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Enzymatic changes induced by Dibutylphthalate in Tilapia *Oreochromis mossambicus*

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Abstract
The influx of phthalate esters into the waterways could cause adverse impact on the fauna and flora inhabiting it. Keeping this in view, the present study was designed to evaluate the risk imposed by DBP (Dibutyl phthalate), which is gauged by the changes in the enzyme (ACP, ALP, SDH) concentration of various tissues (gill, liver and muscle) of Tilapia *Oreochromis mossambicus*. The changes in the enzyme concentration could be an indicator of toxic stress elicited by DBP on fishes. This study reports the modulation in the enzyme concentration of gill, liver and muscle of *Oreochromis mossambicus* on exposure to sub-lethal concentrations of DBP (2.5 ppm and 5 ppm). ACP and SDH level of gill and liver elevated at all concentrations of DBP exposure. On the other hand, elevation in ACP concentration of muscle was evident at higher concentration of DBP (5 ppm). Statistically significant elevation in muscle SDH concentration was registered in all the DBP exposed fishes *Oreochromis mossambicus*. Significant elevation in ALP concentration of gill and muscle and significant decline in ALP of liver was evinced in DBP exposed *Oreochromis mossambicus*. These enzymatic changes of various tissues could be attributed to the mechanism by which *Oreochromis mossambicus* circumvents the toxic stress caused due to DBP exposure.

Keywords: Dibutylphthalate, *Oreochromis mossambicus*, ACP, ALP, SDH.

1. Introduction
Di-n- butyl phthalate (DBP) is used as a coalescing aid in latex adhesive, a plasticizer for cellulose plastic and as a solvent for dyes [6]. Phthalate esters are considered to be a potential carcinogen, teratogen, and mutagen [4, 5]. In our previous work, we have observed that DEP alters the Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathion-S-transferase (GST), Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Sorbital dehydrogenase (SDH) and Lactate dehydrogenase (LDH) content of the gill, liver and muscle of *Oreochromis mossambicus* [12,14]. Keeping this in view, the present study was designed to study the response of various enzymes of tissues of *Oreochromis mossambicus* on exposure to DBP.

2. Materials and Methods
Dibutyl phthalate toxicity was assessed using healthy, live specimens of *Oreochromis mossambicus*, which were collected from local freshwaters. Prior to experimentation, fishes 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. Dibutylphthalate (DBP) 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 BBP (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm) for 96 hours to determine the median lethal concentration (LC_{50}) of DBP with selection exposure concentration of 2.5 and 5 ppm for chronic sub-lethal concentration exposure studies. Water was replaced daily with fresh DBP mixed water to maintain constant level of DBP during exposure period. The LC_{50} value for DBP 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 [14].

2.1 Sample preparation

Gill, liver and muscle of the fishes from the exposed and unexposed groups were dissected carefully and weighed. It was homogenized with chilled sucrose solution (0.25 M) in a glass tube using Teflon coated mechanical tissue homogenizer (MICCRA D-9, Digitronic, Germany). The homogenate was centrifuged at 10000 rpm for 20 min at 4 ^{0}C in a cooling centrifuge. The resulting supernatant was removed and stored (- 40 ^{0}C) for use in tissue enzyme assays Acid Phosphatase (ACP), Alkaline Phosphatase (ALP) and Sorbital dehydrogenase (SDH).

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).

3. Results and Discussion

Table 1: Changes in the Acid Phosphatase of the various tissues of *Oreochromis mossambicus* exposed to Dibutylphthalate

Dibutylphthalate Treatment	Gill (µg/ mg protein)	Liver (µg/ mg protein)	Muscle (µg/ mg protein)
Control	0.5900 ± 0.0115^{c}	0.2467 ± 0.0088^{c}	0.1500 ± 0.0173^{b}
2.5 ppm	1.3500 ± 0.0173^{b}	0.4933 ± 0.0088^{b}	0.1833 ± 0.0066^{b}
5 ppm	2.1667 ± 0.0088^{a}	0.8533 ± 0.0145^{a}	0.2333 ± 0.0120^{a}
F-value	3.649***	761.576***	10.795**

***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 Dibutylphthalate

Dibutylphthalate Treatment	Gill (µg/ mg protein)	Liver (µg/ mg protein)	Muscle (µg/ mg protein)
Control	3.5867 ± 0.0145^{c}	8.7467 ± 0.0202^{a}	12.1433 ± 0.0033^{c}
2.5 ppm	4.8467 ± 0.0088^{b}	6.2133 ± 0.0202^{b}	13.2467 ± 0.0202^{b}
5 ppm	5.4500 ± 0.0173^{a}	5.1600 ± 0.0057^{c}	14.2133 ± 0.0202^{a}
F-value	4.605***	1.192***	3.862***

***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 Dibutylphthalate

Dibutylphthalate treatment	Gill (µg/ mg protein)	Liver (µg/ mg protein)	Muscle (µg/ mg protein)
Control	0.8667 ± 0.0218^{c}	0.2567 ± .01453^{c}	0.1300 ± .0100^{c}
2.5 ppm	0.9600 ± 0.0152^{b}	0.3600 ± .01155^{b}	0.2433 ± .0088^{b}
5 ppm	1.1333 ± 0.0088^{a}	0.4300 ± .01155^{a}	0.3300 ± .0115^{a}
F-value	69.634***	47.744***	97.000***

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

Influence of dibutylphthalate on the acid phosphatase concentration of gill, liver and muscle of *Oreochromis mossambicus* is presented in Table-1. Elevation in acid phosphatase concentration was observed in the gill and liver in DBP exposed fish when compared to the unexposed ones. DBP at 2.5 ppm and 5 ppm registered acid phosphatase concentration of 1.3500 ± 0.0173 µg/mg and 2.1667 ± 0.0088 µg/mg ,respectively, which was found to be significantly (F = 3.649, P < 0.001) higher than the control group (0.5900 ± 0.0115 µg/ mg). Liver acid phosphatase concentration significantly (F = 761.576, P < 0.001) increased on exposure of *Oreochromis mossambicus* to DBP (2.5 ppm: 0.4933 ± 0.0088 µg/mg; 5 ppm: 0.8533 ± 0.0145 µg/ mg) when compared to DBP unexposed ones (0.2467 ± 0.0088 µg/mg). In comparison to the control (0.1500 ± 0.0173 µg/mg) and DBP at 2.5ppm (0.1833 ± 0.0066 µg/mg) concentration, significant (F = 10 .795, P < 0.001) increase in muscle ACP concentration was evinced at higher concentration of DBP exposure (5 ppm : 0.2333 ± 0.0120 µg/mg).

From table -2 it is inferred that DBP alters the ALP activity of gill, liver and muscle of *Oreochromis mossambicus* when compared to the control. All the DBP treated fishes exhibited significant (F = 4605, P < 0.001) increase in ALP concentration of gill (2.5 ppm: 4.8467 ± 0.0088 µg/mg; 5ppm: 5.4500 ± 0.0173 µg/mg) when compared to the control (3.5867 ± 0.0145 µg/mg). Statistically significant (F = 1.1920, P < 0.001) decrease in ALP concentration of liver was evinced in DBP exposed fishes (2.5 ppm: 6.2133 ± 0.0202 µg/mg; 5 ppm: 5.1600 ± 0.0057 µg/mg) when compared to the DBP unexposed ones (8.7467 ± 0.0202 µg/ mg). Muscle ALP activity significantly (F = 3.862, P < 0.001) increased in DBP exposed fishes (2.5 ppm: 13.2467 ± 0.0202 µg/mg: 5 ppm: 14.2133 ± 0.0202 µg/mg) when compared to DBP unexposed fishes (12.1433 ± 0.0033 µg/mg).

Significant (F = 69.634, P < 0.001) elevation in SDH concentration of gill was registered in DBP exposed fishes (2.5 ppm: 0.9600 ± 0.0152 µg/mg; 5 ppm: 1.1333 ± 0.0088 µg/mg) when compared to control fishes (0.8667 ± 0.0218 µg/mg) (table-3). Similarly, liver SDH concentration also significantly (F = 47.744, P < 0.001) elevated in DBP exposed fishes (2.5 ppm: 0.3600 ± 0.01155 µg/mg; 5 ppm: 0.4300 ± 0.01155 µg/mg) when compared to DBP unexposed fishes (0.2567 ± 0.01453 µg/mg). On exposure of *Oreochromis mossambicus* to DBP, muscle SDH activity significantly (F = 97.000, P < 0.001) elevated (2.5 ppm: 0.2433 ± 0.0088 µg/mg; 5 ppm: 0.3300 ± 0.0115 µg/mg) when compared to the DBP unexposed ones (0.1300 ± 0.0100 µg/mg).

The increase in mean ACP concentration of gill, liver and muscle observed in DBP exposed fishes agrees with that of Barse *et al*., [2] who have also noticed increased ACP activity in the muscle tissue of *Cyprinus carpio* exposed to 4-tert-butyl phenol (1.38 and 2.3 mg L^{-1}). Barse *et al*., [3] have noted decrease in ACP activity of muscle of *Cyprinus carpio* exposed to diethylhexylphthalate 20 ppm during 28 days. Experiment, which is contradictory to the present investigation. Increased ACP activity as response of clams exposed to contaminated sites with pollutants such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals have been observed by Tay *et al*., [13]. The present findings partially agrees with that of Nchumbeni Humstoe *et al*., [7] who have evinced pronounced effect of arsenic on ACP, ALP, GOT and GPT activity of liver and muscle tissue of *Rohu carp Labeo rohita*. It has been shown that the liver is the prime location for removing xenobiotics and biocides in fishes [10]. Our results partially coincides with that of Venkateswara rao *et al*., [18] who have noted significant decrease in ALAT,ASAT,ACP and AKP activities in liver of fish, *Oreochromis mossambicus* exposed to RPR-V-(2-butenoic and -3- diethoxy Phosphino thionyl ethyl ester). Acid phosphatase and alkaline phosphatase catalyse the hydrolysis of various phosphate containing compounds and act as transphorylases at acid and alkaline pHs, respectively [1].

The increase in various enzyme concentration of different organs of DBP exposed Tilapia *Oreochromis mossambicus* evinced in this study is in line with the observations of Nte *et al*., [9] who have reported significant (P < 0.05) increase in AST, ALT, ACP and ALP activity in spleen, gill, kidney and muscle of *Sarotherodon melanotheron* exposed to RIVOC industrial effluent for 14 days. Further, they have also observed significant enhancement in AST, ACP and ALP activity of liver and significant (P < 0.05) decline in ACP activity when compared to the control.

We have observed significant elevation in the ACP, ALP and LDH activity of gill, liver and muscle of BBP exposed Tilapia *Oreochromis mossambicus*. In addition, we have also noticed that SDH activity of gill and muscle significantly increased and that of liver significantly decreased and have attributed it to the mechanism by which the fish overcomes the toxic stress [4].

The present observation partially agrees with that of Nivedita Ghorpade [8] who have recorded significant increase in liver and muscle ALP level in DEP (Diethylphthalate) treated fish *Cirrhina mrigala* and have attributed it to increased lysosomal activity in the liver and muscle. The present result gains support from the findings of Barse *et al*., [3] who have reported increase in muscle ALP of *Cyprinus carpio* in response to DEP treatment.

Nivedita Ghorpade *et al*., [8] have evinced significant increase in SDH levels in muscle and have attributed it to involvement of mitochondria in DEP toxicity of *Cirrhina mrigala* on exposure to DEP. This finding lies in parallel with the present observation. The present findings partially agrees with that of our previous observation Umamaheswari and Senthilnathan [17] that ACP activity of gill significantly decreased in the liver and increased in the muscle of Tilapia *Oreochromis mossambicus* exposed to DEP when compared to the control.

The toxic effects of DBP on the SDH levels of gill, liver and muscle partially agrees with that of Sastry *et al*., [12] who have reported quinolphos induced elevation in the activities of SDH in the intestine and inhibition of the same in other tissues (liver, kidney, gill, skeletal muscle, ovary and testis). On contrary, Samuel and Sastry [11] have observed significant decrease in the activities of glucose-6-phosphate, hexokinase, LDH, SDH and MDH in *Channa punctatus* on long term exposure to an organophosphate pesticide.

4. Conclusion

The toxic stress induced by DBP in *Oreochromis mossambicus* is evinced in this study. Significant elevation in ACP and SDH activity of gill, liver and muscle is observed in DBP exposed Tilapia *Oreochromis mossambicus*. On the other hand, ALP concentration significantly elevated in the gill and muscle and declined in the liver of DBP exposed *Oreochromis mossambicus* when compared to the control. The modulation in the enzyme concentration of various tissues observed in this study could be attributed to the mechanism by which fish circumvents the toxic stress induced by DBP.

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