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Effect of *Invivo* chronic exposure to Diethylphthalate on Enzyme activities in *Oreochromis mossambicus* (Tilapia)

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ABSTRACTS

Diethylphthalate is a plasticizer widely used in the manufacture of plastics, cosmetics, cellulose ester films, medical devices and many commercial products. These esters have been detected in the waterways and suspected to exhibit estrogenic activity. Resultantly, diethylphthalate could have adverse impact on the aquatic biota. This research paper reports the changes induced by diethylphthalate on the tissue damaging enzyme activity like Acid phosphatase (ACP), Alkaline phosphatase (ALP), Sorbitol dehydrogenase(SDH) , Lactate dehydrogenase (LDH) of gill , liver and muscle of Tilapia Oreochromis mossambicus . The fishes were exposed to diethylphthalate for a period of seven days at two different concentrations (5ppm and 15ppm) . Significant alternation in the ACP, ALP, SDH and LDH activity of gill,liver and muscle were evinced in diethylphthalate exposed fishes. ACP activity significantly decreased in the gill, whereas significantly elevated in the liver and muscle of Tilapia Oreochromis mossambicus when compared to the control. ALP activity of gill significantly elevated and that of liver and muscle significantly declined in diethylphthalate exposed fishes. SDH activity of gill, liver and muscle significantly declined whereas, LDH activity significantly enhanced in the gill, liver and muscle when compared to unexposed ones. Thus the altered enzyme activity evinced in this study suggests that diethylphthalate could cause metabolic changes in Oreochromis mossambicus .

Keywords: Diethylphthalate; *Oreochromis mossambicus;* Chronic toxicity; ACP, ALP, SDH, LDH,

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INTRODUCTION

Diethylphthalate is an upiquitous contaminant in the environment especially in water ways. Thus DEP gain entry in to the aquatic organisms and causes toxic stress. This toxic stress could be guaged by profiling the enzyme activities in fishes. Alterations in alkaline phosphatase (AKP) and acid phosphatase (ACP) activities in tissues and serum have been reported in fish Jyothi et al ., (2000). The elevated LDH activity serves as a marker enzyme for tissue damage in fish Ramesh et al., (1993). Umamaheswari et al., (2013) have demonstrated that sublethal doses of diethylphthalate causes changes in the haematological parameters of Tilapia and have concluded that diethylphthalate exerts its toxic action even at sub-lethal concentration. Several researchers have reported that treatment of organophosphate to fish inhibits activities of several enzymes such as glucose -6-phosphatases , acid and alkaline phosphatases , pyruvate dehydrogenase (PDH) , succinate dehydrogenase (SDH),acetylcholine esterase (ACHE) , lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and cytochrome oxidase Rao et al ., (1979) ; Sastry et al ., (1982) ; Natarajan et al ., (1984) ; Tripathi et al ., (2006) ; Devarj et al ., (1991); Satyadevan et al ., (1993) ; Kumble et al ., (1999). The displayed hypoxic conditions Das et al., (2004) and muscular harm Balint et al., (1997) serves as good diagnostic tool in aquatic toxicology. The present study aim to scan the changes in the activity

of tissue damaging enzymes of gill, liver and muscle of Tilapia *Oreochromis mossambicus* on exposure to diethylphthalate.

MATERIALS 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 acclimatise 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 (LC50) 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 LC50 value for DEP was 50 ppm. For sub-lethal study, $1/5^{th}$ and $1/10^{th}$ of the LC50 value were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.

Sample Preparation

Gill, liver, muscle were dissected from the experimental fishes and suspended in 50 mM potassium phosphate buffer with 0.5 mM EDTA (pH 7.0). Fifty microliters of Triton X-100 and a few crystals of phenylmethylsulfonyl fluoride (PMSF) were then added to each homogenization tube. Muscle tissues were homogenized on ice using a homogenizer (VirTis) set at 20,000 rpm for 2 min per sample. Both gill and liver tissue were homogenized in microcentrifuge tubes using a plastic hand pestle (gills, liver). After homogenization, all samples were centrifuged at 12,000 rpm for 15 min at 4^0C. The resultant supernatant was removed and stored (- 40^0C) for use in tissue enzyme assays (Acid phosphatase (ACP), Alkaline phosphatase (ALP), Sorbitol dehydrogenase(SDH) , Lactate dehydrogenase (LDH).

Statistical Analysis

Results of the experiment were expressed as mean and standard error of mean of different groups. The differences between the mean values were evaluated by ANOVA (SPSS Version 16.0). The values for $P < 0.001$ were considered significant.

RESULTS

TABLE-1 Changes in the Acid Phosphatase of the various tissues of *Oreochromis mossambicus* exposed to Diethylphthalate

CONTROL	Gill (µmol/mg protein)	Liver (µmol/mg protein)	Muscle (µmol/mg protein)
CONTROL	6.173±0.027 [a]	0.267±0.017 [c]	3.516±0.216 [a]
5PPM	4.163±0.017 [b]	0.816±0.017 [b]	2.393±0.253 [b]
15PPM	3.166±0.008 [c]	1.466±0.088 [a]	1.120±0.005 [c]
F	6.209***	126.798***	38.796***
Significance	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 Changes in the Alkaline phosphatase of the various tissues of *Oreochromis mossambicus* exposed to Diethylphthalate

CONTROL	Gill (µmol/mg protein)	Liver (µmol/mg protein)	Muscle (µmol/mg protein)
CONTROL	0.153±0.014 [c]	4.236±0.014 [a]	6.173±0.027 [a]
5PPM	0.420±0.032 [b]	3.133±0.024 [b]	4.163±0.017 [b]
15PPM	0.556±0.020 [a]	2.253±0.008 [c]	3.166±0.008 [c]
F	76.248***	3.418***	6.209***
Significance	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 Changes in the Sorbital Dehydrogenase of the various tissues of *Oreochromis Mossambicus* exposed to Diethylphthalate

CONTROL	Gill (U/mg protein)	Liver (U/mg protein)	Muscle U/mg protein
CONTROL	0.586±0.014 [a]	1.206±0.014 [a]	12.150±0.015 [a]
5PPM	0.273±0.012 [b]	0.236±0.008 [b]	8.123±0.008 [b]
15PPM	0.143±0.008 [c]	0.123±0.012 [c]	6.140±0.005 [c]
F	359.56***	2.455***	8.168***
Significance	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 Changes in the Lactate Dehydrogenase of the various tissues of *Oreochromis mossambicus* exposed to Diethylphthalate

CONTROL	Gill (U/mg protein)	Liver (U/mg protein)	Muscle (U/mg protein)
CONTROL	5.516±0.024[c]	3.140±0.011 [c]	1.160±0.011 [c]
5PPM	7.170±0.026 [b]	4.233±0.012 [b]	2.166±0.008 [b]
15PPM	8.176±0.020 [a]	6.146±0.014 [a]	3.633±0.012 [a]
F	4.200***	1.421***	1.305***
Significance	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).*

Exposure of *Oreochromis mossambicus* to diethylphthalate at sublethal dosages resulted in significant decline (F=6.209,P<0.001) is acid phosphatase activity of gill (Table-1) . Control group registered acid phosphatase activity of 6.173±0.027 µmol/mg protein which was found to be significantly higher than the treated groups (5ppm: 4.163±0.017 µmol/mg protein; 15ppm: 3.166±0.008 µmol/mg protein). On contrary, liver exhibited significant elevated (F=126.798, P<0.001) acid phosphatase activity in *Oreochromis mossambicus* exposed to diethylphthalate (5ppm: 0.816±0.017 µmol/mg protein; 15ppm: 1.466 ± 0.088 µmol/mg protein) when compared to untreated ones (0.267±0.017 µmol/mg protein). Similar to gills, muscle also elecited significant decline (F=38.796, P<0.001) in acid phosphatase activity in diethylphthalate exposed *Oreochromis mossambicus* (5ppm: 2.393 ± 0.253 µmol/mg protein; 15ppm: 1.120±0.005 µmol/mg protein)

Compared to liver, muscle and gill (Table-2), Alkaline phosphatase activity of *Oreochromis mossambicus* significantly increased on exposure to diethylphthalate . ALP activity of gill of DEP unexposed fish was 0.153± 0.014 µmol/mg protein, which was found to significantly elevated (F=76.248, P<0.001) on exposure to 5ppm (0.420 ± 0.032 µmol/mg protein) and 15ppm (0.556±0.020 µmol/mg protein) diethylphthalate . Thus a dose dependent relationship between the ALP activity of gill and the concentration of DEP was evident.

On exposure to DEP, liver ALP activity significantly (F=3.148,P<0.001) declined (5ppm:3.133±0.024 µmol/mg protein;15ppm:2.253±0.008 µmol/mg protein) when compared to unexposed ones (4.236±0.04 µmol/mg protein) . Similarly, muscle ALP activity declined in the fish *Oreochromis mossambicus* on exposure to diethylphthalate (5ppm: 4.163 ± 0.017 µmol/mg protein; 15ppm: 3.166±0.008 µmol/mg protein) when compared to the diethylphthalate unexposed ones (6.173±0.027 µmol/mg protein).

Sorbital dehydrogenase activity of (Table-3) gill, liver and muscle of *Oreochromis mossambicus* declined significantly on exposure to Diethylphthalate. As the concentration of DEP increased, SDH activity was found to decrease. DEP at 5ppm and 15ppm registered gill SDH activity of 0.273±0.012 U/mg protein and 0.143 ± 0.008 U/mg protein, respectively, On the other hand , control group registered sorbital dehydrogenase activity of 0.586±0.014 U/mg protein (F=359.56, P<0.001). Liver sorbital dehydrogenase activity significantly declined (F=2.455, P<0.001) in DEP exposed *Oreochromis mossambicus* (5ppm: 0.236±0.008 U/mg protein; 15ppm:0.123±0.012 U/mg protein) when compared to the control (1.206±0.014 µ/mg protein) . DEP induced significant decline (F=8.168, P<0.001) in muscle SDH activity (5ppm: 8.123±0.008 U/mg protein; 15ppm: 6.140±0.005 U/mg protein) when compared to the unexposed ones (12.150±0.015 U/mg protein).

Lactate dehydrogenase activity (Table-4) of gill in DEP unexposed fish was 5.516±0.024 U/mg protein, while significantly (F=4.200, P<0.001) increased on exposure of to diethylphthalate. Gill LDH activity at 5ppm and 15ppm were 7.170±0.026 U/mg protein and 8.176 ± 0.020 U/mg protein, respectively. Liver SDH activity significantly (F=1.421, P<0.001) increased (F=1.421, P<0.001) on exposure to diethylphthalate 5ppm: 4.233±0.012

U/mg protein; 15ppm: 6.146±0.014 U/mg protein, whereas, DEP unexposed fishes exhibited liver LDH activity of 3.140±0.011 U/mg protein. Diethylphthalate induced significant (F=1.305, P<0.001) increase in muscle LDH activity (5ppm: 2.166±0.008 U/mg protein; 15ppm: 3.633±0.012 U/mg protein) when compared to the control (1.160±0.011 U/mg protein).

DISCUSSION

The results of the present investigation reflects that DEP has altered ACP, ALP, SDH and LDH activity in the various tissues of Tilapia studied (gill, liver and muscle). Significant decline in the muscle ACP activity observed in this study coincides with that of Barse *et al* ., (2007) who have noted decrease in ACP activity of muscle in *Cyprinus carpio* exposed to DEHP 20ppm during 28 days experiment. Further, they have also observed that at 0.68 mgl-1dose of 4-tert-butylphenol, ALP activity decreased. Increased ACP activity of liver of fish exposed to DEP disagrees with that of Venkateswara rao (2006) who have observed decrease in ACP activity in liver of the fish , *Oreochromis mossambicus* exposed to RPR-V (2-butenoic acid-3- diethoxy phosphinothionyl ethyl ester). The elevation of alkaline phosphatase suggests an increase in the lysosomal mobilization and cell necrosis due to Diethylphthalate toxicity.

Reduced ALP activity was observed in the gill of *Cirrhina mrigala* , *Catla catla* and *Labeo rohita*, while reduced ACP activity was observed in the gill of *Cirrhina mrigala* , *Catla catla* and *Labeo rohita* Das *et al* ., (2004) . This line of data disagrees with the present observations.
The phosphatases (ACP and ALP) are important biomarkers because they are involved in adaptive cellular response to the potential cytotoxicity and genotoxicity of pollutants Leohner et al., (2001). The present result is in good accord with Venkatesan et al., (2012) who have observed that exposure of *Cyprinus carpio* to atrazine and spirulina decreased gill and liver ALP and ACP activity but later recovered. Decline in ALP activity may result from fall in the rate of synthesis of glycogen caused by lowered metabolic demands and electrolytic imbalance due to tissue overhydration.

Decrease in ALP may reflect a change in endoplasmic mass known to occur in the cell membrane Edquist et al., (1992). Since it also functions in the conversion of energy compounds NADPH to NAP Morton (1995), decline in ALP activity could result in biosynthesis shift and energy metabolism pathway of the exposed organism Ovuru (2000). Results from the present work indicate this may happen in wild fish exposed to diethylphthalate.

The present finding is well supported by Nchumbeni Humtsoe et al ., (2007) who have noted a significant decrease in liver ALP activity of the juvenile *Labeo rohita* exposed to arsenic (144µg/L and 96 µg/L). Further, they have also observed that ALP activity significantly declined at arsenic 144µg/L but no significant decline in liver ACP activity at 96 µg/L and have reasoned out that the decreased activities of ACP and ALP indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system.

Our findings agrees with that of Barse et al., (2006) who have demonstrated significant decline (P<0.001) in alkaline phosphatase (ALK) in the muscle of 4-tert-butylphenol exposed fish *Cyprinus carpio*.

In contraction to the present result, reduction in ALP activity was observed with increasing concentration of nitrite 1,2,4,8 and 10.4 mg L^{-1} in serum and brain, as well as in gill of *Cirrhina mrigala* ,*Catla catla* and *Labeo rohita* Das et al., (2004) .The present findings is in good accord with the observation of Nte et al., (2011) who have reported significant (P<0.05) increase in ALP activity in gill of *Sarotherodon melanotheron* exposed to RIVOC industrial effluent for 14 days . On contrary to the present result, Nivedita Ghorpade et al., (2002) have evinced significant increase in liver and muscle ALP level in DEP treated fish *Cirrhina mrigala*

The present findings disagrees with that of Nivedita Ghorpade et al., (2002) who have reported that there was a significant increase in SDH levels in muscle of *Cirrhina mrigala* . The present result is well supported by Samuel and Sastry (1989) who have shown a significant decrease in the activities of SDH *Channa punctatus* on long term exposure to an organophosphate.

Dange and Masurekar (1981) have also found decrease in the succinate dehydrogenase activity of gill, liver and muscle of Tilapia *Sarotherdon mossambicus* Peters exposed to toluene and have explained that it could be due to disturbances in the cellular oxidative processes. This is in consistent with James et al ., (1996) findings that sublethal

levels of mercury inhibits SDH activity of liver ,gill and muscle of *Heteropneustes fossilis* and have ascribed it to impairment of oxidative metabolism and also due to the mitochondrial disruption Brierly and Deung et al ., (1978). Koundinya and Ramamurthy (1978) recorded a significant inhibition in SDH activity and an increase in LDH activity to meet energy demands in the brain , liver, Kidney, intestine , gill and muscle of the fish *Tilapia mossambica* following exposure to fenitrothion.

Tripathi and Shasmal (2011) found that chlorpyriphos significantly reduced the lactate dehydrogenase (LDH) activity 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 present observation coincides with that of Dange et al ., (1981) who have investigated that exposure of Tilapia (*Sarotherodon mossambicus* Peters) to toluene has stimulated Lactate dehydrogenase activity in liver,gill and muscle. Changes in the lactate dehydrogenase and succinate dehydrogenase activity may indicate the facility with which Tilapia can shift to anaerobic metabolism under adverse conditions and it would be interesting to observe if similar enzymatic changes occur more often in the anaerobic type of fish following exposure to pollutants.

CONCLUSION

The waterways serve as an ultimate sink for various kinds of wastes. Diethylphthalate contaminated water could elicit stress in the aquatic biota. The present study is a baseline to assess the impact of diethylphthalate on the tissue damaging enzymes of gill, liver and muscle of Tilapia *Oreochromis mossambicus*. The observation of the present investigation permits us to conclude that diethylphthalate alters the enzyme activity Acid phosphatase (ACP), Alkaline phosphatase (ALP), Sorbitol dehydrogenase(SDH) , Lactate dehydrogenase (LDH) of gill, liver, muscle , which could be attributed to the mechanism by which the fish overcomes toxic stress.

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