Hymenaea stigonocarpa Mart. ex Hayne: A Brazilian medicinal plant with gastric and duodenal anti-ulcer and antidiarrheal effects in experimental rodent models

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ABSTRACT

Ethnopharmacological relevance: Hymenaea stigonocarpa Mart. ex Hayne (Fabaceae) is a medicinal species commonly found in the Brazilian savannah. The stem bark of this medicinal plant, popularly known as "jatobá-do-cerrado", is widely used in tea form to treat gastric pain, ulcers, diarrhoea and inflammation, whereas its fruits pulp is edible.

Aim of the study: The aim of this study was to investigate the antidiarrheal and anti-ulcer effects of a methanolic extract derived from the stem bark (MHs) and diet with fruit pulp of H. stigonocarpa.

Materials and methods: The antidiarrheal action of MHs was measured against the intestinal motility and diarrhoea induced by castor oil in mice. The preventive action of MHs (50, 100, 150 and 200 mg/Kg, by oral route (p.o.)) against peptic ulcers was evaluated in experimental rodent models challenged with absolute ethanol, non-steroidal anti-inflammatory drugs (NSAIDs), ischemia–reperfusion (I/R) (200 mg/Kg, p.o.) and cysteamine (200 mg/Kg, p.o.). The main anti-ulcer mechanisms of action of MHs were analysed as follows: evaluation of the gastric juice parameters, assessment of mucus adherence to the gastric wall, determination of the role of nitric oxide (NO) and sulfhydryl compounds (SH), glutathione (GSH) levels and myeloperoxidase (MPO) activity. The healing effects from MHs (200 mg/Kg) and diet with fruit pulp (10%) against gastric and duodenal ulcers induced by acetic acid were also evaluated by treating rats over 7 or 14 consecutive days of treatment.

Results: The phytochemical profile of MHs and fruit pulp indicated the presence of phenolic compounds (mainly flavonoids and condensed tannins). MHs (200 mg/Kg, p.o.) displayed an antidiarrheal effect and were able to protect gastric mucosa against absolute ethanol (68% protection) and also against the injurious effect of NSAIDs (86% protection) when compared to the group treated with vehicle. These results were accompanied by the prevention of GSH depletion and an inhibition of MPO activity when compared to animals treated with vehicle ($P < 0.05$). MHs markedly protected duodenal mucosa against injuries caused by cysteamine (98%) and also against I/R induced gastric ulceration (80%) when compared to the group treated with vehicle. Furthermore, MHs also prevented the GSH depletion of gastric mucosa relative to the control group treated with vehicle. NO appeared to be involved in this gastroprotective effect. MHs and diet with fruit pulp clearly demonstrated gastric healing actions after treatment for 7 (MHs – 53% inhibition) or 14 days (MHs – 60% inhibition and fruit pulp – 61% inhibition). Treatment with diet with fruit pulp for 7 days demonstrates a significant duodenal healing effect (71% inhibition) without any signs of toxicity.

Conclusions: MHs clearly demonstrate antidiarrheal, gastroprotective and cicatrising effects in experimental gastric and duodenal ulcers, and the diet with fruit pulp displays duodenal healing effects. The observed effects may be associated with the antioxidant effect, which may be due the presence of condensed tannins and flavonoids in the bark and fruit of H. stigonocarpa.

1. Introduction

Peptic ulcer affects 10% of the world population (Zapata-Colindres et al., 2006). Despite great advances in our understanding of this disease, its aetiology has not been completely

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elucidated. Knowledge of the pathophysiology of gastric ulcer disease remains incomplete (Glavin and Szabo, 1992). The peptic ulcer result of an imbalance between aggressive factors, such as acid–pepsin secretion, and protective mechanisms of the stomach, such as the mucosal barrier, mucus secretion, and cell regeneration (Klein et al., 2010). Among various causes of peptic ulcers, lesions due to stress, alcohol consumption, *Helicobacter pylori* infection and the use of non-steroidal anti-inflammatory drugs have all been shown to be mediated largely through the generation of reactive oxygen species, especially hydroxyl radicals (^-OH) (Bandyopadhyay et al., 2002). On the other hand, the mucosal integrity is maintained by protective mechanisms, which include pre-epithelial factors, an epithelial "barrier", continuous cell renewal via proliferation of progenitor cells, continuous blood flow through mucosal microvessels, an endothelial "barrier", sensory innervation, and the generation of prostaglandins and nitric oxide (Laine et al., 2008).

Although many synthetic drugs are available to treat peptic ulcers, most of these drugs have adverse reactions when used long-term (Bandyopadhyay et al., 2002). For this reason, our laboratory has been interested in studying the potential protective effects afforded by natural compounds against peptic ulcers, and special interest was devoted to *Hymenaea stigonocarpa* Mart. ex Hayne. *H.stigonocarpa* (Fabaceae) is a large tree popularly known as "jatobá-do-cerrado" that commonly grows in the Brazilian savannah. Phytochemical studies to date from different parts of the *Hymaenea* plant have demonstrated the presence of sesquiterpenes (Langenheim and Hall, 1983; Aguiar et al., 2010), fatty acids (Matuda and Netto, 2005), diterpenes (Khoo et al., 1973; Cunningham et al., 1973; Cunningham et al., 1974; Marsaioli et al., 1975; Nogueira et al., 2001; Abdel-Kader et al., 2002), and oligosaccharides (Lima et al., 1995). According to ethnopharmacological data, the stem bark and fruit of *H. stigonocarpa* are used in Brazilian folk medicine for the treatment of gastric pain, ulcers, diarrhoea, and as an anti-inflammatory (Grandi et al., 1989). The stem bark, fruit, sap and resin of *H. stigonocarpa* are used in folk medicine in the following forms: decoction, syrup, infusion, sap, poultice, ointment or wine (Grandi et al., 1989). The fruit pulp of *H. stigonocarpa* is also consumed in regional cuisine and the flour used fresh for the preparation of cakes, breads, cookies and hot cereal (Silva et al., 2001). However, despite the ethnopharmacological indications from this medicinal species, there is no pharmacological evidence for their effects in the gastrointestinal tract (for either ulcers or diarrhoea). In this context, the aim of the present work was to investigate the antidiarrheal action of this species and the protective and healing effects of a methanolic extract of the bark of *H. stigonocarpa* and its fruit pulp on gastric and duodenal ulcers.

2. Materials and methods

2.1. Drugs and chemicals

Chemicals were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, USA): absolute ethanol (Sinth, Brazil), lansoprazole (Cruz Vermelha, Brazil), N-nitro-L-arginine methyl ester (L-NAME), indomethacin, carbenoxolone, cimetidine, N-ethylmaleimide (NEM), Alcian Blue, cysteamine hydrochloride, histamine, castor oil, morphine, loperamide, methanol, hexadecyltrimethylammonium bromide, trichloroacetic acid, NaH_2PO_4, Na_2HPO_4, ferrous sulphate, ascorbic acid, thiobarbituric acid, quercetin, ketamine hydrochloride, xylazine hydrochloride, acetic acid and NaOH.

HPLC-grade solvents were obtained from Tedia Company (Phillipsburg, USA). Deionised water (18 MΩ cm) was purified in house using a Milli-Q purification system (Millipore Co., Bedford, USA).

2.2. Plant material

The stem barks and fruits of *H. stigonocarpa* (Hs) were collected in savannah areas near Botucatu city, São Paulo State, in March 2009 by Dr. Leonardo Noboru Seito. Authentication was by the botanist Prof. Dr. Osmar Cavassan. A voucher specimen has been deposited at the Herbarium located at Unesp-Bauru (UNBA number 5691), Brazil.

2.3. Preparation of extracts

After collection, the stem barks were dehydrated, stabilised on an air circulation chamber at 50 °C for 48 h and then milled by a mechanical grinder. The air-dried powdered barks of Hs (1545 g) were exhaustively extracted with methanol (48 h, 1.5 L, three times) at room temperature. Solvents were evaporated at 40 °C under reduced pressure to yield 118.76 g of the methanolic extract of *H. stigonocarpa* (7.6%) (MHs). For the preparation of the fruit flour, fruits were opened to gather the pulp and the seeds rejected. Then, the pulp was dehydrated and stabilised on an air circulation chamber at 40 °C for 24 h. After drying, pulp residue was powdered in a knife mill and sequentially mixed with normal feed Labina-Purine up to 10%, using water as a vehicle. The mixture was automatically pelletised and dried in an air circulation chamber at 40 °C for 48 h. Fingerprints of stem barks and fruits were complementary determined by means of high performance liquid chromatography coupled to photodiode array detection (HPLC–PAD). An aliquot (20 mg) of the bark extract and another one from theof fruit were dissolved in methanol (400 μL) in an ultrasonic bath for 1 min and submitted to clean-up by solid phase extraction. Samples were loaded onto an Agilent SampliQ RP18 cartridge (1 × 1.1 cm d.i.) eluted with 1600 mL of methanol. The eluents were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter and stored in amber vials. The analyses were performed on a HPLC-PAD Jasco system equipped with a PU-2089 quaternary pump, an MD-2010 Plus Photodiode Array Detector, and an AS-2055 Plus autosampler. Separations were achieved with a Phenomenex Synergi Hydro column (C_{18}, 250 × 4.6 mm d.i., 4 μm) equipped with a Phenomenex security guard (C_{18}, 4 × 3 mm d.i.). The mobile phase was composed of acetonitrile (eluent A) and water (eluent B), both acidified with 0.1% trifluoroacetic acid. The mobile phase ranged from 10–23% of B in 35 min to 23–90% of B in 50 min at a flow rate of 1 ml min^{-1}. The software EZCrom Elite version 3.1.7 (Agilent Technologies) was used for data acquisition and processing.

2.4. Animals

Male and female Swiss mice (25–35 g) and male Wistar rats (150–250 g) from the Central Animal House of the UNESP were used. The animals were fed with a certified Labina-Purine diet, with free access to tap water, under standard conditions of 12 h dark-12 h light cycle, humidity (60 ± 1.0%) and temperature (21 ± 1 °C). The animals were fasted before all experimental procedures. Standard drugs and MHs (bark) were administered orally (gavage) using a saline solution (10 mL/Kg) as the vehicle, whereas fruit flour was added to the diet as food. The animals were housed in cages with raised floors made of wide mesh to prevent coprophagy. All experiments were performed in the morning and followed the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993). The UNESP Institutional Animal Care and Use Committee approved all of the employed protocols (42/04-CEEA).

2.5. Antioxidant activity

Additional in vitro experiments were performed to evaluate the antioxidant activity of different concentrations of MHs (1.14–145.45 µg/ml). This in vitro test was evaluated using a thiobarbituric acid-reactive species (TBARS) assay (Witaicenis et al., 2010) modified from the original protocol described by Stocks et al. (1974). Briefly, rat brain samples were obtained from two-month-old male Wistar rats. Tissue samples were homogenised 1:4 (w/v) in buffer containing 19 parts of NaH_2PO_4 (10 mM) and 81 parts of Na_2HPO_4 (10 mM) (pH 7.4) to obtain membrane supernatant after centrifugation ($22{,}176 \times g$/15 min/4 °C). This membrane suspension was diluted 1:10 (v/v) in the above-mentioned buffer solution. Next, buffers (in the assays without inhibitors) or different concentrations of MHs were added. Lipid peroxidation was non-enzymatically induced with 100 mol/L of both ferrous sulphate and ascorbic acid. After incubation at 37 °C for 12 min, the reaction was stopped and malondialdehyde (MDA) determined by 0.5% thiobarbituric acid reaction in 20% trichloroacetic acid. Measurement of the absorbance of the supernatants was read at 532 nm. The resultant products of the thiobarbituric acid reaction were taken as indicators of lipid peroxidation in membrane fractions. The flavonoid quercetin was used as a reference and tested in the same assay system.

2.6. Acute toxicity and hippocratic screening

Male and female Swiss mice were randomly divided into groups ($n=10$) that orally received saline solution (10 mL/Kg) or MHs at the same dose of 5000 mg/Kg. After oral administration, the acute toxicity and behavioural parameters were described according to the methods of Souza Brito (1994). The observations were performed at 30, 60, 120, 240 and 360 min after the oral treatments. For 14 days, the animals were weighed and the number of deaths noted. On the 14th day, mice were sacrificed and the heart, liver, kidney, lung, spleen, testicles (male), ovaries and uterus (female) collected. We compared all parameters from animals treated with MHs with those obtained from the respective control group treated with vehicle (saline).

2.7. Gastrointestinal motility in mice

Male Swiss mice ($n=7$) were weighed and deprived of food for 6 h, but they had free access to water. At time zero, the animals received vehicle (saline) as the negative control, 2.5 mg/Kg of morphine as the positive control or MHs at doses of 100, 150 and 200 mg/Kg by oral gavage. Thirty minutes after treatment, all groups received activated charcoal (0.1 mL/10 g, orally) and were sacrificed 30 min later. The results were expressed as a percentage of the total length of the small intestine. The distance travelled by the charcoal relative to the total length of the small intestine was calculated for each mouse to express the percentage of distance travelled and converted by arcosin (Stickney and Northup, 1959, with modifications).

2.8. Diarrhoea induced by castor oil

Five groups of male Swiss mice ($n=7$) were orally treated with vehicle saline (10 mL/Kg), MHs (100, 150 and 200 mg/Kg) or loperamide (5 mg/Kg) 30 min before castor oil (0.2 mL/animal) administration. Immediately after ingesting castor oil, each animal was kept in an individual cage with a floor lined with blotting paper and observed for 5 h. The following parameters were monitored: time to initial evacuation (min), evacuation classification: 1 (normal stool), 2 (semi-solid stool), and 3 (watery stool) and evacuation index (EI), expressed according to the following

formula: $eI = 1 \times (n^o \text{ stool } 1) + 2 \times (n^o \text{ stool } 2) + 3 \times (n^o \text{ stool } 3)$ (Awounters, 1978, modified by Mukherjee et al., 1998).

2.9. Evaluation of the lesions

Based on their respective specifications, the groups under each experimental model included positive (carbenoxolone, lanzoprazole or cimetidine) and negative (vehicle–saline) controls. Moreover, the animals were kept in cages with raised floors of wide mesh to prevent coprophagy. After each experiment the animals were euthanized with CO_2 gas, the stomachs are excised, cut along the greater curvature, and gently rinsed und tap water pressed onto a glass plate, and scanned so that the lesions could be counted with aid of the AVSoft program. The results were expressed as total ulcerated area (mm^2).

2.10. Evaluation of gastroprotective activity

Ethanol-induced ulcers—This experiment was performed as described by Morimoto et al. (1991) and Glavin and Szabo (1992). Male albino Wistar rats were randomly distributed into 6 groups ($n=8$) and fasted for 18 h prior to receiving an oral dose of the vehicle (saline; 10 mL/Kg), lansoprazole (30 mg/Kg) or MHs (50, 100, 150 or 200 mg/Kg body weight). After 60 min, all groups were treated orally with 1 mL of absolute ethanol to induce gastric ulcers. According to Glavin and Szabo (1992), the ulcers can be examined 1–2 h after ethanol administration. Time course studies revealed that most gastric damage. After another 1 h, the animals were sacrificed, and the extent of the lesions was measured by AvSoft® Bioview Spectra, Brazil. The lesion measurements were expressed as mm^2.

NSAID-induced gastric ulcers in rat—In this model (Guidobono et al., 1997 with modifications), gastric lesions were induced with indomethacin (50 mg/Kg, p.o.) administered to male Wistar albino rats ($n=8$) after an 18 h fast. MHs (50, 100, 150 or 200 mg/Kg body wt.), cimetidine (100 mg/Kg) or vehicle was orally administered 30 min before the induction of the gastric lesions. The animals were sacrificed 6 h after treatment with the ulcerogenic agent. The stomachs were removed, and gastric lesions were measured as described above.

Determination of Gastric Secretion—The assay was performed according to the method of Shay et al. (1945) with a few modifications. Male Wistar albino rats ($n=10$) were randomly divided into three groups and fasted for 18 h with free access to tap water. The determination of gastric secretion was performed by two routes as follows: *oral route*: thirty minutes after oral administration of MHs (200 mg/Kg, which is the dose that provided the best gastroprotective results), cimetidine (100 mg/Kg) as a positive control or vehicle (saline, 10 mL/Kg), pylorus ligature was performed as described by Shay et al. (1945). *Intraduodenal route*: all groups of male rats ($n=10$) were fasted for 18 h with free access to water. Immediately after pylorus ligature, MHs (200 mg/Kg), cimetidine (100 mg/Kg) or saline (10 mL/Kg) was administered by an intraduodenal route. One hour after the ligature, all animals received histamine (100 mg/Kg) subcutaneously. All animals were sacrificed 4 h later, the stomachs removed, the gastric lesions measured (mm^2), and the gastric content collected to determine the total amount of gastric juice acid (mL) and pH values (unit). Distilled water was added until a 10 mL volume was reached, and the resultant solution was centrifuged at $3.000 \times g$ for 10 min. Total acid in the gastric secretion volume was determined in the supernatant by titration to pH 7.0 with 0.01 N NaOH (mEq/mL/4 h).

Assessment of mucus adhered to the gastric wall—The procedure was performed according to the methods described by Come et al. (1974) and modified by Rafatullah et al. (1990). After fasting for

18 h, a longitudinal incision was made below the xiphoid apophysis for the pylorus ligature in anaesthetised rats ($n=8$). Vehicle (saline; 10 mL/Kg), carbenoxolone (200 mg/Kg) or MHs (200 mg/Kg) was orally administered for 1 h before the ligature. Four hours later, the animals were sacrificed and the glandular portion of the stomach separated, weighed and immersed in a solution of Alcian Blue to quantify the mucus. For each sample, the absorbance at 598 nm was measured in a spectrophotometer, and the results are expressed as µg of Alcian blue/g of tissue.

Ethanol-induced gastric lesions in L-NAME- and NEM-pretreated rats—The procedure was performed according to the methods described by Matsuda et al. (1999). Gastric mucosal lesions were induced in male Wistar rats ($n=7$). Male Wistar rats fasted for 18 h were randomly divided into nine groups, three of which each received saline (i.p.), 70 mg/Kg of NO synthase inhibitor (L-NAME) or 10 mg/Kg of sulphydryl depleter (NEM). Thirty minutes after the pretreatment, the oral treatments were applied (saline, carbenoxolone 100 mg/Kg or MHs 200 mg/Kg). Sixty minutes later, 1 mL of absolute ethanol was given to each rat; all animals were sacrificed after 1 h. The stomachs were removed to determine gastric lesion areas (mm^2).

Acute gastric mucosal lesions induced by ischemia-reperfusion in rats—Ischemia–reperfusion (I/R) erosions were produced in three groups of rats ($n=10$) using the method originally proposed by Ueda et al. (1989). The rats were deprived of food for 18 h before the experiments but allowed access to tap water ad libitum. Briefly, under anaesthesia (0.08 mL/100 g of ketamine hydrochloride and 0.04 mL/100 g xylazine hydrochloride), the celiac artery was clamped with a small clamp for 30 min. Then, the clamp was removed, and reperfusion occurred for 60 min. Vehicle (saline, 10 mL/Kg), lansoprazole (30 mg/Kg) or MHs (200 mg/Kg) was administered orally 60 min prior to the experiments. The untreated group comprised animals that were submitted to the abdominal incision but not to I/R. After 60 min of reperfusion, the animals were sacrificed and their stomachs excised for biochemical determinations. The gastric mucosal injury was measured by AvSoft® Bioview Spectra (Brazil) and expressed as mm^2.

2.11. Evaluation of the preventive effect of MHs on duodenal ulcers in rats

Cysteamine duodenal ulcer in rats—Duodenal ulcers were induced in male albino Wistar rats by two oral administrations of cysteamine hydrochloride (400 mg/Kg) in saline solution at an interval of 4 h. MHs (200 mg/Kg), lansoprazole (30 mg/Kg) or vehicle (saline; 10 mL/Kg) was administered 30 min before the first dose of cysteamine. One untreated group was used to evaluate the normal parameters of the duodenum mucosa. All animals were sacrificed 48 h after the first dose of cysteamine. Each duodenum was cut open along the antimesenteric side and rinsed with saline. The duodenal score was determined using the following 4-point scale: 0=no lesion, 1=superficial mucosal erosion, 2=deep ulcer or transmural ulcer, and 3=perforated or penetrated ulcer (Szabo, 1978). The macroscopic duodenal area was recorded using AvSoft® Bioview Spectra (Brazil) and expressed as mm^2.

2.12. Evaluation of curative effect of MHs and fruit pulp of Hymenaea stigonocarpa (Hs) in the gastric and duodenal ulcers induced by acetic acid in rats

The experiments were performed according to the method described by Okabe et al. (1971) and modified by Konturek et al. (1988). Gastric mucosal and duodenal lesions were induced in male albino Wistar rats ($n=8$) that were fasted for 18 h but had free access to water. An incision was made in the abdomen of animals under anaesthesia, and a plastic mould (4.2 mm in diameter) was placed tightly on the stomach in the first experiment and serosal surface of the duodenal wall; the mould was approximately 5 mm distal to the pylorus in the second experiment. Acetic acid (80%, 70 µl) was poured into the mould and allowed to remain for 20 s on the stomach and 10 s on the duodenal wall. The stomach and duodenum were bathed with saline (20 °C) to remove the acetic acid. The abdomen was then closed, and all of the animals were fed normally. The animals were treated for 7 or 14 consecutive days beginning one day after surgery. MHs (200 mg/Kg), lansoprazole (30 mg/Kg) or saline were administered orally and the fruit flour in the diet (10%) was placed in a cage as food (40 g daily for monitoring the consumption, in individual boxes). On the day after the last administration, the rats were sacrificed and their stomachs and duodenum removed. Body weight and food consumption were assessed throughout the experiment. The gastric and duodenal lesions were evaluated by examining the inner gastric and duodenal surfaces with a dissecting magnifying glass. The macroscopic images of the gastric and duodenal ulcers were recorded using AvSoft® Bioview Spectra (Brazil) and expressed as mm^2.

2.13. Evaluation of Hymenaea stigonocarpa subacute toxic activity

We evaluated the subacute toxic activity of these substances in groups of rats treated with MHs and diet with fruit pulp (Hs) (10%). Animals were subjected to daily treatment with MHs and Hs (10%) for 14 days after induction of injury with acetic acid. The parameters studied were body weight and macroscopic analysis of the vital organs: heart, lungs, liver, spleen and kidneys. In addition, the blood of the animals was collected for analysis of biochemical and enzyme parameters, including serum gamma-GT (gamma glutamyltransferase), urea, creatinine, AST (aspartate aminotransferase), ALT (alanine aminotransferase) and glucose. Analyses were quantified using the SBA-200 automatic biochemical analyser and CELM® kinetics and colourimetric kits (Brazil) available for use by the Clinical Analysis laboratory of the Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Science of Unesp, Botucatu, under the guidance of Prof[a]. Dr. Regina Kiomi Takahira.

2.14. Biochemical determinations from gastric and duodenal mucosa

Myeloperoxidase (MPO) activity was measured according to the technique described by Krawisz et al. (1984). Samples were suspended in 1 ml of 50 mM phosphate buffer incorporating 0.5% hexadecyltrimethyl-ammonium bromide (pH 6.0) and minced with scissors for 15 s on an ice-cold plate. The resultant suspension was subsequently diluted to a final 1:20 (w/v) ratio and homogenised for 1 min with an automatic Heidolph homogeniser, then sonicated for 10 s and subjected to three freeze–thaw cycles. The homogenates were then centrifuged at $7000 \times g$ and 4 °C for 10 min, and the supernatants were assayed for MPO activity. The results are expressed as MPO units per gram (U/g) of wet tissue.

Total glutathione content (GSH) was quantified with the recycling assay described by Anderson (1985). Samples were thawed, minced, diluted 1:20 (w/v) in ice-cold 5% (w/v) trichloroacetic acid and homogenised. The homogenates were centrifuged at $7000 \times g$ for 15 min at 4 °C, and the supernatants were used to quantify GSH. The results are expressed as nanomoles per gram (nmol/g) of wet tissue.

2.15. Statistical analysis

The results were expressed as the mean $\pm$ S.E.M., and the differences between groups were determined by analysis of

variance (ANOVA). Significant differences were determined using the Dunnett post-test (for three or more groups) or, for non-parametric results, the Kruskal–Wallis test followed by Dunn's test; $P < 0.05$ considered to be statistically significant.

3. Results and discussion

Acute toxicological studies have showed that an oral administration of 5000 mg/Kg of *H. stigonocarpa* methanolic stem bark extract (MHs) did not produce any sign of acute toxicity in the animals (male and female). Over the 14 days following the oral administration of MHs, none of the animals died and no significant changes in daily body weight or organ weight were observed through the end of this period (data not shown). These results ensured the continuance of pharmacological studies on this species using the oral route and motivated us to proceed with the biological assays.

Based on ethnopharmacological data of the antidiarrheal effects of *H. stigonocarpa* bark (Grandi et al., 1989) and the fact that intestinal motility affects the luminal absorption and regulates the bioavailability of an orally administered drug (Lee et al., 2000), we evaluated the effect of MHs on diarrhoea and on intestinal motility. All mice from the control group (treated with vehicle) produced copious diarrhoea after castor oil administration (Table 1). However, pre-treatment with MHs (100, 150 and 200 mg/Kg) induced a significant delay in the onset of the copious diarrhoea (61, 50 and 53%, respectively, relative to the vehicle)

(Table 1). The decrease in the severity of the diarrhoea as measured by the evacuation index was also observed in a group of animals treated with 100 mg/Kg MHs or loperamide. Treatment with MHs (100, 150 and 200 mg/Kg) before the cathartic agent (castor oil) significantly decreased liquid evacuation similar to the results produced by loperamide ($P < 0.05$).

Although the fruit pulp shows laxative effect (Brandão, 1993), was not tested in this experiment, due to the difficulty of testing the fruit pulp as diet in this experimental model. The antidiarrheal effect of MHs was not accompanied by a reduction in intestinal transit. The effect of MHs on normal intestinal propulsion of mice was tested with different doses of the extract (Table 2). In the MHs-treated group of mice, all doses of extract did not change the normal intestinal propulsive movement relative to the morphine group; morphine produced a greater anti-motility effect ($P < 0.05$).

As a part of the pharmacological study of the gastroprotective effects of MHs, the extract was evaluated in experimental models of gastric ulcers induced by multiple damaging agents (Table 3). Daily, the gastric mucosa is challenged by a variety of both endogenous and exogenous irritants (aggressive factors) including ethanol, gastric acid, pepsin, reactive oxygen species, non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* (Holzer, 1998). In this context, treatment with anti-ulcer compounds that possess anti-secretory, gastroprotective, spasmolytic, immunomodulatory, antibacterial and/or anti-inflammatory effects seems interesting as it may lead to the use of certain compounds with multitarget actions, e.g., medicinal plants (Wagner, 2006). Among the different

Table 1
Effects of methanolic extract of *Hymenaea stigonocarpa* of the bark (MHs) on antidiarrheal induced by castor oil in mice.

Treatment (p.o.)	Dose (mg/kg)	N	Time to initial evacuation (min)	Evacuation classification			Evacuation index (EI)	Inhibition[a] (%)
				Normal	Semi-solid	Liquid		
Vehicle	–	7	59.66 + 7.30	0.50 + 0.34	0.00 + 0.00	7.00 + 1.26	21.50 + 3.99	–
Loperamide	5	7	228.14 + 11.85**	0.20 + 020	0.00 + 0.00	0.80 + 0.80**	2.60 + 2.60**	88
MHs	100	7	77.14 + 14.73	1.00 + 0.31	2.14 + 0.59	1.00 + 0.30*	8.28 + 0.97*	61
	150	7	114.83 + 18.51*	0.50 + 0.22	3.66 + 0.71**	1.00 + 0.36*	10.83 + 2.21	50
	200	7	113.28 + 5.93*	1.00 + 0.22	4.14 + 0.85**	0.28 + 0.18 ***	10.14 + 1.79	53

Data are presented as mean ± S.E.M. ANOVA was employed, followed by Dunnett's test, and significant differences were represented by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control group treated with vehicle. For classification of evacuations and calculation of EI, Kruskal-Wallis followed by Dunn.

[a] Inhibition in relation to evacuation index.
* $P < 0.05$ in relation to the vehicle.
** $P < 0.01$ in relation to the vehicle.
*** $P < 0.001$.

Table 2
Effects of different doses from methanolic extract of *Hymenaea stigonocarpa* of the bark (MHs) on models of gastric induced lesions in rats.

Methods	Treatments (p.o.)	Dose (mg/kg)	N	Gastric Lesion (mm²)	Inhibition (%)	GSH (nmol/g)	MPO (U/g)
Absolute Ethanol	Sham	–	5	–	–	2136.34 + 142.95**	322.60 + 26.58*
	Vehicle	–	12	112.48 + 19.42	–	1583.84 + 61.98	845.97 + 147.53
	Carbenoxolone	100	6	25.31 + 13.22**	77	2032.80 + 101.73**	387.78 + 95.94*
	MHs	50	6	148.36 + 47.46	–	1457.93 + 107.19	1087.48 + 343.86
		100	6	170.38 + 47.65	–	1379.18 + 84.33	572.12 + 81.99
		150	8	23.70 + 5.21**	79	1568.64 + 101.00	371.96 + 44.80*
		200	10	35.52 + 13.90**	68	1855.71 + 88.83*	481.25 + 49.61*
NSAID	Sham	–	6	–	–	2089.40 + 277.49**	323.83 + 13.64**
	Vehicle	–	8	40.12 + 11.42	–	1098.50 + 116.89	798.35 + 101.76
	Cimetidine	100	6	1.17 + 0.98**	97	1883.15 + 68.03**	844.37 + 82.98
	MHs	50	8	24.87 + 10.73	–	1233.59 + 35.84	838.12 + 170.03
		100	7	20.57 + 5.54	–	1243.57 + 83.49	1125.62 + 115.03
		150	8	36.5 + 16.69	–	1502.36 + 100.39	702.6 3 + 110.05
		200	8	5.50 + 2.54**	86	1630.12 + 108.07*	516.60 + 33.43*

Results are expressed in mean ± S.E.M. ANOVA followed by Dunnett's test: gastric lesion, GSH and MPO:
* $P < 0.05$ represents difference in relation to control group treated with the vehicle.
** $P < 0.01$ represents difference in relation to control group treated with the vehicle.

Table 3
Effect of methanolic extract of the bark of *Hymenaea stigonocarpa* (MHs) and fruit pulp of *Hymenaea stigonocarpa* (Hs) (10%) on biochemical parameters of toxicity in rats submitted to the gastric lesions induced by acetic acid.

Treatments (p.o)	AST (U/L)	ALT (U/L)	γ- GT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)	glucose (mg/dL)
Sham	199.00 + 30.20	46.50 + 6.40	0.70 + 0.11	05.4 + 0.04	30.00 + 1.73	149.00 + 4.78
Vehicle	157.14 + 13.37	44.28 + 2.52	0.95 + 0.17[a]	0.55 + 0.01	38.14 + 1.12	136.85 + 7.05
lansoprazole	181.20 + 17.50	48.00 + 5.04	2.02 + 0.55[a]	0.56 + 0.02	39.40 + 5.35	136.40 + 5.80
MHs (barks)	159.12 + 14.34	41.87 + 2.69	1.30 + 0.16	0.58 + 0.03	47.12 + 5.00	144.75 + 11.17
Hs (fruit pulp)	166.00 + 12.44	40.00 + 3.24	1.17 + 0.19	0.55 + 0.04	32.50 + 2.06	148.87 + 7.24

Biochemical parameters from the different experimental groups ($n=8$) after 14 days of daily treatment. Results are mean $\pm$ S.E.M. AST (aspartate aminotransferase), ALT (alanine aminotransferase), gamma-GT (gamma glutamyltransferase), creatinine, urea and glucose.

[a] $P < 0.05$ represents difference in relation to sham group (Kruskall–Wallis, Dunn).

doses of MHs evaluated in this study, the gastroprotective effect was found to be most effective at the 200 mg/Kg dose in both experimental models. The gastric damage in the group treated with MHs was significantly decreased, by 68% in the absolute ethanol model and 86% in the NSAID model when compared to their respective control groups treated with vehicle ($P < 0.05$). These gastroprotective effects of MHs in both experimental models were also accompanied by biochemical changes. The MHs groups demonstrated a restoration of glutathione levels, an important marker of oxidative damage (Buffinton and Doe, 1995) in the gastric mucosa before gastric damage was induced by ulcerogenic agents. This protective effect in MHs groups was accompanied by a significant reduction in MPO activity in the group treated with 200 mg/Kg MHs. The decrease in myeloperoxidase activity in the gastric mucosa of the group pre-treated with MHs might be interpreted as an anti-inflammatory property of a given compound (Veljaca et al., 1995). Therefore, considering both the gastric lesion and the biochemical parameters, MHs at 200 mg/Kg demonstrated a preventive effect against induced damage in the gastric mucosa in the two models used.

The next step of this study was the evaluation the effect of MHs on gastric juice parameters of the extract by either an oral or intraduodenal route through the pylorus ligature mode. We evaluated the systemic effect of MHs in the pylorus ligation model using histamine (secretagogue) and the combination of this secretagogue with extract. The oral and intraduodenal administration of MHs was not able to change the pH of the gastric juice (oral route=MHs -0.6 ± 0.4 vs vehicle -1.0 ± 0.6 and intraduodenal route=MHs -1.2 ± 0.1 vs histamine -1.1 ± 0.1). Therefore, the MHs did not act as an antacid or as buffer solution in contact with gastric mucosa, as indicated by the lack of antisecretory effects in the gastric juice. The gastric mucus is one of the most important protective features for the gastric mucosa, and the effect of MHs in the amount of gastric mucus was also evaluated in pylorus-ligated rats. We observed that the amount of adhered gastric mucus was not augmented by treatment with MHs (200 mg/Kg) when compared to the animals treated with vehicle (MHs-1350.81 $\pm$ 111.59 and vehicle-1524.71 $\pm$ 156.69 µg/g of tissue) (data not shown). These results rule out an increase in the amount of mucus as a potential mechanism of action for the gastroprotective effect of MHs.

We investigated the involvement of endogenous sulphydryls in the gastroprotective effect of MHs using NEM (N-ethylmaleimide), a sulphydryl compound depleter (SHs). Non-protein sulphydryl (NP-SH) compounds are important components of the gastric mucus. NP-SH compounds exert gastroprotective effects by maintaining the integrity of the mucosal barrier and by binding free radicals formed due to the action of noxious agents such as ethanol (Szabo and Vattay, 1990). The involvement of endogenous sulfhydryls compounds was analysed by pretreating with NEM, and we observed that MHs did not change the protective effect (data not shown), eliminating another possible gastroprotective mechanism.
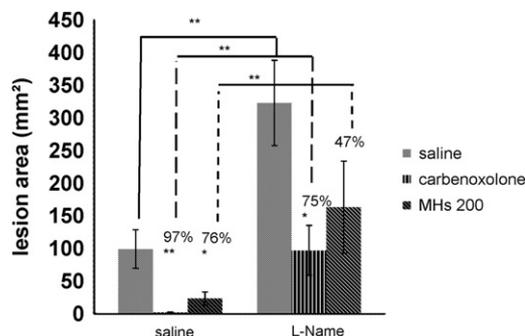

Fig. 1. Effects of methanolic extract of *Hymenaea stigonocarpa* (MHs) on gastric lesions induced by ethanol in rats pretreated with L-NAME (an inhibitor of NO synthase). The bars represent mean $\pm$ S.E.M. for lesion area. The numbers indicate the percentage protection in relation to the control group treated with vehicle. ANOVA was employed, followed by Dunnett's test, and significant differences were represented by $*p < 0.05$ and $**p < 0.01$ vs control group treated with vehicle.

According to Gomes et al. (2009), the absence of NP-SH involvement and the unchanged gastric secretion parameters (as observed in pylorus-ligated rats) strongly indicate the absence of mucus involvement in MHs gastroprotection.

In addition to NP-SH, endogenous nitric oxide (NO) is important for maintaining gastric epithelial integrity and mucus barriers (Tulassay and Herszényi, 2010). Previous studies indicated the involvement of NO in the gastric protection observed experimentally by promoting blood vessel dilatation and reducing lipoperoxidation, acting similarly to an anti-inflammatory drug (Ancha et al., 2003). The gastroprotective effect of MHs was challenged with L-NAME (NG-nitro-L-arginine methyl-ester), a nitric oxide synthase inhibitor. Pretreatment of animals L-NAME markedly increased gastric lesions of animals treated with MHs (Fig. 1), a group that previously had 76% inhibition of gastric lesions. These results demonstrated the involvement of NO in the gastroprotective effect of this extract. The known ability of NO to decrease lipoperoxidation, act as an antioxidant and increase vasodilation enables NO to reverse some important types of tissue injury such as ischemia.

Ischemia/reperfusion (I/R) induced gastric lesions are linked to free radical (FR) formation (El-Abhar et al., 2003). Ischemia followed by reperfusion leads to tissue injury (Piper et al., 2003). The restoration of blood flow (reperfusion) after a period of ischemia initiates a cascade of changes, including the release of local reactive oxygen species (ROS) and an increase in the adhesion of neutrophils to endothelial cells, which causes damage to the integrity of the mucosal lining via free radical release (Rastogi et al., 1998; Abdallah et al., 2009).

When the stomach was subjected to ischemia and reperfusion (I/R), the severity of gastric lesions was significantly increased. The group treated with MHs displayed a significant reduction in gastric

lesions of approximately 5-fold (3.87 ± 1.95 mm^2) when compared to the group control saline (19.6 ± 6.5 mm^2) (Fig. 2). Our results show that MHs markedly protects against gastric ulceration induced by I/R (80%) compared to the control group treated with saline. These effects were accompanied by an ability to maintain the GSH content at high levels when compared to the group treated with vehicle. Kobata et al. (2007) described a marked increase in mucosal MPO activity in the stomach after I/R, confirming the infiltration/activation of neutrophils in the gastric mucosa during I/R. However, MHs did not change the levels of MPO (Fig. 2).

Many studies have reported various mechanisms involved in cysteamine-induced duodenal ulcers, including reduced somatostatin bioavailability and elevated gastrin secretion (Seiler et al., 1983). This model has been considered the standard model for experimental duodenal ulcers and has been used for the biological efficacy assessment of new anti-ulcer drugs. According to Khomenko et al. (2009), animals treated with cysteamine display a significant increase in duodenal lesions due to the generation of free radicals. Fig. 3 shows that duodenal injuries caused by cysteamine were significantly reduced after treatment with MHs (98%) comparing to in the group treated with vehicle. The

glutathione (GSH) levels and myeloperoxidase activity (MPO) in the duodenum of experimental animals are also shown in Fig. 3. The role of GSH in gastric mucosa in counteracting free radical production has been extensively reported (Townsend et al., 2003). Our results reveal the duodenal damage in the saline control group to be biochemically characterised by a 7-fold increase in duodenal MPO activity and by significant duodenal GSH depletion when compared to sham, both consequences of the oxidative stress that takes place in the damaging process. Although animals treated with MHs demonstrated the same MPO activity as that exhibited by the saline control group, the extract prevented the depletion of GSH (2171.68 ± 129.75 nmol/g of tissue) that occurred in the saline control group (1381.36 ± 63.85 nmol/g of tissue) (Fig. 3). Glutathione is essential in controlling the redox state in the cell via several mechanisms including the scavenging of reactive oxygen species and the maintenance of glutathione peroxidase enzymatic levels in a reduced state (Sies, 1999). Thus, the duodenal and gastric protective effects of MHs may be mediated by its antioxidant properties. This property of MHs was also observed in the antioxidant activity assay, in which MHs had an IC$_{50}$ value of 5.25 ± 0.23 µg/mL. The corresponding IC$_{50}$ value of quercetin was 0.33 ± 0.01 µg/mL.

The cicatrisation assay was performed using the acetic acid method, which, according to Okabe and Amagase (2005), most closely approximates human gastric ulcers. New drugs that prevent ulcer relapse and enhance ulcer healing could potentially be developed using this established method. Based on the use of bark in folk medicine against gastric ulcers and also the practice of consuming the fruit pulp of this species as food, we evaluated the healing activity of MHs and diet with fruit pulp in the healing ulcer model induced by acetic acid. Thus, this study determined the effect of MHs (200 mg/Kg) and diet with fruit pulp Hs (10%) during 7 or 14 consecutive days on the healing of gastric (Fig. 4) and duodenal ulcers (Fig. 5) induced by acetic acid in rats. After 7 days of treatment to the gastric mucosa, MHs and lansoprazole improved ulcer healing by shrinking the size of the lesion area compared to the vehicle (53% and 83%, respectively). In the groups treated with lansoprazole and diet with fruit pulp Hs, there was a significant maintenance of glutathione levels (Fig. 4). After the 14-day MHs treatment, lansoprazole and diet with fruit pulp were able to heal gastric ulcers by decreasing the lesion area (83%, 60% and 61%, respectively) in relation to the control group treated with vehicle. However, only treatment with lansoprazole prevented depletion of glutathione (Fig. 4). Fig. 5 shows that lansoprazole and diet with fruit pulp were effective in healing
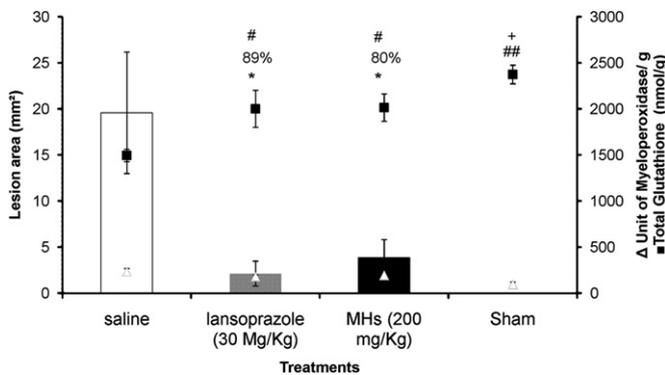
Fig. 2. Gastroprotective activity of methanolic extract of *Hymenaea stigonocarpa* (MHs) barks on levels of total glutathione and myeloperoxidase on model of gastric injury induced by ischemia/ reperfusion in rats ($n=8$–10).The bars represented as mean $\pm$ S.E.M. for lesion area. The numbers indicate the percentage protection in relation to the control group treated with vehicle. ANOVA was employed, followed by Dunnett's test and significant differences were represented by $p^* < 0.05$ for lesion area, $^\#p < 0.05$ and $p < 0.01^{\#\#}$ for total glutathione, $^+p < 0.05$ for myeloperoxidase vs control group treated with vehicle.
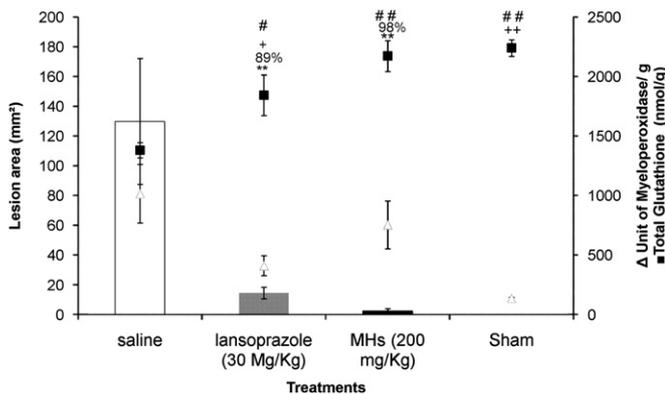
Fig. 3. Effect of methanolic extract of *Hymenaea stigonocarpa* (MHs) on lesion area, level of total glutathione and myeloperoxidase on model of duodenal injury induced by cysteamine in rats ($n=8$). The bars represented as mean $\pm$ S.E.M for lesion area. The numbers indicate the percentage protection in relation to the control group treated with vehicle. ANOVA was employed, followed by Dunnett's test, and significant differences were represented by $^{**}p < 0.01$ for lesion area, $^\#p < 0.05$ and $p < 0.01^{\#\#}$ for total glutathione and $^+p < 0.05$ and $^{++}p < 0.01$ for myeloperoxidase vs control group treated with vehicle.
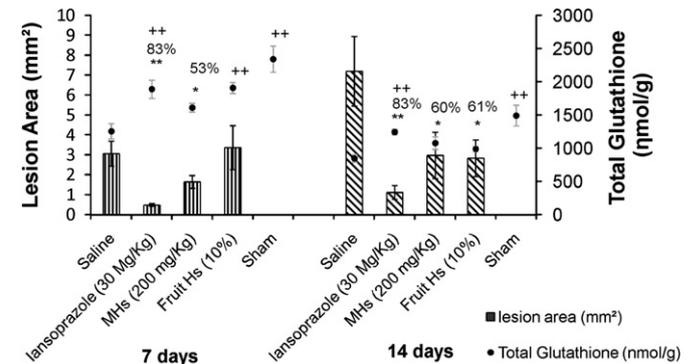
Fig. 4. Gastric healing effect of methanolic extract of *Hymenaea stigonocarpa* (MHs) and fruit pulp by treatment during 7 or 14 days after injury induced by acetic acid in rats ($n=8$). The bars represented as mean $\pm$ S.E.M. for lesion area. The numbers indicate the percentage protection in comparison to the control group treated with vehicle. ANOVA was employed, followed by Dunnett's test, and significant differences were represented by $^*P < 0.05$, $^{**}P < 0.01$ for lesion area; $^{++}p < 0.01$ for total glutathione vs control group treated with vehicle.
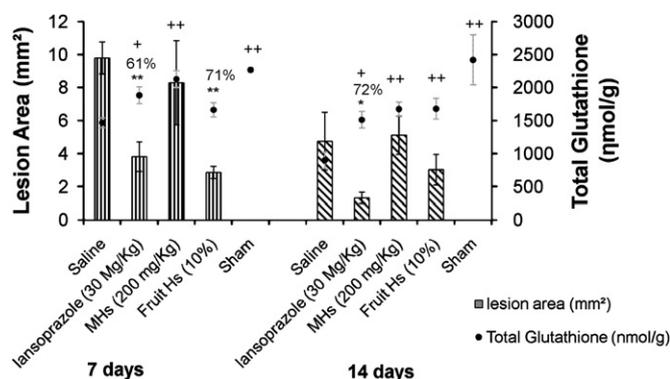
Fig. 5. Duodenal healing effect of methanolic extract of *Hymenaea stigonocarpa* (MHs) and fruit pulp by treatment during 7 or 14 days after injury induced by acetic acid in rats (*n*=8). The bars represented as mean ± S.E.M. for lesion area. The numbers indicate the percentage protection in comparison to the control group treated with vehicle. ANOVA was employed, followed by Dunnett's test, and significant differences were represented by *$P < 0.05$, **$P < 0.01$ for lesion area; $+p < 0.05$, $++p < 0.01$ for total glutathione vs control group treated with vehicle.

duodenal ulcers by decreasing the lesion area compared to the vehicle (61% and 71%, respectively). However, the results in Fig. 5 show that only treatment with lansoprazole for 14 consecutive days was able to reduce the duodenal ulcers (72%). The results in Fig. 5 also demonstrate that treatment with MHs and diet with fruit pulp was able to prevent depletion of glutathione in healing duodenal ulcers within 7 or 14 consecutive days of treatment compared to the control group treated with vehicle. Taken together, these results reinforce the marked effect of MHs and also of diet with fruit pulp in inhibiting gastric and duodenal lesions and in promoting the healing of chronic gastric and duodenal ulcers in rats.

Toxicity analysis performed in the cicatrisation model (14 day) did not show any toxic effects from the MHs and diet with fruit pulp, and no behaviour changes were observed during treatment. The body weight of the animals progressed similarly in all groups, and there was no significant different in the organ weights between groups. Biochemical analysis of AST (unspecific marker of hepatic damage), ALT (specific marker of hepatic parenchymal damage), gamma-GT (initial indicator of hepatic toxicity), creatinine, glucose and urea (markers of renal damage) in the serum of the animals treated for 14 consecutive days with MHs or diet with fruit pulp did not reveal any alterations (Table 3), indicating that during the treatment period neither substance demonstrated any signs of toxicity.

The HPLC-PAD fingerprints of *H.stigonocarpa* bark and fruit pulp are qualitatively similar, given their phenolic composition (Fig. 6A), confirming the manifest polyphenolic nature of both extracts. Peaks eluted in the retention time (R_t) ranging from 0 to 26 min showed a week band approximately 280 nm, a shoulder near 220 nm and a maximum absorption between 205 and 210 nm (Fig. 6B) typical of catechin derivatives (Fig. 6C) (Rohr et al., 2000). In the R_t region of 26–34 min a second group of compounds was eluted that exhibited maximum absorption bands near 216 and 270 nm (Fig. 6D) suggestive of gallic acid derivatives (dag) (Fig. 6E) (Rohr et al., 2000). Moreover, the poorly resolved peak eluted at R_t=39–48 min with a UV pattern characteristic of catechins is closely related to polymeric catechins. The chromatographic profile obtained by HPLC-PAD analysis of *H. stigonocarpa* bark and fruit pulp led to the recognition of flavonoids and condensed tannins.

The qualitative phytochemical screening tests demonstrated that fruits and barks of *H stigonocarpa* are composed primarily

of phenolic compounds such as flavonoids and condensed tannins. The antioxidant activities of the flavonoids and tannins, phytochemical compounds present in MHs, are most likely responsible for these results. Previous studies have highlighted the role of flavonoids in a spectrum of biological activities including anti-ulcer and free radical scavenging (Wach et al., 2007; Havsteen, 2002). The phytochemical composition of MHs (flavonoids) is most likely involved in this result because a study by Rao and Vijayakumar (2007) has already described (+)-catechin, the flavonoid present in MHs, to be an important gastroprotective compound against gastric mucosal injury induced by ischemia–reperfusion in rats. According to Moreira et al. (2004), the antioxidant capacity of the flavonoids has therapeutic potential for treating gastric and duodenal diseases. Tannins are potent scavengers of peroxyl radicals and can also interact with mucus proteins, improving their cytoprotective effect by forming a protein lining over the gastrointestinal mucosa (Okuda, 2005).

However, a central role for NO in the gastroprotective effect of MHs extract may also explain MHs' ability to protect against I/R. Systemic nitrite reduction is greatly enhanced during hypoxic/ischemic conditions (Lundberg and Weitzberg, 2010). Several studies in animal models of ischemia and reperfusion indicate that nitrite and nitrate can modulate hypoxic signalling. Administration of nitrite and nitrate protects against ischemia–reperfusion injury in gastric ulcers (Lundberg and Weitzberg, 2010). Hence, the antioxidant activity and the involvement of NO may be among the most important defensive factors involved in the anti-ulcerogenic effects of MHs.

Extracts prepared from bark of the *Hymenaea courbaril* (jatobá), a medicinal plant from the same genus, contain many bioactive compounds such as procyanidins (Sasaki et al. (2009)). Procyanidins (condensed tannins), a subclass of flavonoids, are widely distributed in a variety of plant species (Santos-Buelga and Scalbert, 2000). Procyanidins have been reported to exhibit a variety of biological activities, including strong antioxidative or free radical scavenging effects (Takahata et al., 2001). Indeed, the fruit protective effect is most likely mediated by diverse nutrients, including antioxidant vitamins, minerals, phytochemicals, and fibre, among others (Marin et al., 2009). Zayachkivska et al. (2005) demonstrated polyphenolic substances to have anti-inflammatory activity in the gastrointestinal tract (GIT), promoting tissue repair though the expression of several growth factors, antioxidant activity and the scavenging of reactive oxygen species (ROS).

The gastroprotective and cicatrising effects of MHs and diet with fruit pulp on peptic ulcers observed in this study can be attributed to the presence of tannins and flavonoids in *H. stigonocarpa* given the antioxidant effects of this species. Thus, to explore new compounds with antioxidant properties from medicinal plants is important, since most of the synthetic drugs available on the market to treat peptic ulcers produces many undesirable side effects, when used long term.

4. Conclusions

Based on these data, we conclude that the methanolic extract of *Hymenaea stigonocarpa* bark (MHs) has antidiarrheal, gastroprotective and cicatrising activities, and the diet with pulp fruits also demonstrate healing effects. The protective action of the extract against various ulcerogenic agents is related to the involvement of NO. The gastroprotective effect of the extract was accompanied by prevention of GSH depletion and inhibition of myeloperoxidase activity in gastric and duodenal mucosa. These observed effects can be attributed to the antioxidant effect due to the presence of condensed tannins and flavonoids in the
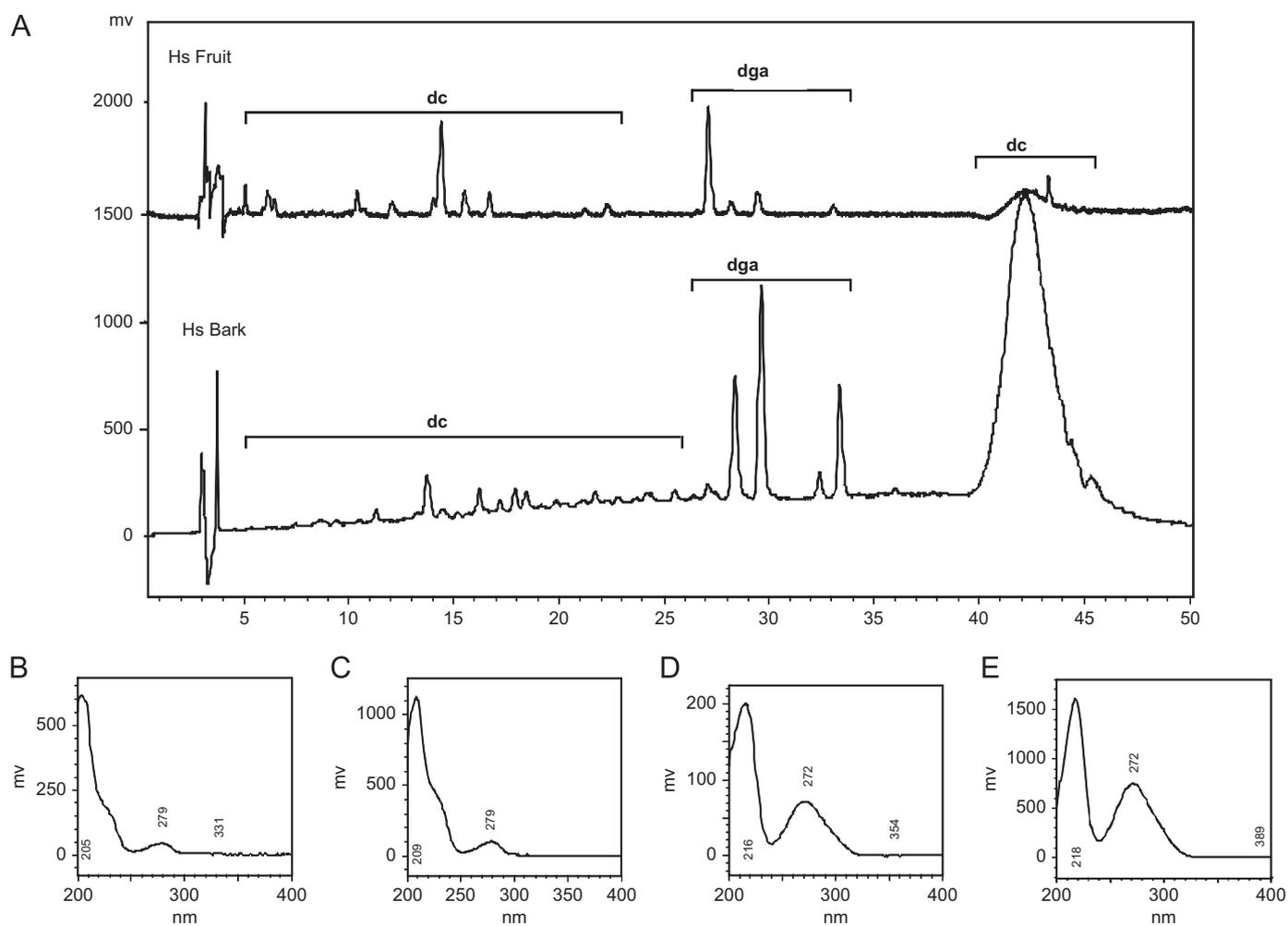
Fig. 6. HPLC-PAD fingerprints of *H. stigonocarpa* barks and pulp fruits (A). UV spectrum pattern derivatives catechin (dc) (B), compounds identified as dc (C), UV spectrum pattern derivatives gallic acid (dga) (D) and compounds identified as dga. Phenomenex Synergi Hydro column (C_{18}, 250×4.6 mm d. i., 4 μm). Eluent A: $H_2O + 0.1\%$ TFA; eluent B: ACN + 0.1% TFA. Ranging from 10 to 23% of B in 35 min, 23–90% of B in 50 min at a flow rate of 1 ml min^{-1}. $\lambda = 254$ nm.

bark and fruit of *H. stigonocarpa*. The cicatrising action of MHs (7 and 14 days) and diet with fruit pulp (14 days) in gastric ulcers and diet with fruit pulp (7 days) in duodenal ulcers demonstrated the powerful healing effect of both the extract and the diet with fruit pulp. Our findings suggest that the fruit pulp of *H. stigonocarpa* may represent an important dietary source for the treatment of peptic ulcers.

Acknowledgements

References

Abdallah, D.M., El- Abhar, H.S., Abdel- Aziz, D.H., 2009. Tempol, a membrane-permeable radical scavenger, attenuates gastric mucosal damage induced by ischemia-reperfusion: a key role for superoxide anion. European Journal of Pharmacology 603, 93–97.

Abdel-Kader, M., Berger, J.M., Slebodnick, C., Hoch, J., Malone, S., Wisse, J.H., Werkhoven, M.C.M., Mamber, S.D., Kingston, G.I., 2002. Isolation and absolute configuration of *ent*-halimane diterpenoids from *Hymenaea courbaril* from the Suriname rain forest. Journal of Natural Products 65, 11–15.

Ancha, H., Ojeas, H., Tedesco, D., Ward, A., Harty, R.F., 2003. Somatostatin-induced gastric protection against ethanol: involvement of nitric oxide and effects on gastric mucosal blood flow. Regulatory Peptides 110, 107–110.

Aguiar, J.C.D., Santiago, G.M.P., Lavor, P.L., Veras, H.N.H., Ferreira, Y.S., Lima, M.A.A., Arriaga, A.M.C., Lemos, T.L.G., Lima, J.Q., De Jesus, H.C.R., 2010. Constituents and larvicidal activity of *Hymenaea courbaril* fruit peel. Natural Product Communications 5, 1977–1980.

Anderson, M., 1985. Determination of glutathione disulfide in biological samples. Methods Enzymmology 113, 548–555.

Awounters, F., 1978. Delay of castor oil diarrhein rats: a new way to evaluate inhibitors of prostaglandin synthesis. Journal of Pharmacy and Pharmacology 30, 41–45.

Bandyopadhyay, D., Biswas, K., Bhattacharyya, M., Reiter, R.J., Banerjee, R.K., 2002. Involvement of reactive oxygen species in gastric ulceration: protection by melatonin. Indian Journal of Experimental Biology 40, 693–705.

Brandão, M., 1993. Plantas medicamentosas de uso popular dos cerrados mineiros. Daphne, Belo Horizonte 3, 11–20.

Buffinton, G.D., Doe, W.F., 1995. Depleted mucosal antioxidant defences in inflammatory bowel disease. Free Radical Biology & Medicine 19, 911–918.

Come, S.J., Morrisey, S.M., Woods, R.J., 1974. A method for the quantitative estimation of gastric barrier mucus. Journal of Physiology 242, 116–117.

Cunningham, A., Martin, S.S., Langenheim, J.H., 1973. Resin acids from two amazonian species of *Hymenaea oblongifolia*. Phytochemistry 12, 633–635.

Cunningham, A., Martin, S.S., Langenheim, J.H., 1974. Labd-13-en-8-ol-15-oic acid in the trunk resin of amazonian *Hymenaea courbaril*. Phytochemistry 13, 294–295.

El-Abhar, H.S., Abdallah, D.M., Saleh, S., 2003. Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. Journal of Ethnopharmacology 84, 251–258.

Glavin, G.B., Szabo, S., 1992. Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. FASEB Journal 6, 825–831.

Guidobono, F., Pagani, F., Ticozzi, C., Sibilia, V., Pecile, A., Netti, C., 1997. Protection by amylin of gastric erosions induced by indomethacin or ethanol in rats. British Journal of Pharmacology 120, 581–586.

Gomes, R.C., Bonamin, F., Darin, D.D., Seito, L.N., Di Stasi, L.C., Dokkedal, A.L., Vilegas, W., Brito, A.R.M.S., Hiruma-Lima, C.A., 2009. Antioxidative action of methanolic extract and buthanolic fraction of Vochysia tucanorum Mart. in the gastroprotection. Journal of Ethnopharmacology 121, 466–471.

Grandi, J.M., Trindade, J.A., Pinto, M.J.F., Ferreira, L.L., Catella, A.C., 1989. Plantas medicinais de Minas Gerais, Brasil. Acta Botanica Brasílica 3, 185–224.

Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics 96, 67–202.

Holzer, P., 1998. Neural emergency system in the stomach. Gastroenterology 114, 823–839.

Klein Jr, L.C., Gandolfi, R.B., Santin, J.R., Lemos, M., Filho, V.C., Faloni de Andrade, S., 2010. Antiulcerogenic activity of extract, fractions, and some compounds obtained from Polygala cyparissias St. Hillaire & Moquin (Polygalaceae). Naunyn-Schmiedeberg's Archives of Pharmacology 381, 121–126.

Khomenko, T., Szabo, S., Deng, X., Ishikawa, H., Anderson, G.J., McLaren, G.D., 2009. Role of iron in the pathogenesis of cysteamineinduced duodenal ulceration in rats. American Journal of Physiology Gastrointestinal and Liver Physiology 296, 1277–1286.

Khoo, S.F., Oehlschlager, A.C., Ourisson, G., 1973. Structure and stereochemistry of the diterpenes of Hymenaea courbaril (Caesalpinoideae) seed pod resin. Tetrahedron 29, 3379–3388.

Kobata, A., Kotani, T., Komatsu, Y., Amagase, K., Kato, S., Takeuchi, K., 2007. Dual action of nitric oxide in the pathogenesis of ischemia/reperfusion- induced mucosal injury in mouse stomach. Digestion 75, 188–197.

Konturek, S.J., Dembinski, A., Warzecha, Z., Brzozowski, T., Gregory, H., 1988. Role of epidermal growth factor in healing of chronic gastroduodenal ulcers in rats. Gastroenterology 94, 1300–1307.

Krawisz, J.E., Sharon, P., Stenson, W.F., 1984. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in the rat and hamster model. Gastroenterology 87, 1344–1350.

Laine, L., Takeuchi, K., Tarnawski, A., 2008. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology 135, 41–60.

Langenheim, J.H., Hall, G.D., 1983. Sesquiterpene deterrence of a leaf-tying lepidopteran, Stenoma ferrocanella, on Hymenaea stigonocarpa in Central Brazil. Biochemical Systematics and Ecology 11, 29–36.

Lee, H.T., Lee, Y.J., Chung, S.J., Shim, C.K., 2000. Effect of prokinetic agents, cisapride and metoclopramide, on the bioavailability in humans and intestinal permeability in rats of ranitidine, and intestinal charcoal transit in rats. Research Communications in Molecular Pathology and Pharmacology 108, 311–323.

Lima, N.N., Rechia, C.G.V., Ganter, J.L.M.S., Reicher, F., Sierakowski, M.R., 1995. Oligosaccharides derived from the xyloglucan isolated from the seeds of Hymenaea courbaril var. stilbocarpa. International Journal of Biological Macromolecules 17, 413–415.

Lundberg, J.O., Weitzberg, E., 2010. NO-synthase independent NO generation in mammals. Biochemical and Biophysical Research Communications 396, 39–45.

Marin, A.M.F., Siqueira, E.M.A., Arruda, S.F., 2009. Minerals, phytic acid and tannin contents of 18 fruits from the Brazilian savanna. International Journal of Food Sciences and Nutrition 60, 180–190.

Marsaioli, A.J., Leitão Filho, H.F., Campello, J.P., 1975. Diterpenes in the bark of Hymenea coubaril. Phytochemistry 14, 1882–1883.

Matsuda, H., Li, Y., Yoshikawa, M., 1999. Gastroprotection of Escins IA, IB, IIA, and IIB on ethanol-induced gastric mucosal lesions in rats. European Journal of Pharmacology 373, 63–70.

Matuda, T.G., Netto, F.M., 2005. Caracterização química parcial da semente de Jatobá-do-cerrado (Hymenaea stigonocarpa Mart.). Ciência e Tecnologia de Alimentos 25, 353–357.

Moreira, A.J., Fraga, C., Alonso, M., Collado, P.S., Zetller, C., Marroni, C., Marroni, N., González-Gallego, J., 2004. Quercetin prevents oxidative stress and NF-kappaB activation in gastric mucosa of portal hypertensive rats. Biochemical Pharmacology 68, 1939–1946.

Morimoto, Y., Shimohara, K., Oshima, S., Sukamoto, T., 1991. Effects of the new anti-ulcer agent kb-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. Japanese Journal of Pharmacology 57, 495–505.

Mukherjee, P.K., Saha, K., Murugesan, T., Mandal, S.C., Pal, M., Saha, B.P., 1998. Screening of antidiarrhoeal profiles of some plant extracts of specific region of West Bengal, India. Journal of Ethnopharmacology 60, 85–89.

Nogueira, R.T., Shepherd, G.J., Laverde Jr, A., Marsaioli, A.J., Imamura, P.M., 2001. Clerodane-type diterpenes from the seed pods of Hymenaea courbaril var. stilbocarpa. Phytochemistry 58, 1153–1157.

Okabe, S., Roth, J.L., Pfeiffer, C.J., 1971. A method for experimental, penetrating gastric and duodenal ulcers in rats. Observations on normal healing. The American Journal of Digestive Diseases 16, 277–284.

Okabe, S., Amagase, K., 2005. An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. Biological and Pharmaceutical Bulletin 28, 1321–1341.

Okuda, T., 2005. Systematics and health effects of chemically distinct tannins in medicinal plants. Phytochemistry 66, 2012–2031.

Olfert, E.D., Cross, B.M., McWilliam, A.A., 1993. Guide to the Care and Use of Experimental Animals. Ottawa, Ontario. Canadian Council on Animal Care, 1–213.

Piper, H.M., Meuter, K., Schafer, C., 2003. Cellular mechanisms of ischemia–reperfusion injury. Annals of Thoracic Surgery 75, S644–S648.

Rafatullah, S., Tariq, M., Al-Yahya, M.A., Mossa, J.S., Ageel, A.M., 1990. Evaluation of tumeric (Curcuma longa) for gastric and duodenal antiulcer activity in rats. Journal Ethnopharmacology 29, 25–34.

Rao, Ch.V., Vijayakumar, M., 2007. Protective effect of (+)-catechin against gastric mucosal injury induced by ischaemia–reperfusion in rats. Journal of Pharmacy and Pharmacology 59, 1103–1107.

Rastogi, L., Patnaik, G.K., Dikshit, M., 1998. Free radicals and antioxidant status following pylorus ligation induced gastric mucosal injury in rats. Pharmacological Research 38, 125–132.

Rohr, G.E., Meier, B., Sticher, O., 2000. Evaluation of different detection modes for the Analysis of procyanidins in leaves and flowers of Crataegus spp. Part I. Diode Array and Electrochemical Detection. Phytochemical Analysis 11, 106–112.

Santos-Buelga, C., Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds–nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture 80, 1094–1117.

Sasaki, K., Matsukura, Y., Shijima, K., Miyake, M., Fujiwara, D., Konishi, Y., 2009. High-performance liquid chromatografhic purification of oligomeric procyanidins, trimers up to nonamers, derived from the bark of jatobá (Hymenaea courbaril). Bioscience Biotechnology and Biochemistry 73, 1274–1279.

Seiler, M., Szabo, S., Ourteff, S., Mccomb, D.J., Kovacs, K., Reichlin, S., 1983. The effect of the duodenal ulcerogen cysteamine on somatostatin and gastrin cells in the rat. Experimental and Molecular Pathology 39, 207–218.

Shay, H., Komarov, S.A., Fels, S.S., Meranze, D., Gruenstein, M., Siplet, H., 1945. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 5, 43–61.

Sies, H., 1999. Glutathione and its role in cellular functions. Free Radical Biology and Medicine 27, 916–921.

Silva, M.R., Silva, M.S., Martins, K.A., Borges, S., 2001. Utilização tecnológica dos frutos de jatobá-do-cerrado e de jatobá-da-mata na elaboração de biscoitos fontes de fibra alimentar e isentos de açúcares. Ciência e Tecnologia de Alimentos 21, 176–182.

Souza Brito, A.R., 1994. Manual de ensaios toxicológicos "in vivo". Ciências médicas. Unicamp, Campinas 15–22.

Stickney, J.C., Northup, D.W., 1959. Effect of gastric emptying upon propulsive motility of small intestine of rat. Proceedings of the Society for Experimental Biology and Medicine 101, 582–583.

Stocks, J., Gutterid, J.M., Sharp, R.J., Dormandy, T.L., 1974. Assay using brain homogenate for measuring the antioxidant activity of biological fluids. Clinical Science and Molecular Medicine 47, 215–222.

Szabo, S., 1978. Duodenal ulcer disease. Animal model: cysteamine-induced acute and chronic duodenal ulcer in the rat. The Journal of Pathology 93, 273–276.

Szabo, S., Vattay, P., 1990. Experimental gastric and duodenal ulcers. Advances in pathogenesis. Gastroenterology Clinical North America 19, 67–85.

Takahata, Y., Ohnishi-Kameyama, M., Furuta, S., Takahashi, M., Suda, I., 2001. Highly polymerized Procyanidins in brown soybean seed coat with a High Radical-Scavenging activity. Journal of Agricultural and Food Chemistry 49, 5843–5847.

Townsend, D., Tew, M., Tapiero, H., K.D., 2003. The importance of glutathione in human disease. Biomedicine and Pharmacotherapy 57, 145–155.

Tulassay, Z., Herszényi, L., 2010. Gastric mucosal defense and cytoprotection. Best Practice and Research Clinical Gastroenterology 24, 99–108.

Ueda, S., Yoshikawa, T., Takahashi, S., Ichikawa, H., Yasuda, M., Oyamada, H., Tanigawa, T., Sugino, S., Kondo, M., 1989. Role of free radicals and lipid peroxidation in gastric mucosal injury induced by ischemia–reperfusion in rats. Scandinavian Journal of Gastroenterology 162, 55–58.

Veljaca, M., Lesch, C.A., Pllana, R., Sanchez, B., Chan, K., Guglietta, A., 1995. BPC-15 reduces Trinitrobenzene sulfonic acid-induced colonic damage in rats. The journal of Pharmacology and Experimental Therapeutics 272, 417–422.

Wach, A., Pyrzynska, K., Biesaga, M., 2007. Quercetin content in some food and herbal samples. Food Chemistry 100, 699–704.

Wagner, H., 2006. Multitarget therapy – The future of treatment for more than just functional dyspepsia. Phytomedicine 13SV, 122–129.

Witaicenis, A., Seito, L.N., Di Stasi, L.C., 2010. Intestinal anti-inflammatory activity of esculetin and 4-methysesculetin in the trinitrobenzenesulphonic acid model of rat colitis. Chemico-Biological Interactions 186, 211–218.

Zapata-Colindres, J.C., Zepeda-Gómez, S., Montaño-Loza, A., Vázquez-Ballesteros, E., Villalobos, J.J., Valdovinos-Andraca, F., 2006. The association of Helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease. Canadian Journal of Gastroenterology 20, 277–280.

Zayachkivska, O.S., Konturek, S.J., Drozdowicz, D., Konturek, P.C., Brzozowski, T., Ghegotsky, M.R., 2005. Gastroprotective effects of flavonoids in plant extracts. Journal of Physiology and Pharmacology 56, 219–231.