ORIGINAL ARTICLE

ANTIULCER EFFECT OF *BIDENS PILOSA* L. AND TOXICOLOGICAL SAFETY IN RODENTS: COMPARATIVE STUDY BETWEEN STEM AND ROOT EXTRACTS

EFEITO ANTIÚLCERA DE BIDENS PILOSA *L. E SEGURANÇA TOXICOLÓGICA EM ROEDORES: ESTUDO COMPARATIVO ENTRE EXTRATOS DO CAULE E DA RAIZ*

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ABSTRACT

Purpose: To investigate and to compare the extracts of the stalks (Bpc-EtOH) and roots (Bpr-EtOH) from *Bidens pilosa* on ethanol-gastric ulcer model rats and acude toxicologial screening in mice. **Methods:** in the ulcer model were accessed the extracts on etanol-induced rat gastric lesions (n = 4-5). To analyze the safety toxicological those extracts in vivo, the acute toxicity screening was perfomed with sigle dose extracts and during three days in mice (n=5). **Results:** the Bpc-EtOH (150 and 500 mg/kg) protected the rat gastric mucosa layer (ULA=138.0 ± 30.5 and 96.3 ± 11.8 mm2, respectivelly). The Bpr-EtOH induced similar effects on all doses (ULA=178.3 ± 10.0; 67.4 ± 9.5 and 35.5 ± 14.3 mm2) and the most efficacy (Emax) than Bpc-EtOH. The omeprazole (ULA=145.7 ± 19.9 mm2) showed also protective effects as expected. The histophatology analyzes corroborated those results. The maximum dose 500 mg/kg (o.r.) of the extracts is safe and do not cause damages to animails because dose 2 g/kg (o.r.) in toxicological screening did fail. **Conclusion:** The stalks and roots from *B. pilosa* also present actives metabolites for antiulcer activity, it may be more concentration in roots or are different than stalks, and both are safe in doses tested.

Keywords: Medicinal plant; Plant extract; Anti-ulcer; Toxicity

RESUMO

Objetivo: Investigar e comparar a atividade antiúlcera de extratos obtidos das raízes (Bpr-EtOH) e do caule (Bpc-EtOH) de *Bidens pilosa* em ensaios de úlceras estomacais induzidas por etanol absoluto em ratos e na triagem toxicológica aguda em camundongos. **Métodos:** Para o ensaio antiúlcera foi utilizado o modelo de úlcera induzida por etanol absoluto em ratos (n = 4 a 5). Para a triagem toxicológica aguda, foram utilizados camundongos machos (n = 5), tratados com dose única dos extratos separadamente com 2 g/kg, via oral ou veículo e monitorados durante três dias. Os valores de $p < 0,05$, pelo teste t ou ANOVA, foram considerados significantes. Todos os procedimentos foram aprovados pelo CEUA/UNINOVE. **Resultados:** O extrato Bpc -EtOH (150 e 500 mg/kg) e o extrato Bpr-EtOH (50, 150 e 500mg/kg) protegeram a mucosa gástrica (ALU = 138,0 ± 30,5 e 96,3 ± 11,8 mm2, respectivamente), porém todas as doses (ALU = 178,3 ± 10,0; 67,4 ± 9,5 e 35,5 ± 14,3 mm2, respectivamente) foram significativamente diferentes do controle e mais eficazes que o extrato Bpc-EtOH. O omeprazol (ALU = 145,7 ± 19,9 mm2) também promoveu efeito semelhante aos extratos. A análise histopatológica das amostras do tecido confirmou a proteção gástrica. A dose máxima de 500 mg/kg dos extratos é segura e não deve causar danos aos animais, uma vez que na triagem toxicológica, utilizando a dose de 2 g/kg, não foram verificados danos importantes nem mortes. **Conclusão:** O caule e raiz de *B. pilosa* também apresentam princípios ativos com atividade antiúlcera, sendo que estão mais concentrados na raiz ou são diferentes daqueles no caule e, ambos, seguros nas doses utilizadas.

Palavras-chave: Planta medicinal; Extrato vegetal; Agente antiúlcera; Toxicidade

INTRODUCTION

The National Policy for Integrative and Complementary Practices in the Sistema Único de Saúde (SUS) and the National Policy for Medicinal Plants and Phytotherapeutics[1,2] encourage research with plants that may result in new treatments or therapies. Plant selection based on ethnopharmacological or chemotaxonomic criteria might lead to discovering medicines that serve as pharmacological tools or models for synthesizing new drugs[3].

The *Asteraceae* family has approximately 23,000 species and holds great medicinal value[4]. *Bidens pilosa* L. is a weed found in all tropical regions worldwide[5], popularly known as "picão-preto" in Brazil. Previous studies demonstrated biological activities for its aerial parts, including antimicrobial, anti-inflammatory, antimalarial, and hypoglycemic properties[6-9]. Also, the leaves of *Bidens pilosa* L. present antisecretory and antiulcerogenic properties[10].

The pharmacological treatment for ulcers relies on inhibiting gastric acid secretion using H2-antagonists and proton pump inhibitors[11]. However, most of those therapies cause adverse reactions[12]. In this sense, this study investigated and compared the effects of extracts obtained from roots (Bpr-EtOH) and stems (Bpc-EtOH) of *Bidens pilosa* on gastric ulcers induced by absolute ethanol in rats and evaluated the acute toxicological safety of these extracts in mice.

METHODS

The animal ethics committee of the Universidade Nove de Julho approved all procedures (AN 0003/12).

We used crude ethanolic extracts (Bpc-EtOH and Bpr-EtOH) obtained from the stem and roots of *B. pilosa*. The extracts were dissolved in TWEEN-20 (0.1%) and diluted in distilled water to obtain the necessary doses for experiments.

To investigate antiulcer activity, Wistar rats fasted for 24 h were separated into groups of five animals and treated orally by gavage with each extract (50, 150, or 500 mg/kg, treated group), 10 mL/kg of distilled water plus TWEEN-20 (negative control group), or 4 mg/kg omeprazole intraperitoneally (positive control group). After 60 minutes, 10 mL/kg of absolute ethanol was administered by gavage[13]. One hour after applying the ulcerogenic agent, animals were euthanized in a CO_2 chamber, stomachs removed and opened along the greater curvature. Ulcerative lesion area (ULA) was calculated according to adapted methodology[14]. Tissue samples were submitted to histopathological analysis with hematoxylin-eosin (HE).

To investigate acute toxicity, groups of five mice received 2 g/kg (PO)[15] of each extract and were monitored for locomotor and behavioral activity every 30 minutes for three consecutive hours. Another group received vehicle PO (distilled water + TWEEN-20 0.1%) as control. Death occurrence was verified after 24, 48, and 72 h to calculate lethal dose 50 (LD_{50})[16]. To observe possible morphological alterations of vital organs (heart, lungs, liver, and kidneys), organs were isolated and properly weighed (mg/g) and samples submitted to histopathological analysis with HE.

Results were expressed as mean ± standard error of the mean. Comparisons were conducted using the t-test or ANOVA followed by Dunnett post-test with significance level of $p < 0.05$. All analyses were performed using GraphPad Prism software, version 5.01.

RESULTS

Figure 1A shows that the Bpc-EtOH extract protected the gastric mucosa dose-dependently ($p < 0.05$) at 150 mg/kg (ALU = 138.0 ± 30.5 mm^2) and 500 mg/kg (ALU = 96.3 ± 11.8 mm^2). The Bpr-EtOH extract induced similar effects at all tested doses: 50 mg/kg (ALU = 178.3 ± 10.0 mm^2), 150 mg/kg (ALU = 67.4 ± 9.5 mm^2), and 500 mg/kg (ALU = 35.5 ± 14.3 mm^2) differed significantly from control (Figure 1B). Bpr-EtOH demonstrated greater efficacy (Emax, $p < 0.05$) than the stem extract (Bpc-EtOH). Omeprazole (ALU = 145.7 ± 19.9 mm^2) provided comparable protection versus control.

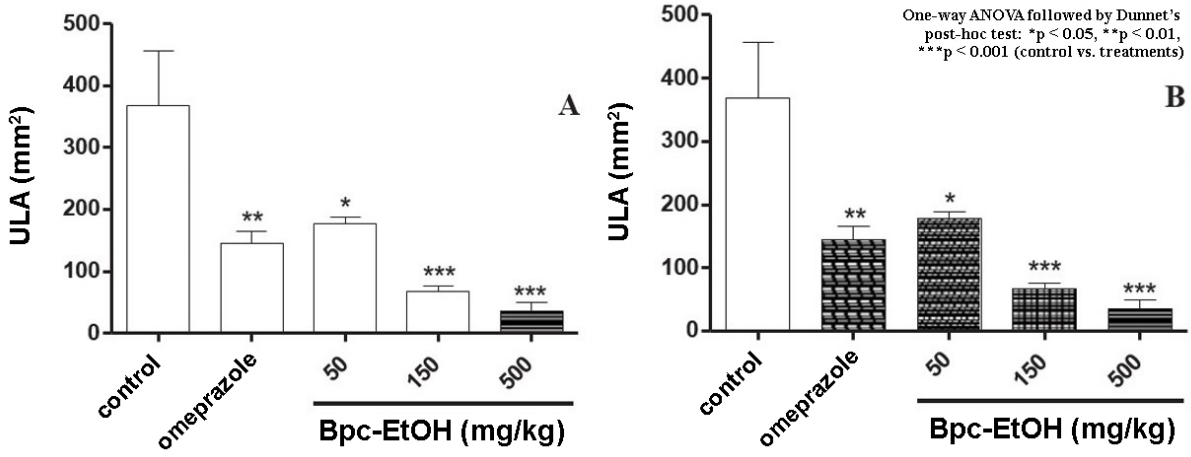

Figure 1. Effect of Bpc-EtOH (A) and Bpr-EtOH (B) extracts and omeprazole against ethanol-induced gastric ulcer (control) in rats (n = 4-5).

In the histopathological analysis of experimental samples, normal gastric mucosa crypts were observed in the control group (Figure 2A). In rats pre-treated with 500 mg/kg (oral) of Bpc-EtOH (Figure 2B) or Bpr-EtOH (Figure 2C) extracts, the products protected superficial mucosal cells, showing no hemorrhagic points or inflammatory cell infiltration, similar to the control group (Figure 2A).

During toxicological evaluation, Bpc-EtOH and Bpr-EtOH extracts did not alter animal ambulation or behavior compared with the control group.

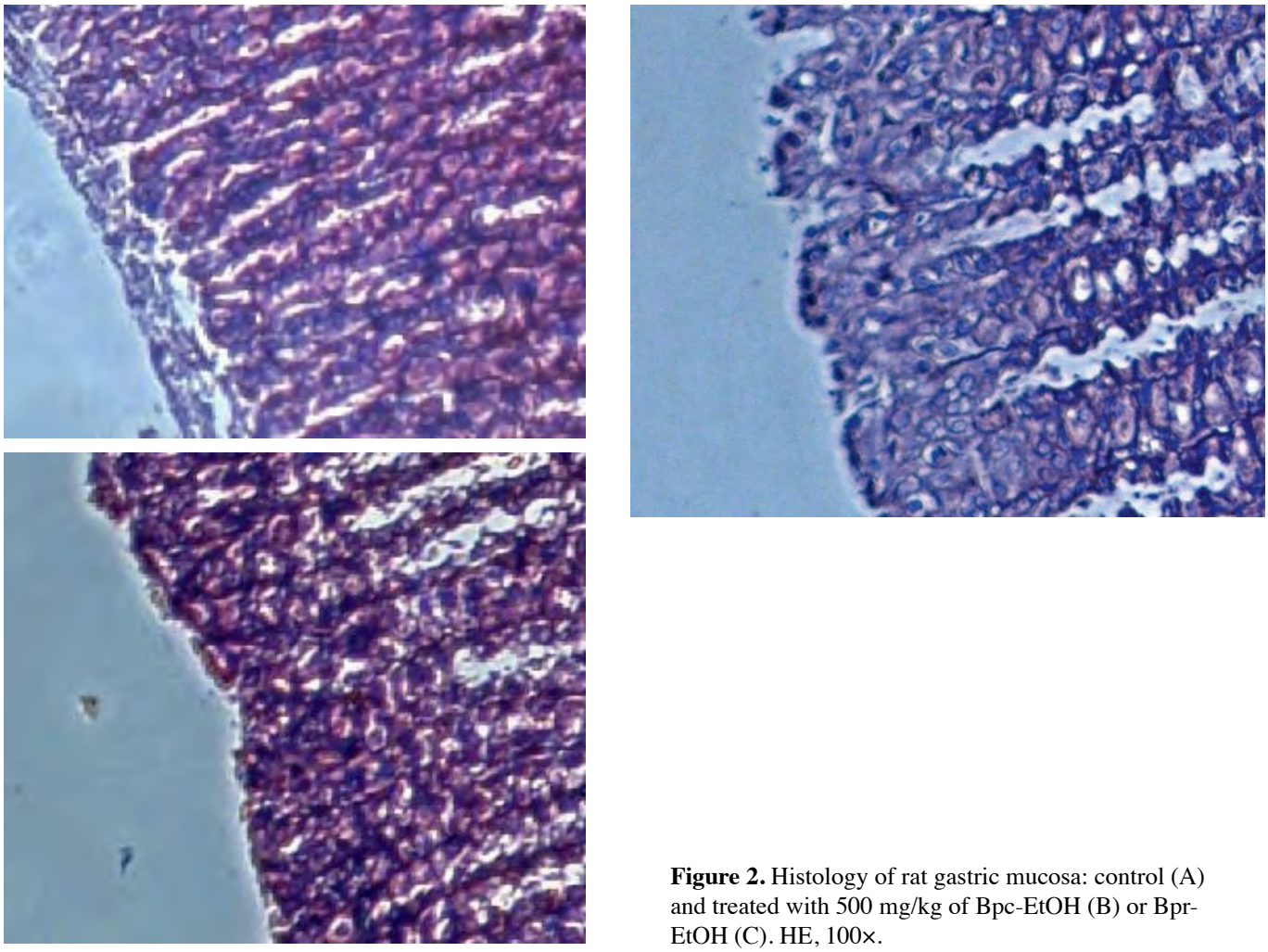

Figure 2. Histology of rat gastric mucosa: control (A) and treated with 500 mg/kg of Bpc-EtOH (B) or Bpr-EtOH (C). HE, 100×.

Mice treated with the Bpc-EtOH extract showed no weight changes after 24, 48, and 72 h of treatment (Table 1). However, Bpr-EtOH significantly reduced animal weight ($p < 0.01$) after 24 and 48 h of administration, accompanied by reduced food intake and increased water intake (data not shown), but animals recovered their weight at 72 h (Table 1).

No animal deaths occurred. Post-euthanasia analysis of vital organs (heart, liver, lungs, and kidneys) revealed that neither extract increased organ weight ratios (Table 2). However, decreased weight ratios were observed in animals treated with Bpr-EtOH extract for the heart and both extracts for the lungs (Table 2). Histopathological analysis revealed no tissue alterations characteristic of organ toxicity.

Table 1. Weight (g) of mice before (control) and 24, 48, and 72 hours after administration of Bpc-EtOH and Bpr-EtOH extracts (p.o., 2 g/kg), n = 5.

Treatment (extract)	Control	24 hours	48 hours	72 hours
Bpc -EtOH	36.6 ± 1.5	36.5 ± 1.4	36.2 ± 1.8	36.9 ± 1.8
Bpr -EtOH	33.6 ± 0.3	30.1 ± 0.7**	30.2 ± 0.9**	32.0 ± 0.6

**p<0.01, one-way ANOVA followed bu Dunnett posthoc (control x Bpr-EtOH)

Table 2. Weight ratio (mg/g) of isolated organs from control animals and animals treated with Bpc-EtOH and Bpr-EtOH extracts (p.o., 2 g/kg), n = 5.

Treatament	Heart	Liver	Lungs	kidneys
Control	0.9 ± 0.09	7.7 ± 0.7	1.9 ± 0.08	2.2 ± 0.1
Bpc -EtOH	0.8 ± 0.06	7.4 ± 0.1	1.3 ± 0.1**	1.9 ± 0.1
Bpr -EtOH	0.6 ± 0.03**	7.5 ± 0.4	1.0 ± 0.04***	1.9 ± 0.05

**p<0.01, ***p< 0.001, t-test (control x extract)

DISCUSSION

Gastrointestinal hyperreactivity can be induced in animal models and corresponds to various conditions that alter gastrointestinal tract physiology. Gastric ulcers occur due to imbalance between defense mechanisms and aggression factors of the gastroduodenal mucosa[17]. The animal model for ethanol-induced gastric lesions involves depression of gastric defense mechanisms and reduction in mucus production, gastric mucosal blood flow, bicarbonate secretion, endogenous glutathione, and prostaglandins[18]. Ethanol increases histamine release, calcium influx, free radical generation, leukotriene production[19], and neutrophil recruitment[20]. The *B. pilosa* extracts (Bpc-EtOH and Bpr-EtOH) protected the gastric mucosa through active principles of *Bidens pilosa*[8] that contributed to local antioxidant and anti-inflammatory activities, providing mucosal protection as observed in Figure 2 (B and C). Antioxidant administration inhibits ethanol-induced gastric lesions in rats[21]. Since the administration of antioxidants inhibits ethanol-induced gastric lesions in rats, and given that the root extract exhibited greater efficacy, it is likely that the active principles responsible for this activity are present at higher concentrations or differ in composition from those in the stem. The antiulcerogenic activity of *B. pilosa* leaves[10] corroborates these findings showing active principles with such properties in the roots and stem of this species.

Acute toxicological screening investigated the safety of extracts used in this study. Motor and behavioral activity of animals in open field showed that extracts demonstrated no anxiolytic, anxiogenic, or sedative effects (data not shown). Although Bpr-EtOH extract reduced rat weight after 24 and 48 hours of administration, this effect reversed at 72 hours. This finding suggests an appetite-moderating effect induced by the extract acting peripherally rather than affecting the central nervous system, since no alteration in animal ambulation was observed. Classic appetite inhibitors such as fluoxetine and sibutramine modulate central neurotransmitters and consequently appetite, reducing food intake[22].

At the end of toxicological screening, no extract increased the organ weight ratio (Table 2), indicating no alterations suggesting toxicity in these organs. However, significant decreases were noted in the heart weight ratio of rats treated with Bpr-EtOH and in the lung weight ratio of animals for both extracts (Table 2). Histopathological analyses showed

no alterations in these tissues (data not shown), ruling out any lesion. The maximum dose of 500 mg/kg of extracts used in the antiulcer effect is safe and should not cause damage to animals. In toxicological screening using 2 g/kg, fourfold higher than that of the pharmacological assay, no important damage or animal deaths were verified; therefore, the LD_{50} was not determined.

In conclusion, *Bidens pilosa* contains active principles with antiulcer properties in roots, stems, and leaves, being safe at the doses used in this study.

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