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Research Article

TOXICOLOGICAL EFFECTS OF *IN VIVO* EXPOSURE TO DIETHYLPHTHALATE ON ANTIOXIDANT ENZYMES IN THE FISH *OREOCHROMIS MOSSAMBICUS* (TIALPIA)

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ABSTRACT

Persistence of Diethyl phthalate (DEP) in the waterways could cause metabolic changes in the fishes. The present investigation was undertaken to elucidate the changes induced by DEP in fish antioxidant system. Thus, in this study *Oreochromis mossambicus* was chronically exposed to DEP for a period of 60 days. Superoxide dismutase (SOD), Catalase(CAT), Glutathione S-transferase (GST) and Glutathione peroxidase (GPx) activity of gill, liver and muscle was assayed in DEP exposed Tilapia. Statistical analysis of the results reveal that DEP significantly declined ($P<0.001$), the enzyme activities in all the organs except, SOD activity of muscle which exhibited significant decline at 15ppm but not at 5ppm when compared to the control. Thus, the results of the present study permits us to conclude that DEP has caused significant disturbance in the antioxidant enzyme system in Tilapia (*Oreochromis mossambicus)*.

Keywords: Chronic Toxicity, Diethylphthalate, *Oreochromis mosambicus*, SOD, CAT,GST,GPx.

INTRODUCTION

The release of toxicants in to the environment from industries, agricultural runoff etc is of great environmental concern, since it could be deleterious to the biota. DEP is widely used as a plasticizer and softner, pharmaceutical coatings and cosmetic additives Page and Lacorix(1995) and has been reported to be highly toxic to fish Umamaheswari and senthilnathan,(2013). Waterways have been the dumping reservoirs of these chemicals, which could cause stress in fishes. In order to assess and evaluate the impact of toxicants , the present study was an attempt to elucidate the changes induced in the enzyme activity of various organs of tilapia exposed to DEP. Diethylphthalate is hydrophilic in nature and undergoes degradation in the environment within 72hours Ross (2004). Despite wide occurence of DEP in marine and freshwater environments , its influence on such system has been meagre. Oxidative enzymes activities of gill, liver and muscle were assayed in the DEP exposed Tilapia to gain insight in to the metabolic disturbances caused by it.

MATERIAL AND METHODS

Diethylphthalate toxicity were assessed using healthy, living specimens of *Oreochromis mossambicus* which were collected from local fresh waters. Prior to Experimentation, fish were allowed to acclimate to laboratory conditions for a month. These adult fishes were reared in aquarium tanks for a period of 30 days at standard environmental conditions and used for further experiments. Diethylphthalate was purchased from Sigma .St.Louis,USA and was dissolved in acetone to form a stock solution and stored at room temperature. 10 fishes were randomly selected from the stock and exposed to different concentrations of DEP (10,20,30,40,50,60,70,80,90 and 100ppm) for 96 hours to determine the median lethal concentration (LC_{50}) of DEP with selection exposure concentration of 5 and 15 ppm for chronic sub-lethal concentration exposure

studies. Water was replaced daily with fresh DEP mixed water to maintain constant level of DEP during exposure period. The LC_{50} value for DEP was 50 ppm. For sub-lethal study, $1/5^{th}$ and $1/10^{th}$ of the LC_{50} value were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.

ENZYME ASSAY

Enzyme activity was studied in gill, liver and muscle tissues of fish (*Oreochromis mossambicus*). After sacrificing the fish, 0.1 g of tissue was immediately placed on ice. It was then homogenized in 2ml of 0.9% chilled saline using a Teflon pestle tissue grinder and centrifuged for 5min at 4°C. The supernatant was used for estimating Total superoxide dismutase (SOD) activity by the method of Marklund and Marklund (1974). The Catalase (CAT) activity was determined by the method of Aebi, (1984) Glutathione S-transferase (GST) activity was determined by the method of Habig *et al*., (1974). Glutathione peroxidase (GPx) activity was assayed according to the method described by Pleban et al., (1982). Enzyme activity was expressed in international units (or milliunits) per mg of protein.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was applied to determine significant differences in results of various groups. P-Values <0.05 were considered significant Subsequently, Tukey's post hoc and Duncun test was applied to determine significant changes between different treatment groups.

The changes induced by Diethylphthalate on the enzyme activity of various tissues of (gill , liver and muscle) of tilapia were analysed statistically. From table-1, it is evident that Superoxide diamutase (SOD) activity of gill significantly declined (F=4.632, P<0.001) in the DEP treated fishes (5ppm: 6.540±0.020 U/mg protein; 15ppm: 4.910±0.011 U/mg protein) when compared to the control (7.856±0.029 U/mg protein). Furthermore, dose dependent relationship was evinced between the concentration of DEP and SOD concentration in the gill and liver. Similar, pattern of decline was observed in SOD activity of liver. In comparision to the DEP unexposed fishes (0.780±0.017 U/mg protein), DEP exposed fishes (5ppm:0.530±0.015 U/mg protein;15ppm:0.450±0.017 U/mg protein) exhibited significant decline (F=106.680,P<0.001) in SOD activity of liver . On contrary, in muscle tissues SOD activity was significantly elevated (F=1.766,P<0.001) in DEP exposed fishes 15ppm(0.503±0.228 U/mg protein) when compared to the control (0.246±0.020 U/mg protein) (Table-1), whereas, diethylphthalate at 5ppm recorded muscle SOD activity of 0.166± 0.012 U/mg protein, which was found to be significantly lower than the SOD activity of DEP unexposed ones.

Significant decline in the gill, liver and muscle Catalase activity was evinced in Diethylphthalate exposed tilapia when compared to DEP unexposed ones. DEP at 5ppm and 15ppm registered 0.546±0.008 U/mg protein and 0.420±0.005 U/mg protein, respectively, which was found to be significantly (F=88.229,P<0,001) lower than the control fishes (0.860±0.040 U/mg protein). Significantly declined (F=89.423., P<0.001) liver Catalase activity in DEP exposed tilapia (5ppm:0.233±0.008 U/mg protein;15ppm:0.166±0.008 U/mg protein) when compared to DEP unexposed ones (0.350±0.011). Muscle Catalase activity significantly (F=192.926; P<0.001) declined in DEP exposed tilapia *Oreochromis mossambicus* (5ppm: 0.773±0.012 U/mg protein 15ppm: 0.656±0.008) when compared to the control group (0.933±0.008 U/mg protein) (Table-2).

Similarly Glutathine-S-transferase (GST) activity of gill, liver and muscle significantly declined in the DEP exposed fishes. The data presented in table-3, indicate that DEP at 5ppm and 15ppm recorded gill GST activity of 15.143±0.014 U/mg protein and 13.236±0.014 U/mg protein, respectively, which was found to be significantly lower (F=3.731,P<0.001) than the control (19.153±0.017). Control fishes recorded Liver GST activity of 21.273±0.012 U/mg protein, which was found to be significantly (F=388.825, P<0.001) higher than the DEP exposed fishes (5ppm:19.153±0.014 U/mg protein ;15ppm:15.376±0.261 U/mg protein). DEP significantly decreased (F=1.657,P<0.001) the muscle GST activity in tilapia (5ppm: 7.393±0.012 U/mg protein; 15ppm: 5.156±0.012 U/mg protein) when compared to DEP unexposed ones (8.126±0.012 U/mg protein) . In comparison to the control, Glutathione Peroxidae (GPx) activity declined in all the DEP exposed fishes, irrespective of the tissues. Moreover, the decrease in GPx activity was found to the concentration dependent. As the concentration of DEP increased, GPx activity declined in all the tissues studied. DEP at 5ppm and 15ppm registered, significantly higher (F=1.178, P<0.001) gill GPx activity (28.136±0.008 U/mg protein and 25.113±0.008 U/mg protein, respectively, whereas, control group registered GPx activity of 31.166±0.008 U/mg protein . Liver GPx activity was found to be 24.340±0.230 U/mg protein in DEP

unexposed fishes, which significantly decreased (F=367.046, P<0.001) on exposure to Diethylphthalate (5ppm: 18.386±0.256 U/mg protein; 15ppm: 15.343± 0.228 U/mg protein). Muscle GPx activity significantly declined (F=5.508,P<0.001) in DEP exposed fishes (5ppm:18.623±0.008 U/mg protein;15ppm:15.163±0.012 U/mg protein) when compared to DEP unexposed ones (20.150±0.011) (Table-4).

RESULTS

Table 1: Diethylphthalate induced changes in the SOD activity of Gill, Liver and Muscle of *Oreochromis mossambicus*

DEP Treatment	Gill U/mg protein	Liver U/mg protein	Muscle U/mg protein
CONTROL	7.856±0.029 a	0.780±0.017 a	0.246±0.020 b
5PPM	6.540±0.020 b	0.530±0.015 b	0.166±0.012 c
15PPM	4.910±0.011 c	0.450±0.017 c	0.503±0.228 a
F	4.632***	106.680***	1.766***
P	0.000	0.000	0.000

*** Significant at P<0.001
In a column, figures having dissimilar letters
differ significantly according to Duncan New Multiple Range Test (DMRT)

Table 2: Diethylphthalate induced changes in the Catalase activity of Gill,Liver and Muscle of *Oreochromis mossambicus*

DEP Treatment	Gill U/mg protein	Liver U/mg protein	Muscle U/mg protein
CONTROL	0.860±0.040 a	0.350±0.011 a	0.933±0.008 a
5PPM	0.546±0.008 b	0.233±0.008 b	0.773±0.012 b
15PPM	0.420±0.005 c	0.166±0.008 c	0.656±0.008 c
F	88.229***	89.423***	192.926***
P	0.000	0.000	0.000

** Significant at P<0.001
In a column , figures having dissimilar letters differ significantly
according to Duncan New Multiple Range Test (DMRT)

Table 3 : Diethylphthalate induced changes in the GST activity of Gill,Liver and Muscle of *Oreochromis mossambicus*

DEP Treatment	Gill U/mg protein	Liver U/mg protein	Muscle U/mg protein
CONTROL	19.153±0.017 a	21.273±0.012 a	8.126±0.012 a
5PPM	15.143±0.014 b	19.153±0.014 b	7.393±0.012 b
15PPM	13.236±0.014 c	15.376±0.261 c	5.156±0.012 c
F	3.731***	388.825***	1.657***
P	0.000	0.000	0.000

*** Significant at P<0.001
In a column, figures having dissimilar letters differ
significantly according to Duncan New Multiple Range Test (DMRT)

Table 4: Diethylphthalate induced changes in the GPx activity of Gill, Liver and Muscle of *Oreochromis mossambicus*

DEP Treatment	Gill U/mg protein	Liver U/mg protein	Muscle U/mg protein
CONTROL	31.166±0.008 a	24.340±0.230 a	20.150±0.011 a
5PPM	28.136±0.008 b	18.386±0.256 b	18.623±0.008 b
15PPM	25.113±0.008 c	15.343±0.228 c	15.163±0.012 c
F	1.178***	367.046***	5.508***
P	0.000	0.000	0.000

*** Significant at P<0.001
In a column , figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

DISCUSSION

Oxidative stress occurs if the activity of the antioxidant defense system such as SOD, CAT, GPx enzymes change by environmental pollution induces the production of reactive oxygen species Li *et al* . (2001). Canada and Calabrese (1989) indicated that the activity of SOD in fish can increase or decrease after exposure to various xenobiotics. In Channel Catfish (*Ictalurus punctatus)* exposed to pollutants , CAT activity increased, but not SOD or GPx , whereas in trout the same pollutant increased SOD as well as CAT activity Marther and Di Giulio,(1991). These observation partially aggress with the present findings. The present result is in parallel with the findings of Guluzar Atli and Mustafa Canli (2010). Who have registered inhibited GST and CAT activities after an acute Cu and GPx activities after an acute Cr exposure and have suggested Sensitivity of antioxidant enzyme to acute exposures. The decreased CAT and SOD activity of gill observed in this study in well supported by Swarna Pandey *et al* , (2008) who have reported a decrease in CAT and SOD activity in gill of *Channa punctatus* Bloch. Roche and Boge (1993) have indicated that an SOD activation is the main feature of compensatory response in fish exposed to short term effect of toxicants at low concentrations or in the field. The present results also gains support from the findings of Alzebera Stara *et al* .(2013) who have reported that exposure of *Cyprinus carpio* to prometryne caused a considerable decrease in SOD activity of gill. They have also observed decline is CAT activity of liver compared to untreated ones.

While a significant increase in CAT activity has been observed in some studies after fish exposure to pesticides Glusczak *et al* .(2011), Oritz *et al* .(2011), Cattaneo *et al* .(2012) Moreas *et al* .(2011) ; Stara *et al* .(2012) , other studies have reported reduced CAT activity in fish organ tissues (Rossi *et al* . (2011) , Toni *et al* . (2011), Pretto *et al* . (2011) .

The present result is in good accord with the findings of Alzebeta Stara *e t al* . (2012) who have evinced significant decrease (P<0.001) in the SOD activity of muscle and CAT activity of muscle and liver after 60 days simazine exposure. In marked contrast with the results of the present study *Jin et al* . (2011) have observed increased SOD activity, especially in the liver of Zebrafish *Danio rerio*, after 14 days of a atrazine exposure. The observation of the present investigation coincides with that of Oruc and Usta (2007) who have observed , a reduction in SOD activity of gill ,muscle and kidney after 60 days diazinon exposure .

Ballesteros *et al*. (2009) stated that the activity of CAT was significantly decreased in liver of the one sided liver bearer (*Jenynsia multidentata*) exposed to endosulfan. Moraes *et al*.(2009) reported a decrease in liver Catalase activity in Teleost fish (*Leporinus obtusidens*) and silver catfish (*Schilbe intermedius)* after exposure to herbicides.

The decline in hepatic antioxidant enzyme activity observed in this study partially agrees with that of Kathya *et al*. (2010) who have observed significant decline in *Roundup transorb* exposed *Prochilodus Lineatus* (1 mgL^{-1} and 5 mgL^{-1}) after 6 and 24 hours of exposure , whereas, for 96 hours exposure no significant alteration in the hepatic SOD and CAT activity. They have further assumed that H_2O_2 is responsible for reduction in SOD activity and also may be due to superoxide which are probably not being neutralized efficiency by SOD.

In parallel to the present findings, GST activity depleted in the brain ,liver and muscle of *Cyprinus carpio* exposed to Quinclorac after 30 days of exposure and for the subsequent 90 days , Cat activity significantly changed Candida Toni *et al* . (2013). They have attributed it to the flux of superoxide radicals due to oxidative stress caused by Quinclorac exposure. The present results agrees with the findings of Toni *et al* . (2010) who have observed decline in hepatic

CAT activity in carp *Cyprinus carpio* exposed to bispyribac-sodium.

Tripathi and Shasmal (2011) found that chlorpyriphos significantly decreased the profiles of CAT and lactate dehydrogenase (LDH) in liver,gill and skeletal muscle of *Heteropneustes fossilis* and have attributed to binding of pesticide or its metabolites with the enzyme molecules or affecting the synthesis and /or degradation of enzyme Sastry *et al* . (1982). Tripathi *et al* . (1990) , Hai *et al* . (1997). This observation agrees with the present findings.

Significant decline in hepatic ,GPx activity observed in this study coincides with Guluzar Atli and Mustafa canli (2010) who have noticed decreased GPx hepatic activity in *Orochromis niloticus* chronically exposed to both Cu, Zn and Fe , On contrary , Basha and Rani (2003) suggested that higher GPx hepatic activity in *Oreochromis mosammbicus* exposed to Cd. The present observation gains support from the findings of Alzebeta Stara *e t al*. (2012) who have evinced that chronic exposure of simazine caused significant decline ($P<0.01$) in hepatic GPx activity in *Cyprinus carpio* L for 14, 28 and 30 days ($4mgL^{-1}$) and for 28 days and 68 days at $2mgL^{-1}$ simazine and have related to O_2^- production or to the direct action of pesticides on enzyme synthesis Bainy *et al* . (1993). Reduction in GPx prevents the formation or radical intermediates by oxygen reduction mechanisms Cheung *et al* .(2001).

In parallel to the present findings, Ballesteros *et al*. (2009) observed inhibition of GPx activity in gill, intestine, liver and muscle of fish *Jenynsia multidentata* exposed to $1.4mgL^{-1}$ endosulfan for 24 hours. Similarly, Elia *et al* . (2002) have noticed decline in hepatic GPx activity in bluegill sunfish exposed to atrazine (6 and 9 mgL-1).

CONCLUSIONS

Indiscriminate release of pollutants into the environment may disturb the delicate ecological balance of the earth. DEP is one such chemical reported to influence the aquatic biota. In this study we have assayed the alteration in the enzyme activities of the gill, liver and muscle of *Oreochromis mossambicus* on exposure to two concentration (5ppm and 15ppm) of DEP. The observation registered in this experiment reveal significant decline in enzyme activity (SOD,CAT,GST,GPx) gill, liver and muscle of DEP treated fishes *Oreochromis mossambicus*. Resultantly, DEP could cause metabolic changes in fishes.

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