Rev Bras Farmacogn (2020) 30:423–426
https://doi.org/10.1007/s43450-020-00007-7

SHORT COMMUNICATION

Gastroprotective activity of punicalagin and *Lafoensia pacari* in mice

Beatriz A. Chaibub[1] · José L. R. Martins[2] · José R. de Paula[1] · Suzana da C. Santos[3] · Elson A. Costa[2] · Maria Teresa F. Bara[1]

Received: 29 May 2019 / Accepted: 9 October 2019 / Published online: 4 June 2020

Abstract
Lafoensia pacari A. St.-Hil., Lythraceae, is used in Brazilian folk medicine to treat ulcer and gastritis; thus, it is important to conduct research so as to better understand its medicinal properties. The aim of this study was to assess the antisecretory and antiulcerogenic activities of lyophilized aqueous fraction and punicalagin isolated from leaves of *L. pacari*. Antisecretory gastric acid activity and antiulcerogenic activity were investigated by pyloric ligation-induced and indomethacin-induced gastric lesion models, respectively. Results show that the lyophilized aqueous fraction of *L. pacari* and punicalagin reduced the volume of gastric secretion, free acidity, and total acidity in mice 4 h after pyloric ligation, as well as the rate of gastric damage following the indomethacin administration model. Reduction percentages varied according to the dose of each treatment and showed statistical difference. This finding indicates that the lyophilized aqueous fraction of *L. pacari* and punicalagin exert an antiulcerogenic effect due to their acid antisecretory activity.

Keywords Antisecretory activity · Antiulcerogenic activity · Bioactivity · Ellagitannin · Punicalagin · Gastroprotective

Introduction

Ethnobotanical studies have shown that *Lafoensia pacari* A. St.-Hil., Lythraceae, is traditionally used in Brazilian folk medicine due to its antiulcer (mostly bark) (Cabral and Pasa 2009) and anti-inflammatory (inner bark) (Jesus et al. 2009) activities.

Studies have shown that some activities of the *L. pacari* stem bark are antinociceptive (Nascimento et al. 2011) and antiulcer (Tamashiro Filho et al. 2012). There are still few studies conducted on the plant's leaves, but an antinociceptive activity has been reported (Guimarães et al. 2010). Its stem bark has pyrrogalic tannins, steroids, triterpenoids, and simple phenols such as ellagic acid (Tamashiro Filho et al. 2012), and its leaves have phenolic compounds (Sampaio et al. 2011) and punicalagin (**1**) (Carneiro et al. 2016). The latter is an ellagitannin that shows biological activities, e.g., antifungal (Silva et al. 2018), chemopreventive, antigenotoxic, and angiogenic (Carneiro et al. 2016).

The gastroprotective potential of natural products has been investigated and has shown promising results (Martins et al. 2015). In view of these findings and of the fact that no studies reporting the antiulcerogenic and antisecretory activities of *L. pacari* leaves and punicalagin have been found in the literature, we carried out this study to evaluate the gastroprotective effect of lyophilized aqueous fraction (LAF) and punicalagin isolated from *L. pacari* leaves in experimental models in mice.

Material and Methods

Lafoensia pacari A. St.-Hil., Lythraceae, leaves were collected in December 2011 in Caldazinha (S 16° 39′ 54.5″; W 49° 00′ 03,9″; 1100 m), Goiás state, Brazil. A voucher specimen was deposited at the herbarium of the Federal University of Goiás (UFG) under no. 47581.

An extract was obtained with 50% acetone water in an ultrasonic bath for 30 min, at about 2% solvent/plant ratio. The acetone was evaporated under reduced pressure at

✉ Maria Teresa F. Bara
mbara@ufg.br

[1] Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil

[2] Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil

[3] Instituto de Química, Universidade Federal de Goiás, Goiânia, GO, Brazil

35 °C, the aqueous residue was filtered, and a liquid-liquid partition was performed with ethyl acetate. The aqueous phase was lyophilized and then named LAF. The isolation of punicalagin (**1**) from *L. pacari* leaves has been described by our research group (Carneiro et al. 2016).

Animals were selected from UFG's Central Animal House. They were kept under controlled conditions of temperature, dark/light cycle, and access to water and food and were acclimatized for 7 days. These experimental protocols were performed in accordance with Brazil's National Council for Animal Experimentation Control and were approved by UFG's Research Ethics Committee under no. 104/08.

As regards the antisecretory gastric acid activity model, pyloric sphincters of adult male Swiss albino mice (30–40 g) were surgically ligated (Visscher et al. 1954). Following a 16 h fast, animals were given vehicle (water, 10 ml/kg), LAF (75, 150, and 300 mg/kg), punicalagin (120 mg/kg), or ranitidine (50 mg/kg), all administered intraduodenally (*i.d.*). This procedure was performed according to Martins et al. (2014). The volume (ml) and value of gastric pH were measured, and gastric acidity was expressed as microequivalents of H^+ per liter for 4 h (mEq[H^+]/l/4 h).

The antiulcerogenic activity model was performed according to Djahanguiri (1969). After a 16 h fast, adult male Swiss albino mice (30–40 g) were given vehicle (water, 10 ml/kg), LAF (150 mg/kg), punicalagin (120 mg/kg), or ranitidine (50 mg/kg), all administered intraduodenally (*i.d.*). One hour after treatment, all animals were given indomethacin (30 mg/kg *s.c.*) to induce gastric ulcer. Treatments were repeated 3 h after indomethacin was administered. This procedure was performed according to Martins et al. (2014), and lesion scores were measured and expressed as the mean rate.

Results were expressed as means ± S.E.M. The data were analyzed statistically by one-way ANOVA, followed by Student-Newman-Keuls as a post hoc test. Statistical analyses were carried out using GraphPad Prism version 5.00. Values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

Lafoensia pacari has been frequently mentioned in ethnobotanical surveys, and its leaves have been investigated with a view to replacing the use of its bark, which causes greater damage to the species.

During the preparation of LAF, a partition with ethyl acetate was performed to separate more polar substances like flavonoids, phenolic acids, and some tannin monomers. Punicalagin (**1**) was the major constituent of LAF, yielding 67% (data not shown). The aqueous fraction was lyophilized and yielded 74.7 g.

In the assessment of the antisecretory activity, three doses were initially chosen for LAF, based on Galdino et al. (2010). LAF (75, 150, and 300 mg/kg) and ranitidine reduced the volume of gastric secretion by 9.64, 11.2, 15.66, and 9.24%, respectively, compared to the control group. Treatment with LAF (75, 150, and 300 mg/kg) and ranitidine reduced free acidity by 76.89, 58.97, 46.66, and 64.14%, respectively, with a significant increase in pH compared to the control group. The same treatment reduced total acidity by 65.30, 71.46, 69.40, and 75.93%, respectively, compared to the control group (Table 1).

As for the pyloric ligation-induced model, secretion and gastric acid accumulation are possibly the most relevant factors among those involved in the pathogenesis of peptic ulcer induced by pyloric ligation (Muthuraman and Soo 2010).

The data showed that the treatment with LAF significantly protects gastric mucosa from damage induced by pyloric ligature at all doses and without a dose-dependent relationship. This is consistent with the findings of Tamashiro Filho et al. (2012), who evaluated the antiulcerogenic activity of methanolic stem bark extract from *L. pacari* (MELP) at doses of 12.5, 50, and 200 mg/kg, using induction models of acute and chronic ulcers. Even though a different part of the plant was analyzed, this extract significantly reduced gastric lesions. Tamashiro Filho et al. identified pyrrogalic tannins, saponins, steroids, triterpenoids, and simple phenols like ellagic acid in MELP.

Table 1 Effects of LAF (75, 150, and 300 mg/kg) and ranitidine (50 mg/kg), administered intraduodenally, on the biochemical parameters of gastric juice obtained from pyloric ligature in mice

Groups	Treatment (i.d.)	Dose (mg/kg)	Volume (ml)	pH	Gastric acidity (mEq[H^+]/l/4 h)
Control	Vehicle	–	2.49 ± 0.08	2.90 ± 0.16	5.36 ± 0.54
	Ranitidine	50	2.25 ± 0.05*	4.76 ± 0.43***	1.29 ± 0.24***
		75	2.25 ± 0.05*	5.13 ± 0.40*	1.86 ± 0.31***
LAF	LAF	150	2.21 ± 0.06*	4.61 ± 0.31**	1.53 ± 0.23***
		300	2.10 ± 0.04***	4.34 ± 0.20**	1.29 ± 0.24***

Results are expressed as mean ± S.E.M. for eight mice. One-way ANOVA followed by Student-Newman-Keuls as post hoc test

*$p \leq 0.05$

**$p \leq 0.01$

***$p \leq 0.001$ compared to the control group

Table 2 Effects of punicalagin (120 mg/kg) and ranitidine (50 mg/kg), administered intraduodenally, on the biochemical parameters of gastric juice obtained from pyloric ligature in mice

Groups	Treatment (i.d.)	Dose (mg/kg)	Volume (ml)	pH	Gastric acidity (mEq[H^+]/l/4 h)
Control	Vehicle	–	2.46 ± 0.07	3.65 ± 0.21	5.36 ± 0.51
	Ranitidine	50	2.17 ± 0.06*	4.47 ± 0.29*	2.52 ± 0.28***
Punicalagin	Punicalagin	120	2.22 ± 0.06*	4.81 ± 0.15*	3.11 ± 0.38**

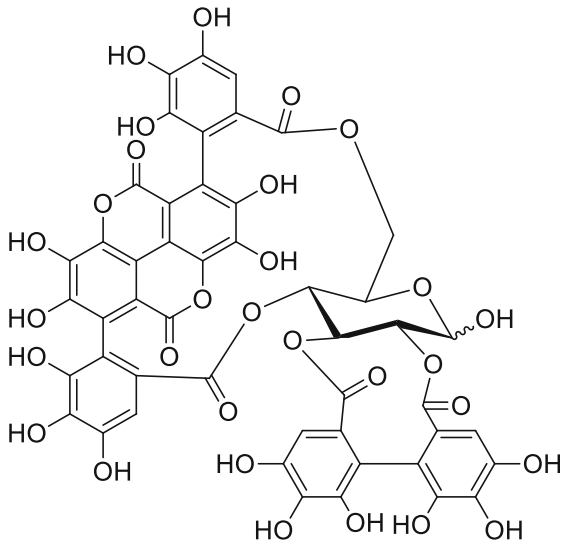
Results are expressed as mean ± S.E.M. for eight mice. One-way ANOVA followed by Student-Newman-Keuls as post hoc test

*$p \leq 0.05$

**$p \leq 0.01$

***$p \leq 0.001$ compared to the control group

Punicalagins were not identified by those authors, which is an important differential in relation to this research study.



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Following, the gastroprotective activity of punicalagin (**1**) was evaluated. As there was no significant difference between the intermediate and highest doses in the pyloric ligature model, we chose to work only with the intermediate dose (150 mg/kg) of LAF, which also met the recommendation of the Ethics Committee for Animal Use to reduce the number of animals tested. Thus, the dose of punicalagin (120 mg/kg) was based on the yield of the extraction process. Its intraduodenal administration reduced the volume of gastric juice by 9.76% and total acidity by 41.98%, while gastric pH increased by 22.47% compared to the control group (Table 2). Intraduodenal administration was used in the pyloric ligation model and supports the idea that the effect of the extract or active principle is due to a systemic action (absorption, distribution, site of action), not to a topical one as may occur in intragastric administration, when the formation of a film on the mucosa or buffering effect could be responsible for pH change and mucosal protection. These data suggest that punicalagin may be responsible or at least contribute to the antisecretory activity of *L. pacari* and thus lead to gastric protection.

Since the LAF and punicalagin (**1**) showed antisecretory activity in the gastric secretion model, we proceeded with assessing antiulcerogenic activity via an indomethacin-induced gastric lesion model. Oral treatment of LAF 150 mg/kg showed a reduction of 29.8% in lesion index when compared to the control group. Ranitidine 50 mg/kg (positive control) reduced lesions by 55.2% in relation to the control group. According to Tamashiro Filho et al. (2012), MELP showed effective gastroprotection against indomethacin, ethanol, and stress-induced cold. Punicalagin, the largest compound in LAF, significantly reduced lesions caused by indomethacin by 49.4%, which suggests that it has a gastroprotective effect (Table 3).

Treatments with LAF and punicalagin were capable of significantly reducing acute gastric lesions. The hypothesis that the mechanism of antiulcerogenic action shown by LAF and punicalagin is only due to the formation of a protective layer on the mucosa can be ruled out; since in

Table 3 Effect of treatment with vehicle (distilled water 10 ml/kg, *p.o.*), LAF (150 mg/kg, *p.o.*), punicalagin isolated from *Lafoensia pacari* (120 mg/kg, *p.o.*), and ranitidine (50 mg/kg, *p.o.*) on indomethacin-induced gastric lesions (30 mg/kg, *s.c.*)

Treatment (*p.o.*)	Dose (mg/kg)	Index lesion	Inhibition (%)
Vehicle	–	11.4 ± 1.50	–
Ranitidine	50	5.10 ± 0.35 **	55,2
LAF	150	8.0 ± 1.08 *	29,8
Punicalagin	120	5.66 ± 0.28 **	49,4

Results are expressed as mean ± S.E.M. for eight mice. One-way ANOVA followed by Student-Newman-Keuls as post hoc test

*$p \leq 0.05$

**$p \leq 0.01$

***$p \leq 0.001$ compared to the control group

the pyloric ligature model, the extract only demonstrates its activity after its absorption and systemic distribution. These results indicate that LAF and punicalagin exert their antiulcerogenic effect as a result of their acid antisecretory activity. Other compounds already described (Tamashiro Filho et al. 2012) may also contribute to this activity. Further research is needed to elucidate the pharmacological mechanism involved.

Authors' Contributions BAC: acquisition, analysis, and interpretation of data. JLRM: acquisition of pharmacological data and statistical expertise. EAC: design of pharmacological tests and provision of materials. SCS: isolation of punicalagin. JRP: provision of materials. MTFB: study design, provision of materials, securing of funding, and writing of article.

Funding Information The authors are grateful to Capes and CNPQ for the financial support provided.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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