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ANIMAL SCIENCE

Toxicological, biochemical and morphophysiological effects of *Serjania erecta* leaf aqueous extract on *Piaractus mesopotamicus*

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Abstract: This study was carried out to determine the toxicity and biochemical and morphophysiological changes caused by *Serjania erecta* leaf aqueous extract in pacu (*Piaractus mesopotamicus*). For acute toxicity testing ($CL_{50\text{-}4h}$), pacu juveniles were exposed during 4 h to *Serjania erecta* aqueous extract concentrations of 2.5, 12.5, 25, 50, 100, and 150 µg mL^{-1}, which were added directly to the water in the tanks. In the control group, the animals were kept in water free from aqueous extract. $CL_{50\text{-}4\ h}$ was estimated at 57.43 µg mL^{-1}. After exposure to the aqueous extract, the highest ($P<0.05$) glucose concentration and the lowest ($P<0.05$) plasma sodium level were when the fish were exposed to the *S. erecta* concentration of 50 µg mL^{-1}. Mortality occurred at *S. erecta* extract levels higher than 50 µg mL^{-1}, and all fish died at concentrations greater than 100 µg mL^{-1}. In addition, exposure to this extract caused severe histological changes in the gills and liver with higher prevalence of necrosis (30.2%), and fatty degeneration (77.4%) respectively. At the concentrations tested here, *S. erecta* aqueous extract causes morphofunctional alterations in this fish species.

Key words: fish, medicinal plant, morphophysiology, pacu, saponins.

INTRODUCTION

Piaractus mesopotamicus, commonly known as "pacu", is a fish of the family Serrasalmidae instead of Characidae (Holmberg 1887). It is a species of great importance to South American countries along the Paraná river basin such as Paraguay, Uruguay, Argentina, and Brazil (Urbinati & Gonçalves 2013). In the year 2017, pacu was one of the main native fish species farmed in Brazil (IBGE 2017).

There has been a growing interest in the effects of plant-derived bioactive compounds, or even medicinal plants, under the purpose of evaluating new natural products with potential for use in aquaculture; e.g. anesthetics, antiparasitic drugs, and antimicrobials (Kavitha et al. 2012, Adesina et al. 2013, Abalaka et al. 2015a, Andrade et al. 2016). This is a consequence of the expansion of this activity and the quest for achieving sustainable production through the use of biodegradable products, which cause the least impact on the environment and also on the health of consumers (Soares & Tavares Dias 2013).

Serjania erecta Radlk. (Sapindaceae), a native Brazilian plant popularly known as "cinco folhas" ("five leaves") or "cipó-cinco-folhas" ("five-leaf vine") (Guarim Neto et al. 2000, Pott et al. 2004), is spread mainly across tropical

regions such as the *cerrado* biome area (Ferrucci 2004). Studies have shown that different species of the genus *Serjania* have antimicrobial (Lima et al. 2006, Cardoso et al. 2013), antiparasitic (De Mesquita et al. 2007, Hernández et al. 2012), antiinflamatory (Gomig et al. 2008), and antioxidant effect (Heredia-Vieira et al. 2015). The toxicological and environmental properties of a plant or compound should be investigated, since the fact that they are natural does not necessarily mean they are safer (Duke 1990). In this regard, studies on acute toxicity help in assessing the risk and determining safe doses (Syngai et al. 2016).

Researchers have examined the toxicity of different species of the genus *Serjania* sp. [e.g. *Serjania marginata* (Périco et al. 2015, Moreira et al. 2019), *Serjania caracasana* (Silva et al. 2017), and *S. erecta* (Castelo et al. 2009, Broggini et al. 2010)] in mice. However, no studies have evaluated the toxicity of *S. erecta* in aquatic organisms. The present study was thus undertaken to evaluate the $CL_{50\text{-}4h}$ acute toxicity and possible biochemical and morphophysiological implications caused by *S. erecta* aqueous extract in pacu (*P. mesopotamicus*).

MATERIALS AND METHODS

Plant material, preparation, and chemical composition of *S. erecta* aqueous extract

The *S. erecta* leaves were collected in the municipality of Aquidauana - MS, Brazil (20° 27” 56.4’ S; 55° 47” 53.2’ W) and identified by Pott et al. (2004). A voucher specimen (HMS 8355) was deposited in the Herbarium of Embrapa Cattle in the state of Mato Grosso do Sul, Brazil.

The aqueous extract was obtained by the method described by Broggini et al. (2010), with adaptations. Leaves were dried in a forced-air circulation incubator with microprocessor and air renewal (Sterilifer - SXCR 40) at 37 ± 2 °C for 48 h and ground through a Wiley mill (TE-680-Tecnal) with a 10-mesh screen. The aqueous extract was obtained by maceration, with 1,000 mL of distilled water used for each 50.0 g of dried and ground plant material. The plant was kept in contact with distilled water for 24 h at room temperature (25 ± 2 °C). Next, the extract was filtered through filter paper and lyophilized for complete water removal.

An extract solution (concentration of 1000 μg mL^{-1} in distilled water) was analyzed for flavonoid content by employing the method described by Lin & Tang (2007), and the result was expressed in milligrams of quercetin equivalents per gram of lyophilized extract. The phenolic compounds were assayed with the same samples used in the quantification of flavonoids, by applying the method described by Lin & Tang (2007)—results were expressed in milligrams of gallic acid equivalents per gram of lyophilized extract. The tannins were quantified using the vanillin reaction, following the method proposed by Broadhurst & Jones (1978) and adapted by Agostini-Costa et al. (1999)—results were expressed in milligrams of catechin equivalents per gram of lyophilized extract. All chemical composition assays were carried out in triplicate.

The presence of saponins in the aqueous extract was determined by qualitative techniques, by applying the foam test in accordance with the methods proposed by the Brazilian Pharmacopeia (2010).

Acute toxicity testing ($CL_{50\text{-}4h}$)

Seventy pacu (*P. mesopotamicus*) juveniles were acquired from a commercial fish farm located in Terenos - MS, Brazil (20°26’1.62”S; 55°17’10.87”W). The animals were kept in tanks with oxygenation at the Experimental Fish Farming Station at the Federal University of Mato Grosso do Sul (20°29’59.04”S; 54°36’52.59”W).

Animal procedures were approved by the Ethic Committee on Animal Use of Federal University of Mato Grosso do Sul (CEUA/UFMS/976/2018).

For the acute toxicity test, the pacu juveniles (average weight 38.68 ± 3.00 g; average length: 13.34 ± 1.0 cm) were randomly distributed, from the same initial lot of fish into 50-L tanks. The fish—10 per experimental unit—were deprived of feed for 48 hours and kept in a static system with constant aeration, as recommended by OECD (1992). The fish were exposed to the following treatments: control (without *S. erecta* extract addition), containing only water from the growing tank; and different concentrations (2.5, 12.5, 25.0, 50.0, 100.0, and 150.0 µg mL^{-1}) of the extract, which were added directly to the tanks, per 4-hour exposure period. After the period of acute exposure to *S.erecta* extract five fish per treatment were used for morphophysiological analyzes.

Physicochemical parameters of water and behavioral alterations

Throughout the experimental period of four hours, the water quality parameters were measured with a YsiLife Sciences® multiparameter instrument and the toxic ammonia level was measured using the Alfakit® colorimetric reagent. The following mean values were obtained: dissolved oxygen = 5.0 ± 0.75 mg L^{-1}; pH = 7.68 ± 0.13; temperature = 27.46 ± 0.14 °C; ammonia = 0.07 ± 0.03 mg L^{-1}; and electric conductivity = 144.73 ± 10.75 µS cm^{-1}. These values are within the recommended range for tropical fish farming according to Boyd (1998).

The following behavioral alterations were observed: erratic swimming, loss of equilibrium, excessive mucus production, aerial respiration and lethargy. These observations were recorded every 30 minutes during the four hours period in which the fish were exposed to the concentrations of the aqueous extract of *S. erecta*. Dead fish were removed at each observation to avoid degradation of water quality parameters.

Biochemical analyses

After the period of acute exposure, five animals per concentration were anesthetized with eugenol (75 mg L^{-1}) and subjected to caudal vessel puncture using 10% EDTA-treated syringes for blood collection. After blood collection, the fish were sacrificed by a quick brain concussion, followed by biometry and necropsy for collection of organs for histological analysis. The animals that survived the different treatments were transferred to clean water and kept in the fish farming sector. The blood was centrifuged at 5000 g for 5 min to obtain the plasma (Fanem Centrimicro, Brazil), which was then stored at – Brazil (Winkaler et al. 2007). Subsequently, total plasma protein, albumin, globulin, chlorine (Cl^{-}), total calcium, and ionized calcium were measured by spectrophotometry (1203 UV, Shimadzu, Japan), using a commercial kit (Labtest®). The sodium (Na^{+}) and potassium (K^{+}) ions were measured by flame photometry (910-M Analyzer) in accordance with the method described by Catani & Paiva Neto (1949). The plasma glucose concentration was determined in each animal, immediately after blood collection, using an Accu check® Advantage portable glucose meter (Roche Diagnóstica, Brazil).

Histological analysis

The fish sacrificed by a quick brain concussion, followed by a detailed macroscopic examination, necropsy, and observation of the cavity. The gills and the liver were dissected and weighed for morphological analysis. The following formula was used to determine the hepatosomatic index: HSI = LW/TW.100, where HSI = hepatosomatic index; LW = liver weight; and TW = total fish weight, according to Vazzoler (1996).

The second left branchial arch and a liver fragment were harvested from three fish per concentration and fixed in 10% formaldehyde buffered with monobasic and dibasic sodium phosphate at a pH of 6.9. The samples were dried in progressive alcohol series, cleared in xylol, and paraffin-embedded, as suggested by Steckert et al. (2018). Subsequently, they were sectioned to 5 μm and stained with Harris' hematoxylin and eosin (HH&E) using a PAT-MR10 microscope (The Pathologist®, Brazil), in accordance with the method of Howard et al. (2004), mounted on Entellan® permanent slides, analyzed, and photographed by the DIC (Differential Interference Contrast) technique using an Axio Imager A2 light microscope (Zeiss®, Germany).

In addition to qualitative description, histological alterations in the gills and liver were assessed using the semi-quantitative method proposed by Steckert et al. (2018), which was adapted to a growing scale of mean alteration values (MAV), according to the degree of lesion severity, namely, 0 (no alterations), 1 (slight alterations or focal process), 2 (moderate alterations or multifocal process), and 3 (serious alterations or diffuse process). Based on this scale, a mean histological alteration value (MAV) was assigned for each lesion, ranging between mild (0.1 to 1.0), moderate (1.1 to 2.0), and intense (2.1-3.0). The prevalence of each lesion was also calculated in accordance with Steckert et al. (2018).

To classify the gill and liver-tissue injuries, a longitudinal section was made in the secondary lamellae and central lobe of the liver, using medium portions of the respective organs. Observations were made in a field covered by a 10x magnifying lens. The following tissue alterations were considered, for the gills: necrosis, vessel and filament dilatation, telangiectasia, epithelial hyperplasia, epithelial displacement, eosinophilic granular inflammatory infiltrate, interlamellar hyperplasia, cellular edema, and lamellar fusion and congestion; and for the liver: fatty degeneration, vacuolization, sinusoidal and vessel and congestion, necrosis, hepatocyte hypertrophy, leukocyte infiltrate, pyknotic nucleus, cellular peripheral nucleus, accumulation of hemosiderin pigments, cord disruption, and melanomacrophage centers.

Statistical analysis

The $CL_{50\text{-}4h}$ value was calculated by the Trimmed Spearman-Karber method (Hamilton et al. 1978), with a confidence interval of 95%. All data were subjected to the Shapiro-Wilk and Levene tests to check for normality and variance homoscedasticity assumptions, respectively. The data which did not show homogeneity of variance were Log_2 (x+1) converted. If the assumptions were met, the data were subjected to single-factor ANOVA and means were separated by Tukey's test. All tests were performed at the 5% significance level, using Statistica 10.0 software. The means were expressed as standard deviation.

RESULTS

Chemical composition of *S. erecta* leaf aqueous extract

Qualitative phytochemical analysis indicated presence of saponins. Quantitative analyses revealed the following amounts of phenolic compounds, flavonoids, and tannins: 386.78 ± 1.6 mg g^{-1}, 212.03 ± 1.2 mg g^{-1}, and 89.7 ± 0.3 mg g^{-1}, respectively.

Acute toxicity and behavioral alteration in *Piaractus mesopotamicus* exposed to *S. erecta* extract

Behavioral alterations such as erratic swimming, loss of equilibrium, excessive mucus production,

aerial respiration, and lethargy were observed in the animals exposed to the concentrations of 25, 50, 100, and 150 μg mL^{-1}. Additionally, mortality occurred at *S. erecta* extract levels higher than 50 μg mL^{-1}, and all fish died at concentrations greater than 100 μg mL^{-1} (Table I).

The concentrations of 100 and 150 μg mL^{-1} caused 100% mortality in the fish within 4 h of exposure. Mortalities occurred at 175 and 158 minutes after exposure to the aqueous extract of *S. erecta*, respectively. At the concentration of 50 μg mL^{-1}, 30% mortality was observed with the occurrence of the first mortality at 200 minutes of exposure to the aqueous extract *S. erecta*.

According to the results, the concentration capable of causing mortality in 50% of pacu juveniles in four hours of exposure to the *S. erecta* aqueous extract was 57.43 μg mL^{-1}, with a lower limit of 46.98 μg mL^{-1} and an upper limit of 70.21 μg mL^{-1}.

Biochemical parameters of *Piaractus mesopotamicus* exposed to S. erecta extract

It was not possible to collect blood from fish at concentrations of 100 and 150 μg mL^{-1} for biochemical analysis because there was 100% mortality before completing 4 hours of exposure to *S. erecta* extract.

Plasma total protein, albumin, and globulin levels and the ionic parameters chlorine (Cl^-), potassium (K^+), sodium (Na^+), total calcium, and ionized calcium did not differ from control group (Table II). The lowest ($P <0.05$) glucose level was obtained in fish submitted to the concentration of 2.5 μg mL^{-1} of *S. erecta*. The lowest ($P <0.05$) plasma sodium level was obtained in fish submitted to concentration of 50 μg mL^{-1} of *S. erecta* (Table II).

Organ morphometry of *Piaractus mesopotamicus* exposed to *S. erecta* extract

Fish weight and length did not differ significantly between the treatments. However, higher ($P<0.05$) liver weight and hepatosomatic index were obtained in the treatment with the extract concentration of 100 μg mL^{-1} compared to control and the other concentrations (except 25 and 50 μg mL^{-1}). Heavier gills ($P<0.05$) were observed in the fish receiving concentrations greater than 12.5 μg mL^{-1} (Table III).

Table I. **Mortality and behavioral alterations observed in *Piaractus mesopotamicus* (n=10) exposed to *Serjania erecta* aqueous extract for 4 h.**

***S. erecta* extract concentration (μg ml^{-1})**	**Percentage of dead animals**	**Erratic swimming**	**Loss of equilibrium**	**Mucus**	**Aerial respiration**	**Lethargy**	**Death**
0	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-
25	-	+ (n=3)[1]	+ (n=2)	+ (n=3)	+ (n=1)	+ (n=2)	-
50	30	++ (n=4)	+ (n=3)	+ (n=3)	++ (n=2)	+ (n=3)	+ (n=3)
100	100	++ (n=7)	++ (n=8)	++ (n=4)	++ (n=5)	++ (n=6)	+ (n=10)
150	100	++ (n=9)	++ (n=10)	++ (n=8)	++ (n=6)	++ (n=8)	+ (n=10)

Behavioral alteration observed: + <60 min and ++ > 60 min.
[1]Number of individuals engaging into the various behavioral alterations.

Table II. Plasma biochemical parameters in *Piaractus mesopotamicus* exposed to different concentrations of *Serjania erecta* aqueous extract for 4 h.

Biochemical parameter	*S. erecta* aqueous extract concentration				
	0 µg mL^{-1}	2.5 µg mL^{-1}	12.5 µg mL^{-1}	25 µg mL^{-1}	50 µg mL^{-1}
Glucose (mg dL^{-1})	75.33 ± 5.03 ^{ab}	73.33 ± 11.68 ^{b}	84.00 ± 13.11 ^{ab}	131.67 ± 35.85 ^{ab}	145.33 ± 43.98 ^{a}
Total protein (mg dL^{-1})^{ns}	3.22 ± 0.18 ^{a}	3.59 ±0.32 ^{a}	3.06 ± 0.45 ^{a}	3.17 ± 0.21 ^{a}	3.18 ± 0.13 ^{a}
Albumin (g dL^{-1}) ^{ns}	1.75 ± 0.88 ^{a}	1.12 ± 0.03 ^{a}	1.08 ± 0.04 ^{a}	1.07 ± 0.08 ^{a}	1.07 ± 0.05 ^{a}
Globulin (g dL^{-1}) ^{ns}	2.19 ± 1.06 ^{a}	2.83 ± 0.35 ^{a}	1.51 ± 0.44 ^{a}	2.2 ± 0.13 ^{a}	2.21 ± 0.10 ^{a}
Chlorine (mE dL^{-1}) ^{ns}	41.51 ± 7.27 ^{a}	46.18 ± 5.96 ^{a}	45.02 ± 3.32 ^{a}	48.05 ± 4.42 ^{a}	50.65 ± 3.89 ^{a}
Calcium (mg dL^{-1}) ^{ns}	9.47 ± 2.88 ^{a}	7.03 ± 2.28 ^{a}	7.11 ± 1.84 ^{a}	6.55 ± 0.32 ^{a}	7.64 ± 1.20 ^{a}
Ionized calcium (mg dL^{-1}) ^{ns}	62.33 ± 17.29 ^{a}	47.66 ± 13.7 ^{a}	48.07±11.11 ^{a}	44.73 ± 1.96 ^{a}	51.26 ± 7.24 ^{a}
Sodium (mg mL^{-1})	3.73 ± 0.23 ^{ab}	4.53 ± 0.06 ^{a}	4.17 ± 0.58 ^{ab}	4.05 ± 0.15 ^{ab}	3.37 ± 0.48 ^{b}
Potassium (mg mL^{-1}) ^{ns}	0.28 ± 0.18 ^{a}	0.22 ± 0.08 ^{a}	0.27 ± 0.03 ^{a}	0.28 ± 0.03 ^{a}	0.35 ± 0.05 ^{a}

Mean ± standard deviation, different letters indicate significant differences between the treatments using one-way ANOVA and Tukey's test (P <0.05). ^{ns} Not significant.

Table III. Morphometric parameters of liver and gills of *Piaractus mesopotamicus* (n=5) exposed to different concentrations of *Serjania erecta* aqueous extract for 4 h.

Parameter	*S. erecta* aqueous extract concentration						
	0 µg mL^{-1}	2.5 µg mL^{-1}	12.5 µg mL^{-1}	25 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	150 µg mL^{-1}
Weight (g) ^{ns}	40.60 ± 6.05 ^{a}	36.13 ± 4.29 ^{a}	39.46 ± 1.72 ^{a}	43.40 ± 3.36 ^{a}	39.0 ± 6.20 ^{a}	38.06 ± 1.90 ^{a}	34.13 ± 6.11 ^{a}
Length (cm) ^{ns}	10.67 ± 1.15 ^{a}	9.83 ± 1.26 ^{a}	11.83 ±0.29 ^{a}	11.67 ±0.58 ^{a}	11.67 ± 1.26 ^{a}	11.67 ± 0.29 ^{a}	12.00 ± 0.50 ^{a}
*HSI	1.52 ± 0.34 ^{b}	1.67 ± 0.5 ^{b}	1.39 ± 0.23 ^{b}	2.51 ± 0.12 ^{ab}	2.63 ± 0.48 ^{ab}	4.46 ± 0.71 ^{a}	2.58 ± 1.88 ^{b}
Gills (g)	1.54 ± 0.16 ^{bc}	1.29 ± 0.15 ^{c}	1.88 ± 0.1 ^{abc}	2.29 ± 0.18 ^{abc}	2.2 ± 0.46 ^{abc}	2.89 ± 0.61 ^{a}	2.46 ± 0.51 ^{ab}

Mean ± standard deviation, different letters indicate significant differences between the treatments using one-way ANOVA and Tukey's test (P <0.05). *HSI - hepatosomatic index. ^{ns} Not significant.

Histopathology in *Piaractus mesopotamicus* exposed to *S. erecta* extract

The following gill alterations were observed: necrosis, dilation of vessels and filaments, telangiectasia, epithelial hyperplasia, epithelial displacement, eosinophilic granular inflammatory infiltrate, interlamellar hyperplasia, cellular edema, lamellar fusion, and congestion (Figure 1). The most prevalent of those was necrosis (30.2%), whereas the least frequent was congestion (1.6%). The mean histological alteration value (MAV) of necrosis was higher (P<0.05) only at the extract concentration of 150 µg mL^{-1}, whereas the MAV of eosinophilic granular inflammatory infiltrate increased (P<0.05) from 50 µg mL^{-1}. The MAV of epithelial hyperplasia was higher at the concentration of 12.5 µg mL^{-1}. The other alterations did not differ between the treatments (Table IV).

The following hepatic alterations were observed: fatty degeneration, vacuolization, sinusoidal and vessel congestion, necrosis, hepatocyte hypertrophy, leukocyte infiltrate, pyknotic nucleus, peripheral cellular nucleus,

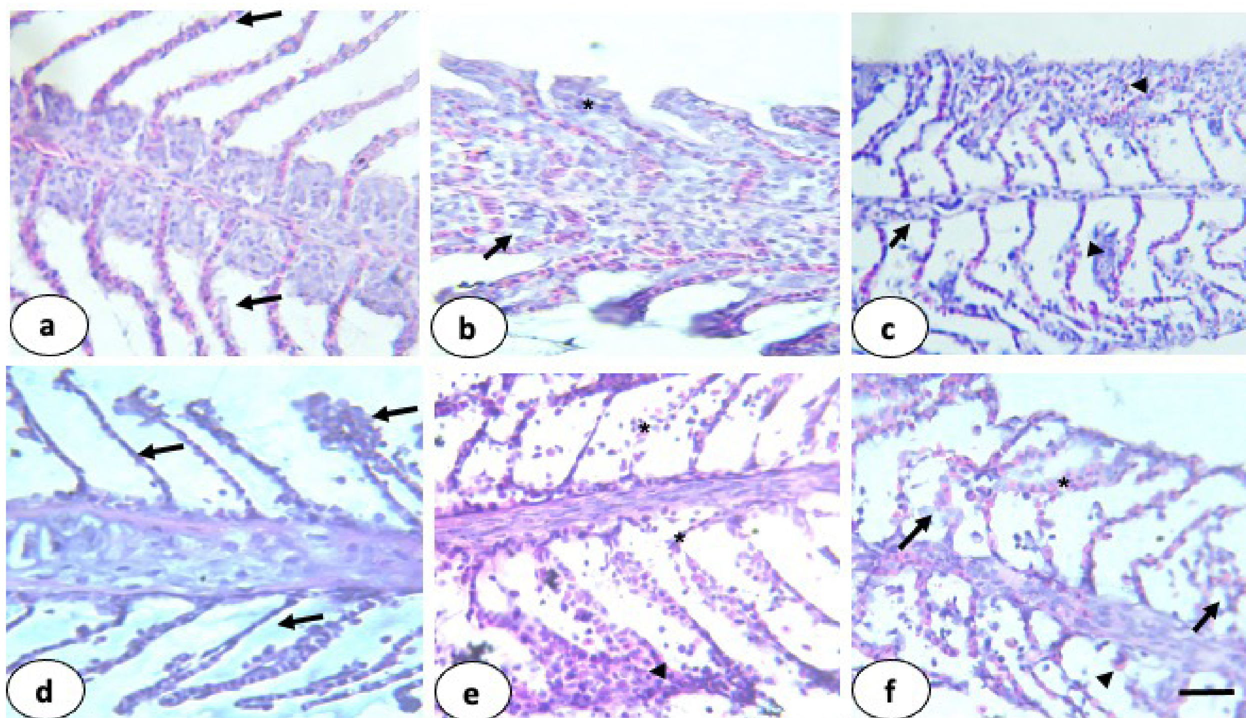

Figure 1. Gills of *Piaractus mesopotamicus* exposed to *Serjania erecta* extract. a = epithelial displacement (2.5 µg mL^{-1}); b = telangiectasia, lamellar fusion (12.5 µg mL^{-1}); c = dilatation of vessels and filaments, interlamellar hyperplasia (25 µg mL^{-1}); d = necrosis (50 µg L^{-1}); e = hemorrhage, necrosis (100 µg mL^{-1}); f = leukocyte infiltrate, hemorrhage, necrosis (150 µg mL^{-1}). Hematoxylin Eosin - 400x. 10-µm scale.

accumulation of hemosiderin pigments, cord structure disruption, and melanomacrophage centers (Figure 2). The most prevalent of these conditions was fatty degeneration (77.4%), and the least prevalent was melanomacrophage (14.3%). The MAV of fatty degeneration and necrosis (P<0.05) from the aqueous extract concentration of 12.5 µg mL^{-1}, whereas nuclear deformity increased (P<0.05) only at levels equal to or higher than 50 µg mL^{-1} and sinusoidal and vessel congestion prevalence increased (P<0.05) after 100 µg mL^{-1}. Alterations in vacuolization, hepatocyte hypertrophy, leukocyte infiltrate, pyknotic nucleus, and cord structure disruption were greater (P<0.05) than in control group only for the extract concentrations of 100.0, 25.0, 12.5, 25.0, and 50 µg mL^{-1}, respectively (Table V).

DISCUSSION

The species of the family Sapindaceae are rich in secondary metabolites such as phenolic compounds, flavonoids, saponins, tannins, triterpenes, diterpenes, isoprenoids, polyphenols, lecithins, and hydrogels (Lima et al. 2006, Gomig et al. 2008, Broggini et al. 2010, Cardoso et al. 2013, Moreira et al. 2019). In the current study, the aqueous extract of *S. erecta* leaves obtained by cold pressing for 24 h exhibited phenolic compounds, flavonoids, tannins, and saponins. Similar results were obtained by Broggini et al. (2010) in *S. erecta* leaf aqueous extract obtained with boiling distilled water and kept at rest for 24 h in the dark. The authors identified saponins, tannins, and flavonoid glycosides. Gomig et al. (2008)

Table IV. Prevalence of alterations in the gills of *Piaractus mespotamicus* exposed to concentrations of *Serjania erecta* aqueous extract.

	Prevalence	***Serjania erecta* aqueous extract concentration**						
Alteration index	(%)	0 μg mL^{-1}	2.5 μg mL^{-1}	12.5 μg mL^{-1}	25.0 μg mL^{-1}	50.0 μg mL^{-1}	100.0 μg mL^{-1}	150.0 μg mL^{-1}
Necrosis	30.2	N.D	N.D	0.03 ± 0.02 ^{a}	0.75 ± 0.37 ^{ab}	1.15 ± 0.42 ^{ab}	1.26 ± 0.40 ^{ab}	1.44 ± 1.03 ^{b}
Telangiectasia ^{ns}	22.2	0.14 ± 0.03 ^{a}	0.11 ± 0.09 ^{a}	0.16 ± 0.07 ^{a}	0.71 ± 0.05 ^{a}	0.53 ± 0.46 ^{a}	0.74 ± 0.12 ^{a}	0.53 ± 0.46 ^{a}
Dilation of vessels and filaments ^{ns}	22.2	0.08 ± 0.03 ^{a}	0.05 ± 0.04 ^{a}	0.21 ± 0.06 ^{a}	0.28 ± 0.08 ^{a}	0.26 ± 0.05 ^{a}	0.68 ± 0.04 ^{a}	0.78 ± 0.10 ^{a}
Epithelial hyperplasia	17.5	0.15 ± 0.07 ^{ab}	0.44 ± 0.22 ^{bc}	0.59 ± 0.17 ^{c}	N.D	N.D	N.D	N.D
Epithelial displacement ^{ns}	14.3	0.17 ±0.04 ^{a}	0.31 ± 0.12 ^{a}	N.D	N.D	N.D	N.D	0.53 ± 0.46 ^{a}
Eosinophilic granular inflammatory infiltrate	14.3	N.D	N.D	N.D	N.D	0.70 ± 0.06 ^{b}	0.76 ± 0.12 ^{b}	0.86 ± 0.08 ^{b}
Interlamellar hyperplasia ^{ns}	12.7	N.D	N.D	0.21 ± 0.06 ^{a}	0.65 ± 0.04 ^{a}	0.30 ± 0.02 ^{a}	0.50 ± 0.44 ^{a}	N.D
Cellular edema ^{ns}	12.7	0.23 ±0.12 ^{a}	0.11 ± 0.09 ^{a}	0.11 ± 0.09 ^{a}	N.D	N.D	0.33 ± 0.08 ^{a}	0.29 ± 0.05 ^{a}
Lamellar fusion ^{ns}	4.8	0.03 ± 0.02 ^{a}	0.10 ± 0.07 ^{a}	0.11 ± 0.09 ^{a}	N.D	N.D	N.D	N.D
Congestion ^{ns}	1.6	0.03 ±0.02 ^{a}	N.D	N.D	N.D	N.D	N.D	N.D

Mean ± standard deviation, different letters indicate significant differences between the treatments using one-way ANOVA and Tukey's test (P <0.05). N.D. (not detected). ^{ns} Not significant.

showed the presence of saponins, flavonoids, triterpenoids, steroids, tannins, and catechins in methanolic extract obtained from stems and leaves of the same plant.

Studies on behavioral and acute toxicity caused by *S. erecta* aqueous extract administered orally and intraperitoneally to mice revealed low toxicity (Broggini et al. 2010). As for the methanolic and chloroformic extracts of *S. erecta* leaves, no acute toxicological effects were observed, either, when they were administered orally to male and female mice (Castelo et al. 2009). At high concentrations, flavonoids may generate reactive oxygen species by autoxidation and redox cycle (Hodnick et al. 1986, Yoshino et al. 1999), whereas tannins have high affinity for proteins in addition to complexing to metal ions (Monteiro et al. 2005). Furthermore, they may trigger hepatotoxic activities and antinutritional effects (Chung et al. 1998).

Studies with guppy (*Poecilia reticulata*) demonstrated the toxicity of serjanoside B at the concentration of 20 μg ml^{-1} obtained from *S. lethalis*, which caused excitatory responses in the initial stage, followed by progressive hypoactivity and death after 5 h of exposure (Teixeira et al. 1984). In the present study, all fish exposed to the *S. erecta* aqueous extract concentrations of 100 and 150 μg mL^{-1} died within 4 h of exposure. Experimental intoxication by saponins in *Poecilia* sp. and *Geophagus* sp. showed that the action of saponins is developed throughout five stages, whose duration varies according to the concentration of the toxic agent, species used, and temperature (Tabarelli Neto & Bonoldi 1945). Plants of the family

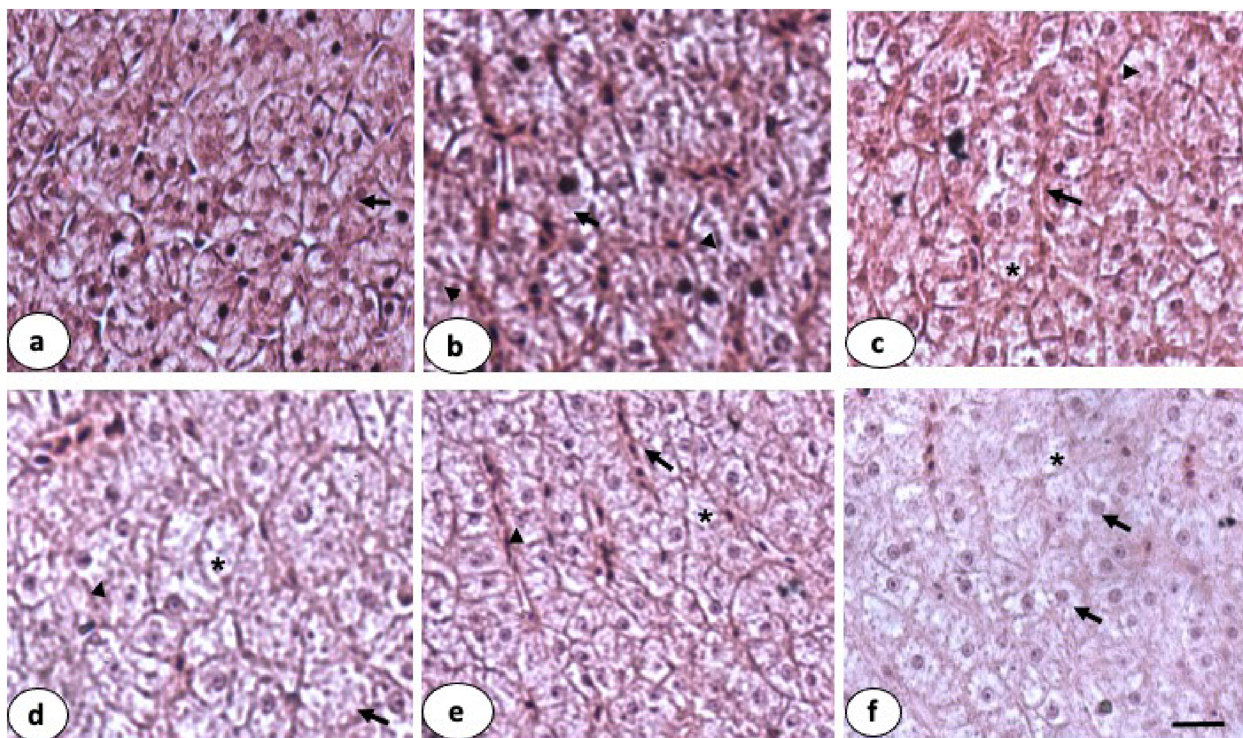

Figure 2. Liver tissue of *Piaractus mesopotamicus* exposed to *Serjania erecta* aqueous extract. a = sinusoidal congestion, peripheral cell nucleus (2.5 μg mL^{-1}); b = hepatocyte hypertrophy, fatty degeneration (12.5 μg mL^{-1}); c = leukocyte infiltrate, necrosis, vacuolization (25 μg mL^{-1}); d = fatty degeneration, loss of cell boundary, necrosis (50 μg mL^{-1}); e = sinusoidal congestion, vacuolization, inflammatory infiltrate (100 μg mL^{-1}); f = necrosis, loss of cell boundary (150 μg mL^{-1}). Hematoxylin Eosin- 400x. 10-μm scale.

Sapindaceae are characterized as abundant sources of saponins (Voutquenne et al. 2002), a compound class present in high amounts in different extracts of *S. erecta* leaves and stems (Gomig et al. 2008, Broggini et al. 2010). On this basis, it can be inferred that the toxic effects of *S. erecta* aqueous extract observed in the present animals are directly related to the concentration of extract used in the water and the presence of saponins in its composition.

Biochemical parameters are important indicators of physiological alterations in fish, and osmoregulation is characterized as an important target for toxic substances (Winkaler et al. 2007). The plasma ionic profile of the fish exposed to *S. erecta* extract did not show significant differences compared to control group, for any of the evaluated parameters. Similar results were obtained with neem (*Azadirachta indica*) leaf extract, which did not interfere with the osmoregulatory capacity of *P. lineatus* (Winkaler et al. 2007). Nevertheless, saponins have hemolytic activity and act on the cell membrane, with specific capacity to form pores and alter cell plasma membrane fluidity (Ma & Xiao 1998). In the present study, no alterations were observed in the blood variables except for glucose levels, which rose with the extract concentration, and plasma Na^{+} level, which declined as the plant extract concentration was increased. This increase in blood glucose can be seen as part of a stress response triggered by the presence of *S.erecta* extract in water. Hyperglycemia has also been found in *P. lineatus* exposed to 2.5 g L^{-1} neem leaf extract (Winkaler et al. 2007). This shows that the observed effects depend on the

Table V. Prevalence of alterations in the liver of *Piaractus mespotamicus* exposed to concentrations of *Serjania erecta* aqueous extract.

	Prevalence	***Serjania erecta* aqueous extract concentration**						
Alteration index	(%)	0 μg mL^{-1}	2.5 μg mL^{-1}	12.5 μg mL^{-1}	25.0 μg mL^{-1}	50.0 μg mL^{-1}	100.0 μg mL^{-1}	150.0 μg mL^{-1}
Fatty degeneration	77.4	0.03 ± 0.02 ^{a}	0.54 ± 0.36 ^{a}	1.77 ± 0.37 ^{b}	1.84 ± 0.77 ^{b}	2.27 ± 0.25 ^{b}	2.71 ± 0.07 ^{b}	2.73 ± 0.10 ^{b}
Vacuolization	45.2	N.D	0.29 ± 0.08 ^{ab}	0.94 ± 0.17 ^{ab}	1.06 ± 0.13 ^{ab}	0.85 ± 0.76 ^{ab}	1.44 ± 0.25 ^{b}	1.20 ± 0.60 ^{ab}
Sinusoidal and vessel congestion	42.7	N.D	0.24 ± 0.06 ^{a}	0.91 ± 0.47 ^{ab}	0.67 ± 0.38 ^{ab}	0.64 ± 0.56 ^{ab}	1.76 ± 0.54 ^{b}	1.71 ± 0.64 ^{b}
Necrosis	40.5	N.D	N.D	0.21 ± 0.19 ^{b}	0.53 ± 0.35 ^{b}	0.43 ± 0.16 ^{b}	1.35 ± 0.65 ^{c}	1.70 ± 0.69 ^{c}
Hepatocyte hypertrophy	39.3	N.D	0.44 ± 0.32 ^{ab}	0.81 ± 0.16 ^{ab}	1.55 ± 0.32 ^{b}	0.33 ± 0.17 ^{ab}	0.67 ± 0.40 ^{ab}	1.34 ± 0.78 ^{ab}
Leukocyte infiltration	32.1	N.D	0.17 ± 0.09 ^{ab}	1.23 ± 0.26 ^{b}	0.70 ± 0.22 ^{ab}	1.11 ± 0.19 ^{ab}	0.54 ± 0.17 ^{ab}	0.88 ± 0.06 ^{ab}
Pyknotic nucleus	25.0	0.10 ± 0.07 ^{a}	0.43 ± 0.04 ^{ab}	0.37 ± 0.31 ^{ab}	1.38 ± 0.70 ^{b}	N.D	0.16 ± 0.08 ^{a}	N.D
Peripheral nucleus ^{ns}	22.6	N.D	0.17 ± 0.03 ^{a}	0.31 ± 0.18 ^{a}	0.93 ± 0.24 ^{a}	0.35 ± 0.11 ^{a}	0.86 ± 0.76 ^{a}	0.16 ± 0.08 ^{a}
Nuclear deformation	22.6	N.D	0.08 ± 0.03 ^{a}	N.D	N.D	0.82 ± 0.37 ^{b}	1.09 ± 0.56 ^{b}	1.07 ± 0.12 ^{b}
Hemosiderosis ^{ns}	16.7	0.04 ± 0.02 ^{a}	N.D	0.33 ± 0.13 ^{a}	0.27 ± 0.24 ^{a}	0.17 ± 0.12 ^{a}	0.68 ± 0.19 ^{a}	0.48 ± 0.12 ^{a}
Cord structure disruption	15.5	N.D	0.03 ± 0.01 ^{a}	0.26 ± 0.24 ^{a}	N.D	1.44 ± 0.24 ^{b}	0.33 ± 0.18 ^{a}	N.D
Melanomacrophage ^{ns}	14.3	0.03 ± 0.02 ^{a}	0.09 ± 0.01 ^{a}	0.29 ± 0.16 ^{a}	0.15 ± 0.26 ^{a}	0.24 ± 0.12 ^{a}	0.33 ± 0.17 ^{a}	0.17 ± 0.09 ^{a}

Mean ± standard deviation, different letters indicate significant differences between the treatments using one-way ANOVA and Tukey's test ($P < 0.05$). N.D. (not detected). ^{ns} Not significant.

concentration of the toxic agent (Tabarelli Neto & Bonoldi 1945, Francis et al. 2002).

Researchers have related the toxicity of a plant extract to tissue alterations in gills (Temmink et al. 1989, Winkaler et al. 2007, Kumar et al. 2010, Abalaka et al. 2015a). When added to water in large amounts, saponins are highly toxic to fish due to damage caused to the respiratory epithelium of the gills, which stems from a reduction of water surface tension (Newinger 1994, Chen et al. 1996, Francis et al. 2002). In addition, they have hemolytic activity, releasing hemoglobin into the plasma, irritating the mucosae in general, and acting on the nervous system with an initial manifestation in the motor system and, later, on the sensory system (Tabarelli Neto & Bonoldi 1945).

In *Heteropneusteus fossilis* exposed to saponin, researchers observed hypertrophy of the branchial epithelium (Hemalatha & Banerjee 1997). The main mechanism of action of saponins is by reducing the surface tension between water and the gills, preventing oxygen absorption and leading the fish to a slow death from oxygen deprivation (Lamba 1970). When oxygen absorption is impeded, the animal metabolism initiates mechanisms to offset it, triggering numerous lesions which could be considered specific signs of the fish defense mechanism (Velasco-Santamaría & Cruz-Casallas 2008). However, when not controlled, this mechanism may incorporate a degenerative nature, resulting in loss of morphofunctional efficiency of the branchial structures in the exposed fish (Hemalatha & Banerjee 1997), as observed in the present study.

Epithelial desquamation and lamellar fusion occur as defensive mechanisms to reduce

the branchial surface area in contact with the aggressor toxic agent (Figueiredo-Fernandes et al. 2007). Such lesions occur whenever the osmoregulatory function of the gills is disturbed (Temmink et al. 1989) and the cell is not able to maintain homeostasis (Mcgavin & Zachary 2013). Similar lesions were reported in the gills of carp (*Cyprinus carpio*) exposed to tannins (Temmink et al. 1989) and in the gills of African sharptooth catfish (*Clarias gariepinus*) exposed to ethanolic extract from the stem-bark of *A. obesum* (Abalaka et al. 2015a). However, these alterations impair gas exchanges in the affected animals, reducing the surface area available for this process (Velasco-Santamaría & Cruz-Casallas 2008), which results in respiratory discomfort and mortality.

The difficulty capturing oxygen leads to hypoxia, which increases respiratory frequency, resulting in behavioral changes (Tiwari et al. 2011) such as the aerial respiration observed in the current study. In an effort to limit the absorption of the toxic agent by the gills, the organism coats the body surfaces by increasing mucus release (Abalaka & Auta 2010), an adaptive response that may heighten the observed behavioral alterations.

The high reactivity of tannins to proteins (Gupta & Haslam 1980) renders these compounds toxic to aquatic organisms such as fish (Temmink et al. 1989). Excessive production of mucus and lesions, which result in secondary lamellae fusion, as observed here, suggests that tannins inactivate the transport of ATPase membranes in the gill epithelium. In *C. carpio* exposed to tannins, big holes were observed in the primary gill epithelium, which might be associated with the ability to bind to hydrogen and low penetration capacity of tannins of high molecular mass (Temmink et al. 1989).

The liver is an important organ in the metabolism of potentially toxic compounds (Moreira et al. 2019). Several plant metabolites, including tannins and flavonoids found in *S. erecta* extract, may be toxic to the liver, even at low concentrations (Chung et al. 1998, Galati & O'Brien 2004, Watjen et al. 2005, Tsuji & Walle 2008). Histological analyses of the liver tissue of the animals in the present experiment demonstrated that *S. erecta* aqueous extract is potentially hepatotoxic, which is reflected in the observed congestion, vacuolization, fatty degeneration, cellular infiltrate, and necrosis. Similar lesions were found in *C. gariepinus* exposed to ethanolic extract from *A. obesum* (Abalaka et al. 2015b). According to those authors, these are pathological responses which may be induced by metabolic disorders resulting from exposure to a toxic agent.

In an *in vitro* study with liver tissue cells from *Oncorhynchus mykiss*, flavonoids influenced hepatocyte cell growth, revealing cytotoxicity to those cells (Tsuji & Walle 2008). In rats, flavonoids induced cytotoxicity and DNA strand breaks (Watjen et al. 2005). According to Galati & O'Brien (2004), flavonoids are toxic due to their pro-oxidative capacity and because they induce mitochondrial dysfunction. This fact can be explained by the presence of activities similar to peroxidase in the hepatocytes of fish, which can activate flavonoids for a toxic species, damaging the cells (Tsuji & Walle 2008). In view of this, it may be inferred that *S. erecta* aqueous extract is potentially toxic to the fish.

CONCLUSIONS

The observed results demonstrate the importance of evaluating functional and morphological responses of fish exposed to plant extracts with different purposes for aquaculture. *Serjania erecta* extract at concentrations higher than 2.5 µg mL^{-1} caused

detrimental morphofunctional alterations in the gills and liver of *P. mesopotamicus*, discouraging the use of this extract at those concentrations for phytotherapeutic purposes in aquaculture.

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