Modulation of Enzyme activity in *Oreochromis mossambicus* (Tilapia) exposed to Butylbenzylphthalate

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ABSTRACT

Wide application of phthalates as plasticizers due to their durability and sustainability has been documented. Phthalates are known to disrupt the enzyme activity of various tissues organs in fishes and rats. The present study focused on the changes elicited by BBP on the enzyme activity (Acid phosphatase, Alkaline phosphatase, Sorbitol dehydrogenase, Lactate dehydrogenase) of the various organs (gill, liver and muscle) of *Oreochromis mossambicus*. The statistical analysis of the data reveal that BBP induced significant modulation in enzyme activity of gill, liver and muscle of the fish *Oreochromis mossambicus*. Significant elevation in the ACP, ALP, LDH activity of gill, liver and muscle were observed in BBP exposed fishes. On the other hand, SDH activity of gill and muscle significantly increased, while that of liver significantly declined. This changes in enzyme activity could be due to the mechanism by which the fish overcomes toxic stress.

Keywords: Butylbenzylphthalate, *Oreochromis mossambicus*, ACP, ALP, SDH, LDH

1. Introduction

Phthalate esters have been detected in highest concentration in urban and rural fish eaters than urban and rural vegeterious [23]. Phthalates are esters of phthalic acid (1,2- benzene dicarboxylic acid) that are primarily synthesized using fisher esterification of phthalic anhydride and the corresponding alcohol [27]. The influence of pollutants an enzymatic activity of fish is one of the most important biochemical parameters which are affected under exposure of toxicants. On exposure to a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted. This increase or decrease in enzyme level is a very accurate index for diagnostic of quantity and quality of toxicant [1].

Man-made Xenoestrogens compounds like Diethylphthalate, Diethylhexylphthalate are known to cause alterations in the enzyme activity of various organ in fishes [7, 14, 3, 29, 30]. Sewage fed fisheries is practiced in many Countries including in India with waste water utilisied for the purpose of culturing fishes [13, 8]. Taking these facts in to consideration, the present study was designed to study the effect of sublethal doses of Butylbenzylphthalate on the enzyme activity of gill, liver and muscle of tilapia *Oreochromis mossambicus*

2. Materials and Methods

Butylbenzylphthalate toxicity were assessed using healthy, living specimens of *Oreochromis mossambicus* which were collected from local freshwaters. Prior to experimentation fishes 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. Butylbenzylphthalate (BBP) 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 (LC50) of BBP with selection exposure concentration of 5 and 15 ppm for chronic sub-lethal concentration exposure studies. Water was replaced daily with fresh BBP mixed water to maintain constant level of BBP during exposure period. The LC50 value for DEP was 50 ppm. For sub-lethal study, 1/5th and 1/10th of the LC50 value were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.
**2.1 Sample preparation**

Tissue homogenate preparation the gill, liver and muscle of the fishes from the exposed and non-exposed 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 (MICRA D-9, Digitronic, Germany). The homogenate was centrifuged at 10000 rpm for 20 min at 4 °C in a cooling centrifuge machine. The resultant supernatant was removed and stored (-40 °C) for use in tissue enzyme assays (Acid phosphatase (ACP), Alkaline phosphatase (ALP), Sorbitol dehydrogenase (SDH), Lactate dehydrogenase (LDH)).

**2.2 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 (16.0). The values for P<0.001 were considered significant. Accordingly, a statistical software package (SPSS) was used.

### 3. Result

#### Table 1: Changes in the Acid Phosphatase of the various tissues of Oreochromis mossambicus exposed to Butyl benzyl Phthalate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gill (µmol/mg protein)</th>
<th>Liver (µmol/mg protein)</th>
<th>Muscle (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.576±0.037</td>
<td>3.150±0.019</td>
<td>7.260±0.025</td>
</tr>
<tr>
<td>5-ppm</td>
<td>1.290±0.056</td>
<td>5.300±0.040</td>
<td>11.400±0.205</td>
</tr>
<tr>
<td>15-ppm</td>
<td>3.213±0.020</td>
<td>7.203±0.594</td>
<td>13.180±0.07</td>
</tr>
<tr>
<td>F-value</td>
<td>1.199E3</td>
<td>34.655</td>
<td>43.266</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**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 Butyl benzyl Phthalate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gill (µmol/mg protein)</th>
<th>Liver (µmol/mg protein)</th>
<th>Muscle (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.426±0.040</td>
<td>21.583±0.023</td>
<td>17.183±0.017</td>
</tr>
<tr>
<td>5-ppm</td>
<td>25.266±0.029</td>
<td>31.213±0.020</td>
<td>21.173±0.026</td>
</tr>
<tr>
<td>15-ppm</td>
<td>31.270±0.047</td>
<td>41.576±0.020</td>
<td>21.073±0.026</td>
</tr>
<tr>
<td>F-value</td>
<td>3.434E4</td>
<td>2.195E5</td>
<td>5.911E3</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**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 Sorbitol Dehydrogenase of the various tissues of Oreochromis mossambicus exposed to Butyl benzyl Phthalate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gill (U/mg protein)</th>
<th>Liver (U/mg protein)</th>
<th>Muscle (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.306±0.049</td>
<td>15.243±0.040</td>
<td>49.34±0.040</td>
</tr>
<tr>
<td>5-ppm</td>
<td>25.266±0.029</td>
<td>31.213±0.020</td>
<td>21.173±0.026</td>
</tr>
<tr>
<td>15-ppm</td>
<td>31.270±0.047</td>
<td>41.576±0.020</td>
<td>21.073±0.026</td>
</tr>
<tr>
<td>F-value</td>
<td>8.243</td>
<td>4.235</td>
<td>1.727E4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.19</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**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 Butyl benzyl Phthalate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gill (U/mg protein)</th>
<th>Liver (U/mg protein)</th>
<th>Muscle (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.473±0.073</td>
<td>28.586±0.026</td>
<td>32.388±0.017</td>
</tr>
<tr>
<td>5-ppm</td>
<td>17.193±0.008</td>
<td>32.183±0.017</td>
<td>37.200±0.026</td>
</tr>
<tr>
<td>15-ppm</td>
<td>19.326±0.046</td>
<td>38.290±0.028</td>
<td>39.226±0.049</td>
</tr>
<tr>
<td>F-value</td>
<td>865.351</td>
<td>3.962E4</td>
<td>1.062E4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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

Chronic exposure of Zebrafish Danio rerio to Butylbenzyl phthalate and the resultant ACP activity of gill, liver and muscle are presented in table 1. Gill ACP activity significantly (F=1.99E3, P<0.001) elevated on exposure of Zebrafish to Butylbenzyl phthalate when compared to BBP unexposed ones (0.576 ± 0.031µmol/mg protein). As the concentration of BBP increased, the ACP activity of the gill also increased 5 ppm: 1.29±0.056 µmol 0/mg protein; 15 ppm: 3.21±0.20 µmol/mg protein. Similarly, liver ACP activity also significantly increased on exposure of Zebrafish Danio rerio to BBP when compared to the unexposed ones 3.150±0.017 µmol/mg protein. BBP at 5 ppm and 15 ppm registered ACP activity of 5.300±0.047 µmol/mg protein and 7.203±0.594 µmol/mg protein, respectively. Muscle also exhibited similar pattern of ACP activity in BBP exposed Zebrafish. BBP elicited significant elevation in (P<0.001) muscle ACP activity 5 ppm: 11.400±0.25 µmol/mg protein; 10 ppm: 13.180±0.07 µmol/mg protein when compared to the DEHP unexposed ones 7.260±0.025 µmol/mg protein. Thus, a dose dependent relationship between the concentration of BBP and ACP activity of Gill, Liver and muscle was evident.

ALP activity of Gill, Liver and Muscle also elevated similar response in the BBP exposed Zebrafish Danio rerio. Control groups recorded gill ALP activity of (15.426±0.049 µmol/mg protein) which was found to be significantly (F=3.434E4, P=0.001 lower than BBP treated fishes 5 ppm: 25.266±0.029 µmol/mg protein; 15 ppm: 31.270±0.047 µmol/mg protein) increased on exposure of Zebrafish to BBP (Table-2). In liver, ALP activity significantly elevated F=2.195E5, P<0.001 in Zebrafish on exposure to BBP: BBP at 5ppm and 10 ppm registered liver ALP activity of 31.213±0.20 µmol/mg protein and 41.576±0.020 µmol/mg protein, respectively. As in the case of gill ACP activity, muscle ALP also elicited dose dependent relationship between the concentration of BBP and enzyme activity. As the concentration of BBP increased, gill, liver and Muscle ALP and ACP activity also significantly increased.

SDH activity of gill and muscle significantly elevated on exposure of the Zebrafish to BBP(Table-3). In comparison to the control 17.306±0.049 U/mg protein BBP treated Zebrafish exhibited significant F=8.243, P=0.19 elevation in gill SDH activity 5ppm:18.213±0.053;U/mg protein 15 ppm:21.183±0.017 U/mg protein BBP exposed fish recorded muscle ALP activity of 18.146±0.034 µmol/mg protein and 21.173±0.026 µmol/mg proteinat 5ppm and 15ppm, respectively. As in the case of gill ACP activity, muscle SDH also elicited dose dependent relationship between the concentration of BBP and enzyme activity. As the concentration of BBP increased, Gill, Liver and Muscle ALP and ACP activity also significantly increased.
recorded SDH activity of 13.123±0.014 U/mg protein which was found to be significantly lower than BBP unexposed ones. BBP induced significant elevation in the LDH activity of gill 865.351 U/mg protein, P<0.001, liver 3.962E4 U/mg protein, P<0.001 and muscle (1.062E4 U/mg protein, P<0.001) in Zebrafish Danio rerio when compared to the control. BBP at 5ppm and 15ppm registered gill LDH activity of 17.193±0.008 U/mg protein and 19.326±0.046 U/mg protein, respectively. On the other hand control group registered gill LDH activity of 16.473±0.073 U/mg protein. Liver LDH activity of BBP exposed fishes were found to the 32.183±0.17 U/mg protein (5 ppm) and 38.290±0.028 U/mg protein (15ppm) which was significantly higher than BBP unexposed ones 28.556±0.026 U/mg protein. Significant elevation in the muscle LDH activity was noticed in the BBP treated fishes (5ppm and 15 ppm; 37.200±0.026 U/mg protein and 39.226±0.049 U/mg protein, respectively, when compared to the control 32.383±0.017 U/mg protein.

The results of this study indicate fluctuations in the enzyme activity (ACP, ALP, SDH, LDH) of gill, liver and muscle tissue of Zebrafish Danio rerio on exposure to sublethal dosages of BBP. Thus this alteration in the enzyme activities could be due to protective mechanism to overcome the toxic stress caused by BBP in Zebrafish Danio rerio.

4. Discussion

The pattern of enzyme activity evinced in this study reflects that BBP induces changes in the enzyme activity of various tissues, resultantly could cause metabolic changes in the fishes Danio rerio. Elevated levels of ACP activity of gill, liver and muscle in the BBP exposed Zebrafish Danio rerio gains partial support from this findings of Barse et al[9] who have reported increased ACP activity in the muscle tissue of Cyprinus carpio exposed to 4-tet butylphenol (1.38 and 2-3mgL^-1). In addition, they have also noticed that at 0.68 mg L^-1 dose of 4-tet butylphenol, ALP activity declined which is contrary to the present findings. On contrary to the present result, Barse et al. [10] have observed decreased muscle ACP activity in Cyprinus carpio exposed to diethyl phthalate 20 ppm dosage 28 days exposure. Increased ACP only was evident in clams exposed to contaminated sites with pollutants such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals Tayet et al. [17]. In our previous findings we have registered decline in gill, liver and muscle ACP activity of Zebrafish Danio rerio on exposure to DEHP. Barse et al. [9]. This observation disagrees with the present result Furthermore, we have also evinced. Elevated liver ACP activity in DEHP exposed Zebrafish Danio rerio which coincides with the increased liver activity of BBP exposed Zebrafish evinced in this study. It has been shown that the liver in the prime location for removing xenobiotics and biocides in fishes Roy. [21]. Similarly, Valavanidis et al. [32] have noted significant elevation in gill ACP and AKP(Alkaline phosphate) activity in Oreoichromis mossambicus exposed to RPR-V (2-butenoeic acid-3- diethoxy phosphinothionyl ethyl ester). On contravery to the elevated liver ACP and ALP only observed in the present investigation, Venkateswara Rao [33] detected significant decrease is liver ACP and AKP activity of Tilapia (Oreoichromis mossambicus). They have also suggested that the elevation of Alkaline phosphatase in due to increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity.

The present findings partially coincides with the reports of Alireza Safahieh et al. [21] who have observed considerable decline in serum ALP activities and significant increase in ACP activities of Yellow fin Sea bream (Acanthopagrus latus) on exposure to mercury (10 μg to 80 μg)

The present findings lies in parallel to the observations of Karanet et al. [14] who have found increased levels of serum ACP on exposure of Carp Cyprinus carpio to copper sulphate for 14 days. It was shown by Poleksic et al. [19] that ACP activity elevates on exposure of Cyprinus carpio to the herbicides Trifuralin.

Our results correlates with Nte et al. [18] who have evinced significant increase in ALP and ACP activity of gill and muscle of Sarotherodon melanotheron exposed to RIVOC industrial effluents for 14 days On the other hand, they have noticed significant decline in liver ACP activity when compared to the control. This report is not in good accord with the present results. Our results are in agreement with that of Ghorpade et al. [7] who have observed significant increase in liver and muscle ALP level in DEP treated fish Cirrhina mrigala and have attributed it to increased lysosomal activity in the liver and muscle. The present observation agrees with that of Barseet et al. [2] who have reported increase in muscle ALP in Cyprinus carpio in response to DEP treatment.

The present observation not in good accord to the observation of Barse et al. [9] who have demonstrated significant decline (P<0.05) in alkaline phosphate in the muscle of 4-tet butyl phenol fish Cyprinus carpio. Increase in the levels of ALP and AST has been shown to reflect liver damage, while rise in the ALP level may be indicative of renal and liver damage Bhattacharya et al. [8] Gillett et al. [9] Guluzar Atli et al. [10] have generalized that ALP activity in the liver, intestine, and serum of Oreoichromis niloticus increased following metal exposure. In contradiction to the present findings, Bernet et al. [4] showed that there was a decrease in serum ALP activity in Salmo trutta exposed to effluent form the sewage treatment compared to tap water. 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. While reduced ACP activity was observed in the gill of Cirrhina mrigala, Catla catla and Labeo rohita Daset al. [6], These observation are contradictory to the present results.

Elevation in SDH activity of gill and muscle of Zebrafish Danio rerio exposed to n-butylbezylphthalate observed in this study coincides with that of Ghorpade et al. [7] who have evinced increased SDH activity of muscle of Cirrhina mrigala and have attributed it to the involvement of mitochondria in DEP toxicity. Further they have also noticed in significant increase in liver activity which disagrees with the present observation. Organophosphate induced reductions in the activities of LDH,MDH,SDH, cytochrome oxidase, glucose 6 phosphatase, acid phosphatase and acetylcholinesterase (ACHE) in various tissues of fishes have been reported by several investigators Rao et al. [20] Sastry et al. [25] Kabeer-Ahammadsahib et al. [15] Natarajan et al. [17], Inbaraj et al. [12] Tripathi et al. [20] Samuel et al. [23] Valavanidis et al. [31] Our results partially agrees with Sastry et al. [24] who have found inhibition in the activity of LDH in liver, kidney, intestine, brain, gill and skeletal muscle of snake headed fish Channa punctatus exposed chronically to
The present study is in good accord with the findings of Hung-Sung attributed it to impairment of lactate utilisation aerobically by these tissues on exposure of Channa punctatus to quinolophs. The present study is in good accord with the findings of Hung-Sung et al. [11] Who have noticed significant elevation in ACP level in haemocyte of green neon shrimp Neocaridina denticulata.

5. Conclusion

The enzyme assay of various tissues of BBP exposed Tilapia Oreochromis mossambicus reveal that BBP has altered the enzyme activity of gill, liver and muscle tissues.

6. Acknowledgement

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7. Reference

27. Tay KL, Doe K, Lee K, Jackman P. Histopathological and histochemical biomarker responses of Baltic clam,


