Biotecnia
Revista de Ciencias Biológicas y de la Salud
http://biotecnia.unison.mx
Universidad de Sonora
"El saber de mis hijos hará mi grandeza"

Antibacterial activity and acute toxicity study of standardized aqueous extract of *Randia monantha* Benth fruit

Estudio de actividad antibacteriana y toxicidad aguda de extracto acuoso estandarizado de fruto de *Randia monantha* Benth

Naida Juárez-Trujillo[1], Fredy Erubiel Tapia-Hernández[2], Mayvi Alvarado-Olivarez[3], César I. Beristain-Guevara[1], Luz Alicia Pascual-Pineda[1], Maribel Jiménez-Fernández[1]*

[1] Centro de Investigación y Desarrollo en Alimentos, Universidad Veracruzana, Xalapa Veracruz, México.
[2] Facultad de Ciencias Químicas, Universidad Veracruzana, Xalapa, Veracruz, México.
[3] Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, México.

ABSTRACT

The fruit of *Randia monantha*, traditionally and empirically used in the preparation of a beverage as antivenom and for the treatment of various diseases has little scientific evidence regarding its antimicrobiological properties and toxicity. The objective of this study was to evalute the antimicrobial effect of the *Randia monantha* pulp aqueous extract on acute toxicity in male Wistar rats, evaluated at different concentrations against pathogenic bacteria. The results of acute oral toxicity revealed no deaths with doses up to 5000 mg/kg body weight. The experimental animals showed no significant changes in the weight and behavior parameters evaluated with respect to the control treatment. Rats did not show significant changes in the hematological parameters, but dosis of 5000 mg/kg body weight rats exhibited the appearance of yellow spots on kidney. The aqueous extract had no antimicrobial activity against pathogenic bacteria tested. These results suggest that the *Randia monantha* fruit aqueous extract can be used with some degree of safety by oral administration but should not be taken in high doses.

Keywords: Acute toxicity, Antimicrobial properties, Crucetillo, *Randia monantha*, Rats.

RESUMEN

El fruto de *Randia monantha* se utiliza tradicional y empíricamente en la preparación de una bebida como antiveneno y para el tratamiento de diversas enfermedades, pero hasta la fecha existe poca evidencia científica sobre sus propiedades antimicrobiológicas y toxicidad. El objetivo de este estudio fue evaluar el efecto de la administración del extracto acuoso de pulpa de *Randia monantha* sobre la toxicidad aguda en ratas Wistar macho y evaluar la actividad antimicrobiana a diferentes concentraciones frente a bacterias patógenas. Los resultados de la toxicidad oral aguda no revelaron muertes en dosis de hasta 5000 mg/kg de peso corporal. Los animales de experimentación no mostraron cambios significativos en los parámetros de peso y comportamiento evaluados con respecto al tratamiento control. Las ratas no mostraron cambios significativos en los parámetros hematológicos, pero las ratas con dosis de 5000 mg/kg de peso corporal mostraron la aparición de manchas amarillas en el riñón. El extracto acuoso no mostró actividad antimicrobiana contra las bacterias patógenas de prueba. Estos resultados sugieren que el extracto acuoso de la fruta *Randia monantha* puede usarse con cierto grado de seguridad por administración oral, pero no debe tomarse en dosis altas.

Palabras clave: Toxicidad aguda, Propiedades antimicrobianas, Crucetillo, *Randia monantha*, Ratas.

INTRODUCTION

Stems, leaves and fruits of several *Randia* genus species contain compounds such as 1-nitropyrene, linoleic acid, palmitic acid, sterols, β-sitosterol, β-sitosterol, campesterol, oleanolic acid acetate, oleanolic acid-3-α-L-arabinoside, and mesembryanthemoidigenic acid, among others, which have been shown to confer biological, antioxidant and various pharmacological properties (Cano-Campos *et al*., 2011; Kandimalla *et al*., 2016; Lapikanon *et al*., 1983). The *Randia monantha* Benth fruit, known as a "crucetillo" because of the cross shape in which the thorns are presented, is used as an antivenom beverage against poisonous animals and to which various pharmacological properties are attributed, since it is used as an anticancer, antidiabetic, antidiarrheal, to relieve stomachache and many other diseases (Gallardo-Casas *et al*., 2012). Currently, the fruit is used in various liquors, predominantly: brandy, ethanol, and Jerez wine. However, it has been reported that the aqueous extract of the pulp and the seed of *Randia monantha* Benth have a higher concentration of polyphenolic compounds, such as chlorogenic acid, rutin, and 4-coumaric acid, which could contribute to their antioxidant activity and other attributed beneficial properties (Juárez-Trujillo *et al*., 2018). On the other hand, there are reports that polyphenol compounds have antimicrobial activity, but there is little information in the literature about the inhibitory capability of *Randia monantha* on pathogenic bacteria. Thus, nowadays people consume it in large quantities and for long periods, considering that the administered dose depends on the bite or sting of the poisonous animal, or the severity of the disease (Mendez and Hernández, 2009). Although in tests conducted with saline artemia there are no toxic effects reported in this species, and as far as we know,

*Autores para correspondencia: Maribel Jiménez Fernández
Correo electrónico: maribjimenez@uv.mx
Recibido: 16 de junio de 2021
Aceptado: 24 de septiembre de 2021

there are no studies on its acute toxicity in experimental animals. Therefore, the objective of this study was to evaluate the acute toxicity of the aqueous extract of the *Randia monantha* fruit in a murine model (male Wistar rats) on behavioral parameters, macroscopic evaluation of liver and spleen, analysis of the pancreatic Langerhans islets, and a histopathological examination of liver, kidney, and pancreas tissue. In addition, we evaluated the effect of the aqueous extract at different concentrations on pathogenic bacteria.

MATERIAL AND METHODS

Obtaining the fruits

To obtain the sample, a batch of 100 fruits (5.73 kg) was selected at random, with a maturity index of 26.60. The fruits were collected at the 2018 January-March period in the town of Actopan, Veracruz, located at the latitude: 19°27´36´´, longitude: 96°49´48´´ west 1060 m.a.s.l. After harvest, fruits were washed and disinfected with 0.01% sodium hypochlorite. Manually obtained pulp (1.04 kg) was lyophilized until it became a powder. The taxonomic identification of the fruit was confirmed by biologist Manuel Carlos Durán Espinosa. A copy of the reference fruit, with Num. of herbarium copy: NJuárezT2 (XAL) was deposited in the Xalapa herbarium of the Institute of Ecology A. C (Juárez-Trujillo *et al*., 2018).

Preparation of experimental animals

The experimental animals used were 21 male Wistar rats, weighing 300 - 359 g and between 3 to 5 months of age from of Neuroethology laboratory of the Universidad Veracruzana. Six groups of *n=3* rats corresponding to the different concentrations (10, 100, 1000, 1600, 2900, and 5000 mg/kg weight body) and a control group (without administration) were randomly formed. The rats were housed in transparent plastic boxes, three rats per box inside the bioterium, with light / dark cycles of 12:12 h at a room temperature of 25 ± 2 ºC, and a humidity between 50 - 60 %. The experimental animals carried a standard diet with water and pelletized food *ad libitum*. They were allowed seven days for acclimatization before the commencement of the research. In this study, all animals were handled in accordance with the guidelines for the Care and Use of Laboratory Animals of Universidad Veracruzana based in the National Institutes of Health.

Acute toxicity study

The dried pulp was mixed at different concentrations with a solution of tween 20 (5%), propylene glycol (5%), and purified water. Doses were prepared according to guide 423, the Organization of Economic Co-operation and Development (OECD, 2001). The groups were administered with extract by intragastric cannulation in a single dose of 5000, 2900, 1600, 1000, 100, and 10 mg/kg body weight for each of the treatment groups, respectively, while the control group received only tween 20, propylene glycol and water used as a vehicle. LD_{50} was estimated according to the guideline 423 from Organization of Economic Co-operation and Development (OECD, 2001).

The animals were monitored during the first 24 hours after cannulation with special emphasis on the first 30 minutes, and with 15-minute videos taken at 2, 4, 6, 8, 12, and 24 hours in search of apparent modification of toxicological behavior (Cavanaugh, 2003). The symptoms analyzed were mucus, ataxia, salivation, diarrhea, seizures, respiratory disorders, hyperactivity, and paralysis (Pérez, 2017). The weight of the rats was recorded daily for 15 days. On day 15, all rats used in the experimental procedures were sacrificed with an overdose of sodium pentobarbital (60 mg/kg).

A blood sample was obtained by cardiac puncture and collected in plastic tubes containing EDTA. Erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and leukocyte count were evaluated using a veterinary hematology analyzer (Abaxis Vatscan HM2). All rats were sacrificed on day 15 and subsequently perfused and dissected; the extraction and weighing of organs, liver, kidneys, pancreas, and spleen were performed.

Histopathology study

Selected organs, including pancreas, liver, kidney, and spleen of the treated and control animal groups, were fixed in 10 % buffered formalin in labeled bottles and subsequently processed for histological examination. The tissue was processed using an automated tissue processor (Leica 820, Germany) and embedded in blocks of paraffin. Stained tissue sections were examined under a light microscope (Leica DM 750, Germany). Number and area of Langerhans islets in the pancreas were determined in at least five islets per section at a 40x magnification. Full images of the selected Langerhans islets were obtained by photomicrographs with Adobe® Photoshop CS6 software, and their analysis was performed using the ImageJ Java® software in which the area, thickness, density, and volume data of the selected islets were obtained according to the methodology reported by Cavanaugh (2003). The numerical concentration of the cells in the islet was determined by manual counting by differential hematoxylin and eosin phloxine (H&E) staining (Coggehall, 1992; Mohammadi and Naik, 2012).

Antimicrobial activity of the aqueous extract of pulp of *Randia monantha* fruit

The *Randia monantha* pulp extract antimicrobial activity was evaluated using the broth dilution method. Active cultures of *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 43895), and *Salmonella typhimurium* (ATCC 14028) were prepared according to Balouri *et al*. (2016). For the above, dilutions were prepared at 0.5, 1.0, 1.5 and 2.0 mg/mL in a liquid growth medium, distributed in tubes containing a volume of 2 mL. Subsequently, each tube was inoculated with a microbial inoculum prepared in the same medium, which was previously standardized to the Mcfarland scale of 0.5. Each tube was then shaken and incubated for 24 hours at 37 °C. After 24 h, the absorbance λ= 600 nm was read.

Statistical analysis

Analysis of variance was carried out to evaluate the results found in the different treatments with pulp extracts, and subsequently, the significant differences were assessed using the Fisher test with a level of significance $p \leq 0.05$, using Minitab program version 17, States United.

RESULTS

Body and organs weight gain

The results of the acute toxicity study indicated that the aqueous extract of the *R. monantha* pulp administered orally to rats at doses between 10-5000 mg/kg body weight in acute toxicity testing methodology did not cause death or significant toxicity in test animals, indicating that the LD_{50} is greater than the doses administered. Table 1 shows that the groups corresponding to an administration of 2900 and 5000 mg/kg body weight presented a slight increase in weight that ranged from 0.25 to 7.94%. However, in relation to the weight of the organs, it was observed that the pancreas, liver, kidneys, and spleen did not show significant differences ($p > 0.05$) compared to the respective organs of the control treatment.

Behavior observations

Consistent with these results, the different treatment groups showed no significant difference in the evaluated clinical signs (mucus, ataxia, salivation, diarrhea, convulsions, hyperactivity, paralysis, and changes in breathing), compared to the control treatment.

Macroscopic evaluation of organs

External macroscopic analysis of liver, spleen, and pancreas did not reveal any alteration nor signs of toxicity, without visual differences with the control treatment, indicating the absence of toxicity. However, the kidneys of the group corresponding to an administration of 5000 mg/kg body weight showed yellow spots. The kidney histopathology examination revealed that the yellow areas presented a minor or no blood supply, with a notable decrease in erythrocytes and a decrease in blood flow than the kidney cuts of the control treatment (Figure 1).

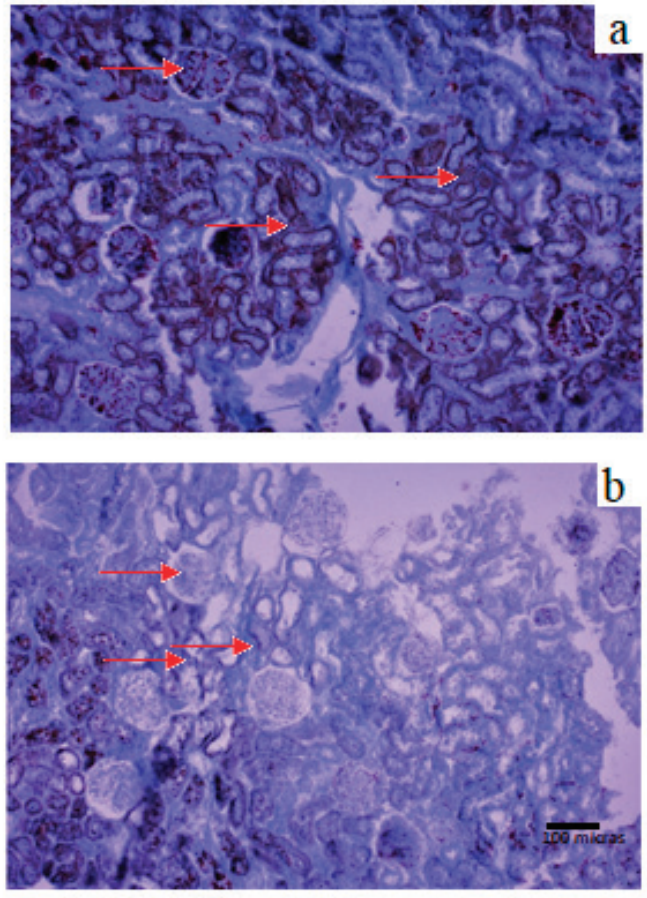

Figure 1. Kidney histological examination. A) Control treatment and B) Treatment with a dose of 5000 mg/kg of weight. The arrows point out the blood supply.
Figura 1. Examen histológico del riñón de A) Tratamiento de control y B) Tratamiento con dosis de 5000 mg/kg de peso. Las flechas señalan el aporte de sangre.

Hematology parameters analysis

The hematology parameters are summarized in Table 2. The hematological analysis of leucocytes, lymphocytes, monocytes, granulocytes, red blood cell, mean cell volume, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, erythrosary distribution index, platelets,

Table 1. Increase in total body weight and in weight of the important vital organs of each treatment group.
Tabla 1. Incremento del peso corporal total y aumento del peso de los órganos vitales importantes de cada grupo de tratamiento.

Doses (mg/kg Body weight)	Increased Total Body Weight (%)	Pancreas	Liver	Kidney	Spleen
0 (Control)	0.05 ± 0.02^{a}	2.99 ± 0.85^{a}	11.01 ± 0.73^{a}	3.34 ± 0.41^{a}	1.40 ± 0.19^{a}
10	0.25 ± 0.19^{a}	2.74 ± 0.68^{a}	10.97 ± 1.33^{a}	2.63 ± 0.27^{a}	1.13 ± 0.24^{a}
100	1.84 ± 0.86^{b}	3.34 ± 0.69^{a}	10.15 ± 1.76^{a}	3.12 ± 0.71^{a}	1.31 ± 0.23^{a}
1000	5.73 ± 0.70^{c}	3.04 ± 0.18^{a}	9.97 ± 1.52^{a}	2.91 ± 0.83^{a}	1.57 ± 0.53^{a}
1600	$4.64 \pm 0.68^{c,d}$	3.27 ± 1.01^{a}	10.69 ± 1.60^{a}	2.89 ± 0.24^{a}	1.30 ± 0.09^{a}
2900	5.39 ± 0.64^{d}	3.80 ± 0.69^{a}	12.45 ± 2.35^{a}	3.04 ± 0.63^{a}	1.25 ± 0.18^{a}
5000	7.94 ± 0.87^{e}	3.74 ± 0.23^{a}	11.79 ± 2.34^{a}	2.87 ± 0.30^{a}	1.56 ± 0.12^{a}

Mean ± SD, n = 3. Different letters in the same column indicate significant differences ($p < 0.05$).

Table 2. Hematological examination of treatments with different dosis (0-5000 mg/kg) of the standardized aqueous extract of *Randia monantha* pulp at 1, 7 and 15 days of administration.
Tabla 2. Examen hematológico de los tratamientos con diferentes dosis (0-5000 mg/kg) del extracto acuoso estandarizado de pulpa de *Randia monantha* a los 1, 7 y 15 días de administración.

Day 1	Dose (mg/kg weight)						
Parameters	**Control**	**10**	**100**	**1000**	**1600**	**2900**	**5000**
Leukocytes (10^9/L)	12.97±1.5	10.21±2.51	16.41±8.15	10.24±2.57	12.23±3.91	14.03±4.18	7.77±0.49*
Lymphocytes (10^9/L)	8.80±0.72	7.88±1.43	9.62±1.29	7.82±2.48	8.34±4.15	6.42±1.59	5.59±1.26*
Monocytes (10^9/L)	0.53±0.34	0.36±0.02	0.75±0.08	0.56±0.01	0.18±0.01	0.29±0.10	0.27±0.23
Lymphocytes (%)	68.00±2.40	76.16±3.26	64.96±19.11	75.33±7.03	64.93±15.34	49.70±26.3	71.56±11.42
Monocytes (%)	4.00±2.26	2.80±0.91	3.46±0.45	0.60±0.00	0.63±0.05	2.10±0.14	2.73±0.10
Granulocytes (%)	28.00±0.14	21.03±4.38	31.56±5.80	24.10±7.00	34.43±5.29	48.20±26.16	25.73±9.57
Red blood rell (10^{12}/L)	9.07±0.35	9.00±0.27	8.35±1.69	9.04±0.31	8.71±0.12	9.86±0.01	9.42±0.83
Hemoglobin (g/dL)	17.50±0.00	17.40±0.30	16.10±2.87	17.36±0.20	16.76±0.81	19.30±0.56	18.43±0.55
Hematocrit (%)	52.69±1.25	53.54±1.66	50.75±9.51	55.27±1.95	51.19±3.89	55.66±1.37	51.84±3.83
Mean cell volumen (fl)	58.50±3.53	59.33±0.57	60.66±1.52	61.33±0.57	58.66±3.78	56.50±0.70	55.00±2.64
Mean corcuspular hemoglobin (g/dL)	33.15±0.77	32.56±0.49	31.80±0.36	31.43±0.87	32.83±0.92	34.60±0.14	35.66±1.76
Erythrosary distribution index (%)	17.75±0.49	16.70±0.20	18.10±1.31	16.50±0.40	18.60±0.95	16.45±0.35	16.60±0.96
Platelets (10^9/L)	620.50±47.37	587.00±60.62	504.66±31.07	754.33±127.44	814.33±88.66	493.00±15.36	20.66 ± 2.03*
Mean platelet volumen (fl)	6.65±0.35	6.56±0.11	6.66±0.15	6.63±0.11	6.76±0.41	6.90±0.42	6.63±0.37
Triglycerides (mg/dL)	100.19±27.83	95.80±31.88	95.80±14.71	62.67±5.53	104.99±18.60	143.11±45.62	73.83±27.29
Glucose (mg/dL)	52.79±12.29	57.89±16.12	54.52±15.44	74.47±7.89	104.23±36.60*	110.40±23.70*	124.08±23.83*
Cholesterol (mg/dL)	80.19±5.80	76.90±6.98	91.40±10.44	75.36±9.05	89.66±12.83	76.08±9.83	86.95±21.16
			Day 7				
Leocucytes (10^9/L)	15.57±4.62	10.39±2.26	16.41±8.15	10.24±2.57	12.23±3.92	10.35±1.01	14.38±7.82
Lymphocytes (10^9/L)	9.20±0.86	7.88±1.43	9.62±1.29	7.81±2.49	8.34±4.15	7.78±0.32	5.47±1.18*
Monocytes (10^9/L)	0.49±0.25	0.26±0.02	0.75±0.09	0.16±0.01	0.18±0.01	0.13±0.02	0.48±0.05
Lymphocytes (%)	61.40±11.55	76.16±3.26	64.96±19.11	75.33±7.03	64.93±15.34	75.75±10.53	247.63±73.57
Monocytes (%)	3.30±0.20	2.80±0.91	3.46±0.45	0.60±0.00	0.63±0.05	1.30±0.98	2.96±0.59
Granulocytes (%)	35.26±12.58	21.03±4.38	31.56±15.80	24.10±7.00	34.43±15.29	23.00±9.45	49.43±6.27
Red blood rell (10^{12}/L)	8.80±0.52	9.00±0.27	8.35±1.69	9.04±0.31	8.71±0.12	9.62±0.35	9.74±0.48
Hemoglobin (g/dL)	17.00±0.86	17.40±0.30	16.10±2.87	17.36±0.20	16.76±0.81	18.75±1.34	18.33±0.73
Hematocrit (%)	50.01±4.73	53.54±1.66	50.75±9.51	55.27±1.95	51.19±3.89	53.46±4.47	51.99±3.20
Mean cell volumen (fl)	57.00±3.60	59.33±0.57	60.66±1.52	61.33±0.57	58.66±3.78	55.50±2.12	53.66±2.08
Mean corcuspular hemoglobin (g/dL)	34.03±1.62	32.56±0.49	31.8±0.36	31.43±0.87	32.83±0.92	35.1±0.56	35.23±0.80
Platelets (10^9/L)	816.00±34.02	587.00±60.62	504.66±31.80	754.33±124.44	814.33±88.66	896.50±255.36	304.66±67.68*
Platelet (%)	0.53±0.02	0.38±0.04	0.33±0.02	0.50±0.09	0.55±0.09	0.62±0.14	0.14±0.02*
Platelet distribution width (%)	31.03±0.28	32.16±0.45	31.66±0.95	31.53±0.57	31.70±0.86	32.6±0.56	33.83±3.35
Triglycerides (mg/dL)	90.61±24.95	113.77±21.85	135.32±4.79	113.97±41.00	103.11±7.85	85.62±14.39	73.05±7.28
Glucose (mg/dL)	59.00±19.75	82.14±10.44	60.51±16.15	65.96±15.15	67.63±14.05	128.36±10.63*	116.00±20.22*
Cholesterol (mg/dL)	47.09±11.17	52.90±11.11	50.20±4.66	50.87±9.00	48.85±2.44	44.93±1.71	39.67±7.49
			Day 15				
Leocucytes (10^9/L)	15.30±2.95	10.05±1.79	12.41±0.60	11.11±1.18	12.73±5.12	10.14±0.71	8.01±3.29*
Lymphocytes (10^9/L)	9.89±1.28	7.01±0.19	9.08±0.50	8.25±1.69	7.78±3.57	8.53±0.73	5.58±2.21
Monocytes (10^9/L)	0.48±0.02	0.19±0.03	0.25±0.01	0.45±0.06	0.44±0.01	0.05±0.00	0.22±0.02
Lymphocytes (%)	65.86±11.61	70.90±10.12	73.40±7.29	73.76±8.21	60.56±11.21	84.10±1.27	70.03±7.03
Monocytes (%)	3.06±0.92	1.66±0.84	1.96±0.36	4.26±0.80	4.36±6.43	0.60±0.00	4.56±0.78

Parameters	Dose (mg/kg weight)						
	Control	10	100	1000	1600	2900	5000
Granulocytes (%)	30.40±10.98	27.43±8.30	24.63±4.93	21.96±4.50	35.06±5.65	15.40±127	25.46±8.98
Red blood rell (10^{12}/L)	8.80±0.33	9.91 0.13	10.10±0.74	8.94±0.41	8.86±0.09	8.98±0.55	9.00±0.49
Hemoglobin (g/dL)	16.06±0.66	19.16±0.36	19.26±0.96	18.53 0.87	17.50±1.01	17.40±0.56	17.36±0.66
Hematocrit (%)	49.81±2.52	59.36±0.61	59.99±4.18	56.42±2.57	53.74±2.54	49.19±1.56	49.03±2.56
Mean cell volumen (fl)	56.66±2.08	60.00±0.00	59.33±1.52	63.00±0.00	60.66±3.05	55.00±1.41	54.33±2.30
Mean corcuspular hemoglobin (g/dL)	32.26±1.92	19.33±0.25	19.10±0.45	32.80±0.69	32.50±1.44	35.45±0.07	35.36±0.90
Platelets (10^9/L)	451.33±46.98	586.66 101.07	552.66±55.62	625.33 87.84	566.00±40.18	951.00±178.00	504.33±83.31
Platelet (%)	0.29±0.02	0.38±0.05	0.37±0.03	0.43 0.06	0.39±0.08	0.65±0.11	0.34±0.06
Platelet distribution width (%)	33.66±4.43	32.13±0.90	32.46±0.23	32.00±0.23	32.43±0.87	32.40±0.28	32.16±0.45
Triglycerides (mg/dL)	89.02±32.93	113.83±41.49	121.33±21.28	113.25±40.87	105.74±34.24	121.49±21.17	89.34±49.82
Glucose (mg/dL)	67.63±29.75	59.88±19.67	66.14±16.32	66.14±16.32	80.94±2.11	128.94±17.74*	131.69±20.20*
Cholesterol (mg/dL)	44.49±6.06	60.69±7.22	48.74±11.06	61.32±11.96	53.14±6.54	42.21±3.66	39.62±2.35

Values are expressed as mean ± SD, n=3.
*Significant differences with the control treatment, $p < 0.05$.

mean platelet volume, and platelet distribution revealed no significant differences ($p > 0.05$) in relation to the control treatment and the days analyzed, the values were within the range considered normal (Tasić *et al*., 2021; Nagamma *et al*., 2019; Dantas *et al*., 2006). The samples did not show significant differences ($p > 0.05$) in the triglycerides and cholesterol concentration with the control group, which should be considered in treating dyslipidemias. Treatments with a dose between 1600 and 5000 mg/kg of body weight exhibited at time zero (first day of administration) had an increase in glucose concentration (116-131.69 mg/dL) compared to the control group (62.79-77.63 mg/dL). However, these values are in the range of values considered normal for laboratory animals (Lapchik *et al*., 2017).

Histopathology examination of pancreatic cells

The size, area, diameter, and volume of the Langerhans islet of the different treatments did not show significant differences ($p > 0.05$) (Table 3), which is important since its parameters directly influence the maintenance of glucose in the blood and their alteration is related to diabetes (Jo *et al*., 2007). However, a significantly lower concentration of langerhans islets (643 cell/mm^2) was observed in the 5000 mg/kg body weight treatment with compared to the other treatments. The groups that were administered 10, 100, 1000, 1600 and 2900 mg/kg body weight of extract exhibited a concentration of cells in the Langerhans islets that varied from 2008 to 3146 cells/mm^2, without significant differences ($p > 0.05$) with the control treatment (3100 cells/mm^2). In contrast, the treatment of 5000 (643 cells / mm^2) mg/kg body weight, showed a significant decrease in the number of cells in relation to the control treatment. Consistent with these results, the pancreas cells of the group administered with 5000 mg/kg body weight of extract showed a lower number of cells (alpha, beta, and delta) in the Langerhans islets compared to the control group (Figure 2).

Antimicrobial properties of aqueous extract

The antimicrobial effects of the *Randia monantha* fruit aqueous extract against Gram (-) and Gram (+) bacteria are shown in Table 4. It can be seen that the aqueous extracts did not present dose-dependent antimicrobial activity in the test concentration range (0.5-2.0 mg/mL) against Gram (+) bacteria, nor Gram (-) test bacteria. These results were similar to those reported for the methanol and ethanol extract of *Randia aculeata* against *Mycobacterium bovis* (García-Cruz, 2018), but different from those reported for the crude extract

Table 3. Cell concentration and morphological parameters of the Langerhans islets of each of the treatments.
Tabla 3. Concentración celular y parámetros morfológicos de los islotes de Langerhans de cada uno de los tratamientos.

Groups (mg/ body weight)	Cell concentration / mm²	Central Islet perimeter (µm)	Central Islet area (µm²)	Islet diameter (µm)	Cell number
0 (Control)	3146 ± 724^b	2436 ± 552^a	255284 ± 49380^{ab}	183.3 ± 50.35^a	780 ± 81^{b,c}
10	2984 ± 296^b	2225 ± 691^a	274342 ± 11183^{ab}	233.3 ± 86.24^a	969 ± 57^{b,c}
100	2585 ± 322^b	2412 ± 277^a	348403 ± 71744^b	243.3 ± 28.91^a	885 ± 93^{b,c}
1000	2622 ± 1000^b	2279 ± 696^a	237570 ± 67146^{ab}	196.6 ± 5.77^a	621 ± 30^b
1600	3311 ± 733^b	2013 ± 156^a	230044 ± 23702^{ab}	210.0 ± 26.53^a	731 ± 88^b
2900	2008 ± 510^b	1748 ± 228^a	178307 ± 52620^a	186.7 ± 49.37^a	375 ± 158^{ab}
5000	643 ± 412^a	2054 ± 489^a	283802 ± 10467^{ab}	206.7 ± 20.81^a	174 ± 109^a

Mean ± SD, n = 3. Different letters in the same column indicate significant differences ($p < 0.05$).

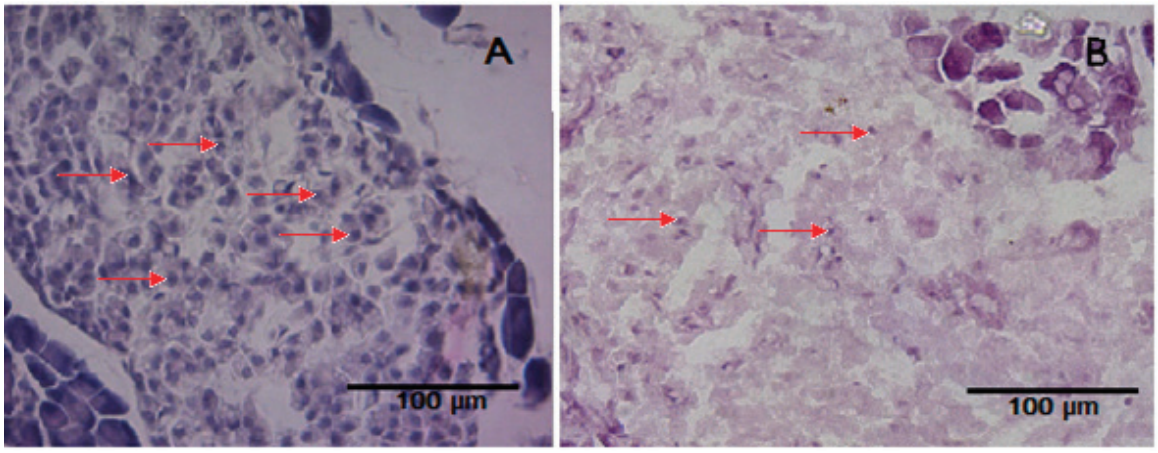

Figure 2. Pancreas histological examination. A) Control treatment and B) Treatment with a dose of 5000 mg/kg of weight. The arrows point out the Langerhans beta cells.
Figura 2. Examen histológico del pancreas. A) Tratamiento de control y B) Tratamiento con dosis de 5000 mg/kg de peso. Las flechas señalan las células beta de Langerhans.

Table 4. Antibacterial activity of aqueous extract of *Randia monantha* fruit.
Tabla 4. Actividad antibacteriana del extracto acuoso de fruto de *Randia monantha*.

Pathogenic bacteria	Control	Dose (mg/mL)			
		0.5	1	1.5	2
Listeria monocytogenes	0.18 ± 0.01^{a}	0.28 ± 0.04^{b}	0.32 ± 0.05^{a,b}	0.42 ± 0.09^{b,c}	0.53 ± 0.03^{c}
Salmonella typhimurium	0.59 ± 0.02^{d}	0.36 ± 0.01^{a}	0.41 ± 0.01^{b}	0.44 ± 0.03^{bc}	0.47 ± 0.01^{cd}
Staphylococcus aureus	0.33 ± 0.05^{b}	0.24 ± 0.05^{a}	0.23 ± 0.03^{a}	0.37 ± 0.05^{b}	0.41 ± 0.03^{b}
Escherichia coli	0.21 ± 0.04^{a}	0.30 ± 0.04^{b}	0.31 ± 0.05^{b}	0.39 ± 0.03^{b,c}	0.46 ± 0.03^{c}

Mean ± SD, n = 3. Different letters in the same column indicate significant differences ($p < 0.05$).

of *Randia dumetorum*, which exhibited significant antimicrobial activity (Kumar *et al*., 2006).

DISCUSSION

In accordance with the OECD criteria under the regulation established by the Globally Harmonized System (GHS) and classification labeling for chemical substances and mixtures, substances with LD_{50} > 5000 mg/kg body weight are category 5 or classified as unclassified (OECD, 2001), suggesting that the concentrations tested in this study do not represent a toxic risk. Similarly, according to Hogde and Sterner (2005), scale compounds with LD_{50} between 5000-15000 mg/kg body weight are considered as practically non-toxic substances. The test animals showed weight gain directly proportional to the extract concentration administered, which could be due to the high concentration of sugars present in the pulp of *Randia monatha* Benth (Juarez-Trujillo *et al*., 2018). This, since the increase in weight regularly relates to alterations in the macronutrient metabolism such as carbohydrates, proteins or fat, and even to hydromineral, or intracellular and extracellular deregulation and these nutrients play an appropriate role in physiological functions (Ezeonwumelu *et al*., 2011). In turn, it has been reported that changes in the weight of the organs are an important and sensitive parameter for the detection of harmful toxicological effects that are often associated with the administration of a chemical substance toxic to the organism (Sellers *et al*., 2007). Despite the increase in body weight, the weight of pancreas, liver, kidneys, and spleen did not show a significant increase with respect to the weight of these organs in the control group. This corroborates that the concentrations tested in this study were not toxic to the organism, since an increase in the weight of the organs suggests the appearance of hypertrophy while the decrease suggests atrophy or necrosis in the target organ (Teo *et al*., 2002).

Hematological parameters are perceptual markers of physiological changes in the health and toxicological status of test animals. The hematological profile suggested no changes in the count of red blood cells, leukocytes, and hemoglobin after the 0, 7, and 15 days of testing compared to the control treatment, suggesting that the aqueous extract has no influence on the hematopoiesis pathway. These results also demonstrated no significant changes in the lipid profile values in the range of tested concentrations, suggesting that this extract does not influence the lipid profile. In turn, the blood test revealed a significant increase in glucose concentration, which is possible since the *Randia monantha* pulp is rich in reducing sugars and total carbohydrates, which explain the increase in blood glucose and changes in the co-

loration of Langerhans cells. However, it has been reported that the soluble melanins and antioxidants present in *Randia echinocarpa* are a potential resource for type II diabetes treatment (Cuevas-Juarez *et al*., 2014) and that the aqueous extract of *Randia nicotica* had satisfactory efficacy in glycemic control in diabetic rats (Alamin *et al*., 2015). It is possible that these differences between the biochemical parameters regarding the antidiabetic effect reported in other species are due to the complexity of the phytochemical compounds present in the aqueous extract.

In general, the human body has several essential organs that are vital and with specific functions, such as liver, pancreas, and kidney, each of them has different functions, either in the metabolism of intake, in the regulation of certain hormones and/or in the excretion of waste products (Umale *et al*., 2013). Therefore, to know the toxicity of a substance it is essential to know the state of these vital organs. In the histopathological study, we observed that the pancreas and liver in all treated groups showed no changes at the macroscopic and cellular level in comparison to the control. However, the macroscopic observation of kidneys from the treatment administered with high doses revealed yellow spots, and histopathology analysis exhibited a noticeable lesser blood supply in the area corresponding to the yellow spots. There are previous reports indicating that the administration of certain extracts can cause hyperemia, inflammation, and kidney damage since this is an organ responsible for disposing of chemical compounds and is more sensitive to damage by xenobiotic compounds (Saleen *et al*., 2017; Meilian *et al.,* 2019).

On other hand, oxidative stress, inflammation, and apoptosis have been reported to be responsible for the degeneration of pancreatic islets, which could negatively affect circulating insulin level and result in a persistent hyperglycemia (Nna *et al*., 2018). All this is a critical parameter to consider in the use of *Randia monantha* Benth extract, since beta cells are responsible for insulin secretion (Levetan, 2010). Thus, our results suggest that at these concentrations, the oral intake of this extract could have a negative health effect, which is a critical factor in the intake of this fruit, considering that people consume it in excess among other things as a remedy for diabetes.

In turn, the aqueous extract did not have an antimicrobial effect against the test pathogenic bacteria, possibly due to the high content of sugars present in the sample, which are a good source of carbon for the bacteria. However, it is necessary to consider other factors such as the concentration of the extract and the incubation time that affect the bacterial effect of the extracts (Othmen *et al.,* 2020).

CONCLUSIONS

According to the hematological parameters obtained in this study, the administration of the *R. monantha* extract in the range of 10-5000 mg/kg body weight concentrations, does not present acute toxicity considering that there was no death in the different experimental groups, with LD_{50} greater than the administered dosis, corresponding to class 5 according to the OECD without toxicity. In general, the experimental organisms did not present significant differences in the hematological analyzes with most of the treatments, and all were within the range considered normal. This was consistent with the microscopic analysis in which doses greater than 2900 mg/kg body weight, caused damage to the pancreas, presenting a decrease mainly in beta cells in the Langerhans islets, in addition to generating alteration in blood supply to the kidneys. According to the LD_{50} and the absence of mortality, the aqueous extract administered orally may indicate that it can be used with some safety degree for diabetes treatment. The microbiological analysis revealed that the extract does not have antimicrobial activity against some pathogenic bacteria and, on the contrary, favors their growth. Therefore, further studies are needed to establish a safe protocol for human clinical trials.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors thank the Veracruzano Council for Scientific Research and Technological Development (COVEICYDET) for their support for the realization of this project.

REFERENCES

Alamin, M.A., Yagi, A.I. and Yagi, S.M. 2015. Evaluaticon of antidiabetic activity of plant used in Waster Sudan. Asian Pacific Journal of Tropical Biomedicine. 5(5): 395-402.

Balouri M., Sadiki M. and Koraichi I.S. 2016. Methods for *in vitro* evaluation antimicrobial activity: a review. Journal of Pharmaceutical Analysis. 6: 71-79.

Cano-Campos M., Díaz S., Uribe M., López G., Montes J., Paredes O. and Delgado F. 2011. Bio-guied fractionation of the antimutagenic activity of methanolic extrac from the fruit of *Randia echinocarpa* (Sessé et Mociño) against 1-nitropyrene. Food Research International. 44: 2087-3093.

Cavanaugh, B. 2003. Nurse's Manual of Laboratory and Diagnostic Tests. FA Davis Company, Philadelphia.

Coggeshall, E. 1992. A consideration of neural counting methods. Agosto 2019, de Biomedical Institute.

Dantas, J.A., Ambiel, C.R., Cuman, R.K.N., Baroni1, S. and Bersani-Amado, C.A. 2006. Valores de referência de alguns parâmetros fisiológicos de ratos do biotério Central da Universidade Estadual de Maringá, Estado do Paraná. Acta Scientiarum Health Science. 28: 165-170.

Ezeonwumelu, J., Julius, A., Muhoho, C., Ajayi, A., Oyewale, A.and Tanayen, J. 2011. Biochemical and histological studies of aqueous extract of *Bidens pilosa* leaves from Ugandan Rift Valley in rats. British Journal of Pharmacology and Toxicology. 2: 302-209.

Gallardo-Casas, Guevara-Balcázar, G., Morales-Ramos, E., Tadeo-Jiménez Y., Gutiérrez Flores, O., Jiménez-Sánchez, N., Valadez-Omaña, M.T. and Castillo-Hernández, M.C. 2012. Ethnobotanic study of *Randia aculeata* (Rubiaceae) in Jamapa, Veracruz, Mexico, and its anti-snake venom effects on mouse tissue. El Diario de Animales Venenosos y las Toxinas Incluyendo Enfermedades Tropicales. 3(18): 287-294.

García-Cruz, N. 2018. Obtaining extracts from the fruit of "crucetillo" (*Randia aculeate*) and evaluating its effects on the growth of *Microbacterium bovis*. Thesis, Universidad Autónoma de México.

Hodge, A. and Sterner, B. 2005. Toxicity Classes. In: Canadian Center for Occupational Health and Safety. http://www.ccohs.ca/oshanswers/chemicals/id50.htm

Jo, J., Choi, M.Y. and Koh, D.S. 2007. Size distribution of mause Langerhans islets. Biophysical Journal. 893: 2655-2666.

Juárez-Trujillo, N., Monribot, J., Alvarado, M., Luna, G. and Jiménez M. 2018. Phenolic profile and antioxidative properties of pulp and seeds of *Randia monantha* Benth. Industrial Crops and Products. 24: 53-58.

Kandimalla, R., Kalita, S., Saikia, B., Choudhury, B., Singh, Y., Kalita, K., Dash, S. and Kotoky, J. 2016. Antioxidant and hepatoprotective potentiality of *Randia dumetorum* Lam. leaf and bark via inhibition of oxidative stress and inflammatory cytokines. Frontiers in Pharmacology. 7: 205.

Kumar, V.P., Chauhan, N.S., Padh, H. and Rajani, M. 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology. 107(2): 182-188.

Lapchik, V.B.V., Mattaraia, V.G.M. and Ko, G.M. 2017. Cuidados e Manejo de Animais de Laboratório, 2a. ed. Atheneu.

Lapikanon, P., Tovivich, P., Woo, W.S. and Choi, J.S. 1983. Phytochemical study on *Randia siamensis*. Archives of Pharmacal Research. 6(1): 29–33.

Levetan, C. 2010. Distinctions between islet neogenesis and b-cell replication: Implications for reversal of Type 1 and 2 diabetes. Journal of Diabetes. 2: 76–84.

Meilian, Y., Zihuan, W., Yudan, W., Guoyin, K., Guy, S. and Shengbao, C. 2019. Acute and subacute toxicity evaluation of ethanol extract from aerial parts of *Epigynum auritum* in mice. Food Chemistry and Toxicology. 131: 110534

Méndez, L.M. and Hernández, M.R. 2009. Evaluación de la toxicidad del fruto de *Randia monantha* Benth. Revista Medica de la Universidad Veracruzana. 9(S1): 42–45.

Monammadi, J. and Naik, P.R. 2012. The histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats. Turkish Journal of Biology. 36: 211-216.

Nagamma, T., Konuri, A., Nayak, C.D., Kamath, S.U., Udupa, P.E.G. and Nayak, Y. 2019. Dose-dependent effects of fenugreek seed extract on the biochemical and haematological parameters in high-fat diet-fed rats. Journal of Taibah University Medical Sciences. 14, (4): 383-389.

Nna, V.U., Bakar, A.B., Md Lazin, M.R.M.L.and Mohamed, M. 2018. Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin–induced diabetic rats. Food Chemistry and Toxicology. 120: 305-320.

OECD. 2001. "Guidelines for the testing of chemicals / section 4: Health effects test no. 423: Acute oral toxicity - Acute toxic class method," Organization for Economic Cooperation and Development, Paris, France.

Othmen, K.B., Elfalleh, W. García-Beltrán, J.M., Esteban, M.A.and Haddad, M. 2020. An in vitro of the effect of carob (*ceratonia siliqua* L.) leaf extracto on gilthead seabream (*Sparus aurata* L.) leucocyte activities. Antioxidant, cytotoxic and bactericidal properties. Fish and Shellfish Immunology. 99: 35-43.

Pérez, S. 2017. Toxicidad por administración contínua (90 días) del extracto clorofórmico de *Calea urticifolia* (juanislama) en ratones de laboratorio. (Tesis de licenciatura). Universidad del Salvador.

Saleen, U., Amin, S., Ahmad, B., Azeem, H., Anwar, F. and Mary, S. 2017. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. Roots in albino mice as per OECD 425 TG. Toxicology Reports. 4: 580-585.

Sellers, R.S., Morton, D., Michael, B., Roome, N., Johnson, J.K., Yano, B.L., Perry, R. and Schafer, K. 2007. Society of toxicologic pathology position paper: organ weight recommendations for toxicology studies. Toxicology Pathology. 35: 751–755.

Tasić, T., Lozić, M., Glumac, S., Stanković, M., Milovanovich, I., Djordjevich, D.M., Trbovich, A.M., Japundžić-Žigon, N. and De Luka, S.R. 2021. Static magnetic field on behavior, hematological parameters and organ damage in spontaneously hypertensive rats. Ecotoxicology and Environmental Safety. 207: 111085.

Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetani, V. 2002. A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Spraguee Dawley rats. Toxicology Journal. 179 (3): 183-196.

Umale, S., Deck, C., Bourdet, N., Dhumane, P., Soler, L., Marescaux, J. and Willinger, R. 2013. Experimental mechanical characterization of abdominal organs: liver, kidney & spleen. Journal of Mechanical Behavior of Biomedical Materals. 17: 22–33.