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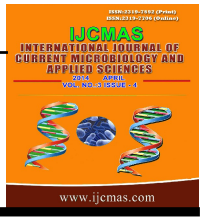


**Original Research Article**

# Antioxidant enzymatic response to Butylbenzylphthalate exposures in a fresh water fish *Oreochromis mossambicus*

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**A B S T R A C T**

Butylbenzylphthalate are a component of building materials, vinylgloves, artificial leather and plastics toys. Only few studies have documented the chronic toxicity of BBP in rats, mice and humans. Since the waterways are the prime reservoir for the various types of wastes contributed by anthropogenic activities, the present investigation was initiated to gain insight into the effect BBP on the enzyme activity (Superoxide Dismutase (SOD), Catalase (CAT) , Glutathione Peroxide (GPx) , Glutathion-S-transperase (GST)) of various organs (gill, liver and muscle) of the fish *Oreochromis mossambicus* . The observation registered in this study reflect that all the enzymes activities were significantly enhanced in all the tissues (Gill, liver and muscle) when compared to BBP unexposed ones. This could be due to the detoxification mechanism exhibited by the fish *Oreochromis mossambicus* on exposure of BBP.

**K e y w o r d s**

Butyl benzyl Phthalate, *Oreochromis mossambicus*, SOD, CAT, GPx, GST

## Introduction

The exposure of fish to Xenoestrogen are known to interfere with the metabolic process by altering enzyme activity in vital organs Ghorpade et al., (2002). Phthalate esters have been suspected to be one of the estrogenic group of compounds (Jopling et al., 1995).

Tilapia is considered to be future of aquaculture, is nicknamed "The aquatic chicken" due to its ability to grow quickly with poor quality inputs (Neeraj kumar et al., 2011). Tilapia is a good biological model for toxicological and immune toxicity studies (Casas Solis et al., 2011; Giron Perez et al 2007; Giron –Perez 2008) due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity and at prolific rate and finally, good tolerance to a wide range of environmental conditions (Fontainhas- Fernandes, 1998).
The antioxidant defense systems (AD) of organisms provides a mean of dealing with oxidative stress and includes several enzymes and vitamins (Filho et al., 1996; Rudern et al., 1997; Kelly et al ., 1998;

Macon and Filho, 1999). A primary role of the antioxidant defense system is protecting cellular compounds from ROS (Reactive Oxygen Species) (Kelly et al., 1998).With this background knowledge, the present study was initiated to gain insight into the oxidative stress caused by Butylbenzylphthalate in Tilapia *(Oreochromis mossambicus)*.

## 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 100ppm) 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, $1/5^{th}$ and $1/10^{th}$ of the $LC_{50}$ value were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.

### 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 (MICCRA D-9, Digitronic, Germany). The homogenate was centrifuged at 10000 rpm for 20 min at $4^0$C in a cooling centrifuge machine. The resultant supernatant was removed and stored (-$40^0$C) for use in tissue enzyme assays (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxide (GPx, Glutathion-S-transperase (GST).

### 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.

## Results and Discussion

The enzyme activity of various tissues were assayed in Tilapia *Oreochromis mossambicus* exposed to BBP. The data displayed in Table-1 indicate that BBP at sublethal concentrations (5ppm:4.160 ± 0.020 U/mg protein ;15ppm: 6.250 ± 0.026 U/mg protein) have significantly elevated ($F = 5.570E3, P < 0.01$) SOD activity of gill when compared to the control (2.240 ± 0.032 U/mg protein). Similarly significant increase $F = 4.651E4$, $P < 0.001$) in SOD activity of liver was evinced in BBP exposed Tilapia (5ppm:8.183 ± 0.034 U/mg protein;15ppm:16.213 ± 0.020 U/mg protein) when compared to the BBP unexposed ones (6.123 ± 0.014 U/mg protein).Muscle SOD significantly (F =

1.431E4) enhanced in Tilapia exposed to 5ppm and 15ppm (10.086 ± 0.020 U/mg protein and 13.180 ± 0.017 U/mg protein, respectively) when compared to the control fishes (8.160 ± 0.025 U/mg protein). Furthermore, dose dependent relationship was evident between BBP concentration and SOD activity of Gill, Liver and Muscle.

As the concentration of BBP increased catalase activity of gill also enhanced significantly (5ppm:15.213 ± 0.020 U/mg protein ;15ppm 19.356 ± 0.024 U/mg protein) (F = 7.340E3, P < 0.001). On the otherhand, BBP unexposed fish registered gill SOD activity of 12.183 ± 0.065 U/mg protein and BBP at 5ppm and 15ppm recorded (20.213 ± 0.037 U/mg protein and 23.913 ± 0.361 U/mg protein, respectively) (table-2). Significantly (F = 1.489E3, P < 0.001) higher. liver SOD activity was registered when compared to the control (8.233 ± 0.056 U/mg protein). From table-2 it is evident the muscle Catalase activity of BBP exposed tilapia *Oreochromis mossambicus* elecited significant increase (F = 3.078E3, P < 0.001) when compared to the control (4.170 ± 0.026 U/mg protein). Similar to SOD, Catalase activity also exhibited a dose dependent relationship with BBP concentrations.

Significant increase (F = 186.75, P < 0.001) in GPx activity of gill, was observed in Tilapia exposed to BBP (5ppm:13.306 ± 0.061 U/mg protein;15ppm:18.656 ± 0.061 U/mg protein) when compared to the control (13.306 ± 0.09 U/mg protein). Liver also elicited similar response to BBP with regard to GPx activity. As the BBP concentration increased, liver GPx activity also elevated (5ppm :18.306 ± 0.049 U/mg protein; 15ppm:21.766 ± 0.014 U/mg protein), whereas, control registered GPx liver avtivity of 16.253 ± 0.038 which were found to significantly lower (F = 5.68E3, P < 0.001) when compared to the BBP treated ones. BBP significantly elevated (F = 1.98E4, P < 0.001) muscle GPx activity in Tilapia when compared to the control fishes (14.193 ± 0.008 U/mg protein).BBP at 5ppm and 15ppm registered muscle GPx activity of 15.386 ± 0.020 U/mg protein and 17.343 ± 0.012 U/mg protein, respectively, table-3.

The data presented in Table-4 reveal significant increase (F = 3.335E4, P < 0.001) in gill GST activity of Tilapia an exposure to BBP (5ppm:31.360 ± 0.015 U/mg protein;15ppm:37.383 ± 0.017 U/mg protein) when compared to the control (27.206 ± 0.042 U/mg protein). Similar patterns of change was evinced in liver GST activity of Tilapia *Oreochromis mossambicus* on exposure to BBP. BBP at 5ppm and 15ppm registered . Liver GST activity of 61.543 ± 0.014 U/mg protein and 72.180 ± 0.017 U/mg protein, respectively, Which was significantly higher (F = 4.046E5,P < 0.001) than the control group (54.476 ± 0.008 U/mg protein). Muscle GST activity also significantly elevated (F = 6.667E3, P < 0.001) on exposure to BBP (5ppm: 41.680 ± 0.020 U/mg protein ; 15ppm:43.680 ± 0.173 U/mg protein) when compared to the control (39.253 ± 0.038 U/mg protein). The present result is in parallel to the observation of Neeraj kumar *et al.,* (2011) who have demonstrated that activities of anti-oxidative enzymes was significantly (P < 0.01) influenced by endosulfan in dose dependent manner in Tilapia *Oreochromis mossambicus* . They have noticed significant (P < 0.01) increase in

**Table.1** Changes in the Superoxide Dismutase of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

| BBP Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | 2.240 ± 0.032$^{c}$ | 6.123 ± 0.014$^{c}$ | 8.160 ± 0.025$^{c}$ |
| 5-ppm | 4.160 ± 0.020$^{b}$ | 8.183 ± 0.034$^{b}$ | 10.086 ± 0.020$^{b}$ |
| 15-ppm | 6.250 ± 0.026$^{a}$ | 16.213 ± 0.020$^{a}$ | 13.180 ± 0.017$^{a}$ |
| F-value | 5.570E3 | 4.651E4 | 1.431E4 |

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

| BBP Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | 12.183 ± 0.065$^{c}$ | 8.233 ± 0.056$^{c}$ | 4.170 ± 0.026$^{c}$ |
| 5-ppm | 15.213 ± 0.020$^{b}$ | 20.213 ± 0.037$^{b}$ | 6.736 ± 0.020$^{b}$ |
| 15-ppm | 19.356 ± 0.024$^{a}$ | 23.913 ± 0.361$^{a}$ | 7.193 ± 0.038$^{a}$ |
| F-value | 7.340E3 | 1.489E3 | 3.078E3 |

*****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 Glutathione Peroxide (GPx) of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

| BBP Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | 13.306 ± 0.061$^{c}$ | 16.253 ± 0.038$^{c}$ | 14.193 ± 0.008$^{c}$ |
| 5-ppm | 16.506 ± 0.336$^{b}$ | 18.306 ± 0.049$^{b}$ | 15.386 ± 0.020$^{b}$ |
| 15-ppm | 18.656 ± 0.020$^{a}$ | 21.766 ± 0.014$^{a}$ | 17.343 ± 0.012$^{a}$ |
| F-value | 186.753 | 5.68E3 | 1.198E4 |

*****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 Glutathion-S-transperase (GST) ) of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

| BBP Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | $27.206 \pm 0.042^{c}$ | $54.476 \pm 0.008^{c}$ | $39.253 \pm 0.038^{c}$ |
| 5-ppm | $31.360 \pm 0.015^{b}$ | $61.543 \pm 0.014^{b}$ | $41.680 \pm 0.020^{b}$ |
| 15-ppm | $37.383 \pm 0.017^{a}$ | $72.180 \pm 0.017^{a}$ | $43.680 \pm 0.173^{a}$ |
| F-value | 3.335E4 | 4.046E5 | 6.667E3 |

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

the activity of CAT, SOD and GST in the gill and liver of Tilapia. The elevated level of SOD and catalase activity of liver of Tilapia *Oreochromis mossambicus* exposed to BBP correlates with the findings of Ezemonye and Erineku (2011) who have reported significant ($P < 0.05$) dose dependent increase in specific activity of superoxide dismutase (SOD) and catalase (CAT) relative to controls in the liver of *Hoplobatrachus occipitalis* exposed to cadmium (0.25,0.5,100 and 2.00 mg $L^{-1}$) for a period of 28 days.

Superoxide dismutase is an antioxidant enzyme involved in the elimination of ROS (reactive oxygen species). The antioxidant enzymes that make up the antioxidant defense system are expected to be intrinsically linked and dependent upon the activity of one another, Therefore, one could expect to see correlative changes in the activity of SOD and CAT ( Filho *et al* ., 1993) . Thus such pattern was observed in their study and correlative activity among Catalase and Superoxide dismutase has been evinced. The present result well supported by Guluzar Atli and Mustafa Canli (2007) who have demonstrated a significant increase in CAT activity in the liver of *Oreochromis niloticus* exposed to 10µM Cd. On the other hand, lower liver CAT activity was evinced at 10µM Zn exposure. The liver was found to be stronger into the face of oxidative stress than the other tissues and an organ with the highest antioxidant enzyme activities (SOD, CAT). Similar response was observed in liver CAT, SOD and GST activity of rainbow trout (*Oncorhynchus mykiss*) exposed to sub lethal concentration of carbosulphan (25 µg $L^{-1}$) for a period of 60 days.

Oxidative stress occurs if the activity of the antioxidant defense systems such as SOD, CAT and GPx (glutathione peroxidase) enzymes change by environmental pollution induces the production of reactive oxygen species (Li *et al*., 2011).

Canada and Calabrese (1989) have reported 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 pollutants increased SOD as well as CAT activity (Marther Minaich and Di Giulio, 1991). The present results partially agrees

with Oruc, (2010) who have evinced decreased GST and increased SOD activity in *Oreochromis niloticus*. In rainbow trout exposed to endosulfan SOD and CAT activities increase while GPx activity did not change compared to the control. Mullet (*Mugil cephalus*) captured from polluted areas had greater SOD, CAT and GPx activities (Rodriguez-Ariza *et al.*, 1993). Conversely, CAT, GST, GR, and SOD activity levels fluctuated in guppy fish (*Poecilia reticulata*) exposed to methyl parathion.

The present finding partially agrees with Menezes *et al.*, (2011) who have showed that SOD and CAT activities recovered from the effect of herbicide clomazone in 8 days while GST remain elevated. In another study, GST activity of *Oreochromis mossambicus* recovered after a recovery period of 7 days (Venkateswara Rao, 2006).The induction of elevated levels of GST ,CAT,SOD and GPx shows a possible shift towards a detoxification mechanism under exposure of BBP.

In contradiction to the present result, (Alzbeta Stara *et al* ., 2013) have reported a considerable decrease in SOD activity of gill of Prometryne exposed *Cyprinus carpio*. In addition, they observed no significant change in the SOD activity of muscle and liver of *Cyprinus carpio* exposed to prometryne . They have also observed decline in CAT activity of liver compared to untreated ones.

Exposure to atrazine was reported to be associated with increased SOD activity , especially in the liver of Zebrafish (*Danio rerio*) Jin *et al* ., (2010). Fifteen days exposure to atrazine at 10.6 mg/ L caused increase of SOD activity in *Channa punctatus* Nwani *et al* ., (2010). Paulino *et al*., (2012) observed increased SOD activity in gill of *Prochilodus lineatus* after 14 days of exposure to 10 µg/l atrazine. Oruc and Usta *et al* ., (2007) reported an increase in SOD activity in gill, muscle, and kidney of *Common carp* after 15 days exposure to diazinon, and after 60 days exposure, observed a reduction in SOD activity.

From the results obtained, it is evident that the enzyme activities superoxide dismutase (SOD), Catalase(CAT), Glutathione peroxidase (GPx) and Glutathione S transperase (GST) of various tissues (gill, liver and muscle) were altered in Tilapia due to BBP exposure. This modulation in enzyme activity could be due to the mechanism by which Tilapia *Oreochromis mossambicus* circumvents the toxic stress caused by BBP.

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