Anti-inflammatory and antinociceptive effects of hydroalcoholic extract from *Pseudobombax marginatum* inner bark from caatinga potiguar

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**Abstract**

*Ethnopharmacological relevance:* The *Pseudobombax marginatum* (St Hil) Rob., Malvaceae, is mentioned in ethnobotanical studies. It is used as anti-inflammatory, for ulcers and gastritis, and back pain. To evaluate anti-inflammatory and antinociceptive activities a hydroalcoholic extract (HE) from inner bark was prepared.

*Materials and methods:* For the anti-inflammatory activity, carrageenan-induced paw edema and peritonitis models, and also myeloperoxidase assay were used. For the antinociceptiva activity acetic acid-induced writhing, hot plate and formalin tests were employed.

*Results:* The HE extract exhibited an intense inhibition in carrageenan-induced edema model and also in myeloperoxidase activity at the doses of 100 and 300 mg/kg. The leukocyte migration into the peritoneal cavity was also inhibited at the doses of 30, 100 and 300 mg/kg. A similar profile was observed against acid-induced abdominal contortions and in formalin second phase test at the doses of 30 and 100 mg/kg, but this treatment did not affect the behavior of animals in the hot plate test.

*Conclusions:* The experimental data of the HE from *Pseudobombax marginatum* show anti-inflammatory and antinociceptive activities, confirming the indication from traditional medicine; however further studies are required to define and isolate the active anti-inflammatory and antinociceptiva components from this active specie.

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1. Introduction

The *Pseudobombax marginatum* (St Hil) Rob., known as “Embiratanha”, is a plant of wide distribution, found in many biomes. Presently, it is classified in the subfamily Bombacoideae from Malvaceae family. This is a diversified family; composed by herbaceous, bushes, trees and lianas. The Bombacoideae subfamily was incorporated to the Malvaceae for the APG system (Souza and Lorenzi, 2008; Oliveira et al., 2009; Roque et al., 2010; Paulino et al., 2011; Paulino et al., 2012). Studies in this area have mentioned the *Pseudobombax marginatum* as a medicinal plant. Brazilian Northeastern communities, mostly “potiguares”, use the bark and inner bark of embiratanha. The preparation is made as decoct or tea and is used as anti-inflammatory, for ulcers and gastritis, and back pain (Agra et al., 2008; Oliveira et al., 2009; Roque et al., 2010; Paulino et al., 2011; Paulino et al., 2012).

Even with those records, there are still very few data about *Pseudobombax marginatum*. The information is limited to cataloging traditional knowledge through ethnobotanical studies. It is important for the submission of this knowledge to experimental tests that verify its applicability and allows its management awareness of the potential offered by this tree.

Inflammation is a physiological process that occurs due to activation of some mechanisms that cause changes in humoral and cellular components after tissue injury (Cruvinel et al., 2010). When it is necessary, anti-inflammatory drugs are the therapeutic approach used to control the inflammatory process. However, long term use of the...
steroidal and non-steroidal anti-inflammatory tend to have serious side effects on the organism (Hilário et al., 2006; Longui, 2007).

In this study, we evaluate the anti-inflammatory and antinociceptive effects of the hydroalcoholic extract (HE) from Pseudobombax marginatum inner bark, to possibly create an alternative for anti-inflammatory therapy with a lot less side effects.

2. Material and methods

2.1. Plant material and extract preparation

The inner bark of Pseudobombax marginatum (St. Hil) Rob, Malvaceae, was collected upon authorization of SISBIO in FLONA (National Forest), at Açu–RN, Brazil, in July of 2011. The plant was identified by professor Doctor Ramiro Camacho Varela, Department of Biology, University of the State of Rio Grande do Norte (UERN), and a voucher specimen (number 13726) was deposited in the Herbarium Dârdano de Andrade Lima (MOS—acronym second Thiers, 2009), located in the Federal University of Semi-Arid (UFERSA). The inner bark was dried and powdered. After maceration it was left for several hours at room temperature with 70% ethanol. The extract was filtered in vacuum, and the solvent was removed using a rotary evaporator. The percentage yield of the hydroalcoholic extract was 5.5%. For biological analysis the plant extract was resuspended in a 0.9% NaCl solution (vehicle).

2.2. Phytochemical screening

For the screening of secondary metabolites present in the hydroalcoholic extract (HE) from Pseudobombax marginatum inner bark, a colorimetric and qualitative method was employed, based on Matos (1997).

2.3. Experimental animals

Wistar rats (120–180 g) and Swiss mice (20–30 g) males were obtained from the Central Biotechnology of the Federal University of Sergipe (São Cristóvão, Brazil). The animals were maintained at controlled room temperature (21 ± 2 °C) with free access to food and water, under a 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 in animal care of the Federal University of Sergipe Ethics Committee for Animal Use in Research (CEPA/UFS), registered at number 98/2011.

2.4. Anti-inflammatory activity

2.4.1. Carrageenan-induced hind paw edema model

The anti-inflammatory activity of the Pseudobombax marginatum HE was studied using the carrageenan (1%, 0.1 mL)-induced paw edema model, which was administered into the subplantar region of the right hindpaw of the rat (Winter et al., 1962). Animals were pre-treated orally (p.o.) with vehicle (saline), Pseudobombax marginatum HE (30, 100, and 300 mg/kg) or subcutaneous injection (s.c.) dexamethasone (2 mg/kg) 1 h before of the edematogenic agent (n=6/group). The paw edema was measured plethysmographically (model 7150, Ugo Basile, Varese, Italy) at 1, 2, 3 and 4 h after the carrageenan. The data obtained were expressed in mL. The percentage inhibition was calculated based on the data of the area under the time-curves (AU(C0–4 h)) using the following formula

\[ \text{Inhibition} \% = \left( 1 - \frac{T}{V} \right) \times 100, \]

where T means test and V means vehicle.

2.4.2. Myeloperoxidase (MPO) assay in rat paws

Myeloperoxidase activity was measured in paw tissue samples obtained from animals after the end of edema measurement. These samples were homogenized in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyl-trimethylammonium bromide. These homogenates were incubated for 2 h at 60 °C to inactivate endogenous catalases. The supernatants were mixed to a solution of o-dianisidine dihydrochloride (0.167 mg/mL, in 50 mM phosphate buffer) containing 0.005% of H₂O₂. The changes of absorbance at 460 nm were measured with a microplate reader (Labsystem Multiskan, Helsinki, Finland). The results were expressed as units of MPO (UMPO)/mg tissue, where one UMPO is defined as the amount of enzyme that degrades 1 μmol of H₂O₂/mim (Bradley et al., 1982).

2.4.3. Leukocyte migration into the peritoneal cavity of mice

Leukocyte migration was induced by injection of carrageenan (1%, 250 μL, i.p.) into the peritoneal cavity of mice (n=6/group) 1 h after the administration of the vehicle (saline, p.o.), HE (30, 100, and 300 mg/kg, p.o.) or dexamethasone (2 mg/kg, s.c.) as previously described by Mendes et al. (2010). The mice were euthanized 4 h after the carrageenan injection and 3 mL of saline containing EDTA (1.0 mM) was injected into the peritoneal cavity. The peritoneal lavages were collected and centrifuged at 1000g, and the cell pellets were resuspended in 1 mL of saline. The total number of cells was counted in a Neubauer chamber, and cytospin preparations were stained with May–Grunwald–Giemsa for the differential leukocyte counts. The results were expressed as the number of leukocytes/mL.

2.5. Antinociceptive activity

2.5.1. Acetic acid-induced abdominal writhes

The abdominal writhes were induced by intraperitoneal (i.p.) injection of acetic acid (0.8%, 0.1 mL/10 g) in mice (Koster et al., 1959) 1 h after the administration of the vehicle (saline, p.o.), Pseudobombax marginatum HE (30, and 100 mg/kg, p.o.) or acetylsalicylic acid (ASA, 300 mg/kg, p.o.) (n=6/group). The abdominal writhes were observed for a period of 20 min, starting 5 min after the injection of the nociceptive agent.

2.5.2. Hot-plate test

Mice (n=6/group) were pre-treated with vehicle (saline, p.o.), Pseudobombax marginatum HE (30, and 100 mg/kg, p.o.) 60 min before, or morphine (3 mg/kg, i.p.) 30 min before being placed on a metal plate warmed to 55 ± 0.5 °C. The time that elapsed between the start of the experiment and the appearance of reactions (latency, in seconds) to the thermal stimulus, such as lifting or licking the paws, was recorded as an index of nociception (Woofle and Macdonald, 1944). Measurements were performed 30, 60, 90, 120 and 150 min after the first thermal stimulus. To avoid damage to the animals, the maximal time standing on the plate was limited to 30 s.

2.5.3. Formalin test

The formalin test was conducted according to the method of Hunskaar and Hole (1987). Mice were pre-treated with vehicle (saline, p.o.), Pseudobombax marginatum HE (30, and 100 mg/kg, p.o.), ASA (300 mg/kg, p.o.), 60 min before the start of the experiment, or morphine (10 mg/kg, i.p.), 30 min before the start of the experiment. An intraplantar injection of 2% formalin solution (20 μL) was given to the right hind paw of the animal (n=7–9/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 (15–30 min) of the test.
2.5.4. Open field test
To evaluate the possible non-specific muscle relaxant or sedative effects of *Pseudobombax marginatum* HE, the mice were submitted to open field test (Capaz et al., 1981). Animals (*n*=5/group) were pre-treated with vehicle (saline, p.o.), *Pseudobombax marginatum* HE (30, and 100 mg/kg, p.o.), 60 min before the start of the experiment, or diazepam (1.5 mg/kg, i.p.), 30 min before the start of the experiment. The animals were individually placed in the open field (Insight®, Ribeirão Preto-SP), after 1 min of ambience, was recorded the frequency of movement of animals during 4 min, which consisted of the act penetrated all 4 animal paws in one of the divisions of the open field arena. Data were expressed as number of crossed fields.

2.6. Statistical analysis

The results are presented as the means ± SEM of *n* animals per group. Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey’s test. *p* Values lower than 0.05 were considered significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening showed that the HE of *Pseudobombax marginatum* inner bark contains tannins, flavonoids and free steroids.

3.2. Anti-inflammatory activity

3.2.1. Carrageenan-induced edema and MPO activity in rat paws

The curve represents the profile of edema during the interval of 4 h, counting from the induction (Fig. 1). After the 2nd hour of evaluation it was possible to notice that the animals treated with extract (100 and 300 mg/kg) exhibited a statistically different edema profile when compared with the one observed in animals treated with vehicle. Through the area under the curve analysis—AUC (Table 1) it was possible to observe that the 100 mg/kg dose caused a 48% inhibition in the edema’s volume when compared with vehicle.

The *Pseudobombax marginatum* HE also caused significant inhibition of MPO activity in the paws tissue from the carrageenan-induced edema (Fig. 2). Groups treated with the 100 and 300 mg/kg doses of extract or by the standard drug dexamethasone, exhibited a statistically lower amount of MPO activity when compared with the groups treated with vehicle, 53.24 ± 10.00 UMPO/mg tissue.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>AUC (mL.h)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>3.7 ± 0.2</td>
<td>–</td>
</tr>
<tr>
<td>HE</td>
<td>30</td>
<td>3.5 ± 0.1</td>
<td>5.19</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.9 ± 0.3**</td>
<td>48.13</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.5 ± 0.2***</td>
<td>32.71</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>2</td>
<td>1.3 ± 0.1***</td>
<td>65.03</td>
</tr>
</tbody>
</table>

Values mean ± SEM.

*** *p* < 0.001 vs. the respective vehicle group in the Tukey test, *n*=6/group.

3.2.2. Carrageenan-induced peritonitis in mice

In the peritonitis model (Fig. 3), the carrageenan injection induced leukocyte migration into the peritoneal cavity during 4 h. The groups treated with HE showed lower polymorphonuclear cell migration to the peritoneal cavity when compared with the ones treated with vehicle, but did not interfere with the mononuclear cells migration. The leukocyte migration inhibition was 59, 63 and 71% for the doses 30, 100 and 300 mg/kg, respectively, and 59% for dexamethasone (2 mg/kg).

Fig. 1. The effect of *Pseudobombax marginatum* HE on rat paw edema. Animals were pre-treated with vehicle, dexamethasone (Dexa, 2 mg/kg), or HE (30–300 mg/kg) before the carrageenan injection. *p* < 0.05 and *** *p* < 0.001 vs. the vehicle group in the Tukey test (*n*=6/group).

Fig. 2. The effect of *Pseudobombax marginatum* HE on MPO activity in the paws of rats. Animals were pre-treated with vehicle, dexamethasone (Dexa, 2 mg/kg), or HE (30–300 mg/kg) before the carrageenan injection. *** *p* < 0.01 vs. the vehicle group in the Tukey test (*n*=6/group).

Fig. 3. The effect of *Pseudobombax marginatum* HE on polymorphonuclear (PMN) and mononuclear (MONO) leukocyte migration. Mice were pre-treated with vehicle, dexamethasone (2 mg/kg), or HE (30–300 mg/kg) before carrageenan-induced peritonitis. *** *p* < 0.001 vs. the respective vehicle group in the Tukey test (*n*=6/group).
3.3. Antinociceptive activity

3.3.1. Acetic acid-induced writhing in mice

The number of writhings caused by acetic acid injection in the peritoneal cavity was significantly lower in the groups treated with Pseudobombax marginatum HE (30 and 100 mg/kg) or ASA (300 mg/kg) (Fig. 4). These values represent a percentage of inhibition of 72.5% and 78.5% to 30 and 100 mg/kg doses of the extract, respectively, and 83.8% to ASA, in comparison to vehicle.

3.3.2. Hot-plate

Observing in Table 2, the treatment with Pseudobombax marginatum HE did not increase the tolerance to pain on the hot-plate (55 °C). Only the animals treated with morphine (positive control group) were able to resist longer to the hot painful stimulation inflicted throughout this test.

3.3.3. Formalin test

In the first phase of the test it was observed that the treatment with the Pseudobombax marginatum HE did not influence the behavior of the animals, therefore they showed the same behavior pattern of that of the control group. Nevertheless, in the second phase, the animals treated with 30 and 100 mg/kg doses of Pseudobombax marginatum HE exhibited a different behavior profile when compared with the ones treated with the vehicle that spent much more time licking/biting their paw (Fig. 5).

Fig. 4. The effect of Pseudobombax marginatum HE on acetic acid-induced nociception. Mice were pre-treated with vehicle, HE (30–100 mg/kg) or acetylsalicylic acid (ASA, 300 mg/kg) before a acetic acid injection. ***p < 0.001 vs. the control group in the Tukey test (n=6/group).

Fig. 5. The effect of Pseudobombax marginatum HE on formalin-induced nociception. Mice were pre-treated with vehicle, HE (30–100 mg/kg), morphine (10 mg/kg) or acetylsalicylic acid (ASA, 300 mg/kg) before a formalin injection. *p < 0.05 and ***p < 0.001 vs. the respective control group in the Tukey test (n=7–9/group).

3.3.4. Open-field test

The open field test shows the spontaneous locomotor activity of mice (Fig. 6). As expected, the animals treated with the vehicle moved between fields and the ones treated with 30 and 100 mg/kg doses of Pseudobombax marginatum HE, exhibited a statistically similar behavior. Only the group treated with diazepam exhibited a diminished intensity of intercrosses compared to the vehicle.

4. Discussion

This study evaluated the effects of Pseudobombax marginatum, Malvaceae, HE using models of acute inflammation and nociception in rodents. The data revealed that P. marginatum HE can modulate the inflammatory response induced by carrageenan. The Pseudobombax marginatum was chosen from an ethnobotanic approach and there are no published data about this tree or other species from the same gender regarding chemical components or biological activity. However, some classes of the metabolites detected by phytochemical screening on Pseudobombax marginatum HE, as flavonoids and tannins, have already been found in other plants that exhibited anti-inflammatory activity. Additionally, it has been demonstrated that a variety of secondary metabolites are responsible, at least in part, by the biological activities of the plant under study. Tannins, flavonoids, and saponins, for example, are able to inhibit the inducible isoforms of cyclo-oxygenase (COX-2) and/or nitric oxide synthase (iNOS) enzymes, as well as other mediators of the inflammatory process (Di Carlo et al., 1999; Ahn et al., 2005; Carvalho, 2004; Soobrattee et al., 2005; Santos et al., 2011).

The COX-2 induction in the epidermis follows the carrageenan edema. This induction elevates levels of prostaglandin (PG)E2 and also causes infiltration of leukocytes and neutrophils (Natel et al., 1999). The results shown by 100 and 300 mg/kg doses of Pseudobombax marginatum HE suggest that the extract interfered with the mechanisms triggered by carrageenan. The inhibitory activity shown by Pseudobombax marginatum HE (mainly the 100 mg/kg dose) over a period of 4 h was especially evident.

Another important factor for evaluation in the inflammatory response is the migration of inflammatory cells, like neutrophils, to the lesion site. The migration of polymorphonuclear leukocytes was also inhibited by pre-treatment with Pseudobombax marginatum HE. The peritoneal fluid obtained from the peritonitis induced by carrageenan also showed significant reduction in cell number, similar to the one evoked by 2 mg/kg dexamethasone.

The results from the MPO assay corroborates with those previous data. This enzyme represents 5% of the dry weight of neutrophils, thereby it can indirectly measure the amount of neutrophil that have migrated to the tissue (Davies, 2011). Through MPO activity, it was possible to notice that the 100 and 300 mg/kg doses of Pseudobombax marginatum HE exhibited a profile of neutrophils migration inhibition similar to the one observed with dexamethasone.

In the inflammatory process, in addition to cellular and vascular events, there is also stimulation and sensitization of nociceptive receptors. Nociception tests were carried out using 30 and 100 mg/kg doses of Pseudobombax marginatum HE, such as abdominal writhing, hot plate and formalin models.

In the writhing test, the acetic acid promotes the release of arachidonic acid by COX pathway, with biosynthesis of PGs, effectors of the inflammatory pain (Franzotti et al., 2002). The pre-treatment with the Pseudobombax marginatum HE (30 mg/Kg and 100 mg/kg doses) was able to inhibit the number of abdominal writhes in the animals, which allows us to infer that the
extract may exert its antinociceptive effect through inhibiting PGs biosynthesis.

In the hot plate test the *Pseudobombax marginatum* HE did not interfere in the extension of pain latency. The nociceptors activated by the test lead the impulse to the dorsal culcoid of the spinal marrow and then to the cortical centers, in a frequency proportional to the sensitization of nociceptors (Dickenson, 1997).

In another assessment, formalin is the model to study nociception induced by formalin. Therefore, we may conclude that the nociception induced by formalin. Therefore, based on the results obtained in this study, we can explain the mechanisms involved with these activities further studies are required.

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction time after first stimulus (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>9.2 ± 1.7</td>
</tr>
<tr>
<td>HE</td>
<td>30</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>HE</td>
<td>100</td>
<td>11.8 ± 1.5</td>
</tr>
<tr>
<td>Morphine</td>
<td>3</td>
<td>30.0 ± 0.0*</td>
</tr>
</tbody>
</table>

Values mean ± SEM.

* p < 0.05 vs. the vehicle group in Tukey test, n = 6/group.

**Fig. 6.** The effect of *Pseudobombax marginatum* HE in open field test. Mice were pre-treated with vehicle, HE (30–100 mg/kg) or diazepam (1.5 mg/kg). *p < 0.01 vs. control group in the Tukey test (n=5/group).

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References


