Journal of Ethnopharmacology 175 (2015) 75–85

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Evidence of gastric ulcer healing activity of *Maytenus robusta* Reissek: In vitro and in vivo studies

Luisa Mota da Silva [a,*], Thaise Boeing [a], Lincon Bordignon Somensi [a], Benhur Judah Cury [a], Viviane Miranda Bispo Steimbach [a], Alessandro Conrado de Oliveira Silveria [b], Rivaldo Niero [a], Valdir Cechinel Filho [a], José Roberto Santin [a], Sérgio Faloni de Andrade [a]

[a] Programa de Pós-Graduação em Ciência s Farmacêuticas (PPGCF), Universidade do Vale do Itajaí (UNIVALI), Itajaí, SC, Brazil
[b] Departamento de Ciências Farmacêuticas, Universidade Regional de Blumenau (FURB), Blumenau, SC, Brazil

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
31 August 2015
Accepted 5 September 2015
Available online 10 September 2015

Keywords:
M. robusta
Healing ulcer
Gastroprotection
Antiulcer
Fibroblast
Antioxidant

ABSTRACT

Ethnopharmacological relevance: *Maytenus robusta* Reissek (Celastraceae) is traditionally used in Brazilian folk medicine to treat gastric ulcer, as a substitute for *M. ilicifolia*, which is almost extinct. The gastroprotective properties of *M. robusta* were demonstrated previously using only preventive approaches, such as acute gastric ulcer models. However, the healing effect of *M. robusta* in gastric ulcers remains unclear.

Aim of the study: The current study was carried out to investigate the healing effectiveness of *M. robusta* hydroalcoholic extract (HEMR) from aerial parts in the acetic acid-induced chronic ulcer model and to determine its effect on cell proliferation, scavenging free radicals, and inflammatory and oxidative damage.

Material and methods: To evaluate the healing properties of HEMR in vivo, chronic gastric ulcer was induced in rats by 80% acid acetic. Next, different groups of animals ($n=6$) were treated orally with vehicle (water plus 1% tween, 1 ml/kg), omeprazole (20 mg/kg), or HEMR (1–10 mg/kg), twice daily for 7 days. At the end of the treatment, the total ulcer area (mm^2) was measured and a sample of gastric tissue was taken for histological and histochemical analysis. Evaluation of GSH and LOOH levels, GST, SOD, CAT and MPO activity was also performed at the site of the lesion. In parallel, radical scavenging activity, cytoprotective effect, and cell proliferation activity in fibroblasts (L929 cells) were determined by in vitro trials. The antisecretory properties were evaluated using the pylorus ligature model in rats, and the anti-*Helicobacter pylori* activity was determined in vitro. Acute toxicity was evaluated by relative organ weight and biochemical parameters in serum. The prokinetic properties were also evaluated in mice.

Results: Oral administration of HEMR (10 mg/kg) reduced the gastric ulcer area by 53%, compared to the vehicle group (120.0 ± 8.3 mm^2), the regeneration of gastric mucosa was evidenced in histological analysis. Moreover, HEMR treatment increased gastric mucin content and reduced oxidative stress and inflammatory parameters at the site of the ulcer. In vitro, HEMR (1–1000 μg/ml) was able to scavenge free radical DPPH and promote cytoprotection against H_2O_2 in fibroblasts at 0.1–100 μg/ml. Moreover, HEMR healing properties also were confirmed by enhancement of proliferation and coverage of scratched wounds in fibroblast monolayer. However, HEMR (10 mg/kg) by the intraduodenal route did not promote changes in volume, pH, total acidity or pepsin activity in the pylorus ligature model, and HEMR up to 2000 μg/ml also did not present considerable activity against *H. pylori*. In relation to gastrointestinal motility, HEMR (10 mg/kg, p.o) did not provoke alterations. It is also important to mention that oral administration of HEMR did not produce any sign of acute toxicity in animals.

Abbreviations: ALFAC, 85% alcohol, 10% formaldehyde and 5% acetic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHT, butylated hydroxytoluene; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DMEM, Dulbecco's modified eagle medium; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMSO, N,N-dimetyl sulfoxide; DTNB, 5,5-dithio-bis-(2-nitrobenzoic acid); ECL, enterochromaffin-like cells; EDTA, ethylenediaminetetraacetic; FBS, fetal bovine serum; FOX2, ferrous oxidation-xylenol orange; GE, gastric emptying; GSH, glutathione reduced; GST, glutathione-S-transferase; HEMR, hydroalcoholic extract of *Maytenus robusta*; HTAB, hexadecyltrimethylammonium bromide; IT, intestinal transit; LOOH, lipoperoxide; MIC, minimum inhibitory concentration; MPO, myeloperoxidase; MTT, 3,4,5-dimethylthiazolyl-2-2, 5-diphenyltetrazolium bromide; Ome, omeprazole; PAS, periodic acid of Schiff; PBS, phosphate buffered saline; ROS, reactive oxygen species; SOD, superoxide dismutase; TMB, 3,3′,5,5′-tetramethylbenzidine; Veh, vehicle

* Correspondence to: Programa de Pós-Graduação em Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade do Vale do Itajaí, RuaUruguai, 458, Centro, 88302-202 Itajaí, SC, Brazil.
E-mail address: lu.isamota@hotmail.com (L. Mota da Silva).

http://dx.doi.org/10.1016/j.jep.2015.09.006
0378-8741/

Conclusions: The data here obtained show that *M. robusta* has evident ulcer healing potential, mainly through the strengthening of protective factors of gastric mucosa, such as mucus layer, antioxidant defenses and cell proliferation. Taking into account the advantages of cultivation and harvesting of *M. robusta* compared to *M. ilicifolia*, and the evidence presented here, it is plausible to conclude that hydroalcoholic extract obtained from aerial parts of *M. robusta* is an interesting source for the development of a phytotherapeutic formulation to treat gastric ulcer.

1. Introduction

Maytenus robusta Reissek (*cafezinho do mato* or *coração de bugre)* is used in traditional folk medicine to gastric ulcer (Balbach, 1980; Cunha, 2003, Niero et al., 2011). Similarly to other *Maytenus* sp, the traditional use of *M. robusta* consist on the intake of its decoction or infusion, prepared with dry and pulverized leaves, up to four times per day (Teske and Trentini, 2001). Based on this, our research group has attempted to find a scientific basis for the development of an alternative phytotherapeutic formulation prepared from *M. robusta*, given that this species is very well adapted to the South of Brazil (Niero et al., 2001). In addition to its traditional use, harvesting of *M. robusta* is favored because this species is a medium-sized tree with a dense crown, and its leaves have no thorns, unlike those of *M. ilicifolia*. It is therefore suitable for sustainable cultivation in order to obtain standardized phytotherapic preparations. Previously, the gastroprotective properties of *M. robusta* were demonstrated by Andrade et al. (2007, 2008) using only preventive approaches, such as acute gastric ulcer models. However, the healing effect of *M. robusta* in gastric ulcer remains unclear, and it is important to emphasize that confirmation of gastroprotective activity is not necessarily an indication of healing activity on existing gastric ulcers (Vasconcelos et al., 2008; Périco et al., 2015).

Gastric ulcer is a lesion characterized by necrosis, neutrophil infiltration, reduction in blood flow, increased oxidative stress, and inflammation (da Silva et al., 2013). It occurs due to an imbalance between aggressive injurious factors (pepsin, HCl) and defensive mucosa-protective factors (prostaglandins, mucus and bicarbonate barrier and adequate blood flow) (Tygat, 2011). Besides, stress (Levenstein et al., 2014), smoking, nutritional deficiencies (Duggan and Duggan, 2006), prolonged ingestion of nonsteroidal-anti-inflammatory drugs (NSAIDs) (Belaiche et al., 2002), and *Helicobacter pylori* infection (Beltrán-Anaya et al., 2014) are all relevant etiological factors for the development of gastric ulcer.

Currently the treatment of gastric ulcer is based on the inhibition of gastric acid secretion by H2-antagonists, such as ranitidine; or proton-pump inhibitors, such as omeprazole (De Vault and Talley, 2009). However, the main problem is that despite a healing rate, ulcers treated with H2-antagonists and proton pump inhibitors can present recurrence within 1 year after the end of treatment (Kangwan et al., 2014). This is mainly due to neutrophil accumulation and ROS production, resulting in an incomplete healing process (Tarnawski et al., 1990). Furthermore, side effects such as osteoporosis (Panday et al., 2014), hypergastrinemia and hyperplasia of enterochromaffin-like cells (ECL) (Sheen and Triadafilopoulos, 2011) are common in the prolonged therapy with antisecretory drugs.

In view of the above, the search for new antiulcer treatments is essential, focusing mainly on the search for agents that promote effective healing of gastric ulcer. The present study was carried out using the acetic acid-induced chronic gastric ulcer as an experimental model. This model is highly similar to human ulcers in terms of pathological aspects and healing process. Consequently, this model is an appropriate tool to evaluate the gastric ulcer healing process of different substances in the gastric tissue (Okabe and Amagase, 2005). The aim of the study was to investigate the effectiveness of healing activity of *Maytenus robusta* hydroalcoholic extract from aerial parts in the acetic acid-induced chronic ulcer model, and to determine its effect on cell proliferation, scavenging free radicals, and inflammatory and oxidative damage regulation.

2. Materials and methods

2.1. Drugs and reagents

The following substances were used: Bovine serum albumin, 2,2-diphenyl-1-picrylhydrazyl, 5,5′-dithiobis (2-nitrobenzoic acid), glutathione, MTT, omeprazole, pyrogallol, xylenol orange (all from Sigma, St. Louis, USA), absolute ethanol, acetic acid, ascorbic acid, hydrochloric acid, diethyl ether, formaldehyde, hydrogen peroxide, magnesium chloride, methanol, sodium acetate, sodium carbonate, sucrose, trichloroacetic acid (Vetec, Rio de Janeiro, RJ, Brazil), dimetilsufoxide and N,N-dimethylformamide (DMSO, Synth, Diadema, SP, Brazil), Dulbecco's Modified Eagle Medium (DMEM, Vitrocell, Campinas, SP, Brazil), and fetal bovine serum (FBS, Gibco).

2.2. Plant material and preparation of the extract

Maytenus robusta was collected at the Morro do Baú Ecological Park, Ilhota, Santa Catarina, and identified by Dr. Ademir Reis (Department of Botany, Universidade Federal de Santa Catarina). A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí-SC), and identified as V.C. Filho 016.

Briefly, air-dried and ground leaves (500 g) were macerated in 70% aqueous ethanol (v/v) at room temperature for 7 days, as described previously (Andrade et al., 2007). The macerated leaves were then filtered, and the solvent was eliminated under reduced pressure. The dried material yielded 38.0 g (7.6%) of the crude hydroalcoholic extract of *M. robusta* (HEMR). The phytochemical profile of the extract used in this work was studied previously, and the main compounds present were identified as pentacyclic triterpenes (Niero et al., 2001, 2006; Andrade et al., 2008).

2.3. Animals

The ulcer experiments were conducted with female Wistar rats (2–3 months, 180–200 g). Swiss female mice (8 weeks, 20–25 g) were used in the gastric emptying and intestinal experiments. The animals were provided by the Universidade do Vale do Itajaí colony and maintained under standard laboratory conditions (12 h light/dark cycle, temperature of 22 ± 2 °C) with free access to standard pellet food (Biobase, Águas Frias/SC, Brazil) and water. The animals were deprived of food (12 h) prior to the experiments. All the experiments were performed after approval by the Institutional Animal Ethics Committee of Universidade do Vale do