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EFFECTS OF ACUTE EXPOSURE TO CHLORFENAPYR ON HEPATIC ENZYME ACTIVITIES AND SERUM LIPID PROFILE OF AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL 1822)

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ABSTRACT

The effects of acute exposure to chlorfenapyr on hepatic enzymes and lipid profile of *Clarias gariepinus* juveniles were investigated following standard protocols. Test organisms were exposed to chlorfenapyr concentrations of 5, 7, 9, 11 and 15 mgL^{-1} for 96 hr exposure period. After exposure, peripheral blood was collected through the caudal vein into plain sterilised tubes and allowed stand for 1 h for clot to form. The clotted blood was then centrifuged at 3000 rpm for 10 min and the serum obtained was used for all the biochemical assay. Cross-sections of the gills were examined for histological aberrations. Results showed significant increase in enzymatic activities of Aspartate amino transferases (AST), Alanine amino transferases (ALT) and Alkaline phosphatase (ALP) ($p < 0.05$) when compared with control. Lipid profile results showed significant ($p<0.05$) increase in Total cholesterol (TC) and Low- density lipoprotein-cholesterol (LDL-cholesterol) in all treated groups when compared with the control. Significant ($p<0.05$) reduction in high density lipoprotein- cholesterol (HDL-cholesterol) and triacylglycerides in all treated groups compared to the control was also recorded. Such alterations in biochemical parameters did not show any relationship with gills histology as the cross-section of the gills provided no evidence of aberrations. The results suggest that exposure to acute concentrations of chlorfenapyr can alter liver enzyme activities and serum lipid profile initiating a jump in the energy requirement of the exposed organism.

KEYWORDS: Chlorfenapyr, hepatic enzymes, Lipid profile, *Clarias gariepinus*

Introduction

Pesticides are used extensively in salvaging households from pest infestations and improving yields in agriculture; and these have brought both beneficial and detrimental impacts to the environment (Aktar *et al*., 2009; Muyesaier *et al*., 2021).Pesticide residue, which can affect different non-target aquatic organisms can reach aquatic ecosystem through various routes and these includes run-off, direct application, spray-drift, leaching, indiscriminate discharge from factories and sewage (Barlas 1999; Katagi 2010). Through the food web, these pesticides can bioaccumulate and get transferred from planktons (producers) through fish (primary consumer) and ultimately to humans (tertiary consumer). According to Matagi *et al.* (1998), fin and shell fishes directly absorb toxicants deposited within aquatic environment using their gills and other exposed bio-membranes. Pesticide may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes, affecting their growth, survival and reproduction (Banaee *et al.,* 2008, 2011; Mhadi, 2012). It is also reported that long-term exposure to pesticide can increase stress in juvenile aquatic organisms, thereby rendering them prone to predation (Ewing, 1999).

Due to their persistence in the environment and resistance posed by several targeted pest species, many pesticides (including the organochlorides, organophosphates, carbamates and pyrethroids) have met with criticisms (Ansari *et al.*, 2011). Such criticisms have led to the synthesis of new chemical pesticides perceived to be more potent and more environmentally friendly. One of such newly developed pesticide is the insecticide, chlorfenapyr (chemical formula $C_{15}H_{11}BrClF_3N_2$; melting point of 101.4 – 102.3ºC), a member of the class known as pyrroles (Black *et al.*, 1994; Raghavendra *et al.*, 2011). According to Ingham *et al.* (2012), it was considered a suitable alternative to synthetic pyrethroids due to a lower toxicity to mammalian and aquatic life. It is a pro-insecticide, which requires activation in *in vivo* by mixed function oxidative elimination of its N-ethoxymethyl group to produce the active compound, disrupting respiratory pathways and proton gradients via uncoupling the mitochondrial oxidative phosphorylations, and interrupting the conversion of ADP to ATP (Black *et al.*, 1994). According to WHO criterion, chlorfenapyr molecule has low mammalian toxicity and is classified as a slightly hazardous insecticide (Tomlin, 2000). It is used in controlling mites and insects (e.g. termites and mosquitoes) (Sheppard *et al.*, 1998; Kamaraju *et al.*, 2011). Unfortunately, pesticides developed to be as much as possible, specific and selective in action against target organisms, rarely achieve their purposes without the wider environment being exposed and susceptible non-target organisms getting affected (Malik *et al.*, 2008). For instance,

chlorfenapyr has been shown to increase glutathione S-transferase in the freshwater protozoan, *Paramecium* sp. (Benbouzid *et al.,* 2015) indicating induction of free radicals in this aquatic fauna; and significant reduction in fecundity in *Geocoris punctipes* by this chemical has also been reported (Elzen and Elzen, 1999). Based on the observations of Zhao and Mu (2018), low lethal concentrations of this pesticide can affect oviposition, population development, digestive activities, detoxification enzymes and nutrients accumulation in *Bradysiao doriphaga*.

Fishes change and adapt to their metabolic functions during acute stress conditions (Malarvizhi *et al.*, 2012). Such adaptations have been realized through alterations in biochemical markers studied as indicators of tissue damage (Webb *et al.*, 2005). Sub-lethal effects of stressors (e.g. pesticides) in fishes can be indicated by significant increase or decrease in enzymatic activities (Harabawy *et al.,* 2014). The liver serves as the main organ of various essential metabolic pathways; hence, adversely affected by chemicals. Foreign compounds are predominantly biotransformed in the liver by the action of metabolizing enzymes including microsomal enzymes, aminotransferases and oxygenases (Stickel *et al.*, 2005). Thus, the liver has a high metabolic flexibility in response to enzyme induction to a variety of metabolites as well as the capacity to regulate the expression of genes coding for the biosynthesis of such enzymes. The disruption of the integrity of biochemical and physiological processes in fish has been monitored by determining the changes in the activities of enzymes in plasma/serum and tissues of the gill, brain, liver, muscle and kidney of the fish (de laTorre *et al.*, 2005, Ayalogu *et al.*, 2001; Gabriel and George, 2005; Leelanvinonthan and Amali, 2005). The liver marker enzymes of common interest are the transaminases: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP).

In addition, there are reports indicating that exposures to environmental stressors such as pesticides have resulted in alteration of the lipid profile of fishes (Hammed *et al.*, 2013). Lipid profile are good indicators of an organism's likelihood of developing cardiovascular attack as a result of blockage of the blood vessels or hardening of the arteries (atherosclerosis). The lipid profile frequently assayed include total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides. Nevertheless, sometimes an extended profile may include very low-density lipoprotein cholesterol (VLDL-C) and non-HDL-C.

Furthermore, such toxic effects can be assayed through morphological aberrations of histological sections of some organs. Exposure and bioaccumulation of pesticides in sensitive organs such as the liver and gills of a fish may cause histological injuries thus leading to physiological disruptions; and such histolological injuries are reflected in the histolological sections of the organ (Xing *et al.*, 2012; Banaee *et al.*, 2014; Maksymiv *et al.*, 2015; Adel *et al.*, 2017; Ghelichpour *et al.*, 2017; Hoseini and Yousefi, 2019).

Histopathological alterations of tissues have been commonly used along with biochemical biomarkers in assessing the health of fish exposed to pollutants *in situ* or *ex situ*; and by extension, deciphering the overall health status of the entire aquatic ecosystem (Drishya *et al.*, 2016; Olaniyi, 2020). Although there is deluge of studies on the effect of pesticides on aquatic organisms, limited studies exist on the sub-lethal effect of chlorfenapyr insecticides on fishes. The aim of this study was to investigate the effect of chlorfenapyr on the activities of liver enzymes and lipid profile of African mud catfish (*Clarias gariepinus).* Additionally, the histological section of the gills was analysed to assay the extent of toxicity caused by the chemical on the fish. *Clarias gariepinus* was selected because it is readily and widely available. It is ecologically important as it serves as effective biomarker to changes in the state of the aquatic environment and can survive under laboratory conditions.

Materials and Methods
Fish Collection and acclimatization
Three hundred juvenile species of *C. gariepinus* Juveniles (Burchell, 1822) (Family: Clariidae, Order: Siluriformes) with an average weight of 172.6 g were procured from a local fish farm in Abak Local Government Area and were transported in well-aerated, open plastic container of fresh water to the Department of Animal and Environmental Biology Laboratory, University of Uyo. The fish were kept in transparent plastic tanks filled with dechlorinated tap water and made to acclimatize in laboratory conditions for two weeks. They were fed *ad libitum* with commercial feed (Coppens commercial feed, Coppens International Helmond, Netherlands) containing 40% crude protein. To maintain hygiene, the water was renewed every 24 h to prevent the accumulation of metabolic wastes and unconsumed food particles.

Experimental Procedure and preparation of stock solutions
After the period of acclimation, the fish were randomly distributed into six plastic aquaria (56 x 28 x 28cm) which were designated as 1 - 6. The control group of fish was kept in aquarium 1 while aquaria 2 - 6 contained the test group of fish reared in water contaminated with varying concentrations of chlorfenapyr. The concentrations were prepared following standard protocol as described in a previous study by Esenowo *et al.* (2021). Each aquarium was made to hold ten fishes in 10 L of dechlorinated water. The experiment lasted for 96 hrs. After completion of treatment, the test animals were removed from aquaria, washed with water, and sacrificed. Feeding of the fishes was stopped 24 hours before the start of the test (Sprague, 1972).

Blood collection, serum preparation and biochemical analysis
After sacrifice, blood was collected through the caudal vein, stored in plain sterile tubes and allowed to stand for 1 hr to clot. The clotted blood samples were centrifuged at 3000 rpm for 10 min to separate the serum. The serum was used for all biochemical assays. The enzymic activities of alanine

transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in the serum were measured using kits purchased from Randox Laboratories Limited, United Kingdom (UK). The ALT activity was measured by monitoring the concentration of pyruvate hydrazine formed with 2.4-dinitrophenylhydrazine at 546 nm.

AST activity was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2.4-dinitrophenylhydrazine at 546 nm.

ALP was determined by measuring the increase in absorbance due to increase in the formation p-nitrophenol reaction in the sample at 405 nm.

Serum total cholesterol was estimated using automatic serum chemistry auto analyser and kit (AUTOPAK supplied by Beyer Diagnostics India) by the enzymatic (Cholesterol Esterase, Cholesterol Oxidase and Peroxidase method of Allain *et al*. (1974). Results obtained were expressed as mgdl^{-1} of serum.

Serum Triglycerides estimation was carried out with the use of the automatic serum analyser and kit (AUTOPAK, Bayer Diagnostics India) by the enzymatic Lipoprotein lipase, Glycerol kinase, Glycerol-3-Phosphate Oxidase method of McGowan *et al*. (1983). Results are expressed as mgdl^{-1} of serum.

Serum high density lipoprotein-cholesterol (HDL-cholesterol) was estimated using the automatic serum analyser and kits (AUTOPAK, Bayer Diagnostics India) by the phosphotungstate method. Results obtained were expressed as mgdl^{-1}of serum.

Very Low-Density Lipoprotein-Cholesterol (VLVD-cholesterol) in serum was estimated using the Friedewald formula (Friedewald *et al*., 1972).

VLVD-Chol = Serum triglycerides $\div$ 5

The results obtained are expressed as mgdL^{-1} of serum.

The serum Low Density Lipoproteins cholesterol (LDL-cholesterol) was estimated mathematically as the difference between the serum totalcholesterol and the sum of the Very Low-Density Lipid cholesterol and the High-Density Lipid cholesterol according to the Friedwald formula (Friedewald *et al*., 1972).

LDL-Chol (mg/dL serum) = Total Chol $-$ (VLDL-Chol + HDL-Chol).

Histopathological Analysis
For this investigation, gills samples were immediately collected from sacrificed animals and rinsed with saline solution (0.9% NaCl) to remove blood mucus and debris and fixed in 10% formaldehyde. The tissues were dehydrated through a graded alcohol series. The dehydrated tissues were cleared in xylene and then embedded in paraffin wax. Tissue sections were cut at 5 µm using a rotary microtome and stained with hematoxylin and eosin. The prepared slides were analyzed under an Olympus optical microscope and

photographed at 40x and 100x magnifications.

Statistical analysis
Data obtained from the experiments were collated and subjected to ANOVA using MS Office Excel Package, version 10 and the differences among means tested at p<0.05. Results were expressed as means ± standard deviation using tables and charts.

Results
Biochemical analysis
The results of the effect of exposure to chlorfenapyr on the liver enzymes of *Clarias gariepinus* are presented in Fig.1. As shown on the chart, activities/levels of AST, ALT and ALP were elevated following exposure to different concentrations of the insecticide when compared with what was recorded for the control. However, this increase in activities was not completely dose dependent as highest values of the enzymes were recorded at 7 mg/l of the toxicant whereas exposure to higher concentrations of the toxicant witnessed a reduction in the activities/levels of the liver enzymes when values were compared to what was recorded for the fish treated with 7mg/l of the toxicant.

The results of lipid profile presented in Table 1 revealed significant (p<0.05) alterations in the lipid profile of the fish when groups exposed to the toxicant (test groups) were compared to those that were free of the toxicants (control group); and even comparison amongst the test groups revealed significant (p<0.05) alterations. Levels of total cholesterol in the fish from the test group was significantly (p<0.05) higher in the groups treated with the toxicant when compared to what was recorded for those in the control group. LDL-cholesterol showed a similar trend. HDL-cholesterol and triacylglycerides were significantly (p<0.05) reduced in the chlorfenapyr treated groups when compared to those in the control group. Comparison amongst the treated groups showed alterations which were dose dependent and were significant (p<0.05) when mean values were compared for all the parameters except for Triacyglycerides where mean values for groups 2 and 3 as well those of groups 4 and 5 showed no significant (p>0.05) differences.
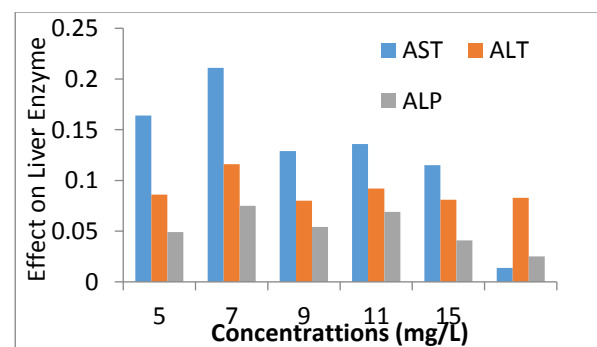

Figure 1: Effect of chlorfenapyr on Liver Enzymes of *Clarias gariepinus*

Table 1: Effect of chlorfenapyr insecticide on the serum lipid profile of *Clarias gariepinus* juveniles following 96hr exposure.

Experimental Groups	Total Cholesterol (Mg/dl)	HDL-Cholesterol (Mg/dl)	Triacylglycerides (Mg/dl)	LDL-Cholesterol (Mg/dl)
Group 1 (Control)	148.76 ± 0.18	101.30 ± 0.28	201.10 ± 7.35	48.14 ± 0.67
Group 2	153.38 ± 0.42^{a}	56.02 ± 1.29^{a}	174.61 ± 2.85^{a}	98.00 ± 0.36^{a}
Group 3	$172.30 \pm 1.59^{a,b}$	$37.56 \pm 0.89^{a,b}$	176.76 ± 1.67^{a}	$104.18 \pm 1.45^{a,b}$
Group 4	$192.22 \pm 2.04^{a,b,c}$	$30.34 \pm 0.63^{a,b,c}$	$161.72 \pm 1.19^{a,b,c}$	$110.26 \pm 0.87^{a,b,c}$
Group 5	$242.34 \pm 0.75^{a,b,c,d}$	$23.78 \pm 0.73^{a,b,c,d}$	$161.35 \pm 0.79^{a,b,c}$	$120.08 \pm 0.81^{a,b,c,d}$
Group 6	$261.10 \pm 0.44^{a,b,c,d,e}$	$20.74 \pm 1.07^{a,b,c,d,e}$	$150.10 \pm 0.96^{a,b,c,d,e}$	$124.90 \pm 0.88^{a,b,c,d,e}$
P-value range	0.000	0.000	0.000 - 1.000	0.000

Values are expressed as Mean $\pm$ SD; N = 5

a = p $\leq$ 0.05 (comparing values from other groups with that of the control)
b = p $\leq$ 0.05 (comparing values from other groups with that of group 2)
c = p $\leq$ 0.05 (comparing values from other groups with that of group 3)
d = p $\leq$ 0.05 (comparing values from other groups with that of group 4)
e = p $\leq$ 0.05 (comparing values from other groups with that of group 5)

Histopathology result
The histological sections of the gills of *C. gariepinus* for the control and treatment groups are shown in fig. 2. In this study, there were no morphological anomalies throughout the experimental period.
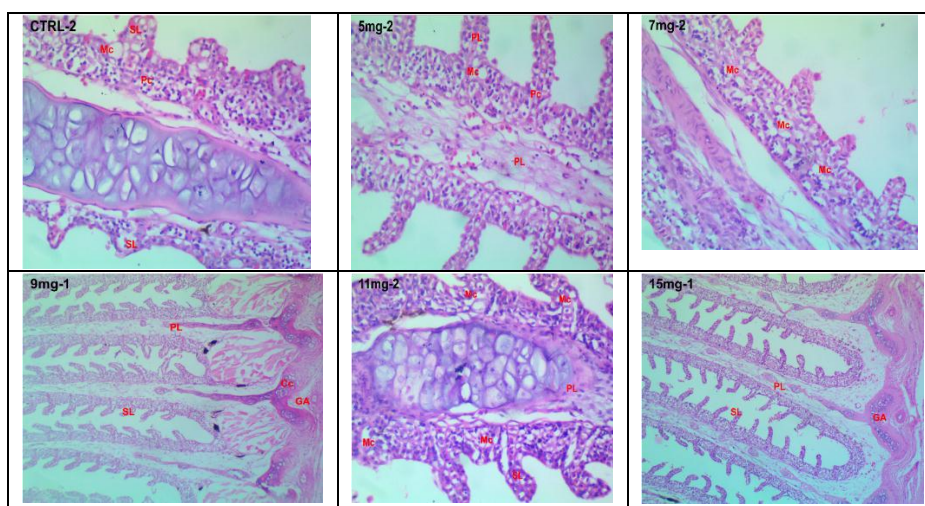

Fig. 2: Photomicrograph of the cross section of the gill of *C. gariepinus* showing: control (CTRL-2) group, H & E x100; 5 mg group, H & E x100; 7 mg group, H & E x100; 9 mg group, H & E x40; 11 mg group, H & E x100; 15 mg group, H & E x40. SL – Secondary lamellae; PL – Primary lamellae; GA – Gill arch cells; Pc – Pilaster (Pillar) cells; Mucous cells – Mc; Chloride cell (Cc)

Discussion
Acute exposure of animals to toxicants may inflict stresses on the mechanisms required for maintaining a healthy physiological state. Several reports have shown that these stresses may result in changes in biochemical, physiological or behavioural processes as well as alterations in molecular patterns of the DNA in exposed organisms (Malarvizhi *et al.*, 2012; Harabawy *et al.,* 2014; Ghelichpour *et al.*, 2017; Ekpo *et al.*, 2018; Hoseini and Yousefi, 2019). In view of this, there has been increasing interest in examining the physiological and biochemical stress response in aquatic vertebrates to protect aquatic life. The liver is susceptible to potential impairment by xenobiotics due to its function in accumulation, biotransformation, degradation, bioinactivation and excretion of contaminants as such, the evaluation of toxicity in animals is usually done through such path (Ross and Wilson, 2018). Alterations in the activities of the transaminases (AST and ALT) and ALP act as markers to illustrate compromised tissue integrity of the liver (Huculeci *et al.*, 2008; Fırat *et al.*, 2011; Banaee, 2013). Such increases could be proportional to compromised cellular integrity resulting from potential increase of cell membrane permeability (Granner and Rodwell, 2006), hepatocellular damage (Bhattacharya and Lun, 2005; Joseph

and Raj, 2011), cholestasis and liver damage (Della Torre *et al.*, 2000). ALP adopts different mode of action, preferentially influenced by exposure dose and duration, to elicit responses to contaminants (Brander *et al.*, 2012; Sun *et al.*, 2007). In our study, there was a significant increase in the activities of AST, ALT and ALP in comparison with the control. Additionally, there are reports indicating that exposures to environmental stressors such as pesticides have resulted in alteration of the lipid profile of fishes (Hammed *et al.*, 2013). Lipid profile are good indicators of an organism's likelihood of developing cardiovascular attack as a result of blockage of the blood vessels or hardening of the arteries (atherosclerosis). The lipid profile frequently assayed include total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides.

In this study, exposure to chlorfenapyr elicited stress actions that precipitated elevated levels/activities of liver enzymes and serum lipid profile in all treated groups when compared with what was recorded for the control. Previous researchers have documented reports of effect of chemicals on liver enzymes of *Clarias gariepinus*. Okonkwo *et al.* (2013) and Kumar *et al.* (2011) reported similar elevations in liver enzymes exposed to cypermethrin. These researchers had suggested that the elevated values in liver enzymes was due to the stepped-up transamination, where amino acids are used to generate intermediates for tricarboxylic acid cycle, in an attempt to handle the energy crisis during stress caused by the toxicants. Karami-mohajeri and Abdollahi (2011) also reported liver damage in *Clarias batrachus* exposed to phorate and carbaryl which was thought to be as a result of the production of lipolytic mitochondrial enzymes that dissolve cell membranes, lysosomal membranes and other hepatocellular organelles, thereby releasing liver enzymes into the blood. However, some of these exposures are long-term in order for observable changes in enzymic activities (Ayanda *et. al*, 2015). Also, the outcome of this present study showed an increase in total cholesterol and LDL-cholesterol level in the blood of test animals compared to their counterparts in the control group. On the other hand, Triacylglycerides and HDL-cholesterol recorded significant (p<0.05) reductions in all chlorfenapyr treated groups when compared with what was recorded for their counterparts in the control group. These alterations in lipid profile were observed to be dose dependent. This rise in cholesterol is an indication and probably suggests a general increase in lipid mobilization. Hypercholestrolemia observed may be due impairment of liver and inhibition of enzymes which convert cholesterol into bile acid (Murray, 1991). Reduced lipoprotein lipase activity plays a role in the increment of plasma lipid (Sharma and Agrawal, 2005). As recorded in Hammed *et al*. (2010), High density lipoproteins (HDLs) remove cholesterol from the walls of arteries, return it to the liver, and help the liver excrete it as bile while Low density lipoproteins (LDLs) transports cholesterol from the liver to the cells leaving plaque-forming cholesterol in the walls of the arteries, clogging the artery walls and setting the stage for heart disease. The higher amount of total cholesterol and

LDL-cholesterol is an indication of the chlorfenapyr's potential to impose cardiovascular health risk in *Clarias gariepinus*. This position is shared by several scholars (Filipiak, 1995; Hammed *et al.*, 2010; Hammed *et al.*, 2013).

In some cases, positive biochemical effects may not reflect positive histological damages. In our study, cross-sections of the gills were analysed to ascertain histological injury to sensitive organs. Gills were perfect for this analysis because they remain in close proximity to the external environment and respond rapidly to changes in the quality of water due to contaminants (Fernandes and Mazon, 2003; Camargo and Martinez, 2007). Our study showed that the histology of all test groups were not strongly altered compared to that of the control group. Although, the presence of mucous cells was well demonstrated at increased doses, there were no signs of degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae in our study. According to Islam *et al.* (2019), lower concentrations of toxicants have no remarkable alterations in vital organs of treated organisms. We can infer that chlorfenapyr concentrations used in our study may pose no danger to the histology of the gills. This is not in agreement with histopathological studies with positive results under the influence of different pollutants recorded by several scholars (Kakuta and Murachi, 1997; Mohamed, 2009; Banaee *et al.*, 2013; Somdare *et al.*, 2015). Some notable pathological gill responses when in contact with contaminants include oedema with epithelial lifting and desquamation, telangiectasia, hyperplasia and hypertrophy of the epithelial cells (Giari *et al.*, 2007). These responses were not observable in our study.

Conclusion
The results of the present investigation indicated that acute exposure of chlorfenapyr induced significant changes in the liver enzymes and lipid profile of *C. gariepinus*. This may infer that exposure to chlorfenapyr could pose deleterious consequences to the ecosystem and will definitely affect the survival of fish. On the contrary, the changes shown in the biochemical parameters in our study did not transmit to any observable aberrations in the histological parameters of the gills. Hence, further studies have to be carried out to evaluate the residual effects of this pesticide in other body tissues of fish in order to provide a better understanding of its physiological significance for a conclusive standpoint.

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