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TOXICITY OF THE HERBICIDE 2, 4-D DIMETHYL AMINE SALT ON BEHAVIORAL, HISTOLOGICAL AND OXIDATIVE STRESS BIOMARKERS OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS

***[1]Hamisu, Y., [1]Auta, J. and *[2]Bawa, B. S.**

[1]Department of Biology Ahmadu Bello University, Zaria Kaduna Nigeria
[2]Department of Fisheries and Aquaculture Ahmadu Bello University, Zaria. Kaduna Nigeria

*Corresponding authors' email: aquablends@gmail.com Phone: +2348069675754

ABSTRACT

The aim of this paper was to assess the toxicological impacts of the Herbicide 2, 4-D Dimethyl amine salt on *Clarias gariepinus* fingerlings. The fishes were exposed to lethal concentration of 0.00, 1.34, 1.42, 1.48, 1.54 and 1.60ml/L for 96 hours as well as sub-lethal concentrations of 0.00, 0.069, 0.138 and 0.276ml/l of 2, 4-D Dimethyl amine salt for 8 weeks. The value of 96 hours LC_{50} was 1.385ml/L. Respiratory disturbance, loss of equilibrium, and sudden fish death were observed in 96hrs of exposed fish, and these varied greatly with increase in concentration of the toxicant, with a trend of significant (p<0.05) increase in mortality with increasing concentrations. As the concentration of 2, 4-D dimethyl amine salt increased the beats of the tail and opercular ventilation increased at 12 and 24 but decreased at 72 and 96 hours. The lethal exposure of *Clarias gariepinus* fingerlings to 2, 4-D dimethyl amine salt indicate significant changes in enzymes activity of liver for SOD and CAT. at 1.36, 1.42, 1.48, 1.54 and 1.6ml/L while sub-lethal show no significant changes. The changes for lethal and sub-lethal in gills were characterized by, inflammation of primary and secondary lamella, vacuolation, filament atropy. The liver showed, necrosis, adipocyte infiltration, and lymphocyte hyperplasia in fish exposed lethal and sub lethal concentrations of 2, 4-D Dimethyl amine salt.

Keywords: *2*, 4-D Dimethyl Amine Salt, Behaviour, Herbicide, Histology, Oxidative stress biomarkers, Toxicity

INTRODUCTION

Herbicides are agrochemicals that are widely used for the control of unwanted plants (weeds) in crops, fruit gardens, aquaculture ponds, and green spaces (Tudi *et al*., 2021). They are widely used in agriculture and represent about 50% of all agrochemicals used throughout the world (Dey and Saha, 2014). Herbicides even at low concentration can cause behavioral changes in fish species. Residues of herbicides and other toxicants have been found to accumulate in fish (Pereira *et al*. 2013) there is need for organism to adjust to external and internal stimuli in order to meet the challenge of surviving in a changing environment as a result of adaptations to environmental variables (Ramesh & Munshawi, 2009).

Significant alterations in histopathology of fish act as important biomarkers in toxicological studies particularly pesticides toxicity (Vali *et al.*, 2022). Therefore, histopathology is suitable to be used as biomarkers as they give an early response or measurable biological event due to exposure to pollutants (Liebel *et al*., 2013). Histopathology is often the easiest method of assessing both short- and long-term toxic effects for field assessment (Reddy, 2012). Histopathological analysis appears to be a very sensitive parameter, crucial in determining the cellular changes occurring in the target organs such as the gills, liver, kidney, brain, spleen, gonads and muscles (Gaber *et al*., 2014).

Antioxidants are important biosensor parameters because they bring additional information on the toxicity mechanisms of pollutants, on an organism. Antioxidant are defensive biomarkers that changes in the activity of key enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Chen *et al*., 2016). The antioxidant capacity of tissues is critical in combating free radicals to less harm compound and ensuring normal metabolic functions in fish (Banaee *et al*., 2015). Residues of herbicides and other toxicants have been found to accumulate and cause hazardous effects on fish health, consequently threatening human health (Pereira *et al*., 2013; Drishya *et al*. 2016). The aim and objectives of this research is to assess the toxicity of the Herbicide 2, 4 -D Dimethyl amine salt on behavioral, histological and oxidative stress biomarkers on *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Source of the fish

Total number of two hundred and fifty (250) fingerlings of African catfish *Clarias gariepinus* were obtained from the Navic Fish farm in Zaria and were brought in a well aerated jerrycan to the Fisheries Laboratory, Ahmadu Bello University Zaria. Prior to the experiment, the fish mean weight (4g ± 2.0) were kept for 2 weeks acclimatization period. Fish were fed twice in a day on commercial diets at a rate of 3% of their body weight.

Source of herbicide

The herbicide used in this study was 2, 4-D dimethylamine salt solution at 720g/L manufactured by Binhaj Economic Development Area, Weifang, Shandog China. Registered by Rainbow Agrosciences Co. Ltd CMD Complex, 183.

Stocking for acute bioassay

Five nominal concentrations were prepared geometrically (1.36, 1.42, 1.48, 1.54 and 1.6ml/L which is T_1, T_2, T_3, T_4 and T_5 respectively) with T_0=0.00 as control. Fish were exposed in batches of ten (in rectangular-shaped plastic containers of 20 L of test medium) to varying concentrations of 2, 4-D dimethylamine salt solution with two replicates for each test concentration along with the control sets. Water medium were replaced every 48 hours followed by an addition of required concentration of the test solution. The mortality at 12, 24, 48, 72 and 96 was observed and recorded to determine LC_{50}.

Behavioral studies
After exposure of the fish to various concentrations of the toxicant, observations were carried out on the behavioural and morphological responses of the fish at 12, 24, 48, 72 and 96 hours (Drummond *et al*., 1986). The opercula ventilation count and tail fin movement rates were determined, timing was carried out using stop watch at 12, 24, 48, 72 and 96 hours per minutes. Three fish were used for the counting per tank and the average count were taken.

Stocking for sub-lethal bioassay
Fraction of 1/5, 1/10, and 1/20 as T_1=0.069, T_2=0.138, and T_3=0.276ml/L respectively along with T_0= 0.00 as control, was taken for sub-lethal test from the results obtained for LC_{50} from the acute toxicity of 96 hours exposure. The experiment lasted for 8 weeks. Eight (8) plastic aquaria were used with two replicates per treatment. Ten groups of fish were exposed to 3 sub-lethal concentrations of 2, 4-D dimethyl amine salt. The fish were fed pelleted feeds containing 35% crude protein. Two fish per replicate were sacrificed biweekly to isolate gill and liver for pathological studies.

Tissue examination
The fish samples for all various concentrations both for acute and sub-lethal exposures were carefully collected and dissected to isolate some organs (gill and liver) and fix in formal saline solution (Alan *et al*., 1983). The harvested organs were conveyed to the Department of Anatomy, Ahmadu Bello University, Zaria. The tissues were washed in running water to remove traces of formalin, followed by dehydrating using successive percentages of graded alcohol (30, 50, 70, 90 and 100%). This was cleared through gradual increase in the concentration of chloro-alcohol (50, 75 and 100%) and then embedded in paraffin wax at 58ºC to 60ºC melting point. Sections was cut at 5µm thickness, this was done by using Bright rotary microtome. Harris's haematoxylin and acetic eosin was used as general stain by (Slaoui & Fiette, 2014). There after examining all the slides under the light microscope (NILCON 300) and photomicrographs were taken at X10, X20, or X40 magnification with a digital camera at its highest resolution.

Determination of oxidative stress
The following parameters were assayed by homogenating the organs (gill and liver) of fish in phosphate buffer of 7.0 pH by using homogenizer (IKA-WERKE, DI 18 BASIC). Samples were homogenized under ice bath, and the homogenates were centrifuged at 3000 rpm, for 5 min, at 4 °C. The supernatant was used for determination of SOD and CAT activities. The protein concentrations (mg mL−1) were determined by the Lowry *et al.* (1951) method using bovine serum albumin as standard. Enzymatic activity of SOD was measured through a commercial kit (Cayman Chemicals). The SOD assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase, read at 540 nm. One unit (U) of SOD activity was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The CAT activity was performed according to Beers and Sizer (1952). In this method, H_2O_2 was used as a substrate, and the decomposition of H_2O_2 by CAT was measured using UV-Vis spectrophotometer at 240 nm for 3 min the reaction occurred on 20 µL of sample supernatants, using 980 µL of H_2O_2 10 mM. Malondialdehyde (MDA) was determined according to the method of Nair and Turner (1984). MDA derived from lipid peroxidation was determined with thiobarbituric acid (TBA). 0.5 ml homogenate without filtration was taken and 4.5 ml of TBA reagent was added. The mixture was heated using boiling water bath for 20 min, centrifuged at 2500 r.p.m for 10 min. The absorbance of supernatant was recorded at wave length 525nm. MDA results were expressed as µ mol of MDA per g.

Data analysis
Minitab version 15 was used to determine LC_{50}. Data were subjected to one-way analysis of variance (ANOVA) to test for the significant differences between control treatments means using statistical package for social science (SPSS) software version 4.2. The Duncan's Multiple Range Test (DMRT) was used to separate the significance different means. Level of significant was set at P<0.05. /95% confident limit.

RESULTS AND DISCUSSION

Behavioral response of *Clarias gariepinus* to acute exposure of 2, 4-D dimethyl amine salt
Clarias gariepinus exhibited some behavioral changes while exposed to 2, 4-D dimethyl amine salt herbicide. After the fish were introduced into the tank containing 2, 4-d dimethyl amine salt herbicide at concentrations of 1.36, 1.42, 1.48, 1.54 and 1.6ml/L, they became restless and agitated, fishes came to the surface of water much more frequently. Treated fish exhibited increased mucous secretion and progressively became sluggish and lethargic. As the time of exposure increased fish stood in vertical position with their heads above the water surface eventually the fish were found dead.

Opercula ventilation beat and Tail fin beat
The *Clarias gariepinus* fingerlings exposed to 2, 4-D dimethyl amine salt, showed increased opercular ventilation and tail fin beat with increase in the concentration of the toxicant for 1.36, 1.42, 1.48, 1.54 and 1.6ml/L respectively. The result of opercular ventilation and tail fin beat means with different superscript across treatments are significant different (P<0.05). The opercular beat and tail fin beat of the exposed fish to the toxicant at 12 and 24 hours were higher compare to control group. As the concentration of 2, 4-d dimethyl amine salt increased the beats of the operculum and tail fin increases except at the 48th hours when the beats started to decrease. Further duration of exposure led to more decrease in the opercula ventilation and tail fin beat of the fish. By the 96th hour the opercula ventilation and tail fin beat rates of the exposed fish were significantly (p<0.05) lower than those of the control group. Figure 1 and 2.

Mortality rate of LC_{50} and threshold
The mortality rate of LC_{50} was determined by the antilog of 0.1413ml/L. The result of the acute toxicity showed that 2, 4-d dimethyl amine salt was toxic to African catfish fingerlings with LC_{50} value of 1.385ml/L as shown in Figure 3.

Oxidative stress biomarkers for acute exposure of gill to 2, 4 –D dimethyl amine salt
Superoxide dismutase (SOD) Catalase (CAT) activity and Manoldialdehyde (MDA) lipid peroxidation indicator, for acute exposure of 2, 4 –D dimethyl amine salt concentration on gill of *Clarias gariepinus*, the values for SOD are 10.3 which is highest followed 10.05, 9.85, 9.7, 9.45, 8.95u/mgpro and that of CAT are 1.9, 1.7, 1.65, 1.3, 1.1 and o.95u/mgpro respectively This indicates that as the concentration increased the activity increased i.e. the activity was dose dependent. By the observation the differences were not significant (p>0.05). There was no significant difference in gill MDA that is number lipid peroxidation discharge (p>0.05) through values ranged from 0.00 to 1.54ml/L but increase with increase in concentration As presented in Table

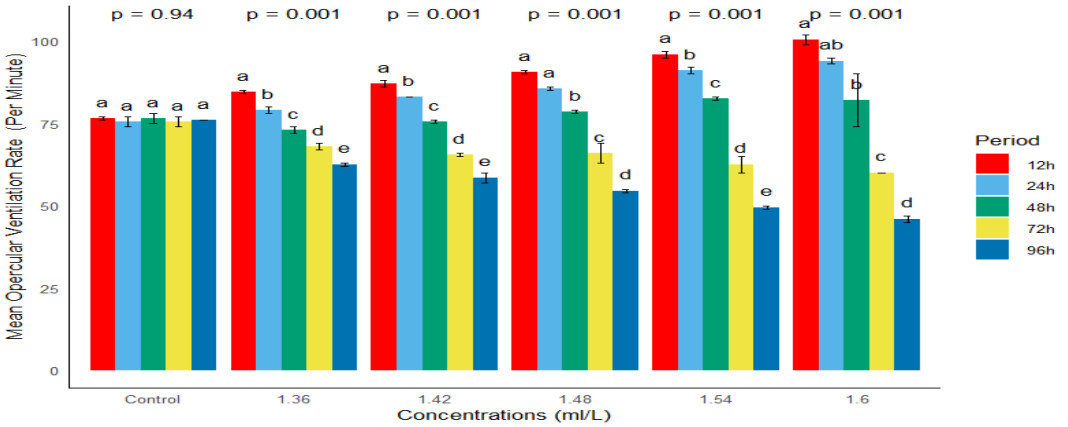

Figure 1: Opercular ventilation rate of *Clarias gariepinus* fingerlings exposed to acute concentration of 2, 4-d dimethyl amine salt

There was significant difference ($p \leq 0.05$) in liver SOD and CAT activity across treatments (Table 2) with 1.6ml/L having the highest activity followed by 1.54, 1.48, 1.42, 1.36 and 0.00 respectively. The value for SOD are 26.5 which is highest followed by 22.4, 21.3, 18.35, 17. 4 and 15.00u/mgpro while tha of CAT are 6.7 which the highest followed by 6.3, 4.6, 4.2, 4.1 and 3.10u/mgpro. This indicates that as the concentration increase the activity increases i.e. the activity is dose dependent there was no significant difference in liver MDA activity across treatments (Table 2), with 1.54ml/L having the highest activity followed by 1.60, 1.48, 1.42, 1.36 and 0.00, respectively.

Oxidative stress biomarkers to sub-acute exposure 2, 4 – D dimethyl amine salt

Gill

Catalase and Superoxide dismutase activity, quantity of manoldialdehyde by product following eight weeks exposure to sub-lethal concentrations of 2, 4 – D dimethyl amine salt for gills of African catfish fingerlings (Table 3). There was no significant difference in CAT, SOD and MDA of the gill ($p > 0.05$) for 8 weeks as the values have the same superscript across the row with 0.138ml/L having relatively highest activity followed by 0.069, 0.00 and 0.276ml/L.

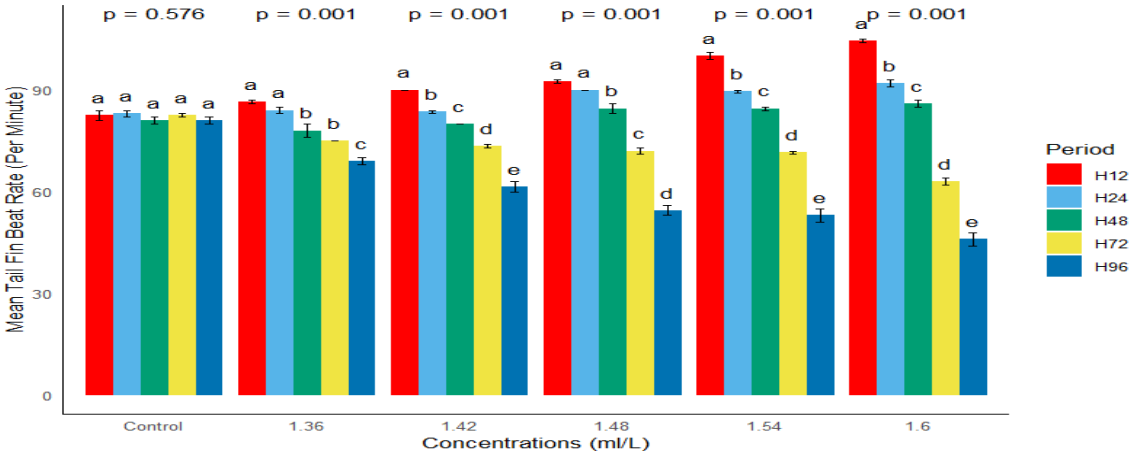

Figure 2: Tail Fin Beat of *Clarias gariepinus* Fingerlings Exposed to Acute Concentration of 2, 4-D Dimethyl Amine Salt

Liver

CAT, SOD and MDA in liver of *Clarias gariepinus* fingerlings exposed for eight weeks to sub-lethal concentration of 2, 4 – D dimethyl amine salt is shown in Table 4. No significant difference ($p > 0.05$) in liver for SOD and CAT and MDA for eight weeks as 0.276ml/l treatment (T3) having the highest value followed by 0.138, 0.069 and 0.00ml/l (T2, T1, and T0) respectively.

Histopathlogy of Acute and Sub-Acute

Histology of Gill

The gills of the control i.e T_0 and 1.36ml/L i.e T_1 concentration (T0 and T1) for acute and those of the treated fish exposed to different concentrations of 2, 4 –D dimethyl amine salt showed marked difference. The gills of the control fish had normal structure of the filament and lamellae (Plate I) the finger-like projection of secondary lamellae are intact on each side, for both acute and sub-acute while the lamellae of treated fish at various concentrations were distorted (Plate I) also, T1, T2, and T3 (Plate III), for sub-acute. The gill of the fish with concentration had marked distortion of the architecture. The treatments concentrations showed reduction in size of filament, filament atrophy (FA), and Filament Necrosis (FN).

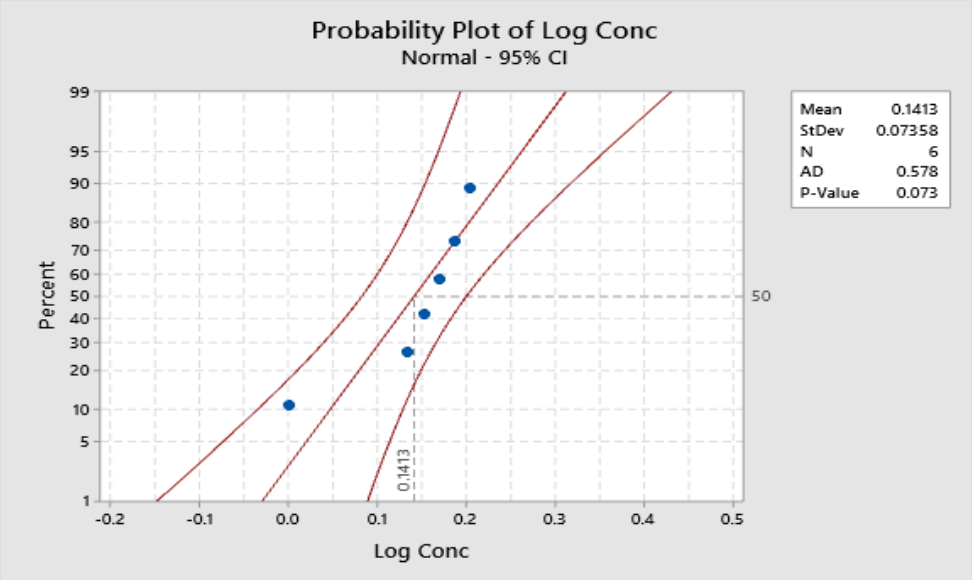

Figure 3: The Graph for 96hrs LC_{50} of 2, 4-D Dimethyl Amine Salt Herbicide on of *Clarias gariepinus* Fingerlings

Liver

The liver of the exposed fish to different concentration of the herbicides 2, 4 – D dimethyl amine salt were observed to have adipocyte infiltration i.e. a group of cells specialized for the storage of fat, found in connective tissue (Plates II and IV). Kupffers cell degeneration, also hepatic necrosis (HN), hyperplasia of inflammatory cell (LH). The liver from the control group (T0) for both lethal and sub lethal showed no sign of hepatic cells damage and no irregularities were observed (Plates II and IV).

Table 1: Oxidative Stress Biomerkers for Acute Exposure of 2, 4 –D Dimethyl Amine Salt Concentration on Gill of *Clarias gariepinus* Fingerlings

Parameters	Treatments						*p*-value
	T_0	T_1	T_2	T_3	T_4	T_5	
SOD(u/mg pro)	8.95±1.0	9.45±0.25	9.65±0.3	9.85±0.25	10.05±0.3	10.30±0.4	0.53
MDA(nmol/mg	93.8±3.3	98.25±67	101.4±1.6	108.6±1.0	107.5±2.8	107.6±6.1	0.197
CAT(u/mg pro)	0.95±0.2	1.10±0.1	1.30±0.1	1.65±0.15	1.7.0±0.8	1.90±0.2	0.506

Means with the same superscript along the columns are not significantly different (P>0.05).
Key: T1= 0.00, T_1=1.36, T_2=1.42, T_3=1.48, T_4=1.54, and T_5=1.60ml/L

Table 2: Oxidative Stress Biomarkers for Acute Exposure of 2, 4 –D Dimethyl Amine Salt Concentration on Liver of *Clarias gariepinus* Fingerlings

Parameters	Treatments						*p*-value
	T_0	T_1	T_2	T_3	T_4	T_5	
SOD (u/mg pro)	15.00 ± 0.6^{e}	17.4 ± 0.1^{de}	18.35 ± 1.2^{cd}	21.3 ± 0.8^{bc}	22.35 ± 0.65^{b}	26.5 ± 1.5^{a}	0.01
MDA (nmol/mg)	128.9 ± 6.5^{a}	134.00 ± 8.3^{a}	$143.\pm23.8^{a}$	147.0 ± 9.5^{a}	159.0 ± 3.7^{a}	165.5 ± 10.0^{a}	0.382
CAT (u/mg pro)	3.10 ± 0.2^{b}	4.10 ± 0.69^{ab}	4.60 ± 0.9^{ab}	4.20 ± 1.1^{ab}	6.3 ± 1.4^{a}	6.7 ± 0.3^{a}	0.046

Means with the same superscript across rows are not significantly different (P>0.05).

Table 3: Mean (±SE) CAT, SOD and MDA (U/mg protein) for Eight Weeks Exposed to Sub- Lethal Concentration of 2, 4 –D Dimethyl Amine Salt on Gill of *Clarias gariepinus* Fingerlings

Parameters	Treatments				*p*-value
	T_0	T_1	T_2	T_3	
SOD(u/mg pro)	7.8 ± 1.5^{b}	9.6 ± 1.1^{a}	9.7 ± 1.3^{a}	10.21 ± 1.8^{a}	0.708
MDA(nmol/mg	$118\pm16.^{ab}$	129 ± 11.7^{ab}	146 ± 14.0^{a}	167 ± 19.6^{a}	0.67
CAT(u/mg pro)	4.2 ± 0.5^{a}	4.9 ± 0.1^{a}	5.16 ± 0.5^{a}	5.4 ± 0.6^{a}	0.42

Means with the same superscript across rows are not significantly different (P>0.05).
Key: T1= 0.00, T_1=0.069, T_2=0.138, T_3=0.276ml/L

Table 4: Mean (±SE) CAT, SOD and MDA (U/mg protein) for Eight Weeks Exposed to Sub-Lethal Concentration of 2, 4 –D dimethyl Amine Salt on Liver of *Clarias gariepinus* Fingerlings

Parameters	Treatments				*p*-value
	T_0	T_1	T_2	T_3	
SOD (u/mg pro)	8.9 ± 2^{b}	10.6 ± 1.5^{ab}	10.73 ± 1.3^{ab}	12.2 ± 2.1^{a}	0.688
MDA (nmol/mg	141 ± 5.3^{a}	149 ± 3.8^{a}	163 ± 5.5^{a}	156 ± 11^{a}	0.443
CAT (u/mg pro)	4.7 ± 76^{b}	5.7 ± 0.7^{a}	5.66 ± 0.7^{a}	5.7 ± 0.8^{a}	0.074

Means with the same superscript across the rows are not significantly different (P>0.05)
Key: T_0= 0.00, T_1=0.069, T_2=0.138, T_3=0.276ml/L

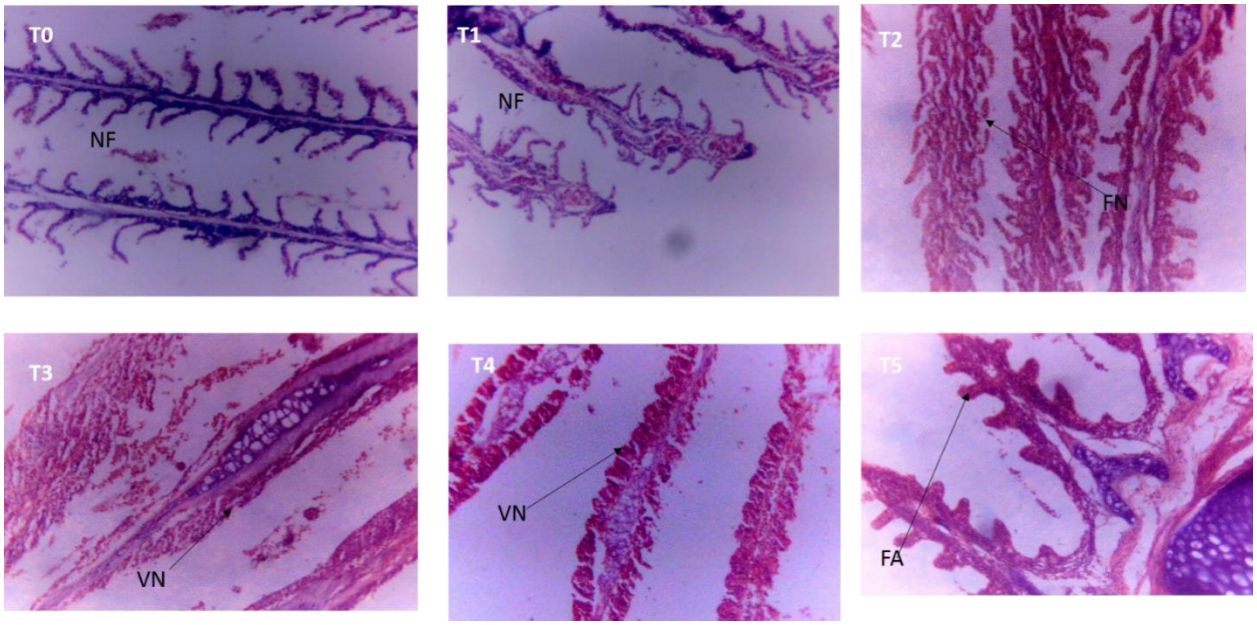

Plate 1: T. S of the gill filament of *Clarias gariepinus* exposed to acute concentration 2, 4 – D dimethyl amine salt
Note: NF, normal features, FN is filament necrosis, VN is villi necrosis and FA is filament atropy

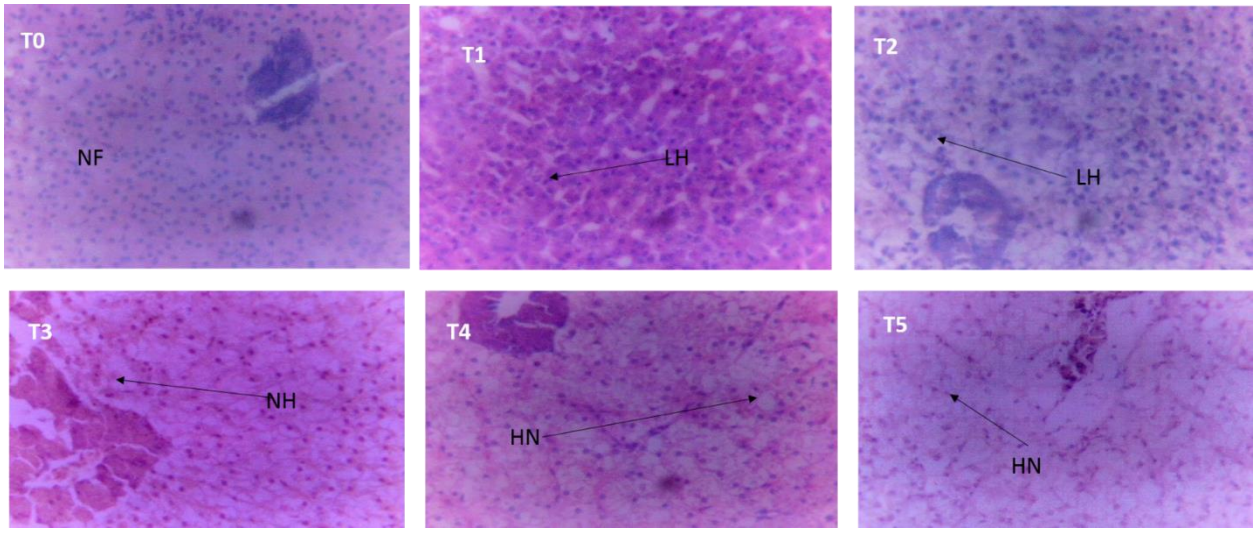

Plate 2: T. S of the liver of *Clarias gariepinus* for acute exposure to 2, 4 – D dimethyl amine salt
Note NF is normal features, LH is hyperplasia ofiInflammatory cell, and HN is hepatic necrosis

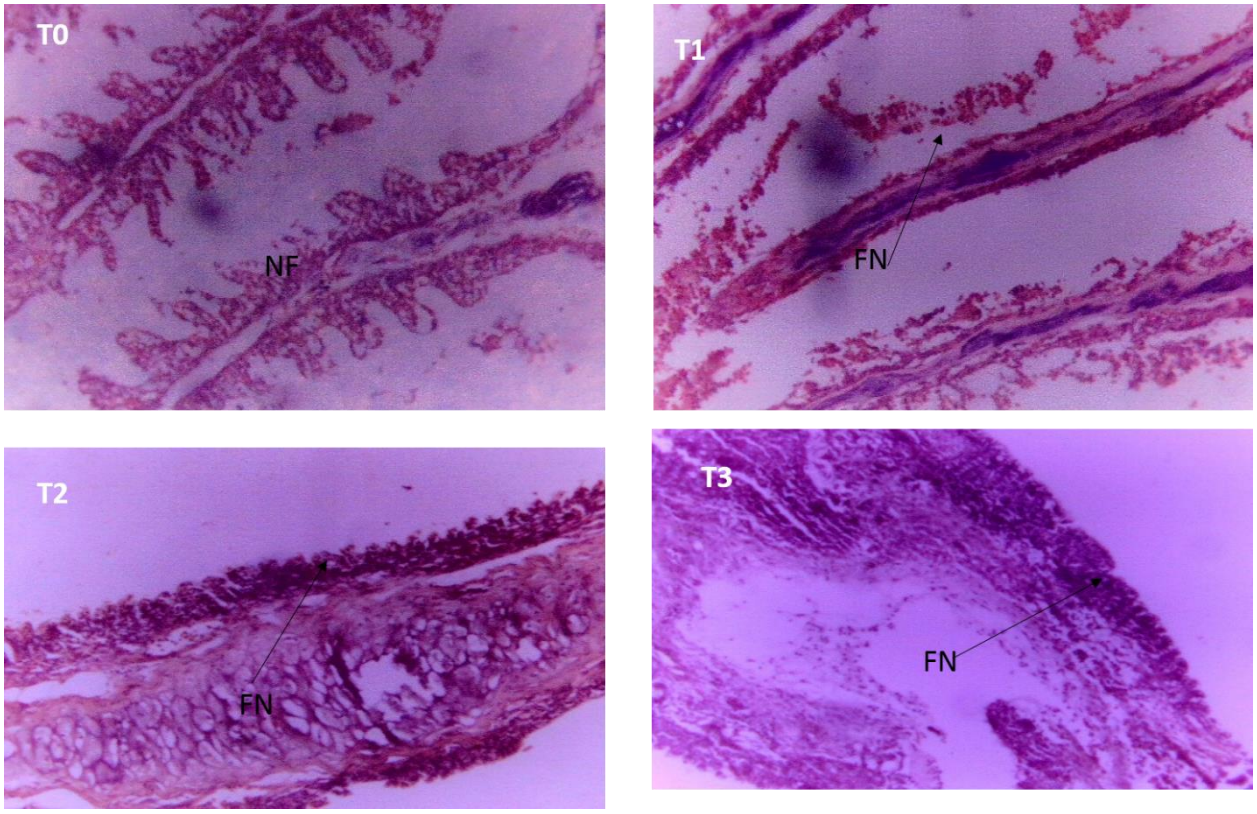

Plate 3: T. S of the gill filament of *Clarias gariepinus* exposed to sub lethal concentration of 2, 4 – D dimethyl amine salt
Note: NF, normal features, FN is filament necrosis, and FA is filament necrosis

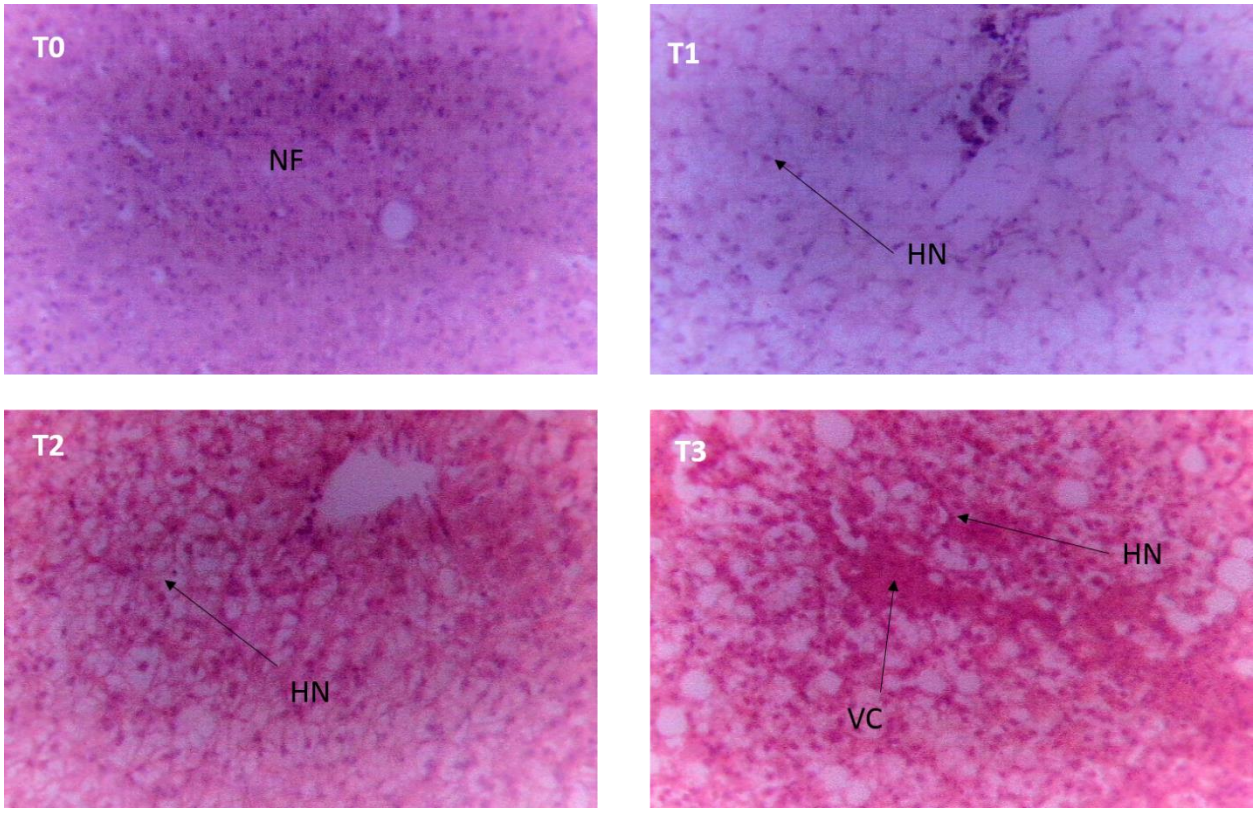

Plate 4: T. S of the liver of *Clarias gariepinus* for sub lethal exposure to 2, 4 – D dimethyl amine salt
Note LH is Hyperplasia of Inflammatory Cell, HN is Hepatic Necrosis and VC is Vacoulization

Discussion

Behavioural Responses

The present research showed that exposure of *Clarias gariepinus* to 2, 4 –D di methyl amine salt in water evoked a lot of abnormal behavior of the fish. The observation were made in the fish immediately post-exposure to the acute concentration. The fish exhibited loss of equilibrium, frequent surfacing, agitation and erratic swimming, hyperactivity and finally death. These abnormal behaviors or hyperactivity could be that the fish is trying to suppress the effect of toxicant substances or trying to find a safer place due to insufficient oxygen in aquaria tank containing the herbicide. Similar research were reported by El-Sharkawy *et al.* (2011) that jumping of the fish in aquaria tank containing toxicant substances to gulp an air could be attributed to either oxygen depletion as a result of pollution caused by herbicides or other toxic chemicals caused irritation by dermal contact. Similar research was carried out by Okayi *et al.* (2010) who reported that fish showed various abnormalities upon exposure to propanil with the immediate reaction being erratic swimming and tendency to jump out of the test bowl. Introduction of fishes to test solution usually showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and color fading were prominent after 24 hrs exposure of glyphosate (Ranjan & Kumar, 2022). Fish exposed to higher concentrations of the pesticide showed abnormal behaviour and tried to avoid the test water by swimming very fast, jumping and displaying erratic with vigorous jerky movements, faster opercula movement, hyper excitation, surfacing and gulping of air (Chris *et al*., 2022)

Opercula ventilation and Tail fin beat

The opercular ventilation and Tail fin beat frequency of *Clarias gariepinus* fingerlings to the various toxicant concentrations of 1.36, 1.42, 1.48, 1.54 and 1.6ml/L were higher at 12th and 24th hours compared to control fish. The rates tend to stabilize at 48th hour but thereafter 72nd and 96th hour; there was decline with increase in exposure time and toxicant concentration. This could be due to insufficient oxygen in which fish found no alternative way than to increase in rate of opercular ventilation so as to meet its metabolic demand for oxygen. Similar case were reported by Salim *et al.* (2021) that the results of opercular ventilation and tail fin beats mean values showed that the tail fin beats of the exposed groups were significantly higher ($p<0.05$) than that of the control by the 12th hour. At the 24th and 48th hour post-exposure, these parameters decreased in the exposed groups compared to the control. By the 72nd and 96th hours the tail fin beats of the control group were significantly higher than the exposed groups, the decrease of tail fin beats at the 72nd and 96th hours were also dose-dependent.

Oxidative Stress Biomarker for Gill and Liver

Gill

The antioxidant activity for acute and subacute toxicity shows that catalase, superoxide dismutase activity, and quantity of lipid peroxidation for malondaildehyde, that there is increase in activity with increase in concentration. The increase in activities indicates that the fish is stressed as a result of the toxicant levels. This could be that fish is trying to protect itself against the damage that might be caused by free radicals, these antioxidants are widely accepted as biological indicators of xenobiotic-induced peroxidative injury in fish tissues, and have been used in diagnosing the negative impacts of xenobiotics in aquatic environment (Lasheen *et al*., 2012). However, manifestation of lipid peroxidation was not observed during the early days of exposure in MDA value between the exposed fish and the control until after 45 days at higher toxicant concentrations. Increased levels of MDA are indicative of lipid components' vulnerabilities to the reaction of free radicals, thereby increasing lipid peroxidation. The increase in MDA levels may be explained by the disproportionate generation of ROS (Owolabi, & Abdulkareem, 2021).

Liver

The Liver is a uniform organ that most of antioxidant activity of enzymes such as catalase, super oxide dismutase can be found. This is because of the position of liver and multiple oxidation reaction produce free radicals is maximum (Gul *et al*., 2004), the liver in fish is an organ that performs various functions associated with the metabolism of xenobiotics (Owolabi & Abdulkareem, 2021).

Histology of Fish Gill and Liver

In the present investigation, the gills and liver of the fish exposed to 2, 4 – D dimethyl amine salt exhibited marked histopathological changes. The main features observed in exposed fish to lethal and sub-lethal concentrations of the toxicant include degeneration of epithelium of secondary gill lamellae, shrunken filaments and secondary lamella, fusion of secondary gill lamellae. This suggests that the effect of the pesticide is damaging to the respiratory and osmoregulatory function of the fish and also cause vacuolization. Similar dose-response degenerative lesions were recorded when *Heterobranchus bidorsalis* was exposed to acute toxicity of Cypermethrin. The alterations in the gill architecture is adaptation, necessary for the fish to reduce the rate of absorption of toxic substances (Olufayo & Alade, 2012). The liver of the fish exposed to 2, 4 – D dimethyl amine salt exhibited marked histopathological changes. The main features observed in exposed fish to acute and subacute concentrations of the toxicant include hepatic necrosis and hyperplasia of inflammatory cells. This could be that liver is prone to damage by toxic chemicals due to its role as a vital organ in breaking down chemicals (Ladipo *et al*., 2011). This agrees with the research findings that the liver sections of fish exposed to sublethal concentration of Bisphenol-A for 14 days showed various histopathologic changes including ruptured central vein, lipid-like vacuolization, macrophage and lymphocytes infiltration, ruptured and degenerated hepatocytes (Faheem & Lone, 2017). Similarly, Bawa & Idris (2021) reported *Ricinus communis* hepatotoxicity as being reflected by pathological lesions, disintegration of cells, hydropic degeneration and eventually necrosis.

CONCLUSION

2, 4-D dimethyl amine salt is toxic to *Clarias gariepinus* fingerlings, at LC_{50} value of 1.385 ml/L eliciting behavioral responses such as restlessness and erratic swimming, opercular beat, tail fin beat, increased gulping of air to cope with the oxygen deficiency. The lethal exposure of *Clarias gariepinus* fingerlings to 2, 4-D dimethyl amine salt indicate significant changes in enzymes activity of liver for SOD at 1.36, 1.42, 1.48, 1.54 and 1.6ml/L and CAT. The lethal and sub-lethal exposure of *Clarias gariepinus* fingerlings to 2, 4-D dimethyl amine salt indicate significant changes in histology of fish gill due to villi necrosis, fusion of gill lamellae and that of liver are hephatic necrosis, vocoulization, hyperplasia of inflammatory cell across all the treatments excluding control. Further research should be carried out to investigate effects of 2, 4 - D dimethyl amine salt herbicides on kidney and oxidative stress biomarker on GSH of *Clarias*

gariepinus. Investigation into non-chemical based herbicides or bio-herbicides should be intensified.

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