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# Actions of Crude Hydroalcoholic Extract of *Pfaffia* sp on Gastrointestinal Tract

**Cristina Setim Freitas, Maria Fernanda Rodrigues de Paula, Lia Rieck and Maria Consuelo Andrade Marques***

*Departamento de Farmocologia; Setor de Ciências Biológicas; Universidade Federal do Paraná; Centro Politécnico; C. P. 19031; Jardim das Américas; 81531-990; Curitiba - PR - Brazil*

## ABSTRACT

*The plants that compound the Pfaffia genus are used in folk medicine to treat gastric disturbances. This study examined the effects of a crude hydroalcoholic extract of Pfaffia sp on the gastrointestinal tract. Female Wistar rats were pretreated orally (p.o.) with the hydroalcoholic extract of Pfaffia (0.5, 1 and 2 g.kg$^{-1}$) before the induction of ulcer with hypothermic restraint stress (HRS), ethanol (ET) or indomethacin (IND). Control animals received water (C) or ranitidine (60mg/kg) p.o. The hydroalcoholic extract of Pfaffia (0.5, 1 and 2 mg.kg$^{-1}$) protected rats against HRS and ET – induced ulcers, but was not able to protect the gastric mucosa against IND - induced ulcers. When injected into the duodenal lumen, the hydroalcoholic extract of Pfaffia inhibited basal and stimulated acid secretion in pylorus-ligated rats. These results indicate that this plant has a protective action against gastric lesions of the mucosa involving the reduction of gastric acid secretion.*

**Key words:** *Pfaffia*, Amaranthaceae, ulcers, acid secretion

## INTRODUCTION

Brazil is the most important center in harvesting of *Pfaffia* (Amaranthaceae) in America (Siqueira, 1988). This plant popularly known as "paratudo" ("for everething"), is used in folk medicine for scar and to treat gastric disturbances (Teske and Trentini, 1995). *Pfaffia* genus occurs in Guiana, Bolívia, Argentina and Brazil, mainly in states of São Paulo, Paraná, Mato Grosso and Goiás (Smith and Downs, 1972). After the discovery of pfaffic acid in *Pfaffia paniculata* Kuntze roots, have been grown the interest in the study of *Pfaffia* species due their antitumoural activity (Nishimoto et al, 1984). This study examined the effects of a crude hydroalcoholic extract of *Pfaffia* sp on gastrointestinal tract, seeking to validate the gastrointestinal effects of this plant.

## MATERIALS AND METHODS

**Animals:** Adult female Wistar rats (180-250 g) were kept under controlled temperature (20 ± 2°C) and lighting (12:12h light/dark) conditions, with free access to water and food. When necessary animals were deprived from food 15 –18 h before the experiments.

**Plant material and preparation of extract:** The hydroalcoholic extract of *Pfaffia* sp roots (EHP) was provided by FUNDAÇÃO HERBARIUM LABORATÓRIO BOTÂNICO

* Author for correspondence

LTDA, Colombo, Paraná, Brasil (lot nº 112795 - setember/1995 and lot nº 062236 - february/1997). It was obtained after 8 days maceration in percolator (2.2 kg of *Pfaffia* sp dust in 70% alcohol - 10.0 l). The extract was filtered and the remainder was macerate 24 h with 70% alcohol and again filtered. The pH of the filtrate was adjusted to 7.67 with KOH 14 %. The aqueous extract was concentrated under vacuum to ¼ volume, lyophilized and stored under light protection and low temperature (- 5 ºC).

**Antiulcer activity:** Fasting rats were treated with the EHP (0.5, 1 and 2 $g.kg^{-1}$ *per os – p.o.*). After 1 h gastric lesions were induced by either stress (restraint for 3 h at 4°C), 70% ethanol (0.5 ml, *p.o.*) or indomethacin (20 $mg.kg^{-1}$, subcutaneous - *s.c.*). Animals were killed after either 3 h restraint stress at 4°C, 1 h ethanol administration or 6 h indomethacin injection. The stomach was dissected out, the mucosal side was gently washed to remove remaining food and examined under a stereoscope to determine the number of ulcer and to score the index of mucosal damage (IMD). Color, edema and hemorrhage in the gastric folds, as well pethechyae, ulcer numbers and ulcer sizes were taken into consideration to determine IMD index. Ranitidine treated animal were used for positive control (Senay and Levine, 1967; Robert et al, 1979; Djahanguiri, 1969).

**Determination of gastric secretion and peptic activity:** A pylorus ligature was carefully done in rats under ether anesthesia and the EHP (0.5, 1 and 2 $g.kg^{-1}$) was injected into the duodenal lumen (*i.d.*). After 4 h the animals were killed and the gastric secretion was collected with a pipette. After washing the mucosal side of the stomach with 2 ml of distilled water, gastric secretion volume and pH were determined. Total acidity of the gastric juice was titrated with 0.1N NaOH using phenolphthalein (2%) as indicator. The EHP was also tested on gastric secretion induced in rats by bethanechol (2.5 $mg.kg^{-1}$, *s.c.*) or histamine (10 $mg.kg^{-1}$, *s.c.*) injected 1 h after surgery (Shay et al, 1945; Domer, 1971). Aliquots of 20 µl of the gastric content were incubated with 500 µl of albumin solution (5 $mg.mL^{-1}$ in 0.06 N hydrochloric acid) at 37 ºC for 10 minutes. The reaction was stopped with 200 µl of 10% trichloroacetic acid and the samples were centrifuged at 1500 *g* for 20 minutes. The supernatant was alkalinized with 2.5 mL of 0.55 M sodium carbonate and 400 µl of 1.0 N Folin's reagent was added to the tubes, which were incubated for 30 minutes at room temperature. The absorbance of the samples was determined by spectrophotometry at 660 nm and interject in a standard curve of tirosine for the determination of the concentration of pepsine in µg $tirosin^{-1}$ (Anson, 1938).

**Statistics Analysis:** Data were expressed as means ± s.e.m. Statistical significance of the results was determined using a one-way-analysis of variance followed by the Tukey Kramer test. Results were considered different at a significance level of $P < 0.05$.

## RESULTS

**Gastric protection against stress, ethanol or indomethacin:** Rats immobilized in the cold (4 ºC) for 3 h developed ulcers and other signs of gastric damage. The IMD index score was 7.71 ± 1.57. Previous treatment with 0.5 or 2 $g.kg^{-1}$EHP decreased the IMD to 3.80 ± 0.49 and 3.50 ± 0.43, respectively (Fig. 1 - *A*). Intragastric administration of 70% ethanol (0.5 ml) produced in vehicle-treated rats an IMD of 23.3 ± 5.03 after 60 min. Pretreatment with 1 or 2 $g.kg^{-1}$ EHP decreased the IMD to 8.83 ± 2.82 and 1.83 ± 0.60, respectively. (Fig. 1 - *B*). When control rats were treated with indomethacin (20 $mg.kg^{-1}$, *s.c.*), the gastric mucosa presented after 6 h IMD of 14.50 ± 4.50. Pretreatment of the animals with the EHP was not able to alter the IMD.

**Effect on gastric secretion and peptic activity:** In control rats after 4 h of EHP inoculation, the volume of gastric juice secreted by control rats, over 4 h, was 9.2 ± 0.6 ml with a pH 1.8 ± 0.05, total acidity of 0.031 ± 0.003 $mEq[H^+].mL^{-1}$ and peptic activity of 127.0 ± 4.2 µg $tirosin.ml^{-1}$. The EHP administration decreased the gastric juice volume to 5.9 ± 0.4 and 4.8 ± 0.4 ml, the total acidity to 0.016 ± 0.003 and 0.004 ± 0.001 $mEq[H^+].mL^{-1}$ and increased the pH to 2.8 ± 0.4 and 3.7 ± 0.3. The peptic activity was reduced with all the doses tested (111.0 ± 5.4, 104.0 ± 3.3 and 87.0 ± 6.0 µg $tirosin.ml^{-1}$) (Fig. 2). Injection of bethanechol (2.5 $mg.kg^{-1}$, *s.c.*) increased the

volume of basal acid secretion volume in pylorus-ligated rats from 7.1 ± 0.3 to 12 ± 0.9 ml, the total acidity from 0.029 ± 0.005 to 0.063 ± 0.006 mEq[$H^+$].$mL^{-1}$ and reduced the pH from 1.8 ± 0.04 to 1.4 ± 0.05. The peptic activity was not altered. Pretreatment with 0.5, 1 or 2g.$kg^{-1}$ EHP inhibited gastric juice volume induced by bethanechol to 8.3 ± 0.6, 8.7 ± 0.4 and 6.2 ± 0.8 ml, respectively. It pretreatment also inhibited the total acidity of stomach secretion induced by bethanechol to 0.042 ± 0.003, 0.04 ± 0.005 and 0.019 ± 0.003 mEq[$H^+$].$mL^{-1}$. The pH was increased to 2.0 ± 0.08, when 2.0 g.$kg^{-1}$ bethanecol was used (Fig. 3).

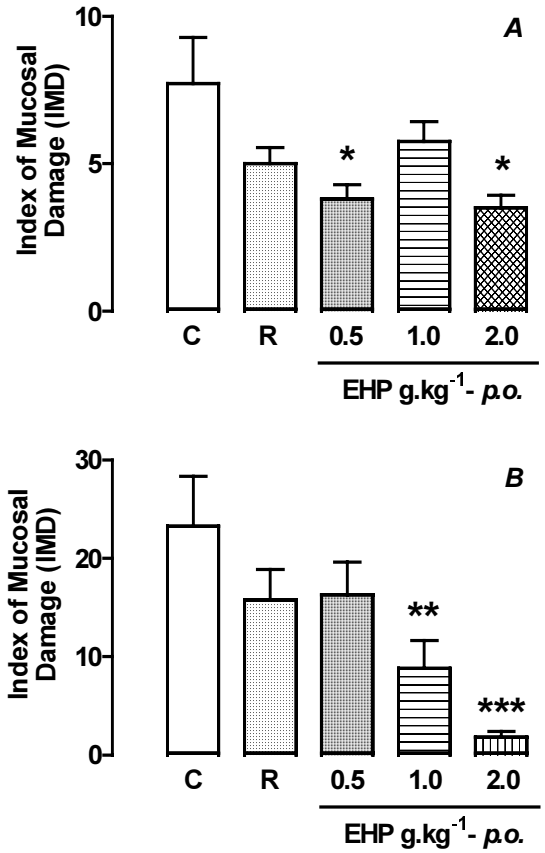


**Figure 1 -** Protective effect of the hydroalcoholic extract of *Pfaffia* – EHP- (C: control water, 0.5 ml.100$g^{-1}$; R: ranitidine, 60 mg.$kg^{-1}$ - p.o.) against gastric lesions induced by hypothermic restraint stress (*A*) and 70% ethanol (*B*). The data are means ± s.e.m., n=6 in all groups. * different from the control group at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Similarly, injection of histamine (10 mg.$kg^{-1}$, *s.c.*) increased the volume of basal acid secretion in pylorus-ligated rats from 6.5 ± 0.5 to 9.1 ± 0.8 ml, the total acidity from 0.03 ± 0.007 to 0.05 ± 0.007 mEq[$H^+$].$mL^{-1}$. Pretreatment with 2.0 g.$kg^{-1}$ of EHP prevented this effect, reducing the volume of gastric juice volume to 4.3 ± 0.2 ml and the total acidity to 0.007 ± 0.001 mEq[$H^+$].$mL^{-1}$. The pH was increased to 2.5 ± 0.1 (Fig. 4).

## DISCUSSION

In the present study, the hydroalcoholic extract of *Pfaffia* sp was tested to verify its action on the gastrointestinal tract reported in folk medicine. The results indicated that the extract (EHP) protected against gastric mucosal damage induced by ethanol, indicating an important cytoprotective action against the direct necrosing action of ethanol. Ethanol can cause injury to the gastric mucosa by damaging and breaking the gelatinous layer composed of mucus and bicarbonate that protects the stomach (Szabo, 1991; Glavin et al, 1992).

The EHP also protected against hypothermic restraint stress-induced ulcers (HRS), suggesting that active principles extract with ethanol from *Pfaffia* sp can oppose the increase in parasympathetic tonus promoted by HRS, with consequent stimulation of type $M_3$ muscarinic receptors of parietal cells, increased levels of gastrin regulator peptide (Glavin et al, 1991; Pachaly et al, 1993).

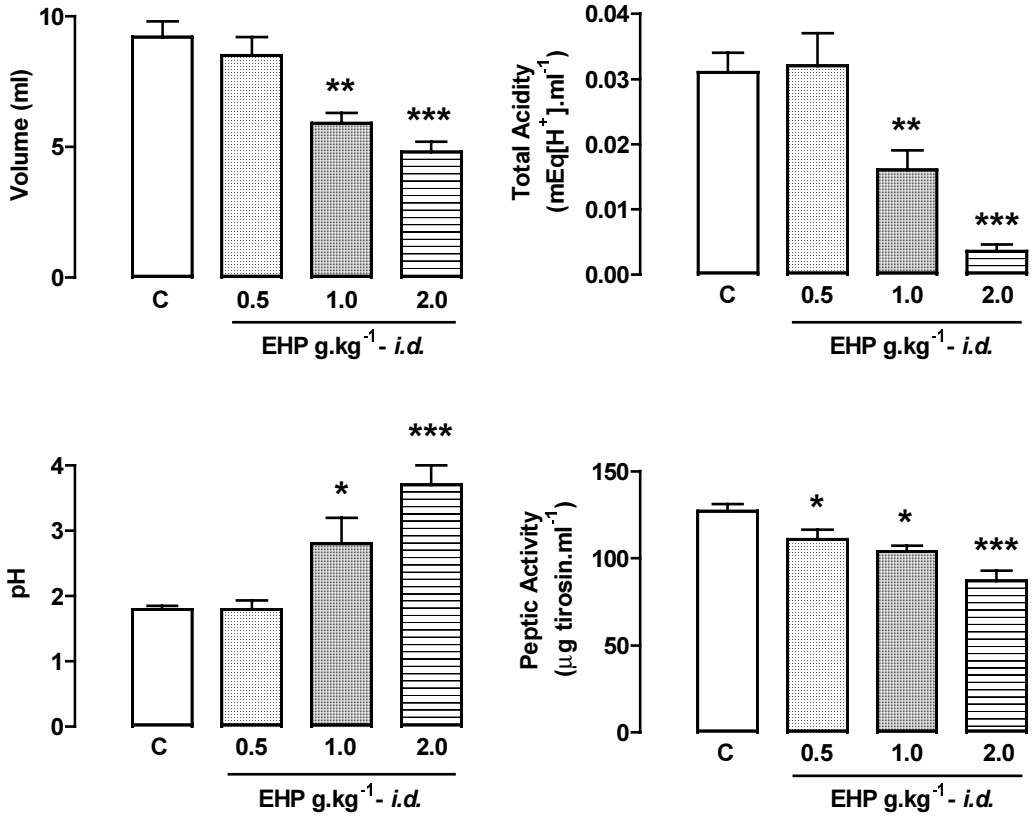


**Figure 2 -** Effects of the hydroalcoholic extract of *Pfaffia* – EHP - (C: control water, 0.5 ml.100g$^{-1}$) on the volume, total acidity, pH and peptic activity of basal gastric acid secretion after 4 hours of pylorus ligature in female rats. The data are means ± s.e.m., n=6 in all groups. * different from the control group at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

The EHP also can oppose to the histamine release stimulation by enterochromaffin cells and reduction of gastric mucosa blood flow also promoted by HRS (Hersey and Sachs, 1995; Brodie et al, 1962). The treatment with EHP can modify all of them factors that promote increase of gastric secretion and reduction of protective factors, with consequent protection against necrosis, hemorrhage, erosions and ulcers in the gastric mucosa. The EHP also reduced basal gastric acid and bethanechol- or histamine-induced secretion. These effects could not be attributed to a topic effect on the gastric mucosa since the extract was always injected in the duodenal lumen. On the other hand, a specific blockade of different receptors of the physiological secretagogues is unexpected. Taking into account that muscarinic agonists and gastrin may stimulate histamine release to induce acid secretion *in vivo* (Schubert, 1997), future studies should be done to test whether the extract of *Paffia* sp inhibits the histaminergic pathway of acid secretion *in vitro*, considering either the $H_2$ receptors, cAMP production or the proton pump. These studies may be done with a specie of *Paffia* with known origin and correct botanical nomenclature.

In conclusion, the hydroalcoholic extract obtained from *Pfaffia* sp roots effectively protected the gastric mucosa and inhibited gastric acid secretion in rats. These results confirmed folk information regarding the use of the plant in gastric upset.

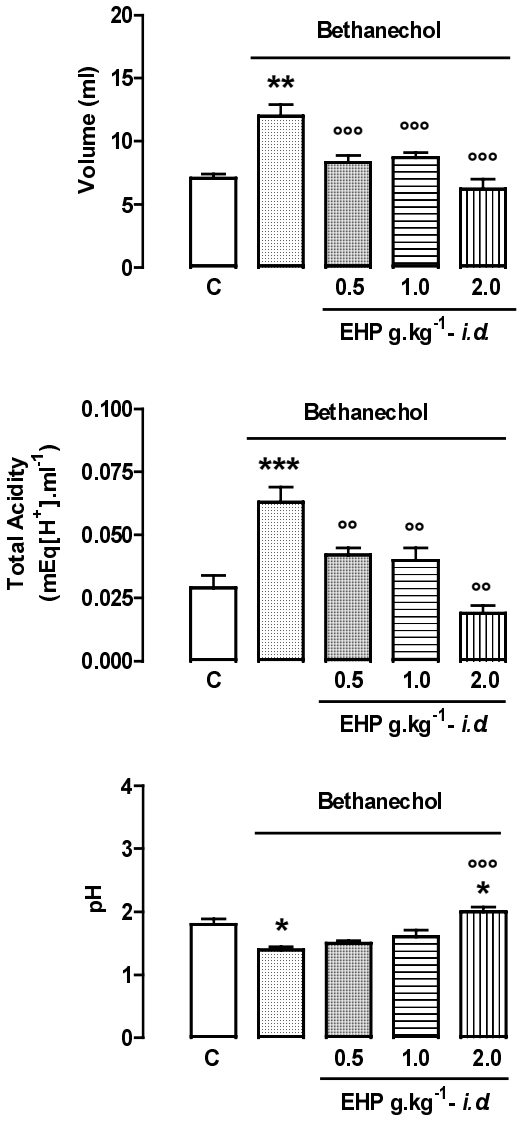


**Figure 3 -** Effects of the hydroalcoholic extract of *Pfaffia* – EHP - (C: control water, 0.5 ml.100g$^{-1}$) on the volume, total acidity and pH of gastric acid secretion induced by bethanechol (2.5 mg.kg$^{-1}$, *s.c.*), collected after 4 hours of pylorus ligature in female rats. The data are means ± s.e.m., n=6 in all groups. * different from the control group at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; °° different of bethanechol group at $p < 0.01$ and °°° $p < 0.001$.

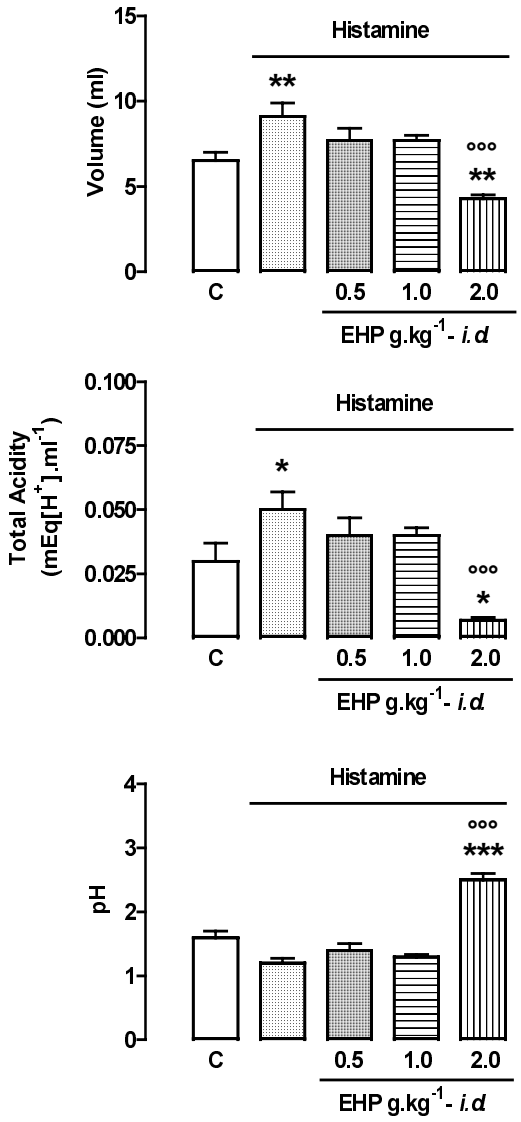


**Figure 4 -** Effects of the hydroalcoholic extract of *Pfaffia* – EHP - (C: control water, 0.5 ml.100g$^{-1}$) on the volume, total acidity and pH of gastric acid secretion induced by histamine (10 mg.kg$^{-1}$, *s.c.*), collected after 4 hours of pylorus ligature in female rats. The data are means ± s.e.m., n=6 in all groups. * different from the control group at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; °°° different of histamine group at $p < 0.001$.

## RESUMO

As plantas que compõem o gênero *Pfaffia* são utilizadas na medicina popular para tratar distúrbios gástricos. O presente estudo verificou os efeitos de um extrato bruto hidroalcoólico de *Pfaffia* sp sobre o trato gastrointestinal. Ratos Wistar fêmeas, foram pré-tratados por via oral

(*p.o.*) com o extrato hidroalcoólico de *Pfaffia* sp (0,5; 1,0 e 2,0 g/kg) 1 hora antes da indução de úlceras com estresse por imobilização e frio, com etanol 70% ou indometacina.

Animais controle receberam água (C) ou ranitidina (R: 60mg/kg) – *p.o.* O tratamento com o extrato protegeu a mucosa gástrica contra o aparecimento de úlceras induzidas por estresse e etanol, mas não foi capaz de proteger a mucosa contra úlceras induzidas por indometacina. Quando injetado pela via intraduodenal (na luz do duodeno – *i.d.*), o extrato hidroalcoólico de *Pfaffia* sp inibiu tanto a secreção ácida gástrica basal quanto a estimulada com histamina e betanecol (drogas agonistas), em ratas com ligadura de piloro. Os resultados obtidos neste trabalho indicam que esta planta apresenta ação protetora da mucosa gástrica, envolvendo a redução da secreção gástrica.

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