

Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*)

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Abstract

In this study, teleostean fish *Leporinus obtusidens* (piava) were exposed to different concentrations of Roundup, a glyphosate (acid equivalent) herbicide: 0 (control), 3, 6, 10, and 20 mg/L for 96 h (short-term). Acetylcholinesterase (AChE) activity was verified in brain and muscle tissues. Metabolic parameters in the liver and muscle (lactate, glycogen, glucose, protein, and ammonia), as well as some hematological parameters, were determined. Unexposed fish exhibited significantly higher brain AChE activity when compared to that of the muscle ($P < 0.05$) (13.8 ± 0.76 and 6.1 ± 1.31 $\mu\text{mol}/\text{min}/\text{g}$ protein, respectively). Results indicated that AChE activity significantly decreased in the brain of fish exposed to all glyphosate concentrations tested, but in the muscle this parameter was not altered. In addition, fish exposed to all glyphosate concentrations showed a significant increase in hepatic glycogen and glucose, but a significant reduction in muscle glycogen and glucose. Lactate and protein of fish exposed to all glyphosate concentrations presented a significant decrease in the liver, but did not change significantly in the muscle. Levels of ammonia in both tissues increase in fish at all glyphosate concentrations. Exposure to this herbicide produced a decrease in all hematological parameters tested. These results indicate that AChE activity as well as metabolic and hematological parameters may be good early indicators of herbicide contamination in *L. obtusidens*.

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1. Introduction

Herbicide and pesticide contamination of surface waters derived from agricultural practices is a problem of worldwide importance due to aquatic contamination by these products (Oruç and Üner, 1999). Roundup (glyphosate 48%), a commercial formula containing an active ingredient which is the acid equivalent of the isopropylamine salt of glyphosate (*N*-phosphonomethyl glycine), is a nonselective and postemergent herbicide used for controlling aquatic weeds (Abdullah et al.,

1995; Jiraungkoorskul et al., 2002). Glyphosate concentrations used in rice and soybean cultures in Southern Brazil range from 0.36 to 2.16 mg/L. The water solubility of this herbicide is 15,700 mg/L, and its half-life in soil is 30–90 days (Rodrigues and Almeida, 1998). A limited amount of information is available on the toxic characteristics of glyphosate, the surfactants used in its formula, and its effects on fish. According to Giesy et al. (2000), glyphosate does not bioaccumulate in terrestrial or aquatic animals and is widely used in the world due to its high efficiency, its cost effectiveness, and the fact that it is practically nontoxic and readily degradable in the environment.

The piava (*Leporinus obtusidens*) was chosen for this study, since the effect of glyphosate herbicide on fish

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species, particularly on this one, has scarcely been studied. It was also chosen because it is a native freshwater fish of Southern Brazil with good potential for cultivation and acceptance by consumers (Andrian et al., 1994). Teleost fish may be good indicators of contamination by pollutants because their biochemical responses are quite similar to those found in mammals. The response of some aquatic organisms to pollutants has been studied through the measurement of hematological and physiological parameters (Begum, 2004; Gimeno et al., 1995; Sancho et al., 1998, 2000). Studies of glyphosate toxicity conducted on rodents, dogs, rabbits, aquatic invertebrates, and fish showed that glyphosate, while highly toxic to plants, is relatively nontoxic to animals including fish (Williams et al., 2000). The measurement of acetylcholinesterase (AChE) activity, present in the cholinergic synapses and motor end plates, has been used by different authors as a biomarker to monitor carbamate and organophosphate effects on higher vertebrates, such as fish (Chuiko, 2000; De La Torre et al., 2002; Fernández-Vega et al., 2002). AChE activity is extremely important for many physiological functions of fish, such as prey location, predator evasion, and orientation toward food. When AChE activity decreases Ach is not broken and accumulates within synapses, which therefore cannot function in a normal way (Dutta and Arends, 2003).

The aim of this study was to investigate the effects of short-term glyphosate exposure on AChE activity and metabolic and hematological parameters in *L. obtusidens*, as possible early indicators of herbicide toxicity.

2. Materials and methods

Piava of both sexes were obtained from the Santa Maria Federal University (UFSM) fish farm (RS, Brazil). Fish (weight, 12.0 ± 1.0 g; length, 8.0 ± 1.0 cm) were acclimated in laboratory conditions for 10 days. They were kept in continuously aerated tanks (250 L) with a static system and with a natural photoperiod (12 h light–12 h dark). Throughout the experimental period, the water quality was as follows: temperature 22 ± 0.5 °C, pH 7.4 ± 0.05 units, dissolved oxygen 7.2 ± 0.2 mg/L. Fish were fed once a day with commercial fish pellets (42% crude protein, Supra, Brazil). Feces and pellet residues were removed daily by suction. Acute toxicity assays were made in a static way for a 96-h, according to Antón et al. (1994). We chose 96 h test for this study because this studies are usually an initial step in the evaluation of the toxic characteristics of a substance. Previous experiments carried out in our laboratory were not able to obtain a lethal concentration (LC₅₀) of glyphosate at 96 h, because all the fish survived even at the highest concentration tested (100 mg/L) and showed normal swimming and feeding

behavior. Therefore, experimental glyphosate concentrations were chosen considering that the recommended nominal concentration for use in agriculture ranges from 0.36 to 2.16 mg/L. After the acclimation, groups of 8 fish were placed in 45-L continuously aerated glass tanks and exposed for 96 h to 0 (control), 3, 6, 10, or 20 mg/L of Roundup (48% acid equivalent, Monsanto, St Louis, MO). All tests were carried out in triplicate and fish did not receive food during the experimental period. The herbicide was added to the water only at the beginning of the experiment. Water quality did not change throughout the experimental period.

At the end of the exposure period (96 h), all fish were sampled and blood was collected from the caudal vein with a 1-ml heparinized syringe. Total blood was used for determination of the hematocrit, hemoglobin, total erythrocyte, and leucocyte counts according to Barcellos et al. (2004). One blood aliquot was centrifuged (10 min, 1500g) for protein determination according to Lowry et al. (1951). Tissues (brain, liver, and muscle) were removed and placed on ice, frozen in liquid nitrogen, and then stored at -20 °C. Liver and muscle glycogen were determined according to Bidinotto et al. (1997). Tissue protein was estimated according to Lowry et al. (1951). Tissue samples were homogenized with 10% trichloroacetic acid using a motor-driven Teflon pestle and centrifuged at 1000g for 10 min. Deproteinized supernatant was used for the determination of lactate (Harrower and Brown, 1972), sugar reducer (Park and Johnson, 1949), and total ammonia (Boyd and Tucker, 1992). AChE (EC 3.1.1.7) activities were assayed as described by Ellman et al. (1961) and modified by Villescas et al. (1981). A suitable amount (50–100 µl) of homogenate was incubated at 25 °C for 2 min with 0.8 mM acetylthiocholine as substrate and 1.0 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as chromogen. Protein content was determined according to Bradford (1976). Enzyme activity is expressed as µmol of acetylthiocholine hydrolyzed per min per gram of protein.

One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used. Data ($n = 3$) were expressed as mean $\pm$ standard deviation (SD) and mean differences were considered significant at $P < 0.05$.

3. Results and discussion

The brain of unexposed control fish showed higher AChE activity than the muscle (13.8 against 6.1 µmol/min/g of protein, respectively). AChE activity in the brain of fish exposed to glyphosate was lower than that in the control group (reduction of activity ranging from 17% in 3.0 mg/L up to 42% in 20 mg/L). However, exposure to glyphosate did not provoke significant alterations of AChE activity in the muscle (Fig. 1). Brain

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