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Evaluation of the anti-inflammatory efficacy of *Passiflora edulis*

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Abstract

The aim of this study was to investigate the anti-inflammatory effect of the aqueous extract of *Passiflora edulis* forma *flavicarpa* Degenerer leaves in the air pouch inflammation model induced by carrageenan, histamine or substance P, in mice. An aqueous extract was prepared by infusion and lyophilized. Part of the extract was fractionated with *n*-butanol, resulting in the butanolic and aqueous residual fractions. The anti-inflammatory activities of the lyophilized extract and these two derived fractions were evaluated. In the inflammation induced by carrageenan, aqueous extract (100 mg/kg, i.p.), butanolic fraction (50 mg/kg, i.p.), aqueous residual fraction (100 mg/kg, i.p.) and dexamethasone (0.5 mg/kg, i.p.) inhibited the leukocyte, neutrophil, myeloperoxidase, nitric oxide, and interleukin-1 beta (IL-1β) levels ($p < 0.05$). The aqueous extract and butanolic and aqueous residual fractions, but not dexamethasone, decreased macrophage inflammatory protein-2 (MIP-2) levels ($p < 0.05$). Only dexamethasone inhibited mononuclear cells ($p < 0.01$). In the inflammation induced by histamine, the aqueous extract, butanolic and aqueous residual fractions, and dexamethasone inhibited total and differential leukocytes ($p < 0.01$). In the inflammation induced by substance P, the aqueous extract, butanolic and aqueous residual fractions, and dexamethasone also inhibited total leukocytes and mononuclears ($p < 0.01$). Neutrophils were only inhibited by aqueous extract, butanolic fraction, and dexamethasone ($p < 0.05$). In conclusion, the active principle(s) present in the *P. edulis* aqueous extract and its two fractions showed pronounced anti-inflammatory properties, inhibiting cell migration, proinflammatory cytokines, enzymes and mediators.
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Keywords: *Passiflora edulis*; Anti-inflammatory activity; Air pouch; Mediators of inflammation

1. Introduction

The genus *Passiflora*, comprising about 400 species, is the largest in the Passifloraceae family. The species of this genus are well distributed in the warm temperate and tropical regions of the New World, but they are much rarer in Asia, Australia, and tropical Africa (Sacco, 1980). In Brazil, species of the genus *Passiflora* are known as maracujás (Pio Corrêa, 1978). Two types of *Passiflora edulis* are grown commercially, a purple form (*Passiflora edulis* Sims) and a yellow form (*P. edulis* forma *flavicarpa* Degener) (Carvalho-Okano & Vieira, 2001). In Brazil, *P. alata* and its leaf extract, as well as the leaf extracts of other *Passiflora* species, are included as an active component in many phytopharmaceutical preparations (González Ortega, Schenkel, Athayde, & Mentz, 1989).

Yellow passion fruit juice is commonly used for commercial pure or sweetened (due to its slight acidic taste) juice production. The *P. edulis* is very popular, not only because of their fruits (passion fruit), but also because the tea of their leaves has been largely used in American and European countries, in popular medicine, as a sedative

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or tranquilizer, and also against cutaneous inflammatory diseases (Pio Corrêa, 1978). Most of the pharmacological investigations of *P. edulis* have been directed towards its activities in the central nervous system, such as its anxiolytic, anticonvulsant and sedative actions (De Paris et al., 2002; Dhawan, Kumar, & Sharma, 2001; Maluf, Barros, Frochtengarten, Benti, & Leite, 1991; Petry et al., 2001). A part from a recent description on our part of the anti-inflammatory properties of *P. edulis* (Montanher, Zucolotto, Schenkel, & Fröde, 2007), there is only a single report in the literature dealing with the anti-inflammatory activity of *Passiflora incarnata* (Borreli et al., 1996). The aim of this study was therefore to confirm and extend the investigation of anti-inflammatory effects of aqueous extract obtained from leaves of *P. edulis* forma *flavicarpa* Degener, as well as the butanolic and aqueous residual fractions of this extract, in a murine air pouch model. Specifically, we evaluated the effects upon leukocytes in the air pouch induced by different phlogogens (carrageenan: Cg, histamine: HIS, and substance P: SP) and also the effects of myeloperoxidase, nitric oxide, interleukin-1 and macrophage inhibitory protein-2 levels on the inflammation induced by carrageenan. These results were compared to those obtained with a classical steroidal anti-inflammatory drug (dexamethasone).

2. Materials and methods

2.1. Plant material

The leaves of *P. edulis* forma *flavicarpa* Degener (Passifloraceae) (*P. edulis*) were collected in Antônio Carlos located in Santa Catarina State, Brazil. They were collected in October of 2003 and identified by the botanist Prof. Dr. Daniel Falkenberg of the Department of Botany at the Federal University of Santa Catarina, Florianópolis, SC, Brazil. A voucher specimen is deposited in the Herbarium at the same university (ICN 33886).

2.2. Extract preparation

Passiflora edulis leaves were air-dried at room temperature for 14 days. The powdered leaves were extracted using hot water at 90 °C (plant: solvent, 1:10, w/v) under infusion for 10 min. Thereafter, the extract was filtered and an aliquot was lyophilized (Edward® E-C Micromodulyo Freezer Dryer, USA). Part of the aqueous extract was extracted with *n*-BuOH three times (300 ml per occasion), resulting in the butanolic and aqueous residual fractions. These extracts were evaporated under reduced pressure at a temperature below 50 °C, yielding dry residues, considered as the butanolic fraction and aqueous residue.

2.3. Chromatographic analysis

Analytical TLC aluminium sheets coated with silica gel GF254 (Merck) were used. The flavonoids were analyzed using ethyl acetate:formic acid:water (80:10:10, v/v/v) as the mobile phase and methanol solutions of diphenylboryloxyethylamine (1%) (NP Reagent) as the colour reagent. The spots were observed under short- and long-wave UV light. The flavonoids vitexin, isovitexin, orientin and isoorientin (Extrasynthèse, France) were used as reference substances.

2.4. Animals

Swiss mice, weighing 18–25 g, were housed under standardized conditions (room at constant temperature (25 °C) with alternating 12-h periods of light and darkness), humidity 50–60%, and they were fed on a standard mouse diet with water *ad libitum* before use. This study was approved by the Committee for Ethics in Animal Research of our university and experiments were carried out in accordance with the norms of the Brazilian College of Animal Experimentation.

2.5. Experimental protocol

In this experimental protocol, different groups of animals received air injection on three alternate days to induce the air pouch. On the sixth day, the animals received different phlogistic agents (Cg: 1%, HIS: 10 μmol or SP: 40 nmol) administered by subcutaneous route (s.c.), and 24 h later they were sacrificed by an overdose of aether (Da Cunha et al., 2001; Fröde-Saleh, Calixto, & Medeiros, 1999; Saleh, Calixto, & Medeiros, 1996). The animals were fixed on a surgical table and an incision in the dorsal skin was made to perforate the air pouch. The cavity was washed with 1.0 mL of sterile PBS (pH 7.6, composition mmoL: NaCl 137, KCl 2.7 and phosphate buffer salts 10) containing heparin (20 IU/mL). Cell migration was evaluated 24 h after phlogogen administration. Myeloperoxidase (MPO), nitrate/nitrate concentrations (NO^x), interleukin-1 beta (IL-1β) and macrophage inflammatory protein-2 (MIP-2) levels were evaluated only in the protocols of carrageenan-induced inflammation.

Initially, to establish a standard for the *P. edulis* aqueous extract and its two derived fractions (butanolic and aqueous residual fraction), together with the doses and timings to be used in the experiments, different groups of animals were treated (0.5 h before) with different doses (25–100 mg/kg) of aqueous lyophilized extract (AE), butanolic fraction (BuOH) or aqueous residual fraction (AR) administered by intraperitoneal route (i.p.) or treated with sterile saline administered by subcutaneous route (s.c.), and the cell migration was analyzed 24 h after carrageenan injection (Cg 1%, s.c.). In another set of experiments, different groups of animals were pre-treated with a single dose of AE (100 mg/kg), BuOH (50 mg/kg) or AR (100 mg/kg) at different time points (0.5–4 h), and the same inflammatory parameters were evaluated 24 h after the induction of inflammation. According to this protocol, AE (100 mg/kg), BuOH (50 mg/kg) or AR (100 mg/kg) of *P. edulis*,

administered 0.5 h before inflammation induction, were selected as the doses to be used in the experiments below.

Animals were randomly allocated to 16 groups: (1) control-treated with sterile saline (NaCl, 0.9%, s.c.), (2) Cg (1%, s.c.), (3) Cg (1%, s.c..) plus AE (25–100 mg/kg, i.p.), (4) Cg (1%, s.c.) plus BuOH (25–100 mg/kg, i.p.), (5) Cg (1%, s.c.) plus AR (25–100 mg/kg, i.p.), (6) Cg (1%, s.c.) plus dexamethasone (0.5 mg/kg, i.p.), (7) HIS (10 μmol, s.c.), (8) HIS (10 μmol, s.c.) plus AE (100 mg/kg, i.p.), (9) HIS (10 μmol, s.c.) plus BuOH (50 mg/kg, i.p.), (10) HIS (10 μmol, s.c.) plus AR (100 mg/kg, i.p.), (11) HIS (10 μmol, s.c.) plus dexamethasone (0.5 mg/kg, i.p.), (12), SP (40 nmol, s.c.), (13) SP (40 nmol, s.c.) plus AE (100 mg/kg, i.p.), (14) SP (40 nmol, s.c.) plus BuOH (50 mg/kg, i.p.), (15) SP (40 nmol, s.c.) plus AR (100 mg/kg, i.p.), and (16) SP (40 nmol, s.c.) plus dexamethasone (0.5 mg/kg, i.p.).

The *P. edulis* extract (AE) and two derived fractions (BuOH and AR) were administered 0.5 h prior to inflammation induction. In relation to dexamethasone, the animals were treated with two injections of dexamethasone (0.5 mg/kg, i.p.) separated by an interval of 12 h (the first of which was applied 0.5 h before the phlogogens).

2.6. Quantification of cell migration

After killing the animals (24 h after inflammation induction), samples from the air pouch exudates were collected for determinations of total and differential leukocyte contents. Total leukocyte counts were performed in a Neubauer chamber after diluting the exudates with Türk solution (1:20) and cytospin preparations of exudates were stained with May-Grünwald Giemsa for the differential leukocyte count, which was performed under an oil immersion objective (Saleh et al., 1996).

2.7. Quantification of nitrate/nitrite concentrations in carrageenan-induced inflammation

Nitric oxide was measured as its breakdown products nitrite (NO_2^-) and nitrate (NO_3^-) using the Griess method (Green et al., 1982). Samples of the exudation obtained from control animals (treated with sterile saline), carrageenan-treated animals and animals pre-treated with either *P. edulis* aqueous extract or the two derived fractions (BuOH and AR) of *P. edulis* or dexamethasone were collected, separated and stored at −20 °C. The levels of nitrate/nitrite were determined as previously described by Saleh, Calixto, and Medeiros, 1999. Results were expressed as μM.

2.8. Quantification of myeloperoxidase activity in carrageenan-induced inflammation

In-house assays of MPO were employed according to the methods developed by Rao, Currie, Shaffer, and Isakson (1993). Using conventional reagents, the concentration of enzyme was estimated by means of colourimetric measurements (absorbances at 450 nm) in an ELISA plate reader (Organon Tecknica). One unit of MPO is defined as the activity of the enzyme that oxidizes 1 mol of H_2O_2/min. Detailed descriptions of these assays have been published previously (Fröde & Medeiros, 2001). Results were expressed as mU/ml.

2.9. Quantification of IL-1β and MIP-2 levels in carrageenan-induced inflammation

Samples of the air pouch exudate obtained from control animals (treated with sterile saline), carrageenan-treated animals, and animals pre-treated with AE, BuOH, AR or dexamethasone, were collected and immediately prepared for the analysis of cytokine levels. In this protocol, commercially available kits were used with monoclonal specific antibodies for each cytokine. The cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA), using Immuno Biological Laboratories kits according to the manufacturer's instructions. The ranges of the values detected by these assays were: IL-1β (100–6400 pg/mL) and MIP-2 (1–64 pg/mL). The intra- and interassay coefficients of variation (CV) for IL-1β and MIP-2 were: intra CV: IL-1β: $= 6.2 \pm 0.4\%$, MIP-2 $= 13.99 \pm 9.6\%$, inter CV: IL-1β $= 5.1 \pm 0.6\%$ and MIP-2 $= 12.09 \pm 8.86\%$, sensitivities of IL-1β $= 1.67$ pg/ml and MIP-2 $= 0.36$ pg/mL. All cytokine concentrations were estimated by means of colourimetric measurement at 450 nm on an ELISA plate reader (Organon Teknika, Roseland, NJ, USA) by interpolation from a standard curve.

2.10. Drugs and reagents

The following drugs were used: carrageenan (degree IV), histamine, substance P, human polymorphonuclear leukocyte myeloperoxidase, diphenylboryloxyethylamina (Sigma Chemical Co., St. Louis, CO, USA), dexamethasone (Prodome Química e Farmacêutica Ltda., Campinas, SP, Brazil), May-Grünwald dye (Newprov, Pinhais, PR, Brazil), Giemsa dye (Laborclin, Pinhais, PR, Brazil), and enzyme-linked immunosorbent assay (ELISA: EIA) for quantitative determination of rat interleukin-1beta (IL-1β) and macrophage inflammatory protein-2 (MIP-2) (IBL – Immuno Biological Laboratories Co. Ltd., Fujioka-city, Gunma, Japan). For chromatographic analysis, we used diphenylboryloxyethylamina as the colour reagent, reference compounds vitexin, isovitexin, orientin and isoorientin (Extrasynthèse, France) and aluminium sheets coated with silica gel GF 254 (Merk, Darmstadt, Germany). Other reagents used were of analytical grade and were obtained from different commercial sources.

2.11. Statistical analysis

Data is reported as the mean ± standard error of the mean (SEM). Significant differences between groups were

determined by analysis of variance (ANOVA) complemented with Dunnett's and/or Student's *t* tests. $p < 0.05$ was considered as indicative of significance.

3. Results

3.1. Chromatographic analysis

The chromatographic analysis of the *P. edulis* leaf aqueous extract, butanolic fraction and aqueous residual fraction using TLC showed mainly spots with the same characteristics as the flavonoid C-glycoside reference compounds. According to the intensity of the fluorescence observed after revelation with NP Reagent, the presence of these compounds was predominant in the butanolic fraction. Comparison with the flavonoid C-glycoside reference compounds indicated the presence of isovitexin, vitexin, orientin and isoorientin, compounds already described for *P. edulis* (Petry, 1999).

3.2. Inflammation induced by carrageenan

3.2.1. Effects of Passiflora edulis and dexamethasone on cell migration

Passiflora edulis aqueous lyophilized extract (AE), solely at the dose of 100 mg/kg, i.p., administered 0.5 h before carrageenan, significantly reduced the leukocyte migration (% of inhibition: 32.3 ± 5.6) ($p < 0.05$) (Fig. 1a). This reduction occurred by neutrophil decrease (% of inhibition: 48.0 ± 5.9) ($p < 0.01$) (Fig. 1b). On the other hand, AE (25 and 50 mg/kg, i.p.) enhanced the leukocytes (% of enhancement: 25 mg/kg: 50 ± 7.5 and 50 mg/kg: 48 ± 8.7) ($p < 0.01$) and neutrophils (% of enhancement: 25 mg/kg: 56 ± 7.6 and 50 mg/kg: 47 ± 11.5) ($p < 0.05$) (Fig. 1a and b). The dose of 25 mg/kg did not modify mononuclears ($p > 0.05$), whereas doses of 50 mg/kg, i.p. or 100 mg/kg, i.p. of this aqueous extract increased these cells (% of enhancement: 50 mg/kg: 57 ± 4.1 and 100 mg/kg: 62 ± 4.4) ($p < 0.05$) (Fig. 1c).

The *P. edulis* butanolic fraction (BuOH: 50 and 100 mg/kg, i.p., 0.5 h before) significantly reduced the leukocyte migration (% of inhibition: 50 mg/kg: 39.2 ± 1.2 and 100 mg/kg: 40.2 ± 4.3) ($p< 0.05$) (Fig. 2a) and neutrophils (% of inhibition: 50 mg/kg: 58.2 ± 1.5 and 100 mg/kg: 45.2 ± 5.6) ($p < 0.05$) (Fig. 2b), and failed to change mononuclears ($p > 0.05$) (Fig. 2c). The dose of 25 mg/kg, i.p. of the BuOH did not modify the total or differential leukocyte levels ($p > 0.05$) (Fig. 2a–c).

Finally, the *P. edulis* aqueous residual fraction (AR) reduced the leukocyte migration only at the dose of 100 mg/kg, i.p., 0.5 h before carrageenan inflammation (% of inhibition: 10.2 ± 4.3) ($p < 0.05$) (Fig. 3a). This reduction occurred by neutrophil decrease (% of inhibition: 29.2 ± 6.8) ($p < 0.05$) (Fig. 3b). The aqueous residual fraction (25–50 mg/kg, i.p.) did not modify the leukocytes or neutrophils ($p > 0.05$). (Fig. 3a and b). Also, AR did not change the mononuclears at any of the studied doses ($p > 0.05$) (Fig. 3c).

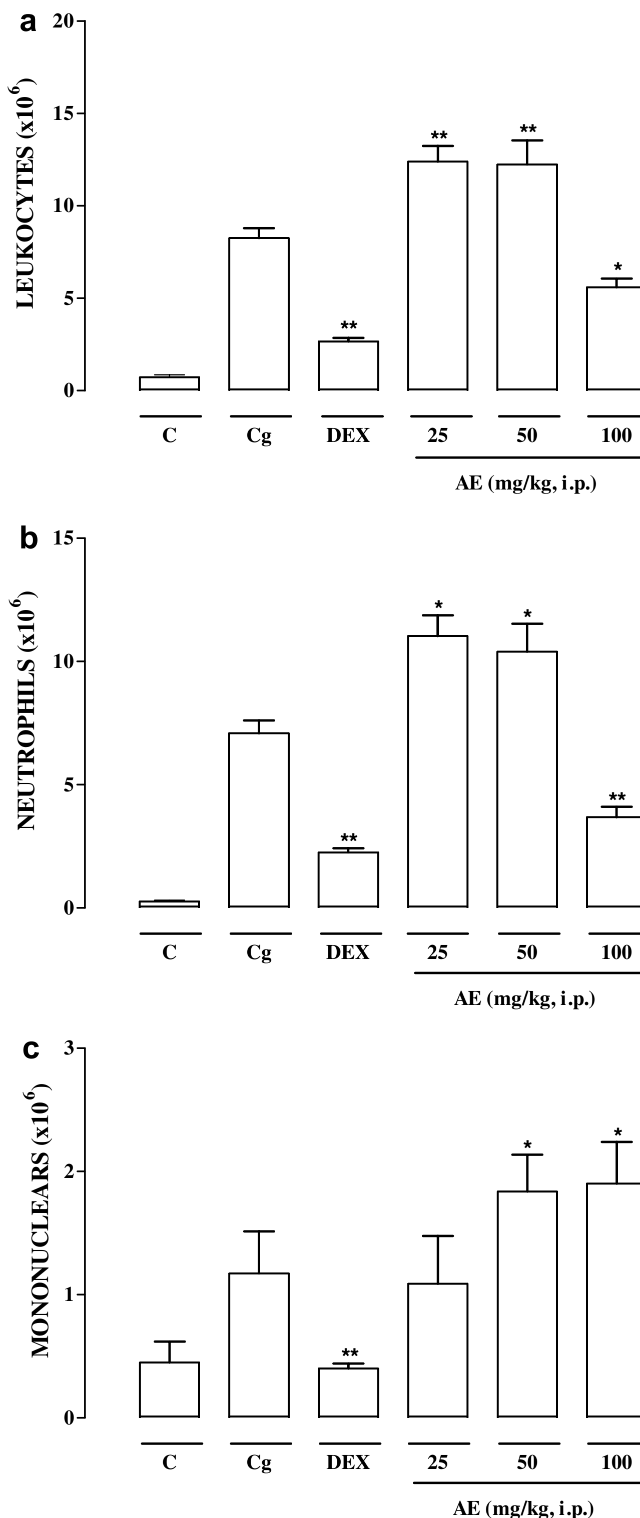

Fig. 1. Effects of different doses of aqueous lyophilized extract (AE: 25–100 mg/kg, i.p.) on the inflammation induced by carrageenan (Cg, 1%) in the mouse model of air pouch. Effects of AE upon leukocytes (a), neutrophils (b) and mononuclears (c). C = control = responses in animals treated only with sterile saline (NaCl, 0.9%), Cg = responses in animals treated only with carrageenan, DEX = responses in animals treated with dexamethasone (0.5 mg/kg, i.p.) plus carrageenan. Each group represents the mean of 3–6 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented with Dunnett's or Student's *t* tests. $^{*}p < 0.05$, $^{**}p < 0.01$.

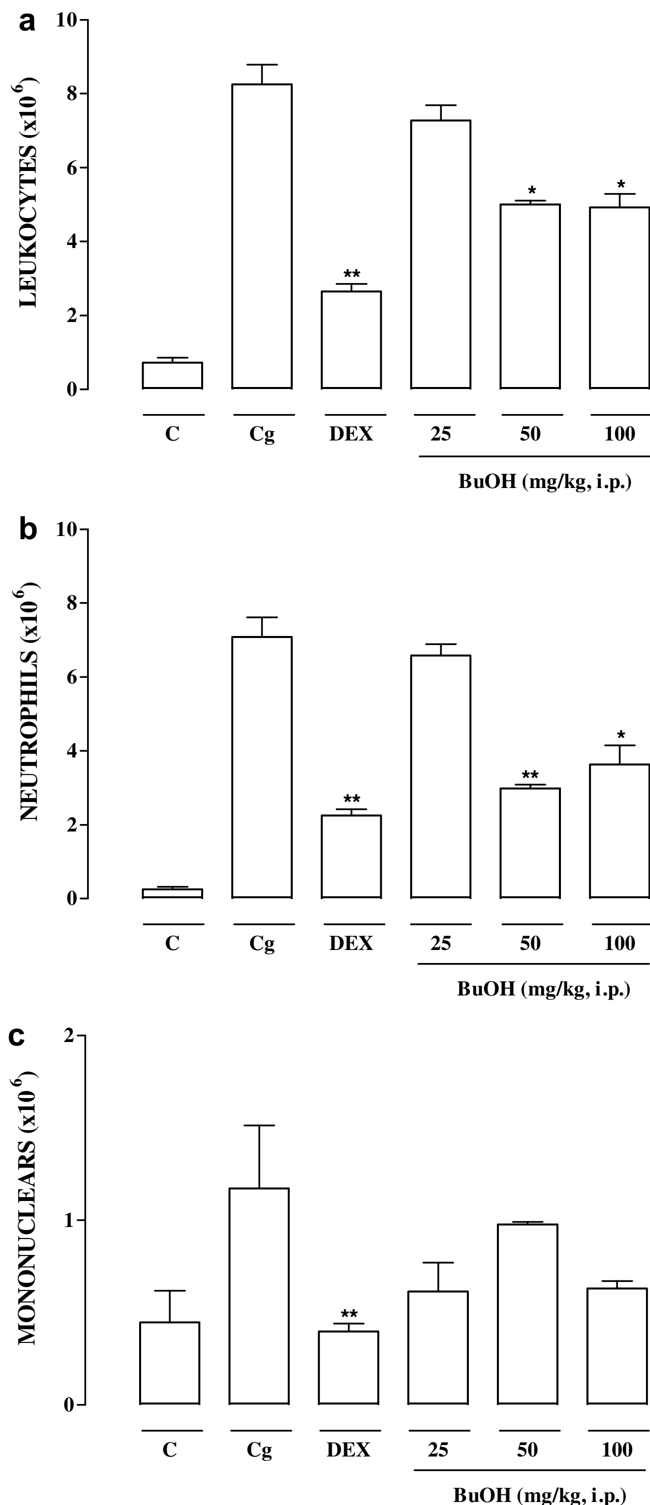

Fig. 2. Effects of different doses of butanolic fraction (BuOH: 25–100 mg/kg, i.p.) on the inflammation induced by carrageenan (Cg 1%) in the mouse model of air pouch. Effects of BuOH upon leukocytes (a), neutrophils (b) and mononuclears (c). C = control = responses in animals treated only with sterile saline (NaCl, 0.9%), Cg = responses in animals treated only with carrageenan, DEX = responses in animals treated with dexamethasone (0.5 mg/kg, i.p.) plus carrageenan. Each group represents the mean of 3–6 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented with Dunnett's or Student's *t* tests. $^{*}p < 0.05$, $^{**}p < 0.01$.

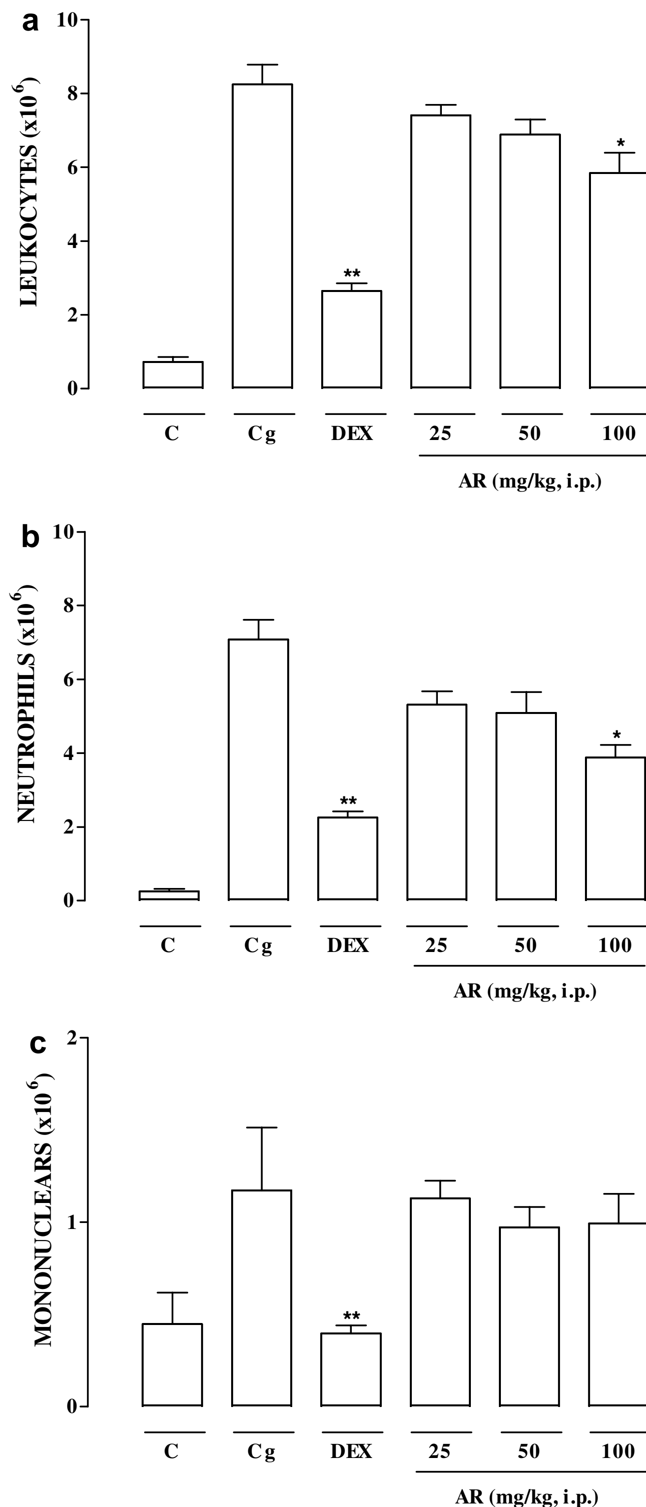

Fig. 3. Effects of different doses of aqueous residue (AR: 25–100 mg/kg, i.p.) on the inflammation induced by carrageenan (Cg 1%) in the mouse model of air pouch. Effects of AR upon leukocytes (a), neutrophils (b) and mononuclears (c). C = control = responses in animals treated only with sterile saline (NaCl, 0.9%), Cg = responses in animals treated only with carrageenan, DEX = responses in animals treated with dexamethasone (0.5 mg/kg, i.p.) plus carrageenan. Each group represents the mean of 3–6 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented with Dunnett's or Student's *t* tests. $^{*}p < 0.05$, $^{**}p < 0.01$.

In the time course profile study, the results showed that the *P. edulis* aqueous lyophilized extract (100 mg/kg, i.p.) and butanolic (50 mg/kg, i.p.) and aqueous residual (100 mg/kg, i.p.) fractions significantly decreased the leukocyte migration only when they were administered 0.5 h before carrageenan (results not shown).

Dexamethasone (0.5 mg/kg, i.p.), which is considered to be an important steroidal anti-inflammatory drug, decreased leukocyte (% of inhibition: 67.9 ± 2.5) ($p < 0.01$) (Figs. 1a, 2a and 3a), neutrophil (% of inhibition: 68.2 ± 2.4) ($p < 0.01$) (Figs. 1b, 2b and 3b) and monocluclear migration (% of inhibition: 65.9 ± 3.6) ($p < 0.01$) (Figs. 1c, 2c and 3c).

The dose–response and time–course profiles indicated that the inhibitory effects of the *P. edulis* leaf aqueous extract and derived fractions were AE: 100 mg/kg, BuOH: 50 mg/kg and AR: 100 mg/kg when they were administered 0.5 h before carrageenan.

Thus, these doses and time of pre-treatment were chosen for the analysis of other inflammatory parameters such as myeloperoxidase, nitric oxide and cytokine levels in the inflammation induced by carrageenan, as well as cellular migration in the inflammation induced by histamine or substance P.

3.2.2. Effect of Passiflora edulis on myeloperoxidase and nitric oxide levels

In this protocol, *P. edulis* aqueous lyophilized extract (AE: 100 mg/kg), butanolic (BuOH: 50 mg/kg) and aqueous residual (AR: 100 mg/kg) fractions, as well as dexamethasone (0.5 mg/kg, i.p.), were effective in inhibiting both MPO and NO levels (MPO: % of inhibition: AE: 30.9 ± 3.2, BuOH: 48.2 ± 3.8, AR: 30.5 ± 4.1 and dexamethasone: 68.9 ± 3.2) ($p < 0.01$) (Fig. 4a), (NO: % of inhibition: AE: 41.2 ± 8.4, BuOH: 61.0 ± 1.0, AR: 61.7 ± 2.3 and dexamethasone: 50.2 ± 3.6) ($p < 0.01$) (Fig. 4b).

3.2.3. Effect of Passiflora edulis on MIP-2 and IL-1β levels

The results demonstrated that *P. edulis* aqueous lyophilized extract (AE: 100 mg/kg) and butanolic (BuOH: 50 mg/kg) and aqueous residual (AR: 100 mg/kg) fractions were also effective in inhibiting the IL-1β and MIP-2 levels (IL-1β: % of inhibition: AE: 38.5 ± 3.5, BuOH: 67.0 ± 3.0 and AR: 74.0 ± 6.0) ($p < 0.05$) (Fig. 5a), (MIP-2: % inhibition: AE: 55.5 ± 7.5, BuOH: 31.0 ± 7.0, and AR: 78.0 ± 3.0) ($p < 0.05$) (Fig. 5b). Dexamethasone inhibited IL-1β (% of inhibition: 55.3 ± 4.1) ($p < 0.01$) but not MIP-2 levels (Fig. 5a and b).

3.3. Inflammation induced by histamine and substance P

3.3.1. Effects of Passiflora edulis on cell migration

Passiflora edulis aqueous lyophilized extract (AE: 100 mg/kg), like butanolic (BuOH: 50 mg/kg) and aqueous residual (AR: 100 mg/kg) fractions, significantly decreased the leukocytes and mononuclears induced by HIS and SP and neutrophils induced by HIS ($p < 0.01$) (Table 1). AE and BuOH, but not AR, inhibited neutrophils inducible by SP ($p < 0.05$) (Table 1).

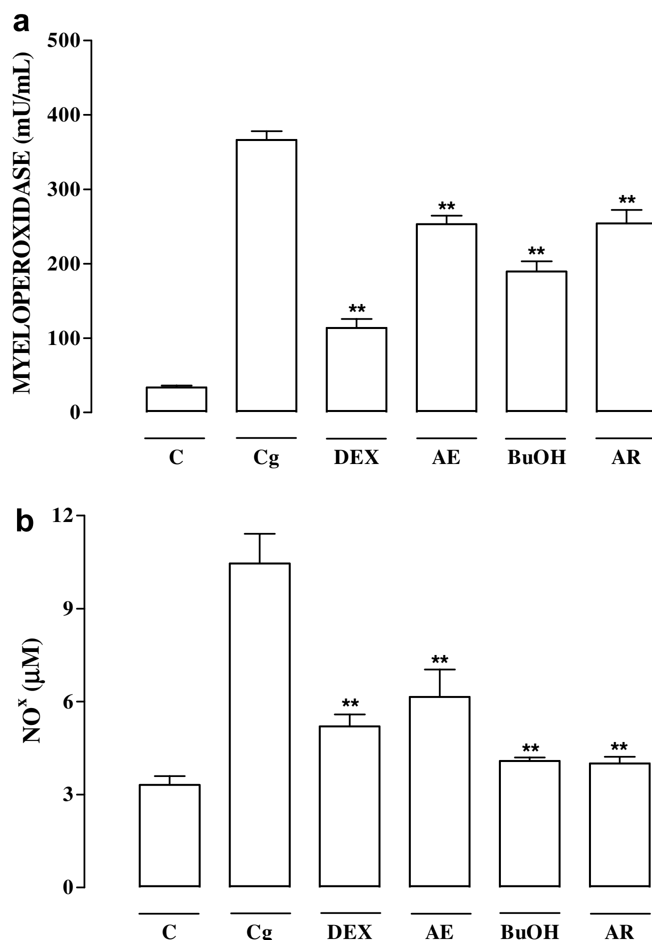

Fig. 4. Effects of aqueous lyophilized extract (AE: 100 mg/kg, i.p.), butanolic fraction (BuOH: 50 mg/kg, i.p.), aqueous residue (AR: 100 mg/kg, i.p.) administered 0.5 h before carrageenan administration in the mouse model of air pouch. Effect of AE, BuOH and AR upon myeloperoxidase (a) and nitric oxide (b) levels. C = control = responses in animals treated only with sterile saline (NaCl, 0.9%), Cg = responses in animals treated only with carrageenan, DEX = responses in animals treated with dexamethasone (0.5 mg/kg, i.p.) plus carrageenan. Each group represents the mean of 3–6 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented with Dunnett's or Student's *t* tests. $^{**}p < 0.01$.

A similar result was found in the animals pre-treated with dexamethasone (0.5 mg/kg), which significantly decreased leukocyte migration, neutrophils, and mononuclears in the inflammation induced by histamine and substance P ($p < 0.01$) (Table 1).

4. Discussion

In the literature, there is one report concerning the anti-inflammatory activity of the *P. incarnata* (Borreli et al., 1996) in addition to our previous report (Montanher et al., 2007). The results of the present study establish that the aqueous extract of *P. edulis* leaves and two derived fractions (butanolic and aqueous residue) exhibited potent anti-inflammatory action in the experimental model *in vivo*.

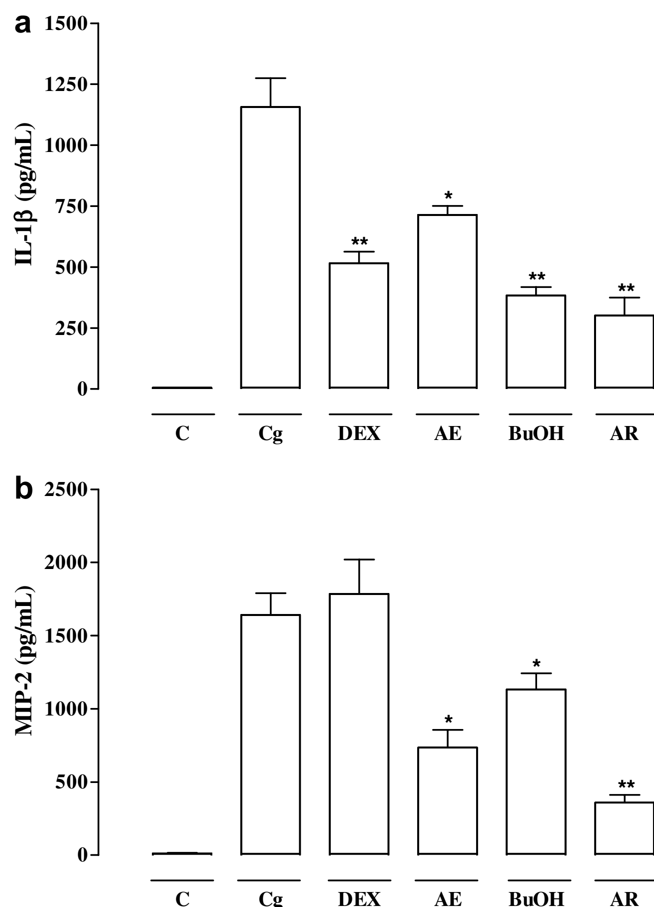

Fig. 5. Effects of aqueous lyophilized extract (AE: 100 mg/kg, i.p.), butanolic fraction (BuOH: 50 mg/kg, i.p.), aqueous residue (AR: 100 mg/kg, i.p.) administered 0.5 h before carrageenan administration in the mouse model of air pouch. Effect of AE, BuOH and AR upon IL-1β (a) and MIP-2 (b) levels. C = control = responses in animals treated only with sterile saline (NaCl, 0.9%), Cg = responses in animals treated only with carrageenan, DEX = responses in animals treated with dexamethasone (0.5 mg/kg, i.p.) plus carrageenan. Each group represents the mean of 3–6 animals and the vertical bars the SEM. Significant differences determined by ANOVA complemented with Dunnett's or Student's *t* tests. $^{*}p < 0.05$, $^{**}p < 0.01$.

These results allowed us to characterize a marked inhibitory profile upon cell migration, regarding both neutrophil and mononuclear influxes, besides myeloperoxidase activity, nitric oxide, interleukin-1 beta and macrophage inhibitory protein-2 levels in the air pouch exudate. Since dexamethasone demonstrated a similar profile, it is possible that both *P. edulis* and dexamethasone were acting via common pathways.

Among the different kinds of compounds already reported for *P. edulis* leaves (e.g. triterpenes, saponins, cyanogenic glycosides), the C-glycosides of flavonoids are possibly involved, considering that they are the predominant compounds in *P. edulis* leaves (De Paris et al., 2002; Petry, 1999) and also considering the anti-inflammatory properties described for some of these compounds. *In vitro* studies have shown that luteolin, isoorientin and other luteolin-derived C-glycosides are able to inhibit thromboxan and leukotriene synthesis (Odontuya, Hoult, & Houghton, 2005). In relation to isovitexin, an apigenin C-glycoside, it has been shown that this substance causes the inhibition of the inducible nitric oxide mRNA expression from LPS-activated macrophages (RAW 264.7) (Lin et al., 2005). Furthermore, this inhibition seems to be via inhibition of nuclear factor kappa B (NF-κB) activation by way of its interference with the phosphorylation of IκB (Lin et al., 2005). There are also *in vivo* studies reporting antiedematogenic activity for isoorientin in the carrageenan-induced hind paw oedema model in mice (Kuepeli, Aslan, Guerbuez, & Yesilada, 2004).

In this context, the results obtained in our studies suggested that C-glycosylflavonoids can possibly be the active compounds of the crude extract. In our study, the *P. edulis* leaf aqueous extract, like the butanolic and aqueous residual fractions, as well as dexamethasone, significantly inhibited cell migration by decreasing the influxes of neutrophils, the most representative cells of carrageenan inflammation, and also of neutrophils and mononuclears, in the inflammation caused by both histamine and substance P (Da Cunha et al., 2001; Fröde-Saleh et al., 1999; Saleh et al., 1996; Wallace, Chapman, & Mcknigh, 1999). This inhibitory effect was more evident in animals pre-treated with butanolic fraction, since the dose of 50 mg/kg of this fraction had the same effect in inhibiting leukocytes as the doses of 100 mg/kg of *P. edulis* aqueous extract or aqueous residual fraction. Furthermore, analysis of their effects on this model showed that the decrease of neutrophil influx in the carrageenan inflammation was also associated with an inhibition of myeloperoxidase, corroborating their inhibitory effect upon neutrophils (Saleh et al., 1999). Also, the butanolic fraction showed a more potent effect than the *P. edulis* aqueous extract or aqueous residual fraction. Whether the decrease in number of neutrophils and mononuclear cells in the exudate was the primary effect on the leukocyte function, or a secondary effect of the inhibition of local production of chemotactic molecules, remains to be determined.

In our study, we also demonstrated that the *P. edulis* aqueous extract and its derived fractions are effective in treating experimental inflammation, and this was accompanied by a decrease in IL-1β and MIP-2 levels. It has been reported that neutrophils and monocytes/macrophages produce large amounts of IL-1β and MIP-2 which are potent triggers of many of the actions involved in leukocyte migration (Baggioloo, Dewald, & Moser, 1999; Kasama et al., 2005; Lomas-Neira, Chung, Wesche, Perl, & Ayala, 2005; Zhang, Wang, Liu, & Thorlacius, 2001). Furthermore, IL-1β can stimulate the release of other cytokines, including IL-6 and chemokines, as well as the expression of adhesion molecules and inducible enzymes (Brandolini et al., 1997; Calkins et al., 2002; Matsukawa, Yoshimura, Miyamoto, Ohkawara, & Yoshinaga, 1997).

On the other hand, distinct effect was observed with dexamethasone in relation to pro-inflammatory cytokines. This important steroidal anti-inflammatory drug inhibited IL-1 β but not MIP-2 levels. Therefore, it is suggested that

Table 1
Effects of *Passiflora edulis* and dexamethasone on cell migration in the inflammation induced by histamine or substance P

Groups/dose	Absolute values	
	Histamine (10 μmol/cav.)	Substance P (40 nmol/cav.)
Leukocytes (×10⁶)		
C[a]	1.25 ± 0.10	0.87 ± 0.04
AE (100 mg/kg)[b]	0.59 ± 0.05**	0.35 ± 0.02**
AR (100 mg/kg)[b]	0.51 ± 0.04**	0.44 ± 0.04**
BuOH (50 mg/kg)[b]	0.42 ± 0.05**	0.40 ± 0.03**
DEX (0.5 mg/kg)[b]	0.16 ± 0.03**	0.08 ± 0.01**
Neutrophils (×10⁶)		
C[a]	0.64 ± 0.05	0.21 ± 0.01
AE (100 mg/kg)[b]	0.28 ± 0.03**	0.08 ± 0.01**
AR (100 mg/kg)[b]	0.21 ± 0.01**	0.18 ± 0.02
BuOH (50 mg/kg)[b]	0.18 ± 0.03**	0.15 ± 0.01*
DEX (0.5 mg/kg)[b]	0.07 ± 0.10**	0.02 ± 0.01**
Mononuclears (×10⁶)		
C[a]	0.71 ± 0.05	0.64 ± 0.02
AE (100 mg/kg)[b]	0.31 ± 0.03**	0.27 ± 0.02**
AR (100 mg/kg)[b]	0.32 ± 0.04**	0.27 ± 0.03**
BuOH (50 mg/kg)[b]	0.25 ± 0.02**	0.24 ± 0.03**
DEX (0.5 mg/kg)[b]	0.09 ± 0.02**	0.62 ± 0.01**

Aqueous lyophilized extract, aqueous residue, butanolic fraction of *Passiflora edulis* or dexamethasone (0.5 mg/kg, i.p.) administered to different groups of animals 0.5 h before inflammation induction by histamine or substance P. C = control = responses in animals treated only with histamine (10 μmol/cav.) or substance P (40 nmol/cav.). AqE, AqR, BuOH = responses in animals pretreated with aqueous lyophilized extract (100 mg/kg, i.p.), aqueous residue (100 mg/kg, i.p.) or butanolic fraction (50 mg/kg i.p.) of *Passiflora edulis*, respectively. DEX = responses in animals pretreated with dexamethasone (0.5 mg/kg, i.p.). Each value represents the mean ± SEM of 5–6 animals. $^{*}p < 0.05$ and $^{**}p < 0.01$. *, ** indicate statistical significance. *a* = administered by subcutaneous route, *b* = administered by intraperitoneal route.

different pathways were activated in the observed effects of the *P. edulis* leaf aqueous extract and its derived fractions.

We also noted that the *P. edulis* aqueous extract and its derived fractions, like dexamethasone, exhibited inhibitory effects on nitrate/nitrite levels, an effect that involves, among other actions, the activation of endothelial nitric oxide synthase (Barnes & Adcock, 2003). Furthermore, the *in vitro* inhibition of nitric oxide synthase by flavonoid C-glycosides such as isovitexin has already been reported by Lin et al. (2005). Also in this model, the butanolic fraction inhibited this parameter at the dose of 50 mg/kg, while the same effect was observed at the 100 mg dose of the *P. edulis* aqueous extract or aqueous residual fraction.

In conclusion, the *P. edulis* aqueous extract and its derived fractions inhibited inflammation induced by carrageenan, histamine and substance P in the mouse air pouch model and the production of a variety of pro-inflammatory cytokines, mediators and enzymes at the inflammatory sites. These effects were generally observed at a lower dose (50 mg/kg) of the butanolic fraction than the one required for the *P. edulis* aqueous extract (100 mg/kg) or aqueous residual fraction (100 mg/kg). These results can be explained by the predominance of C-glycoside flavonoids as observed through the TLC analysis.

Additional studies will be necessary to identify whether the predominant compounds in the butanolic fraction, identified as isovitexin, vitexin, orientin and isoorientin, isolatedly have the same anti-inflammatory properties.

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