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TOXIC EFFECT OF GLYPHOSATE-BASED HERBICIDE ON ANTIOXIDANT DEFENSE SYSTEM AND HEAT SHOCK PROTEIN 70 EXPRESSION IN *Monopterus cuchia* (Hamilton)

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ABSTRACT

The present study was aimed to evaluate the toxicological effect on antioxidant defense system in *Monopterus cuchia* induced by commercial formulation of Glyphosate EXCEL MERA 71. In the study, fish were exposed to sub-lethal concentrations of 1 and 2 mg L^{-1}. LC_{50} value for glyphosate at 96 h exposure could not be determined because no fish mortality was observed even at the maximum concentration tested 200 mg L^{-1}. After 6, 24, 96 and 168 h of exposure, there were significant decrease in the level of red blood cells, haemoglobin concentration and a significant increase in white blood cells count in both treated groups. The enzyme alanine aminotransferase decreased whereas aspartate aminotransferase and alkaline phosphatase showed increase in comparison to control which were analyzed in serum. The fish showed transient decrease in superoxide dismutase and catalase activities and the non-enzymatic antioxidant reduced glutathione showed repressed activity after glyphosate exposure. However, glutathione-S-transferase activity significantly increased in kidney and intestine and decreased in liver in treated groups. Moreover, the present study also investigated the possible stimulation of heat shock protein 70 (Hsp70) mRNA and glyphosate exposure led to a significant increase in the levels of mRNA transcript in liver and kidney.

Key words: Antioxidant, glyphosate, Hsp70, hematology

INTRODUCTION

The continuous discharge of agricultural effluents into water bodies has led to the accumulation of a wide variety of toxic pollutant (Mason, 1999). Different types of structural and cellular alteration of cells, tissues and physiology of aquatic organisms are the hallmark of toxicological stress response (Parvez and Raisuddin, 2005). Herbicides when present in aquatic system contaminate fish and aquatic plants and animals (Sarikaya and Yilmaz 2003; Fonseca *et al*., 2008). Owing to the continuous use of certain chemicals, the chemicals having same mode of action or belonging to the family create selection pressure and enhance weed races inducing a shift in weed population (Owen *et al*., 2008). Herbicides applied on fields are absorbed by crop plants and weeds. Most herbicides are entirely metabolized while others like paraquat, dinoseb-acetate, etc. are not (Hatzios and Penner, 1982; Pline and Hatzios, 2003). The herbicide residues are sometimes retained in plant materials especially applied late crop growth stages. Some herbicides pollute groundwater through leaching (Vizantinopoulos and Lolos, 1994). Leaching of herbicides may be high with salt formulated herbicides used or in treated sandy soils under heavy irrigation or rain.

Among the most extensively used broad-spectrum herbicides, glyphosate herbicides are of great concern due to their frequent use on corn and soybean crops which are modified by genetic engineering to tolerate it. The major commercial formulation of glyphosate contains active component N-(phosphonomethyl) glycine, in which glyphosate is formulated as isoproylamine salt and a surfactant, POEA, escalating the efficacy of herbicide (Tsui and Chu, 2004; Releya 2005). Glyphosate may be released into surrounding environment by agriculture, anthropogenic or industrial activities and are commonly detected in overland runoff and bed sediment in rural suburban/ urban areas due to close proximity or rate of recurrence of their use or their association with sewage and domestic sewage, causing the risks of human and environmental accumulations (Li *et al*., 2016). Glyphosate-based herbicides could cause hepatorenal, teratogenic and tumorigenic effects by endocrine disruption and oxidative stress (Mesnage *et al*., 2015). Fish in general is an excellent bioindicator of pollutant contamination as their biochemical responses are analogous to those found in other mammals. Fish species are inadvertently affected by glyphosate contamination such as Pseudoplatystoma sp. (Sinhorin *et al*., 2014), *Prochilodus lineatus* (Langiano and Martinez, 2008), *Cyprinus carpio* (Cattaneo *et al.*, 2011) and *Rhamdia quelen* (de Menezes *et al*., 2011).

Hematological parameters like haemoglobin (Hb) content, number of red blood cells (RBC) and white blood cells (WBC) provide toxicity indicators in assessing the ecotoxicological impact on aquatic organisms (Barcellos *et al*., 2003). Glyphosate containing herbicides induce reactive oxygen species (ROS) formation, such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (OH) or other nitrogen species which cause imbalance between pro-oxidant and antioxidant defence mechanisms and ensue oxidative damage (Modesto and Martinez, 2010a; Glusczak *et al*., 2011). Fish has an antioxidant defence system, composed of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and also a detoxificant enzyme glutathione-S-transferase (GST) for neutralizing ROS (Modesto and Martinez, 2010a; de Menezes *et al*., 2011). As a result of oxidative damage, lipid peroxidation indicates alterations in cellular membrane, cohesion, flow, permeability and metabolic role thus leading to cellular instability with subsequent cell damage and death (Langiano and Martinez, 2008; de Menezes *et al*., 2011). Other alternatives for observing exposure to pesticides in fish are through laboratory analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alanine aminotransferase (ALP) because these enzymes specify liver damage or hepatotoxicity (Ferreira *et al*., 2010).

Organisms have evolved diverse mechanisms to support their uninterrupted adaptation and physiological acclimation to natural environmental variations and stress responses, including the creation of stress proteins called heat shock proteins (Wang *et al*., 2007a). Hsp reportedly are prospective biomarkers under environmental stress in fish (Iwama *et al*., 1998; Sinha *et al*., 2012). When cells are exposed to stresses like toxic metal contamination, hypoxia/anoxia, chemical shock, salinity stress, thermal and transportation stress, rapid amplification in abundance of Hsps improve cell viability by minimizing harmful effects of these stressors (Gornati *et al*., 2004; Wang *et al*., 2007b; Poltronieri *et al*., 2008). Glyphosate harmfully affect the aquatic ecosystem so this emphasizes on having proper remedial measures. Using of ecofriendly herbicide control the weed population in specified crop. Moreover, during hot weather conditions high volatile herbicides should be avoided, since chemical drift in vapour form or crop damage is highly possible. Instead less or non-volatile forms are recommended. In addition, to keep weed population under control herbicide rotation is essential; any variation from this system may result in weed population shift, development of weed races, crop- relative weeds and herbicide resistance.

It is appealing to note that glyphosate toxicity has almost not been conducted on *M. cuchia*, which has been frequently used as a model animal for eco-toxicological studies since it can easily acclimatize to laboratory condition. The fish inhabit low lying areas like swamps, paddy fields and shallow areas of ox-bow lakes. Thus, in order to gather additional information about the peril imposed by the commercial formulation of glyphosate to *M. cuchia*, this work was aimed to assess the toxicity effect induced by glyphosate and to evaluate the responses of this fish at haematological and biochemical levels after acute exposure to sub-lethal concentrations of herbicide.

MATERIALS AND METHODS

Animals

The study was performed in the year 2017. Healthy fishes (mud eel, *Monopterus cuchia*) {200±10 g body mass} were collected from a single local commercial source in Guwahati (India). The collected species of fish were then acclimatized in laboratory for 1 month at 28±2ºC in a glass aquarium, using tap water (pH, 7.4±0.1), and exposed to 12**:**12 h day/night photoperiods before use in experiments. Dried fish powder and rice bran were given as food; and water was consistently changed on alternate days. Fish were used for experiment when mortality rates became zero and food consumption was normal. Food was withdrawn 24 h prior to experimentation.

Experimental design

The commercial formulation of Excel Mera-71 (Excel Corporation Care Ltd., India), a systemic herbicide (ammonium salt of glyphosate 71) and 12.5% POEA as a surfactant, was evaluated at two sub-lethal concentrations of 1 and 2 mg L^{-1}. The concentration selection was based on acute toxicity test. For this, five fish, irrespective of their sex, were placed in 20 L plastic buckets filled with dechlorinated and constantly aerated water. Toxicity test was run during each experimental period (6, 24, 96 and 168 h) in which one group of fish (N = 10) was exposed to water only (control), while the other 5 groups were exposed to 30, 50, 70, 140 and 200 mg L^{-1} of ammonium salt of glyphosate. All toxicity tests were conducted in duplicate following the OCED guidelines (OECD, 1992). during experiment, water parameters were monitored continuously. The temperature and pH were maintained at 28±2ºC and 7.4±0.1, respectively. The LC_{50} for glyphosate at 96 h could not be determined, because no fish mortality was observed even at the maximum concentration of 200 mg L^{-1}. Therefore, experimental roundup concentrations were selected considering that the concentration range of 0.36-2.16 mg L^{-1} is used in agriculture (Rodrigues *et al*., 1998). Thus, sub-lethal concentration (1 and 2 mg L^{-1}) were chosen for this study. After 6, 24, 96 and 168 h exposure, the animals in each plastic bucket were sampled. The fish were removed from plastic bucket, straight away anesthetized with benzocaine (0.1 g L^{-1}) and blood drawn immediately from caudal vein with the help of a heparinized syringe for estimating haematological parameters. The fish were killed by removal of spinal cord at the back of operculum and tissues (liver, kidney and intestine) were quickly removed and placed on ice and dipped in liquid nitrogen and frozen at -80ºC for further use.

Hematogical parameters

RBC and WBC count in blood samples. after diluting in Hi-media RBC and WBC diluting fluid (Grower's), were made using Improved Neubauer Haemocytometer. The RBC and WBC counts were calculated as 10^6 mm^{-3} and mm^3 x 10^3 (Wintrobe, 1967). Whole blood Hb was estimated using Sahli´s hemoglobinometer. The serum ALT and AST were measured as per the method of Reitman and Frankel (1957) and enzymes expressed in U L^{-1}. The ALP in serum was measured as per King's method (Hansen, 1966) and expressed in U L^{-1}.

For enzyme assay, the tissue samples (liver, kidney and intestine) were weighed, crushed in liquid nitrogen, homogenized (10% volume) in 20 mM tris HCL buffer (pH = 7.4) and triton X, centrifuged (25 min, 15000 g at 4ºC). The supernatant obtained was analyzed for biochemical parameters. SOD activity was assayed as per Flohe and Otting (1984) which is based on the method of measuring the inhibition of decreased rate of cytochrome c by the superoxide radical at 550 nm. SOD activity was expressed in U SOD mg $protein^{-1}$, with one unit of SOD equivalent to the quantity of enzyme that promoted the inhibition of 50% of reduction rate of cytochrome c. Catalase activity was estimated as per Beutler (1975) and the rate of breakdown of H_2O_2 was monitored from decrease in absorbance at 240 nm (Systronics UV-VIS Spectrophotometer 117) and the activity expressed in µg mL^{-1} min^{-1}. GST activity was ascertained as per Keen *et al.* (1976) following the

complexation of GSH with 1-chloro-2, 4-dinitrobenzene. The change in absorbance was recorded at 340 nm and the activity expressed as µg mL^{-1} min^{-1}.

For non-enzymatic antioxidant assay, GSH level was estimated as per Beutler *et al.* (1963), Monterio *et al*. (2009) and Thomaz *et al*. (2009) using 5, 5-dithiobis-2-nitrobenzoic acid (DTNB). The supernatant of extract was added to 0.25 mM DTNB in 0.2 M potassium phosphate buffer (pH 8.0) and the formation of thiolate anion was estimated at 412 nm against a reduced glutathione (GSH) standard curve. The GSH content was expressed as µg g^{-1} wet tissue weight.

RNA extraction and cDNA synthesis

Total RNA was isolated from 50 mg each of liver and kidney tissues by means of TRI reagent (Sigma) as per Rio *et al.* (2010). The RNA was further purified using the RNeasy MinElute™ Cleanup Kit (Qiagen, Germany). Purified RNA was quantified using QIAxpert (Qiagen, Germany), and integrity was verified by electrophoresis on 1% agarose gel. First-strand cDNA was synthesized from 400 ng total RNA (DNase I treated, Invitrogen, USA) in a total volume of 20 µL with Verso cDNA synthesis kit (Thermo Scientific, USA) as per the manufacture's protocol.

Quantitative real-time PCR (qPCR)

The qPCR was performed in a StepOnePlus Real-Time PCR (Applied Biosystems, USA) with DyNAmo™ Flash SYBRGreen qPCR kit (Thermo Scientific). The reaction mixture of 10 µL each contained 5 µL SYBR Green/ROX PCR Master Mix, 0.5 µL cDNA, 0.5 µL each primer and 3.5 µL MilliQ H_2O. The assay conditions were an initial denaturation at 95ºC for 7 min, followed by 40 cycles at 95ºC for 10 sec, 60ºC for 25 sec and 72ºC for 30 sec. After this, melt curve analysis was done to assess amplification specificity post PCR with fluorescence acquisition transition rate of 1º per step. The qPCR was performed in triplicate, and negative controls with no cDNA were also run for each gene. Melting curve analysis was done to reconfirm amplification of only a single PCR product. Fold changes of hsp70 gene in treated and untreated controls were calculated using modified delta-delta CT method (Livak and Schmittgen, 2001). The primer pairs used were forward 5'–3'CACTGCTTGCGAGAGGGCCA and reverse 5'–3' GCCCTGGGGAGTGAGGTGT A (Hangzo *et al*. 2017).

Statistical analysis

The experiments were conducted in duplicates. All haematological and enzymatic parameters were presented as mean± SE. All values were compared statistically with control using one-way ANOVA with the help of Microsoft Excel and ORIGIN 6.1. The level of significance was established at $P_{0.05}$.

RESULTS AND DISCUSSION

In present study, haemato-biochemical parameters were assessed to explore the potential toxic effects of long-term glyphosate exposure on *Monopterus cuchia*. Glyphosate exposure upset the hematological parameter where a significant decrease in Hb and RBC count and elevation in WBC count was observed. As a result, the antioxidant capacity of organisms was reduced as evidenced by decreased levels of SOD, GST and GSH. The study revealed that glyphosate destroyed the internal metabolic networks of tissues. Significant perturbations in oxidative stress were observed in glyphosate dosed fish, which were associated with the toxicity of glyphosate.

Haematological parameters of M. cuchia exposed to glyphosate

The haematological parameters i.e. RBC, WBC and Hb of *M. cuchia* are shown in Table 1. The alterations observed in haematological parameters were significant over control. Significant variants were observed between various haematological parameters with different concentrations of herbicide (1 and 2 mg L^{-1}) after 6, 24, 96 and 168 h exposure. The study revealed a decrease in Hb and total erythrocyte count when *M. cuchia* was exposed to 1 and 2 mg L^{-1} of glyphosate as compared

Table 1: Blood parameters of *Monopterus cuchia* exposed to ammonium salt of glyphosate

Parameters	Exposure time (h)	Control	Concentration of glyphosate	
			1 mg L^{-1}	2 mg L^{-1}
RBC ($x10^6$ mm^{-3})	6	6.29 ± 0.110	5.17 ± 0.341*	2.94 ± 0.381*
	24	6.89 ± 0.225	5.86 ± 0.335*	3.96 ± 0.381*
	96	7.64 ± 0.046	5.90 ± 0.035*	3.63 ± 0.065*
	168	8.34 ± 0.173	6.60 ± 0.050*	4.90 ± 0.029*
WBC ($x10^6$ mm^{-3})	6	5.76 ± 0.047	7.73 ± 0.062*	9.86 ± 0.067*
	24	5.96 ± 0.024	7.82 ± 0.035*	9.98 ± 0.006*
	96	6.39 ± 0.043*	9.69 ± 0.032*	11.79 ± 0.029*
	168	8.95 ± 0.021	12.93 ± 0.032*	14.41 ± 0.052*
Hb (g dL^{-1})	6	16.00 ± 1.200	12.50 ± 1.50*	11.82 ± 0.507*
	24	16.60 ± 1.300	13.97 ± 1.098*	8.00 ± 1.800*
	96	18.33 ± 0.822	15.00 ± 0.577*	12.93 ± 0.032*
	168	23.20 ± 0.857	19.00 ± 0.577*	14.32 ± 0.708*

" * " = Significantly different at $P_{0.05}$ level as compared to their controls, determined by one way ANOVA).

to control. Similar findings have been reported by Glusczak *et al*. (2006) on *L. obtusidens* exposed to different concentrations of Roundup original (3, 6, 10 and 20 mg L^{-1}) which were similar to those of Jerald Felix *et al.* (2015) who found a significant decrease in total number of erythrocytes, Hb, hematocrit, mean corpuscular volume (MCV), and platelet count on *Catla catla* exposed to Glyphosate Roundup (41%). However, present study disagrees with Kathya *et al*. (2010) who reported increase in hematocrit and erythrocytes on *Prochilodus lineatus* exposed to the concentration of Roundup Transorb (RDT). Reduction in RBC count observed in this study may be attributed to the destructive action of herbicides on peripheral red cell thus affecting the cell viability (Farlaner and Smith, 1981). This leads to anaemia, induced by herbicide glyphosate, due to the hydrolysis of acetylcholine in body fluids by cholinesterase in erythrocytes thereby showing detrimental effects on erythrocytes in terms of decrease in red cell production.

Significant increase in total WBC count was found in this study when *M. cuchia* was exposed to glyphosate. The increase in WBC is line with Jerald Felix *et al*. (2015) in *C. catla* and Kathya *et al*. (2010) in *P. lineatus* exposed to Glyphosate Roundup (41%). The possible reason for increase in the number of leucocytes in present study in comparison to control group might be the development of immunological response. An increase in WBC count in treated fish suggests that fish immune system tried to become stimulated and get adapted to the stress induced by herbicide (Jerald Felix *et al*., 2015).

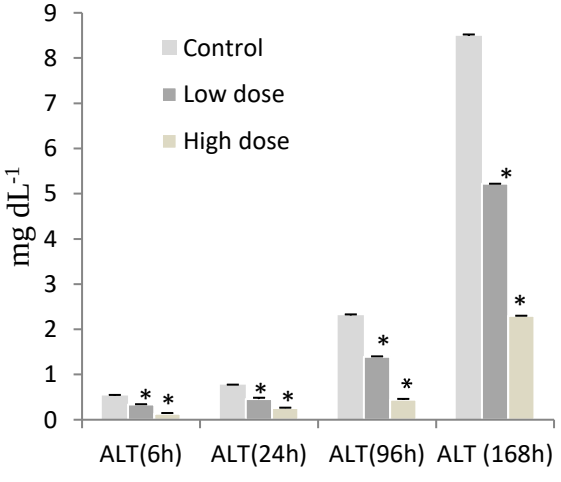

Fig. 1: ALT activity in the serum of *Monopterus cuchia* exposed to glyphosate; Values are significant at $P_{0.05}$. ("* ": significantly different at $P_{0.05}$ level as compared to their controls.

A set of biomarkers is essential for considering the reaction of an organism when exposed to herbicides. Thus, biomarkers that signify oxidative damage, antioxidant enzymes and plasma enzymes were included in this study. These biomarkers may forecast the toxicity of compound and be helpful in aquaculture and ecological peril evaluation.

Transaminases activities

The ALT activity was inhibited at both test concentrations of glyphosate. Serum ALT level for 6, 24, 96 and 168 h decreased by 1.58, 1.69, 1.66 and 1.61 folds in low dose and by 3.96, 2.99, 5.2 and 3.7 folds for high dose over control, respectively (Fig. 1). These findings are

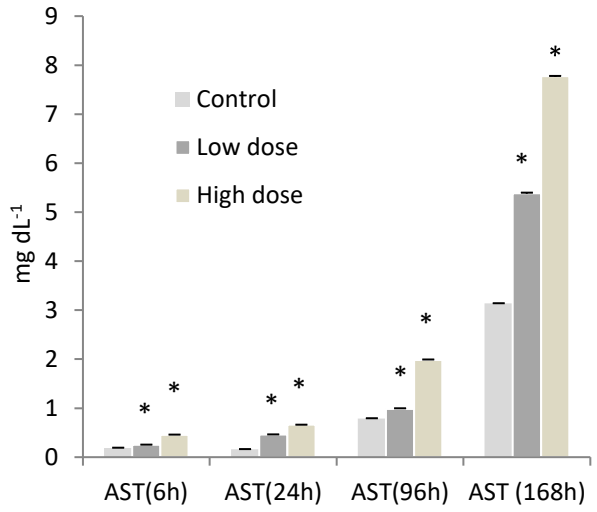

Fig. 2: AST activity in the serum of *Monopterus cuchia* exposed to two concentrations of glyphosate. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

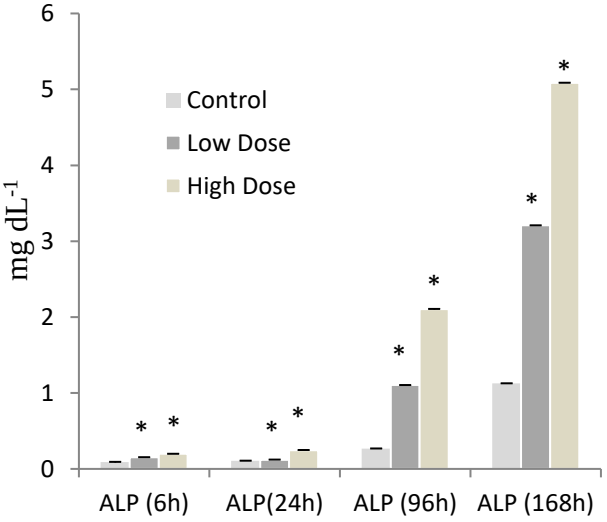

Fig. 3: ALP activity in serum of *Monopterus cuchia* exposed to two concentrations of glyphosate. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

found decreased ALT activity in the plasma of silver cat fish (*R. quelen*) exposed to different glyphosate formulations. Therefore, decrease in ALT activities in the serum of *M. cuchia* is mainly due to the deficiency of accessible amino acids, leading to decline in alpha keto acids (Camila *et al*., 2016).

The AST activity in serum increased by 1.33, 2.85, 1.25 and 1.7 folds ($P_{0.05}$) in low dose and by 2.4, 4.0, 2.5 and 2.5 folds in high dose as compared to the control in all treatment groups (Fig. 2). Similar findings have been reported by Camila *et al*. (2016) exposed to glyphosate. Increased AST activity may be the indication of damage to hepatocytes where excessive production of enzyme occurs, or may be as a result of necrosis leading to the disruption of membranes, releasing them into the blood (Camila *et al*., 2016).

Alkaline phosphatase activity

In present study, ALP activity significantly increased in serum by 1.7, 1.12, 4.0 and 3.0 folds ($P_{0.05}$) in low dose and by 2.0, 2.3, 7.9 and 4.5 folds ($P_{0.05}$) in high dose when compared to control value **(**Fig**.** 3). Similar findings have also been reported by Okonkwo *et al*. (2010) in cat fish *Clarias albopunctatus* exposed to glyphosate. Cholestasis is one of the possible reasons in the elevation of serum ALP concentration (Ejike *et al*., 2008). Such increases in markers of toxicity may be as a consequence of stepped-up transamination in effort to survive with the energy crisis during exposure to hassle as a result of a toxicant (Philips and Rajasree, 1996; Okonkwo *et al*., 2010).

Enzyme assay

The undesirable effects of ROS are effectively unbiased by an antioxidant defense system and a well-known balance is maintained between pro-oxidant and antioxidant processes. Fish have evolved an enzymatic antioxidant defense mechanism which comprise of SOD, CAT and many other molecules so as to reduce oxygen radical formation, one of the potential ways to reduce adverse effects of ROS (Zelikoff *et al*., 1996).

Catalase and SOD activity

CAT and SOD activities were significantly low in liver, kidney and intestine of the groups (6, 24, 96 and 168 h) exposed to low and high concentrations of herbicide as compared to control (Fig. 4

and 5). Decrease in these primary antioxidant enzymes is in tune with Kathya *et al*. (2010) and Modesto and Martinez (2010a) who observed inhibition in liver CAT activity in *P. lineatus* exposed to RDT and surfactant POEA. However, these findings disagree with do Carmo Langiano and Martinez (2008) and Navarro *et al*. (2014) who found enhanced hepatic SOD and CAT activities in *P. lineatus* exposed to POEA and a glyphosate-based herbicide for a specific time period.

In present study decreased SOD and CAT activities signify that large amounts of H_2O_2 are generated which contributed to decrease in SOD activity and induced more generation of superoxide anion responsible for decreased CAT activity (Bagnyukova *et al*., 2006). Similarly, superoxide ions failed to be neutralized efficiently by SOD thereby leading to the reduction in CAT activity. Antioxidant enzymes like CAT-SOD provide first line defense against oxidative actions due to the inhibitory effects on the development of oxyradicals (Pandey *et al*., 2003) and these enzymes are used as biomarkers, indicating the generation of ROS (Monteiro *et al*., 2006).

GST activity

GST is an important antioxidant enzyme involved in catalyzing the conjugation of diverse electrophilic metabolites to reduce the activity glutathione thereby protecting cells against the action of xenobiotics (Ferrari *et al*., 2007). In present study, hepatic GST activity decreased significantly in

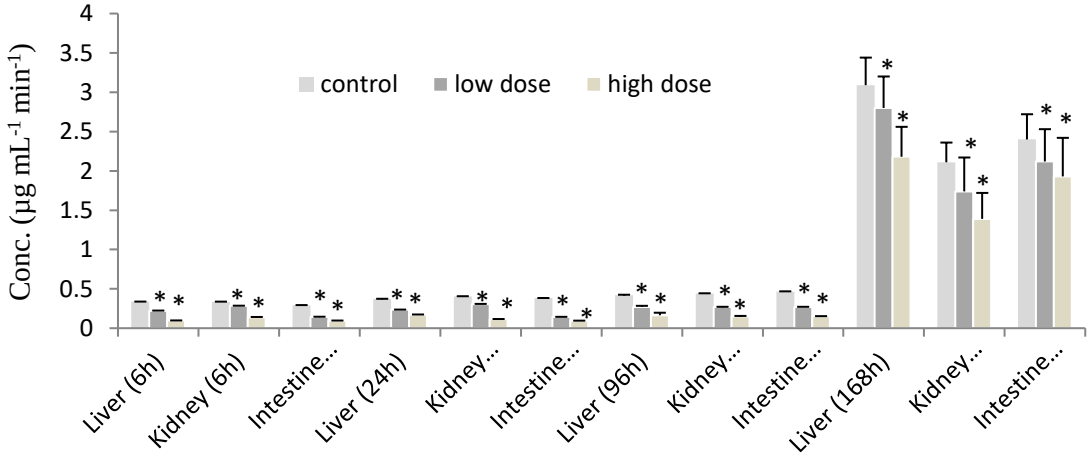

Fig. 4: CAT activity in liver, kidney and intestine under control and different concentrations of glyphosate exposure. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

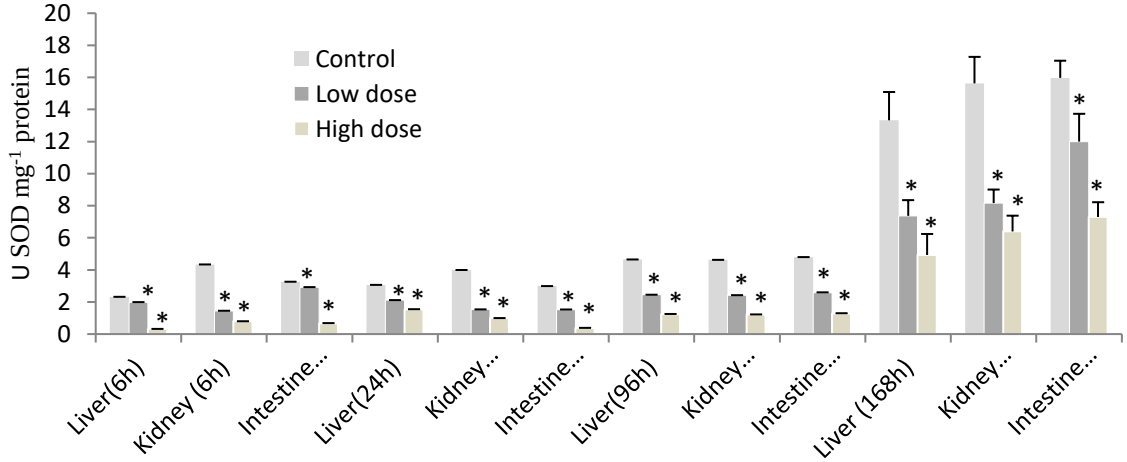

Fig. 5: SOD activity in liver, kidney and intestine under control and glyphosate exposure. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

fish exposed to 1 and 2 mg L^{-1} glyphosate but considerably increased in kidney and intestine in all the groups exposed to both the concentration of glyphosate (Fig. 6). The results are in line with de Menezes *et al*. (2011) who found decreased GST in liver of *R. quelen* exposed to RDT. Decreased GST activity suggest that the existence of oxidants leads to inactivation of enzymatic activity (Bagnyukova *et al*., 2006) revealing that GST is responsive to products of Haber-Weiss reaction (Hermes-Lima *et al*., 1993). However, since there was an increase in GST activity in intestine and kidney in glyphosate treated fish, it may be concluded that the exposed fishes tried to show some degree of defense against the herbicide.

Non-enzymatic antioxidant assay

A decreased GSH level in all the tissues of fish exposed to both the concentrations of glyphosate in all the treated groups (Fig. 7). This finding disagrees with the results reported by Kathya *et al*. (2010) who observed a momentary decrease after 24 h exposure followed by an increase in fish *P.lineatus* exposed to the highest concentration of the herbicide(5mg/L of RDT). Decreased GSH activity observed in our present study work may be due to its increased consumption and its conversion into oxidized glutathione and an inefficient GSH regeneration (Monteiro *et al*., 2006) thereby producing an oxidative imbalance leading to the oxidative damages of cells. Moreover,

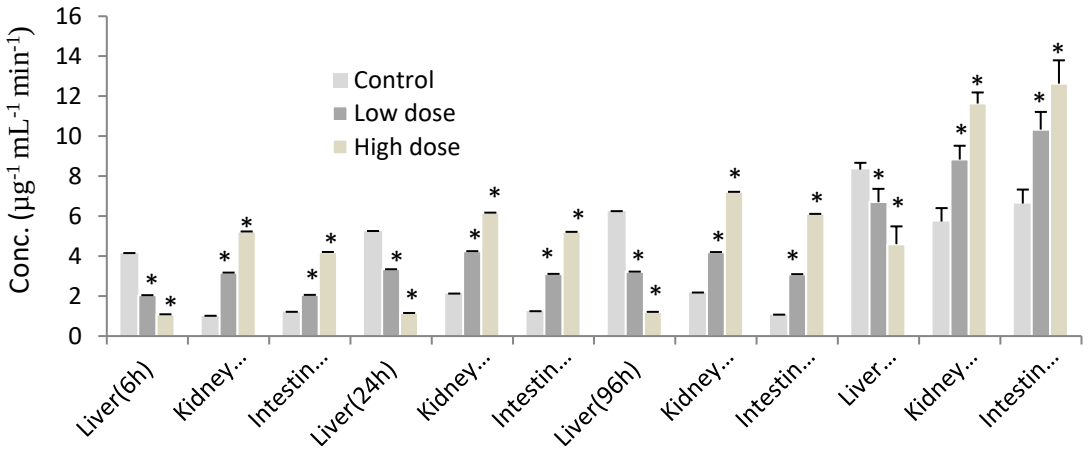

Fig. 6: GST activity in liver, kidney and intestine under control, and different concentrations of glyphosate exposure. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

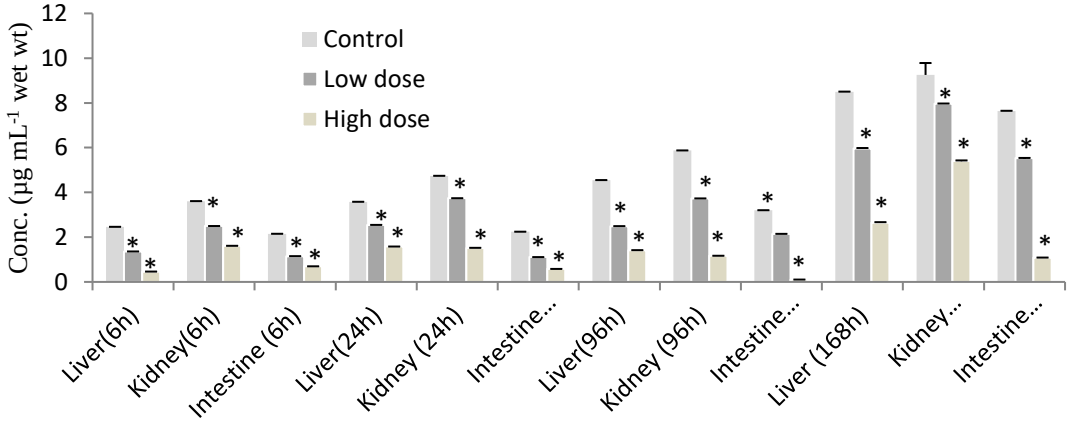

Fig. 7: GSH activity in liver, kidney and intestine in control and glyphosate exposed fish. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

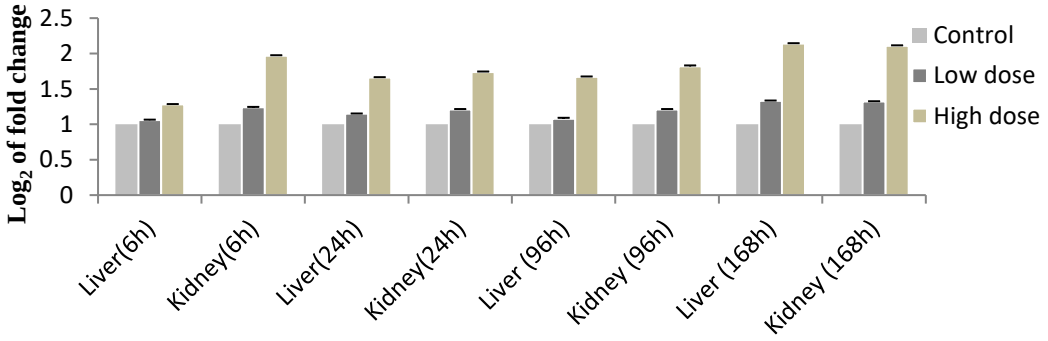

Fig. 8: qPCR analysis showing the levels of relative expression of mRNAs of hsp70 gene in different tissues of mud eel following glyphosate exposure. Values are significant at $P_{0.05}$. ("* ": Significantly different at $P_{0.05}$ level as compared to respective controls)

glutathione, being the major non-protein thiol of cells, is involved in providing cellular defense mechanism against the toxic action of oxyradicals (Schuliga *et al.*, 2002). This low molecular mass thiol can be effectively oxidized serving as a sink for free radicals and other reactive species (Hermes-Lima, 2004).

Expression patterns and changes in the abundance of Hsp70 protein

Glyphosate stress led to a significant increase in the abundance of hsp70 mRNA transcript in both l tissues (liver and kidney) after 6, 24, and 96 h exposure, followed by an additional increase till 168 h. It increased maximally by 1.06, 1.15, 1.08 and 1.33 folds (6, 24, 96 and 168 h) in low dose in liver ($P_{0.05}$), 1.28, 1.66, 1.67 and 2.14 folds in high dose in liver; 1.24, 1.21, 1.21 and 1.32 folds in low dose in kidney, 1.97, 1.74, 1.82 and 2.11 folds (6, 24, 96 and 168 h) in high dose in kidney as compared to control (Fig. 8). Hsp70 is the mostly expressed protein and enables the fish to adapt to ecological stressors together with temperature and osmotic stress and exposure to a multiplicity of xenobiotic compounds. Hsps trigger immune response through activities that occur both inside the cell (intracellular) and outside the cell (extracellular).

Conclusion: The present study revealed that the use of glyphosate herbicide is toxic to *Monopterus cuchia* and causes hematologic and enzymatic changes in different organs like liver, kidney and intestine. Such hindrance noticeably has deleterious fitness consequences for individual fish, and in long run may negatively affect fish population. The information has a great significance in terms of preservation, considering that glyphosate-based herbicides are prevalent at sublethal concentration in natural environments. Therefore, the use of herbicide on/close to fish farms or in the area near to aquatic surroundings should be discouraged.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical statement: The study was approved by the Institutional Animal Ethics Committee (IAEC) Ref: IAEC/ PER/ 2017/ RF/ BBC/ AS/ 2016-28 of Gauhati University, Guwahati, India.

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