

# Gastroprotective Effect of an Ethanolic Extract from *Neoglaziovia variegata* (Arruda) Mez (Bromeliaceae) in Rats and Mice

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This study investigates the gastroprotective effect of a crude ethanolic extract of *Neoglaziovia variegata* (Arruda) Mez (Bromeliaceae), designated Nv-EtOH, in experimental models of gastric ulcer. In the ethanol-induced gastric ulcer model, Nv-EtOH showed gastroprotection at doses of 200 and 400 mg/kg body weight (BW) (57.0% and 79.7%, respectively). Nv-EtOH also significantly reduced the formation of gastric lesions induced by ethanol/HCl (31.6% and 63.5%), ibuprofen (70.0% and 74.3%), or ischemia/reperfusion in rats (65.0% and 87.0%) at 200 and 400 mg/kg BW when compared with the vehicle group. In the antioxidant activity assessment, Nv-EtOH (400 mg/kg BW) increased the catalase activity and sulfhydryl groups (SH) levels, respectively. Moreover, gastroprotection against ethanol damage was decreased after ibuprofen pretreatment. Nv-EtOH (400 mg/kg BW) promoted a significant increase in the content of gastric wall mucus. The Nv-EtOH effect was significantly reduced in mice pretreated with *N*<sup>G</sup>-nitro-L-arginine (L-NOARG) or glibenclamide, inhibitors of nitric oxide synthase and  $K_{ATP}$  channel activation, respectively, suggesting the involvement of these mechanisms in the Nv-EtOH-induced gastroprotective effect. Nv-EtOH decreased the total acidity, but did not modify other gastric juice parameters. Nv-EtOH was also effective in promoting the healing process in chronic gastric ulcer induced by acetic acid in rats.

**Key words:** Gastroprotection, *Neoglaziovia variegata*, Bromeliaceae

## Introduction

Gastric ulcer is a disease caused by an imbalance between protective (mucus, bicarbonate, adequate blood flow, NO, prostaglandins, among others) and aggressive factors (pepsin, HCl, H<sub>2</sub>O<sub>2</sub>, OH<sup>-</sup>, O<sub>2</sub><sup>-</sup>, among others). Furthermore, this pathology can also be caused and/or exacerbated by exogenous predisposing factors related to health life conditions such as stress, smoking, alcohol, use of non-steroidal anti-inflammatory drugs (NSAIDs), some foods, and the presence of the pathogen *Helicobacter pylori* (Wallace and Granger, 1996; Wallace, 2001)

Medicinal plants currently represent a promising alternative treatment for gastric injuries. Recent studies have shown a large variety of chemical

compounds isolated from herbs and plant extracts that exhibit therapeutic activity in experimental models of gastric ulcer, which indicates the important potential of plants and their active metabolites in the discovery of novel therapies against peptic ulcers (Schmeda-Hirschmann and Yesilada, 2005).

The Bromeliaceae family has been found all over the neotropic ecosystem with wide distribution in the American continent. In Brazil, many endemic species of this family occur, some of them are used in folk medicine against gastrointestinal disorders, such as *Bromelia laciniosa* Mart. ex Schult. f., a root decoction of which is used against hepatitis and intestinal diseases, and *Tillandsia recurvata* (L.) L. which is used against ulcers and hemorrhoids as a decoction of a small whole plant (Agra *et al.*, 2007).

The species *Neoglaziovia variegata* (Arruda) Mez [synonyms: *Agallostachys variegata* (Arruda) Beer; *Billbergia variegata* (Arruda) Schult. f.; *Bromelia linifera* hort. ex Beer; *Bromelia variegata* (Arruda); and *Dyckia glaziovii* Baker (Smith and Downs, 1979)] is popularly known as “caroá” and possesses an important role in the northeastern economy due to the production of a resistant fiber similar to sisal (*Agave sisalana* Perrine), widely used in the textile industry. Regarding its biological properties, the crude ethanolic extract obtained from aerial parts of *N. variegata*, designated Nv-EtOH, has been reported to have antioxidant properties and low toxicity, as well as antinociceptive activity, probably by interacting with opioid mechanisms (Lima-Saraiva *et al.*, 2012a, b; Mayo, 1992).

A phytochemical screening of the Nv-EtOH by high-performance liquid chromatography (HPLC) revealed the presence of cinnamic acid, coumarin, and flavonoid derivatives, compounds which possess potent biologic activities (Lima-Saraiva *et al.*, 2012b). Likewise, a previous study on flavonoids in the Bromeliaceae family reported the presence of quercetin derivatives in *N. variegata* (Williams, 1978).

The lack of additional information on the biological activities of *N. variegata* encouraged us to investigate the gastroprotective effect of its ethanolic extract in different gastric ulcer models in rodents. In addition, the role of endogenous NO, sulfhydryl groups, catalase, and prostaglandins in the gastroprotective effect was evaluated in order to provide information on the mechanisms involved in this effect.

## Material and Methods

### Plant material

The leaves of *Neoglaziovia variegata* (Arruda) Mez were collected in the region of Sub-medium São Francisco (BR 428, Km 152, municipality of Lagoa Grande, Pernambuco, 45 km from the city of Petrolina, PE, Brazil) in January 2009. The plant material was subjected to taxonomic identification at the Federal University of Vale do São Francisco, Petrolina, PE, Brazil. A voucher specimen (2889, M.M. Coelho 92) has been deposited in the Vale do São Francisco Herbarium in the same institution. The plant material was dried in an oven with circulating air at the average temperature of 40 °C for 3 d. The dried and pulver-

ized plant material (1174 g) was macerated with ethanol (95%) in a stainless steel container. Successive extractions were performed, followed by evaporation of the solvent, resulting in a crude ethanolic extract denominated Nv-EtOH [yield 45 g or 3.83% (w/w)].

### Animals

Male Swiss albino mice (20–25 g) and Wistar rats (180–220 g) were obtained from the Sectorial Vivarium of the Medicinal Plants Research Center of the Federal University of Piauí, Teresina, PI, Brazil. They were fasted over a period of 18 h and acclimatized to the test environment for 2 h before the experimentation. All experiments followed the experimental protocols submitted and approved by the Ethics Committee of the Federal University of Piauí (No. 48/10).

### Chemicals and drugs

The following drugs and chemicals were used: absolute ethanol (Quimex, São Paulo, SP, Brazil), carbenoxolone (Sigma-Aldrich, St. Louis, MO, USA), ibuprofen (Sigma-Aldrich), cimetidine (Glaxo Smith Kline, Rio de Janeiro, RJ, Brazil), *N*<sup>G</sup>-nitro-L-arginine (L-NOARG) (Sigma-Aldrich), L-arginine (L-ARG) (Sigma-Aldrich), diazoxide (Sigma-Aldrich), glibenclamide (Sigma-Aldrich), ethylenediaminetetraacetic acid (EDTA) (Reagen, Colombo, PR, Brazil), Tween 80 (Sigma-Aldrich, Seelze, Germany), *N*-acetylcysteine (NAC) (Sigma-Aldrich), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich), sodium hydroxide (Cristália, São Paulo, SP, Brazil), and acetic acid (Vetec, Duque de Caxias, RJ, Brazil).

The Nv-EtOH was first solubilized in 1.0% (v/v) Tween 80 in distilled water and then diluted in saline solution (0.9% NaCl, w/v). Other drugs were dissolved either in saline solution or distilled water. Nv-EtOH and drug concentrations were adjusted for the treatment to yield 10 mL/kg body weight (BW).

### Absolute ethanol- and HCl/ethanol-induced gastric ulcer

Acute gastric lesions were induced in mice by oral administration (0.2 mL/animal) of absolute ethanol or acidified ethanol (60% ethanol/0.3 M HCl). Vehicle, Nv-EtOH (50, 100, 200, and 400 mg/kg BW), or carbenoxolone (100 mg/kg

BW) were orally administered 1 h before application of the ulcerogenic agent. Animals were euthanized 30 min after ethanol or 1 h after ethanol/HCl administration, respectively, stomachs were removed and opened along the greater curvature, and the area of gastric lesions was measured by planimetry (mm<sup>2</sup>) (Robert *et al.*, 1979; Mizui and Douteuchi, 1983).

#### *Ibuprofen-induced gastric ulcer*

In this model, mice were orally treated with vehicle, cimetidine (100 mg/kg BW), or Nv-EtOH (50, 100, 200, and 400 mg/kg BW). After 60 min, all groups were treated with ibuprofen (400 mg/kg BW). The animals were euthanized 6 h after ibuprofen administration, the stomachs were removed and opened along the greater curvature, and the area of gastric lesions was measured by planimetry (mm<sup>2</sup>) (Bhargava *et al.*, 1973).

#### *Ischemia and reperfusion-induced gastric ulcer*

Wistar rats ( $n = 6$ ) were orally (p.o.) treated with the vehicle, *N*-acetylcysteine (200 mg/kg BW) or Nv-EtOH (50, 100, 200, and 400 mg/kg BW). After 30 min, under anesthesia of sodium thiopental [25 mg/kg BW, by intraperitoneal (i.p.) route], the celiac artery blood flow was interrupted by a clamp. After 30 min the clamp was removed and the reperfusion was established. Then, animals were euthanized 1 h after induction of the reperfusion. Stomachs were removed and opened along the greater curvature, and the area of gastric lesions was measured by planimetry (mm<sup>2</sup>) (Yoshikawa *et al.*, 1989).

#### *Gastric lesions induced by acetic acid*

For induction of gastric ulcer in rats, a glass tube (8 mm in diameter and 2 cm long) was used in contact with the stomach serosa to limit the area that would be injured. Inside the tube, 70  $\mu$ L of 80% acetic acid were added, which remained in contact with the serosa for 1 min. The stomach was accommodated in the abdominal cavity, and the abdominal region was sutured. One d after ulcer induction, daily oral treatment was started with: vehicle, cimetidine (100 mg/kg BW) or Nv-EtOH (400 mg/kg BW) for 14 d. After the chronic treatment the animals were euthanized. The calculation of the ulcerated area (mm<sup>2</sup>) was performed by measuring its length and height.

#### *Quantification of sulfhydryl groups*

Stomachs of mice previously treated with absolute ethanol and Nv-EtOH (400 mg/kg BW) were used to analyse the role of sulfhydryl groups (SH) in the Nv-EtOH-induced gastroprotective effect. The amount of SH in the gastric mucosa was measured according to Sedlak and Lindsay (1968). A standard calibration curve was prepared using reduced glutathione (GSH). The absorbance of all samples was measured spectrophotometrically at 412 nm within 5 min after the addition of 0.05 mL of DTNB, 0.01 M in methanol, and the results were expressed as  $\mu$ g of GSH/g tissue.

#### *Catalase activity*

Stomachs of mice previously treated with absolute ethanol and Nv-EtOH (400 mg/kg BW) were used to analyse the role of catalase (CAT) in the Nv-EtOH-induced gastroprotective effect. The determination of the CAT activity was measured according to the method described by Beers and Sizer (1952). The absorbance was measured at 240 nm, and the CAT activity was defined as the amount of enzyme required to decompose 1 mmol of H<sub>2</sub>O<sub>2</sub> per min during 6 min. The results were expressed as mmol/(min 100 mg tissue).

#### *Evaluation of the role of prostaglandins in the Nv-EtOH-induced gastroprotective effect*

Mice were pretreated with vehicle or ibuprofen. Vehicle was administered 30 min before administration of Nv-EtOH. Ibuprofen (100 mg/kg BW, p.o.) was administered 1 h before administration of vehicle, Nv-EtOH (400 mg/kg BW, p.o.), or carbenoxolone (100 mg/kg BW, p.o.). After 1 h of treatment, all animals received absolute ethanol (0.2 mL) for the induction of lesions (Olinda *et al.*, 2008).

#### *Determination of gastric wall mucus*

Glandular segments from rat stomachs were removed and weighed. Each segment was immediately transferred to 0.25% Alcian Blue (in 0.16 M sucrose, buffered with 0.05 M sodium acetate, pH 5.8). The free dye was removed by rinsing in 0.25 M sucrose solution. The gastric mucus-bound dye was extracted with 0.5% magnesium chloride. A 4-mL sample of the blue extract was then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged,

and the absorbance was recorded at 598 nm. The quantity of Alcian Blue extracted/g glandular tissue was calculated.

#### *Effects of L-arginine (L-ARG) and N<sup>G</sup>-nitro-L-arginine (L-NOARG) on Nv-EtOH gastroprotection*

The role of nitric oxide in the Nv-EtOH-induced (400 mg/kg BW) gastroprotective effect in mice was assessed according to the method described by Olinda *et al.* (2008), using an appropriate inhibitor, L-NOARG (70 mg/kg BW, i.p.), and the substrate, L-ARG (600 mg/kg BW, i.p.), of nitric oxide synthase (NOS). In each case, animals were pretreated with the specific substance 30 min before the treatment with Nv-EtOH.

#### *Role of K<sub>ATP</sub> channels in the gastroprotective effect of Nv-EtOH*

The role of K<sub>ATP</sub> channels in the gastroprotective effect of Nv-EtOH (400 mg/kg BW) was assessed in mice according to the method described by Olinda *et al.* (2008), using an appropriate K<sub>ATP</sub> channel blocker, glibenclamide (5 mg/kg BW, i.p.), or activator, diazoxide (3 mg/kg BW, i.p.). In each group, animals were pretreated 30 min before the treatment with Nv-EtOH (Olinda *et al.*, 2008).

#### *Determination of gastric secretion by pylorus-ligated rats*

Rats were anesthetized with sodium thiopental (45 mg/kg BW, i.p.), and then their abdomen was incised and the pylorus ligated. Nv-EtOH (400 mg/kg BW), cimetidine (100 mg/kg BW), or vehicle were administered intraduodenally after pylorus ligation. Four h after treatment, rats were euthanized, stomachs were removed, and gastric juice solution was collected and centrifuged at 4465 x g for 30 min. The content (in mL) was measured, then pH value and total acidity were determined by titration with 0.1 M NaOH in a pH-meter (WTW 330i; Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) and expressed as mEq/h (Shay *et al.*, 1945).

#### *Statistical analysis*

The results are expressed as means ± standard error of the mean (S.E.M.). The statistical significance for differences between groups was

calculated by analysis of variance (ANOVA) and Tukey's post test. The differences between groups were regarded as significant at  $p < 0.05$ . All analyses were performed using GraphPad Prism™ 5.0 (GraphPad Software, San Diego, CA, USA).

## **Results**

#### *Effect of Nv-EtOH on gastric ulcer induced by absolute ethanol or HCl/ethanol*

In the ethanol-induced gastric ulcer model, oral administration of Nv-EtOH (200 and 400 mg/kg BW) and carbenoxolone (100 mg/kg BW) decreased the area of lesions by 57.0%, 79.7%, and 84.4%, respectively, when compared with the vehicle group. Likewise, in the HCl/ethanol-induced ulcer model, Nv-EtOH and carbenoxolone at the same doses also significantly decreased the area of lesions by 31.6%, 63.5%, and 82.0%, respectively, when compared with the vehicle group (Table I).

#### *Effect of Nv-EtOH on gastric ulcer induced by ibuprofen*

In the ibuprofen-induced gastric ulcer model, Nv-EtOH (100, 200, and 400 mg/kg BW, p.o.) and cimetidine (100 mg/kg BW, p.o.) inhibited gastric ulcer formation by 50.0%, 70.0%, 74.3%, and 91.0%, respectively (Table I).

#### *Effect of Nv-EtOH on ischemia and gastric ulcer induced by reperfusion*

The administration of Nv-EtOH (200 and 400 mg/kg BW, p.o.) and *N*-acetylcysteine (200 mg/kg BW, p.o.), 30 min before the induction of gastric lesions by ischemia and reperfusion, decreased the lesion areas by 65.0%, 87.0%, and 77.4%, respectively (Table I).

#### *Effect of Nv-EtOH on gastric lesions induced by acetic acid*

In the model of gastric lesions induced by acetic acid, daily oral treatment for 14 d showed that Nv-EtOH (400 mg/kg BW) promotes healing of gastric ulcers in rats (Fig. 1). Nv-EtOH (400 mg/kg BW) or cimetidine (100 mg/kg BW) significantly reduced the main lesion area [ $(2.51 \pm 2.17)$  mm<sup>2</sup> and  $(1.70 \pm 0.76)$  mm<sup>2</sup>] in comparison with

Table I. Effect of Nv-EtOH, carbenoxolone, cimetidine, and *N*-acetylcysteine (NAC) in different acute gastric lesion models in rodents.

Gastric lesion model	Treatment	Dose (mg/kg BW)	Lesion area [mm <sup>2</sup> ]	Inhibition (%)
Ethanol (mice)	Control	–	11.83 ± 0.74	0.0
	Nv-EtOH	50	11.50 ± 1.45	2.8
		100	9.38 ± 1.42	20.7
		200	5.09 ± 0.97***	57.0
		400	2.40 ± 0.45***	79.7
		100	1.85 ± 0.32***	84.4
Ethanol/HCl (mice)	Control	–	9.74 ± 0.91	0.0
	Nv-EtOH	50	9.00 ± 0.76	7.6
		100	7.84 ± 0.88	19.5
		200	6.66 ± 0.77**	31.6
		400	3.56 ± 0.26***	63.5
		100	1.75 ± 0.24***	82.0
Ibuprofen (mice)	Control	–	7.42 ± 0.54	0.0
	Nv-EtOH	50	6.20 ± 0.64	16.5
		100	3.74 ± 0.57***	50.0
		200	2.24 ± 0.29***	70.0
		400	1.91 ± 0.68***	74.3
		100	0.68 ± 0.27***	91.0
Ischemia/reperfusion (rats)	Control	–	11.08 ± 1.72	0.0
	Nv-EtOH	50	14.71 ± 1.01	0.0
		100	7.93 ± 1.12	28.5
		200	3.90 ± 0.75***	65.0
		400	1.43 ± 0.35***	87.0
		200	2.50 ± 0.28***	77.4
NAC	200	2.50 ± 0.28***	77.4	

Values represent the mean ± S.E.M. of 8 animals per group; \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with respective control group.

the vehicle group [(12.87 ± 2.17) mm<sup>2</sup>] (Okabe *et al.*, 1971).

#### Participation of SH groups in the gastroprotective effect of Nv-EtOH

Oral administration of Nv-EtOH (400 mg/kg BW) and carbenoxolone (100 mg/kg BW) to mice previously treated with absolute ethanol prevented the decrease in GSH levels in gastric mucosa (Fig. 2).

#### Catalase activity

Compared with non-treated animals ethanol treatment reduced the gastric wall catalase activity by more than 50%, and this decrease was largely prevented by oral administration of 400 mg/kg BW Nv-EtOH or 100 mg/kg BW carbenoxolone (Fig. 3).

#### Participation of prostaglandins in the gastroprotective effect of Nv-EtOH

Absolute ethanol-induced gastric lesions were significantly decreased to 16 and 13% after pretreatment with Nv-EtOH (400 mg/kg BW) or carbenoxolone (100 mg/kg BW), respectively (Fig. 4). Pretreatment with ibuprofen (100 mg/kg BW), a non-selective cyclooxygenase (COX) inhibitor, partially reversed the Nv-EtOH- and carbenoxolone-induced compared with the vehicle group (Fig. 4).

#### Effects of Nv-EtOH on gastric mucus content

The effects of Nv-EtOH on the gastric wall mucus content in pylorus-ligated rats are shown in Fig. 5. The intraduodenal administration of Nv-EtOH (400 mg/kg BW) elicited a significant 54% increase in the gastric mucus content compared

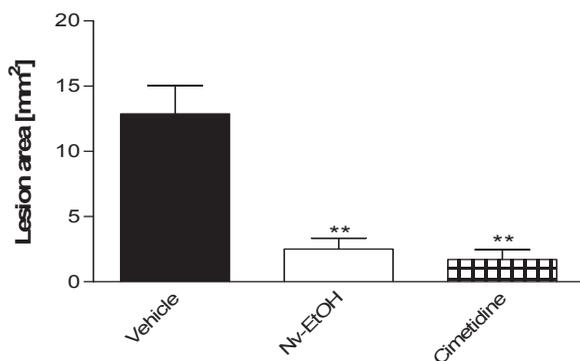


Fig. 1. Effect of Nv-EtOH (400 mg/kg BW) and cimetidine (100 mg/kg BW) on a model of acetic acid-induced chronic gastric ulcer in rats. Data are expressed as means  $\pm$  S.E.M.; \*\* $p$  < 0.01 compared with vehicle group;  $n$  = 6 for each group.

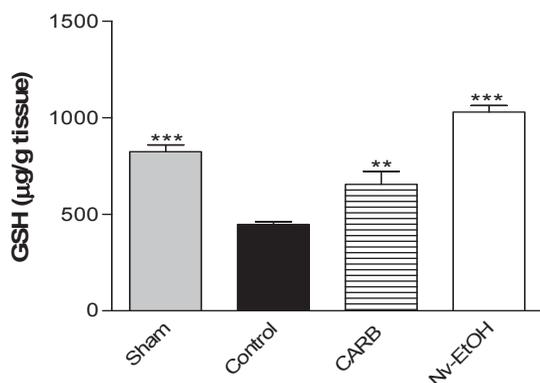


Fig. 2. Effect of Nv-EtOH (400 mg/kg BW) and carbenoxolone (CARB, 100 mg/kg BW) on the GSH levels in the gastric wall of mice treated with absolute ethanol. The results are expressed as means  $\pm$  S.E.M.; \*\*\* $p$  < 0.001 and \*\* $p$  < 0.01 compared with control;  $n$  = 8 for each group.

with the vehicle, while carbenoxolone (100 mg/kg BW) increased the gastric mucus by 80%.

#### Participation of NO synthase in the gastroprotective effect of Nv-EtOH

Prior administration of the nitric oxide synthase inhibitor L-NOARG significantly abolished the Nv-EtOH (400 mg/kg BW)- or L-ARG (600 mg/kg BW)-induced gastroprotection, suggesting the participation of nitric oxide in this response (Fig. 6).

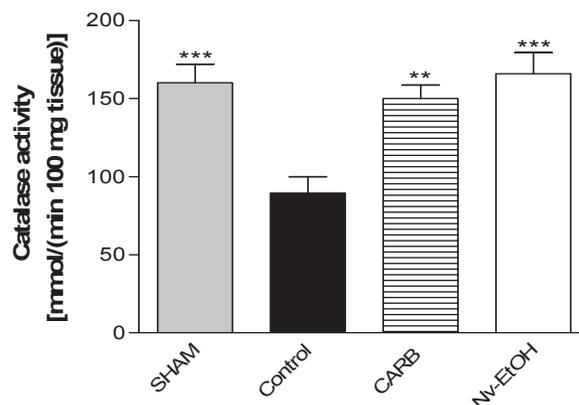


Fig. 3. Effect of Nv-EtOH (400 mg/kg BW) and carbenoxolone (CARB, 100 mg/kg BW) on the catalase activity in the gastric wall of mice treated with absolute ethanol. The results are expressed as means  $\pm$  S.E.M.; \*\*\* $p$  < 0.001 and \*\* $p$  < 0.01 compared with control;  $n$  = 8 for each group.

#### Participation of $K_{ATP}$ channels in the gastroprotective effect of Nv-EtOH

Pretreatment with glibenclamide (5 mg/kg BW, i.p.), a  $K_{ATP}$  channel blocker, significantly reduced the gastroprotection provided by Nv-EtOH (400 mg/kg BW) or diazoxide (3 mg/kg BW, i.p.) (Fig. 7), suggesting the participation of these channels in the Nv-EtOH-induced gastroprotective effect.

#### Determination of gastric secretion by pylorus-ligated rats

Biochemical parameters of stomach contents and gastric juice, such as volume, pH value, and total acidity, after intraduodenal administration of vehicle, Nv-EtOH (400 mg/kg BW), or cimetidine (100 mg/kg BW) were evaluated. Nv-EtOH promoted only alterations in total acidity, while in animals pretreated with cimetidine all parameters were significantly changed (Table II).

#### Discussion

In the present study, the antiulcer effect of the crude ethanolic extract from leaves of *Neoglaziovia variegata* (Arruda) Mez (Nv-EtOH) was investigated as well as the possible mechanisms involved. The results showed that Nv-EtOH is an effective antiulcerogenic agent. This gastroprotective activity is probably due to

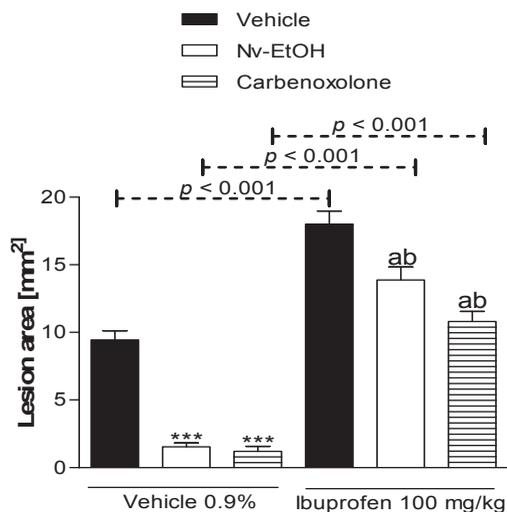


Fig. 4. Effect of Nv-EtOH (400 mg/kg BW) and carbenoxolone (100 mg/kg BW) after pretreatment with ibuprofen (100 mg/kg BW) on the absolute ethanol-induced gastric lesions in mice. Data are expressed as means  $\pm$  S.E.M.; \*\*\* $p$  < 0.001 compared with vehicle + vehicle group; <sup>a</sup> $p$  < 0.01 compared with vehicle + ibuprofen group; <sup>b</sup> $p$  < 0.001 compared with vehicle + Nv-EtOH;  $n$  = 6 for each group.

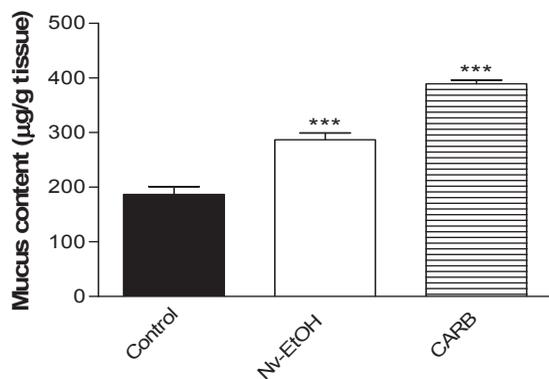


Fig. 5. Effect of Nv-EtOH (400 mg/kg BW) and carbenoxolone (CARB, 100 mg/kg BW) on the gastric wall mucus content in the pylorus-ligated model in rats. The results are expressed as means  $\pm$  S.E.M.; \*\*\* $p$  < 0.001 compared to control;  $n$  = 6 for each group.

the activation of antioxidant systems, likely involving prostaglandins (PGs) and the nitric oxide synthase (NO synthase) pathway.

Ethanol causes gastric lesions; it solubilizes mucus constituents in the stomach, increases the release of pepsin, and destabilizes mast cells, thus inducing the release of histamine, which reduces

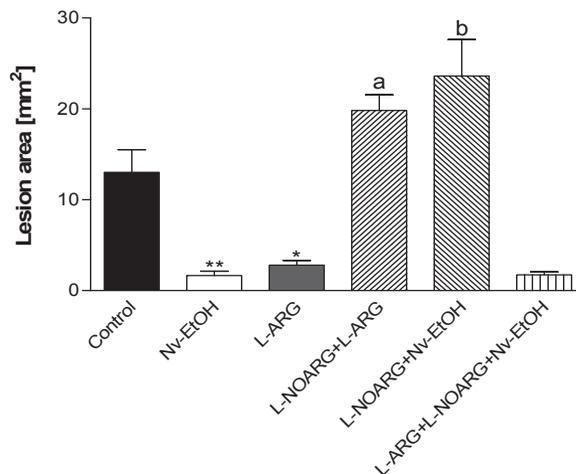


Fig. 6. Participation of NO synthase in the gastroprotective effect on the absolute ethanol-induced gastric ulcers in mice. The results are expressed as means  $\pm$  S.E.M.; \* $p$  < 0.05, \*\* $p$  < 0.01 compared with vehicle; <sup>a</sup> $p$  < 0.001 compared with L-ARG; <sup>b</sup> $p$  < 0.001 compared with Nv-EtOH;  $n$  = 8 for each group.

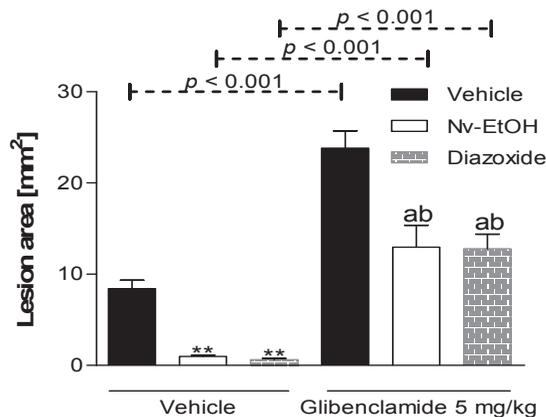


Fig. 7. Effect of Nv-EtOH (400 mg/kg BW) and diazoxide (3 mg/kg BW) after pretreatment with glibenclamide (5 mg/kg BW) on the absolute ethanol-induced gastric lesions in mice. Data are expressed as means  $\pm$  S.E.M.; \*\* $p$  < 0.01 compared with vehicle + vehicle group; <sup>a</sup> $p$  < 0.001 compared with vehicle + glibenclamide group; <sup>b</sup> $p$  < 0.001 compared with vehicle + Nv-EtOH;  $n$  = 8 for each group.

the blood flow to injured tissues and decreases the gastric defence mechanism. Thus, this irritant agent causes the formation of gastric ulcer. The presence of HCl enhances and accelerates this process (Laine and Weinstein, 1988; Guslandi, 1987; Szabo, 1987).

Table II. Effect of Nv-EtOH on pH value, total acidity, and volume of gastric juice in pylorus-ligated rats.

Parameter	Treatment	Dose (mg/kg BW)	Gastric secretion (mean ± S.E.M.)
pH	Control (saline)	–	2.03 ± 0.35
	Nv-EtOH	400	3.22 ± 0.73
	Cimetidine	100	7.00 ± 0.66***
Total acidity [mEq/h]	Control (saline)	–	1.65 ± 0.39
	Nv-EtOH	400	0.29 ± 0.14***
	Cimetidine	100	0.05 ± 0.03***
Volume [mL]	Control (saline)	–	10.30 ± 1.49
	Nv-EtOH	400	7.28 ± 0.77
	Cimetidine	100	4.48 ± 0.39**

Values represent the mean ± S.E.M. of 8 animals per group; \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with respective control group

The results of the investigations of ethanol-induced and ethanol/HCl-induced ulcers revealed a significant Nv-EtOH-induced gastroprotective effect at higher doses. Lower doses appeared to cause a small reduction of gastric lesions compared with the vehicle group, but no statistical significance was observed (Table I).

In these gastric ulcer models, Nv-EtOH exhibited cytoprotective activity, with a possible increase in the release of endogenous protection factors, such as secretion of mucus, bicarbonate, and antioxidative agents. The role of PGs in this effect was investigated. Promotion of gastric ulcers by non-steroidal anti-inflammatory drugs involves the inhibition of cyclooxygenases I and II. This causes a reduction in the production of PGs and decreases the protective barrier of the gastric mucosa which is composed of mucus and bicarbonate, thus facilitating the formation of lesions by gastric hydrochloric acid and enzymes (Wallace, 2001). Nv-EtOH had a significant gastroprotective effect on ulcers induced by an anti-inflammatory drug like ibuprofen, suggesting involvement of PGs in this response (Table I). PGs play important roles in modulating the gastric mucosal integrity and various functions of the gastrointestinal tract (Ferreira *et al.*, 2011). The ability of PGs to stimulate mucus and bicarbonate secretion may also significantly contribute to the promotion of ulcer healing (Wallace, 2008)

Ischemia promotes injuries and leads to cell death. Moreover, the reperfusion of ischemic tissue promotes several tissue changes, which eventually leads to the appearance of gastric lesions. Among these changes, capillary permeability and the production of oxygen free radicals increase

which then attack cell membranes, causing damage, attract neutrophils, and the inflammatory response is initiated (Yoshikawa *et al.*, 1989).

Administration of Nv-EtOH reduced ischemia and reperfusion-induced gastric lesions, suggesting a likely antioxidant effect (Table I). There are many protective factors in the gastric mucosa which capture ROS (reactive oxygen species) and prevent their destructive action, such as SH groups (GSH), that scavenge free radicals (Ueda *et al.*, 1989), and the enzyme catalase (CAT), which accelerates the degradation of H<sub>2</sub>O<sub>2</sub> into water and oxygen (Kwiecien *et al.*, 2002).

A possible antioxidant effect of Nv-EtOH (400 mg/kg BW) after induction of gastric lesions was evaluated. An increase in GSH as well as CAT activities was observed, indeed suggesting an antioxidant activity of Nv-EtOH (Figs. 2 and 3). According to Szabo and Vattay (1990), SH groups are key components in the protection of the gastric mucosa against ethanol-induced damage.

A previous report (Andreo *et al.*, 2006) showed that the methanolic extract from *Mouriri pusa* Gardn. (Melastomataceae) increased levels of GSH in the gastric mucosa. This action can probably be related to the presence of flavonoids, a group of compounds with high antioxidant properties which also occur in *Neoglaziovia variegata*.

We considered participation of PGs, formation of gastric mucus, involvement of NO, and activation of ATP-sensitive potassium channels (K<sub>ATP</sub>) as factors in the mediation of the gastroprotective effect of Nv-EtOH.

The involvement of PGs in the Nv-EtOH-induced gastroprotective effect was deduced from the attenuation of the effect by a pretreatment

with ibuprofen, a non-specific cyclooxygenase inhibitor (Fig. 4). These results corroborate those observed for ibuprofen-induced gastric ulcers and suggest the possible involvement of PGs in the gastroprotective effect of Nv-EtOH.

Considering the significant role of PGs in the mucosal integrity by stimulating mucus secretion (Ferreira *et al.*, 2011), the mucus content was also assessed. Mucus is an important protective factor of gastric mucosa and is formed by water and mucin-like glycoproteins which can be detected by Alcian Blue staining (Bolton *et al.*, 1978). Nv-EtOH significantly increased the mucus content of gastric mucosa in rats subjected to a pylorus ligation (Fig. 5), suggesting that mucus formation is a component in the gastroprotective response.

The results observed in these two models are consistent with a previous study conducted by Nunes *et al.* (2009) which suggested the participation of PGs and an increase in mucus secretion in the gastroprotective activity of the ethanolic extract from *Combretum leprosum* Mart. & Eiche (Combretaceae). A phytochemical screening of this species revealed the presence of triterpenes, flavonoids, tannins, and saponins. All these chemical constituents are also present in *Neoglaziovia variegata*, which probably explains the similarity between the results of these studies. According to Muscara and Wallace (1999), NO is partially involved in mucus and bicarbonate secretion. Sugita *et al.* (2003) reported cytoprotective effects for this compound on ethanol-induced gastric lesions. In our study, the application of L-NOARG, an inhibitor of the enzyme NO synthase which enhances ethanol-induced gastric lesions (Aly, 1995), made an involvement of NO in the gastroprotective effect of Nv-EtOH likely (Fig. 6). NO synthase also participates in the gastroprotection induced by extracts from *Encholirium spectabile* Mart. (Bromeliaceae) (Carvalho *et al.*, 2010).

NO can also increase the gastric blood flow by activation of ATP-sensitive potassium channels ( $K_{ATP}$ ) (Murphy and Brayden, 1995). To evaluate the role of  $K_{ATP}$  in the gastroprotection by Nv-EtOH, a pretreatment with glibenclamide (5 mg/kg BW), a well-known blocker of these channels,

was performed in the ethanol-induced gastric lesion model. The drug was found to abolish the gastroprotective effect of Nv-EtOH (Fig. 7). The ability of glibenclamide and diazoxide, activators of  $K_{ATP}$  channels, to modify the gastroprotective effect of some drugs, is considered as evidence for the involvement of  $K_{ATP}$  in the gastroprotection (Standen *et al.*, 1989).

The gastroprotective effect of Nv-EtOH may involve inhibition of gastric acid secretion, which is partially sufficient to decrease the number of lesions induced by ethanol (Mizui and Douteuchi, 1983). Therefore, the antisecretory activity of Nv-EtOH was evaluated by analysis of gastric juice biochemical parameters (gastric secretory volume, titratable acidity, and pH value) in pylorus-ligated rats. Only acidity was reduced in animals treated with Nv-EtOH, suggesting that mucosa protection by the extract does not involve the inhibition of gastric acid secretion (Table II).

The gastroprotective activity of Nv-EtOH was also evaluated using a model of acetic acid-induced chronic gastric ulcers. Nv-EtOH enhanced the healing process of chronic gastric ulcer by reducing the injured area (Fig. 1). How this effect is mediated is not fully understood, but the involvement of PGs in the gastroprotective effect of Nv-EtOH shown above, as well as the previously reported ability of PGs to reduce gastric acid secretion, would contribute to the acceleration of ulcer healing (Wallace, 2008).

Thus, we have established a promising gastroprotective effect of Nv-EtOH and provided evidence for the involvement of NO, PG, mucus, sulfhydryl groups, catalase, and  $K_{ATP}$  channels, factors that play an important role in gastric cytoprotection.

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