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Alterations in the Enzyme Activities in the Plasma of *Clarias gariepinus* Exposed to Benzalkonium Chloride

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Abstract

This study was carried out to assess the effects of Benzalkonium chloride on the plasma enzymes in juveniles of Clarias gariepinus. One hundred and fifty (150) Clarias gariepinus of mean length 11.74±2.64cm and mean weight 256.68±1.81g were exposed to the chemical at 0.00, 0.05, 0.10, 0.15 and 0.20mg/L for 96 hours. During the experimental period, physico-chemical parameters of water in the exposure tanks were evaluated. At the end of the experiment, blood was taken from the exposed fish and analyzed for changes in its enzymes profiles. Blood was extracted from the fish at 0hr, 24hrs, 48hrs, 72hrs, and 96hrs, in a static non-renewal water system. The collected blood samples were stored in heparinized bottles for enzymes analysis. The plasma was assayed for liver enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), acid phosphate (ACP), alkaline phosphate (ALP) and lactate dehydrogenase (LDH) in the exposed fish. The data obtained from the study was subjected to analysis of variance (ANOVA).The results revealed a significant reduction ($P<0.05$) in the values of dissolved oxygen from 6.68±0.77 in the control to 4.99±0.54 at 0.20mg/L concentration of the chemical. Also, significant ($P<0.05$) increase with increasing concentrations of the chemical were however recorded in the values of nitrite and ammonia. While other parameters such as temperature and pH were within the same range in all concentrations of the chemical. Also, the values of all the enzymes in the exposed fish were significantly elevated ($P<0.05$) when compared to the control. Changes in enzymes response were concentration dependent ($p<0.05$), with increased concentration of Benzakonium chloride. The chemical caused some alterations in the enzymes composition of C,gariepinus, which was more pronounced at higher concentrations of 0.15 and 0.20mg/L. Based on the results of this study, the disinfectants can be applied at 0.05mg/L in the culture medium without significant alterations to the physiology of the fish.

Keywords: *Enzymes, Fish, Disinfectants, Toxicology. Aquatic pollution*

INTRODUCTION

In the quest to control some common biological problems such as aquatic disease infestation, proliferation of wild fish and improvement of water quality, the use of chemicals and some substances have been used in aquaculture practice [1]. A few chemicals have been ecologically tested in Nigeria for safety in spite of their environmental and ecological impact. These chemicals can cause some deleterious effects to the environment when used indiscriminately [2]. Surface waters that can be harnessed for use in aquaculture systems may contain some pathogenic organisms which may originate from the areas over which the water flows. The direct use of such open water supplies without adequate treatment should be avoided. Disinfection of the surface water is very important so as to avoid the pathogen transmission and contamination from the environment into the aquaculture systems. The effective control of infectious diseases using environmentally safe disinfectants is a critical need in aquaculture [3].The adverse effect of this disinfectant is widely studied in fish, mammals and human [4]. Fish is ubiquitous to aquatic systems and can serve as a good bio-accumulator, therefore a good candidate for pollution studies. When exposed to certain chemical pollutants, it may damage and weaken the organs or defense mechanism concerned thereby leading to pathological, physiological and biochemical alterations in the fish [5].

Enzymes play an important role in food utilization and metabolism in a living organism. But this system may get altered under the stress and influence of toxicants because cells in organisms contain enzymes which perform different function. Consequently, when the integrity of the cell is disrupted through external interference by toxicants, enzymes escape into the plasma in the blood stream where their activities can be measured as a useful tool of cell integrity. The response of aquatic organisms to pollution is expressed through several key biochemical activities involving enzymes which are concerned with the biotransformation system and these biomarkers give early warning signs of aquatic pollution [6]. Evaluation of enzyme activities in the serum or plasma has frequently been used as investigative tool in fish toxicology all over the world. In addition, enzyme activities have also been comprehensively utilized to predict chemical toxicity especially disinfectants in aquatic animals. Moreover, transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver specific enzymes that give a more insightful appraisal of liver and kidney damage that can be assessed within a short time [7]. However Teuten *et al.* [8] reported that alterations in the values of ALT and AST suggest tissue damage in some important organs such as liver, kidney, muscle and gill. Likewise, variations in phosphatases, which include alkaline phosphatise (ALP) and acid phosphatase (ACP) activities in tissues, organs and plasma have been reported in fish exposed to toxicants in varying concentrations and have been used as biomarker for tissue damage in fish and a good diagnostic tool in toxicological studies [9].

Clarias gariepinus is a popular species in warm water aquaculture and it is indigenous to Africa. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, hardiness, high yield, tolerance to poor water quality, omnivorous feeding habit, fine flavour, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available [10]. *C. gariepinus* is an important fish for aquaculture in Nigeria because it meets up a partial

solution for the increasing demand of protein. It has been artificially reproduced and cultured under various Nigerian aquaculture systems. *Clarias* species have high adaptability, fast growth rate in the natural and cultured environments and has proved to be successful aquaculture species, [11]. It is an important fish species in both aquaculture and capture fisheries [12]. The African catfish is found in most water bodies, ranging from swamps, temporary pools, rivers and lakes [13]. The wide distribution of African catfish is attributed to its capacity to: utilize atmospheric oxygen and hence survive in habitats with low dissolved oxygen, burrow into mud and survive extended periods of desiccation. The aim of this study is to evaluate the effects of Benzalkonium chloride on some enzymes in juveniles of *Clarias gariepinus*.

MATERIAL AND METHODS

Experimental Location

The experiment was conducted at the Wet Laboratory in the Department of Fisheries and Aquaculture Management, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Source of Experimental Fish

One hundred and fifty (150) juvenile *Clarias gariepinus* of similar size (mean length 12.85±7.88cm and mean weight 251.97±30.78g) were sourced from House Tully Fish Farms, Opunno, Awka, Anambra State, Nigeria.. They were transferred in two 50liter plastic tanks to the laboratory for acclimation process.

Acclimation and Feeding Of Fish

The experimental fish were acclimated in four 150L capacity circular plastic tanks containing 150L de-chlorinated water, for 7 days to experimental conditions at room temperature netted materials with central slits was tied to the tops of the tanks to prevent escape of fish. Water renewal was done every two days. The fish were fed with top feed, a commercial feed at 5% body weight throughout this period.

Experimental Design

The experimental design was a completely randomized design (CRD) with four treatments levels and a control with each level having three replicates.

Procurement and Preparation of Test Solution

A newly introduced pond disinfectants "GAB Disinfectants" (Benzalkonium Chloride-80.0%; Acetic Acid-10.0%; Glutaraldehyde- 5.0%; Activants-5.0%) used in cleaning of both earthen ponds and concrete tanks was purchased off shelf, from Gabrovic Agric Nig. Ltd, Rumuodara, Port Harcourt, Rivers State, Nigeria. The solution of the chemical in water was prepared by serial dilution.

Exposure of Fish to Benzalkonium Chloride (BKC)

Five *C. gariepinus* each were introduced individually into 15, aquaria tanks of 1.5m x 1m x 0.5m dimension, containing 0.00 (control), 0.50, 1.00, 1.50, and 2.00mg/l of Benzalkonium Chloride. Each treatment(s) and control were replicated three times and the experimental duration lasted for a period of 96 Hours. The tanks were covered with netted materials and supported with heavy objects to prevent the fish from jumping out.

Physico-Chemical Parameters of Water

During the experiment, the following water quality parameters namely: Temperature, pH, Dissolved Oxygen, Nitrate and Ammonia levels of control and other treatment exposures were determined and the readings taken at 0, 24, 48, 72 and 96hr intervals in three replicates. Temperature was determined using the mercury-in-glass thermometer, which was inserted in water and the temperature (°C) reading was taken after four minutes.

pH was determined using a Jenway® type pH meter (Model 3015). The probe was first inserted in the buffer for 5 minutes to standardize the meter to pH 7, thereafter, it was dipped into the water and the static pH was read 60 seconds later. Dissolved oxygen was measured by Winklers method APHA, (2005). The determination of nitrate, and ammonia was determined by automation using a multi-parameter photometer (Hanna instrument H183200).

Blood Sample Collection and Preservation

The blood was drawn from caudal vein known as *Vena cava* Fish were caught individually with a hand net. Blood samples were obtained with 5ml disposable syringes and 21-gauge hypodermic needle. During collection the head of each fish was covered with a piece of cloth for physical restriction with minimal stress. The needle was inserted perpendicularly into the vertical surface of the fish at a point slightly above the openings in the genital papilla. As the needle pierced the vein, blood flowed easily into the syringe and 3ml of blood was taken before the needle was withdrawn. The needle was then detached from the syringe and the 1.5ml blood was transferred into labeled herparinized bottles. The blood samples were analyzed in the Laboratory.

Analysis of Enzymes in Juveniles of *C. gariepinus*

Commercial kits manufactured by Randox laboratories, Bangalore, India were used for the determination of the enzymes activities in the plasma of the exposed fish. The analyzed enzymes include: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP).

Statistical Analysis

Date obtained from the experiments were collated and subjected to ANOVA using Statistical Package for the social Sciences, (SPSS) version 22, differences among means were separated by Turkeys Comparative Test at 0.05%.

RESULTS

The effect of Benzalkonium chloride on the physio-chemical parameter of water in the experimental tanks

Table 1shows the results of the physiochemical parameters of water in the tanks *of C gariepinus* exposed to different concentrations of benzalkonium chloride (0.00, 0.05, 0.10, 0.15, 0.20mg/l) respectively for 96hrs. The results indicated significant reduction ($p<0.05$) in the values of dissolved oxygen from 6.67 0.25 in the control to 4.03+0.99 at 0.20mg/l concentration of the chemical. Also, significant ($p<0.05$) increase with increasing concentration of the chemical were however recorded in the values of nitrite and ammonia. While other parameters such as temperature and pH were within the same range comparable to the control in all concentrations of the chemical.

Changes in Enzymes Levels in the Plasma of *C gariepinus* Exposed to Different Concentrations of Benzalkonium Chloride for 96hrs.

The enzymes in the plasma of *C gariepinus* exposed to acute concentrations of Benzakonium chloride for 0hour are presented in the Table 2. Generally the values of the enzymes (AST, ALT, ALP, ACP, LDH) in the plasma of the exposed *C gariepiinus* were within the same range with no significant differences in all concentration. At 24hours of exposure, (Table 3), slight increase was observed in the AST, ALT, and ALP, while there was significant increase in the value of LDH. However the values of ACP were within the same range with no significant difference ($p>0.05$) in all concentration. At 48hours of exposure of *C gariepinus* to varying concentrations of Benzakonium Chloride (Table 4) there was significant increase in AST, ALT, ALP and LDH while the values of ACP was within the same range. At 72 and 96hours (Table 5 and 6), there was significant increase in the values of AST, ALT, ALP, ACP, LDH with increasing concentration.

Comparative Values of Benzakonium Chloride on Enzyme Activities for 96 Hours

Comparative values of Aspartate transaminase (AST) into the plasma of *C. gariepinus* exposed to Benzakonium chloride for 96hours is shown in Figure 1. The values of Aspartate transaminase increased as the experimental period increased with the value of (21.37± 5.09) observed at the control, and (29.12±7.89) in 0.20mg/l at 24hours. Comparatively, the value of Alanine transaminase (ALT) as shown in Figure 2 indicated that the values of alanine transaminase in *C. gariepinus* exposed to varying concentrations of Benzakonium chloride were elevated progressively as the experimental period increased and peaked at 96hours for all concentrations. The highest value of (75.33± 9.65) was recorded in the fish exposed to 0.20mg/l of the chemical at 96hours, while the lowest value of (47.507±4.88) was observed at the control (Figure 3) increased considerably as the experimental period increased ; this was more pronounced at the concentration of 0.10, 0.15, and 0.20mg/l concentration of the chemical. The value of acid phosphate (ACP) (Figure 4) were within the same range with no much significant difference in all concentrations of exposure with the value of (10.251±.65) observed at the control, and (10.99± 0.83) in 0.20mg/l at 48hours. The value of lactate dehydrogenase (LDH) is shown in (Figure 5). The value increases significantly as the experimental period progressed from 24 to 96hours. However the LDH records its highest value in the concentration of 0.20mg/l at 96 hours with a value of (477.023± 9.45).

Table 1: Physicochemical Parameters of Water in Tanks of *C. gariepinus* exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 96 Hours .

Concentrations (mg/L)	Physico- Chemical Parameters of Water				
	Temperature	pH	DO	Nitrate	Ammonia
0.00	28.01±1.22[a]	6.62±0.12[a]	6.68±0.77[a]	0.01±0.00[a]	0.02±0.01[a]
0.50	28.38±2.39[a]	6.60±0.17[a]	6.54±0.90[a]	0.06±0.01[b]	0.23±0.04[b]
1.00	28.73±1.31[a]	6.68±0.18[a]	5.47±0.77[b]	0.06±0.00[b]	0.30±0.01[c]
1.50	28.60±1.88[a]	6.67±0.11[a]	5.18±0.41[b]	0.07±0.00[c]	0.30±0.02[c]
2.00	28.72±1.66[a]	6.68±0.16[a]	4.99±0.54[c]	0.07±0.00[c]	0.32±0.02[c]

Means within the same column with different superscript are significantly different (P<0.05)

Table 2: Enzymes in the Plasma of *C. gariepinus* Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 0 Hours (Mean ± S.D)

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ALP	ACP	LDH
0.00	20.66 ± 7.44[a]	42.54 ± 5.11[a]	12.75 ± 1.88[a]	10.88 ± 2.99[a]	302.99 ± 11.02[a]
0.05	20.77 ± 2.02[a]	42.60 ± 3.62[a]	12.71 ± 0.98[a]	10.76 ± 1.88[a]	301.41 ± 13.03[a]
0.10	20.81 ± 4.18[a]	42.52 ± 2.03[a]	12.79 ± 1.34[a]	10.88 ± 3.99[a]	301.33 ± 10.74[a]
0.15	20.71 ± 3.55[a]	42.57 ± 3.66[a]	12.72 ± 1.17[a]	10.73 ± 2.76[a]	302.11 ± 10.03[a]
0.20	20.68 ± 5.18[a]	42.61 ± 5.09[a]	12.71 ± 1.08[a]	10.72 ± 3.77[a]	301.04 ± 10.77[a]

Means within the same column with different superscript are significantly different (P<0.05)

Table 3: Enzymes in the Plasma of *C. gariepinus* Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 24 Hours (Mean ± S.D)

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ALP	ACP	LDH
0.00	20.68 ± 7.11[a]	42.60 ± 3.90[a]	12.79 ± 3.03[a]	10.85 ± 1.08[a]	304.88 ± 19.77[a]
0.05	20.99 ± 8.02[a]	44.03 ± 6.02[a]	12.89 ± 2.77[a]	10.87 ± 1.06[a]	306.88 ± 24.55[a]
0.10	22.04 ± 4.77[b]	50.09 ± 6.88[b]	12.99 ± 0.71[b]	10.88 ± 1.21[a]	309.65 ± 22.08[a]
0.15	25.77 ± 5,03[b]	51.77 ± 4.44[b]	13.04 ± 2.11[b]	10.96 ± 0.75[a]	322.04 ± 16.03[b]
0.20	27.03 ± 5.03[b]	53.04 ± 5.23[b]	13.91 ± 3.43[b]	10.99 ± 0.88[a]	330.52 ± 12.77[b]

Means within the same column with different superscript are significantly different (P<0.05)

Table 4: Enzymes in the Plasma of *C. gariepinus* Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 48 Hours (Mean ± S.D)

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ALP	ACP	LDH
0.00	20.71 ± 6.11[a]	42.68 ± 4.03[a]	12.78 ± 4.11[a]	10.85 ± 1.77[a]	306.11 ± 23.99[a]
0.05	21.03 ± 3.02[a]	45.66 ± 7.44[a]	12.99 ± 2.05[a]	10.89 ± 1.04[a]	310.09 ± 25.11[b]
0.10	23.03 ± 5.77[a]	54.04 ± 4.51[b]	14.01 ± 3.63[b]	10.90 ± 1.99[a]	318.09 ± 22.89[b]
0.15	27.99 ± 7.12[a]	60.11 ± 5.33[c]	14.05 ± 2.03[b]	10.97 ± 0.98[a]	325.88 ± 28.03[b]
0.20	30.02 ± 8.13[b]	65.12 ± 9.04[c]	15.11 ± 3.84[c]	10.99 ± 0.53[a]	335.77 ± 25.99[c]

Means within the same column with different superscript are significantly different (P<0.05)

Table 5: Enzymes in the Plasma of *C. gariepinus* Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 72 Hours (Mean ± S.D)

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ALP	ACP	LDH
0.00	20.80 ± 6.03[a]	42.88± 4.32[a]	12.79 ± 3.05[a]	10.88 ± 1.04[a]	307.58 ± 29.54[a]
0.05	23.13 ± 2.04[a]	47.11 ± 9.11[a]	14.04 ± 4.04[b]	10.95 ± 1.55[a]	311.11 ± 23.90[b]
0.10	25.54± 4.43[b]	57.66 ± 7.98[b]	14.99 ± 5.44[b]	11.12 ± 1.04[b]	329.05 ± 22.98[b]
0.15	29.07 ± 8.12[b]	62.18 ± 4.97[c]	15.34 ± 2.09[c]	11.39 ± 2.55[b]	339.02 ± 23.90[b]
0.20	32.09 ± 5.05[c]	67.03 ± 7.12[c]	15.87 ± 3.09[c]	11.56 ± 1.04[b]	340.02 ± 24.09[b]

Means within the same column with different superscript are significantly different (P<0.05)

Table 6: Enzymes in the Plasma of *C. gariepinus* Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 96 Hours (Mean ± S.D)

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ALP	ACP	LDH
0.00	20.90 ± 7.22[a]	42.90 ± 8.91[a]	12.80 ± 6.33[a]	10.89 ± 1.09[a]	308.29 ± 11.93[a]
0.05	25.99 ± 2.54[a]	49.82 ± 8.11[a]	14.88 ± 2.89[b]	10.99 ± 3.12[b]	318.72 ± 15.90[b]
0.10	31.07± 7.42[b]	51.44 ± 9.05[b]	14.99 ± 7.50[b]	11.66 ± 1.75[b]	327.92 ± 19.04[b]

0.15	35.79 ± 9.86^{b}	62.66 ± 7.03^{c}	16.90 ± 2.34^{b}	11.75 ± 2.74^{b}	343.09 ± 48.72^{c}
0.20	40.77 ± 9.11^{c}	65.99 ± 9.77^{c}	17.04 ± 9.89^{b}	12.85 ± 1.67^{c}	377.88 ± 37.66^{c}

Means within the same column with different superscript are significantly different (P<0.05)

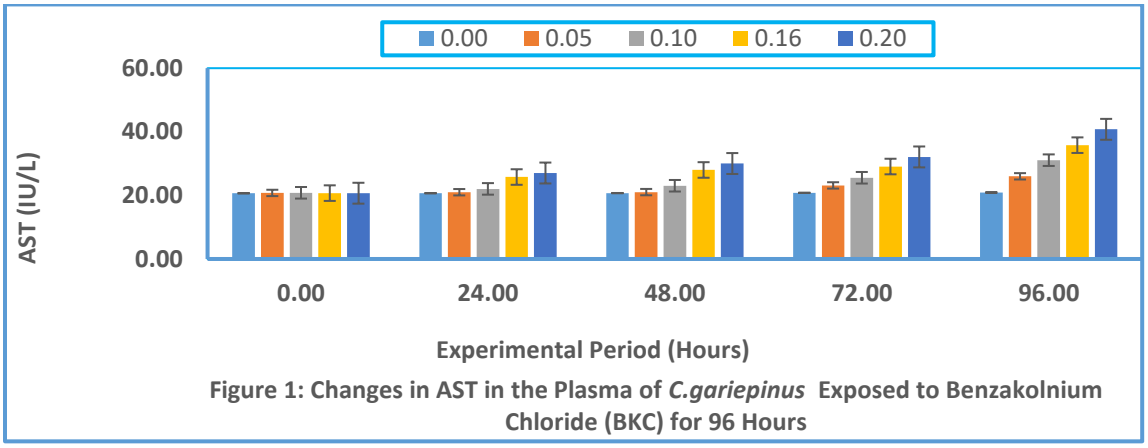

Figure 1: Changes in AST in the Plasma of *C.gariepinus* Exposed to Benzakolnium Chloride (BKC) for 96 Hours

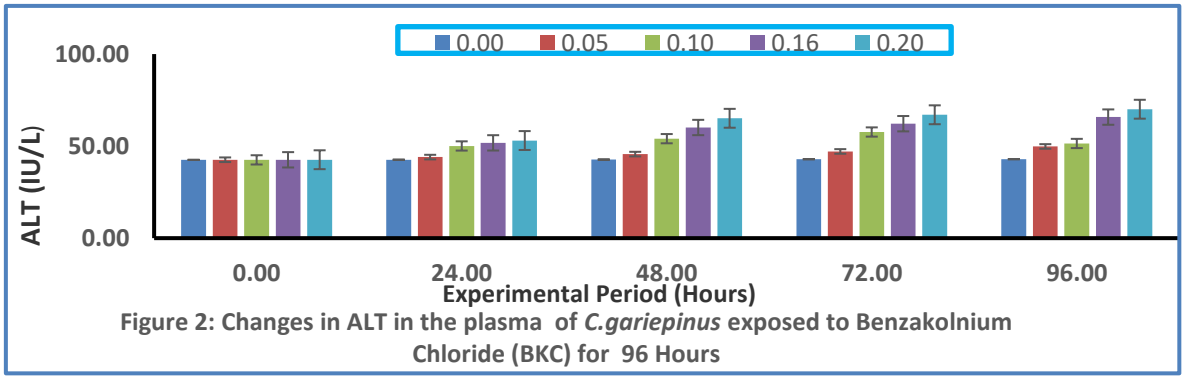

Figure 2: Changes in ALT in the plasma of *C.gariepinus* exposed to Benzakolnium Chloride (BKC) for 96 Hours

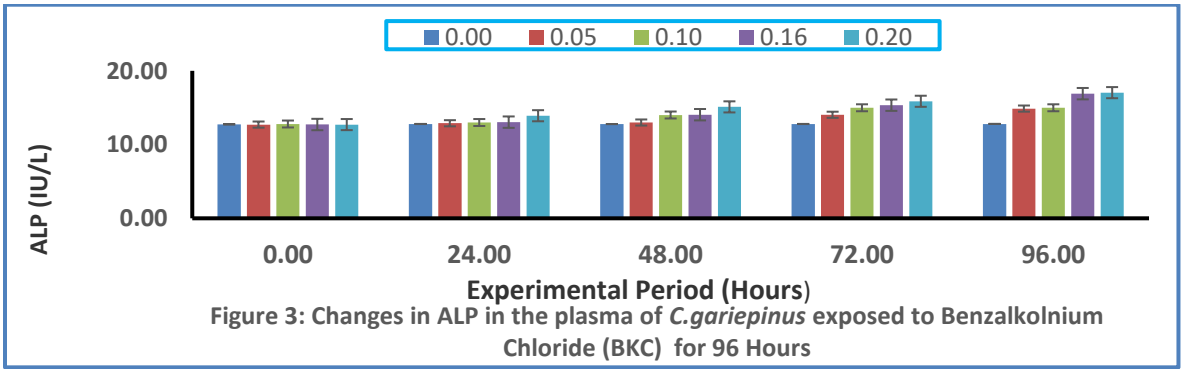

Figure 3: Changes in ALP in the plasma of *C.gariepinus* exposed to Benzalkolnium Chloride (BKC) for 96 Hours

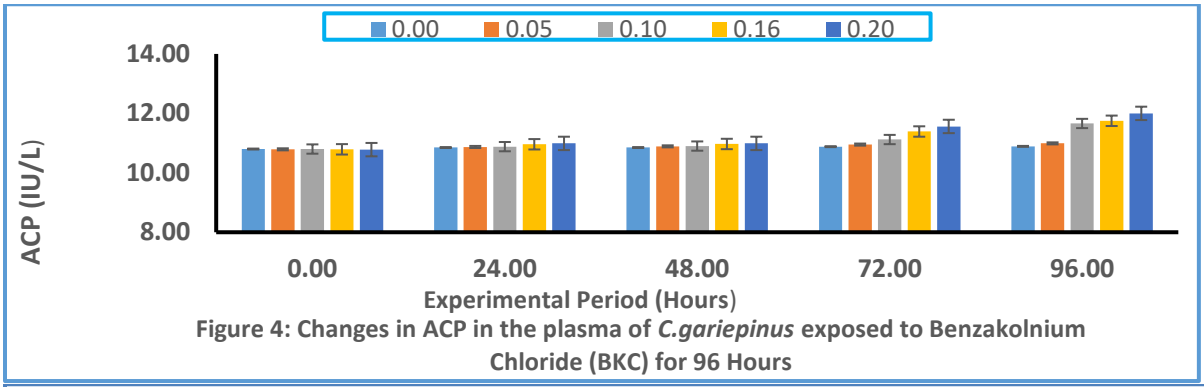

Figure 4: Changes in ACP in the plasma of *C.gariepinus* exposed to Benzakolnium Chloride (BKC) for 96 Hours

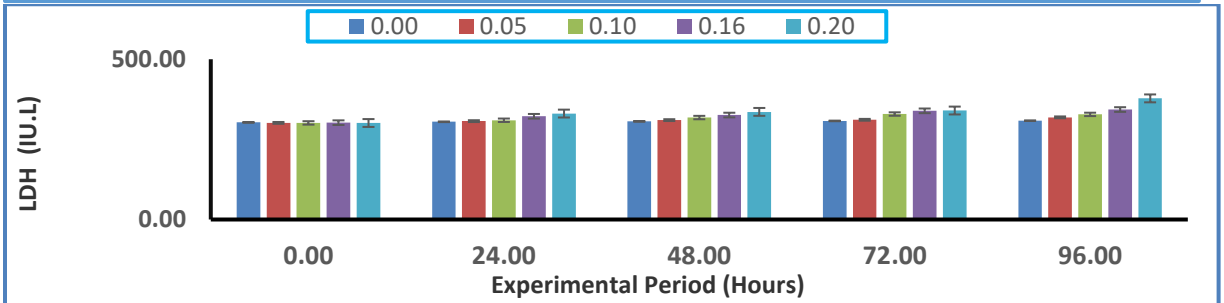

Figure 5: Changes in LDH in the plasma of *C.gariepinus* exposed to Benzakolnium Chloride (BKC) for 96 Hours

DISCUSSION

Water quality parameter such as temperature, dissolve oxygen, pH ammonia and nitrate evaluated in this study are critical and influence fish health, growth and reproduction in the aquatic medium. The values of the parameters were within the same range except dissolved oxygen that reduced with increasing concentrations of the chemical. In this study, enzymes assay such as ALP, AST, ALT, ACP are parts of routine laboratory analysis test to identify anomaly in the physiological status of aquatic animals. Alterations in any of these enzymes resulting from pollutants consequences in the plasma of fish have been reported by various authors [14]. Such alterations in fish are aimed at maintaining equilibrium in the presence of these toxicants which are known to disrupt physiological and biochemical processes. In this study, activities of these enzymes increased, as the concentration of Benzalkonium chloride increased in the plasma of the exposed fish, this result agrees with the report of Das *et al* [15] in India catfish exposed to nitrite toxicity. They suggested that the increase of transferases is as a result of diversion of alpha-amino acids in the tricarboxylic acid (TCA) cycle as keto acids to augment energy production in the cell of the fish.

Moreover, cellular toxicity of toxicants has been attributed to an increase in AST, ALT and ALP in the plasma of *Sarotherodon melanotheron* exposed to industrial effluents as reported by Nte *et al.* [16]. In this study AST activity was elevated in the plasma of the exposed fish probably to enable the fish cope with the energy demand during stress condition. Similar findings, suggest that this energy demand could be satisfied through amino acid. ALP activity [17]. In this work, ALT and ACP activities increased in *Clarias gariepinus* is exposed to Benzalkonium Chloride an

increase in these enzymes are indications that some vital organs of the fish have been impaired. This view was supported by Gabriel *et al*. [18] when *C. gariepinus* was exposed to cypermethrin in the laboratory. ALT activity reflects a change in endoplasmic reticulum mass; it is also known to occur in the cell membrane and may be involved in metabolic activities. This increase may denote an increase in metabolic transport which may eventually result in a shift in biosynthesis and the energy metabolism pathway of the exposed organism [19]. While an elevation in ACP, as observed in this study suggests an increase in lysosomal mobilization and cell necrosis due to effluent toxicity. The lowered activity of ALT in this study showed the inactive transamination and oxidative deamination occurred [20].

CONCLUSION AND RECOMMENDATIONS

This study revealed that the exposure of *Clarias gariepinus* to sub lethal levels of Benzalkonium Chloride produced significant changes in the physiological composition of the fish as revealed by alterations in the enzyme activities in the study. Continual exposure through pollution by disinfectant in aquatic environments can lead to mortality of *Clarias gariepinus* and economic loss. So there should be adequate disposal of disinfectants so as to prevent ecological challenges in the aquatic medium. However, the disinfectants can be applied at 0.05mg/L in the culture medium without any damage to the physiology of the fish.

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