

## Research Article

# Investigations on the anti-inflammatory and anti-allergic activities of the leaves of *Uncaria guianensis* (Aublet) J. F. Gmelin

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**Abstract.** *Uncaria guianensis* (Aublet) J. F. Gmelin is an herbal medicine from tropical areas of South and Central America. We investigated the anti-inflammatory and anti-allergic properties of an ethanolic extract of *U. guianensis* leaves, containing alkaloids, flavonoids and phenol carboxylic acids, as revealed by thin layer chromatography (TLC). Oral pre-treatment with *U. guianensis* inhibited zymosan-induced paw oedema (500 mg/paw) and pleural exudation (100 mg/kg) within 4 h (25–200 mg/kg). *U. guianensis* (100 mg/kg) inhibited total leukocyte and neutrophil numbers in the pleural cavity 4 h after zymosan stimulation. Pre-treatment with *U. guianensis* (100 mg/kg, p.o.) inhibited total leukocyte, neutrophil and eosinophil recruitment into the pleural cavity 24 h after LPS (250 ng/cavity, i.t.). Pre-treatment with *U. guianensis* inhibited paw oedema (25–200 mg/kg) induced by ovalbumin (OVA) within 1 h, and neutrophil and eosinophil recruitment into the mice pleural cavity 24 h after OVA (100 mg/kg). *In vitro* data revealed that *U. guianensis* impaired LPS-induced nitric oxide and CXCL8 generation by murine peritoneal macrophages, as well as OVA-induced interleukin-5 synthesis by previously sensitized spleen cells. These results demonstrate that *U. guianensis* leaves provide effective anti-inflammatory and anti-allergic activities.

**Key words:** *Uncaria guianensis*; Anti-inflammatory; Anti-allergic; Pleurisy, Paw oedema; Nitric Oxide; Cytokines

## Introduction

*Uncaria guianensis* (Aublet) J. F. Gmelin and *U. tomentosa* (Willd.) DC. are closely related species of the Rubiaceae

family, popularly known as cat's claw (unha-de-gato or uña de gato in South America). Both species are large woody vines occurring at the Amazon rain forest and other tropical areas of South and Central America, and are interchangeably used in traditional medicine, since they share the same ethnomedical applications (reviewed by Heitzman et al., 2005). The decoction of their barks is used for the treatment of diabetes, cancer, intestinal affections (Vilches, 1997; Laus & Keplinger, 2003) and inflammatory conditions such as arthritis (reviewed by Heitzman et al., 2005). The dried and powered leaves of *U. guianensis* are used to treat wounds and abscesses (Jones, 1995). The chemical characterization of such species revealed so far alkaloids (whole plant), flavonols (bark) and triterpene glycosides (bark) (Laus & Keplinger, 2003; Sandoval et al., 2002; Yépez et al., 1991).

Despite the broad use of *U. guianensis* in traditional medicine, very few studies have assessed its therapeutic properties. Previous studies using a decoction of the barks supported its anti-inflammatory effects *in vitro* through different mechanisms. Sandoval and coworkers (2002) showed that water extract of the barks of *U. guianensis* was able to inhibit TNF- $\alpha$  mRNA and protein synthesis in stimulated RAW cells *in vitro*, nitrite formation and apoptosis. Moreover, the anti-oxidant activity of the barks of *U. guianensis* has also been demonstrated (Sandoval et al., 2002; Piscocoya et al., 2001). A clinical study has demonstrated the efficacy of *U. guianensis* to treat patients with osteoarthritis of the knee, a phenomenon associated to its ability to inhibit TNF- $\alpha$  and PGE<sub>2</sub> synthesis (Piscocoya et al., 2001).

In the present study we have assessed the anti-inflammatory activity of the ethanolic extract of *U. guianensis* leaves, using murine models of pleurisy and paw oedema. We also investigated the ability of such extract to inhibit the allergic response to antigenic challenge in previously sensitized mice.

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## Materials and Methods

### Plant material and preparation of extracts

The leaves of *U. guianensis* were collected in Jurueña, Mato Grosso, Brazil. A voucher specimen was deposited in the Central Herbarium of Universidade Federal do Mato Grosso (UFMT), Brazil, under the number 24,715, identified by the botanist Pierro Delprete (New York Botanic Garden). The leaves were air-dried at room temperature and milled in a blender. The extract was prepared by exhaustive extraction of the dried and powered leaves (30.0 g) with ethanol at room temperature, followed by filtration and evaporation of the solvent at low pressure yielding 3.30 g of the dried extract (11%).

### Thin layer chromatography profiles of the extract

In order to obtain preliminary information of the chemical constituents of the leaves extract, the oxindole alkaloid and polyphenol chromatographic profiles were made through thin layer chromatography (TLC). The analyses were carried out by applying 10 µl of a methanol solution (c=50 mg/ml) of the extract to pre-coated silica-gel 60F<sub>254</sub> plates (Merck), in two different systems. For the oxindole alkaloid profile was used the method described by Valente et al., 2005 with hexane-ethyl acetate 5:95 as mobile phase and visualization of the spots by UV irradiation at 254 nm. The crude extract profile was compared at the same chromatographic condition to a reference sample with the six current biomarker pentacyclic oxindole alkaloids and to extracts of leaves and bark of *U. tomentosa*. For the polyphenolic profile, chloroform-methanol-formic acid 3:1:0.1 was used as mobile phase and visualization was done by UV irradiation at 254 nm followed by spraying with NP/PEG reagent and observation under UV light at 365 nm (Wagner & Bladt, 1996).

### Animals

Male Swiss Webster (20–30 g) and C57Bl/6 mice (18–20g), from the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) colony were lodged in a room with controlled temperature (23 ± 2 °C) and lighting (lights on from 7 a.m. – 7 p.m.), with free access to lab chow and tap water. All experimental procedures were performed according to the Ethics Committee for Animal Care and Use (CEUA/FIOCRUZ, license 0050/00) and to the ethical guidelines of International Association for the Study of Pain (Zimmermann, 1983).

### Treatments

Twelve hour-fasted animals were treated orally (p.o.) with 25, 50, 100 or 200 mg/kg of *U. guianensis* ethanolic extract diluted in filtered water, 1h before stimulation. In designed experiments, diclofenac (100 mg/kg in filtered water, p.o., 1h prior) and promethazine (30 mg/kg in sterile saline, intraperitoneally, i.p.) were administered 1 h prior stimulation. In another set of experiments, dexamethasone (2 mg/Kg, i.p.) was used as reference inhibitor, and administered 24 and 1 h before stimulus. In all instances, the final volume injected was 200 µL. Control groups received the same volume of the corresponding vehicle alone.

### Paw oedema

Paw oedema was induced as described by Henriques and co-workers (1987). Briefly, animals received an intraplantar (i.pl.) injection of 50 µL of zymosan (500 µg/paw) into one hind paw. The contralateral paw was injected with the same volume of vehicle (sterile saline) and used as control. The volumes of each hind paw were measured by using a digital plethysmometer (Ugo Basile, Italy) 4h after stimulation. The oedema

was estimated as the difference of volume (µL) between the stimulated and non-stimulated paws. Allergic paw oedema was induced in animals previously sensitized with a subcutaneous injection of 50 µg of ovalbumin (OVA) plus 5 mg of Al(OH)<sub>3</sub>, in a final volume of 100 µL. Fourteen days later, oedema was triggered by an i.pl. stimulation with OVA (3 µg/paw).

### Pleurisy

Pleurisy was induced as described by Henriques and coworkers (1990). Briefly, an adapted needle (13 × 5 gauge) was inserted into the right side of the thoracic cavity to enable the intrathoracic (i.t.) administration of zymosan (100 µg/cavity) or LPS (250 ng/cavity), diluted in sterile saline to a final volume of 100 µl per cavity. Allergic pleurisy was induced by an i.t. injection of OVA (12.5 µg/cavity) in 14 days previously sensitized mice by a subcutaneous (s.c.) injection (100 µl) of an emulsion of complete Freund's adjuvant containing 200 µg of OVA). Control groups received an i.t. injection of the same volume of vehicle. At different time points after stimulation, mice were killed by inhalation of carbon dioxide and their thoracic cavities were washed with 1 ml of PBS containing heparin (20 IU/mL). Total leukocyte counts were performed in an automated particle counter (Z1; Coulter-California). Differential leukocyte counts were determined in cytocentrifuged smears stained with May-Grünwald-Giemsa dye using an oil immersion objective (100 X). For quantification of protein extravasation in the pleural cavity, animals received Evans blue dye (25 mg/kg) intravenously (i.v.) 24h before stimulation. Pleural washes were centrifuged (1,155 g, 10 min) to remove cells, and the Evans blue in the supernatant was quantified by measuring the absorbance at 600 nm using a plate reader (Spectramax 190, Molecular Devices, CA). Results are expressed as µg of Evans blue dye per cavity.

### Cell Viability Assay

The effect of *U. guianensis* extract on the viability of C57Bl/6 peritoneal macrophages was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann, T., 1983). The yellow tetrazolium salt MTT is cleaved by the mitochondrial activity of viable cells, forming a purple formazan derivative. Cells (2.5 × 10<sup>5</sup>/well) were incubated with *U. guianensis* extract (0.5, 5, 50 and 500 µg/mL) for 24 h at 37 °C, and then DMSO (dimethyl sulfoxide) was added to dissolve formazan crystals. Analysis was performed at 540 nm in a microplate reader (Molecular Devices).

### Nitric Oxide Production

Peritoneal macrophages recovered from C57Bl/6 mice were resuspended in complete RPMI (RPMI 1640 supplemented with 10% fetal bovine serum and gentamicin 25 µg/mL). Macrophage viability was determined by trypan blue exclusion. Cells were plated onto 96-well flat culture plates (2.5 × 10<sup>5</sup> cells per well) and allowed to adhere for 1 h. Adherent cells (98% macrophages) were incubated with different concentrations of *U. guianensis* (0.5, 5, 50 and 500 µg/mL) in a final volume of 200 µL per well, in the presence or in the absence of LPS (30 ng/mL), at 37 °C/5% CO<sub>2</sub>. Dexamethasone (100 µg/mL) was used as reference inhibitor. Twenty-four h later, the amount of NO<sub>2</sub> was evaluated in the supernatant by the Griess method (Green et al., 1982). Equal volume of the culture supernatant treatment groups and Griess reagent (1% sulphanilamide and 0.1% N-[naphthyl] ethylenediamine; 1:1) were mixed and the absorbance was measured on a microplate reader at the wavelength of 540 nm. The amount of nitrite was calculated from a NaNO<sub>2</sub> standard curve.

### Enzyme-linked immunosorbent assay

Levels of IL-5 were analysed *in vitro* in the supernatant of splenocytes recovered from previously sensitized (50 mg OVA/5 mg Al(OH)<sub>3</sub>, s.c.)

C57Bl/6 mice. Briefly, spleens were pressed through a stainless steel mesh, and suspended in RPMI 1640 medium. Red blood cells were lysed by hypotonic shock. Erythrocyte-free spleen cells were then washed and cell viability was determined by trypan blue exclusion. Cells were added to a 24 well plate ( $5 \times 10^6$  cells/mL) in RPMI 1640 supplemented with 10% fetal bovine serum, incubated with *U. guianensis* extract (0.5, 5, 50 and 500  $\mu\text{g/mL}$ ), or dexamethasone (100  $\mu\text{g/mL}$ ) for 1 h at 37 °C at 5%  $\text{CO}_2$ , and stimulated with OVA (10  $\mu\text{g/mL}$ ) for 6 h. Analysis was performed in the supernatant by sandwich enzyme-linked immunosorbent assay (ELISA) using matched antibody pairs from PharMingen (San Diego, CA), according to the manufacturer's instructions.

## Material

Zymosan, lipopolysaccharide (LPS, from *Escherichia coli* serotype 055:B5), ovalbumin, dexamethasone, RPMI 1640, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), gentamicin, trypan blue, thioglycolate, Griess reagent (sulfanilamide and N-(1-naphthyl, 10:1) ethylenediamine dihydrochloride, BSA (bovine serum albumin), *o*-phenylenediamine dihydrochloride (OPD), red blood cell lysing buffer and phosphate-buffered saline (PBS) were purchased from Sigma Chemical Co. (St. Louis, MO). Promethazine hydrochloride (i.e. Fenegan®) was obtained from Aventis (Brazil). Streptavidin-conjugated horseradish peroxidase was purchased from Zymed (USA). Aluminum hydroxide was obtained from Sanofi Synthelabo (USA). Diclofenac potassium and saline solution were obtained from the Institute of Drug Technology – Farmanguinhos (Fiocruz, Brazil). Evans blue dye was purchased from Merck (Germany). Sodium heparin (Liquemine®) was obtained from Roche (Brazil). Ethanol (PA grade), used for the extraction, dimethyl sulfoxide (DMSO) and  $\text{H}_2\text{SO}_4$  was purchased from VETEC, Brazil. The following antibodies were purchased from PharMingen (San Diego, CA): purified anti-murine IL-5 mAb, purified anti-murine TNF- $\alpha$  mAb, purified anti-murine CXCL1 (KC) mAb, biotinylated anti-IL-5 mAb, biotinylated anti-TNF- $\alpha$  mAb, biotinylated anti-CXCL8 mAb, as well as the respective murine recombinant IL-5, TNF- $\alpha$  and CXCL-8 (KC).

## Statistical Analysis

Data were reported as the mean  $\pm$  S.E.M. and were analyzed statistically by means of analysis of variance (ANOVA) followed by Newman-Keuls-Student test or Student t test. Values of  $p < 0.05$  were regarded as significant.

## Results

### Alkaloid and polyphenol chromatographic profile of the extract of the leaves of *U. guianensis*

The TLC alkaloid profile of the extract of the leaves of *U. guianensis* seems to present speciophylline, uncarine F, pteropodine and isopteropodine along with other non-identified alkaloids all at low concentration as judged by comparison of the  $R_f$  value and the intensity of their spots with those of the reference sample and also with those of the leaves and bark of *U. tomentosa* (Valente et al., 2005). The TLC polyphenolic profile of the plant extract showed the presence of flavonoids and phenol carboxylic acids by analyzing the color of the spots after detection with NP/PEG-UV (Wagner & Bladt, 1996).

### Effect of the ethanolic extract of the leaves of *U. guianensis* on zymosan-induced paw oedema and pleural exudation

The anti-inflammatory properties of the *U. guianensis* leaf were assessed by the zymosan-induced paw edema and pleurisy in mice previously treated with *U. guianensis* extracts (25, 50, 100 and 200 mg/kg, p.o.). As shown in figure 1, the i.pl. injection of zymosan induced a significant paw oedema in Swiss mice within 4 hours. Pre-treatment with the anti-inflammatory compound used as reference inhibitor (diclofenac, 100 mg/kg, p.o.) was able to inhibit 45% of oedema formation induced by zymosan stimulation. The oral pre-treatment with *U. guianensis* extract significantly inhibited paw oedema triggered by zymosan at all doses tested (25–200 mg/kg), with maximal inhibition of 31% achieved with 100 mg/kg. Table 1 demonstrates that the i.t. injection of zymosan (100  $\mu\text{g/cavity}$ ) induced a significant protein extravasation into the mice pleural cavity 4 h after stimulation. Pre-treatment with diclofenac (100 mg/kg, p.o., 1 h

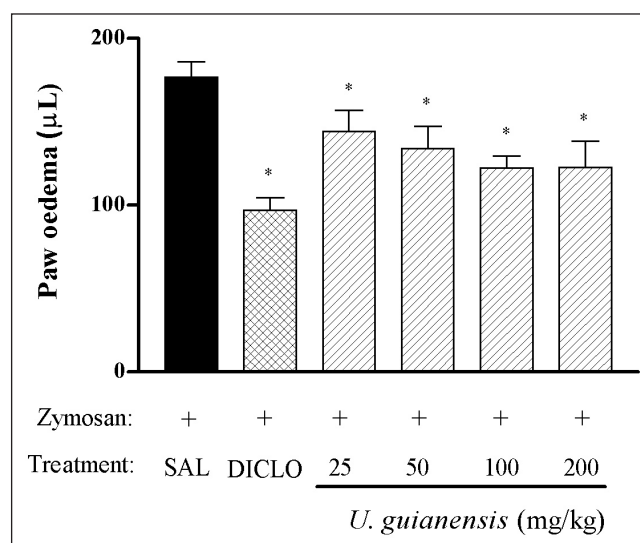


**Fig. 1.** Effect of the pre-treatment with *U. guianensis* ethanolic extract (25–200 mg/kg, p.o., 1 h before stimulation) on paw edema induced by the i.pl. injection of zymosan (500  $\mu\text{g/paw}$ ). Diclofenac (100 mg/kg, p.o.) was used as reference inhibitor. Analysis was performed 4 h after stimulation. Results are expressed as the mean  $\pm$  SEM of at least 7 animals. Statistically significant differences ( $p \leq 0.05$ ) between treated and non-treated groups are indicated by an asterisk.

**Table 1:** Effect of ethanolic extract of *U. guianensis* upon zymosan-induced plasma pleural protein extravasation

| Groups               | Protein ( $\mu\text{g/mL}$ ) |
|----------------------|------------------------------|
| Saline               | 1.22 $\pm$ 0.21              |
| Zymosan              | 3.29 $\pm$ 0.79 *            |
| Diclofenac           | 1.07 $\pm$ 0.13 +            |
| <i>U. guianensis</i> | 1.39 $\pm$ 0.30 +            |

Results are expressed as mean  $\pm$  EPM of three representative experiments. \*  $p \leq 0.05$  compared to saline; +  $p \leq 0.05$  compared to zymosan (ANOVA followed by Neman-Keuls test)

prior zymosan stimulation) and *U. guianensis* extract (100 mg/kg, p.o.) significantly inhibited protein extravasation into the pleural cavity (67% and 58%, respectively). Such result supports the anti-edematogenic activity of *U. guianensis* ethanolic extract, observed in paw oedema.

#### *Effect of the ethanolic extract of the leaves of U. guianensis on zymosan-induced leukocyte accumulation*

According to previous data, the i.t. injection of zymosan induced a marked increase in total leukocyte numbers in mice pleural cavity within 4 h after stimulation (figure 2A), due to neutrophil accumulation (figure 2B; Henriques et al., 1990; Sampaio et al., 2000a). The oral administration of *U. guianensis* extract (100 mg/kg, p.o.) significantly diminished total leukocyte numbers (44%), due to the inhibition of neutrophil

influx (57%; figures 2A, B) into the mice pleural cavity. The reference anti-inflammatory drug diclofenac (100 mg/kg, p.o., 1 h prior) also inhibited zymosan-induced leukocyte influx into the mice pleural cavity (66% and 78% of inhibition of total leukocytes and neutrophils, respectively).

#### *Effect of the ethanolic extract of the leaves of U. guianensis on LPS-induced leukocyte recruitment*

It has been demonstrated that the LPS i.t. stimulation does not induce pleural protein extravasation, but induces an important leukocyte accumulation in the pleural cavity, due to an early neutrophil influx, followed by eosinophil and mononuclear cells accumulation (Bozza et al., 1994a; Penido et al., 1997). In fact, LPS stimulation induced a significant increase in total leukocyte, neutrophil and eosinophil numbers in the mice pleural cavity within 24 h (figure 3), a phenomenon inhibited by dexamethasone (2 mg/kg, i.p., 24 and 1 h prior LPS i.t. injection). We observed that the pre-treatment with *U. guianensis* ethanolic extract (100 mg/kg, p.o.) also significantly inhibited total leukocyte accumulation (33% of inhibition; figure 3 A) due to an impairment of neutrophil and eosinophil recruitment (50% and 41%; figures 3B, C, respectively), confirming the anti-inflammatory activity of *U. guianensis*.

#### *Effect of the ethanolic extract of the leaves of U. guianensis on paw oedema induced by antigenic challenge*

The anti-allergic property of *U. guianensis* was evaluated on paw oedema triggered by the OVA. As illustrated in figure 4, the anti-histaminic compound promethazine was able to inhibit significantly (40%) the oedema formation induced by antigenic challenge. The oral pre-treatment with the extract also significantly inhibited the oedema formation induced by OVA, at all doses tested (25–200 mg/kg, p.o.), in a dose-dependent manner ( $R=0.96$ ), with maximal inhibition of 61%. Such result suggests a potent anti-edematogenic and anti-allergic activity of such extract.

#### *Effect of the ethanolic extract of the leaves of U. guianensis on leukocyte influx during allergic pleurisy*

The murine model of allergic pleurisy was used to assess the anti-allergic activity of *U. guianensis* leaf extract. According to previous published data, we observed an important leukocyte accumulation in the mice pleural cavity 24 h after i.t. antigenic challenge, mainly due to eosinophil and neutrophil influx (Xanthoudakis et al., 1996; Sampaio et al., 2000b) (figure 5). The previous treatment with *U. guianensis* (100 mg/kg, p.o.) markedly reduced the total leukocyte (A), neutrophil (B) and eosinophil (C) migration into pleural cavity. It is noteworthy that the inhibition of neutrophil and eosinophil accumulation by *U. guianensis* (81% for neutrophils and 77% for eosinophils, respectively) was similar to the observed after dexamethasone pre-treatment (84% for neutrophils and 80% for eosinophils, respectively).

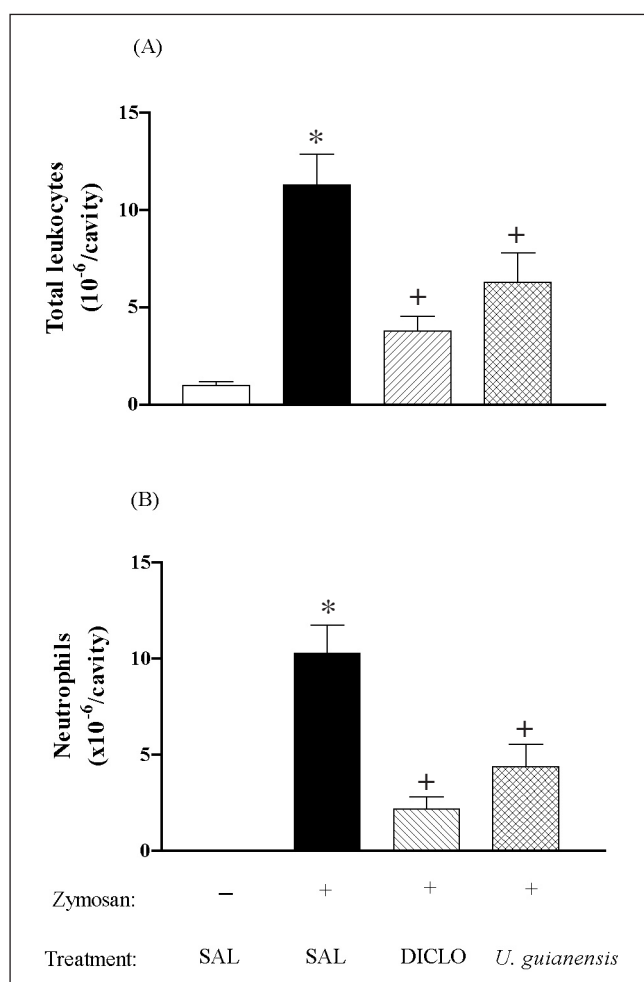


**Fig. 2.** Effect of the *U. guianensis* ethanolic extract (100 mg/kg, p.o.) on zymosan (100 µg/cavity, i.t.)-induced total leukocyte (A) and neutrophil recruitment (B) into the mice pleural cavity. Diclofenac (100 mg/kg, p.o.) was used as reference inhibitor, and administered 1 h before zymosan i.t. injection. Analysis was performed 4 h after stimulation. Results are expressed as the mean  $\pm$  SEM of at least 7 animals. Statistically significant differences ( $p \leq 0.05$ ) between stimulated and non-stimulated groups are indicated by an asterisk, whereas + indicates differences between treated and non-treated groups.

### Assessment of cell viability in mice peritoneal macrophages

In order to investigate the effect of *U. guianensis* upon the production of inflammatory mediators, *in vitro* models were used. Therefore, we first investigated the cytotoxic effects of *U. guianensis* extract on C57Bl/6 mice peritoneal macrophages by MTT assay. As shown in table 2, the incubation of macrophages with *U. guianensis* extract for 24 h was not cytotoxic at all concentrations analyzed (0.5–500 µg/mL).

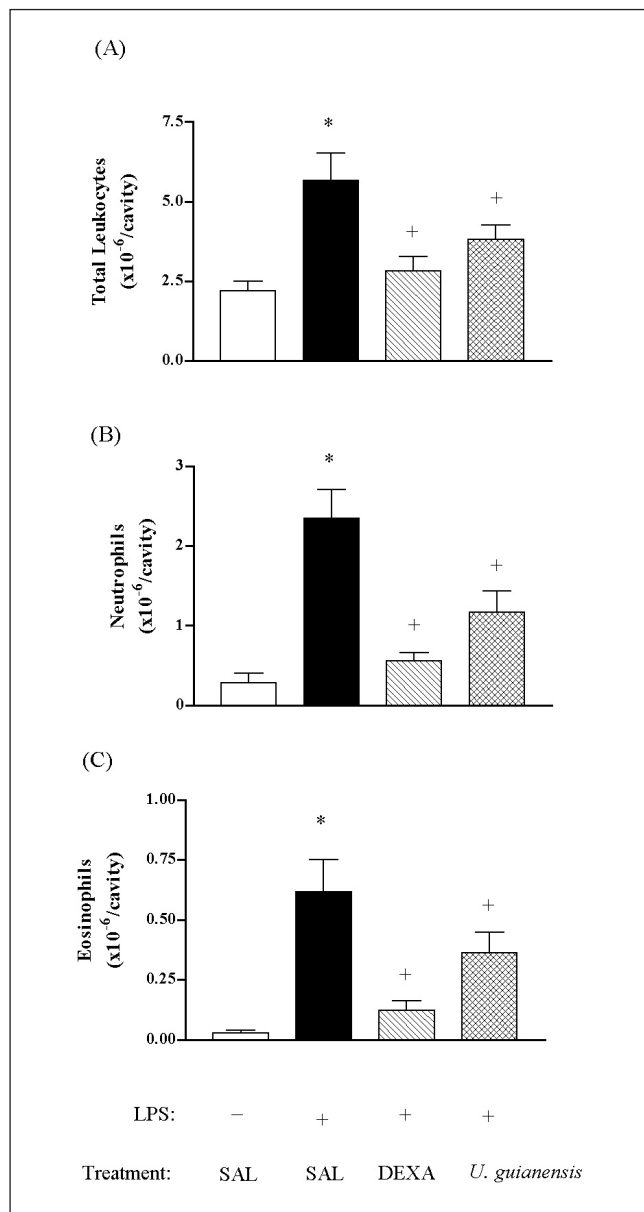


**Fig. 3.** Effect of *U. guianensis* ethanolic extract (100 mg/kg, p.o.) on total leukocyte (A), neutrophil (B) and eosinophil (C) recruitment into the mice pleural cavity induced by LPS (250 ng/cavity, i.t.). Dexamethasone (2 mg/kg, i.p.) was used as reference inhibitor, and administered 24 and 1 h before stimulation. Analysis was performed within 24 h. Results are expressed as the mean  $\pm$  SEM of at least 7 animals. Statistically significant differences ( $p \leq 0.05$ ) between stimulated and non-stimulated groups are indicated by an asterisk, whereas + indicates differences between treated and non-treated groups.

### Effect of the ethanolic extract of the leaves of *U. guianensis* on LPS-induced nitric oxide, CXCL1 and TNF- $\alpha$ production by mouse peritoneal macrophages

LPS stimulation (30ng/mL) was able to induce significant increase in CXCL8, TNF- $\alpha$  and NO generation by murine peritoneal macrophages, as observed in figure 6. The pre-treatment with *U. guianensis* extract (0.5–500 µg/mL) was able to significantly inhibit CXCL8 production, with maximal inhibition of 24%, at 0.5 µg/mL, in the same extent as dexamethasone (20% at 100 µg/mL) (figure 6A). The pre-treatment with such extract also inhibited NO production (5–500 µg/ml), in a dose-dependent manner ( $R=0.96$ ; with maximal inhibition of 61%, at 500 µg/mL; figure 6C). By contrast, *U. guianensis* extract failed to inhibit on TNF- $\alpha$  production (figure 6B) by LPS-stimulated murine peritoneal macrophages.

**Table 2:** Macrophages viability after incubation with ethanolic extract of *U. guianensis*

| Groups                          | Viability (%) |
|---------------------------------|---------------|
| Control                         | 100%          |
| <i>U. guianensis</i> (0.5µg/mL) | 100%          |
| <i>U. guianensis</i> (5µg/mL)   | 100%          |
| <i>U. guianensis</i> (50µg/mL)  | 100%          |
| <i>U. guianensis</i> (500µg/mL) | 100%          |

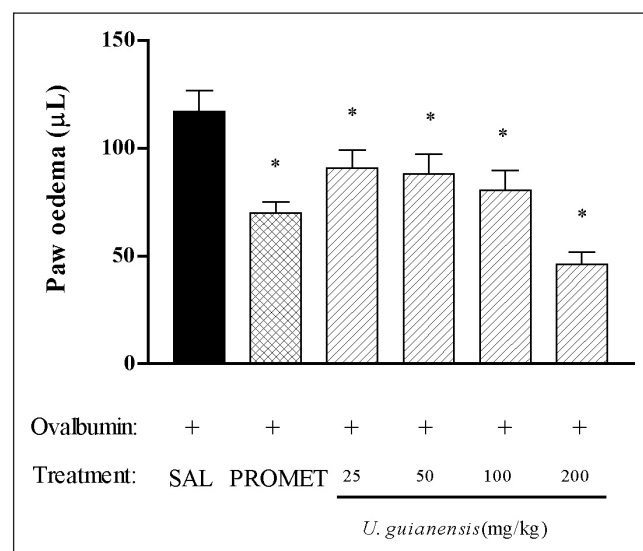


**Fig. 4.** Effect of the pre-treatment with *U. guianensis* ethanolic extract (25–200 mg/kg, p.o., 1 h before stimulation) on paw edema induced by the i.pl. injection of OVA (3 µg/paw) in previously sensitized mice. Promethazine (30 mg/kg, p.o.) was used as reference inhibitor. Analysis was performed 1 h after stimulation. Results are expressed as the mean  $\pm$  SEM of at least 7 animals. Statistically significant differences ( $p \leq 0.05$ ) between treated and non-treated groups are indicated by an asterisk. The dose-effect relationship between doses was detected by linear regression, and the correlation coefficient was  $R=0.96$ .

*Effect of the ethanolic extract of the leaves of U. guianensis on IL-5 production by murine splenocytes*

ELISA assay demonstrated increased levels of IL-5 in the supernatant of OVA-stimulated splenocytes recovered from previously sensitized C57Bl/6 mice (table 3). The pre-treatment of cells with *U. guianensis* extract (0.5–50 µg/mL) was able to inhibit on IL-5 production, with maximal inhibition of 39%, at 50 µg/mL).

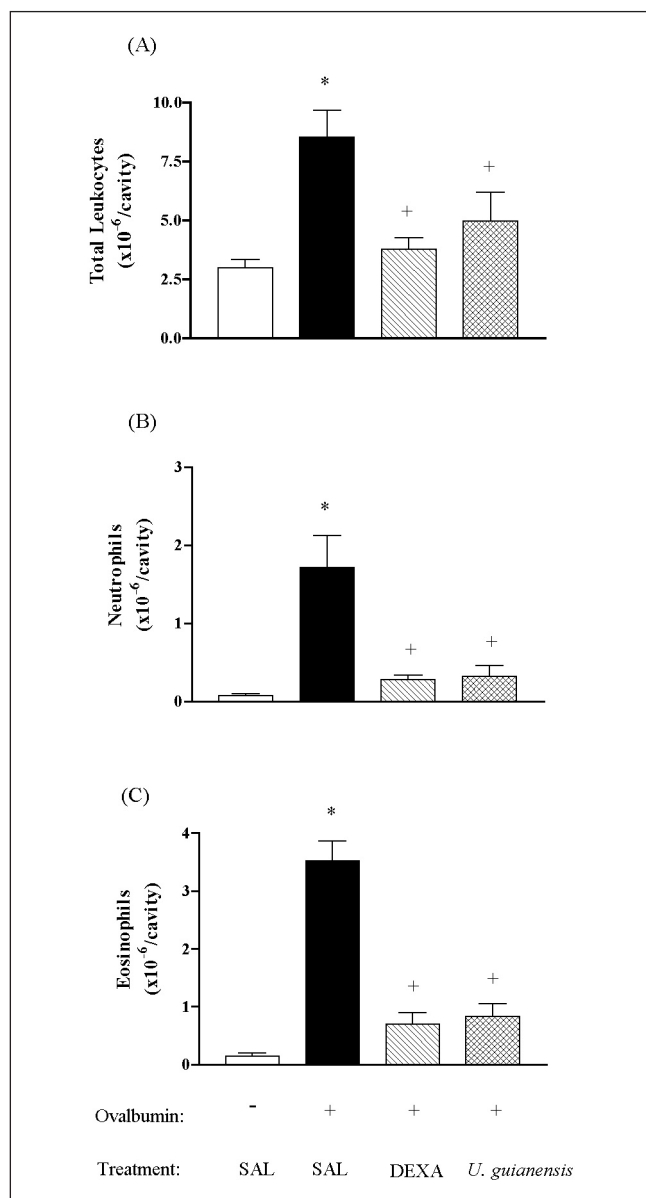


**Fig. 5.** Effect of the oral pre-treatment with *U. guianensis* ethanolic extract (100 mg/kg, 1 h prior stimulus) on total leukocyte (A), neutrophil (B) and eosinophil (C) recruitment induced by OVA (12.5 µg/cavity, i.t.) into the pleural cavity of previously sensitized mice. Dexamethasone (2 mg/kg, i.p.) was used as reference inhibitor, and administered 24 and 1 h before challenge. Analysis was performed within 24 h. Results are expressed as the mean ± SEM of at least 7 animals. Statistically significant differences (p ≤ 0.05) between stimulated and non-stimulated groups are indicated by an asterisk, whereas + indicates differences between treated and non-treated groups.

## Discussion

To date, very few studies have assessed the therapeutic properties of *U. guianensis*. The results of the present work contribute to validate the ethnobotanical use of the leaves of *U. guianensis* as anti-inflammatory agent, and also revealed its anti-allergic potential. The present results clearly

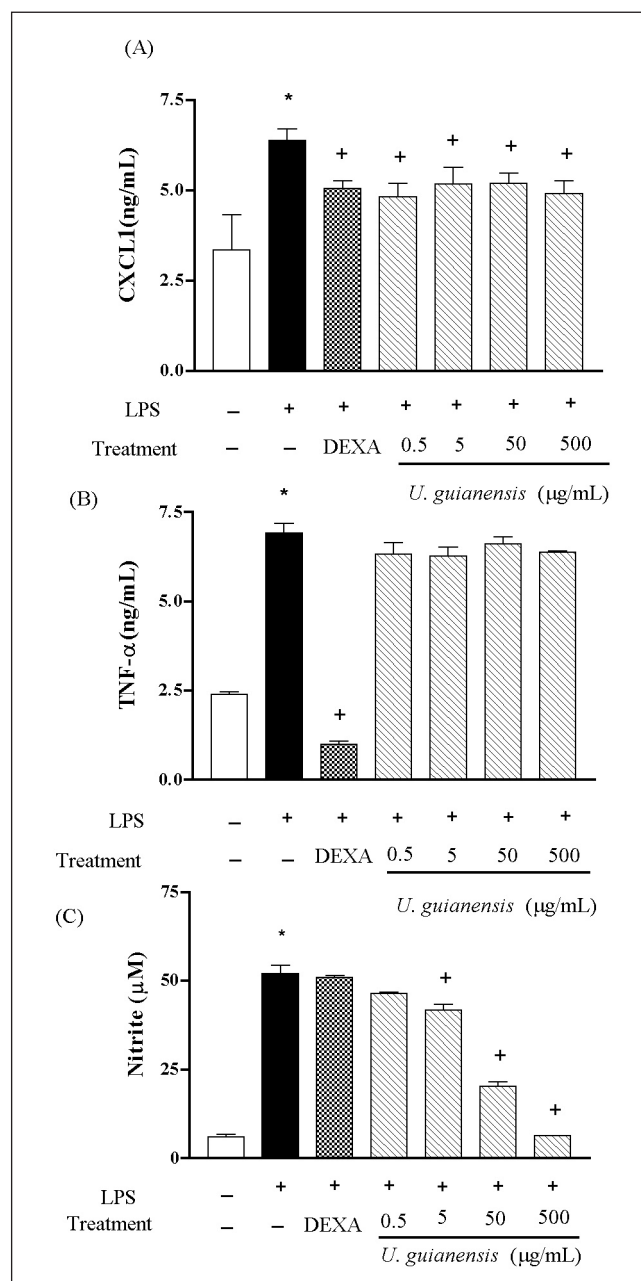


**Fig. 6.** Effect of the ethanolic extract of *U. guianensis* (0.5–500 µg/ml) on CXCL1 (A), TNF-α (B) and nitric oxide (C) production by mouse peritoneal macrophages stimulated with LPS (30 ng/ml). Cells were pre-treated with the extract for 1 h and analysis was performed within 4 or 24 h. Results are expressed as mean ± SEM of two experiments with three samples each. Statistically significant differences (p ≤ 0.05) between stimulated and non-stimulated groups are indicated by an asterisk, whereas + indicates differences between treated and non-treated groups.

**Table 3:** Effect of ethanolic extract of *U. guianensis* upon IL-5 production by splenocytes stimulated with ovalbumin

| Stimulus                        | IL-5 (pg/mL) |
|---------------------------------|--------------|
| Saline                          | 0.0±0.0      |
| Ovalbumin                       | 2.3±20 *     |
| Dexametasone                    | 0.0±0.0 +    |
| <i>U. guianensis</i> (0.5µg/mL) | 2.4±1+       |
| <i>U. guianensis</i> (5µg/mL)   | 1.7±1+       |
| <i>U. guianensis</i> (50µg/mL)  | 1.4±1+       |

Results are expressed as mean ±EPM of three experiments. \*  $p \leq 0.05$  compared to saline; +  $p \leq 0.05$  compared to ovalbumin (ANOVA followed by Neman-Keuls test)

demonstrate the anti-inflammatory activity of the ethanolic extract of *U. guianensis* leaves in different *in vivo* models. The anti-oedematogenic properties of *U. guianensis* was first investigated, since vascular permeability is an important feature of early inflammatory reactions, leading to plasma extravasation and consequent oedema formation at sites of injury. The oral pre-treatment with *U. guianensis* extract showed a marked anti-edematogenic activity, by inhibiting the paw oedema and plasma exudation in the pleural cavity of mice induced by zymosan. These data are similar to the results obtained by Aguilar and colleagues (2002), with *U. tomentosa*, which was also able to inhibit paw oedema induced by carrageenan in mice.

The present report also shows, for the first time, that *U. guianensis* leaves presents an important anti-allergic activity, since it was able to inhibit paw oedema induced by antigenic challenge in previously sensitized mice. Although the production of oedematogenic mediators was not directly investigated, the hypothesis that *U. guianensis* leaves is somehow able to inhibit their effects, might be put forward. Since histamine plays a major role in vascular permeability enhancement during allergic responses, it is especially noteworthy that the extent and potency of the inhibition provided by *U. guianensis* extract (at 200 mg/kg) against OVA-induced paw edema was similar to that of promethazine, the reference anti-histaminic compound tested, suggesting the anti-histaminic property of *U. guianensis*. However, further investigation is necessary to elucidate the mechanisms by which *U. guianensis* is exerting its anti-oedematogenic activity.

Leukocyte influx from blood into the inflammatory site represents a crucial step in inflammatory responses. Early inflammatory reactions are usually characterized by the influx of neutrophils into the inflamed tissues, whereas at late time points, an intense influx of eosinophils and mononuclear cells is usually observed. According to previous data, our results showed a significant neutrophil accumulation in the pleural cavity 4 h after zymosan stimulation (Sampaio et al., 2000a). Such accumulation was significantly inhibited by the *U. guianensis* extract. Cisneros et al. (2005) reported that mice treated with *U. tomentosa* also showed reduced neutrophil numbers in bronchoalveolar lavage (BAL) fluid and bronchioles of inflamed lungs, suggesting that the inhibition of neutrophil accumulation might be one of the important mechanisms by which such species elicit its anti-inflammatory effects.

In accordance to previously published data, LPS and OVA challenge induced a marked increase in total leukocyte numbers in the pleural cavity within 24 h, due to an influx of mononuclear cells, neutrophils and eosinophils (Bozza et al., 1994a,b; Larangeira et al., 2001). *U. guianensis* extract was also able to inhibit the influx of total leukocytes into the pleural cavity triggered by LPS and OVA within 24 h, due to the inhibition of neutrophils and eosinophils, with no changes observed in mononuclear cell numbers (data not shown). It is noteworthy that the marked effect of *U. guianensis* extract pre-treatment in LPS and allergen-induced leukocyte recruitment was also very close to the one induced by dexamethasone.

No studies have previously reported the effects of *U. guianensis* or *U. tomentosa* on the production of neutrophil-tactic mediators. The increased levels of the chemokine CXCL1 in the inflammatory site has been implicated in the mobilization of neutrophils in different conditions and experimental models, such as during the course of rheumatoid and experimental arthritis and allergic inflammation (López-Armada et al., 2002; Danahay et al., 1997). Here, we show that the *U. guianensis* pre-treatment was able to inhibit CXCL1 synthesis by murine macrophages stimulated with LPS *in vitro*, suggesting it might be an important mechanism by which *U. guianensis* impairs neutrophil influx *in vivo*. In addition to that, *U. guianensis* extract also inhibited NO generation, according to previous publish data (Sandoval-Chacón et al., 1998), suggesting an additional mechanism for the reduction of neutrophil influx (Farsky et al., 2004; Salvemini et al., 1996).

It is plausible that the anti-allergic property of *U. guianensis*, marked by its ability to inhibit eosinophil influx, be due to a decreased synthesis of eosinophil-tactic mediators. It is known that interleukin (IL)-5 plays a central role in the orchestration of eosinophil infiltration, and that during allergic responses IL-5 cooperates with eotaxin for eosinophil migration *in vivo* and *in vitro* (Collins et al., 1995 and unpublished data). We have observed an important increase in IL-5 synthesis by splenocytes after antigenic challenged *in vitro*. Such phenomenon was inhibited by *U. guianensis* extract, supporting the *in vivo* inhibition of allergic eosinophilia in the mice pleural cavity. It has been demonstrated that LPS-induced eosinophil accumulation depends on a neo-synthesized protein not yet identified, but it occurs through a mechanism independent of IL-5 or eotaxin (Bozza et al., 1994a; 1994b), therefore, the mechanisms by which *U. guianensis* inhibits LPS-induced eosinophil accumulation has to be further investigated. It is interesting to note that *U. tomentosa* extracts have been shown to inhibit NF-κB activation (Sandoval-Chacón et al., 1998), an activity that can respond for various effects found in that species, since NF-κB coordinates the expression of multiple genes that encode inflammatory cytokines and chemokines, including IL-5 and eotaxin (Yang et al., 1998). However, no data have been published concerning NF-κB inhibition by *U. guianensis*.

Pre-clinical studies on *U. tomentosa* extracts are much more frequent and reported then studies on *U. guianensis*. On the other hand, it has been previously demonstrated that both cat's claw species present a similar qualitative chemical profile (Vilches, 1997) and that is probably the reason why both species are reciprocally used for the same purposes (re-

viewed by Heitzman et al., 2005). Six pentacyclic oxindole alkaloids, speciophylline, mitraphylline, pteropodine, uncarine F, isopteropodine and isomitraphylline, are currently considered to be their biochemical markers and are used to standardize commercial herbal medicines. These alkaloids are found in several parts of cat's claw species, and are usually more abundant in *U. tomentosa* than in *U. guianensis* (Laus & Keplinger, 2003). Sandoval et al. (2002) reported that the aqueous extract of the barks of *U. guianensis* showed a greater antioxidant and anti-inflammatory activity than the similar extract of *U. tomentosa* despite its lower alkaloid and flavanol content. However, comparison of the anti-inflammatory activity of the bark hydroalcoholic extract of *U. tomentosa* against its bark aqueous extract revealed that the first, with higher alkaloid content, was significantly more active than the last one (Aguilar et al., 2002). The bioassay guided fractionation of the anti-inflammatory active extract of *U. tomentosa* led to the isolation of a triterpenoid acid glycoside as a bioactive compound (Aquino et al., 1991). On the other hand Gonçalves et al. (2005) provided some evidences for an antioxidant mechanism underlying the anti-inflammatory activity of the aqueous extract of the barks of *U. tomentosa* that could be correlated to polyphenol compounds present in the extract. All these data point towards, as commented in Heitzman et al. (2005), a possible synergic action of different compounds in the anti-inflammatory activity. However, efforts are being done in order to elucidate the chemical composition of the ethanolic *U. guianensis* extract used in the present work.

In conclusion, the present results indicate that leaves of *U. guianensis* presents a marked anti-inflammatory activity, supporting its use in traditional medicine. In addition, an important anti-allergic effect of *U. guianensis* is here described for the first time. Such effects rely on the inhibition of nitric oxide, CXCL1 and IL-5 synthesis.

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