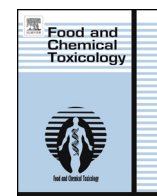Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Acute and subacute (28 days) oral toxicity assessment of the oil extracted from *Acrocomia aculeata* pulp in rats

CrossMark

Giseli Karenina Traesel [a], Juliane Coelho de Souza [a], Aline Lima de Barros [b],
Marcos Alexandre Souza [a], Wanderley Onofre Schmitz [c], Rozanna Marques Muzzi [d],
Silvia Aparecida Oesterreich [a], Arielle Cristina Arena [a,b,*]

[a] *School of Health Sciences, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil*
[b] *Department of Morphology, Institute of Biosciences of Botucatu, UNESP, Univ. Estadual Paulista, Botucatu, São Paulo, Brazil*
[c] *University Hospital, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil*
[d] *School of Exact Sciences and Technology, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil*

ABSTRACT

Acrocomia aculeata, popularly known as "bocaiúva", is a species used for nutritional purposes and for the treatment of various diseases, as it has, among other things, high levels of antioxidant compounds. This study aimed to assess the toxicological profile of *A. aculeata*, through acute and subacute toxicity tests. Male and female rats (Wistar) received by gavage 2000 mg/kg of oil extracted from the pulp of *A. aculeata* (OPAC) for the acute toxicity test and 125, 250, 500 or 1000 mg/kg of OPAC for subacute toxicity test. In the acute toxicity study no mortality or behavioral changes were observed in rats treated with 2000 mg/kg, indicating that the LD_{50} is higher than this dose. In the subacute toxicity test, the tested doses produced no significant changes in hematological, biochemical or histopathological parameters in the animals exposed. These results demonstrate the absence of acute and subacute toxicity after oral exposure to *A. aculeata* oil in rats. However, further studies in animals and in humans are needed in order to have sufficient safety evidence for its use in humans.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Natural products have been the target of numerous studies for obtaining active molecules with therapeutic potentials. Besides the diversity of medicinal species found, the use of plants with phytotherapic properties has increased due to the belief that everything that is "natural" does not cause adverse health effects (Oliveira et al., 2011). However, some species selected for medicinal use underwent chemical, toxicity or pharmacological studies to evaluate their safety and efficacy (Melo et al., 2011; Reyes-García, 2010).

Acrocomia aculeata (Jacq.) Lodd. ex Mart., popularly known as "bocaiúva" or "macaúba", is a plant widely distributed in the Midwest region of Brazil (Lorenzi, 2006). Their use has recently increased due to important features in the prevention of diseases. Besides its

medicinal use, the pulp of *A. aculeata* can be used as food, cosmetics and to produce energy (Ramos et al., 2008). According to popular knowledge, this medicinal plant has been used to treat respiratory diseases, and has laxative, analgesic and restorative properties (Lorenzi, 2006). In addition, pharmacological studies have shown its reductive effects on glucose and total cholesterol (Silva, 2012).

Regarding the chemical composition, studies have shown high levels of β-carotene (Ramos et al., 2008; Rocha et al., 2013; Sanjinez-Argandoña and Chuba, 2011), α-tocopherol (Coimbra and Jorge, 2011, 2012) and oleic acid (Amaral et al., 2011; Mariano et al., 2011) in the *A. aculeata* pulp performed by high-performance liquid chromatography and open column procedures. Though widely used by the population and favored for its nutritional and medicinal-chemistry purposes, little information is available in the literature on the therapeutic or toxic effects of this species. Thus, the aim of this study is to evaluate the toxicological profile of the oil extracted from the pulp of *A. aculeata* after a single oral administration or 28 consecutive daily administrations.

2. Materials and methods

2.1. Plant material and preparation of oil

Mature fruits of the palm *A. aculeata* were collected (latitude 22°13'18.54" south and longitude 54°48'23.09" west) in September 2013. A voucher specimen

---

was authenticated by Dr. LS Nogueira, and deposited (No. 2169) in the Herbarium of the Federal University of Grande Dourados. Access to the plant samples used for research has been authorized by the National Counsel of Technological and Scientific Development, Brazil (No.010653/2013-9).

The fruits of *A. aculeata* were washed, peeled and the pulp scarified. The pulps were then oven dried with ventilation at 50 °C for 2 hours. After drying, 500 g of sample were weighed and added as a solvent 1 L of hexane, leaving the mixture to stand under artificial light for 7 days. The hexane mixture was then filtered and concentrated at 57 °C in a rotary evaporator (Oetterer et al., 2006).

2.2. Determination of the acid values, transesterification and characterization of methyl monoesters

The OPAC (200 g) was weighed and mixed with methanol (60 mL) and KOH (2 g). The mixture was stirred and heated to 60 °C. The reaction was followed by thin-layer chromatography (TLC). After the oil consumption, the solution was decanted for 1 hour to separate the glycerin phase. The methanolic phase was treated with distilled water (10 mL) plus 10% NaCl (10 mL), dried with MgSO4, and finally filtered and concentrated in a rotaevaporator (Silva, 2012).

Gas chromatography (GC), using detector-mass spectrometry (DMS) and a flame ionization detector (FID), was performed to determine the chemical composition of the methyl fatty acid monoesters. The GC/DMS was performed using the chromatograph Varian model 431-GC210MS, according to the following conditions: Column: ZB-5 ms 30 m × 0.25 mm; Carrier gas: Helium 99.99%; Flow gas: 3.0 mL/min; Mass Spectrometry: ionization electron-impact mode SCAN-m/z 40–700; Oven temperature: 50 °C/1 min – 5 °C/1 min – 248 °C/6 min; Sample injection: mode split 50:1; Sample volume: 0.1 µL; and standard fatty acid methyl esters. The chromatographic conditions of the GC/FID were as follows: Column: ZB-Wax (5% phenyl Dimethylpolysiloxane); Carrier gas: Helium 99.99%; Flow gas: 3.0 mL/min; Temperature of detector: 250 °C; Oven temperature: 50 °C/1 min – 5 °C/1 min – 248 °C/6 min; Sample injection: mode split 50:1; Sample volume: 0.1 µL; and standard fatty acid methyl esters.

Values were determined based on the comparison between the times of retention and the profile of fragmentation of fatty acid methyl esters of samples analyzed under the same conditions. Quantification was performed by converting the percentages of peak areas in relation to the percentage of mass (% fatty acid = peak area of each fatty acid × 100/sum of all peak areas of fatty acids) (Silva, 2012).

2.3. Animals

Adult male and female Wistar rats (8–12 weeks, weighing 170–270 g) from the Federal University of Mato Grosso do Sul, were maintained under controlled temperature (23 °C), with a constant 12 h light–dark cycle and free access to food and water. Ten animals (females) were used for acute toxicity test and 50 animals (females and males) for the subacute toxicity test (OECD, 2008a, 2008b). The experimental procedures were in accordance with the Ethical Principles in Animal Research and approved by the Committee for Ethics in Animal Experimentation at the University Federal of Grande Dourados (number: 028–2013).

2.4. Toxicity studies

Acute and subacute toxicity studies were based on the OECD (Organisation for Economic Co-operation and Development) – Guidelines 425 and 407 (OECD, 2008a, 2008b).

2.4.1. Acute toxicity

The oil extracted from the pulp of *A. aculeata* (OPAC) was administered by gavage, at a dose of 2000 mg/kg, to one female (OECD, 2008a) under fasting for 8 hours. Sequentially, at intervals of 48 hours, the same dose was administered to four females, totaling five treated animals (Group: OPAC 2000 mg/kg). In parallel, five females were treated with vehicle (saline with Tween® 80 2%) in order to establish a comparative negative control group (OECD, 2008a).

The animals were observed periodically during the first 24 hours after administering the oil and then once a day for 14 days. The five parameters of the Hippocratic screening (Malone and Robichaud, 1962) were analyzed: conscious state (general activity); activity and coordination of motor system and muscle toning (response to tail touch and grip, straightening, strength to grab); reflexes (corneal and headset); activities on the central nervous system (tremors, convulsions, straub, sedation, anesthesia and ataxia) and activities on the autonomic nervous system (lacrimation, cyanosis, ptosis, salivation and piloerection). The water and feed consumption and body weight were also recorded daily (OECD, 2008a).

At the end of the observation period, all animals were anesthetized (Ketamina and xylazine, 25 and 10 mg/kg, respectively) and organs (heart, lung, spleen, liver, kidney, uterus and right ovary) were removed, weighed and examined macroscopically.

2.4.2. Subacute toxicity

The animals were divided into five experimental groups (n = 10 animals/group, five males and five females). Three different doses of OPAC (125, 250 or 500 mg/kg) were administered per group, orally (gavage), daily for 28 consecutive days. The control group received only the vehicle (saline with Tween® 80 2%). Another group (Satellite) received the maximum dose of 1000 mg/kg of OPAC for 28 days and remained untreated for 14 more days. It is important to use a satellite group for observation of reversibility, persistence, or delayed occurrence of toxic effects related to the administration of the test substance. The doses were chosen based on the Guideline 407 from OECD (Repeated Dose 28-Day Oral Toxicity Study in Rodents) (OECD, 2008b).

During treatment, daily body weight, food and water consumption, and possible signs of toxicity were observed and recorded, following the hippocratic screening. Clinical examination was performed once daily. At the end of the observation period, all animals were anesthetized (Ketamina and xylazine, 25 and 10 mg/kg, respectively). Blood samples were collected by cardiac puncture for subsequent hematological and biochemical analysis.

The following biochemical parameters were analyzed: bilirubin direct and total, cholesterol, electrolytes (sodium, potassium and calcium), markers of renal function (blood urea nitrogen and creatinine) and liver (alanine aminotransferase and aspartate aminotransferase) and protein profile (albumin, total protein and globulin) using Cobas Integra 400 Plus using commercial kits (Roche). Hematological analysis measured leukocyte count total and differential, erythrocytes and platelets, besides the levels of hemoglobin, hematocrit and red cell distribution width, with the unit XT-4000i (Sysmex).

After collecting blood, the vital organs (heart, lung, kidney, liver and spleen) and reproductive organs (testis, epididymis, vas deferens, prostate, uterus and ovary) were weighed. Samples of all organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin and examined under light microscopy (Martey et al., 2010). Histological analysis aimed to assess tissue integrity of the organs. The parameters analyzed were: degeneration, necrosis, apoptosis, leukocyte infiltration, congestion, extravasation of blood and fibrosis (Cunha et al., 2009).

2.5. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Student's t-test was used for comparison between two experimental groups (acute toxicity). The differences between groups of subacute toxicity were determined by analysis of variance (one-way ANOVA) followed by Dunnett's test. Differences were considered significant at p < 0.05.

3. Results and discussion

Vegetable oils rich in bioactive compounds are receiving growing interest due to their role in disease prevention through diet improvement (Coimbra and Jorge, 2011). However, toxicological studies are required to determine the toxicity and thus establish criteria for the selection of a safe dose (Berenguer-Rivas et al., 2013; Farsi et al., 2013). The present study may represent the first study that demonstrates the absence of toxicity of the oil extracted from the pulp of *A. aculeata*, which can contribute to the safe use of this species.

Our research group has determined the chemical composition of fatty acid of methyl monoesters of the oil obtained from the pulp of *A. aculeata* (OPAC) through gas chromatography (GC) using methods of mass spectrometry detector (MS) and flame ionization detector (FI) (Traesel et al., 2014). The results are expressed in Table 1.

According to Mariano et al. (2011), the pulp oil of *A. aculeata* presents higher levels of monounsaturated fatty acids than those found

Table 1
Monounsaturated fatty acids composition of oil extract of *A. aculeata* pulp.

Monounsaturated fatty acids	% Area ± standard error (GC/MS)	% Area (CG/FI)
Lauric	0.184 ± 0.019	0.250
Myristic	0.243 ± 0.086	0.070
Palmitoleic	2.016 ± 0.203	2.031
Palmitic	–	20.587
Linoleic	2.605 ± 0.341	2.050
Linolenic	–	0.311
Oleic	61.092 ± 2.731	71.305
Stearic	2.978 ± 0.631	2.614
Arachidonic	1.087 ± 0.194	0.154

Table 2
Body weight gain and food and water consumption of rats treated orally with oil extract of *A. aculeata* pulp.

	Acute toxicity		Subacute toxicity				
	Control	2000 mg/kg	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female							
Initial weight (g)	200.60 ± 8.08	200.00 ± 8.21	199.00 ± 6.98	176.50 ± 2,40	179.60 ± 2.32	174.75 ± 1.18	189.20 ± 8.61
Final weight (g)	223.00 ± 13.69	228.40 ± 14.95	240.60 ± 16.53	214.20 ± 8.01**	214.60 ± 8.53**	212.80 ± 8.07**	225.40 ± 18.00*
Body weight gain (%)	11.14 ± 4.51	14.22 ± 8.46	21.01 ± 4.17	23.78 ± 8.28	19.51 ± 4.20	23.05 ± 5.54	19.34 ± 5.91
Food intake (g/day)	130.21 ± 6.70	124.21 ± 7.27	118.67 ± 7.17	107.10 ± 13.62*	106.96 ± 10.81*	106.85 ± 15.78*	109.75 ± 9.97*
Water intake (mL/day)	201.42 ± 25.22	196.42 ± 24.44	195.00 ± 13.33	180.80 ± 18.85**	178.46 ± 10.82**	179.17 ± 9.89**	192.50 ± 18.33
Male							
Initial weight (g)			252.80 ± 10.39	250.40 ± 9.64	248.80 ± 8.22	253.00 ± 9.98	
Final weight (g)			333.00 ± 24.62	308.00 ± 29.89	311.20 ± 23.30	323.00 ± 29.53	248.80 ± 1.93
Body weight gain (%)			28.82 ± 4.42	22.92 ± 2.90	25.14 ± 4.49	28.35 ± 5.89	316.20 ± 5.16
Food intake (g/day)			148.46 ± 10.23	134.92 ± 7.53*	147.77 ± 9.93	147.71 ± 12.63	27.10 ± 2.01
Water intake (mL/day)			232.32 ± 20.02	205.71 ± 15.13**	230.00 ± 32.75	229.64 ± 22.84	147.46 ± 11.96

Values expressed as mean ± SEM, n = 5 animals/group.
  * $p < 0.05$ (ANOVA/Dunnett's test) compared with control group;   ** $p < 0.01$ (ANOVA/Dunnett's test) compared with control group.

in other vegetable oils such as olive oil, soy, corn, cottonseed, sunflower and flaxseed. Amaral et al. (2011) determined the presence of 69.07% of oleic acid in the pulp oil of *A. aculeata*. These data corroborate the chemical characterization performed on the oil sample used in this study, in which the predominance of oleic acid was identified by the GC/MS and GC/FI methods and points out the important chemical composition of the oil studied. The higher levels of mono-unsaturated fatty acid found in the present oil demonstrate a potential to become a good source of oleic acid, which makes this pulp oil the most suitable for human consumption, due to its properties compatible with other vegetable oils (Coimbra and Jorge, 2011).

Although we have not determined the carotenoids composition here, studies conducted by Hiane and Penteado (1989) and Ramos et al. (2008) indicated large amount of carotenoids in the pulp of *A. aculeata*. The authors emphasized the high potential of β-carotene as precursor of vitamin A and as antioxidant, assisting in the process of oxidative stress. Later, Coimbra and Jorge (2011) identified the presence of carotenoids (β-carotene) and also tocopherols (α-tocopherol) in *A. aculeata*.

A few edible oils extracted from vegetal species were evaluated in relation to their toxicity. Some oils obtained from *Piper aduncum* and *Copaifera reticulate* presented low toxicity after acute and/or subacute exposure (Sachetti et al., 2009; Souza et al., 2008). However, others presented some toxicity, such as the oils obtained from *Azadirachta indica*, *Drimys angustifolia* and *Drimys*

brasiliensis (Deng et al., 2013; Gomes et al., 2013), which reinforces the need for toxicological evaluation of oils of medicinal interest.

Changes in body and organ weights are a clear indicative of damage caused by the substance test (Berenguer-Rivas et al., 2013), while the hippocratic screening provides a general estimate of pharmacological and toxicological nature (Lucio et al., 2000). After the acute toxicity test, the dose of 2000 mg/kg (limit test – OECD, 2008a) of OPAC did not cause the death of any animal. The female rats exposed presented no behavioral changes during the treatment period, as well as no changes in water and food consumption and ponderal evolution, in relation to the control group (Table 2). No abnormality was found in the organs at necropsy. There was no statistical difference in the organs' relative weights between treated and control groups (Table 3). Thus, the oil tested falls in Class 5 (a substance with oral lethal dose (LD_{50}) higher than 2000 mg/kg), hence considered of low toxicity (OECD, 2008a). The absence of any sign of toxicity in the satellite group at the end of the 14-day observation period indicated that the OPAC showed no late toxic effects in the test animals. Similar results were found of Copaiba oil-resin obtained from *C. reticulate*. In this study, it was observed that the lethal oral toxicity of this oil was estimated to be higher than 2000 mg/kg, classified as category 5 according to OECD Guide 423, indicating a certain safety margin associated with the use of copaiba as therapeutic agent (Sachetti et al., 2009).

Table 3
Relative organ weight (g/100 g of body weight) of rats treated orally with oil extract of *A. aculeata* pulp.

	Acute toxicity		Subacute toxicity				
	Control	2000 mg/kg	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female							
Liver	4.61 ± 0.26	4.58 ± 0.39	3.88 ± 0.12	3.69 ± 0.17	3.64 ± 0.22	3.90 ± 0.14	3.63 ± 0.19
Kidney	0.45 ± 0.03	0.48 ± 0.05	0.39 ± 0.02	0.37 ± 0.01	0.39 ± 0.01	0.37 ± 0.00	0.37 ± 0.01
Spleen	0.20 ± 0.02	0.21 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
Heart	0.38 ± 0.02	0.38 ± 0.02	0.34 ± 0.01	0.37 ± 0.01	0.37 ± 0.02	0.37 ± 0.01	0.38 ± 0.02
Lung	0.57 ± 0.06	0.57 ± 0.11	0.52 ± 0.02	0.56 ± 0.01	0.50 ± 0.03	0.56 ± 0.04	0.54 ± 0.03
Ovary	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Uterus	0.20 ± 0.01	0.22 ± 0.04	0.19 ± 0.04	0.18 ± 0.06	0.30 ± 0.12	0.19 ± 0.09	0.22 ± 0.10
Male							
Liver			3.91 ± 0.10	3.73 ± 0.15	3.66 ± 0.25	3.88 ± 0.28	3.69 ± 0.17
Kidney			0.40 ± 0.01	0.41 ± 0.02	0.39 ± 0.01	0.39 ± 0.02	0.38 ± 0.03
Spleen			0.16 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
Heart			0.34 ± 0.01	0.31 ± 0.03	0.37 ± 0.01	0.34 ± 0.01	0.36 ± 0.02
Lung			0.44 ± 0.02	0.46 ± 0.03	0.46 ± 0.01	0.43 ± 0.01	0.46 ± 0.02
Testis			0.39 ± 0.04	0.45 ± 0.04	0.45 ± 0.03	0.44 ± 0.04	0.46 ± 0.03
Epididymis			0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.00	0.16 ± 0.00	0.17 ± 0.00
Vas deferens			0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Prostate			0.19 ± 0.02	0.19 ± 0.04	0.18 ± 0.03	0.19 ± 0.04	0.18 ± 0.01

Values expressed as mean ± SEM, n = 5 animals/group. $p > 0.05$ (ANOVA/Dunnett's test).

Toxicological evaluations after repeated exposures are required by regulatory agencies to characterize the toxicological profile of any substance (OECD, 2008b). In the present study, after subacute exposure, the animals were active and responsive to stimuli, with no clinical signs that could be associated with local or systemic toxic effects observed. There were no deaths and the behavior of animals remained normal for the species. However, the consumption of water and food for the female groups treated with OPAC, at all doses, diminished when compared to the control group. Although the mean intake differed statistically, no biological importance was assigned to this, since the weight gain values (Table 2) did not differ among groups. Similarly, in the present study, the relative weights of all organs examined did not vary significantly among groups (Table 3), corroborating the hypothesis of low toxicity of the oil after subacute exposure.

Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, should be performed and under certain circumstances may provide useful information. Some enzymes and proteins can be used as indicative of hepatocellular effects (such as ALT, AST, gamma-glutamyltransferase, bilirubin) (OECD, 2008b; Brandt et al., 2009), and others as biomarkers of nephron functional injury (creatinine, blood urea nitrogen) (Lameire et al., 2005). In this study, there was no statistical difference in liver or renal parameters (ALT, AST, bilirubin levels, creatinine and blood urea nitrogen) between the treated and control groups (Table 4). Some parameters (total protein, albumin, calcium and cholesterol) were statistically different when compared to the control group. However, this increase has no clinical significance. According to Giknis and Clifford (2006), the values found in this study are within the normal range for healthy rats at this age, indicating the absence of liver or renal toxicity.

The hematopoietic system is one of the most susceptible targets to toxic substances and is an important parameter for assessing the physiological and pathological status in humans and animals (Li et al., 2010). Although some parameters observed in this study (erythrocyte count, hematocrit and platelets) showed a statistical difference in the groups treated with OPAC, the other hematological parameters were similar among groups (Table 5). Similarly as in the biochemical analysis, the observed variations are not biologically meaningful, since the values are within the normal range for the species (Giknis and Clifford, 2006), indicating that the OPAC provided no adverse effects on circulating blood cells or on their production.

The assessment of pathological changes in the organs of treated animals, both macro and microscopically, is the basis of a safety assessment (Prabu et al., 2013). In this study, the macroscopic analysis, the OPAC, at all doses tested, produced no changes in the treated animals' vital and reproductive organs in the qualitative analysis. Similarly, in the histopathological analyses there were no findings suggestive of toxic effects (Fig. 1). These results proved to be consistent with biochemical analyses, confirming the safety of using the OPAC.

4. Conclusion

Previous findings from our group indicated that OPAC did not exhibit cytotoxic, genotoxic or mutagenic effects in rats (Traesel et al., 2014), which corroborates with the results of the present study. These results demonstrate the absence of acute and subacute toxicity as a consequence of the oral treatment with *A. aculeata* oil, suggesting that their oral LD_{50} is higher than 2000 mg/kg. The data obtained in this study are relevant as they provide for the use of a

Table 4
Biochemical parameters of rats treated orally with oil extract of *A. aculeata* pulp.

| | Subacute toxicity | | | | |
	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female					
Aspartate aminotransferase (U/L)	75.60 ± 2.50	68.80 ± 6.90	74.40 ± 14.97	72.60 ± 13.10	80.00 ± 6.01
Alanine aminotransferase (U/L)	39.80 ± 5.93	38.00 ± 2.91	40.20 ± 6.30	45.00 ± 6.96	37.60 ± 2.07
Direct bilirubin (mg/dL)	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Total bilirubin (mg/dL)	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
Total protein (g/dL)	6.14 ± 0.35	6.00 ± 0.29	6.16 ± 0.32	6.06 ± 0.16	6.01 ± 0.30
Albumin (g/dL)	4.38 ± 0.08	4.36 ± 0.20	4.48 ± 0.04*	4.40 ± 0.10	4.36 ± 0.20
Globulin (g/dL)	1.72 ± 0.27	1.64 ± 0.28	1.74 ± 0.29	1.64 ± 0.19	1.64 ± 0.26
Albumin/globulin ratio	2.62 ± 0.40	2.70 ± 0.49	2.64 ± 0.37	2.70 ± 0.30	2.70 ± 0.40
Blood urea nitrogen (mg/dL)	56.98 ± 7.53	54.24 ± 5.69	57.92 ± 6.59	51.14 ± 0.90	50.98 ± 1.90
Creatinine (mg/dL)	0.23 ± 0.05	0.20 ± 0.01	0.24 ± 0.05	0.20 ± 0.01	0.24 ± 0.05
Sodium (mmol/dL)	142.44 ± 1.13	141.06 ± 2.34	140.22 ± 0.80	141.42 ± 51.14	139.96 ± 3.90
Potassium (mmol/L)	5.32 ± 0.22	5.22 ± 0.88	5.60 ± 0.53	4.96 ± 0.71	5.07 ± 0.57
Calcium (mg/dL)	10.78 ± 0.87	10.94 ± 0.93	11.10 ± 0.54	10.36 ± 0.31	9.72 ± 0.33*
Cholesterol (mg/dL)	68.98 ± 7.56	71.66 ± 8.61	69.18 ± 7.79	78.84 ± 6.95*	66.71 ± 8.12
Male					
Aspartate aminotransferase (U/L)	81.40 ± 7.89	72.00 ± 11.64	73.80 ± 7.31	75.80 ± 7.79	75.00 ± 4.47
Alanine aminotransferase (U/L)	46.80 ± 2.28	40.00 ± 6.04	39.60 ± 5.31	43.20 ± 3.11	43.40 ± 3.36
Direct bilirubin (mg/dL)	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.02	0.04 ± 0.01	0.02 ± 0.01
Total bilirubin (mg/dL)	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.12 ± 0.01
Total protein (g/dL)	5.84 ± 0.08	5.78 ± 0.26	5.28 ± 0.25**	5.70 ± 0.33	6.16 ± 0.75
Albumin (g/dL)	4.14 ± 0.08	4.04 ± 0.21	3.68 ± 0.20*	4.00 ± 0.20	4.36 ± 0.20
Globulin (g/dL)	1.68 ± 0.08	1.74 ± 0.25	1.60 ± 0.12	1.72 ± 0.21	1.74 ± 0.29
Albumin/globulin ratio	2.42 ± 0.16	2.42 ± 0.43	2.30 ± 0.21	2.38 ± 0.29	2.64 ± 0.85
Blood urea nitrogen (mg/dL)	43.90 ± 7.90	45.00 ± 4.40	44.20 ± 4.93	43.90 ± 5.79	46.76 ± 5.29
Creatinine (mg/dL)	0.20 ± 0.02	0.20 ± 0.10	0.30 ± 0.00	0.25 ± 0.07	0.22 ± 0.04
Sodium (mmol/dL)	142.50 ± 0.99	142.80 ± 1.14	139.68 ± 1.33	138.32 ± 5.01	141.58 ± 2.74
Potassium (mmol/L)	5.08 ± 0.32	5.20 ± 0.67	5.03 ± 0.43	4.56 ± 0.19	4.73 ± 0.23
Calcium (mg/dL)	10.54 ± 0.20	10.30 ± 0.14	9.92 ± 0.23**	10.00 ± 0.35**	10.10 ± 0.15**
Cholesterol (mg/dL)	77.20 ± 9.72	70.00 ± 7.80	69.48 ± 5.10	72.75 ± 14.11	70.96 ± 5.73

Values expressed as mean ± SEM, n = 5 animals/group.
 * p < 0.05 (ANOVA/Dunnett's test) compared with control group; ** p < 0.01 (ANOVA/Dunnett's test) compared with control group.

Table 5
Hematological parameters of rats treated orally with oil extract of *A. aculeata* pulp.

	Subacute toxicity				
	Control	125 mg/kg	250 mg/kg	500 mg/kg	Satellite
Female					
Leukocytes ($10^3/\mu$L)	3.45 ± 1.80	3.07 ± 1.39	3.49 ± 1.68	3.92 ± 1.64	3.82 ± 1.65
Erythrocytes ($10^6/\mu$L)	7.84 ± 0.18	7.99 ± 0.36	8.05 ± 0.27	7.88 ± 0.22	7.96 ± 0.38
Hemoglobin (g/dL)	13.68 ± 0.37	13.62 ± 0.97	14.22 ± 0.64	13.86 ± 0.42	13.44 ± 0.47
Hematocrit (%)	44.58 ± 1.59	44.30 ± 2.80	44.24 ± 2.00	42.62 ± 1.81	41.98 ± 1.17
Platelets ($10^3/\mu$L)	625.80 ± 95.04	547.20 ± 78.26	439.60 ± 124.84	586.00 ± 146.40	517.40 ± 89.54
Red Cell Distribution Width (%)	13.44 ± 0.43	13.86 ± 0.73	13.80 ± 1.28	12.86 ± 0.49	13.22 ± 1.86
Neutrophils (%)	19.64 ± 3.40	19.06 ± 4.98	17.80 ± 3.84	20.56 ± 6.18	20.24 ± 6.67
Lynphocytes (%)	72.86 ± 9.59	75.85 ± 6.36	77.48 ± 5.68	74.14 ± 8.30	73.58 ± 7.32
Monocytes (%)	5.40 ± 6.36	2.98 ± 1.01	3.08 ± 2.76	3.42 ± 3.00	3.92 ± 3.25
Eosinophils (%)	1.44 ± 0.30	1.82 ± 0.98	1.4 ± 0.25	1.58 ± 0.42	2.04 ± 0.81
Basophils (%)	0.66 ± 0.66	0.32 ± 0.30	0.24 ± 0.20	0.3 ± 0.2	0.22 ± 0.17
Male					
Leukocytes ($10^3/\mu$L)	4.43 ± 2.02	4.44 ± 1.35	3.98 ± 2.22	4.82 ± 0.36	4.55 ± 1.48
Erythrocytes ($10^6/\mu$L)	8.92 ± 0.32	8.75 ± 0.11	8.38 ± 0.23*	8.72 ± 0.13	8.76 ± 0.27
Hemoglobin (g/dL)	15.10 ± 0.56	14.64 ± 0.18	14.30 ± 0.45	14.90 ± 0.38	15.15 ± 0.64
Hematocrit (%)	47.24 ± 1.87	45.56 ± 0.43	43.54 ± 1.57**	44.96 ± 1.58	44.98 ± 1.66
Platelets ($10^3/\mu$L)	611.80 ± 137.77	647.20 ± 55.16	556.60 ± 83.61**	594.80 ± 58.97	612.80 ± 71.07
Red Cell Distribution Width (%)	14.24 ± 0.91	13.74 ± 1.07	13.54 ± 0.29	14.18 ± 1.07	14.80 ± 1.07
Neutrophils (%)	17.52 ± 3.86	21.98 ± 12.37	23.56 ± 5.37	24.12 ± 3.80	14.76 ± 2.38
Lynphocytes (%)	79.32 ± 3.66	74.98 ± 13.06	71.46 ± 8.31	72.66 ± 3.97	82.36 ± 2.28
Monocytes (%)	2.14 ± 0.46	2.06 ± 1.94	3.56 ± 2.92	1.98 ± 0.61	1.52 ± 0.37
Eosinophils(%)	0.84 ± 0.33	0.82 ± 0.16	1.22 ± 0.51	1.2 ± 0.58	1.16 ± 0.16
Basophils (%)	0.18 ± 0.10	0.16 ± 0.14	0.20 ± 0.10	0.04 ± 0.08	0.20 ± 0.20

Values expressed as mean ± SEM, n = 5 animals/group.
  * p < 0.05 (ANOVA/Dunnett's test) compared with control group; ** p < 0.01 (ANOVA/Dunnett's test) compared with control group.

species of great economic and medical importance. However, other studies based on protocols elaborated by regulatory agencies should be performed (such as studies of chronic toxicity, reproductive toxicity, and others) in order to evaluate the total safety of this plant in humans.
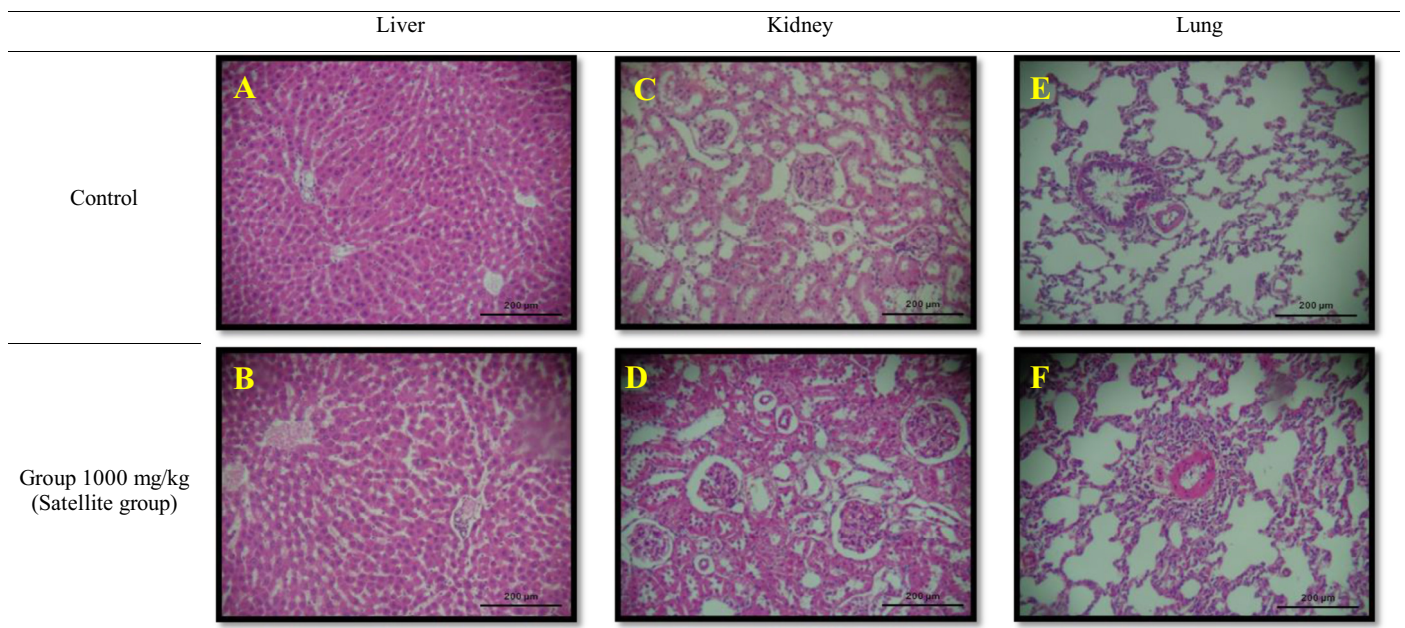
Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

Liver	Kidney	Lung

Fig. 1. Histopathological analysis of organs treated with oil extract of *A. aculeata* pulp in the subacute toxicity (H&E ×100). (A and B) liver; (C and D) kidney; (E and F) lung.

References

Amaral, F.P., Broetto, F., Batistell, C.B., Jorge, S.M.A., 2011. Extraction and qualitative characterization of pulp and almonds oils of macauba fruits [*Acrocomia aculeata* (Jacq) Lodd. ex Mart] collected in the region of Botucatu, SP. Rev. Energ. Agric. 26, 12–20.

Berenguer-Rivas, C.A., Castillo, A.A., Martínez, H.S., Zapata, E.P., Hernández, J.B., Tassé, Y.M., 2013. Acute oral toxicity of *Azadirachta indica* (Neem Tree). Rev. Cubana Plant Med. 18, 502–507.

Brandt, A.P., Oliveira, L.F.S., Fernandes, F.B., Alba, J., 2009. Evaluation of prospective hypocholesterolemic effect and preliminary toxicology of crude extract and decoction from *Vitex megapotamica* (Spreng) Moldenke (*V. montevidensis* Cham.) *in vivo*. Rev. Bras. Farmacogn. 19, 388–393.

Coimbra, M.C., Jorge, N., 2011. Proximate composition of guariroba (*Syagrus oleracea*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*) palm fruits. Food Res. Int. 44, 2139–2142.

Coimbra, M.C., Jorge, N., 2012. Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). J. Sci. Food Agric. 92, 679–684.

Cunha, L.C., Azeredo, F.S., Mendonça, A.C.V., Vieira, M.S., Pucci, L.L., Valadares, M.C., et al., 2009. Acute and subacute toxicity studies of the latex and of the ethanolic extract of the leaves of *Synadenium umbellatum* Pax in rats. Rev. Bras. Farmacogn. 19, 403–411.

Deng, Y.X., Cao, M., Shi, D.X., Yina, Z.Q., Jiaa, R.Y., Xua, J., et al., 2013. Toxicological evaluation of neem (*Azadirachta indica*) oil: acute and subacute toxicity. Environ. Toxicol. Pharmacol. 35, 240–246.

Farsi, E., Shafaei, A., Hor, S.Y., Ahamed, M.B., Yam, M.F., Asmawi, M.Z., et al., 2013. Genotoxicity and acute and subchronic toxicity studies of a standardized methanolic extract of *Ficus deltoidea* leaves. Clinics (Sao Paulo) 68, 865–875.

Giknis, M.L.A., Clifford, C.B. 2006. Clinical laboratory parameters for Crl:CD (SD) rats. Charles River Laboratories.

Gomes, M.R.F., Schuh, R.S., Jacques, A.L.B., Dorneles, G.G., Montanha, J., Roehe, P.M., et al., 2013. Biological assessment (antiviral and antioxidant) and acute toxicity of essential oils from *Drimys angustifolia* and *D. brasiliensis*. Rev. Bras. Farmacogn. 23 (2), 284–290.

Hiane, P.A., Penteado, M.V.C., 1989. Carotenoids and provitamin A values of fruit and flour of "Bocaiúva" (*Acrocomia mokayayba* Barb. Rodr.) from Mato Grosso do Sul. Rev. Far. Bioq. Un. 25 (2), 158–168.

Lameire, N., Van Biesen, W., Vanholder, R., 2005. Acute renal failure. Lancet 365, 417–447.

Li, X., Luo, Y., Wang, L., Li, Y., Shi, Y., Cui, Y., et al., 2010. Acute and subacute toxicity of ethanol extracts from *Salvia przewalskii* Maxim in rodents. J. Ethnopharmacol. 131, 110–115.

Lorenzi, G.M.A.C. 2006. Acrocomia aculeata (Jacq.) Lodd. ex Mart. – arecaceae: bases para o extrativismo sustentável. Tese (Doutorado em Ciências Agrárias): Curitiba.

Lucio, E.M.R.A., Rosalen, P.L., Sharapin, N., Souza Brito, A.R.M., 2000. Avaliação toxicológica aguda e screening hipocrático da epilsopilosina, alcalóide secundário de *Pilocarpus microphyllus* Stapf. Rev. Bras. Farmacogn. 9, 23–25.

Malone, M.H., Robichaud, R.C., 1962. A Hippocratic screen for pure or crude drug materials. Llordya 25, 320–331.

Mariano, R.G., Moura, C., Couri, S., Nogueira, R.I., Freitas, S.P., 2011. Partition of free fatty acids in deacidification of macaúba pulp oil by liquid-liquid extraction using ethanol/water as solvent. Def. Dif. Forum 312, 554–559.

Martey, O.N., Armah, G., Okine, L.K.N., 2010. Absence of organ specific toxicity in rats treated with Tonica, an aqueous herbal haematinic preparation. Afr. J. Trad. Complement. Alternat. Med. 7, 231–240.

Melo, J.G., Santos, A.G., Amorim, E.L., Nascimento, S.C., Albuquerque, U.P., 2011. Medicinal plants used as antitumor agents in Brazil: an ethnobotanical approach. Evid. Based Complement. Alternat. Med. 2011, 365359.

Oetterer, M., Regitando-D'Arce, M.A.B., Spoto, M.H.F., 2006. Fundamentos de Ciência e Tecnologia de Alimentos, 1st ed. Manole, Barueri.

OECD, 2008a. Guidelines for testing of chemical. Acute oral toxicity – up-and-down-procedure. OECD (Ed.): Paris.

OECD, 2008b. Guidelines for testing of chemical. Repeated dose 28-day oral toxicity study in rodents. OECD (Ed.): Paris.

Oliveira, A.K.M., Oliveira, N.A., Resende, U.M., Martins, P.F.R.B., 2011. Ethnobotany and traditional medicine of the inhabitants of the Pantanal Negro sub-region and the raizeiros of Miranda and Aquidauna, Mato Grosso do Sul, Brazil. Braz. J. Biol. 71, 176–179.

Prabu, P.C., Panchapakesan, S., Raj, C.D., 2013. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. Phytother. Res. 27, 1169–1178.

Ramos, M.I.L., Ramos Filho, M.M., Hiane, P.A., Braga Neto, J.A., Siqueira, E.M.A., 2008. Nutritional quality of the pulp of bocaiuva *Acrocomia aculeata* (Jacq.) Lodd. Sci. Tec. Alim. 28, 90–94.

Reyes-García, V., 2010. The relevance of traditional knowledge systems for ethnopharmacological research: theoretical and methodological contributions. J Ethnobiol. Ethnomed. 6, 32.

Rocha, M.S., Figueiredo, R.W., Araújo, M.A.M.A., Moreira-Araújo, R.S.R., 2013. Physical and chemical characterization and antioxidant activity (*in vitro*) of fruit of the Piaui savanna. Rev. Bras. Frutic. 35, 4.

Sachetti, C.G., Fascineli, M.L., Sampaio, J.A., Lameir, O.A., Caldas, E.D., 2009. Assessment of the neurotoxic potential and acute toxicity of copaiba (*Copaifera reticulata* Ducke, Fabaceae). Rev. Bras. Farmacogn. 19 (4), 937–941.

Sanjinez-Argandoña, E.J., Chuba, C.A.M., 2011. Biometrical, physical and chemical characterization of bocaiuva (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart) Palm fruits. Rev. Bras. Frutic. 33, 3.

Silva, P.V.B.S. 2012. Caracterização química e avaliação do potencial antidiabético e citotóxico de óleo extraído de Acrocomia aculeata (macaúba). Dissertação (Mestrado): Dourados.

Souza, P.J.C., Barros, C.A.L., Rocha, J.C.S., Lira, D.S., Monteiro, G.M., Maia, J.G.S., 2008. Toxicological evaluation of the essential oil of *Piper aduncum* L. Rev. Bras. Farmacogn. 18 (2), 217–221.

Traesel, G.K., Castro, L.H.A., Silva, P.V.B., Muzzi, R.M., Kassuya, C.A.L., Arena, A.C., et al., 2014. Assessment of cytotoxic, genotoxic and mutagenic potential of *Acrocomia aculeata* in rats. Genet. Mol. Res, *in press*.