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Original Article

Anti-inflammatory activity and acute toxicity studies of hydroalcoholic extract of *Herissantia tiubae*

Ana L.A. Lima[a], Adriano F. Alves[a], Aline L. Xavier[a], Talissa Mozzini-Monteiro[a], Theresa R.R. Oliveira[b], Fagner C. Leite[a], Wemerson N. Matias[a], Marianna V.S.C. Branco[a], Maria F.V. Souza[a], Marcia R. Piuvezam[a,*]

[a] *Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
[b] *Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil*

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ABSTRACT

Hydroalcoholic extract of aerial parts of *Herissantia tiubae* (K. Schum.) Bricicky, Malvaceae, was evaluated in experimental models of inflammation and toxicity. For toxicity assays, male and female Swiss mice were orally treated with hydroalcoholic extract of *H. tiubae* (2000 mg/kg) and analyzed by consumption of water and food, body weight, mortality and rates of major organ weights, as well as biochemical and hematological indexes. For anti-inflammatory effect, phlogistic agents such as carrageenan or acetic acid were used to evaluate paw edema, cell migration and cytokine production. It was also investigated the hydroalcoholic extract of *H. tiubae* in RAW 264.7 macrophage lineage by nitric oxide and cytokine productions. Swiss mice treated with hydroalcoholic extract of *H. tiubae* showed low toxicity and (50 or 100 mg/kg) was able to reduce significantly ($p < 0.01$, $p < 0.001$) polymorphonuclear cell migration, TNF-α and IL-1β production in the carrageenan-induced peritonitis. However the hydroalcoholic extract of *H. tiubae* (50, 100 or 200 mg/kg) did not reduce carrageenan-induced paw edema. Additionally, hydroalcoholic extract of *H. tiubae* did not present cytotoxicity at concentrations of 6.25, 12.5, 25 or 50 μg/ml but induced significantly decrease of NO, TNF-α and IL-6 production in macrophage lineage. This study suggests that hydroalcoholic extract of *H. tiubae* has anti-inflammatory activity by inhibiting cell migration mainly by decreasing the inflammatory cytokine levels at the inflamed site independently of the anti-edematogenic effect.

Introduction

Malvaceae family has a wide variety of natural compounds with pharmacological properties such as anti-inflammatory, analgesic, anti-rheumatic, among others (Falcão-Silva et al., 2009). *Herissantia tiubae* K. Schum.) Bricicky is one of the species of this botanic family largely found in tropical regions of South America, especially in northeastern Brazil and it is popularly known as "mela-bode" or "lava-prato". The plant is used in folk medicine to treat influenza and fever (Albuquerque et al., 2007). Phytochemical investigation of *H. tiubae* demonstrated the presence of polyoxygen flavonoids, triterpenes, steroid, phenolic compounds and two glycosylated flavonoids (kaempferol 7-*O*-α-L-rhamnopyranoside and 4′,5-dihydroxy-3,6,7,8,3′-pentamethoxyflavone) (Silva et al., 2009). Therefore, the aim of this study was to investigate the anti-inflammatory activity of the hydroalcoholic extract of the aero parts of the *H. tiubae* (HtE) and its toxicity using different experimental models.

Material and methods

Animals

Male and female Swiss mice ($n = 6$/per group, 6–8 weeks, 25–30 g) were used throughout the study. The animals were provided from Prof. Thomas George Vivarium of the Biotechnology Center (CBiotec) from Federal University of Paraíba (UFPB), PB, Brazil. All experimental protocols were approved and performed in accordance with the recommendations of Commission of Ethics for Use of Animals (CEUA) from UFPB, which was recorded under number 0508/12. Animals were kept in polypropylene cage, at room

* Corresponding author.
E-mail: mrpiuvezam@ltf.ufpb.br (M.R. Piuvezam).

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temperature (25 ± 2 °C), under 12 h light/dark cycle, and free access to food and water.

Plant material and preparation of hydroalcoholic extract

Aerial parts of *Herissantia tiubae* (K. Schum.) Brizicky, Malvaceae, were collected in January 2010 in the city of Juazeirinho, Paraiba, Brazil. It was identified by Dr. Maria de Fatima Agra from UFPB. A voucher specimen (n° 2434) is deposited in the Herbarium Lauro Pires Xavier – JPB at the same University. The aerial parts of *H. tiubae* (1 kg) were dried at 40 °C in a circulating air oven for 96 h and ground to powder. Dried and powdered plant material was submitted to extraction by maceration with ethanol–water (70:30) as a solvent at room temperature for 72 h. The ratio of plant material:solvent was 20:80 (w/v) and at the final extraction process the material was filtered and concentrated in rotaevaporator, thus obtaining the HtE.

Chromatography of the HtE

HtE was successively partitioned with hexane, $CHCl_3$, EtOAc and butanol. Kaempferol was isolated from the ethyl acetate extract and subjected to a Sephadex LH-20 gel column eluted with MeOH. Kaempferol was quantified by means of High Performance Liquid Chromatography (HPLC) with ultraviolet detection. Calibration curves to kaempferol were constructed by using the standard addition method. The separation of kaempferol was achieved using a Prominence Chromatographic System (Shimadzu®, Tokyo, Japan) equipped with LC-20AT multi solvent delivery system, degassing system DGU-20A5, autoinjector SIL-20A, oven CTO-20A column and detection by electron spectroscopy in the ultraviolet-visible region with diode array SPD-M20A UV-VIS. Data were collected and integrated through software Class VP V6.14 SP1. The mobile phase consisted of a mixture of methanol:water:H_3PO_4 (1:1:0.01, v/v) pH controlled at 3.1 and the flow rate of 1.2 ml/min in the gradient mode, where the proportion of the organic phase constitutions by 47% for 18 min, from 80% in 23 min and returning to 47% after 28 min. To perform the chromatographic runs, we used a C18 column (Phenomenex®) dimensions 25 cm × 4.6 mm × 5 μm, the UV detector with the wavelength of 351 nm, injection volume of 10 μl temperature 50 °C.

Treatment with HtE

For *in vivo* experiments, the HtE in 2% Tween 20 (Vetec®) and distilled water (vehicle) was orally (*p.o.*) administered at doses of 50, 100 or 200 mg/kg. The untreated control group received an equal volume of the vehicle. For *in vitro* experiments, the HtE was dissolved in dimethylsulfoxide (DMSO), the stock solution was sterilized using a disposable filter unit of 0.22 mm in porosity (Millipore Millex™) and used in the follow range of concentrations 0, 6.25, 12.5, 25, 50, 100, 200 or 400 μg/ml.

Acute toxicological test

Groups of male and female ($n = 6$) Swiss mice were treated orally with HtE (2000 mg/kg) or vehicle. The animals were observed for signs of general toxicity in intervals of 0, 15, 30 and 60 min, 4 and 24 h later and daily for 14 days (Hibbs et al., 1988). During these times, occurrence of central nervous system changes was analyzed: hyperactivity, irritability, aggressiveness, tremors, convulsions, catatonia, analgesia, anesthesia, ptosis, decreased touch response, ambulation, cleaning capacity, raise, and autonomic nervous system changes: diarrhea, constipation, defecation, urination, muscle tone, among others (Almeida et al., 1999). Throughout the experiment, the consumption of water and food intake and weight gain were observed. On day 14th, the treated animals and non-treated animals were euthanized by anesthetic: sodium thiopental (Thiopentax R, Cristalia – Pharmaceutical Chemicals) and organs were removed: heart, liver, kidneys, spleen and thymus to determine its indexes. The weight gain for each animal was determined using the formula:

$$\%\ \text{of weight gain} = \left(\frac{\text{animal weight on first challenge}}{\text{animal weight on last challenge}}\right) - 1 \times 100$$

The index of the weight organs was calculated following the formula below:

$$\text{Index} = \frac{\text{organ weight (mg)}}{\text{animal weight (g)}}$$

Evaluation of biochemical and hematological parameters

On the 14th day animals fasted for 6 h were anesthetized with sodium thiopental and orbital sinus blood was collected using a heparinized Pasteur pipette and transferred into tubes (Eppendorf). The blood was analyzed for hematological (erythrocyte and leukocyte counts) and biochemical parameters (urea, creatinine, uric acid, alanine transaminase-ALT, aspartate transaminase-AST, albumin, total protein, triacylglycerides, glucose and total cholesterol).

Carrageenan-induced mice paw edema

Groups of Swiss mice ($n = 6$) were treated (*p.o.*) with vehicle, indomethacin (10 mg/kg-Roche®) or HtE (50, 100 or 200 mg/kg) 1 h before administration of carrageenan at 2.5% (Sigma-Aldrich®) injected subcutaneously into the plantar region of the left hind paw and phosphate buffer saline (PBS) in right hind paw. Negative control group received 20 μl PBS injections in both paws. Paw diameter was measured with a digital micrometer at 1, 2, 3, 4, 6 and 24 h after stimulation. Results were expressed as difference between the diameter of left and right paws (De Vasconcelos et al., 2011).

Carrageenan induced peritonitis

Mice ($n = 6$) were orally treated with HtE (50 or 100 mg/kg), indomethacin 10 mg/kg or vehicle 1 h before carrageenan (1%) intraperitoneal injection. The basal group received saline. After 4 h the animals were euthanized by xylazine and ketamine overdose and the peritoneal cavity washed with 2 ml of sterile cold PBS, followed by a one-min massage and collection of the fluid (Guerra et al., 2011; Pinheiro et al., 2013). Exudates were centrifuged (10 min, 266 g, 4 °C) and the pellet of cells resuspended in 1 ml of PBS (4 °C), diluted in Turk solution in the ratio of 1:40 and total cells were counted in at Neubauer camera under optical microscope (Nikon E200, Melville, NY – EUA). Differential cell measurement was made in cytocentrifuge – 254 × *g*, 15 min (Cytospin – Bio Research, Washington – USA), slide stained in Fast Panoptic (RenyLab) and counted under optical microscope (100× objective). For each slide a minimum of 100 cells were counted in optical microscope under 1000 magnification (Sousa et al., 2010).

Cytotoxic assay

RAW 264.7 macrophage lineage (ATCC®, Rockville, MD, USA) was cultured in RPMI-1640 medium (streptomycin 10 mg/ml; penicillin 6 mg/ml, kanamycin 2 mg/ml, fetal bovine serum 10% – Gibco®, without phenol red). The cytotoxic effect of the extract were evaluated by MTT assay as first described by Mosmann (1983) with the modifications suggested by Denizot and Lang (1986). Cells were seeded in 96-well plates (2×10^5 cells/well) and incubated for 4 h. After this period, cells were treated with HtE (0, 6.25, 12.5,

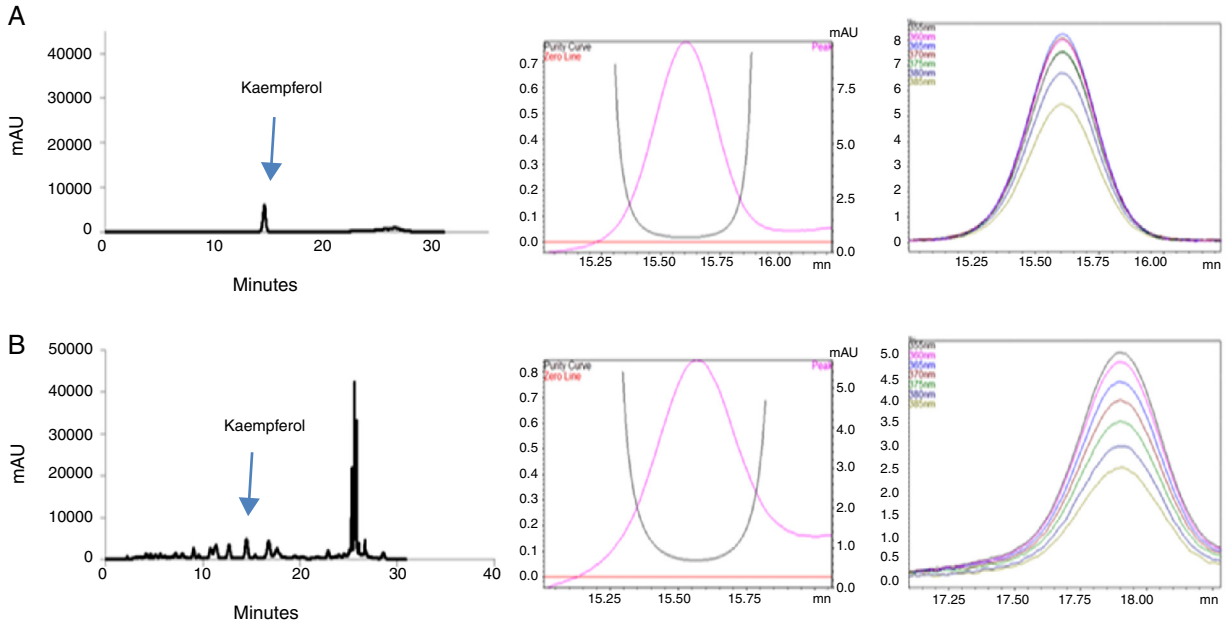

Fig. 1. Chromatographic profile at 370 nm of (A) sample solution of kaempferol standard USP and your DAD spectrum and (B) sample of the hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) and your DAD spectrum.

Table 1
Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on toxical parameters of male and female Swiss mice.

Parameter (unit)	Male (control)	Male (HtE)	Female (control)	Female (HtE)
Feed intake (g)	41.88 ± 2.98	38.13 ± 1.15	37.08 ± 2.73	42.79 ± 1.36
Water consumption (ml)	67.50 ± 3.89	59.29 ± 3.05	40.00 ± 4.42	52.29 ± 2.56**
Initial weight (g)	28.60 ± 1.26	25.50 ± 1.29	30.05 ± 0.98	28.17 ± 1.01
Final weight (g)	38.05 ± 3.22	39.98 ± 1.27	33.14 ± 2.31	33.15 ± 1.07
Weight gain (%)	41.06 ± 6.15	50.19 ± 5.03	14.43 ± 3.46	17.79 ± 1.48
Index heart (mg/g)	3.93 ± 0.08	4.01 ± 0.17	4.14 ± 0.18	4.08 ± 0.16
Index liver (mg/g)	61.27 ± 3.71	56.89 ± 1.11	56.53 ± 2.15	56.25 ± 1.78
Index kidneys (mg/g)	12.66 ± 1.22	11.86 ± 0.99	11.09 ± 0.24	10.70 ± 0.32
Index spleen (mg/g)	5.59 ± 0.21	6.45 ± 0.53	7.56 ± 0.59	6.97 ± 0.62
Index thymus (mg/g)	2.94 ± 0.34	2.84 ± 0.24	3.33 ± 0.23	3.89 ± 0.27

Data are expressed as mean ± S.E.M. and were subjected to analysis of variance of Student's *t*-test.
** $p < 0.01$ when compared to female control group. The data are representative of two experiments with $n = 6$ (per group).

25, 50, 100, 200 or 400 μg/ml) at 37 °C in 5% CO_2 for 24 h. After 24 h, 100 μl of 5 mg/ml MTT solution (Sigma-Aldrich®) was added to each well, followed by incubation for 4 h. The medium was aspirated, and the formazan crystals were dissolved in 100 μl of DMSO for 15 min. The optical density of each well was measured at 570 nm in a microplate reader (Bio-Rad model 550, Japan). Treated cells were compared to non-treated cells.

NO production

The production of NO was determined by measuring the accumulated level of nitrite on RAW 264.7 macrophage supernatants. After pre-incubation of cells (2×10^5 cells/well) at 37 °C in 5% CO_2 for 4 h, the plant extract (0, 6.25, 12.5, 25 or 50 μg/ml) was added and co-stimulated or not with lipopolysaccharide-LPS (1 μg/ml) plus INF-γ (10 ng/ml). The cells were further incubated for 24 h. Amounts of nitrite were measured using Griess reagent. Briefly, 50 μl of cell culture medium was mixed with 100 μl of Griess reagent. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader (Bio-Rad model 550, Japan). The quantity of nitrite was determined from a sodium nitrite standard curve (Green et al., 1982).

Cytokine assays

IL-1β, TNF-α and IL-6 from cell supernatants and peritoneal lavages were measured by ELISA, using the recommended protocol from the antibodies' suppliers. Antibody pairs and standard recombinant cytokines for ELISA assay were purchased from eBiosciences.

Statistical analysis

Data were analyzed by Student's *t*-test, ANOVA followed by Tukey post-test using software GraphPad Prism (GraphPad, San Diego, CA). Values were expressed as mean ± standard error of mean (S.E.M.), and results were considered significant when $p < 0.05$.

Results and discussion

Fig. 1 shows the chromatogram of the HtE (Fig. 1B) and a solution of kaempferol (Fig. 1A), demonstrating that separation of kaempferol was achieved without any interferes from the extract at the same retention time. We performed the validated chromatographic method to confirm the HtE using kaempferol as the chemical marker in all subsequent experiments. Due to the absence

Table 2
Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on biochemical and hematological parameters of male and female *Swiss* mice.

Parameters (unit)	Male (control)	Male (HtE)	Females (control)	Females (HtE)
Biochemical				
Glucose (mg/dl)	123.00 ± 18.61	87.17 ± 7.99	149.00 ± 6.37	150.50 ± 16.76
Urea (mg/dl)	59.18 ± 4.423	50.88 ± 6.22	54.10 ± 3.78	44.42 ± 1.75
Creatinine (mg/dl)	0.46 ± 0.01	0.50 ± 0.07	0.45 ± 0.08	0.37 ± 0.01
Total Cholesterol (mg/dl)	88.02 ± 4.86	98.48 ± 1.06	83.20 ± 3.05	64.84 ± 16.47
Triacylglycerides (mg/dl)	82.00 ± 10.45	97.4 ± 10.25	64.50 ± 5.58	55.00 ± 16.02
Uric acid (mg/dl)	3.43 ± 0.54	2.45 ± 0.65	2.08 ± 0.19	2.00 ± 0.27
AST (U/l)	175.40 ± 19.56	139.0 ± 14.52	184.90 ± 14.16	195.00 ± 7.82
ALT (U/l)	79.66 ± 2.44	115.1 ± 30.80	103.3 ± 12.25	84.12 ± 8.53
Total proteins (g/dl)	5.04 ± 0.30	4.99 ± 0.18	4.99 ± 0.24	5.22 ± 0.21
Albumin (g/dl)	2.53 ± 0.10	2.31 ± 0.19	2.79 ± 0.08	2.52 ± 0.18
Hematological				
Red blood (10^6/mm^3)	9.28 ± 0.78	8.90 ± 0.43	8.15 ± 0.32	8.77 ± 0.14
Hemaglobin (g/dl)	13.77 ± 0.85	13.98 ± 0.23	12.82 ± 0.45	13.67 ± 0.24
Hematrocit (%)	43.30 ± 2.82	44.54 ± 1.48	39.56 ± 1.72	43.38 ± 0.49
VCM (fm^3)	47.00 ± 0.96	48.80 ± 2.31	48.40 ± 0.40	49.80 ± 0.58
HCM (pg)	14.98 ± 0.43	15.96 ± 0.38	15.78 ± 0.14	15.58 ± 0.15
CHCM (g/dl)	31.85 ± 0.35	31.72 ± 0.35	32.30 ± 0.53	31.22 ± 0.19
Leukocytes (10^3/mm^3)	3.73 ± 0.32	3.76 ± 0.38	4.15 ± 0.93	4.28 ± 0.69
Neutrophil (%)	35.67 ± 6.87	21.60 ± 6.95	18.40 ± 4.69	30.00 ± 1.78
Lymphocytes (%)	58.67 ± 6.08	73.00 ± 6.77	74.60 ± 4.93	65.33 ± 1.33
Monocytes (%)	5.66 ± 1.80	4.20 ± 0.73	6.80 ± 1.39	7.16 ± 1.56

Data are expressed as mean ± S.E.M. and were subjected to analysis of variance of Student's *t*-test. The data are representative of two experiments with $n = 6$ (per group). Band, basophil and eosinophil cells were not found in the differential count.

of information about pre-clinical toxicity of this plant, preliminary toxicological evaluation was carried out. HtE (2000 mg/kg) acute oral treatment did not induce signs of general toxicity (Table 1). However, it was observed a significant increase ($p < 0.01$) in water consumption in the female treated group as compared to the female control group. This result may be associated with susceptibility and differential sensitivity to drugs between genders (Anderson, 2008). In addition, no significant changes in biochemical and hematological parameters were observed in the treated animals as compared with the control animals, as shown in Table 2.

We also evaluate the anti-inflammatory effect of HtE considering its use in folk medicine to treat diseases with inflammatory characteristics (Albuquerque et al., 2007). The paw edema induced by carrageenan is widely used to determine anti-edematogenic activity of compounds. In this model, carrageenan promotes an immediate phase (range from 0 through 6 h) that involves several

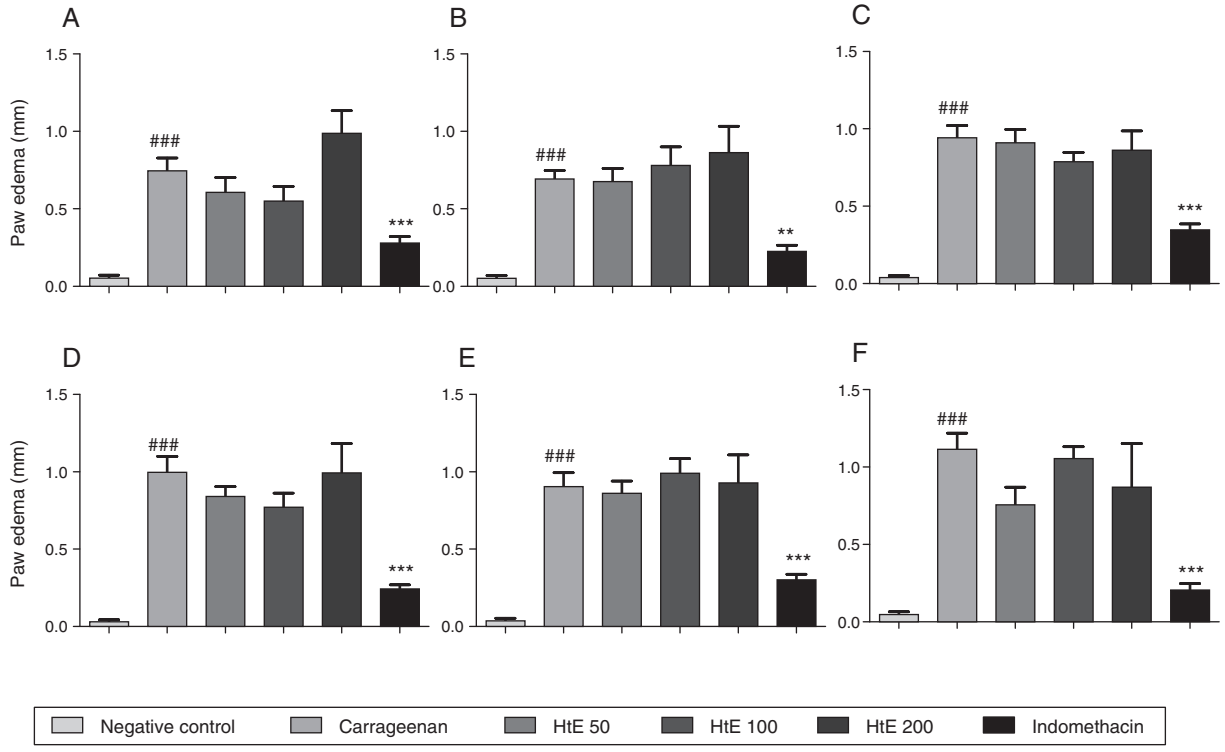

Fig. 2. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on paw edema induced by carrageenan in different times. The data represent the mean ± S.E.M. of the difference between the paws measured in 1 (A), 2 (B), 3 (C), 4 (D), 6 (E) and 24 h (F). $^{###}p < 0.001$ *vs* negative group, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* positive control group (carrageenan) after analysis by ANOVA one way followed by Tukey post-test. The data are representative of three independent experiments ($n = 6$ per group).

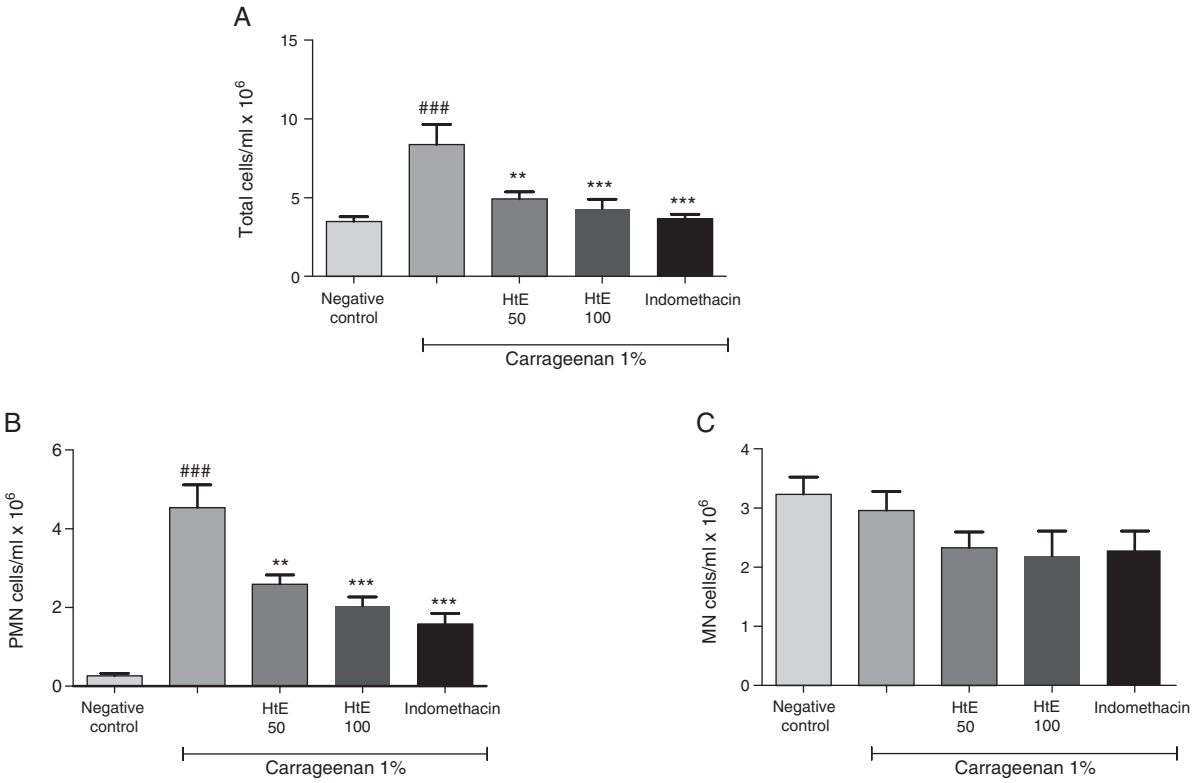

Fig. 3. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) in the carrageenan-induced peritonitis model. Determination of the total cellularity (A), polymorphonuclear cells (PMN) (B) and mononuclear cells (MN) (C). The data represent the mean ± S.E.M. of total and differential cell counts. $^{###}p < 0.001$ *vs* negative group, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* positive control group (carrageenan) after analysis by ANOVA one way followed by Tukey post-test. The data are representative of two experiment with $n = 6$ (per group).

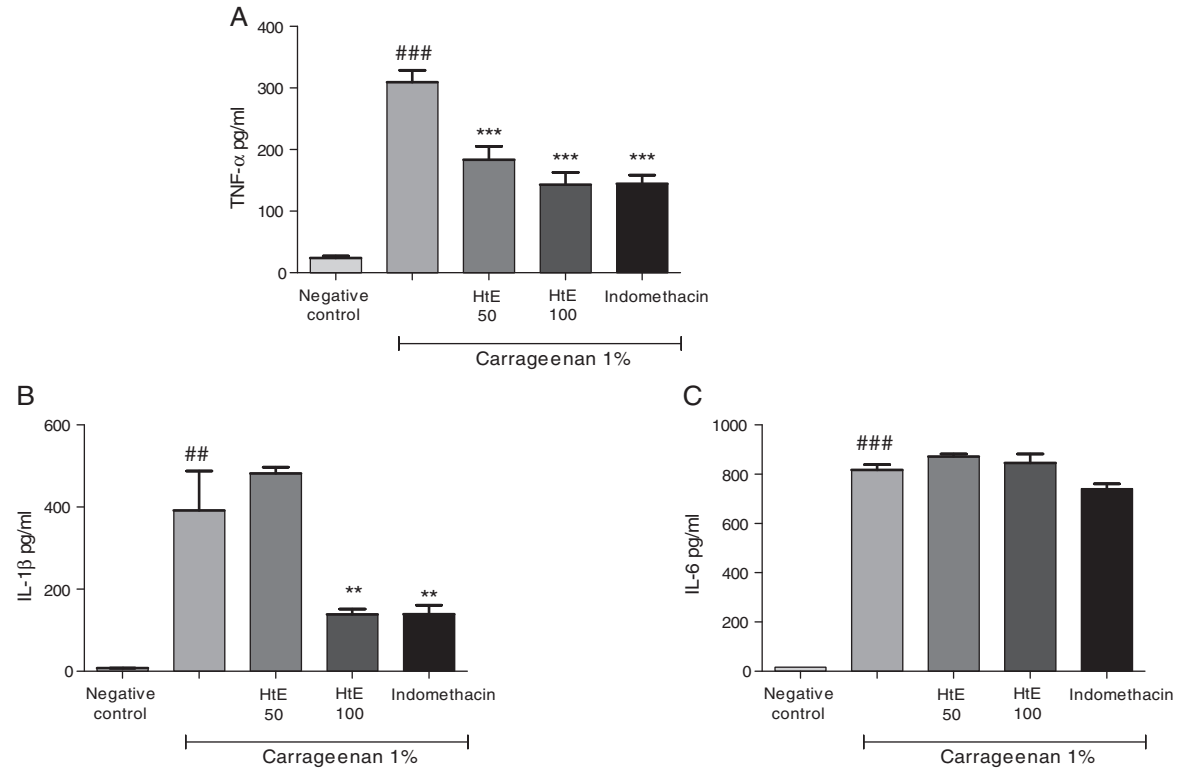

Fig. 4. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on cytokine TNF-α (A) IL-1β (B) and IL-6 (C) levels in peritoneal lavage. The data represent the mean ± S.E.M. of cytokine levels. $^{###}p < 0.001$ and $^{##}p < 0.01$ *vs* negative control group, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* positive control group (carrageenan) after analysis by ANOVA one way followed by Tukey post-test. The data are representative of two experiment with $n = 6$ (per group).

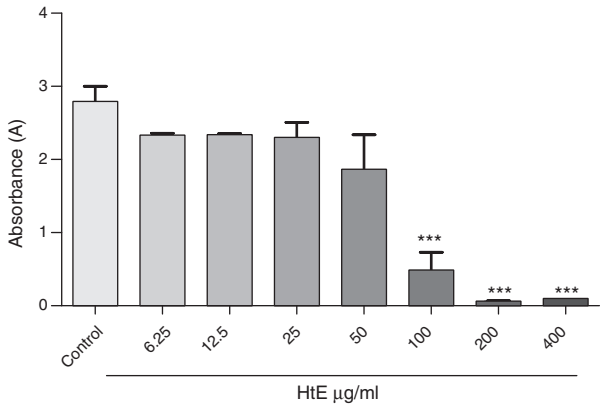

Fig. 5. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on RAW 264.7 macrophages. The data represents mean ± S.E.M. optical densities of cell lysate according to the treatments. ***$p < 0.001$ *vs* control after analysis by ANOVA one way followed by Tukey post-test. The data are representative of two independent experiments.

mediators (histamine, cytokines and NO) and, a late phase (range from 6 through 96 h) where it is observed the leukocyte migration (Posadas et al., 2004). HtE (50, 100, or 200 mg/kg) did not reduce the edema at both phases demonstrating no anti-edematogenic effect (Fig. 2A–F).

In order to study the HtE effect on cell migration to the inflamed site, the carrageenan-induced peritonitis was performed. The mechanism of action by which carrageenan induces the inflammatory processes is a synergism among several mediators (bradykinin, serotonin, prostaglandins, leukotriene B4) (Pinheiro et al., 2013). As shown in Fig. 3A–C, the HtE (50 or 100 mg/kg) significantly reduced ($p < 0.01$ and $p < 0.001$, respectively) the leukocytes and polymorphonuclear cell (PMN) numbers into the peritoneal but did not change mononuclear cell number (MN). These findings suggested that HtE presents anti-inflammatory effect by inhibiting cell migration to the inflamed site without decreases the edema process. Similar results were described by Paiva and colleagues (2013) where *Pseudobombax marginatum*, Malvaceae, extract also inhibited the migration of PMN without affect the MN cell migration.

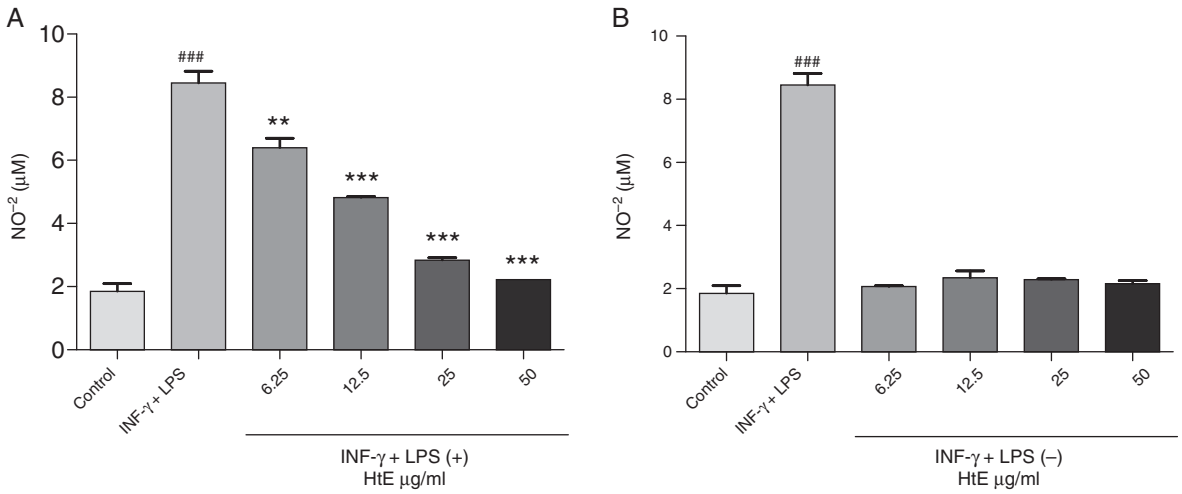

Fig. 6. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on NO production by RAW 164.7 macrophages exposed (A) or not (B) to INF-γ (10 ng/ml) + LPS (1 µg/ml). The data represent mean ± S.E.M. concentrations of nitrite. The data represent two independent experiments. ***$p < 0.001$ and **$p < 0.01$ *vs* the INF-γ + LPS group, ###$p < 0.001$ *vs* control group after analysis by ANOVA one way followed by Tukey post-test. The data are representative of two experiments in triplicate.

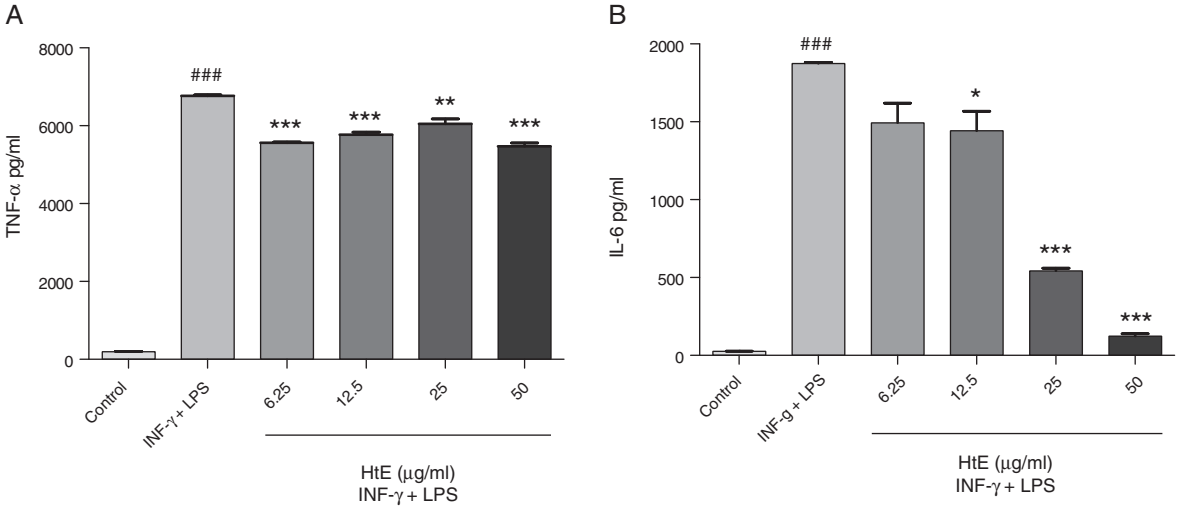

Fig. 7. Effect of hydroalcoholic extract of the aero parts of the *Herissantia tiubae* (HtE) on cytokine (TNF-α (A) and IL-6 (B)) production by RAW 164.7 macrophages exposed (A) or not (B) to INF-γ (10 ng/ml) + LPS (1 µg/ml). The data represent the mean ± S.E.M. of cytokine levels. ###$p < 0.001$ *vs* control, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ *vs* IFN-γ + LPS after analysis by ANOVA one way followed by Tukey post-test. The data are representative of two experiments in triplicate.

The carrageenan-induced peritonitis also involves increases of TNF-α, IL-1β and IL-6 levels at the peritoneal fluid, which presents a key role in inflammatory processes (Loram et al., 2007). In this regard, we analyzed the HtE effect on the cytokine levels at the peritoneal exudate induced by carrageenan injection. Fig. 4A and C shows the HtE (50 or 100 mg/kg) decreased significantly ($p < 0.001$) the levels of TNF-α without reducing IL-6, respectively. However, only HtE at dose of 100 mg/kg was able to reduce the amount ($p < 0.01$) of IL-1β in peritoneal fluid (Fig. 4B). The reduction of IL-1β and TNF-α levels by HtE may be responsible for the inhibition of leukocyte migration, since these pro-inflammatory cytokines promote expression of endothelium adhesion molecules involved in permeability and leukocyte transendothelial migration (Schmidt et al., 2013).

To better understand the anti-inflammatory effect of the extract and its mechanisms of action we used *in vitro* anti-inflammatory assay by measuring the production of NO and cytokines in RAW 264.7 macrophages. First of all, we demonstrated (Fig. 5) that HtE was not toxic for the cells at concentrations ranging from 6.25 to 50 μg/ml. As shown in Fig. 6, the HtE (6.25–50 μg/ml) significantly ($p < 0.01$ – $p < 0.001$) decreased NO production by activated cells (Fig. 6A). In addition, macrophages in presence of HtE did not produce NO (Fig. 6B), demonstrating the absence of endotoxins or other agents capable of initiating an inflammatory response. As shown in Fig. 7, the HtE (12.5 or 50 μg/ml) was able to inhibit significantly ($p < 0.001$ – $p < 0.01$) the production of TNF-α (Fig. 7A) and IL-6 (Fig. 7B). The HtE at 6.25 μg/ml was able to reduce significantly ($p < 0.001$) only TNF-α production (Fig. 7A). These data corroborate with the *in vivo* results and with Park and colleagues (2012) results where the *Wercklea insignis*, Malvaceae, extract also reduced the inflammatory cytokines (IL-6, IL-1β and TNF-α).

In addition, species in the Malvaceae family are known to produce phenolic compounds which exhibit antioxidant action, therefore they can be used to treat several diseases with inflammatory characteristics (Oliveira et al., 2012) and kaempferol which has been described to possess potent anti-inflammatory properties (Devi et al., 2015; Kadioglu et al., 2015). Therefore, these phyto-constituents present in HtE could be contributing to anti-inflammatory activity presented in this study.

In summary, we showed for the first time that HtE presented low toxicity following oral administration at a dose of 2000 mg/kg and also presented anti-inflammatory activity (50 and 100 mg/kg) by modulating inflammatory cells *in vivo* and *in vitro*. Thus, these results can contribute to the main popular use of the *H. tiubae*. However, the mechanisms of action of compounds of HtE on inflammatory processes will be explored in future studies.

Authors' contributions

ALAL carried out the study, participated in the toxicological and anti-inflammatory assays and wrote the paper; MFVS provided the plant; WNM has performed the chemical studies; ALX, TMM and MVSCB provided assistance in the acute toxicity; FCL, AFA, TRRO participated in the anti-inflammatory experiments; MRP supervised the work and corrected the manuscript for publication. All the authors have read the final manuscript and approved the submission.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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