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Anti-inflammatory, anti-hyperalgesic, antiplatelet and antiulcer activities of *Byrsonima japurensis* A. Juss. (Malpighiaceae)

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ABSTRACT

Ethnopharmacological relevance: Decoctions or infusions of the stem bark of *Byrsonima japurensis* A. Juss. (Malpighiaceae) are widely used as an anti-inflammatory drug in folk medicine of Amazonas State (Brazil).
Aim of the study: To evaluate the pharmacological potential of an aqueous extract of the stem bark of *Byrsonima japurensis* (BJEA) to scientifically verify of its traditional use.
Materials and methods: Anti-inflammatory, antihyperalgesic and antiulcer activities were evaluated in Wistar rats, a Hippocratic screening was performed in Swiss mice to evaluate the toxic effects, and antiplatelet evaluation was performed in human platelet rich plasma assay. Additionally, antioxidant activity was evaluated by superoxide radical scavenging method and β-carotene bleaching test.
Results: Anti-inflammatory, antihyperalgesic and gastroprotective activities were observed in rats treated orally with different doses of BJEA. While signals of toxicity were observed in the mice treated with a very high dose of extract (5000 mg/kg), no death occurred. BJEA also showed expressive antiplatelet and antioxidant activities *in vitro*.
Conclusion: According to our results, it was concluded that stem bark of *Byrsonima japurensis* has significant and safe anti-inflammatory activity, which is closely related with their potent antioxidant activity, supporting the folk medicinal use of this species.

1. Introduction

Inflammation is a nonspecific response of the microcirculation to tissue injury caused by physical, chemical or biological stimuli, or some combination of these. However, when there is loss of homeostatic control of this process of defense, inflammation plays a damaging role that contributes to the appearance and worsening of diseases (Nathan, 2002). The non-steroidal anti-inflammatory drugs (NSAIDs) utilized in treatment of inflammation are one the most widely used classes of drugs throughout the world, but their undesirable side effects on gastric mucosa and cardiovascular system are well know (Wallace and Vong, 2008). Therefore, the search for more effective anti-inflammatory drugs is still very relevant.

The use of plants as medicine dates back to early man. Often this folk medicine is the only source of access for certain populations for treating their diseases (Rates, 2001). However, the traditional use of plants is not sufficient for ethical validation of their therapeutic effects, requiring scientific studies to verify their real pharmacological potential (McChesney et al., 2007).

The *Byrsonima* genus (Malpighiaceae) has about 150 species with remarkable neotropical distribution. In various regions of Brazil, several species of this genus are widely used in the treatment of gastrointestinal complications. Various studies confirmed different biological activities in these plants, especially antioxidant, antimicrobial, including action against bacteria, fungi and protozoa and topical and systemic anti-inflammatory activities. Phytochemically, this genus has being characterized by the presence of flavonoids and triterpenoids (Guilhon-Simplicio and Pereira, 2011).

Byrsonima japurensis A. Juss. (Malpighiaceae) is a tree found in lowland areas of the Amazonian region used in folk medicine in rural areas of Amazonas State (Brazil), where it is popularly known as "saratudo" and considered a potent anti-inflammatory, being used against pathologies of gastrointestinal and genitourinary tracts. Normally, half a glass of tea prepared by decoction or infusion done with about 5.0% of fragmented stem bark is consumed once a day until the reduction of the symptoms of inflammation, and, in more severe cases, twice. Alcoholic beverages are also used in the preparation of folk medicines by maceration of

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the plant. For both cases, the raw material is previously dried in the shade and its outer layer is scraped for removal of impurities. However, there is no present scientific data to support these uses.

Therefore this study intends to investigate pharmacological activities of *Byrsonima japurensis*, to contribute to the scientific validation of its popular use. For this, we evaluate the antiedematogenic, antihyperalgesic, antiplatelet, antiulcer and antioxidant activities of an aqueous extract of stem bark of this plant. In addition, acute toxic potential was evaluated in animal experiments in order to verify the safety of oral administration for therapeutic purposes.

2. Materials and methods

2.1. Plant material

The collection of samples was authorized by the Managing Council of the Genetic Patrimony (CGEN), chaired by the Ministry of Environment (Brazil), under the registration 034/2008. Stem bark of *Byrsonima japurensis* was collected in Careiro-Castanho, Amazonas, Brazil, on March 2, 2007. The plant material was taxonomically identified and authenticated by José Lima of the Herbarium of the Instituto Nacional de Pesquisas da Amazônia, where a voucher specimen (224415) was deposited.

2.2. Samples of human blood

The collection of samples of human blood was authorized by the Ethical Committee on Research with Humans of the Universidade Federal do Amazonas, under the registration 038/2006. The samples were obtained from healthy adult volunteers of both genders.

2.3. Animals

The tests with experimental animals were performed in accordance with the Brazilian National Council for the Control of Animal Experimentation. Female Wistar rats weighing 100–220 g and male albino Balb/c mice weighing 20–30 g were used in this study. The animals were housed in polypropylene cages at 25 ± 2 °C with 12 h of a light-and-dark cycle and acclimatized in the experimental environment for at least 24 h before the tests. Water and a balanced diet were continually provided *ad libitum*, but food was withdrawn 12 h before the tests.

2.4. Drugs and chemicals

Arachidonic acid, adenosine diphosphate, epinephrine and λ-carrageenan was purchased from Fluka Chemical Company (Buchs, Switzerland); nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) and phenazine methylsulfate (PMS) were purchased from Chrono-Log Corporation (Havertown, PA, USA); butylated hydroxytoluene (BHT), linoleic acid and β-carotene was purchased from Sigma–Aldrich (St. Louis, MO, USA). Other reagents were purchased locally.

2.5. Preparation of extract

In this study, the aim was to replicate the folk medicine usage of the species when obtaining the crude extract. The stem bark was dried at 40 °C in a stove (WHO, 2003), and then scraped and pulverized using a mechanical grinder. The extract was obtained by infusion with distilled water at 5% of plant material for 15 min and lyophilized for further use. The yield of extraction was 10.5%. The extract obtained is named BJAE in this work.

2.6. Hippocratic screening

The maximal dose of 5000 mg/kg of BJAE dissolved in normal saline were administered orally to a group of five mice, observing abnormal morphological and behavior signs of toxicity during the first 4 h after administration of extract and at every 24 h for 14 days (Malone and Robichaud, 1962).

2.7. Anti-inflammatory activity

Doses of 100, 200 and 400 mg/kg of BJAE in normal saline were administered orally in different groups of 6 rats. Two other groups received indomethacin at 10 mg/kg (positive control) and normal saline (negative control). After 1 h, a solution of λ-carrageenan 1% in normal saline was injected in the right hind paw of all animals, and the paw edema progression was measured using a digital plethysmometer (LE-7500, Panlab, Barcelona, Spain), in hourly intervals, for 5 h (Winter et al., 1962).

2.8. Anti-hyperalgesic activity

Different groups of 6 rats received were 100, 200 and 400 mg/kg of BJAE in normal saline and indomethacin at 10 mg/kg (positive control) and normal saline (negative control) utilized in each analysis. After 1 h, a solution of λ-carrageenan 1% in normal saline was injected in the right hind paw of all animals and pressure stimuli were applied on the inflamed paw at hourly intervals for 4 h, with the maximal load borne by each animal registered by a digital analgesimeter (EFF301, Insight, Ribeirão Preto, Brazil), in grams (Lapa et al., 2008).

2.9. Antiulcer activity

Rats were organized in groups of six animals. Water was used as a vehicle and negative control and ranitidine (50 mg/kg, by oral route) was used as a positive control. Doses of 100, 200 and 400 mg/kg of BJAE were administered in three different groups. After 1 h, indomethacin (10 mg/kg) was subcutaneously administered to all the animals. After 3 h, all the rats were sacrificed with excess of anesthetic ether and their stomach was cut open along the lesser curvature, cleared of residual matter with saline and the inner surface was examined for ulceration and the ulcer index was calculated (Djahanguiri, 1969).

2.10. Antiplatelet activity

This analysis was performed in accordance with methodology of Born (1962), where different concentrations of BJAE are incubated with platelet rich plasma obtained from human blood at 37 °C for 2 min. Arachidonic acid, adenosine diphosphate and epinephrine were used as platelet inductors of platelet aggregation. Acetylsalicylic acid was used as a positive control. The reading of results was done in a Born aggregometer (PA-04, Qualiterm, São Paulo, Brazil).

2.11. Antioxidant activity

2.11.1. Superoxide radical scavenging test

Different concentrations of BJAE dissolved in 50% ethanol were incubated for 5 min in darkness with NBT, NADH and PMS in a 96-well plate, in triplicate. Ascorbic acid, gallic acid and α-tocopherol were used as positive controls. Ethanol at 50% was used as a negative control. The absorbance was recorded at 560 nm to check the bleaching of mixture, indicating the radical scavenging (Ozturk et al., 2007).

2.11.2. β-Carotene bleaching test

Different concentrations of BJAE dissolved in dimethyl sulfoxide (DMSO) were incubated with an emulsion of linoleic acid and β-carotene in water saturated with oxygen, for 15 min at 50 °C in a 96-well plate, in triplicate. BHT was used as positive control and DMSO was used as a negative control. The absorbance was recorded at 492 nm every 15 min to check the bleaching of b-carotene, indicating peroxidation (Miller, 1971).

2.12. Statistical analysis

The results are presented as mean ± standard deviation, when applicable. Results of groups in antiedematogenic, anti-hyperalgesic and antiulcer tests were compared with a paired Student–Newman–Keuls test, considering values of $p < 0.05$ statistically significant. The median inhibitory concentrations (IC_{50}) of the antioxidant and antiplatelet activities were calculated by linear regression method, and the statistical significance of their differences was measured with Student's *t*-test.

3. Results

3.1. Hippocratic screening

The following symptoms were observed in all animals during Hippocratic screening of BJAE: decrease in motor activity, increase in respiratory and cardiac frequency, dyspnea, palpebral ptosis, pilomotor erection, cyanosis, and decrease in defecation, urination and appetite. However, no deaths occurred during the observation period of 14 days and the animals recovered the normal parameters within 48–72 h.

3.2. Anti-inflammatory and antihyperalgesic activities

BJAE presented significant antiedematogenic activity with 100, 200 and 400 mg/kg, in early phase (first hour) and late phase (third hour) of inflammatory response induced by carrageenan, but the dose of 400 mg/kg of extract presented significantly higher antiedematogenic activity than the standard drug in the second phase of carrageenan-induced inflammatory response (Fig. 1). In the analysis of anti-hyperalgesic activity, only the dose of 400 mg/kg showed activity in both phases of carrageenan-induced inflammatory response, with greater activity than the in positive control (Fig. 2).

3.3. Anti-ulcer activity

The results of our test for evaluation of anti-ulcer activity showed that doses of 100, 200 and 400 mg/kg of BJAE have significant gastroprotective action against indomethacin-induced gastric lesion. However, there were no differences within doses. Furthermore, ranitidine 50 mg/kg tested in same conditions presented better protective effect on the gastric mucosa (Fig. 3).

3.4. Antiplatelet activity

BJAE presented antiplatelet activity against different inductors of aggregation, but its activity was not greater than acetylsalicylic acid (AAS) tested in the same conditions. The IC_{50} of BJAE when arachidonic acid, adenosine diphosphate and epinephrine were used as inductors of aggregation was 178.5 ± 1.6, 117.7 ± 4.5 and 97.5 ± 7.9 μg/mL, respectively. For AAS, the results were 32.5 ± 0.2, 21.5 ± 1.4 and 11.7 ± 0.5, respectively. The difference between the results is statistically significant, obtaining *p*-values smaller than 0.01 in all the cases.

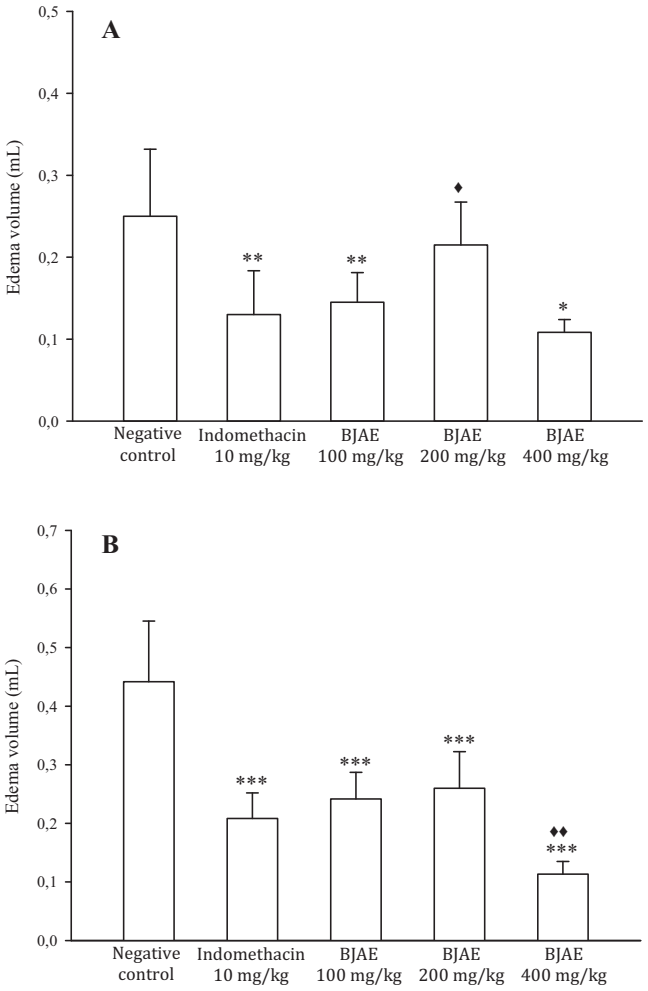

Fig. 1. Anti-inflammatory activity of *Byrsonima japurensis*. (A) In early phase of carrageenan-induced paw edema and (B) in late phase of carrageenan-induced paw edema; *p*-values versus negative control: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and *p*-values versus positive control: $^{\blacklozenge}p < 0.05$, $^{\blacklozenge\blacklozenge}p < 0.01$.

3.5. Antioxidant activity

BJAE present significant antioxidant activity in the superoxide radical scavenging test. In this test, their IC_{50} was 42.5 ± 1.3 μg/mL, significantly lower than α-tocopherol (180.0 ± 14.2 μg/mL) and ascorbic acid (32.9 ± 1.6 μg/mL) and higher than Gallic acid (7.8 ± 1.2 μg/mL). Its inhibition of lipid peroxidation was greater than BHT tested in the same conditions, in which IC_{50} was 45.7 ± 3.1 and 81.6 ± 7.0 μg/mL, respectively. The difference between the results is also statistically significant, obtaining *p*-values smaller than 0.01 in all the cases.

4. Discussion

The inflammatory response produced by carrageenan is divided into two parts: the first phase occurs within first hour after polysaccharide injection and is mainly related to the release of histamine, serotonin, bradykinin, platelet activating factor and leukotrienes; the second phase occurring within the first and third hours (peak of the inflammatory response) and is due to the release of prostanoids, especially prostaglandins and prostacyclins, by the action of 1 and 2-cyclooxygenases (COX-1 and COX-2) (Vinegar et al., 1969). During any inflammatory response, usually innocuous stimuli produce

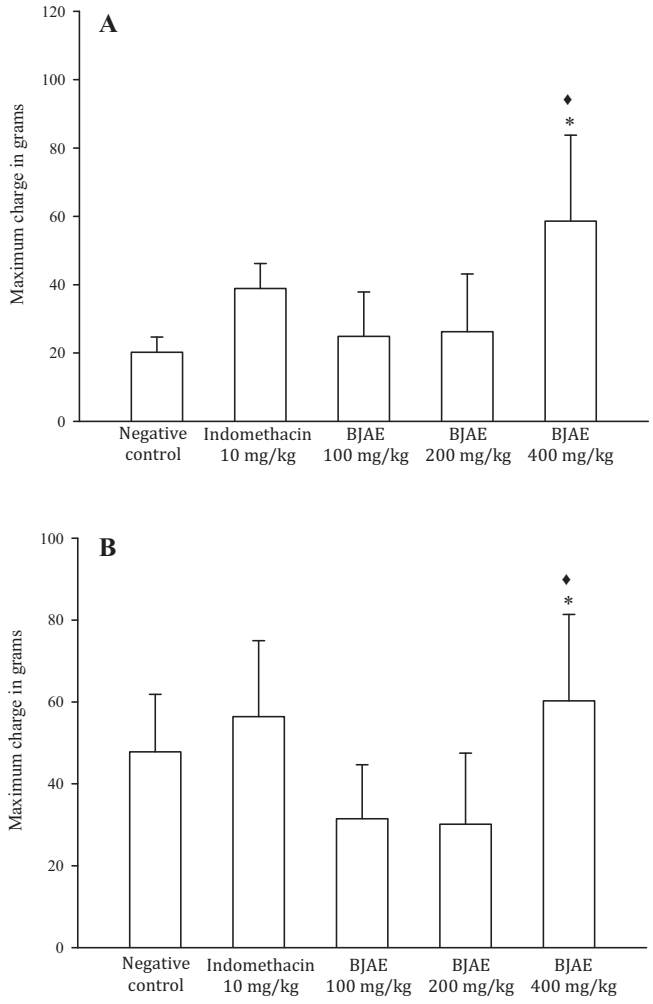

Fig. 2. Anti-hyperalgesic activities of *Byrsonima japurensis*. (A) In early phase of carrageenan inducing paw edema and (B) In late phase of carrageenan inducing paw edema; *p*-values versus negative control: $^{*}p < 0.05$, and *p*-values versus positive control: $^{\blacklozenge}p < 0.05$.

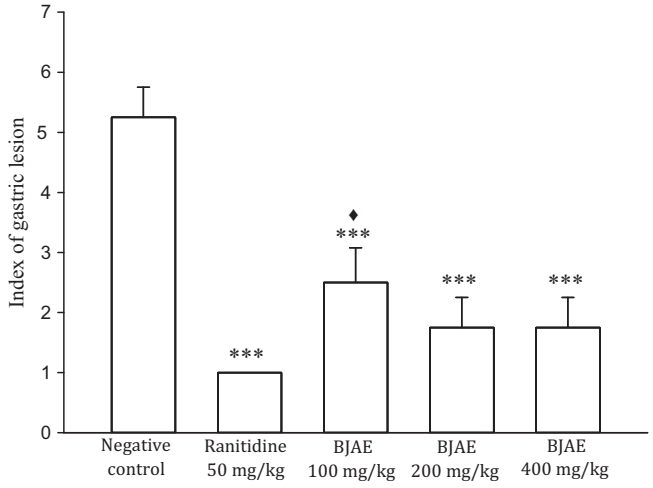

Fig. 3. Effect of extract on the formation of gastric lesions by action of the indomethacin; *p*-values versus negative control: $^{***}p < 0.001$, and *p*-values versus positive control: $^{\blacklozenge}p < 0.05$.

pain, which is caused by the stimulation of afferent nervous fibers by action of kinins, serotonin, histamine, prostanoids, protons and reactive species of oxygen (ROS) released during inflammation (Scholz and Woolf, 2002).

Superoxide is the first reduction product of molecular oxygen, and it is an important source of hydroperoxides and deleterious free radicals (Chauhan and Chauhan, 2006). This ROS is involved in degenerative diseases of aging, including Alzheimer's disease, cancer and in the worsening of inflammatory diseases, such as rheumatoid arthritis and atherosclerosis, through DNA and protein damage or lipid peroxidation (Ames et al., 1993; Wiseman and Halliwell, 1996). It has been shown that lipid peroxidation is related to the aggravation and acute and chronic inflammatory responses. *In vivo*, this phenomenon is due mainly to the formation of peroxynitrite from the combination of superoxide radicals and nitric oxide released during the inflammatory response (Salvemini et al., 2006).

In humans and other species, the COX-1 is expressed constitutively throughout the gastrointestinal tract (GI), where the production of prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) has cytoprotective effects on the mucosa by reducing gastric acid secretion by parietal cells in the stomach, increasing mucosal blood flow, and stimulating the release of viscous mucus (Rao and Knaus, 2008). Gastric or duodenal ulcers develop in 15–30% of patients who regularly take a non-steroidal anti-inflammatory drug (NSAID), which inhibit the action of COX-1 (Borer and Simon, 2005).

The selective inhibitors of COX-2, the inducible isoform of cyclooxygenase, were intended to be a safer alternative of NSAID to gastrointestinal tract. However, these agents have been associated with undesirable effects on the cardiovascular system, such as heart attacks and strokes, since that they do not inhibit thrombosis associated with release of thromboxane A_2, a powerful inductor of platelet aggregation, produced by COX-1 constitutively present in all platelets, after platelet activation by collagen, adenosine diphosphate (ADP), epinephrine, platelet activating factor, arachidonic acid metabolites, polycations, serotonin and thrombin, among other substances involved in inflammatory response (Weyrich et al., 2003; Sohn and Krötz, 2006).

The results of tests of anti-inflammatory and anti-hyperalgesic activities showed that the effects of BJAE have a pattern similar to the effect of indomethacin, an NSAID not selective for COX-1 or COX-2, which also presented reduction of edema and pain in both phases of carrageenan-induced inflammatory response, suggesting a common mechanism of action between the drugs. This observation is reinforced by the results from the antiplatelet test, in which BJAE was shown to have a good inhibitory effect against different platelet inductors, suggesting that the extract is acting by a mechanism of inhibition common to all the inductors, such as inhibition of COX-1 (Meadows and Bhatt, 2007).

The gastroprotective action against indomethacin-induced gastric lesions suggests that the anti-inflammatory and anti-hyperalgesic activities presented by BJAE are not only related to the inhibition of cyclooxygenases, since the gastric lesions induced by non-steroidal anti-inflammatory drugs (NSAIDs) depend of the inhibition of both COX-1 and COX-2 (Wallace et al., 2000; Wallace and Vong, 2008).

Therefore, the action of aqueous extract as a radical scavenger of superoxide may also contribute to its anti-inflammatory and anti-hyperalgesic effect. Some studies showed that the removal of superoxide has significant anti-inflammatory effects, both preclinically and clinically and it is a good strategy to inhibit peripheral and central sensitization associated with several pain states (Wang et al., 2004; Salvemini et al., 2006). Indeed, it is known that superoxides play a critical role in mediating the formation of peroxynitrite in both early and late phases of the carrageenan-induced inflammatory response (Salvemini et al., 1996; Cuzzocrea et al., 1998).

The signals of toxicity and/or pharmacological activity observed in Hippocratic screening of BJAE are very similar to those presented by *Byrsonima crassifolia* in two previous similar analyses (Guilhon-Simplicio and Pereira, 2011). In the respective papers, the authors suggest that the species has an effect on the central nervous system (CNS). This hypothesis should be further investigated, since the critical role of nicotinic acetylcholine receptors in the regulation of inflammation has been established (Wang et al., 2003).

The results observed in this study can be partially explained by the presence of different class of flavonoids that we detected in the species by phytochemical screening, high performance liquid chromatography, nuclear magnetic resonance and infrared spectrometry fingerprint (unpublished results). The capacity of these substances to inactivate enzymes involved in inflammatory response, such phospholipases and cyclooxygenases and their expressive radical scavenging activity is well known (Middleton et al., 2000). The increase in respiratory and cardiac frequency observed in Hippocratic screening can be explained by the presence of cardioactive heterosides which was also detected in the same study.

5. Conclusion

This work met its goal of scientifically verifying the popular use of stem bark of *Byrsonima japurensis*, whose tea is widely consumed in folk medicine of Amazonas State (Brazil) as an anti-inflammatory drug. The aqueous extract obtained by infusion at 5% of this vegetal drug showed good antiedematogenic and anti-hyperalgesic potential after oral administration in animal experiments. Indeed, this extract also showed good antiplatelet and anti-ulcer activities that may have fewer side effects compared to traditional NSAIDs. The expressive antioxidant action of extract, in addition to the already well-known benefits to health, may be contributing to its mechanism of anti-inflammatory effect. It is important to highlight that no death occurred after exposure to very high doses of BJAE, which confirmed the safety of its use for therapeutic purposes and for advancement of studies *in vivo* with this raw material. Therefore, this species is a promising object for further studies aiming to support its mechanism of pharmacological activities presented in this work.

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