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Antinociceptive and anti-inflammatory activities of the hexane extract from *Hortia brasiliana* Vand. leaves on experimental animal models

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Antinociceptive and anti-inflammatory activities of the hexane extract from *Hortia brasiliana* Vand. leaves were investigated by experimental animal models. Guaiol (9.72%), nonacosane (25.57%) and eicosane (10.80%) were the most abundant components identified by GC/MS. The extract, with LD_{50} of 2.40 g/kg, reduced the number of abdominal contortions by 13.86 (100 mg/kg) and 18.51% (200 mg/kg). Doses of 100 and 200 mg/kg inhibited both phases of the time paw licking: First phase (9.44% and 16.32%) and the second phase (11.97 and 23.49%), respectively. The extract increased the reaction time on a hot plate at doses of 100 (24.92%) and 200 mg/kg (55.69%) after 90 min of treatment. The paw edema was reduced by the hexane extract at doses of 100 (10.42 and 8.23%) and 200 mg/kg (15.62 and 17.65%) after 3 to 4 h of application of carrageenan, respectively. At the dose of 200 mg/kg, the extract reduced the exudate volume by 19.44%, while the leucocyte migration was inhibited at the doses of 100 and 200 mg/kg (6.13 and 13.84%, respectively). These results suggest that *H. brasiliana* can be an active source of substances with antinociceptive and anti-inflammatory activities supporting the use in the Brazilian folk medicine.

Key words: *Hortia brasiliana*, Rutaceae, antinociceptive activity, anti-inflammatory activity.

INTRODUCTION

One of the cardinal features of the inflammatory states is that normally innocuous stimuli produce pain (Stankov, 2012). The physiological mechanisms of pain involve peripheral sensitization and neuroplasticity in the perpetuation of this event, with action through chemical mediators on nociceptive pathways (Stankov, 2012). Based on the origin of these mediators, these substances can contribute to the cycle of pain causing hyper-sensitivity by synthesis of cytokines (White et al., 2005). Usually, the pain is treated with opioids and non steroidal anti-inflammatory drugs (NSAIDs). However, the adverse effects as constipation and respiratory depression and irritation of gastric mucosa and ulcer, water retention and nephrotoxicity for opioids and NSAIDs, respectively, have

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prevented the application of these therapeutic agents (Kawada et al., 2012; Benyamin et al., 2008). Another important option in the treatment of pain and inflammation is the use of medicinal plants that is a common practice worldwide (Shah et al., 2011). Therefore, the evaluation of the pharmacological effects of these plants can be used as a strategy and support to find new drugs with scientific sustainability in the treatment of many disorders.

Hortia brasiliana Vand. (Rutaceae), proposed as synonyms *Hortia arborea* Engl., *Hortia badinii* M. Lisboa and *Hortia colombiana* Groppo, is distributed from Panama to the state of São Paulo, Brazil, most of them occurring in the Amazonian region (Groppo, 2010). In Brazilian folk medicine reports, plants of the genus *Hortia*, including *H. brasiliana*, possess excellent stimulant, stomachic, sedative, hypotensive, antiulcerogenic, analgesic and anti-inflammatory properties (Sobrinho et al., 2011).

In addition, ruteacarpine, an alkaloid found in plants of Rutaceae family as *H. brasiliana*, has demonstrated cardiovascular, antithrombotic, anticancer, anti-inflammatory, analgesic, antiobesity and thermoregulatory activities and effects on endocrine and smooth muscle (Lee et al., 2008), as well as cyclooxygenase-2 inhibition (Liao et al., 2011).

Considering the phytochemical and pharmacological aspects, *H. brasiliana* has been described as a large source of alkaloids, coumarins, flavonoids, and limonoids (Suárez et al., 1998; Severino et al., 2009a, 2009b, 2012). Five alkaloids were isolated from wood and identified as flindersine, N-methylflindersine, γ-fagarine, skimmianine and 2,4-dimethoxyquinoline (Suárez et al., 1998). A tetranortriterpenoid and two dihydrocinnamic acid derivatives, as well as alloxanthoxyletin, nerolidol, epoxynerolidol, three known dihydrocinnamic acid derivatives and two amides were obtained from the wood (Suárez et al., 2002). Four dihydrocinnamic acid derivatives, limonoid guyanin and furoquinoline alkaloid dictamnine, obtained from roots, showed antibacterial activity (Severino et al., 2009a). Rutaecarpine, found in leaves and wood of *H. brasiliana*, has demonstrated different pharmacological properties, including analgesic and anti-inflammatory (Shin et al., 2007; Lee et al., 2008; Liao et al., 2011).

From leaves were identified guyanin, rutaecarpin and dictamnine and dihydrocinnamic acid derivatives, together with the new cinnamic acid derivative, been observed plasmocidal and trypanosomidal activities (Severino et al., 2009b). Moreover, several limonoids were identified in the dichloromethane extracts obtained from taproots and stems (Severino et al., 2012).

In the present study, *H. brasiliana* was selected because it is one plant among Brazilian biodiversity commonly used as traditional medicine to treat abdominal pain, headache, inflammation, swelling and rheumatism. However, extracts from leaves have not been evaluated systematically for pharmacological properties to corroborate the traditional uses of this species in folk medicine. In this investigation, we evaluated the antinociceptive and anti-inflammatory activities of *H. brasiliana* in experimental animal models using hexane extract.

MATERIALS AND METHODS

Plant material and extraction

Leaves of *Hortia brasiliana* Vand. were collected in the city of Muriaé, Minas Gerais State, Southeast region of Brazil, in September 2010. The species was identified by Dr Milton Groppo Júnior and a voucher specimen (number 59192) was deposited in the Herbarium CESJ, Federal University of Juiz de Fora, Brazil. Dried and cut leaves (20 g/100 ml) were exhaustively extracted in hexane by static maceration at room temperature with renewal of solvent every day. The hexane was filtered and evaporated under a rotary evaporator at controlled temperature (40 to 45°C).

Gas chromatography/mass spectrometry analysis (GC/MS)

This analysis was carried out using a Hewlett-Packard 6890 gas chromatograph equipped with a fused silica capillary column (HP-5, 30m × 0.25 mm, 0.25 µm film thickness), helium as carrier gas with a flow rate 1.0 ml/min; temperature programming from 70°C to 290°C (2°C/min), coupled to a Hewlett-Packard 5972 mass spectrometer. The MS operating parameters were: 70 eV, ion source 250°C equipped with EI. The compound identifications were carried out by comparison of their retention indices (RI) with literature values; and the MS data with those from Wiley 275.1 mass spectral data base besides literature records (Adams, 1995). The retention indices were calculated using a GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C22.

Chemicals

Drugs and reagents used in this study (and their sources) were as follows: Acetic acid and hexane (Vetec Química Farm Ltda, Rio de Janeiro, RJ, Brazil), formaldehyde and acetylsalicylic acid (Reagen Quimibrás Ind. Química S.A., Rio de Janeiro, RJ, Brazil), morphine hydrochloride (Merck Inc., Whitehouse Station, NJ, USA), naloxone and indomethacin (Sigma Chemical Co, St Louis, MI, USA).

Animals

Male Wistar rats (90-110 days) weighing 200 to 240 g and male Swiss albino mice (50-70 days) weighing 25 to 30 g were used in the experiments. The animals were provided by the Central Biotery of the Federal University of Juiz de Fora. The animals were divided into groups and kept in plastic cages (47 × 34 × 18 cm) under a 12 h light/12 h dark cycle at room temperature (22 ± 2 °C), with free access to Purina® rations and water. Animal care and the experimental protocol followed the principles and international guidelines suggested by the Brazilian College of Animal Experimentation (COBEA) and were approved by the local ethical committee (protocol number 036/2010).

Acute toxicity

Groups of ten mice received oral doses of 0.5, 1, 1.5, 2 and 3 g/kg of hexane extract from *H. brasiliana*, while the control group received the vehicle (saline). Due to solubility of the extract, the volume changed from 0.3 to 0.9 ml. After administration, the groups were observed for 48 h and 50% lethal dose (LD_{50}) was the mortality at the end of this period was recorded for each group (Lorke, 1983). The LD_{50} determined by probit test using a log plot of percentage death *versus* dose (Litchfield and Wilcoxon, 1949).

Acetic acid-induced writhing test

The acetic-acid writhing test is used for the evaluation of the analgesic activity (Schmidt et al., 2010). Mice (n = 8 per group) were injected (i.p.) with 0.6% acetic acid (10 ml/kg body weight), and the intensity of nociception was quantified by counting of the total writhes number that occurred between 10 and 30 min after injection. Animals received hexane extract (50, 100 or 200 mg/kg, p.o.) or sterile saline (control group, 0.9%, w/v) 60 min before acetic acid injection. Acetylsalicylic acid (200 mg/kg, p.o.) and Indomethacin (10 mg/kg, p.o.) were administered 60 min before acetic acid as reference compounds.

Formalin test

Groups of mice (n = 8) were treated (p.o.) with hexane extract (50, 100 or 200 mg/kg) or sterile saline (0.9%) 60 min before formalin injection. Twenty microliters of 1% formalin was administered (i.pl.) in the mouse's right paw and the licking time was recorded from zero to 5 min (phase 1, neurogenic) and from 20 to 25 min (phase 2, inflammatory) (Hunskaar and Hole, 1987). Morphine (1 mg/kg, s.c.) and indomethacin (10 mg/kg, p.o.) were also administered 60 min before the formalin injection and used as reference compounds.

Hot plate test

Animals were placed on a hot-plate (Model LE 7406, Letica Scientific Instruments, Barcelona, Spain) heated at 55 ± 1°C (Eddy and Leimbach, 1953). Three groups of mice (n = 8) were treated (p.o.) with hexane extract (50, 100 or 200 mg/kg; 0.1 ml per 10 g body weight); the control group received sterile saline (10 ml/kg). Measurements were performed at zero, 30, 60 and 90 min after drug administration, with a cut-off time of 40 s to avoid lesions in the animals' paws. The effect of pretreatment with naloxone (1 mg/kg, subcutaneously) on the analgesia produced by the hexane extract (200 mg/kg) was determined in a separate group of animals. Morphine (1 mg/kg, s.c.), in the absence and presence of naloxone (1 mg/kg, s.c.) treatment, was used as a reference.

Carrageenan-induced rat paw edema

Anti-inflammatory activity was assessed on the basis of inhibition of paw edema induced by the injection of 0.1 ml of 2% carrageenan (an edematogenic agent) into the subplantar region of the right hind paw of the rat (Winter et al., 1962). Male Wistar rats were divided into groups of six animals which received (p.o.) doses of hexane extract (50, 100 and 200 mg/kg; 0.1 ml per 10 g body weight), saline or indomethacin (10 mg/kg) 1 h before the injection of carrageenan. In the left paw, used as a control, 0.1 ml of sterile saline was injected. 1, 2, 3 and 4 h after injection of carrageenan, the measure of edema was made by the difference between the volume displaced by the right paw and the left paw using a plethysmometer (model LE 7500, Letica Scientific Instruments, Barcelona, Spain).

Carrageenan-induced pleurisy in rats

Pleurisy was induced in male Wistar rats by intrapleural administration of 0.5 ml 2% carrageenan suspension in saline solution between the third and fifth ribs on the right side of the mediastinum (Vinegar et al., 1973). Hexane extract (50, 100 or 200 mg/kg), saline or indomethacin (10 mg/kg) (p.o.) were given 60 min before injection of the irritant agent. Animals were killed 4 h after carrageenan injection, and the skin and the pectoral muscles were retracted. A longitudinal incision was made between the third and fifth ribs on each side of the mediastinum. The exudate was collected and transferred to a 15 ml conical centrifuge tube and the total volume determined. A 20 µl aliquot was used to determine the total leucocyte count by Neubauer chambers.

Statistical analysis

Data were expressed as mean ± S.E.M. Statistical significance was analysed by the one-way analysis of variance followed by the Student Newman-Keuls test. *P* values below 0.05 were considered significant.

RESULTS

Chemical constituents

According Table 1, based on the fragmentation pattern obtained in the mass spectrometry, constituents belonging to the classes of sesquiterpenes (20.28), diterpenes (3.94%), hydrocarbons (36.37%) and pentacyclic triterpenes (35.12%) were detected in the hexane extract from *H. brasiliana* leaves. Among the identified compounds, eight were sesquiterpenes and two hydrocarbons. Guaiol (9.72%), nonacosane (25.57%), eicosane (10.80%) and one pentacyclic triterpene (18.49%) were the most abundant components found in the extract.

Acute toxicity

At the doses administered per oral route (p.o.), the hexane extract from *H. brasiliana* leaves was toxic to animals with LD_{50} of 2.40 g/kg (95% confidence intervals 1.51-3.83 g/kg). However, in the evaluated period, the animals did not show cyanosis, piloerection, writhing, ptosis, tremors, convulsions, ataxia, hypnosis, red urine or diarrhea. The parameters like motor activity, respiration, corneal reflex, righting and withdrawal, and body tone were not affected.

Table 1. Constituents of the hexane extract from *Hortia brasiliana* leaves.

Compound	Chemical class	Retention time	Concentration (%)
Alpha-curcumene	Sesquiterpene	18.67	2.70
Beta-bisabolene	Sesquiterpene	19.32	0.86
Beta-sesquiphellandrene	Sesquiterpene	19.72	1.16
Caryophyllene oxide	Sesquiterpene	21.27	0.94
Guaiol	Sesquiterpene	21.50	9.72
Dihydrolinalool	Sesquiterpene	23.47	1.61
Trans-alpha-bergamotene	Sesquiterpene	23.63	1.30
Trans-bergamotol	Sesquiterpene	23.99	2.53
Unidentified	Diterpene	24.67	1.62
Unidentified	Diterpene	26.10	2.32
Nonacosane	Hydrocarbon	44.33	25.57
Eicosane	Hydrocarbon	46.95	10.80
Unidentified	Pentacyclic triterpene	50.33	5.12
Unidentified	Pentacyclic triterpene	50.56	2.73
Unidentified	Pentacyclic triterpene	51.05	18.49
Unidentified	Pentacyclic triterpene	54.89	8.78
Total			96.25

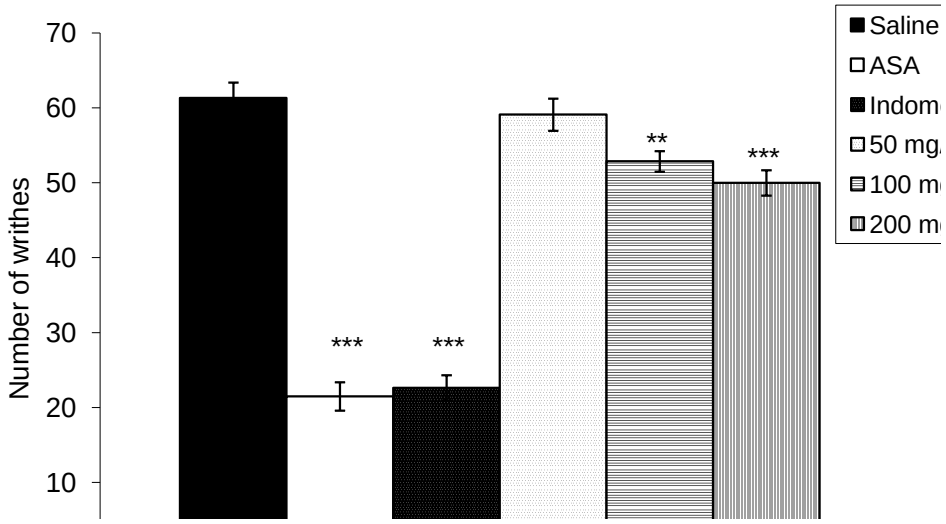

Figure 1. Effects of the hexane extract from *H. brasiliana* leaves on acetic acid-induced writhing in mice. ASA, Acetylsalicylic acid. Data are mean ± S.E.M. of eight mice. **p < 0.01; ***p < 0.001 *vs* control group (saline).

Acetic acid-induced writhing response in mice

The treatment of animals with hexane extract (100 and 200 mg/kg, p.o.) produced a significant ($p < 0.01$ and $p < 0.001$, respectively) and dose-dependent inhibition in abdominal writhes induced by acetic acid (Figure 1). The control group caused 61.36 ± 2.05 abdominal contortions. Acetylsalicylic acid (200 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) decreased the abdominal contortions by 64.96 and 63.13%, respectively, when compared with the control group.

Formalin-induced paw licking in mice

The Figure 2 shows that pretreatment with morphine (1 mg/kg) or with the hexane extract of *H. brasiliana* (100 and 200 mg/kg, p.o.) produced significant changes of

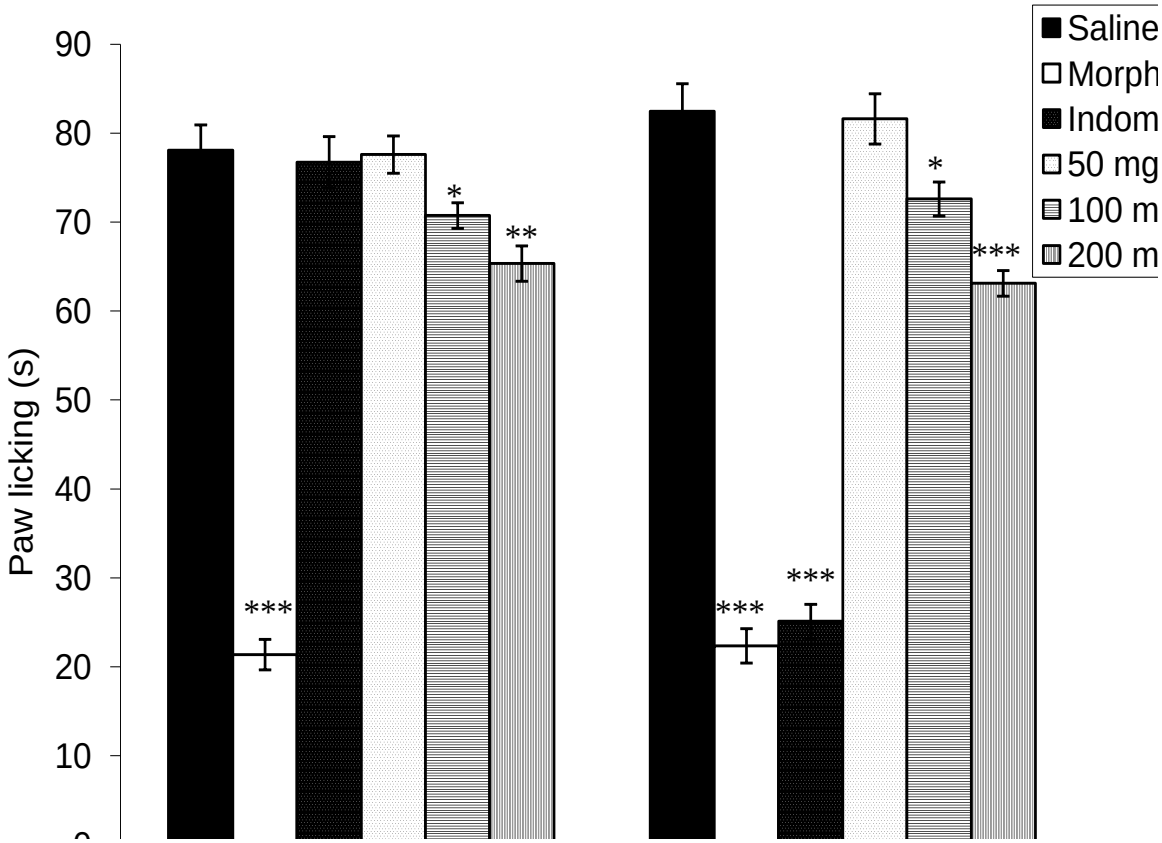

Figure 2. Effects of the hexane extract from *H. brasiliana* leaves on formalin-induced nociception in mice. Data are mean ± S.E.M. of eight mice. *p < 0.05; **p < 0.01; ***p < 0.001 *vs* control group (saline).

paw licking time in the first phase of pain response. In the second phase, a dose-dependent and significant ($p < 0.05$ or $p < 0.001$) reduction was observed in mice treated with extract (100 and 200 mg/kg, p.o.) as well as with indomethacin (10 mg/kg, p.o.) and morphine (1 mg/kg, s.c.). For the control group, the time spent was 78.12 ± 2.82 s and 82.50 ± 3.10 s in the first and second phases, respectively.

Effects on hot-plate latency assay in mice

In consequence of the analgesic effect observed in the first phase of formalin test, we decided to evaluate the hexane extract using hot plate test, a model of central antinociceptive activity.

After 60 min of treatment, doses of 200 mg/kg ($p < 0.01$) increased significantly the latency time in the respective control group (Table 2). Morphine (1 mg/kg) proved to be a potent analgesic increasing the latency time within the evaluated periods. Naloxone (1 mg/kg, s.c.), an opioid antagonist, blocked the effect of morphine (1 mg/kg, s.c.) and extract (200 mg/kg, p.o.).

Effects on carrageenan-induced edema in rats

The anti-inflammatory effect of the hexane extract from *H. brasiliana* evaluated by the paw edema method induced by carrageenan is shown in Table 3. Edema inhibition was observed 3 h after carrageenan application in the doses of 100 (0.86 ± 0.02; 10.42%; $p < 0.05$) and 200 mg/kg (0.81 ± 0.02; 15.62%; $p < 0.01$). Four hours after carrageenan injections, the doses of 100 (0.78 ± 0.02; $p < 0.05$) and 200 (0.70 ± 0.02; $p < 0.001$) reduced the paw edema in 8.23 and 17.65%, respectively. Indomethacin (10 mg/kg), reference drug, was active from 2 h of treatment.

Effects on carrageenan-induced pleurisy in rats

The anti-inflammatory effect of the hexane extract from *H. brasiliana* was confirmed by a decrease in exudate volume and leucocyte migration to the pleural cavity of rats. The pleurisy effects demonstrated that dose of 200 mg/kg (0.87±0.05; $p < 0.05$) of the extract significantly reduced the exudate volume by 19.44% when compared

Table 2. Effects of the hexane extract from *H. brasiliana* leaves on the latency time of mice exposed to the hot plate test.

Group	Dose (mg/kg)	Time after drug administration (s)			
		0 min	30 min	60 min	90 min
Control	Saline	6.12±0.64	6.37±0.60	6.25±0.65	6.50±0.60
	50	6.50±0.50	6.50±0.57	7.12±0.58	7.50±0.42
Extract	100	6.75±0.65	6.87±0.51	7.50±0.42	8.12±0.40*
	200	6.62±0.65	7.37±0.60	8.87±0.48**	10.12±0.51***
Morphine	1	6.25±0.65	10.25±0.67***	12.37±0.86***	15.25±0.80***
Naloxone+morphine	1 + 1	6.00±0.71	9.15±0.64**	8.75±0.67*	7.87±0.80
Naloxone+extract	1 + 200	6.62±0.50	7.12±0.72	8.12±0.44*	7.75±0.53

Data are mean ± S.E.M. of eight mice. *p < 0.05; **p < 0.01; ***p < 0.001 *vs* control group (saline).

Table 3. Effects of the hexane extract from *H. brasiliana* leaves on carrageenan-induced paw edema in rats.

Group	Dose (mg/kg)	Volume of hind paw (ml)			
		1 h	2 h	3 h	4 h
Control	Saline	0.55±0.02	0.76±0.02	0.96±0.03	0.85±0.02
Indomethacin	10	0.53±0.02	0.56±0.02***	0.62±0.02***	0.47±0.01***
	50	0.56±0.02	0.77±0.02	0.94±0.03	0.84±0.02
Extract	100	0.56±0.01	0.76±0.02	0.86±0.02*	0.78±0.02*
	200	0.54±0.02	0.75±0.02	0.81±0.02**	0.70±0.02***

Data are mean ± S.E.M. of six rats. *p < 0.05, **p < 0.01, ***p < 0.001 *vs* control group (saline).

to the control group (Table 4). The number of total leucocytes was inhibited significantly at the doses of 100 ($12.55 \pm 0.22 \times 10^3$ cells/mm^3; $p < 0.05$) and 200 mg/kg ($11.52 \pm 0.25 \times 10^3$ cells/mm^3; $p < 0.001$) (Table 4). Indomethacin (10 mg/kg) reduced the exudate volume and the leucocyte migration.

DISCUSSION

Considering the phytochemicals, the major components identified in *H. brasiliana* were the alkaloids and these are described as chemical markers of the species (Suárez et al., 1998; Shin et al., 2007; Lee et al., 2008; Severino et al., 2009a; Liao et al., 2011). However, based on the polarity of the compounds extracted in the hexane extract, sesquiterpenes, diterpenes, hydrocarbons and pentacyclic triterpenes were detected in the hexane extract from *H. brasiliana* leaves and these chemical classes may be responsible for the pharmacological activities reported in the present study.

The acute toxicity assay showed that the tested doses of the hexane extract were toxic to mice. However, the largest dose administered (200 mg/kg) is less than the lowest dose applied for determination of the LD_{50} (0.5 g/kg or 500 mg/kg). Probably, the toxic effect was due to the presence of compounds, such as terpenes, detected in the hexane extract, as well as identified substances from this species (Suárez et al., 2002). In the present study, the LD_{50} was used to define the doses that were administered to the animals.

Intraperitoneal injection of acetic acid has been reported to significantly increase level of prostanoids, particularly PGE_2 and $PGF_{2\alpha}$ as well as lipoxygenase products in the peritoneal fluid (Ahmed et al., 2011; Ricciotti and FitzGerald, 2011). Regarding this test, our results clarified that the hexane extract from *H. brasiliana* (Figure 1) presented antinociceptive property by reducing abdominal writhing based on this explanation.

The hexane extract also produced significant inhibition in the both phases of formalin-induced pain. The formalin test is a valid and reliable model for nociception investigation and it is sensitive for several classes of analgesic drugs. This important experiment produces a distinct biphasic response that is characterized by two phases (first and second phases) (Zouikr et al., 2013). Centrally acting drugs such as opioids inhibit both phases equally, while peripherally acting drugs such as nefopam and ketoprofen only inhibit the second phase (Girard et al., 2008). According to the Figure 2, the effect observed

Table 4. Effects of the hexane extract from *H. brasiliana* leaves on pleural exudation and number of leucocytes in carrageenan-induced pleurisy in rats.

Group	Dose (mg/kg)	Exudate volume (ml)	Nº Leucocytes (× 10^3 cells/mm^3)
Control	Saline	1.08±0.06	13.37±0.24
Indomethacin	10	0.62±0.07***	9.27±0.53***
	50	1.07±0.07	13.42±0.27
Extract	100	1.03±0.08	12.55±0.22*
	200	0.87±0.05*	11.52±0.25***

Data are mean ±S.E.M. of six rats. *p < 0.05; ***p < 0.001 *vs* control group (saline).

with the hexane extract suggests that antinociceptive activity may be resulting from central and peripheral actions confirming the writhing test.

In the hot plate procedure, a central model that has a selectivity for opioid-derived analgesics, the oral treatment with hexane extract exerts an antinociceptive action confirming the central activity observed in the first phase of formalin test (100 and 200 mg/kg). This experiment is also considered to be sensitive to drugs acting at the supraspinal modulation level of the pain response (Little et al., 2012) suggesting at least a modulatory effect of the investigated extract. Our results indicated that the analgesia induced by the extract could be dependent on the opioid system, since previous treatment with naloxone reversed the effect (Table 2). In addition, it is possible that this effect was due to a synergistic action of the constituents presented in the hexane extract.

The anti-inflammatory activity of the hexane extract from *H. brasiliana* suggested in the formalin test was confirmed by the carrageenan-induced paw edema model through the reduction on the displaced volume (Table 3). This experiment is a suitable model for evaluating anti-inflammatory drugs, which has frequently been used to assess the anti-edematous effect of natural products (Omar et al., 2012). Moreover, carrageenan-induced rat paw edema is associated with three distinct phases (Patel et al., 2012). The first phase is early mediated by mast cell degranulation and histamine and serotonin release (1 h), the second phase (60 to 150 min) is characterized by bradykinin release and pain, and further eicosanoid (prostaglandins) production in the late phase (3-4 h) (Moore et al., 2010; Patel et al., 2012). The treatment with the hexane extract from *H. brasiliana* reduced the paw edema demonstrating a possible inhibition of the inflammatory mediators in the late phase (Table 3).

Carrageenan-induced pleurisy in rats is considered to be an excellent acute inflammatory model in which fluid extravasation, leukocyte migration and the various biochemical parameters involved in the inflammatory response can be measured easily in the exudate (Patel et al., 2012). Therefore, this method assesses the inflammatory infiltrate and confirms the paw edema results. Non-steroidal anti-inflammatory drugs inhibit the accumulation of exudate and mobilization of leucocytes between 3 and 6 h after the application of carrageenan (Vinegar et al., 1973). By reducing the both volume of exudate and leucocyte migration (Table 4), the hexane extract from H. brasiliana reinforced the anti-inflammatory effect observed in the formalin (Figure 2) and paw edema tests (Table 3).

Antinociceptive and anti-inflammatory activities verified in the present study were also reported with plants belonging Rutaceae family (Lee et al., 2008; Liao et al., 2011). Probably, similar components detected in our experiments could be responsible for these properties, because the chemical analysis of the hexane extract from *H. brasiliana* demonstrated the presence of sesquiterpenes, diterpenes, hydrocarbons and pentacyclic triterpenes, suggesting a synergistic biological action (Heras and Hortelano, 2009). Interestingly, compounds like triterpenes, common in hexanic extracts, have been shown to possess antinociceptive and anti-inflammatory activities (Heras and Hortelano, 2009; Gomes et al., 2010). Based on the classes of compounds detected in *H. brasiliana,* mechanisms of action could be applied to explain the activities observed with hexane extract. For example, the anti-inflammatory activity of extracts from *H. brasiliana* could be associated with the inhibitory effect of triterpenes on the nuclear factor-κB (Harikumar et al., 2010). The anti-inflammatory mechanisms of asiatic acid, a pentacyclic triterpene, have been related to the decrease in the level of MDA, iNOS, COX-2, and NF-κB in the paw edema via increasing the activities of CAT, SOD, and GPx in the liver (Huang et al., 2011).

Therefore, the hexane extract from *H. brasiliana* leaves showed antinociceptive and anti-inflammatory effects as demonstrated by well established methods suggesting a potential alternative for therapeutic purposes and supporting the use of this plant in the Brazilian folk medicine. However, further studies need to be conducted to ensure the safe use.

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