CARDIOSPERMUM CORINDUM L. (SAPINDACEAE) HAS GASTROPROTECTIVE AND ANTISPASMODIC EFFECTS IN RODENT MODEL

CARDIOSPERMUM CORINDUM L. (SAPINDACEAE) TEM EFEITO PROTETOR GÁSTRICO E ANTIESPASMÓDICO EM MODELO DE ROEDORES

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ABSTRACT

Objectives: To investigate the effects of the hydroethanolic extract of aerial parts of *Cardiospermum corindum* (Cc-EtOH) in the ethanol-induced gastric ulcer assay in rats, its oral toxicity in mice, and its antispasmodic activity in isolated rat ileum *in vitro*. **Methods:** The anti-ulcerative activity of Cc-EtOH was evaluated in ethanol-induced rat gastric lesions. Oral toxicity was assessed in mice following a single acute treatment and monitored for 14 days. The antiespasmodic effect of Cc-EtOH in isolated rat ileum was analyzed *in vitro*. **Results:** Ethanol-induced gastric lesions in rats (ulcer index = 367.5 ± 89.3 mm^2) were prevented by 50, 150, and 500 mg/kg Cc-EtOH (ulcer index = 210.0 ± 55.8, 119.2 ± 39.3, and 47.7 ± 13.2 mm^2, respectively), with efficacy comparable to omeprazole. Histological findings corroborated these results. A single oral dose of 2 g/kg Cc-EtOH induced transient sedation in mice after 60 min (n = 5), which was reversed within 30 min. No acute toxicity was observed, as evidenced by the absence of animal mortality, changes in body weight, or alterations in water or food intake over 14 days. Cc-EtOH reduced *in vitro* phasic contractions of isolated rat ileum elicited by carbachol or KCl (IC_{50} = 236 ± 59 and 201 ± 43 mg/mL, respectively). **Conclusion:** The aerial parts of *C. corindum* contain bioactive compounds that exert effective gastric protection in rats, show no evidence of acute toxicity, and display antispasmodic effect *in vitro*.

Keywords: Medicinal plant; Plant extract; Anti-ulcer Agent; Antispasmodic effect; Toxicity

RESUMO

Objetivos: Investigar os efeitos do extrato de etanol bruto obtido das partes aéreas C. corindum (Cc-EtOH) sobre o teste de úlcera gástrica induzido pelo etanol em ratos, sua toxicidade aguda oral em camundongos e sua atividade antiespasmódica em íleo isolado de rato. **Métodos:** O Cc-EtOH foi avaliado em lesões gástricas induzidas por etanol. Para verificar a segurança deste extrato in vivo, o teste de toxicidade aguda foi realizado durante 14 dias em camundongos. Finalmente, os efeitos do extrato no íleo do rato foram analisados. **Resultados:** O extrato Cc-EtOH (50, 150 e 500 mg/kg) protegeu significativamente a camada de mucosa gástrica do rato (área da úlcera = 210,0 ± 55,8; 119,2 ± 39,3 e 47,7 ± 13,2 mm^2, respectivamente) das lesões induzidas pelo etanol (área da úlcera = 367,5 ± 89,3 mm^2), tão efetivamente como omeprazol. As análises histológicas também corroboraram seu efeito protetor. Além disso, tratamento de 60 minutos com uma dose única de 2g/kg v.o., o extrato de Cc-EtOH promoveu a sedação dos camundongos (n = 5), que se reverteu após 30 minutos, sem causar nem a morte do animal, nem a massa corporal, a ingestão de água, nem as alterações da alimentação, durante 14 dias, mostrando não toxicidade aguda. O efeito antiespasmódico do extrato Cc-EtOH foi evidenciado no componente fásico das contrações induzidas por CCh e KCl (IC_{50} = 236 ± 59 e 201 ± 43 mg/mL, respectivamente) em íleo isolado do rato. **Conclusão:** As partes aéreas do C. corindum contêm compostos químicos que são capazes de promover proteção gástrica efetiva em ratos, na ausência de qualquer toxicidade aguda, combinada com o efeito antiespasmódico in vitro.

Palavras-chave: Planta medicinal; Extrato vegetal; Agente anti-úlcera; Efeito antiespasmódico; Toxicidade

INTRODUCTION

Gastric hyperacidity and gastroduodenal ulcers are frequent conditions that currently represent a major worldwide public health concern[1]. *Cardiospermum corindum L.* (*Sapindaceae*), popularly known as *balãozinho,* occurs in the northeastern, southeastern, and southern regions of Brazil[2]. In traditional medicine, particularly in the Brazilian northeast, infusions prepared from C. *corindum* are used to treat liver disorders and rheumatism, as a memory tonic, diuretic, and emmenagogue[3]. Several compounds have been isolated and chemically identified from the leaves and other aerial parts of *C. corindum*[4,5]. Despite its widespread folk use for stomach ailments, the biological activity of its compounds has not yet been scientifically described.

The present study aimed to investigate the effects of the hydroethanolic extract obtained from the aerial parts of *C. corindum* on ethanol-induced gastric ulcers in rats, its acute toxicity in mice, and its antispasmodic activity in isolated rat ileum.

METHODS

The aerial parts of *Cardiospermum corindum L.* were collected during the flowering period at the base of Pico do Jabre, Paraíba, Brazil. The plant was identified, and a voucher specimen (No. M.F. Agra et al. 6898) was deposited in the Prof. Lauro Pires Xavier herbarium at Universidade Federal da Paraíba. The crude hydroethanolic extract (Cc-EtOH) was prepared according to previous report[5].

The Cc-EtOH extract was dissolved in Tween-20 (0.32 mg/mL) and diluted in distilled water to final doses. Wistar rats (200 - 300 g) were stratified into six groups (n = 5) and pretreated with 50, 150, 250, or 500 mg/kg Cc-EtOH extract (gavage), 4 mg/kg omeprazole (intraperitoneal), or vehicle (distilled water-Tween-20, 10 mL/kg). One hour later, 1 mL of absolute ethanol was orally administered to each animal. After one hour, animals were euthanized in a CO_2 chamber, and their stomachs were collected and dissected along the greater curvature[6]. The ulcer index (UI) was assessed by quantifying the extent of erosion and experimental gastric lesions[7]. Samples were processed for histological analysis and stained with hematoxylin and eosin. This protocol was approved by the ethics committee on animal use of Universidade Nove de Julho (opinion AN 0002/11).

Swiss male mice (25 - 30 g) were divided into two groups (n = 5) and orally treated with either a single dose of 2 g/kg Cc-EtOH extract ("treated-group") or vehicle (10 mL/kg distilled water-Tween-20; "control group"). Animals were observed for 120 min at 30 min intervals to assess toxicity signs and motor activity (spaces roamed [S]). Reflexes (grooming or piloerection) were assessed after 24 h. Mortality, body weight, and water and food intake were concurrently monitored for 14 days. At the end of this period, animals were euthanized in a CO_2 chamber, and the heart, lung, liver, and kidney of animals were dissected and weighed (normalized to body weight[9]), and processed for hematoxylin-eosin histological analysis. Histopathological images were obtained using a camera-equipped microscope and analyzed with NS-Elements D software. This study was approved by the ethics committee on animal use of Universidade Nove de Julho (opinion # AN 0003/11).

Fasting (18 h) Wistar rats (250 - 350g) were decapitated, and the ileum was carefully isolated and dissected[8]. Tissue strips (1.5 cm) were mounted in organ baths containing 5 mL of modified Krebs-Henseleit solution at 37 °C with 11.0 mM glucose, and continuously aerated with O_2[10]. Strips were connected to a force transducer and amplifier, and contraction data were recorded using AQCD software. A Cc-EtOH stock solution (10 mg/mL) was prepared in 0.1% cremophor and diluted in Mili-Q water. After 30 min of equilibration period, strips were contracted with 1 μM carbachol (CCh) or 40 mM KCl, following 15 min of incubation with Cc-EtOH (27-730 μg/mL) or vehicle. Contractile responses to CCh or KCl were compared in the absence and presence of Cc-EtOH extract. The IC_{50} of the Cc-EtOH extract in CCh- or KCl-induced contraction were calculated by non-linear regression[11]. This protocol was approved by the ethics committee on animal use of the Universidade Federal de São Paulo (opinion number 4295060514/14).

Results were expressed as mean ± SEM. Student T-test or one-way ANOVA test followed by post-hoc Dunnett test for multiple comparisons were used when applicable. Differences were considered significant at $p < 0.05$.

RESULTS

Oral administration of ethanol induced stomach lesions (UI = 368 ± 89 mm^2; Figure 1). Pretreat-

ment with 50 mg/kg Cc-EtOH extract (UI = 210.0 ± 55.8 mm^2) did not prevent the ethanol-induced lesions compared with the control group. However, treatments with 150 and 500 mg/kg Cc-EtOH prevented ethanol-induced lesions in a dose-dependent manner (UI = 119 ± 39 and 48 ± 13 mm^2, respectively), similar to the effect observed with 20 mg/kg omeprazole (UI = 146 ± 20 mm^2).

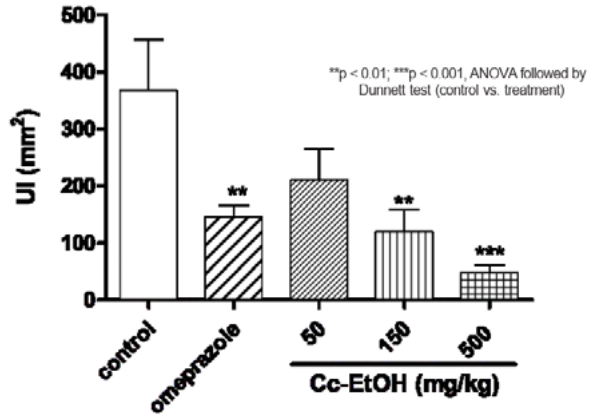

Figure 1. Ethanol-induced lesions (control) in the presence of omeprazole (20 mg/kg) or Cc-EtOH extract (50, 150, and 500 mg/kg) on rat stomachs (n = 6).

Histological analysis confirmed epithelial damage and inflammatory infiltration in the control group (Figure 2A), consistent with the characteristic mucosal injury in the ethanol-induced ulcer lesion model. However, the single-dose treatment with 500 mg/kg Cc-EtOH extract prevented ethanol-induced gastric lesions (Figure 2B). Pretreatment also attenuated the inflammatory response, suggesting regeneration and re-epithelization of the gastric mucosa.

In subsequent experiments, oral administration of 2 g/kg Cc-EtOH extract did not induce mortality or changed body weight, water or food intake (data not shown). However, treated animals showed significantly reduced ambulation between 30 and 60 min after treatment (S = 47.8 ± 4.4 and 34.4 ± 3.1 units, respectively) compared with the control group (Figure 3). Normalized organ weights are presented in Table 1.

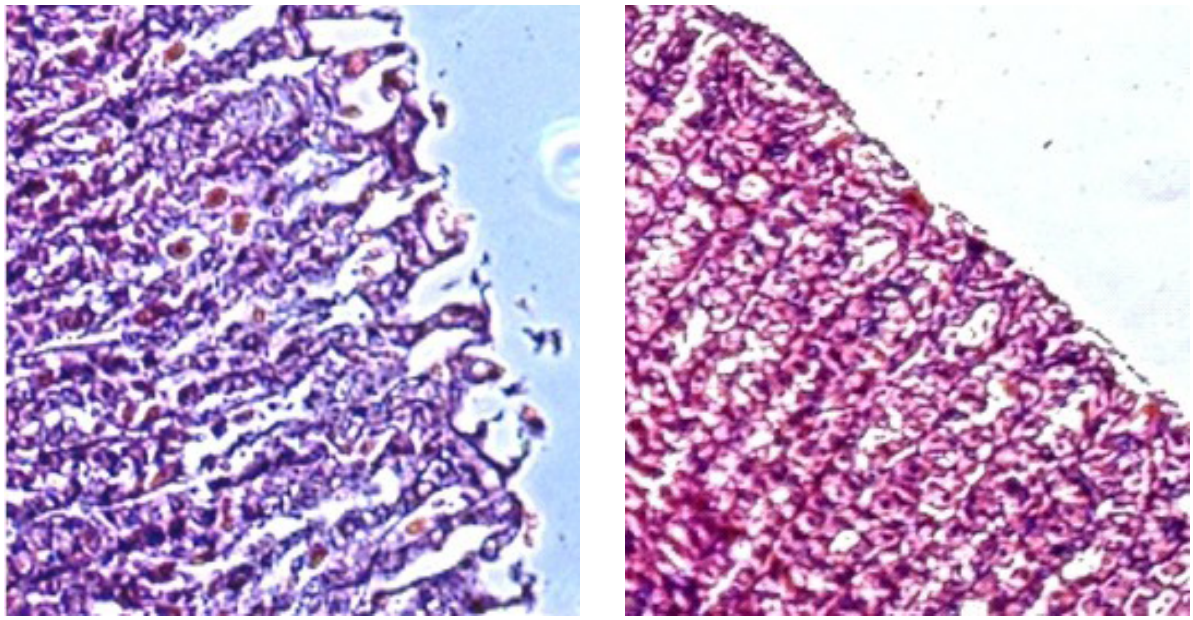

Figure 2. Effects of Cc-EtOH on ethanol-induced rat gastric lesions stained with hematoxylin and eosin. Panel A shows a photomicrograph (100 x) of epithelial lesion and inflammatory cell infiltration in the gastric mucosa after oral administration of ethanol. Panel B shows the effect of pretreatment with 500 mg/kg Cc-EtOH extract, preventing the instauration of epithelial lesions or inflammatory infiltration in the gastric mucosa.

Table 1. Weight of the indicated organs isolated from mice treated or not (control) with Cc-EtOH extract.

	Weight ± SEM (mg/g)			
Treatment	**Liver**	**Heart**	**Lung**	**Kidney**
Control	7.7 ± 0.7	0.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.1
Cc-EtOH	6.5 ± 0.3	0.6 ± 0.1*	1.0 ± 0.2**	2.0 ± 0.1

Data are presented as mean ± SEM. (n = 5, t-test *p < 0.05; **p < 0.01)

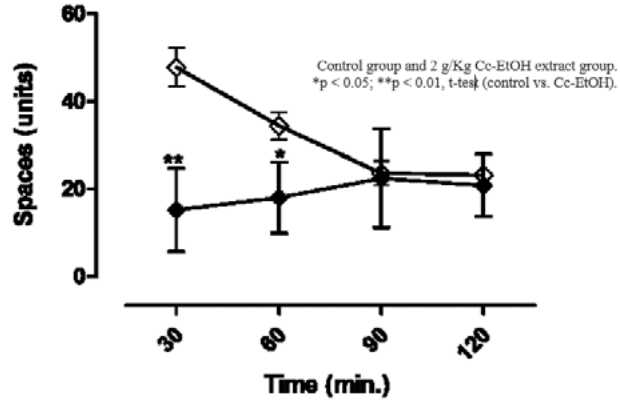

Figure 3. Effect of Cc-EtOH extract on mice ambulation from 30 min to 120 min of Cc-EtOH treatments (n = 5).

No significant changes were observed in liver or kidney weights, whereas heart and lung weights were reduced in treated animals compared with control group.

Preincubation with Cc-EtOH extract equipotently antagonized the CCh- and KCl-induced phasic contractions (Figure 4A and B, respectively). No significant differences were observed between the calculated IC_{50} for the Cc-EtOH extract in CCh-induced contraction (236 ± 59 μg/mL) and KCl-induced contractions (201 ± 43 μg/mL).

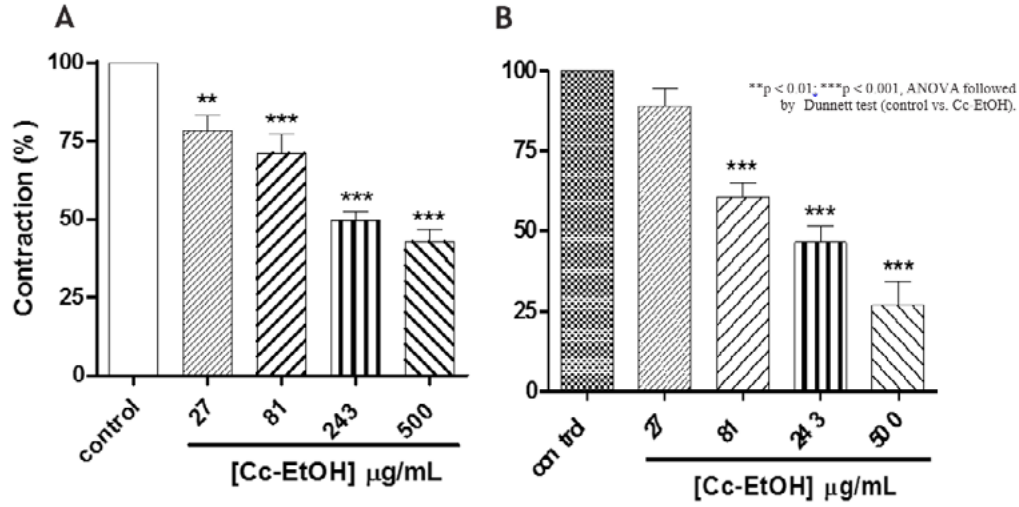

Figure 4. Effects of Cc-EtOH extract on phasic contractions induced by 1 µM CCh (panel A) or 40 mM KCl (panel B) of rat isolated ileum (n = 4).

DISCUSSION

The Cc-EtOH extract exhibited anti-ulcer activity in rats, showed no acute toxicity in mice, and presented antispasmodic effects in isolated rat ileum *in vitro*.

Considering the traditional use of C. corindum (*balãozinho*) in Bahia, Brazil, for its anti-ulcerative proprieties, we investigated its activity using an ethanol-induced stomach ulcer model in rats[8]. The Cc-EtOH extract protected the gastric mucosa from ethanol-induced lesions with efficacy comparable to omeprazole, a proton pump inhibitor (Figure 1). This protective effect is consistent with previous findings in *C. halicacabum*[11] and *Serjania caracasana*, two other *Sapindaceae* species[13]. Thus, the anti-ulcer proprieties of *C. corindum* may be associated with its chemical composition, including triterpenes, fla-

vonoids, and steroids[4,5], also reported in related species[13].

No toxicological data were previously available for *C. corundum*, underscoring the need for *in vivo* assays. In the present study, acute oral toxicity of Cc-EtOH extract was investigated in mice, an appropriate model for initial toxicity screening[14]. No toxicity was detected, as no mortality or tissue damage was observed during the 14-day evaluation period. However, transient sedative effects were evidenced by decreased locomotor activity (Figure 3), consistent with earlier reports of central nervous system effects of this plant[15]. Nevertheless, this reduction in ambulation was limited to the first 60 min following oral administration. Organ weight analysis revealed decreased heart and lung weights (Table 1), although no histological alterations were observed. While the physiological relevance of these evidence remains unclear, the absence of morphological changes supports the safety of a single 2 g/kg oral dose of Cc-EtOH extract. Notably, this dose was four-fold higher than the effective anti-ulcer dose (500 mg/kg; Figure 1) and did elicit putative toxicity.

Further investigation in the gastrointestinal tract demonstrated that Cc-EtOH extract inhibited CCh- or KCl-induced phasic contractions in isolated ileum strips *in vitro* with similar potency, suggesting effects on two distinct transduction pathways involved in intestinal contraction. These contractile responses, whether pharmaco-mechanical or electromechanical couplings rely on Ca^{2+} influx through voltage-dependent Ca^{2+}-channels[16].

The observed *in vivo* gastroprotective may, at least in part, rely on the antispasmodic proprieties of Cc-EtOH extract *in vitro*, similar to agents such as atropine (i.e., reducing gastrointestinal motility) and prostaglandins (i.e., protecting gastric mucosa)[17].

In conclusion, Cc-EtOH extract promoted gastric protection in rats and antispasmodic effects *in vitro*, without evidence of acute toxicity. Together, these findings strongly support the traditional use of *balãozinho* in folk medicine for the treatment of gastric ulcers.

CONFLICTS OF INTEREST

Nothing to declare.

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