EXPOSURE TO ATRAZINE ALTERED POSTEMBRYONIC ORGANS DEVELOPMENT, FUNCTIONS AND GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS CATFISH JUVENILES*

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ABSTRACT

Atrazine is a selective pre- and post-emergence herbicide for the control of weeds. Decades after being banned, atrazine remains the most abundant pesticide in water bodies. This study evaluated the toxic effects of atrazine on the post-embryonic development of African catfish (Clarias gariepinus). Catfish juveniles of 0.89 ± 0.06 cm and with an average weight of 0.01 ± 0.005 g were exposed to five different concentrations (0, 0.03, 0.3, 3.0 and 30 μgL^{-1}) of atrazine in three replicates. The catfish juveniles mortality were significantly increased with increasing atrazine concentrations ($p<0.05$). Probit analysis showed 48 hours LC_{50} of atrazine at 0.68 μgL^{-1} with 100 % mortality at 30 μgL^{-1}. Significant reduction ($p<0.05$) was observed in the specific growth rate (SGR) and the relative growth rate (RGR) with increasing concentration of Atrazine treatment. Histological assessment revealed disintegration of the nervous tissues, vacuolization of the epithelium of the anterior intestine, loss of gill cytoarchitecture and distortions of the intestine in all atrazine-treatment groups. Our results show that environmentally realistic concentrations (0.30 – 30.00 μgL^{-1}) of atrazine in the aquatic environment may adversely affect the post-embryonic development and survival of African catfish.

Keywords: Atrazine herbicide, Post-embryonic development, *Clarias gariepinus,* Acute toxicity

INTRODUCTION

Increased industrialization and agricultural production in Nigeria in recent years has intensified pollution of the environment with the introduction of anthropogenic compounds that are foreign to living systems. The quest for food security has raised a lot of questions on how to feed the growing population which is in excess of 200 million inhabitants. This has become a topical issue of discourse in government circle since the introduction of the Millennium Development Goals (MDGs) at the beginning of the twenty-first century (Onoja and Adione, 2020).

The need to produce a greater quantity and quality of food to adequately feed the teeming population of the country has left the government with no choice but to promote intensified agriculture beyond subsistence scale. Agricultural mechanization however, cannot be separated from the use of pesticides and other agricultural chemicals to promote and improve the quantity and quality of farm produce. This has made pesticides an indispensable tool in large scale agricultural production in Nigeria. These

pesticides are applied with the sole aim of controlling weeds but are used indiscriminately and they tend to persist in the environment and end up in the aquatic environment where they affect non-target organisms like fish. They may lead to fish kill, affect fish behaviour, feeding, growth and ultimately reduce fish productivity (Gill and Garg, 2014). Bioaccumulation of these chemicals in the fish tissues may occur overtime and become hazard to the long-term survival of fishes by disrupting the ecological interactions between organisms and loss of biodiversity (Morel *et al.*, 1998; Rajeshkumar and Li, 2018). Long-term exposure of pesticides induces physiological disorder, behavioural changes, histopathological damages, haematological alterations, biochemical changes, immune-suppression, hormone disruption, reduced intelligence, reproductive aberrations and cancer in fishes (Bunton, 1996; Mishra *et al.*, 2006; 2008; Madhuri *et al.*, 2012; Ullah *et al.,* 2014; Pandey *et al.,* 2014; Ullah and Zorriehzahra, 2015). Fishes serve as important bio-indicators for aquatic contamination. Recent studies indicated that fishes are rapidly becoming scarce owing the growing use of chemical pesticides in fields. Since fishes are important sources of proteins and lipids, health of fishes is very essential for human beings (Srivastava and Singh, 2013).

The herbicide atrazine, an endocrine disrupting chemical (EDC), frequently contaminates potable water supplies and aquatic ecosystems. Regarded as moderately toxic to aquatic species, atrazine is mobile in the environment and is among the most detected pesticides in streams, rivers, ponds, reservoirs and ground waters (Battaglin *et al.*, 2003; 2009). The prolonged use of atrazine and its persistence involves the risk of its retention in crops and soils. Likewise, these compounds may also pass from surface to ground waters (Mudiam *et al.,* 2012). Atrazine is an endocrine disruptor (Birnbaum and Fenton, 2003), meaning that it can interfere with the balance of hormones in an organism.

The acute toxicity values for freshwater fish ranged from a 96-hour LC_{50} of 4300 µg/L for the guppy (*Poecilia reticulata* Peters, 1859, Cyprinodontiformes: Poeciliidae) to a 96-hour LC_{50} of >100,000 µg/L for the carp (*Carassius carassius* Linnaeus, 1758, Cypriniformes: Cyprinidae) (Giddings *et al.*, 2005). Chronic non-observable-effect concentration (NOEC) values for fish ranged from 65 µg/L for brook trout (*Salvelinus fontinalis* Mitchill, 1814, Salmoniformes: Salmonidae) to 4300 µg/L for channel catfish (*Ictalurus punctatus* Rafinesque-Schmaltz, 1818, Siluriformes: Ictaluridae) (Giddings *et al*., 2005). Hostovsky *et al.* (2014) assessed the overall effects of triazine herbicides on the physiology of fish. The study indicated that acute exposure of fish to triazines affects reproductive development in fish, while long term exposure to low concentrations did not affect fish behaviour. Most studies have addressed either catfish juvenile or adult exposures, with very few investigations regarding a developmental origin hypothesis in well studied species such as zebrafish (*Danio rerio* Hamilton, 1822, Cypriniformes: Cyprinidae) (Hedayatirada *et al.,* 2020). Several studies on atrazine have been reported in Nigeria. Nwani *et al.* (2010) reported the toxicity and effects of a commercial formulation of atrazine (Rasayanzine) on lipid peroxidation and the antioxidant enzyme system of *Channa punctatus* Bloch, 1793 (Anabantiformes: Channidae). George *et al.* (2017) studied the effects of atrazine and metalachlor on the haematological parameters in *Clarias gariepinus* Burchell, 1822 (Siluriformes: Clariidae) juveniles, while Olatoye *et al.* (2021) investigated the presence of atrazine residues in fish feed and catfish (*C. gariepinus*) fillets from commercial aquaculture farms in Southwestern Nigeria, and the effects of atrazine on the endocrinology and histoarchitecture of the testes of *C. gariepinus* juveniles was reported recently by Opute *et al.* (2021).

However, there is data paucity on the effects of atrazine on organs development of

indigenous Nigerian catfish species such as *C. gariepinus.* This study investigates the effects of atrazine on the post-embryonic development and histoarchitecture of the cephalic region and intestinal tissues of *C. gariepinus.*

MATERIALS AND METHODS

Experimental Protocol: The post-embryonic toxicity test was conducted using a modified method of the short term toxicity test on fish Sac-Fry Stages – OECD guideline 212 (OECD, 1998). *C. gariepinus* was used as a suitable model organism (Erhunmwunse *et al*., 2021). *C. gariepinus* juveniles of about 5 days old were obtained from Faculty of Agriculture Fish Farm in Benin City, Edo State, Nigeria, and transported in plastic containers to the Animal Unit of the Animal and Environmental Biology Department, University of Benin, Benin. 450 individual catfish juveniles were randomly selected and weighed (average total length of 0.89 ± 0.06 cm and an average weight of 0.01 ± 0.01 g) into five circular holding tanks of 85 litres with 40 litres of de-chlorinated water containing 0.00, 0.03, 0.3, 3, 30 μgL^{-1} atrazine (Sigma-Aldrich, Czech Republic; chemical purity ≥ 99 %) for 72 hours and were allowed to recover in toxicant free medium for another 25 days (Halappa and David, 2009).

Water quality properties (temperature, pH, total dissolved solids, electrical conductivity and dissolved oxygen (Table 1) of the test solution and the control were assayed (APHA, 2005) to ensure they are within suitable range for the growth of the fish.

The behavioural response of the exposed fishes was observed every 6 hours from the point of exposure until the end of the exposure regime. Swimming behaviour, balance, colouration, and vigour were all monitored throughout the exposure. These behavioural characteristics were obtained for three fishes in each tank and the mean was recorded.

Thirty catfish juveniles were used for each group; the test was performed in triplicate. The control group was exposed only to atrazine-free dechlorinated water and was done for each substance and their mixture. Test solutions in all groups were replaced daily by gently draining each chamber and adding new solution slowly to avoid disturbing of the embryos. The temperature during the test was 26.00 ± 1.00 °C. The experiment was conducted under natural day light and darkness.

All experimental tanks including the control tank was observed daily for mortality, this was done daily till zero mortality was recorded. Catfish juveniles were considered dead if they fail to respond to nudging. Behavioural responses were monitored throughout the period of the experiment.

Growth Rate: Growth rate was calculated using the formula: Growth rate = Final weight – Initial weight / Time (days) x 100.

Specific Growth Rate (SGR): The SGR was calculated as described by the formula reported by Bwala and Omoregie (2009) thus: SGR = In Final weight – In Initial weight / Time (days) x 100.

Condition Factor: The condition factor was measured using the formula: Condition factor = Total weight of fish / $(Length)^3$ x 100.

Length-Weight Relationships: The determination of the Length-weight relationships was worked out in accordance to cube law projected by Le Cren (1951). The total length (cm) and body weight (g) was transformed to logarithms and the relationship was represented by linear regression analysis and scatter chart. The equation for estimating the relationship was: $W = aL^b$, where 'a' is a constant and 'b' is a regression coefficient, then after conversion of the length and weight to logarithms, it was applied thus: log W = log a + b log L, estimate by the least square regression method.

Histology: Histology was done at the Histopathological Laboratory of the University of Benin Teaching Hospital (UBTH), Edo State, Nigeria, using an Automated Tissue Processor (ATP). The specimens were placed in cassette and put in a basket of the ATP. In the automated tissue processor, the specimens were passed through graded concentrations of alcohol 50, 70, 90, 96 % and absolute alcohol (100 %) for the purpose of dehydration. They were cleared in two jars of xylene and then impregnated with wax. The specimens were embedded in paraffin and sections of 5 microns thick were obtained using a microtome. Ribbons were floated in water bath and mounted on clear albumenized slides and dried in an oven at 60°C for 15 – 20 minutes. The ribbons were dewaxed using xylene and hydrated using decreasing concentrations of alcohol (100, 96, 90, 70, 50 %) and water. The slides were then stained with hematoxylin and counterstained with eosin.

Data Analysis: Data on the physiochemical properties and growth parameters were analyzed for any significant difference using Analysis of Variance (ANOVA). The differences between means were partitioned with Duncan's Multiple Range Test. All analyses were done using Statistical Package for Social Sciences (SPSS) version 21.

RESULTS

Physiochemical Properties of Water: The temperature ranged between 26.0 – 26.80 ^{o}C (26.73 ± 0.13 ^{o}C), pH value ranged from 7.40 – 8.00 (7.65 ± 0.06), dissolved oxygen values obtained ranged from 4.00 – 5.90 mg/L (4.52 ± 0.19 mg/L), while electronic conductivity values ranged from 50.00 – 80.0 μScm^{-1} (64.4 ± 3.57 μScm^{-1}) (Table 1). These values recorded for the physicochemical parameters of the test water were within the suitable range necessary for the culture, survival and standard growth of *C. gariepinus.*

Behavioural Performance: Abnormal behavioural changes were observed in the treatment tanks characterized by erratic/agitated swimming, loss of equilibrium, frequent surfacing and hanging on water surface and discolouration (Table 2). The discolouration was observed in the form of pale colouration which persisted after the period of exposure (48 hours), although the intensity of paleness reduced in the lower concentrations with dark spots observed on the body of the exposed catfish juveniles.

Survival: Mortality of the catfish juveniles was observed in all treatment groups and the percent survival is presented in Figure 1. The rate of mortality was highest in catfish juveniles treated with 30 µg/L concentration of atrazine, while least mortality was recorded in catfish juveniles exposed to 0.03 µg/L of atrazine. It was observed that at 24 hours of exposure, only five catfish juveniles were alive in the 30 µg/L concentrations, and at 48 hours of exposure, all catfish juveniles in the group were confirmed dead. The rate of mortality of catfish juveniles was affected by the level of toxicity, thus, mortality increased with increase in atrazine concentration.

Growth Performance: The average total length (TL) and average total body weight (TBW) were uniform at the beginning of the research, but divergence was observed during the period of exposure with significant difference ($p<0.05$) between some of the groups (Table 3). The catfish juveniles were found to vary within a range of 0.80 – 3.70 cm (TL) and 0.10 – 0.40 g (TBW) in the control group. There was significant retardation in the growth as decrease in the mean total length and weight of the catfish juveniles were observed with increase in concentration of atrazine. Significant decline was seen in the specific growth rate (SGR) and the relative growth rate (RGR) with increasing concentration of Atrazine treatment.

Table 1: Physiochemical properties of test water

Parameter	Control	0.03 µgL^{-1}	0.30 µgL^{-1}	3.00 µgL^{-1}	30.00 µgL^{-1}
Temperature (^{0}C)	27.37 ± 0.39	27.39 ± 0.39	27.41 ± 0.37	27.38 ± 0.35	27.46 ± 0.35
pH	7.02 ± 0.09	7.00 ± 0.08	7.00 ± 0.11	7.03 ± 0.12	7.04 ± 0.13
Conductivity (µS/cm)	102.2 ± 0.92	102.3 ± 0.95	102.0 ± 0.95	102.2 ± 0.92	101.1 ± 0.88
Turbidity (mg/l)	0.23 ± 0.02	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.02	0.23 ± 0.02
Alkalinity (mg/l)	17.43 ± 0.43	17.55 ± 0.36	17.53 ± 0.43	17.46 ± 0.49	17.50 ± 0.44
Hardness (mg/l))	32.52 ± 0.37	32.57 ± 0.40	32.48 ± 0.33	32.46 ± 0.33	32.50 ± 0.34
DO (mg/l)	8.11 ± 0.12	8.13 ± 0.13	8.12 ± 0.13	8.14 ± 0.10	8.12 ± 0.14

Table 2: Behavioural observations of *Clarias gariepinus* juveniles during 28 days exposure to varying concentrations of atrazine

Behaviour	Control	0.03 µgL^{-1}	0.3 µgL^{-1}	3.0 µgL^{-1}	30.0 µgL^{-1}
Erratic swimming	-	-	-	+	++
Loss of balance	-	+	+	++	+++
Discolouration	-	+	+	++	+++
Lethargy	-	+	+	++	+++
Hanging on surface of water	-	+	++	++	+++

(+) low response, (++) moderate response, (+++) high response, (-) no response

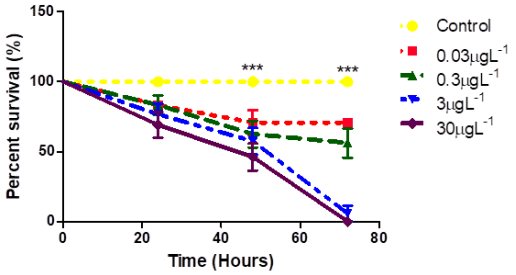

Figure 1: Kaplan–Meier survival curve analysis of catfish juvenile fish exposed to atrazine (n = 30 per group) (log-rank test, $p < 0.001$). (Chi-squared test, mean ± SE *** $p < 0.0001$)

Table 3: Summary of growth performance of *Clarias gariepinus* catfish juveniles exposed to varying concentrations of atrazine

Growth parameters	Control	0.03 µgL^{-1}	0.3 µgL^{-1}	3.0 µgL^{-1}	30.0 µgL^{-1}
IBW (g)	0.06 ± 0.01^c (0.05 – 0.07)	0.03 ± 0.01^b (0.02 – 0.04)	0.03 ± 0.01^b (0.02 – 0.04)	0.02 ± 0.01^a (0.01 – 0.03)	0.00 ± 0.00 (0.00 – 0.00)
FBW (g)	0.10 ± 0.02^c (0.07 – 0.12)	0.05 ± 0.01^4 (0.03 – 0.06)	0.04 ± 0.02^a (0.02 – 0.07)	0.03 ± 0.01^a (0.02 – 0.04)	0.00 ± 0.00 (0.00 – 0.00)
ITL (cm)	1.58 ± 0.15^d (1.30 – 1.80)	1.25 ± 0.14^b (1.00 – 1.50)	1.28 ± 0.15^c (1.10 – 1.60)	1.15 ± 0.14^a (0.90 – 1.40)	0.00 ± 0.00 (0.00 – 0.00)
FTL (cm)	1.87 ± 0.21^c (1.60 – 2.30)	1.60 ± 0.17^b (1.30 – 1.90)	1.34 ± 0.24^a (1.10 – 1.90)	1.36 ± 0.16^a (1.10 – 1.60)	0.00 ± 0.00 (0.00 – 0.00)
GR (%)	0.50 ± 0.13^c (0.29 – 0.57)	0.23 ± 0.07^b (0.14 – 0.29)	0.10 ± 0.09^a (0.00 – 0.29)	0.12 ± 0.05^a (0.00 – 1.43)	0.00 ± 0.00 (0.00 – 0.00)
SGR (%)	6.46 ± 0.98^c (4.80 – 7.70)	5.90 ± 1.11^b (4.11 – 7.30)	3.1 ± 2.81^a (0.00 – 5.79)	5.88 ± 2.96^b (0.00 – 9.90)	0.00 ± 0.00 (0.00 – 0.00)
K	1.48 ± 0.24^b (0.99 – 1.71)	1.14 ± 0.14^a (0.87 – 1.36)	1.16 ± 0.25^a (0.58 – 1.50)	1.1 ± 0.22^a (0.89 – 1.50)	0.00 ± 0.00 (0.00 – 0.00)

Figure in parenthesis = range, IBW - Initial Body Weight, FBW– Final Body Weight , ITL– Initial Total Length, FTL– Final Total Length, GR– Growth Rate, K– Condition Factor

Table 4: Final length-weight relationship of *Clarias gariepinus* Catfish juveniles exposed to varying concentrations of atrazine for 4 weeks

Tank	FAW (g)	FSGR (%)	Logarithm Equation Log	'R'	'R^2'	'FAK'	'b'
Control	0.12 ± 0.06^e	11.18 ± 6.63^e	Log W=Log– 1.62 + 2.18 Log L	0.99	0.97	1.29	2.18
0.03 µgL^{-1}	0.08 ± 0.06^d	8.39 ± 7.15^d	Log W=Log– 1.74 + 2.29 Log L	0.97	0.93	1.38	2.29
0.30 µgL^{-1}	0.06 ± 0.05^c	7.56 ± 7.95^c	Log W=Log– 1.76 + 2.33 Log L	0.97	0.94	1.27	2.33
3.0 µgL^{-1}	0.02 ± 0.01^b	5.78 ± 3.64^b	Log W=Log– 1.76 + 2.33 Log L	0.97	0.94	1.23	2.33
30.0 µgL^{-1}	0.00 ± 0.00^a	0.00 ± 0.00^a	Log W=Log– 0.00 ± 0.00 Log L	0.00	0.00	0.00	0.00

FAW-Final average weight; FSGR-Final average specific growth rate; R-Correlation coefficient; R^2- Coefficient of determination; FAK-Final average condition factor; b-Slope

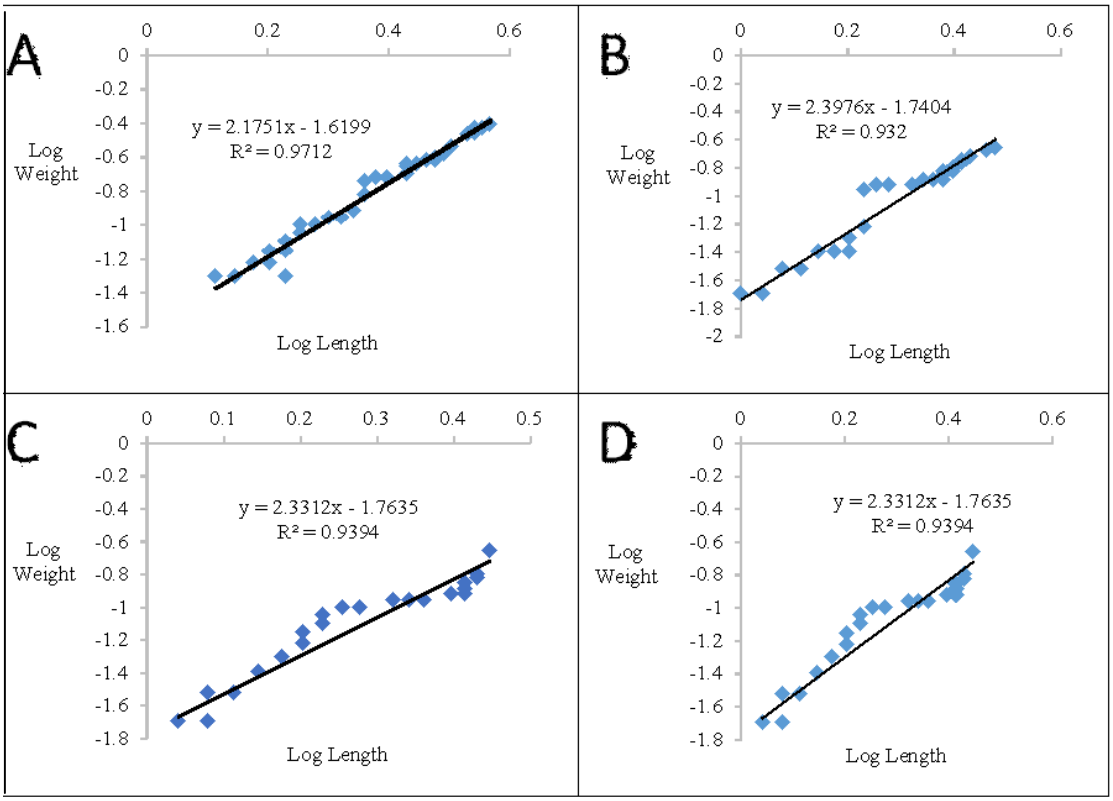

Figure 2: Regression between total length and body weight of *Clarias gariepinus* catfish juvenile for 4 weeks (A- Control cistern; B- AT_1 cistern; C- AT_2 cistern; D- AT_3 cistern)

Significant decrease ($p<0.05$) across all treatments was recorded. The specific growth rate of *C. gariepinus* juveniles exposed to atrazine remained lower than those of the control fish throughout the duration of the study. The length-weight relationship is summarized in Table 4. The control group as well as all the treatment groups showed negative allometric growth with b values of 2.18, 2.29, 2.33 and 2.33 respectively (Figure 2).

Histopathological Effects

Organs development: Significant alterations were observed in gills, muscle tissues and intestines which were not present in the control group (Figures 3 – 5). The gill of *C. gariepinus* juveniles exposed to atrazine showed loss of gill cytoarchitecture (Figure 4). These damages observed in the gill architecture may have been responsible for impairment of the respiratory and regulatory functions of the gills and hence

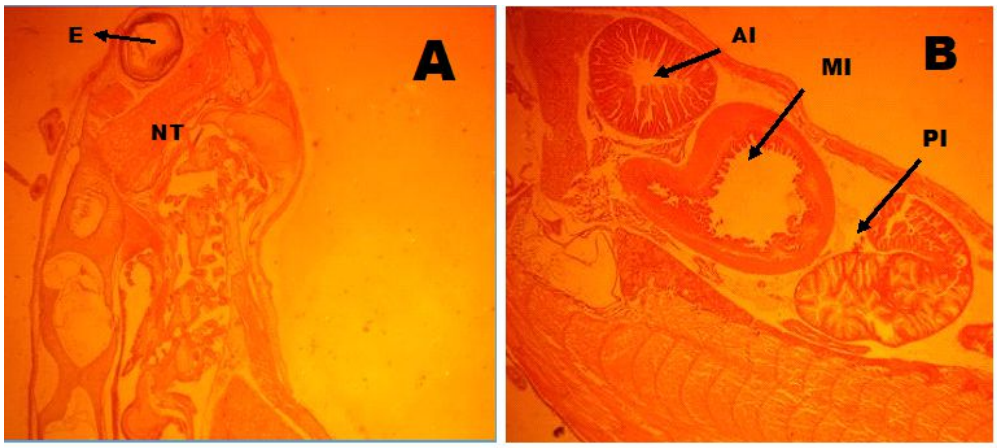

Figure 3: Normal histological structure of a section of the cephalic region of *Clarias gariepinus* juvenile in the control group: (E) eye; (NT) nerve tissues; AI: anterior intestine; MI: middle intestine; PI: posterior intestine. H&E (x100)

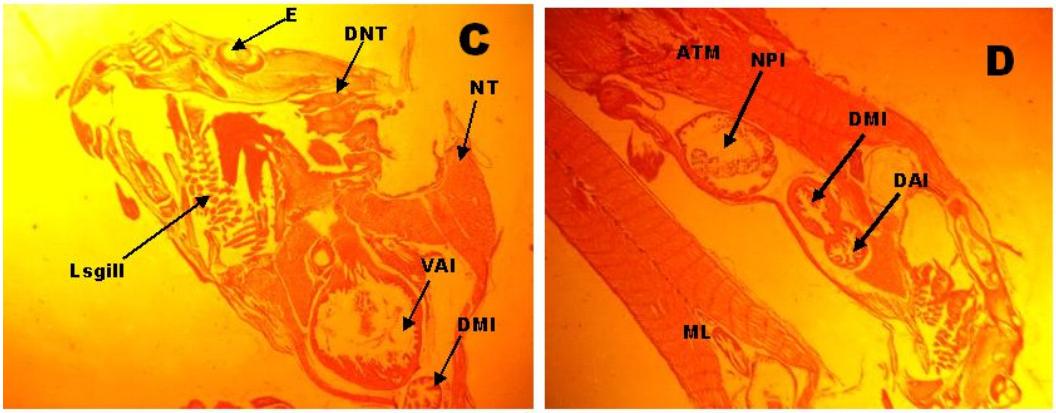

Figure 4: Representative section of the cephalic region of *Clarias gariepinus* in the treatment group (juvenile) 28 days post exposure to atrazine (AT_1) showing atrophic myocyte (ATM); disintegration of the nervous tissues (DNT); vacuolization of epithelium of the anterior intestine (VAI) & loss of gill cytoarchitecture (Lsgill); degeneration of the middle intestine (DMI); myocyte losses (ML); necrosis of the posterior intestine (NPI) and degeneration of the anterior intestine (DMI and DAI) (H & E, x 100)

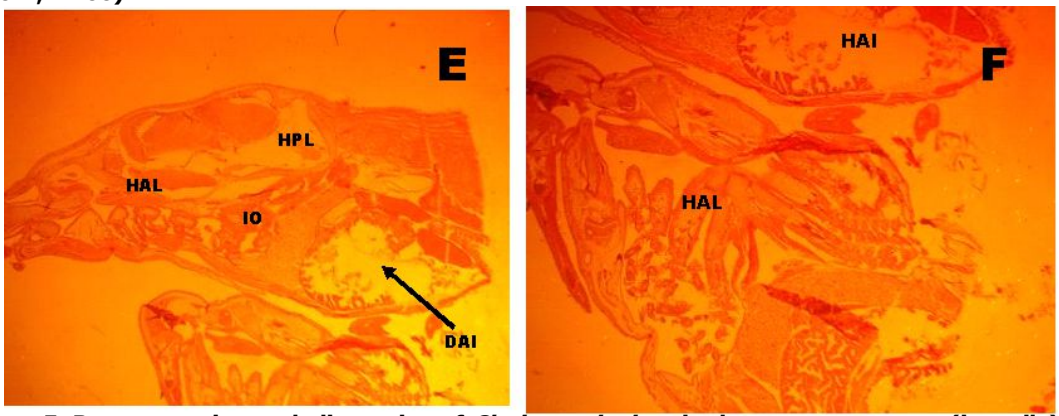

Figure 5: Representative cephalic section of *Clarias gariepinus* in the treatment group (juvenile) 28 days post exposure to atrazine (AT_2) showing histoarchitectural loss (HAL); histopathological lesions (HPL); intercellular oedema (IO); degeneration of the anterior intestine and hyperplasia of the anterior intestine (DAI) (H & E, x 100)

resulted in the death of fishes in higher concentrations. Alterations in the structure of muscle of catfish juveniles exposed to atrazine were manifested in atrophic myocytes (Figure 4). Degeneration of intestines was observed in treatment groups (Figures 4 and 5). Hyperplasia of anterior intestine was also observed in catfish juveniles exposed to atrazine (Figure 5).

DISCUSSION

The abnormal behavior of catfishes reported in this study indicated the toxic effect of atrazine on the central nervous system (CNS) and cardiovascular system which was in agreement with the findings of Ramesh *et al*. (2009) on the effects of atrazine on carp. The behavioural responses were also similar to the findings of Chattopadhyay *et al*. (2006), who reported erratic behavioral patterns of fish exposed to herbicides. Furthermore, the normally dark pigmented *C. gariepinus* was changed to light pigmentation. Ikele *et al*. (2011) reports the same for juveniles of *C. gariepinus* exposed to diethyl phthalate.

The rate of mortality was dose-dependent giving credence to the report by Obiezue *et al*. (2014), where there was a direct relationship between mortality in *C. gariepinus* and concentration of diethyl phthalate. Earlier, Waring and Moore (2004) report very high mortality rates for Atlantic salmon exposed to atrazine.

The gill of the catfish juveniles exposed to atrazine showed loss of gill cytoarchitecture. These damages observed in the gill architecture may have been responsible for impairment of the respiratory and regulatory functions of the gills and hence resulted in the death of fishes in higher concentrations. Erkmen *et al*. (2000) reported the lifting of epithelial layer from the gill lamellae, necrosis and degeneration of secondary lamellae, shortening of secondary lamellae and clup-shaped lamellae in the gills of *Lepistes reticulatus* exposed to cyphenothrin.

Alterations in the structure of the muscle of the catfish juveniles exposed to atrazine were manifested in atrophic myocytes.

This was similar to the report of Murali *et al*. (2018) where they discovered some alterations in muscle tissues such as necrosis, abnormalities in the muscle fibre, atrophic myocytes, myocyte losses and intercellular edemea in *Oreochromis mossambicus* Peters, 1852 (Cichliformes: Cichlidae) after aluminum nanoparticles exposure. Abbas and Ali (2007) had reported the destruction and vacuolation of the muscle cells in *Oreochromis* spp. exposed to chromium. Mohamed (2009) observed the degeneration of muscle bundles with aggregation of inflammatory cells between them and focal areas of necrosis. Anshu *et al*. (1995) studied the sub-lethal effect of quinolphos and padan on the muscle tissues of common carp - *Cyprinus carpio* Linnaeus**,** 1758 (Cypriniformes: Cyprinidae). Glycogen content was found to be depleted in the tissues as concentrations of toxicants increased.

Degeneration of intestines was observed in the toxicant exposed groups. The uptake of pollutants occurs mainly via the gills but may also occur via the intestinal epithelium (Mohamed, 2008). Toxic pollutants enter the digestive tract of fish via the food and water they consume, causing structural and functional deterioration of the intestine (Banerjee and Bhattacharya, 1995). Murali *et al*. (2018) reported degenerated intestines in *O. mossambicus* exposed to aluminum nanoparticles where intestinal tissues showed disturbed structural deformities such as swelling of goblet cells, occurrence of hyperplasia, vacuolation and necrosis. Hyperplasia of anterior intestine was also observed in catfish juveniles exposed to atrazine. This is considered as a protective mechanism from environmental irritant which works by decreasing the respiratory surface and increasing the toxicant–blood diffusion distance (Meissner and Diamandopoulos, 1977).

Conclusion: Forty-two hours atrazine (30 µgL-1) exposure caused 100 % mortality in the early life stage of African catfish *C. gariepinus.* Fish-specific organs were susceptible to the toxicity of Atrazine pesticide. Atrazine exposure altered the growth and the physiology of catfish.

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