

Gastroprotective effect of the ethanolic extract of *Parkia platycephala* Benth. leaves against acute gastric lesion models in rodents

Hélio B Fernandes¹, Francilene V Silva¹, Flávia Franceli B Passos¹, Roosevelt D S Bezerra², Mariana H Chaves², Francisco A Oliveira¹ and Rita C Meneses Oliveira¹ *

Medicinal Plants Research Center¹, Department of Chemistry², Federal University of Piauí, Teresina, PI, Brazil.

ABSTRACT

Parkia platycephala Benth. (Leguminosae - Mimosoideae), popularly known as “visgueira”, fava bean tree or “fava-de-bolota”, is widely found in the Northern and Northeastern regions of Brazil. Its pods are used as cattle food supplement in the drought period. Compounds with a gastroprotective activity were obtained from the genus *Parkia*. Therefore, this study aimed at investigating the gastroprotective effect of the ethanolic extract of *Parkia platycephala* Benth. leaves (Pp-EtOH), as well as evaluating its possible mechanisms of action in experimental ulcer induction models. Lesions were induced by absolute ethanol, ethanol-HCl, ischemia-reperfusion and indomethacin in rodents. Pp-EtOH showed a protective effect in the lesion models (66, 48 and 52 %, respectively), but it was not able to protect gastric mucosa against indomethacin-induced lesions. Results show a possible participation of the NO-synthase pathway in the gastroprotection and an antioxidant activity, by the increase of the catalase activity. The participation of prostaglandins and potassium channels sensitive to ATP in the gastroprotective effect of Pp-EtOH seems less likely to occur. More comprehensive studies, therefore, should be carried out to elucidate the antiulcerative effects of this promising natural product against this gastrointestinal disorder.

Key terms: antioxidant, ethanol, gastroprotection, nitric oxide, *Parkia platycephala*, ulcer.

1. INTRODUCTION

Ulcer is a deep mucous lesion, with the destruction of the components of the epithelial and conjunctive tissues, including subepithelial myofibroblasts, smooth muscle cells, vessels and nerves [Milani and Calabro, 2001]. Ulcers are likely to result from different pathogenic mechanisms and, non-dependant on its etiology, are formed when an imbalance between the aggressive factors of the mucous membrane, endogenous (chloride acid and pepsin) or exogenous (non-steroidal anti-inflammatory, smoking and alcohol) and the gastric mucous membrane protective factors (mucus, bicarbonate, prostaglandins, blood flow, nitric acid) occur [Glavin and Szabo, 1992; Wallace and Granger, 1996].

Usually, antiulcerogenic compounds obtained from plants exert their effects by stimulating the protective factors of gastric mucosa due to increased synthesis of prostaglandins, by stimulating the secretion of mucus and bicarbonate, through inhibition of acid secretion by interacting with different receptors, or regulating enzymes or hormones involved in the secretory process (Borrelli and Izzo, 2000).

Parkia platycephala Benth. (Leguminosae - Mimosoideae), popularly known as “visgueira” or “fava-de-bolota”, is an arboreal species found all over the Northern and Northeastern part of Brazil, and its pods are widely used in cattle breeding as food [Alves, 2004; Nascimento and Machado, 2007].

There are no studies or popular indications proving the activity of this species against gastrointestinal disorders. However, antioxidant and gastroprotective compounds have been verified in some species of the genus *Parkia*: triterpenes β -amirin and lupeol have been isolated from the *Parkia*

pendula, and campesterol, stigmaterol, β -sitosterol, ferulic acid, catechin and epicatechin have been isolated from *Parkia biglobosa* [Tringali, Spatafora and Longo, 2000; Akintayo, 2004]. Therefore, the objective of this work is to investigate the presence of gastroprotective activity in the ethanolic extract of *Parkia platycephala* Benth. (Pp-EtOH) leaves and evaluate the possible action mechanisms.

To study Pp-EtOH-induced gastroprotection, we used ethanol and HCl/ethanol models of acute gastric lesions. These models allowed us to assess whether the studied extract stimulates or maintains the resistance of the gastric mucosa against the lesions produced by exogenous substances, a mechanism which can be attributed to many factors present in the stomach, known as protective factors of gastric mucosa (Wallace, 2001). Lesions caused by ethanol, HCl/ethanol or ischemia-reperfusion injuries are hemorrhagic, resulting from intense production of free radicals causing damage to gastric epithelial cells. This damage leads to constriction of gastric mucosa veins and arteries, producing congestion, inflammation and tissue injury (Repetto and Liesuy, 2002). As well, ischemia weakens gastric mucosa resistance to acid and increases its susceptibility to damage. After reperfusion, ROS promote lipid peroxidation, which results, in combination with gastric acid secretion, in damage to the gastric mucosa (Brzozowski et al., 2000).

One of the most important components of mucosal defense is the blood flow response to irritants. Endothelial cells from these beds produce potent vasodilators, such as nitric oxide (NO) and prostacyclin (PGI₂), which protect the gastric mucosa. Sugita (2003) reports the cytoprotective effects of nitric oxide (NO) on intestinal lesions caused by ethanol. In order to assess nitric oxide cytoprotective effect

* Corresponding author: Rita de Cássia Meneses Oliveira, Medicinal Plants Research Center, Federal University of Piauí, Minister Petrônio Portela Campus, 64049-550 Teresina-PI, Brazil. Phone: +55 86 3215 58 72, E-mail: menesesoliveira@gmail.com, ritameneses@ufpi.br

in gastric injury, it is used a NO-synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME). Nitric oxide (NO) has been recognized as a key mediator in gastric defense mechanisms, because it stimulates mucus production, inhibits neutrophil adherence to endothelial cells and especially increases blood flow to the gastric mucosa (Coruzzi et al., 2000).

The ATP dependent potassium channels (K_{ATP}) belong to a large family of membrane proteins and are activated by ligands. In the vascular system, they are implicated in vascular smooth muscle relaxation, playing a role in blood pressure control. The literature shows that K_{ATP} channels are involved in a variety of pathophysiological functions in the stomach, for example, regulation of blood flow and gastric acid secretion (Toroud et al., 1999).

The ulcer induced by NSAIDs, such as indomethacin is a process that involves multiple mechanisms. Indomethacin promotes depletion of cytoprotective prostaglandins in gastric mucosa by inhibiting the enzyme cyclooxygenase, microcirculatory disturbances with reduced blood flow, gastric hypermotility, topical irritation and interference in tissue repair (Filaretova et al., 2002). In this model there is an increased vascular permeability and neutrophils infiltration in the development of lesions (Jainu and Devi, 2006).

2. MATERIALS AND METHODS

2.1. Animals

All experiments were performed on male Swiss mice (20-30 g) and Wistar rats (180-200 g) with access to food and water, in a 12-hour day-night cycle at 24 ± 2 °C obtained from the Vivarium-NPPM, UFPI, Teresina city, Piauí State, Brazil. Before each experiment, the animals fasted for 24 hours with free access to water. The UFPI *Research Ethics Committee* approved all of the applied protocols (23/08).

2.2. Vegetal material and extract preparation

Leaves of plants were collected in Timon, Maranhão State, and identified with the help of the Biology Department. Voucher specimens have been deposited at the herbarium Graziela Barroso - UFPI, Teresina - Piauí State (Brazil), number TEPB - 15.553. Air-dried and powdered leaves (2.263.2 g) were extracted by EtOH 95% and macerated at room temperature. The solvent was evaporated and the extract concentrated (Pp-EtOH), resulting in 547 g (24.2 % yield).

2.3. Study of potential toxic effects of *Parkia platycephala* in vivo and in vitro

To determine acute toxicity, we used the method described by Miller and Tainter [1944]. Male and female mice ($n = 10$) received Pp-EtOH at a single dose of 2000 mg/kg and the control group received saline (10 mL/kg) by gavage. Mortality, body weight and behavioral screening were recorded daily for 14 days after the treatment. To assess cellular toxicity in rat erythrocytes, the method proposed by Rangel et al. [1997] was used with modifications. The rat blood was collected and added to a $CaCl_2$ 10 mM saline solution. The erythrocytes were washed twice by

centrifugation at 3000 rpm/3min. The last centrifugation sediment was resuspended in saline and bubbled with carbogen air. Crescent concentrations of Pp-EtOH (1-1000 µg/ml) were added to test tubes in triplicate. In positive control 100 µL of Triton X-100 1% (100% hemolysis) was added. Some 100 µL of washed erythrocytes was added to each test tube and the volume completed at 0.5 mL for 1 hour with constant shaking. The samples were then centrifuged (3000 rpm/3min) and the hemolysis was assayed on a spectrophotometer at 540 nm.

2.4. Study of the anti-ulcer action of *P. platycephala* in the ethanol induced ulcer model

Mice were pretreated orally with vehicle (NaCl 0.9 %), positive control (carbenoxolone 100 mg/kg) or Pp-EtOH (62.5, 125 or 250 mg/kg). After one hour, they were treated by absolute ethanol (0.2 mL/animal, p.o.) according to Robert et al. [1979]. After 30 minutes, the animals were killed and their stomachs removed and opened along the greater curvature to determine the lesion area (mm²). The lesion area was calculated by the following formula: lesion area (%) = lesion area (mm²) × 100/total area (mm²)

2.5. Role of nitric oxide and K_{ATP} channels in the gastroprotective effect of Pp-EtOH in the ethanol induced ulcer model

Mice were pretreated with vehicle, positive control (L-arginine, 600 mg/kg, i.p.) and Pp-EtOH (250 mg/kg, p.o.) alone, or in their combinations with L-NAME (20 mg/kg, i.p.) prior to induction of gastric damage with absolute ethanol. While Pp-EtOH was administered 1 hour before, L-NAME and L-arginine were given 30 min prior to ethanol [Olinda et al., 2008].

In another study, mice were pretreated with vehicle, positive control diazoxide (3 mg/kg, i.p.), Pp-EtOH (250 mg/kg, p.o.) alone, or in their combinations with glibenclamide (5 mg/kg, i.p.) prior to the oral administration of absolute ethanol. Pp-EtOH was given 1 h before, whereas diazoxide was administered 30 min prior to ethanol or glibenclamide. Glibenclamide was administered 30 min before Pp-EtOH [Olinda et al., 2008].

2.6. Study of the anti-ulcer action of *P. platycephala* in the HCl/ethanol induced ulcer model

Groups of mice were pretreated orally with vehicle, carbenoxolone (100 mg/kg) or Pp-EtOH (62.5, 125 or 250 mg/kg). After one hour, the animals were treated with an HCl/ethanol solution, according to Mizui and Doteuchi [1983]. Past 30 minutes, the mice were sacrificed and their stomachs removed and opened along the greater curvature to determine the lesion area (mm²).

2.7. Study of the anti-ulcer action of *P. platycephala* in the ischemia-reperfusion induced ulcer model

The method proposed by Yoshikawa [1989] was used. Rats were treated with vehicle, positive control (N-acetyl cysteine (NAC), 750 mg/kg, i.p.) or Pp-EtOH (250 mg/kg p.o.). After one hour, ischemia-reperfusion erosions were produced in rats. Briefly, under tiopental anesthesia (50 mg/kg, i.p.), the

celiac artery was clamped with a small device for 30 minutes. After this, the clamping device was removed to obtain the reperfusion. After one hour of reperfusion, the animals were killed and their stomachs removed and opened along the greater curvature to determine the lesion area (mm²).

2.8. Study of the anti-ulcer action of *P. platycephala* in the indomethacin induced ulcer model

In this model, rats were treated orally with vehicle, positive control (cimetidine 100 mg/kg) and Pp-EtOH (250 mg/kg). One hour after this treatment, indomethacin, suspended in 5% bicarbonate water, was administered in a dose of 30 mg/kg s.c. (0.1 mL/100 g) [Bhargava, Gupta and Tangri, 1973]. After 6 hours of indomethacin treatment, the stomachs of the animals were removed, rinsed with formalin 1% and studied according to the standard procedure [Szabo et al., 1985].

2.9. Catalase activity on the anti-ulcer action of *P. platycephala* in the ethanol induced ulcer model

Stomachs previously submitted to absolute ethanol [Robert et al., 1979] were used to assess the role of catalase (CAT) in Pp-EtOH-induced mucosa protection. The determination of CAT activity present in the gastric mucosa was assayed by a modification of the spectrophotometric method of Beers and Sizer [1952]. Mice were pretreated with vehicle, positive control (N-acetyl cysteine (NAC), 750 mg/kg i.p.) or Pp-EtOH (250 mg/kg, p.o.). They were then treated with absolute ethanol (0.2 mL/animal, p.o.) [Robert et al., 1979]. The animals were killed and their stomachs removed and put in a homogenate ice-cold buffer (KH₂PO₄/K₂HPO₄, pH=7.4) and then centrifuged at 3000 rpm for 15 minutes. An approximately 0.05 M solution of hydrogen peroxide substrate was prepared with 0.05 M KH₂PO₄ buffer, pH 7.4 in distilled water. Aliquots of 0.1 ml of supernatant were added into 1.9 ml of substrate solution and the disappearance of hydrogen peroxide was followed spectrophotometrically at 240 nm.

2.10. Statistical analysis

The results are presented as the mean \pm S.E.M. 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.

3. RESULTS

3.1. Study of potential toxic effects of *Parkia platycephala* in vivo and in vitro

The investigation of the potential toxic effects of extracts obtained from leaves of *Parkia platycephala* Benth. aimed at establishing a safe and effective dose in the investigation of gastroprotective activity of the extract in models of acute gastric lesions.

The ethanolic extract obtained from the leaves of *Parkia platycephala* Benth. (Pp-EtOH) in the dosage of 2 g/kg, orally, did not demonstrate any sign of evident toxicity and it did not cause the deaths of the animals, within 72 hours. In the

evaluation of the cytotoxicity in rat erythrocytes, lytic activity was not evidenced up to the concentration of 100 μ g/mL of Pp-EtOH. In the greater concentration used, hemolysis in the erythrocytes was not greater than 5%, as compared to Triton-X-100 (100%). In the group of saline, as expected, there was no hemolysis (0 %), assuring a good margin of safety for the extract.

3.2. Study of the anti-ulcer action of *P. platycephala* in the ethanol induced ulcer model

It is known that the damage caused by ethanol in the gastric mucosa result from disturbances in microcirculation, ischemia and the appearance of free radicals, release of endothelin, degranulation of mast cells, inhibition of prostaglandin synthesis and consequent reduction in mucus production (Samonina et al, 2004). According to the results, Pp-EtOH (62.5, 125 or 250 mg/kg, p.o.) reduced the lesion area in the gastric mucous membrane dose-dependently (62.2 \pm 4.6; 35.1 \pm 7.0 and 22.3 \pm 8.7 mm², respectively). Pp-EtOH in the dosage of 250 mg/kg provided greater protection, compared to the control group (65.5 \pm 3.8 control vs. 22.3 \pm 8.7 mm² (Pp-EtOH), corresponding to 66% of lesion inhibition. (Fig. 1). The positive control did not show significant difference between Pp-EtOH 250 mg/kg (19.3 \pm 3.8 vs 22.3 \pm 8.7 mm², respectively) with inhibition of 70.22 %.

3.3. Role of nitric oxide and K_{ATP} channels on the gastroprotective effect of Pp-EtOH in the ethanol induced ulcer model

In mechanistic studies, separate experiments were realized to examine the role of nitric oxide and K⁺_{ATP} channel activation on the gastroprotective effect of *P. platycephala* (Pp-EtOH), using appropriate agonists (Olinda et al., 2008).

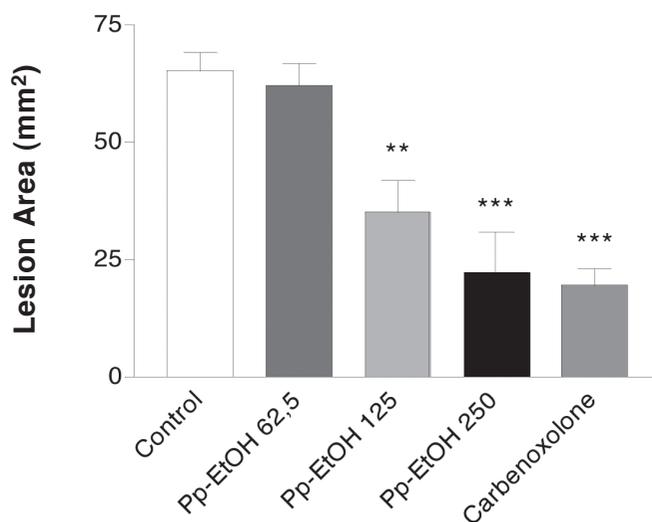


Fig. 1: Effect of Pp-EtOH (62.5, 125 and 250 mg/kg, p.o.) on ethanol absolute-induced gastric ulcer model. Data are presented as mean \pm S.E.M. From 6 - 8 animals. ** p < 0.01 vs. control (vehicle); *** p < 0.001 vs. control (vehicle) (ANOVA followed by Tukey's test).

The treatment with NG-nitro-L-arginine methyl ester (L-NAME) an inhibitor of nitric oxide synthase (20 mg/kg, i.p.) inhibited the protection of the mucous membrane offered by Pp-EtOH (250 mg/kg), increasing the lesion area significantly (22.4 ± 8.7 to 70.4 ± 11.7 mm²), with no significant difference in the control group (67.2 ± 6.1 mm²), as was observed in positive control, L-Arginine 600 mg/kg (8.9 ± 1.50 mm² to L-Arginine vs. 29.4 ± 2.9 mm² to L-Arginine + L-NAME), suggesting the participation of this method in the gastroprotection of this extract (Fig. 2).

After the treatment with glibenclamide, a K⁺ATP channel blocker (5 mg/kg i.p.), Pp-EtOH (250 mg/kg) reduced ethanol-induced lesions, without significant difference comparing to the non-blocked group (27.9 ± 5.7 vs. 22.4 ± 8.7 mm², respectively). In positive control (diazoxide 3 mg/kg) after the blockage with glibenclamide, an increase in gastric lesions (12.5 ± 1.6 to 22.6 ± 1.0 mm²) was observed, suggesting that these channels are probably not involved in the gastric protection evidenced by Pp-EtOH (Fig. 3).

3.4. Study of the anti-ulcer action of *P. platycephala* in the HCl/ethanol induced ulcer model

The presence of HCl in ethanol solution accelerates the progress of ulcerogenesis and enhances gastric injury (Sing et al., 2008). In this model, Pp-EtOH only promoted a significant level of gastroprotective activity with the dose of 250 mg/kg (50.3 ± 3.5 mm²) in comparison to the control group (97.5 ± 12.8 mm²), corresponding to a 48% inhibition of the lesion area (Fig. 4). Carbenoxolone, the positive control, has a significant reduction in lesion area compared to the control group (35.35 ± 7.89 vs 97.84 ± 12.97 mm², respectively).

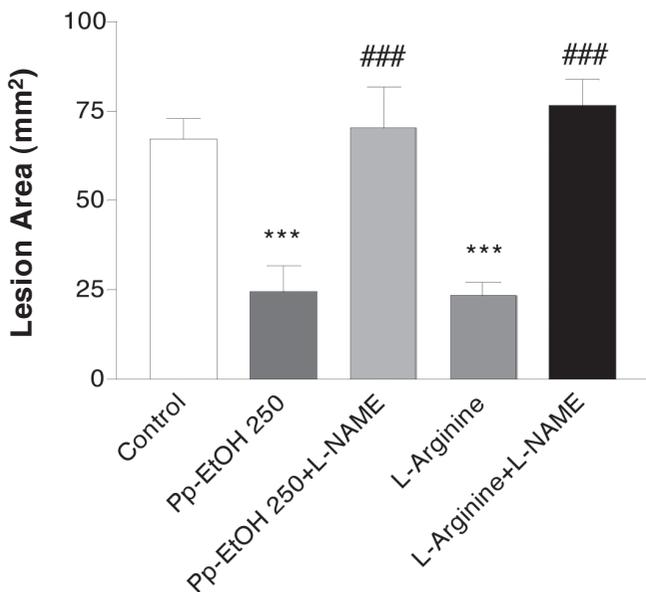


Fig. 2: Involvement of NO-synthase in the gastroprotective effect of Pp-EtOH (250 mg/kg) against ethanol-induced gastric damage in mice. Data are presented as mean ± S.E.M From 6 - 10 animals. *** p<0.001 vs. control (vehicle); ### p<0.001 vs. Pp-EtOH or L-Arginine alone (ANOVA followed by Tukey's test).

3.5. Study of the anti-ulcer action of *P. platycephala* in the ischemia-reperfusion induced ulcer model

Ischemia-reperfusion (I/R) induces gastric injury as a result of excessive formation of reactive oxygen species (ROS), neutrophils infiltration and gastric microvascular dysfunction (Derin et al., 2006). The Pp-EtOH extract (250 mg/kg, p.o.) reduced the ischemia-reperfusion-induced lesion area (40.9 ± 9.7 mm²) compared to the control group (89.6 ± 8.2 mm²), corresponding to a 52% inhibition. The positive NAC control (750 mg/kg, i.p.) was also effective in reducing the lesion areas (42.8 ± 7.6 mm²) (Fig. 5).

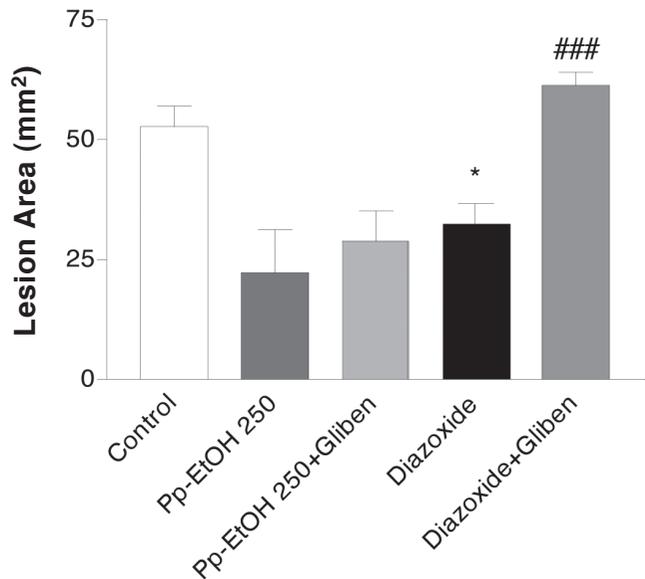


Fig. 3: Role of K⁺ATP channels in the gastroprotective effect of Pp-EtOH (250 mg/kg) against ethanol-induced gastric damage in mice. Data are presented as mean ± S.E.M From 6 - 9 animals. * p<0.05 vs. control (vehicle); ### p<0.001 vs. diazoxide alone (ANOVA followed by Tukey's test).

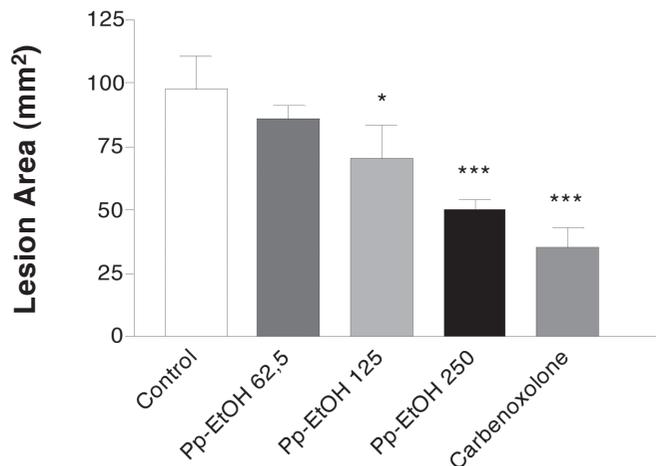


Fig. 4: Effect of Pp-EtOH (62.5, 125 and 250 mg/kg, p.o.) on HCl/ethanol-induced gastric ulcer model in mice. Data are presented as mean ± S.E.M. From 5 - 8 animals. * p < 0.05 vs. control (vehicle) ; *** p<0.001 vs. control (vehicle) (ANOVA followed by Tukey's test).

3.6. Study of the anti-ulcer action of *P. platycephala* in the indomethacin induced ulcer model

Indomethacin, a potent inhibitor of prostaglandin production, acts on gastric mucosa to promote an increase in acid production and decrease the formation of cytoprotective mucus, which may lead to ulcer formation (Walace and Devchand 2005). In this protocol, the indomethacin-induced gastric ulcers in the control group reached the values 5.2 ± 0.6 . Pp-EtOH (250 mg/kg, p.o.) was not able to protect the gastric mucous membrane from the ulcer lesions (5.4 ± 0.6). Cimetidine (100 mg/kg, p.o.), the positive control for the test, reduced the values of the lesions (0.7 ± 0.1) (Fig. 6).

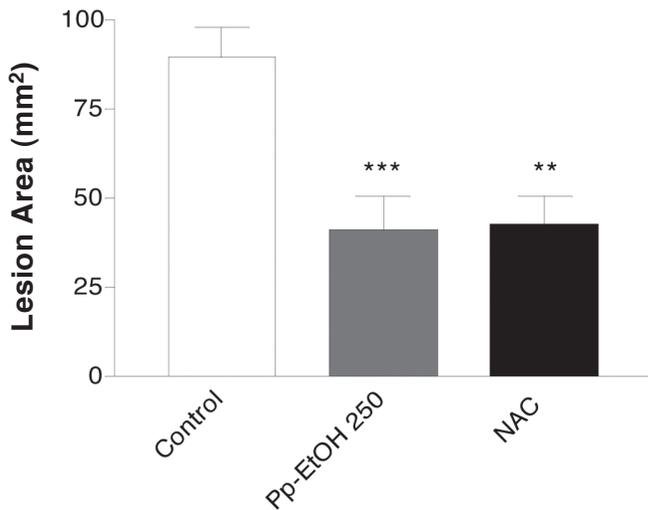


Fig. 5: Effect of Pp-EtOH (250 mg/kg, p.o.) on ischemia-reperfusion-induced gastric lesion model in rats. Data are presented as mean \pm S.E.M. From 8 - 10 animals. ** $p < 0.01$ vs. control (vehicle); *** $p < 0.001$ vs. control (vehicle) (ANOVA followed by Tukey's test).

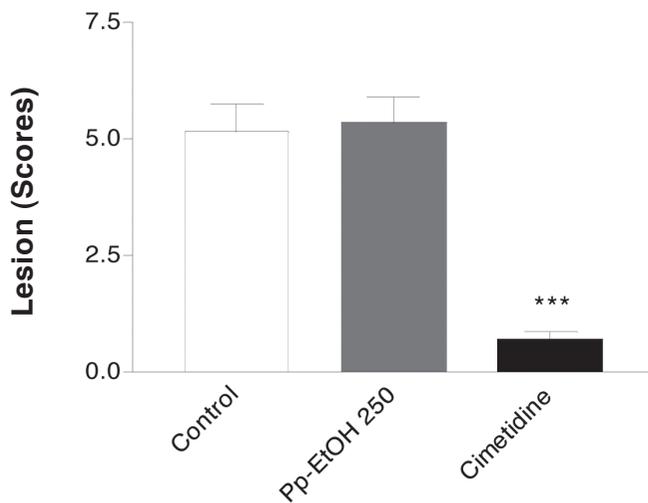


Fig. 6: Effect of Pp-EtOH (250 mg/kg, p.o.) on indomethacin-induced gastric ulcer model in rats. Data are presented as mean \pm S.E.M. From 6 animals. *** $p < 0.001$ vs. control (vehicle) (ANOVA followed by Tukey's test).

3.7. Anti-oxidant catalase activity of *P. platycephala* in the ethanol induced ulcer model

The release of oxygen-derived free radicals (ROS) has drawn attention as a possible pathogenic factor of gastric mucosal injury associated with ethanol consumption. Preventive antioxidants, such as catalase (CAT) and others, are the first line of defense against ROS (Alimi et al., 2010). Therefore, in the present study the activity of CAT in rat stomach tissue was studied to explore the effects of Pp-EtOH on oxidative damage. The Pp-EtOH extract (250 mg/kg) increased the activity of this enzyme significantly, in comparison to the control group (205.2 ± 17.3 vs. 67.9 ± 6.5 , respectively). The positive control (NAC, 750 mg/kg, i.p.) also increased catalase activity significantly to mean values similarly to the non-treated (SHAM) group (189.1 ± 17.8 vs. 162.7 ± 13.0 mM/min.100 mg of tissue, respectively) as shown in Fig. 7.

4. DISCUSSION

In the current study, the main findings reveal that the ethanolic extract obtained from the leaves of *Parkia platycephala* Benth. (Pp-EtOH) presents gastroprotective activity in acute models of gastric lesions in mice and rats.

The absence of acute toxicity in mice and cytotoxicity in rat erythrocytes for the Pp-EtOH extract ensure a good margin of safety for the doses of this extract in the experimental protocols of gastric lesions, as well as in the models used for the elucidation of the action mechanism.

Ethanol and acidified ethanol are among the most widely used agents in experimental models for the evaluation of the antiulcerative activity in mice [Robert et al., 1979; Mizui and Douteuchi, 1983].

Ethanol necroses the superficial cells of the gastric mucous membrane by precipitation of the cytoplasmic components, interrupting the function of the cell mucous membranes, with the participation of vasoactive mediators released, such as leukotrienes C₄ (LTC₄) and histamine [Jamal et al., 2006]. These mediators cause the submucous membranes to constrict with a subsequent blood flow stasis

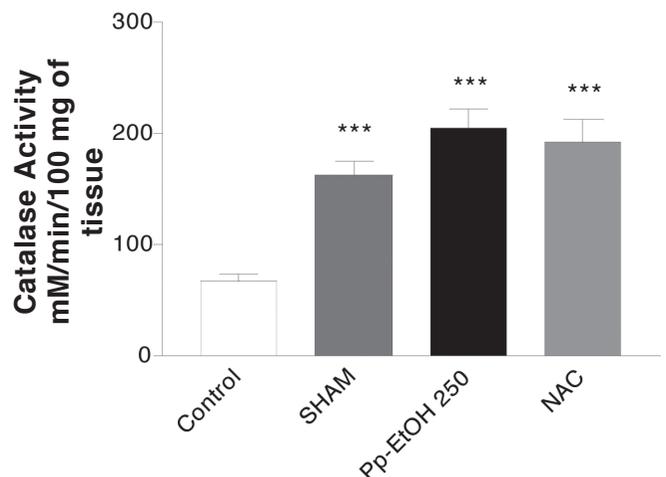


Fig. 7: Role of catalase in the anti-oxidant activity of Pp-EtOH (250 mg/kg) - against ethanol-induced gastric damage in mice. Data are presented as mean \pm S.E.M. From 10 animals. *** $p < 0.001$ vs. control (vehicle) (ANOVA followed by Tukey's test).

of the microcirculation in the mucous membrane, with the formation of edema, which may contribute to the increase of lesions in this model [Konturek et al., 1988; Wallace, 2001]. Ethanol induces the solubilization of the mucous constituents in the stomach, increases the flow of sodium and potassium in the lumen, increases the pepsin released, and decreases the tissue levels of DNA, RNA and proteins, leaving the mucous membrane unprotected, leading to an injury in the tissue [Robert et al., 1979]. HCl further deepens the necrosis and increase tissue injury [Singh et al., 2010]. In the results obtained, it was observed that Pp-EtOH reduced the lesions caused by these agents, suggesting the presence of gastroprotective activity in this extract.

Anti-inflammatory drugs, such as indomethacin, injure the gastric mucous membrane through the inhibition of the gastric cyclooxygenase enzyme, resulting in the decrease of the production of prostaglandins [Wallace et al., 2000]. Prostaglandins, such as E₂ and prostacyclins, act on the synthesis of mucus and bicarbonate, on the regulation of the acid secretion and on the blood flow of the gastric mucous membrane, and its inhibition critically compromises the gastric cytoprotection [Whittle, Oren-Wolman and Guth, 1985]. The increase of the production of these prostanoids provides greater resistance of the gastric mucous membrane against several agents, such as anti-inflammatory drugs [Wallace, 2001; Arun and Asha, 2008; Nguielefack et al., 2008]. According to the results obtained by the extract in the indomethacin-induced lesion model (Fig. 6), the prostaglandins are not likely to participate in the gastroprotection of Pp-EtOH, since this extract could not reduce the gastric lesion area in relation to the control.

The nitric oxide (NO), produced by the NO-synthase pathway, has been recognized as a fundamental mediator in the gastric defense mechanisms because it stimulates mucus production, inhibits the adherence of neutrophils to the endothelial cells, and especially because it increases the blood flow of the gastric mucous membrane [Coruzzi et al., 2000; Olinda et al., 2008]. The inhibition of the production of NO by the use of L-NAME interferes in the gastroprotective activity of several vegetal extracts [Ferreira et al., 2008; Gomes et al., 2009]. In this study, the pre-treatment with L-NAME, an NO-synthase inhibitor, reversed the gastroprotective effect of Pp-EtOH (Fig. 2). Since the NO-synthase pathway is important for the development of the protective activity, it is suggested that the NO may be mediating the gastroprotection of this extract.

Studies have reported that the activation of potassium channels sensitive to ATP (K⁺_{ATP}) reduces gastric lesions and that the use of glibenclamide, a blocker of these channels, intensifies them. The activation of these channels may occur in several ways, such as through the action of NO [Murphy and Brayden, 1995; Chandranath, Bastaki and Singh, 2002]. Treatment with glibenclamide did not inhibit the gastroprotective effect of Pp-EtOH, and the participation of K⁺_{ATP} in this effect is less likely to occur.

Gazzieri [2007] has demonstrated that ethanol increases the production of reactive oxygen species (ROS). In ischemia and reperfusion experiments, lesions appear in the cells of the gastric mucous membrane due to the formation of ROS [Ueda, Yoshikawa and Takahashi, 1989]. Among the most common ROS are the superoxide and the hydroxyl, responsible for lipid peroxidation and chemotaxis of

inflammatory cells with injuries [Yu et al., 2002; Ribeiro and Yoshida, 2005]. The cell is provided with several antioxidant mechanisms, such as the superoxide dismutase enzymes (SOD), catalase (CAT), and glutathione peroxidase, which detoxifies these ROS and reduces oxidative damage [Bafna and Balaraman, 2004; Alimi et al., 2010]. Pp-EtOH reduced the ischemia-reperfusion-induced lesions with an increase of catalase activity, showing an antioxidant effect, suggesting its efficacy in preventing the damages caused by ROS.

Preliminary phytochemical studies have reported the presence of a high percentage of phenolic compounds in the extract (Pp-EtOH) from leaves of *Parkia platycephala* Benth [Bezerra, Carvalho and Chaves, 2009]. Phenolic compounds are special metabolites that have significant antioxidant activity (Chun et al., 2005). It is reported in the literature that phenolic compounds participate significantly in the gastroprotection of a great number of vegetal extracts [Almeida et al., 2002]. Based on these facts, these compounds, also identified in the Pp-EtOH extract, may be part of the gastroprotective activity evidenced.

Pp-EtOH showed gastroprotective effects in the ethanol- and ethanol-HCl- and ischemia-reperfusion-induced gastric ulcer models, possibly due to the participation of the NO-synthase pathway and an increase of the catalase enzyme activity, with no apparent toxic effects. More comprehensive studies are necessary to explain the mechanisms of action and safety profile of this extract, aimed at the development of new anti-ulcerative therapies derived from this product.

In conclusion, the results of this study indicate that Pp-EtOH shows gastroprotective activity against gastric damage induced by ethanol and ethanol-HCl, but not by indomethacin, which is possibly mediated, in part, by the nitric oxide release, although there is no involvement of the activation of K⁺_{ATP} or of prostaglandins. This extract also exhibits protection against lesions by ischemia-reperfusion and an antioxidant effect, in part, by an increase in catalase activity. Further studies are necessary to elucidate the mechanisms of action and the safety profile of this extract.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support received from the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq/MCT/Projeto Universal (03/2009), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/Brazil.

The authors thank Edison S. Ferreira Filho and João Batista for translation of the text to English.

REFERENCES

- AKINTAYO ET, (2004) Characteristics and composition of *Parkia biglobbosa* and *Jatropha curcas* oils and cakes. *Biores Technol.* 92: 307-310.
- ALIMI H, HFAIEDH N, BOUONI Z, HFAIEDH M, SAKLY M, ZOURGUI L, RHOUMA KB. (2010). Antioxidant and antiulcerogenic activities of *Opuntia ficus indica* f. *inermis* root extract in rats. *Phytomedicine*, in press.
- ALMEIDA FRC, FONTAO DFS, OLIVEIRA RCM, CRUZ FJSM, NUNES PHM (2002) Antiulcerogenic activity of aqueous and ethanolic extract of bark of *Cenostigma macrophyllum* Tul. var. *acuminata* Teles Freire. In: Fesbe annual reunion, Salvador/Ba. Abstract book of XVII Fesbe annual reunion.
- ALVES AA (2004) Nutritive value of fava bean pod (*Parkia platycephala*

- Benth.*) to ruminants. Teresina: SAPIÊNCIA FAPEPI (2004) Avaluável in: <http://www.fapepi.pi.gov.br/sapiencia/resumo1.php>.
- ARUN M, ASHA VV. (2008) Gastroprotective effect of *Dodonaea viscosa* on various Experimental Ulcer Models *Bhopharmacol* 460-465.
- BAFNA PA, BALARAMAN R. (2004) Anti-ulcer and antioxidant activity of DHC-1, a herbal formulation. *J. Ethnopharmacol* 90: 123-127.
- BEER RJ, SIZER IW (1952) Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. *J Biol Chem.* 195: 133-140.
- BEZERRA RDS, CARVALHO AA, CHAVES MH (2009) Total phenolics and antioxidant activity of extract of leaves of *Parkia Platycephala* Benth. In: 32^a Brazilian Chemical Society Reunion. Fortaleza - CE.
- BHARGAVA KP, GUPTA MB, TANGRI KK (1973) Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. *Eur J Pharmacol*, 22: 191-195.
- BORRELLI, F, IZZO AA (2000) The plant kingdom as a source of anti-ulcer remedies. *Phytother Res* 14: 581-91.
- BRZOWSKI T, KONTUREK PCh, KONTUREK SJ, DROZDOWICZ D, KWIECIEŃ S, PADJO R, BIELANSKI W, HAHN EG (2000) Role of gastric acid secretion in progression of acute gastric erosions induced by ischemia-reperfusion into gastric ulcers *Eur J Pharmacol* 398: 147-158.
- CHANDRANATH SI, BASTAKI SM, SINGH J. (2002) A comparative study on the activity of lansoprazole, omeprazole and PD-136450 on acidified ethanol- and indomethacin-induced gastric lesions in the rat. *Clin Exp Pharmacol Physiol* 29: 173-180.
- CHUN SS, VATTEM DA, LIN YT, SHETTY K (2005) Phenolic antioxidants from cloral oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. *Process Biochem.* 40: 809-816.
- CORUZZI G, ADAMI M, MORINI G, POZZOLI C, CENA C, BERTINARIA M (2000) Antisecretory and gastroprotective activities of compounds endowed with H2 antagonistic and nitric oxide (NO) donor properties. *J Physiol Paris*, 94: 5-10.
- DERIN N, AGAC A, BAYRAM Z, ASAR M, IZGUT-YUSALIA VN (2006). Effects of L- carnitine on neutrophil-mediated ischemia-reperfusion injury in rat stomach. *Cell Biochem Funct.*, 24:437-442.
- FERREIRA MP, NISHIJIMA CM, SEITO LN, DOKKEDAL AL, LOPES-FERREIRA M, DI STASI LC, VILEGAS W, HIRUMA-LIMA CA (2008) Gastroprotective effect of *Cissus sicyoides* (Vitaceae): Involvement of microcirculation, endogenous sulphydryls and nitric oxide. *J Ethnopharmacol*, 117: 170-174.
- FILARETOVA L, TANAKA A, MIYAZAWA T, KATO S, TAKEUCHI K (2002) Mechanisms by which endogenous glucocorticoid protects against indomethacin-induced gastric injury in rats. *Am. J Physiol Gastrointest Liver* 283:1082-1089.
- GAZZIERI D (2007) Substance P released by TRPV1-expressing neurons produces reactive oxygen species that mediate ethanol-induced gastric injury. *Free Radic Biol Med*, 43: 581-589.
- GLAVIN G B, SZABO S (1992) Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. *Faseb J*, 6: 825-831.
- GOMES RC, BONAMINA F, DARIN DD, SEITO L (2009) Antioxidative action of methanolic extract and buthanolic fraction of *Vochysia tucanorum* Mart. in the gastroprotection. *J Ethnopharmacol*, 121: 466-471.
- JAINU M, DEVI C.S.S. (2006) Gastroprotective action of *Cissus quadrangularis* extract against NSAID induced gastric ulcer: role of proinflammatory cytokines and oxidative damage. *Chem.-Biol Interaction.* 161: 262-270.
- JAMAL A, KALIM JAVED, ASLAMA M, JAFRI MA (2006). Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats. *J Ethnopharmacol* 103: 149-153.
- KONTUREK SJ, BRZOWSKI T, DROZDOWICZ D, BECK G (1988) Role of leukotrienes in acute gastric lesions by ethanol, taurocholate, aspirin, platelet activating factor and stress in rats. *Dig Dis Sci* 33: 806-813.
- MILANI S, CALABRÒ A (2001) Role of growth factors and their receptors in gastric ulcer healing. *Micros Res Tech*, 53: 360-37.
- MILLER LC, TAINTER ML (1944) Estimation of the LD₅₀ and its error by means of logarithmic probit graph paper. *Proc Soc Exp Biol Med.* 57: 261-264.
- MIZUI T, DOUTEUCHI M (1983) Effect of polyamines on acidified ethanol gastric-lesions in rats. *Jpn J Pharmacol*, 33: 939-945.
- MURPHY EM, BRAYDEN JE (1995) Nitric oxide hyperpolarized rabbit mesenteric arteries via ATP-sensitive potassium channels. *J Physiol (London)*, 486: 47-58.
- NASCIMENTO MSB, MACHADO FA (2005) Native trees - a free richness. Embrapa. Available in: http://www.embrapa.br/noticias/artigos/folder.2005-02-02.1550581232/artigo.2005-12-05.7898292197/mostra_artigo.
- NGUELEFACK TB, FEUMEBO CB, ATEUFACK G, WATCHO P, TATSIMO S, ATSAMO AD, TANE P, KAMANYI A (2008) Anti-ulcerogenic properties of the aqueous and methanol extracts from the leaves of *Solanum torvum* Swartz (Solanaceae) in rats. *J Ethnopharmacol* 119: 135-140.
- OLINDA TM, LEMOS TLG, MACHADO LL, RAO VS, SANTOS FA (2008) Quebrachitol-induced gastroprotection against acute gastric lesions: Role of prostaglandins, nitric oxide and K⁺_{ATP} channels. *Phytomedicine* 15: 327- 333.
- RANGEL M, MALPEZZI ELA, SUSINI SMM, FREITAS JC (1997) Hemolytic activity in extracts of the diatom *Nitzschia*. *Toxicon*, 35: 305-309.
- REPETTO MG, LLESUY SF (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz J Med Biol Res* 35:523-534.
- RIBEIRO ME, YOSHIDA WB (2005) Reperfusion injury after intestinal ischemia: pathophysiology and experimental models. *J Vasc Br*, 4: 183-194.
- ROBERT A, NEZAMIS JE, LANCASTER C, HANCHAR AJ (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* 77: 433-443.
- SAMONINA GE, KOPYLOVA GN, LUKJANZEVA GV, ZHUYKOVA SE, SMIRNOVA EA, GERMAN SV, GUSEVA AA (2004) Antiulcer effects of amylin: a review *Pathophysiology* 11: 1-6.
- SINGH S, KHAJURIA A, TANEJA SC, KHAJURIA RK, SINGH J, JOHRI RK, QAZI GN (2008) the gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from *Boswellia serrata*, in rats. *Phytomedicine* 15: 408-415.
- SUGITA H, UENO T, SHIMOSEGAWA T, YOSHIMURA T (2003) Direct detection of nitric oxide and its roles in maintaining gastric mucosal integrity following ethanol induced injury in rats. *Free Radic Res.* 37: 159-169.
- SZABO S, TRIER JS, BROWN A, SCHNOOR J (1985) Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*, 88: 228-236.
- TOROUD HP, RAHGOZAR M, BAKHTIARIAN A, DJAHANGUIRI (1999) Potassium channel modulators and indomethacin-induced gastric ulceration in rats. *Scand J Gastroenterol* 34(10): 962-966.
- TRINGALI C, SPATAFORA C, LONGO OD (2000) Bioactive constituents of the bark of *Parkia biglobosa*. *Fitoterapia*, 71: 118-125.
- UEDA S, YOSHIKAWA T, TAKAHASHI S (1989) Role of free radicals and lipid peroxidation in gastric mucosal injury induced by ischemia-reperfusion in rats. *Scand J Gastroenterol.* 24: 55-58.
- WALLACE JL (2001) Mechanisms of protection and healing: current knowledge and future research. *Am J Med* 110:19-23.
- WALLACE JL, GRANGER DN (1996) The cellular and molecular basis of gastric mucosal defense. *Faseb J* 10:731 - 740.
- WALLACE JL, MCKNIGHT W, REUTER BK, VERGNOLL N (2000) NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology*, 119: 706-714.
- WALLACE JL, DEVCHAND PR (2005) Emerging roles for cyclooxygenase-2 in gastrointestinal mucosal defense. *Brit J Pharmacol*, 145: 275-282.
- WHITTLE BJ, OREN-WOLMAN N, GUTH PH (1985) Gastric vasoconstrictor actions of leukotriene C₄,PGF₂ alpha, and thromboxane mimetic U-46619 on rat submucosal microcirculation in vivo. *Am J Physiol Gastrointest Liver Physiol* 248: 580-586.
- YOSHIKAWA T, UEDA S, NAITO Y, TAKAHASHI S, OYAMADA H, MORITA Y, YONETA T, KONDO M (1989) Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia-reperfusion in rats. *Free Radic Res Commun*, 7: 285.
- YU JH, LIM JW, NAMKUNG W, KIM H, KIM KH. (2002). Suppression of cerulein-induced cytokine expression by antioxidants in pancreatic acinar cells. *Lab Invest.* 82: 1359-1368.

