

Toxicity studies of *Cordia salicifolia* extract

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ABSTRACT. This study was carried out to determine the acute toxicity of the whole *Cordia salicifolia* extract (LD₅₀) after oral and intraperitoneal administration in mice, and its effect on certain biochemical parameters in the plasma of rats after 90 days of administration. The oral LD₅₀ value of the extract was higher than 2000 mg/kg while the LD₅₀ by intraperitoneal injections was about 920 mg/kg. A daily oral administration of extracts at 20, 100, 200 and 400 mg/kg doses for 90 days did not cause significant changes in the body weight gain, organs weight or biochemical assays and hematology in the animals. The results showed that the administration of the extract for a prolonged period did not produce toxic effects in the animals.

Key words: *Cordia salicifolia*, porangaba, medicinal plants, toxicity.

RESUMO. Estudo da toxicidade do extrato de *Cordia salicifolia*. Este estudo foi realizado para determinar a toxicidade aguda do extrato total de *Cordia salicifolia* (DL₅₀) após administração oral e intraperitoneal em camundongos, assim como os efeitos do extrato sobre alguns parâmetros bioquímicos no plasma de ratos após um tratamento prolongado (90 dias). A DL₅₀ do extrato administrado por v.o. foi maior que 2000 mg/Kg, enquanto a DL₅₀ por via i.p. foi aproximadamente 920 mg/Kg. A administração oral diária do extrato nas doses de 20, 100, 200 e 400 mg/kg por um período de 90 dias não causou modificações no ganho de peso corporal, no peso dos órgãos, nos parâmetros hematológicos e bioquímicos dos animais. Estes resultados indicam que a administração do extrato por um período mais prolongado não provocou efeitos de toxicidade nos animais.

Palavras-chaves: *Cordia salicifolia*, porangaba, planta medicinal, toxicidade.

Introduction

Since ancient times, plants have commonly been used in folk medicine for the treatment of various ailments. However, the pharmacology and toxicity of these plants have begun to receive attention from scientists only recently: for the verification of the claimed pharmacological and/or therapeutic properties of the isolations of their active constituents, and for the investigation of their possible toxicities.

Cordia salicifolia popularly known as porangaba or bugre's tea, which grows abundantly in the central and northeast regions of Brazil and in tropical forest areas of Argentina and Paraguay, has been commonly used as a diuretic, an anorexigen and to reduce weight gain (Cruz, 1995).

Despite the popularity of porangaba in Brazil very little has been done to analyze the phytochemicals in the plant. At present it is known to contain caffeine, potassium, allantoin and alantoic acid. The red fruits or berries of porangaba (resembling a coffee bean) contain caffeine (Saito, 1986).

Some studies report that the ethanolic extract of leaves of *Cordia salicifolia* provokes a reduction of viral herpes type 1 replication and a cytotoxic effect to neoplastic cells. (Hayashi *et al.*, 1990; Arisawa, 1994; Matsunaga *et al.*, 1997).

Notwithstanding the widespread use of *Cordia salicifolia* in Brazilian folk medicine, no study of the toxic effects of these preparations has been performed so far.

The present study was undertaken to provide a minimum set of data on the safety of the extract of *Cordia salicifolia*.

Material and methods

Animals

Adult male Wistar rats (200-250 g), and male Swiss mice (25-30 g) were used. They were housed at a temperature of 22 ± 2°C under a 12h dark-light cycle and given a standard pelleted diet and water *ad libitum* throughout the experimental period. The protocol for these experiments was approved by the Animal Ethics Committee of the University of

Maringá under approval number 080/02 - CEEA.

Preparation of solutions extracts

The *Cordia salicifolia* extract was obtained from Steviafarma Industria in Brazil.

The solutions were prepared immediately before use, with the plant extract being dissolved in saline solution 0.9% with carboxymethylcellulose (CMC) 0.05%.

Acute toxicity

Male mice were fasted overnight and orally or intraperitoneally administered with the *Cordia salicifolia* extract. Doses were increased progressively for the determination of the lethal dose (LD₅₀) (Miller and Tainter, 1944). The mice were observed following a 7 days treatment, and all signs of toxicity, their latencies and deaths were recorded. All animals that died during the observation period and all the surviving ones (put down by cervical dislocation) were subject to necropsy.

Chronic toxicity

Rats were randomly divided into six groups. Animals of Groups I and II were administered orally with saline and carboxymethylcellulose respectively, while animals of Groups III, IV, V and VI were administered orally with the *Cordia salicifolia* extract at daily doses of 20, 100, 200 and 400 mg/kg, for 90 days. Food and water were freely available during the experiment. The body weights of the rats were measured every day and recorded on day 90. Animals were anesthetized with ether and the blood was collected directly from the aorta into tubes for the evaluation of biochemical analysis.

Biochemical assays

Plasma was used for the determination of the concentration of glucose, urea, creatinine, alkaline phosphatase (ALP), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride and cholesterol using appropriate kits supplied by manufacturers.

Organ body weight ratio

Vital organs such as the liver, kidneys, heart and lungs were quickly removed and weighed individually. The macroscopic appearance of the organs was recorded and the organ weight ratio by 100 g of the body weight was calculated.

Hematology

Blood was collected from the tail in small heparinized tubes on day 0 (the start of the extract administration) and on day 90 (just before putting down the rats). The total leukocyte counts were determined in

a Neubauer chamber. For differential cell count smears were prepared, air-dried and fixed with Rosenfeld stain (Rosenfeld, 1947).

Statistical analysis

The data were expressed as mean values \pm SEM and tested with analysis of variance followed by the Tukey multiple comparison test.

Results and discussion

The acute oral toxicity of the *Cordia salicifolia* extract was very low in mice. No deaths and no other signs or symptoms of toxicity were observed up to the dose tested (2000 mg/kg of body weight). The necropsy of these mice 7 days after oral treatment with the extract did not reveal any gross pathological alteration either. Lethality was noted only after intraperitoneal (i.p.) administration of 920.45 mg/kg body weight. Except for hypoactivity and contortion, no other adverse effect preceding death was noted.

Repeated oral administration (chronic toxicity) of different doses of extract (20, 100, 200 mg/kg) for 90 days did not produce characteristic signs of toxicity. There were no deaths of animals at any dose over 90 days. Animals treated with a dose of 400 mg/kg showed some alterations. As can be observed in Figure 1, body weight gain in animals treated with the extract in doses of 20, 100, 200 mg/kg was comparable to controls. However, animals treated with the dose of 400 mg/kg showed relatively lower weight gain. This reduction may be due to a lower gastrointestinal absorption of food, or to an increase in the gastrointestinal transit, or further, to satiation caused by the extract.

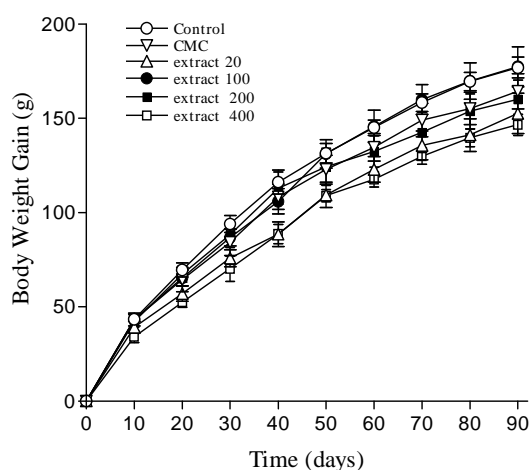


Figure 1. Body weight gain of rats treated p.o. with *Cordia salicifolia* extract for 90 days. Saline solution (control) or carboxymethylcellulose (CMC) also was administered p.o. for 90 days. The values are means \pm SEM of 12 animals/group.

The hematological data from animals after 90 days of oral exposure to the extract at the doses of 20, 100 and 200 mg/kg did not show significant changes in total and differential leukocyte counts, although a slight reduction in the number of total leukocytes and mononuclear cells at the end of the experiment with the dose of 400 mg/kg did occur (Table 1).

The biochemical findings are shown in Table 2. The plasma concentrations of glucose, urea, creatinine, total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride and cholesterol were unchanged compared to controls. The levels of alkaline phosphatase (ALP) were only slightly increased in the animals that received the dose of 400 mg/kg. However, the change was within the historical control range for the rat strain (Wolfort *et al.*, 1986; Kang *et al.*, 1995).

The relative organ weights (organ to body weight ratio) of treated animals also did not indicate changes. The values are shown in Table 3.

The alterations observed in the animals that received the extract at the dose of 400 mg/kg of body weight were of doubtful toxicological significance since they were very slight and occurred with a much larger dose than that recommended for an adult human weighing 70 kg (20 times larger).

Conclusion

Although toxicity studies in experimental animals cannot always be totally extrapolated to humans (Van Miert, 1989), it should be borne in mind that people often self-administer the plant extract.

In conclusion, the results showed that the oral toxicity of the *Cordia salicifolia* extract is low and no evidence was found that it poses risks after prolonged usual doses.

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Table 1. Hematological findings in rats treated with *Cordia salicifolia* extract for 90 days.

GROUPS	CELLS/ mm ³ x 10 ³					
	Prior treatment			After treatment		
	Leucocyte	Mononuclear	Polymorphs	Leucocyte	Mononuclear	Polymorphs
Control (12)	10.37 ± 0.53	8.66 ± 0.43	1.71 ± 0.20	9.16 ± 0.43	6.73 ± 0.45	2.43 ± 0.22
CMC (12)	10.20 ± 0.55	8.04 ± 0.38	2.16 ± 0.30	8.84 ± 0.54	6.43 ± 0.53	2.41 ± 0.22
Extract (mg/kg)						
20 (12)	9.72 ± 0.58	7.82 ± 0.50	1.90 ± 0.21	8.36 ± 0.52	5.69 ± 0.40	2.67 ± 0.22
100 (12)	10.05 ± 0.43	7.75 ± 0.31	2.30 ± 0.24	9.27 ± 0.55	5.99 ± 0.39	3.28 ± 0.39
200 (12)	10.38 ± 0.62	7.92 ± 0.32	2.46 ± 0.33	8.26 ± 0.49	5.78 ± 0.48	2.48 ± 0.30
400 (12)	10.18 ± 0.83	8.16 ± 0.62	2.02 ± 0.33	7.34 ± 0.49	5.34 ± 0.52	2.00 ± 0.17

• The extract, or (CMC) was administered, p.o. during 90 days. • Values are means ± SEM. • (n) = number of animals tested.

Table 2. Serum biochemical findings in rats treated with *Cordia salicifolia* extract for 90 days.

Groups	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl x 10 ²)	Cholesterol (u/l)	Triglycerides (u/l)	ALP (u/l)	ALT (u/l)	AST (u/l)	TP (g/l)
Control (12)	120.6 ± 3.2	46.4 ± 1.8	0.54 ± 0.02	107.8 ± 5.2	49.7 ± 3.3	88.9 ± 5.4	60.6 ± 4.2	73.3 ± 1.8	73.5 ± 2.3
CMC (12)	117.4 ± 4.9	46.9 ± 2.3	0.53 ± 0.02	96.0 ± 7.3	54.3 ± 6.6	92.4 ± 6.1	59.8 ± 2.8	75.5 ± 3.9	70.9 ± 1.9
Extract (mg/kg)									
20 (12)	103.6 ± 4.9	50.8 ± 1.3	0.56 ± 0.02	92.9 ± 5.0	55.5 ± 2.9	93.4 ± 7.5	71.4 ± 1.2	81.0 ± 2.9	68.6 ± 1.1
100 (12)	105.0 ± 3.4	50.9 ± 1.8	0.62 ± 0.02	95.4 ± 5.6	58.9 ± 4.8	86.3 ± 6.9	67.1 ± 1.8	76.3 ± 2.4	65.5 ± 1.0
200 (12)	117.0 ± 3.5	43.2 ± 2.0	0.51 ± 0.01	100.3 ± 5.5	50.8 ± 1.7	84.0 ± 6.1	56.0 ± 4.1	75.3 ± 2.7	77.0 ± 1.0
400 (12)	133.3 ± 7.3	48.6 ± 2.9	0.47 ± 0.01	105.4 ± 6.6	45.6 ± 2.0	107.2 ± 7.3	59.5 ± 1.8	75.1 ± 4.1	78.1 ± 2.9

• The *Cordia salicifolia* extract, or saline (control), or carboxymethylcellulose (CMC) was administered, p.o. during 90 days. • Values are means ± SEM. (n) = number of animals tested. • ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3. Wet Organs weight (g/100 g body weight).

Groups	Heart	Kidney	Liver	Lung
Control (12)	0.32 ± 0.01	0.61 ± 0.01	2.9 ± 0.09	0.53 ± 0.02
CMC (12)	0.31 ± 0.01	0.61 ± 0.01	2.8 ± 0.05	0.49 ± 0.02
Extract (mg/kg)				
20 (12)	0.34 ± 0.01	0.65 ± 0.01	3.1 ± 0.09	0.53 ± 0.021
100 (12)	0.33 ± 0.02	0.65 ± 0.01	3.4 ± 0.08	0.49 ± 0.023
200 (12)	0.32 ± 0.01	0.60 ± 0.01	2.7 ± 0.08	0.49 ± 0.010
400 (12)	0.31 ± 0.01	0.63 ± 0.01	2.8 ± 0.09	0.50 ± 0.014

• The extract, or (CMC) was administered, p.o. during 90 days. • Values are means ± SEM. • (n) = number of animals tested.

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