JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1413–1419

Received April 9, 2007
Accepted June 28, 2007
DOI 10.1211/jpp.59.10.0012
ISSN 0022-3573

# Gastroprotective activity of the hydroalcoholic extract obtained from *Polygala paniculata* L. in rats

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**Funding:** The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio a Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), Programa de Apoio aos Núcleos de Excelência (PRONEX) and Universidade Federal do Paraná for financial support.

## Abstract

The possible gastroprotective effects of the hydroalcoholic extract of *Polygala paniculata* in rats have been evaluated. We have investigated the effects of this hydroalcoholic extract on acute lesions induced by ethanol (70%, p.o.) and indometacin (20 mg $kg^{-1}$, s.c.). Its influence on mucus secretion was investigated, measured as the amount of Alcian blue dye estimated by colorimetry, and antisecretory effects were assessed in the pylorus ligature model. The treatment of rats with a crude hydroalcoholic extract of *P. paniculata* (HEPP; 30, 100, 300 mg $kg^{-1}$, p.o., or 3, 10 and 30 mg $kg^{-1}$, i.p.) decreased the ulcer index, and maintained the gastric mucus production in acute gastric lesions caused by ethanol 70%. In addition, the extract partially protected the mucosa against indometacin-induced lesions. The extract did not change the volume and acidity of gastric secretion in the pylorus-ligated rat. An additional antioxidant activity of the extract and its isolated flavonoid compound rutin, in the DPPH free radical scavenging assay, was observed. In conclusion, HEPP exhibited marked gastroprotection; these effects may have involved prostaglandins and be related to cytoprotective factors, such as antioxidant activity and maintenance of mucus production.

## Introduction

*Polygala paniculata* Linneu (Polygalaceae) is a native small bush that is widespread on the Brazilian Atlantic coast. This plant is popularly known as "barba-de-são-joão", "bromil", "vassourinha branca" or "mimosa" (Newall et al 1996; Lorenzi & Matos 2002).

*P. paniculata* L. is traditionally used in folk medicine for the treatment of several inflammatory diseases, such as asthma, bronchitis, arthritis, stomach pain, diarrhoea, and other pathologies, including kidney disorders (Newall et al 1996). Apart from these medicinal uses, there have been reports showing antipsychotic (Chung et al 2002), antitumoral (Dall'acqua et al 2002), anti-inflammatory (Kou et al 2003), antinociceptive (Campos et al 1997; Meotti et al 2006), and antispasmodic (El Sayah et al 1999) activity of some *Polygala* species.

Previous phytochemical investigations on different plants of the genus *Polygala* yielded several compounds, including cytotoxic lignans (Dall'acqua et al 2002), saponins (Chung et al 2002), xanthones, coumarins and flavonoids (Pinheiro et al 1998). Likewise, Cristiano et al (2003) reported the characterization of xanthones, coumarin, the flavonol rutin and two sterols, spinasterol and delta25-spinasterol, from *P. paniculata* L. In addition, several studies of plant-derived extracts have shown the presence of compounds with gastroprotective activity such as coumarins and/or flavonoids (Konturek et al 1986; Motilva et al 1994; Reyes et al 1996; Rao et al 1997; Blankson et al 2000; Gonzalez & Stasi et al 2002; Biguetti et al 2005; Brzozowski et al 2005; Zayachkivska et al 2005). However, no pharmacological study has been carried out with this species concerning its gastroprotective activity. In this study we have examined the possible antisecretory activity and gastroprotective action of the hydroalcoholic extract of *P. paniculata* L. (HEPP).

## Materials and Methods

### Animals

Female Wistar rats (180–200 g) were kept on a constant 12-h light/dark cycle with controlled temperature (22 ± 2°C). Standard pellet food (Nuvital, Curitiba, PR, Brazil) and water were freely available. The institutional Ethics Committee of the Universidade Federal do Paraná approved all procedures adopted in this study (approval number: 41685/04-46/2004 – 110).

### Preparation of hydroalcoholic extract, isolation and identification of its active compounds

*P. paniculata* L. was collected on Daniela beach (Santa Catarina State, Brazil) and was classified by Olavo Araújo Guimarães (Universidade Federal do Paraná, Curitiba, Brazil). A voucher specimen of this plant was deposited in the herbarium of the Botany Department of the Universidade Federal do Paraná. The dried whole plant (3500 g) was minced and submitted to exhaustive extraction by maceration with ethanol:water (80:20) in a closed container. After maceration, the extract was passed through a paper filter and the solvent was evaporated under reduced pressure (50°C) in a rotatory evaporator. The respective crude hydroalcoholic extract (yield 50 g) was obtained.

Using chemical and spectroscopic methods (EIMS, IR, $^{1}H$ and $^{13}C$ NMR, NOE difference spectroscopy), the structures of three xanthones (1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone 1,5-dihydroxy-2,3-dimethoxyxanthone and 1-hydroxy-2,3,5-trimethoxyxanthone) and the sterol spinasterol were established, together with the coumarin murragatin and the flavonoid rutin. Moreover, using high resolution gas chromatography coupled to mass spectrometry (HRGC-MS) it was possible to characterize a minor sterol, delta 25-spinasterol (Cristiano et al 2003). However, due to limited quantities of these compounds, it was not possible to test their possible gastroprotective effects in-vivo.

### Assessment of gastroprotective effect of HEPP

*Induction of acute gastric lesions by ethanol*

Rats deprived of solid food for 15 h were treated with vehicle (water 0.1 mL/100 g, p.o. or i.p.: non-lesion control (NLC)) or HEPP (30, 100, 300 mg $kg^{-1}$, p.o. or 3, 10, 30 mg $kg^{-1}$, i.p.). One hour later different groups of animals received ethanol 70% (0.5 mL, p.o.). The animals were killed by cervical dislocation 1 h after ethanol treatment. The stomachs were removed and examined for quantification of lesions to score the ulcer index (Robert et al 1979).

*Determination of gastric mucus secretion*

The gastric mucus secretion was determined using segments of the gastric mucosa after induction of acute gastric lesions by ethanol 70%. The segments (accurately weighed) were incubated for 2 h in a 0.1% Alcian blue solution. The excess of Alcian blue fixed in the gastric wall was removed with two successive washes with 0.25 mol $L^{-1}$ sucrose (15 and 45 min, respectively). The residual Alcian blue complexed with mucus in the gastric wall was removed with 0.5 mol $L^{-1}$ $MgCl_2$, shaking the segments for 1 min every 30 min for a total of 2 h. Approximately 3 mL blue supernatant solution was transferred to tubes together with 3 mL diethyl ether and centrifuged at 684 *g* for 10 min, to separate the aqueous phase for determination of Alcian blue absorbance (598 nm). Standard curves of Alcian blue were used for calculations (Corne et al 1974).

*Induction of acute gastric lesions by indometacin*

Rats deprived of solid food for 15 h were treated with vehicle (NaCl 0.9%, 0.1 mL/100 g, p.o. or s.c.) or HEPP (30, 100, 300 mg $kg^{-1}$, p.o.). One hour later different groups of animals were treated with indometacin (20 mg $kg^{-1}$, s.c.) dissolved in 5% sodium bicarbonate. The animals were killed by cervical dislocation 6 h after indometacin treatment. The stomachs were removed and examined for quantification of lesions to score the ulcer index (Djahanguiri 1969).

### Study of antisecretory activity of HEPP

Rats submitted to a 15-h fast were anaesthetized with ether and a pylorus ligature was produced with sutures. Four hours after suture of the abdominal wall the animals were killed by cervical dislocation (Shay 1945). The mucosa was washed with 3 mL water and the gastric contents were transferred to tubes for later centrifugation at 286 *g* for 30 min.

The volume of the gastric juice supernatant was determined and the gastric acidity was measured by simple titration with 0.1 M NaOH using 2% phenolphthalein as acid–base indicator.

### Involvement of nitric oxide in protective effect of HEPP

To assess the involvement of nitric oxide in the protective effect of HEPP observed in the ethanol 70% model of acute gastric lesion, animals were treated with $N^{G}$-nitro-L-arginine methyl ester (L-NAME, 70 mg $kg^{-1}$, i.p.), an inhibitor of nitric oxide synthase, and after 30 min the rats received HEPP (100 mg $kg^{-1}$, p.o.). Sixty minutes after this treatment, ethanol 70% (0.5 mL per animal) was given orally and 60 min later the animals were killed by cervical dislocation. The stomachs were removed and the mucosa was examined for quantification of lesions and determination of gastric mucus secretion (Arrieta et al 2003).

### DPPH free radical scavenging assay

The DPPH free radical is a stable free radical that has been widely used as a tool to estimate the free radical scavenging activity of antioxidants. The free radical scavenging activity of HEPP (1, 3, 10, 30, 100 and 300 mg $mL^{-1}$) and its isolated flavonoid rutin (0.664, 2.0, 6.64, 20.0 and 66.4 $\mu$g $mL^{-1}$) on the DPPH radical was determined using the method described by Blois (1958) and Chen et al (2004), with some modifications. Samples of the extract and rutin (0.75 mL) were mixed with 0.25 mL DPPH radical solution in methanol. The decrease in absorbance at 517 nm was measured at each predetermined checkpoint. For all the experiments, the vehicle of

the extract and fractions was used as negative control and ascorbic acid (50 $\mu$g mL$^{-1}$) was used as positive control.

### Statistical analysis

All the results were presented as mean ± s.e.m., except the ID50 (i.e. the dose of extract necessary to reduce the response by 50% relative to the control value) or IC50 (i.e. the concentration of compound necessary to reduce the response by 50% relative to the control value), which were reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance of differences between groups was determined by analysis of variance followed by Newman–Keuls multiple comparison test. *P* values less than 0.05 were considered as indicative of significance. The ID50 values were determined by linear regression from individual experiments with linear regression Instat Software.

## Results

The HEPP treatment (30, 100 or 300 mg kg$^{-1}$, p.o.) caused a dose-related reduction in gastric lesions induced by ethanol 70%, decreasing the ulcer index mainly at doses of 100 and 300 mg kg$^{-1}$ (Figure 1A), with a mean ID50 value of 101.6 (78.8–130.8) mg kg$^{-1}$ and inhibition of 97 ± 1% at 300 mg kg$^{-1}$.

Intraperitoneal (i.p.) administration of HEPP (3, 10 or 30 mg kg$^{-1}$) also decreased the ulcer index of gastric mucosa at doses of 10 and 30 mg kg$^{-1}$ (Figure 1B), with a mean ID50 value of 13.0 (7.8–21.8) mg kg$^{-1}$ and inhibition of 74 ± 3% at 30 mg kg$^{-1}$.

In addition, this extract reduced partially, although not significantly, the lesions induced by indometacin (20 mg kg$^{-1}$, s.c.), with inhibition of 34 ± 9%, 32 ± 13%, and 42.3 ± 15% at doses of 30, 100 and 300 mg kg$^{-1}$, respectively (Table 1). This suggested that prostaglandins might have been involved in the protective effect of HEPP. However, the HEPP administered by the intraduodenal route (i.d.), immediately after pylorus ligation, did not alter the volume nor the acidity of the gastric contents over 4 h (results not shown).

HEPP, at 100 and 300 mg kg$^{-1}$ orally and 10 and 30 mg kg$^{-1}$ intraperitoneally, maintained the gastric mucus production. Treatment with the extract by oral or intraperitoneal route maintained mucus production compared with values of 69.0 ± 3.1 and 49.3 ± 3.6 $\mu$g Alcian blue mL$^{-1}$ (g tissue)$^{-1}$ for the non-lesioned control groups (NLC), respectively (Figure 2A and B). At doses of 100 and 300 mg kg$^{-1}$ HEPP, orally, the values were 73.0 ± 5.6, 80.3 ± 6.2 $\mu$g Alcian blue mL$^{1}$ (g tissue)$^{-1}$, respectively (Figure 2A). For 10 and 30 mg kg$^{-1}$ HEPP, intraperitoneally, the values were 56.6 ± 10.3 and 69.1 ± 8.0 $\mu$g Alcian blue mL$^{1}$ (g tissue)$^{-1}$, respectively (Figure 2B).

The results depicted in Table 2 show that pretreatment with L-NAME (70 mg kg$^{-1}$, i.p.) plus ethanol 70% increased the index of mucosa ulceration to 119.0 ± 11.3 when compared with the lesioned group treated only with ethanol 70%, which presented an index of ulceration of 78.0 ± 5.4. Furthermore, treatment with L-NAME did not inhibit mucus production or gastric protection promoted by HEPP (100 mg kg$^{-1}$, p.o.) (Table 2).

In this study HEPP and its isolated flavonoid rutin presented antioxidant activity, verified in the DPPH free-radical scavenging assay (Table 3). The free-radical scavenging activity of HEPP was observed at 30, 100 and 300 mg mL$^{-1}$ with a mean IC50 value of 61.2 (27.95–134.4) mg mL$^{-1}$ and inhibition of 86 ± 1% at 300 mg mL$^{-1}$. The flavonoid rutin isolated from HEPP presented a similar DPPH radical scavenging activity at doses of 0.664, 2.0, 6.64, 20.0 and 66.4 $\mu$g mL$^{-1}$. At a dose of 66.4 $\mu$g mL$^{-1}$, rutin had a mean IC50 value of 4.0 (3.3–4.6) $\mu$g mL$^{-1}$ and inhibition of 98 ± 2%.

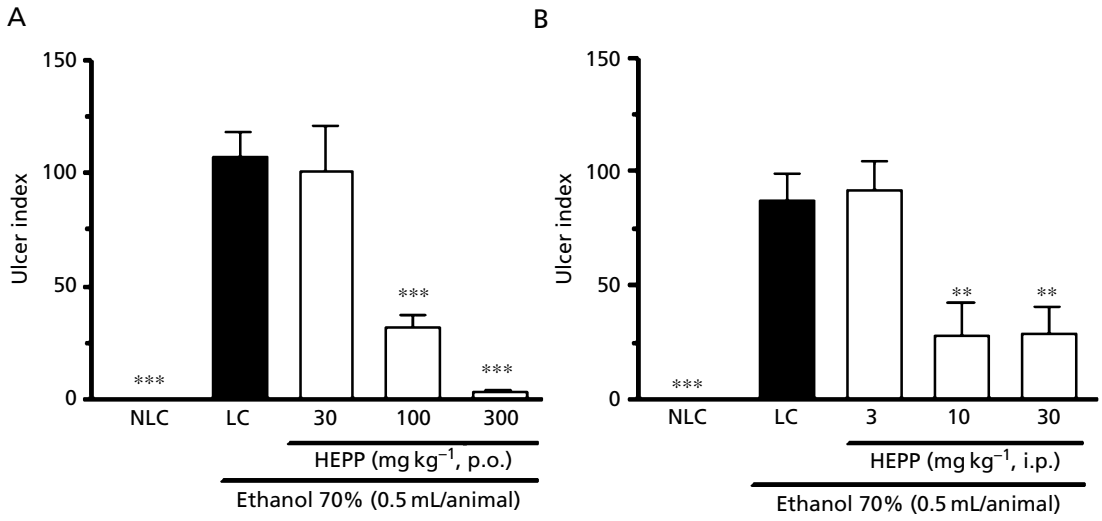


**Figure 1** Protective effect of HEPP when administered by (A) oral or (B) intraperitoneal route against gastric lesions induced by ethanol 70%. NLC, non-lesioned control–water 0.1 mL per 100 g; LC, lesioned control– ethanol 0.5 mL per animal. The result are expressed as mean ± s.e.m.(n=6). The differences between groups were determined by analysis of variance followed by Newman–Keuls multiple comparison test. $^{**}P < 0.01$ and $^{***}P < 0.001$, when compared with lesioned control group (LC).

**Table 1** Protective effect of HEPP by the oral route when assessed against gastric lesions induced by indometacin in rats

| Treatment | Dose | Ulcer index |
|---|---|---|
| Vehicle (p.o.) | 0.1 mL/100 g | 0.0 |
| Indometacin (s.c.) | 20 mg kg$^{-1}$ | 47.2 ± 8.7 |
| HEPP (p.o.) + indometacin (20 mg kg$^{-1}$, s.c) | 30 mg kg$^{-1}$ | 37.1 ± 5.5 |
| | 100 mg kg$^{-1}$ | 43.5 ± 7.5 |
| | 300 mg kg$^{-1}$ | 37.7 ± 8.4 |

Values represent mean ± s.e.m. (n = 6). The difference between groups was determined by analysis of variance followed by Newman–Keuls multiple comparison test.

## Discussion

Phytochemical studies carried out with the hydroalcoholic extract of *P. paniculata* have demonstrated the presence of xanthones, the coumarin murragatin, the flavonol rutin, spinasterol and delta25-spinasterol (Cristiano et al 2003). Furthermore, it has been reported that plant-originated flavonoid and coumarin substances present cytoprotective and anti-ulcer activity (Konturek et al 1986; Motilva et al 1994; Reyes et al 1996; Rao et al 1997; Blankson et al 2000; Gonzalez & Stasi et al 2002; Biguetti et al 2005; Brzozowski et al 2005; Zayachkivska et al 2005). This work has shown for the first time that the HEPP administered orally was able to protect the gastric mucosa against lesions induced by ethanol 70%. It is well established that intragastric administration of ethanol causes acute haemorrhagic erosion of the gastric mucosa in animals and man (Iaquinto et al 2003). In rats, endothelial injury and increased vascular permeability preceded the occurrence of haemorrhagic gastric erosion and the mediators of this vascular damage in the gastric mucosa included histamine, leukotrienes, platelet-activating factor (PAF), and endothelin (Szabo et al 1985; Whittle & Esplugues 1988; Yonei & Guth 1991).

In this study, the extract was given by two routes (oral and intraperitoneal) to evaluate whether the observed effect was related to an adherent property of the extract on the gastric

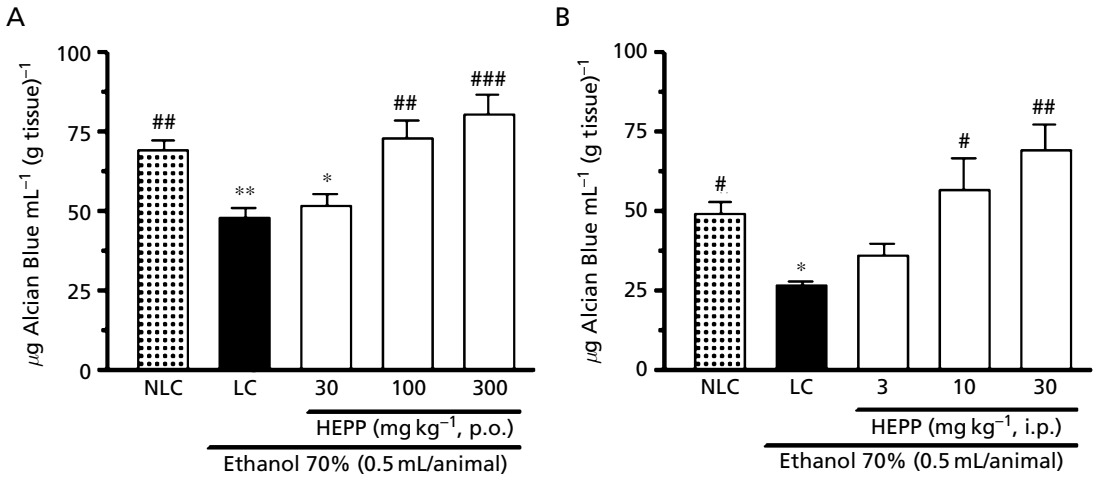


**Figure 2** Quantification of gastric mucus in μg Alcian Blue mL$^{-1}$ (g of tissue)$^{-1}$ after (A) oral or (B) intraperitoneal administration of HEPP in the ethanol model of gastric lesions. NLC, non-lesioned control – water 0.1 mL per 100 g; LC, lesioned control – ethanol 0.5 mL per animal. The results were expressed as mean ± s.e.m. (n=6). The differences between groups were determined by analysis of variance followed by Newman–Keuls multiple comparison test. *$P$<0.05, **$P$<0.01 when compared with non-lesioned control group (NLC) and $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 when compared with lesioned control group (LC).

**Table 2** Influence of HEPP on the effect of pretreatment with L-NAME (70 mg kg$^{-1}$, i.p.) plus ethanol 70% on the ulcer index and quantification of gastric mucus in μg Alcian blue mL$^{-1}$ (g tissue)$^{-1}$

| Treatment | Dose | Ulcer index | Alcian blue (μg Alcian blue mL$^{-1}$ (g tissue)$^{-1}$) |
|---|---|---|---|
| Vehicle (p.o.) | 0.1 mL/100 g | 0.0 | 0.0 |
| Ethanol 70% (p.o.) | 0.5 mL/animal | 78.7 ± 5.4 | 111.9 ± 6.2 |
| L-NAME (i.p.) + ethanol 70% (p.o.) | 70 mg kg$^{-1}$ + 0.5 mL/animal | 118.9 ± 11.3 | 100 ± 5.8 |
| Ethanol 70% (p.o.) + HEPP (p.o.) | 0.5 mL/animal + 100 mg kg$^{-1}$ | 27.6 ± 5.8*** | 151.4 ± 6.1*** |
| L-NAME (i.p.) + ethanol 70% (p.o.) + HEPP (p.o.) | 70 mg kg$^{-1}$ + 0.5 mL/animal + 100 mg kg$^{-1}$ | 27.6 ± 6.6*** | 139.8 ± 7.2*** |

The results were expressed as mean ± s.e.m. (n = 6). The difference between groups was determined by analysis of variance followed by Newman–Keuls multiple comparison test. ***$P$ < 0.001, when compared with the group treated with ethanol 70% or ethanol 70% plus L-NAME.

**Table 3** Effect of HEPP, the flavonoid rutin and the positive control ascorbic acid upon DPPH radical scavenging

| Experimental group | | Absorbance (517 nm) |
|---|---|---|
| HEPP | Control | 0.157 ± 0.07 |
| | Ascorbic acid (50 $\mu$g mL$^{-1}$) | 0.046 ± 0.0*** |
| | 3 mg mL$^{-1}$ | 0.160 ± 0.001 |
| | 10 mg mL$^{-1}$ | 0.131 ± 0.02 |
| | 30 mg mL$^{-1}$ | 0.083 ± 0.003*** |
| | 100 mg mL$^{-1}$ | 0.062 ± 0.007*** |
| | 300 mg mL$^{-1}$ | 0.023 ± 0.001*** |
| Rutin | Control | 0.165 ± 0.01 |
| | Ascorbic acid (50 $\mu$g mL$^{-1}$) | 0.059 ± 0.004*** |
| | 0.664 $\mu$g mL$^{-1}$ | 0.070 ± 0.016*** |
| | 2.0 $\mu$g mL$^{-1}$ | 0.090 ± 0.001*** |
| | 6.64 $\mu$g mL$^{-1}$ | 0.032 ± 0.014*** |
| | 20.0 $\mu$g mL$^{-1}$ | 0.007 ± 0.006*** |
| | 66.4 $\mu$g mL$^{-1}$ | 0.002 ± 0.002*** |

Values represent mean ± s.e.m. (n = 3). The difference between groups was determined by analysis of variance followed by Newman–Keuls multiple comparison test. ***$P < 0.001$, when compared with the control group (vehicle of the extract and isolated compound was used as negative control) incubated only with DPPH.

mucosa, forming a protective barrier against the aggressive effects of ethanol. Our results showed that when given by the intraperitoneal route, HEPP exhibited an important cytoprotective effect, similar to the one seen when the extract was given orally. This suggested that the pharmacological mechanism did not have any relationship with an adherent property of the extract.

This study also showed that HEPP, when administered orally or intraperitoneally, maintained or slightly increased (300 mg kg$^{-1}$, p.o. or 30 mg kg$^{-1}$, i.p.) the production of gastric mucus, and this effect was likely to be responsible for its protective activity. Moreover, the maintenance of gastric mucus protection seen against ethanol effects might have been related to changes in the quality of the mucus, improving its viscoelastic properties. The mechanisms involved in this maintenance or increase require investigation.

The gastric mucus is one of the main defensive elements of the mucosa against aggressive agents. It serves as a physical barrier over the mucosa and is continuously secreted by epithelial cells (Mózsik et al 1997; Bi & Kaunitz 2003). The production of gastric mucus can be stimulated by a wide range of mediators including growth factors, acetylcholine, nitric oxide (NO) and prostaglandins (Ichikawa et al 2000). Prostaglandins are one of the most important protective factors in gastric mucosa and are involved in the maintenance of gastrointestinal integrity, enhancing gastrointestinal continuity, mucus production and vascular homeostasis (Smith & Langenbach 2001; Whittle 2004). In addition, non-steroidal anti-inflammatory drugs (NSAIDs) such as indometacin are known to induce gastric damage due to unspecific inhibition of cyclooxygenase-1 (COX-1) and COX-2, and markedly reduce prostanoid synthesis decreasing mainly mucosal $PGE_2$ levels (Kataoka et al 2000; Wallace et al 2000). On the other hand, endogenous prostaglandin deficiency alone does not induce visible gastric lesions (Okada et al 1989; Takeuchi et al 1997) and the pathogenesis of NSAIDs also involves luminal acid (Elliot & Wallace 1998) and neutrophil activation (Wallace et al 1990). Our results have shown that HEPP was not able to significantly protect the mucosa against lesions induced by indometacin, but reduced the ulcer index with inhibition of 34 ± 9%, 32 ± 13% and 42.3 ± 15% at oral doses of 30, 100 and 300 mg kg$^{-1}$, respectively. This suggested that prostaglandins were involved in the protective effect of HEPP, although other factors may have contributed to this effect. We cannot discard the possibility of an active compound present in HEPP having a prostaglandin-like activity, partially reducing the damage in the mucosa.

We demonstrated recently the presence of several constituents in HEPP, including the flavonol rutin (Cristiano et al 2003). Several studies have shown that plant-derived extracts that contain flavonoids exhibit gastroprotection, similar to HEPP, reducing the gastric injury induced by ethanol and partially attenuating indometacin injury, predominantly as a result of an increase in the mucus secretion, thereby suggesting the involvement of prostaglandins (Motilva et al 1994; Zayachkivska et al 2005). In addition, recent studies found that flavonoids such as catechin, epicatechin and naringenin, isolated from grapefruit seed extract, exhibited antioxidant activity, scavenging reactive oxygen species (ROS), and this effect was related to gastroprotective effects (Yimaz & Toledo et al 2004; Zayachkivska et al 2005).

These statements were in agreement with our result, which showed the antioxidant activity of HEPP and its isolated flavonoid rutin, verified in the DPPH free-radical scavenging assay. This finding suggested that an antioxidant effect of the extract might have been related to its protective effects against gastric lesions and that the flavonoid rutin contributed to this activity. The antioxidant properties of the *Polygala* extract were also observed in a recent study from our group where HEPP exerted protective effects against methylmercury-induced neurotoxicity (Farina et al 2005).

Following these findings, additional experiments were carried out with the objective of verifying the involvement of nitric oxide (NO) in the mechanism of gastric protection of HEPP. MacNaughton et al (1989) showed that authentic NO or NO donors could markedly reduce the severity of damage to the gastric mucosa induced by topical application of ethanol. These findings are underscored by evidence that NO, a potent vasodilator, is one of the key mediators regulating mucosal blood flow responses both under basal conditions and in response to irritants such as ethanol, through regulation of the gastric mucosal microcirculation (Elliot & Wallace 1998). In addition, pretreatment with $N^{\omega}$-nitro-arginine (L-NA) or $N^{G}$-nitro-L-arginine-methyl ester (L-NAME), both nitric oxide synthesis inhibitors, significantly increased the gastric damage caused by ethanol (Elliot & Wallace 1998).

The results of this study also showed that pretreatment of rats with L-NAME did not reverse the cytoprotection and mucus production promoted by oral HEPP administration. This suggested that the participation of NO in this mechanism was unlikely.

The principal drugs able to protect the gastric mucosa commercialized today (e.g. cimetidine, ranitidine and omeprazol) decrease gastric acid secretion by different mechanisms ($H_2$-receptor antagonists and $H^+/K^+$/ATPase pump). To

verify whether the gastroprotective effects of HEPP were related to one of these mechanisms, thereby reducing acid secretion, the pylorus ligature model was employed. Our results showed that the extract (30, 100 and 300 mg $kg^{-1}$), administered intraduodenally, did not alter the acidity or volume of gastric secretion during 4-h pylorus ligation. This result suggested that the gastroprotective action of HEPP was not related to inhibition of acid secretion.

It has been recognized that anatomic and functional integrity of the gastric mucosa depends on the balance between aggressive and defensive mechanisms. The success of pharmacological treatments in preventing or healing ulcerative lesions may not depend only on the blockade of acid secretion, but also on the enhancement of mucosal protective factors (Peskar & Maricic 1998; Dajani & Klamut 2000). Mucus, in combination with bicarbonate secreted by the surface epithelial cells, has long been thought to play a key role in protecting the gastric epithelium from damage induced by acid and pepsin (Wallace 2001). In addition, mucus plays an important role in preventing bacterial colonization and translocation to the luminal surface; furthermore, it plays an important role in the prevention of mechanical injury to the epithelium, providing a microenvironment over sites of superficial injury in which rapid repair can occur (Wallace 2001). The results showed the relevant cytoprotective activity of HEPP involving the maintenance of mucus secretion, a protective factor, and that this effect was not related to inhibition of an aggressive factor such as acid secretion, a mechanism shared by all the main drugs used for the treatment of gastric ulcers today, although it might be related to prostaglandins or an antioxidant effect of *Polygala* extract. The precise mechanisms of action involving these protective factors need to be studied.

## Conclusion

HEPP markedly inhibited gastric mucosal lesions induced by ethanol 70%. In addition, intraduodenal administration of HEPP did not alter the acidity or the volume of gastric acid secretion. This suggested that the gastroprotective action of HEPP was not related to inhibition of acid secretion. According to our results, the protective effect of HEPP may have been related to a slight increase or maintenance of gastric mucus secretion, since this was maintained after lesions were induced by ethanol. This effect was not related to NO because L-NAME did not reverse the cytoprotection and mucus production promoted by oral HEPP administration. Additional studies evaluating the participation of endogenous prostaglandins and SH groups in protection of the mucosa against ethanol, as well as the protective effects of active isolated compounds present in this plant, may have contributed to clarifying the mechanism involved in cytoprotection. Furthermore, this study has provided a pharmacological basis for *P. paniculata* use in folk medicine and has shown that this plant has therapeutic potential for the development of new phytomedicines with gastroprotective properties.

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