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Acute and sub-chronic pre-clinical toxicological study of *Averrhoa carambola* L. (Oxalidaceae)

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***Averrhoa carambola* L., a species belonging to the Oxalidaceae family, is associated with neurological symptoms in individuals with renal diseases. The objective of this work was to accomplish a pre-clinical toxicological study of the hydroalcoholic extract (HE) from *A. carambola* leaves. Wistar rats and Swiss mice, both male and female, were used in these experiments. The rats were used in the acute toxicity assessment, with the extract administered at doses of 0.1 to 8.0 g/kg (oral route), and 0.5 to 3.0 g/kg (via intraperitoneal route). The mice received the extract in doses of 0.5 to 5.0 g/kg (via oral and intraperitoneal routes) and were observed for 14 days. Rats were also used in the sub-chronic toxicity evaluation, and divided into three groups (n=10): control group, HE 0.125 g/kg and HE 0.25 g/kg. These animals received HE for a 60 day period, at the end of which a macroscopic analysis of selected organs was performed with biochemical analysis of the blood. The acute toxicity assessment revealed that the HE of *A. carambola* L. presented low toxicity in the mice and rats. Furthermore, no signs of toxicity were present in the sub-chronic assessment.**

Key words: *Averrhoa carambola* L., Oxalidaceae, acute toxicity, sub-chronic toxicity.

INTRODUCTION

The use of medicinal plants is common in popular culture, where it represents the result of centuries of accumulation of empirical knowledge on the action of plants by diverse ethnic groups. Some of the most valuable and most widely used medicines were developed from the accumulation of this knowledge (Simoes, 1989). The species *Averrhoa carambola* L., popularly known in Brazil as “carambola” (starfruit), belongs to the Oxalidaceae family. This family is comprised of eight genera distributed predominantly in the southern hemisphere, in the tropical and subtropical zones, and made up of trees cultivated for ornamental purposes because of their fruit (Carreira and Schatzmayr, 1982; Joly, 1979). Two species are present in this genus: *A. carambola* L. and *Averrhoa bilimbi* L. The assessment of the physicoche-mical characteristics of the fruits of *A. bilimbi* L. showed the presence of oxalic acid and ascorbic acid (Lima et al., 2001). Hypolipidemic and hypoglycemic activities were observed using this plant species in Sprague-Dawley rats with induced diabetes (Tan et al., 2005) and the methanol extract from the leaves was shown to contain antioxidant activity (Abas et al., 2006).

Popularly, *A. carambola* L. is used as an anti-thermal, against the bites of poisonous animals, and as an anti-dysentery and anti-scurvy treatment. Chemical and phytochemical analyses performed on the plant revealed

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Abbreviations: MPO, Myeloperoxidase; **HE,** hydroalcoholic extract; **CT,** control animals; **HDL,** high density lipoprotein; **ALT,** alanine aminotransferase; **AST,** aspartate amino transferase.

the presence of oxalic acid, vitamin C and tannins (Prance, 1975), moderate amounts of sugar, fiber, and calcium, as well as a low phosphorus and iron content (Oliveira et al., 1989), isoforms of the galactoside enzymes (Balasubramanian et al., 2005), volatile compounds (Macleod and Ames, 1990) and carotenoids (Gross et al., 1983). Several studies have reported that the ingestion of *A. carambola* L. fruit is related to the appearance of neurological symptoms in individuals affected by renal insufficiency, with symptoms including intractable hiccups, vomiting, paresthesias, paresis of the upper and lower limbs and several different disturbances related to consciousness present in varying degrees (Moysés et al., 1998; Tse et al., 2003; Moysés and Mep, 2004; Lo et al., 2001; Wu et al., 2002; Yap et al., 2002; Chan et al., 2002). Provasi et al. (2001) investigated the acute toxicity of *A. carambola* L. in doses of 3.5 g/kg in mice and 1.14 g/kg in rats by intra-gastric route and did not observe any deaths among treated animals in the 4 h following the administration of the extract. On the other hand, Signate et al. (2009) reported the cases of two patients with undiagnosed chronic renal insufficiency who developed severe encephalopathy after ingestion of star fruit. The two patients developed intractable hiccups, vomiting, impaired consciousness and status epilepticus. Diffusion-weighted magnetic resonance imaging showed cortical and thalamic hyperintense lesions related to epileptic status.

The use of insoluble fibers of the *A. carambola* L. fruit had beneficial effects on intestinal activity, leading to a decrease in the pH in the cecum, together with a decrease in the cecal and fecal ammonia levels (Chau and Chen, 2006). In addition to their effects on digestive system function, the insoluble fibers present in the fruit, when evaluated against glycemia *in vitro* demonstrated potential hypoglycemic activity, besides inhibiting the enzyme alpha amylase (Chau et al., 2004). Provasi et al. (2001) observed that a 30 mg/kg dose of extract from the leaves of *A. carambola* L. had anti-hypoglycemic activity in rats and when the authors examined the effect of the crude hydroalcoholic extract and isolated fractions of leaves on the glucose metabolism in Wistar rats treated for two weeks with a 20 mg/kg dose, they found a reduction in glycemia. However, when the extract's activities were investigated in isolated muscle, there was no stimulation of the production of glycogen and lactate (Provasi et al., 2005). In a study on the effect of the hydroalcoholic extract of the leaves on glycemia during fasting in rats treated orally (20 mg/kg), we found that the *A. carambola* L. extract lowered glycemia in comparison with the control group, while this reduction was not caused by inhibition of hepatic gluconeogenesis nor by an increase in glucose uptake by muscle (Ferreira et al., 2008).

Anti-inflammatory activity was observed in the skin of mice treated with the crude ethanolic extract of *A. carambola* leaves, as well as its hexane, ethyl acetate, and butanol fractions and two isolated flavonoids. The activity was measured using a croton oil-induced ear edema model of inflammation, and topically applied ethanolic extract reduced edema in a dose-dependent manner, with a maximum inhibition of 73±3% and an ID_{50} value of 0.05 (range: 0.02–0.13) mg/ear. Myeloperoxidase (MPO) activity was also inhibited by the extract, with a maximum inhibition of 60±6% (0.6 mg/ear). All of the fractions tested inhibited edema formation as well as MPO activity, although treatment with the ethyl acetate fraction was the most effective, resulting in inhibition of 75±5 and 54±8% for edema formation and MPO activity, respectively. However, treatment of mice with isolated compounds [apigenin-6-Cb- L-fucopyranoside and apigenin-6-C-(200-O-a-L-rhamnopyranosyl)-b-L-fucopyranoside] did not yield notable results, with the latter compound causing only a mild reduction in edema formation (28± 11%) (Cabrini et al., 2011).

In another work, Khoo et al. (2010) examined the toxic effect of *A. carambola* juice, kept under different storage conditions, in Sprague Dawley (SD) rats, observing no deaths and consequently being unable to determine the LD_{50} for the juice. The authors did report an increase in alanine aminotransferase (ALT) levels ($P < 0.05$) in those rats treated with *A. carambola* juice stored for 3 h.

Studies that examine the potential toxicity of *A. carambola* L. remain scarce, which explains the importance of assessing its toxic effects in experimental studies. The objective of this work was to assess the acute and sub-chronic toxicity of the hydroalcoholic extract of *A. carambola* L. leaves in rats and mice.

MATERIALS AND METHODS

Plant material and extract preparation

The *A. carambola* L. leaves were collected from the municipal area of Paço do Lumiar, in the state of Maranhão, Brazil in February 2006, and identified at the "Atico Seabra" herbarium (Federal University of Maranhão), where a voucher specimen was registered under number 0561 SLS/MA. The hydroalcoholic extract (HE) was obtained from the dehydrated and ground leaves by maceration in 70% ethanol for 72 h. Next, the extract was concentrated in a rotary evaporator at a reduced pressure and at a temperature of 50°C. Finally, the dry weight of the extract was determined.

Animals

The experiments were carried out in Wistar rats (*Rattus norvegicus*), between 60 and 90 days of age, weighing between 220 and 360 g (male) and between 135 and 260 g (female), and Swiss mice (*Mus musculus*), between 60 and 90 days of age, weighing between 26 and 32 g (male) and between 21 and 28 g (female), supplied by the Central Animal Facility of the Federal University of Maranhão. These animals were divided into groups of 6 to 10, including both males and females, according to the experiment performed. The control animals (CT, rats and mice respectively) received water at 0.1 ml/100 g or 0.1ml /10g body weight and the remaining animals received HE at varying doses. The results obtained with the HE are compared to the control animals. Animals were handled based on the guidelines of the animal ethics committee of the Universidade Estadual do Maranhão (UEMA), who approved this research (license number 002/ 2008).

Assessment of acute toxicity

The experimental protocols for the assessment of acute toxicity were mainly based on the Specific Resolution (SR 90/2004) of the National Sanitary Vigilance Agency (ANVISA; Brazil, 2004). Rats and mice were divided into five or six experimental groups with ten animals each (five male and five female), and increasing doses of extract were administered. The rats received the following via intraperitoneal route (i.p.): water (CT group), *A. carambola* L. hydroalcoholic extract at doses of 0.5 g/kg (group HE 0.5), 1.0 g/kg (group HE 1.0), 2.0 g/kg (group HE 2.0) and 3.0 g/kg (group HE 3.0). By oral route (v.o.), they were given: water (CT group), *A. carambola* L. HE at doses of 1.0 g/kg (group HE 1.0), 2.0 g/kg (group HE 2.0), 5.0 g/kg (group HE 5.0) and 8.0 g/kg (group HE 8.0). After administration of the HE, the animals remained under observation for a 14-day period to monitor signs of toxicity, according to criteria established by Malone (1997). The animals were weighed weekly by group (sex/dose), with the mean value for each group being recorded. At the end of this period, the surviving animals were sacrificed, and had the kidneys, heart, liver and spleen weighed and subjected to a macroscopic analysis.

Assessment of sub-chronic toxicity

The rats (*R. norvegicus*) were divided into three groups of six animals each (three male and three female). The groups were treated (v.o) with *A. carambola* L. HE at doses of 0.125 g/kg (HE 0.125) or 0.25 g/kg (HE 0.25), or water, for 60 days. During this interval, the body weight and consumption of food were determined three times a week, and the average weekly values of each group (sex/dose) were recorded. The behavioral parameters were also measured daily. At the end of the treatment period, the animals were subjected to a 12 h fast for subsequent blood collection and analysis of the following biochemical parameters: Glucose, urea, creatinine, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, albumin, total proteins, aspartame amino transferase, alanine amino transferase and alkaline phosphatase. Afterwards, the animals were sacrificed and selected organs (kidneys, lungs, heart, liver, spleen and pancreas) were analyzed macroscopically for the detection of possible alterations caused by the *A. carambola* L. extract.

Statistical analysis

The results of the experiments were expressed as mean ± standard error of the mean and submitted to analysis of variance (ANOVA), followed by Newman Keuls test, considering significant differences between the groups when $p \leq 0.05$. The analyses were done with the aid of the GraphPad Prism 3.0 program (GraphPad Prism, 1999).

RESULTS

Preparation of hydroalcoholic extract (HE)

The HE prepared from the *A. carambola* L. leaves presented a dark green color, with a residue that was easily soluble in water and a yield of 17.6%.

Assessment of acute toxicity

Mice

Among the animals treated by oral route who received the lowest doses (0.5 and 1.0 g/kg), no significant changes were observed, similar to the animals in the control group. In relation to the animals treated with the doses of 2.0 and 5.0 g/kg, in the first few hours following treatment, some signs of toxicity were observed including convulsions and tachycardia (Table 1). From the second day, the animals were found to be sedated, although the intensity of this effect was not proportional to the dose employed. These effects occurred in both sexes, although the only animals that died were both female, one at each dose of HE over the course of the 14 days of observation (Table 1). No change in body weight was seen in the animals at any of the doses employed (Table 2). As a result of this profile of mortality, the mean lethal dose (LD_{50}) could not be determined in these animals. Among the mice treated with the HE by intraperitoneal route that received the lowest doses (0.5 and 1.0 g/kg), no significant changes were noted; however, in the animals treated with the doses of 2.0 and 5.0 g/kg, some signs of toxicity such as abdominal pain, grouping, con-vulsions and tachycardia were observed in the first few hours after treatment, and these can be seen in Table 1. From the second day onwards, the animals exhibited sedation and death proportional to the dose employed. These effects occurred in both sexes, although mortality only occurred in females at a dose of 2.0 g/kg, and in males and females at a dose of 5.0 g/kg, as shown in Table 1. Once again, as a result of the profile of mortality in these animals, the mean lethal dose (LD_{50}) could not be determined. Significant weight loss was not seen in the animals (Table 2). Macroscopic examination of the kidneys, heart, liver and spleen of the surviving animals did not reveal any relevant changes. Furthermore, there were no changes in the weights of organs as shown in Table 3.

Rats

The rats that received higher doses of HE by the intraperitoneal route (2.0 and 3.0 g/kg) presented a mortality index proportional to the dose, as can be seen from Table 4. The LD_{50} of the HE of *A. carambola* L. in rats treated by this route was 1.49 g/kg. Among the treated animals that were given the lowest doses (0.5 and 1.0 g/kg), no significant behavioral changes were observed, which was a similar result to that seen in the control group. In relation to the rats treated with the doses of 2.0 and 3.0 g/kg, in the first few hours after treatment, some signs of toxicity were observed which are presented in Table 4; these were piloerection, writhing, sedation and depression. With this treatment the animals died within a few hours of the administration of the extract. Among the rats treated by oral route no behavioral changes were noted and none of the animals died (Table 4). The acute toxicity in rats was not associated with any detectable macroscopic changes in the organs analyzed (kidneys, liver, spleen, heart and pancreas) after the 14 days of observation. Small superficial inclusions of extract were found in the liver of the only surviving animal treated by

Table 1. Effect of single oral and intraperitoneal dose of *Averrhoa carambola* L. extract in mice.

Route – dose (g/kg)	Gender	D/T	Latency (h)	Symptom
Oral route				
Control	M	0/5	-	None
	FM	0/5	-	None
0.5	M	0/5	-	None
	FM	0/5	-	None
1.0	M	0/5	-	None
	FM	0/5	-	None
2.0	M	0/5	4-48h	Tachycardia, convulsions and sedation
	FM	1/5	4-48h	Tachycardia, convulsions and sedation
5.0	M	0/5	4-48h	Tachycardia, convulsions and sedation
	FM	1/5	4-48h	Tachycardia, convulsions and sedation
LD_{50}= -				
Intraperitoneal route				
Control	M	0/5	-	None
	F	0/5	-	None
0.5	M	0/5	-	None
	F	0/5	-	None
1.0	M	0/5	-	None
	F	0/5	-	None
2.0	M	0/5	2-48	Grouping, writhing, tachycardia, convulsions
	F	1/5	2-48	Grouping, writhing, tachycardia, convulsions
5.0	M	3/5	2-48	Grouping, writhing, tachycardia, convulsions
	F	4/5	2-48	Grouping, writhing, tachycardia, convulsions
LD_{50}= -				

All treated mice (n=10 in each group; 5 males and 5 females) were carefully examined for up to 14 days after the treatment for adverse effects including behavioral changes, lethality and latency of death. D/T, number of dead mice / number of treated mice; none, no symptoms observed during the observation period; latency, time to onset of effects after the treatment; M, male; FM, female.

intraperitoneal route with the dose of 2.0 g/kg. No changes in organ weights were observed.

Assessment of sub-chronic toxicity

The animals treated with the HE of *A. carambola* L. for 60 days at the doses of 0.125 and 0.25g/kg did not present any behavioral changes during the period of treatment. In addition, no deaths were recorded during the treatment. In relation to the body weight of the animals, treatment with the HE of *A. carambola* L. did not cause any change in this parameter (Table 5). The profile of food consumption was also found to be unaltered in the treated animals, when compared to the control group. Treatment with the extract of *A. carambola* L. did not lead to any macroscopic changes in the vital organs (kidneys, lung, heart, liver, spleen and pancreas) or to changes in the

Table 2. Effect on body weight in mice after single oral administration of *Averrhoa carambola* L. extract.

Group	Sex	D_0 (g)	D_7 (g)	D_{14} (g)
Control	M	26.26± 1.09	28.00± 1.32	27.74± 1.14
	F	19.86± 0.78	19.98± 0.70	20.46± 0.75
A. carambola (0.5 g/kg)	M	24.68± 1.41	26.10± 1.66	26.60± 1.69
	F	21.16± 0.89	21.42± 0.90	22.12± 0.92
A. carambola (1.0 g/kg)	M	26.80± 2.91	27.42± 2.16	27.74± 2.24
	F	22.44± 1.90	22.88± 1.80	23.24± 1.76
A. carambola (2.0 g/kg)	M	27.80± 2.46	28.07± 2.32	28.35± 2.15
	F	20.70± 0.71	21.15± 0.47	21.73± 0.49
A. carambola (5.0 g/kg)	M	26.75± 1.65	28.00± 1.95	27.70± 2.25
	F	21.05± 1.30	21.80± 1.37	22.60± 1.38

All treated mice (n=10 in each group; 5 males and 5 females) were carefully examined for up to 14 days after the treatment. Data are expressed as mean ± S.E.M. No significant difference between control and the extract (P≤0.05).

Table 3. Weight of organs from surviving mice 14 days after administration of a single dose of the extract of *Averrhoa carambola* L.

Group	Kidney (g)	Heart (g)	Liver (g)	Spleen (g)
Control	0.41± 0.02	0.13± 0.004	1.64± 0.09	0.10± 0.006
A. carambola (0.5 g/kg)	0.41± 0.02	0.14± 0.004	1.64± 0.10	0.10± 0.006
A. carambola (1.0 g/kg)	0.41± 0.02	0.13± 0.005	1.68± 0.12	0.10± 0.006
A. carambola (2.0 g/kg)	0.40± 0.09	0.12± 0.005	1.29± 0.10	0.11± 0.01
A. carambola (5.0 g/kg)	0.40± 0.08	0.14± 0.01	1.37± 0.04	0.11± 0.007

The values represent the means of the organ weights (in grams) ± S.E.M. All treated mice (n=3-10 in each group; males and females) were carefully examined for up to 14 days after the treatment. No significant difference was between control and the extract (P≤0.05).

Table 4. Effect of single oral and intraperitoneal dose of *Averrhoa carambola* L. extract in rats.

Route – dose (g/kg)	Sex	D/T	Latency (h)	Symptom
Oral route				
Control	M	0/5	-	None
	FM	0/5	-	None
1.0	M	0/5	-	None
	FM	0/5	-	None
2.0	M	0/5	-	None
	FM	0/5	-	None
5.0	M	0/5	-	None
	FM	0/5	-	None
8.0	M	0/5	-	None
	FM	0/5	-	None
LD_{50}= -				
Intraperitoneal route				
Control	M	0/5	-	None
	FM	0/5	-	None
0.5	M	0/5	-	None
	FM	0/5	-	None
1.0	M	0/5	-	None
	FM	0/5	-	None

Table 4. Contd.

2.0	M	4/5	2-4	Piloerection, Sedation, Writhing, Convulsions
	FM	5/5	2-4	Piloerection, Sedation, Writhing, Convulsions
3.0	M	5/5	2-4	Piloerection, Sedation, Writhing, Convulsions
	FM	5/5	2-4	Piloerection, Sedation, Writhing, Convulsions
LD_{50}= 1.49 g/kg				

All treated rats (n=10 in each group; 5 males and 5 females) were carefully examined for up to 14 days after the treatment for adverse effects including behavioral changes, lethality and latency of death. D/T, Number of dead rats / number of treated rats; none, no symptoms observed during the observation period; Latency, time to onset of effects after the treatment; M, male; FM, female.

Table 5. Effect on body weight in rats after daily oral administration of *Averrhoa carambola* L. extract.

Group	Sex	D_0	D_{15}	D_{30}	D_{45}	D_{60}
Control	M	234.6± 14.44	235.4± 15.34	238.9± 13.77	257.0± 18.96	278.6± 14.26
	FM	212.4± 12.07	213.5± 12.07	217.4± 11.54	241.2± 11.70	257.5± 11.88
Averrhoa carambola (0.125 g/kg)	M	245.5± 4.58	248.6± 5.11	252.1± 0.73	242.3± 6.63	276.8± 9.67
	FM	215.6± 4.62	218.7± 4.92	225.4± 3.94	212.4± 6.63	246.9± 9.65
Averrhoa carambola (0.25 g/kg)	M	229.4±7.93	231.5±8.17	235.1±7.98	262.2±6.69	271.6±8.36
	FM	202.3± 5.24	204.9± 5.62	199.5± 3.59	232.5± 6.81	241.9± 8.43

All treated mice (n=6 in each group; 3 males and 3 females) received the extract orally at daily doses of 0, 0.125 and 0.25 g/kg for up 60 days. Data are expressed as mean ± S.E.M. No significant difference was between control and the extract (P≤0.05).

Table 6. Effects of daily oral administration of *Averrhoa carambola* L. extract for up 60 days on the biochemical parameters of rats.

Parameter	Control	0.125 g/kg	0.25 g/kg
COL – T (mg/dL)	57.4±5.62	57.5±5.50	75.7±7.51
GLI (mg/dL)	152.2±14.43	109.5±11.50	186.3±24.6
ALB (g/dL)	2.89±0.18	2.74±0.46	2.78±0.12
TRI (mg/dL)	73.50±15.61	49.33±21.79	88.00±23.64
AST (U/l)	100.3±6.65	130.5±31.50	107.7±20.10
ALT (U/l)	36.25±4.75	39.00±8.00	22.00±7.00
URE (mg/dL)	878.8±50.01	848.5±8.50	778.7±41.33
CRE (mg/dL)	1.03±0.11	1.25±0.05	1.00±0.057
PT (d/dL)	9.08±1.12	7.75±0.95	7.20±0.40
ALP (U/l)	44.25±10.09	29.00±1.00	48.67±7.42
COL – HDL (mg/dL)	9.75±1.65	4.33±2.60	6.00±2.65

Data are expressed as mean ± S.E.M (n=6). COL-T, Total cholesterol; GLI, glucose; ALB, albumin; TRI, triglyceride; AST, aspartate aminotransferase; ALT, alanine amino transferase; URE, urea; CRE, creatinine; PT, total protein; ALP, alkaline phosphatase; COL- HDL, high density lipoprotein. *p≤0.05 compared with the control group. No significant difference was between the control and the *Averrhoa carambola* L. extract (p≥0.05).

weights of the organs. At the end of the treatment, analysis of the blood of these animals showed no statistically significant changes in the selected biochemical parameters (Table 6).

DISCUSSION

In many low and middle income countries, folklore or herbal medicine often represents the popular therapeutic

system to which people are referred for their primary health care. The use of herbal remedies is further substantiated by its affordability, knowledge of medicinal plants and the belief that they are harmless. The increase in the number of users as opposed to the scarcity of scientific evidence on the safety of medicinal plants has raised concerns regarding toxicity and detrimental effects of these remedies. Medicinal plants commonly contain various bioactive principles which have the potential to cause beneficial and/or harmful effects. To optimize their safe use as plant-based medicines, we should, despite the historical experience of application in humans and animals, evaluate the toxicity of these medicinal herbs (Li et al., 2010). The assessment of the extract of *A. carambola* L. in this study was prompted by the fact that this form of plant medicine closely mimics the traditional dosage form, while it also represents a convenient form that can be standardized in terms of its chemical or physical constituents, and also is easily stored. Thus, the assessment of the safety of this dosage form of *A. carambola* L. is greatly facilitated. The present study was designed to investigate the pre-clinical toxicological effects of the hydroalcoholic extract of the leaves of *A. carambola* L. administered by oral (acute and subchronic effects) and intraperitoneal (acute effect) routes in order to determine the safety of the extract.

The goal of tests of acute toxicity is to define the lethal dose of a drug administered in a single dose or in several doses intercalated over a short period of time, as part of the initial pharmacological triage during which the action of the drugs on important functions is observed (Déciga-Campos et al., 2007). In addition, toxicity assessments are carried out with the objective of determining the potential of new substances and products to cause harm to human health. Tests of acute systemic toxicity are used to classify and appropriately label substances according to their lethal or toxic potential as established by relevant legislation (Valadares, 2006).

Among the animals in the control groups (rats and mice), no significant physiological changes were observed in either of the sexes. The mice that received the HE at lower doses (0.5 or 1.0 g/kg) also did not present significant changes. By contrast, the mice treated with HE at doses of 2.0 or 5.0 g/kg presented signs of toxicity, such as convulsions and tachycardia, in the first four hours after the treatment. From the second day on, the animals appeared sedated. These effects occurred in both sexes; however, the only animals that died were two females (Table 1). In the mice that received the HE via intraperitoneal route, behavioral changes were observed with doses of 2.0 or 5.0 g/kg HE in the first four hours after treatment, including writhing, grouping and convulsions. On the second day after treatment, the animals presented sedation and death proportional to the dose employed (Table 1). No significant change in body weight was observed in these animals (Table 2). Macroscopic examination of the kidneys, heart, liver and spleen of the surviving animals did not reveal any relevant changes; likewise, there was no significant change in the weight of these organs (Table 3). The study by Provasi et al. (2001) did not record any death among mice treated with a 3.5 g/kg dose; however, in that study, the period of monitoring of the animals was limited to four hours. This is important since although toxic effects can appear in multiple systems and organs, it is the liver that is most heavily affected both because of its anatomical position and because its cells concentrate the compounds to be metabolized, together with the resulting metabolites and the enzymes responsible for the metabolic process (Mendes, 1988).

In the rats treated orally, there were no behavioral changes nor deaths with the doses employed (Table 4), nor were there macroscopic or weight changes in the organs analyzed after 14 days of observation. The rats that received HE via the intraperitoneal route at doses of 2.0 and 3.0 g/kg presented an index of mortality proportional to the dose used (Table 4), and the mean lethal dose (LD_{50}) of HE for the rats treated via intraperitoneal route was 1.49 g/kg. The signs of toxicity presented by these animals were piloerection, writhing, sedation and depression. Moreover, the animals died in the first two hours after receiving the extract. We know that the value of the mean lethal dose as a fundamental parameter for the definition of chemical toxicity is limited, since this test measures only the acute toxicity produced by a single dose, and not long-term toxicity, and also does not measure individual reactions (Rang et al., 2003). Besides this, doses in excess of the maximum dose for the evaluation of this parameter, which is 2 g/kg (Larini, 1997), were used in this work.

Studies of sub-chronic toxicity are also performed in the pre-clinical evaluation of drugs, in an attempt to identify any damage that the use of the drug under consideration could cause to biological systems in the medium- and long-term (Sorrentino et al., 2006). The animals treated with the HE of *A. carambola* L. leaves for 60 days at doses of 0.125 and 0.25 g/kg did not present any behavioral alterations or deaths in the treatment period. No significant changes in the weight of the animals were noted (Table 6); likewise, there were no significant changes in food consumption in the groups that received HE compared to the control group. There was no apparent effect of the *A. carambola* L. HE on the selected organs, nor did they show any macroscopic alterations or changes in weight.

In the analysis of blood samples, no statistically significant changes were observed in the biochemical parameters in the animals treated with HE, compared to the control group (Table 6). The assessment of the subchronic toxicity of *A. carambola* L. involved examining liver function by assay of ALT, aspartate amino transferase (AST) and alkaline phosphatase (ALP), with no changes being found in relation to the control group at the doses employed. These data are similar to those

reported by Provasi et al. (2001), who did not find significant changes in the levels of ALT and AST in rats. In the small animal clinic, abnormalities are commonly detected in the serum levels of these enzymes, which are considered sensitive indicators of hepatobiliary disturbances (Sharon and Center, 1995).

A rise in serum enzyme activities may result from reversible or irreversible changes in cell permeability, the induction of microsomal enzymes or structural damage associated with necrosis, cholestasis or hepatocellular ischemia. Numerous pathological processes involving the liver can lead to proportionally distinct rises in liver enzymes, due to variation in the distribution of each specific enzyme in the hepatic lobule (Sharon and Center, 1995).

The transaminases are enzymes that catalyze the interconversion of amino acids and alpha-keto acids by transfer of the amino group. They are widely distributed throughout the tissues, with AST predominantly found in the liver, heart, cardiac muscle, striated muscle, kidney and pancreas, while ALT is predominantly seen in the liver, kidney and heart. AST is present in the cytosol as well as in the mitochondria of hepatocytes. The plasma activity of this enzyme is controlled by an enzymatic release mechanism localized to the cell membrane of the hepatocyte. The rate of excretion of the transaminases is variable, with AST being eliminated more quickly than ALT (Miller and Gonçalves, 1999). The increased levels of amino transferases in the serum of animals treated with the extract may have resulted from the release of enzymes from the cells of the organ affected, or from a change in cell permeability (Rebecca et al., 2002). With regard to *A. carambola* L., to our knowledge, only one study in the literature has investigated the effect of acute toxicity of the hydroalcoholic extract of the leaves in rats and mice, with the authors showing that the hepatic enzymes ALT) and AST were unchanged in relation to the control group (Provasi et al., 2001).

ALP is a phosphohydrolase enzyme found in various tissues, with higher concentrations in the liver, the epithelium of the biliary tract and in the bones. In the liver, ALP is secreted by the hepatocytes and by the cells of the mucosa of the biliary tract. In general, any active hepatopathy can increase the values of ALP, but the greatest increases in the levels of the enzyme occur in cases of obstruction of the biliary tract. In the case of hepatotoxic drugs, there is a smaller rise in enzyme levels (Miller and Gonçalves, 1999).

Urea is produced in the liver and excreted by the kidneys. When kidney filtration is insufficient, urea accumulates in the blood. Some drugs are capable of enzyme induction, and can consequently increase toxicity. According to literature, biochemical assays that show increased urea and creatinine in animals subjected to treatment with plant-based drugs may indicate that the effect of the drug interfere with the metabolism and function of the kidney (Baliga et al., 2004). Raised levels of total cholesterol and triglycerides or low levels of HDL are related to the risk of cardiovascular disease. Subchronic treatment with the HE of *A. carambola* L. did not alter these parameters. This result may be explained by the fact that rats are resistant to the development of hypercholesterolemia and atherosclerosis, possibly as a result of the increased conversion of cholesterol to biliary acids in the liver (Soares et al., 2005).

It should be emphasized that the interpretation of laboratory exams is much more complex than a simple comparison with reference values, classifying the test results as normal or abnormal according to the limits for the reference, and then comparing the results with standards that indicate the presence of certain diseases (Ravel, 1997). With regard to reference data in laboratory animals, the analysis and interpretation of the results are even more difficult, since the scientific literature lacks reference values for laboratory analyses in the animal species used in the present work. Consequently, the results obtained following the treatment of animals with the HE of *A. carambola* L. were compared by statistical methods with the data obtained in the control groups, according to methodology employed by Daher et al. (2006) and Thanabhorn et al. (2006), who assessed the toxicity of plants in animals and used their own control groups as references of normality in the analysis of their results.

Conclusion

The present acute and sub-chronic pre-clinical toxicological study in Wistar rats and Swiss mice using the hydroalcoholic extract of *A. carambola* L. leaves demonstrated that the plant species investigated has relatively low sub-chronic and acute toxicity in these animals.

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