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Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma sp*)

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ABSTRACT

The aim of this study was to investigate the effects of acute glyphosate (active ingredient) exposure on the oxidative stress biomarkers and antioxidant defenses of a hybrid surubim (*Pseudoplatystoma sp*). The fish were exposed to different herbicide concentrations for 96 h. The thiobarbituric acid-reactive substances (TBARS), protein carbonyls and antioxidant responses were verified. The 15 mg a.p L^{-1} of herbicide resulted in the death of 50% of the fish after 96 h. An increase in liver and muscle TBARS levels was observed when fish were exposed to the herbicide. The protein carbonyl content was also increased in the liver (4.5 mg a.p L^{-1} concentration) and brain (2.25 mg a.p L^{-1} concentration). The antioxidant activities decreased in the liver and brain after exposure to herbicide. Levels of ascorbic acid in the liver (2.25 mg a.p L^{-1} and 4.5 mg a.p L^{-1} concentrations) and brain (2.25 mg a.p L^{-1} concentration) were increased post-treatment. Levels of total thiols were increased in the liver and brain (2.25 mg L^{-1} and 7.5 mg a.p L^{-1}, respectively). Glyphosate exposure, at the tested concentrations affects surubim health by promoting changes that can affect their survival in natural environment. Some parameters as TBARS and protein carbonyl could be early biomarkers for Roundup exposure in this fish species.

1. Introduction

In recent years the frequent use of agricultural pesticides has been cited as one of the factors contributing to environmental contamination. Hundreds of pesticides of varying chemical structures are extensively used to control a wide variety of agricultural pests and can contaminate aquatic habitats due to leaching and water runoff from treated areas. These pesticides may result in an immense disruption of the ecological balance causing widespread damage to non-target organisms, including fish of commercial importance (Oruç et al., 2004).

Pesticide exposure can cause an increase in the production of reactive oxygen species (ROS) and also alter the antioxidant defenses. ROS are highly reactive substances that cause damage to lipids, proteins, carbohydrates, and nucleic acids (Monserrat et al., 2007). The oxidative damage involves the peroxidation of unsaturated fatty acids and the corresponding increase in tissue malondialdehyde levels, which serve as an indicator of possible damage to lipids. The reactive species may also cause damage to proteins, which is frequently measured by protein carbonyl levels. Protein carbonyl formation is a result of protein oxidation that can lead to the loss of sulfhydryl groups in addition to the modification of amino acids, ultimately contributing to the formation of carbonyls and other oxidized moieties (Parvez and Raisuddin, 2005). Some authors considered that both the levels of lipid peroxidation (LPO) and protein carbonyls have been used as biomarkers for fish species exposed to pesticides (Menezes et al.,

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2011). The antioxidant system is comprised of several enzymes and low-molecular-weight antioxidant substances such as ascorbic acid and non-protein thiols (Winston and Di Giulio, 1991; Van der Oost et al., 2003). Ascorbic acid acts as an antioxidant and detoxifies numerous peroxide metabolites, thus protecting cell-sensitive structures and processes against oxidation (Fracalossi et al., 2001). The antioxidant glutathione (GSH), the most abundant non protein thiol is involved in many cellular processes in fish, including protects cells against oxidative injury. Another role is detoxicates xenobiotics and or their metabolites through glutathione peroxidase activity and also GSH is important to glutathione S-transferase in fish (Al-Ghais, 2013).

Superoxide dismutase (SOD) is an important enzyme in the line of the antioxidant defenses and catalyzes the conversion of the superoxide anion into hydrogen peroxide. In addition, catalase (CAT) converts hydrogen peroxide into water and oxygen. Studies have shown that pesticide exposure alters the activities of these enzymes in fish tissues (Modesto and Martinez, 2010a, 2010b).

The production of large monocultures for export, such as soybeans, corn and cotton in the state of Mato Grosso has been associated with an intensive use of pesticides (MAPA, 2010). For soybeans, the most important herbicide is the glyphosate-based formulation Roundup®, used mainly in control of weeds in genetically modified crops. The directions for use of Roundup® indicate the application of 0.5 L Ha^{-1} of the herbicide, diluted in 100 L of water, which represents 1800 mg L^{-1} of glyphosate (INDEA, 2008, 2010). The Roundup® formulation contains glyphosate as the active ingredient along with polyethoxylene amine (POEA), a non-ionic surfactant, added to increase the efficiency of the active ingredients by promoting the penetration of the herbicide through plant cuticles (Brausch and Smith, 2007). The toxicity of the herbicides and the risks for humans, other mammals and birds were analyzed in detail by Williams et al. (2000), who concluded that "under present and expected conditions of use, Roundup® herbicide does not pose a health risk for humans." However, Dores and De-Lamonica-Freire (2001) demonstrated that glyphosate used in monocultures in Mato Grosso state presents mobility in the environment and it reaches groundwaters corroborating the potential of the glyphosate to pollute aquatic environments. The current literature showed that aquatic organisms, particularly fish, could be more sensitive to glyphosate than mammals. Studies have demonstrated the potentially adverse effects of Roundup® on fish and also some evidences of metabolic, oxidative, and hematological impairments after Roundup® exposure (Lushchak et al., 2009; Modesto and Martinez, 2010a, 2010b; Menezes et al., 2011).

The hybrid fish popularly known as surubim is the result of an artificial cross breeding between two large Neotropical catfish species, pintado, *Pseudoplatystoma corruscans* and cachara, *Pseudoplatystoma reticulatum* (Campos, 2005; Ibama, 2008). This fish was chosen for this study due to its economic importance, mainly in Mato Grosso state. The fish farms are generally located near agricultural fields and, during the planting of soybeans, the herbicide Roundup Original® can contaminate the rivers and streams that supply the hatchery tanks, mainly because of its great solubility in water (USEPA, 1993; Dores and De-Lamonica-Freire, 2001). According to Rodrigues and Almeida (2005) values of glyphosate used in agricultural areas in Brazil ranging from 0.36 to 2.16 mg L^{-1}. Specifically in the north of Mato Grosso this date is scarce, besides the highest use of Roundup original® in our region and proximity of fish farms from agricultural fields. Based in literature data we chose the Roundup original® concentrations (Glusczak et al., 2006; Langiano and Martinez, 2008; Lushchak et al., 2009). We used the measured concentrations of 2.25, 4.5, 7.5 and 15 mg a.p L^{-1} to test in hybrid fish. Considering that there is no information available about toxicity of this pesticide and there are available some data about effects on fish of commercial importance, the aim of the present study was to investigate the effect of glyphosate-based herbicide on oxidative parameters and antioxidant defenses in hybrid surubim.

2. Materials and methods

2.1. Chemicals

The herbicide used in this study (Roundup original®, Monsanto, St. Louis, MO) was dissolved in water. We consider the active ingredient (a.p) for all exposure groups. Bovine serum albumin, Triton X-100, hydrogen peroxide (H_2O_2), 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 2,4-dinitrophenylhydrazine (DNPH) and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals management

Juvenile surubim (55.5 ± 10.0 g and 17.0 ± 2.0 cm) were obtained from a fish farm Nativ (Sorriso, MT, Brazil). Prior to experimentation, the fish were acclimated to laboratory conditions for 10 days in 300-L fiberglass tanks containing dechlorinated and aerated water and held under natural photoperiod conditions (12 h:12 h light to dark). During this period, fish were fed once a day with commercial fish food containing 42% crude protein. The water conditions were as follows: temperature 26 ± 1.0 °C, pH 6.75 ± 0.4, dissolved oxygen 6.31 ± 0.5 mg L^{-1}, hardness 18 ± 2.0 mg L^{-1} $CaCO_3$, nonionized ammonia 0.09 ± 0.01 μg/L, and nitrite 0.06 ± 0.01 mg L^{-1}. Feces and pellet residue were removed every other day by suction.

2.2.1. Exposures

After the acclimation period, the fish were distributed among the non-exposure group (control) or four exposure groups (2.25, 4.5, 7.5 and 15 a.p mg L^{-1} of Roundup original®). These concentrations were chosen based on studies that also investigated the effects of the acute exposure of other fish species to Roundup®, using similar concentrations (Glusczak et al., 2006; Langiano and Martinez, 2008). Fish was placed in 50-L glass tanks, which were continuously aerated and contained 5 fish/tank. All tests were carried out in triplicate and the fish did not receive food during the experimental period according to previous experiments and considering literature data (Aguiar et al., 2004; Glusczak et al., 2006, 2011). The water quality did not change throughout the experimental period and fish was maintained in a static system. Following exposure, the fish were removed from the aquaria, immediately anesthetized with benzocaine (0.08 g L^{-1}), and the caudal vein blood drawn with a heparinized syringe. The animals were sampled by medullar sectioning, measured and weighed. Samples of liver, brain and muscle were removed by dissection. The samples were frozen at −80 °C until biochemical assays. The study was approved by the Committee guidelines (Ethics in Animal Research of the Federal University of Mato Grosso), Reference number: 23108.053066/10-7. The glyphosate concentrations were measured, and monitored at the beginning and end of the experimental period by liquid chromatography with previous derivatization (Hidalgo et al., 2004). The results are listed in Table 1. The nominal concentrations used were (2.25, 4.5, 7.5 and 15 mg a.p L^{-1} of Roundup original®) obtained from the stock solution (100 mg a.p L^{-1}) of the herbicide.

Table 1
Glyphosate concentration (mg a.p L^{-1}) in water of the experimental tanks (96 h) following of % of glyphosate (Gly) reduction.

Day	Glyphosate [2.25]	% reduction (Gly)
1	2.24 ± 0.05	0
4	2.01 ± 0.03	10.26
Day	Glyphosate [4.5]	% reduction (Gly)
1	4.52 ± 0.06	0
4	3.85 ± 0.04	14.82
Day	Glyphosate [7.5]	% reduction (Gly)
1	7.55 ± 0.07	0
4	6.64 ± 0.05	12.05
Day	Glyphosate [15]	AMPA
1	14.8 ± 0.09	0
4	13.9 ± 0.085	6.08

2.2.2. Oxidative damage parameters: TBARS and protein carbonyl levels

For the analysis of the LPO, liver, brain and muscle samples were homogenized in four volumes (w/v) of a 1.15% (w/v) KCl solution using a T10 Basic-IKA homogenizer, centrifuged at 1000*g* at 4 °C, and the supernatant was removed for subsequent analysis. The LPO in the liver, brain and muscle was estimated by determining spectrophotometrically the levels of thiobarbituric acid reactive substances (TBARS). The TBARS concentration was expressed as nmol MDA mg protein^{-1} following calibration curve for MDA according to methods described in Konn and Liversedge (1944).

For protein carbonyl assay, liver, brain and muscle samples were homogenized in 10 volumes (w/v) of 10 mM Tris–HCl buffer (pH 7.4) using a T10 Basic-IKA homogenizer. The protein carbonyl content was determined spectrophotomrtrically after DNPH derivation by the method described by Yan et al. (1995), with some modifications. The total carbonylation content was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} and expressed as nmol carbonyl per mg of protein^{-1}.

2.2.3. Antioxidant enzymes and non-enzyme antioxidants

SOD activity was measured in liver and brain tissues and was based on inhibiting the radical superoxide reaction with epinephrine as described by Misra and Fridovich (1972). In this method, the SOD present in the sample competes with the detection system for radical superoxide. A unit of SOD is defined as the amount of enzyme that inhibits the velocity of epinephrine oxidation by 50%. SOD activity is determined by measuring the velocity of adrenochrome formation, as observed at 480 nm in a reaction medium containing glycine–NaOH (50 mM, pH 10.0) and epinephrine (1 mM). The activity was expressed in UI SOD mg protein^{-1}. CAT activity was assayed according to Nelson and Kiesow (1972). Liver and brain tissues were homogenized in 20 mM potassium phosphate buffer, pH 7.5 (1:20 dilution and 1:10, respectively) using a T10 Basic-IKA homogenizer and centrifuged at 10,000*g* for 10 min at 4 °C. The assay mixture consisted of 1.0 mL potassium phosphate buffer (50 mM, pH 7.0), 0.025 mL H_2O_2 (0.3 M), and 0.025 mL homogenate. Change of H_2O_2 absorbance in 60 s was measured spectrophotometrically at 240 nm and expressed as μmol H_2O_2 min^{-1} mg protein^{-1}, using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}. The levels of ascorbic acid (ASA) and total thiols were studied as non-enzymatic antioxidants. The ASA content in the liver and brain tissues were determined as described by Roe (1954). Liver tissue was homogenized with 1.5 mL Tris HCl 50 mM pH 7.5 followed by centrifugation at 3000*g* for 10 min. An aliquot of the supernatant (1.0 mL) was mixed with 1.0 mL trichloroacetic acid (10% v/v) followed by centrifugation. For the determination of ASA content, an aliquot of the supernatant (300 μL) was combined with 2,4-dinitrophenylhydrazine (4.5 mg/mL), 0.6 mg/mL thiourea, $CuSO_4$ (0.075 mg/mL) and trichloroacetic acid (13.3% v/v) and incubated for 3 h at 37 °C. After this, H_2SO_4 was added to the medium for a final concentration of 65% (v/v). The ASA levels were determined spectrophotometrically at 520 nm using a calibration curve with ASA and expressed as μmol ASA g^{-1} tissue. Total thiols were determined according to Sedlack and Lindsay (1968) using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Liver and brain samples were homogenized in 10 volumes (w/v) with 20 mM EDTA. A 2.0 mL aliquot of supernatant was mixed with 4.0 mL of Tris–HCl (0.4 M and pH 8.9) and was added to 0.01 M DTNB in 0.05 M phosphate buffer, pH 8.0. The formation of the thiolate anion was determined at 412 nm against a cistein standard curve. Total thiols were expressed as mmol g^{-1} tissue.

2.2.4. Protein levels determination

Protein was determined by the Coomassie Blue method using bovine serum albumin as a standard. Absorbance of samples was measured at 595 nm according to Bradford (1976).

2.2.5. Statistical treatment

The mean ± S.E.M. (standard error) of each group was calculated using one way (ANOVA) following by the Student Newman Keuls (SNK) test. The statistical significance was accepted at $P < 0.05$.

3. Results

The water glyphosate concentration ranging from 2.01 until 13.9 mg a.p L^{-1} and the glyphosate reduction was 10.26–2.25%, 14.82–4.5%, 12.05–7.5% and 6.08–15% mg a.p L^{-1} (Table 1). The 15 mg a.p L^{-1} concentration of Roundup original® caused deaths in 50% of fish during the 96 h of exposure, the death situation that was not observed with other concentrations. The results of the oxidative stress biomarkers showed a significant increase in TBARS levels in the liver (53% at 7.5 mg a.p L^{-1} concentration) and muscle (81.5% at 4.5 mg a.p L^{-1} of herbicide) compared to the control group. In contrast, TBARS levels were not altered in the brain of fish over the entire range of herbicide levels tested. The exposure of the surubim to pesticide promoted a significant increase in the protein carbonyl levels in the liver (56% at 4.5 mg a.p L^{-1} of Roundup herbicide) and brain (21% at 2.25 mg a.p L^{-1} of herbicide) in comparison to the control group (Table 2). The protein carbonyl content of muscle was not changed compared to control groups after Roundup original® exposure (Table 2).

The SOD activity in the liver decreased (25.4%) after exposure to the 2.25 mg a.p L^{-1} of Roundup original® (Fig. 1A). The SOD activity in the brain was found to significantly decrease after exposure to 4.5, 7.5, 15 mg a.p L^{-1} of Roundup original® as compared to the control values (29.7%, 30% and 27.8%, respectively) (Fig. 1B). Hepatic CAT activity was decreased after exposure to 4.5, 7.5, 15 mg a.p L^{-1} of herbicide (32%, 36.9%, 36.3%, respectively) as compared to the control values (Fig. 2A). In addition, the CAT activity in brain also diminished after exposure to 4.5, 7.5, 15 mg a.p L^{-1} of herbicide (68.4%, 55.8%, 65%, respectively) (Fig. 2B).

With respect to non-enzymatic antioxidants, the ASA levels increased in surubim livers exposed to 2.25 and 4.5 mg a.p L^{-1} of Roundup original® (49% and 45%, respectively); ASA levels were also increased in the brains (14.4%) of fish exposed to 2.25 mg a.p L^{-1} of Roundup original®. GSH levels were found to increase in the livers of fish exposed to 2.25 mg L^{-1} of herbicide (35.8%) (Table 3). Accordingly, total thiols levels were also found to increase in brains of fish exposed to 7.5 mg a.p L^{-1} of herbicide (21.4%) as compared to the control values (Table 3).

4. Discussion

This is the first study analyzing the changes on the oxidative stress parameters of surubim, hybrid fish species, acutely exposed

Table 2
Levels of TBARS (nmol MDA mg^{-1} protein) and protein carbonyl (nmol carbonyl mg^{-1} protein) content in liver, brain, and muscle of surubim exposed to different concentrations of Roundup original® (mg a.p L^{-1}).

	Control	2.25 mg L^{-1}	4.5 mg L^{-1}	7.5 mg L^{-1}	15 mg L^{-1}	Statistics
TBARS						
Liver	12.23 ± 0.38	12.62 ± 1.47	12.92 ± 0.40	18.71 ± 2.04*	14.16 ± 2.65	P=0.0386 $F(4,25)$=2.998
Brain	31.41 ± 11.40	33.37 ± 8.02	43.90 ± 10.19	32.70 ± 3.95	NA	P=0.7176 $F(3,20)$=0.453
Muscle	12.29 ± 1.49	10.21 ± 1.19	22.31 ± 2.56*	17.20 ± 3.12	15.64 ± 2.11	P=0.0114 $F(4,25)$=4.023
Protein carbonyl						
Liver	9.04 ± 1.83	10.41 ± 0.67	14.06 ± 1.35*	10.71 ± 0.87	8.25 ± 0.85	P=0.0221 $F(4,25)$=3.459
Brain	10.28 ± 0.74	12.44 ± 0.33*	10.86 ± 0.42	10.07 ± 0.47	NA	P=0.0288 $F(3,20)$=3.742
Muscle	11.71 ± 1.28	10.80 ± 0.99	10.72 ± 0.72	10.63 ± 0.91	10.70 ± 0.99	P=0.9350 $F(4,25)$=0.202

Data represent the mean ± S.E.M. (n=6). Asterisks indicate a difference between groups and control values. We consider the $P < 0.05$. as the significant level. NA (not available).

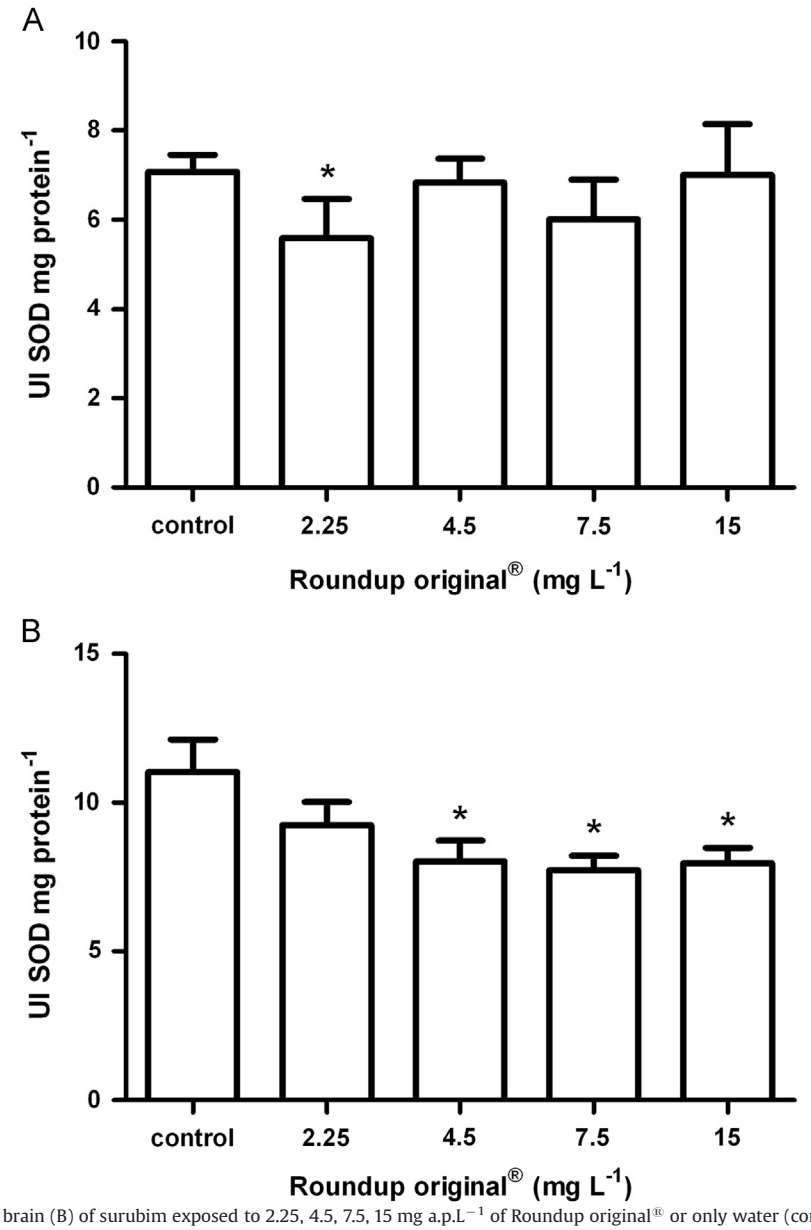

Fig. 1. SOD activity in liver (A) and brain (B) of surubim exposed to 2.25, 4.5, 7.5, 15 mg a.p.L^{-1} of Roundup original® or only water (control), for experimental period 96 h. Data are means ± S.E.M. ($n=6$). *Different from respective control, significant level of $P<0.05$.

to Roundup original®, attempting to verify toxic effects of this herbicide in hybrid fish. The residual glyphosate measured in water showed reduction of approximately 11% as compared with initial measured concentrations. Considering that the percent of reduction was observed only in water with fish (exposure period), the loss of herbicide suggests a possible absorption by the tissues of fish. Although, more studies are needed to monitor glyphosate and metabolites derived to it in water and fish tissues to confirm the presented hypothesis. In this study, the acute toxicity of Roundup original® on the surubim resulted in 50% fish death during 96 h of exposure at the 15 mg L^{-1} concentration used, although other concentrations did not cause such widespread death in the fish. Our results could be a clear indication that the hybrid fish is less resilient to glyphosate-based herbicide exposure than other fish species previously studied (96 h), such as *Leporinus obtusidens* that survived even at the highest concentrations of Roundup® tested (100 mg L^{-1}, 48% acid equivalent) (Glusczak et al., 2006). In line with this, Jiraungkoorskul et al. (2002) observed a variation of 2 until 55 mg L^{-1} in LC_{50} values to Roundup considering different fish species, life stage and test conditions. In addition, rainbow trout exposed to Roundup showed LC_{50} values of 52–55 mg L^{-1} (Hildebrand et al., 1982). The differences observed at different fish species could be attributed also to different Roundup® commercial formulations available around the world.

The increased levels of TBARS in the liver and muscle at 7.5 and 4.5 mg L^{-1}, respectively following Roundup original® exposure is a clear indication of LPO in the fish. However, TBARS levels in the brain did not exhibit any changes at the tested concentrations. The increased levels of TBARS in the liver and muscle following Roundup original® exposure were similar to those observed in *Rhamdia quelen* acutely exposed to the same herbicide (Menezes et al., 2011). On the other hand, Ferreira et al. (2010) observed that TBARS levels increased in the liver of *Rhamdia quelen* following exposure to tebuconazole and methyl-paration, but not to Roundup®. Our results indicated that acute exposure to sublethal concentrations of glyphosate-based herbicide causes oxidative damage in liver and muscle of surubim. The measurements of TBARS levels at different tissues could be proposed as biomarker to indicate lipid damage.

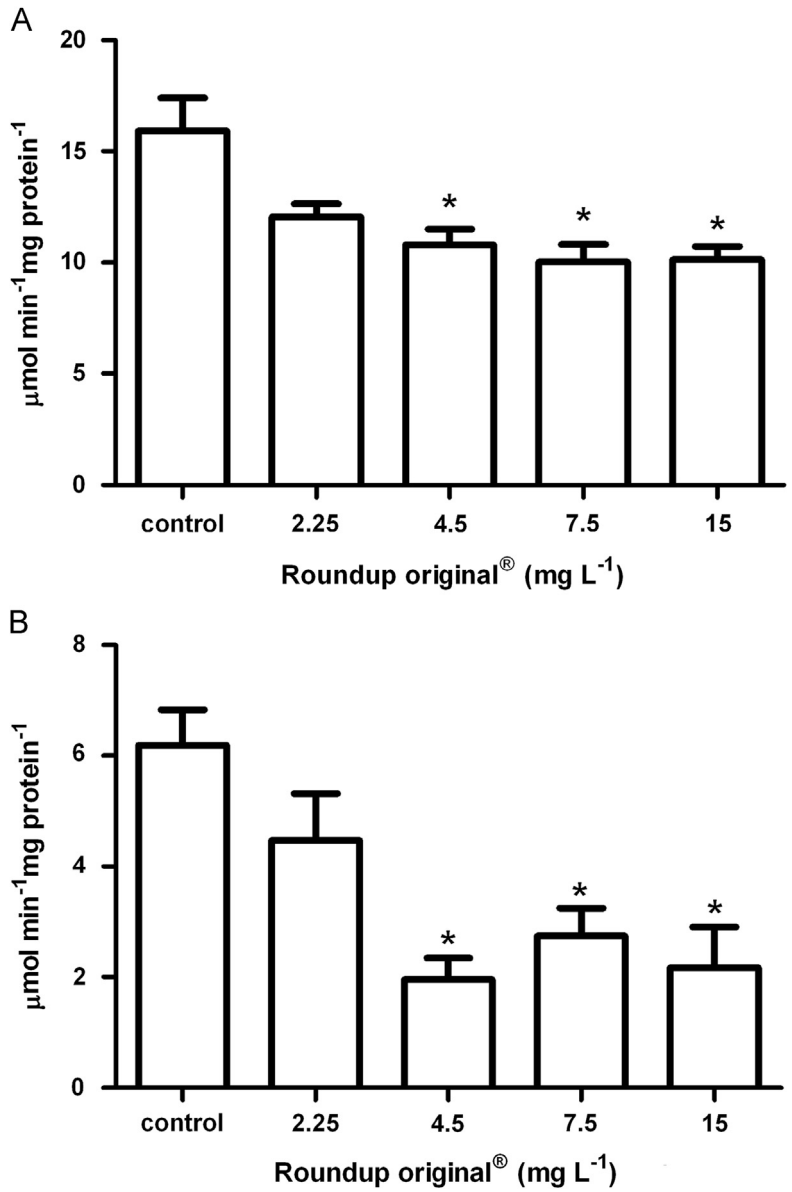

Fig. 2. CAT activity in liver (A) and brain (B) of surubim exposed to 2.25, 4.5, 7.5, 15 mg a.p L^{-1} of Roundup original® or only water (control), for experimental period 96 h. Data are means ± S.E.M. ($n=6$). *Different from respective control, significant level of $P<0.001$.

Table 3
Levels of ascorbic acid (µmol ASA g^{-1} tissue) and total thiols (mmol g^{-1} tissue) contents in liver and brain of surubim exposed to different concentrations of Roundup (mg a.p L^{-1}) original® (96 h).

	Control	2.25 mg L^{-1}	4.5 mg L^{-1}	7.5 mg L^{-1}	15 mg L^{-1}	Statistics
ASA						
Liver	0.40 ± 0.03	0.60 ± 0.04*	0.58 ± 0.02*	0.46 ± 0.02	0.50 ± 0.02	$P=0.0007$ $F(4,25)=6.959$
Brain	0.63 ± 0.02	0.72 ± 0.01*	0.65 ± 0.01	0.59 ± 0.02	NA	$P=0.0055$ $F(3,20)=7.028$
TOTAL THIOLS						
Liver	2.14 ± 0.07	2.88 ± 0.17*	2.53 ± 0.17	2.49 ± 0.09	2.55 ± 0.13	$P=0.0313$ $F(4,25)=3.267$
Brain	0.82 ± 0.02	0.93 ± 0.03	0.90 ± 0.04	1.06 ± 0.03*	NA	$P=0.0022$ $F(3,20)=7.844$

Data represent the mean ± S.E.M. ($n=6$). Asterisks indicate a difference between groups and control values, significant level of $P<0.05$. NA (not available).

In this study, acute Roundup original® exposure caused protein oxidation in liver and brain of surubim at 4.5 and 2.25 mg a.p L^{-1}, respectively. For other side, did not cause protein oxidation in muscle. These data suggest that the measurement of carbonyl groups may provide a convenient parameter for detecting and quantifying the oxidative modification of proteins during oxidative stress induced by herbicide in fish. In line with our work, Menezes et al. (2011) also observed an increase in protein carbonyl in the liver of *Rhamdia quelen* exposed to glyphosate herbicide. Glusczak et al. (2011) working with *Leporinus obstusidens* also found liver

protein carbonyl increased after Roundup® exposure. The assay was performed as in the present study at 96 h. Differently, Li et al. (2010), using another pesticide class, observed increased protein carbonyl content in brain of rainbow trout *Oncorhynchus mykiss* exposed to propiconazole. There are few studies about the effects of glyphosate on the protein carbonyl content in fish, and the available literature consider the liver the main site of protein carbonyl production. The Roundup original® herbicide may induce oxidative stress, thereby increasing free radical production that could participate to the oxidative damage of lipids and proteins in tissues from surubim acutely exposed to Roundup original®, contributing to an overall mechanism for the herbicide toxicity. Considering that protein carbonyl formation is non-reversible and result in conformational changes to protein structure (Zhang et al., 2008), it can be suggested that carbonyl formation could be a cause of the loss of activity and breakdown of antioxidant enzymes.

Among the key enzymes involved in detoxifying the ROS of various organisms, it can be cited SOD and CAT. In the present study, all tested concentrations of Roundup® caused a decrease in SOD activity in the liver and brain of surubim. Decreased SOD activity was also observed by Modesto and Martinez (2010a, 2010b) in liver from *Prochilodus lineatus* exposed to Roundup® and Roundup Transorb. Lushchak et al. (2009) observed SOD activity decrease in liver and brain of *Carassius auratus* exposed to Roundup. Our study demonstrated that the activity of CAT was significantly decreased after exposure to 4.5, 7.5, 15 mg a.p L^{-1} of herbicide, in both the liver and brain. These results are in agreement with Ferreira et al. (2010), who reported liver catalase inhibition in *Rhamdia quelen* following exposure to glyphosate. In addition, CAT activity in liver was also inhibited in *Prochilodus lineatus* following exposure to Roundup Transorb (Modesto and Martinez, 2010b). This decrease in both CAT and SOD activities could be explained by the increased flux of superoxide radicals and H_2O_2, respectively, which have been previously reported to inhibit enzymatic activity (Lushchak et al., 2009). Specifically changes in the present study could be related with the increase of protein carbonyl that is responsible to changing protein and then reducing enzyme activities. This fact could explain the reduced activity of SOD and CAT in tissues of surubim after glyphosate exposure. Taken together, the impaired activities of CAT and SOD suggest a disruption of normal antioxidant response in liver and brain of suburim exposed to Roundup®. These antioxidant enzymes are essential for the conversion of ROS to harmless metabolites and may be activated or inhibited under chemical stress.

With respect to non-enzymatic antioxidants, the levels of ascorbic acid were increased in liver and brain of acutely-exposed surubim to Roundup original®. It has been observed that when normal enzymatic defenses are stressed, others non enzymatic defense such as ASA and total thiols levels are activated preventing auto-oxidation reactions. ASA has long been recognized as an essential factor to oppose against some of the toxic effects of oxygen radicals (Sayeed et al., 2003). In the present study, ASA levels were increased in the liver and brain of herbicide-exposed surubim. Interestingly, most teleosts are unable to synthesize ASA because of the lack of L-gulonolactone oxidase (EC 1-1-3-8) (Fracalossi et al., 2001). Therefore, an exogenous source of ASA is required in fish diets. The elevated levels of ASA found in surubim probably can be due the vitamin stored. Elevated ASA levels have been also reported in the livers of *Rhamdia quelen* exposed to glyphosate, methyl-paration and tebuconazole (Ferreira et al., 2010) or clomazone (Menezes et al., 2011). Sayeed et al. (2003) observed an increase in the ASA levels in the kidney and liver of the *Channa punctatus* after deltamethrin exposure.

In the present study, the levels of total thiols were found to increase in the liver and brain of fish acutely exposed to Roundup®. Glyphosate increase GSH levels in liver of *Rhamdia quelen* (Ferreira et al., 2010). The variations in non protein thiol were observed in different fish species and glyphosate treatments as follow: Lushchak et al. (2009) found reduced levels of total thiols by 26–29% in brain and liver of goldfish after glyphosate treatment. Similar results were recorded by Menezes et al. (2011) where liver non protein thiols decreased when *Rhamdia quelen* were exposed to 0.95 mg a.p L^{-1} of Roundup®. In the present study total thiols and ASA increased levels could be important to improve antioxidant defenses of surubim.

5. Conclusion

This is the first study that investigates the toxicity of commercial formulation containing glyphosate in hybrid fish. We concluded that surubim is very sensible at the pesticide because it presented alterations in biomarkers of oxidative stress, such as TBARS and carbonyl protein, in different tissues, as well as in important SOD and CAT enzymatic antioxidants. This study also pointed out the important role of non-enzymatic antioxidants ASA and total thiols to protect glyphosate-induced damage in specific tissues. The results of this study could suggest that the levels of TBARS and protein carbonyl can be used as early biomarkers of exposure to glyphosate.

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