



Antiarthritic property and toxicological evaluation of the tea prepared from *Ximenia americana* barks

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Abstract

Ximenia americana L. (Olacaceae), popularly known as wild plum, northern plum or Brazilian plum, is used in the folk medicine to treat several inflammatory conditions. This study evaluated the effect of the tea, containing total carbohydrates (44.8%), polyphenols (28 gallic acid equivalent - GAE) and proteins (1.3%), and the polysaccharide fraction (FII) of *X. americana* barks (Tea-Xa) on rat zymosan-induced arthritis, along with the acute toxicity of Tea-Xa given *per oral*. Female rats ($N=36$; 200–220 g) received Tea-Xa (30–300 mg/kg; *p. o.*) or FII (1 mg/kg; *i.v.*) 2 h after zymosan-induced arthritis, being evaluated for hypernociception, edema and articular leukocyte influx, histopathological alterations (inflammatory signs, tissue necrosis and disorganization, edema). For toxicity (OECD-423) female rats ($N=9$; 170–200 g) received *per oral* Tea-Xa (300 or 2000 mg/kg) and were observed for behavioral (14 days) and hematological, biochemical and histopathological parameters (day 14). Tea-Xa and FII reduced joint hypernociception and edema (47%), articular cavity neutrophils (83%) and nitrite (55%). Joint histopathological analysis revealed that Tea-Xa (30–300 mg/kg) and FII reduce tissue disorganization, and Tea-Xa attenuates the inflammatory infiltrate, necrosis and tissue disorganization. Tea-Xa (LD₅₀ greater than 2000 mg/kg/body weight) did not alter body weight, relative organ mass, liver and kidney function markers, or tissue structure. No alterations were observed of most animal behavioral parameters. The polysaccharides of *X. americana* possess anti-inflammatory and antinociceptive effects on zymosan-induced arthritis and Tea-Xa did not produce adverse effects or mortality.

Keywords Acute oral toxicity · Experimental arthritis · OECD 423 · Wild plum · Zymosan

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic and autoimmune disease of unknown etiology, in general related to environmental and genetic factors, that affects the joints (Guo et al. 2018) of approximately 1% the worldwide population, impacting their work capacity and life quality,

showing prevalence in women and increasing with age (Radu and Bungau 2021).

RA is characterized by synovial tissue proliferation, pannus formation, cartilage destruction, edema, and joint pain (Jang et al. 2022). The acute and chronic phases of RA involve leukocyte activity, mainly of neutrophils, via release of proteolytic enzymes, formation of inflammatory mediators from activated resident cells (Cecchi et al. 2018), such as macrophages and lymphocytes involved in the leukocyte migration, tissue disorganization and necrosis (Veale et al. 2017).

Zymosan, a polysaccharide derived from *Saccharomyces cerevisiae*, is experimentally used to mimic RA, whose intra-articular administration promotes inflammation characterized by severe synovitis, necrosis and joint tissue disorganization. The acute phase lasts until the 3rd day after induction and is characterized by increase in vascular

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permeability, edema and migration of leukocytes, mainly neutrophils, followed by mononuclears at the 4th day, and production of inflammatory mediators including cytokines and nitric oxide (NO) that lead to nociceptors sensitization (Bringel et al. 2020).

The pharmacological therapy for the control and relief of RA signs and symptoms includes non-steroidal anti-inflammatory drugs, glucocorticoids, and the disease-modifying anti-rheumatic drugs (Brown et al. 2024). However, the pain evoked from RA is difficult to relieve, either with pharmacological or non-pharmacological strategies (Aliverini et al. 2022). Moreover, despite its effectiveness, the pharmacological therapy produces adverse effects, such as hypertension, hyperglycemia, immunosuppression, depression, anxiety, gastrointestinal and cardiovascular disorders, that restrict its long-term use (Brown et al. 2024).

Ximenia americana L. is a tropical plant belonging to the Olacaceae family, popularly known as wild plum, northern plum or Brazilian plum, being widely distributed in the Brazilian Northeast, Africa, India, New Zealand, Central America and South America (Souza et al. 2014). In folk medicine this plant is used for the treatment of several inflammatory conditions, such as stomach pain, cancer, mouth infections, rheumatism (Brasileiro et al. 2008), fever, sore throats, asthma, abdominal pain, mouth ulcers and inflamed joints (Lall et al., 2014). In Brazil, the tea prepared from *X. americana* barks is popularly used to treat dermatitis, wound healing, flu, antipyretic, headache and diabetes (Souza et al. 2014).

Experimental data demonstrated that *per oral* administration of the tea obtained from *X. americana* barks inhibits neutrophil infiltration in the in mice model of gastritis induced by indomethacin (da Silva-Pantoja et al. 2018), and the paw edema induced by zymosan (Marques et al. 2020). This tea had been characterized for the content of total carbohydrates (uronic acid), polyphenols and proteins by chemical methods, including infrared spectroscopy (FT-IR) (da Silva-Leite et al. 2017; Marques et al. 2020).

The polysaccharides of *X. americana* barks are composed by arabinose, galactose, rhamnose and high content of uronic acid, being the fraction FII, isolated by DEAE-cellulose, characterized by similar content of total carbohydrates (FII: 44.5; tea: 44.8%), high content of uronic acid (FII: 25; tea: 10.4%), low proteins (FII: 0.8; tea: 1.34%) and polyphenols (FII: 1.50; tea: 28.3 GAE) (da Silva-Leite et al. 2017; Marques et al. 2020). Accordingly, the polysaccharide nature of FII was confirmed by infrared spectroscopy (FT-IR), showing peaks in the carbohydrate range (COO^-) (da Silva-Leite et al. 2017). In addition, the polysaccharide-rich extract of *X. americana* barks was shown to be well tolerated in mice, without causing changes in renal, hepatic and hematological markers or body weight (da Silva-Leite et al.

2017). Besides, these polysaccharides present antinociceptive effects in behavioral models (da Silva-Leite et al. 2017) and antiinflammatory in pancreatitis model (da Silva-Leite et al. 2018).

This study aimed to evaluate the effect of the tea from *X. americana* barks (Tea-Xa) and the polysaccharide fraction FII on zymosan-induced arthritis in rats, along with the acute toxicity of Tea-Xa given *per oral* to rats.

Materials and methods

Tea Preparation and polysaccharide extraction and fractioning

The barks of *X. americana* (voucher 46794/Herbarium Prisco Bezerra - Federal University of Ceará) were collected in Custódio-Quixadá, Ceará, Brazil (SISGEN-A8B2C79). For the tea preparation three grams of dry barks were immersed in distilled water (1 L), boiled (5 min) and filtered, being the decoction lyophilized and named Tea-Xa (da Silva-Pantoja et al. 2018).

For the polysaccharide obtention barks were dried (40 °C), ground into fine powder (5 g), depigmented and extracted (0.1 M NaOH), neutralized (1 M HCl), and precipitated (ethanol). The total polysaccharides were fractioned by ion exchange chromatography (DEAE-cellulose) and eluted (0.25 M NaCl), providing the polysaccharide fraction FII (Pereira et al. 2016; da Silva-Leite et al. 2017).

To remove remaining proteins both Tea-Xa and FII were precipitated with trichloroacetic acid (TCA) up to pH 3.0, over 4 h at 4 °C. The protein precipitate was separated by centrifugation (2496 g, 30 min, 25 °C) and the supernatant dialyzed and lyophilized (Marques et al. 2020).

The chemical analysis of *X. americana* tea revealed the following composition: total carbohydrates (44.8%); uronic acid (10%), polyphenols (28 gallic acid equivalent - GAE) and proteins (1.3%), that were corroborated by the FTIR spectra showing peaks related to carbohydrate range, uronic acid (group COOH), phenolic compounds and flavonoids (Marques et al. 2020).

Animals

Female Wistar rats (*Rattus norvegicus*) ($N=45$; 170–220 g) were housed in a climate-controlled (22–26 °C; 12 h light/dark cycle, fed with standard chow, receiving water *ad libitum*). The experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of Ceará (CEUA/UECE 4153018/2018). Female rats were chosen, since males exhibit lower response to

pain, affecting the initiation and maintenance of inflammatory nociception (Strickland et al. 2012).

Arthritis induction and treatment

Zymosan (500 µg/25 µL saline) or 0.9% NaCl (sterile saline; 25 µL) was injected intra-articular (i.art.) into the right tibio-tarsal joints of animals under sedation (xylazine 10 mg/kg + ketamine 80 mg/kg, i.m.) (Bringel et al. 2020). The *per oral* (p.o.) administration of Tea-Xa (30–300 mg/kg) or indomethacin (5 mg/kg), and the intravenous (i.v) injection of the polysaccharide fraction FII (1 mg/kg) were performed 2 h after zymosan and compared to the non-treated animals that had received saline. Animals were evaluated for the following parameters: hypernociception; articular leukocyte influx; articular edema (joint diameter); histopathological analysis (inflammatory signs, tissue necrosis and disorganization, edema).

Hypernociception and joint edema

Animals were placed individually in acrylic boxes with malleable mesh floor. A wide area probe (4.15 mm²) coupled to a digital algometer (EFF302, Insight®) was used for application of a force to the center of the paw to cause joint flexion. The reduced mechanical threshold (paw withdrawal) was considered as hypernociception, being the nociceptive response measured within 24 h and the basal response 24 h before saline or zymosan injection. The articular diameter (joint edema) was measured by digital paquimeter (da Silva Nascimento et al. 2021).

Joint leukocyte influx

Animals were euthanized under terminal anesthesia (xylazine 10 mg kg⁻¹ + ketamine 100 mg kg⁻¹ i.p.). Joint cavities were washed with phosphate-buffered saline (PBS) containing EDTA (100 µL; 10 mmol L⁻¹) and the total leukocyte influx was measured under microscopy in Neubauer chamber. The fluid was centrifuged (670×g), and the pellet resuspended with PBS (50 µL) for preparation of smears slides, stained with hematoxylin and eosin (H&E) for differential leukocyte count (Bringel et al. 2020).

Joints histopathological analysis

The dissected joints were fixed in formaldehyde 10% v/v and demineralized in buffered solution containing EDTA 10% w/v (pH = 7.35). Specimens were embedded in paraffin

for preparation of H&E stain slides of 5 µm (5 sections per specimen) and double-blind examined under optical microscopy. For the histopathological analysis of the periarticular tissue, inflammatory scores were considered for: (a) presence or absence of inflammatory cells (0 - no inflammatory infiltrate; 1 - polymorphonuclear leukocytes; 2 - mononuclear leukocytes). (b) intensity of inflammation (0 - few or no leukocytes, no inflammatory reaction; 1 < 25 leukocytes, with mild inflammatory reaction; 2–25 to 125 leukocytes, with moderate inflammatory reaction; 3 ≥ 125 leukocytes, with severe inflammatory reaction). (c) granulation tissue and fibrosis (0 - absent; 1 - thin; 2 - thick). Dystrophic calcification was quantified in percentages (Souza et al. 2021).

Acute toxicity evaluation of Tea-Xa

Rats were randomly divided into three groups (*n* = 3) and maintained fasting 2 h before receiving *per oral* (gavage) single doses of Tea-Xa. Group I received sterile saline (10 mL/kg; control group); Group II and III received Tea-Xa at 300 and 2000 mg/kg, respectively. Sixty minutes after treatment, animals were evaluated for acute toxicity during 4 h in the first day, and daily during fourteen consecutive days, according to the Guideline 423 of the Organization for Economic Co-operation and Development (OECD 2002). The parameters (exploratory activity, changes in the autonomic and central nervous systems, somatosensory activity) were evaluated daily for 14 days. The body weight of the animals was measured on days 1, 7 and 14. The relative organ masses, haematological and histopathological parameters were evaluated on day 14.

Exploratory activity

The exploratory activity was evaluated in the open field test, using an acrylic structure (100 × 50 × 90 cm) made of transparent walls and black floor, divided into nine equal quadrants. After 1 min of environmental habituation, the following parameters were recorded for 5 min: number of crossings (spontaneous locomotor activity), grooming (self-cleaning) and rearing (elevations on two paws without leaning on the walls of the floor) (Archer 1973).

Nervous system changes

The Hippocratic screening for toxic reactions was performed according to Malone's reports (Malone and Robichaud 1962) for observations of the following parameters: (a) autonomic nervous system (respiratory rate, fecal excretion, diarrhea, sialorrhea, diuresis, tears and cyanosis);

(b) central nervous system (self-cleaning, stereotyped movements, agitation, escape reaction, catatonia, tremors, seizure); (c) somatosensory activity (locomotor activity patterns, licking behavior, vibrissa movement, changed march, prostration, exophthalmia, sedation, analgesia). The toxicity signals (initiation, intensity, duration, progression time) were classified according to the scores: 0 (absent), 1 (rare), 2 (discrete), 3 (moderate) and 4 (intense).

Body weight and organs relative mass

Immediately after euthanasia, the relative mass of organs (brain, liver, stomach, kidneys, spleen) was measured using the relation: relative organ mass (%) = organ mass/body weight $\times$ 100.

Haematological and biochemical evaluation

Animals were sedated with xylazine (10 mg/kg) and ketamine (90 mg/kg) before blood collection and euthanasia by CO₂ inhalation. Blood samples were collected in EDTA for hematological analysis: erythrocytes (RBC), mean corpuscular volume (MCV), hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red cell distribution (RDWc), platelets, white blood cells (WBC), number and percentage of total lymphocytes (LYM), number and percentage of medium cells (monocytes, basophils and eosinophils - MID), leukocytes (WBC), number and percentage of granulocytes (GR). Cells number was determined on automatic cellular hematology analyzer (SDH-3, Labtest). The serum levels of urea, creatinine and the enzymatic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using Randox kits.

Organs histopathological evaluation

Kidney, stomach, spleen, liver, lung and brain were removed from rats and the tissues fixed in 10% formalin and embedded in paraffin, sectioned (5 μ m) and stained with H&E. The slides were analyzed under light microscopy and magnified images captured to assess presence and extension of morphological alterations.

Statistical analysis

Results were expressed as mean $\pm$ standard error (SEM) or median (IQR). Comparison between two groups was analyzed by t test, whereas that of three or more groups by one/two-way ANOVA, followed by Bonferroni's test. For the Malone Hippocratic screening were used Kruskal-Wallis followed by Dunn's test. Histological categorical variables

were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. $p < 0.05$ was considered statistically significant.

Results

Tea-Xa inhibits inflammatory parameters of zymosan-induced arthritis

Zymosan induced hypernociception, leukocyte influx, edema and increased the levels of nitrite 6 h after being injected. Tea-Xa at 30 and 100 mg/kg increased the mechanical threshold, respectively in 1.8-fold (21.5 ± 2.4 g) and 1.7-fold (20.1 ± 0.7 g) compared to zymosan (11.4 ± 0.5 vs. saline: 45.2 ± 2 g) (Fig. 1a). Tea-Xa also reduced other inflammatory parameters: joint edema by 47% at 100 mg (1.6 ± 0.1 vs. zymosan: 3.0 ± 0.1 vs. saline: 0.1 ± 0.0 mm) (Fig. 1b); leukocyte influx, mainly neutrophils by 82% at 30 mg/kg (9691 ± 3224 neutrophils), by 83% at 100 mg/kg (9032 ± 1442 neutrophils) and by 82% at 300 mg/kg (9865 ± 1333 neutrophils) compared to zymosan (54525 ± 8673 vs. saline: 74 ± 30 neutrophils) (Fig. 1c). Furthermore, Tea-Xa at 100 mg/kg, the dose that showed better efficacy to inhibit the joint edema, reduced the nitrite levels by 55% (10.0 ± 1.5 μ M/ml) compared to zymosan (22.5 ± 4.9 vs. saline: 7.3 ± 1.6 μ M/ml) (Fig. 1d).

Duration of Tea-Xa effect on hypernociception and leukocyte influx of zymosan-induced arthritis

Zymosan reduced the nociceptive threshold in response to the pressure applied to the rat paws during the entire time-course of hypernociception. Tea-Xa increased the mechanical threshold of the animals 3 h after treatment by 2-fold (24.9 ± 2.1 vs. zymosan: 13.0 ± 1.5 g); at 6 h 2-fold (26.0 ± 3.5 vs. zymosan: 14.2 ± 1.0 g); 9 h 1.7-fold (33.8 ± 2.9 vs. zymosan: 20.1 ± 2.2 g); 10 h 1.7-fold (38.3 ± 2.9 vs. zymosan: 23.0 ± 0.9 g) and at 24 h 2-fold (27.7 ± 2.5 vs. zymosan: 13.8 ± 1.8 g) (Fig. 2a). Furthermore, 24 h after, zymosan elicited leukocyte influx, mostly of neutrophils (1723 ± 305 vs. saline: 21 ± 3 neutrophils), which was reduced (68%) by Tea-Xa (547 ± 178 vs. zymosan: 1723 ± 305 neutrophils) (Fig. 2b).

Inhibitory effect of FII on inflammatory parameters of zymosan-induced arthritis

The evaluation of the inflammatory parameters elicited by zymosan evaluated 6 h after stimulation, showed reduction in the animal nociceptive threshold, increase in joint diameter and leukocyte influx (Fig. 3). The reduction in the

Fig. 1 Inhibitory effect of Tea-Xa on inflammatory parameters of zymosan-induced arthritis. Zymosan (500 µg; i.art.) or saline (i.art.) was administered to the rat tibiotarsal joints 2 h before Tea-Xa (30, 100, 300 mg/kg; *p.o.*). Six hours after arthritis induction were evaluated: **a** mechanical threshold, **b** joint edema, **c** leukocyte influx, **d** nitrite levels. Mean±SEM (*n*=8). ANOVA and Bonferroni **p*<0.05 versus saline. # Tea-Xa versus zymosan. Tea-Xa: tea of *X. americana* barks

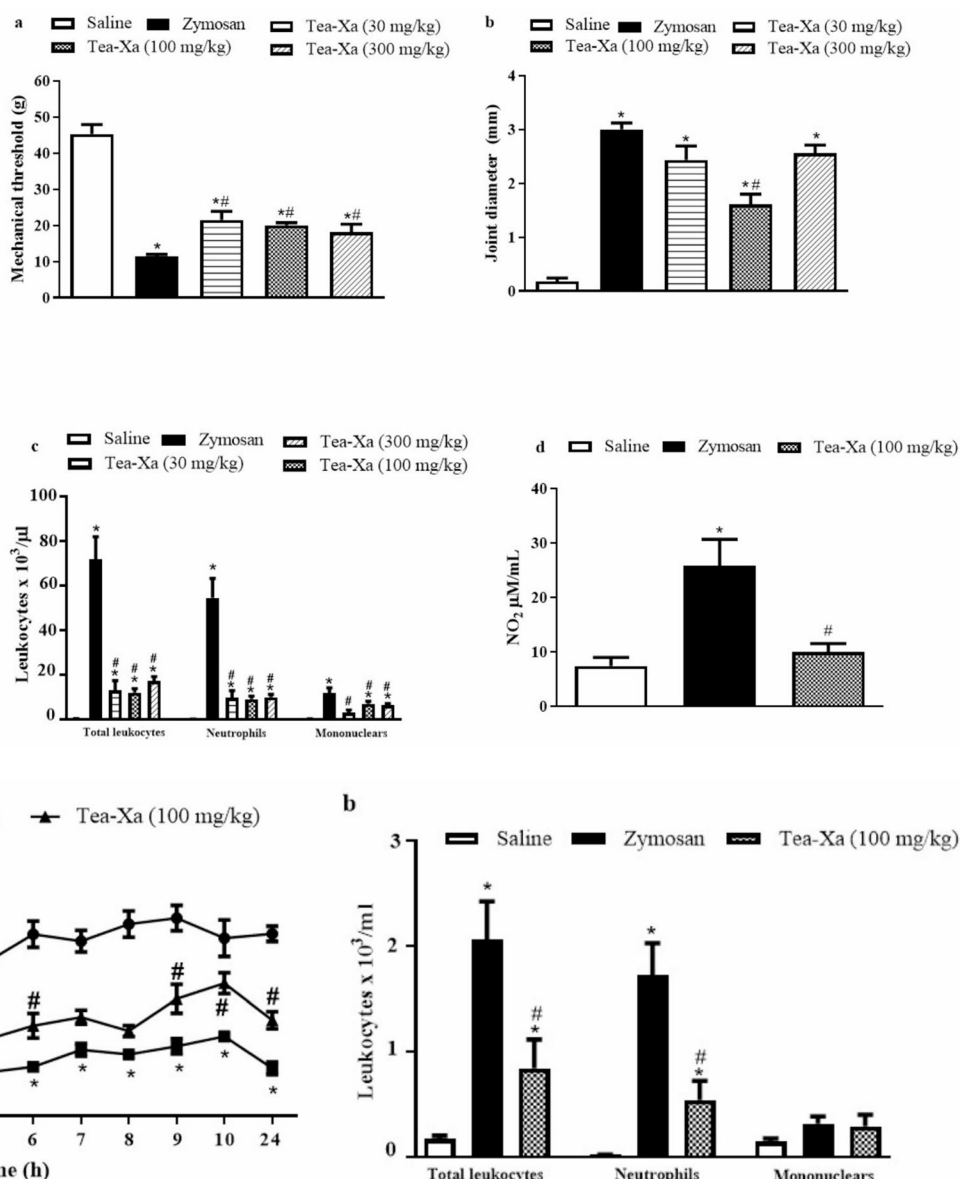


Fig. 2 Duration of the inhibitory effect of Tea-Xa on hypernociception and leukocyte influx of zymosan-induced arthritis. Zymosan (500 µg; i.art.) or saline (i.art.) was administered to the rat tibiotarsal joints 2 h before Tea-Xa (30, 100, 300 mg/kg; *p.o.*). Animals were evaluated

from 1 to 24 h later. **a** mechanical threshold, **b** leukocyte influx (24 h). Mean±SEM (*n*=8). ANOVA and Bonferroni **p*<0.05 versus saline. # *p*<0.05 versus zymosan. Tea-Xa: tea of *X. americana* barks

mechanical threshold was increased by fraction FII in 3-fold (31.6 ± 3 g) compared to zymosan (11.6 ± 0.8 vs. saline: 50.2 ± 3.4 g) (Fig. 3a). The increase in joint diameter was reduced (43%) by FII (1.6 ± 0.2 mm) compared to zymosan (2.8 ± 0.1 vs. saline: 0.1 ± 0.1 mm) (Fig. 3b). In respect to the increased leukocyte influx, markedly of neutrophils, it was reduced (74%) by FII (15008 ± 5794 neutrophils) compared to zymosan (57300 ± 14659 vs. saline: 79 ± 44 neutrophils) (Fig. 3c). The reference drug indomethacin enhanced the mechanical threshold in 4-fold (44.5 ± 3.1 g) and reduced the joint diameter by 30% (2.1 ± 0.0 mm) and the leukocyte

influx by 85% (8411 ± 2133 neutrophils) of the animals stimulated with zymosan (Fig. 3).

Tea-Xa and FII attenuate the histopathological inflammatory profile of zymosan-induced arthritis

The articular surfaces of the control group presented articular discs without microscopic alterations in joint spaces, showing capsules over the tibiotarsal joint containing chondrocytes and chondroblasts inside. Zymosan induced intense polymorphonuclear inflammatory infiltration on the

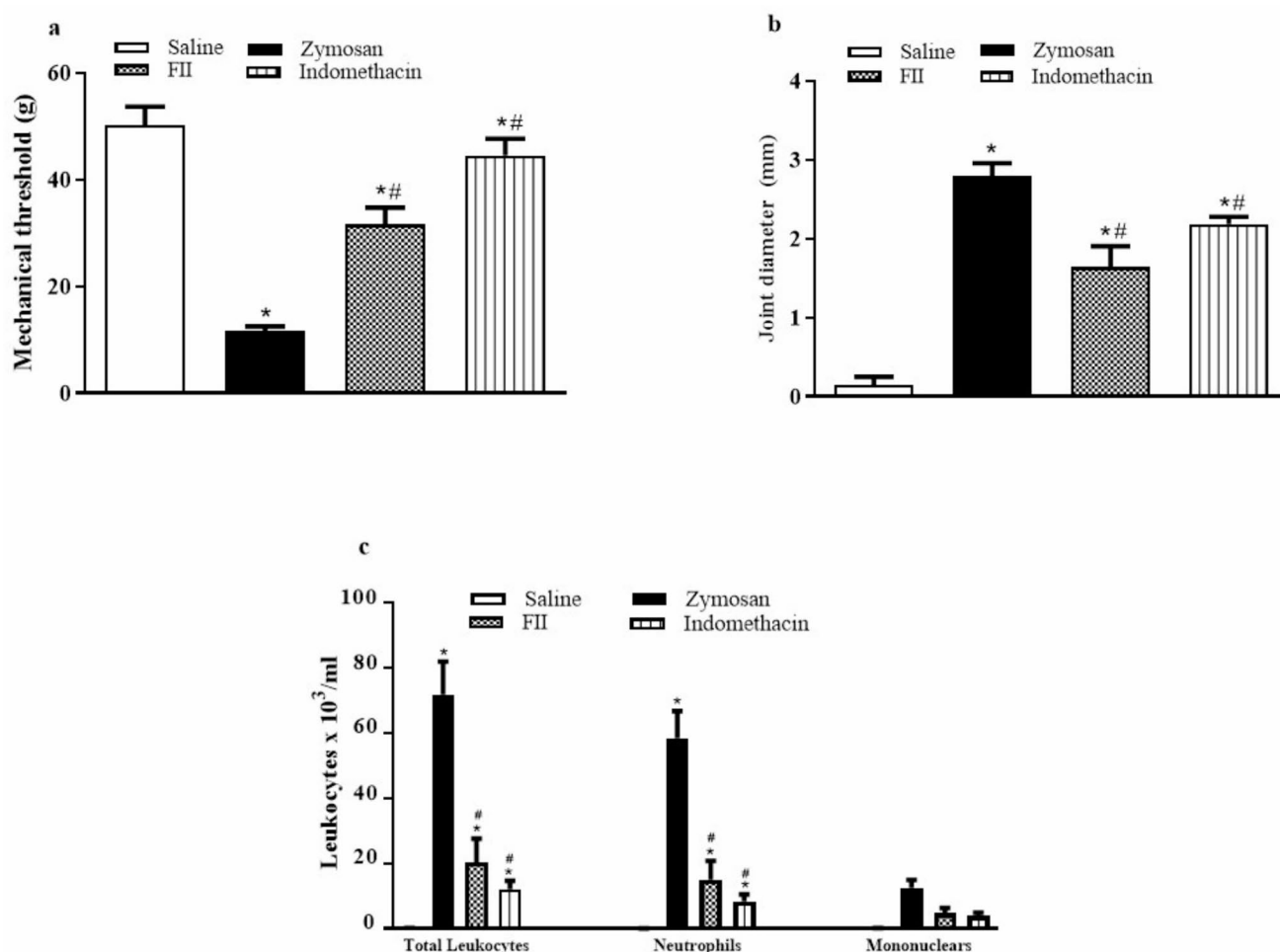


Fig. 3 Inhibitory effect of the FII on inflammatory parameters of zymosan-induced arthritis. Zymosan (500 μg ; i.art.) or saline (i.art.) was administered to the rat tibiotarsal joints 2 h before FII (1 mg/kg; i.v.) or indomethacin (5 mg/kg; *p.o.*). The evaluation was per-

formed six hours after arthritis induction for: **a** mechanical threshold, **b** joint edema, **c** leukocyte influx. Mean $\pm$ SEM ($n=8$). ANOVA and Bonferroni * $p<0.05$ versus saline. # $p<0.05$ versus zymosan. FII=polysaccharide fraction

upper articular surface showing areas of edema and effacement of the supradiscal space. Tea-Xa treatment attenuated the inflammatory infiltrate, but the areas of edema and effacement of the supradiscal space remained (Fig. 4).

Zymosan induced intense polymorphonuclear inflammatory infiltrate ($p<0.001$), necrosis ($p=0.027$), extensive areas of edema ($p<0.001$) and joint surface disorganization ($p<0.001$). The treatment with Tea-Xa (30 mg/kg) showed slight reduction of polymorphonuclear infiltrate, areas of edema, and morphological disorganization of the joint surface. Tea-Xa (100, 300 mg/kg) reduced polymorphonuclear infiltrate, edema ($p<0.001$), incidence of tissue necrosis ($p=0.027$) and disorganization of joint tissue ($p<0.001$), while the treatment with FII reduced only tissue necrosis ($p=0.027$). Indomethacin reduced the tissue disorganization ($p<0.001$). ***p-value*** highlights the statistical significance found for each parameter (Fig. 4) (Table 1).

Effect of Tea-Xa on acute toxicity parameters

Behavioral patterns and body weight

The oral treatment with Tea-Xa (300 and 2000 mg/kg) caused no significant changes in the animal's exploratory activity at 60 min. However, on days 7 (at 300 and 2000 mg/kg) and 14 (at 2000 mg/kg) Tea-Xa induced significant changes on the number of crossing but did not alter the number of grooming or rearing when compared to the control group (Table 2). In addition, no animal death or significant changes were observed by the Malone's Hippocratic triage (Table 3). Furthermore, Tea-Xa (300 and 2000 mg/kg) did not cause, on any day, significant changes in the animals body weight (Table 4) or organs relative weight (Table 5).

Fig. 4 Representative photomicrographs of zymosan-induced arthritis in rat tibiotarsal joint. Zymosan (500 ug) or saline was administered to tibiotarsal joints 2 h before indomethacin (5 mg/kg; p.o.), Tea-Xa (100 mg/kg; p.o) or FII (1 mg/kg; i.v). Evaluation was performed 6 h after zymosan. H&E. Magnification (x 400).

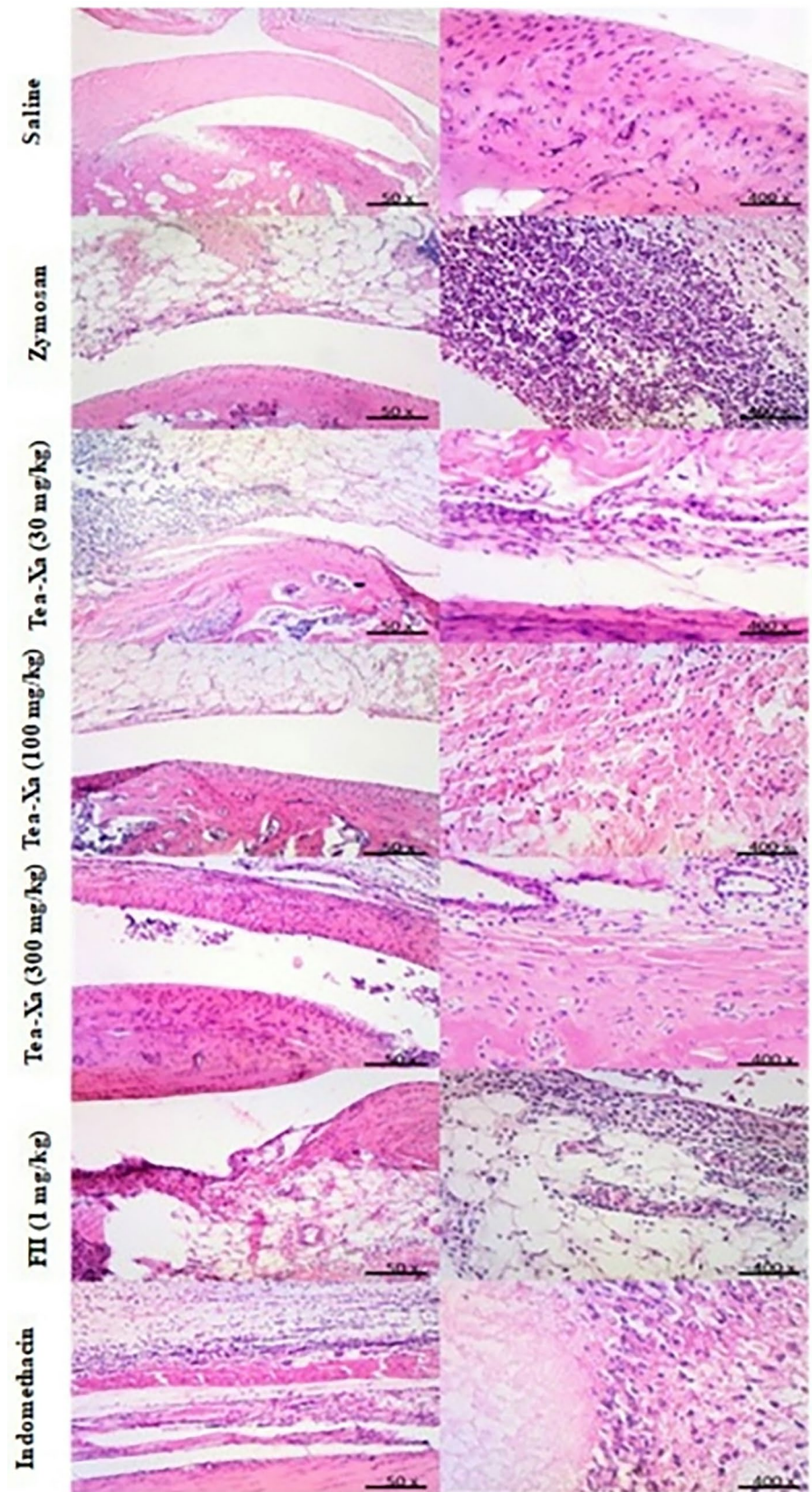


Table 1 Histopathological parameters of rat joints treated with Tea-Xa or FII

	Saline	Zymosan	Tea-Xa ^{&} 30 mg/kg	Tea-Xa 100 mg/kg	Tea-Xa 300 mg/kg	FII (1 mg/kg)	Indomethacin	p-Value
Inflammatory profile								
Absent	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001
Acute	0 (0.0%)	6 (100.0%)*	6 (100.0%)*	6 (100.0%)*	6 (100.0%)*	5 (100.0%)*	5 (100.0%)*	
Chronic	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Inflammation scores								
Absent	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001
=25 cels/field	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)*	
>26 / <125 cels/field	0 (0.0%)	0 (0.0%)	1 (16.7%)	3 (50.0%)*	5 (83.3%)*	1 (20.0%)	2 (40.0%)	
>125 cels/field	0 (0.0%)	6 (100.0%)*	5 (83.3%)*	3 (50.0%)	1 (16.7%)	4 (80.0%)*	2 (40.0%)	
Presence of necrosis	0 (0.0%)	4 (66.7%)*	3 (50.0%)*	0(0.0%) [†]	0(0.0%) [†]	1 (20.0%)	2 (40.0%)*	0.027
Presence of tissular desorganization	0 (0.0%)	6 (100.0%)*	6 (100.0%)*	2(33.3%) [†]	0(0.0%) [†]	0(0.0%) [†]	0(0.0%) [†]	< 0.001
Presence of edema zones	0 (0.0%)	6 (100.0%)*	6 (100.0%)*	6 (100.0%)*	6 (100.0%)*	5 (100.0%)*	5 (100.0%)*	< 0.001

* $p < 0.05$ versus saline; [†] $p < 0.05$ versus Zymosan; Fisher's exact test or Pearson's chi-square ($n = 6$); [&]Tea of *X. americana* barks

Table 2 Behavioral parameters

	Grooming			Rearing			Crossing		
	Tea-Xa ^{&} (mg/kg)			Tea-Xa (mg/kg)			Tea-Xa (mg/kg)		
	Control	300	2000	Control	300	2000	Control	300	2000
Day 1	3.3±2.4 ^a	7.3±0.8	5.0±1.0	7.6±0.3	6.6±0.6	9±2.0	51.3±3.3	48±2.0	59.3±7.8
Day 7	2.6±1.2	2.3±0.8	3.0±1.1	5.0±1.1	8.3±1.4	12.3±2.9	22±3.4	57±2.8*	63±4.5*
Day 14	3.6±1.4	1.6±0.3	2.6±0.3	7±1.2	4±1.8	4.5±2.8	37±8.0	45±10	68±9.7*

^aMean ± SEM ($n = 3$); * $p < 0.05$ versus control; one-way ANOVA; [&]Tea of *X. americana* barks

Table 3 Malone's hippocratic triage

Parameter	Tea-Xa ^{&} (mg/kg)		
	Control	300	2000
Respiration	N	N	N
Fecal excretion	N	N	N
Diarrhea	N.D	N.D	N.D
Micturition	N	N	N
Sialorrhea	N.D	N.D	N.D
Lacrimation	N.D	N.D	N.D
Cyanosis	N.D	N.D	N.D
Stereotyped movement	N.D	N.D	N.D
Agitation	N.D	N.D	N.D
Escape reaction	N.D	N.D	N.D
Convulsions/Tremors	N.D	N.D	N.D
Pilomotor erection	N.D	N.D	N.D
Death	N.D	N.D	N.D

N.D, Not detected, N, Normal; [&]Tea of *X. americana* barks

Table 4 Animals body weight

Groups	Body weight (g)		
	Day 1	Day 7	Day 17
Control	182±10.81 ^a	181±11.35	184±9.29
Tea-Xa ^{&} (300 mg/kg)	196±13.11	196±13.11	200±13.20
Tea-Xa (2000 mg/kg)	187±2.5	187±2.6	189±2.5

^aMean ± SEM ($n = 3$); one-way ANOVA; [&]Tea of *X. americana* barks

Table 5 Organs relative weight

Organs	^{&} Tea-Xa (mg/kg)		
	Control	300	2000
Brain	1.06±0.04 ^a	1.01±0.03	1.05±0.10
Liver	3.67±0.34	3.08±0.46	2.35±1.45
Stomach	1.54±0.20	1.08±0.29	1.63±0.84
Kidney	0.68±0.15	0.72±0.12	1.61±1.77
Spleen	0.27±0.02	0.28±0.01	0.31±0.05

^aMean ± SEM ($n = 3$); [&]Tea of *X. americana* barks

Haematological and biochemical

Tea-Xa caused no significant changes on the haematological parameters at any doses evaluated after 14 days of treatment, except for the number of total leukocytes and lymphocytes that were raised at the dose of 300 and 2000 mg/kg compared to control animals (Table 6). Moreover, the treatment with Tea-Xa (300 and 2000 mg/kg) exhibited normal profile of the serum biochemical markers of liver function (alanine aminotransferase-ALT, aspartate aminotransferase-AST), or renal function (creatinine, urea) after 14 days (Table 7).

Histopathological analysis of organs

Histological sections taken from the kidneys, spleen, stomach, brain and liver showed no structural abnormalities of

Table 6 Haematological parameters

Parameters	Unit	Tea-Xa ^{&} (mg/kg)		
		Control	300	2000
Red Blood Cells (RBC)	$\times 10^6/\text{mm}^3$	5.37 $\pm$ 0.65 ^a	8.26 $\pm$ 0.11	6.59 $\pm$ 1.41
Haemoglobin (Hbg)	g/dL	9.36 $\pm$ 1.58	13.67 $\pm$ 0.17	13.43 $\pm$ 0.33
Hematocrit (Hct)	%	36.34 $\pm$ 4.15	53.40 $\pm$ 1.64	42.91 $\pm$ 8.42
Mean corpuscular volume (MCV)	M	68.0 $\pm$ 1.00	64.67 $\pm$ 1.20	65.67 $\pm$ 1.76
Mean corpuscular hemoglobin (MCH)	Pg/cell	1.76 $\pm$ 1.35	16.57 $\pm$ 0.42	23.33 $\pm$ 6.98
Mean corpuscular hemoglobin concentration (MCHC)	g/dl	25.50 $\pm$ 1.90	25.70 $\pm$ 1.01	35.03 $\pm$ 9.48
Red cell distribution width (RDW)	%	13.57 $\pm$ 0.58	13.33 $\pm$ 0.26	15.93 $\pm$ 1.38
Total leukocytes	$\times 10^3/\text{mm}^3$	4.38 $\pm$ 1.22	9.20 $\pm$ 1.83*	20.55 $\pm$ 9.53*
Lymphocytes (LYM)	$\times 10^3/\text{mm}^3$	3.59 $\pm$ 1.06	8.31 $\pm$ 1.63*	11.06 $\pm$ 3.38*
Lymphocytes (LYM)	%	2.23 $\pm$ 1.63	2.96 $\pm$ 0.58	4.20 $\pm$ 2.08
Monocytes, basophils and eosinophils	$\times 10^3/\text{mm}^3$	2.23 $\pm$ 1.63	2.96 $\pm$ 0.58	4.20 $\pm$ 2.08
Granulocytes	mg/dL	0.68 $\pm$ 0.21	0.59 $\pm$ 0.11	8.91 $\pm$ 6.28
Medium cells	%	0.10 $\pm$ 0.08	0.29 $\pm$ 0.09	0.58 $\pm$ 0.27
Granulocytes (GR)	%	15.73 $\pm$ 2.60	6.50 $\pm$ 0.56	33.40 $\pm$ 10.74
Platelets	$\times 10^3/\text{mm}^3$	511.7 $\pm$ 161.3	871.3 $\pm$ 46.39	580.3 $\pm$ 90.64
Platelets (%)	%	0.26 $\pm$ 0.08	0.50 $\pm$ 0.02	0.31 $\pm$ 0.04
Mean platelet volume	MPV	5.33 $\pm$ 0.29	5.76 $\pm$ 0.08	5.40 $\pm$ 0.10
Platelet distribution	%	27.17 $\pm$ 0.77	28.87 $\pm$ 0.50	27.43 $\pm$ 0.46

^aMean $\pm$ SEM ($n=3$); * $p<0.05$ versus control; one-way ANOVA; [&]Tea of *X. americana* barks

Table 7 Serum biochemical markers

Markers	Unit	Tea-Xa ^{&} (mg/kg)		
		Control	300	2000
Creatinine	mg/dL	0.54 $\pm$ 0.03 ^a	0.57 $\pm$ 0.03	0.51 $\pm$ 0.01
Urea	mg/dL	29.33 $\pm$ 1.33	25.67 $\pm$ 1.85	28 $\pm$ 0.0
ALT	U/L	59.33 $\pm$ 4.63	47.33 $\pm$ 3.84	54.67 $\pm$ 2.72
AST	U/L	109.07 $\pm$ 9.28	109.3 $\pm$ 4.66	103.7 $\pm$ 12.91

^aMean $\pm$ SEM ($n=3$); [&]Tea of *X. americana* barks

the organs from animals treated with Tea-Xa (300, 2000 mg/kg) when compared to those of control animals, excepted for the liver, that showed moderate portal congestion signs in animals treated with a dose of 2000 mg/kg (Fig. 5).

Discussion

This study demonstrated the antinflammatory effect of Tea-Xa and polysaccharide fraction FII obtained from *X. americana* barks in the rat model of arthritis induced by zymosan in the tibiotarsal joint. It also demonstrated that Tea-Xa given *per oral* in single doses (300, 2000 mg/kg) to rats lacked to produce mortality or toxicological signs.

Arthritis induced by zymosan is an established experimental model that mimics the clinical and pathophysiological features of rheumatoid arthritis, such as articular edema, hypernociception and leukocyte influx, mainly of neutrophils (Bringel et al. 2020). Our results demonstrated the inhibitory effect of Tea-Xa, rich in polysaccharides, on joint edema, nociception and leukocyte influx elicited by zymosan. The effect on nociception and leukocyte influx was sustained up to 24 h after arthritis induction, indicating a long-lasting effect. The anti-inflammatory effect of the polysaccharides from *X. americana* barks had been previously demonstrated in animal models, such as gastritis (da Silva-Pantoja et al. 2018), pancreatitis (da Silva-Leite et al. 2018), and in the models of paw edema and peritonitis (Marques et al. 2020). The antinociceptive effects had also been demonstrated for these polysaccharides (da Silva-Leite et al. 2017, 2018; Marques et al. 2020).

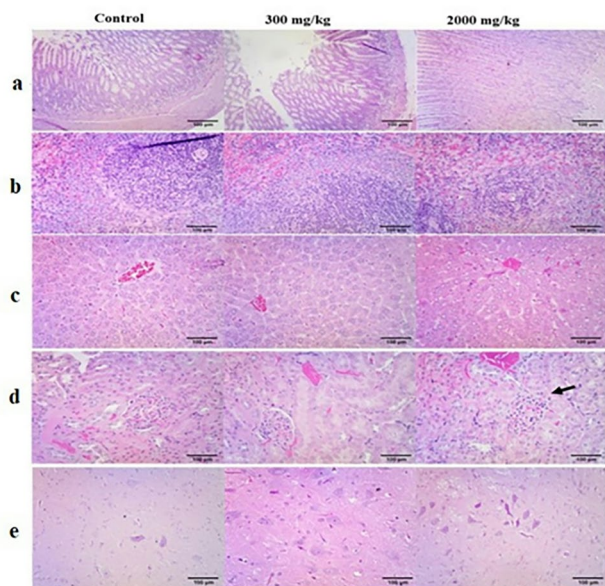


Fig. 5 Histological profile of vital organs from animals treated with Tea-Xa. **a** stomach, **b** spleen, **c** liver, **d** kidney and **e** brain. Photomicrographs of transverse sections: column 1: control; column 2: Tea-Xa (300 mg/kg); column 3: Tea-Xa (2000 mg/kg). Tea-Xa: Tea of *X. americana* barks

It is well known that the inflammatory mediator nitric oxide (NO) induces vasodilation, increase in vascular permeability, nociceptors sensitization in articular inflammation in animals (Spiller et al. 2019; Bringel et al. 2020) and humans (Tsikas and Mikuteit 2022). In addition to the inhibitory effect of Tea-Xa on the inflammatory parameters mediated by NO, Tea-Xa also reduced the nitrite concentration in the articular fluid. Therefore, it is possible to suggest that NO is involved in the Tea-Xa effect on the inflammatory events present in the zymosan-induced arthritis. In this line, the total polysaccharides obtained from *X. americana* barks reduced nitrite levels in the experimental model of gastritis (da Silva Pantoja et al. 2018).

In this study, it was also demonstrated that the fraction FII, possessing similar quantity of polysaccharides compared to Tea-Xa, inhibited inflammatory parameters (joint edema, hypernociception, leukocyte influx) manifested in the zymosan-induced arthritis, suggesting the important role of polysaccharides in this effect. The anti-inflammatory effect of FII is in accordance with those demonstrated in other inflammation models (da Silva-Leite et al. 2018; da Silva-Pantoja et al. 2018; Marques et al. 2020). Moreover, other plant-derived polysaccharides had shown protective effect in articular inflammation models (da Silva Nascimento et al. 2021; Kuang et al. 2023).

Previous study of our research group had demonstrated reduction on leukocyte rolling and adhesion for the polysaccharides of *X. americana* barks (da Silva-Pantoja et al. 2018). Considering the high carbohydrate content present both in Tea-Xa and FII, it would be possible that the inhibition on neutrophil migration elicited by these polysaccharides occur due to their binding to adhesion proteins, alleviating the joint inflammatory process. Accordingly, the inhibition on neutrophil adhesion was previously shown for other plant-derived polysaccharides (Tong et al. 2014; Araujo et al. 2024).

Histopathological analysis of the joints revealed that *X. americana* polysaccharides reduce the leukocyte influx, necrosis and tissue disorganization induced by zymosan. The effect on tissue damage induced by the inflammatory process had been demonstrated in other studies for plant derived-polysaccharides (da Silva-Leite et al. 2018; da Silva-Pantoja et al. 2018; da Silva Nascimento et al. 2021). The protective effects of *X. americana* polysaccharides on tissue injury, such as necrosis and edema, involve reduction of neutrophil migration and myeloperoxidase activity (Silva-Pantoja et al. 2018).

It is known that joint tissue damage triggers nociception and that plant-derived polysaccharides decrease joint nociception via attenuation of tissue injury (da Silva Nascimento et al. 2021). Furthermore, previous studies showed that these polysaccharides alleviate inflammatory response

in the joint tissue cells, including chondrocytes (Wu et al. 2022).

Moreover, the treatment with Tea-Xa given per oral in single doses to rats did not produce toxicological signs or mortality, when evaluated according to the OECD guideline 423. There were no significant changes in the breathing, somatomotor activity, convulsions, tremors or itching. Furthermore, no abnormal food or water intake, or significant variations in body weight were identified. It suggests the normal processing of lipids, carbohydrates and protein metabolism within the body of animals as such nutrients play important roles in different physiological functions (Olsen et al. 2021). These data are in line with previous study that demonstrated no signs of systemic toxicity for the polysaccharide extract obtained from *X. americana* barks (da Silva-Leite et al. 2017). Our findings also reinforce the literature on the lack of toxicity of plant polysaccharides (Xie et al. 2016).

On the other hand, changes in exploratory activity were observed on the 7th and 14th days after treatment, being more evident at 2000 mg/kg. It is well established that the increase in locomotion can be considered a stimulating effect, while its decrease is related to sedation or prostration. In the open field, an increase in locomotion or time spent in the central part of the device and vertical exploration can be interpreted as anxiolytic effect, while the opposite is associated with anxiogenic effect (Prut and Belzung 2003). Changes in exploratory activity have been already reported for other plant polysaccharides (Nonato et al. 2018).

The hematopoietic system is a primary target for toxic substances; therefore, several haematological parameters can be analyzed in sub-acute toxicity study, providing information specially to the functioning of the immune system and revealing pathologies in humans and animals (Li et al. 2010). In general, the administration of Tea-Xa did not cause changes on haematological parameters (hemoglobin, red blood cells, platelets), which reflects lack of adverse effects on the hematopoietic system, as previously demonstrated for the total polysaccharide extract of *X. americana* barks in mice (da Silva-Leite et al. 2017) and for other plant decoctions (Deyno et al. 2020).

The leukogram showed that Tea-Xa at 300 and 2000 mg/kg stimulates leukocytosis and neutrophilia. Since Tea-Xa contains in its composition immunostimulant molecules such as proteins, phenols and mostly polysaccharides (Marques et al. 2020), it may be stimulating bone marrow, a phenomenon that can be attributed to the immunomodulator role of plant polysaccharides (Ferreira et al. 2015).

In general, the assessment of some parameters such as body and organs weight, tissue injury, enzyme activity and other physiological disorders after pharmacological treatment may indicate animal health status (Michael et al.

2007). Our data showed no significant macroscopic changes either in body weight or relative organ mass, corroborating previous studies with the polysaccharidic extracts of *X. americana* (Silva-Leite et al. 2017; Cho et al. 2020).

The liver is the major target of toxic substances administered per oral. Changes in serum enzymes levels ALT and AST may indicate hepatocellular toxicity and liver damage (Singh et al. 2016) and the increased levels of serum creatinine and urea is indicative of changes in kidneys, an important organ for drugs elimination (Travlos et al. 1996). The *per oral* treatment with Tea-Xa in rats did not cause significant alterations in AST and ALT activities, or in the content of creatinine and urea, suggesting no alteration in the renal and hepatic systems, as already described in other studies with plant decoction (Deyno et al. 2020), and with the polysaccharide extracts of *X. americana* (Silva-Leite et al. 2017).

The histopathological analysis showed signs of portal congestion, signaling possible hepatotoxic effect. However, as previously described, this toxicity can be questioned by the absence of alteration in the liver enzymes (ALT, AST). Furthermore, the literature reports that this effect may be related to the hormones, as there are differences in the development of liver lesions between male and female rats, which means that the animal's gender may have influenced (Dongmo et al. 2019; Miller 2001).

In view of the exposed data, which suggest the safety of the consumption of *X. americana* barks decoction (Tea-Xa) in the folk medicine, along with the pharmacological effects of its polysaccharides described in the literature, Tea-Xa can be pointed to an alternative or adjuvant therapy for the treatment of inflammatory and painful conditions, especially in acute arthropathies.

Conclusion

The oral treatment with *Ximenia americana* barks decoction (Tea-Xa), rich in polysaccharides, inhibits inflammatory parameters of the tibiotarsal arthritis induced by zymosan in rats without causing major adverse effects or mortality, showing LD50 greater than 2000 mg/kg.

Author contributions Assreuy AMS, Castro RR and Marques GFO conceived and designed the research; Marques GFO and da Silva Nascimento FG conducted the in vivo experiments; Pereira MG performed the polysaccharides chemical analysis; da Silva Nascimento FG, de Barros Silva PG, Alves APNN and Mota MRL performed the histopathological analysis. All authors contributed to the manuscript writing.

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Data availability Availability is consented by all authors.

Declarations

Conflict of interest Authors declare no conflict of interest.

Ethical approval Authors have followed all applicable international, national, and/or institutional guidelines for the care and use of animals. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of Ceará (CEUA/UECE 4153018/2018).

Consent for publication The consent of all the authors has taken to publish the research in this journal.

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