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Original Article

Polysaccharide rich fractions from barks of *Ximenia americana* inhibit peripheral inflammatory nociception in mice
Antinociceptive effect of *Ximenia americana* polysaccharide rich fractions

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ABSTRACT

Ximenia americana L., Olacaceae, barks are utilized in folk medicine as analgesic and anti-inflammatory. The objective was to evaluate the toxicity and antinociceptive effect of polysaccharides rich fractions from *X. americana* barks. The fractions were obtained by extraction with NaOH, followed by precipitation with ethanol and fractionation by ion exchange chromatography. They were administered *i.v.* or *p.o.* before nociception tests (writhing, formalin, carragenan-induced hypernociception, hot plate), or during 14 days for toxicity assay. The total polysaccharides fraction (TPL-Xa: 8.1% yield) presented 43% carbohydrate (21% uronic acid) and resulted in two main fractions after chromatography (FI: 12%, FII: 22% yield). FII showed better homogeneity/purity, content of 44% carbohydrate, including 39% uronic acid, arabinose and galactose as major monosaccharides, and infrared spectra with peaks in carbohydrate range for COO^- groups of uronic acid. TPL-Xa (10 mg/kg) and FII (0.1 and 1 mg/kg) presented inhibitory effect in behavior tests that evaluate nociception induced by chemical and mechanical, but not thermal stimuli. TPL-Xa did not alter parameters of systemic toxicity. In conclusion, polysaccharides rich fractions of *X. americana* barks inhibit peripheral inflammatory nociception, being well tolerated by animals.

Introduction

The use of medicinal plants as analgesic agents is a common practice that has prompted ethnopharmacological studies. Among plant constituents, polysaccharides are found in large quantities and show low toxicity (Ovodov, 1998).

Ximenia americana L., Olacaceae, is distributed in tropical and temperate regions, being popularly known in the Northeast Brazil as "ameixa-do-mato", "ameixa-brava" or "ameixa-do-sertão" (Silva et al., 2008). In Brazilian folk medicine as in other countries, *X. americana* barks are utilized as anti-cancer, analgesic for headaches, gastric and back pains and other inflammatory conditions (de Albuquerque et al., 2007; Le et al., 2012). The phytochemical analysis of *X. americana* barks extracts revealed the presence of alkaloids, anthraquinones, glycosides, flavonoids, saponins, tannins terpenoids (Maikai et al., 2010) and carbohydrates (James et al., 2007). Experimental studies performed with the aqueous extracts of this plant had demonstrated antinociceptive activity (Soro et al., 2009).

The immunomodulatory role of plant polysaccharides is already well described (Schepetkin and Quinn, 2006), including the anti-inflammatory effect (Pereira et al., 2012a,b). However, the effect of plant polysaccharides in the nociception process is scarce, although recent studies had demonstrated the antinociceptive activity in mice for *Thladiantha dubia* crude polysaccharides (Wang et al., 2011), and for arabinogalactan (do Nascimento et al., 2015) and

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galactoarabinoglucuronoxylan from *Solanum betaceum* fruit (do Nascimento et al., 2013). In this study polysaccharide rich fractions of *X. americana* barks were evaluated in mice for its toxicity and antinociceptive effect.

Materials and methods

Animals

Male Swiss mice (20–25 g), 5–6 weeks of age, were maintained with free access to water and food at 22–26 °C, 12/12 h light/dark cycle. The experimental protocols were approved by the Animal Care and Use Committee of the State University of Ceará (n° 12783679-9/2012).

Drugs and reagents

DEAE-cellulose, indomethacin, bovine serum albumin (BSA), λ-carrageenan (Cg) and monosaccharides (Sigma Chemical Co., St. Louis, MO, USA); agarose (Bio Rad Laboratories); *N*-cetyl-*N*-*N*-*N*-trimethylammonium bromide (Cetavlon) (British Drug House Chemical, Ltd.); chondroitin-6-sulfate, heparin sulfate and dermatan sulfate (Seikagau Kogyo Co); morphine (Dimorf®, Cristalia, SP, Brazil); diazepam (Teuto S/A, GO, Brazil); formaldehyde and acetic acid (Isofar, Rio de Janeiro, RJ, Brazil); ketamine and xylazine (König S/A, Argentina). The remaining drugs and reagents were of analytical grade.

Plant collection, polysaccharides extraction and fractioning

Ximenia americana L., Olacaceae, was collected at Custódio-Quixadá, Ceará (Brazil) and a voucher specimen (n° 46794) was deposited in the Herbarium Prisco Bezerra of Federal University of Ceará. Barks of *X. americana* were washed, dried at 40 °C and macerated into powder (5 g). The powder was suspended in methanol (1:50, w/v, 76 °C, 2 h), to remove pigments, and filtered (step repeated twice). The insoluble residue was added to 0.1 M NaOH (1:50, w/v, 97 °C, 2 h), filtered (step repeated three times) and centrifuged (2496 × *g*; 15 min, 25 °C). Alkaline supernatants were pooled, neutralized in 1 M HCl and precipitated in ethanol (1:4 (w/v); 24 h, 4 °C). The mixture was centrifuged and the pellet was dialyzed (cut-off 14,000 Da; 72 h) against distilled water and re-centrifuged (Pereira et al., 2016). The final supernatant was lyophilized and named total polysaccharides of *X. americana* (TPL-Xa).

TPL-Xa (1:2, w/v) was dissolved in distilled water and applied to ion exchange chromatography – DEAE-cellulose. Column (9.8 × 2.0 cm) was equilibrated and eluted with distilled water for removal of neutral polysaccharides, and the acidic polysaccharides were eluted (1 ml/min) with NaCl (0.1, 0.25, 0.5, 0.75, 1.0 M). Polysaccharide fractions were monitored for the carbohydrate content by the method of phenol–sulfuric acid (DuBois et al., 1956).

Polysaccharides characterization

Polysaccharides were quantified for total carbohydrate (DuBois et al., 1956), uronic acid (Dische, 1947) and soluble protein (Bradford, 1976), using arabinose and galactose (3:1), D-galacturonic acid and BSA (albumin serum bovine) as respective standards.

Agarose 0.5% gel electrophoresis: polysaccharides (6 mg/ml, 15 μl) were applied and run in 0.05 M 1,3-diaminopropane-acetate buffer (pH 9.0) for 60 min at 110 V and fixed with 0.1% Cetavlon for 24 h. Gel was dried and stained with Stains-All (5 mg Stains-All; 100 ml of 50% ethanol, w/v) and washed with distilled water (Dietrich and Dietrich, 1976; Souza et al., 2015). The glycosaminoglycans chondroitin 6-sulfate (~60 kDa), dermatan sulfate (~30 kDa) and heparan sulfate (~15 kDa) were used as standards.

The monosaccharide composition was analyzed by gas–liquid chromatography coupled to mass spectrometry (GC–MS). Polysaccharide fractions (5 mg) were hydrolyzed with trifluoroacetic acid (1 mol/l; 96 °C; 5 h) evaporated in rota evaporator (Buchi RE 11, Switzerland), extensively washed with water and reduced with sodium borohydride (1 h, r.t.). The reaction was interrupted with acetic acid until neutralization. The resulting boric acid was removed as trimethyl borate with methanol (3 × 5 ml) in the rota evaporator and acetylation carried out with acetic anhydride–pyridine (1:1 (v/v); 100 °C; 1 h). The resulting alditol acetate was extracted with chloroform (5 ml) and analyzed by GC–MS HP-Ultra 2 column (Kircher, 1960).

Polysaccharides were analyzed by infrared (FTIR) spectroscopy (Bruker – Vertex 70), coupled to Pike Miracle single-bounce attenuated total reflectance (ATR) cell equipped with a ZnSe single crystal module. The spectral region examined extended of 500–4000 cm^{-1} using a resolution of 3 cm^{-1}. All spectra are the average measurements with 124 scans each.

Toxicity assay

Mice were weighed before and after treatment for 14 days with TPL-Xa (10 mg/kg, *i.v.*). Blood was collected after anesthesia intraperitoneal (*i.p.*) with ketamine 90 mg/kg and xylazine 10 mg/kg for hematological analysis (erythrocytes, leukocytes and platelets), serum content of urea and creatinine and enzymatic activity of alanine transaminase (ALT) and aspartate transaminase (AST). Heart, spleen, stomach, kidney and liver were removed and weighed (wet weight/body mass).

Behavioral tests

Mice ($n = 6–8$ per group) were treated 30 min before tests with polysaccharides *i.v.* (0.1, 1, 10 mg/kg) or *p.o.* (100 mg/kg), sterile saline (0.9% NaCl; 0.05 ml/10 g body mass; *i.v.*), morphine (5 mg/kg, *s.c.*), indomethacin (10 mg/kg, *i.p.*) or diazepam (5 mg/kg, *i.p.*). The protocols were conducted in a double-blind manner.

Formalin test: formalin (2.5% v/v; 20 μl) was injected *s.c.* in the hind animal paws and the time (s) in which they spent licking its paws in response to chemical stimuli was recorded in the initial (P1: 0–5 min) and late (P2: 15–30 min) phases (Le Bars et al., 2001).

Writhing test: acetic acid (0.8% (v/v); 0.1 ml/10 g body mass) was injected *i.p.* and the number of writhes (typical contractions of the abdominal musculature followed by hind limb stretches), elicited in response to chemical stimuli, was counted from 10 to 30 min post-injection (Le Bars et al., 2001).

Carrageenan-induced paw hypernociception: carrageenan was injected by intraplantar route (500 μg/paw, *s.c.*). Animals were placed in clear acrylic boxes with raised platforms of wire mesh to allow access to the ventral surface of hind paws from 15 to 30 min before evaluation. For this, the frequency of paw withdrawal was quantified at 10 s intervals after six applications of stimuli (100%), using 0.8 g flexible von Frey filaments, at time zero and from 1-3 h after stimulation with carrageenan (Le Bars et al., 2001).

Hot plate test: animals were placed on a hot plate at 55 ± 0.5 °C and the time delayed before behavioral responses (shaking, licking paws or jumping) was recorded at baseline and after 30, 60–150 min. Animals reaction time higher than 10 s 24 h prior test was excluded.

Rota-rod test: animals were selected 24 h prior test, excluding those that did not remain on the Rota-rod (22 r.p.m.) for at least two

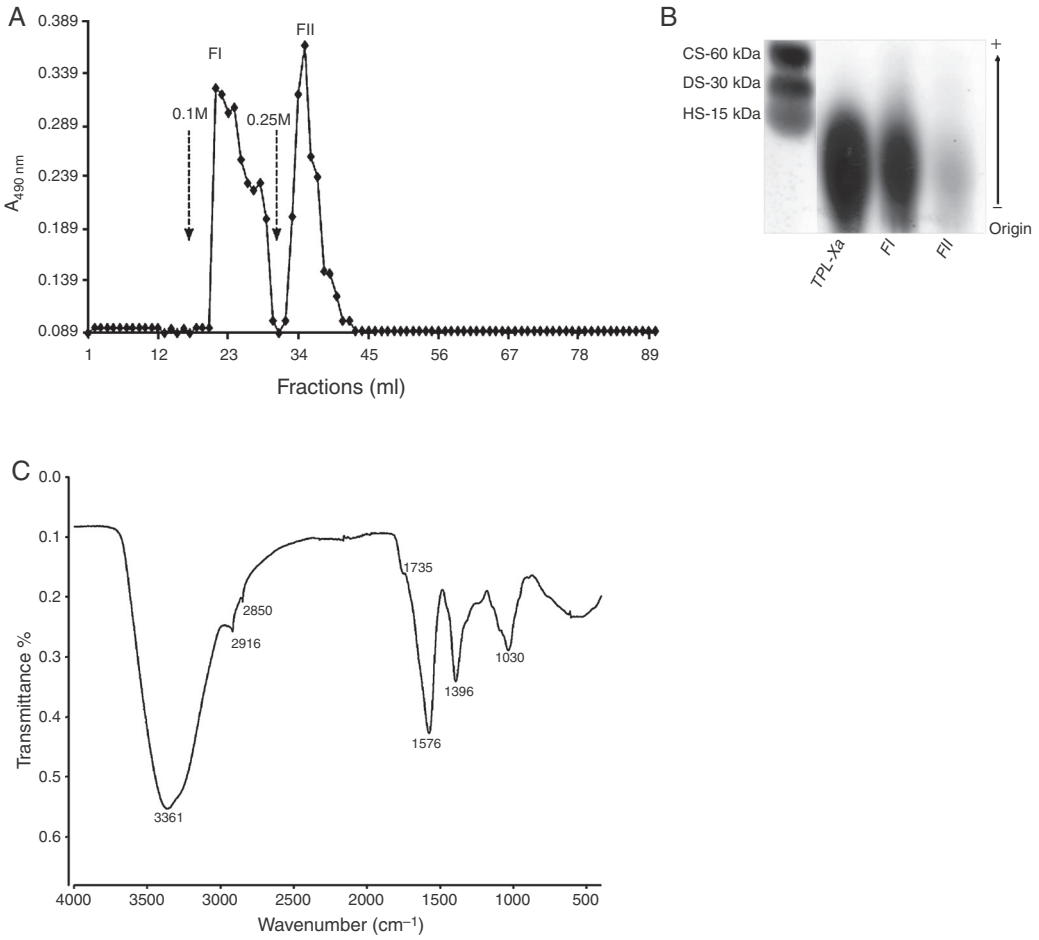

Fig. 1. Partial purification and characterization of polysaccharides from *Ximenia americana* barks. (A) TPL-Xa (10 mg) was applied to DEAE-cellulose column (9.8 cm × 2.0 cm) and resin eluted with water. The acidic polysaccharide fractions were eluted (1 ml/min) by step wise in NaCl (↓) and monitored for total carbohydrates at A_{490nm} (♦) by the phenol-sulfuric acidic method. (B) TPL-Xa, FI or FII (6 mg/ml, 15 μl) were applied in 0.5% agarose gel 0.05 M 1,3-diaminopropane:acetate pH 9.0 (110 V, 60 min) and stained with Stains-All. Chondroitin 6-sulfate (CS); Dermatan sulfate (DS) and Heparan sulfate (HS). (C) FII was analyzed by infrared (FTIR) spectroscopy. The spectral region examined was from 500 to 4000 cm^{-1} with resolution of 3 cm^{-1}.

consecutive periods of 60 s. The permanency time in apparatus was quantified (D'amour and Smith, 1941).

Statistical analysis

Results are presented as mean ± S.E.M and analyzed by One-way ANOVA and Bonferroni test (Prism 5.0, GraphPad Software Inc., California, USA). Values of $p < 0.05$ were considered significant.

Results and discussion

The extraction of total polysaccharides from *X. americana* barks (TPL-Xa) revealed 8.1% yield and presented high carbohydrate content (43%, including 21% uronic acid) with low protein (6.5%). The extraction of TPL-Xa showed higher yield and similar carbohydrate content compared to those obtained from other terrestrial Angiosperm, whose primary cell walls are composed by pectic polysaccharides, such as *Azadirachta indica* (1.3%, 54%), *Caesalpinia ferrea* (2.8%, 31%) and *Erigeron canadensis* (1%, 34.1%), extracted by similar procedures (Pereira et al., 2012a,b; Pawlaczyk et al., 2011).

Fractioning of TPL-Xa (DEAE-cellulose) resulted in two major peaks eluted at 0.1 (FI: 12% yield) and 0.25 M NaCl (FII: 22% yield). FII presented better yield and highest resolution compared to FI (Fig. 1A). Chemical analysis of the polysaccharide fractions revealed high content of carbohydrate in FII (44% total carbohydrate, containing 39% uronic acid) compared to FI (20% total carbohydrate, containing 8% uronic acid) (Table 1). In addition, the content of proteins was still inferior, especially in FII (1.6%) compared to that of FI (2.4%) and TPL-Xa.

The carbohydrate content of FII was similar to TPL-Xa and superior to FI and to FII of *C. ferrea* (Pereira et al., 2012a) and FII of *A. indica* (Pereira et al., 2012b). In both fractions the protein contaminant was lower compared to that of TPL-Xa.

The agarose gel electrophoresis revealed polydisperse bands typical of polysaccharides (Fig. 1B) after staining with Stains-All, suggesting better purity for FII and indicative of uronic acid presence. Similar feature was demonstrated for the polysaccharides obtained from *Geoffroea spinosa* barks (Souza et al., 2015). In addition, the monosaccharide composition by GC–MS demonstrated that polysaccharide fractions are composed mainly by arabinose (FI: 39%; FII: 57%) and galactose (FI: 16%; FII: 20%), however, FI also presented 35% glucose (Table 1). The monosaccharide composition of FII, showing better homogeneity than FI, corroborates its relative purity and was similar to other pectic polysaccharides isolated from *Ilex latifolia* (Fan et al., 2014), *E. canadensis* (Pawlaczyk et al., 2011) and *G. spinosa* (Souza et al., 2015).

FTIR-ATR spectra of FII (Fig. 1C), the major polysaccharide fraction (containing high content of carbohydrate and uronic acid), revealed absorption peaks in the region of 1200–1000 cm^{-1}, corresponding to carbohydrate range (Souza et al., 2015); signals at 3361 cm^{-1}, assigned to –OH stretching vibration (Li et al., 2014) and signals at 2916–2850 cm^{-1}, derived from stretching and angular

Table 1
Carbohydrate content and monosaccharide composition of polysaccharide fractions of *Ximenia americana* barks, FI and FII.

Fractions	Carbohydrate (%)	Uronic acid (%)	Ara	Rha	Gal	Glc	Xyl	Man
FI	20	8	39	4	16	35	4	2
FII	44	39	57	7	20	7	9	–

Arabinose (Ara), Rhamnose (Rha), Galactose (Gal), Glucose (Glc), Xylose (Xyl), Mannose (Man) in molar percentage.

vibration of C–H linkage, especially methyl (CH_3) group. Also, it was detected peaks at 1750–1396 cm^{-1}, resonances of COO^{-1} groups of uronic acid (Zhao et al., 2007; Pawlaczyk et al., 2011; Souza et al., 2015) and at 1735 cm^{-1} originated from the C=O stretching vibration, confirming the presence of uronic acid (group COOH) in the fraction FII (Li et al., 2014). Besides, the lack of signals at 1240 cm^{-1} indicated the absence of sulfate esters in FII (Souza et al., 2015). The FTIR-ATR of FII corroborates the high content of uronic acid demonstrated either in the agarose gel electrophoresis or by chemical analysis.

Some studies have being establishing a correlation between the presence of uronic acid, an important feature of pectic polysaccharides of plant cell walls (Drozdova and Bubenchikov, 2005; Pereira et al., 2012b), and biological activities, such as antitussive, antioxidant, anti-inflammatory and anticoagulant (Nosál'ová et al., 2000; Yoon et al., 2002; Chen et al., 2004). This correlation would also be associated with the antinociceptive activity demonstrated in our study.

The *i.v.* treatment of animals with TPL-Xa produced antinociception in the behavioral tests that evaluate chemical (Formalin and Writhing) and mechanical (carrageenan-induced hypernociception), but not thermal (Hot Plate) stimuli. TPL-Xa showed inhibitory effect in the first phase (neurogenic) of formalin test, characterized by direct excitation of nociceptive afferent fibers (Le Bars et al., 2001), inhibiting the licking time by 48% (23.0 ± 4.4 s) at 1 mg/kg and by 78% (12.8 ± 3.5 s) at 10 mg/kg. TPL-Xa also inhibited the formalin second phase (inflammatory), characterized by release of inflammatory mediators (Le Bars et al., 2001), by 50% (58.9 ± 16.9 s) at 0.1 mg/kg and by 93% (8.1 ± 5.2 s) at 10 mg/kg compared to saline (128.7 ± 11.4 s) (Fig. 2A). FII decreased the licking time

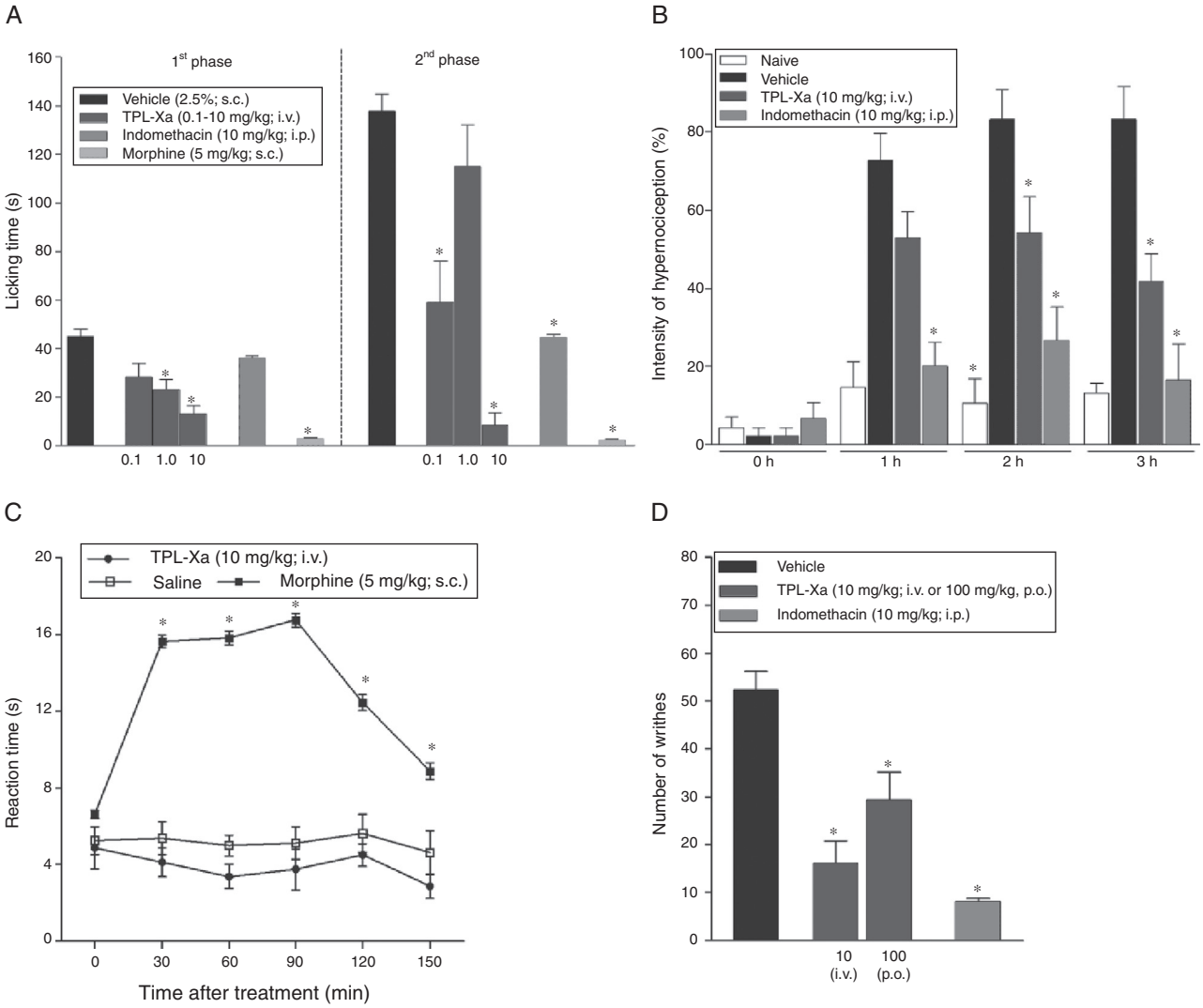

Fig. 2. TPL-Xa antinociceptive effect. Mice were pre-treated with saline (*i.v.*), morphine (5 mg/kg; *s.c.*), indomethacin (10 mg/kg; *i.p.*), TPL-Xa (0.1–10 mg/kg; *i.v.*) or TPL-Xa (100 mg/kg; *p.o.*). (A) Formalin (2.5%; *s.c.*); (B) Carrageenan-induced hypernociception (500 μg/paw; *s.c.*); (C) Hot plate (55 ± 0.5 °C); (D) Writhes (0.8% acetic acid; *i.p.*). Mean ± S.E.M. (n = 6–8). One-way ANOVA and Bonferroni test. *$p < 0.05$ compared to nociceptive stimuli.

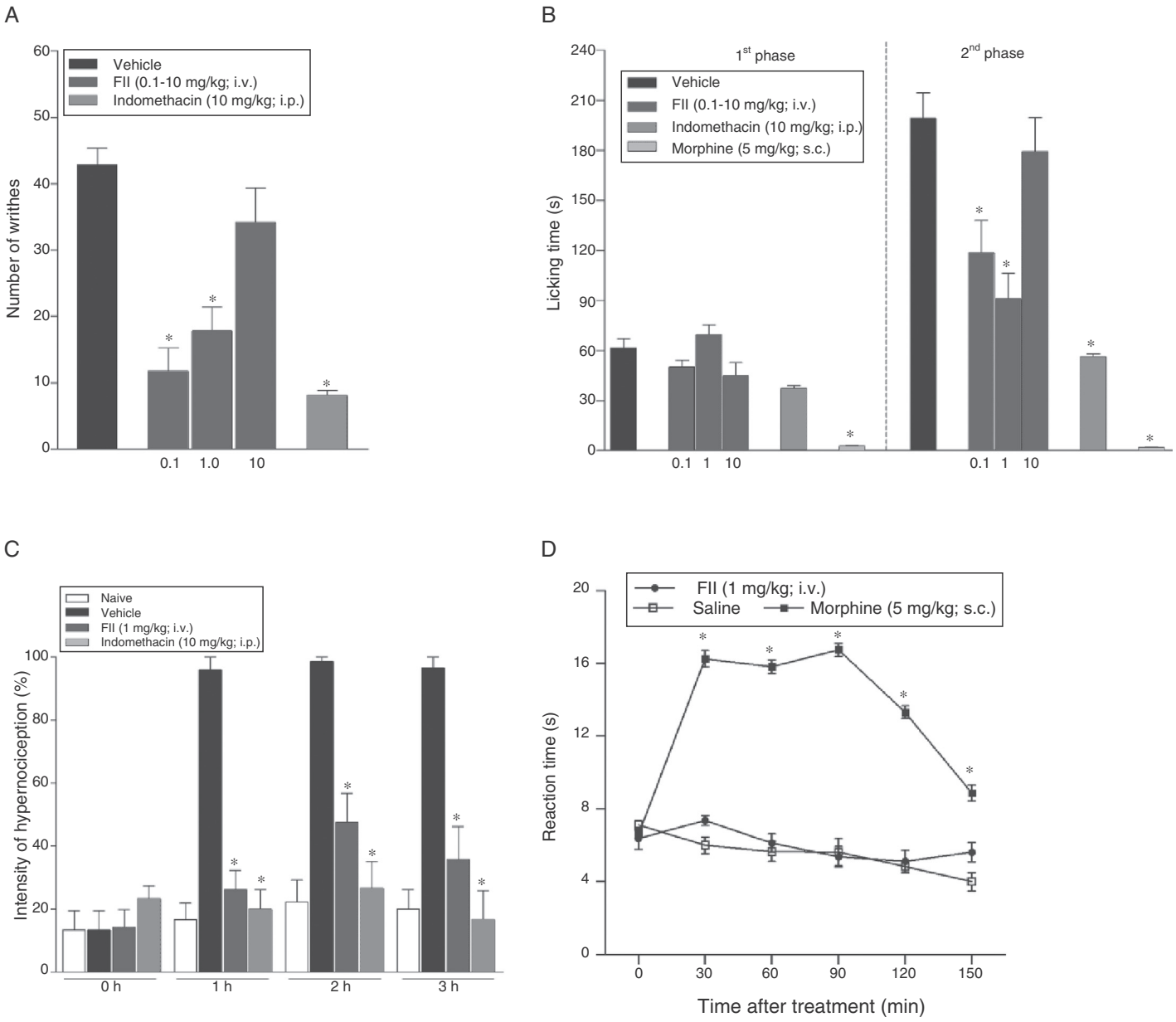

Fig. 3. FII antinociceptive effect. Mice were pre-treated with saline (*i.v.*), indomethacin (10 mg/kg; *i.p.*), morphine (5 mg/kg; *s.c.*), FII (0.1; 1 or 10 mg/kg; *i.v.*). (A) Writhes (0.8% acetic acid; *i.p.*); (B) Formalin (2.5%; *s.c.*); (C) Carrageenan-induced hypernociception (500 μg/paw; *s.c.*); (D) Hot plate (55 ± 0.5 °C). Mean ± S.E.M. (n = 6–8). One-way ANOVA and Bonferroni test. *$p < 0.05$ compared to nociceptive stimuli.

only in the second phase by 41% at 0.1 mg/kg (118.5 ± 19.4 s) and 60% at 1 mg/kg (90.7 ± 15.7 s) compared to saline (199.4 ± 15.3 s) (Fig. 3B). The analgesic opioid control morphine reduced the licking time in the first and second phases by 93% (2.6 ± 0.4 s) and 98% (2.1 ± 0.3 s), respectively, differing from the anti-inflammatory control indomethacin, that inhibited only the second phase by 68% (44.3 ± 1.3 s) (Fig. 2A). The inhibitory effect of FII (rich in uronic acid) only in the second phase suggests that TPL-Xa inhibits the release of endogenous inflammatory mediators partially mediated by acidic polysaccharides of fraction FII, and that the purification process increases the selectivity to inhibit inflammatory nociception.

Corroborating these data, TPL-Xa and FII reduced the hypernociceptive response induced by carrageenan. In mice, the injection of carrageenan into animal paws induces hypernociception characterized by the release of inflammatory cytokines, especially TNF-α and KC (keratinocyte-derived chemokine), which activate the release of IL-1β (Cunha et al., 2005) and prostanoids, involved in the pain sympathetic component (Nakamura and Ferreira, 1987).

In this test, TPL-Xa (10 mg/kg) inhibited the frequency of paw withdrawal at the 2nd h by 35% (TPL-Xa: 54.1 ± 11.1% *vs.* Vehicle: 83.2 ± 7.7%) and at the 3rd h by 50% (TPL-Xa: 41.6 ± 8.5% *vs.* Vehicle: 83.2 ± 11.7%) (Fig. 2B). FII (1 mg/kg) was also inhibitory at the 1st h by 69% (FII: 26.1 ± 6.1% *vs.* Vehicle: 86.6 ± 9.7%), at the 2nd h by 52.8% (FII: 47.2 ± 9.2% *vs.* Vehicle: 100 ± 0.0%) and at the 3rd h by 63% (FII: 35.6 ± 10.5% *vs.* Vehicle: 96.6 ± 3.3%) (Fig. 3C). Indomethacin inhibited the paw withdrawal at all times (1st h: 19.9 ± 6.3; 2nd h: 26.6 ± 8.5; 3rd h: 16.6 ± 9.1) (Fig. 2B).

In the Writhing test TPL-Xa injected either *i.v.* or *p.o.* inhibited the number of acetic acid-induced abdominal writhes (52.2 ± 3.8) by 69% (16.1 ± 4.6) at 10 mg/kg (*i.v.*) and 44% (29.3 ± 5.8) at 100 mg/kg (*p.o.*) (Fig. 2D). FII *i.v.* (0.1 and 1 mg/kg) was also inhibitory (42.8 ± 2.4) by 72% (11.8 ± 3.4) and 58% (17.8 ± 3.6), respectively (Fig. 3A). Indomethacin decreased the number of

Table 2
Markers of hepatic, renal and hematological function of animals treated with TPL-Xa.

	[a]Treatment (50 μl/10 g)	
Parameters	Saline	TPL-Xa (10 mg/kg)
AST (U/l)	65.91 ± 6.15	68.00 ± 3.89
ALT (U/l)	35.45 ± 2.10	35.17 ± 2.14
Urea (mg/dl)	40.31 ± 1.65	38.92 ± 1.59
Creatinine (mg/dl)	0.33 ± 0.01	0.31 ± 0.00
Hematocrit (%)	49.85 ± 1.98	45.15 ± 1.13
Hemoblobin (g/dl)	13.23 ± 0.95	11.50 ± 0.36
Erytrocyte (10^6 ml^{-1})	8.47 ± 0.24	7.51 ± 0.24
Platelet (10^3 ml^{-1})	1136 ± 88.68	1213 ± 97.89
Lymphocyte (%)	78.83 ± 1.92	82.00 ± 1.47
Monocyte (%)	0.50 ± 0.34	0.250 ± 0.25
Eosinophil (%)	0.66 ± 0.21	0.0 ± 0.00
Neutrophil (%)	20.14 ± 1.84	16.40 ± 1.36
MCV (fL)	58.81 ± 0.83	60.00 ± 0.43
MCH (pg)	16.65 ± 0.29	16.10 ± 0.12
MCHC (g/dl)	28.38 ± 0.70	26.65 ± 0.41

[a] Mice were treated daily in single doses with TPL-Xa (10 mg/kg) or saline (0.9%) during 14 days; Mean ± S.E.M ($n = 7$); One-way ANOVA and Bonferroni test; $^*p < 0.05$ compared to saline. ALT, alanine transaminase; AST, aspartate transaminase; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

writhes (84.4%) (Fig. 2D). This model assesses different nociceptive mechanisms, including release of inflammatory mediators such as histamine, serotonin, bradykinin and PGE_2 (Le Bars et al., 2001). These results are in accordance with the anti-inflammatory and antinociceptive effects of the polysaccharides extracted from *Thladianta dubia* (Wang et al., 2011) and with the anti-inflammatory activity of other pectic polysaccharides (Salman et al., 2008).

The suggestion of peripheral effects of TPL-Xa and FII were confirmed by the lack of effect in the Hot plate test (Figs. 2C and 3D), that evaluate medullar spinal nociceptive pathways (Le Bars et al., 2001).

It is important to highlight that TPL-Xa (10 mg/kg), different from the sedative agent (diazepam: 20.1 ± 6.4 *vs.* saline: 36.4 ± 4.7 s), did not alter the animals-fall-latency in the Rota-rod test (TPL-Xa: 42.5 ± 5.9 s *vs.* saline: 36.4 ± 4.7 s). This data suggests that TPL-Xa does not alter animals motor activity, a side effect commonly associated with the use of analgesic drugs. In addition, mice treatment with TPL-Xa during 14 days did not alter the following parameters: renal, hepatic and hematological markers (Table 2) or the animal body mass (initial weight: 29.1 ± 1.2 *vs.* final weight: 31.2 ± 1.4) compared to saline (initial weight: 28.8 ± 1.0 *vs.* final weight: 32.0 ± 1.4). The wet weigh of kidney (TPL-Xa: 6.9 ± 0.3 *vs.* Saline: 6.5 ± 0.2), stomach (TPL-Xa: 8.7 ± 0.5 *vs.* Saline: 9.6 ± 0.4), liver (TPL-Xa: 44.6 ± 0.8 *vs.* Saline: 42.2 ± 1.0) and heart (TPL-Xa: 4.4 ± 0.1 *vs.* Saline: 4.7 ± 0.2), except for the spleen (TPL-Xa: 4.1 ± 0.3 *vs.* Saline: 2.5 ± 0.1), was not altered. These data corroborate the well-known low toxicity of plant polysaccharides.

In conclusion, polysaccharides rich fractions of *X. americana* barks, containing high levels of uronic acid, arabinose, galactose and glucose, inhibit peripheral inflammatory nociception, being well tolerated by animals.

Author contributions

KESSL, LEAD, LFM and AFP conducted animal experiments; KESSL, MGP and PASM conducted the extraction, isolation and characterization of polysaccharides; MGRQ performed the hematologic and biochemical analysis; KESSL, AFP, AMSA and MGP supplied critical input to experimental design and data interpretation; KESSL provided statistical analysis and interpretation; KESSL, AFP, AMSA and MGP were responsible for writing the manuscript. All authors have read and approved the submission of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Chen, H., Zhang, M., Xie, B., 2004. Quantification of uronic acids in tea polysaccharide conjugates and their antioxidant properties. J. Agric. Food Chem. 52, 3333–3336.
Cunha, T.M., Verri, W.A., Silva, J.S., Poole, S., Cunha, F.Q., Ferreira, S.H., 2005. A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. Proc. Natl. Acad. Sci. U. S. A. 102, 1755–1760.
D'amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.
de Albuquerque, U.P., Muniz de Medeiros, P., de Almeida, A.L.S., Monteiro, J.M., Machado de Freitas Lins Neto, E., Gomes de Melo, J., dos Santos, J.P., 2007. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: a quantitative approach. J. Ethnopharmacol. 114, 325–354.
Dietrich, C.P., Dietrich, S.M.C., 1976. Electrophoretic behaviour of acidic mucopolysaccharides in diamine buffers. Anal. Biochem. 70, 645–647.
Dische, Z., 1947. A new specific color reaction of hexuronic acidas. J. Biol. Chem. 167, 189–198.
do Nascimento, G.E., Corso, C.R., de Paula Werner, M.F., Baggio, C.H., Iacomini, M., Cordeiro, L.M.C., 2015. Structure of an arabinogalactan from the edible tropical fruit tamarillo (*Solanum betaceum*) and its antinociceptive activity. Carbohydr. Polym. 116, 300–306.
do Nascimento, G.E., Hamm, L.A., Baggio, C.H., Werner, M.F.de P., Iacomini, M., Cordeiro, L.M.C., 2013. Structure of a galactoarabinoglucuronoxylan from tamarillo (*Solanum betaceum*), a tropical exotic fruit, and its biological activity. Food Chem. 141, 510–516.
Drozdova, I.L., Bubenchikov, R.A., 2005. Composition and antiinflammatory activity of polysaccharide complexes extracted from sweet violet and low mallow. Pharm. Chem. J. 39, 197–200.
DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Fan, J., Wu, Z., Zhao, T., Sun, Y., Ye, H., Xu, R., Zeng, X., 2014. Characterization, antioxidant and hepatoprotective activities of polysaccharides from *Ilex latifolia* Thunb. Carbohydr. Polym. 101, 990–997.
James, D.B., Abu, E.A., Wurochekke, A.U., Orji, G.N., 2007. Phytochemical and antimicrobial investigation of the aqueous and methanolic extracts of *Ximenia americana*. J. Med. Sci. 7, 284–288.
Kircher, H.W., 1960. Gas-liquid partition chromatography of methylated sugars. Anal. Chem. 32, 1103–1106.
Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacol. Rev. 53, 597–652.
Le, N.H.T., Malterud, K.E., Diallo, D., Paulsen, B.S., Nergård, C.S., Wangensteen, H., 2012. Bioactive polyphenols in *Ximenia americana* and the traditional use among Malian healers. J. Ethnopharmacol. 139, 858–862.
Li, X., Jiang, J., Shi, S., Bligh, S.W.A., Li, Y., Jiang, Y., Huang, D., Ke, Y., Wang, S., 2014. A RG-II type polysaccharide purified from *Aconitum coreanum* alleviates lipopolysaccharide-induced inflammation by inhibiting the NF-κB signal pathway. PLoS One 9, e99697, http://dx.doi.org/10.1371/journal.pone.0099697.
Maikai, V.A., Kobo, P.I., Maikai, B.V.O., 2010. Antioxidant properties of *Ximenia Americana*. African J Biotechnol 9, 7744–7746.

Nakamura, M., Ferreira, S.H., 1987. A peripheral sympathetic component in inflammatory hyperalgesia. Eur. J. Pharmacol. 135, 145–153.
Nosál'ová, G., Kardosová, A., Franová, S., 2000. Antitussive activity of a glucuronoxylan from *Rudbeckia fulgida* compared to the potency of two polysaccharide complexes from the same herb. Pharmazie 55, 65–68.
Ovodov, I.S., 1998. Polysaccharides of flower plants: structure and physiological activity. Bioorg. Khim. 24, 483–501.
Pawlaczyk, I., Czerchawski, L., Kuliczkowski, W., Karolko, B., Pilecki, W., Witkiewicz, W., Gancarz, R., 2011. Anticoagulant and anti-platelet activity of polyphenolic-polysaccharide preparation isolated from the medicinal plant *Erigeron canadensis* L. Thromb. Res. 127, 328–340.
Pereira, L., de, P., da Silva, R.O., Bringel, P.H., de, S.F., da Silva, K.E.S., Assreuy, A.M.S., Pereira, M.G., 2012a. Polysaccharide fractions of *Caesalpinia ferrea* pods: potential anti-inflammatory usage. J. Ethnopharmacol. 139, 642–648.
Pereira, L., de, P., Mota, M.R.L., Brizeno, L.A.C., Nogueira, F.C., Ferreira, E.G.M., Pereira, M.G., Assreuy, A.M.S., 2016. Modulator effect of a polysaccharide-rich extract from *Caesalpinia ferrea* stem barks in rat cutaneous wound healing: role of TNF-α, IL-1β, NO, TGF-β. J. Ethnopharmacol. 187, 213–223.
Pereira, L., de, P., Silva, K.E.S.da, Silva, R.O.da, Assreuy, A.M.S., Pereira, M.G., 2012b. Anti-inflammatory polysaccharides of *Azadirachta indica* seed tegument. Rev. Bras. Farmacogn. 22, 617–622.
Salman, H., Bergman, M., Djaldetti, M., Orlin, J., Bessler, H., 2008. Citrus pectin affects cytokine production by human peripheral blood mononuclear cells. Biomed. Pharmacother. 62, 579–582.
Schepetkin, I.A., Quinn, M.T., 2006. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. Int. Immunopharmacol. 6, 317–333.
Silva, G.G.da, Souza, P.A.de, Morais, P.L.D.de, Santos, E.C.dos, Moura, R.D., Menezes, J.B., 2008. Caracterização do fruto de ameixa silvestre (*Ximenia americana* L.). Rev. Bras. Frutic. 30, 311–314.
Soro, T.Y., Traore, F., Sakande, J., 2009. Analgesic activity of the aqueous extract from *Ximenia americana*. C. R. Biol. 332, 371–377.
Souza, R.O.S., Assreuy, A.M.S., Madeira, J.C., Chagas, F.D.S., Parreiras, L.A., Santos, G.R.C., Mourão, P.A.S., Pereira, M.G., 2015. Purified polysaccharides of *Geoffroea spinosa* barks have anticoagulant and antithrombotic activities devoid of hemorrhagic risks. Carbohydr. Polym. 124, 208–215.
Wang, L., Zhao, D., Di, L., Xu, T., Lin, X., Yang, B., Zhou, X., Yang, X., Liu, Y., 2011. The analgesic and anti-rheumatic effects of *Thladiantha dubia* fruit crude polysaccharide fraction in mice and rats. J. Ethnopharmacol. 137, 1381–1387.
Yoon, S.-J., Pereira, M.S., Pavão, M.S.G., Hwang, J.-K., Pyun, Y.-R., Mourão, P.A.S., 2002. The medicinal plant *Porana volubilis* contains polysaccharides with anticoagulant activity mediated by heparin cofactor II. Thromb. Res. 106, 51–58.
Zhao, M., Yang, N., Yang, B., Jiang, Y., Zhang, G., 2007. Structural characterization of water-soluble polysaccharides from *Opuntia monacantha* cladodes in relation to their anti-glycated activities. Food Chem. 105, 1480–1486.