Evaluation of anti-ulcerogenic activity in an Aqueous Extract obtained from Bauhinia forficata leaves

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ABSTRACT

Bauhinia forficata Link, popularly known as pata-de-vaca, unha-de-vaca, casco-de-vaca, has been widely used in traditional medicine to treat several diseases. Leaves of B. forficata are used in popular medicine as a diuretic, hypoglycemic, tonic and cleanser, and to combat elephantiasis. However, despite the wide range of ethnopharmacological data surrounding the plant, there are no scientific data demonstrating a probable anti-ulcerogenic activity conferred by use of that species. The present study aimed to evaluate the antiulcer properties of an infusion of fresh leaves of B. forficata Link. From the leaves of B. forficata, an Aqueous extract (AqE) was obtained and the phytochemical analysis showed the presence of flavonols in this extract. In the gastric ulcer induced by administration of HCl-Ethanol model performed with four different doses of AqE (125, 250, 500 and 1000 mg.Kg⁻¹), the AqE showed significant preventive activity (*p<0.01) at doses of 1000 mg.Kg⁻¹. The antiulcer activity of AqE (1000 mg.Kg⁻¹) could also be demonstrated in experimental models of NSAID-bethanechol (**p<0.001) and absolute ethanol (**p<0.001). Moreover, AqE (1000 mg.Kg⁻¹) promoted a significant increase (**p<0.001) in the amount of gastric mucus. The data presented here demonstrated the potential gastroprotective activity from AqE, possibly attributed to the presence of flavonols in this extract. These results may serve as a support for the development of new treatments related to the pathology of gastric ulcer.

Keywords: Gastric ulcer. Cytoprotection. Flavonoids. Bauhinia forficata.

INTRODUCTION

Peptic ulcer is a term used to describe a group of ulcerative disorders that occur in areas of the upper gastrointestinal tract that are exposed to acidic secretions and pepsin. This pathology is characterized by mucosal damage and is predominantly caused by Helicobacter pylori, antiplatelet agents such as acetylsalicylic acid, nonsteroidal anti-inflammatory drugs (NSAIDs) such as oral biphosphonates, potassium chloride, immunosuppressive medications, serotonin reuptake inhibitors, alcohol consumption, and cigarette smoking (Golbabapour et al., 2013).

Peptic ulcer represents a chronic health problem. Approximately 10% of the population have or will develop a peptic ulcer (Brito Júnior et al., 2013). In Brazil, despite the absence of epidemiological records on ulcer cases, it is known that there are numerous cases involving this disease, meaning that it is a significant public health problem that has prompted a search for new substances with anti-ulcerogenic activity. Although a large arsenal of drugs with anti-ulcerogenic activity is already on the market, none results in 100% remission of ulcers with minimal side effects and without compromising the patient’s wellbeing, which usually results in the chronic use of these drugs. Published studies have reported the widespread identification of new drugs derived from natural anti-ulcerogenic sources (Jamal et al., 2006).

According to the World Health Organization, 80% of people in developing countries depend on traditional medicinal practices to meet and/or supplement their basic health needs (Pires et al., 2011). Currently, despite marketing and encouragement from the pharmaceutical industry during the development of allopathic medicines, a large segment of the population in many countries continues to utilize complementary practices for their health care (Bonifácio et al., 2014).

Brazil is an important reference point for the study of medicinal plants. With more than 56,000 species (excluding fungi), Brazil has one of the richest floras in the world – nearly 19% of the world’s flora (Giulietti et al., 2013).
Antiulcerogenic activity of Bauhinia forficata

2005). Among this wide range of plant species, particularly relevant in the present context is Bauhinia forficata Link (Caesalpinioideae). This species corresponds to one of about 300 species of Bauhinia, and is also popularly known as Unha-de-Vaca, Pata-de-Vaca and Casco-de-Vaca. It is an ornamental tree because its showy white flowers are widely used in landscaping and urban forestry. Native to the Atlantic forest, this species is found from Rio de Janeiro to Rio Grande do Sul; therefore, its presence is very common on the coast of Santos city (Lorenzi, 2002).

B. forficata is a species that has been used in folk medicine in Africa, Asia and America for the treatment of various diseases (Pizzolatti, 2003). Moreover, an infusion of the leaves of B. forficata is used in popular medicine as a diuretic, hypoglycemic, tonic and cleanser, and to combat elephantiasis (Martins et al., 1988). However, despite the wide range of ethnopharmacological data surrounding the plant, our literature review in databases of biomedical scientific information such as PubMed and Google Scholar showed that there are no data regarding a probable antiulcerogenic activity conferred by use of B. forficata.

Considering the need to search for new resources for the treatment of gastric ulcers and the exponential growth in scientific research about medicinal plants, the present study aimed to evaluate the antiulcer properties of an infusion of fresh leaves of B. forficata Link.

MATERIALS AND METHODS

Plant Specimen and Extraction

B. forficata Link leaves were obtained from Peruíbe, São Paulo, Brazil (-24.267948, -46.959276 (latitude/longitude)) in March 2007 and were identified by Botanical Ms Paulo Salles Penteado; a voucher specimen with number 4651 was deposited in the herbarium of the Universidade Santa Cecília (HUSC). The fresh leaves were subjected to an infusion extraction according to Pepato et al. (2002). In this process, the infusion with the leaves of this species was performed at a ratio of 150 g of fresh leaves/liter of water. Subsequently, the extracts were filtered and concentrated under vacuum to obtain an Aqueous Extract (AqE) from B. forficata leaves.

Phytochemical Analysis

Tests for the presence of flavonoids in the AqE were carried out according to previously reported methods (Shinoda test, alkaline reagent test, ferric chloride test and oxalo-boric acid reaction) (Costa, 1982; Harborne, 1973; Harborne, 1998; Zuanazzi & Montanha, 2010).

Animals

Male Wistar rats (150–250 g) and male Swiss mice (25–35 g), both obtained from the breeding facility of the Santa Cecilia University (UNISANTA), were used. The animals were fed a certified Nuvilab® (Nuvital) diet with free access to tap water under standard conditions of 12 h dark–12 h light, humidity (60 ± 1.0%) and temperature (21 ± 1°C). Moreover, the animals were kept in cages with raised floors of wide mesh to prevent coprophagy. The protocols were approved by the Santa Cecilia University Institutional Animal Care and Use Committee (CEUA – UNISANTA) under protocol number 53/07.

HCl/Ethanol-Induced Ulcer

The anti-ulcerogenic activity of the AqE from B. forficata leaves was assessed in mice using this model as described by Mizui and Doteuchi (1983). Mice were divided into 3 groups which were fasted for 24 h prior to oral dosing with the vehicle, 0.9% Saline (10 ml.Kg⁻¹), Lansoprazole (30 mg.Kg⁻¹), or AqE (125, 250, 500 and 1000 mg.Kg⁻¹). Fifty minutes after the treatments, all animals received 0.2 ml orally of a 0.3 M HCl/60% EtOH solution. Animals were killed 1 h after the administration of HCl/ EtOH solution; the stomachs were excised, inflated by an injection of saline (2 ml) and opened along the greater curvature. Then the stomachs were fixed in 5% formalin for 30 min and the ulcerative lesion index (ULI) was calculated by the methodology of Szelenyi and Thiemer (Szelenyi and Thiemer, 1978).

NSAID-Induced Gastric Ulcers in Cholinomimetic-Treated Mice

The experiment was performed according to the method of Rainsford, 1978. In this model, gastric ulcer was induced using indomethacin (30 mg.Kg⁻¹, s.c.) and bethanechol (5 mg.Kg⁻¹, i.p.) administered to mice after a 24 h fast. AqE (1000 mg.Kg⁻¹), Lansoprazole (30 mg.Kg⁻¹) or 0.9% Saline (10 mL.Kg⁻¹) were administered orally 30 min before the induction of a gastric ulcer. The animals were killed by cervical dislocation 4 h after treatment with the ulcerogenic agents; the stomachs were removed and inflated with 4% formalin in buffered saline, and the gastric damage was determined by the methodology of Szelenyi and Thiemer (1978).

Ethanol-induced gastric ulcer

Rats were subjected to an ethanol-induced ulcer assay (Morimoto et al., 1991). The rats were fasted for 24 h (free access to water) before the experiment. The animals were then randomly divided and received either oral AqE (1000 mg.Kg⁻¹), Lansoprazole (30 mg.Kg⁻¹) or 0.9% Saline (10 mL.Kg⁻¹). After 30 minutes, all animals received 1 mL of absolute ethanol orally. One hour later, the animals were killed by cervical dislocation and their stomachs were removed to determine their ulcer index (Szelenyi & Thiemer, 1978).

Determination of Mucous in Gastric Contents

This assay was performed according to the methodology described previously by Curtis et al. (1995), with some modifications. Mice were fasted for 24 h under anesthesia, with the abdomen incised and the pylorus ligated. Either AqE (1000 mg.Kg⁻¹), Carbenoxolone (200 mg.Kg⁻¹) or vehicle (10 mL.Kg⁻¹) were administered...
intraluminal administration of a vehicle of the experiment. The animals were killed by cervical dislocation 4 h after the drug treatments. The stomach contents were immersed in 10 mL of 0.02% Alcian Blue in 0.16 M sucrose/0.05 M sodium acetate, pH 5.8 and incubated for 24 h at 20°C. The Alcian Blue binding extract was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 615 nm using a spectrophotometer U/2000 (Hitachi, Japan). The free mucous in the gastric contents was calculated from the amount of Alcian Blue Binding (mg/wet tissue (g)).

Statistical Analysis

Results were expressed as the mean± (S.E.M.) and statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test, with the minimum level of significance set at p<0.05.

RESULTS

Some studies have shown that B. forficata extract presented flavonoid compounds such as Kaempferol (a flavonol compound). The phytochemical screening of this study showed that AqE from B. forficata leaves presented flavonoids in four different analyses (Table 1).

We can observe that the AqE from B. forficata leaves at doses of 1000 mg.Kg⁻¹ by the oral route prevented the gastric lesions induced by the administration of HCl Ethanol (*p<0.01), absolute ethanol (***p<0.001) and NSAIDS-Bethaneocol (**p<0.01) (Table 2).

Still searching for a possible gastroprotective mechanism for the AqE from B. forficata leaves, we investigated the effects of this extract (1000 mg.Kg⁻¹) on experimental protocols of mucus production. Pretreatment with AqE and Carbenoxolone (200 mg.kg⁻¹) significantly (***p<0.001) increased the amount of adherent mucus in the gastric mucosa when compared to the control group (Figure 1).

DISCUSSION

Flavonoids are some of the most common phenolics, widely distributed in plant tissues and often responsible, together with carotenoids and chlorophylls, for their blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonoids, anthocyanins, anthocyanidins, proanthocyanidins and catechins (Ferreira & Pinho, 2012; Rong, 2010).

These phenolic compounds (Flavonoids) belong to the recently popularized phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. The therapeutic effects of many traditional medicines may be related in many cases to the presence of these polyphenols (Manach et al., 1997). For example, a wide variety of pharmacological activities have been reported for these substances, including antiviral (Critchfield et al., 1996), anti-allergic (Cheong et al., 1998), antiplatelet (Carotenuto et al., 1997), anti-estrogenic, anti-carcinogenic, anti-inflammatory, anti-proliferative, antiangiogenic, and antioxidant properties, and their ingestion typically produces no or very little toxicity (Havsteen, 2002). Flavonoids are also reported to act in the gastrointestinal tract, having antisapmodic, anti-secretory, anti-diarrheal and anti-ulcerative properties (Mota et al., 2009). In our phytochemical screening, we found that AqE from B. forficata leaves presented flavonoids in four different analyses (Table 1).

Experimental animals play an important role in the search for new drugs with protective properties. Considering that the etiology of ulcer is multifactorial, lesions in the gastric mucosa can be induced in different experimental

| Table 1 - Phytochemical screening of Aqueous Extract (AqE) from B. forficata leaves
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| Table 2 - Effects of an aqueous extract (AqE) obtained from B. forficata Link leaves in different models of acute gastric lesion induced in rats or mice. |
|---|---|---|---|
| Gastric Ulcer Models | Treatment (p.o.) | n | Dose (mg.Kg⁻¹) | ULI (mm) | Inhibition (%) |
| HCl-Ethanol in mice | 0.9% Saline | 07 | 10 mL.Kg⁻¹ | 36.9 ± 11.0 | --- |
| Lansoprazole | 08 | 30 mg.Kg⁻¹ | 12.7 ± 1.7** | 65.6% |
| AqE | 07 | 125 mg.Kg⁻¹ | 36.9 ± 3.8 | --- |
| AqE | 07 | 250 mg.Kg⁻¹ | 30.0 ± 3.5 | --- |
| AqE | 07 | 500mg.Kg⁻¹ | 29.7 ± 3.0 | --- |
| AqE | 07 | 1000 mg.Kg⁻¹ | 25.0 ± 3.3* | 32.2% |
| Ethanol in Rats | 0.9% Saline | 07 | 10 mL.Kg⁻¹ | 94.6 ± 8.0 | --- |
| Lansoprazole | 07 | 30 mg.Kg⁻¹ | 59.4 ± 7.4** | 37.2% |
| AqE | 07 | 1000 mg.Kg⁻¹ | 75.7 ± 6.1** | 20% |
| NSAIDS | 0.9% Saline | 08 | 10 mL.Kg⁻¹ | 22.0 ± 2.1 | --- |
| Bethaneocol in mice | Lansoprazole | 10 | 30 mg.Kg⁻¹ | 11.0 ± 1.4** | 50% |
| AqE | 08 | 1000 mg.Kg⁻¹ | 16.0 ± 1.9** | 27.3% |

Data are presented as mean± S.D (n=7–10). ANOVA followed by Dunnett’s test with *p < 0.01; ** p < 0.001
models through various mechanisms (Samonina, 2004). Some of the most widely used acute models for the evaluation of anti-ulcerogenic substances in animals are models of gastric lesion induced by HCl-ethanol, absolute ethanol and NSAIDS-bethanecol. In this work, we used these methodologies to evaluate the pharmacological activity of an AqE from *B. forficata* leaves.

Lee *et al.* (2010) related that the administration of HCl-ethanol produces ulcerative lesions and increases lipid peroxidation in the gastric mucosa, which plays a significant part in the pathogenesis of the mucosal lesions. Moreover, HCl-ethanol instillation induces severe epithelial desquamation, deepmucosal necrosis and submucosal edema associated with leukocyte accumulation. Brito Júnior *et al.* (2013) showed that the gastric lesions induced by absolute ethanol occur predominantly in the glandular portion of the stomach, as the result of a direct necrotizing action and, moreover, a lack of defense mechanisms such as secretion of bicarbonate and mucus and increased oxidative stress. The suppression of prostaglandin synthesis by nonsteroidal anti-inflammatory drugs (NSAIDS), such as indomethacin, is known to result in the increased susceptibility to mucosal injury and gastroduodenal ulceration. Indomethacin inhibits both cyclooxygenase-1 (COX-1) and (COX-2) as well as the production of prostaglandins in the stomach and intestines to maintain the mucus lining of the gastrointestinal tract. Indomethacin, therefore, like other non-selective COX inhibitors, can cause peptic ulcers. Cholinomimetic agents (bethanecol) administered in association with NSAIDS have a synergistic effect on the gastric injury induced by increased secretion of acid and pepsin in the stomach (Lee *et al.*, 2010).

In our protocols, we observed that the AqE (1000 mg.Kg⁻¹) by the oral route prevented gastric lesions induced by the administration of HCl Ethanol (**p<0.01), absolute ethanol (**p<0.001) and NSAIDS-Bethanecol (**p<0.001) (Table 2).

Several works have shown that substances with antioxidant properties, such as flavonoids, may protect against the gastric-damaging effects caused by HCl-ethanol, absolute ethanol and NSAIDS-Bethanecol (Matsumoto *et al.*, 2004; Dekanski *et al.*, 2009). Hirano *et al.* (1994) demonstrated that a flavone protected the gastric mucosa of rats from injuries induced by ethanol. Moreover, Izzo *et al.* (1994) also obtained positive results in the treatment of gastric ulcer by utilizing flavonoids. Most likely, the flavonoids present in the AqE from *B. forficata* leaves are responsible for the anti-ulcerogenic activities evident in the experimental protocols demonstrated in this work.

In a review by Mota *et al.* (2009), the mechanisms of action of flavonoids such as flavonoids in gastric protection were reported to include increased gastric blood flow and stimulated mucus synthesis in the gastric mucosa (**p<0.001) (Figure 1). Most probably, the increase in adherent gastric mucus apparent after administration of the AqE from *B. forficata* leaves is possibly associated with the presence of flavonoids in this extract. Considering the lack of the cytoprotective treatments against gastric ulcers, new sources of cytoprotective treatments are vital. Thus, the data shown have an important bearing on new studies about probable cytoprotective treatments for gastric ulcers.

In conclusion, the AqE from *B. forficata* leaves showed preventive anti-ulcerogenic activity in mice in 3 ulcer models. Mucus secretion is involved in the gastroprotection exerted by this species, probably due to the flavonoids (flavonols) present in the plant. These results are important for the development of future cytoprotective treatments for gastric ulcers.

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**CONFLICTS OF INTEREST**

The authors of this article declare no conflict of interest regarding the data presented in the study.

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