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Toxicity effect of atrazine on histology, haematology and biochemical indices of *Clarias gariepinus*

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Abstract

The impact of short term exposure to herbicides, atrazine on *Clarias gariepinus* juveniles was evaluated using standard methods that assessed fish histology, haematological and biochemical. Histology analysis of the fish organs examined revealed varying degrees of pathological alterations to the gill and liver in the study. The gill of fish showed alterations like thickening of lamella and sloughing off. The liver exhibited changes such as vacuolar degeneration of hepatocytes, hepatocyte swelling and necrosis amongst other were observed. The assessment of biochemical parameters revealed significant increase ($p<0.05$) in the activities of enzymes, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) in all experimental groups compared with control exposures. There was also a significant increase ($p<0.05$) in the value of blood glucose of the fishes. All experimental groups showed significant different ($p<0.05$) in the values across the treatments for PCV, HB, WBC, MCHC, MCV and MCH. The study therefore revealed that atrazine exposure had a toxic effect on *C. gariepinus*.

Keywords: Atrazine, *C. gariepinus*, toxicity, histology, haematology and biochemical indices

Introduction

Herbicide at high concentration are known to reduce the survival, growth and reproduction of fish and can produce many visible effects on them (Rahman *et al.* 2002) [1]. Toxicity testing of herbicides on animal has been used for a long time to detect the potential hazards posed by herbicides to man (Rahman *et al.* 2002) [1]. Bioassay technique has been the cornerstone of programmes on environmental health and herbicide safety (Oshode *et al.* 2008) [2]. Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and to find the relationship between toxicant concentration and effect on aquatic life organisms (Olaifa *et al.* 2003) [3]. The application of environment toxicology studies on aquatic organism especially fish is rapidly expanding for the evaluation of the effects of environmental contamination by noxious compounds (Ayoola, 2008) [4].

Atrazine is a widely-used herbicide in many countries for the control of broadleaved and grassy weeds in agricultural crops. The prolonged use of Atrazine and its persistence involves the risk of its retention in crops and soils. Moreover, these compounds may also pass from surface to ground waters (Mundiam *et al.* 2011) [5]. The chemicals through surface run off may reach unrestricted areas like ponds and rivers and alter the physio-chemical properties of water and consequently affect aquatic organisms (Kamble *et al.* 2000) [6].

Fishes are the most useful bio indicator of environmental quality and fish erythrocytes are a potential biomarker for in situ monitoring of water quality of an aquatic environment because of their close contact with water (De Flora *et al.* 1993) [7]. Toxicity evaluation of pollutants in fish is of great concern due to their potential adverse effects on human health after consumption. Thus toxicity studies are essential for determine sensitivity of animals to toxicants and also useful for evaluating the degree of damage to target organs and the consequent physiological, biochemical and behavioural disorders. To supplement risk assessment studies of this herbicide, it is important to obtain information on their toxicity and effects on some local species of fish.

Clarias gariepinus is a genus of clariid (order Siluriformes) of the family Clariidae, the air breathing catfish (Froese *et al.* 2011) [8]. It 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, tolerance to poor water quality

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parameters, omnivorous in its feeding habit, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available (Osman *et al.* 2006) ^[9]. For sustainable fish production in Nigeria, the ecotoxicology monitoring programmes need to incorporate proper management programmes for herbicide use and disposal in aquatic habitat. This study was therefore aimed to determine if atrazine is toxic to *C. gariepinus* juveniles.

Materials and Methods

A total of 300 apparently healthy Juveniles of *Clarias gariepinus* were purchased from Jacular Fish Farm Akure and transported to the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State Nigeria in March 2017. The length and weight of the fish were taken to indicate age. The *Clarias* species averaging 15.5±0.5cm standard length and body weight of 20±0.6g were used for the study. The fish were transported in a well aerated container containing water from the fish farm to the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State Nigeria. The fishes were acclimated for one weeks in concrete tanks of 1x2x1 (m³) dimension half filled with fresh water. During this period, the fishes were fed with commercial feed containing 35% crude protein twice per day at 4% body weight. The experiment lasted for 96 hours.

At the end of the experiment, five live fish per concentration were sampled for histological analysis. The gills and livers were examined after dissecting the fish. They were fixed in 10% formalin for two days to preserve the organs. Fixed organs were dehydrated in graded levels of alcohol (50%, 70%, 90%, 100%) after which they were immersed in 50/50 mixture of alcohol and xylene for three hours followed by cleaning in 100% xylene for three hours after which they were embedded in petri dishes with wax. The specimen was later mounted on wooden blocks and sectioned with the aid of a microtome to 7µm sections before staining in haematoxylin and eosin. The stained specimen was observed under a light microscope blood profile analyses were carried out at FUTA Health Centre. Blood samples were collected for haematology by inserting 5ml disposable heparinized syringes into caudal vein of the experimental fish. The blood was stored in 10ml Ethylene diamine tetra-acetic acid (EDTA) bottle to prevent coagulation. The following blood parameters were measured using standard laboratory procedures: Total erythrocyte count, Total leucocytes count, Packed cell volume (PCV), Haemoglobin determination, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC). Serum was obtained from heparinized blood samples, and was used for the determination of selected biochemical indices. Biochemical indices in blood serum included glucose and enzymes. Data obtained from haematological parameters were analysed by one factor analysis (ANOVA) where mean were significantly different, New Duncan Multiple Range Test were used for follow up analysis using MINITAB 14 software.

Results and Discussion

Histological changes observed in juveniles *C. gariepinus* exposed to different concentrations (0.0ml, 0.10ml, 0.15ml, 0.20ml, 0.25ml and 0.30ml) of atrazine is presented in plate 1-12. Juveniles *C. gariepinus* exposed to atrazine had

histological alteration in the gills and livers.

Gill

This study shows that sublethal concentrations of atrazine is toxic to *Clarias gariepinus*. This is in line with previous researcher (Ayoola, 2008) ^[4]. Who observed that herbicides are toxic to fish. Changes in fish gills lead to an overall reduction in the efficiency of gill filaments to aid in diffusion of oxygen across the gill lamellae which resulting in the development of a hypoxic condition within the fish (Elahee and Bhagwant, 2007) ^[10] and further confirm the deleterious effects some environmental pollutants may have effect on major fish organs.

The gills of fish exposed to sublethal concentrations of atrazine showed severely eroded in gill mucosa, marked proliferation of mucus secreting cells, severe erosion of the gill arch, thickening of secondary lamella and loss of epithelial cells. The degree of alterations in fish gill were less severe compared to the findings of Ayoola (2008) ^[4] who reported severe damage to the gills of *Clarias gariepinus* treated with acute concentrations of glyphosate. The overall toxicity of commercial formulations of atrazine partly depends on the toxicity of its associated compounds, especially surfactants (Peixoto, 2005) ^[11]. There is a slight probability that the percentage of the poly ethoxylated tallow amine (POEA) surfactants used in the herbicide formulations in this study differs from the report of Ayoola (2008) ^[4] which was more toxic.

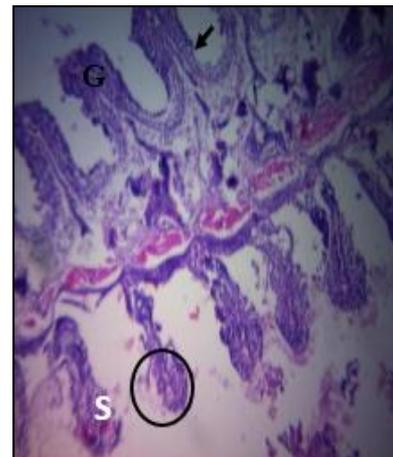


Plate 1: gill treated with 0.0ml/L of atrazine. No visible lesions observed

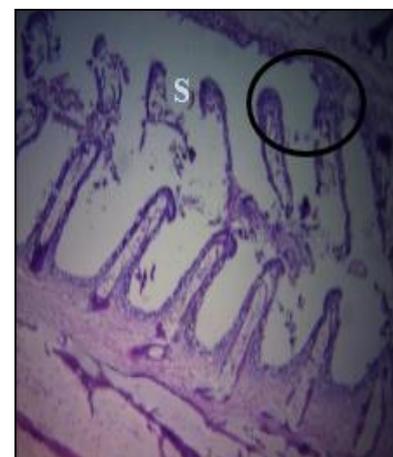


Plate 2: Gill treated with 0.10ml/L of atrazine. There was marked proliferation of mucous secreting cells

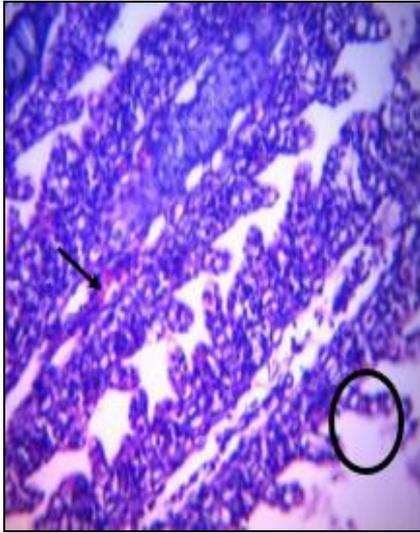


Plate 3: gill treated with 0.15ml/L of atrazine. There was severe vacuolation (arrow) of the gill architecture, alteration of the primary structure of the gill filament and hyperplasia (circle) of the gill epithelium

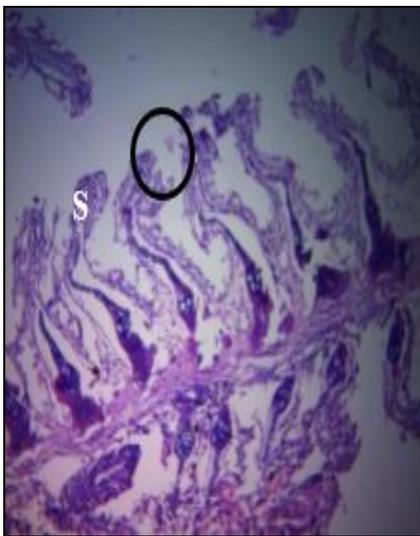


Plate 4: gill treated with 0.20ml/L of atrazine. There was a moderate to severe of the secondary lamella SL (circle), erosion of the gill arch, hypertrophy of primary lamella.

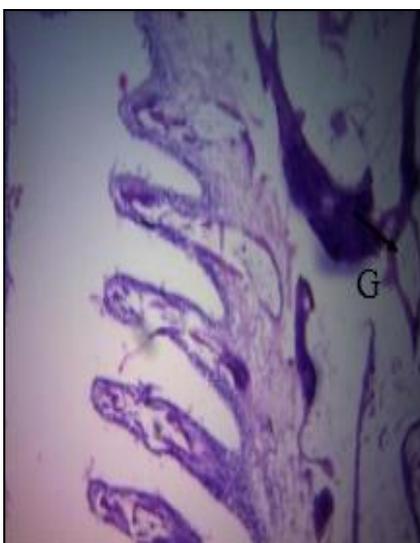


Plate 5: gill treated with 0.25ml/L of atrazine. There was a severe erosion of the arch (arrow), alteration of the primary structure of the gill filament and loss of epithelial cells.

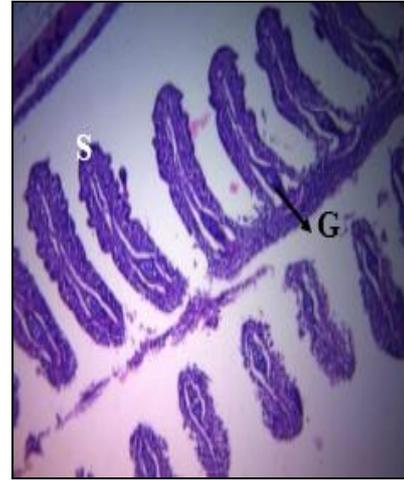


Plate 6: gill treated with 0.30ml/L of atrazine. There was a mild to severe erosion of the gill arch GA (arrow), thickening of secondary lamella, alteration of the primary structure of gill filament and loss of epithelial cell.

Liver

Histology changes in liver indicate that atrazine is hepatotoxic to *Clarias gariepinus*. No pathological alteration, vacuolations of the hepatocytes and hepatic cell was recorded in liver of control fish, indicating that observed damage can only have occurred as a result of exposure of fish to polluted water. Vacuolar degeneration of hepatocytes and disintegration of the sinusoids were the major alterations observed in fish liver in this study. Vacuoles in the cytoplasm of the hepatocytes contain lipids and glycogen, which is related to the normal metabolic function of the liver (Wilhelm -Filho *et al.* 2001) ^[12]. Thus, vacuolar degeneration will result in a depletion of the glycogen reserves in the hepatocytes (Wilhelm -Filho *et al.* 2001) ^[12]. Vacuolar degeneration will result in stress to fish because glycogen acts as a reserve of glucose to supply higher energetic demand occurring in such situation (Panepucci *et al.* 2001) ^[13]. Also, vital processes like detoxification and biotransformation are performed by the liver and 80% of these functions are carried out by the hepatocytes. This implies that if the integrity of the hepatocytes is compromised, the liver becomes inefficient to carry out these vital processes. Furthermore, (Pacheco and Santos 2002) ^[14] reported that increased vacuolisation of hepatocytes in fish exposed to contaminated water is a sign of degenerative process which itself suggests metabolic damage. Some other studies (Hued *et al.* 2012, Altinok *et al.* 2010 ^[15], Ayoola, 2008) ^[13, 4]. Have reported liver necrosis as a result of exposure of fish to acute concentrations of pollutants.

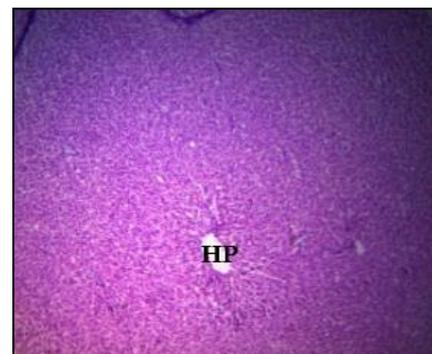


Plate 7: liver treated with 0.0ml/L of atrazine. There is normal vacuolations of the hepatocytes and hepatic cell, the hepatic portal vein was not congested and no pathological lesion observed in the liver cells

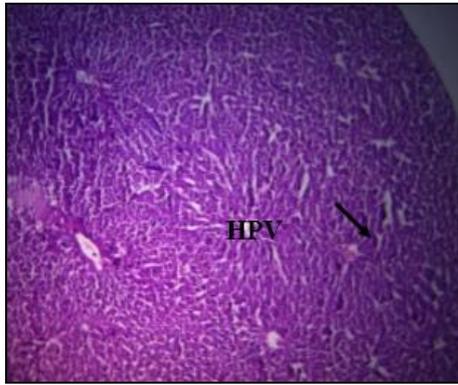


Plate 8: liver treated with 0.10ml/L of atrazine. There was vacuolation in the hepatocytes and mild erosion of the sinusoids

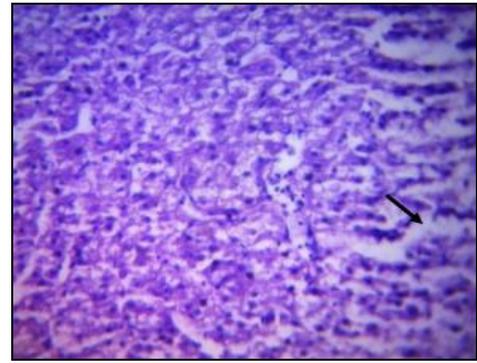


Plate 11: liver treated with 0.25ml/L of atrazine. There was a severe diffuse vacuolation of hepatocytes, erosion of sinusoids (arrow) and destruction of normal architecture of the liver. Reduced number and karyolysis of nucleus as a result of necrosis was observed.

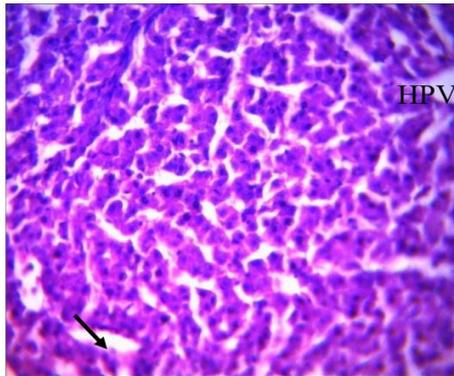


Plate 9: liver treated with 0.15ml/L of atrazine. There was mild to severe vacuolation of hepatocytes seen in the parenchyma of the liver cells, there as disintegration of the sinusoids.

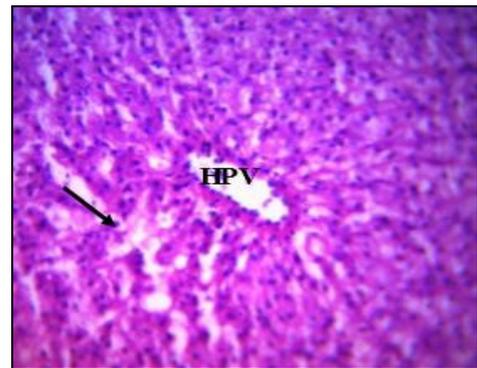


Plate 12: liver treated with 0.30ml/L of atrazine. There was destruction of the normal architecture of the liver cells, destruction of sinusoids (arrow) and centre of necrosis in the parenchyma of the liver cells

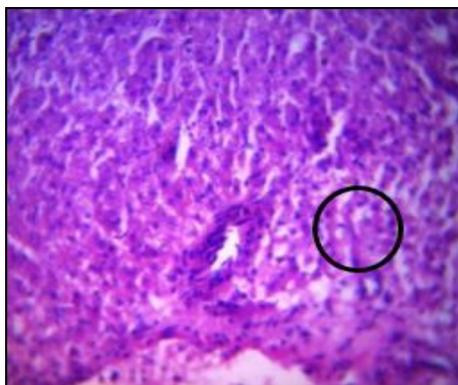


Plate 10: liver treated with 0.20ml/L of atrazine. There was a moderate central venous congestion, disintegration of the sinusoids with vacuolation (circle)

The results of the haematological parameters of *Clarias gariepinus* exposed to varying concentrations of atrazine is presented in Table 1. In this study, pack cell volume (PCV) of the fishes in control were significantly better and higher than the fishes exposed to atrazine. This could be due to response to stress imposed on them by atrazine. According to Oyewoye and Ogunkunle, (1988) [16] reduction in the concentration of PCV in blood usually suggests the presences of toxin factor, example is haemagglutin which has adverse effect on blood formation. Changes in the WBC profile indicate the response of fish to stress reaction. White Blood Cell (WBC) is involved in the regulation of immunological function and their alterations could be connected with immunotoxic potential of substances (Solomon *et al.* 2008) [17].

Table 1: Haematological parameters of *C. gariepinus* juveniles during exposure to atrazine

Parameter	0.0ml/L	0.10ml/L	0.15ml/L	0.20ml/L	0.25ml/L	0.30ml/L
PCV (%)	30.33±0.88 ^a	23.67±0.33 ^b	20.33±2.60 ^c	19.00±1.15 ^d	18.33±2.91 ^d	16.67±0.88 ^c
HB (g/dl)	10.47±0.39 ^a	7.93±0.16 ^b	7.03±0.89 ^c	6.86±0.40 ^c	6.07±0.98 ^d	5.20±0.11 ^e
WBC (mm ³)	8.73±0.27 ^a	7.60±1.27 ^b	7.57±1.01 ^b	6.23±0.81 ^c	6.03±0.33 ^c	5.17±0.38 ^d
RBC (mm ³)	3.40±0.11 ^a	2.73±0.14 ^b	2.53±0.37 ^b	2.50±0.37 ^b	1.97±0.15 ^c	1.93±0.14 ^c
MCHC (pg)	33.50±0.21 ^a	33.37±0.23 ^b	33.33±0.21 ^b	33.30±0.150 ^b	32.97±0.43 ^c	32.40±0.00 ^c
MCV (fl)	91.97±1.72 ^a	91.40±1.72 ^a	90.23±1.47 ^b	89.87±1.20 ^b	89.40±0.32 ^b	86.73±1.01 ^c
MCH (%)	30.77±0.79 ^a	30.74±1.15 ^a	30.33±0.83 ^a	30.07±0.87 ^a	30.10±0.98 ^a	28.16±0.79 ^b

Means with similar superscripts on the same row are not significantly ($P>0.05$) different. TRT 1: Treatment 1 with 0.1ml/L of atrazine, TRT 2: Treatment 2 with 0.15ml/L of atrazine, TRT 3: Treatment 3 with 0.2ml/L of atrazine, TRT 4: Treatment 4 with 0.25ml/L of atrazine, TRT 5: Treatment 5 with 0.3ml/L of atrazine.

The Red Blood Cell (RBC) profile revealed decreased in values with increasing concentration of atrazine. A significant decreased in RBC in the study can be interpreted as a compensatory response that reduces the oxygen carrying capacity to maintain gas exchange in the damaged gill lamellae. The result is in accordance with Ramesh *et al.*

(2009) [18], who assessed the effects of selected herbicides on haematology profile of fish blood. The values of MCHC, MCV and MVH were marginally decreased across the treatment exposed to atrazine. The blood glucose result revealed increased in values across the treatment (atrazine). However, there were significance differences in the treatment. A significant increase of glucose level in the blood stream as a conventional indicator of liver injury in fishes exposed to different atrazine concentrations. Similar results were found

by (Neskovic *et al.* 1993) [19], who assessed effects of subacute (14 days) exposure to atrazine at range of 1.5-6mg/L⁻¹ in common carp.

The results of the blood glucose of *garipepinus* exposed to varying concentrations of atrazine are presented in Figure 1 which shows an increase in glucose levels in all experimental group after 96 hours of atrazine. The result indicated significant increase ($p>0.05$) across the treatments.

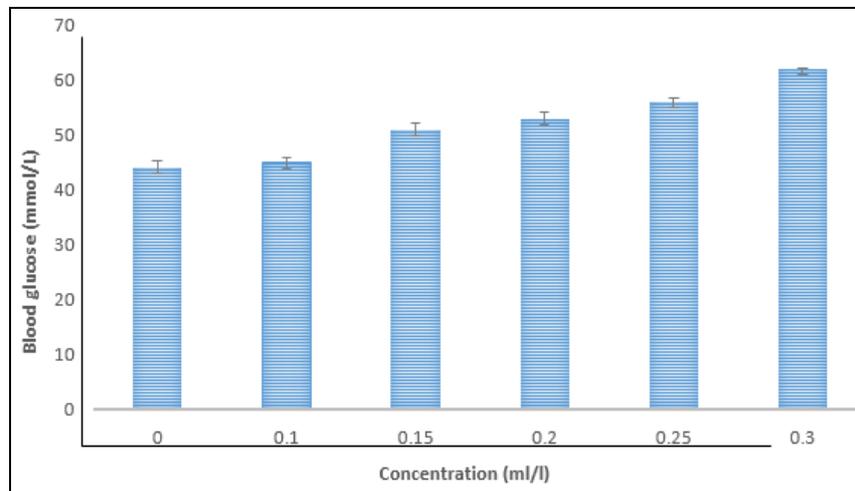


Fig 1: Blood glucose in *Clarias garipepinus* exposed to varying concentration of Atrazine.

The Blood glucose has been shown to be a sensitive indicator of environment stress for any chemical pollutant including herbicide according to Banae (2012) [20]. The increase in the values of glucose compared to the control indicated that *C. garipepinus* generated more glucose to produce the energy used in combating the stress induced on fish by atrazine. Increase in glucose level that was observed might have resulted from increase in glycogenesis and glycogenolysis as well as inhibition of glycogenolysis and glycogenesis during stress as reported by Iwama *et al.* (1999) [21]. As the respiratory metabolism is being depressed, stored intracellular glycogen is utilised under such condition; hyperglycaemic hormone is released for the degradation of glycogen and glucose thus leaked into the blood causing hyperglycaemia by Bhattacharya *et al.* (1975) [22]. In this study, sub lethal

exposure to atrazine at 0.1, 0.15, 0.20, 0.25 and 0.30ml/L resulted in significance increase in serum glucose concentration, demonstrating the response of exposed fish to stress. Similar observations were reported by Crestani *et al.* (2006) [23]. In silver catfish *Rhamdia quelen*, Velisek *et al.* (2009) [24]. In common carp, and Adedeji (2010) [24]. In catfish *C. garipepinus*.

The results of the enzymes activities in fish exposed to various concentrations of atrazine are presented in Table 2. The results showed that the aspartate aminotransferase (AST) increased at a very high rate at each successive increasing level of the atrazine. However, there was no significant ($P>0.05$) differences in the values recorded across the treatments.

Table 2: Enzymes Response of *C. garipepinus* during exposure to atrazine

Parameters	0.0ml/L	0.10ml/L	0.15ml/L	0.20ml/L	0.25ml/L	0.30ml/L
AST	8.63±0.25 ^f	17.50±0.51 ^e	23.73±0.61 ^d	34.12±0.61 ^c	49.78±0.70 ^b	58.59±0.50 ^a
LDH	7.66±0.12 ^f	8.46±0.22 ^e	9.38±0.16 ^d	10.60±0.09 ^c	11.61±0.24 ^b	14.54±0.15 ^a
ALT	16.90±0.96 ^e	20.57±0.16 ^d	25.46±0.68 ^c	27.67±0.91 ^c	31.16±0.70 ^b	34.98±0.85 ^a

Means with similar superscripts on the same row are not significantly ($P>0.05$) different.

Physiological stress biomarkers can be used as a criterion when defining effect of atrazine on aquatic organism, as it serves as indicator for toxicity effect of pollutant on fish (Conn *et al.* 2009) [25]. In the present study, blood biomarkers were analysed for AST, LDH and ALT. The result inferred that, atrazine administration led to increase levels of blood markers (AST, LDH, and ALT). The values recorded for all the enzymes increased across the treatment with significant ($p<0.05$) differences across the treatment. The significantly higher AST, LDH, and ALT activities in the fish exposed to increasing atrazine across the treatment when compared with control groups were as a result of leakage of aminotransferase

(ALT) enzymes from injured liver cells. These results were similar to that of Konstantinova and Russanov, (1999) [26] who studied paraquat induced oxidative stress in rat liver.

Conclusion

Based on the data and evidence recorded in this study, atrazine is found to be toxic and posed stress to *Clarias garipepinus* and the effect increased with increase concentration of atrazine. Histology observation further revealed that sublethal concentrations of atrazine damage the gills and livers of fish. The results of the biochemical and haematological parameters assayed showed that *C. garipepinus* was seriously affected by atrazine.

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