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RESEARCH ARTICLE OPEN ACCESS

POLYSACCHARIDE-RICH EXTRACT OF *GENIPA AMERICANA* LEAVES: ACUTE TOXICITY STUDY

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ABSTRACT

The leaves of *Genipa americana* L. (Rubiaceae) are utilized in folk medicine to treat diarrhea, syphilis and gastritis. The objective was to evaluate the acute toxicity of polysaccharides rich extract from leaves of *G. americana*. They were administered orally during 14 days in rats for toxicity assay based on the Organization for Economic Co-operation and Development (OECD) guideline 423, to identify signs of toxicity, the Malone Hippocratic Screening Scale was applied during the evaluation period. Blood samples were collected for biochemical, hemogram, leukogram and platelet parameters. Polysaccharide rich extract from leaves of *Genipa americana* at a dose of 2000 mg/kg did not produce mortality. SGOT, SGPT, Creatinine phosphokinase, LDH, urea and creatinine were elevated in 300 and 2000 mg/kg doses. In conclusion, though there was no mortality, the extract of *G. americana* higher than 300 mg/kg can produce signs of biochemical and histopathological toxicity in liver and kidney. It is recommended that lower doses than the studied ones should be used for treatment.

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INTRODUCTION

In the last few years, polysaccharides obtained from higher plants have been shown wide spectrum of pharmacological properties, such as immunomodulator (Yi, 2011), anti-inflammatory (Chen, 2011; Pereira, 2012; Pereira, 2016) analgesic (Wang, 2011) anticoagulant and antiplatelet (Souza, 2015; Madeira, 2018), antidepressant, [8]neuroprotectiveand antioxidant (Zhou, 2010; Lin et al., 2014; Ai, 2013; Nonato, 2018). In addition, these polysaccharides are considered potential therapeutic alternative, since mostly is devoid of toxicity (Liu, 2018). *Genipa americana* L. (Rubiaceae)is distributed along riversin Brazil northeast semi-arid regions, being popularly known as "jenipapo" or "jenipapeiro". (Delprete, 2005; Mesquita, 2011). In the folk medicine, *G. Americana* leaves are used to treat fever, diarrhea, syphilis and liver disorders in the form of macerate or decoction (Agra, 2008). Scientific studies demonstrated for the polysaccharide-rich extract of *G. americana* leaves trypanocidal activity in all parasite developmental forms (Souza, 2018), and antioxidant and anticonvulsant effects in the model of PTZ-induced seizures (Nonato, 2018). Besides, its isolated glycoconjugates presented antiplatelet, anticoagulant and antithrombotic activity, being devoid of hemorrhagic risk (Madeira, 2018). The polysaccharide-rich extract (PRE) of *G. americana* leaves containing 54.6% total carbohydrates, including 21.1% uronic acid present in its structural composition a heteropolysaccharide (Madeira, 2018), demonstrated by infrared spectra and magnetic resonance (Nonato, 2018). Considering the atoxicity of polysaccharides of higher plants, along with the therapeutical potential of the polysaccharides from *G. americana* leaves, the aim of this study was to investigate the toxicity of the polysaccharide-rich extract from *G. americana* leaves after rat *peroral* treatment based in 425 OECD guideline.

MATERIALS AND METHODS

Animals: Nine female Wistar non-pregnant rats (170-200g; 5-6 weeks), divided into 3 groups of 3 animals each, were maintained with free access to water and food at 22-26 °C,

12/12 h light/dark cycle. Experimental protocols were approved by the Animal Care and Use Committee of the State University of Ceara (nº 2204175/2016).

Chemicals and reagents: **Ketamine and xylazine were purchased** from Sigma (St. Louis, MO, USA) and clinical diagnostic kits from Labtest Diagnóstica S.A., Brazil. All other chemicals and reagents were of analytical grade.

Plant collection, identification and polysaccharides extraction:
Leaves (voucher n° 46794-Herbarium Prisco Bezerra - Federal University of Ceará, Brazil) were dried and grounded into powder (5 g), suspended in absolute methanol for partial remotion of methanol-soluble material, and residue was suspended at 0.1M NaOH for extraction of polysaccharides. Supernatants were pooled, neutralized, precipitated with 96% ethanol and centrifuged. The pellet was dialyzed against distilled water for 48 h, re-centrifuged and the supernatant, containing 54.6% total carbohydrates (including 21.1% uronic acid), 12% protein and 3.58 mg/g polyphenols (gallic acid equivalent) was named PRE (polysaccharide-rich extract; 6.5% yield)

Acute toxicity assay: Rats were maintained without food for 3–4 h, but with access to water *ad libitum* before receiving per oral (*p.o.*) single dose of PRE (300 or 2000 mg/kg). The animal's body weight was measured at 1, 7 and 14 days after PRE treatment. Animals were also evaluated in the behavioral tests: Open Field (crossing; rearing) and Malone Hipocratic (toxicity; mortality) according to 425OECDGuidelines for the Testing of Chemicals n° 425.[18]Blood samples were collected for evaluation of biochemical and hematological parameters and afteranimal's sacrifice, organs were excised for histopatho logical and morphometric evaluation.

Behavioral tests: *Open field.* Mice were individually placed in the open-field apparatus, an acrylic box (30 × 30 × 15 cm) with the floor divided into 9 squares. The number of rectangles crossed with all paws (crossing) and the number of rearing were counted during 6 min (Montgomery, 1955). Animals were treated with saline or PRE 60 min before the first evaluation and evaluated again after 7 and 14 days of administration.

Malone Hippocratic scale: The intensity of autonomic (breathing,fecal excretion, diuresis, sialorreia exploratory activity, prostration, exophthalmia, sedation, analgesia) and central (grooming, catatonia, shiver, seizures) behavioral parameters was classified as absent (-), rare (+), discrete (++), moderate (+++) and intense (++++) (Malone, 1969). Rats were treated with PRE and evaluated in the open field apparatus at day 1 (60, 120, 180, 240 min)and once a day at the same time from day 2 until day 14.

Biochemical andhaematologicalassays: At day 14 after PRE treatment, blood samples were collected in tubes containing potassium EDTA for evaluation of hematological and biochemical parameters. The hematological evaluation included erythrocyte, leukocyte and platelet counts; hemoglobin (Hb); hematocrit (Ht); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); hemoglobin mean concentration (MCHC) and red distribution width (RDW). The plasma biochemical parameters analyzed (diagnostic kits from Labtest, Brazil) included glucose; total cholesterol; triglycerides; total protein; albumin; alanine aminotransferase (ALT); aspartate aminotransferase (AST); direct bilirubin (DB); total bilirubin (TB); alkaline phosphatase (AP); creatinine; amylase; urea and uric acid.

Histopathological and Morphometric analysis: For histopathological analysis liver, brain, kidney cortex were removed, weighed, fixed during 24 h in 10% buffered formaldehyde, transferred to 70% ethanol, trimmed byan automated histotechnique (Leica®), embedded in paraffin, sectioned (thickness: 3-5 µm), stained (H&E) and photomicrographed (microscope Nikon Eclipse Nis). For morphometric analysis, the following parameters were observed: hepatic (hepatocyte nucleus), renal (leukocyte infiltrate, tubular necrosis, hemorrhage areas, tubular atrophy), splenic (hyperplasia, cells with fused nuclei, granulomas) and brain (presence of black neurons, hemorrhage areas). The total area of pathological changes was calculated by dividing the area of a square formed with the points of intersection (between two perpendicular lines), multiplied by the total sum of squares observed (Image Pro Plus 4.5®) (Mandarim-de-Lacerda, 2003).

Statistical analysis: Results are presented as mean ± S.E.M and analyzed by ANOVA and Newman-Keuls tests. Values of $p< 0.05$ were considered significant.

RESULTS

PRE reduces the n° of crossings and rearing in the open field test: PRE reduced the n° of crossing in the 1sthour at 300mg/kg (8.33 ± 3.48) and 2000 mg/kg (2.66 ± 2.66) compared to saline (38.67 ± 7.31), and in the 7th day at 300 mg/kg (21.67 ± 4.41) and 2000 mg/kg (19.00 ± 2.64) compared to saline (39.00 ± 5.19), but did not alter in the 14th day (Fig. 1A, B, C). PRE also reduced the n° of rearing in the 1st hour at 2000 mg/kg (1.66 ± 1.20*vs*. saline: 14.00 ± 2.51) and in the 7thday at both doses: 300 mg/kg (9.67± 0.88) and 2000 mg/kg: (7.33 ± 1.20) compared to saline (14.67 ± 1.76), without alteration in the 14th day (Fig. 1 D, E, F).

Effect of PRE in the behavioral parameters of Malone Hippocratic scale: Most of the behavioral parameters were unaltered by PRE. However, the exploratory activity was reduced in the first day of treatment from moderate to discrete at 300 mg/kg and from moderate to rare at 2000 mg/kg, being recovered in the following days. In addition, prostration was increased along PRE treatment, especially in the first day (Table 1).

Effect of PRE on body mass and organs weight of rats after 14 day-treatment: Rats treated with PRE (300 and 2000 mg/kg; p.o.) for 14 days survived and showed no alterations in water or food consumption. The weight of most organs were not altered, except for the kidney, which was increased by 28.5% (Table 2). Along PRE treatment, the body mass was reduced by 12% and 13% at 300 and 2000 mg/kg, respectively (data not shown). In addition, no clinical abnormalities, that would suggest toxicity, were observed.

PRE does not alter hematological or biochemical parameters of rats evaluated 14 days after p.o. treatment: The hematological parameters were unaltered by PRE treatment (Table 3), being the erythrocytes normocytic and norm chromic (data not shown). In addition PRE did not induce alterations in the function biomarkers of pancreas (α-amylase),

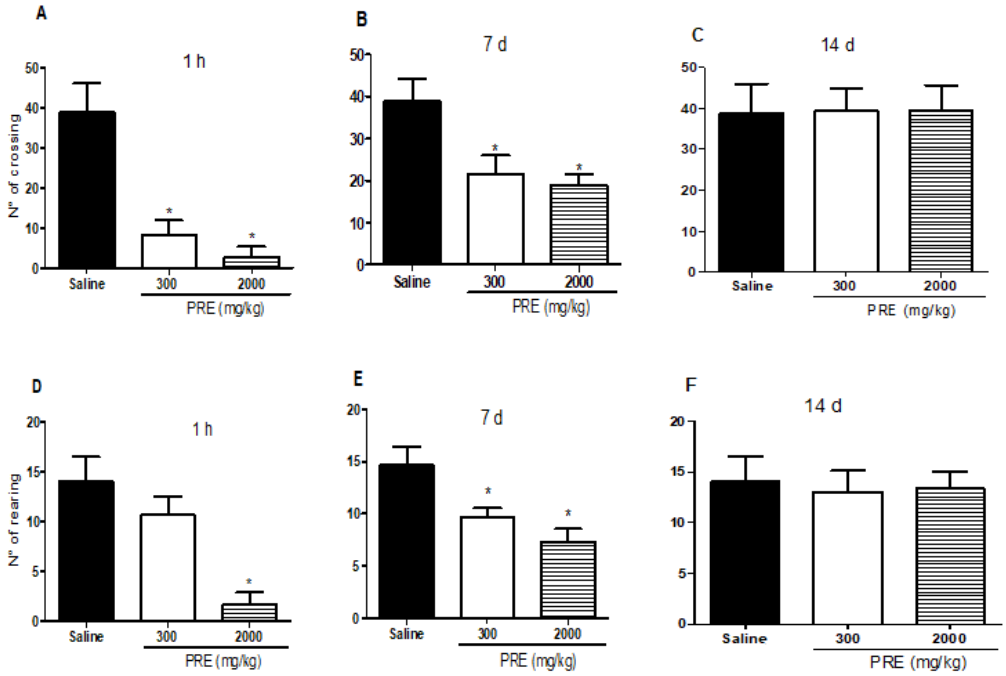

Figure 1.PRE reduces the n° of crossings and rearing in the open field test. Rats received p.o. 0.9% NaCl (Saline) or PRE (300, 2000 mg/kg). The evaluation was performed 1 h, 7 and 14 days after treatment. N° of crossing (A, B, C) and n° of rearing (D, E, F). Mean ± SEM (n=8). ANOVA and Newman-Keuls.*p<0.05 *vs.* Saline

Table 1. Effect of PRE in the rat behavioral parameters of Malone Hippocratic scale

Parameters		Time(min)			Time (days)													
		60	120	180	240	2	3	4	5	6	7	8	9	10	11	12	13	14
Breathing	G1[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
	G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fecal excretion	G1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G2	+	+	-	-	++	+	+	+	+	+	-	-	-	-	-	-	-
	G3	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+
Diuresis	G1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G2	+	-	-	-	+	+	+	+	-	-	+	-	-	+	+	+	++
	G3	++	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-
Sialorreia	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Exploratoryactivity	G1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	G2	++	++	++	+	++	++	++	++	++	++	++	+++	++	+++	+++	++	++
	G3	+	++	+	+	++	+++	++	++	++	++	+	++	+	+++	++	++	+
Prostration	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	+	++	++	++	+	+	++	++	++	++	++	++	++	++	++	++	++
	G3	++	++	+++	+++	+	+	+++	+	+	+	+	+	++	++	++	+	-
Exophthalmia	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G3	+	+	-	-	++	+	+	-	-	-	-	-	-	-	-	-	-
Sedation	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Analgesia	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grooming	G1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G2	-	-	-	-	+	++	++	++	-	+	+	+	++	+	+	+	+
	G3	++	+	-	-	++	+	-	-	-	+	+	+	+	++	++	++	-
Shiver	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	++	++	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	G3	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]G1 (Saline); G2 (PRE 300 mg/kg); G3 (PRE 2000 mg/kg), scores: Absent (-); Rare (+); Discrete (++); Moderate (+++); Intense (++++)

Table 2. Effect of PRE on rat organs weight after 14 day-treatment

	Organs weight (g)			
Group	Liver	Spleen	Brain	Kidney
Saline	4.24[a] ± 0.02	0.37 ± 0.01	0.94 ± 0.02	0.77 ± 0.04
300 mg/kg	3.89 ± 0.21	0.34 ± 0.01	1.01 ± 0.05	0.89 ± 0.02
2000mg/kg	3.72 ± 0.13	0.37 ± 0.04	1.01 ± 0.04	0.99 ± 0.03*

[a]Mean ± S.E.M. (n=10). *P≤ 0.05 *vs*. Saline (ANOVA and Newman-Keuls).

Table 3. PREdoes not alter rat haematological parameters after 14 day-treatment.

Parameters	Saline	300 mg/kg	2000 mg/kg
Erythrocytes x10^6	7.79 ± 0.26	7.42 ± 0.29	8.19 ± 0.08
Hb (g/dl)	14.37 ± 0.44	13.00 ± 0.45	14.63 ± 0.18
Ht (%)	46.18 ± 0.64	42.62 ± 1.21	46.35 ± 0.61
MCV (μm3)	59.33 ± 1.20	59.67 ± 0.33	56.33 ± 1.20
MCH (pg)	18.43 ± 0.17	17.33 ± 0.38	17.80 ± 0.36
MCHC (g/dl)	31.13 ± 0.63	30.07 ± 0.49	31.60 ± 0.32
RDW (%)	14.40 ± 0,10	13.33± 0,65	13.43 ± 0,16
Leukocytes/mm³	9.93 ± 2.85	7.40 ± 2.10	13.28 ± 3.41
Neutrophils (%)	7.23 ± 1.73	7.02 ± 2.75	9.66 ± 2.13
Monocytes (%)	0.68 ± 0.43	0.45 ± 0.26	0.97 ± 0.47
Lymphocytes (%)	74.83 ± 3.91	66.75 ± 5.85	73.83 ± 3.38
Plateletsx 10³/mm³	811.0 ± 93.82	651.0 ± 265.0	748.0 ± 137.0

Hb: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscularhemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width. Mean ± S.E.M. *p≤0.05 *vs*. control (ANOVA and Newman-Keuls).

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Table 4. Effect of PRE on rat biochemical blood parameters after 14 day-treatment

Parameters	Saline	300 mg/kg	2000 mg/kg
ALT (U/L)	42.67 ± 3.18	47.00 ± 9.29	52.67 ± 6.48
AST (U/L)	45.47 ± 11.46	50.95 ± 16.02	41.09 ± 20.21
DB (mg/dL)	0.05 ± 0.01	0.03 ± 0.00	0.05 ± 0.00
TB (mg/dL)	0.17 ± 0.02	0.18 ± 0.10	0.54 ± 0.43
Creatinine (mg/dL)	0.37 ± 0.05	0.45 ± 0.02	0.38 ± 0.03
AP (U/L)	232.00 ± 94.07	114.00 ± 6.11	181.30 ± 57.65
Cholesterol (mg/dL)	49.33 ± 1.20	56.67 ± 5.81	63.67 ± 3.38
Triglycerides (mg/dL)	78.67 ± 2.18	78.67 ± 6.93	156 ± 9.28**
Glucose (mg/dL)	126.70 ± 4.33	144.00 ± 9.45	164.30 ± 6.88*
Amylase (U/L)	427.70 ± 10.48	466.00 ± 7.00	464.30 ± 18.41
Albumin (g/dL)	3.67 ± 0.05	4.33 ± 0.04**	4.01 ± 0.13
TP (g/dL)	7.48 ± 0.12	8.27 ± 0.22*	8.05 ± 0.19
Uricacid (mg/dL)	0.73 ± 0.02	0.95 ±0.05*	1.11 ± 0.06**
Urea (mg/dL)	32.00 ± 4.58	34.00 ± 2.64	34.33 ± 3.38

ALT: alanine amino transferase; AST: aspartate aminotransferase; DB: direct bilirubin; TB: total bilirubin; AP: alkaline phosphatase; TP: total protein. Mean ± S.E.M. *p≤0.05, **p≤0.001 *vs*. Saline (ANOVA and Newman-Keuls).

kidney (urea and creatinine), and liver (ALT, alanineaminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; TB, total bilirubin; DB, direct bilirubin and cholesterol). However, PRE increased at 300 and/or 2000 mg/kg the serum biomarker of liver function (albumin and TP, total protein) and pancreas function (glucose and triglycerides) still increase uric acid, the serum biomarker of kidney function (Table 4).

Histological pattern of organs from mice treated with PRE: Liver presented normal-architecture with lobular organization and normal hepatocyte cords permeating normal-looking hepatic sinusoids without dilationor degeneration, such as cellular vacuolization or steatosis. Brain showed normal hippocampus neurons, loose chromatin nuclei with some visible nucleoli, and organized amphophilic cytoplasm, adjacent to a fibrillar area, containing normal glial cells.

Kidney presented an organized cortical area with renal corpuscles of normal size, containing glomeruli of normal morphology.

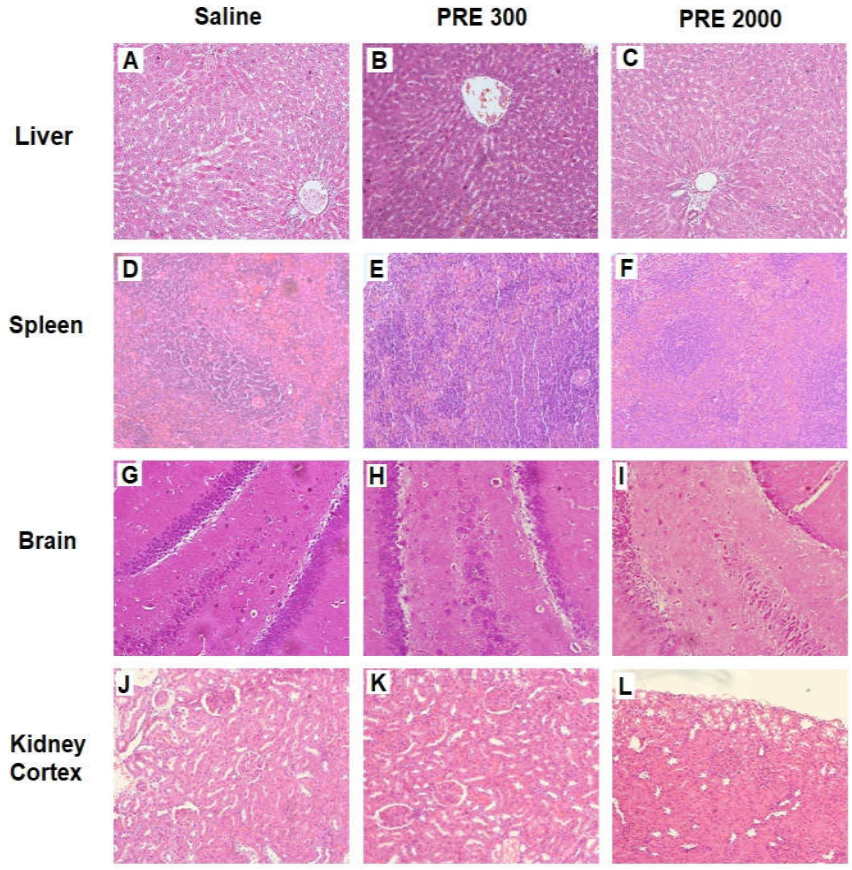

Figure 2. Histological pattern of rat organs after PRE 14 day-treatment. Liver (A, B, C); brain (D, E, F); kidney cortex (G, H, I). H&E Microscope Nikon Eclipse Nis, Nis Software 4.0®. Rats received *p.o.* 0.9% NaCl (Saline) or PRE (polysaccharide-rich extract of *G. americana*).

DISCUSSION

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions.[22] In general, natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Newman, 2003). The investigation of PRE (p.o.) systemic toxicity in rats, assessed by several parameters (survival, water and food consumption, body mass, absolute and relative organs weight, blood biochemical dosage, hemogram and organs morphology), demonstrated that all animals survived and no major toxicity was observed in most of the organs analyzed. PRE reduced exploratory activity in open field, in accordance that demonstrate by Nonato (Nonato, 2018), it was decreased the number of crossing and of rearing but did not promote sedative effect, which most sedative drugs promote as an adverse effect. The toxic outcomes of drugs on vital body organs are exposed by clinical signs and symptoms which are principal observations among various other toxicity indicators (Subramanion, 2011). No animal was found dead while some changes in behavioral pattern in Autonomic Nervous System like breathing, fecal cake, diuresis, sialorreia, exploratory, prostration, analgesia, sedation, exophthalmia and in central nervous system like Grooming, Catatonia, Tremors, Seizures were observed in treatment group in first 24 h and during 14 days. In general in vivo toxicity study is the toxicological analysis of many medicinal plants and its potency to evaluate qualitatively and quantitatively by histopathology and oral acute toxicity studies. Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose (Sasidharan, 2008). A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products (Moshi, 2007). PRE does not alter hematological parameters of rats, however increased the serum biomarker of liver function and pancreas function and serum biomarker of kidney function. In accordance *C. fistula* Also there were no any significant elevations observed in the biochemical analysis of the blood serum. Further, histopathological examination revealed normal architecture and no significant adverse effects observed on the kidney, heart, liver, lung and spleen.[24]

Conclusion

In conclusion, though there was no mortality, the extract of *G. americana* higher than 300 mg/kg can produce signs of biochemical and histopathological toxicity in liver and kidney. It is recommended that lower doses than the studied ones should be used for treatment.

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

Agra MF, Silva KN, Basílio JLD, Freitas PF, Barbosa-Filho JM. 2008. Survey of medicinal plants used in the region Northeast of Brazil. Revista Brasileira de Farmacognosia: OrgaoOficial Da Sociedade Brasileira de Farmacognosia.; 18(3): 472–508.

Ai S, Fan X, Fan L, et al. 2013. Extraction and chemical characterization of *Angelica sinensis* polysaccharides and its antioxidant activity. *Carbohydrate Polymers*.; 94(2): 731-736.

Chen J, Chen X, Qin J. Effects of polysaccharides of the *Euphoria longan* (Lour.) Steud on focal cerebral ischemia/reperfusion injury and its underlying Mechanism. Brain Injury. 2011; 25(3): 292–299.

Delprete PG, Smith LB, Klein RM. Flora Ilustrada Catarinense: Rubiáceas. Herbário Barbosa Rodrigues, Itajaí. 2005.

Humber JM. The role of complementary and alternative medicine: Accomodating pluralism. J. Am. Med. Assoc. 2002; 288, 1655-1656.

Lin X, Huang Z, Chen X, et al. Protective effect of *Millettia pulchra* polysaccharide on cognitive impairment induced by d-galactose in mice. Carbohydrate Polymers. 2014; 101 533-543.

Liu J, Bai R, Liu Y, Zhang X, Kan J, Jin C. Isolation, structural characterization and bioactivities of naturally occurring polysaccharide–polyphenolic conjugates from medicinal plants-A reivew review. *Int J Biol Macromol.* 2018; 107:2242–2250.

Madeira JC, Silva GVL, Batista JJ, et al. An arabino galactan-glycoconjugate from *Genipa americana* leaves present anticoagulant, antiplatelet and antithrombotic effects. Carbohydrate polymers. 2018; 202: 554-562.

Malone MH, Robichaud RC. A hippocratic screen for pure or crude drug materiais. Lloydia. 1962; 25 (4):320-331

Mandarim-de-Lacerda CA. Stereological tools in biomedical research. An Acad Bras Cienc. 2003; 75 (4):469-486.

Mesquita JB, Santos MJCD, Ribeiro GT, Moura AO. Avaliação da composição de substratos em recipientes na produção de mudas de jenipapo (*Genipa americana* L.). Natural Resources. 2011; 1(1): 47–58.

Montgomery KC. The relation between fear induced by novel stimulation and exploratory behavior. J Comp Physiol Psychol. 1955; 8:254-60.

Moshi MJ. Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *Afr.* J. Tradit. Complement. Altern. Med. 2007; (4) 219-225.

Newman DJ, Cragg, GM, Snader KM. Natural Products as Sources of New Drugs. J. Nat. Prod. 2003; 66, 1022-1103.

Nonato DTT, Vasconcelos SMM, Mota, MRL, et al. The anticonvulsant effect of a polysaccharide-rich extract from *Genipa americana* leaves is mediated by GABA receptor. Biomedicine & Pharmacotherapy. 2018;101: 181–187.

OECD, Acute oral toxicity: Up and down procedure, Guideline for the Testing of Chemicals 425 OECD. 2008; 1–2.

Pereira LP, Mota MRL, Brizeno LAC, Nogueira FC ; Ferreira EGM ; Pereira MG, Assreuy AMS. Modulator effect of a polysaccharide-rich extract from *Caesalpinia ferrea* stem barks in rat cutaneous wound healing: Role of TNF-α, IL-1β, NO, TGF-β. Journal of Ethnopharmacology. 2016; 187: 213-223.

Pereira LP, Silva RO, Bringel PHSF, et al. Polysaccharide fractions of *Caesalpinia ferrea* pods: potential anti-inflammatory usage. J Ethnopharmacol. 2012; 139 (2): 642-648.

Sasidharan S, Darah I, Jain K. *In vivo* and *in vitro* toxicity study of *Gracilariachangii*. Pharm.Biol. 2008; 46, 413-417.

Souza ROS, Assreuy AMS, Madeira JC, et al. Purified polysaccharides of *Geoffroea spinosa* barks have anticoagulant and antithrombotic activities devoid of hemorrhagic risks. Carbohydr Polym. 2015;124:208-215.

Souza ROS, Sousa PL, Paula RR, et al. Trypanocidal activity of polysaccharide extract from *Genipa americana* leaves. J. Ethnopharmacol. 2018; (10) 311–317.

SubramanionLJ, Zuraini Z, Yeng C, Yee LL, Lachimanan YL, Sreenivasan S. Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Molecules. 2011; 16: 5268-5282.

Wang J, Flaisher-Grinberg S, Li S, et al. Antidepressant-like effects of the active acidic polysaccharide portion of *ginseng* in mice. *J Ethnopharmacol*. 2010; 132 (1): 65-69.

Wang L, Zhao D, Di L, et al. The analgesic and anti-rheumatic effects of *Thladiantha dubia* fruit crude polysaccharide fraction in mice and rats. *J Ethnopharmacol*. 2011;137(3): 1381-1387.

Yi Y, Liao ST, Zhang MW, et al. Physicochemical characteristics and Immunomodulatory Activities of three polysaccharide-protein complexes of *Longan pulp.* Molecules. 2011; 16(7): 6148-6164.

Zhou ZY, Tang YP, Xiang J, et al. Neuroprotective effects of water-soluble *Ganoderma lucidum* polysaccharides on cerebral ischemic injury in rats. *J Ethnopharmacol*. 2010; 131(1): 154–164.

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