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Gastroprotective xanthones isolated from *Garcinia achachairu*: Study on mucosal defensive factors and H^+, K^+-ATPase activity

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ABSTRACT

The present study was designed to investigate the gastroprotective effect of xanthones 7-preniljacareubin (PJB), 1,3,5,6-tetrahydroxy xanthone (THX), 3-demethyl-2-geranyl-4-prenylbellidypholine (DGP) and 1,5,8-trihydroxy-4′, 5′-dimethyl-2H-pyrane (2,3:3,2)-4-(3-methylbut-2-enyl) xanthone (TDP) isolated of branches from *G. achachairu*. Their structures were identified through the spectroscopic analysis in comparison with previously reported data. The xanthones were tested at dose of 10 mg/kg against ethanol 60%/HCl 0.3 N-induced gastric ulcer in female swiss mice. The xanthones PJB, THX, DGP and TDP exhibit gastroprotective effect after intraperitoneal treatment, but only the first two displayed anti-ulcer activity after oral administration. Both PJB and THX augmented the antioxidative capacity of tissue by an increase in glutathione levels, as well as were able to prevent an increase in myeloperoxidase activity and tumor necrosis factor level. On the other hand, only THX showed an *in vitro* free radical scavenger activity, and only PJB avoided mucus depletion on gastric mucosa, which was not associated with an increase in mucin production at glandular level. In addition, PJB and THX inhibited the *in vitro* H^+K^+-ATPase activity at similar range as omeprazole. Together, these results demonstrate the anti-ulcer efficacy of xanthones isolated from *G. achachairu*, which can contribute for future directions in the development of effective strategies to improve gastric diseases.

1. Introduction

Gastric ulcer is a common disease of gastrointestinal tract with a high clinical incidence, which may result in severe superior gastrointestinal bleeding [1]. It involves an imbalance between defensive and aggressive factors of gastric mucosa that can be disrupted by diet, stress, excessive alcohol intake, free radicals, nonsteroidal anti-inflammatory drugs and *Helicobacter pylori* infection [2,3].

Nowadays, the options available to treat gastric ulcers are grounded on the use of proton pump inhibitors and H_2 receptor antagonists [4]. However, there are several gaps in the treatment because acid suppression or eradication of *H. pylori* are insufficient for completely healing process, and thus increase the probability of ulcer recurrence after the treatment. Thus, the continued use of antisecretory drugs becomes indispensable, which may cause a diversity of side effects such as increased susceptibility to pneumonia, bone fractures, thrombocytopenia, vitamin B12 and iron deficiency, hypergastrinemia and gastric cancer [5–8].

These problems have led to the recognition that gastric ulcer's treatment requires better and more specific agents. In the last years several studies have shown a wide of variety of naturally compounds with potential gastroprotective effect, including alkaloids, saponins, tannins, triterpenoids and Xanthones [9].

Xanthones are an important class of compounds by possessing several biological properties, which are associated with the

Abbreviations: AA, ascorbic acid; CBX, Carbenoxolone; CMEB, crude methanolic extract of branches; DGP, 3-demethyl-2-geranyl-4-prenylbellidypholine; DPPH, 2,2-diphenyl-1- picrylhydrazyl; GSH, reduced glutathione; H/E, hematoxylin and eosin; MPO, myeloperoxidase; NV, naive; OME, omeprazol; OUA, ouabain; PJB, 7-preniljacareubin; ROS, reactive oxygen species; TDP, 1,5,8-trihydroxy-4′, 5′-dimethyl-2H-pyrane (2,3:3,2)-4-(3-methylbut-2-enyl) xanthone; THX, 1,3,5,6-tetrahydroxy xanthone; TLC, thin-layer chromatography; TNF, tumor necrosis factor; VEH, vehicle.

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structure, and related with the position, number and type of substituents attached to the basic nucleus of the structure [10]. Some of the main pharmacological effects reported in the literature for natural or synthetic derivatives of xanthones are antimicrobial, anti-inflammatory, antioxidant, neuroprotective, cardioprotective and gastroprotective effects [11,12].

In this context, some species of the genus *Garcinia* are of great interest by the pharmaceutical industry, given the presence of chemicals such as benzophenones and xanthones with relevant biological effects [13–16]. One of the species that deserve attention is *Garcinia achachairu* Rusby (Clusiaceae), used in folk medicine to treat rheumatism, inflammation, pain and gastric disorders [17,18], which was target of the present study.

2. Materials and methods

2.1. Drugs

The following substances were used: Bovine serum albumin, 2,2-diphenyl-1-picrylhydrazyl, 5,5′-dithiobis (2-nitrobenzoic acid), carbenoxolone, glutathione reduced form, omeprazole, ouabaine, xylenol orange (all from Sigma, St. Louis, USA). Absolute ethanol, ascorbic acid, hydrochloric acid, diethyl ether, formaldehyde, hydrogen peroxide, magnesium chloride, methanol, hexane, acetone, chloroform, dichloromethane, ethyl acetate, sucrose, sodium acetate, trichloroacetic acid (Vetec, Rio de Janeiro, RJ, Brazil), *N*,*N*-dimethylformamide (Synth, Diadema, SP, Brazil), alcian blue solution (Merck, Billerica, MA, USA), Mouse TNF ELISA kit (BD Biosciences, Franklin Lakes, NJ, USA).

2.2. Plant material

The branches of *G. achachairu* were collected in Camboriú city, state of Santa Catarina, Brazil, at August 2014 (S27°04′24.7″, W48°42′45.1″), and identified by Dr. Oscar B. Iza (University of Vale of Itajai, UNIVALI, Itajai, SC, Brazil). A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajai-SC) under number HBR 52,637.

2.3. Extraction and isolation

Air-dried powdered branches (1.78 kg) of *G. achachairu* were exhaustively extracted with methanol (3 L × 2) at room temperature for seven days. The macerated was filtered and concentrated under reduced pressure in a rotatory evaporator, yield a dark-brown residue 120 g named of crude methanolic extract of branches (CMEB). The extract was suspended in a methanol:water (60:40) mixture (500 ml) and subjected to liquid-liquid partition using solvents of increasing polarity: dichloromethane and ethyl acetate, respectively (300 ml; 4 × each).

Part of the soluble dichloromethane fraction (7.0 g) of CMEB was subjected to column chromatography (0.063–0.20 mm, 72.0 g, 3.0 × 50 cm, Merck) over silica-gel and eluted with hexane: acetone (100:0 → 0:100) in increasing order of polarity to afford 100 fractions, which were combined based on TLC profiles. The fraction 49–56 (34.96 mg), presented as a yellow crystal, was identified as 7-preniljacareubin (**PJB**) by nuclear magnetic resonance (NMR), ^{13}C NMR spectrum using polarization transfer (Dept), heteronuclear multiple bond correlation (HMBC) date in comparison with those reported previously by Delle Monache et al. [19].

Part of the soluble ethyl acetate fraction (3.3 g) of CMEB was subjected to column chromatography (0.063–0.20 mm, 108.0 g, 3.0 × 50 cm, Merck) over silica-gel and eluted with chloroform: methanol (100:0 → 0:100) in increasing order of polarity to afford 90 fractions, which were combined based on TLC profiles. The fraction 43–50 (81.6 mg), presented as a yellow crystal, was identified as 1,3,5,6-tetrahydroxy xanthone (**THX**) by NMR, Dept, HMBC, date in comparison with those reported previously by Guat-Lee et al. [20]. Moreover, the compounds 3-demethyl-2-geranyl-4-prenylbellidypholine (**DGP**) and 1,5,8-trihydroxy-4′, 5′-dimethyl-2H-pyrane (2,3:3,2)-4-(3-methylbut-2-enyl) xanthone (**TDP**), were obtained as previously described by Mariano et al., [21].

2.4. Animals

Female swiss mice (25–30 g) were used in these experiments. One adult albino rabbit (*Oryctolagus cuniculus*) weighing ~2 kg was used for gastric H^+,K^+-ATPase activity determination. The animals were provided by UNIVALI and kept in a temperature- and light-controlled room (22 ± 2 °C; 12-h light/dark cycle), with free access to water and food. All experimental procedures adopted in this study were approved by the Institutional Ethics Committee of UNIVALI (Process 034/15).

2.5. Ethanol/HCl induced ulcer

The method employed was described by Morimoto et al. [22], with a few modifications. The animals were divided in groups of six animals each pretreated with vehicle (VEH: water plus 1% tween, 1 ml/kg, p.o.), carbenoxolone (CBX: 200 mg/kg p.o., a positive control used in clinic for the treatment of peptic ulcer) or xanthones at dose of 10 mg/kg. One hour later all the groups received 0.2 ml of ethanol 60%/HCl 0.3 N in order to induce gastric ulcer. After 1 h of the ethanol administration the animals were euthanized, the stomachs were removed and opened along the greater curvature, and then area of lesion (mm^2) was measured using EARPs software. The stomachs were also used to the analysis of mucin content, oxidative stress and inflammatory parameters.

2.6. Histological evaluation

Tissue samples of gastric lesions were evaluated microscopically as previously described [23] with minor modifications. The tissues were fixed in Alfac (85% ethanol 80%; 10% formaldehyde and 5% acetic acid) for 24 h. Subsequently, the tissues were dehydrated with alcohol and xylene, embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin/eosin (HE). The material was analyzed and photographed using a stereo microscope with magnification of 10×.

2.7. Determination of mucin content

The histochemistry for the mucin content was assessed accordingly with Mowry and Winkler [24]. The gastric tissue sections were deparaffinized, rehydrated, oxidized in periodic acid (0.5%) for 5 min and washed in distilled water. The sections were then stained with Schiff's reagent for 20 min and washed with sulphurous water and tap water. After that, the slides containing the material were counterstained with hematoxylin for 20 s and dehydrated with alcohol. The glycoproteins (mucin) stained by Periodic acid-Schiff (PAS) were quantified using ImageJ® software, in accordance to Pereira et al. [25].

2.8. Determination of mucus adhering in the gastric mucosa

Glandular gastric segments from mice were collected to measure the gastric wall mucus as previously described by Corne et al. [26]. The gastric tissues were weighed and stained for 2 h with 0.1% Alcian Blue solution prepared in 50 mM sucrose and 0.16 mM sodium acetate (pH 5.0) at room temperature. Two successive rinses