Original Research Article

Effects of Altered pH on Antioxidant Defence System of *Monopterus cuchia* (Hamilton, 1822) in Ex-situ Modulated Environment pH Condition.

Amilee Konwar, Manas Das*, Debjani Dey, Priyambada Chutia
Department of Zoology, Animal Physiology and Biochemistry Laboratory, Gauhati University, Guwahati, Assam, India.
Dr. Manas Das, Assistant Professor, Gauhati University
Correspondence: manasdasne@gmail.com
Received: April 11, 2018; revised: May 22, 2018; accepted: May 27, 2018

Abstract: Water pollution is brought about mostly by industrial, domestic and other such anthropogenic activities. Whatever be the source of the pollutants, the effect is widespread in the water body and its biotic components. The first effect of pollutants may have on water is to change its natural pH to either a more acidic or alkaline pH. The present study investigated the effect of experimentally maintained acidic (pH 6.4) and basic (pH 8.0) conditions on the activities of alkaline transferase (ALT), aspartate transaminase (AST), the anti-oxidant enzymes and non- enzymatic and anti-oxidant assays in the liver, kidney and intestine of the mud eel (*Monopterus cuchia*). Fishes were exposed to high and low pH for the particular period, anti-oxidant marker enzymes like catalase (CAT), glutathione-s-transferase (GST) concentration showed a decreasing pattern whereas superoxide dismutase (SOD) showed a subsequent increase initially followed by a marked diminution. The non enzymatic marker reduced glutathione (GSH) markedly decreased in experimental condition. In the plasma, ALT and AST and the lipid peroxidation marker namely malondialdehyde (MDA) showed significant increase as compared to the control. Thus the physiological changes observed during the study period establish that the fishes exposed to both the measures of pH were under acute stress.
Key words: Catalase (CAT), Glutathione-s-transferase (GST), Glutathione (GSH), Lipid peroxidation, Oxidative stress

Introduction

Aquatic life is constantly exposed to chemical contamination by an increasing variety of anthropogenic activities that can induce many different mechanisms of toxicity, each contributing to varying degrees to the final overall deleterious effect (Correia *et al.*, 2003). pH affect the solubility and toxicity of chemicals and heavy metals in water. Extreme pH levels usually increase the solubility of elements and compounds, making toxic chemicals more "mobile" and increasing the risk of absorption by aquatic life (EPA, 2012).

The pH of freshwater ecosystems can fluctuate considerably within daily and seasonal timeframes, and most freshwater animals have evolved to tolerate a relatively wide environmental pH range. However, animals can become stressed or die when exposed to a sudden change in pH even if the change occurs within a pH range that is normally tolerated (Tucker and Abramo, 2008).

Fishes are a useful indicator of environmental water quality because of their differential sensitivity to pollution. Changes in climate on a global scale have been noticed since the onset of the industrial revolution which indicates that global climate change has an anthropogenic element to it (Kenneth *et al.*, 2011). Rising atmospheric CO_2 concentrations resulting from human activities is playing a central role in anthropogenic ocean acidification (IPCC, 2011). Global atmospheric CO_2 concentrations have increased by 40% from 278 to 390.5 ppm in a time period stretching from 1750 to 2011 (Ballantyne *et*

al., 2012). Basically, this development has been paralleled with human activities such as industrialization, fossil fuel combustion and agricultural activities. About 30% (155 ± 30 PgC) of this anthropogenic CO_2 in the atmosphere has been reported to be taken up by oceans, consequently causing a change in pH especially in the ocean surface (Sabine, 2004, Khatiwala, 2013).

The mud eel (*Monopterus cuchia*) (family: Synbranchidae; order: Synbranchiformes; class: Actinopterygii) is an obligatory air-breathing bony fish that lacks paired fins and has a scale-less long cylindrical body (Graham,1997). It is mainly found distributed in tropical and subtropical freshwaters of India, South China, Malaysia and Indonesia. It inhabits stagnant, slow-flowing and shallow water bodies such as swampy areas, muddy ponds, canals, rice fields, and sometime in ditches and temporary pools, where it burrows in moist earth during rainy season and has a capacity of surviving for about 4–5months under semidry conditions during summer (Davidson 1975; Tay *et al.,* 2003). Therefore this species is quite prone to ground water seepage that alters the pH of its surroundings.

Acid-base status influences several physiological and biochemical processes in both vertebrates and invertebrates; for example acid-base parameters such as pH play a regulatory role in metabolic processes and overall rate of energy turnover (Reipschlager and Pörtner, 1996). This explains why maintenance of acid-base steady state is important for normal functionality and survival. As the level of hydrogen ions increases, metal cations such as Al^{3+}, Pb^{2+}, Cu^{2+} and Cd^{2+} are released into the water instead of being absorbed into the sediment. As the concentrations of heavy metals increase, their toxicity also increases. Aluminium can limit growth and reproduction while increasing mortality rates at concentrations as low as 0.1-0.3 mg/L. In addition, mobilized metals can be taken in by organisms during respiration, causing physiological damage (Osmond *et al.*, 1995).

The altered pH is likely to cause changes in anti-oxidant defence mechanisms, plasma enzyme concentrations and lipid peroxidation marker enzyme, malondialdehyde. Biochemical constituents, like lipid peroxidation and antioxidant enzymes, are the potential biomarkers of pollutants exposure in different organisms (Livingstone 2003; Regoli *et al.*, 2004; Bechard *et al.*, 2008). They have the advantage of being more sensitive, less variable, highly conserved between species and often easier to measure as stress indices (Agrahari *et al.*, 2007). The LPO, as induced by heavy metal pollution in aquatic organisms, is expressed by malondialdehyde (MDA) formation, which represents the secondary LPO product with the thiobarbituric acid reactive substance test (Draper *et al.*, 1993; Janero 1990). These enzymatic systems associated with non-enzymatic systems can prevent the formation of oxyradicals or intercept oxidative propagation systems promoted by oxyradicals (Bainy *et al.*, 1995). When the pro-oxidant forces overwhelm the anti-oxidant defences (enzymatic and non-enzymatic) a cellular oxidative stress is established (DiGuieseppi and Fridovich, 1983).

Materials and methods

Experimental Fishes

Mud eels (*Monopterus cuchia*, weighing 150–170 g body mass) were purchased from a local fish market of Guwahati, Assam. All the fishes were of uniform size of a body mass of 150-170g. The fishes were then acclimatized in the Aquaculture and Biodiversity Centre, Gauhati University for 4 weeks at a temperature of about 28±2° C, maintained in cemented tanks (1.5m length X 1 m wide X 1 m height) containing tap water with a layer of mud of approximately 30 cm height at the bottom of each tank with a normal pH of 7.4 ± 0.1. Small dried fish and earthworms were given as food on alternate days. The water was changed on daily basis. The fishes were taken for study only after the mortality rates were zero and food consumption was normal. No sex differentiation was done in the fishes in the study period. Food was withdrawn 24 h prior to experiments.

Experimental design

The fishes were categorised into five groups for experimentation. The control batch of fishes were kept in water with normal pH of 7.4 (N =9). The other four groups of fishes were exposed to a low pH of 6.4 and high pH of 8.0. The acidity and alkalinity

of the water was induced by the addition of hydrochloric acid (HCl) and sodium hydroxide (NaOH) respectively. Teleost fish can withstand high and low pH without being killed (Tucker and Abramo, 2008). Therefore the fishes in pH 6.4 and 8.0 consisted of two groups each that were studied for a period of 5 days (N= 6) and 10 days (N =6) respectively were selected for the experiment. It is to be noted that prior to exposing the fishes to the low pH of 6.4 and high pH of 8 the fishes were tested under more acidic pH of 5.4, 6.0 and alkaline pH of 8.5 but the fishes failed to survive for more than two days. Eventually the fishes were exposed to 5 and 10 days under acidic (6.4) and alkaline (8.0) conditions so that a better understanding of their adaptational strategies can be explored. In this context no such direct literature was available to follow. The water was changed consistently on a daily basis. The temperature of the water was maintained at 28 ± 2^0 C. After the planned exposure the fishes were removed from the tanks and immediately sacrificed. Blood samples of fish from all groups were taken from caudal vein and processed immediately according to (Lied *et al*., 1975). Sera were separated and immediately frozen at -20^0C for further estimations of biochemical parameters. The targeted tissues i.e. kidney, intestine and liver were removed and preserved in -20^0C freezer for the experimental studies.

Plasma enzyme assay

ALT and AST: Serum ALT and AST were measured spectrophotometrically according to the standardized method described by IFCC using kits manufactured by Aspen Laboratories.

Antioxidant anzyme assay

After sacrificing the fish the targeted tissues i.e. liver, kidney and intestine were weighed, homogenized (10% volume) in 1mM trisHCl buffer (pH 6.8) and diluted in 1% triton X and then centrifuged (at 15000g for 10 minutes at 4^OC). Then the supernatant was collected for the analysis of biochemical parameters.

Catalase (CAT)

CAT activity was determined according to the technique described by Abei *et al.,* (1984). The reaction mixture contained 1.98 ml of 50Mm of potassium phosphate buffer (pH 7.0) and 1ml of 30 mM H_2O_2. The reaction was initiated by adding 20 ìl of enzyme extract. The CAT activity was determined by measuring the rate of H_2O_2 at 240nm. CAT activity was expressed as ìg/ml/min.

Glutathione-s-transferase (GST)

The activity of GST was determined spectrophotometrically at 25°C following the formation of GSH conjugate with 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm using extinction co-efficient of 96 Mm-1cm-1 (Habig *et al.,* 1974). The reaction mixture contained 3 ml volume of 0.1 M potassium phosphate buffer, 30 mM GSH, 30 mM CDNB in ethanol and was expressed as ìg/ml/min.

Superoxide dismutase (SOD)

Tissue SOD was analyzed using the method described by Kakkar *et al.,* (1984). Reaction mixture contained 1.2 ml (0.052 mM) potassium pyrophosphate buffer, 0.1 ml (186 mM) Phenazine Methosulphate (PMS) and 0.3 ml (300 mM) Nitro Blue Tetrazolium (NBT). Reaction was initiated by adding 0.2 ml NADH (780 mM). Colour intensity was measured at 560 nm. SOD activity was expressed as unit/ml.

Reduced glutathione (GSH)

GSH level was estimated according to the methodology by Beautler *et al.,* (1963) using DTNB (5, 5-dithiobis-2-nitrobenzoic acid, Ellman's reagent) following Monteiro *et al.,* (2009) and Thomaz *et al,.* (2009). Supernatant of the extract was added to 0.25mM DTNB in 0.2 potassium phosphate buffer and the thiolate ion formation was determined at 412 nm absorbance against a GSH standard curve. GSH was expressed as ìg/ml.

Lipid peroxidation

Lipid peroxide was measured in tissues according to the method of Satoh. It is based on the ability of thiobarbituric acid (TBA)

to react with malondialdehyde (MDA) in acidic medium at temperature of 95^{O}C for 30 min to form TBA reactive product. The absorbance of the resultant pink product was measured at 534 and 600 nm.

Statistical analysis

The obtained data were statistically analyzed by ANOVA using Origin 6.1 software. The graphs were plotted using Microsoft Excel 2007. The data were reported as mean± SEM.

Results

Serum enzyme assay

ALT and AST

The serum enzymes ALT and AST are basically liver specific enzymes and it showed an elevated increase in the treated group of fishes while equated with the control. The ALT value is 2.3 folds higher (pH 6.4) and 1.2 folds higher (pH 8) in the 5 day treated group and 2.7 folds higher (pH 6.4) and 3.2 folds higher (pH 8) in the 10 day treated group as compared to the 0 day control. The ALT value is 2.3 folds higher (pH 6.4) and 1.2 folds higher (pH 8) in the 5 day treated group when compared with the 5 day control group. Consequently, the ALT value is 2.9 folds higher (pH 6.4) and 3.4 folds higher (pH 8) in the 10 day treated group when compared to the 10 day control group (Fig. 1).

Similarly, the AST value is 3.4 folds higher (pH 6.4) and 2.8 folds higher (pH 8) in the 5 day treated group and 8.5 folds higher (pH 6.4) and 5.4 folds higher (pH 8) in the 10 day treated group as compared to the 0 day control. The AST value is 3.2 folds higher (pH 6.4) and 2.5 folds higher (pH 8) in the 5 day treated group when compared with the 5 day control group. Consequently, the AST value is 9.18 folds higher (pH 6.4) and 5.8 folds higher (pH 8) in the 10 day treated group when compared to the 10 day control group (Fig. 2).

Enzymatic and non-enzymatic antioxidant assay

Catalase

The primary anti-oxidant enzyme i.e. catalase (CAT) showed a decrease in activity in the treated group of fishes. In the 5 day exposed group the CAT activity decreased by 2.6, 1.9 and 2.7 folds (pH 6.4) and 1.6, 1 and 1.3 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control. Similarly, in the 10 day exposed group the CAT activity decreased by 10, 14.2 and 5.5 folds (pH 6.4) and 7.3, 3.5 and 5.3 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control (Fig. 3).

The CAT activity decreased in the 5 days exposed fishes by 3, 2.1 and 3.2 folds (pH 6.4) and 1.8, 1.1 and 1.6 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 5 day control group. Similarly, the CAT

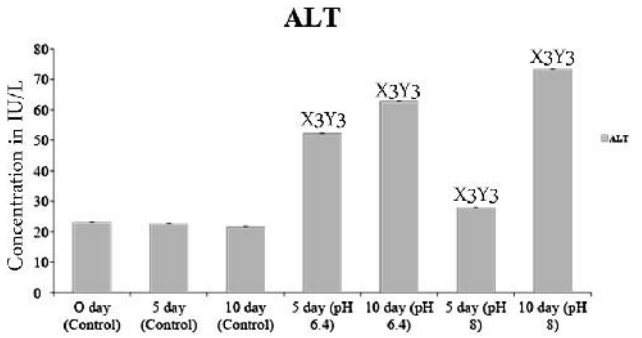

Fig. 1. ALT activity in blood plasma of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

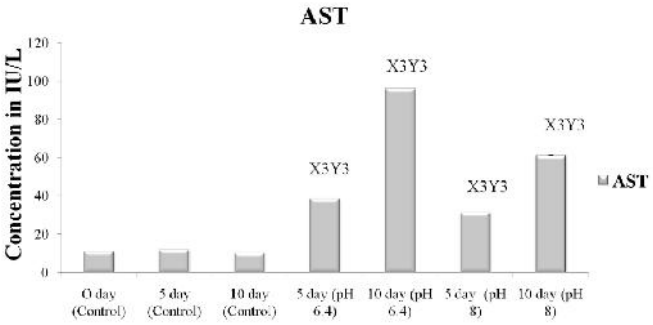

Fig. 2. AST activity in blood plasma of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

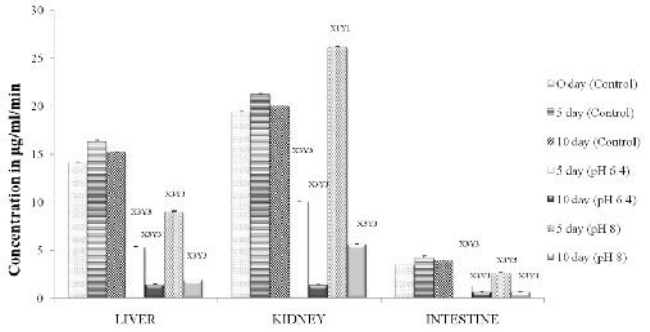

Fig. 3. CAT activity in liver, kidney and intestine of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

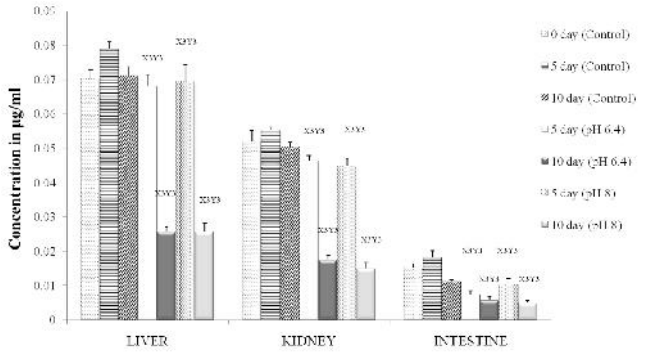

Fig. 4. GST activity in liver, kidney and intestine of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

activity decreased in the 10 days exposed fishes by10.7, 14.7 and 6.2 folds (pH 6.4) and 7.9, 3.6 and 5.9 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 10 day control group (Fig. 3).

Glutathione-s-transferase (GST)

The enzyme GST showed a slight decrease in its activity in the fishes. In the 5 day exposed group the GST activity decreased by 1, 1.1 and 2 folds (pH 6.4) and 1.0, 1.2 and 1.5 folds (pH 8) in the liver, kidney and intestine respectively when compared

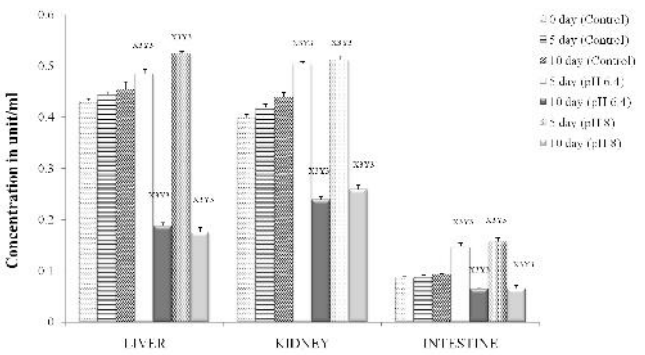

Fig. 5. SOD activity in liver, kidney and intestine of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

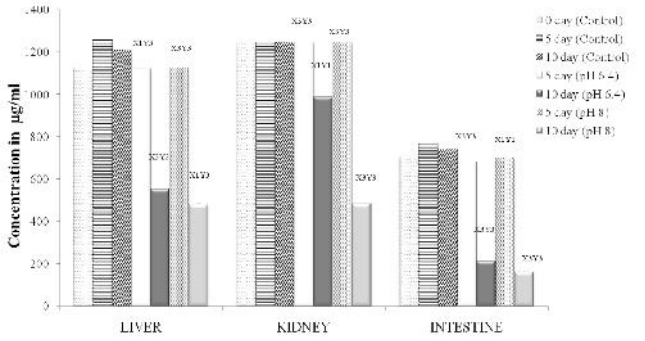

Fig. 6. GSH activity in liver, kidney and intestine of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively.

to the 0 day control. Similarly, in the 10 day exposed group the GST activity decreased by 2.7, 3 and 2.7 folds (pH 6.4) and 2.8, 3.5 and 3.1 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control (Fig. 4).

The GST activity decreased in the 5 days exposed fishes by 1.1, 1.2 and 2.5 folds (pH 6.4) and 1.1, 1.2 and 1.7 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 5 day control group. Similarly, the GST activity decreased in the 10 days exposed fishes by 2.7, 2.9 and

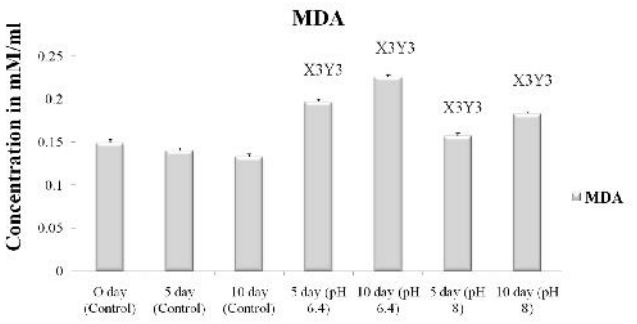

Fig. 7. MDA activity in blood of *M. cuchia* under control (pH 7.4) and treated conditions (pH 6.4 and pH 8) following 5 and 10 days of exposure. Values are mean ± S.E. (N=3). X: significant difference from 0 day control group (X1: $p \leq 0.05$, X2: $p \leq 0.01$, X3: $p \leq 0.001$) and Y: significant difference from 5 day and 10 day control group (Y1: $p \leq 0.05$, Y2: $p \leq 0.01$, Y3: $p \leq 0.001$) as compared to 5 day treated and 10 day treated group respectively determined by one way ANOVA analysis.

2 folds (pH 6.4) and 2.8, 3.3 and 2.3 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 10 day control group (Fig. 4).

Superoxide dismutase (SOD)

The primary antioxidant enzyme SOD showed a significant increase in the enzyme activity of fishes exposed to acidic as well as alkaline water for duration of 5 days but showed a significant decrease in the enzyme activity exposed in the same water but for duration of 10 days when compared with the normal pH.

In the 5 day exposed group the SOD activity increased by 1.1, 1.3 and 1.7 folds (pH 6.4) and 1.2, 1.2 and 1.8 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control. Similarly, in the 10 day exposed group the SOD activity decreased by 2.3, 1.7 and 1.4 folds (pH 6.4) and 2.4, 1.5 and 1.3 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control (Fig. 5).

The SOD activity increased in the 5 days exposed fishes by 1, 1.2 and 1.6 folds (pH 6.4) and 1.2, 1.2 and 1.7 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 5 day control group. Similarly, the SOD activity decreased in the 10 days exposed fishes by 2.4, 1.8 and 1.4 folds (pH 6.4) and 2.6, 1.7 and 1.4 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 10 day control group (Fig. 5).

Reduced glutathione (GSH)

The level of the non-enzymatic antioxidant GSH, significantly decreases. The enzyme GSH showed a slight decrease in its activity .In the 5 day exposed group the GSH activity decreased by 1.0, 1.0 and 1.0 folds (pH 6.4) and 1.0, 1.0 and 1.0 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control. Similarly, in the 10 day exposed group the GSH activity decreased by 2.0, 1.2 and 3.3 folds (pH 6.4) and 2.3, 2.6 and 4.3 folds (pH 8) in the liver, kidney and intestine respectively when compared to the 0 day control (Fig. 6).

The GSH activity decreased in the 5 days exposed fishes by 1.1, 1.0 and 1.1 folds (pH 6.4) and 1.1, 1.0 and 1.1 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 5 day control group. Similarly, the GSH activity decreased in the 10 days exposed fishes by 2.1, 1.2 and 5.6 folds (pH 6.4) and 2.5, 2.6 and 4.5 folds (pH 8) in the liver, kidney and intestine respectively when compared with the 10 day control group (Fig. 6).

Lipid peroxidation assay

Malondialdhyde (MDA)

The lipid peroxidation marker MDA showed an elevated increase in the treated group of fishes while equated with the control. The MDA value is 1.3 folds higher (pH 6.4) and 1.0 folds higher (pH 8) in the 5 day treated group and 1.5 folds higher (pH 6.4) and 1.2 folds higher (pH 8) in the 10 day treated group as compared to the 0 day control. The MDA value is 1.4 folds higher (pH 6.4) and 1.1 folds higher (pH 8) in the 5 day treated group when compared with the 5 day control group. Consequently, the MDA value is 1.6 folds higher (pH 6.4) and 1.4 folds higher (pH 8) in the 10 day treated group when compared to the 10 day control group (Fig. 7).

Discussion

The specific liver enzymes alanine transaminase (ALT) and aspartate transminase (AST) are important markers for the detection of liver injury, damage or hepatic necrosis. In the present study, an elevated serum ALT and AST enzyme activity was seen in the treated groups as compared to control. The

increase in serum AST and ALT activities is in harmony with the findings of Kumar *et al.,* (2016) in *Oreochromis mossambicus* on stress induced by "endosulfan pesticides" and by Prusty *et al.,* (2011) in *Labeo rohita* fingerlings exposed to fenvalerate. Amin and Hashem (2012) and Sayed and Saad (2007) also reported similar findings in the plasma in deltamethrin induced oxidative stress in *Clarius gariepinus* and *Oreochromis niloticus* respectively.

The primary anti-oxidant enzyme present in the vertebrates is Catalase, located in the peroxisomes of the liver protects the cells from oxidative damage by reactive oxygen species (ROS) and destructive hydrogen peroxide produced as a by-product of cellular metabolism. In the present study, Catalase activity was significantly reduced in the kidney, liver and intestines of the fishes exposed to altered pH in comparison to control one; the decline being more in the fishes exposed for longer duration. The decrease in CAT activity might be caused by an inhibition of Catalase synthesis and it suggests a decrease in oxidative defence. This reduction could also be because of the influx of super oxide radicals, which have been reported to decrease CAT activity. Bainy *et al*., 1996 showed an inhibition of the CAT activity in *Oreochromis niloticus* from polluted areas. This result is also in agreement with findings of Hashem and Amin (2012) in deltamethrin induced stress in *C. gariepinus*.

In the present study, the GST was found to be diminished in the treated groups in comparison to the control. GST activity indicates the response of the bio-protection system against the formation of oxyradicals failing which, there will be an oxidative stress. Hence, this finding further confirms an increase in oxidative stress in the fishes exposed to high and low pH. The GST activity was found to be lowered in the intestine, liver and kidney of the treated fish. Decreased GST activity signifies a disrupted antioxidant defence mechanism that fails to provide defence against oxidative stress. The results of the present findings are in accordance with the findings of Luschak *et al*., (2009) where an inhibition in GST activity was noticed in the liver of gold fish under chromium induced stress.

In cells, GSH is the dominant intracellular thiol and has an important function in the cellular defence against oxidative injury. Conjugation with GSH is an important detoxification reaction for electrophilic xenobiotics. Due to the significant lower levels of GSH in the liver of pH altered *M. Cuchia* compared to control fish, the liver in the treated fish may be less capable in the transformation and excretion of xenobiotics from the body and consequently, a reduction in detoxifying capacity of the liver might lead to enhanced risk of xenobiotic damage of this organ. Similar reports were also made by Hjeltnes *et al.,* (1992) in Atlantic salmon (*Salmo salar)* suffering from salmon anaemia.

The elevation of lipid peroxidation (MDA) in the liver in response to the exposure to altered pH as observed in the present investigation suggests that there is increased production of ROS. It may be due to the imbalance between ROS generation and removal which results in the damage of DNA, lipids and proteins; and in the peroxidation of membrane lipids of the liver. The observed LPO resulting from generated ROS may lead to cell apoptosis as ROS and oxidative stress have been known to trigger apoptosis. In the present work, the increase in SOD activity in the 5 day treated fish suggests the effects of reduced oxygen concentration on the activities of enzyme systems. The higher rate of SOD also suggests a higher rate of formation of H_2O_2 which is to be decomposed by catalase or such antioxidant enzymes. The finding of this present work are in accordance with the finding of Markovic *et al*., (2002) on liver of *Cyprinus carpio* and Bainy *et al*., (1996) on liver, kidney and erythrocytes of *Oreochromis niloticus* collected from a polluted site. Similarly Lopez-Lopez *et al.,* (2011) found that *Goodea atripinnis* exposed to polluted water displayed decreased SOD activity. They concluded that the decline in SOD activity might reflect damage to the SOD protein due to ROS overproduction.. It can also be inferred that in the 5 day treated fish there was a conversion of superoxide radical to hydrogen peroxide and water but as catalase activity was found to be quite low the hydrogen peroxide remained likewise and in the 10 day treated fish when there was a low SOD activity there might be an excess superoxide and hydrogen peroxide.

SOD, CAT, GST and GSH are among the most important antioxidants protecting from oxidative attacks by

active oxygen species such as LPO. They act as a reducing agent and free-radical trapper. Therefore the diminishing SOD, CAT, GST activity and GSH level in the fishes treated to low and high pH may demonstrate the inefficiency of this tissue in combating the impact of ROS, resulting in increased LPO. Sinha *et al.*, (2014) reported increased production and accumulation of MDA and H_2O_2 as a consequence of ammonia induced oxidative stress in some salmonid and cyprinid species.

Acknowledgements

The authors are thankful to the Head, Department of Zoology, DST SERB, UGC SAP, BIF Gauhati University, for providing the necessary facilities and equipments for conducting the study. The authors acknowledge the Assam Science Technology and Environment Council, (No. ASTEC/ S&T/ 206/ 2017-2018/ 1066) for the financial support.

References

Aebi, H. 1974. Catalase. In: Bergmeyer HV, editor. Methods in Enzymatic Analysis. New York: Academic Press. Vol.2. Pp: 674-684.

Afonso C. D. Bainy, Erika Saitob, Paulo S. M. Carvalho, Virginia B. C. Junqueirab.1996:Oxidative stress in gill, kidney, erythrocytes and liver of Nile tilapia *Oreochromis niloticus* from a polluted site. Aquatic toxicology. 34(2) : 151-162

Amin, A. K. and Hashem, S. K. 2012. Deltamethrin induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarius gariepinus*) : anti-oxidant defense and role of alpha- tocopherol. BMC veterinary research. 8(1): 45.

Hamilton, B. 1822. An account of the fishes found in the river Ganges and its branches.Edinburgh (Reprinted). Pp: 405.

Correia, A. D., Costa, H. M., Luis, O. J. and Livingstone, D. R. 2003. Age-related changes inantioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body *locusta* (Crustacea: Amphipoda).J. Exp. Mar. Biol. Ecol. 289, 83-101.

El-Sayed, Saad, T. T. and El-Bahar, S. M. 2007. : Acute intoxication of Deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical biochemical and haematological effects. Environmental toxicology and pharmacology. 24(3): 212-217.

Hjeltnes, B. K., Samuelsen, O. B. and Svardel, A. M. 1992. Changes in plasma and liver glutathione levels in Atlantic salmon *Salmo salar* suffering from infectious salmon anaemia (ISA) . Brage.bibsys.no

Kakkar, P., Ballah, D. and Viswanathan, P. N. 1984: A modified spectrophotometric assay of superoxide dismutase. Ind. J.*Biochem. Biophys.* 21, pp 130-132.

Koudinya, P. R, Ramamurthy, R. 1979: Effect of organophosphate sumithion on some aspects of carbohydrate metabolism in freshwater fishes *Sarotherodon mossambicus* and *Tilapia mossambicus* (Peters). Journal of experimental biology. 15: 1632-1633.

Lied, E., Gjerde, J. and Braekkan, O. R. 1975: Simple and rapid technique for repeated blood sampling in rainbow trout. J. Fish. Res. Board Canada, 32: 699-701 . DOI: 10.1139/175-089

Lopez-Lopez, E., Sedeno-Diaz, J. E., Soto, C. and Favari, L. 2011: Responses of antioxidant enzymes, lipid peroxidation, and Na^+/K^+-ATPase in liver of the fish *Goodea atripinnis* exposed to Lake Yuriria water. Fish *Physiol Biochem*. 37:511–522.

Lushchak, V., Kubrak, O. I., Storey, M. J., Lushchak, Vi. 2009: Low toxic herbicide Roundupinduces mild oxidative stress in goldfish tissues. Chemosphere. 76(7).

Markovic, M. D., Zikic, R. V., Stajn, A. S., Ognjanovic, B. I., Pavlovic, S. Z., Radojicic, R. and Saicic, J. S. 2002. Some blood parameters and antioxidant defense enzyme activities in the liver of carps (*Cyprinus carpio* L.) under acute hypoxic conditions. Kragujevac Journal of Science. 24: 111-120.

Mishra, J. and Srivastava, A. K. 1983. Malathion induced haematological and biochemical changes in the Indian catfish *Heteroterus fossilis. Environ. Res.* 30: 399-401

Nath, R. and Banerjee, V. 1995. Effects of various concentrations of leadnitrate on Haematological parameters on air breathing fish, *Clarius batrachus.* (1995). Freshwater Biol. 7: 267-268.

Olaifa, F. E., Olaifa, A. K. and Onwude, T. E., 2004: Lethal and sub-lethal effects of copper to the African catfish (*Clarius gariepinus*). African Journal of Biomedical research 7(2).

Ordonez-Ortiz, E., Uria- Galicia, E., Trejo, Y. H., Sedeno-Diaz, J. E., Lopez- Lopez, E. 2011. Effect of yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis.* Archives of environmental contamination and toxicology. 61(3): 443-452.

Pandey, S., Ahmad, I., Parvez, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S. 2001. Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch: 1. Protection against lipid peroxidation in liver by copper preexposure. *Arch. Environ. Contam. Toxicology*. 41: 345-352.

Pereira, L., Fernandez, N. M., Martinez, B. R. C., 2013. Haematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. Environmental toxicology and pharmacology. 36(1): 1-

Regoli, F. and Principato, G. 1995: Glutathione, glutathione dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers Aquatic Toxicology. 31 143-164. 8.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Rizwanul, H. and Raisuddin, S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch, *Ecotoxicol. Environ. Saf.* 56: 295-301.

Santhakumar, M., Balaji, M. and Ramudu, K. 1999. Effects of sub-lethal concentrations of Monocroptophos on erythropoietic activity and certain Haematological parameters of fish *Anabas testudineus* (Bloch). Bulletin of environmental contamination and toxicology. 63(3): 379-384.

Saravanan, M., Kumar, P. K. and Ramesh, M. 2011. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii : Cypriniformes) during acute and sub-lethal exposure to lindane. Pesticide biochemistry and Physiology. 100(3): 206-211.

Sinha, A. K., AbdElgawad, H., Giblen, T. *et al.,* 2014. Anti-oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia-induced oxidative stress. PLoS ONE 9:e95319. doi:10.1371/journal.pone. 0095319

Tucker, S. C. and D'Abramo, L. R. 2008. Managing high pH in freshwater ponds. Southern regional aquaculture centre, Mississippi State University.

Wepener, V., Van Vuren, J. H. J. and DuPreez, H. H. 1992: Effect of manganese and iron at a neutral and acidic pH on the hematology of the banded tilapia (*Tilapia sparrmani*). Bulletin of environmental contamination and toxicology. 49(4): 613-619

Yekeen, T. A., Fawole, O. O. 2011. Toxic effects of endosulfan on haematological and biochemical indices of *Clarius gariepinus*.