

Original Article

The efficacy of the tea and polysaccharide-extract from *Cissus sicyoides* leaves to inhibit acute inflammation mediators in mice

O chá e o extrato polissacarídico das folhas de *Cissus sicyoides* apresentam eficácia inibitória sobre medidores da inflamação aguda em camundongos

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Abstract

Cissus sicyoides leaves are used in the Brazilian folk medicine to treat inflammatory processes and its polysaccharide-rich extract possesses healing effect. This study aimed to obtain and perform chemical analysis of the polysaccharide-rich extract (PE-Cs) and the tea (Tea-Cs) from *C. sicyoides* leaves and to investigate its anti-inflammatory effect. Mice received PE-Cs or Tea-Cs before inflammatory stimuli. Paw edema and hypernociception were induced by carrageenan, histamine, PGE₂, TNF- α or L-arginine and analyzed by plethysmometry/histopathology and analgesimetry. Both peritoneal cavity and naïve peritoneal macrophages were incubated with PE-Cs before being stimulated with carrageenan, and fluids evaluated for inflammatory and oxidative stress markers. PE-Cs (0.86% yield; 25.4% total carbohydrates, including 9.8% uronic acid) at 0.01 mg/kg inhibited the edema induced by carrageenan (58%), histamine (37%), TNF- α (46%) and L-arginine (73%). Histological analysis showed reduction of leukocyte infiltration and hemorrhage. PE-Cs and Tea-Cs [56% yield; 40.1% carbohydrates (5.1% uronic acid)] inhibited the carrageenan-edema by 60%. In the peritoneal fluid, PE-Cs reduced total protein (27%), neutrophil migration (92%), NO₂⁻ (20%), and MPO (1.8x), while increased GSH (2.2x). *In vitro*, PE-Cs reduced NO₂⁻ (30%) released by activated macrophages. PE-Cs and Tea-Cs inhibit acute inflammatory mediators elicited by carrageenan in mice.

Keywords: acute inflammation, carrageenan, “cipó pucá”, macrophage, oxidative stress, plant polysaccharide.

Resumo

As folhas de *Cissus sicyoides* são empregadas na medicina popular brasileira e o extrato polissacarídico obtido destas apresenta efeito cicatrizante. Este estudo objetivou, extrair, caracterizar quimicamente o extrato rico em polissacarídeos (PE-Cs) e o chá (Tea-Cs) das folhas de *C. sicyoides* e avaliar seu efeito anti-inflamatório. Camundongos foram pré-tratados com PE-Cs ou Tea-Cs antes dos estímulos inflamatórios. O edema de pata e a hipernociceção foram induzidos com carragenana, histamina, PGE₂, TNF- α ou L-arginina e analisados por pletismometria/histopatologia e analgesimetria. A cavidade peritoneal e os macrófagos peritoneais foram incubados com PE-Cs antes da estimulação com carragenana, e os fluidos avaliados quanto aos teores de marcadores inflamatórios e de estresse oxidativo. O PE-Cs (rendimento: 0,86%; carboidratos: 25,4%, incluindo ácido urônico: 9,8%) inibiu o edema induzido por carragenana (58%), histamina (37%), TNF- α (46%) e L-arginina (73%). A análise histológica revelou inibição do infiltrado leucocitário e de hemorragia. Tanto o PE-Cs quanto o Tea-Cs [rendimento: 56%; carboidratos: 40,1% (ácido urônico 5,1%)] inibiram o edema induzido por carragenana em 60%. No fluido peritoneal, o PE-Cs diminuiu proteínas totais (27%), migração de neutrófilos (92%), NO₂⁻ (20%) e MPO (1,8x), enquanto elevou GSH (2,2x). *In vitro*, o PE-Cs reduziu a liberação de NO₂⁻ (30%) por macrófagos ativados. Conclui-se que PE-Cs e Tea-Cs inibem mediadores de inflamação aguda estimulada por carragenana em camundongos.

Palavras-chave: inflamação aguda, carragenana, cipó pucá, macrófago, estresse oxidativo, polissacarídeo de planta.

1. Introduction

Acute inflammation is a physiological response to injury or infection involving blood vessels, inflammatory mediators and immune cells, playing essential role in vascular and cellular events (Larsen and Henson, 1983). Macrophages

modulate the neutrophil migration induced by inflammatory stimuli via release of cytokines, and reactive species of oxygen (malondyaldeide-MDA) and nitrogen (nitric oxide) (Cighetti et al., 1999; Cinelli et al., 2019).

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It is well known that the acute inflammatory reaction induced by carrageenan is characterized by exudation of fluid accompanied by plasma proteins and migration of leukocytes, via macrophage activation and release of chemotactic factors from neutrophils, such as PGE₂, TNF- α , NO (Nacife et al., 2000).

Plant polysaccharides are molecules presenting heterogeneous and complex structures, being known for its modulator effects in the immune system, such as inflammation, nociception and oxidative stress, as well as its relatively low toxicity (Yin et al., 2019).

Anti-inflammatory and antinociceptive effects of plant polysaccharide extracts have been extensively demonstrated in rodent models. The polysaccharides of *Caesalpinia ferrea* pods and barks, and *Ximenia americana* barks present inhibitory effect in the acute inflammation models of paw edema and peritonitis (Pereira et al., 2012; Marques et al., 2020; Holanda et al., 2021) and that from barks and pods of *C. ferrea* (Pereira et al., 2016; Mota et al., 2022; Assreuy et al., 2023), and cladodes of *Opuntia ficus-indica* (Adjafre et al., 2024) in the model of cutaneous wound healing. The polysaccharide-rich extract of *X. americana* barks inhibits peripheral inflammatory nociception (Silva-Leite et al., 2017), prevents indomethacin-induced gastrointestinal damage (Pantoja et al., 2018) and attenuates visceral hypernociception in acute pancreatitis induced by caerulein (Silva-Leite et al., 2018). Moreover, the leaf polysaccharide extract of *Genipa americana* inhibits oxidative stress in classical (Araújo et al., 2024) and neuro-inflammation models (Nonato et al., 2018, 2024).

Cissus sicyoides (L.) Klein ex. Steud or *Cissus verticillata* (L.) Nicolson & C.E.Jarvis (Vitaceae family) is distributed in Neotropical regions, popularly known as “anil-trepador” or “cipó-pucá”, being part of the Caatinga (semi-arid) vegetation of Northeast Brazil. Its leaves are traditionally used in the Brazilian folk medicine as decoction to treat inflammatory disorders, such as rheumatism, epilepsy, stroke, abscesses, arthritis and diabetes (Silva et al., 2012). Besides, experimental studies reported for the aqueous extract of *C. sicyoides* leaves the antinociceptive effect (Almeida et al., 2006), and for its polysaccharide-rich extract cutaneous healing (Souza et al., 2024) and antioxidant effect in ovarian tissue culture (Aguar et al., 2025).

This study aimed to obtain the polysaccharide-rich extract, and the tea prepared from *C. sicyoides* leaves, to investigate its inhibitory effect in mice models of acute inflammation (paw edema, peritonitis) and in isolated macrophages, focusing on the participation of inflammatory mediators.

2. Materials and Methods

2.1. Plant material

C. sicyoides leaves were collected in the District of Custódio-Quixadá, Ceará and a voucher specimen deposited at the Herbarium Prisco Bezerra of Federal University of Ceará (n° 61902). The assessment activity was registered in the National System for the Management of the Genetic Heritage and Associated Traditional Knowledge (Code AF8E9ED).

2.2. Polysaccharides extraction and chemical analysis

Leaves were washed with distilled water, dried at 40 °C and grounded into fine particles. Absolute MeOH was added to 5 g of powder (1:50, w/v, 76 °C) for removal of pigments (procedure repeated twice). The insoluble portion was extracted (3 times) with 0.1 M NaOH at 97 °C and centrifuged (2496 × g; 25 °C). The supernatant containing the alkaline polysaccharides was pooled, neutralized in 1 M HCl, precipitated with 4 volumes of EtOH and centrifuged. The precipitate was resuspended and dialyzed in distilled water followed by deproteination with trichloroacetic acid (TCA; pH 3.0, 4 h, 4 °C) and centrifugation (Pereira et al., 2012; Souza et al., 2024). The supernatant was dialyzed and lyophilized, providing the polysaccharide-rich extract of *Cissus sicyoides* (PE-Cs). For the tea preparation, 3 g of entire dry leaves were immersed in 1 L of distilled water, boiled for 5 min, filtered, lyophilized and named Tea-Cs.

The chemical analysis of PE-Cs and Tea-Cs was performed by the quantification of total carbohydrates (Dubois et al., 1956), uronic acid (Dische, 1947), proteins (Bradford, 1976) and polyphenols (Siddiqui et al., 2017). D-Galactose (A₄₉₀ nm), D-galacturonic acid (A₅₂₅ nm), bovine serum albumin (A₅₉₅ nm) and gallic acid (A₇₆₀ nm) were used as standards.

2.3. Animals

Female Swiss mice (25-35 g) were maintained at 22-25 °C, 12/12 h light/dark cycle, receiving water and food *ad libitum*. The experimental protocols were performed following the guidelines of the Brazilian College of Animal Experimentation (COBEA) and Animal Care and Use Committee of the State University of Ceará (n° 5748564/2015), Fortaleza, CE, Brazil.

2.4. Evaluation of PE-Cs and Tea-Cs in acute inflammation models

Mice (n=8 per group) received by intravenous (i.v.) route PE-Cs (0.001 - 0.1 mg/kg), N(gamma)-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg) or sterile saline (0.9% NaCl; 0.1 mL/100 g) 30 min before injection of the inflammatory stimuli. PE-Cs or Tea-Cs (0.1 mg/kg) were also given *per oral* (p.o.) 60 min before stimulation. PE-Cs was evaluated in the experimental models of paw edema and peritonitis for the inflammatory parameters edema, hyperalgesia, leukocyte migration, vascular permeability, oxidative and nitrosative stress markers, such as NO₂⁻, myeloperoxidase (MPO), reduced glutathione (GSH), and histopathological alterations.

Paw edema was induced by subcutaneous (s.c.) injection of λ -carrageenan (300 μ g), or inflammatory mediators involved along the edema time-course elicited by carrageenan, such as histamine (100 ng/paw), prostaglandin E₂ (PGE₂; 30 μ g/paw), tumor necrosis factor alfa (TNF- α ; 5 nmol/paw) or L-arginine (15 nmol/paw). Edema was measured by plethysmometry before (zero time) and at 30, 60, 120-300 min thereafter inflammatory stimuli, calculated and expressed as the difference in paw volume displacement (μ l) or area under curve-AUC (arbitrary units) (Pereira et al., 2012).

The hypernociceptive reaction (paw withdrawal) was quantified (g) before (zero time) and 60-240 min thereafter stimulation with carrageenan (s.c.). For this, 30 min before evaluation, animals were placed in clear plexiglass boxes of

elevated wire mesh to allow access to the ventral surface of hind paws, in which six consecutive mechanical pressures were applied (polypropylene tip - 0.5 mm diameter coupled to digital analgesimeter) (Cunha et al., 2005).

Histopathological analysis of subplantar tissue fragments from euthanized animals was performed 4 h after carrageenan injection. Tissues were fixed overnight in 10% buffered paraformaldehyde embedded in paraffin, sliced (5 µm thickness) and stained with hematoxylin-eosin (H&E). Morphological changes were observed by light microscopy and graded as follows: (0) Normal tissue: absence of edema, inflammatory infiltrate or hemorrhagic areas; (1+) Discrete, (2+) Moderate (3+) or Severe tissue changes: edema, inflammatory infiltrate and focal areas of hemorrhage (Lim et al., 2010). Polymorphonuclear cells were counted in 10 fields (Software Nis 4.0.) at 400x magnification and quantified by Image J software (NIS, USA).

Animals received intraperitoneal (i.p.) injection of carrageenan (500 µg/100 µL) 4 h before being euthanized. The peritoneal fluid was collected with 3 mL saline (5 IU heparin) for quantification of total and differential leukocytes (Pereira et al., 2012), total protein (A_{595nm}) (Bradford, 1976), NO_2^- (A_{540nm}) (Green et al., 1982), GSH (A_{412nm}) (Sedlak and Lindsay, 1968), and MPO (A_{405nm}) (Bradley et al., 1982).

2.5. Evaluation of PE-Cs in isolated macrophages

Naive peritoneal macrophages were collected under aseptic conditions, washed twice with RPMI by centrifugation (100 × g, 5 min), and pellets were resuspended in 1 mL culture medium (RPMI) containing 10% BSA, penicillin (100 U/mL) and streptomycin (100 U/mL). Macrophage monolayers were prepared by addition of 1 mL/well (10^6 macrophages) to 24-well culture plates, for cell adhesion (24 h, 37°C, 5% CO_2). Plate-adhered macrophages were incubated (60 min, 37°C) with PE-Cs (40 or 80 µg/mL) or RPMI, followed by additional incubation (60 min) with carrageenan (30 µg/mL) or RPMI. Macrophage monolayers were washed and incubated for 3 h with 1.5 mL RPMI for the release of chemotactic factors. Supernatants were collected, centrifuged during 5 min for NO_2^- dosage. Cell viability was analyzed by trypan blue exclusion (Cunha and Ferreira, 1986).

2.6. Statistical analysis

Data was expressed as mean ± S.E.M. and analyzed by ANOVA and Bonferroni tests (Prism 5.0, GraphPad Software Inc., California, USA). Histopathological results were expressed as Median (maximum and minimum) and analyzed by Kruskal-Wallis and Dunn's test. Significance was set at $p < 0.05$.

3. Results

3.1. Chemical analysis of the polysaccharide-rich extract from *C. sicyoides* leaves (PE-Cs) and Tea-Cs

PE-Cs, presenting 0.86% yield, showed high content of total carbohydrates (including uronic acid) compared to the low content of polyphenols and proteins. Tea-Cs

(56% yield) showed higher content of total carbohydrates (PE-Cs: 25.4% vs. Tea-Cs: 40.1%), polyphenols (PE-Cs: 0.97% vs. Tea-Cs: 1.14%) and proteins (PE-Cs: 0.32% vs. Tea-Cs: 0.54%) compared to PE-Cs. The carbohydrate/uronic acid ratio was higher in PE-Cs (0.38%) compared to Tea-Cs (0.12%), as well as the percentage of uronic acid (PE-Cs: 9.8% vs. Tea-Cs: 5.1%).

3.2. PE-Cs inhibits the paw edema and hypernociception induced by carrageenan

Carrageenan induced paw edema that lasted from 1-5 h after injection, being inhibited by PE-Cs at 0.01 mg/kg and 0.1 mg/kg (Figure 1A). PE-Cs inhibited this edema (70.7 ± 1.8 vs. saline: 13.5 ± 2.0 AUC) from 0-5 h at 0.01 mg/kg (13.2 ± 1.7 AUC) and 0.1 mg/kg (12.9 ± 0.5 AUC) by 57% and 58% respectively, showing maximal effect at 0.01 mg/kg, but lacking in efficacy at 0.001 mg/kg (Figure 1B). The inhibitory effect of PE-Cs was observed at the edema initial phase (0-2 h), mainly in the late phase (2-4 h). At the initial phase PE-Cs was inhibitory by 60% at 0.01 mg/kg (43 ± 8.6 AUC) and 57% 0.1 mg/kg (46.5 ± 2.5 AUC) compared to carrageenan (Cg: 105.5 ± 8 AUC) (Figure 1C). At the second phase PE-Cs was inhibitory by 42% at 0.001 mg/kg (109.7 ± 13.4 AUC), with maximal effect by 62% at 0.01 mg/kg (72 ± 17 AUC) versus carrageenan (187 ± 12 AUC) (Figure 1D). PE-Cs (0.01 mg/kg) also inhibited the hypernociception induced by carrageenan (17.62 ± 0.37 AUC), increasing (1.18x) the nociceptive threshold (20.79 ± 0.81 AUC) in response to mechanical stimulation (Figure 1E).

The anti-edematogenic effect of PE-Cs (0.01 mg/kg; i.v.), by 60%, was reproduced by its *per oral* administration (128.8 ± 17.37 vs. Cg: 318.8 ± 22.5 AUC) (Figure 2A-B). Besides, this effect (62%: 20 ± 7.303 vs. Cg: 48.57 ± 17.38 µL) was maintained up to 24 h. Also, Tea-Cs *p.o.* (0.1 mg/kg) inhibited by 46% the paw edema induced by carrageenan (86.5 ± 17.5 vs. PE-Cs: 37.2 ± 12.9 AUC), an effect that was maintained up to 24 h (Cg: 87.5 ± 16.4 vs. PE-Cs: 10 ± 5.3 µL) (Figure 2C-D).

3.3. PE-Cs reduces inflammatory paw-tissue alterations induced by carrageenan

Carrageenan (400 µg/paw) elicited intense leukocyte infiltration, with presence of hemorrhagic areas and congestive vessels in the animal paw-tissues [median score: 3 (+3; +2) vs. saline: 1 (+2; +1)]. PE-Cs reduced significantly these alterations [median score: 2 (+2; +1)] (Figure 3).

3.4. PE-Cs inhibits the effect of inflammatory mediators that participate in the carrageenan-induced edema

PE-Cs (0.01 mg/kg; i.v.) reduced the edematogenic effect elicited either by initial or late-phase inflammatory mediators involved in the carrageenan-induced edema. PE-Cs inhibited by 37% (116 ± 15 AUC) the paw edema elicited by histamine (182.9 ± 20.7 vs. saline: 116 ± 15.04) (Figure 4A), by 46% (145 ± 51.1 AUC), the edema induced by TNF- α (267 ± 52.1 vs. saline: 83 ± 36.6 AUC) (Figure 4C), and by 73% (172 ± 47.6 AUC) that induced by L-arginine (633 ± 82.6 vs. saline: 165 ± 67.5 AUC). The PE-Cs inhibitory effect on L-arginine-induced edema was of similar efficacy (78%) compared to L-NAME (138 ± 36.2 AUC) (Figure 4D).

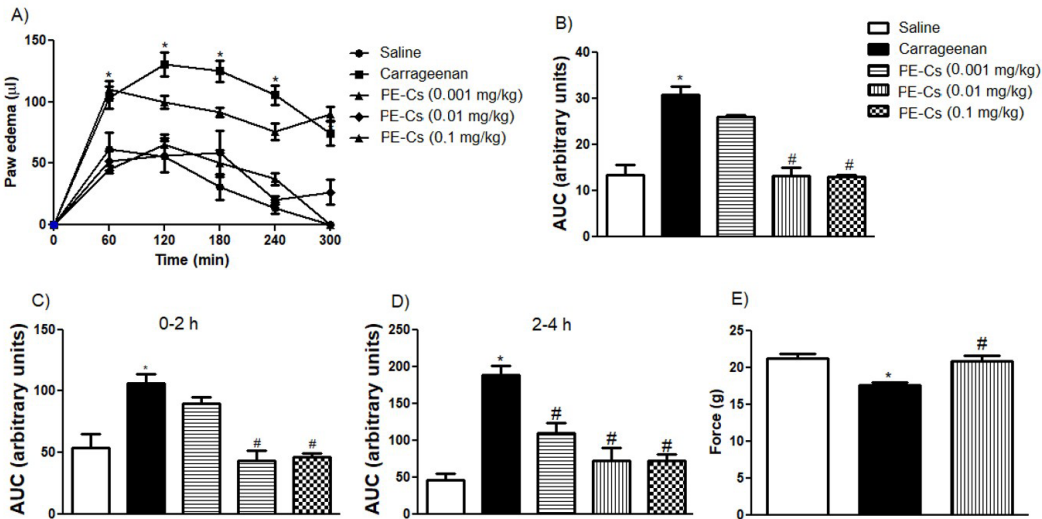


Figure 1. Intravenous treatment with PE-Cs inhibits paw edema and mechanical hypernociception induced by carrageenan. Mice received PE-Cs (0.001–0.1 mg/kg i.v.) 30 min before carrageenan (300 µg/i.p.). Paw edema was measured by hydroplethysmometry. (A) Edema time-course (mL); (B) Area under curve-AUC (arbitrary units); (C) Paw edema (AUC: 0–2 h); (D) Paw edema (AUC: 2–4 h); (E) Paw withdrawal reaction (g). Mean $\pm$ S.E.M. (n=8). One-way ANOVA/Bonferroni. * p <0.05 vs. Saline, # p <0.05 vs. carrageenan.

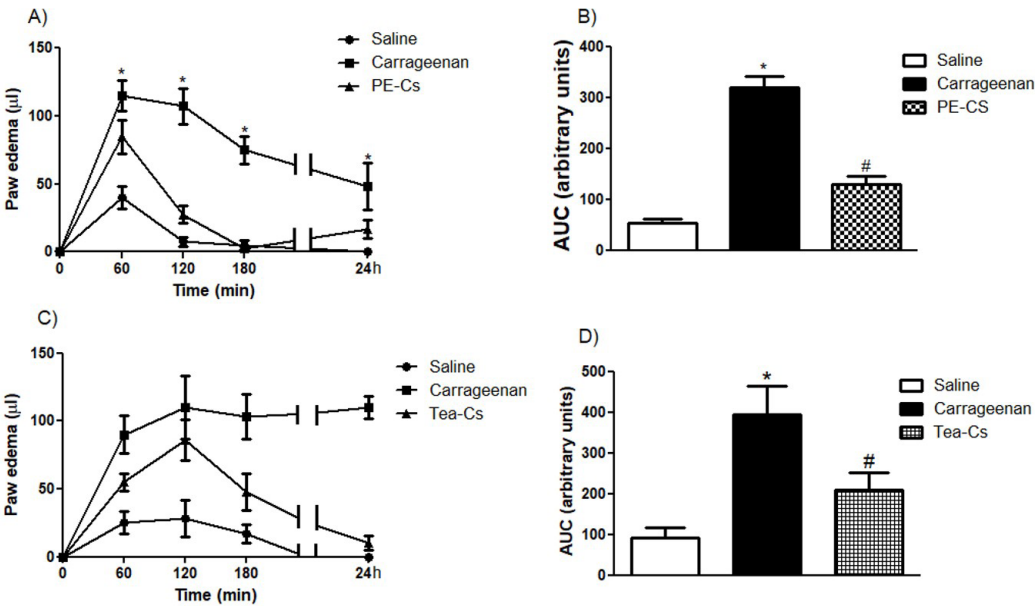


Figure 2. Per oral treatment with PE-Cf and Tea-Cf inhibits paw edema induced by carrageenan. Mice received PE-Cs (0.01 mg/kg p.o.) or Tea-Cs (0.1 mg/kg p.o.) 60 min before stimuli. Paw edema was measured by hydroplethysmometry. (A and C) Edema time-course (mL); (B and D) Area under curve-AUC (arbitrary units). Mean $\pm$ S.E.M. (n=8). One-way ANOVA/Bonferroni. * p <0.05 vs. Saline, # p <0.05 vs. carrageenan.

However, PE-Cs did not alter the edema induced by PGE_2 (Figure 4B).

3.5. PE-Cs alters peritoneal leukocyte migration and oxidative stress markers in the peritoneal fluid stimulated with carrageenan

PE-Cs (0.01 mg/kg) reduced by 52% ($2286 \pm 137 \times 10^3$ cell/mL) the total leukocytes migration to peritoneal cavities

4 h after carrageenan ($4760 \pm 279 \times 10^3$ vs. saline: $1410 \pm 121 \times 10^3$ cell/mL). Neutrophils were the most inhibited cells (92%) by PE-Cs ($150 \pm 28 \times 10^3$ cell/mL vs. Cg: $2026 \pm 134 \times 10^3$ vs. saline: $88.2 \pm 18.5 \times 10^3$ cell/mL) (Figure 5A). The total proteins were also reduced (27%) by PE-Cs ($0.24 \pm 0.02 \times 10^3$ mg/mL vs. Cg: $0.34 \pm 0.02 \times 10^3$ mg/mL vs. saline: $0.17 \pm 0.02 \times 10^3$ mg/mL) (Figure 5B).

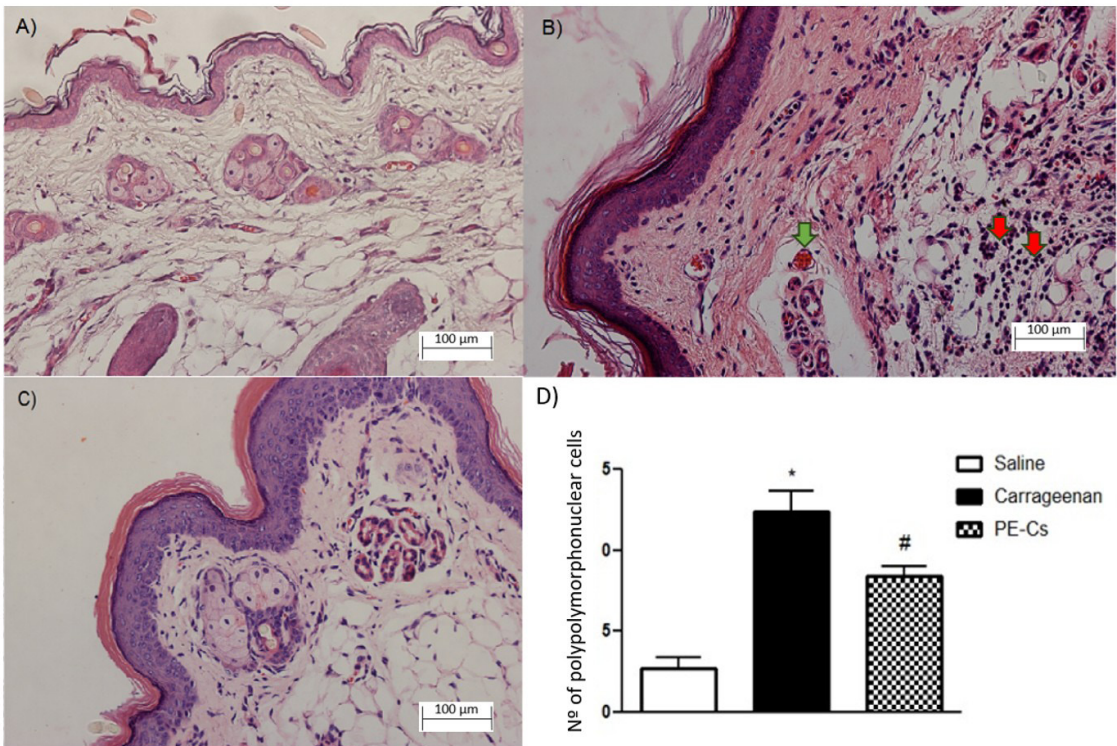


Figure 3. PE-Cs reduces inflammatory events in the paw edema induced by carrageenan. Paw tissues were collected after 4 hours with paw edema induction, submitted to the stages of fixation, dehydration, diaphanization and embedded in paraffin. The samples were sectioned to a thickness of 3 µm, stained in HE and analyzed by optical microscopy. (A) Saline: absence of edema, inflammatory infiltration, hemorrhagic areas or congestive vessels; (B) Carrageenan (1%; 400 µg/paw): intense leukocyte infiltration (red arrows), hemorrhagic areas and congestive vessels (green arrow); (C) PE-Cs: discrete inflammatory infiltration, hemorrhagic areas and congestive vessels. HE 400x. Nikon Eclipse Nis, Software Nis 4.0.

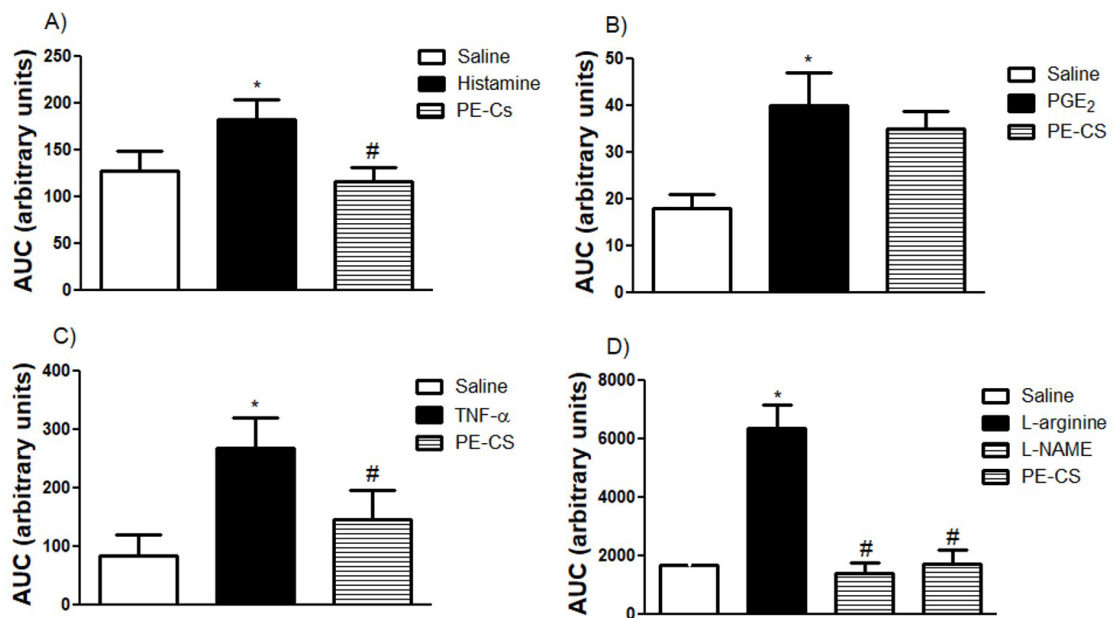


Figure 4. PE-Cs inhibits paw edema induced by histamine, PGE₂, TNF-α and L-arginine. Mice received PE-Cs (0.01 mg/kg i.v.) 30 min before stimuli and paw edema measured by hydroplethysmometry. (A) Histamine (100 ng/paw); (B) PGE₂ (30 nmol/paw); (C) TNF-α (15 ng/paw); (D) L-arginine (15 nmol/paw). Mean ± S.E.M. (n=8). One-way ANOVA/Bonferroni. *p<0.05 vs. Saline, #p<0.05 vs. inflammatory stimuli.

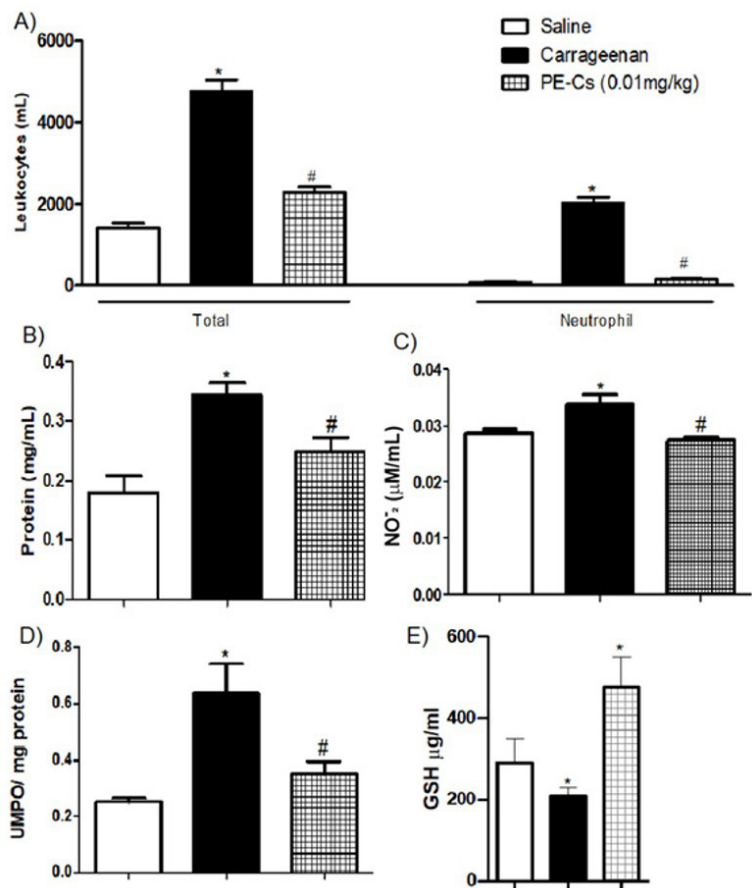


Figure 5. PE-CS inhibits neutrophil migration, protein leakage and alters stress oxidative markers. Mice received PE-Cs (0.01 mg/kg; i.v.) 30 min before carrageenan (500 μ g i.p.). The evaluation was performed 4 h after stimulation. (A) Total leukocytes and neutrophils; (B) Total protein (A_{595nm}); (C) NO₂⁻ (A_{540nm}); (D) Activity of myeloperoxidase (MPO) (A_{405nm}); (E) GSH (A_{412nm}). Mean $\pm$ S.E.M. (n=8). One-way ANOVA/Bonferroni. *p<0.05 vs. Saline, #p<0.05 vs. Carrageenan.

The evaluation of peritoneal fluid 4 h after peritonitis induction showed that the increase of NO₂⁻ induced by carrageenan (0.03 ± 0.001 vs. saline: 0.02 ± 0.0007 /mL) was reduced (20%) by PE-Cs (0.02 ± 0.0005 /mL) (Figure 5C) and in 1.8x (0.34 ± 0.04 U/mL) the MPO activity (Cg: 0.63 ± 0.1 vs. saline: 0.25 ± 0.01 U/mL) (Figure 5D). Besides, the levels of GSH were increased in 2.2x (477.2 ± 33.3 ng/mL vs. Cg: 208 ± 8.7 vs. saline: 290.9 ± 26.2 ng/mL) (Figure 5E).

3.6. PE-Cs reduces NO₂⁻ in the supernatant from cultured macrophages stimulated with carrageenan

The concentration of NO₂⁻ in the supernatant of macrophages stimulated with carrageenan was increased in 3.2x (0.59 ± 0.05) compared to the control using the culture medium RPMI (0.18 ± 0.04). The pre-incubation of PE-CS at 80 μ g/mL, but not at 40 mg/mL (0.64 ± 0.05), inhibited by 31% (0.41 ± 0.04) this relation. On the other hand, the incubation of non-stimulated macrophages with PE-Cs either at 40 μ g/mL (0.17 ± 0.04) or 80 μ g/mL (0.18 ± 0.007) did not alter NO₂⁻ in the supernatant, being similar to RPMI (Figure 6).

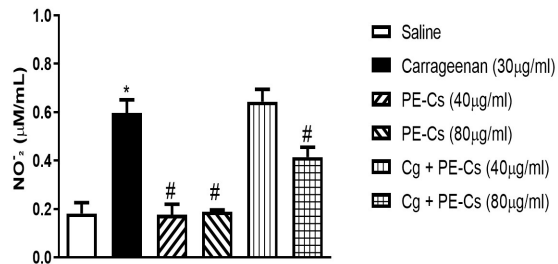


Figure 6. PE-Cs reduces NO₂⁻/NO₃⁻ of the supernatant from cultured macrophages stimulated with carrageenan. Isolated peritoneal macrophages were pre-incubated with PE-Cs (40 or 80 μ g/mL) or RPMI 30 min before stimulation during 1 h with carrageenan (30 μ g/mL). After 3 h NO₂ was measured by the Griess method. Mean $\pm$ S.E.M. (n=4). One-way ANOVA/Bonferroni. *p<0.05 vs. Saline, #p<0.05 vs. Carrageenan.

4. Discussion

This study demonstrated that the polysaccharide-rich extract obtained from the leaves of *C. sicyoides*

(PE-Cs), presenting high content of carbohydrates and low polyphenols and proteins, similar to that demonstrated previously (de Souza et al., 2024) inhibits acute inflammatory parameters *in vivo* and *in vitro*. Furthermore, the tea preparation from its leaves (Tea-Cs), containing higher total carbohydrates, and low content of polyphenols and proteins, also inhibited the paw edema elicited by carrageenan.

The development of paw edema induced by carrageenan, firstly described in rodents, is a biphasic event (Levy, 1969). The first phase (1–2 h) is characterized by release of histamine, serotonin and bradykinin, while the second phase (2–4 h) has been correlated to production of PGE₂, leukotrienes, the cytokines TNF- α , interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β), and reactive oxygen and nitrogen species (Vinegar et al., 1969).

PE-Cs, injected *i.v.* or *p.o.*, presented inhibitory effect on both phases of carrageenan-induced edema, as well as Tea-Cs *p.o.*, that showed maximal effect in the cell infiltrate phase. However, we cannot exclude the vascular effect of PE-Cs, since it showed inhibitory response on paw edema elicited by histamine and L-arginine. It is important to highlight the great efficacy of PE-Cs anti-inflammatory effect (*i.v.*) at 0.01 mg/kg in comparison with the polysaccharide extract obtained by similar protocols from *C. ferrea* pods (*i.v.*), that had its maximum effect at a dose one-hundred higher (1 mg/kg) in the model of rat paw edema induced by carrageenan (Pereira et al., 2012). This great efficacy was also observed for the *per oral* treatment with Tea-Cs (0.1 mg/kg), showing maximal anti-edematogenic effect compared to PE-Cs (0.01 mg/kg), given at dose 10x higher. Moreover, the efficacy of Tea-Cs was shown to be similar to that of the tea preparation of *X. americana* barks at a dose 1000x higher (100 mg/kg, *per oral*) (Marques et al., 2020). These data indicate that PE-Cs contains bioactive substances present in the tea used in folk medicine to treat inflammatory processes. The anti-inflammatory effect of PE-Cs *i.v.* was confirmed by the histological analysis that showed intense reduction in the number of polymorphonuclear cells in the paw-tissues.

PE-Cs also inhibited, at different degrees, the paw edema induced by inflammatory and hyperalgesic mediators involved in the time-course of the edema induced by carrageenan, such as histamine, TNF- α , and nitric oxide (NO), but not PGE₂. In this line, there is experimental evidence suggesting a modulator crosstalk between the nitric oxide and prostaglandins pathways (Salvemini and Masferrer, 1996), which is in accordance with the present study, showing a clear inhibitory selectivity of PE-Cs for the nitric oxide pathway.

As well known, carrageenan stimulates a behavioral response to thermal or mechanical stimuli, in which the animals develop a state of hyperexcitability, clinically expressed as hyperalgesia (Cunha et al., 2005). In our study, PE-Cs inhibited the paw-withdrawal response to carrageenan, an effect that reinforces the inhibition of paw edema induced by the nociceptive mediators TNF- α and L-arginine, being in line with the important role of NO in the nociception modulation (Cury et al., 2011).

In addition to the inhibitory effect on edema and hypernociception, PE-Cs reduced protein, total leukocytes and neutrophil migration to mice peritoneal cavities stimulated with carrageenan. The reduced protein content in the peritoneal fluid is coherent with the inhibitory effect of PE-Cs in the osmotic phase (0–2 h) of carrageenan-induced edema, as well as with the inhibition of paw edema induced by histamine, a potent vasodilator mediator (Posadas et al., 2004). Our results demonstrated significant decrease in leucocyte migration and myeloperoxidase activity, corroborating the histological findings.

Oxidative stress involves an excessive amount of reactive oxygen and nitrogen species that exceed the neutralizing capacity of endogenous antioxidants, leading to the oxidation of enzymes, proteins, DNA, and lipids being present in the inflammatory reaction induced by carrageenan (Nacife et al., 2000; Levy, 1969). Besides, some free radicals formed during oxidative stress may be degraded/converted by antioxidant substances, such as superoxide dismutase (SOD) and reduced glutathione (GSH) (Picón-Pagès et al., 2019). GSH not only acts in the elimination of oxygen-reactive species but also in the removal of toxic electrons and heavy metals (Calabrese et al., 2017). MPO, an enzyme highly expressed in neutrophils, plays essential role in the inflammatory response, catalyzing the formation of reactive species involved in microbial killing (Galijašević, 2013). In our study PE-Cs increased the concentration of reduced glutathione (GSH), decreased the activity of MPO and NO₂⁻ concentration in the peritoneal fluid of animals stimulated with carrageenan. These data are in accordance with the inhibitory action of PE-Cs on the edema induced by L-arginine, suggesting an antioxidant effect.

In physiological conditions, NO promotes hemostasis via vasodilatation and regulation of vascular tonus, anti-oxidative activity and inhibition of neutrophil activation. However, at high concentrations NO induces apoptosis, oxidative stress, neutrophil activation, DNA damage and inhibition of mitochondrial respiration, promoting inflammation as consequence of oxidative pathways, leading to the release of pro-inflammatory cytokines, such as TNF- α and the activation of induced nitric oxide synthase (iNOS) (Guzik et al., 2003; Sharma et al., 2007). In our study the concentration of NO₂⁻ was detectable in the supernatant of macrophages stimulated with carrageenan, that was inhibited by PE-Cs previous incubation. This data is in line with the literature, since NO is a mediator produced in high quantities by carrageenan-stimulated macrophages and that plant polysaccharides promote NO production in macrophages (Yin et al., 2019). Thus, it is possible to be suggested that the anti-inflammatory effect of PE-Cs involves the NO pathway.

Summarizing, our results revealed that the polysaccharides obtained from *C. sicyoides* leaves could inhibit the paw edema induced by carrageenan and that of the inflammatory mediators NO, histamine and TNF- α . Moreover, as a naturally produced biomolecule, PE-Cs did not display cytotoxic effects in cultured macrophages. Therefore, these polysaccharides have the potential to be used to treat acute inflammation, validating the popular use of the tea for inflammatory disorders.

5. Conclusion

The polysaccharide-extract (PE-Cs) and the tea (Tea-Cs) prepared from *Cissus sicyoides* leaves inhibit mice acute inflammation, an effect assigned to the negative modulation of inflammatory mediators, mainly nitric oxide, involved in the acute carrageenan-induced inflammation.

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Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available due to institutional restrictions and ethical considerations involving animal experimentation, but are available from the corresponding author upon reasonable request.

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