Antiinflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from *Macrosiphonia velame* (A. St.-Hil.) M. Arg. in animal models

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Macrosiphonia velame (Apocynaceae), popularly known as "velame-branco", is mainly used for treating inflammatory conditions. The antiinflammatory, antinociceptive and antipyretic effects of the hydroethanolic extract of the xylopodium from *M. velame* (HE*Mv*) were evaluated using several animal models. HE*Mv* showed low acute oral toxicity with LD_{50} of 4.176 ± 218.5 mg/kg in mice. In tests of carrageenan and dextran-induced paw edema and carrageenan-induced pleurisy in rats, and croton oil-induced cutaneous dermatitis in mice, HE*Mv* presented systemic and topical antiinflammatory activities. In experiments of nociception induced by acetic acid, formalin and capsaicin in mice, the HE*Mv* evidenced an antinociceptive effect, being active against both inflammatory and neurogenic pain. Additionally, the HE*Mv* prevented brewer's yeast-induced pyrexia in rats**.** It is likely that the pharmacologic mechanism of HE*Mv* may involve the inhibition of different mediators of the inflammatory response, such as histamine, serotonin, prostaglandins and leukotrienes. A preliminary phytochemical study was also undertaken on HE*Mv*, which revealed the presence of flavonoids, phenolic compounds, pentacyclic triterpenoids, saponins, coumarins, catechins, tannins, and alkaloids. Taken together, these results suggest that *M. velame* extract has antiinflammatory, antinociceptive and antipyretic properties and further validate the traditional use of this plant in inflammatory conditions.

Uniterms: *Macrosiphonia velame*/antiinflammatory effect. *Macrosiphonia velame*/antinociceptive effect. *Macrosiphonia velame*/antipyretic effect. *Macrosiphonia velame*/pharmacognosy/experimentel study.

Macrosiphonia velame (Apocynaceae), conhecida popularmente como velame-branco, é utilizada no tratamento de inflamações. Avaliou-se nesse estudo, os efeitos antiinflamatório, antinociceptivo e antipirético do extrato hidroetanólico do xilopódio de *M. velame* (HE*Mv*) em modelos animais. O HE*Mv* apresentou baixa toxicidade aguda oral, com $DL_{50} = 4.176 \pm 218,5$ mg/kg nos camundongos. Nos testes de edema de pata por carragenina e dextrana e pleurisia por carragenina em ratos e dermatite cutânea por óleo de croton em camundongos, o HE*Mv* apresentou atividade antiinflamatória sistêmica e tópica. Nos experimentos de nocicepção induzida por ácido acético, formalina e capsaicina em camundongos, o HE*Mv* inibiu a resposta nociceptiva nos três modelos, mostrando-se ativo tanto na dor de origem inflamatória como neurogênica. Adicionalmente, o HE*Mv* bloqueou a hipertermia induzida por levedura de cerveja nos ratos. É provável que os efeitos farmacológicos observados para o HE*Mv* decorram da inibição de mediadores da resposta inflamatória produzidos pelas vias da COX e/ou LOX bem como da inibição da liberação de histamina e serotonina. A análise fitoquímica do HE*Mv* revelou a presença de flavonóides, compostos fenólicos, triterpenóides pentacíclicos, saponinas, cumarinas, catequinas, taninos catéquicos e alcalóides. Estes resultados validam sob o ponto de vista pré-clínico, o uso popular de *M. velame* em processos inflamatórios.

Unitermos: *Macrosiphonia velame*/efeito antiinflamatório. *Macrosiphonia velame*/efeito antinociceptivo. *Macrosiphonia velame*/efeito antipirético/*Macrosiphonia velame*/farmacognosia/estudo experimental.

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INTRODUCTION

Macrosiphonia velame (A. St.-Hil.) M. Ar*g*., popularly known as "velame-branco", "velame do campo" or "losna do campo", is one of the most used plants as a medicinal resource by rural populations of central Brazil plateau (Guarim-Neto, Morais, 2003).

In interviews carried out with 15 herbal sellers from Goiania-Goias State, Brazil, *M. velame* was cited by 90% of respondents as an effective anti-inflammatory, depurative and antisyphilitic agent (Santos *et al*., 2004). In Mato Grosso, home preparations made with "velame-branco" are used for different purposes, especially as a treatment for inflammation, skin diseases and as a blood depurative (Macedo, Ferreira, 2004; De La Cruz, 2008).

M. velame occurs naturally in soils with gravel cover in areas of Brazilian cerrado. The collection of this plant in an indiscriminate manner coupled with habitat loss due to intensive agriculture, have made this plant rare in the region. In spite of its high usage and risk of extinction, no chemical or pharmacological studies on the plant are available in the literature. Therefore, the aim of the present study was to investigate the anti-inflammatory, antinociceptive and antipyretic effects of hydroethanolic extract of the xylopodium of *M. velame* using experimental animal models.

MATERIAL AND METHODS

Plant material and extraction

The xylopodium of *M. velame* was collected in 2007 in Acorizal, Mato Grosso State, Brazil (GPS coordinates: S15° 04´17.3 W56°20´55.5) and taxonomically identified by Dr Rosilene Rodrigues Silva, a researcher from the Herbarium of the Federal University of Mato Grosso (UFMT). The voucher specimen has been preserved at the UFMT Herbarium under the reference number 38.289. The collection was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA).

1 kg of xylopodium was, cleaned and dried under shade for 7 days at room temperature and shredded in an electric mill (sieve with a mesh size of 5 mm) until powder was obtained. Water-ethanol 75% (1:3 w/v) was added to the powder, which was macerated for 7 days, concentrated in a rotary evaporator under reduced pressure (600 mmHg), at a temperature of 50ºC, with the residual solvent being eliminated in an oven at 40º C, obtaining 60.1 g (yield 6%) of hydroethanolic extract 75% from *Macrosiphonia velame* (HE*Mv*).

Animals

Male Wistar rats and male Swiss-albino mice, supplied by the UFMT Animal House, were used in the experiments. The animals were grouped and housed in propylene cages, receiving a standard dry pellet diet (Purina®, Labina) and water *ad libitum*, under controlled temperature conditions (22±2 °C) and a 12 h light-dark cycle. For the assays, animals were randomly assigned into groups of 6-8, except for the hippocratic and LD_{50} tests. Prior to the experiments, the animals were fasted for 18 h. The experimental protocols were previously approved by the Committee for Ethics in Animal Experimentation (CEPA/UFMT).

Preliminary phytochemical analysis

Phytochemical analysis HE*Mv* of was carried out based on color and precipitation tests, according to Matos (1988).

Acute toxicity assay

Three mice per group were used and treated orally (p.o.) with doses of 500 to 5,000 mg/kg of HE*Mv*. A control animal was used for each dose, receiving vehicle only (10 mL/kg). All animals were individually observed in open field after the extract or vehicle was administered, at 0, 5, 10, 15 and 30 min; 1, 2, 4 and 8 h and, once every day, for 14 days. The results for the general behavioral observations were recorded in a table adapted from Malone (1977). To calculate the LD_{50} of the extract, HE*Mv* at 3,000, 4,000, 4,500 and 6,000 mg/kg doses were administered orally, in groups of 10 mice each (5 male and 5 female mice). After 24 h, the number of dead animals was verified and LD_{50} was calculated according to Miller, Tainter (1944).

Rat paw edema induced by carrageenan and dextran

Rats (180-200g) were treated orally, with vehicle (10 mL/kg), HE*Mv* (20 and 200 mg/kg) and indomethacin (10 mg/kg) in the carrageenan test, or vehicle, HE*Mv* (50, 200 and 800 mg/kg) and cyproheptadine (5 mg/kg) in the dextran test. After 1 h of treatment, the edema was induced by subplantar injection of 100 µL of lambda carrageenan (1% w/v in saline 0.9%) or dextran (1.5% w/v in saline 0.9%) in the left posterior paw of the rats. The contralateral paw received the same volume of saline 0.9%. The volume of the edema of the paw was measured in a plestimograph (7150 model, Ugo Basile®) at 0, 60, 120, 180 and 240 min after injection of carrageenan or 0, 30, 60 and 120 min after injection of dextran (Winter *et al.,* 1962).

Carrageenan-induced pleurisy

Pleurisy was produced in rats (180-200 g) by the intrapleural injection of 0.1mL carrageenan at 2%. The animals received the vehicle, HE*Mv* (50, 200 and 800 mg/kg) or 0.5 mg/kg of dexamethasone orally, 1 h before the injection of the phlogistic agent. After 6 h, the animals were anesthetized with ethyl ether, their thoracic cavities opened, pleural exsudate collected by aspiration, the volume measured (mL) and the total number of leukocytes quantified in a Neubauer hemocytometer (Vinegar *et al*., 1981).

Croton oil-induced cutaneous dermatitis

Mice (25-30 g) were used, topically treated in each ear with 20 µL of vehicle, HE*Mv* (1, 10 and 100 µg) or dexamethasone (5 µg) incorporated in hostacerin® NCB formulation. After 6 h of the injection of the irritant substance, the animals were euthanized by cervical displacement and both ears were extirpated at the base (6 mm diameter) and weighed on analytical laboratory scales (Swingle *et al*., 1981).

Acetic acid-induced writhing test

Mice (25-30 g) were treated orally with vehicle, HE*Mv* (20 and 200 mg/kg) or indomethacin (5 mg/kg). After 1 h, 10 mL/kg of acetic acid 0.6% diluted in saline 0.9% was injected intraperitoneally into each animal and the animal's contortions (writhings) were counted during the subsequent 30 min (Koster *et al*., 1959).

Formalin test

Mice (25-30 g) received vehicle, HE*Mv* (50, 200, 800 mg/kg), indomethacin (10 mg/kg) or morphine (10 mg/kg) orally. After 1 h, each animal received 20 µL formaldehyde solution 2.5% in saline 0.9% in the subplantar region of the left posterior paw. The time spent licking and / or biting the injected paw was timed during the first 5 min (1st phase) and during the 20 – 30 min (2nd phase) interval which followed the injection of the noxic agent (Hunskaar *et al*., 1985).

Capsaicin test

Mice (25-30 g) were pre-treated orally 1 h before the test with vehicle and HE*Mv* (50, 200 and 800 mg/kg), and with morphine 30 min before administration of the noxic agent. Animals subsequently received 20 µL capsaicin (1.6 µg/paw), injected into the subplantar region of the left posterior paw, and the time period the animal kept licking and / or biting the paw over the 5 min that followed was timed and recorded (Sakurada *et al*., 1992).

Brewer's yeast induced pyrexia

Rats (160-180 g) received, subcutaneously, 5 mg of a 20% brewer's yeast suspension in sterile saline 0.9% (w/v). After 21 h (zero time), the rectal temperature was monitored for 1 min using a clinical thermometer introduced 3 cm into the rectal ampoule. Subsequently, vehicle, HE*Mv* (50, 200 and 800 mg/kg) or diclophenac sodium (30 mg/kg) were administered, and the rectal temperature taken again 1, 2 and 3 h after treatment. Another group received 5 mL/kg sterile saline 0.9%, instead of brewer's yeast. After the recommended time, the vehicle was administered orally and rectal temperature was taken (Al-Ghamdi, 2001).

Rota-rod performance test

Male mice (25-30 g) were pre-selected 24 h in advance to eliminate those animals which could not stay on the bar for a period of 60 s. Animals were treated orally with vehicle, HE*Mv* (50, 200 and 800 mg/kg) or intraperitoneally with diazepam (5 mg/kg). The maximum period of time was 60 s, with up to 3 reintroductions to the rota-rod (Letica LI 8200) were allowed (Rosland *et al*., 1990).

Statistical analysis

Results for the parametric tests were expressed as mean ± standard error of the mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Student-Newman–Keuls post-test, with p values <0.05 considered significant.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical study carried out with HE*Mv* showed the presence of alkaloids, catechins, phenolic compounds, coumarins, flavonoids, flavonones, saponins, condensed tannins and pentacyclic triterpenoids.

Acute toxicity test

Doses up to 1,000 mg/kg caused no behavioral alteration in the animals. Reduction in mobility and respiratory frequency was noted at 2,000 and 5,000 mg/kg

of HE*Mv*, as well as mild analgesia (evidenced by tail compression), causing death of 2/3 of the animals at the highest dose, with $LD_{50} = 4{,}176 \pm 218.5$ mg/kg, p.o.

Carrageenan and dextran-induced paw edema

In the carrageenan-induced paw edema, animals which received the vehicle presented a slow edema evolution, reaching peak inflammatory response in the 3rd hour (0.60 ± 0.03 mL). The 20 and 200 mg/kg of HE*Mv* reduced the paw edema in the 1st h (30 and 26.6%, p<0.01, respectively), whereas only the lowest dose was active in the 3rd h, with 21.6% (p<0.01) edema inhibition. Indomethacin (10 mg/kg) was more active than HE*Mv*, inhibiting edemas at all periods of time and reaching maximum effect in the 3rd h (0.38 ± 0.03 mL, p<0.001) (Figure 1).
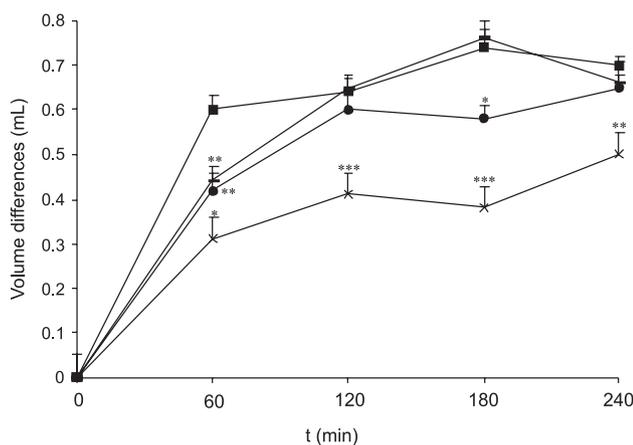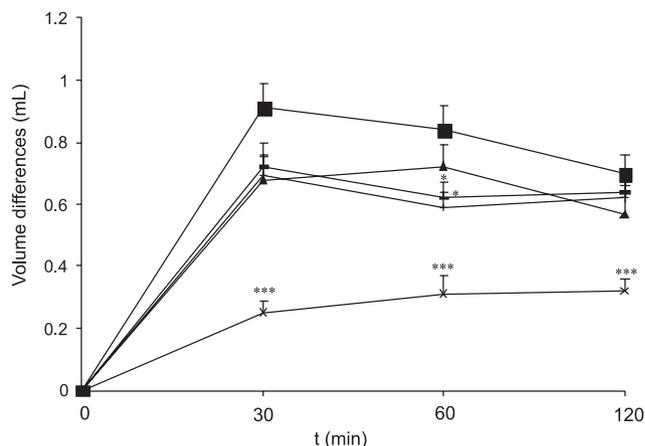

FIGURE 1 - Effect of oral treatment with vehicle (■), hydroethanolic extract of *M. velame* (HE*Mv*) at doses of 20 (♦) and 200 mg/kg (●), or 10 mg/kg of indomethacin (×), on carrageenan-induced paw edema in rats. Each value is the mean ± S.E.M. of 6-8 rats. *p<0.05, **p<0.01, ***p<0.001 compared with control. One-way ANOVA followed by Student-Newman–Keuls.

In the dextran-induced paw edema, rats treated with vehicle developed edema rapidly from the onset with intensity, reaching its peak within 30 min (0.84 ± 0.8 mL). HE*Mv* (200 and 800 mg/kg) reduced the edema by 26.2% and 29.8% (p<0.05), respectively, after 60 min of the phlogistic agent injection. Cyproheptadine (5 mg/kg, p.o.) caused intense edema inhibition, starting from 30 min and maintaining inhibition up to the 2nd hour (54.3%, p <0.01) of the experiment (Figure 2).

Carrageenan-induced pleurisy

In the control group, the intrapleural injection of carrageenan 2% caused plasma leakage (1.74 ± 0.06 mL) and leukocyte infiltration (81.4 ± 1.8 x 10^6 cells).

FIGURE 2 - Effect of oral treatment with vehicle (■), hydroethanolic extract of *M. velame* (HE*Mv*) at doses of 50 (▲), 200 (-) and 800 (+) mg/kg, or 5 mg/kg of cyproheptadine (×), on dextran-induced paw edema in rats. Each value is the mean ± S.E.M. of 7-8 rats. *p<0.05 and ***p<0.001 compared with control. One-way ANOVA followed by Student-Newman–Keuls.

HE*Mv* (50, 200 and 800 mg/kg) reduced plasma exudation and cell migration, in a dose-dependent manner, reaching its strongest effect with an 800 mg/kg dose (17.8% - p<0.01 and 40.3 % – p<0.001, respectively). Dexamethasone (0.5 mg/kg) reduced both exudation (74.1%, p<0.001) and leukocyte infiltration (71.6%, p<0.001) more intensively than HE*Mv* (Figure 3).

Croton oil-induced cutaneous dermatitis

The topical administration of 100 µg croton oil caused an increase of 6.07 ± 0.50 mg in the weight of control mice ears. The local treatment with HE*Mv* (100 µg/ear) in hostacerin® NCB reduced ear edema by 50.4 % (p<0.05), whereas 5 µg dexamethasone reduced edema by 69.2% (p<0.01) (Figure 4).

## Acetic acid-induced writhing in mice

The intraperitoneal injection of acetic acid (0.6%) caused strong nociceptive response in the control group, with 43.3 ± 2.8 abdominal contortions. Treating animals with HE*Mv* (20 and 200 mg/kg) inhibited writhings caused by acetic acid by 57 and 72% (p<0.001), respectively. Indomethacin (5mg/kg), the standard for this kind of experiment, reduced contortions by 91% (p<0.001) (Figure 5).

Formalin test

The injection of formalin (2.5%) coused in the control

group a characteristic biphasic response (Figure 6). The duration of licking was 68.0 ± 2.6 s in the 1st phase (0-5 min) and $70.6 \pm 4.9$ s in the second phase (20-30 min). The results show that, in the 1st phase, HE*Mv* was active only at an 800 mg/kg dose, reducing the nociceptive response by 54.7% (p<0.001), whereas in the 2nd phase HE*Mv* was active for all
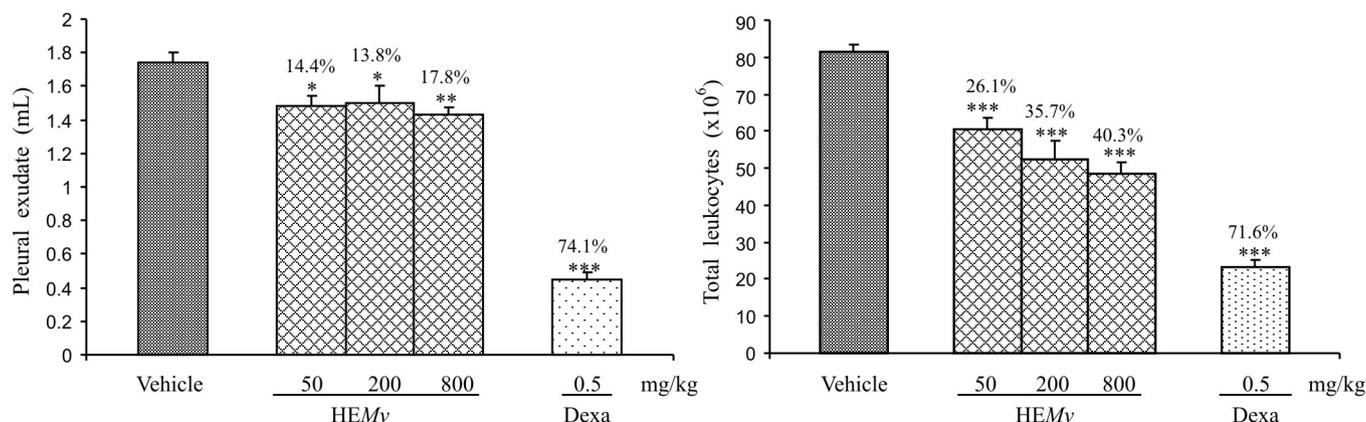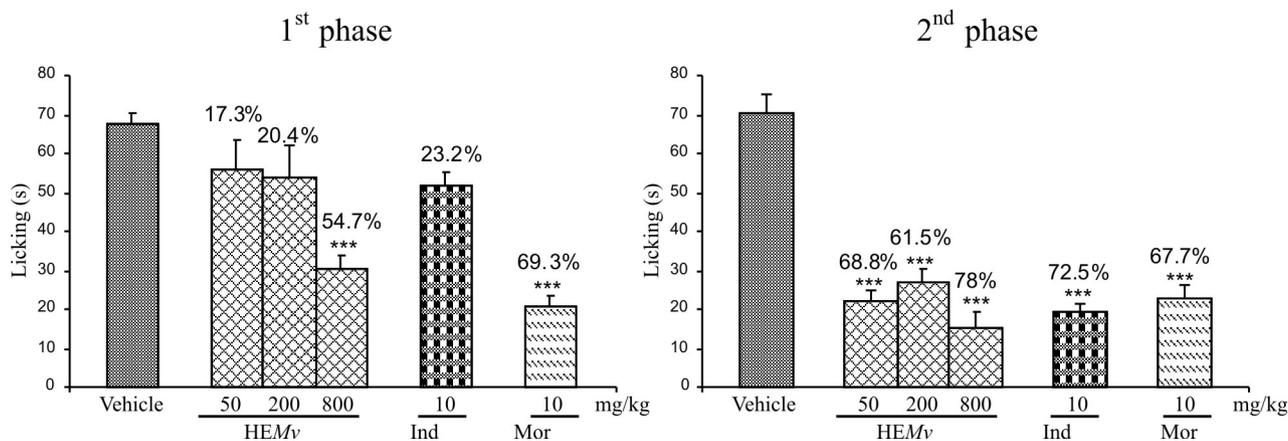

FIGURE 3 - Effect of hydroethanolic extract of *M. velame* on exudate and cell migration carrageenan-induced in rats. Each value is the mean ± S.E.M. of 7-8 rats. *p<0.05, **p<0.01, ***p<0.001 compared with control. One-way ANOVA followed by Student-Newman–Keuls.
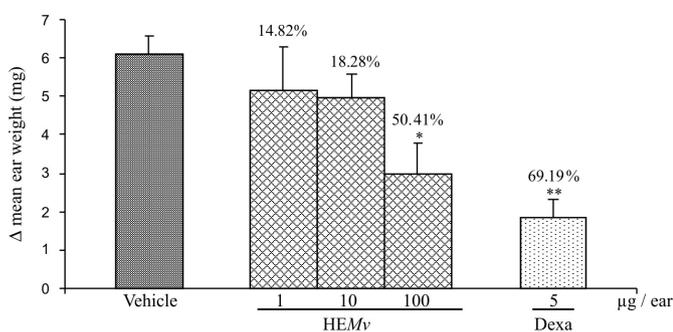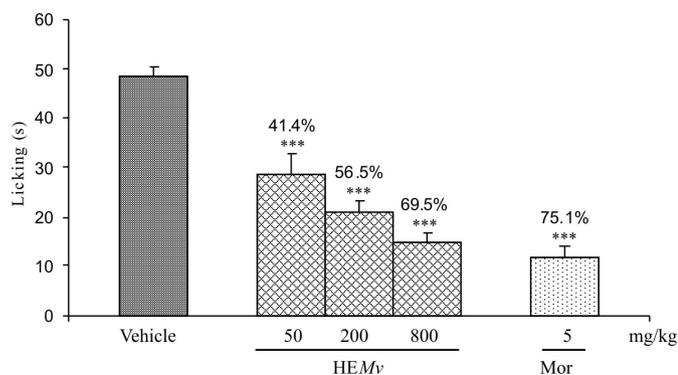

FIGURE 4 - Effect of hydroethanolic extract of *M. velame* in hostacerin® on croton oil-induced cutaneous dermatitis in mice. Each value is the mean ± S.E.M. of 7-8 rats. *p<0.05 and **p<0.01 compared with control. One-way ANOVA followed by Student-Newman–Keuls.
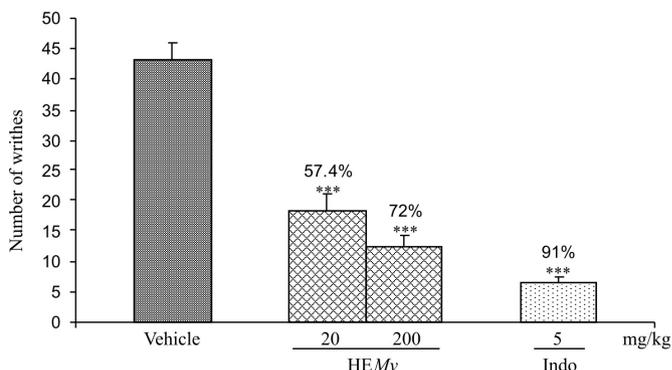
FIGURE 5 - Effect of hydroethanolic extract of *M. velame* on acetic acid-induced writhing in mice. Values are expressed as mean ± S.E.M. (n = 7-8). One-way ANOVA followed by Student-Newman–Keuls test. Significantly different to control, ***p<0.001.

FIGURE 6 - Effect of hydroethanolic extract of *M. velame* on the 1st and 2nd phases of pain induced by formalin injection in mice. Each value is the mean ± S.E.M. of 8 rats. ***p<0.001 compared with control. One-way ANOVA followed by Student-Newman–Keuls.

FIGURE 7 - Effect of hydroethanolic extract of *M. velame* on capsaicin induced nociception in mice. Each value is the mean ± S.E.M. of 7-8 rats. ***p<0.001 compared with control. One-way ANOVA followed by Student-Newman–Keuls.

doses (50, 200 and 800 mg/kg), reaching its maximum effect at the highest dose (78%, p<0.001), exceeding even the 10 mg/kg indomethacin effect (72.5%, p<0.001). Morphine (10 mg/kg, p.o.) was shown to be equally effective in inhibiting the pain response, both in the 1st and 2nd phases, with 69.3 and 67.7% (p<0.001) reductions, respectively.

Capsaicin test

The results presented in Figure 7 showed that HE*Mv* (20, 200 and 800 mg/kg, p.o.) caused significant and dose-dependent capsaicin-induced neurogenic pain inhibition. At the highest HE*Mv* dose, responses reached a similar intensity (69.5%, p<0.001) to that obtained with 5 mg/kg morphine (75.1%, p<0.001), administered subcutaneously.

Brewer's yeast-induced pyrexia

The subcutaneous injection of 20% brewer's yeast suspension substantially increased the rectal temperature of the rats 21 h after administration (38.3 ± 0.13 °C vs. 37.1 ± 0.16 °C, p<0.001). HE*Mv* treatment at 200 and 800 mg/kg doses significantly reduced the rectal temperature of the animals in the 2nd and 3rd hours after administration, reaching peak antipyretic effect at the highest dose in the 2nd hour (37.5 ± 0.08 °C, p<0.001) compared to controls (38.5 ± 0.01 °C). Diclophenac sodium treatment (30 mg/kg) caused significant reduction in rectal temperature at all periods of time, reaching peak antipyretic effect in the 2nd hour among all groups (37.0 ± 0.09, p<0.001), compared to control (38.3 ± 0.08 °C) (Figure 8).

Rota-rod performance test

Treating mice with HE*Mv* (50, 200 and 800 mg/kg)
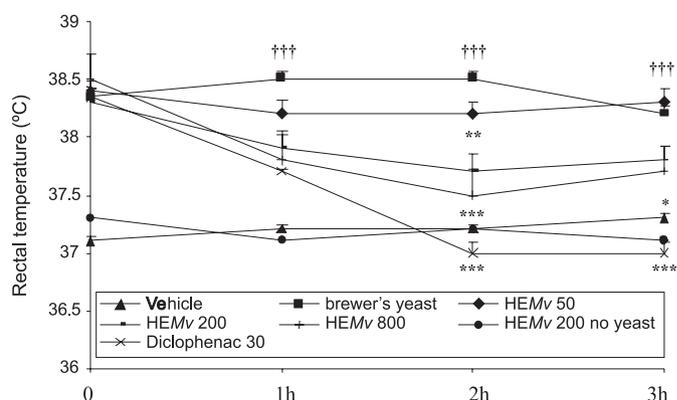
FIGURE 8 - Effect of hydroethanolic extract of *M. velame* on brewer's yeast induced pyrexia in rats. Each value is the mean ± S.E.M. of 6-8 rats. *p<0.05, **p<0.01, ***p<0.001 compared with control or †††p<0.001 compared with yeast. One-way ANOVA followed by Student-Newman–Keuls.

presented no significant alterations (p>0.05) in motor coordination of the mice on the experimental spinning bar at any of the observed times, unlike the animals which received diazepam (5 mg/kg), whose time of permanence on the spinning bar was lower (33.4 ± 5.3 s, p<0.001) compared to the control group (60 ± 0 s).

DISCUSSION

With the aim of providing the first scientific evidence for the popular use of *M. velame*, the pharmacological effects of HE*Mv* were investigated, particularly those related to the inflammatory process.

The carrageenan-induced paw edema model has frequently been used to evaluate the antiedematogenic effect of most anti-inflammatory drugs in routine clinical use (Carvalho *et al.*, 1999). Reports describe that several mediators are released through the injection of carra-geenan in the rat's paw; while histamine, serotonin and bradykinin are released in the initial phase (0-1h), in the later phase (1-6 h) there is an increase in the production levels of prostaglandins (PGs) through the activation of cyclooxygenase-2 (COX-2) and release of nitric oxide (NO) (Silva *et al.*, 2005).

In the carrageenan test, HE*Mv* (20 and 200 mg/kg) exerted significant edema inhibition, albeit to a lesser degree than indomethacin (10 mg/kg), a non-selective COX-inhibitor that decreases PG production (Farsam *et al.*, 2000).

It is known that histamine and serotonin are the principal inflammatory mediators involved in the dextran-induced paw edema (Lo *et al.*, 1982). HE*Mv* caused sig-nificant inhibition of dextran-induced edema, suggesting

that it could interfere with either the degranulation of mast cells, and the consequent release of histamine and serotonin, or with their receptor actions.

Pleurisy and paw edema induced by carrageenan are considered the most appropriate experimental models for evaluating anti-inflammatory substances (Otterness, Gans, 1988). Leukocyte migration to the inflammation site is a key event in the inflammatory process. The injection of carrageenan in the pleural cavity of rodents causes an acute inflammatory response, characterized by accumulation of fluids, and a large number of polymorphonuclear leukocytes (PML) in the pleural cavity. This causes lipid peroxidation and increased production of PGs, leukotrienes, reactive oxygen species and cytokines such as TNF-α and IL1-β (Di Paola *et al.*, 2004). Our results suggest that the plant extract may impact the production of these mediators thereby exerting an anti-inflammatory effect in this pleurisy model.

Croton oil is an irritant agent that induces an inflammatory response when applied to the ear skin through activation of phospholipase A_2 and by initiation of arachidonate metabolism. The released arachidonic acid in turn is metabolized by COX and LOX enzymes promoting the production of eicosanoids and leukotrienes. As a consequence, there is an increase in vascular permeability and hydrostatic pressure, resulting in edema formation and migration of neutrophils to the damaged area. The ear edema reaches its peak in the 6th hour, as a result of the formation of the arachidonic acid metabolites and other pro-inflammatory mediators (Swingle *et al.*, 1981). This experimental model is used to detect COX and or LOX *in vivo* inhibition activity, being very sensitive to steroidal antiinflammatory drugs (SAIDs) and less sensitive to NSAIDs (Swingle *et al.*, 1981; Young *et al.*, 1984). In this model, topical application of HE*Mv* (100 µg) produced a significant antiedematogenic effect, similar in intensity to the result observed with 5 µg dexamethasone. Although the present study established the efficacy of HE*Mv* as an anti-inflammatory agent, its exact mechanism and the mediators involved have yet to be elucidated.

Besides antiinflammatory activity, HE*Mv* demonstrated antinociceptive properties in the experimental models of nociception induced by acetic acid, formalin and capsaicin.

The abdominal contortions test induced by acetic acid – a visceral pain model – despite its high sensitivity and low specificity, is described as a typical model to relieve inflammatory pain (Braggio *et al.*, 2002). The local irritation caused by intraperitoneal administration of this agent, unleashes the release of several mediators, such as bradykinin, substance P and PGs, as well as cytokines

such as IL-1β, TNF-α and IL-8. These mediators activate chemosensitive nociceptors which contribute to the development of inflammatory pain. HE*Mv* (20 and 200 mg/kg) inhibited the mice nociceptive response to acetic acid in a significant and dose-dependent manner, suggesting suppression of inflammatory pain through the inhibition of release/activity of inflammatory mediators (Sekiya, Okuda, 1982).

The formalin test is more specific and frequently used in the study of nociception. The injection of formalin produces a particular biphasic response: the 1st phase involves the release of glutamate excitatory amino acid and sensorial neuropeptides, such as the substance P released from spinal cord sensorial neurons, whereas the 2nd phase depends basically on the liberation of inflammatory mediators and on functional changes in the spinal cord dorsal horn (Otuki *et al.*, 2001). The HE*Mv* blocked the licking response in both phases (the first phase response at the highest dose and 2nd phase at all doses), indicating that the extract is also effective against formalin-induced nociception.

Previous studies have shown that capsaicin induces the release of neurokinins, neuropeptides, excitatory amino acids (glutamate and aspartate), NO and pro-inflammatory peripheral mediators, besides promoting the transmission of nociceptive information to the spinal cord and the activation of vanilloid receptors (Caterina, Julius 2001). The inhibition of capsaicin-induced nociception suggests that HE*Mv* may possibly interfere with the release of neuropeptides or their receptor activation.

The possibility of the HE*Mv* antinociceptive effect being the result of motor deficits or CNS depression can be ruled out since the extract did not impair the animals' motor performance on the rota-rod apparatus.

These results The results in nociceptive tests indicate that HE*Mv* has antinociceptive potential but does not define the exact mechanism, and further does not delineate whether its action is central or peripherally mediated.

The brewer's yeast-induced pyrexia test is of great relevance, as most NSAIDs inhibit the hyperthermal response. The injection of 20% brewer's yeast causes release of pro-inflammatory cytokines which, upon reaching circulation, stimulate the synthesis of PGE_2 in the surroundings of the hypothalamic thermoregulator centers (Chan, Fiscus, 2004). HE*Mv* (200 and 800 mg/kg) reduces the yeast-induced hyperthermia.

HE*Mv* does not appear to cause any kind of acute toxicity when administered through orogastric gavage in the pharmacological assays, presenting a high LD_{50} (4,176 ± 218.5 mg/kg, p.o.), suggesting low oral toxicity.

The preliminary phytochemical studies with HE*Mv* revealed the presence of alkaloids, catechins, phenolic

compounds, coumarins, flavonoids, saponins, condensed tannins and pentacyclic triterpenoids, having been previously described for its many pharmacological effects, including antiinflammatory, antinociceptive and antipyretic activities (Safayhi, Sailer *et al*., 1997; Silva *et al*., 2002, Tuñón *et al*., 2009).

Although the exact nature of the antiinflammatory, antinociceptive and antipyretic activity mechanisms of the phytoconstituents have not been elucidated, the results of the present study validate from a pre-clinical point-of-view, the popular use of this medicinal plant in the treatment of inflammatory diseases.

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