Anti-Inflammatory and Antinociceptive Effects of Sterculia striata A. St.-Hil. & Naudin (Malvaceae) in Rodents

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ABSTRACT The present work reports the anti-inflammatory and antinociceptive activities of the ethanol extract obtained from the stem bark of Sterculia striata A. St.-Hil. & Naudin (Ss-EtOH) in the experimental models of edema induced by carrageenan, dextran, or histamin and nociception induced by chemical stimuli, such as acetic acid, formalin, capsaicin, or glutamate. The Ss-EtOH (50 mg/kg) promoted a marked inhibition on the hind paw edema induced by carrageenan or dextran (30% and 73%, respectively). Besides, Ss-EtOH (25 mg/kg) exhibited a slight activity (30%) on the hind paw edema induced by histamin. The Ss-EtOH (12.5 and 25 mg/kg) showed the antinociceptive activity on chemical stimuli induced by acetic acid (65.59% and 38.37%, respectively), formalin, in the initial (35.08% and 31.5%, respectively) and late phases (44.09% and 83.57%, respectively), capsaicin (43.77% and 51.31%, respectively), and glutamate (36.6% and 52.12%, respectively). Regarding the possible mechanism involved in the antinociceptive effect, Ss-EtOH (12.5 mg/kg) showed a decrease in the antinociceptive effect (65.8%) in the acetic acid model after pretreatment with naloxone. Thus, opioid mechanisms might be underlying this response.

KEY WORDS: anti-inflammatory • antinociceptive • carrageenan • opioid • paw edema • Sterculia striata

INTRODUCTION

IN FOLK MEDICINE, plants have played an important role in human life since ancient times, not only as a food source but also in the treatment of various diseases. Hence, several herbs have been used as a form of therapy for pain relief along the history. The study of plants used in traditional medicine as anti-inflammatory or analgesic has represented an important research strategy in the development for new analgesics and anti-inflammatory drugs.1

Some species of Northeastern Brazil have demonstrated their anti-inflammatory activity in animal models, such as the essential oil of Lippia sidoides Cham. (Verbenaceae), known as “alecrim pimenta,”2 and antioxidant and orofacial antinociceptive properties of aqueous extract of leaves of Hyptis pectinata L. Poit (Lamiaceae), popularly known as “sambacaita” and found in the states of Sergipe and Alagoas (Brazil).3

Species from the Malvaceae family have been investigated due to their providing of several secondary metabolites, such as triterpenes,4 flavonoids,5 alkaloids,6 and coumarins.7 Some species of the genus Sterculia have several constituents and present several biological activities, as Sterculia foetida L. whose anti-inflammatory and central nervous system depressant activities have been reported.8 Beyond the pharmacological activities, there is also pharmaceutical interest in the biotechnological application of natural gums derived from the family Malvaceae exudates, such as gum karaya (Sterculia urens)9–11 and chicha gum (Sterculia striata),12,13 in stabilizing or improving the dissolution of formulas and the release of active ingredients.

S. striata A. St.-Hil. & Naudin is popularly known as “chíchá,” “Pau-rei,” “Mendubi-guaçu,” “Arachachá,” “Chechá-do-norte,” and “Castanheiro-do-mato.” It is a large and widespread tree spread from the Amazon Region toward Piauí, Mato Grosso, Minas Gerais, and Rio Grande do Sul, whose seeds are consumed by humans.14 In folk medicine, the leaves have been used topically with hot butter or olive oil for the treatment of boils.15

Preliminary phytochemical investigations of the oil obtained from seeds of S. striata detected the presence of
cyclopropenoid fatty acids, and the ethanol extract obtained from stem barks of *S. striata* (Ss-EtOH) provided a mixture of sitosterol and stigmasterol (0.8%), sitosterol-3-O-β-D-glucopyranoside (0.24%), and four pentacyclic triterpenoids: lupeol (5.2%), 3-b-O-acetyl-lupeol (0.04%), lupenone (0.32%), and betulinic acid (0.09%). Lupeol is the major chemical constituent obtained. In the literature, there are reports of anti-inflammatory and gastroprotective activities for some of these chemical constituents.

A previous study has demonstrated the Ss-EtOH-induced gastroprotective activity in different models of gastric mucosal injury. In this context, several studies concerning plants-derived products and their anti-inflammatory and gastroprotective properties have been reported.

Toxicological results demonstrate that the stratum is safe. Oral administration of Ss-EtOH (2000 mg/kg) and intraperitoneal administration (1000 mg/kg) did not result in the death of any animal nor did it cause damage to internal organs.

Thus, the data presented about the Malvaceae family and the reported phytochemical and pharmacological profiles of *S. striata* lead us to investigate the anti-inflammatory and antinociceptive effects of the ethanol extract from stem barks of *S. striata* (Ss-EtOH) in rodents.

**MATERIALS AND METHODS**

**Plant material and extraction**

The species was collected in the city of Teresina (−5° 2′ 45.51″, −42° 47′ 18.42″), state of Piauí, Brazil, determined, and deposited in the Herbarium Graziela Barroso from the Federal University of Piauí (voucher specimen No. TEPB 13,870). Stem barks were dried at room temperature and then powdered (1800 g). The powder was exhaustively extracted in 95% ethanol under Ultrasonic apparatus for 45 min. This procedure was repeated four times. The ethanol solution was concentrated in rotary evaporator at 60°C yielding 66.9 g (yield 3.71%, w/w) of crude ethanol extract (Ss-EtOH).

**Animals**

Male Swiss mice (25−30 g) and Wistar rats (180−220 g) were housed at 24°C±2°C under a 12 h light−12 h dark cycle, and they had free access to standard pellet diet and water. All experiments followed experimental protocols submitted to and approved by the Animal Research Ethics Committee of the Federal University of Piauí (no. 12/2008).

**Drugs and reagents**

Dexamethasone, dextran, cyproheptadine, histamine, capsaicin, l-carrageenan, indomethacin, MK801, naltrexone, and Tween80® were purchased from Sigma-Aldrich (St. Louis, MO, USA), and morphine was obtained from Cristália (São Paulo, SP, Brazil). The Ss-EtOH extract was previously solubilized in 1.0% Tween 80 and then diluted in saline solution (0.9% NaCl). Other drugs were dissolved either in saline solution or in distilled water. Ss-EtOH extract and drug concentrations were adjusted for oral treatment to 1 mL/100 g body weight.

**Anti-inflammatory activity**

Paw edema induced by carrageenan. Male Wistar rats (n = 8) were orally pretreated with vehicle (1 mL/100 g), Ss-EtOH (25, 50, or 100 mg/kg), and indomethacin (5 mg/kg). Paw edema was induced by subplantar injection of 1.0% carrageenan (0.1 mL, i.pl.) into the right hind paw. Their right hind paws were measured with a pachymeter (in millimeters) before (Ti) and every 60 min for 6 h after induction of edema. The difference in the paw edema diameter was calculated for each group and compared with the control group.

Paw edema induced by dextran or histamine. Male Swiss mice (n = 8) were orally pretreated with vehicle (0.05 mL/paw, i.pl.) in the left paw and with dextran or histamine (0.05 mL/paw, i.pl.) in the right paw. After 60 min, the animals were orally treated with vehicle, cyproheptadine (10 mg/kg), or Ss-EtOH (25, 50, or 100 mg/kg). Then, after 1 or 2 h of edema induction, the animals were euthanized by cervical dislocation and their paws were removed for the measurement of edema (in grams).

**Antinociceptive activity**

Writhing induced by acetic acid. In this experiment, male Swiss mice (n = 8) were orally pretreated with vehicle or Ss-EtOH (6.25, 12.5, or 25 mg/kg) 60 min before the intraperitoneal administration of acetic acid (0.75%, i.p.). Then, the total number of writhings was counted during 20 min. The elicited antinociceptive effect was compared with the response induced by morphine (2.5 mg/kg, i.p.) administered 30 min before the acetic acid injection.

Formalin test. Nociception was induced by the injection of formalin (2%, 20 μL, i.pl.) in the right hind paw of male Swiss mice (n = 8). Ss-EtOH (6.25, 12.5, and 25 mg/kg, p.o.), morphine (5 mg/kg, p.o.), and vehicle were orally administered 30 or 60 min before formalin. The time during which the animal licked the paw that received the stimulation for 0−5 min (acute pain) and 15−30 min later (inflammatory pain) was measured and compared between the groups.

Capsaicin test. In this experiment, male Swiss mice (n = 8) were orally treated with vehicle, Ss-EtOH (6.25, 12.5, and 25 mg/kg, p.o.), or morphine (2.5 mg/kg, p.o.). One hour after those treatments, the right hind paw was injected with capsaicin (20 μL, 2 mg/paw), and nociception was observed and measured by paw biting or licking time during 5 min, with further comparison between the groups.

Glutamate test. Glutamate-induced nociceptive response is promoted by an injection of the amino acid glutamate (20 μL, 10 μmol/paw, i.pl.) on the ventral surface of the right hind paw of male Wistar rats (n = 8). Vehicle,
Ss-EtOH (6.25 or 12.5 mg/kg p.o.), or MK801 (0.03 mg/kg, i.p.) were administered. Then, 30 or 60 min later, they received glutamate. The number of times in which the animals licked the stimulated paw was quantified during 5 min.

Investigation of possible mechanism involved in Ss-EtOH-induced antinociceptive effect

To investigate the possible participation of the opioid system on the antinociceptive effect, male Swiss mice (n=8) were subcutaneously pretreated with naloxone (2 mg/kg, s.c.) 15 min before the treatment with Ss-EtOH (12.5 mg/kg, p.o.) or morphine (2.5 mg/kg, i.p.). Then, animals were subjected to the already-described writhing test.34

Data analysis

The results were analyzed by one-way ANOVA followed by Tukey’s, Dunnett’s, or Bonferroni’s test and are expressed as mean ± SEM. The results were considered significant when P<.05. All analyses were performed using GraphPad Prism software, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Anti-inflammatory activity

Paw edema induced by carrageenan. Ss-EtOH at a dose of 50 mg/kg was able to reduce the paw edema induced by carrageenan in rats only at the third and fifth hour when compared with the vehicle group and indomethacin group. This response was more effective at the third hour (30%). The same result was not observed at a dose of 25 mg/kg (Fig. 1).

Paw edema induced by dextran or histamine. The paw edema induced by dextran was decreased by 73% after oral treatment with Ss-EtOH (50 mg/kg). Besides, Ss-EtOH at a dose of 25 mg/kg decreased by 30% the paw edema induced by histamine. All results were compared with the vehicle group. Cyproheptadine (10 mg/kg) significantly inhibited the edema formation (56%) when compared with the vehicle group (Figs. 2 and 3).

Antinociceptive activity

Writhing induced by acetic acid. In the writhing test, Ss-EtOH (12.5 or 25 mg/kg) reduced the number of writhes by 65.59% and 38.37%, respectively, when compared with the vehicle group. Morphine (2.5 mg/kg) markedly reduced the number of writhes (90.55%) when compared with the vehicle group (Fig. 4).
Formalin test. Ss-EtOH (12.5 and 25 mg/kg) significantly decreased (35.08% and 31.5%, respectively) the hind paw licking time during the early phase (Fig. 5A). During the late phase, Ss-EtOH (12.5 and 25 mg/kg) also exhibited a dose-dependent effect (44.09% and 83.57%, respectively) due to a marked decreasing of the hind paw licking time (Fig. 5B).

Capsaicin test. On capsaicin-induced neurogenic nociception, Ss-EtOH at doses of 12.5 and 25 mg/kg promoted a significant inhibition by 43.77% and 51.31%, respectively (Fig. 6).

Glutamate test. In the glutamate test, Ss-EtOH at doses of 6.25 and 12.5 mg/kg significantly inhibited the number of times the animal lifted the paw stimulated with glutamate by 36.6% and 52.12%, respectively (Fig. 7).

Investigation of possible mechanisms involved in Ss-EtOH-induced antinociceptive effect

When used alone, naloxone (2 mg/kg, i.p.), an opioid-receptor antagonist, failed to modify the acetic acid-induced nociceptive responses. However, naloxone was able to antagonize the Ss-EtOH-induced (12.5 mg/kg, p.o.) and morphine-induced (2.5 mg/kg, i.p.) antinociceptive effect by 65.8% and 85.3%, respectively (Fig. 8).

**DISCUSSION**

Recent studies have shown that natural products, such as medicinal plants from Northeastern Brazil, represent an important source for the development of new therapeutic alternatives. Furthermore, novel studies regarding essential oils and herbal extracts with antimicrobial and antinociceptive activities have been increasing.35–37

Several natural products that possess anti-inflammatory and antinociceptive activities have been identified and isolated from plants, whereas some of them are currently synthesized and available for commercial use. Hence, the major goal of this work is to report the effects of Ss-EtOH on experimental models of edema and nociception since data on the bioactivity regarding the *S. striata* species are scarce.

Inflammation is a protective and defense mechanism of the body and occurs in two distinct phases. An acute phase characterized by local vasodilatation, increased capillary permeability, and release of inflammatory mediators such as histamine, serotonin, and prostaglandins. A chronic phase is characterized by infiltration of leukocytes and phagocytic cells.38

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation, and it has been increasingly used to test new anti-inflammatory drugs.39 That model is characterized by the production of a biphasic edema. In the relatively rapid early phase (1–2 h), edema formation is mediated by histamine and serotonin (5-HT), and in the late phase (3–4 h), kinins and prostaglandins contribute to the edema.40 Ss-EtOH inhibited the paw edema between the first and fifth hour and then demonstrated the action on inflammatory mediators released during this period (e.g., histamine, 5-HT, or prostaglandins). Therefore, the positive results of the effect of Ss-EtOH on experimental models of edema induced by dextran or histamine (which were used as complementary tests to the first phase of edema triggered by carrageenan) are consistent with such data.

Histamine promotes a vasodilator response and then an increase in vascular permeability, promoting an edema
formation in minutes. Dextran is a polysaccharide that promoted the release of histamine and serotonin from mast cells during the formation of edema, interacting with their respective receptors (H₁/H₂ and 5-HT₂, respectively) on endothelium layer of microvessels. Therefore, it is reasonable that antiedematogenic or anti-inflammatory effect of Ss-EtOH probably involves the action of Ss-EtOH compounds on the release of histamine and/or inhibition of its binding to its pharmacological receptors as a competitive or noncompetitive antagonism.

The acetic acid-induced writhing reaction in mice is described as a typical model for inflammatory pain. Therefore, it is used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents and, in most cases, as a model to study the peripheral antinociceptive effect of chemical compounds. Such a model of nociception is suggested to represent the stimulation of peripheral mechanism since the administration of phlogogen leads to an increase in the levels of cyclooxygenase (COX) and lipooxygenase, and then, several endogenous nociceptive mediators (e.g., prostanoids of the PGE₂ and PGF₂α types, serotonin, histamine, cytokines, and eicosanoids) are produced and released.

The positive effect of Ss-EtOH in reducing the number of contortions may indicate its ability to inhibit the action of inflammatory mediators released by COX. Accordingly, these data are consistent with those observed in the second phase of paw edema induced by carrageenan. Furthermore, the model demonstrated that the Ss-EtOH induces the antinociceptive activity as well, due to nociception induced by acetic acid, besides involving inflammatory mediators and the direct stimulation of the low pH of nociceptive nerve fibers.

The stimulation of nociceptive nerve endings by C-fibers or A-fibers activation transmits the painful stimuli. Then, signals transmitted through nociceptors (or “pain receptors”) are interpreted as pain in the cognitive centers of the brain. The brain and spinal cord play a major role in central pain mechanisms. The capsaicin test involves the participation of afferent C-fibers, which trigger neuropathic pain when activated. This information suggests, as a response to the positive effects of Ss-EtOH about this experimental model of nociception, the involvement of these fibers and vanilloid receptors (TRPV1), a nonselective ligand-gated ion channel, that lead to membrane depolarization and increased cation influx and then triggering the noxious stimulus.

Another experimental model of nociception that has been widely used to further support the evaluation of the antinociceptive effect induced by several new compounds is the formalin test. Subcutaneous injection of formalin induces a distinct biphasic nociception. The first phase immediately begins after injection and lasts for 5 min. This phase reflects...
a direct effect of formalin on nociceptors (neurogenic pain). The second phase is marked by a return to high levels of nociception beginning 15–20 min after administration of formalin and may continue up to 60 min, characterized as an inflammatory pain.\textsuperscript{51} The Ss-EtOH inhibited both phases of formalin-induced nociception, probably acting on a central level of nociception, a mechanism characteristic of opioid drugs (e.g., morphine), whereas drugs acting peripherally (e.g., nonsteroidal anti-inflammatory drugs) inhibit only the late phase.\textsuperscript{16,50,51} Likewise, the possible participation of opioid mechanism underlying the Ss-EtOH-induced antinociceptive effect might be reasonable, considering that naloxone, a nonspecific opioid antagonist, was able to reverse its effect.\textsuperscript{52}

In the experimental model of glutamate-induced nociception, Ss-EtOH also induced a significant nociceptive response, probably involving the activation of receptors at the peripheral level, especially NMDA receptor, with subsequent release of nitric oxide.\textsuperscript{53} Thus, these previous findings might indicate that the Ss-EtOH-induced antinociceptive effect could be due to a possible interaction with the glutamatergic system and the inhibition of NO production.

Hence, the Ss-EtOH possesses the antiedematogenic activity, probably due to the presence of bioactive compounds that seem to interfere with the release of inflammatory mediators. Besides, the Ss-EtOH also induces a significant antinociceptive response in chemical-induced nociception models, probably underlying opioid participation and inhibition of glutamatergic and vanilloid receptors, although further studies are necessary to elucidate additional mechanims involved in the observed effects. This work raises the knowledge about \textit{S. striata} as a rich source of bioactive compounds and reinforces the development of new natural-based pharmaceutical products.

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**AUTHOR DISCLOSURE STATEMENT**

The authors declare that they have no conflicts of interest.

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