Advances in Environmental Health Sciences and Toxicology (AEHST)

A publication of the University of Medical Sciences, Ondo City, Ondo State, Nigeria

URL: https://journals.unimed.edu.ng/index.php/JEMT/index

Vol. 1 No 2 June 2025, Pp. 22 - 32

ISSN: 1595 - 1189 (Online)

CYPERMETHRIN EXPOSURE IMPACTS BIOCHEMICAL PARAMETERS AND THE METABOLISM OF LIVER ENZYMES IN AFRICAN CATFISH (*Clarias gariepinus*)

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Submitted 20 December, 2023

Accepted 03 June, 2025

ABSTRACT

Background: Pesticides present in aquatic settings pose a threat to aquatic species as well as to people who rely on the ecosystem.

Objective: The effect of Cypermethrin on some biochemical parameters of the widely consumed African catfish*, Clarias gariepinus* was evaluated.

Methods: Healthy juvenile *Clarias gariepinus* (n = 60, mean, 198.42 ± 5.4g and 28 ± 1.23 cm) were procured from a farm and acclimatized for 14 days before exposure to sub-lethal concentrations of Cypermethrin. The water was changed once every two days. Six fish each in groups A–E with replicates were exposed to 0.00, 1.25, 2.5, 3.75, and 5.0 ml/L of toxicant, respectively, for 96 hours. Water quality parameters were monitored. Blood was drawn from the caudal circulation, separated into plasma by centrifugation, and then the plasma's biochemistry was determined spectrophotometrically using Randox kits.

Results: The water's obtained physicochemical parameter values fell within the ranges necessary for freshwater fish viability. Group E had the highest total protein, albumin, and cholesterol values (10.40.36g/dl, 4.270.40g/dl, and 228.68mg/dl, respectively), while Group A without any toxicant had the lowest values (6.702.04g/dl, 2.001.32 g/dl, and 193.67g/dl, respectively). The experimental fish showed a dose-dependent rise in serum aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) with an increase in the quantity of the toxicant ($P < 0.05$).

Conclusions: Fish with elevated transaminase levels typically have liver cells that are deteriorating or destroyed. An increase in the pesticide concentration in any body of water could cause abnormal biochemical dysfunction in the health and general condition of the fish.

Keywords: *Cypermethrin*, Cholesterol, *Clarias gariepinus*, liver enzymes, total plasma protein

INTRODUCTION

The aquatic environment faces significant pollution challenges, primarily due to human activities that introduce contaminants into water bodies. The use of pesticides has also increased over the years to help reduce the damage caused by pests to crops on land (Bashir et *al.,* 2020). Pesticides have significantly contributed to the increased food production in modern times through the means of pest control and weed control. However, this impact comes with its attendant ecotoxicological implications.

The injudicious and unregulated discharge of agricultural chemicals especially pesticides into aquatic bodies has caused ecological problems for all classes of aquatic organisms including fish (Weldeslassie *et al*., 2018). Many agricultural fields and homes are constantly sprayed with pesticides. This constitutes the major source of pollution to the aquatic environment hence many non-target organisms including fish are affected.

Exposure to low levels of pesticides has attested to cause profound effects on non-target organisms. Pesticides may also find their way into the food chain and cause functional damage (Lushchak *et al*., 2018), and affect and alter the performance of the organism (Zacharia, 2011). Their residues often sink to the bottom of the water body where they exert effects on aquatic life, particularly fish. They generate a main menace because of their injuriousness persistence and propensity to gather in the organism (Sabra and Mehana, 2015). Different level of pollution in biotopes causes the different accumulation of xenobiotics in fish tissues and this may change fish physiology and biochemistry (Lushchak, 2016).

Cypermethrin is a synthetic pyrethroid used as an insecticide on a large scale, and used extensively in households, industrial, and agricultural fields for the control of several insect pests. Cypermethrin is highly toxic to fish, bees, and aquatic insects as reported by Ullah *et al.* (2018).

Cypermethrin makes its entrance into natural water bodies through erosion/agriculture runoff as a result of indiscriminate use. Several non -target aquatic organisms were ultimately affected. The growth of the aquatic animals is inhibited. Several metabolic activities of crustaceans are affected, most times affects fish metabolism, biochemistry, and hematology, thus adversely affects fish quality and fish population (Andem *et al*., 2016).

Cypermethrin is reported to be less toxic to mammals, and birds however reported to be highly toxic to fish (Kumar et *al*., 2010). Fishes are unable to metabolize the pyrethroids efficiently. Cypermethrin also affects the biochemical enzyme by mode of neurotransmitters (Farag *et al*., 2021). Increasing contamination of water bodies by pesticides and other xenobiotics is of great concern not only to the public health implications but also to ecosystem health concerns. Billions of people, mostly in developing countries depend on fish as a primary source of animal protein (Béné *et al*., 2015). The model projects that the total fish supply will increase from 154 million tons in 2011 to 186 million tons in 2030, with aquaculture entirely responsible for the increase was predicted (Kobayashi *et al*., 2015).

Fish farming continues to make substantial contributions to Nigeria's animal protein requirements. *Clarias gariepinus* (African catfish) is considered to be a freshwater species that is widely cultivated. *Clarias gariepinus* is an important species in commercial aquatic farming in Nigeria. It responds promptly to environmental alterations and it is widely tolerant to many different habitats. It survives in floodplains, slow-flowing rivers, lakes, dams, and even the upper reaches of estuaries (Oyieng, 2014). It can endure extremely harsh conditions.

It can tolerate waters high in turbidity and low in dissolved oxygen. *Clarias gariepinus* is often the last or only fish species found in remnant pools of drying rivers. It can even survive for considerable period of time out of the water, via the use of a specialized supra branchial organ (Maina, 2018).

This organ is a large paired chamber with branches above the gill arches specifically adapted for air-breathing, and allows it to move over land even when not forced to do so by drought (Damsgaard *et al*., 2020).

Water temperatures between 8°C and 35°C, salinities of 0 to 10% and a wide pH range are all tolerated *C. gariepinus* (Ndubuisi *et al*., 2015). *C. gariepinus* exhibits high growth rates between temperature of 25°C and 33 °C, with optimum growth recorded at 30°C (Suleiman and Solomon, 2017). The ability of the *C. gariepinus* to be able to tolerate these extreme conditions allows it to survive even in moist sand or in borrows with an air-water interface (Reebs, 2009). Rudneva *et al*. (2012) pointed out that the health of fish can be determined by evaluating blood biochemistry parameters. Biochemical parameters in fish are sensitive for detecting potential adverse effects of metal, pesticides, and or other xenobiotics buildups. Higher or lower values than normal ranges established for organisms of variables such as total protein, albumin, globulin, and A-G (Albumin-Globulin) ratio are indications of serious health conditions. The activities of various enzymes are considered to be sensitive biochemical indicators before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants (Hedayati, 2018). Aminotransferase activity could be reduced due to the structural liver alternations, and this increases plasma protein concentration with a simultaneous decrease in deamination capability (Lawrence and Steiner, 2017). Determination of enzymes, such as AST (Aspartate transaminase), ALT (Alkaline transaminase) ALP (Alkaline phosphatase), and lactate dehydrogenase (LDH), is considered a useful biomarker to determine pollution levels during acute and chronic exposure to hazardous agents. Hence, this study was carried out to assess and contribute to knowledge on the biochemical alterations in *Clarias gariepinus* by exposure to different sub-lethal concentrations of Cypermethrin.

Materials and Methods

Experimental fish

Sixty (60) mixed-sex juveniles *Clarias gariepinus* (mean weight=198.42 ± 5.4g, mean length= 28 ± 1.23cm) were procured from a fish pond in the Lipakala area of Ondo City in Ondo State, Nigeria. They were transported to the Biology Laboratory, University of Medical Sciences, Ondo City, Ondo State. The fish were separated into 10 different transparent plastic tanks filled with clean borehole water. The fish were maintained and managed under the ethical guidelines for handling experimental aquatic animals for 14 days. The experimental fish were fed *ad libitum* with commercially pelleted fish feed, Ecofloat fish feed, 4mm in size: (protein 36%, fat 8%, ash%, crude fiber 4.5%, moisture 8%, calcium 2%, phosphorus 0.85%, sodium 0.3%) during the acclimation period and throughout the exposure period. The aquaria were cleaned and water was renewed daily. Unconsumed feed and fecal were removed, and water were replenished regularly.

Experimental design for toxicity studies

The toxicant used for the study was Avesthrin containing 10% Cypermethrin. The toxicant 1 ml stock solution was prepared aseptically using the serial dilution method to get a 10^{-2} concentration. Fish were randomly allotted six (6) fish per group (A, B, C D, and E) set up in duplicates. Five pairs of plastic aquaria (20cm×10cm×12cm) filled with 25 liters of borehole water were used. Group A served as control without any toxicant while groups B to E were polluted with 1.25, 2.5, 3.75, and 5.0ml/L of the prepared toxicant, respectively. The fish were kept for 4 days under continuous renewable bioassay and were observed for behavioral changes. Physicochemical parameters of the water used in the aquaria were also continually monitored, and measured during the 4-day trial using the methods described by Kim *et al*. (2017). At the end of the 4 days of exposure to a sub-lethal concentration of Cypermethrin, blood was drawn from the experimental fish. Before the blood collection, the fish were anesthetized with ms-222 (Tricaine methane sulfonate) to reduce stress. Blood was collected from the caudal vein, using 5ml plastic syringes and stored in small EDTA bottles. The blood was then centrifuged for 20 minutes in a cooling centrifuge to obtain the plasma used for the estimation of the biochemical parameters.

Biochemical analysis

Total Protein (Calorimeter Biuret Method)

Principle: - Cupric ion is an alkaline medium that intermingles with protein peptide bonds resulting in the formation of the colored complex. **Procedure**: - Twenty microliter of serum was added into One thousand microliter of Biuret reagent test tube. Twenty microliter of the standard was added into One thousand microliter of Biuret reagent, mixed, and incubated for 10 minutes at room temperature at 20°- 25°C. The absorbance of the sample and the standard was measured against the blank on the spectrophotometer at 540nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

Albumin (Bromocresol Green Calorimetric Method)

Procedure: - Ten microliter of serum was added into 3000L of BCG reagent in a test tube, ten microliter of the standard was added into three thousand microliter of BCG reagent, mixed, and incubated for 10mins at room temperature at 20°C - 25°C. The absorbance of the sample and the standard was measured against the blank on the spectrophotometer at 630nm

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

BCG reagent consists of succinate buffer and bromocresol green.

Globulin:

Calculation= Total protein – Albumin

A.G ratio:

$$\text{Calculation} = \frac{\text{Albumin}}{\text{Globumin}}$$

Glucose:

Oxidase peroxidase enzymatic colorimetric method:

Procedure: - Ten microliter of the sample was added into one hundred microliter of glucose oxidase reagent, mixed, and incubated for 10 minutes at room temperature 20^0C-25^0C. The absorbance of the sample and of the standard were measured against the blank on the spectrophotometer at 54nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

Cholesterol:

CHOD- POD Enzymatic Colorimetric Method:

Procedure: -Ten microliter of serum sample was added into 1000 microliter of cholesterol oxidase in test tube mixed and incubated for

10 minutes at room temperature 20^0c-25^0c. The absorbance of the sample and of the standard was measured against the blank on the spectrophotometer at 540nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

Aspartate Aminotransferase (AST): (Colorimetric Method)

AST is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine.

Procedure:-One hundred microliter of the sample was added to five hundred microliter of buffer reagent, mixed and incubated at 37°C in the water bath for 30mins, removed from the water bath, add five hundred microliter of 2,4 dinitrophenylhydrazine mixed, and incubated for 20mins, removed from the water bath add five thousand microliter 0f 16% sodium hydroxide solution. The absorbance of the sample and of the standard was measured against the blank on the spectrophotometer at 540nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

ALP (Alkaline phosphatase): (Colorimetric Method)

Procedure: - Twenty microliter of serum sample was added into 1000 microliter of buthanolamine buffer and p-nitrophenyl phosphate into the test tube mixed and incubated at 37^0c for 45mins in the water bath, removed, and allowed to cool. Add 5000 microliter of 16% sodium hydride solution. The absorbance of the sample was measured on the spectrophotometer at 410nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

ALT is measured by monitoring the concentration of pyruvate hydrozone formed with 2, 4 dinitrophenylhydrazine

Procedure:- One hundred microliter of serum sample was added into500µl of buffer reagent mixed and incubated at 37°C in the water bath for 30mins, removed from the water bath, add 500 microliter of 2,4 dinitrophenylhydrazine mixed and incubated for 20mins, removed from the water bath add 5000 microliter 0f 16% sodium hydroxide solution. The absorbance of the sample and the standard was measured against the blank on the spectrophotometer at 540nm.

$$\text{Calculation} = \frac{\text{A sample}}{\text{A standard}} \text{ X concentration of standard}$$

Statistical analysis

Analysis of variance (ANOVA, one way) and descriptive statistics were employed to analyze all data with Graph Pad Prism Software Version 5.1. The results were expressed as mean ± standard error (SE). A level of P value less than 0.05 was considered to be significant ($P < 0.05$)

Ethical Approval

Permission was sought and obtained from the University of Medical Sciences, Ondo City, Ondo State, Nigeria Ethical Committee to use live animals for the study on pesticides. All the experimental fish were handled humanely under the ethical guidelines for the use of animals in scientific research.

Results and Discussion

Exposure of animals to sub-lethal levels of toxicants may inflict stress on the mechanisms required for maintaining a healthy physiological state. These stresses may result in changes in biochemical, physiological, or behavioral processes. Given this, there has been increasing interest in examining the physiological and biochemical stress response in aquatic vertebrates to protect aquatic life. A handful of biochemical blood parameters are routinely used to assess health status and aid in the diagnosis of diseases in man and animals. These tools in most cases have less frequently been applied in studies dealing with ecotoxicology or related disciplines. In this study, an attempt was made to investigate biochemical blood parameters in *C. gariepinus* following exposure to sub-lethal concentrations of Avesthrin (10% Cypermethrin) under laboratory conditions.

Mean values of the water quality parameters recorded during the exposure of *Clarias gariepinus* to the prepared toxicant as shown in Table 1 shows that temperature ranged from 26.4°C to 26.7°C, pH ranged from 6.5 to 6.6, conductivity ranged from 0.31mS/cm to 0.39mS/cm while dissolved oxygen (DO) ranged from 5.17 to 7.00 mg/L. Statistical analysis indicates that there was no significant difference ($P > 0.05$) in water quality parameters between the control group and the treated group. The values of the physicochemical parameters obtained were within the ranges for the survival of freshwater fishes as described by

Table 1: Water quality parameters (mean ± SE) in experimental aquaria of the *Clarias gariepinus* exposed to Cypermethrin

Parameters	Group A	Group B	Group C	Group D	Group E
Temperature	26.50 ± 0.28[a]	26.40 ± 0.00[a]	26.50 ± 0.00[a]	26.70 ± 0.14[a]	26.65 ± 0.07[a]
pH	6.55 ± 0.35[a]	6.50 ± 0.00[a]	6.55 ± 0.00[a]	6.60 ± 0.00[a]	6.60 ± 0.00[a]
Conductivity (mS/cm)	0.32 ± 0.01[a]	0.39 ± 0.06[a]	0.31 ± 0.04[a]	0.34 ± 0.04[a]	0.37 ± 0.01[a]
Dissolved Oxygen (mg/L)	7.00 ± 0.03[a]	5.20 ± 0.01[a]	5.20 ± 0.01[a]	5.17 ± 0.1[a]	5.17 ± 0.01[a]

Values with different superscripts along the rows differ significantly with the control and the various treated groups.
Groups: A (control), water was not polluted with Cypermethrin; B water was polluted with 1.25m/L of Cypermethrin; C water was polluted with 2.5m/L of Cypermethrin; D water was polluted with 3.75m/L of Cypermethrin; E water was polluted with 5.00m/L of Cypermethrin

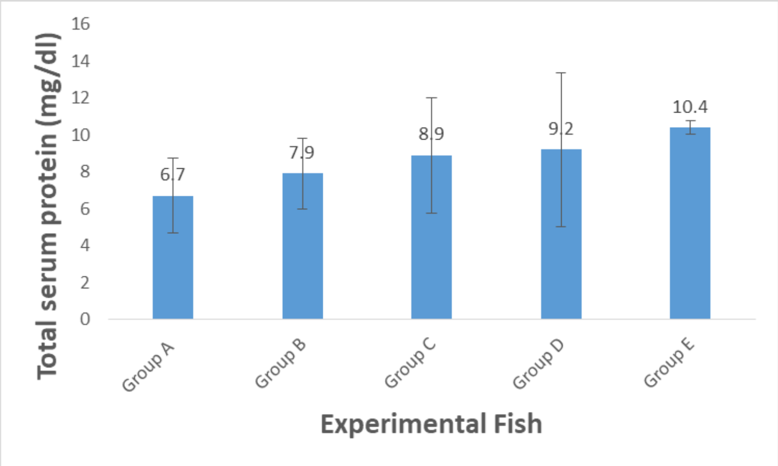

Figure 1: Total serum protein (mg/dl) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

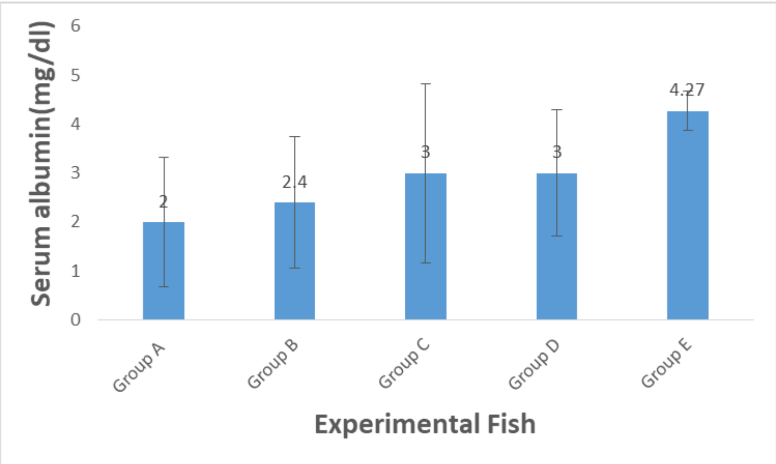

Figure 2: Serum albumin (mg/dl) of post-juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

Keremah *et al.* (2014). However, in all treatments, a reduction was observed in the value of dissolved oxygen compared with the control, but the slight changes in the water quality were within the range of tolerance level for fish survival and growth as reported by Bhatnagar and Devi, (2013). The rate of DO in the water body is one of the important parameters in culturing fish. Low dissolved oxygen in the water body makes fishes that inhabit such type of water physiologically weak and easily susceptible to diseases (Zhang, *et al*., 2013). A dissolved oxygen level of 5 mg/L or more is desirous for fish production while levels equal to or lesser than 3 mg/L are regarded as hazardous for fish production (Meade, 2012). The pH measures the acidity and alkalinity condition of the water, described as the efficiency index of a water body, and is one of the essential elements in fish cultivation. The pH values observed in all the aquaria in this experiment were slightly alkaline, an indication of good pH conditions for fish husbandry. The pH ranges from 6.5 to 9.0 is appropriate for fish culturing according to Bassey and Ajah, (2010). Aquaria water used for the experiment was not turbid due to feces, food particles, and sand particles and this could be a result of frequent changes of the water in the aquaria. The parameters recorded showed that the toxicant used to contaminate the water did not significantly affect the water.

Serum protein increased with an increase in the concentration of the toxicant (Cypermethrin). The highest value of 10.4 ± 0.36g/dl was obtained for the group E treatment while the lowest value of 6.70±2.04g/dl was recorded in group A as shown in Figure 1. Serum albumin increased with an increase in the concentration of the toxicant. The highest value of 4.27 ± 0.40g/dl was obtained for the group E treatment while the lowest value of 2.00 ± 1.32 g/dl was recorded in group A as shown in Figure 2. The highest value for serum globulin of 6.20 ± 1.91g/dl was obtained for the group D treatment which was slightly higher than the 6.13 ± 0.12 g/dl value obtained in the group E with a higher concentration of the toxicant while the lowest value of 4.70 ± 0.72 g/dl was recorded in the group A as shown in Figure 3. The albumin-globulin (A-G) ratio increased with an increase in the concentration of the toxicant. The highest value of 0.70 ± 0.10 g/dl was obtained for the group E treatment while the lowest value of 0.37 ± 0.21 g/dl was recorded in group A as shown in Figure 4. Glucose increased with an increase in the concentration of the toxicant (Cypermethrin). The highest value of 305.33 ± 5.51g/dl was obtained in the group D treatment while the lowest value of 286.67 ± 18.72 g/dl) was recorded in group A as shown in Figure 5. Cholesterol increased with an increase in the concentration of the toxicant (cypermethrin). The highest value of 228 ± 68.15mg/dl was obtained for the group D treatment while the lowest value of 193.67 ± 15.82 was recorded in group A as shown in Figure 6.

The biochemical parameters recorded with the highest values were observed to be in the group with contaminated media but the lowest values obtained in the group with uncontaminated media. This observation could be due to the stress exerted by the toxicant and this could cause reversible and irreversible changes in the homeostasis of the affected fish. The biochemical parameters investigated and recorded in the treatment groups were not significantly increased (P>0.05) however the values obtained were increased when compared to the control groups. The increase in serum protein recorded in this work agrees with Oruç and Üner, (1999) observation that revealed an increase in liver protein following exposure of fish to 2, 4 Diamine for 30 days. This observation was equally supported by Singh *et al*., (2006), who explained that proteins are mainly involved in the architecture of the cell. During chronic periods of metabolic stress, protein also serves as a source of energy. There is an increase in both Albumin and Globulin recorded in this work. Velisek *et al*. (2011) reported that Cypermethrin caused the lowering of albumin and globulin in both Trout and Carp, while Deltamethrin exposure caused elevated values of these parameters in both fish. Protein depletion in experimental animals (including fish) has been reported to be a physiological strategy in the animals to adapt to alterations in their metabolic systems. This leads to degenerated processes such as proteolysis and utilization of degraded products for increased metabolism (Yadav *et al*., 2003). Yadav *et al*. (2003) reported that decreased total protein content and albumin level might be due to the destruction or necrosis of cells and, consequently, impairment in the protein synthesis mechanism however the total protein increased in this study but not significantly. This observation could be due to the fact that the introduced toxicant into the media was mild. The quantity of protein has been shown to be dependent on the rate of protein synthesis or its degradation. Protein quality may also be affected by impaired incorporation of amino acids into polypeptide chains.

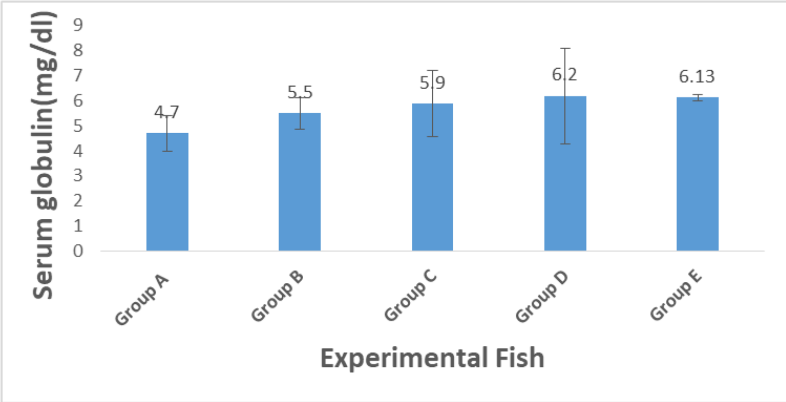

Figure 3: Serum globulin (mg/dl) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

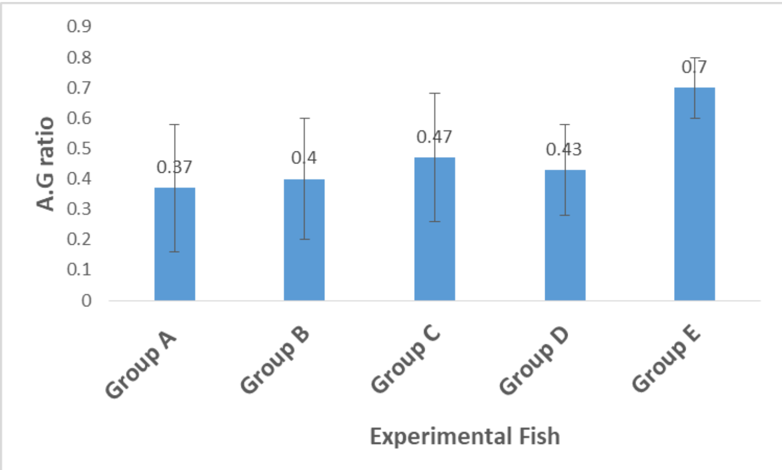

Figure 4: Serum A.G ratio (mg/dl) of post juvenile *Clarias gariepinus* exposed to exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

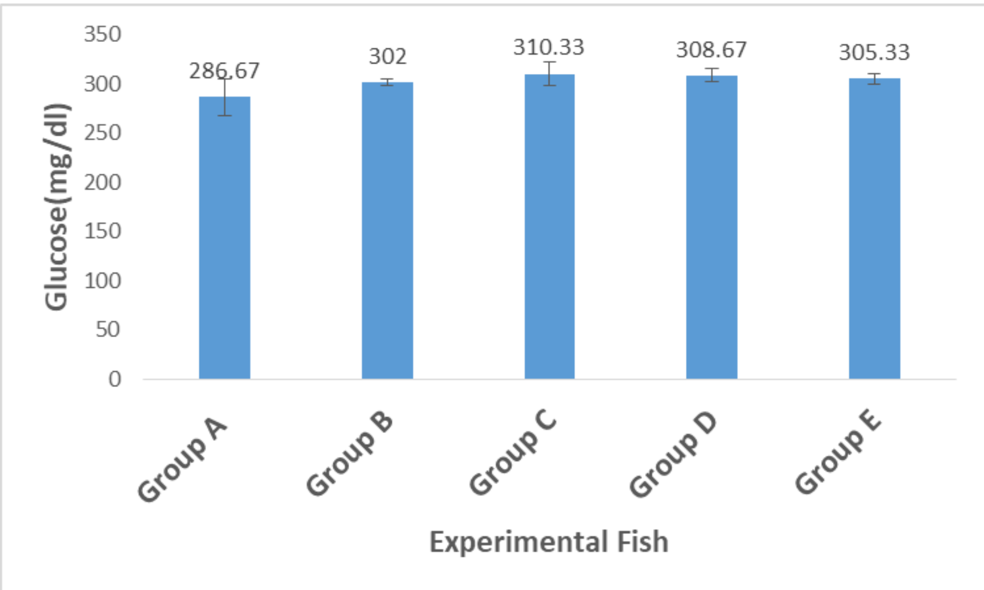

Figure 5: Glucose (mg/dl) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

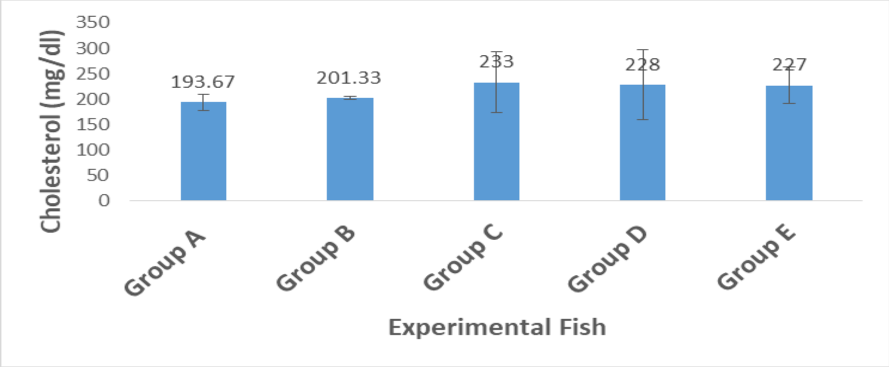

Figure 6: Cholesterol (mg/dl) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

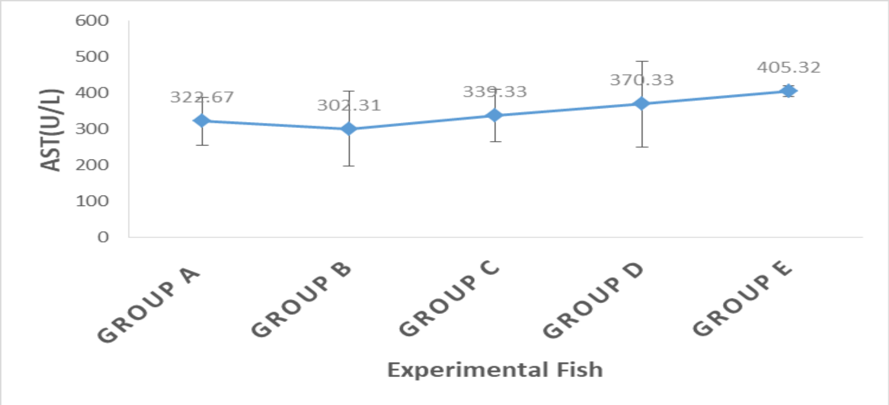

Figure 7: AST (u/L) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

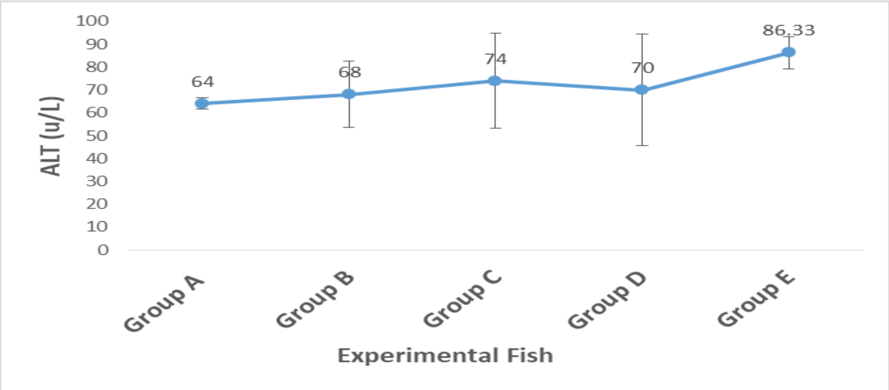

Figure 8: ALT u/L) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

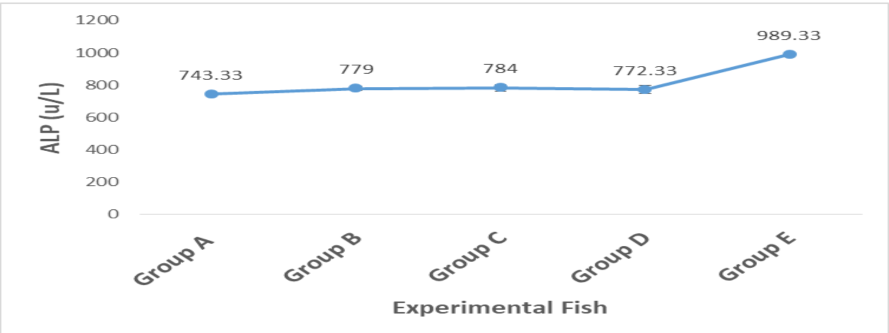

Figure 9: ALP (u/L) of post juvenile *Clarias gariepinus* exposed to sub-lethal concentration of Avesthrin (10% Cypermethrin) for 96 hours

The result also shows that the level of serum Aspartate transaminase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) of *Calaris gariepinus* increased ($P < 0.05$) with an increase in the concentration of the toxicant, depicting a dose-dependent relationship. For AST, the highest value of (405.32 ± 4.93 u/L) was obtained in group E while the lowest value of (302.31 ± 103.50 u/L) was recorded for group B as shown in Figure 7, for ALT, the highest value of (86.33 ± 7.09 u/L) was obtained in group E while the lowest value of (64.00 ± 2.49 u/L) was recorded for group A as shown in Figure 8. The activities of ALP followed the same pattern, the highest value of (989.33 ± 7.02u/L) was obtained in group E while the lowest value of (743.33 ± 122.32u/L) was recorded in group A as shown in Figure 9. Aspartate transaminase (AST), Alkaline phosphatase (ALP), and Alanine aminotransferase (ALT) enzymes are used to evaluate the toxic effects of pollutants (Fathy *et al*., 2012), hence are considered important diagnostic tools. The higher values of alanine aminotransferase (ALT) suggested that the blood serum enzyme in the experimental fish efficiently utilized amino acids for metabolic purposes, confirming the observation of Xiao *et al*. (2020). Increased levels of transaminases in the blood serum of fish are usually associated with dying or damaged liver cells while a decrease could suggest leakage of enzymes into the serum (Gaim *et al*., 2015; Ozovehe, 2013).

Conclusion

Results showed that Cypermethrin exposure impacts the energy and protein metabolisms in *C. gariepinus*. Measurement of these parameters could be used in bio-monitoring studies and the data could provide the basis for developing a framework for establishing safe levels of pesticides in water bodies. In conclusion, it's evident from this study that increasing the concentration of the chemical additives effluent when present in any water body could lead to biochemical dysfunction in fish health and general condition. Hence, there is a need for preventive measures to be taken to prevent the indiscriminate discharge of Cypermethrin derived products into nearby streams and ponds. Man is the final recipient of toxic bio-accumulated chemicals via the food chain and environment, It is therefore recommended that Avesthrin widely used in controlling pests, especially among cocoa farmers in Ondo state should not be used indiscriminately to reduce pesticides intended for target pests but travel into the environment instead, and damage the land, water, and air.

Acknowledgements

The authors wish to acknowledge the head of the Department of Biosciences and Biotechnology and the technical staff of the General Biology Laboratory of University of medical Sciences for their assistance in the course of this project.

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