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Antiulcer and gastric antisecretory effects of dichloromethane fraction and piplartine obtained from fruits of *Piper tuberculatum* Jacq. in rats

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ABSTRACT

Ethnopharmacological relevance: *Piper tuberculatum* Jacq. (Piperaceae) is medicinally used as an analgesic and as a treatment for gastric complaints. Thus, the current study aimed to investigate the gastroprotective and antisecretory properties of the dichloromethane fraction of the fruit of *Piper tuberculatum* (DFPT) and piplartine, a compound isolated from the DFPT, in rats.

Materials and methods: Gastric ulcers were induced in fasted rats by oral administration of absolute ethanol and then mucus content and glutathione (GSH) levels were measured. Mechanisms underlying the antisecretory action were studied through gastric H^+,K^+-ATPase activity of highly purified rabbit gastric microsomes and pylorus ligature method in rats.

Results: In the acute toxicity test the values of estimated LD_{50} for oral and intraperitoneal administration of DFPT were 1.6266 and 0.2684 g/kg, respectively. The DFPT (ED_{50} = 29 mg/kg, p.o.) and piplartine (4.5 mg/kg, p.o.) promoted gastroprotection against acute lesions induced by ethanol, effect that could be related with the maintenance of GSH levels in the gastric mucosa. However, only DFPT stimulated gastric mucus secretion. *In vitro*, the DFPT and piplartine inhibited the H^+,K^+-ATPase activity and, *in vivo* DFPT and piplartine also reduced basal gastric acid secretion, as well as that stimulated by pentagastrin.

Conclusions: These results demonstrate that DFPT and piplatine cause marked gastroprotective effects accompanied by the increase and maintenance of gastric mucus and GSH levels, as well as a reduction in gastric acid secretion through the gastrinergic pathway.

1. Introduction

Peptic ulcer is a common and multi-etiologic disease that can be induced by physical or chemical agents and stress in human populations. The gastric mucosal damage and the development of peptic ulcers were attributed to an imbalance in the equilibrium between aggressive (HCl, pepsin) and protective (blood supply, mucus, prostaglandins, etc.) factors as well as stress due to trauma, infection caused by *Helicobacter pylori*, excessive use of cigarettes and alcohol and chronic treatment with steroidal and non-steroidal drugs (Laine et al., 2008; deFoneska and Kaunitz, 2010). Many current rodent models are utilized to produce various types of damage to the stomach. Experimental gastric ulcers can be triggered by ethanol because of its direct toxic action, generation of free radicals and inflammatory mediators, leading to hemorrhage and necrosis (Nassini et al., 2010). Moreover, pylorus ligature induces the retention of gastric acid secreted through cholinergic, histaminergic and gastrinergic pathways, which culminates in the activation of the parietal cell H^+,K^+-ATPase (proton pump) (Schubert, 2011).

Antisecretory drugs such as histamine H_2 receptor antagonists (ranitidine) and irreversible proton pump inhibitors (omeprazole) are well known to be effective for treating gastric ulcers (Jain et al., 2007). However, ulcers frequently relapse even in the presence of maintenance therapy with these drugs, and in general, long-term treatment with antisecretory agents can cause serious side effects, such as osteoporosis, hypergastrinemia, resulting in hyperplasia of enterochromaffin-like (ECL) cells and development of carcinoids in gastric mucosa (Poynter et al., 1985; Penston and Wormsley, 1986;

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Eom et al., 2011; Sheen and Triadafilopoulos, 2011). Considering the present clinical scenario, new, more effective and safer treatments with fewer side effects are necessary. In this context, natural products emerge as interesting therapeutic resources for the development of new drugs to treat gastric ulcers.

The plant family Piperaceae is a promising source of diverse phytochemicals because its members have been employed for various medicinal applications. *Piper tuberculatum* Jacq., which belongs to this family, is commonly known in Brazil as "jaborandi falso" or "pimenta darta". Pharmacologically, its leaves and fruits infusions are widely used in folk medicine as an analgesic, sedative, antidote for snake bites and treatment for digestive disorders, whereas other references just mention that dried fruits are prepared as a food spice to improve the flavor of beans (Chaves et al., 2003; Felipe et al., 2007). Despite the extensive review and to the best of our knowledge, there were no such reports available in support of the doses used in traditional medicine for the gastric-ulcer protective activity.

Moreover, pharmacological studies have revealed the antinociceptive, antifungal and trypanocidal activities of extracts and fractions of *Piper tuberculatum* (Regasini et al., 2009a, 2009b; Rodrigues et al., 2009). Particularly, phytochemical analyses of *Piper tuberculatum* have identified the presence of a variety of amides with different biological activities (Navickiene et al., 2000, 2003; Duarte et al., 2004; Furlan et al., 2009). Among these compounds, the most commonly studied amide alkaloid isolated from *Piper tuberculatum* is piplartine, which displays several biological actions, such as anti-platelet, antifungal, schistosomicidal, cytotoxic, genotoxic, antitumor, antinociceptive, anxiolytic and antidepressant effects (Navickiene et al., 2003; Bezerra et al., 2005, 2006, 2009; Felipe et al., 2007; Fontenele et al., 2009; Rodrigues et al., 2009; Moraes et al., 2011). Interestingly, some *Piper* species have also been demonstrated to have antisecretory properties and gastroprotective activity against experimental ulcers induced by ethanol, nonsteroidal anti-inflammatory drugs and stress (Agrawal et al., 2000; Majumdar et al., 2003; Morikawa et al., 2004; Trabadela et al., 2008; Quilez et al., 2010; Lima et al., 2012).

Because plants of the genus *Piper* have been used for gastric complaints and its antiulcer activity has been demonstrated on experimental models, the present study sought to investigate the antisecretory and gastroprotective actions of the dichloromethane fraction (DFPT) obtained from the crude extract of *Piper tuberculatum* and its isolated alkaloid piplartine in animal models.

2. Material and methods

2.1. Preparation of the dichloromethane fraction and isolation of the active compound

Piper tuberculatum fruits were collected in September 2005 at Porto Velho, Rondônia, Brazil. A voucher specimen was identified and deposited at the Herbarium of Instituto de Pesquisa da Amazonia (INPA), under the number 211724.

The dried fruits of *Piper tuberculatum* (1.1 kg) were powdered and extracted with ethanol (3 L × 3) at room temperature. The solvent was fully evaporated under reduced pressure to yield a brown solid (36.0 g). Part of the extract (30.0 g) was chromatographed on a silica gel column and eluted with hexane, dichloromethane, ethyl acetate and methanol, giving the respective fractions: 22.9% of hexane fraction (6.9 g), 21.3% of dichloromethane fraction (DFPT, 6.4 g), 22.3% of ethyl acetate fraction (6.7 g) and 30.0% of methanol fraction (8.9 g).

The dichloromethane fraction (5.1 g) showed a marked antinociceptive activity in previous studies (Rodrigues et al., 2009) and for this reason was chromatographed using a silica gel column eluted with a mixture of hexane and dichloromethane with increasing polarity. Elution with hexane and dichloromethane (20:80 v/v) yielded a white amorphous solid (87.6 mg), identified as piplartine [*N*-(3', 4', 5'-trimethoxycinnamoyl-Δ3-pyridin-2-one] (Fig. 1), by comparing spectral analysis of ^{1}H and ^{13}C NMR uni- and bi-dimensional, mass spectra and comparison with ^{1}H and ^{13}C NMR literature data (Facundo et al., 2005, 2008; Rodrigues et al., 2009).

2.2. Animals

Female Wistar rats (180–220 g) and female Swiss mice (25–35 g) were obtained from the Biotery of Federal University of Parana. Experimental groups consisted of 8 rats or 6 mice per group. They were housed at 22 ± 2 °C under a 12-h light/dark cycle (lights on at 07:00 h), with free access to laboratory chow and tap water. The rats were deprived of food in the 24 h period prior to starting the experimental ulcer models whereas mice were deprived of food in the 12 h period prior to acute toxicity studies. One adult albino rabbit (*Oryctolagus cuniculus*) weighing ~2 kg and purchased from the Biotery of Pontifícia Universidade Católica do Paraná (PUC-PR) was used for gastric H^+,K^+-ATPase assay. The experiments were conducted in accordance with the Ethical and Practical Principles of the Use of Laboratory Animal Guidelines, and the experimental procedures were previously approved by the Institutional Ethics Committee of the Federal University of Paraná (CEUA/UFPR; approval number 446).

2.3. Hippocratic screening (test of general activities) and acute oral toxicity

In order to study any possible toxic effect or changes on the general behavior of conscious animals, female Swiss mice ($n=6$) were fasted overnight (12 h) with free access to water prior to administration of single oral or intraperitoneal doses of DFPT (0.005, 0.05, 0.5 and 5 g/kg). The signs and symptoms associated with the DFPT administration were observed in freely moving animals at 0, 30, 60, 120, 180, 240 and 300 min afterwards and thereafter daily up to 7 days. At the end of the period the acute toxicity for each administration route was expressed by the required dose in g/kg body weight to cause death in 50% of the animals tested (LD_{50}) (Litchfield and Wilcoxon, 1949).

2.4. Acute gastric lesion induced by ethanol

The experiments were carried out according to the method described by Robert et al. (1979). Rats were treated with vehicle [C; water or saline plus 0.5% Tween 80 (1 ml/kg) per os (p.o.) or by the intraperitoneal (i.p.) route], DFPT (10, 30 and 100 mg/kg, p.o.; or 1, 3 and 10 mg/kg, i.p.), piplartine (4.5 mg/kg, p.o.) or omeprazole (40 mg/kg, p.o.), 60 min (p.o.) or 30 min (i.p.) before oral administration of ethanol P.A. (0.5 ml/200 g). 1 h later, the animals were sacrificed, their stomachs were removed, and the area of the gastric lesion (mm^2) was measured using the program Image Tool 3.0® as previously described (Potrich et al., 2010). It is important

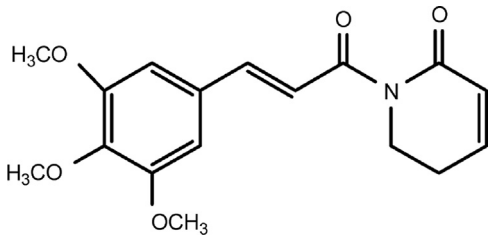

Fig. 1. Chemical structure of piplartine.