.E.M.; *p <0.05, significantly different from control; ANOVA followed Dunnet's test ($n = 8$, per group).

DISCUSSION

In the current investigation, we have clearly demonstrated that orally administration of ethanolic extract of the trunk bark of *Amburana cearensis* (EEA) dose dependently produces an antinociceptive effect in the mouse by using formalin paw test (second phase) and reduced the number of writhing in higher doses.

The mice treated with EEA (400 or 800 mg/kg, *p.o.*) presented behavioural alterations as reduction of the ambulation decrease, palpebral ptosis and sedation. These signals show possible evidence that the effects on CNS are similar to drugs that reduce the CNS activity (Morais et al., 2004; De Sousa et al., 2007).

Both tests are proven to be effective in evaluating the antinociceptive activity (Tjolsen et al., 1992). EEA inhibited acetic acid-induced writhing in mice hence it can be suggested that the analgesic effect of the extract is also peripherally mediated (Okpo et al., 2001). In acetic acid-induced abdominal writhing, pain is elicited by the injection of an irritant such as acetic acid into the peritoneal cavity which produces episodes of characteristic stretching (writhing) movements and inhibition of number of episodes by analgesics is easily quantifiable. Furthermore, these results support the hypothesis of EEA participation in the inhibition of prostaglandin synthesis since the nociceptive mechanism of abdominal writhing induced by acetic acid involves the process or release of arachidonic acid metabolites via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte et al., 1988; Melo et al. 2008). In this regard, in formalin test peripheral inflammatory processes are involved in the second phase (Tjolsen et al., 1992). We figured out that 100, 200 and 400 mg/kg (*p.o.*) was able to

inhibit the inflammatory processes dose dependently (second phase).

The tail-flick response is believed to be a spinally mediated reflex (Chapman et al., 1985). Morever, Grumbach (1966) has shown that the effectiveness of analgesic agents in tail-flick pain model is highly correlated with relief of human pain. However, EEA was ineffective in producing antinociception behavior in the tail-flick test.

Antinociceptive, anti-inflammatory, bronchodilator, and antimalarial activities of hidroalcoholic extract of stem bark of the *A. cearensis* have been reported (Leal et al., 2000; Mariath et al., 2009). Additionally, others studies carried out with the stem bark from *A. cearensis* (*T. cearensis*) demonstrated the presence of several compounds, including coumarins and flavonoids (Bastos, 1983; Matos, 1994). According Leal et al. (2000) antinociceptive and antiinflamatory properties of *A. cearensis* was attributed by intraperitoneal route to presence of coumarins and flavonoids. Therefore, flavonoids may interact directly with the prostaglandin system (Recio et al., 1995).

It is concluded that ethanolic extract of the trunk bark of *Amburana cearensis* (EEA) possesses oral analgesic effect probably to interact of arachidonic acid metabolites via cyclooxygenase (COX), and prostaglandin biosynthesis. The extract will, therefore, be of potential benefit in the management of inflammatory pain disorders. Further studies currently in progress will enable as to understand the mechanisms of action underlying the effects observed in this investigation.

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