IMPACT OF BENZYL BUTYL PHTHALATE ON HAEMATOLOGY AND METABOLIC ENZYMES OF *Clarias gariepinus*

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ABSTRACT

Benzyl butyl phthalate (BBP) is used in plastic industry and leaches into the environment through effluent and indiscriminate dumping of plastic wastes with possible deleterious effects on fish and other aquatic animals. Standard solution of 100 mg/ L was prepared from 10 mg of BBP stock solutions dissolved in acetone as carrier agent, and the used treatments were determined after conducting range finding test. Toxicity of various treatments: 0.1, 0.2, 0.8 and 1.6 µg/ L and control were investigated using haematology and metabolic enzymes as indicators in 150 juveniles of Clarias gariepinus. Each treatment was arranged in triplicates containing 10 fish samples each. Data obtained were subjected to descriptive and inferential statistics and tested at 0.05 significant level. Results showed that the dissolved oxygen in the fish tanks reduced gradually over the 4 days (96 h) of the experiment. There were increases in the levels of red blood cell (2.32-3.57 x10^{12}/ L) and lymphocytes (58.1-63 %) but decrease in eosinophils (3.00-2.00 %). Levels of all the four metabolic enzymes gradually reduced (AST: 1001-890, ALT: 45.2-18.5, ALP: 62.60-17.15, and LDH: 925-906 U/ L) as the BBP treatment levels increased when compared to the control. Such observations might result from the efforts of the experimental fishes to subdue the toxicological effects of the BBP treatments. Usage of BBP in plastic manufacturing industries because of its cost-effectiveness should be replaced with environment-friendly alternatives such as thermoplastic elastomers.

Keywords: Fish toxicology, Melanomacrophage, Plastic pollution, Range finding test

INTRODUCTION

Benzyl butyl phthalate (BBP) is an organic chemical compound regularly used in the manufacture of plastic products. It is a major hazard to the environment, with low molecular weight, high boiling point, low melting point, high flash point, and low solubility (NCBI, 2020). The compound (BBP) gives off pungent smoke and irritating fumes if heated to decomposition (Lewis, 2004). Solubility of BBP in water (at a temperature of 25°C) was found to be relatively low at 2.7 mg/ dm^3 (CMA, 1999). BBP is not persistent in environmental matrices (water, sediments, or soil) under aerobic conditions and has a half-life of a few days (WHO, 1999).

Usage of phthalate in polyvinyl chloride (PVC) additives, insect repellents and cercaricides had been recorded more than a decade ago (Yen *et al.*, 2011) and continues to be an integral part of human necessities today (Andrady and Neal, 2009). The commonly used phthalate compounds such as BBP do not bind chemically with other polymers but they migrate easily into the natural environment to dissipate through their matrices (Jarmolowicz *et al.*, 2015). It leaches into the aquatic environment

through indiscriminate dumping of plastics and the release of effluent from industries. Studies had shown possible quantification of BBP in aquatic environments such as in Nigeria (Oghenekohwiroro *et al.,* 2016; Olujimi *et al.,* 2012), United State of America (Gledhill *et al.,* 1980), Italy (Vitali *et al.,* 1997), and Norway (NIWR, 1996). The compound (BBP) had been detected previously in aquatic bodies at different quantities and proven to disrupt endocrine activities in fish (Sakamoto *et al.,* 2011). Oehlmann *et al.* (2009) confirmed existence of phthalate in aquatic environments and their accumulation in both invertebrates and vertebrates. The bioaccumulation of phthalate is possible to naturally affect the organisms. Call *et al*. (2001) expressed that lower molecular weight phthalates such as BBP are acutely toxic at different levels of organisms depending on the consumed or exposed concentrations.

Ecological niche determines the rate of exposure of aquatic lives to phthalates through water column, food and sediments (Peijnenburg and Struijs, 2006). Bioaccumulation of the compound in fish had been demonstrated previously (Staples *et al*., 1997). Influence of BBP had been linked with anti-oestrogenic and anti-androgenic effects (Sohoni and Sumpter, 1998). The common mechanism of action of phthalates is binding to and modulation of molecular pathway (Fan *et al.,* 2004). However, recording adverse effects of BBP is solvent-dependent (Corton and Lapinskas, 2005). One of the sensitive and indicative techniques to examine the effects of pollutants on fish in an aquatic habitat is haematological examination for changes in blood parameters.

The use of haematological examination of fish has provided researchers with the opportunity to assess the environmental influence of toxic substances on the physiology of a fish (Sharma, 2012). African mud catfish (*Clarias gariepinus*) is highly adaptable to a wide range of fresh water habitats and can survive severe environmental conditions. These attributes enhance its increased acceptability for aquaculture among the fish farmers in Nigeria.

Consideration of the *Clarias gariepinus* as a bio-indicator for research purposes had been outlined by Holt and Miller (2010) to include adequate distribution of the fish species, its measurable response and considerable stability to environmental disturbance, and its taxonomic documentation. The fish is ecologically understood. It is also economically and commercially important to the fish farmers. Sharma *et al.* (2012) described the fish to be a good bio-indicator for toxicological study. Besides, a good bio-indicator is an organism with high possibility of giving an indication of the health of an ecosystem. Therefore, this research was carried out to determine the deleterious effects of BBP on haematology and metabolic enzymes of *C. gariepinus*.

MATERIALS AND METHODS

Study area

The study was carried out at the Environmental Management and Toxicology laboratory of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. It lies nearly on latitude 7° 30' N and longitude 3° 54' E, and within the humid lowland rainforest region. The area experiences both wet season (March to October) and dry

season (November to February) with average rainfall of above 1200 mm/ year as well as monthly temperature which could range from 22.9 to 36.32 ˚C in August and March respectively while the relative humidity may be between 75.52 and 88.15 % in February and July respectively (Aiboni, 2001).

Collection of experimental fish and test chemical

A total of 150 juvenile catfish with mean weight 9.44 ± 0.31 g were purchased from the fish hatchery of FUNAAB, Ogun State, Nigeria. The fish were acclimatized under laboratory conditions for 7 days and randomly stocked in fifteen glass tanks (60 x 30 x 30 cm^3) at a rate of ten fishes per tank. The fishes were fed with 2mm Coppens feed at 0700 and 1800 h daily and feeding was stopped 24 h prior to the exposure study. Standard stock solutions were made by dissolving 10 mg of BBP in acetone as the carrier solvent (Method 8061A) and the volume was adjusted to 100 µg/mL (Application News, 2010) using BBP with 98.5 % standard purity (Sigma Aldrich, South Africa), CAS Number 85-68-7. Standard solutions were further prepared by mixing and diluting the stock solutions with distilled water to obtain the concentrations used as described by Sun *et al.* (2014).

Water quality determination and fish exposure study

The water was sourced from FUNAAB borehole, analysed and certified to conform to NIS 977: 2017. Water quality parameters: Temperature (^oC), Electrical conductivity (µS/cm) and pH were monitored using Hanna Scientific$^{(R)}$ kit: HI 98130 while Dissolved oxygen was analysed according to APHA (1998). After conducting range finding test (Rand, 2008), experimental fish were exposed to BBP at 0, 0.1, 0.2, 0.8 and 1.6 µg/L in triplicates for 96 h, 0 was the control experiment which contained only water. Addition of the BBP concentrations was renewed every 24 h of the experimental duration. Static bioassay method was employed without feeding the experimental fish to prevent build-up of metabolic wastes.

Haematological test

Blood samples were collected by cardiac puncture (Blaxhall and Daisley, 1973) in heparinized microhaematocrit tubes covered with plasticine at one end and centrifuged for 5 min at 3,000 rpm. Blood samples were analysed for red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), and packed cell volume (PCV) as described by Kori-Siakpere *et al.* (2007). The WBC count was determined using visual counting method (Safina *et al.*, 2013). The WBC differential counts were carried out according to the method described by WHO (2010). The RBC count was estimated using Neubauer counter chamber (Erhunmwunse and Ainerua, 2013). The red blood cell indices were calculated as described by Sepperumal and Saminathan (2013). Haemoglobin concentration was measured with Van Slyke apparatus and application of Hufner's factor (Akinrotimi *et al.*, 2012). The PCV was evaluated using Haematocrit reader (Jiro *et al.*, 2008).

Metabolic enzyme assay

Metabolic enzyme extractions were carried out according to the method described by Ramakrishnan *et al.* (2013). The extracted enzyme supernatant fractions were then analysed for Aspartate transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Lactate

Dehydrogenase (LDH) as described by Agbafor *et al.* (2014).

Statistical analysis

The data obtained were subjected to descriptive statistics (Means and Standard error). Further statistical test was performed with inferential statistics (one-way-ANOVA and Duncan Multiple Range Test) at $p < 0.05$ using IBM SPSS Version 22.

RESULTS AND DISCUSSION

Water quality in the experimental tanks of the *C. gariepinus* exposed to BBP for 96h

The days 1 and 4 of the experiment recorded reduction in temperature values when compared to the control (Table 1). The least value was traced to day 4, when 0.1 µg/ L of BBP treatments influenced the highest temperature reduction when compared to the control. There was no significant ($p > 0.05$) difference in the temperature values of BBP treated water and the control group from days 1 to 4, except on day 3. Dissolved oxygen (DO) of the experimental tanks was observed to reduce significantly ($p < 0.05$) compared to the control as the levels of BBP increased except on day1, when DO values slightly varied in 0.20 µg/ L (5.17 mg/ L). Levels of DO in the BBP treated water reduced gradually from day1 to day4 and were significantly ($p < 0.05$) different from the control. The values were lower than the expected DO value of 5 mg/ L (Towers, 2015). The culture pH was observed to be lower in the BBP treatments than in the control (6.88) on day 1, with ranged from 6.6 to 6.7 on day 2, 6.3 to 6.8 on day 3 and 6.64 to 6.9 on day 4. However, the culture was near neutral (6.50 to 8.50) (NIS 977: 2017) throughout the 96 h experimental periods which implied that BBP treatments were neither acidic nor alkaline. The EC µS/ cm for days 1, 2, 3 and 4 ranged from 0.27 to 0.31, 0.29 to 0.31, 0.27 to 0.29, and 0.27 to 0.30 respectively. This showed moderate presence of BBP in *C. gariepinus* culture during the 96 h periods.

The EC (Electrical Conductivity, µS/ cm) of the treated and control water under study was low with no significant ($p > 0.05$) difference. The pH of BBP treated water had no significant ($p > 0.05$) difference from days 1 to 4 when compared to the control. Higher levels of EC in the control occurred in 0.1 µg/ L on day 2 (0.31>0.29 µS/ cm) and 0.8 µg/ L on day 4 (0.30>0.29 µS/ cm). The water could have maintained the ambient temperature as the low soluble BBP had no thermal effects on the experimental setup (Ayotunde *et al.*, 2010). Reduction in dissolved oxygen values could have been from the influence of chemical oxidation and respiration of the test fish since it was observed that the higher the BBP concentrations the lower the DO level. This is in line with the findings of Adebayo *et al*. (2013) who reported toxicological effects of diazinon on *Clarias anguillaris*. Presence of the BBP, as applicable to the phthalates when leached into the environment, made it to interact with water under study. The interaction changed the water chemical behaviour. Possible influences were not only by a range of processes such as hydrolysis, photolysis, and biodegradation but also the ambient physical and chemical conditions. The hydrophilic nature of the alkyl phthalate ester varied inversely with the length of the alkyl side chain while their levels of degradation are governed by their molecular weight. The longer alkyl side chains exhibited longer half-lives under the given media, and the decrease in the DO below 4 mg/ L could cause fish mortality.

The pH level of culture water indicated that BBP was neither acidic nor alkaline but maintained the neutral nature of the water used. The pH range recorded in this study is similar to the tolerable range reported by Weiner (2010) for fish survival. Chemical compound in water is affected by pH variation that could make the medium toxic to the experimental fish (Tucker and D'Abramo, 2008). The neutral pH condition of the treatments could be associated with low EC. Neutral pH of the test water showed absence of impurities while low EC indicated low possible active ions. The EC of a test water/solution is dependent on the summation of all the cations and anions concentration present in it; so, the lower the ions concentrations, the lower the EC in this study. The observed EC in this study conformed to the observation of Reboucas *et al.* (2015) that monitored EC of acidic water used in the culture of Nile Tilapia. As the BBP dissolves gradually in water, it may reduce the water quality especially the DO in the water column and pose serious threat to fish and other aquatic organisms.

Haematological responses in *C. gariepinus* exposed to BBP for 96h

The packed cell volume (PCV) was observed to range from 21.1 to 39 %. Levels of PCV determined in the treated test fish were observed to be higher than in the control fish, except in 0.2 µg/ L treatment, which was below the value for the control (Table 2). The incremental pattern of PCV was followed by the variations of haemoglobin (Hb). Levels of the determined Hb was highly significant ($p < 0.05$) in highest BBP treatment (1.6 µg/ L) with the least level in the control.

Table 1: Water quality parameters in the experimental tanks of the *C. gariepinus* exposed to BBP for 96 h

Treatment	Temp. (^{O}C)	DO (mg/ L)	EC (µS/ cm)	pH
		Day 1		
Control	26.6 ± 0.03^{a}	5.50 ± 0.15^{a}	0.31 ± 0.01^{a}	6.88 ± 0.28^{a}
0.1 µg/ L	26.3 ± 0.18^{a}	5.10 ± 0.06^{b}	0.27 ± 0.01^{b}	6.37 ± 0.03^{bc}
0.2 µg/ L	26.6 ± 0.03^{a}	5.17 ± 0.03^{b}	0.29 ± 0.01^{ab}	6.53 ± 0.03^{bc}
0.8 µg/ L	26.6 ± 0.03^{a}	5.00 ± 0.06^{b}	0.29 ± 0.01^{ab}	6.77 ± 0.09^{ab}
1.6 µg/ L	26.4 ± 0.23^{a}	5.07 ± 0.03^{b}	0.28 ± 0.01^{ab}	6.30 ± 0.00^{c}
		Day 2		
Control	26.7 ± 0.03^{a}	5.17 ± 0.18^{a}	0.29 ± 0.01^{ab}	6.65 ± 0.00^{a}
0.1 µg/ L	26.7 ± 0.00^{a}	4.87 ± 0.03^{ab}	0.31 ± 0.01^{a}	6.70 ± 0.08^{a}
0.2 µg/ L	26.6 ± 0.06^{a}	4.50 ± 0.21^{bc}	0.29 ± 0.01^{b}	6.77 ± 0.10^{a}
0.8 µg/ L	26.7 ± 0.02^{a}	4.47 ± 0.03^{bc}	0.29 ± 0.00^{b}	6.74 ± 0.12^{a}
1.6 µg/ L	26.7 ± 0.00^{a}	4.30 ± 0.06^{c}	0.29 ± 0.00^{ab}	6.60 ± 0.10^{a}
		Day 3		
Control	26.7 ± 0.03^{ab}	4.80 ± 0.12^{a}	0.29 ± 0.01^{a}	6.72 ± 0.03^{a}
0.1 µg/ L	26.3 ± 0.18^{b}	3.80 ± 0.10^{b}	0.27 ± 0.01^{a}	6.80 ± 0.08^{a}
0.2 µg/ L	26.6 ± 0.06^{ab}	3.50 ± 0.21^{bc}	0.29 ± 0.01^{a}	6.43 ± 0.09^{b}
0.8 µg/ L	26.8 ± 0.03^{a}	3.33 ± 0.09^{c}	0.29 ± 0.01^{a}	6.76 ± 0.10^{a}
1.6 µg/ L	26.4 ± 0.15^{ab}	2.93 ± 0.03^{d}	0.27 ± 0.00 ^{a}	6.92 ± 0.00^{a}
		Day 4		
Control	26.5 ± 0.19^{a}	4.03 ± 0.09^{a}	0.29 ± 0.01^{ab}	6.79 ± 0.06^{a}
0.1 µg/ L	26.2 ± 0.12^{a}	3.27 ± 0.12^{b}	0.28 ± 0.00^{b}	6.91 ± 0.15^{a}
0.2 µg/ L	26.4 ± 0.07^{a}	2.97 ± 0.03^{b}	0.28 ± 0.01^{b}	6.67 ± 0.13^{a}
0.8 µg/ L	26.5 ± 0.03^{a}	2.63 ± 0.12^{c}	0.30 ± 0.00^{a}	6.64 ± 0.15^{a}
1.6 µg/ L	26.3 ± 0.03^{a}	1.73 ± 0.09^{d}	0.27 ± 0.00^{b}	6.72 ± 0.11^{a}

Mean ± S.E. Values with the same superscript down each column were not significantly different ($p > 0.05$).
Temp: Temperature; DO: Dissolved oxygen; EC: Electrical conductivity; pH: Hydrogen ion concentration.

The BBP treatments triggered red blood cell (RBC) levels in the experimental fishes to increase above the level of RBC in the control. The higher the BBP treatments the higher the levels of PCV, Hb and RBC significantly ($p < 0.05$) across the 96 h period of experimentation. Influence of BBP on the test fish could have led to increase in PCV level which conforms to the previous studies of Binukumari and Vasanthi (2013) who reported increase in PCV of *Labeo rohita* exposed to an herbal insecticide. Lower Hb level of the blood could hinder blood potency to supply tissues with oxygen, thereby resulting in a condition called hypoxia (Oellermann *et al.*, 2015). This corresponds to the finding of Mekkawy *et al.* (2013) who reported increase in Hb level of the fish exposed to different concentrations of phthalate treatments.

High levels of both Hb and RBC could have been adaptive strategies against the stress imposed by the BBP concentrations. The observed increase in RBC count could be a resultant effect of increase in the blood cell reserves coupled with cell contractions (Ololade and Oginni, 2010) due to respiratory activities of the fish to survive the stress imposed by BBP concentrations. This buttressed the findings of Sepperumal and Saminathan (2013) who observed significant increase in RBC level in the blood of *Oreochromis mossambicus* exposed to different doses of phthalate. However, these results are in contrast with Obiezue *et al.* (2013) who reported decrease in the PCV, Hb and erythrocyte in *C. gariepinus* exposed to phthalate.

The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin count (MCHC), and mean corpuscular volume (MCV) increased gradually as BBP treatments increased above that of control fish. The observed values of MCH and MCHC tripled MCV in all the treatments. This was the same in the control treatments. Levels of white blood cell (WBC) and the lymphocytes (LYM) gradually increased significantly ($p < 0.05$) above that of the control treatments while eosinophils (EOS) contents decreased steadily. However, there were no significant ($p > 0.05$) differences in the levels of both EOS and monocytes (MON) when compared with their respective control treatments. Increasing levels of MCH, MCHC and MCV depicted an adaptive measure to counter the toxicological effects of the BBP on the test fish. This corroborated the study of Rao (2010) who observed increase in the level of MCH, MCHC and MCV in the blood of common carp exposed to a stressor. Increases in the level of MCHC, MCH and MCV had been attributed to alterations in the synthesis of haemoglobin and impairment in the red blood cell membrane causing haemolysis (Kayode and Shamusideen, 2010).

Increase in the levels of both WBC and LYM indicated the readiness of the test fish to antagonise toxicological threats posed by the BBP in water media. This result agreed with the observation of Moharram *et al.* (2011) who reported higher levels of LYM in the blood of Nile perch in water bodies polluted with mixture of stressors (heavy metals). Davis *et al.* (2008) reported significant increase in the level of WBC and their indices in the blood of monosex tilapia raised in water containing introduced toxicant (methyltestosterone). *Cyprinus carpio* developed a defensive mechanism to overcome stress posed by cypermethrin (Masud and Singh, 2013).

Activities of metabolic enzymes in C. gariepinus exposed to BBP for 96h

Levels of the four enzymes increased with the introduction of BBP treatments and decreased as the levels of BBP increased (Table 3). The effects of BBP treatments on metabolic enzyme levels were significantly ($p < 0.05$) higher in the experimental fishes than in the control. High levels of both AST and ALT indicated exhaustion of the fish while energetically thriving against the effects posed by the BBP treatments. Levels of AST and ALT in the four BBP treatments were significantly higher than in the control.

Table 2: Haematological response in *C. gariepinus* exposed to BBP for 96 h

Parameters	0.1 µg/ L	0.2 µg/ L	0.8 µg/ L	1.6 µg/ L	Control
PCV (%)	25.0 ± 1.15^{b}	19.3 ± 1.20^{c}	26.7 ± 0.88^{b}	39.0 ± 0.58^{a}	21.1 ± 0.02^{c}
Hb (g/ dL)	8.37 ± 0.38^{b}	6.47 ± 0.39^{c}	8.80 ± 0.29^{b}	13.0 ± 0.20^{a}	6.40 ± 0.45^{c}
RBC ($x10^{12}$/ L)	2.32 ± 0.12^{b}	2.35 ± 0.09^{b}	2.37 ± 0.08^{ab}	3.57 ± 0.04^{a}	1.13 ± 0.14^{c}
MCH (pg)	36.0 ± 0.18^{cd}	37.4 ± 0.45^{a}	37.1 ± 0.05^{ab}	36.3 ± 0.18^{bc}	35.1 ± 0.20^{d}
MCHC (g/ dL)	33.4 ± 0.04^{a}	33.5 ± 0.06^{a}	33.2 ± 0.00^{a}	33.2 ± 0.03^{a}	$32.3 \pm 0.15^{\mathbf{b}}$
MCV (fl)	10.8 ± 0.04^{a}	11.2 ± 0.14^{a}	11.2 ± 0.01^{a}	10.9 ± 0.05^{a}	9.94 ± 0.05^{b}
WBC ($x10^{3}$/ L)	12.7 ± 0.26^{c}	16.3 ± 0.23^{a}	13.9 ± 0.12^{b}	16.9 ± 0.26^{a}	11.8 ± 0.00^{c}
LYM (%)	58.1 ± 1.01^{ab}	59.7 ± 2.03^{b}	62.0 ± 1.73^{ab}	63.0 ± 0.00^{a}	56.0 ± 0.16^{b}
EOS (%)	3.00 ± 0.00^{a}	2.70 ± 0.25^{a}	2.67 ± 0.33^{a}	2.00 ± 0.58^{a}	2.00 ± 0.00^{a}
MON (%)	0.67 ± 0.33^{a}	0.33 ± 0.33^{a}	0.67 ± 0.33^{a}	0.67 ± 0.33	2.67 ± 0.33

Mean ± S.E. Values with the same superscript across each row were not significantly different ($p > 0.05$). PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cell; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; MCV: Mean corpuscular volume; WBC: White blood cell; LYM: Lymphocyte; EOS: Eosinophil; MON: Monocyte.

Table 3: Activities of metabolic enzymes in *C. gariepinus* exposed to BBP for 96 h

Parameters	AST (U/ L)	ALT (U/ L)	ALP (U/ L)	LDH (U/ L)
0.1 µg/ L	1001 ± 9.00^{a}	45.2 ± 0.77^{a}	62.6 ± 1.90^{a}	925 ± 5.00^{a}
0.2 µg/ L	982 ± 3.00^{a}	30.2 ± 2.37^{b}	26.3 ± 1.79^{b}	923 ± 5.25^{a}
0.8 µg/ L	$920 \pm 14.00^{\mathbf{b}}$	20.2 ± 1.27^{c}	17.9 ± 1.04^{c}	911 ± 3.41^{b}
1.6 µg/ L	890 ± 6.00^{c}	18.5 ± 0.77^{c}	17.6 ± 1.53^{cd}	906 ± 3.00^{b}
Control	766 ± 2.00^{d}	11.9 ± 2.43^{d}	11.9 ± 2.43^{d}	875 ± 29.33^{d}

Mean ± S.E. Values with the same superscript down each column were not significantly different ($p > 0.05$).
AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.

The BBP treatment might have triggered the glutamate transaminase activities and injured the fish according to Obiezue *et al.* (2013), thereby causing tissue repair and increasing the fish respiration through protein turn over. Both partake in the gluconeogenesis and their high levels could depict possible hepatic necrosis. Increase in the BBP concentrations might influence reduction in the levels of AST. High levels of LDH might indicate reduction in mobility proteins which the fish could have released to replace the damaged cells and tissues influenced by BBP toxicity. It was generally observed that levels of the four metabolic enzymes reduced gradually as concentrations of BBP increased in the treatments. Thus, the damaging effects possibly caused by BBP treatments might

result in reduced growth, reproduction and immunity.

Increase in LDH level in fish is attributable to distortion of liver and gill tissues as previously reported by Latif *et al.* (2014). Level of LDH in *Sarotherodon mossambicus* exposed to different treatments of mercury resulted in imbalance in the activity of mitochondrial membrane processes (Srivastava and Singh, 2013). Hepatocellular defects could be linked to high level of ALT. Increase in the level of AST in fish had been observed to cause damage to the liver and gill tissues (Ramesh and Saravanan, 2008; Singh *et al.*, 2011). The trend in this study conforms to the findings of Yildrim *et al.* (2006) who reported increase in the level of AST of *O. niloticus* treated with deltamithrin while the ALT values of fish corroborated the finding of Latif *et al.* (2014) which indicated higher value of ALT in *L. rohita* subjected to naphthalene treatment and suggested gill and liver tissue impairment. Increases demand to produce more WBCs might have influenced more ALP for effective trunk blood resolution. The present findings corroborated the work of Akinrotimi *et al.* (2013) which found that increase in the activity of ALP of *C. gariepinus* exposed to anaesthetic metomidate caused necrosis of the liver tissue and distortion of gill filaments.

CONCLUSION

Continuous usage of benzyl butyl phthalate in plastic manufacturing could pose deleterious effects on aquatic lives as observed from this study. Eco-friendly alternative material to phthalate is needed to be formulated for usage in the plastic making industries. There is the need to encourage the plastic making industries to control indiscriminate discharge of their wastes into water bodies as these could have harmful effects on the aquatic organisms.

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