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# Polysaccharide fraction isolated from *Passiflora edulis* inhibits the inflammatory response and the oxidative stress in mice

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**Keywords**
anti-inflammatory; cytokine; neutrophil; oxidative stress

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## Abstract

**Objectives** The aim of the study was to investigate the anti-inflammatory, antioxidant and antinociceptive actions of PFPe, a polysaccharide fraction isolated from the dried fruit of the *Passiflora edulis*.

**Methods** Animals were pretreated with PFPe (0.3, 1 or 3 mg/kg, i.p.) 1 h before induction of paw oedema by carrageenan, histamine, serotonin, compound 48/80 or prostaglandin $E_2$ (PGE2). Neutrophil migration and vascular permeability were measured after carrageenan injection into the peritoneum, and the action of the PFPe on the tumour necrosis factor-alpha, interleukin-1 beta (IL-1β), myeloperoxidase (MPO), glutathione (GSH) and malondialdehyde (MDA) levels was also evaluated. To assay nociception, we examined acetic acid-induced writhing, formalin-induced paw licking and response latency in the hot plate test.

**Key findings** Pretreatment with PFPe significantly inhibited carrageenan-induced paw oedema. PFPe also reduced paw oedema induced by compound 48/80, histamine, serotonin, and PGE2 and compound 48/80-induced vascular permeability. In addition, PFPe significantly reduced the MPO activity, MDA and GSH concentrations, and IL-1β level. In the nociception tests, PFPe reduced acetic acid-induced writhing and formalin-induced paw licking and did not increase the response latency time.

**Conclusions** Our results suggest that PFPe administration reduces the inflammatory response by modulation of the liberation or synthesis of histamine and serotonin, by reduction of neutrophil migration, IL-1β levels, and oxidative stress and nociception.

## Introduction

*Passiflora edulis*, commonly known as 'Maracujá', is a member of the Passifloraceae family, originated in the tropical and subtropical regions of the Americas and widely distributed in northern and northeastern Brazil.[1] In popular medicine, *P. edulis* has been used for its effects on the central nervous system (CNS) as a sedative and a tranquilizer.[2] Another important application is its use as a poultice or lotion for the treatment of infection and inflammation of the skin.[3] Studies have reported various pharmacological activity of extract, fruit pulp and polysaccharide fraction from *P. edulis*, including anxiolytic,[4] antihypertensive,[5] antioxidant,[6] antitumour[7] effects and prevention of inflammatory processes in the colon.[8]

Inflammation is a primary protective response of the body, which, acting as a defence mechanism, aims to

 

remove or destroy noxious stimuli and restore normal tissue structure and function. A complex cascade of inflammatory mediator is released, including cytokines, i.e. interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), chemokines, and growth factors.[9] In addition, there is an intense recruitment of leucocytes to the injured site and increased vascular permeability, culminating in the accumulation of plasma proteins.[10] Another important component of inflammation is oxidative stress, which occurs when reactive oxygen species (ROS) are overproduced, exceeding the capacity of the endogenous antioxidant system and leading to tissue injury.[11]

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the clinic to reduce both the early and late responses of the inflammatory process.[12] However, NSAID use is limited due to tolerance, dependence, gastrointestinal complications (e.g. gastritis and ulcers) and cardiovascular problems.[13] In recent years, interest by pharmaceutical companies for new drugs with fewer side effects has grown considerably, particularly with respect to molecules derived from natural products, especially those isolated from plants.[14,15] In this context, polysaccharides extracted from plants may reduce inflammatory processes by interfering with specific molecules or mechanisms.[16,17] The advantages of herbal medicines include significant efficacy, a low incidence of side effects, low cost and relative safety.

In view of the popular use of *Passiflora* spp. for treatment of inflammation and the few pharmacological investigations on polysaccharides isolated from *P. edulis* and their mechanisms of action, we investigated the anti-inflammatory and antinociceptive potential of PFPe, a polysaccharide fraction isolated from dried fruit of the *P. edulis*, in several experimental models of acute inflammation.

## Materials and Methods

### Drugs and reagents

λ-Carrageenan, histamine, serotonin, compound 48/80, prostaglandin $E_2$ (PGE2), acetic acid and formaldehyde were purchased from Sigma Chemical (St Louis, MO, USA). Heparin and morphine were provided by Merck (São Paulo, SP, Brazil). All drugs were dissolved in sterile 0.9% NaCl (saline).

### Polysaccharide extraction

The extraction methods and chemical characterization of the polysaccharide were previously described by our research group.[7] Samples of a product from the peel of *P. edulis* fruits commercially purchased from Pectina do Brasil based at the Technology Development Park PADETEC (Parque de Desenvolvimento Tecnológico do Ceará) in Fortaleza, Brazil, were utilized to obtain carbohydrate fraction. The polysaccharide fraction from *Passiflora edulis* (PFPe) was obtained without acidic extraction and characterized by infrared spectroscopy, nuclear magnetic resonance (NMR) and high-performance size-exclusion chromatography (HPSEC).

### Monosaccharide composition

The monosaccharide contents of the polysaccharide were determined after hydrolysis with 4 M trifluoroacetic acid for 5 h at 96°C. Methanol was added to the hydrolysed material, and trifluoroacetic acid (TFA) was eliminated by rotoevaporation of the methanol/TFA mixture. The monosaccharides were converted into alditol acetate by successive reduction with $NaBH_4$, followed by acetylation with pyridine-$Ac_2O$. Gas–liquid chromatography analysis was performed on a 3800 Varian Gas Chromatograph connected to a 2000 R-12 Varian Ion-Trap Mass Spectrometer, with He as the carrier gas (2.0 ml/min). A DB-23 capillary column was used with a heating programme of 40°C/min up to 220°C (constant temperature). The resulting derivatives were identified by their typical electron impact breakdown profiles.

### High-performance size-exclusion chromatography

The peak molar mass of the PFPe sample was determined by HPSEC using a Shimadzu LC-10AD chromatograph with a refractive index (RID-6A) detector at room temperature using an Ultrahydrogel linear column (7.8 × 300 mm). A flow rate of 0.5 ml/min, polysaccharide solution concentration of 0.1% (w/v), water as the solvent and 0.1 mol/l $NaNO_3$ as the eluent were used. The sample volume was 50 µl. The molar mass of the polysaccharide was estimated using a Pullulan standard. The correlation between the Pullulan standard molar mass and the elution volume is given by equation (1):

$$\log M = 14.016 - 0.951\,Ve \quad R = 0.991 \tag{1}$$

where Ve is the elution volume.

### Infrared spectroscopy

FTIR spectra were recorded with KBr pellets on an fourier transform infrared spectroscopy (FTIR) Shimadzu 8300 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) with a resolution of 2-$cm^{-1}$. The spectra were obtained from 30 scans.

### Nuclear magnetic resonance

$^1H$ and $^{13}C$ broadband spectra of 3% (wt/v) solutions in $D_2O$ at 70°C were recorded on a Fourier transform Bruker Avance DRX 500 spectrometer with an inverse

  

multinuclear gradient probe-head equipped with z-shielded gradient coils and with silicon graphics. Sodium 2,2-dimethylsilapentane-5-sulfonate (DSS) was used as the internal standard (0.00 ppm for $^1$H).

## Animals

Male Swiss mice weighing 25–30 (6–7 weeks) were housed at a temperature of 25 ± 2°C under a 12/12 h light/dark cycle with food and water *ad libitum*. All experimental protocols were performed in accordance with the Guide for Care and Use of Laboratory Animals (National Institute of Health, Bethesda, MD, USA) and approved by the Ethics Committee in Research of the Federal University of Piauí (Protocol No. 020/12).

## Paw oedema induced by carrageenan

Mice were pretreated with saline (untreated control) or PFPe (0.3, 0.1 or 3 mg/kg), injected intraperitoneally. After 1 h, the oedema was induced by carrageenan (50 µl; 500 µg/paw) into the right hind paw. Paw volume was measured immediately before ($V_o$) and at 1, 2, 3 and 4 h after carrageenan treatment ($V_t$) using a plethysmometer (Panlab, Barcelona, Spain) as previously described.[10]

## Paw oedema induced by several agents

Mice received histamine (100 µg/paw), serotonin (1% w/v), compound 48/80 (12 µg/paw) or PGE2 (3.0 nmol/paw) into the right hind paw. Saline (untreated control) or PFPe (3 mg/kg, i.p.) was injected 1h before the phlogistic agents. Paw volume was measured immediately before ($V_o$) and at 30, 60, 90 and 120 after stimuli, as described above.

## Effect of PFPe on compound 48/80-induced vascular permeability

Initially, the mice received PFPe (3 mg/kg), after 1 h was administrated compound 48/80 (12 µg/paw), and vascular permeability measured 30 min later (peak of oedema). The animals received Evans blue (25 mg/kg; 150 µl, i.v.) 1 h before sacrifice. Next, the paws were excised, weighed and placed in formamide (at 37°C for 72 h). The vascular permeability was evaluated using a spectrophotometer at 570 nm and expressed as µg of Evans blue/g of paw based on a standard curve.

## Evaluation of cell migration

Mice received saline (untreated control) or PFPe (3 mg/kg, i.p.), and 1 h later was injected carrageenan (250 µl; 500 µg/cavity; i.p.). The animals were euthanized 4 h after carrageenan administration, and the peritoneal cavity was washed with 1.5 ml of heparinized phosphate-buffered saline. Total cell counts were performed in Neubauer chamber, and differential cell counts (100 cells total) were carried out on cytocentrifuge slides stained with H&E. Results are presented as the number of cells per millilitre of peritoneal exudate. Aliquots were collected for evaluation of myeloperoxidase (MPO) activity, glutathione (GSH) levels and malondialdehyde (MDA) concentration.

## Myeloperoxidase activity

Aliquots of the peritoneal exudates were centrifuged at 40 000 × *g* for 7 min at 4 °C. The pellet was resuspended, and MPO activity was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride and 1% hydrogen peroxide.[18] MPO activity is reported as units per millilitre of peritoneal exudates (units/ml).

## Cytokine measurements

The levels of TNF-α and IL-1β were evaluated in the peritoneal fluid 4 h after carrageenan (250 µl; 500 µg/cavity; i.p.) injection, and measured using sandwich ELISA as described previously.[19] The results were expressed as picograms per millilitre of homogenate (pg/ml).

## Glutathione levels

Aliquots of the peritoneal exudates were centrifuged at 3000 rpm for 15 min at 4°C. Next, 400 µl of each supernatant was mixed with 800 µl of Tris buffer (0.4 M, pH 8.9) and 20 µl of 0.01 M 5,5-dithio-bis (2-nitrobenzoic acid). Subsequently, the samples were stirred for 3 min and read on a spectrophotometer at 412 nm.[20] GSH levels are expressed as micrograms per millilitre of exudates (µg/ml).

## Malondialdehyde concentration

Aliquots peritoneal exudates were centrifuged at 3000 rpm for 15 min at 4°C. Next, 250 µl each supernatant was added to 1.5 ml of 1% phosphoric acid ($H_3PO_4$) and 0.5 ml of 0.6% thiobarbituric acid. After this, the mixture was stirred and heated in a boiling water bath for 45 min. The mixture was then cooled immediately in an ice water bath followed by the addition of 4 ml of n-butanol. This mixture was shaken and the butanol layer was separated by centrifugation at 1200 × *g* for 10 min and read on a spectrophotometer 535 and 520 nm.[21] MDA concentrations are expressed as nanomoles per millilitre of exudates (nmol/ml).

## Writhing test

Mice received saline, PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c., reference control). After 1 h, they were administered 0.6% acetic acid (10 ml/kg body weight, i.p.).

After 10 min, the number of constrictions, including abdominal muscle contractions and hind paw extension, was recorded over 20 min as previously described.[22]

### Formalin test

Mice received saline, PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c.; reference control). After 1 h, they were administered 2.5% formalin (20 µl) in the intraplantar region into the right hind paw. Licking time was recorded from 0 to 5 min (phase 1, corresponding to a direct chemical stimulation of nociceptors) and 20–25 min after formalin injection (phase 2, involving release of inflammatory mediators).[23]

### Hot plate test

Mice received saline, PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c.; reference drug). Measurements were performed before (zero time) and 30, 60, 90 and 120 min after treatment, with a cut-off time of 45 s to prevent development of paw lesion.[24] Each animal was dropped on a heated plate (55 ± 1°C) and evaluated the control reaction time (licking of a paw or jumping), recorded as the response latency on a hot plate (Insight, Ribeirão Preto, São Paulo, Brazil; model EFF-361).

### Rota-rod test

Rota-rod test was performed to evaluate the possible muscle relaxation and sedative effects of PFPE, using a method previously described.[25] Mice were selected 24 h before test, eliminating those that do not remain on the bar for three consecutive periods of 60 s. The animals ($n = 6$) received saline (untreated group), PFPe (3 mg/kg, i.p.) or diazepam (5 mg/kg, i.p.; positive control).

### Statistical analysis

Results are expressed as means ± standard error of the mean ($n = 6$–7 animals per group), and statistical analysis was performed using one-way analysis of variance followed by the Newman–Keuls post-hoc test. Statistical significance was set at $P < 0.05$.

## Results

### Polysaccharide characterization

The characterization of the polysaccharide fraction from *P. edulis* was described previously.[7] The data in the monosaccharide composition suggest that the PFPe has linear homogalacturonan (HG) and neutral sugar-branched rhamnogalacturonan-1 (RG-1) structures. The low galacturonic/rhamnose ratio indicates that RG-1 is present in a higher proportion than HG. The major component in PFPe was galacturonic acid (44.2 g/100 g) linked by connections (1→4) (esterified and unesterified). Neutral sugars such as arabinose (11.8 g/100 g), glucose (11.8 g/100 g), rhamnose (10.6 g/100 g), mannose (9.0 g/100 g), fucose (1.6 g/100 g), xilose (3.6 g/100 g) and ribose (1.3 g/100 g) were found. HPSEC chromatograms of the PFPe using the refractive index detector have shown only one broad peak, with a maximum elution volume at 9.78 ml appearing. The molar mass of the polysaccharide was estimated using equation (1) and the value was $6.0 \times 10^4$ g/mol.

The protein content of the PFPe sample is lower than that obtained for the passion fruit flour extracted with acetic acid (4.05%). This protein content may be due to the presence of a polysaccharide-protein complex as observed in other polysaccharides. The methodology used for the extraction of PFPe was efficient at reducing the protein content of the passion fruit rind, which has a much higher protein content (8.9 to 12.8%).[26]

The dates of infrared spectroscopy and nuclear magnetic resonance demonstrated that the PFPe structure has a linear chain, composed of HG and RG-1 with a low galacturonic/rhamnogalacturonan ratio and a low esterification level (26.2 ± 0.8 mol%).

### Effect of PFPe on carrageenan-induced paw oedema

Carrageenan (500 µg/paw) elicited an intense time-dependent paw oedema that reached a maximum level after 3 h (0.076 ± 0.008 ml) (Table 1). Treatment with PFPe at doses of 0.3 mg/kg, 1 mg/kg or 3 mg/kg intraperitoneally produced a significant reduction ($P < 0.05$) in paw oedema formation at 3 h to 0.050 ± 0.005 ml (34.2% inhibition), 0.046 ± 0.003 ml (39.5% inhibition) and 0.024 ± 0.004 ml (60.6% inhibition), respectively. Given that the 3 mg/kg dose provided the greatest protection against the inflammatory effects caused by carrageenan, it was selected for subsequent studies.

### Effect of PFPe on paw oedema induced by several agents

Injection of several phlogistic agents into the paw of mice promoted a marked increase in paw volume, as compared with the saline group (Figure 1), with the peak effect occurring at 30 min. However, pretreatment with PFPe (3 mg/kg, i.p.) significantly decreased ($P < 0.05$) the inflammatory response caused by serotonin (5-HT) (0.050 ± 0.005 ml vs 0.021 ± 0.007 ml; 58% reduction) (Figure 1a), histamine (Hist) (0.041 ± 0.006 ml vs 0.006 ± 0.003; 85.3% reduction) (Figure 1b), compound 48/80 (0.101 ± 0.009 ml vs 0.041 ± 0.003 ml; 59.4% reduction) (Figure 1c) or PGE2 (0.090 ± 0.009 ml vs 0.034 ± 0.008 ml; 62.2% reduction) (Figure 1d), measured at the oedema peak.

**Table 1** Effect of PFPe on carrageenan-induced paw oedema in mice

| Treatment | Dose (mg/kg) | Paw oedema (ml) | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| Saline | | 0.025 ± 0.008 | 0.012 ± 0.004 | 0.008 ± 0.003 | 0.010 ± 0.004 |
| Carrageenan | | 0.058 ± 0.003* | 0.061 ± 0.006* | 0.076 ± 0.008* | 0.052 ± 0.004* |
| PFPe | 0.3 | 0.043 ± 0.008 (25.8%) | 0.050 ± 0.005 (18.0%) | 0.050 ± 0.005# (34.2%) | 0.037 ± 0.004 (28.8%) |
| | 1 | 0.040 ± 0.005 (31.0%) | 0.060 ± 0.005 (1.6%) | 0.046 ± 0.003# (39.5%) | 0.040 ± 0.005 (23.0%) |
| | 3 | 0.028 ± 0.001# (51.7%) | 0.027 ± 0.002# (57.7%) | 0.024 ± 0.004#† (60.6%) | 0.040 ± 0.005 (23.0%) |

PFPe, polysaccharide fraction isolated from *Passiflora edulis*. Oedema was measured 1, 2, 3 and 4 h after carrageenan administration. Values of paw oedema expressed as mean ± SEM, $n = 6–7$, $^*P < 0.05$ vs saline group, $^\#P < 0.05$ vs carrageenan group, $^\dagger P < 0.05$ vs PFPe 0.3 and 1 mg/kg. Per cent inhibition of paw oedema is indicated in parentheses.

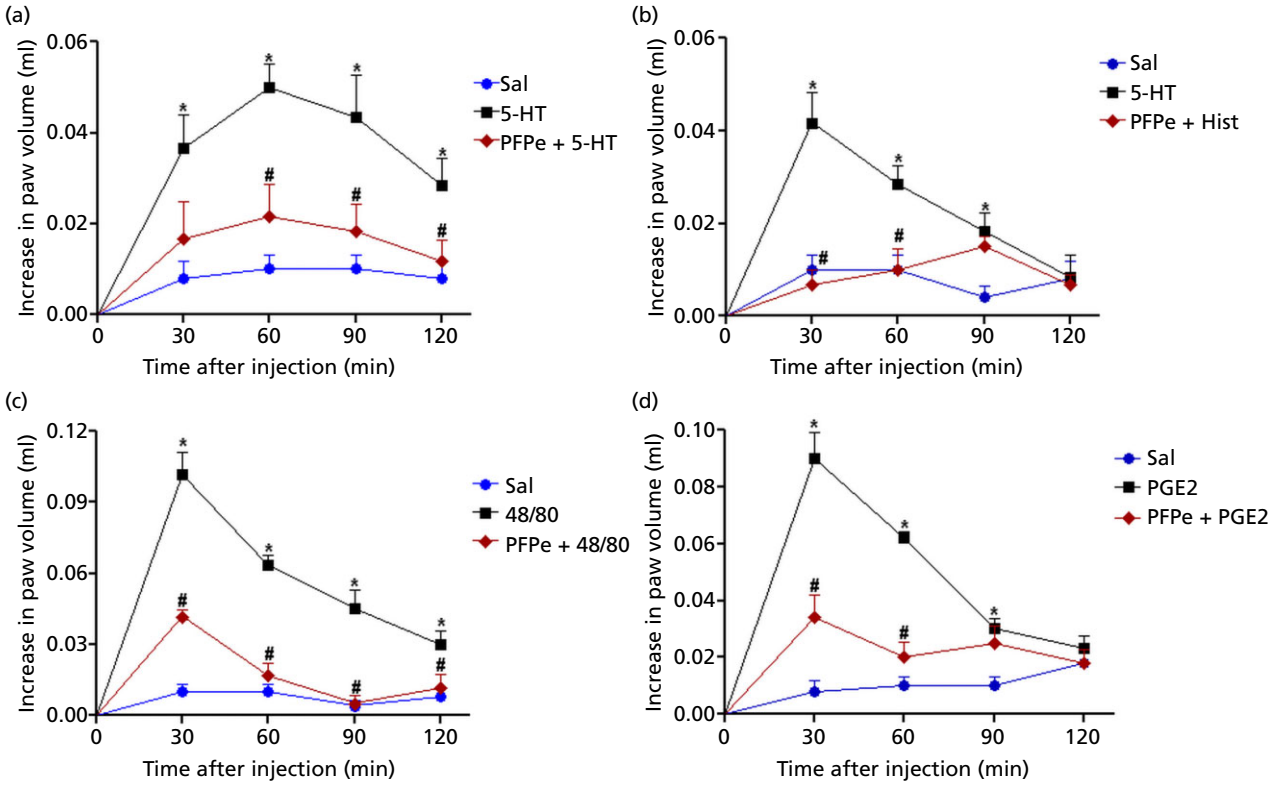


**Figure 1** Effects of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on paw oedema induced by several agents. Oedema was induced by (a) histamine (Hist) (100 μg/paw), (b) serotonin (5-HT) (1% w/v), (c) compound 48/80 (48/80) (12 μg/paw) or (d) prostaglandin $E_2$ (PGE2) (3 nmol/paw). Each point represents the mean ± standard error of the mean, $n = 6–7$, $^*P < 0.05$ vs saline, $^\#P < 0.05$ vs stimuli. Saline (untreated control) or PFPe (3 mg/kg, i.p.) was injected 1h before the phlogistic agents.

## Effect of PFPe on compound 48/80-induced vascular permeability

Compound 48/80 (12 μg/paw) elicited a significant increase in vascular leakage (0.215 ± 0.009 μg of Evans blue/mg of paw), compared with the saline group (0.039 ± 0.004 μg of Evans blue/mg of paw) (Figure 2). Pretreatment with PFPe (3 mg/kg, i.p.) significantly decreased ($P < 0.05$) compound 48/80-induced vascular leakage (0.179 ± 0.005 μg of Evans blue/mg of paw).

## Effect of PFPe on carrageenan-induced peritonitis

Intraperitoneal injection of carrageenan induced leucocyte migration into the peritoneal cavity during a period of 4 h,

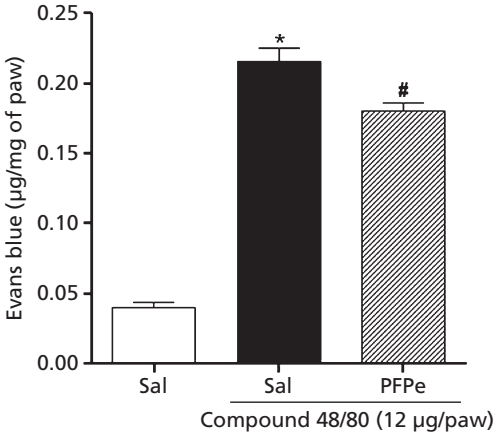


**Figure 2** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on compound 48/80-induced vascular permeability. PFPe (3 mg/kg, i.p.) was administered 1 h before compound 48/80 (12 µg/paw). The animals received Evans blue (25 mg/kg; i.v.) 1 h before sacrifice and expressed as Evans blue (µg/g paw wet weight). $^{*}P < 0.05$ vs saline (Sal), $^{\#}P < 0.05$ vs stimulus.

with total leucocytes being $9.19 \pm 1.05 \times 10^6$/ml of exudates (Figure 3a) and neutrophils comprising $7.32 \pm 1.00 \times 10^6$/ml of exudates (Figure 3b). Pretreatment with PFPe (3 mg/kg, i.p.) significantly decreased ($P < 0.05$) the influx of leucocytes ($4.55 \pm 0.78 \times 10^6$/ml of exudates) and also lowered neutrophil migration ($1.22 \pm 0.19 \times 10^6$/ml of exudates) (Figure 3).

## Effect of PFPe on myeloperoxidase activity, glutathione levels and malondialdehyde concentration

Table 2 shows the MPO activity and antioxidant potential, measured as GSH and MDA concentrations, in mice pretreated with PFPe. After carrageenan administration, MPO activity ($9.18 \pm 0.91$ U/ml) and MDA levels ($41.83 \pm 1.78$ nmol/ml) were greater than that of the saline group ($2.79 \pm 0.50$ U/ml and $22.88 \pm 3.07$ nmol/ml, respectively). In addition, GSH levels ($93.67 \pm 44.68$ µg/ml) were less than those of the saline group ($410.70 \pm 47.78$ µg/ml). Treatment with PFPe (3 mg/kg, i.p.) significantly reversed all of these parameters, decreasing MPO activity ($5.38 \pm 0.78$ U/ml) and MDA levels ($29.32 \pm 3.26$ nmol/ml), and increasing GSH levels to $93.67 \pm 44.68$ µg/ml (Table 2).

## Effect of PFPe on interleukin-1 beta and tumour necrosis factor-alpha levels

The IL-1β ($1046.00 \pm 34.53$ pg/ml) and TNF-α levels ($170.40 \pm 25.46$ pg/ml of exudates) after carrageenan administration were significantly greater than those of the saline group (IL-1β = $52.26 \pm 11.26$ pg/ml and TNF-α = $46.26 \pm 1.56$ pg/ml). Treatment with PFPe (3 mg/kg, i.p.) significantly reduced ($P < 0.05$) IL-1β levels ($297.30 \pm 44.47$ pg/ml; Figure 4a) but not TNF-α levels ($144.00 \pm 6.38$ pg/ml; Figure 4b).

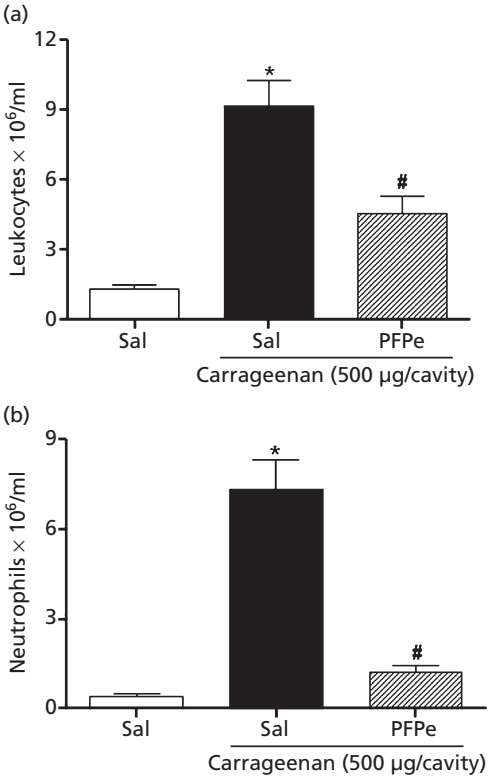


**Figure 3** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on cell migration in the carrageenan-induced peritonitis. The animals received saline (Sal) or PFPe (3 mg/kg) and were injected with carrageenan (500 µg/cavity, i.p.) 1 h later. Cell migration was evaluated after 4 h. (a) Total counts and (b) differential counts. Data are expressed as the mean ± standard error of the mean, $n = 6$–7, $^{*}P < 0.05$ vs saline, $^{\#}P < 0.05$ vs carrageenan.

## Effect of PFPe on acetic acid-induced writhing test

Pretreatment with PFPe (3 mg/kg, i.p., 1 h before acetic acid) produced a significantly decreased ($P < 0.05$) abdominal writhing response ($22.00 \pm 2.41$ writhing), compared with the group treated with acetic acid alone ($29.50 \pm 1.32$ writhing) (Figure 5). Morphine (5 mg/kg, s.c.), an opioid receptor agonist, had a potent analgesic effect ($0.66 \pm 0.42$ writhing).

## Effect of PFPe on formalin-induced paw licking time

Formalin administration triggered a nociceptive process, increasing paw licking time in mice in the first

  

**Table 2** Effect of PFPe on MPO activity, GSH levels and MDA concentration

| Treatment | Dose (mg/kg) | Parameters | | |
|---|---|---|---|---|
| | | MPO (U/ml) | GSH (μg/ml) | MDA (nmol/ml) |
| Saline | | 2.79 ± 0.50 | 410.70 ± 47.78 | 22.88 ± 3.07 |
| Carrageenan | | 9.18 ± 0.91* | 93.67 ± 44.68* | 41.83 ± 1.78* |
| PFPe | 3 | 5.38 ± 0.78# | 435.47 ± 4.86# | 29.32 ± 3.26# |

PFPe, polysaccharide fraction isolated from *Passiflora edulis*; MPO, myeloperoxidase; GSH, glutathione; MDA, malondialdehyde. Values of the biochemistry parameters are means ± SEM, $n = 5–6$, $^*P < 0.05$ vs saline group, $^{\#}P < 0.05$ vs carrageenan group.

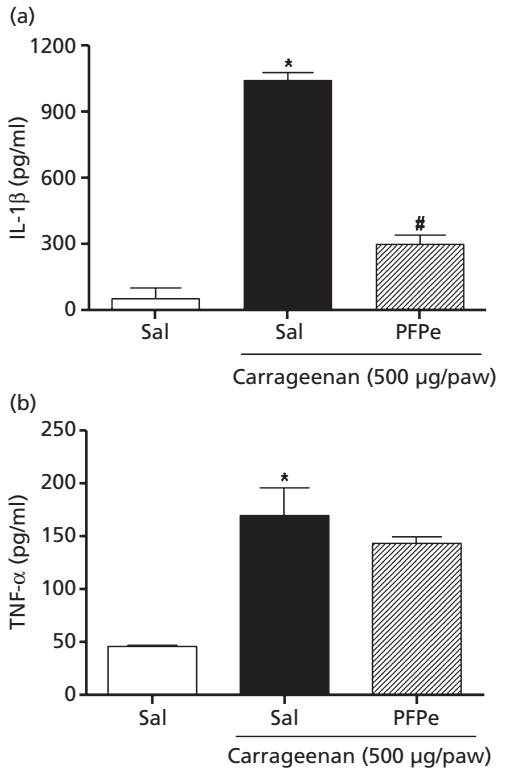


**Figure 4** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on carrageenan-induced cytokine production. Levels of tumour necrosis factor-alpha (a) and interleukin-1 beta (b) in the peritoneal cavity were measured 4 h after carrageenan injection. Mice were received saline (Sal) or PFPe (3 mg/kg, i.p.) and 1 h later carrageenan (500 μg/cavity, i.p.). Data are expressed as the mean ± standard error of the mean, $n = 6–7$, $^*P < 0.05$ vs saline, $^{\#}P < 0.05$ vs carrageenan.

(58.50 ± 8.87 s) and second phase (49.50 ± 11.87 s) of the test (Figure 6). Pretreatment with PFPe (3 mg/kg, i.p.) significantly inhibited ($P < 0.05$) licking during the early phase (neurogenic pain; 37.00 ± 4.60 s) and the late phase (inflammatory pain; 5.40 ± 2.76 s) of nociception ($P < 0.05$). Treatment with morphine (5 mg/kg, s.c.) also inhibited the response to formalin, decreasing ($P < 0.05$) the licking times in the first phase (20.20 ± 3.99 s) and second phase (2.00 ± 2.00 s).

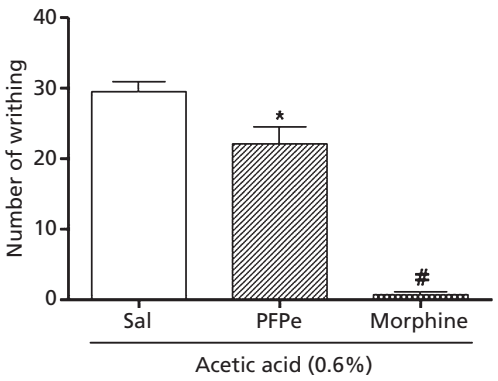


**Figure 5** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on the writhing response induced by acetic acid in mice. Mice received saline (Sal), PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c.; reference control), and 1 h later 0.6% acetic acid (250 μl/cavity; i.p.). Data are expressed as the mean ± standard error of the mean, $n = 6–7$, $^*P < 0.05$ or $^{\#}P < 0.05$ vs acetic acid group.

### Effect of PFPe on hot plate test

Pretreatment with PFPe (3 mg/kg, i.p.) did not increase the response latency of the animals in the hot plate test over that observed at time zero (Figure 7). On the other hand, treatment with morphine (5 mg/kg, s.c.) induced a significant increase ($P < 0.05$) in response latency throughout the experimental period.

### Rota-rod test

The animals treated with PFPe (3 mg/kg, i.p.) did not alter the performance in the rota-rod test compared with saline group (156.4 ± 12.1 s vs 166.0 ± 7.8 s, respectively). However, diazepam (5 mg/kg, i.p., reference drug) significantly reduced (44.0 ± 6.1 s) the residence time in the bar (Figure 8).

## Discussion

Data in the literature have shown that the fruit of plant *Passiflora edulis* is used traditionally in the popular medicine to treat inflammatory disorders and infections of the skin.[3] Thus, this study validated experimentally, using well

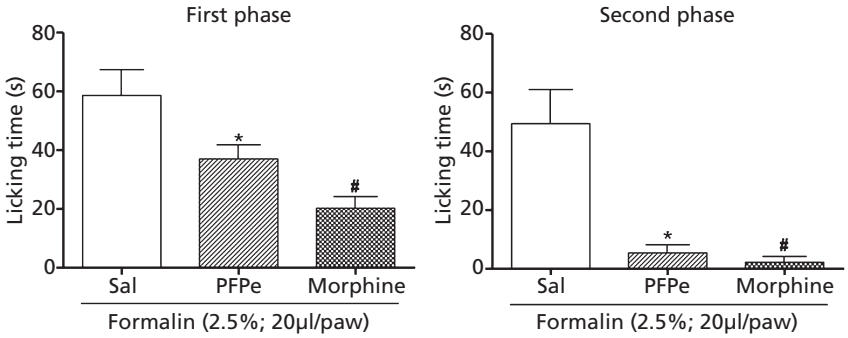


**Figure 6** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on the formalin test. The time spent licking was determined during the first 0–5 min (first phase; panel a) and 20–25 min (second phase; panel b) after injection with 2.5% formalin. Saline (Sal), PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c.; positive control) was administered 1 h before administration of formalin. Data are expressed as the mean ± standard error of the mean, $n = 6–7$, $^*P < 0.05$ vs formalin group.

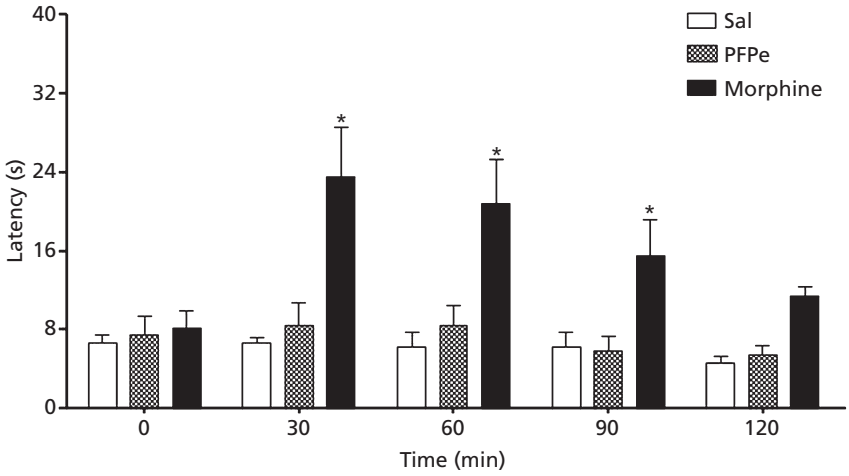


**Figure 7** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on reaction times to thermal stimuli (hot plate). Mice received saline (Sal), PFPe (3 mg/kg, i.p.) or morphine (5 mg/kg, s.c.). Data are expressed as the mean ± standard error of the mean, $n = 6–7$, $^*P < 0.05$ vs time zero.

characterized models, the use of fruit in reducing of the inflammatory response. Our results demonstrated that PFPe, a polysaccharide fraction isolated from *P. edulis*, possesses anti-inflammatory effect-dependent inhibition of the synthesis/release of important inflammatory mediators, such as histamine, serotonin, PGE2 and IL-1β levels. Furthermore, reduction of the neutrophils migration and oxidative stress is of great importance in the observed effects. In addition, we demonstrated its antinociceptive potential by modulating painful response by peripheral mechanisms.

Carrageenan-induced paw oedema is characterized as a biphasic inflammatory model. The early phase (1–2 h) is mainly mediated by increased synthesis of histamine, serotonin and prostaglandins in the damaged tissue. The late phase is sustained by the release of bradykinin and leukotrienes, neutrophils infiltration,[10,27] and production of

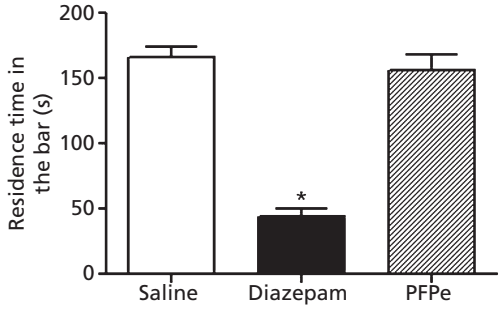


**Figure 8** Effect of polysaccharide fraction isolated from *Passiflora edulis* (PFPe) on rota-rod test. The animals were pretreated 1 h before test with saline, PFPe (3 mg/kg, i.p.) or diazepam (5 mg/kg, i.p.). Data are expressed as the mean ± standard error of the mean, $n = 8$, $^*P < 0.05$ vs saline group).

  

free radicals.[28] In this study, PFPe reduced the carrageenan-induced paw oedema in both the early and late phases of inflammation, suggesting that its anti-oedematogenic effect is mediated by the inhibition of several chemical mediators, as well as by a reduction of neutrophil migration.

To investigate the effect of the PFPe on the actions of specific inflammatory mediators during the acute inflammatory process, paw oedema was induced by PGE2, compound 48/80, histamine and serotonin. Prostaglandins are molecules derived from arachidonic acid metabolism and induce paw oedema by increased vascular permeability and production of chemo-attractants, such as IL-8 and IL-1β.[29,30] Our results showed that PFPe inhibited the formation of PGE2-induced paw oedema, which supports the hypothesis that the anti-inflammatory effect of PFPe is dependent, at least in part, on the inhibition of synthesis/action of these mediators.

Compound 48/80 triggers mast cell activation, resulting in the release of inflammatory mediators, such as histamine and serotonin, which are potent vasodilators.[31,32] In this study, we demonstrated that PFPe inhibited compound 48/80-induced paw oedema, suggesting that its anti-oedematogenic effect is due, partly, to the prevention of mast cell degranulation. These data corroborate the results from experiments with histamine- and serotonin-induced paw oedema, which showed that pretreatment with PFPe also inhibited the inflammatory process triggered by these phlogistic agents. In addition, our results showed that PFPe reduced the compound 48/80-induced vascular permeability. These results corroborate the previous results showing that compound 48/80-induced paw oedema was inhibited by pretreatment with PFPe.

Leucocyte aggregation at the site of injury is another important event in the inflammatory response. Carrageenan, when administered intraperitoneally, leads to inflammation of the peritoneal cavity, followed by neutrophil influx,[33] which is dependent of cytokines, like IL-1β and TNF-α.[34] Our results showed that PFPe significantly inhibited the migration of leucocytes and neutrophils to the peritoneal cavity after carrageenan-induced peritonitis. In addition, corroborating with these results, the administration of PFPe decreased MPO activity and is a biomarker for neutrophil infiltration,[35] confirming that the inhibition of neutrophil migration is an important component of the anti-inflammatory activity of PFPe.

Neutrophil migration to an injury site results in the overproduction of ROS, and leads to oxidative stress and damage of important biomolecules, such as proteins or DNA.[36] Evidence has implicated intracellular ROS production modulating the release of mediators of inflammation.[37] ROS can also regulate adhesion molecule expression on the endothelium, directly influencing the increase of cell recruitment to inflammation sites.[37]

We analysed the antioxidant potential of PFPe by two commonly used methods, MDA concentration and GSH levels. MDA is the major final product of lipid peroxidation, and its concentration reflects an imbalance between the oxidative and antioxidant systems. Elevated MDA levels are due to ROS-induced plasma membrane injury and have been shown in several models of inflammation. Molecules with the potential to decrease MDA concentration have been targets for the discovery of new anti-inflammatory drugs.[38] The tripeptide GSH is the main intracellular thiol antioxidant of living organisms.[39] A decrease in GSH concentration leads to increased ROS, failure of the immune response and increased susceptibility to infection.[40] Our results showed that pretreatment with PFPe increased GSH levels and reduced MDA concentration, which had been altered by carrageenan administration. These results indicate that this substance may protect tissue from ROS, thereby reducing inflammation.

Increased oxidative stress during inflammation also seems to be caused by the induction of cytokines, such as TNF-α and IL-1β, and activation of their target, nuclear factor-κB (NF-κB), leading to the production of ROS.[41] Cytokines constitute a link between cellular damage and cell migration, oedema, and hyperalgesia.[42] IL-1β might act directly on the target cells or through the induction of other inflammatory mediators involved with cellular recruitment, such as IL-6, IL-8, adhesion molecules and chemokines.[43–45] Pretreatment with PFPe reduced IL-1β levels in the peritoneal fluid, which had been increased by carrageenan administration. Given the important role of IL-1β in the inflammatory response, we suggest that the anti-inflammatory effects of PFPe may be associated, at least in part, with its ability to inhibit the synthesis or release of IL-1β.

The connection between the development of the inflammatory response and pain has been well characterized. Therefore, we assessed the antinociceptive activity of PFPe in three experimental models of pain: acetic acid-induced writhing, formalin-induced paw licking and the hot plate test.

In this study, we showed that pretreatment with PFPe promoted an antinociceptive effect in the model of acetic acid-induced visceral pain, a commonly used test to evaluate the antinociceptive potential of natural products.[31,46] In this line, acetic acid causes a painful response in the abdomen of the animals through the release of important inflammatory mediators (e.g. bradykinin, histamine and serotonin), cytokines (e.g. TNF-α and IL-1β) and chemokines that excite the nerve endings.[47] Thus, the inhibitory effect of PFPe observed in this model is due in part to modulation of the release of these inflammatory mediators and stabilization of mast cell membrane, and corroborates with our previous results of paw oedema, vascular permeability and IL-1β levels.

Formalin-induced paw licking is a model of persistent pain with two distinct phases.[48] The first phase (0–5 min) is neurogenic, primarily due to the direct activation of the nociceptive afferent C fibres, reflecting a central pain, which in turn leads to the release of bioactive amines. The second phase (15–20 min) involves the release of inflammatory mediators at the inflammatory sites and peripheral tissue irritation.[49] Our results show that PFPe reduced nociception in both phases, an effect similar to that of morphine, an analgesic opioid drug. This is consistent with our data showing that PFPe can modulate the oedema response induced by several mediators of inflammation. These molecules are also critical to the nociceptive response.

The pulp fruit from *Passiflora edulis* is used commonly in the popular medicine as tranquilizer because of the possible effects on CNS. Drugs that act as CNS depression may reduce the motor coordination in animals, interfering with pain behaviour and thus compromise the evaluation nociception test.[50] In this sense, we performed the rota-rod test to evaluate the possible action of PFPe on the locomotor activity of the animals. Our results show that PFPE did not alter the mobility, indicating that the reaction of animals in the nociception tests has not been compromised by a possible action of PFPE on CNS.

Finally, we performed the hot plate test to differentiate the central and peripheral nociceptive actions. This model measures a response to a thermal stimulus and is mediated by integration of central neuronal inputs that are susceptible to opioids.[51] We showed that pretreatment with PFPe did not increase the reaction time to a thermal stimulus, suggesting that the antinociceptive activity of the compound is related to peripheral mechanisms and excluding the possibility of involvement of central opioid receptors.

## Conclusions

In conclusion, PFPe administration reduced the inflammatory response by modulation of the synthesis or release of histamine and serotonin; by reduction of neutrophil migration, IL-1β levels and oxidative stress; and by scavenging free radicals. In addition, we show that PFPe decreased the nociceptive response induced by formalin and acetic acid, and that this effect is dependent, at least in part, from peripheral effects. Thus, our results reinforce the ethnopharmacological use of *P. edulis* in popular medicine.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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