Anti-inflammatory and analgesic activities of *Hypericum brasiliense* (Willd) standardized extract

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ABSTRACT: The anti-inflammatory and antinociceptive activities of the standardized leaves extract (HBSE) of *Hypericum brasiliense* (Guttiferae) were evaluated in animal models. Male Wistar rats were treated with *H. brasiliense* extract (50, 250 and 500 mg/kg, p.o.) in 3% Tween 80 0.9% saline solution. The treatment of the edema induced by carrageenin with HBSE (500 mg/kg) showed significant inhibition when compared to the control group. At this dose, the edema decreased by 31.25% in the third hour after treatment (edema peak), but the dose of 50 mg/kg has inhibited the edema by 53.13% (p < 0.05). At the dose of 50 mg/kg, the decrease of the edema induced by dextran was similar to that caused by cyproheptadine. The decrease of the formation of granulomatous tissue (6.6%) was comparable to the control group. The HBSE inhibited the abdominal constriction induced by acetic acid. At a dose of 50 mg/kg, the inhibition of the abdominal constrictions (46.4%) was comparable to that produced by indomethacin (42.9%). A dose of 250 mg/kg inhibited these contractions by 70.66% when compared to control (p < 0.001). In the hot-plate test, an increase in the latency time was observed at a 50 mg/kg dose. These data suggest that HBSE has anti-inflammatory activity on acute process, developed principally by arachidonic acid derivates and analgesic effect due to its probable involvement in the Central Nervous System.

Keywords: *Hypericum brasiliense*, Guttiferae, anti-inflammatory, antinociceptive, CNS.
INTRODUCTION

Plants belonging to the genus Hypericum (Guttiferae) are well known for their use as antidepressant (Harrer & Schulz 1994; Linde et al., 1996; Vorbach et al., 1997; Wheatly, 1997; Volz, 1997), analgesic (Apaydin et al., 1999), spasmylytic (Jakovljevic et al., 2000; Viana et al., 2007), among other applications (Nepomuceno et al., 2005; Cordeiro et al., 2005; Fritz et al., 2007).

Hypericum brasiliense is an annual bush, known in Brazilian folk medicine as “milfacadas” or “alecrim bravo”. Phloroglucinols, flavonoids and xanthones were found in this specie, as well as in H. perforatum (Rocha et al., 1995).

The aerial parts of H. perforatum have been described to show anti-inflammatory properties due to their inhibitory effects in the expression of pro-inflammatory genes like cyclooxygenase-2 (COX-2), interleukin-6 and inducible nitric-oxide synthase (iNOS), by down-regulation of the DNA binding of the transcription factor signal transducer and activator of transcription-1α (STAT-1α), but not of nuclear factor-κB (Tedeschi et al., 2003).

Miller (1998) showed that out of many species used traditionally as anti-inflammatory, only a few have been known for this action. Hyperforin, the major lipophilic compound found in H. perforatum and other Hypericum species, has been shown to be able to inhibit 5-lipoxygenase (5-LO) formation (IC50 values of about 1-2 μM) and suppressed products with equal potency to the well-documented 5-LO inhibitor zileuton (Dana et al., 2002). Hyperforin, at nanomolar concentrations, induced significant inhibition of various ion channels (such as P-type Ca2+ channels) via interaction with calmodulin or through calmodulin-activated pathways involving at least one second messenger (Krishnal et al., 2001). H. perforatum significantly inhibited the edema induced by carrageenin and PGE2 in rats (Shipchliev et al., 1981), may be due to the mechanisms explained above. Western blot analyses showed that in-vivo treatment with H. perforatum extract could modulate lipopolysaccharide and interferon-γ induced COX-2 and inducible iNOS expression in peritoneal macrophages (Raso et al., 2002).

Dried and lyophilized methanol extracts of other species, H. lalandii (Recio et al., 1995) and H. triquetrifolium (Ozturk et al., 2002), were able to decrease the edema induced by carrageenin in rats as well. H. emperifolium methanol extract (Trovato et al., 2001) exhibited significant anti-inflammatory and analgesic effects, showing that this extract was active against inflammatory pain.

Pre-clinical studies have demonstrated that the extract of Hypericum brasiliense has low toxicity at doses under 1,000 mg/kg (Riel et al., 2002).

In this paper, we report the evaluation of possible anti-inflammatory and antinociceptive activities of a standardized extract of Hypericum brasiliense (Willd).

MATERIAL AND METHODS

Hypericum brasiliense Willd standardized extract

Leaves of Hypericum brasiliense (Willd) were collected in the State of Rio de Janeiro, Brazil, identified by Dr. Leandro Machado Rocha, of Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil. The standardized extract was prepared by selective extraction of flavonoid compounds and xanthone derivates as described earlier (Rocha et al., 1995).

Animals

Wistar male rats (Rattus norvegicus) and Swiss male albino mice (Mus musculus) weighing between 180 g - 200 g and 20 g - 25 g respectively, were used in the experiments for anti-inflammatory and analgesic activities. The animals were acquired from the Biotery of the Universidade do Amapá (UNIFAP) and were kept in polyethylene boxes (n = 5), in acclimatized environment (25 ± 4 ℃), light/dark control each 12 hours (7 a.m. to 7 p.m.). They were kept without food 12 hours prior to the experiments, and water was ad libitum. The local Bioethical Committee approved the assay protocols.

Anti-inflammatory evaluation

Carrageenin-induced rat paw edema

The method used was described by Winter et al. (1962) and modified by Carvalho et al. (1999). Thirty minutes after oral administration of HBSE (50 and 500 mg/kg p.o.), 0.1 ml (1000 μg/paw) of carrageenin (Iota-Fluka-Biochemika Co.) was injected into the right paw and 0.1 ml of saline 0.9% solution into the left paw. The measurement of paw volume was taken during a four-hour period after the stimulus application, using a Plethysmometer (Ugo Basile, Mod. 7540). The inhibition of the inflammation was calculated by measuring the volume difference between the right and left paws in comparison to the control group (3% Tween 80 0.9% saline solution, p.o.) and the group treated with indomethacin (10 mg/kg, p.o., MSD Co.), used as a standard drug for anti-inflammatory activity.

Dextran-induced rat paw edema

The method used was described by Carvalho et al. (1999). Thirty minutes after oral administration of HBSE (50 and 500 mg/kg p.o.), 0.1 ml (100 μg/paw) of dextran (T-70 MW, 70000 Pharmacia) was injected into the right paw and 0.1 ml of saline solution (0.9%) into the left paw. The measurement of paw volume was taken at the second hour after the stimulus. The inhibition of the
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Inflammation was calculated by measuring the volume difference between the right and left paws in comparison to the control group (3% Tween 80 0.9% saline solution, p.o.) and the group treated with cyproheptadine (10 mg/kg, p.o.), used as a standard drug for anti-inflammatory activity.

**Granulomatous tissue formation**

This assay was described by Niemegeers et al. (1975), Swingle & Shideman (1972) and Meier et al. (1950). Pellets weighing approximately 40 mg each were made with 5mm of dental cotton tampon. The pellets were sterilized and then impregnated with 0.4 ml ampicillin aqueous solution at the moment of implantation. Having the animals anaesthetized, the pellets were subcutaneously introduced, through abdominal skin incision. The HBSE group was daily treated (50 and 250 mg/kg), for six consecutive days. The second group was treated, during a six-day period, with dexamethasone (2 mg/kg, topically, MSD Co.). The third group (control) was treated daily, for six consecutive days, with 0.5 ml of distilled water (p.o.). This treatment was initiated 2 hours following the implantation of pellets and continued until the sixth day. In the seventh day, the animals were sacrificed and the granulomas removed, dried for 24 hours (60 °C) and the weigh determined. The difference between the initial and final weighs was considerate the weigh of the produced granulomatous tissue.

**Analgesic activity evaluation**

**Writhing test in mice**

This test was based on the method described by Koster et al. (1959). Groups of mice (n = 8) were orally treated with 50 and 250 mg/kg HBSE doses 30 minutes before intraperitoneal injection of 0.6% (v/v) acetic acid solution (0.25 ml/animal). Indomethacin was used as the standard drug (10 mg/kg, p.o.). The number of muscular contractions was counted during 20 minutes, starting on the 5th minute after the stimulus. Data represent average of the total number of writhes observed.

**Hot-plate test**

The hot-plate test described by Jacob et al. (1974) was used. The animals were placed on an aluminum plate with controlled temperature (50 ± 0.5 °C, Model DS37 Socrel, Ugo Basile - Italy). The reaction time was noted by observing either the licking of the hind paws or the rotation movements at 0, 30, 60, 90 and 120 min after oral administration of HBSE in the doses of 50 and 250 mg/kg. Morphine (4 mg/kg, p.o., Cristália Co.) was used as reference drug.

**Statistical analysis**

The statistical analysis was carried out using ANOVA followed by Dunnet’s test, with p < 0.05. Data are expressed as Media ± S.E.M. n = 6/group.
RESULTS

Rat paw edema

In the assay induced by carrageenin, the treatment with 500 mg/kg of HBSE produced a statistically significant reduction when compared with the control (p < 0.05). In the third hour (edema peak), the reduction was 31.25%. The 50 mg/kg dose decreased the volume by 53.13% when compared to the control (p < 0.05), as shown in Figure 1.

In the edema induced by dextran, HBSE decreased the edema with 50 mg/kg dose when compared to the control. This result was not statistically significant when compared to the group treated with cyproheptadine (Figure 2).

Granulomatous tissue induction

The treatment with HBSE did not reduce the granulomatous tissue formation. The 50 mg/kg dosage inhibited the granulomatous tissue formation by 6.6% when compared to control group (Figure 3).

Writhing test in mice

The HBSE inhibited the writhing process induced by acetic acid in a dose-dependant manner. The 50 mg/kg dose produced an inhibition comparable to that caused by indomethacin, and these were 46.4% and 42.9% respectively (p < 0.01). The 250 mg/kg dosage inhibited the writhing by 70.66% when compared to the control group (p < 0.001) as shown in the Figure 4.

Hot plate test

HBSE, at a dose of 50 mg/kg, increased the latency time of the animals by 42.4% (30 min), 68.1% (60 min) and 55.6% (90 min). Morphine, used as the positive control, increased the latency time by 64.3% (30 min), 97.6% (60 min) and 52.1% (90 min), as shown in the Figure 5.

Rota-rod test

In the rota-rod assay, there was no significant difference (p > 0.05) between the group treated with HBSE and the control group at the dose tested during the observation period (3 hours).

DISCUSSION

The genus Hypericum has been used worldwide to treat pain, inflammation and specially depression.
(Harrer & Schulz, 1994; Linde et al., 1996; Vorbach et al., 1997; Wheatly, 1997; Volz, 1997; Apaydin et al., 1999; Jakovljevic et al., 2000). It is a promising anti-inflammatory drug in chronic diseases (Apaydin et al., 1999), as well as in acute process, which could be in part related to their modulation of COX-2 expression (Shipochliev et al., 1981). The major chemical compounds found in this species, such as dianthrones, naphthodianthrene, anthraquinone, emodin, hyperforin, hypericin and pseudohypericin caused inhibition of 12-lipooxygenase activity (Bezakova et al., 1999).

The *Hypericum brasiliense* standardized extract (HBSE) showed anti-inflammatory activity in the carrageenin-induced rat paw edema, which is an in vivo model commonly used for the assessment of this kind of activity (Winter et al, 1962; Carvalho et al., 1999). The HBSE 50 mg/kg dose inhibited the edema formation in the third hour after the stimulus application, when the arachidonic acid derivatives are actuating, principally PGE\(_2\) (Carvalho et al., 1999). This result could be due to an inhibitory effect on the expression of pro-inflammatory genes like COX-2, interleukin-6 and inducible iNOS (Rocha et al., 1995). The edema induced by dextran is characterized by histamine and serotonin liberation in the first hour after stimulus application, increasing vascular permeability by binding in H\(_1\) and 5-HT\(_2\) receptors located in endothelium (Trowbridge & Emling, 1996). Non-steroidal substances are not able to inhibit this edema due to its mechanisms, which involves arachidonic acid derivatives (Merlos et al., 1990). At a dose of 50 mg/kg, HBSE efficiently reduced this process. Raso et al. (2002) described the modulation of lipopolysaccharides production, interferon-\(\gamma\) induced COX-2 and inducible iNOS expression in peritoneal macrophages by hyperforin, may explain the results found for HBSE.

The granulomatous tissue formation is a model to study the chronic effects of anti-inflammatory drugs (Ismail et al., 1997) and also allows quantifying this process (Swingle & Shidem, 1972). The HBSE treatment did not inhibit the tissue formation, suggesting that this drug is not able to actuate in this experimental chronic process.

The HBSE showed peripheral analgesic activity by the results obtained with the writhing test and central effects by the data obtained with the hot-plate test. This inhibition should be due to central effects, but this did not induce loss of motor coordination (Trovato et al., 2001), as our group found on the rota-rod test. An antinociceptive effect by *H. caprifolatum* and *H. polyanthemum* mediated by the opioid system had been described (Viana et al., 2003). Moreover, this activity seems to depend on at least two chemical substances, which could be flavonoids belonging to quercetin group or dianthrones (Kumar et al., 2001).

Thermal stimulation excites the skin receptors, activating thermosensible and nociceptive fibers (Le Bars et al., 2001). The treatment with HBSE has shown during the first 60 minutes, a central action in any dose and this effect has been more effective with the animals treated with 50 mg/kg doses. It is possible to suggest an analgesic action due to a central action with the data obtained with the hot-plate test after the treatment with HBSE and previous literature data.

The data presented above suggest that HBSE has an anti-inflammatory activity in acute process with the involvement of arachidonic acids derivatives. This can be attributed to the presence of major compounds such as flavonoids, dianthrones and hyperforin (which were detected in thin layer chromatography analysis) and analgesic action due to a possible central action.

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