Cardiospermum corindum L. (Sapindaceae) has gastroprotective and antispasmodic effect on rodent model

Cardiospermum corindum L. (Sapindaceae) tem efeito protetor gástrico e antiespasmódico em modelo de roedores

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ABSTRACT

Aim: *To investigate the effects of crude ethanol extract obtained from the aerial parts Cardiospermum corindum (Cc-EtOH) on the ethanol-induced gastric ulcer assay in rats, its oral acute toxicity on mice, and its antispasmodic activity on the isolated rat ileum.* ***Methods:*** *The Cc-EtOH was evaluated on ethanol-induced rat gastric lesions. To verify the safety this extract in vivo, the acute toxicity assay was performed during 14 days in mice. Finally, effects of extract on rat ileum were analyzed.* ***Results:*** *The Cc-EtOH extract (50, 150 and 500 mg/kg) significantly protected the rat gastric mucosa layer (ULA = 210.0 ± 55.8; 119.2 ± 39.3 and 47.7 ± 13.2 mm^2, respectively) from lesions induced by ethanol (ULA = 367.5 ± 89.3mm^2), as effectively as omeprazole. The histological analyses also corroborated its protective effect. In addition, 60-min treatment with a single dose 2 g/kg p.o. of Cc-EtOH extract promoted mice sedation (n = 5), which reversed after 30 min., without causing neither animal death, nor body mass, water ingestion, or feed alterations, during 14 days thus showing no acute toxicity. The antispasmodic effect of the Cc-EtOH extract was evidenced on the phasic component of the isolated rat ileum contractions elicited by CCh and KCl (IC50 = 236 ± 59 and 201 ± 43mg/ml, respectively).* ***Conclusion:*** *the aereal parts of C. corindum contain chemical compounds which are able to promote effective gastric protection in rats, in the absence of any acute toxicity, combined with the 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 (ULA = 210,0 ± 55,8; 119,2 ± 39,3 e 47,7 ± 13,2 mm^2, respectivamente) das lesões induzidas pelo etanol (ULA = 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 2 g / 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 (IC50 = 236 ± 59 e 201 ± 43mg/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 ulcer are usual and global problems today nowadays[1]. *Cardiospermum corindum* L. (Sapindaceae) occurs in the Northeast, Southeast and Southern regions of Brazil where the plant is known by the popular name "balãozinho"[2]. Infusion of any part from *C. corindum* is used by folks in Brazilian Northeast to treat liver disturbs and rheumatism, as tonic for memory, as diuretic,

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and emmenagogue[3]. A number of compounds have been isolated and chemically identified from leaves and the aerial parts of *C. corindum*[4,5]. In spite of the common use of *C. corundum* as a medicine against stomach problems no biological activity from any of its compounds has yet been described.

The aim of this study was to investigate the effects of the hydroethanolic extract from the aerial parts of *C. corindum* on ethanol-induced rat gastric ulcer, its antispasmodic effect on isolated rat ileum, together with its acute toxicity.

METHODS

The aerial parts of *Cardiospermum corindum* L. were collected at the base of Pico do Jabre, Paraiba, Brazil, during floration period. The plant was identified and a voucher specimen (No. M.F. Agra et al. 6898) was deposited in the Herbarium Prof. Lauro Pires Xavier (JPB), in the same University. The crude extract was prepared and referred as Cc-EtOH extract throughout the paper[5].

The Cc-EtOH extract was dissolved in Tween-20 (0.32 mg/mL) and diluted in distilled water to the final designed doses. Wistar rats (200-300 g) were stratified in 6 groups of five animals. Each group was pretreated by oral route (gavage) with Cc-EtOH extract 50, 150, 250 and 500 mg/kg; omeprazole 4 mg/kg (i.p.); or vehicle (distilled water-Tween-20, 10 mL/kg). One hour after treatments, 1mL of absolute ethanol was administred by gavage to each animal. One hour later, all animals were euthanized in CO_2 chamber, their stomachs were removed and opened along the greater curvature[6]. Evaluation of the ulcer index (UI) was done by the quantitative method of gauging the extent of erosion and experimental gastric ulcers[7]. Sample tissues were prepared on blades and stained with hematoxylin and eosin technique. This study was approved the Commission of Ethics on the Use of Animals (CEUA) of Nove de Julho University (approval # AN 0002/11).

The Swiss male mice (25-30 g) were divided in two groups of five animals. They were orally (gavage) treated with either a unique dose of Cc-EtOH extract (2 g/kg) (treated group) or vehicle (distilled water-Tween-20, 10 mL/kg) (control group) separately. The animals were observed for 120 min at 30 min interval after the treatment and for any toxic signs and motor activity, measure by spaces roamed (S), and reflexes (gruming or piloerection) after 24h. Animal death, body weight, water intake and food ingestion were concurrently evaluated. Animals were euthanized at CO_2 chamber after 14 days treatment and the heart, lung, liver and kidney were isolated and weighted in relation to total weight anima[19]. Samples of these organs were prepared on blades and stained with hematoxylin-eosin technique. The histopathological images were acquired and analyzed through a camera coupled to microscopy using the software NS-Elements D. The clearance for conducting the study was obtained from CEUA of Nove de Julho University (approval # AN 0003/11).

Wistar rats (250-350g) on fasting (18 h) were decapitated, and the ileum was carefully isolated and cleaned[8]. Tissue strips of 1.5cm were then suspended in organ bath (5 mL), containing modified Krebs solution[10], glucose 11.0, at 37° C and bubbled with O_2. Ileum strips were connected to force transducer, amplifier and the contractions were captured and recorded by the data AQCD system. After 30 min equilibrium to experimental conditions, two single similar contractions (control) were induced by administration of either carbachol 1 μM or KCl 40 mM. The Cc-EtOH extract stock solution (10 mg/mL) was prepared in cremofor 0.1% and solubilized MiliQ water. At the moment of the experiment, the following Cc-EtOH extract concentrations (27-730 μg/mL) were incubated during 15min in distinct ileum strips. The % inhibition of the phasic component of the contractile responses to either CCh or KCl was determined by comparing the responses in the absence and presence of the indicated Cc-EtOH extract concentration. The IC50 was determined for each Cc-EtOH extract concentration tested by non-linear regression[11]. The procedures done in isolated rat ileum were approved and licensed (number 4295060514/14) by the Commission of Ethics on the Use of Animals (CEUA) of Federal University of São Paulo.

All values were expressed as mean SEM. t-Test or ANOVA followed by Dunnett test for multiple comparisons were used and differences were considered significant when $p < 0.05$.

RESULTS

The oral administration of ethanol induced stomach lesions which has UI of 368 ± 89mm^2 (Fig. 1). Pretreatment of the animals with Cc-EtOH extract 50mg/kg (UI=210.0 ± 55.8 mm^2) did not reduced the lesions in a significant manner in

this ulcer-model in comparison with the control group, 150 and 500 mg/kg were able to protect from gastric ulcer in a dose-dependent manner (UI = 119 ± 39 and 48 ±13 mm^2, respectively) similar to the protection observed with omeprazole 20 mg/Kg (UI = 146 ± 20 mm^2).

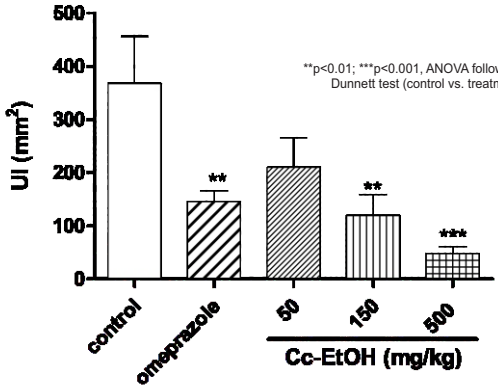

Figure 1. Bar graphs of the effects of ethanol (control) in the presence of omeprazole (20 mg/kg) or Cc-EtOH extract (50, 150 and 500 mg/kg) on rat stomachs (n = 6).

Histological analysis to the vehicle group (control) showed epithelial damage and cells inflammatory infiltrate (Fig. 2A), thus confirming the expected mucosal injury described in the ethanol-induced ulcer lesion model. Fig. 2B clearly illustrates that those gastric lesions were reversed by previous treatment of the animal with a unique dose of Cc-EtOH extract (500 mg/kg), by avoiding the inflammatory response leading to an apparent regeneration of the mucosa and its epithelization even after animal exposure to ethanol.

Administration of Cc-EtOH plant extract (2 g/kg) did not cause animal mortality, in body weight, water intake or feed ingestion changes (data not shown), but significantly decreased animal ambulation from 30 to 60 minutes (S = 47.8 ± 4.4 and 34.4 ± 3.1 units, respectively) as compared to the control group (Fig. 3).

Relative organ weights of the Cc-EtOH (2 g/kg) treated group are shown in Table 1.

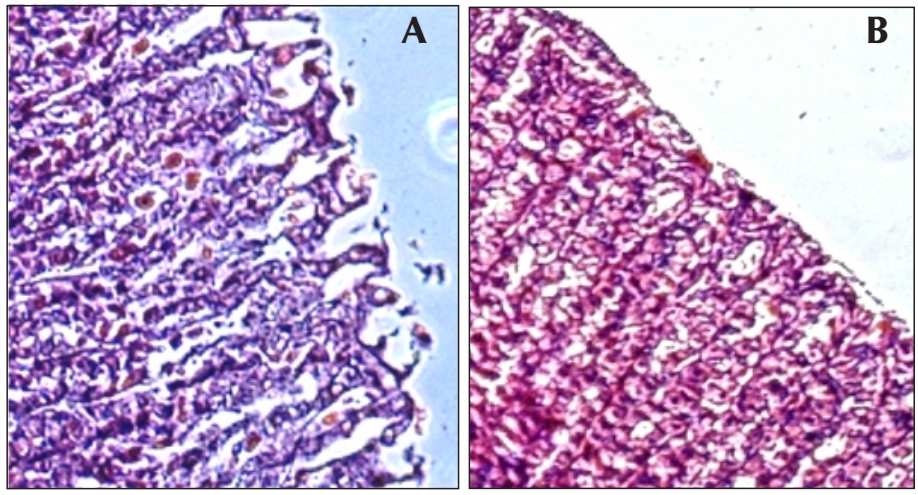

Figure 2. Effects of Cc-EtOH on ethanol-induced rat gastric lesions stained with hematoxylin and eosin.A) Photomicrograph of ethanol-induced rats showing epithelial lesions of gastric mucosa and inflammatory cell infiltration. B) Photomicrograph of ethanol-induced rats pretreated with Cc-EtOH (500 mg/kg p.o.). Note the absence of any lesions and cell inflammatory infiltration in the gastric mucosa. (100x)

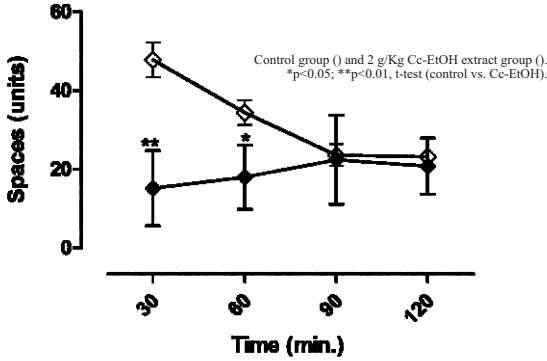

Figure 3. Effect of Cc-EtOH extract on mice ambulation from 30 up to 120 min of Cc-EtOH treatments (n = 5).

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

Treatment	Weight ± s.e.m. (mg/g)			
	Liver	Heart	Lung	Kidney
control	7.7 ± 0.7	0.9 ± 0.09	1.9 ± 0.08	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 ± s.e.m. (n = 5, t-test *p < 0.05; **p < 0.01)

There were not significant changes in the liver and kidney weights of the treated animals compared with those from the control. However, statistically significant decreases in heart and lungs weights were observed in treated animals in comparison to the control.

Pre-incubation of Cc-EtOH extract antagonized in an equipotent manner the phasic contractions induced either by CCh and KCl (Fig. 4A and B, respectively). The extract Cc-EtOH presented IC50 of 236 ± 59 and 201 ± 43μg/mL for contractions induced by CCh and KCl, respectively ($p \geq 0.05$).

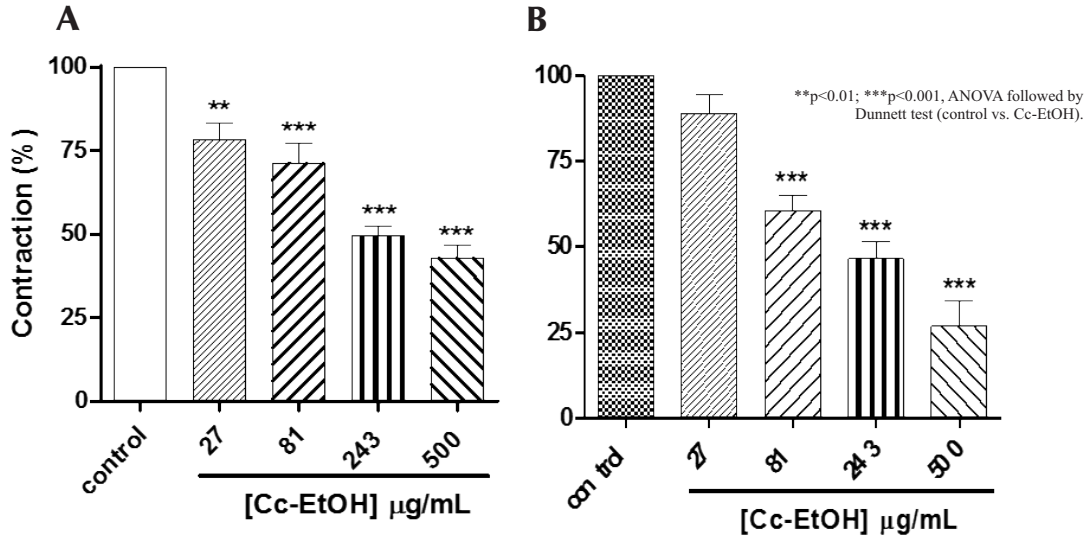

Figure 4. Effects Cc-EtOH of extract on phasic contractions induced by CCh 1 μM (A) or KCl 40 mM (B) of the rat isolated ileum (n = 4).

DISCUSSION

The ethanol crude extract from aerial parts of *Cardiospermum corindum*, (Cc-EtOH) presents effect antiulcer in rats, without toxicity, associate to antispasmodic effects in vitro on ileum isolated rat.

Considering the popular use of the herbal plant "balãozinho" at Bahia state, Brazil as having anti-ulcerative substances, we further explore this possibility by studying that activity in ethanol-induced stomach ulcer model in rodents[8]. The Cc-EtOH herbal aerial extract protected the gastric mucosa from the lesions induced by ethanol with similar efficacy as omeprazole, a proton pump inhibitor (Fig. 1). This protective effect caused by ethanol extract from *C. corindum* was very similar to those described for *C. halicacabum*[11] and *Serjania caracasana* another *Sapindaceae species*[13]. Based on that similarity it is quite possible to suggest that the antiulcer effect of C. corindum results from the chemical composition of its aerial extract containing triterpenes, flavonoids and steroids as active principles[4,5] like those reported for other species[13].

As far as we know, no toxicological studies have been done about *C. corindum*, so it was very important to investigate its toxicity mainly in vivo assays. We thus investigated acute oral toxicity of Cc-EtOH on mice, since this animal species is more appropriate for initial evaluation due to the small body size[14]. The absence of toxicity was evidenced by the observations of neither animal death nor any damage throughout the 14 days of Cc-EtOH extract administration. On the other hand, we corroborate previous data of a sedative effect of this plant on the central nervous system together with a decreased locomotor activity[15] as a lower level of animal ambulation was detected (Fig. 3). However, this effect was detected only within 60-min period of the oral administration of the Cc-EtOH extract. Analysis of toxicity at organ level, revealed a significantly decrease in the heart and lung weights (Table 1), despite any histological signs of tissue damage. The physiological importance of these effects are not clear to us, but the absence of morphological alterations support that administration of a single dose Cc-EtOH (2 g/kg o.r.), which is four times higher than the dose used to determine the anti-ulcer effect of the plant extract (500 mg/kg), did not show putative toxicity.

In order to further explore actions of the plant extract on the gastrointestinal tract, its

effects on the isolated rat ileum was investigated. Interestingly, the Cc-EtOH extract from *C. corindum* inhibited the phasic contractions induced by both the muscarinic (carbachol) and the depolarizing (KCl) agents with a similar potency (Fig. 4), thus presenting quite similar IC50 values. These data suggest a modification at a common step of the transduction mechanisms responsible for triggering intestine contraction. Since the contractile response induced either by pharmacomechanical or electromechanical couplings is due to increased Ca2+-influx by the opening of voltage-dependent Ca2+-channels[16].

The antispasmodic effects of the Cc-EtOH extract in vitro seems to underlie the observed gastric protective effect in vivo, similar to gastrointestinal modulators such as atropine and prostaglandins which decrease gastrointestinal motility and protect gastric mucosa, respectively[17].

In conclusion, we provide evidence that aerial parts of *Cardiospermum corindum* promote gastric protection in rats, and antispasmodic intestinal effects in vitro, without causing any acute toxicity. Altogether, these data strongly justify the use of "balãozinho" in folk medicine for gastric ulcer treatment.

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