

Gastroprotective effect of *Lagenocarpus rigidus* (Kunth) Ness (tirica-do-nativo) leaf extract against indomethacin-induced gastric injury

Efecto gastroprotector de *Lagenocarpus rigidus* (Kunth) Ness (tirica-do-Nativo) extracto de hoja contra las lesiones gástricas inducidas por indometacina

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ABSTRACT

Introduction: species of the family Cyperaceae are commonly used by the population to treat gastric disorders. However, there are a few ethnopharmacological studies about this family *Lagenocarpus rigidus* (Kunth) Ness, Cyperaceae, is one of the most widespread swamp species.

Objective: evaluate the gastric activity of *L. rigidus* and its chemical characterization.

Methods: ethanolic extract of *L. rigidus* (ELR) leaves prepared by percolation was subjected to total polyphenol and flavonoid quantification, as well as HPLC quantification of some flavonoids. Angiotensin converting enzyme (ACE) inhibition was determined by colorimetric assays. The gastric effects of ELR were tested in male Wistar rats (n = 6 each group) treated with different doses (600, 60 and 6 mg/kg i.p.) ELR. Gastric lesions were induced by administration of indomethacin (30 mg/kg s.c.). The number of ulcers and the index of mucosal damage (IMD) were determined taking into account the color, edema and bleeding of gastric lesions, the number of petechiae, and the number and size of the ulcers. Statistical analysis of data was performed with one-way ANOVA followed by Tukey's test; significance was p < 0.05.

Results: ELR inhibited the ACE ($68.5 \pm 18.1\%$) at a concentration of 100 mg/mL. Oral administration of ELR (6, 60 and 600 mg/kg) showed protective activity against indomethacin-induced gastric injury. Total polyphenols in ELR were 157.7 ± 5.8 mg pirogalol/mg equivalent flavonoids and 66.9 ± 3.1 μ g equivalent quercetin/mg.

Conclusion: *L. rigidus* protects against acute gastric damage induced by indomethacin in an independent dose manner.

Key words: Cyperaceae, anti-ulcerogenic, flavonoids, *L. rigidus*, phenolic compound.

RESUMEN

Introducción: las especies de la familia Cyperaceae popularmente se utilizan para tratar trastornos gástricos. Sin embargo, hay pocos estudios etnofarmacológicos sobre esta familia. *Lagenocarpus rigidus* (Kunth) Ness, Cyperaceae, es una de las especies más grandes de población en pantano.

Objetivo: evaluar la actividad gástrica de *L. rigidus*, junto a su caracterización química.

Métodos: el extracto etanólico de hojas de *L. rigidus* (ELR), preparado por percolación fue objeto de cuantificación de polifenoles y flavonoides totales, cuantificación por HPLC de algunos flavonoides. La inhibición de la enzima convertidora de angiotensina (ECA) se realizó por ensayos colorimétricos. Los efectos gástricos de ELR se llevó a cabo en ratas Wistar macho ($n = 6$, cada grupo), el tratamiento con diferentes dosis (600, 60 y 6 mg/kg ip) de ELR. Las lesiones gástricas se indujeron mediante la administración de indometacina (30 mg/kg sc). El número de úlceras y signos de puntuación del índice de lesión de las mucosas (IMD) se evaluó teniendo en cuenta el color, edema y hemorragia de las lesiones gástricas, el número de petequias, y el número y tamaño de las úlceras. El análisis estadístico de los datos se realizó mediante ANOVA 1 vía, seguido por el test de Tukey y significación fue de $p < 0,05$.

Resultados: ELR inhiben la ACE ($68,5 \pm 18,1 \%$) a una concentración de 100 mg/mL. La administración oral de ELR (6, 60 y 600 mg/kg) mostró un efecto protector gástrico contra inducido por indometacina. Los polifenoles totales de ELR fue $157,7 \pm 5,8$ mg equivalente de pirogalol/mg de flavonoides y $66,9 \pm 3,1$ μ g equivalentes de quercetina/mg.

Conclusiones: *L. rigidus* protege contra el daño gástrico agudo inducido por la indometacina en una organización independiente de la dosis.

Palabras clave: Cyperaceae, anti-ulcerogénicos, flavonoides, *L. rigidus*, compuesto fenólico.

INTRODUCTION

A large variety of plant species are popularly used to treat gastric disorders, such as *Euterpeedulis* Mart. (Palmito), *Baccharis trimera* DC (Carqueja), *Macrosiphonia velame* (St. Hil.) M. Arg (Velame Branco), *Qualea grandiflora* Mart. (Pau Terra),

Speranthera adoratissima St. Hil. (Manacá) and some Cyperaceae species: *Cyperus esculentus* L. (Junça) and *C. rotundus* (Tiririca).¹⁻⁴

The species of Cyperaceae are endemic and well represented in the five geopolitical regions of Brazil.⁵ However, there are a few ethnopharmacological studies using this family as source. The lack of taxonomic studies for the family Cyperaceae also contributes to the low number of studies.⁶ *Lagenocarpus rigidus* (Kunth) Ness, Cyperaceae, is distributed in herbaceous swamp and its chemical composition and biological activity are not well known.⁶

Although the renin-angiotensin system (RAS) operates classically in the cardiovascular system, recent studies showed that inhibitors of angiotensin converting enzyme (I-ACE) seems to have gastroprotective effect by enhancing level of endogenous prostaglandins. Besides, an application of I-ACE reduces angiotensin II formation and activates renin-kallicrein-kinin system resulting in NO formation that is in its turn an important component of reparative process of mucous of gastrointestinal tract.⁷ Angiotensin Converting Enzyme (ECA, E.C. 3.4.15.1), an important enzyme of the RAS, plays a substantial role in regulating the homeostatic mechanism of mammals by modulating the RAS.

To our knowledge, there were no scientific studies in literature on *L. rigidus* extract regarding its gastrointestinal activity. Thus, the aim of this study is evaluated the in vitro ACE inhibition and gastroprotetor effects of the ethanolic extract of *L. rigidus*.

METHODS

Plant material

Leaves of *L. rigidus* were collected from the Paulo César Vinha State Park, Guarapari, Espírito Santo, Brazil, in July 2010 (Protocol permission number 629/09, IEMA). A voucher specimen has been deposited at the Herbarium of the Federal University of Espírito Santo (VIES 26084). After drying at 40 °C for 48h, the plant material was grained (200.0 g) and submitted to ultrasound maceration (15min, 40 Hz) at room temperature for 7 days for preparation of ethanolic extract. This extract was evaporated at low pressure and 40°C, to yield 10.7 g of crude ethanolic extract. Aliquots of the extract were dissolved in saline, to be used for the ACE inhibition assay and the analysis of the hypotensive effect.

HPLC Analysis

A Waters 1515 system (USA) is composed of a binary pump, UV/VIS detector (model 2489), and manual a sampler and Breeze software were used for data processing. The analyses were performed on aXBridge TM C-18 column (150 x 4.6 mm i.d., 3.5 µm, Waters) in combination with XBridge TM C-18 guard column (20 x 4.6 mm i.d., 3.5 µm, Waters), at room temperature and flow rate of 0.80 mL/min. UV detection was performed at 254 nm and 365 nm. An isocratic elution of MeOH: H₂O (9:1) was employed. Solvents used were of HPLC grade (Merck, Germany), water was ultrapure (ELGA 18.2 Ω) and degassed by sonication before use. Standards and samples were dissolved in MeOH to final concentrations of 2 and 10 mg/mL, respectively, for standards (rutin, apigenin) and extract of leaves of *L. rigidus* (ELR). After centrifugation at 8.400g for 5 min, the sample

solutions (20 μ L) were manually injected into the apparatus. Standard stock solution of rutin was prepared by dissolving 10 mg of rutin in methanol, yielding 10 ml of a concentration 1.00 mg/mL. Series of dilutions were prepared to yield 10 mL of standard solutions containing 1.95, 3.90, 7.80, 15.6, 31.3, 62.5, 125.0, and 250 μ g/mL of rutin, apigenin and quercetin. All samples were analyzed in triplicate.

Spectrophotometric quantification of total polyphenols and flavonoids and non-adsorbed

The determination of polyphenols and flavonoid were performed as previously described⁸ Quantification of total polyphenols was performed by reading the absorbance at 715 nm of the extract solution of the product with the Folin-Denis. And the determination of tannins by the difference in this quantitation by absorbance performed with the extract solution added to the PVPP. The result was calculated from the equation of the linear curves with pyrogallol.

Quantitation of flavonoids was performed by reading the absorbance at 425 nm of the complex flavonoid-aluminum chloride. The result was expressed as a percentage of total flavonoid quercetin calculated as anhydrous, obtained from the equation of the line of standard curves.

Animals

Male Wistar rats weighing 300 to 350 g were obtained from the breeding facility of the University of Vila Velha. They were housed at 22 ± 3 ° C under a 12 h light/12h dark cycle and had free access to standard pellet diet (ration Probiotério, Windmill Primor SA) and tap water. The animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and were approved by the Ethics Committee of the University of Vila Velha (protocol N^o150/2011 CEUA/UVV).

Gastric damage induced by indomethacin

Fasted rats were given 10 % glucose water for 24 hours. After the groups were treated with the root extracts (6, 60 and 600 mg/kg, body wt., i.p.). After 1 h, gastric lesions were induced by indomethacin (30 mg/kg, body wt., s.c.). The animals were killed under ether anesthesia (thiopental, 100 mg/kg) after 3h after indomethacin injection. The stomach was dissected out, and the mucosal side was gently washed to remove remaining food and inspected under magnification to determine the area of gastric hemorrhagic ulcers (mm²). The photographs of the stomach were digitized and converted to binary images through gray scale imaging, using the National Institute of Health (NIH) image-J software.

ACE activity measurement

The effect of ELR (100 μ g/mL) against angiotensin converting enzyme in vitro was determined by measuring Gly-Gly (glycyl-glycine) cleavage product of Hip-Gly-Gly by ACE. The assay was performed as previously described.⁹

Statistical analysis

The results are presented as the mean \pm S.E.M. of six animals per group. Statistical analysis was carried out using the one way analysis of variance (ANOVA) followed

by Tukey post hoc test for multiple comparisons. P-values less than 0.05 ($p < 0.05$) were considered as indicative of statistical significance.

RESULTS

ELR showed a polyphenols content of 157.7 ± 5.8 mg equivalent of pirogalol/g of dry extract and flavonoids content of 66.9 ± 3.1 mg/g of dry extract, quantified in quercetin. Apigenin and quercetin were not identified by HPLC while rutin was detected, but below the limit of detection.

ELR inhibit in vitro the ACE in 68.5 ± 18.1 %. The oral administration of ELR (6, 60 and 600 mg/kg) exhibited a protective effect against indomethacin-induced gastric lesions in comparison to the vehicle group. The inhibition percentages were significantly for the respective doses employed, 50.9, 37.0, and 36.3 % (Table).

Table. Effect of *L. rigidus* extract (ELR) on gastric damage induced by indomethacin in rats

Treatment	Dose	pH	Gastric Lesion Area (mm ²)
Vehicle	-	3.0±1.0	22.6±2.3
LR	6 mg/kg	4.5±1.0	11.2±1.8*
	60 mg/kg	3.5±1.2	14.2±0.8*
	600 mg/kg	4.8±0.8	14.4±1.8*

Data are mean ± S.E.M. of 6 animals in each group. * $p < 0.05$ vs. vehicle (ANOVA followed by Tukey's test)

DISCUSSION

The results of this study show that ELR affords a pronounced gastroprotection against indomethacin (Table). The mechanisms of NSAID-induced gastric injury are not well understood, it is widely accepted that both cyclooxygenase dependent and -independent mechanisms are involved, such as the stimulation of gastric acid production, inflammatory cells infiltration, cytokines, mucosal blood flow and free radicals production.¹⁰ In this context, the gastroprotection may be justified for ACE inhibition and by presence of antioxidants in the ELR extract.

There are other factors associated with the development of gastric disorders, among of them the indiscriminate use of anti-inflammatory, unbalanced diet, smoking and alcohol are habits that may cause the imbalance between acid secretion and the production of substances for the mucous protection as mucous secretion, bicarbonate and prostaglandins.¹¹

The significant role of angiotensin-converting enzyme (ACE) in the pathogenesis of gastric damage and the beneficial effects of their inhibitors have been demonstrated in many investigations.^{7,11,12} ACE inhibitors may improve the gastroprotection by several mechanisms including: lowering of angiotensin II and an increase in bradykinin with consequently enhancement of endogenous prostaglandins and consequently increment of mucosal blood flow and secretion of mucus and bicarbonate and also reduction gastric acid secretion. Besides, the ACE inhibition can improve the nitric oxide formation that is in its turn an important component of reparative process of mucous of gastrointestinal tract also acting as a scavenger of free radicals.^{7,13} The role of reactive oxygen species in the

pathogenesis of indomethacin have been demonstrated in a recent studies being reported positive effects with antioxidants.^{7,11-13} Thus, the gastroprotective effect of ELR can be justified by a potentiation of the intrinsic antioxidant properties and by ACE inhibition.

Some studies have been conducted involving the species of the genus *Maytenus*, commonly called *Espinheirasanta*, one of the most known and popularly used for gastric disorders and various authors have related to gastric protection with the elevation of gastric pH and the presence of flavonoids, polyphenols and triterpenoids.¹⁴⁻¹⁷

In conclusion, *L. rigidus* protects against acute gastric damage induced by indomethacin in an independent dose-manner.

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