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Effects of Benzalkonium Chloride on Metabolic Activities in *Clarias Gariepinus* Juvenile

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Abstract: *Benzalkonium Chloride, a commonly used disinfectant, has been implicated in a myriad of adverse effects on aquatic organisms. This study therefore investigated changes in some metabolites in the plasma of Clarias gariepinus exposed to Benzalkonium Chloride. A total of 150 juvenile of C, gariepinus (mean length 12.85±7.88cm and mean weight 251.97±30.78g) were exposed to different concentrations (0.00mg/L-control; 0.05; 0.10; 0.15 and 0.20mg/L) of benzalkonium chloride for 96 hours. During the experiment, some physico-chemical parameters of the water in the exposure tanks were evaluated. Blood was taken from the exposed fish at 0, 24, 48, 72 and 96 hours and analyzed for changes in its metabolic profiles. The results indicated 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 concentration of the chemical were 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. At zero hour the values of all the metabolites (Urea, Creatinine, Total bilirubin, Albumin, and Total protein) in the plasma of the exposed C. gariepinus were within the same range with no significant difference ($p>0.05$). At 24, 48. 72, and 96 hours of exposure of C. gariepinus to varying concentrations of Benzalkonium Chloride, there was significant reduction ($P<0.05$) in the values of total bilirubin, creatinine, albumin and total protein. While the values of urea increased significantly ($P<0.05$) with increasing concentrations of the chemical. This chemical caused some changes in the metabolic profiles of the fish metabolites. Hence, caution should be exercised when applying this chemical in aquatic medium.*

Keywords: *Aquaculture, Toxicity, Benzalkonium Chloride, Metabolites.*

1. INTRODUCTION

Benzalkonium chloride (BKC) is a widely used quaternary ammonium compound with antiseptic and antimicrobial properties. It is commonly found in various household products such as disinfectants, hand sanitizers, and cosmetics. Despite its effectiveness in killing bacteria and viruses, there is growing concern over its potential adverse effects on human health, including metabolic changes [1]. There is growing evidence to suggest that BKC may have adverse effects on metabolic health. Further research is warranted to fully understand the mechanisms underlying these effects and to assess the potential risks associated with BKC exposure in various populations [3]. Metabolic changes play a crucial role in determining the toxicity of chemicals within the body. When foreign substances enter our system, our metabolism kicks into gear to process and eliminate them. This process involves a series of biochemical reactions aimed at transforming these substances into less harmful forms that can be excreted from the body [4]. The metabolic pathways vary widely depending on the chemical structure of the substance. Some chemicals are broken down quickly and efficiently, while others may undergo bioactivation, transforming into even more toxic intermediates before being neutralized [5]. Understanding the metabolic pathways of chemicals is essential for assessing their potential toxicity and designing strategies for detoxification or minimizing exposure. This knowledge informs regulatory decisions, medical treatments, and public health interventions aimed at protecting human health from the harmful effects of chemical exposure [6].

Metabolic changes in *Clarias gariepinus* can serve as important indicators of toxicity when exposed to certain chemicals. These changes often manifest in alterations to biochemical pathways, enzyme activities, and physiological responses [7].Exposure to toxic chemicals can disrupt biochemical pathways involved in metabolism, leading to changes in metabolite profiles. Metabolomics, the comprehensive analysis of metabolites within an organism, can reveal shifts in metabolic pathways induced by chemical exposure. By comparing the metabolite profiles of exposed and unexposed fish, researchers can identify biomarkers indicative of toxicity. [8]. The goal of this study is to investigate the acute toxicity effects of Benzalkonium Chloride in catfish, *Clarias gariepinus*, using some of the biomarkers employed in determining stressed conditions in fish. According to Ayoola [9], knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges. As a result, juveniles of *C. gariepinus* were exposed to acute concentrations of Benzalkonium Chloride in order to determine their sensitivity to these chemicals, using metabolites as endpoint. After the acute testing, biochemical analyses of the fish plasma were carried out to the endpoint of understanding how these chemicals impact the fish physiology. This study aims at examining the effects of Benzalkonium Chloride based detergent on the metabolites in *Clarias gariepinus* juveniles.

2. Material And Methods

2.1. Experimental Location and Source of Experimental Fish

The experiment was carried out at the Wet Laboratory in the Department of Fisheries and Aquaculture Management, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. One Hundred and Fifty (150) *Clarias gariepinus* of the same size range (mean length 12.85±7.88cm and mean weight 251.97±30.78g) were sourced from House Tully Fish Farms, Okpunno, Awka, Anambra State, Nigeria. They were transferred in two 50-litre plastic tanks to the laboratory for the acclimation process.

2.2. Acclimation and Feeding of Fish

The experimental fish were acclimated in four 150 L 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 a commercial feed at 5% body weight throughout this period.

2.3. Experimental Design

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

2.4. 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 method described by Ganriel and Edori [10].

2.5. Exposure of Fish to Benzalkonium Chloride (Bkc)

Ten *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 escaping.

2.6. 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 values of DO, nitrate, and ammonia were determined by the method of APHA [11].

2.7. Determination of Blood Plasma Metabolites

A 2ml sample of fresh blood was taken at the conclusion of each experimental period by puncturing the caudal artery with a tiny needle and pouring the sample into heparinized sample vials. Serum was separated by centrifugation in a TG20-WS Tabletop High Speed Laboratory Centrifuge for 5-8 minutes at 10,000 rpm. After centrifugation the blood samples were divided into 2 sections in the heparinized bottle. The plasma on top which was yellowish in colour and the serum, which was dark red. The plasma was extracted. Following the guidelines provided by APHA [11], the samples were examined for the metabolites creatinine, total bilirubin, total urea, and total protein. There were three copies of each test run. The methods APHA [11] were also used to determine water quality parameters.

2.8. 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%.

3. Results

The results of the physico-chemical properties of the water in exposure tanks in *C. gariepinus* exposed to Benzalkonium Chloride presented in Table 1. The results indicated a significant reduction ($p<0.$) in the values of dissolve 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 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. Comparative values of Total bilirubin in the plasma of *C. gariepinus* exposed to Benzalkonium Chloride for 96 hours is shown in Figure 1. The values of Total bilirubin reduced as the experimental period increased, with the highest value of 11.55 observed at the control, while the lowest (7.43) at 96 hours. Comparatively, the values of urea as shown in Figure 2, indicated that the values of urea in *C. gariepinus* exposed to varying concentrations of Benzalkonium Chloride were elevated progressively as the experimental period increased and peaked at 96 hours for all concentrations. The highest value of 17.99 was recorded in the fish exposed to 0.20mg/L of the chemical at 96 hour, while the lowest value of 5.19 was observed in the control (Figure 3). The values of creatinine reduced considerably as the experimental period increased, this was more pronounced at the concentration of 0.10, 0.15 and 0.20mg/l concentrations of the chemical. The values of Albumin (Figure 4) slightly reduced when compared to the control value in all concentrations of exposure. Comparative the value of Total protein is shown in Figure 5. The values of Total protein reduced as the experimental period progressed from 24 to 96 hours. However, a sharp decline was observed in the concentration of 0.20mg/l at 96 hours.

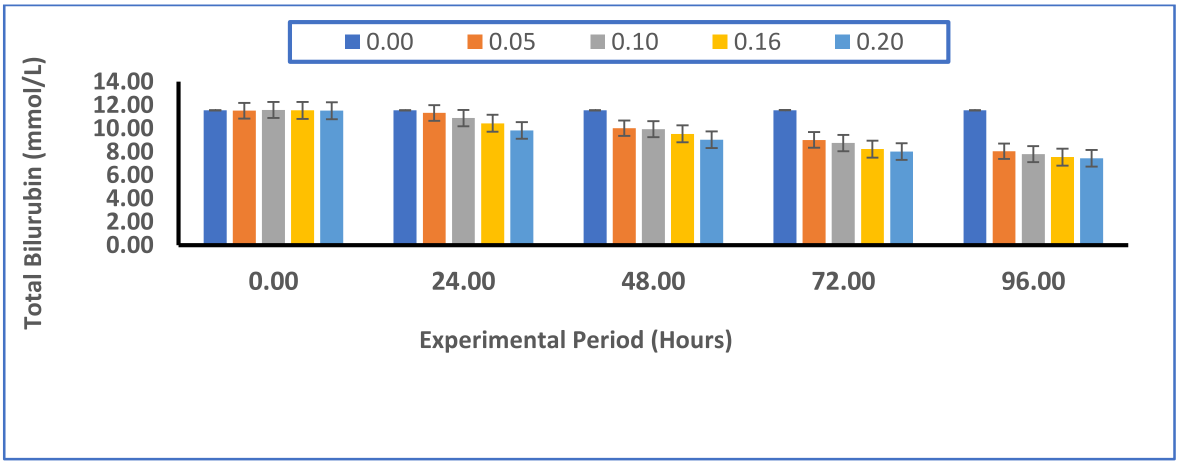

Figure 1. *Variations inthe values of Total bilirubinin the plasma of C.gariepinus exposed to Benzakolnium Chloride (BKC) for 96 hours*

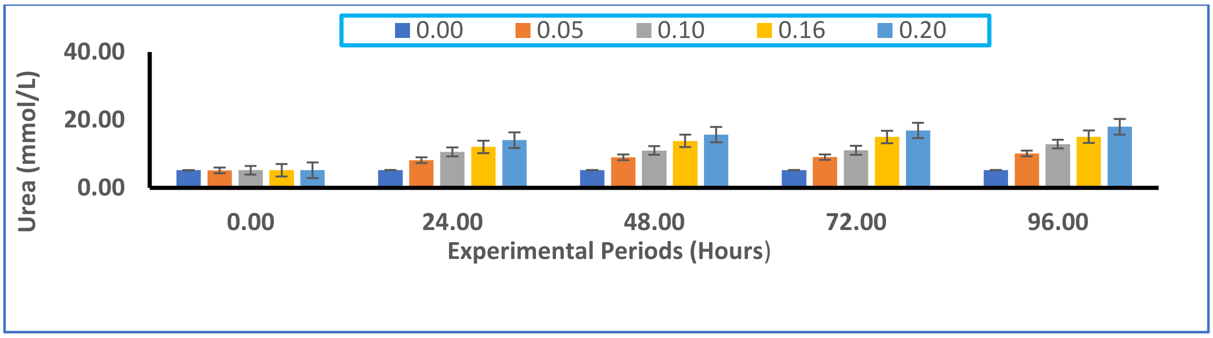

Figure 2. *Variations in the values of Urea in the plasma of C.gariepinus exposed to Benzakolnium Chloride (BKC) for 96 hours*

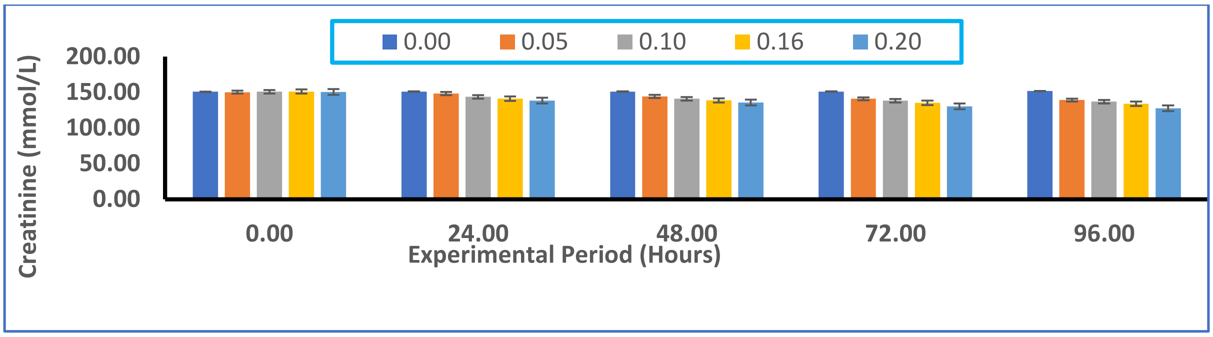

Figure 3. *Variations in the values of Creatinine in the plasma of C.gariepinus exposed to Benzakolnium Chloride (BKC) for 96 hours*

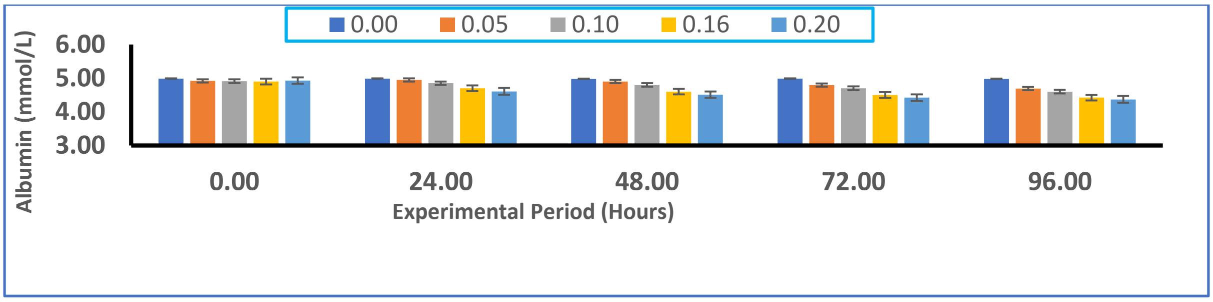

Figure 4. *Variations in the values of Albumin in the plasma of C.gariepinus exposed to Benzakolnium Chloride (BKC) for 96 hours*

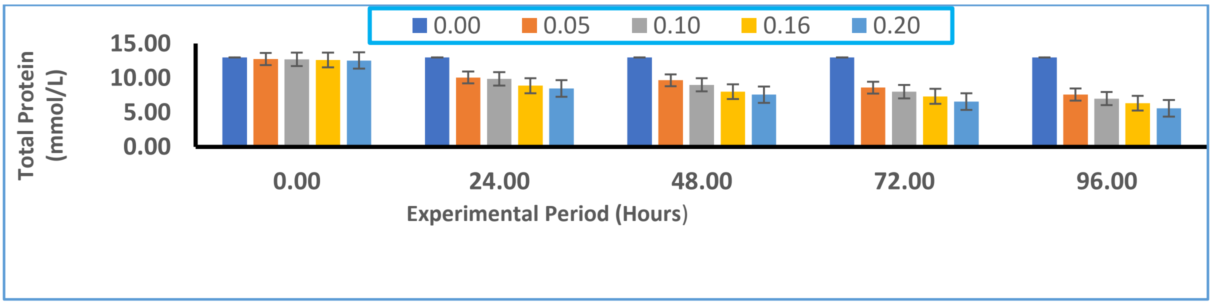

Figure 5. *Variations in the values of Total Protein in the plasma of C.gariepinus exposed to Benzakolnium Chloride (BKC) for 96 hours*

Table1. *Physicochemical Parameters of Water in Tanks of C. gariepinus Exposed to Acute Concentrations of Benzalkonium Chloride (BKC) for 96 Hours*

Concentrations	Temperature	pH	DO	Nitrite	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)

4. Discussion

The mean values of the water quality metrics did not change significantly ($p>0.05$) over the course of the investigation. The measured results were similar to the control values with the exception of a decrease in dissolved oxygen at Benzalkonium Chloride concentrations of 0.20 mg/l. This outcome is consistent with the research done by Akinrotimi *et al.*, [12] on the effects of cypermethrin exposure on *Clarias gariepinus*. At exposure of the toxicant for 96 hours, the recorded increase in nitrite, ammonia, and decrease in dissolved oxygen and the fluctuations that occur between the various concentration and time could be due to the fact that these parameters are highly unstable. Although chemicals cause changes in the quality of water in and around exposed areas and decrease the dissolved oxygen in the water which may pose a threat to the survival of fish species, the result of the present study indicates that Benzalkonium Chloride application does not result in significant changes in the physicochemical parameter to a point that is capable of causing visually observable negative impacts in fish.

In this study, there was the reduction in the values of total protein in plasma of *C. gariepinus* exposed to Benzalkonium Chloride, This results agree with the findings of Akinrotimi *et al.* [13] in *C.gariepinus* exposed to sodium carbonate. Protein and carbohydrate play a major role as energy precursors for fish under stress conditions. Changes in each of these blood components have been employed as useful general indicators of stress in teleosts [14]. Some of the other possible reasons for protein reduction in response to the stressor might be due to haemolysis and shrinking of the erythrocytes, which must have caused dilution of the plasma volume, contributing to some extents in such a reduction. It could also result from the blocking of protein synthesis or protein denaturation or interruption in the amino acid synthesis due to the increase in cortisol, inhibiting protein synthesis and stimulation of protein catabolism]. Similar significant reduction in protein level in plasma were reported in *Clarias batrachus* exposed to cypermethrin, in rainbow trout and in *Cyprinus carpio* exposed to diazinon[15].

Albumin slightly decreased with increased concentration of Benzalkonium Chloride and this agrees with the findings of Ogundiran *et al.* ([16] who observed that necrosis which occurred with increase in concentration of detergents resulted in the reduction of albumin in the liver. This could be due to the inability of fish to regenerate new liver cells. It was also observed that the physiological changes in the plasma of the exposed fish might have caused metabolic problems. Urea has been used as important indices for the evaluation of the effects of chemicals on kidney rand plasma. In this study, the values of urea in the plasma were elevated as the concentrations of the toxicant increased. This is in agreement with John [17] who opined that the influence of toxicants on the plasma is a function of its strength. It could be said that the irregular response of urea as concentration increased may imply that the glomerular filtration rate of the kidney had a slight stress. The activities of bilirubin dropped below the control values. The decrease in total bilirubin activities as concentration increased in this study may probably cause some stress or damage to the organs concerned and this is in agreement with the reports of Affonso *et al.* [18] in *Labeo rohita* exposed to Carbofuran in the laboratory. Similarly, the decrease in creatinine levels in fish exposed to Benzalkonium Chloride compared to the control suggests that creatinine was completely used up by the muscle as a result of the stress induced by the toxicant. On the other hand, the general decrease in the levels of the metabolic parameters of *C. gariepinus* in the cause of exposure to Benzalkonium Chloride conformed with the reports by Kori-Siakpere *et al.* [19] when *C. gariepinus* was exposed to sub-lethal concentrations of petroleum.

5. Conclusion And Recommendations

The effects of Benzalkonium chloride on the metabolic changes in *Clarias gariepinus* highlight its potential as a significant stressor impacting various physiological processes. From altered metabolic rates, these changes underscore the importance of understanding the impact of environmental pollutants on aquatic organisms. Though, it was proven from this research that it does not impact negatively on the physicochemical parameters of the water, as all the values falls within the tolerance range for *Clarias gariepinus*. The use of Benzalkonium Chloride at riverside and coastal areas should be strongly controlled and carefully monitored to avoid exposure to aquatic environments. If there is any need for the application of this disinfectant it should be applied at the rate of 0.05mg/l.

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