In vivo/*in vitro* Studies of the Effects of the Type II Arabinogalactan Isolated from *Maytenus ilicifolia* Mart. ex Reissek on the Gastrointestinal Tract of Rats

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Type II arabinogalactan (AG) is a polysaccharide found in *Maytenus ilicifolia* (Celastraceae), a plant reputed as gastroprotective. Oral and intraperitoneal administration of the AG protected rats from gastric ulcers induced by ethanol. No alteration of mechanisms related to acid gastric secretion and gastrointestinal motility were observed. *In vitro*, the AG showed a potent scavenging activity against the radical of DPPH (2,2-diphenyl-1-picrylhydrazyl) with an IC_{50} value of 9.3 μM. However, the mechanism of the gastroprotective action remains to be identified.

Key words: Maytenus ilicifolia, Arabinogalactan, Gastroprotective

Introduction

Maytenus ilicifolia, a plant popularly known in Brazil as "espinheira santa", is extensively used to treat stomach disorders (Cruz, 1982; Macaubas *et al*., 1988). The gastroprotective properties of various extracts of *M. ilicifolia* have been shown in experimental ulcer models using rodents (Baggio *et al*., 2007; Ferreira *et al*., 2004; Jorge *et al*., 2004; Souza-Formigoni *et al*., 1991; Tabach and Oliveira, 2003). Our laboratory identified inhibition of gastric acid secretion and modulation of nitric oxide in the mechanism of activity of a flavonoid-rich extract containing galactitol (25%), epicatechin (3.1%), and catechin (2%) as the major components (Baggio *et al*., 2007).

The polysaccharide arabinogalactan is found as an essential structural polymer of the cell wall of plants and as a major component of many gums and exudates (Delgobo *et al*., 1998; Fincher *et al*., 1983). Several plants have been reported to contain polysaccharides of this type, and its presence has been correlated with a variety of biological activities such as antiviral, antitumour, immune-stimulating, anti-inflammatory, anticoagulant, hypoglycemic, and antiulcer (Capek *et al*., 2003; Nergard *et al*., 2005; Srivastava and Kulshreshtha, 1989; Yamada, 1994). Furthermore, our laboratory showed a potent antiulcer activity of this compound in the ethanol-induced gastric injury model (Cipriani *et al*., 2006).

In this study, we screened the effects of the arabinogalactan against the experimental models of gastric hypersecretion, ulcer, and gastrointestinal motility in which *Maytenus ilicifolia*, from which this compound has been isolated, showed potent gastroprotective activity.

Material and Methods

Plant material

Leaves of *Maytenus ilicifolia* Mart. ex Reissek (Celastraceae) were collected in October 2003, at Curitiba (Paraná, Brazil), and provided by the Central de Produção e Comercialização de Plantas Medicinais, Aromáticas e Condimentares do Paraná Ltda, Curitiba, PR, Brazil. A voucher specimen was deposited in the herbarium of the Botany Department of the Federal University of Paraná, Curitiba, PR, Brazil, under number 30842.

Extraction and purification of the type II arabinogalactan (AG)

Isolation and identification of the AG has been described in detail elsewhere (Cipriani *et al.*, 2006). The content of this purified AG in *M. ilicifolia* was 0.38% w/w and its average molar mass (M) was 11400 g/mol.

Animals

Female Wistar rats (180–200 g) and female Swiss mice (25–30 g) were from UFPR colony and were maintained under standard laboratory conditions [12 h/12 h light/dark cycle, (22 ± 2) °C]. Standard pellet food (Nuvital®; Quimtia, Curitiba, PR, Brazil) and water were *ad libitum*. The rats and mice were deprived of food for 16 h and 6 h, respectively, prior to the start of experiments. All experimental protocols using animals were performed according to the "Principles of Laboratory Animal Care" (NIH Publication 85–23, revised 1985) and after approval of the respective protocols by the Committee of Animal Experimentation of Federal University of Paraná, Curitiba, PR, Brazil (CEUA/BIO-UFPR, protocol 167).

Induction of acute gastric lesions in rats

The experiment was carried out according to the method described by Robert *et al.* (1979). Rats (n = 6) were treated with vehicle [control: water or saline, 0.1 ml/100 g body weight (BW), *per os* (p.o.) or intraperitoneal (i.p.), respectively], arabinogalactan (AG: 10 mg/kg BW, p.o. or i.p.) or omeprazole (Ome: 40 mg/kg BW, p.o.) 60 min (p.o. treatment) or 30 min (i.p. treatment) before administration of 80% ethanol (0.5 ml/200 g BW, p.o.). Animals were sacrificed 1 h later, the stomachs were removed and gastric lesion extension measured as the total injured area (mm^2) [= length (mm) · width (mm) of injury] (Baggio *et al.*, 2007).

Induction of hypersecretion by pylorus ligature in rats

A pylorus ligature according to the method of Shay *et al.* (1945) was carefully done in fasted female rats (n = 6) under anaesthesia. Either the vehicle [water, 0.1 ml/100 g BW, intraduodenal (i.d.)] or arabinogalactan (AG: 10, 30, and 100 mg/kg BW, i.d.) was administered immediately after pylorus ligation to the respective groups. Omeprazole (Ome: 40 mg/kg BW, p.o.) was given 1 h before surgery. After 4 h of pylorus ligature, animals were killed, the stomachs were opened, and gastric secretions collected. Volume and total gastric acidity were measured immediately (Baggio *et al.*, 2007).

Determination of gastrointestinal motility

Fasted female Swiss mice (n = 8) were treated with vehicle (control: water, 0.1 ml/10 g BW, p.o.), arabinogalactan (AG: 10, 30, and 100 mg/kg BW, p.o.) or atropine [A: 3 mg/kg BW, subcutaneous (s.c.)]. After 1 h, animals received 0.5 ml of a semisolid solution of 0.05% phenol red in 1.5% methylcellulose. After 15 min, the animals were killed and the stomach and small intestine quickly removed. Gastric emptying (GE) was measured as the amount of marker that remained in the stomach at the end of the experiment. Each stomach was homogenized with 7 ml distilled water and centrifuged at 1300 x g for 15 min. Equal amounts (1 ml) of supernatant and 0.025 M NaOH were mixed and the absorbance measured using a spectrophotometer at 560 nm. GE (%) was calculated using the equation: $\%GE = 100 - (X \cdot 100/Y)$, where X is the absorbance of phenol red recovered from the stomach of animals sacrificed 15 min after the administration of marker, and Y is the mean (n = 8) absorbance of phenol red recovered from the stomachs of control animals (killed immediately after administration of the marker).

Intestinal transit (IT) was measured as the distance travelled by the marker in the small intestine. Briefly, the small intestine was dissected from the pylorus to the ileocaecal junction. The total length of the small intestine and the distance travelled by phenol red were then measured. IT was calculated as: $\%IT = X/Y \cdot 100$, where X is the distance travelled by phenol red, and Y is the total length of the small intestine (Suchitra *et al.*, 2003).

DPPH free radical scavenging assay

The free radical scavenging activity of arabinogalactan using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined with a slightly modified method described by Blois (1958). Aliquots of arabinogalactan (0.75 ml, to give final concentrations of 0.3, 3, and 30 μM) were mixed with 0.25 ml DPPH radical solu-

tion in methanol. The decrease in absorbance at 517 nm was measured after 5 min. For all experiments, the vehicle (distilled water) of arabinogalactan was used as negative control; ascorbic acid (300 μM) was used as a reference control. Experiments were performed in triplicate. Concentrations of DPPH were calculated using an extinction coefficient on 0.999 M/cm.

Statistical analysis

Data were expressed as means ± standard error of mean (S.E.M.) Statistical significance of the results was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test. Data were considered different at a significance level of $P < 0.05$. The inhibitory concentration or dose 50 (IC_{50} or ID_{50}) were calculated by fitting the data to the equation: $Vi/Vo = 1/(1 + [I]/IC_{50})$ using the KhaleidaGraph 3.0 for Windows program (Synergy Software, Reading, PA, USA), where *Vi* is the total activity, *Vo* is the remaining activity, and [I] is the inhibitor concentration.

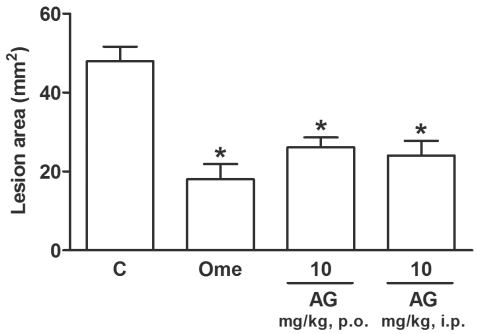

Fig. 1. Comparison of the gastroprotective effects of the arabinogalactan administered by two routes. Similar gastroprotective potency was observed in the ethanol-induced gastric injury model when the arabinogalactan was administered by oral (p.o.) or intraperitoneal (i.p.) route. The animals received vehicle (C: water or saline, 0.1 ml/100 g BW, p.o. or i.p., respectively), omeprazole (Ome: 40 mg/kg BW, p.o.), and arabinogalactan (AG: 10 mg/kg BW, p.o. or i.p.) 60 min (p.o. treatment) or 30 min (i.p. treatment) before oral administration of 80% ethanol (0.5 ml/200 g BW, p.o.). The results are expressed as mean ± S.E.M. ($n = 6$). *Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

Results

Effects on acute gastric lesions

Oral treatment of animals with the arabinogalactan (10–100 mg/kg BW) protected the mucosa against gastric lesions induced by ethanol in a dose-related manner. The ID_{50} value was 9.3 mg/kg BW when administered orally (Cipriani *et al.*, 2006). Intraperitoneal administration of arabinogalactan (10 mg/kg BW) reduced the ethanol-induced gastric lesions by 50% [injured control group value = (47.9 ± 3.7) mm^2] (Fig. 1). Omeprazole (40 mg/kg BW, p.o.), used as a positive control, reduced the gastric lesions induced by ethanol by 62% (Fig. 1).

Effects on gastric acid secretion

Hypersecretion induced by pylorus ligature for 4 h was not altered by any tested dose of the arabinogalactan up to 100 mg/kg BW (i.d.). Omeprazole, positive control of the test, inhibited the gastric volume and total acidity by 44 and 92%, respectively (Table I).

Effects on gastrointestinal motility

No alteration on gastric emptying or intestinal transit as measured by the semisolid solution propulsion was observed with doses up to 100 mg/kg BW (p.o.) arabinogalactan. Atropine, used as positive control, reduced gastric emptying by 47% and the intestinal transit by 70% (Table I).

Effects on DPPH scavenging activity

Arabinogalactan scavenged DPPH radicals, with an IC_{50} value of 9.3 μM when compared with the control [(0.305 ± 0.005) M] (Fig. 2). Ascorbic acid (300 μM), the reference compound, scavenged DPPH radicals by 68% when tested under the same experimental conditions.

Discussion

The results of this study show the potent anti-ulcer effect of the arabinogalactan isolated from *M. ilicifolia* against irritant actions of ethanol-induced gastric injury. Ethanol destroys the protective factors of the mucosa, such as mucus barrier (Hirschowitz, 1989) and non-proteic sulfhydryl (NP-SH) groups (Siegmund *et al.*, 2003). Increase of oxygen-derived free radicals (Pihan *et al.*, 1987) and of vascular permeability (Szabo *et al.*, 1985) as well as apoptosis of gastric cells (Piotrowski *et al.*, 1997) are also known processes involved

Table I. Effects of the arabinogalactan (AG) from *M. ilicifolia* on acid secretion and gastrointestinal motility.

Test	Acid secretion		Gastric emptying (%)	Intestinal transit (%)
	Volume [ml]	Total acidity [mEq[H^+]/l]		
Control	8.2 ± 0.8	71.8 ± 6.2	52.1 ± 1.8	54.2 ± 3.0
AG 10 mg/kg BW, p.o.	6.7 ± 0.6	60.6 ± 8.8	50.0 ± 2.9	65.1 ± 2.2
AG 30 mg/kg BW, p.o.	8.5 ± 0.6	69.8 ± 3.9	48.2 ± 4.1	68.9 ± 3.8
AG 100 mg/kg BW, p.o.	5.8 ± 0.5	63.1 ± 4.9	47.2 ± 2.9	63.3 ± 2.3
Omeprazole 40 mg/kg BW, p.o.	4.6 ± 0.6 *	5.6 ± 2.6 *	NA	NA
Atropine 3 mg/kg BW, s.c.	NA	NA	27.6 ± 1.5 *	16.1 ± 4.7 *

NA, not applicable.
* Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

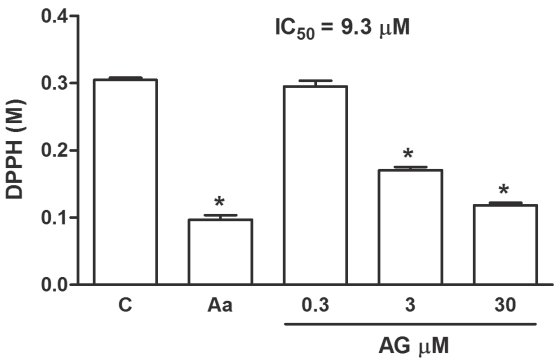

Fig. 2. DPPH scavenging activity of the arabinogalactan from *M. ilicifolia*. Ascorbic acid (Aa: 300 μM) was used as a positive control. The results are expressed as mean ± S.E.M. All experiments were performed in triplicate. *Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

in ethanol injury. In this study, the effects of the arabinogalactan on the most common pathways of gastroprotection during gastric injury including antioxidant activity, gastric secretion, and motility were determined. The antioxidant potential (DPPH free radical scavenging activity) of the arabinogalactan was moderate. Similar results were previously shown for an arabinogalactan isolated from *Tinospora cordifolia* (Subramanian *et al.*, 2002).

Intraduodenal administration of the arabinogalactan in animals with gastric hypersecretion induced by pylorus ligature did not alter the gastric acid secretion of the animals indicating that the histaminergic and muscarinic pathways as well as activity of the gastric ATPase were not involved in the gastroprotection provided by the arabinogalactan. Confirmatory experiments *in vitro* using isolated H^+,K^+-ATPase showed weak activity (IC_{50} = 3.4 mg/ml, data not shown). A topical effect of the compound on the gastric mucosa could explain the gastroprotection against ethanol injury, but since the arabinogalactan maintained its protective activity against ethanol when administered intraperitoneally discards this hypothesis. The effects of the same dose administered either orally or intraperitoneally were comparable (Fig. 1). An attempt to explain this observation through pharmacokinetics is difficult as no relevant information is currently available. A few pharmacokinetics studies of arabinogalactans showed that the amount of arabinogalactan absorbed following an oral dose remains unclear. Animal studies for larch arabinogalactan using intravenous administration resulted in about 53% of the dose being present in the liver and 30% in the urine 90 min after dosing. Non-absorbed larch arabinogalactan is actively fermented by the intestinal microflora and is particularly effective in increasing beneficial anaerobes such as *Bifidobacteria* and *Lactobacillus* (Groman *et al.*, 1994).

Similarly, no effects were observed in the gastric emptying and intestinal transit models after oral administration of the arabinogalactan. Since the gastric emptying rate is related to a neurohumoral mechanism, which depends on an intact vagal innervation and therefore on the action of several neurotransmitters with acetylcholine as the major regulator (reviewed by Hansen, 2003), failure of the compound to alter the gastric emptying indicates that cholinergic pathways are not involved in the antiulcer properties of the compound. This result is in agreement with findings in humans where no effect on transit time frequency,

fecal weight or pH value and short-chain fatty acids, blood lipids or blood insulin were observed after a three-weeks exposure to arabinogalactan (15 to 30 g) (Robinson *et al.*, 2001).

The results indicate that the arabinogalactan protects the gastric mucosa against irritant agents such as ethanol with a potency that is the highest observed until now for a constituent of *M. ilicifolia*. Although the mechanism of action of the arabinogalactan in gastric protection is still unknown, we can conclude that these effects do not occur through alterations of the gastrointestinal motility or acid secretion which are the major effects observed with *M. ilicifolia*. Further studies on the antioxidant capacity need to be performed to determine if this mechanism is the key to the gastroprotective effects of arabinogalactan and hence of *M. ilicifolia*.

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