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Oxidative stress in the hybrid fish jundiara (*Leiarius marmoratus* × *Pseudoplatystoma reticulatum*) exposed to Roundup Original®

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HIGHLIGHTS

- We examined the effects of Roundup Original® herbicide on oxidative stress parameters in a hybrid fish.
- Our results confirm that the glyphosate-based herbicide promotes oxidative stress in these fish.
- Reduction of AChE activity can be used as a suitable indicator of herbicidal toxicity.

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ABSTRACT

The aim of this study was to investigate the effects of Roundup Original®, a glyphosate-based herbicide, against biochemical parameters including thiobarbituric acid-reactive substances (TBARS), protein carbonyl, enzymatic and nonenzymatic antioxidant responses and acetylcholinesterase (AChE) of jundiara fish (*Leiarius marmoratus* × *Pseudoplatystoma reticulatum*) at a sublethal concentration of 1.357 mg L^{-1}. Fish exposed to the herbicide for different periods (6–96 h) showed a significant increase of both hepatic and muscular TBARS and protein carbonyl. Enzymatic antioxidant activity was decreased in the liver and brain after 48 h of exposure. Glutathione-S-tranferase (GST) had its levels raised in the brain and gills, probably as a toxicity event response. Non protein thiols (GSH) demonstrated a reduction after 6 and 24 h of exposure in the hepatic tissue, followed by an increase at 48 and 96 h in the same tissue. GSH brain levels, however, increased only after 96 h. AChE activity in muscle decreased for all the times tested (26.5, 45, 38 and 14% for 6, 24, 48 and 96 h respectively), but only at 96 h (34%) in the brain. We found that Roundup Original® is able to trigger important changes in the biochemical parameters tested, showing it can be a potential threat for the health and survival of fish in the environment.

1. Introduction

Water contamination is a current problem in several locations around the world, and a huge variety of pesticides are involved in this process (Smiley et al., 2014). Herbicides are widely used in agricultural fields in order to enhance productivity in terms of biomass, although all together they represent an environmental threat, affecting non-target organisms, including fish (Dey et al., 2016; Samanta et al., 2014). Glyphosate is a post-emergent, broad spectrum systemic herbicide, used all over the world since the 1970s, and its first and best known formulation, Roundup®, is used in agriculture, garden maintenance, horticulture and other purposes (Yusof et al., 2014).

Glyphosate (*N*-(phosphonomethyl)glycine) is a highly polar substance that solubilizes in water very easily and whose formulations are not considered to bioaccumulate in terrestrial or aquatic animals. On the one hand, it is a highly efficient substance (Giesy et al., 2000), capable of inhibiting plant growth and interfering on the production of essential amino acids by reducing enolpyruvylshikimate phosphate synthase activity (Lushchak et al., 2009). On

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the other hand, its repeated use is observed to significantly increase the quantity of residual run-off into water bodies adjacent to productive lands (Samanta et al., 2014; Nwani et al., 2013). The Roundup formulation was proposed in 1974 and contained glyphosate as the active ingredient and a non-ionic surfactant named polyoxyethylene amine (POEA), as discussed by Lushchak et al. (2009). The acute toxicity of Roundup (particularly glyphosate) is recognized to be low as reported by data from the World Health Organization (WHO, 1994). Nonetheless, according to Giesy et al. (2000) the toxicity of Roundup to aquatic organisms may be caused mainly by POEA.

Soil availability and appropriate climate conditions bolstered the development of monocultural crops, such as the transgenic glyphosate-tolerant soybean in Western, Southern and Southeastern Brazil (Shiogiri et al., 2012). In the state of Mato Grosso, located in the Central-Western portion of Brazil, soybean and cotton production is widely associated with the use of pesticides, including Roundup Original® (Ministério da Agricultura Pecuária e Abastecimento, 2010). Dores and De-Lamonica-Freire (2001) reported that glyphosate-based herbicides used in Mato Grosso reach groundwaters and could contaminate water environments and aquatic organisms. According to Rodrigues and Almeida (2005), values of glyphosate found near agricultural areas in Brazil correspond to the range of 0.36–2.16 mg L^{-1}, although these data are particularly scarce in the north of Mato Grosso. According to Queiroz et al. (2011) glyphosate transport in runoff and leaching waters in agricultural soil was of 1.7% (w/w) and 15.4% (w/w), respectively, in state of Santa Catarina; and in a study by Aparicio et al. (2013) in Argentina, the presence of glyphosate and aminomethylphosphonic acid, its metabolite, was detected in surface waters and agricultural basin soil. Regarding this kind of contamination, fish are reported as the largest and most diverse group of non-target organisms that are continuosly exposed to chronical pesticide poisoning events (Samanta et al., 2016).

Fish are usually sensitive to the presence of pollutants in the environment and have an important position when aquatic toxicology is discussed (Di Giullio and Hinton, 2008). Diverse environmental xenobiotics or pollutants, including herbicides, are able to form reactive oxygen species (ROS) via several mechanisms (Braz-Mota et al., 2015). The production of ROS caused by the presence of toxic substances can induce an oxidative stress response; this mechanism, considered to be an adaptative response of living beings to cope with damage, has been found in aquatic organisms when contaminated with pesticides (Oropesa et al., 2009). Lipid peroxidation (LPO) determined by TBARS levels, as well as protein oxidative damage determined by protein carbonylation, are often used to investigate herbicide-induced damage in fish (Braz-Mota et al., 2015). As discussed by Sinhorin et al. (2014), enzymatic and nonenzymatic antioxidants are responsible for preventing oxidation of fish cells caused by glyphosate-based herbicides, thus protecting them from ROS. Modesto and Martinez (2010a), for instance, mentioned the role of the major enzymatic antioxidant responses, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which catalyze the conversion of radicals into less toxic substances, as well as ascorbic acid (ASA) and nonprotein thiols (GSH), which compose the nonenzymatic defense system (Menezes et al., 2011). Glutathione-S-tranferase is responsible for the dexotification of many xenobiotics, and its action requires GSH (Al-Ghais, 2013). AChE has a notably important activity in many physiological functions of fish metabolism, and, as it may be affected by the action of toxic substances on the organism, it is considered useful in toxicologic experiments (Menéndez-Helman et al., 2012). Therefore, this complex system in fish can be influenced by the presence of a contaminant (Menezes et al., 2011).

In recent years there has been a reduction of available fish in the environment for human consumption, motivated by many factors including deforestation and water pollution (Sinhorin et al. (2014). Because of that, breeding and raising hybrid fish is becoming more and more common, especially when taken into consideration the good acceptance from both national and international markets, decreased costs of production and easier management (Pádua et al., 2012).

The hybrid fish locally known as jundiara or pintado-da-Amazônia (*Leiarius marmoratus* × *Pseudoplatystoma reticulatum*) is the result of the crossing between a male jundiá-da-Amazônia (*L. marmoratus*) and a female cachara (*P. reticulatum*) (Ventura et al., 2013). This species was chosen to carry out the present study due to its economical appeal in the North of Mato Grosso, and also because there is no information about the toxicological risk of Roundup Original® to farmed fish. This herbicide has been widely used in several agriculture crops and applied as a non-selective herbicide around aquaculture fish tanks to control aquatic weed plants (Araújo et al., 2008). Thus, this work set out to evaluate the effects of Roundup Original® on oxidative stress biomarkers and AChE activity of jundiara after acute exposure to a sublethal concentration of the herbicide. The results of this study may provide information concerning the use of indicators to evaluate the sublethal toxicity of herbicide on hybrid fish.

2. Material and methods

2.1. Chemicals

The herbicide used in this study was Roundup Original® (480 g L^{-1} containing isopropylamine salt of glyphosate, 360 g L^{-1} acid equivalent, *N*-(phosphonomethyl) glycine (glyphosate) and 684 g L^{-1} of inert ingredients, MAPA SOB Nº 00898793), Monsanto, St. Louis, MO, USA). Bovine serum albumine, hydrogen peroxide (H_2O_2), sodium dodecyl sulfate (SDS), reduced glutathione, Bradford reagent, trichloroacetic acid (TCA), monobasic potassium phosphate, dibasic potassium phosphate, monobasic sodium phosphate, dibasic sodium phosphate, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), Trizma® hydrochloride solution, 1-chloro-2,4-dinitrobenzene (CDNB), Triton X-100, 2-thiobarbituric acid (TBA), 5,5'-dithio-bis(2-nitro-benzoic acid) (DTNB), malondialdehyde (MDA), 2,4-dinitrophenylhydrazine (DNPH) and the other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) at a high purity percentage (95–99%).

2.2. Fish

Juvenile jundiara (85.0 ± 10.0 g and 18.0 ± 2.0 cm) were obtained from a fish farm in Sinop, Mato Grosso, Brazil. The fish were acclimated to laboratory conditions for 7 days in 300-L fiberglass tanks containing aerated and dechlorinated water, under natural photoperiod conditions (12 h light/12 h dark) before the experimentation. During this period the fish were fed once a day with commercial fish pellets containing 42% crude protein. Water quality parameters were measured every day (mean ± SD): temperature 27 ± 1.0 °C, pH 6.84 ± 0.4, dissolved oxygen 6.31 ± 0.5 mg L^{-1}, hardness 18 ± 2.0 mg L^{-1} $CaCO_3$, nonionized ammonia 0.07 was detected 0.03 and nitrite 0.06 ± 0.01 mg L^{-1}. Pellet residue and feces were removed by suction and filtration every other day.

2.3. Experimental design

Previous laboratory experiments determined the lethal dose at LC_{50} at 13.57 mg L^{-1} of Roundup Original® (nominal concentration of glyphosate), which was estimated by trimmed Sperman-Karber