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RESEARCH ARTICLE

Deltamethrin-induced nuclear erythrocyte alteration and damage to the gills and liver of *Colossoma macropomum*

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Abstract

Deltamethrin is one of the most commonly used pyrethroids in the world, and it has a high toxic potential, mainly on aquatic organism. Thus, the purpose of this study was to evaluate LC_{50} values of deltamethrin on tambaqui (*Colossoma macropomum*) fingerlings and to investigate genotoxic effects and histopathological responses. Fish were exposed to different concentrations of deltamethrin (0, 6.16×10^{-3}; 6.44×10^{-2}; 1.34×10^{-1}, and 1.93×10^{-1} mg L^{-1}) for 96 h. In addition, a genotoxicity analysis was carried out on peripheral blood erythrocytes and histopathological changes were classified by the severity degree of damage and organ functioning. The 96 h LC_{50} value for tambaqui was estimated at 5.56×10^{-2} mg L^{-1} using a static test system. Nuclear abnormalities in exposed fish included micronuclei, blebbed, notched, 8-shaped, and binucleated nuclei forms. Deltamethrin significantly induced a notched nucleus compared to other abnormalities. A histopathological examination showed hepatic lesions and gill damage. Deltamethrin was found to be highly toxic; it induced genotoxicity and caused liver and gill inflammation in tambaqui.

Keywords Acute toxicity · Genotoxicity · Histology · Pathology · Pyrethroid · Tambaqui

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Introduction

Pyrethroids are synthetic insecticides widely used in pest control; they are less persistent in the environment compared to organochlorine, organophosphate, and carbamate (Aydin et al. 2005; Pimpão et al. 2007; Singh and Singh 2008). Among the pyrethroids, deltamethrin presents the cyan (CN) group in the phenoxybenzyl portion, which allow in the effect of the pyrethroid pesticide permethrin, your predecessor, making it an insecticide that is stable to light, moisture, and air (Verschoyle and Aldridge 1980; Nasuti et al. 2003).

Deltamethrin applications are widespread in the areas of public health, veterinary medicine, and especially in agriculture, making it one of the widely used pyrethroids globally (Santos et al. 2007; WHO, 2010). This widespread use is a factor of environmental concern because it may seriously damage water resources given its extreme toxicity to aquatic organisms (Datta and Kaviraj 2003).

In India and Spain, deltamethrin has already been reported in rivers in the range of 0.000033×10^{-3} to 0.000045×10^{-3} mg L^{-1} and 0.002×10^{-3} to 0.0588×10^{-3} mg L^{-1}, respectively (Feo et al. 2010; Robles-Molina et al. 2014; Mahboob et al. 2015), and is considered a risk factor for the species that live there. In South America, few studies have been conducted to detect the presence of these compounds in water bodies. In a study conducted in a stream in Cintra, São Paulo, Brazil, deltamethrin was detected at a concentration of 4×10^{-3} mg L^{-1} (Belluta et al. 2010); however, the Brazilian Government does not set maximum allowable limits for this pyrethroid in water.

Deltamethrin is easily absorbed through fish gills due its lipophilic characteristic (Santos et al. 2007; Al-Ghanbousi et al. 2012). Even at low concentrations (0.15×10^{-3} mg L^{-1}), it can promote behavioral changes in the zebrafish (*Danio rerio*) (Huang et al. 2014). During the intoxication process, fish may become hyperactive, show a lack of coordination, and have seizures due to a blocking of sodium channels and inhibition of the enzyme acetylcholinesterase and gamma-aminobutyric acid (GABA) receptors (Bradbury and Coats 1989; Ren et al. 2016).

Given that fish have a direct contact with the water and that deltamethrin is easily absorbed, the gill is the most quickly affected organ, and hyperplasia, edema, congestion, and lamellar fusion were verified in tilapia exposed to a concentration of 5×10^{-3} mg L^{-1} (Yildirim et al. 2006). Reactive oxygen species (ROS) are generated in the process of product metabolism, resulting in oxidative damage that can affect different macromolecules such as DNA, giving rise to genotoxic and cytotoxic effects (Parvez and Raisuddin 2005; Patel et al. 2006). These effects are also reflected in the liver, causing hydropic degeneration and blood congestion in hepatocytes, as well as the presence of micronucleus and nuclear abnormalities in erythrocytes (Ansari et al. 2009; Marques et al. 2014; Sahoo et al. 2017).

The alterations mentioned above are used as environmental biomarkers since they present fast responses to chemical products; however, there are still no reports for the tropical fish species tambaqui (*Colossoma macropomum*). The tambaqui has a high market value and is widely cultivated in Northern South American regions near agricultural crops where pyrethroids are used as insecticides (MAPA 2016; Valladão et al. 2016). The present study aimed to evaluate tissue changes in the gills, liver, and erythrocytes of tambaqui following exposure to deltamethrin.

Material and methods

Chemicals

The goal was to assess the effect of deltamethrin [(S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromvinyl)-2,2-dimethylcyclo-propancarboxylate] on tambaqui. It was tested in the form of the Decis 25 EC pesticide, the active substance of which is deltamethrin, at a concentration of 25 g L^{-1}. A stock solution of deltamethrin was prepared by dissolving Decis 25 EC (1 mL) in water (999 mL). Four predefined concentrations were inserted into different receptors for deltamethrin extraction by dispersive microextraction according to Boonchiangma et al. (2012). The analyses were performed using a liquid chromatography Shimadzu DGU-20A3 HPLC system equipped with a diode array detector and a *Shim-Pack*® C18–250 × 4.6 mm, 5 μm column connected to precolumn *Phenomenex*® C18–30 × 4 mm, 4 μm. Ultrapure water (A) and acetonitrile (B) were used as the mobile phase. The analyses were performed with a gradient orientation system, starting with 70% B for 1 min, 70–95% B for 1–2 min, 95–100% B for 2–10 min, 100–70% B for 10–15 min, returning as initial conditions and completing the analyzes. The mobile phase flow was 0.8 mL/min and the injection volume of the 20 μL samples. The analyses were performed under a temperature of 25 °C and the wavelength for a detection of compounds of 210 nm.

Test organisms

The experiment was conducted at an Aquaculture Laboratory located at Embrapa Tabuleiros Costeiros, Aracaju, Sergipe, Brazil. The fish were purchased from CODEVASF, located in the city of Porto Real do Colégio, Alagoas, Brazil. Fish were acclimated in 500 L tanks under continuous aeration with a natural photoperiod and average water temperature of 27 ± 2 °C. Fish were fed a commercial feed (Nutripiscis TR 4 mm 32% protein floor) twice daily. After this period, the fish used in the final test had an average weight of 9.15 ± 1.49 g

and an average length of 6.87 ± 1.45 cm. This study was approved by the Animal Ethics Committee (CEUA-Unit; Protocol number 030514).

Acute toxicity tests

Prior to the final test, a preliminary test lasting 96 h was conducted to determine the approximate deltamethrin harmful action range. For the final test, five deltamethrin concentrations were used (0, 6.16×10^{-3}, 6.44×10^{-2}, 1.34×10^{-1}, and 1.93×10^{-1} mg L^{-1}) with three replications and four fishes for each experimental unit. The test was conducted in 9 L containers.

Fish behavior and survival were monitored every 6 h during the first 24 h and then every 24 h until the end of the experiment at 96 h. Dissolved oxygen and temperature were recorded daily using a YSI meter (model 55-12FT), pH was measured with a pH meter (AKROM KR20), conductivity was measured using a YSI meter (model 30–10 FT), and total ammonia was measured before and at the end of the experiment by a photo colorimeter (HANNA®).

The trimmed Spearman Karber method (Hamilton et al. 1977) was used to calculate the $CL_{50-96\ h}$. After obtaining the $CL_{50-96\ h}$, data were compared with Extension Toxicology Network (1996) data for a toxicity classification. Once data were obtained, a linear regression was performed using BioEstat 5.0.

Moribund fish with minimum opercula beat, lethargy, loss of balance, and desensitization to external stimuli were removed from the treatment and then recorded as dead to calculate the $CL_{50-96h.}$ The blood from these individuals was collected by puncturing the caudal fin for a hematological analysis. They were subsequently stunned and euthanized by a spinal section for the collection of gills and liver for a histological analysis. Samples were then fixed in 10% formalin for 48 h.

Micronucleus and nuclear anomaly analyses

For micronucleus and nuclear abnormalities analyses, a blood smear of each fish was fixed with 100% methanol (P.A.) and stained with Giemsa 10%. A total of 2000 erythrocytes were counted, and deficiencies and micronuclei were counted and classified according to Carrasco et al. (1990) and Fenech et al. (2003). Micronucleus data were subjected to a non-parametric Kruskal–Wallis test and Dunn's test (5%).

Histological analysis

For the histological analysis, the organs were dehydrated in graded alcohol solutions after fixation, diaphanized in xylol, and the tissue was embedded in paraplast. Histological sections 5 μm thick were obtained by a microtome and then stained with hematoxylin and eosin (Behmer et al. 1976). Organs were evaluated for diseases, the degree of which was determined following Cengiz and Unlu (2006) criteria, and the progressive stage of each organ was classified according to Poleksic and Mitrovic-Tutundžic (1994).

Results

During the final deltamethrin toxicity test, fish were maintained in water at 28.6 ± 0.46 °C, pH 7.6 ± 0.1, 0.3 ± 0.1 mg L^{-1} total ammonia, 6.5 ± 0.37 mg L^{-1} dissolved oxygen, and 129.3 ± 2.9 μs cm^{-1} conductivity. Fish exposed to deltamethrin at concentrations of 1.34×10^{-1} and 1.93×10^{-1} mg L^{-1} had a 100% mortality rate in the first 6 and 12 h of experiment, respectively. At a concentration of 6.44×10^{-2} mg L^{-1}, the mortality rate was 33.3%, and 0% mortality was observed at a concentration of 6.16×10^{-3} mg L^{-1} and in the control. Based on these data, a $CL_{50-96\ h}$ of 5.56×10^{-2} mg L^{-1} was determined with an upper limit of 0.09 mg L^{-1} and lower limit of 0.04 mg L^{-1}, resulting in the regression equation $y = 585.7x - 0.19$, $R^2 = 0.9239$. In the first hour of the experiment, exposure to the two highest concentrations of deltamethrin resulted in reactions such as tremors, erratic swimming, presence on the water surface, and increased opercula beating.

Nuclear changes, such as the presence of micronucleus and nuclear erythrocyte changes determined as notched, lobbed, blebbed, binuclear, and 8-shape format, were observed in all deltamethrin treatments (Fig. 1). Fish exposed to deltamethrin showed a higher amount of nuclear abnormalities when compared to the control group (Fig. 2). The notched and 8-shape format abnormalities were the most prevalent (Fig. 3). The influence of exposure time was also observed in defect formations (Fig. 4), with increased anomaly formation after 6 and 12 h of exposure.

Exposure to deltamethrin resulted in histopathological effects in the gills, such as lamellar fusion, hyperplasia, edema, necrosis, and aneurysm (Fig. 5 and Table 1). At lower concentrations, moderate and/or low changes were observed, and hyperplasia and necrosis were only recorded at a concentration of 6.44×10^{-2} mg L^{-1}, the highest concentration at which fish survived for 96 h. The highest concentration used in the experiment (1.93×10^{-1} mg L^{-1}) resulted in severe edema (stage I), lamellar fusion (stage II), and a moderate occurrence of aneurysm (stage II) based on an exposure time of 6 h when there was also 100% mortality. Whereas at a concentration of 1.34×10^{-1} mg L^{-1}, a more severe degree of edema and aneurysm was observed at 12 h.

Blood congestion, steatosis, necrosis, and pyknotic nuclei were observed in the livers of tambaqui exposed to deltamethrin (Fig. 6 and Table 2). Necrosis was more serious at the higher concentrations of 1.93×10^{-1} and 1.34×10^{-1} mg L^{-1} at 6 and 12 h, respectively. And at the end of 96 h at a concentration of 6.44×10^{-2} mg L^{-1}, an increase in lesion severity was observed.

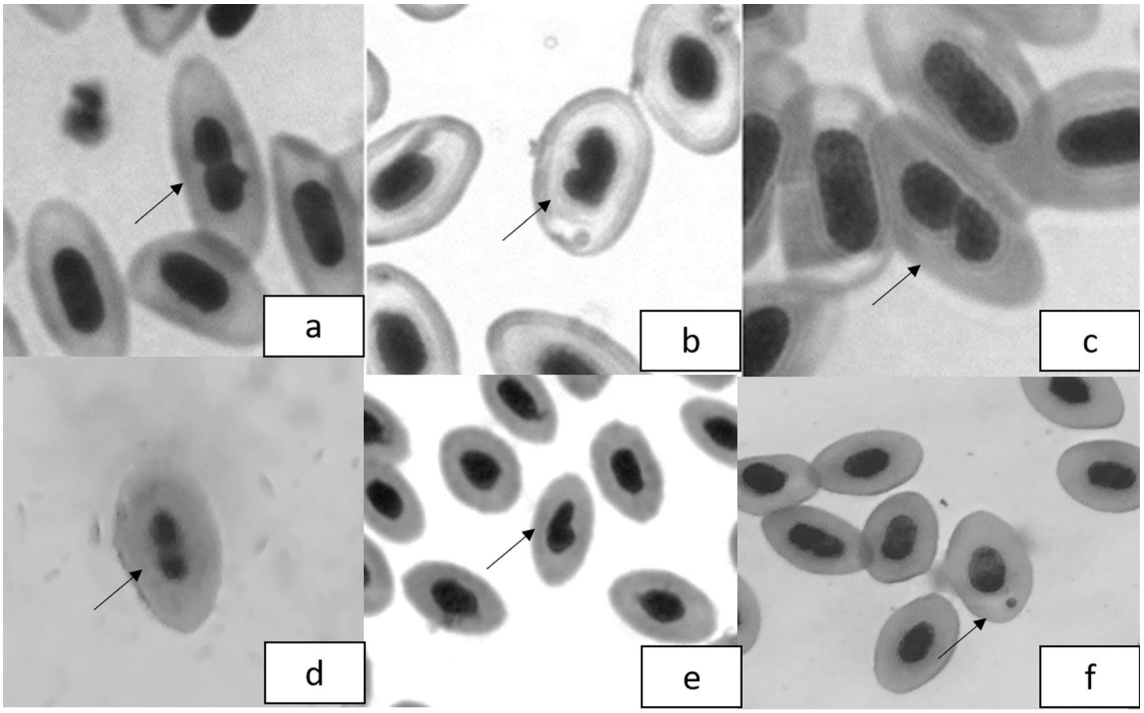

Fig. 1 Different types of abnormalities found in the erythrocytes of tambaqui exposed to deltamethrin. **a** Eight-shape. **b** Notched. **c** Blebbed. **d** Binucleated. **e** Lobbed. **f** Micronuclei. Giemsa 10% 1000×

Discussion

The pyrethroid group ranks third in terms of global use as an insecticide in both public health and agricultural practices (Li et al. 2016). A low toxicity was observed in mammals and they have little persistence in water (Hirata 1995), but toxicity is high for non-target aquatic organisms. The US Department of Agriculture National Agricultural Pesticide Impact Assessment Program EXTOXNET (1996) determined that the acute fish toxicity range of deltamethrin was CL_{50} 1–10 μg L^{-1}. However, a lower sensitivity was observed for tambaqui in this study (CL_{50} 5.56×10^{-2} mg L^{-1}). This difference in sensitivity may be due to difference in responses between temperate and tropical fish to the intoxication process. There are several factors that may explain this difference, such as different modes of action and detoxification as well as temperature-influenced metabolism (Kwok et al. 2007).

The Nile tilapia (*Oreochromis niloticus*) proved to be more resistant than tambaqui. Yildirim et al. (2006) observed that the CL_{50} of Nile tilapia with an average weight of 15 g was

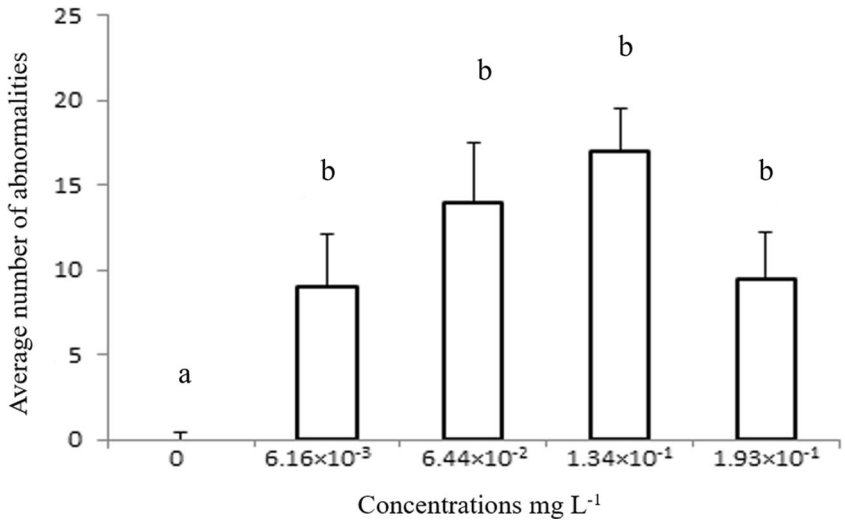

Fig. 2 Frequency of nuclear abnormalities (micronuclei, notched, lobbed, blebbed, binucleated, 8-shape) in tambaqui erythrocytes exposed to different concentrations of deltamethrin. Similar letters indicate no significant difference based on a Kruskal–Wallis test and Dunn's test (5%)

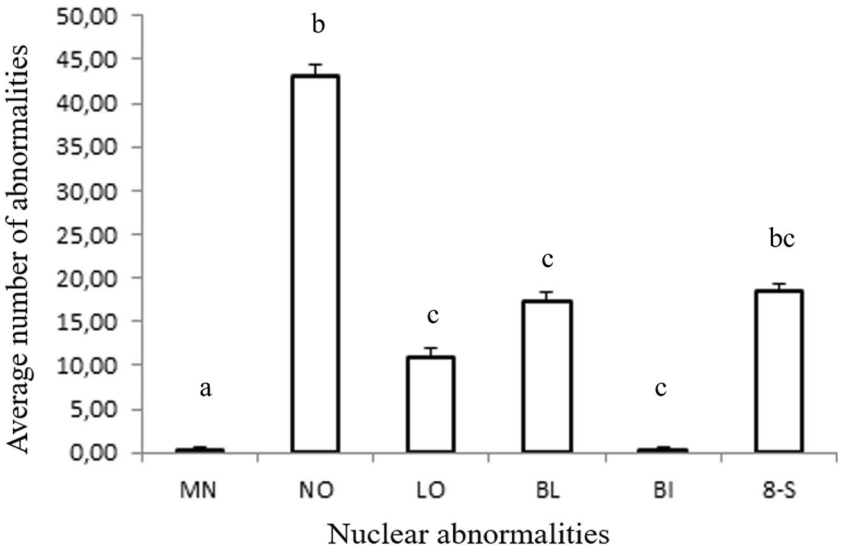

Fig. 3 Frequency of nuclear abnormalities in tambaqui erythrocytes exposed to deltamethrin over a 96-h period. Similar letters indicate no significant difference based on a Kruskal–Wallis test and Dunn's test (5%). MN micronuclei, NO notched, LO lobbed, BL blebbed, BI binucleated, and 8-S 8-Shape

4.85×10^{-3} mg L^{-1} and is approximately 12 times more sensitive than tambaqui.

Despite the fact that tambaqui were maintained under ideal breeding conditions with adequate water quality parameters for this species of tropical fish (Kochhann et al. 2015; FAO 2017), mortality was recorded after 6-h exposure to a 1.93×10 mg L^{-1} concentration of deltamethrin, which classifies this as highly toxic substance according to Extension Toxicology Network (1996). The averages of the water parameters were within those recommended for rearing tambaqui, indicating that they had no effect on the experiment results.

Deltamethrin is easily absorbed by the gills due to its lipophilic character, which provokes respiratory and swimming changes in tambaqui, similar to that described by Yildirim et al. (2006) for Nile tilapia fingerlings exposed to deltamethrin for 45 min. These swimming changes were also due to the inhibitory effect of deltamethrin on GABA, which causes hyperexcitability in fish (Soderlund 2010).

The branchial conditions of tambaqui observed in this study were induced by concentration and exposure time, as fish that survived pesticide exposure over 96 h showed a greater number of pathologies compared to fish that survived for 6 and 12 h. This could be because mortality at higher concentrations occurred very early, and the fish were unable to develop different defense mechanisms (Freitas et al. 2013). This scenario can be explained by the fact that fish exposed to a high concentration of deltamethrin showed a collapse of the pillar cells in the gills, which resulted in a release of blood and caused an elevation and in some cases epithelial disruption of the epithelium and consequently internal hemorrhage in the gills (Hinton and Laurén 1990). This may have hindered gas exchange in tambaqui, leading to fish staying on the surface

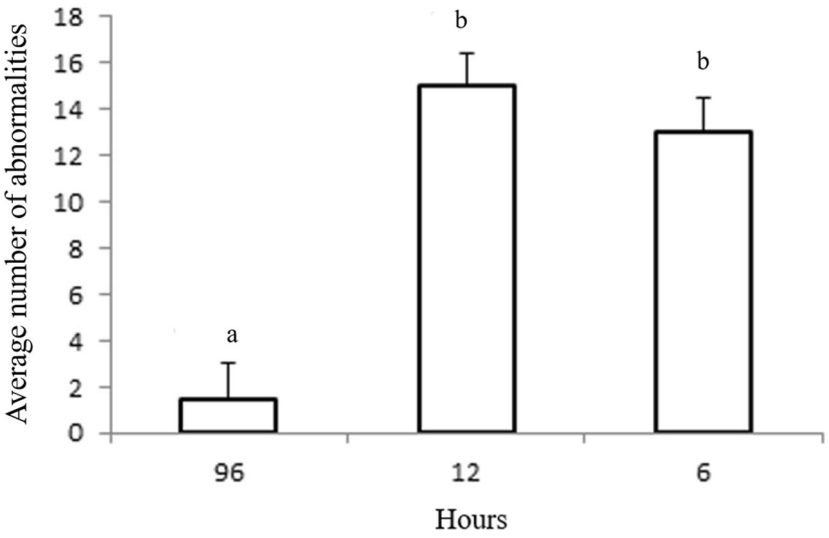

Fig. 4 Frequency of nuclear abnormalities (micronuclei, notched, lobbed, blebbed, binucleated, 8-Shape) in tambaqui erythrocytes based on exposure time to deltamethrin. Similar letters indicate no significant difference based on a Kruskal–Wallis test and Dunn's test (5%)

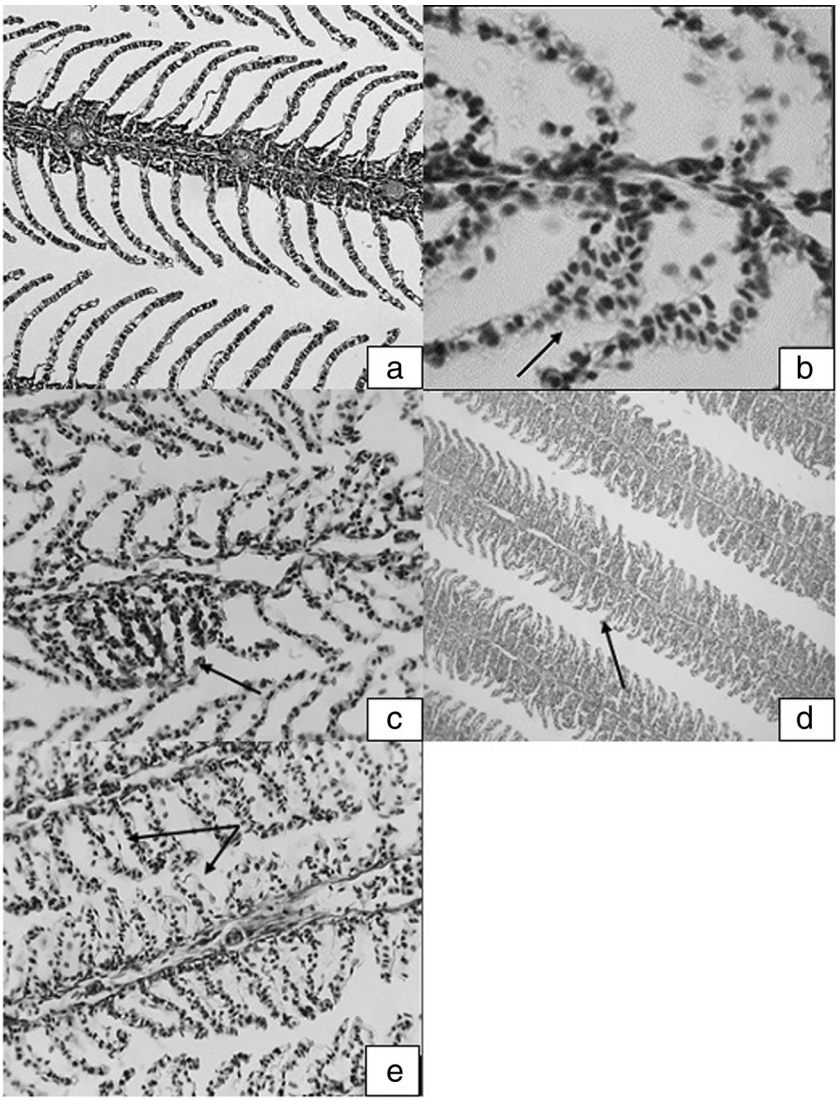

Fig. 5 Histopathology of tambaqui gills exposed to deltamethrin. **a** Control group, 400×. **b** Aneurysm following exposure to 1.34×10^{-1} mg L^{-1}, 1000×. **c** Lamellar fusion following exposure to 1.93×10^{-1} mg L^{-1}, 400×. **d** Hyperplasia following exposure to 6.44 × 10–2 mg L^{-1}, 100×. **e** Edema following exposure to 1.93×10^{-1} mg L^{-1}, 400×

and contributing to death in the early hours of the experiment. Further, the gill proved to be an efficient biomarker organ since these responses occurred in the first hour after exposure.

Deltamethrin is absorbed through the gills and is further metabolized by the liver (Florio and Souza 2006); during the metabolizing process, ROS are generated resulting in oxidative damage that can affect different macromolecules such as DNA, resulting in cytotoxic and genotoxic effects (Jha 2008; Taju et al. 2014). In this study, deltamethrin may have compromised the integrity of the liver, as steatosis, necrosis, picnosis, and congestion were observed (Poleksic and Mitrovic-žic Tutund 1994; Azzalis et al. 1995). Sublethal concentrations also induced liver damage in tambaqui, similar to that reported by Cengiz and Unlu (2006) for mosquitofish (*Gambusia affinis*) exposed to sublethal deltamethrin concentrations over 10, 20, and 30 days. This probably occurred due to alterations in enzymatic activities which causes cell damages as well as the fish take longer to metabolize pyrethroids compared to mammals and birds, so the liver is constantly contaminated and they develop different types of pathologies, as observed in this study at a concentration of 6.44×10^{-2} mg L^{-1} (Casilhas et al. 1983; Bradbury and Coats 1989).

As seen in the gills, liver histopathology showed the tissue alterations after just a few hours of exposure, but higher

Table 1 Histopathologic effects in tambaqui gills exposed to different concentrations of deltamethrin. – no anomaly, + low occurrence, ++ moderate occurrence, +++ severe occurrence. *ET* exposure time, *LF* lamellar fusion , *H* hyperplasia , *A* aneurysm , *N* necrosis

Concentration mgL^{-1}	ET (h)	LF	H	E	A	N
Control	96	–	–	–	–	–
6.16×10^{-3}	96	+	–	+	+	–
6.44×10^{-2}	96	++	+	++	+	+
1.34×10^{-1}	12	+	+	+++	+++	–
1.93×10^{-1}	6	+++	–	+++	++	+

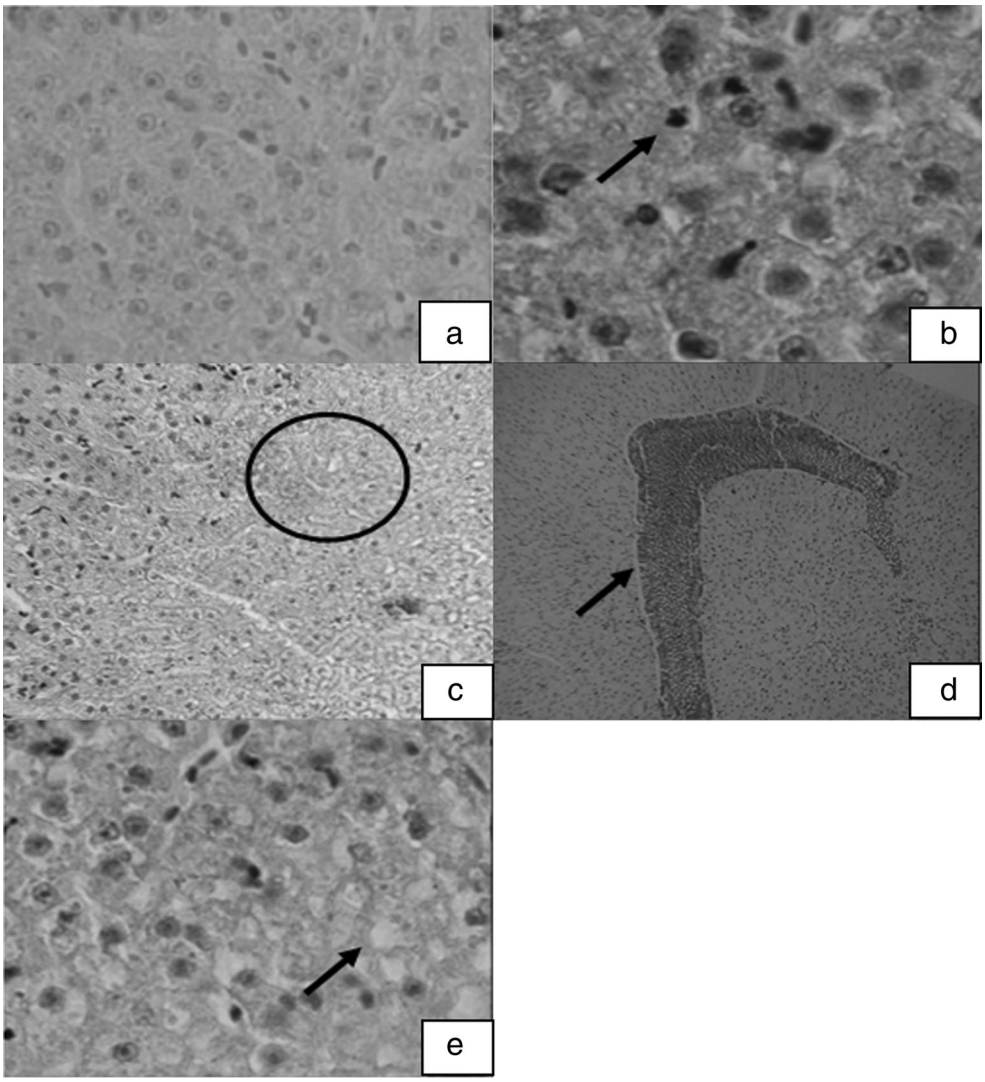

Fig. 6 Histopathology of tambaqui livers exposed to deltamethrin. **a** Control group, 1000×. **b** Pyknotic nuclei following exposure to 6.44×10^{-2} mg L^{-1}, 1000×. **c** Necrosis following exposure to 1.93×10^{-1} mg L^{-1}, 400×. **d** Blood congestion following exposure to 6.16×10^{-3} mg L^{-1}, 400×. **e** Steatosis following exposure to 6.44×10^{-2} mg L^{-1}, 1000×

concentrations generated irreversible organ damage such as necrosis. In addition to this histological damage in the gills and liver, the genotoxic effect of deltamethrin was observed due to the clastogenic capacity of deltamethrin (Marques et al. 2014). This product probably affected the ability of tambaqui to expel the damaged DNA or condensed chromatin inducing failures in cell division and finally cell death (Fenech 2000; Lindberg et al. 2007). This resulted in nuclear abnormalities as the micronucleus showed a notched, lobbed, blebbed, binuclear, and 8-shape format (Carrasco et al. 1990; Fenech et al. 2003).

Table 2 Histopathologic effects in the liver of tambaqui following exposure to different concentrations of deltamethrin. – no anomaly, + low occurrence, ++ moderate occurrence, +++ severe occurrence. *ET* exposure time, *BC* blood congestion, *S* steatosis, *N* necrosis, *PN* pyknotic nuclei

Concentration mg L^{-1}	ET	BC	S	N	PN
Control	96	–	–	–	–
6.16×10^{-3}	96	++	–	++	+
6.44×10^{-2}	96	+++	+++	++	++
1.34×10^{-1}	12	++	++	+++	++
1.93×10^{-1}	6	+	++	+++	+

Many studies have used the micronucleus test and nuclear abnormality evaluation, and it has been proven that these are responses to genotoxic agents (Ayllón and Gaber-Vazquez 2000; Çavas and Ergen-Gözükara 2003); however, it is unclear the effect of deltamethrin in the formation process of the nuclear abnormalities (Bolognesi and Hayashi 2011). Type II pyrethroid induced genotoxic effects of abnormal nuclei in the European eel (*Anguilla anguilla*) after 3-day exposure and in the spotted snakehead (*Channa punctata*) under different sublethal concentrations (1.75×10^{-2} and 3.5×10^{-2} mg L^{-1})

(Ansari et al. 2009; Muranli and Mr. Güner 2011; Khan et al. 2012; Marques et al. 2014).

In the present work, the observed nuclear abnormalities provided a rapid response to deltamethrin contamination at higher concentrations. Ansari et al. (2009) also observed the time-concentration relationship for the spotted snakehead at lower concentrations of deltamethrin (0.4×10^{-3}, 0.8×10^{-3}, and 1.2×10^{-3} mg L^{-1}), where a higher number of abnormalities were observed after 72 h compared with those after 48 h.

These results reinforce the consensus of Barsiene et al. (2012), that abnormalities are efficient as an environmental tool that has to be rapid, non-invasive, and reproducible. The toxic effect of deltamethrin has been observed in several species, but there are no studies on tambaqui, a native species from the Amazon region that is grown in different parts of the world. The results of this work confirm that the tambaqui is a potential indicator species of environmental contamination. Further studies are needed to investigate whether deltamethrin has transitory or permanent effects in tambaqui in order to avoid health risks. And others studies are recommended to investigate deltamethrin toxicity on adult and early life stages of fish to assess possible environmental risk.

Conclusion

We conclude that deltamethrin is highly toxic to tambaqui inducing nuclear erythrocytes alteration and damage to the gills and liver, compromising fish survival, even at low concentrations. Although the present study provides important answers, future studies, such as enzymatic changes and oxidative stress, are necessary to broaden our scope of knowledge and make possible the development of an efficient biomarker for deltamethrin.

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Compliance with ethical standards

This study was approved by the Animal Ethics Committee (CEUA-Unit; Protocol number 030514).

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