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Article

Therapeutic benefits of *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, in experimental models of pain and inflammation

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RESUMO: "Benefícios terapêuticos da *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, em modelos experimentais de dor e inflamação". A *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, é utilizada na medicina popular em processos dolorosos e inflamatórios. Para avaliar as atividades analgésica e anti-inflamatória desta planta, estudos foram realizados com o extrato etanólico (EE) da entrecasca (100-400 mg/kg). O tratamento oral único dos animais com o EE inibiu (200 e 400 mg/kg, p<0,01) o efeito do ácido acético e reduziu (100, 200 e 400 mg/kg, p<0,001) o efeito da formalina na segunda fase; entretanto, não apresentou efeito no teste da placa quente. A formação de edema e a migração de leucócitos para a cavidade peritoneal induzidas pela carragenina foram reduzidas pelo tratamento com o EE (100, 200 e 400 mg/kg, p<0,001). Desta forma conclui-se que o EE da *Sideroxylon obtusifolium* apresenta atividades antinociceptiva e anti-inflamatória, suportando seu uso popular no tratamento da dor e de doenças inflamatórias.

Unitermos: anti-inflamatória, antinociceptiva, *Sideroxylon obtusifolium*, Sapotaceae.

ABSTRACT: *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, is a plant with analgesic and anti-inflammatory activities used in folk medicine. In order to evaluate the actions of this plant, studies were performed on antinociceptive and anti-inflammatory activities with the ethanol extract (EE) of inner bark (100-400 mg/kg). Oral treatment with the EE elicited inhibitory activity (200 and 400 mg/kg, p<0.01) on acetic acid effect and reduced (100, 200, and 400 mg/kg, p<0.001) the formalin effect at the second-phase, however it did not elicit any inhibitory effect on hot-plate test. Carrageenan-induced oedema formation and leukocyte migration into the peritoneal cavity were reduced with the EE at 100, 200, and 400 mg/kg (p<0.001). In conclusion, the EE of *Sideroxylon obtusifolium* shows antinociceptive and anti-inflammatory activities, supporting the folkloric usage of the plant to treat pain and various inflammatory diseases.

Keywords: anti-inflammatory, antinociceptive, *Sideroxylon obtusifolium*, Sapotaceae.

INTRODUCTION

Inflammation is a major threat to human health and plays an important role in the development of various infections and non-infections diseases. Natural compounds or compounds derived from natural leads can be used for the treatment of inflammation and pain.

The plant *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, known as "quixabeira", "quixaba" or "rompe-gibão", is a medicinal plant species from the *caatinga* widely known with a distribution pattern, and used in folk medicine and for commercial manufacturing of phytotherapeutic products (Albuquerque et al., 2007). *S. obtusifolium* has been used in commercial manufacturing processes in Brazil and it is considered one of native species more important

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according to their use-values of the local therapeutic indications (Albuquerque & Oliveira, 2007).

Sideroxylon obtusifolium is threatened with extinction mainly by the extraction to use in popular medicine. The bark is used to blow, pains in general, duodenal ulcer, gastritis, heartburn, chronic inflammation, genital injury, ovarian inflammation, adnexitis, colic, renal problems, cardiac problems, diabetes, healing and as expectorant (Filipoy, 1994; Albuquerque et al., 2007). The entire plant is also used against ovarian inflammations and diabetes (Beltrão et al., 2008).

In this study, we evaluate the antinociceptive and anti-inflammatory effects of the ethanol extract (EE) from *Sideroxylon obtusifolium* inner bark.

MATERIALS AND METHODS

Plant material and extraction of *Sideroxylon obtusifolium* inner bark

Inner bark of *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, was collected in the municipality of Canindé de São Francisco, Sergipe State, Brazil, in March 2009 (09º66'00"S, 37º78'94"W). The plant was authenticated by Professor Ana Paula Prata, Department of Biology, Federal University of Sergipe, and a voucher specimen deposited in the Federal University of Sergipe Herbarium, number ASE 13.163). The dried inner bark at 37 ºC in a forced air oven for seven days was powdered (2,800 g), extracted by maceration at room temperature with 90% ethanol for five days. The extract was filtered in vacuum and the solvent was removed using a rotary evaporator (50 °C). The percentage of yield of the EE was 16.4% (459.6 g).

Phytochemical screening

The methods of Harborne (1984) were used to screen the EE of *Sideroxylon obtusifolium* inner bark used in this study for its chemical constituents.

Chemicals and drugs

The following chemicals and drugs used were: acetylsalicylic acid (ASA), carrageenan, dexamethasone, and morphine hydrochloride from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid from Merck (Damstadt, Germany). Formalin from Baker (Santo Amaro-SP, Brazil). Solvents from Vetec (Rio de Janeiro-RJ, Brazil). All substances used were dissolved in 0.2% Tween 80 in 0.9% NaCl solution.

Animals

Wistar rats (120-180 g) and Swiss mice (20-30 g) of both sexes, young adults, were obtained from the Central Biotery of the Federal University of Sergipe (São Cristóvão, Brazil). Animals were maintained in plastic boxes at controlled room temperature (21±2 °C) with free access to food (Purina®) and water, under a 12:12 h light/dark cycle. All the experimental procedures were carried out during the light period of the day (8:00 a.m. to 5:00 p.m.) and complied with the guidelines on animal care of the Federal University of Sergipe Ethics Committee for Animal Use in Research (CEPA/UFS 47/07).

Acetic acid-induced abdominal writhes

Abdominal writhes were induced by intraperitoneal (*i.p.*) injection in mice of acetic acid (0.6% solution, 0.1 mL/10 g), the nociceptive agent (Koster et al., 1959). The animals were pre-treated with *Sideroxylon obtusifolium* inner bark EE (100, 200, or 400 mg/kg) orally (*p.o.*) 60 min before initiating algesic stimulation, or with acetylsalicylic acid (ASA, 300 mg/kg, *p.o.*, 60 min beforehand), used as positive control (n=6/group). The abdominal writhes were observed, in separate individual chambers, for a period of 20 min, starting after administration of acetic acid.

Hot-plate test

Mice were pre-treated with *Sideroxylon obtusifolium* inner bark EE (100, 200, or 400 mg/kg, *p.o.*, 60 min beforehand), or with morphine (10 mg/kg, *i.p.*, 30 min beforehand), and after they were placed individually on a metallic plate warmed to 55±0.5 °C (n=6/group). The time elapsed until the appearance of reactions (latency, in s) to the thermal stimulus, such as lifting or licking of the paws was recorded as an index of nociception (Woolfe & Macdonald, 1944). Measurements were performed at time 0, 30, 60, 90, and 120 min after the first thermal stimulus. In order to avoid damage to the animal's paws the maximal time standing on the plate was limited to 30 s.

Formalin test

The formalin test was applied according to the method of Dubuisson & Dennis (1977), modified by Hunskaar & Hole (1987). Mice were pre-treated with *Sideroxylium* inner bark EE (100, 200, or 400 mg/kg, *p.o.*, 60 min beforehand), vehicle (0.1 mL/10 g, *p.o.*, 60 min beforehand), morphine (10 mg/kg, *i.p.*, 30 min beforehand), or ASA (300 mg/kg, *p.o.*, 60 min beforehand), before intraplantar injection of 1% formalin solution (20 µL) into the right hindpaw of the animal (n=6/group). The time that the animal spent licking or biting its paw was measured during the first-phase (0-5 min) and the second-phase (20-25 min) of the test.

Measurement of paw oedema in rats

The anti-inflammatory activity was studied using the paw oedema model induced by 1% carrageenan, administrated at volume of 0.1 mL/animal into the subplantar region of the right hindpaw of the rat (Winter et al., 1962). The volume of the paw was measured by the removal of the water column using a hydroplethysmometer (model 7150, Ugo Basile, Varese, Italy), at the time 0 and the intervals of 1, 2, 3, and 4 h immediately after the subplantar injection of carrageenan.

The EE of *S. obtusifolium* (100, 200, or 400 mg/kg), ASA (300 mg/kg) or vehicle were administrated *p.o.* 1 h before the oedematogenic agent (n=6/group). The data obtained for the various groups were reported as means±s.e.m. and expressed in mL. The percentage inhibition on oedema experiment was calculated based in the area under the curves (AUC) after 4 h.

Leukocyte migration into the peritoneal cavity

The leukocyte migration was induced by injection of carrageenan (500 µg/cavity, *i.p.*, 500 µL) into the peritoneal cavity of rats 1 h after administration of the EE (100, 200, or 400 mg/kg, *p.o.*, n=6) or dexamethasone (2 mg/kg, *s.c.*, n=6) by modification of the technique previously described by Bastos et al. (2007). The animals were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and were euthanized by cervical dislocation 4 h after carrageenan injection. Shortly after, saline containing ethylenediaminetetraacetic acid (EDTA, 1 mM, *i.p.*, 10 mL) was injected. The total cells were counted in a Neubauer chamber, under optic microscopy. The results were expressed as the number of leukocytes/mL. The percentage of the leukocyte inhibition = (1 – *T/C*) x 100, where *T* represents the treated groups leukocyte counts and *C* represents the control group leukocyte counts.

Statistical analysis

The results are presented as the mean±s.e.m. of n animals per group. Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey's test. GraphPad Prism® Software, version 5.00, was used for all statistical analyses. *P* values less than 0.05 were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening showed that the EE of *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, inner bark contain flavonols, flavanones, flavononols, phenols, saponins, steroids, tannins, triterpenes, and xanthones.

Acetic acid-induced writhing in mice

The writhes evoked by *i.p.* injection of 0.6% acetic acid (28.8±2.7 writhes) were markedly reduced by the pre-treatment with *Sideroxylon obtusifolium* EE given *p.o.* (100-400 mg/kg, n=6/group) 1 h beforehand in 49.7% ($p<0.01$) and 61.3% ($p<0.001$) at 200 and 400 mg/kg, respectively (Figure 1). ASA (300 mg/kg, n=6) exhibited significant inhibition (79.2%, $p<0.001$) of the control writhes in the acetic acid-induced writhing.

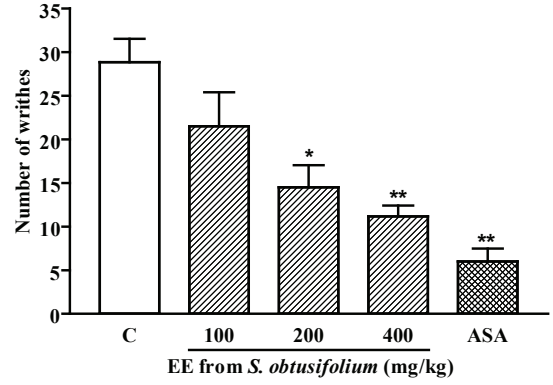

Figure 1. Effect of *S. obtusifolium* EE on acetic acid-induced abdominal writhing. Groups of rats (n=6/group) were pre-treated with vehicle (C), acetylsalicylic acid (ASA, 300 mg/kg) or EE (100-400 mg/kg), *p.o.*, 60 min before irritant agent. Each column represents the mean±s.e.m. *$p<0.01$ and **$p<0.001$, in relation to control group. ANOVA followed by Tukey's test.

Hot-plate reaction time in mice

The treatment of animals with morphine (10 mg/kg, *i.p.*, 30 min beforehand, n=6) caused a marked increase in latency ($p<0.001$) at all analyzed periods according to assessment in the hot-plate test. The EE of *Sideroxylon obtusifolium* inner bark (100, 200, or 400 mg/kg, n=6/group) did not significantly influence the reaction time of mice to the hot-plate (data not shown).

Formalin reaction time in mice

As shown in Figure 2, the intraplantar injection of 1% formalin solution (20 µL) produced nociception on both the first- and second-phases (48.0±3.9 and 37.3±3.4 s, respectively, n=6). *Sideroxylon obtusifolium* EE (100-400 mg/kg, 1 h beforehand, n=6/group) produced marked inhibition (81.7, 92.7, and 95.8% at 100, 200, and 400 mg/kg, respectively, $p<0.001$) on intraplantar injection of formalin in mice only against the inflammatory pain

(second-phase) (Figure 2). Similarly, ASA (300 mg/kg, *p.o.*, 60 min beforehand) caused inhibition of 96.7% on second-phase of formalin-induced nociception ($p<0.001$, n=6, Figure 2). Morphine (10 mg/kg, *i.p.*, 30 min beforehand) caused significant inhibition of 60.9 and 100.0% on the first- and second-phases of formalin-induced nociception, respectively ($p<0.001$, n=6, Figure 2).

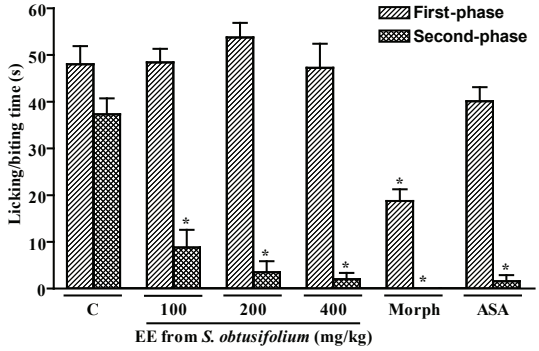

Figure 2. Effect of *S. obtusifolim* EE on formalin-induced nociception. Groups of mice (n=6/group) were pre-treated with vehicle (C, *p.o.*, 60 min beforehand), acetylsalicylic acid (ASA, 300 mg/kg, *p.o.*, 60 min beforehand), morphine (Morph, 10 mg/kg, *i.p.*, 30 min beforehand), or EE (100-400 mg/kg, *p.o.*, 60 min beforehand). Each column represents the mean±s.e.m. *$p<0.001$, in relation to control group. ANOVA followed by Tukey's test.

Carrageenan-induced paw oedema in rats

As observed in Figure 3, the single oral treatment of rats with the EE of *Sideroxylon obtusifolium* inner bark at 100, 200, and 400 mg/kg (1 h beforehand, n=6/group) was capable of reducing ($p<0.05$) the oedema formation induced by carrageenan (1%, 100 µL/paw), an effect observed at 2, 3, and 4 h after the administration of this phlogistic agent. Likewise, ASA (300 mg/kg, *p.o.*, 1 h beforehand, n=6) significantly inhibited ($p<0.001$) the oedematogenic response evoked by carrageenan in rats, at 1, 2, 3, and 4 h (Figure 3).

In the assay with the EE of inner bark the mean AUC found in carrageenan-treated rats was 2.01±0.06 mL x h (n=6). Based on AUC values, the EE at 100, 200, and 400 mg/kg caused 26.4, 28.4, and 28.9% ($p<0.001$) of inhibition on the oedema response, respectively (n=6/group). ASA at 300 mg/kg (n=6) caused an inhibition of 40.8% ($p<0.001$) in the assay with the EE.

Carrageenan-induced peritonitis in rats

The carrageenan injection (500 µg) in control animals induced leukocyte migration into the peritoneal cavity 4 h after stimulus of 13.7±0.9 leukocytes x 10^6/mL. Figure 4 shows the inhibitory effects of the EE of *Sideroxylon obtusifolium* inner bark (43.5, 57.9, and 50.9% at 100, 200, and 400 mg/kg, respectively, $p<0.001$, n=6/group) on carrageenan-induced response. The control drug dexamethasone (2 mg/kg, *s.c.*, 1 h beforehand, n=6) inhibited (59.8%, $p<0.001$) the carrageenan-induced leukocyte migration (Figure 4).

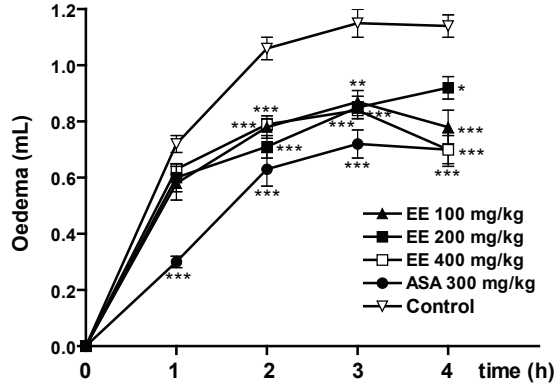

Figure 3. Effect of *S. obtusifolim* EE on paw oedema. Groups of rats (n=6/group, *p.*o.) were pre-treated with vehicle (control), acetylsalicylic acid (ASA, 300 mg/kg), or EE (100-400 mg/kg) 60 min before carrageenan injection (1%, 100 µL). Each value represents the mean±s.e.m. *$p<0.05$, **$p<0.01$, and ***$p<0.001$, in relation to control group. ANOVA followed by Tukey's test.

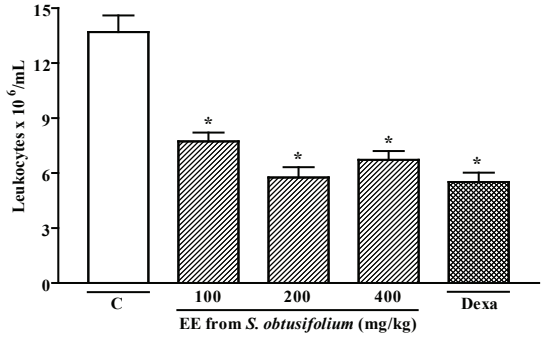

Figure 4. Effect of *S. obtusifolim* EE on leukocyte migration. Groups of rats (n=6/group) were pre-treated with vehicle (C, *p.o.*), dexamethasone (Dexa, 2 mg/kg, *s.c.*), or EE (100-400 mg/kg, *p.o.*) 60 min before carrageenan (500 µg/cavity, *i.p.*)-induced peritonitis. Each value represents the mean±s.e.m. *$p<0.001$, in relation to control group. ANOVA followed by Tukey's test.

DISCUSSION

We demonstrated in this study that *Sideroxylon obtusifolium* (Humb. ex Roem. & Schult.) T.D. Penn., Sapotaceae, EE displays analgesic and anti-inflammatory actions, and provides some evidence on the mechanisms

implicated in these effects.

In relation to nociception, this work shows that *S. obtusifolium* EE *p.o.* produces significant antinociception according to assessment of the abdominal writhes. In the acetic acid-induced abdominal writhing (visceral pain model) the processor releases arachidonic acid via cyclo-oxygenase (COX) and prostaglandins (PGs) biosynthesis plays an important role (Duarte et al., 1988). This method is sensitive to non-steroidal anti-inflammatory drugs (NSAID) and to narcotics and other centrally acting drugs (Reichert et al., 2001).

S. obtusifolium EE at the doses which inhibited the nociception caused by acetic acid, has no effect on the hot-plate test (data not shown). Although the hot-plate test is commonly used to assess narcotic analgesics, other centrally acting drugs, including sedatives and muscle relaxants or psychotomimetics have shown activity in this test (Eddy & Leimbach, 1953).

Our results have also shown that morphine is largely effective in preventing both the first- and second-phases of formalin-induced pain. The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. The formalin test consists of two different phases that are separated in time: the first phase measures direct chemical stimulation of nociceptors, whereas the second phase is dependent on peripheral inflammation and changes in central processing. Some previous studies demonstrated that substance P and bradykinin participate in the first phase, whereas histamine, serotonin, PGs, nitric oxide (NO) and bradykinin were involved in the second phase of the formalin test (Hunskaar & Hole, 1987; Tjolsen et al., 1992). Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the second phase only (Shibata et al., 1989). The second phase is an inflammatory response with inflammatory pain that can be inhibited by NSAIDs (Hunskaar & Hole, 1987). The present study has shown that *S. obtusifolium* EE *p.o.* produce inhibition on the second-phase (inflammatory nociception) of the formalin test. These data suggests that the EE can produce antinociceptive action through inhibition of COX and prostaglandin synthesis.

With the goal of proving the anti-inflammatory property of *S. obtusifolium*, we evaluated the effects of the EE treatment on the carrageenan-induced paw oedema in rats, which is characterized by a biphasic response with marked oedema formation resulting from the rapid production of several inflammatory mediators such as histamine, serotonin and bradykinin (first-phase), which is subsequently sustained by the release of PGs and NO (second-phase) with peak at 3 h, produced by inducible isoforms of COX (COX-2) and NO synthase (iNOS), respectively (Seibert et al., 1994; Nantel et al., 1999). In the present study, previous oral treatment with the EE of *S. obtusifolium* was effective in reducing the oedematogenic response evoked by carrageenan in rats between the second and the fourth hours after the injection.

The carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, as well as the release of other neutrophil-derived mediators (Dawson et al., 1991). The inflammation induced by carrageenan involves cell migration, plasma exsudation and production of mediators, such as NO, PGE_2, interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α (Salvemini et al., 1996; Loram et al., 2007). These mediators are able to recruit leukocytes, such as neutrophils, in several experimental models. The EE of inner bark inhibited leukocyte migration induced by *i.p.* injection of carrageenan (in peritonitis model).

Previous study has shown that *S. obtusifolium* is effective as free radical scavenger, inhibitor of DNA damage and lipid peroxidation, and suggests that this antioxidant activity may play an important role in the anti-inflammatory activity (Desmarchelier et al., 1999).

The phytochemical study of the EE of *Sideroxylon obtusifolium* inner bark detected the presence of flavonoids, which are reported to be good antioxidants and antinociceptive agents. Furthermore, much attention has been given to the relationship between the antioxidant and anti-inflammatory properties of flavonoids, *in vitro* and *in vivo* (Nijveldt et al., 2001; Meotti et al., 2006; Willain-Filho et al., 2008). Flavonoids have been shown to inhibit COX-2, iNOS, lipooxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADPH oxidase, all involved in reactive oxygen species generation (Odontuya et al., 2005; Soobrattee et al., 2005). Others anti-inflammatory properties of flavonoids are their suggested ability to inhibit neutrophil degranulation and reduce complement activation, thereby decreasing the adhesion of inflammatory cells to the endothelium and in general resulting in a diminished inflammatory response (Nijveldt et al., 2001). It has been demonstrated that tannins are also able to inhibit COX-2 and/or iNOS enzymes, as well as other mediators of the inflammatory process (Carvalho, 2004).

In conclusion, the results of the present study showed for the first time that the EE of *Sideroxylon obtusifolium* exhibit antinociceptive and anti-inflammatory actions. The present findings support the folkloric use of *Sideroxylon obtusifolium.* However, further studies are necessary to clarify the precise mechanism of action of this plant.

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