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Toxicity of Aqueous Extract of Ichthyotoxic Plant *Millettia griffoniana Baill.*, *a*nd its effect on Haematological, Histopathological, and Proximate Composition of the Carcass of African Catfish (*Clarias gariepinus*) (BURCHELL, 1802) Juvenile

[1*]Ezekiel Olatunji Ayotunde, [2] Christina Onyemachi Amadi-Ibiam, [3] Sampson Obumneke Ohaturuonye [2], Simeon Agwudele Elom

[1]Department of Fisheries and Aquaculture, Faculty of Agriculture and Forestry, University of Cross River State, PMB 102 Obubra Campus. Cross River State. Nigeria

[2]Department of Fisheries and Aquaculture, Faculty of Agriculture and Natural Resources Managements, Ebonyi State University, Abakaliki Ebonyi State, Nigeria.

[3]Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi Azikwe University Awka. Anambra State. Nigeria

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Abstract: *This research investigated the effect of Ichthyotoxic Plant Milletia grifoniana Baill leaves extract on African Catfish Clarias gariepinus under static bioassay laboratory conditions. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC_{50} were 2.5mg/l, 2.3mg/l, 1.8mg/l, 1.5mg/l, 1.2mg/l respectively, with the range of maximum admissible concentration (MATC) were 0.25 – 0.025mg/l, 0.23 – 0.023mg/l, 0.18 – 0.018mg/l, 0.1 – 0.015mg/l, 0.12 – 0.012mg/l respectively. The mortality rate in the Milletia grifoniana is concentration dependent, the higher the concentration of toxicant the higher the mortality of fish. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. Fish showed initial disturbed swimming movements, rapid opercular movements, loss of balance incessant gulping of air, and fish settling at the bottom motionless with slow opercular movements. There were no obvious changes in fish behaviour in the lower concentrations. The abnormal behaviors displayed by the fish increased with increasing concentrations of Ichthyotoxic PlantsMilletia grifoniana(leaves) in water and time of exposure. Histopathological examination of the gill, liver and kidney of the treated fish showed, The Milletia grifoniana shows severe effect on the skin layer with severe superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain severe melanin laden macrophages (MLM). The liver cells revealed hepatic tissue with severe ground glass (GG) appearance of the cytoplasm and intra hepatic hemorrhage (IHH). The gill showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI), while the kidney revealed severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in same areas. The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. The result of this study calls for the need to discourage the use of toxic plants for catching fish in Nigeria water bodies.*

Key words: Ichthyotoxic Plant, *Milletia grifoniana* Baill, African Catfish (*Clarias gariepinus*); Behavioural responses, Aqueous Extract.

INTRODUCTION

The wild plants have played a significant role in the livelihood of the tribal communities as they mainly prove to be of dietary and medicinal importance, (Dhore *et. al*, 2012; Yesodharan and Sujana, 2007; Oak *et. al*, 2015; Deshmukh and Waghmode, 2011). Fishing involves all the processes of taking the fish from the water.Plant poisoning occurs when an animals consume plant which is poisonous or it is externally applied to it. Poisonous plants are those which result in health and some may eventually cause animal's death, Lavaud (1994), Kawanishi and Raffauf (1986).Miles *et al*., (1989), (Cox 1979). The active components may be found in the root, bark, stem, or leaves of the identified plant. In addition, other plants with fish poisoning properties have been discovered in plants closely related to known piscicidal species. Plant poisons can be classified bacterially, chemically or according to their physiological activity. There are five main groups into which plant poisons can be classified withrespect to their physiological activity. These are irritants, surface/contact poisons, blood poisons,muscular poisons and nerve poisons.

Milletia grifoniana are widely available in the tropics and their leaves have been used as natural piscicides in artisanal fisheries and aquaculture ponds in Nigeria Ayotunde and Igbang (2024). The active substances of the majority of ichthyotoxic Plant are resin, tannin, rotenone, saponin alkaloid, diterpenes, cynogenic/cynogenetic glycosides, nitro containing compounds, oxalates, resins and certain proteins and or amino acidsDeshmukh and Waghmode, 2011). Chemical narcosing of fish was found to be one, of the fishing techniques used for harvesting fish stocks in northern Cross River.

African Sharp tooth catfish *Clarias* gariepinus indigenous from Africa. The African Sharp tooth catfish is a large, eel-like fish, usually of dark gray or black colouration on the back, fading to a white belly. It has an adult length of 1-1.5 m and reaches a maximum total length of 1.7 m and can weigh up to 60 kg. C. gariepinus is one of the most important tropical catfish species for aquaculture in spite its commanding presence in the wild Abalaka (2013). In Nigeria, it is widely cultured in ponds and occurs freely in natural freshwater. The fish has hardiness with high resistance to handling and stress Okechi (2004). *C. gariepinus* has high adaptation for low dissolved oxygen in water especially by fishes above 14 days old with functionally developed accessory respiratory organs Ogundiran *et al.,* (2009)]. It has long tolerance for drought but cannot survive long in water temperature below 9-10°C. These qualities account for its wide application in aquaculture and increased importance in ecotoxicological studies, hence its choice as test organism for the present study Abalaka (2013).The African Catfish (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria. The demand for this fish by large population of Nigerian has necessitated its harvest with the use of poisons by the locals (Abubakar *et al.*, 2022).Among the various animal groups, fishes have been identified as being very sensitive to pollutants and have been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment. There is dearth of work on the acute toxicity levels of *Milletia grifoniana* on fish in Nigeria but extensively used by fishermen of the Cross River State to harvest fish in Cross River. Therefore, otherwise its indiscriminate use could seriously affect the biodiversity

The African Sharp tooth catfish *Clarias gariepinus*, indigenous from Africa, it is a large, eel-like fish, usually of dark gray or black colouration on the back, fading to a white belly. It has an adult length of 1-1.5 m and reaches a maximum total length of 1.7 m and can weigh up to 60 kg. *C. gariepinus* is one of the most important tropical catfish species for aquaculture in spite its commanding presence in the wild Abalaka (2013). In Nigeria, it is widely cultured in ponds and occurs freely in natural freshwater. The fish has hardiness with high resistance to poor handling and stress Okechi (2004). *C. gariepinus* has high adaptation for low dissolved oxygen in water especially by fishes above 14 days old with functionally developed accessory respiratory organs Ogundiran *et al.*, (2009). *Clarias gariepinus* will be used as test organism in this study due to its obtainability, it is commonly cultured for commercial purposes in Nigeria, and it is also easy to maintain under laboratory conditions. It has long tolerance for drought but cannot survive long in water temperature below 9-10°C. These qualities account for its wide application in aquaculture and increased importance in ecotoxicological studies, hence its choice as test organism for the present study.

MATERIALS AND METHOD

Location of Study: The research was conducted in the Fisheries Research Unit of Ebonyi State University, Abakaliki, in the Department of Fisheries and Aquaculture Research and Teaching Farm. The area is located in the South East of Nigeria and has a prevailing tropical climate with mean annual rainfall of about 1500 mm. The average ranges of ambient temperature of 240C to 380C with a yearly average of 340C. The average relative humidity ranges from 60 to 94 percent with a year average of about 83 percent. Abakaliki lies between latitude 50 and 60 N and between longitudes 70 and 80 E at an elevation of 59m above sea level within the South Eastern Agricultural Zone of Nigeria (GPS, 2018).

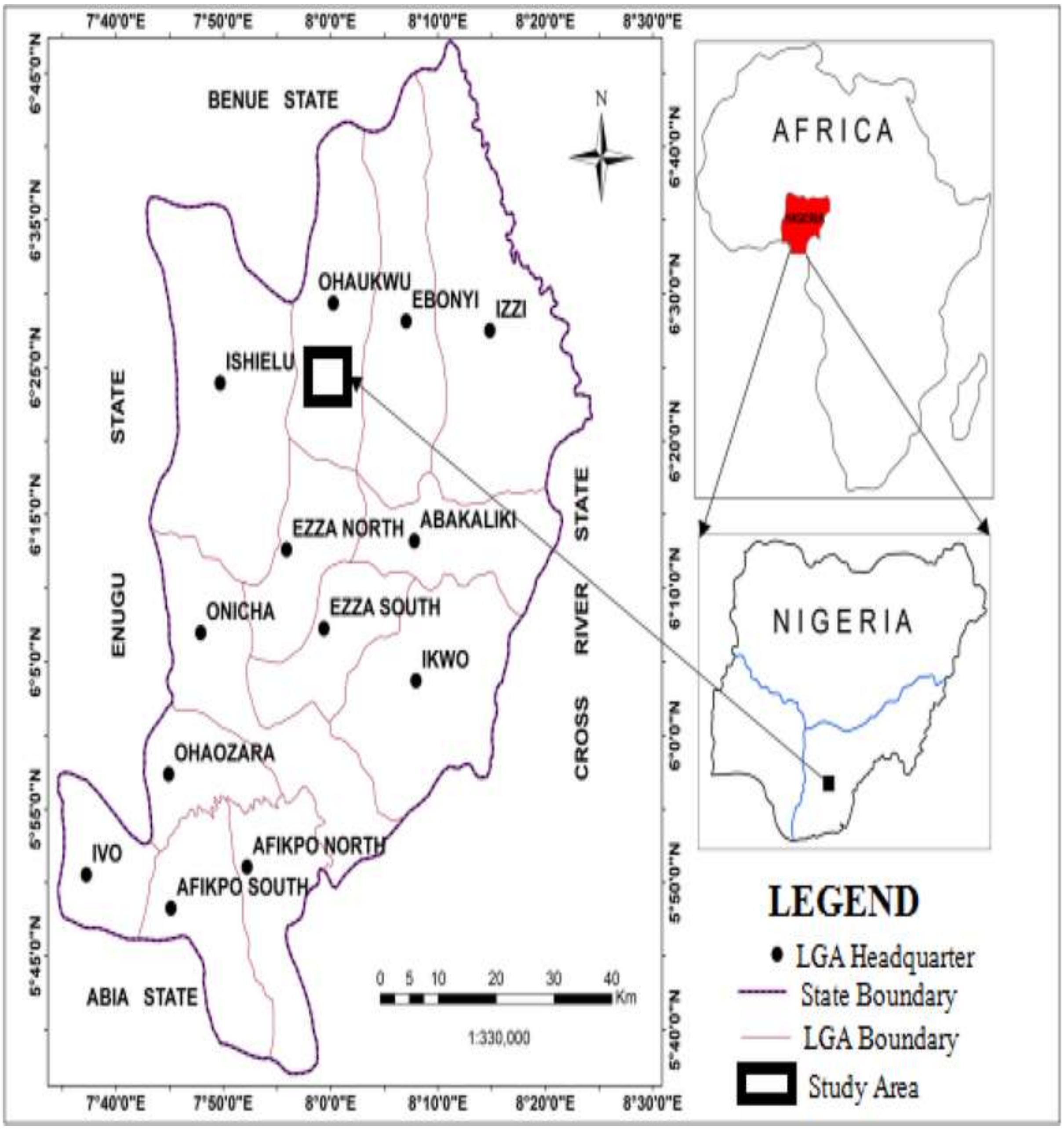

Figure 1: Map of Ebonyi state showing Abakalik the study area

Collection and preparation of plant samples

The Ichtyotoxic plants *Milletia grifoniana*, specifically its leaves, were collected from the abundant plant populations around the communities at Ohaukwu Local Government Area of Ebonyi State, Nigeria. The collection exercise was done during the early hours of the day, between 6:00am and 9:00am. Subsequently, the collected plants were transported to the Herbarium unit of the Department of Crop and Landscape Management (C.L.M) at CAS campus, Ebonyi State University, Abakaliki for proper and accurate identification. The leaf

Milletia grifoniana was pulverized using an electric blender. The powder of each plant sample was sieved through a 100 µm sieve to obtain fine powder and transferred into air-tight sterile bottles, labelled, and stored at 4˚C until further analyses.

Aqueous extraction of Ichthyotoxins

Ichthyotoxins from the plant samplewas extracted by soaking 100 g of the powder from each sample in 1L of distilled water. The solutions were left for 72 h to undergo fermentation and stirred once, morning and evening during this period. After the fermentation period, the solution of each sample was filtered through a Whatman (No.1) filter paper to obtain the aqueous extracts (Fafioye 2005).

Physico-chemical Parameters Determination

Water quality was monitored prior to the commencement of the experiment, during the experiment (once a week), and at the end of the experiment. Water quality parameters determined include: pH, dissolved oxygen concentration, temperature, acidity, alkalinity, ammonia, nitrate, nitrite, general hardness and turbidity.

Toxicity Experiment

Test organism/ Acclimation

African Catfish *Clarias gariepinus* was used as test organism in this study. *C. gariepinus* juveniles (4-6 weeks old) were purchased from Amazons Fish hatcheries Ugwuachara and transported in oxygenated polythene bags, to the Fisheries wet Laboratory, Department of Fisheries and Aquatic Science, CRUTECH Obubra. The juveniles were acclimated separately for fourteen days in holding tanks, half filled with unchlorinated well water. They were fed with commercially prepared fish feed (Vital Fish Feed, Nigeria) at 3% body weight during this period and water in the tanks were changed once every other day to avoid pollution by fish metabolic wastes and food remnants. Feeding was discontinued 24h before the commencement of experiments.

Stock solution of Ichthyotoxins

Stock solutions of the extractwas prepared by dissolving 100g of each extract in 1L of distilled water to give a solution of 100 g/L. The stock solutionwas serially diluted 1:100 (water content: toxicant) depending on required concentrations, for use in toxicity testing studies.

Acute toxicity studies (Range Finding Test)

The acute toxicity studies wasconducted under standard static bioassay procedure (Reish and Oshida, 1987, American Public Health Association (APHA) (1995). Twenty one (21) (75cm x 45cm x 45cm) glass tanks of 121.5 litres capacity each were filled with 50 litres aerated unchlorinated well water. Ten juvenile of the test organism were batch-weighed with a top-loading mettler balance (Mettler Toledo (K), and distributed randomly in triplicate per treatment. The glass tanks were covered, there was no aeration, no water change nor feeding throughout the test. This was done prior to the introduction of the toxicant. *C. gariepinus* juvenileswere exposed to 10, 20, 30, 40, 50, 60Mg/L and 0 mg/L as control, of each of the plant extracts for 24 hours.

Sub-acute toxicity studies (Definitive Test)

African Catfish *Clarias gariepinus* juveniles were exposed to sub-acute concentrations (96 h LC_{50}) of the concentration earlier determine during acute toxicity studies (Range Finding Test). These series of experiment was carried out for a period of 96hours and the semi-static bioassay method was employed to avoid changes in concentration of toxins via evaporation and excessive reduction in dissolved oxygen level. The maximum admissible toxicant concentration (MATC) was determined by multiplying 96 hours LC_{50} with a factor 0.1 – 0.01 according to Koesomadimata (1980).

Haematological Examinations

At the end of 96hours experiment one fish was collected randomly from each treatments for blood analysis. 5 – 10 ml blood per fish was collected from vertebral blood vessel using 2ml EDTA treated disposable syringes and needle. The method of blood sampling follows the method described by (Svobodova *et al.*, 1991). All haematological parameters was analysis at Haematological Unit of the University of Calabar Teaching Hospital, using automated haematology analyzer (SYSMEX KX – 21N™).

Carcass Composition (Proximate) Analysis

The carcass composition of the experimental fish was run to determine the Crude Protein (CP), crude Lipid (CL), Crude Fiber (CF), Moisture (M), Ash and Nitrogen Free Extract (NFE), using standard methods (AOAC, 1990). Nitrogen was determined by the micro-kjedahl method and the crude protein was taken as N% x 6.25 (constant factor) where N is equal to Nitrogen content per 100g sample. Total carbohydrate was determined using the phenol-sulphuric acid method. The crude fibre was obtained by dry ashing of the sample at 550°C dissolved in 10% HCl (25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

HISTOLOGICAL EXAMINATION OF TEST ORGAN

At the end of the experiment, one fish per treatment, that is, three fish per concentration were sampled after 96hours of exposure for histological analysis, the test organism waskilled with a blow on the head, using a mallet and was dissected to remove the vital organs (gill, liver and skin). The organs werefixed in 10% formalin for three days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry, (1976), in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding.

The organs were embedded in malted wax. The tissue was sectioned into thin sections (5-7µm), by means of a rotatory microtome and was dehydrated and stained with Harris haematoxyllin-eosin (H&E) stain, Bancroft and Cook, (1994), using a microtone and each section were cleared by placing in warm water (38°C), where it was picked with clean slide and oven-dried at 58°C for 30 minutes to melt the wax. The slide containing sectioned materials/tissue were cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each. The section was stained in haematoxyline eosin for ten minutes. The stained slide wasobserved under a light microscope at varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

Statistical analysis

The dose-response data obtained from the acute toxicity studywas analysed using SPSS (Statistical Package for Social Sciences) version 20.0. Indices of measuring acute toxicity (lethal concentration affecting a percentage of exposed organisms) and their 95 % confidence limits was reported. Data obtained from haematological studies were analysed using one-way analysis of variance (ANOVA) and where a significant difference ($p < 0.05$) exist, Duncan new multiple range tests were used to detect the source of the difference.

RESULTS

The result of Length-weight relationship and condition factors of *Clarias gariepinus* Juvenile exposed to ichthyotoxic plant *Milletia grifoniana* is presented in table 1. The weight (g) varies from (57.75±3.6 - 69.20±0.2) – (49.75±3.6 - 67.27±5.0) and standard length (18.57 ±0.5 - 20.96±1.3) – (18.57±0.4 - 20.95±1.3) with the condition factor range from (0.8 – 0.9) - (0.7 – 0.9) for range finding test and definitive test respectively, this result indicates that the experimental fish are in good conditions of health. Table 2 summarizes the 96 hours LC_{50} values of Ichthyotoxic Plants *Milletia grifoniana* (leaves) of different time intervals and The mortality rate in the *Milletia grifoniana* is concentration dependent, the higher the concentration of toxicant the higher the mortality of fish as shown in tables 3 and 4. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC_{50} were 2.5mg/l, 2.3mg/l, 1.8mg/l, 1.5mg/l, 1.2mg/l respectively, with the range of maximum admissible concentration (MATC) were 0.25 – 0.025mg/l, 0.23 – 0.023mg/l, 0.18 – 0.018mg/l, 0.1 – 0.015mg/l, 0.12 – 0.012mg/l respectively as presented in Table 2 Figure 1. The percentage cumulative mortality is presented in tables 2 and 3, mortality increases with increases in concentration and time of exposure. The 100% mortality was observed in the group fish exposed to 0.30 mg/l. No mortality was observed in the group of fish in the control experiment.

Table 5 summarizes the range of the physicochemical parameters of control and treated fish observed during the experimental period. There was no significant difference among the different treatments in terms of physicochemical parameters measured. Alkalinity significantly increased throughout the experiment. The result of the physicochemical parameters of the experimental media indicated a significant difference ($P<0.05$) in the values obtained in the level of dissolved oxygen, content free carbon dioxide and alkalinity with the control. Nevertheless, there were no significant difference ($P>0.05$) between the values of temperature and pH of test media with the control. The abnormal behaviour observed in fish exposed to the extract included respiratory distress, loss of balance, gulping of air, settling at the bottom motionless, and erratic swimming.

Table 1.Length-weight relationship of *Clarias gariepinus* Juvenile

Range finding test				Definitive test			
Conc.	Weight (g)	Standard length (cm)	Condition factor: K = 100w/l^3	Conc.	Weight (g)	Standard length (cm)	Condition factor: K = 100w/l^3
0.00	57.75±3.6^a	18.60±0.5^{ab}	0.9	0.00	49.75±3.6^a	18.60±0.5^{ab}	0.8
10.0	60.58±6.3^a	18.57±0.4^{ab}	0.9	0.05	58.58±6.3^a	18.57±0.4^{ab}	0.9
20.0	67.27±5.0^{bc}	19.57±1.0^{ab}	0.9	0.10	67.27±5.0^{bc}	19.57±1.0^{ab}	0.9
30.0	69.20±0.2^c	20.96±1.3^b	0.8	0.15	62.20±0.2^c	20.95±1.3^b	0.7
40.0	62.63±6.1^{abc}	19.10±0.8^a	0.9	0.20	62.63±6.1^{abc}	19.10±0.8^a	0.9
50.0	59.50±1.9^a	18.66±0.7^{ab}	0.9	0.25	60.50±1.9^a	18.66±0.7^{ab}	0.9
60.0	62.82±5.9^a	19.24±1.1^{ab}	0.9	0.30	61.82±5.9^a	19.24±1.1^{ab}	0.9

Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

Table 2: The LC_{50} values of *Clarias gariepinus* Juvenile exposed to Ichthyotoxic Plants *Milletia grifoniana* (leaves).

S/N	TIME(Hrs)	LC_{50}	MATC (mg/l)
1	24	2.5	0.25 – 0.025
2	36	2.3	0.23 – 0.023
3	48	1.8	0.18 – 0.018
4	72	1.5	0.15 – 0.015
5	96	1.2	0.12 – 0.012

Table 6 and 7 show the behavioural changes of *Clarias gariepinus* Juvenile exposed to different concentration of Ichthyotoxic Plants *Milletia grifoniana* leaves. Fish showed initial disturbed swimming movements, rapid opercular movements, loss of balance incessant gulping of air, , and fish settling at the bottom motionless with slow opercular movements. There were no obvious changes in fish behaviour in the lower concentrations. The abnormal behaviors displayed by the fish increased with increasing concentrations of Ichthyotoxic Plants*Milletia grifoniana*(leaves) in waterand time of exposure.

Table 9 present the result of toxicity of aqueous extract of *Milletia grifoniana*on haematological parameters of *Clarias gariepinus* Juvenile. There was reduction in nearly all the results of blood parameters of Sharp tooth Catfish Clarias gariepinus Juvenile exposed to ichthyotoxic plant *Milletia grifoniana* in the respective treatments as presented in table 8. During the 96 hours chronic toxicity bioassay, Results from blood analysis show that the parameters such as the White Blood Cell (ul)), Red Blood Cell (ul), Haemoglobin (g/dl), Haematocrit (%),Lymphocytes (ul), Mean Cell Volume (fl), Mean Cell Haemoglobin (pg), from $1.65x10^2±21.9$, $2.37x10^6±1.0$, 9.07±0.9, 23.25±2.3, 96.60±1.1, $1.16x10^2±2.3$, 48.28±3.5 and 45.40±10.4 to $1.51x10^2±22.9$, $2.01x10^6±7.5$, 6.53±4.2, 16.94±8.7, 90.43±1.0, $1.11x10^2±5.6$, 47.98±9.5 respectively when compared with the control, while there was an increase in Platelet (ul) and Mean Cell Haemoglobin Concentration from $2.64x10^4±1.5$, 45.40±10.4 to $2.83x10^4±2.0$, 49.07±12.9 respectively, when compared with the control.

Variation occurred in the values obtained in the results of Proximate composition of the carcass of*Clarias gariepinus* juvenile exposed to aqueous extract of *Milletia grifoniana*Table 9,There was no significant difference (P<0.05) in Crude Protein (CP) (Mg/l), Energy K(cal)/mg, and Crude Fiber (CF), from 26.25±5.0, $1.36x10^2±5.3$, $1.36x10^2±5.3$, and 0.00±0.0 to 26.87±2.1, $1.36x10^2±9.4$ and 0.00±0.0respectively when compared with the control; Crude Lipid (CL), Ash and Nitrogen Free Extract (NFE) reduce from 3.34±0.6, 0.92±0.1 and 0.49±0.2 to 26.87±2.1, 0.16±0.2 and 0.43±0.2respectively when compared with the control, there is an increase in the value of Moisture (M) from 64.84±3.3 to 67.47±5.0when compared with the control.

Table 10 presented the Histopathological examinations of the test fish showed some pathological disruptions. The liver cells revealed necrosis and vacuolation of the liver cell, the gill showed oedema of the gill lamella while the kidney revealed degeneration of the kidney tubules. The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank.

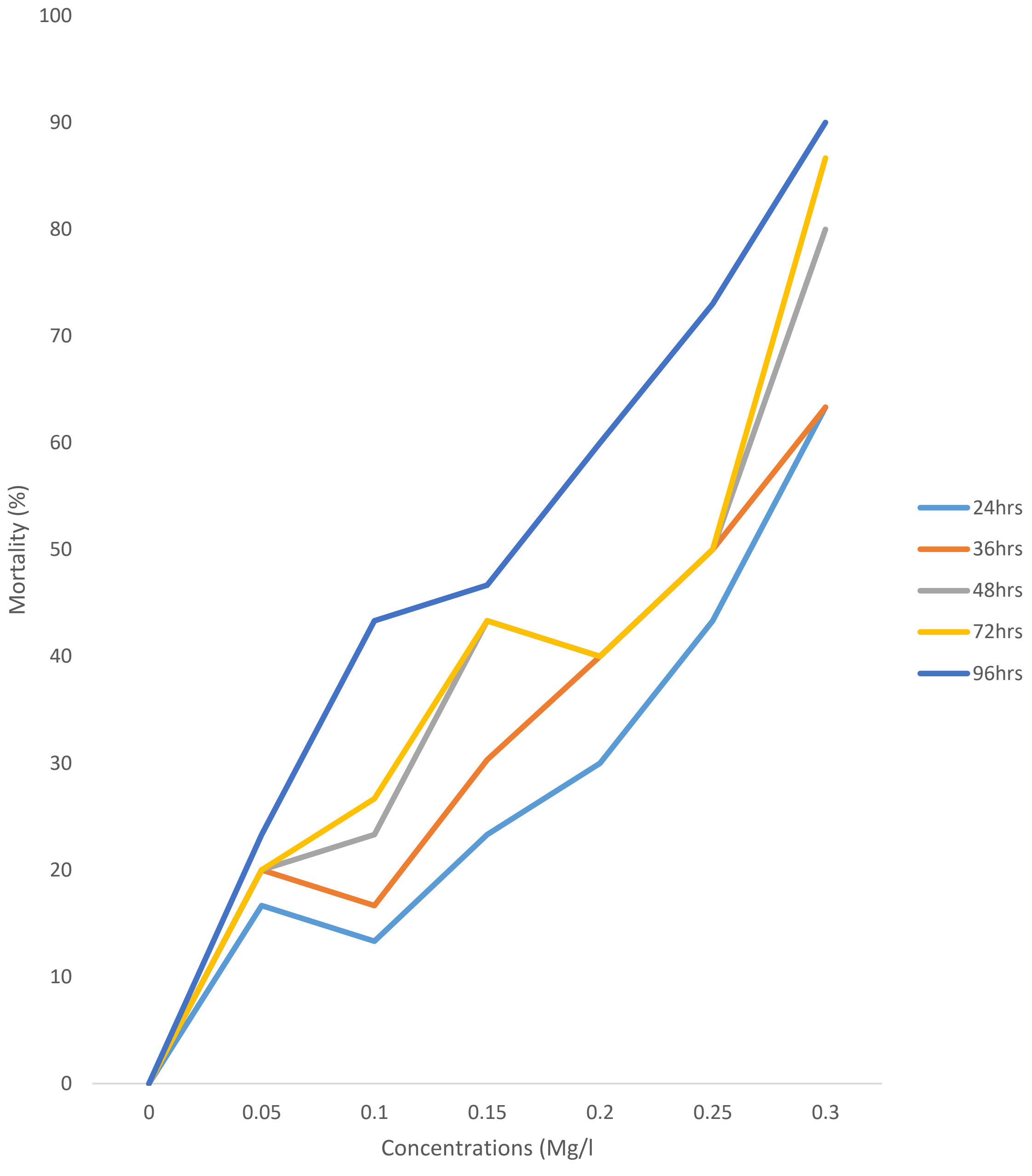

Figure 1: Determination of LC_{50} Using Probit (Graphical) Method (Finney 1971; USEPA 2000).

Table 3: Mean percentage cumulative mortality of *Milletia grifoniana***to *C. gariepinus* adult (Range Finding Test)**

Conc. (mg/L)	15 mins	30 mins	45mins	1hrs	2h	3h	4h	8h	12h	16h	20h	24h
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10.0	0.00	0.00	0.00	6.67	13.33	20.00	20.00	20.00	20.00	20.00	26.67	26.67
20.0	0.00	0.00	0.00	16.67	23.33	23.33	36.67	36.67	40.00	50.00	56.67	60.00
30.0	0.00	6.67	16.67	20.00	20.00	20.00	30.00	33.33	43.33	43.33	46.67	66.67
40.0	0.00	0.00	16.67	30.00	40.00	40.00	46.67	53.33	53.33	60.00	70.00	70.00
50.0	20.00	23.33	23.33	36.67	60.00	60.00	60.00	73.33	73.33	76.67	76.67	83.33
60.0	20.00	23.33	23.33	36.67	60.00	60.00	60.00	73.33	73.33	76.67	100.00	100.00

Table 4: Mean percentage cumulative mortality of *Milletia grifoniana* to *C. gariepinus* Juvenile (Definitive Test)

Conc. (mg/L)	1hr	2hrs	3hrs	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs	36hrs	48hrs	72hrs	96hrs
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.05	0.00	0.00	0.00	0.00	3.33	60.00	10.00	10.00	16.67	20.00	20.00	20.00	23.33
0.10	0.00	3.33	3.33	6.67	6.67	6.67	13.33	13.33	13.33	16.67	23.33	26.67	43.33
0.15	0.00	0.00	0.00	3.33	6.67	13.33	20.00	23.33	23.33	30.33	43.33	43.33	46.67
0.20	3.33	3.33	13.33	13.33	23.33	23.67	26.67	30.00	30.00	40.00	40.00	40.00	60.00
0.25	13.33	16.67	20.00	20.00	56.67	36.67	36.67	36.67	43.33	50.00	50.00	50.00	73.00
0.30	16.67	20.00	23.33	26.67	46.67	46.67	46.67	60.00	63.33	63.33	80.00	86.67	90.00

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Table 5: Summary of water quality parameter of *Milletia grifoniana* to *Clarias gariepinus* (mean ± SD)

RANGE FINDING TEST					DEFINITIVE TEST				
Conc.	Temp(°C)	Conductivity	DO_2 (Mg/l)	pH	Conc.	Temp(°C)	Conductivity	DO_2 (mg/L)	pH
0.00	26.67±1.5^{a}	41.00±4.1^{ab}	3.89±0.5^{ab}	6.97±0.4^{ab}	0.00	26.67±1.5^{a}	41.00±4.1^{ab}	3.89±0.5^{ab}	6.97±0.4^{ab}
10.0	25.17±1.0^{a}	36.50±11.3^{ab}	4.12±0.7^{b}	6.02±1.6^{b}	0.05	25.17±1.0^{a}	36.50±11.3^{ab}	4.12±0.7^{ab}	6.02±1.6^{b}
20.0	25.00±0.6^{a}	33.33±13.4^{a}	4.50±1.1^{b}	8.11±1.5^{c}	0.10	25.00±0.6^{a}	33.33±13.4^{c}	4.50±1.1^{a}	8.11±1.5^{bc}
30.0	25.50±0.8^{a}	42.33±3.4^{b}	3.70±0.5^{a}	7.63±1.0^{ac}	0.15	25.50±0.8^{a}	42.33±3.4^{c}	3.70±0.5^{b}	7.63±1.0^{ac}
40.0	26.17±1.6^{a}	47.33±5.5^{b}	3.55±0.7^{a}	6.90±0.2^{a}	0.20	26.17±1.6^{a}	47.33±5.5^{ab}	3.55±0.7^{b}	6.90±0.2^{a}
50.0	26.00±1.1^{a}	38.33±8.3^{b}	3.71±0.7^{b}	9.93±0.5^{ab}	0.25	26.00±1.1^{a}	38.33±8.3^{ab}	3.71±0.7^{b}	9.93±0.5^{ab}
60.0	25.58±1.2^{a}	39.81±9.1^{ab}	3.91±0.7^{ab}	7.09±1.5^{b}	0.30	25.58±1.2^{a}	39.81±9.1^{ab}	3.91±0.7^{ab}	7.09±1.5^{ab}

Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

Table 6. General behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Milletia grifoniana* (Range Finding Test)

Behaviour/exposure time	12hrs							16hrs							20hrs							24hrs						
Concentration (mg/L)	**0.00**	**1.10**	**0.20**	**0.30**	**0.40**	**0.50**	**0.60**	**0.00**	**1.10**	**0.20**	**0.30**	**0.40**	**0.50**	**0.60**	**0.00**	**1.10**	**0.20**	**0.30**	**0.40**	**0.50**	**0.60**	**0.00**	**1.10**	**0.20**	**0.30**	**0.40**	**0.50**	**0.60**
Air gulping	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Erratic swimming	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Loss of balance	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Excessive mucus secretion	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Operculum movement	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Abnormal Tail movement	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Moulting	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Barbell deformation	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Loss of reflex	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+

Key
+ = present
- = Not present

Table 7: General behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Milletia grifoniana* (Definitive test)

Behaviour/exposure time	24hrs							48hrs							72hrs							96hrs						
Concentration (mg/L)	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.00	0.05	0.10	0.15	0.20	0.25	0.30
Air gulping	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Erratic swimming	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Loss of balance	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Excessive mucus secretion	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Operculum movement	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Abnormal Tail movement	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Moulting	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Barbell deformation	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
Loss of Reflex	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+

Key

+ = present

- = Not present

Table 8: The summary of toxicity of aqueous extract of *Milletia grifoniana* on haematological parameters of *Clarias gariepinus* Juvenile (mean±SD)

Conc. (mg/L)	White Blood Cell (ul))	Red Blood Cell (ul)	Haemoglobin (g/dl)	Haematocrit (%)	Platelet (ul)	Lymphocytes (ul)	Mean Cell Volume (fl)	Mean Cell Haemoglobin (pg)	Mean Cell Haemoglobin Concentration
0.00	$1.65x10^2±21.9^b$	$2.37x10^6±1.0^a$	$9.07±0.9^a$	$23.25±2.3^b$	$2.64x10^4±1.5^a$	$96.60±1.1^b$	$1.16x10^2±2.3^{ab}$	$48.28±3.5^a$	$45.40±10.4^a$
0.05	$159x10^2±12.3^{ab}$	$1.93x10^6±7.7^a$	$8.93±2.8^a$	$19.52±2.2^{ab}$	$3.49x10^4±1.7^a$	$90.58±9.8^{ab}$	$1.06x10^2±10.8^{ab}$	$43.31±1.4^a$	$47.75±10.1^a$
0.10	$1.53x10^2±14.6^{ab}$	$2.12x10^6±1.1^a$	$8.48±4.2^a$	$13.12±7.6^{ab}$	$3.84x10^4±2.8^a$	$88.98±4.3^a$	$1.10x10^2±13.6^{ab}$	$54.95±1.9^a$	$62.85±11.6^b$
0.15	$1.42x10^2±12.5^{ab}$	$1.73x10^6±4.9^a$	$9.37±3.7^a$	$11.37±8.7^a$	$2.23x10^4±2.4^a$	$79.80±1.8^a$	$160x10^2±7.8^{ab}$	$50.60±6.4^a$	$46.10±10.5^a$
0.20	$1.33x10^2±13.7^a$	$1.10x10^6±5.6^a$	$7.03±0.9^a$	$17.87±1.6^{ab}$	$2.45x10^4±2.2^a$	$93.67±3.8^b$	$1.19x10^2±0.9^a$	$46.93±5.8^a$	$39.60±5.0^a$
0.25	$1.53x10^2±41.2^{ab}$	$1.90x10^6±5.1^a$	$6.30±0.9^b$	$16.53±1.1^{ab}$	$2.29x10^4±1.2^a$	$92.93±4.6^b$	$97.83x10^2±3.8^{ab}$	$44.77±7.7^a$	$53.30±7.5^b$
0.30	$1.51x10^2±22.9^{ab}$	$2.01x10^6±7.5^a$	$6.53±4.2^a$	$16.94±8.7^{ab}$	$2.83x10^4±2.0^a$	$90.43±1.0^b$	$1.11x10^2±5.6^{ab}$	$47.98±9.5^a$	$49.07±12.9^b$

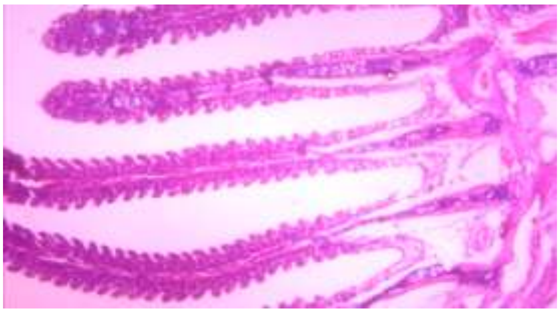
Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

Table 9: The summary of toxicity of aqueous extract of *Milletia grifoniana* on Carcass/Proximate composition of *Clarias gariepinus* Juvenile (Mean±SD)

Conc. (mg/L)	Crude Protein (CP) (Mg/l)	Crude Lipid (CL)	Energy K(cal)/mg	Crude Fiber (CF)	Moisture (M)	Ash	Nitrogen Free Extract (NFE)
0.00	26.25±5.0[a]	3.34±0.6[a]	1.36x10^2±5.3[a]	0.00±0.0	64.84±3.3[ab]	0.92±0.1[b]	0.49±0.2[a]
0.05	26.54±1.2[a]	3.31±0.4[a]	1.42x10^2±1.3[a]	0.00±0.0	67.08±3.1[abc]	0.67±0.3[a]	0.48±0.1[ab]
0.10	27.16±0.3[a]	3.49±0.8[a]	1.42x10^2±3.2[a]	0.00±0.0	71.11±7.8[c]	0.88±0.3[b]	0.57±0.3[b]
0.15	26.98±0.5[a]	3.16±0.2[a]	1.34x10^2±8.5[a]	0.00±0.0	69.49±1.5[bc]	0.91±0.1[b]	0.29±0.1[b]
0.20	26.72±0.5[a]	2.93±0.7[a]	1.33x10^2±1.4[a]	0.00±0.0	68.55±1.3[abc]	0.80±0.1[a]	0.42±0.1[ab]
0.25	27.10±1.8[a]	3.47±0.7[a]	1.33x10^2±3.4[a]	0.00±0.0	63.73±5.4[a]	0.87±0.7[b]	0.37±0.1[ab]
0.30	26.87±2.1[a]	3.29±0.6[a]	1.36x10^2±9.4[a]	0.00±0.0	67.47±5.0[a]	0.16±0.2[b]	0.43±0.2[b]

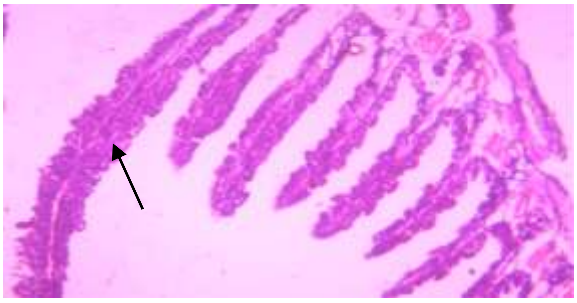
Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

Plate A: (Fig 2-8) Histological change observed in the gill of Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Milletia grifoniana*.

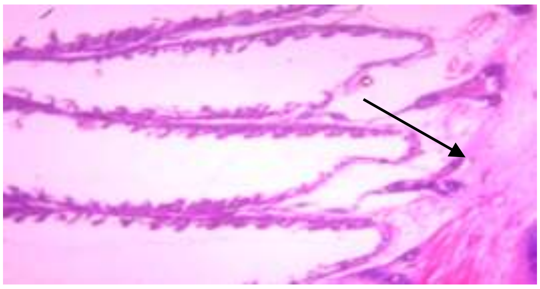


Photomicrograph of T0 control section of gill (X150)(H/E) shows normal gill architecture with well projected filament (PF) , gill epithelium (GE) and cartilages (C).

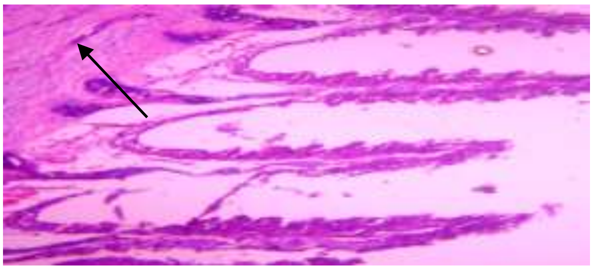
Photomicrograph of T1 section of gill (X150)(H/E) shows section of gill with active filaments (F)



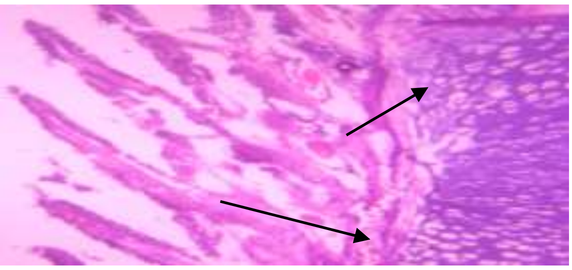
Photomicrograph of T2 section of gill (X150)(H/E) shows moderate effect on the gill architecture with moderate hypertrophy (H) of the



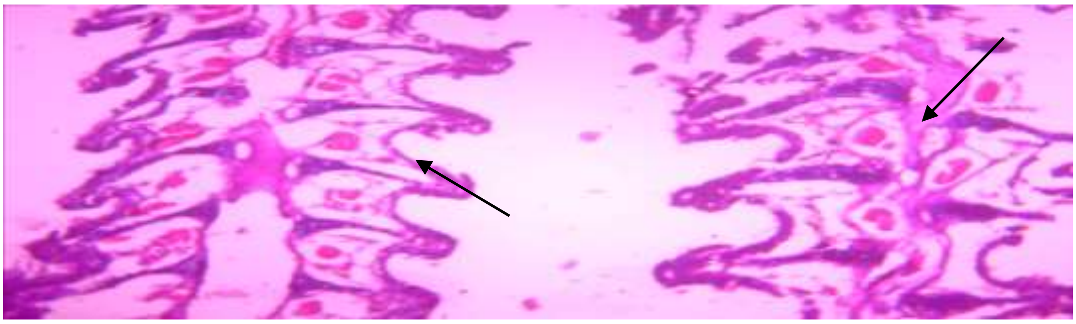
Photomicrograph of T3 section of gill (X150)(H/E) shows mild effect on the gill architecture with mild focal area of hemorrhage (H) otherwise normal,



Photomicrograph of T 4 section of gill (X150)(H/E) shows mild effect on the gill architecture with mild hypertrophied (H) filament

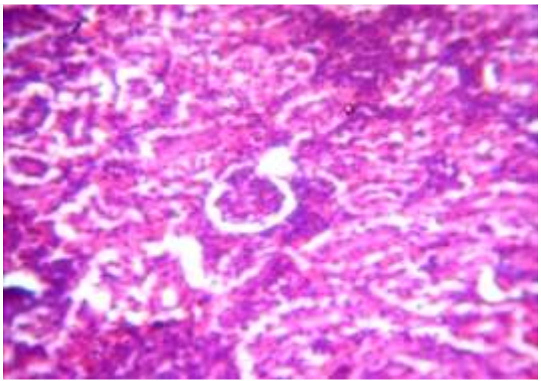


Photomicrograph of T 5 section of gill (X150)(H/E) shows severe effect on the gill architecture with severe areas necrotic filament (NF)

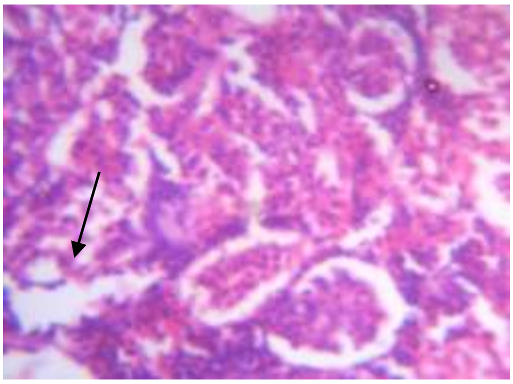


Photomicrograph of T 5 section of gill (X150)(H/E) shows severe effect on the gill architecture with severe clumping ,necrotic (N) filament (C) and areas of hemorrhage (H)

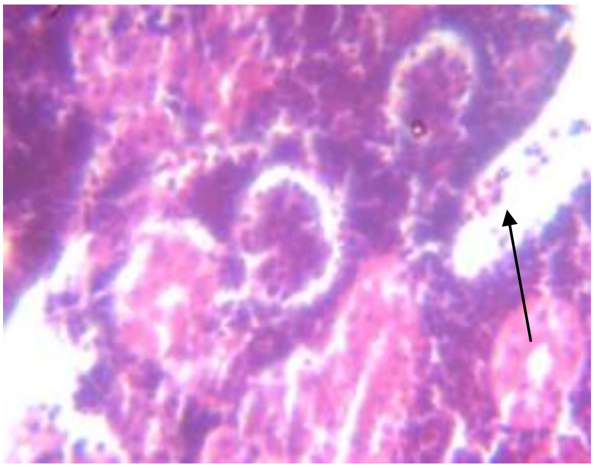
Plate B: (Fig 9-15) Histological change observed in the kidney of Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Milletia grifoniana*.



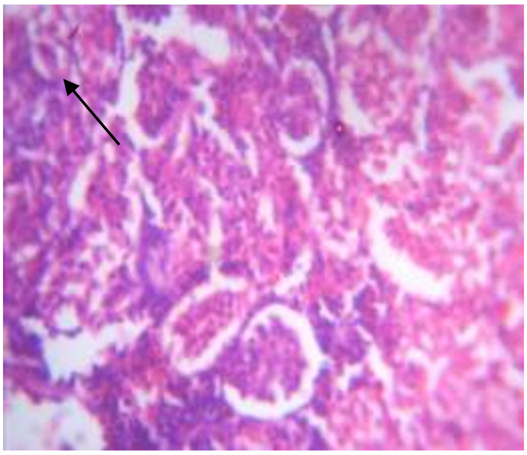
Photomicrograph of T0 control section of kidney(X150)(H/E) shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC)



Photomicrograph of T1 section of kidney(X150)(H/E) shows renal tissue with mild areas of inflammation (I)



Photomicrograph of T2 section of kidney(X150)(H/E) shows renal tissue with severe aggregate of inflammation (AI) with eosinophilic (E) tubules



Photomicrograph of T3 section of kidney(X150)(H/E) shows renal tissue with moderate aggregate of inflammation (AI) with eosinophilic (E) tubules .

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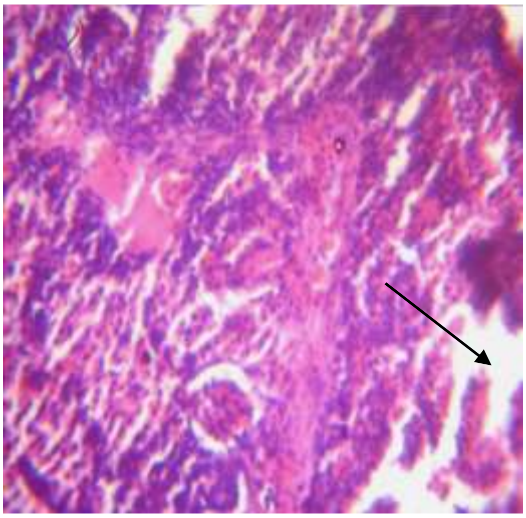
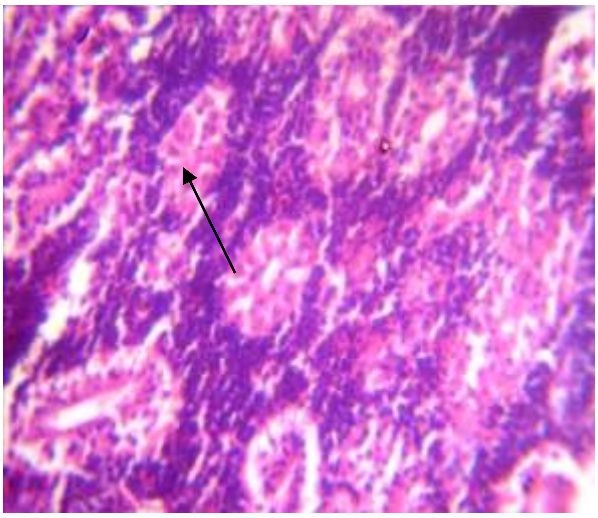
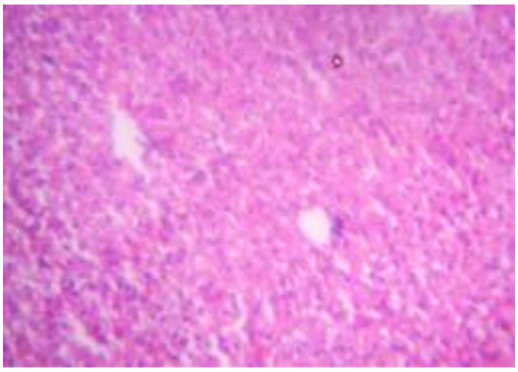


Photomicrograph of T 5 section of kidney(X150)(H/E) shows renal tissue with moderate aggregate of inflammation (A I)

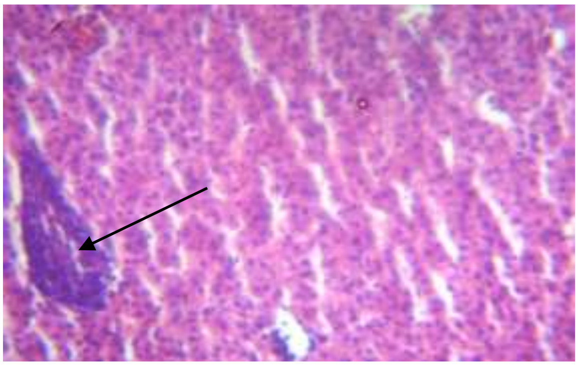


Photomicrograph of T 7 section of kidney(X150)(H/E) shows renal tissue with moderate intra renal inflammation (AI) with active tubules.

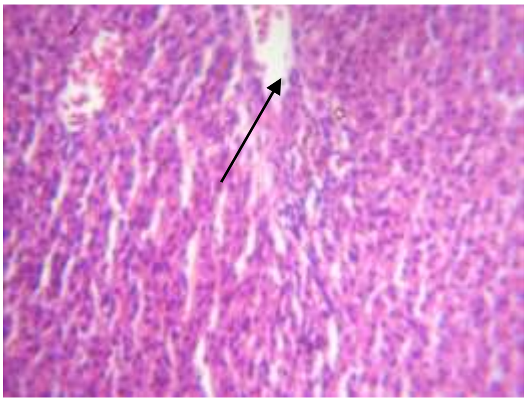
Plate A: (Fig 17-22) Histological change observed in the liver of Juvenile Catfish *Clarias gariepinus* treated with different concentration of plant *Milletia grifoniana* .



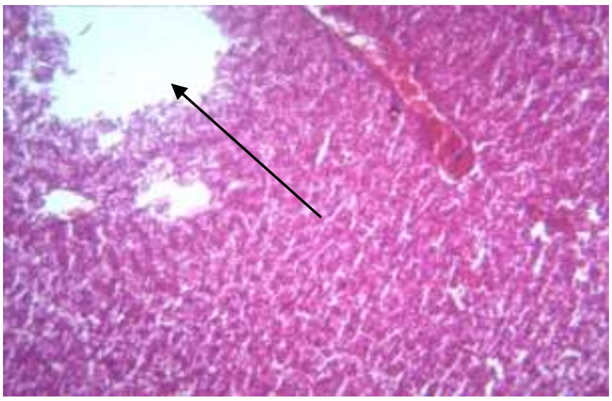
Photomicrograph of T0 control section of liver (X100)(H/E) shows normal hepatic architecture with normal hepatocyte (H) and central vain (C).



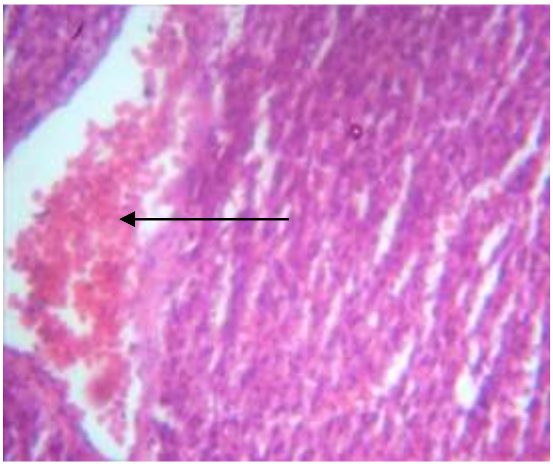
Photomicrograph of T1 section of liver (X100)(H/E) shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI) The hepatocyte are active.

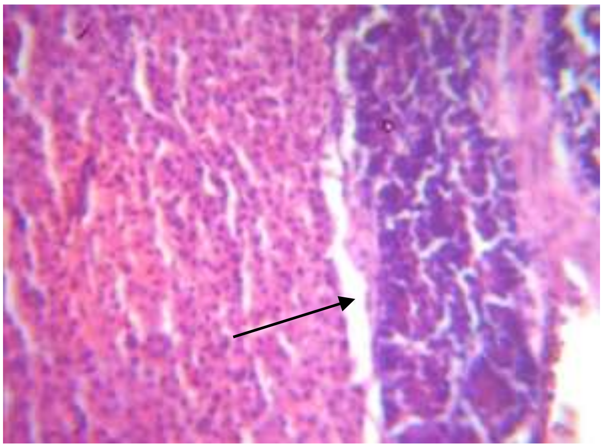


Photomicrograph of T2 section of liver (X100)(H/E) shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI) and congestion of the central vain (CC). The hepatocyte are moderately active.



Photomicrograph of T3 section of liver (X100)(H/E) shows moderate effect on the hepatic tissue with .moderate focal area of hemorrhage (H)

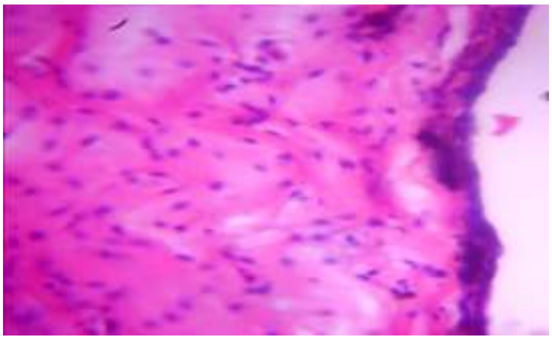


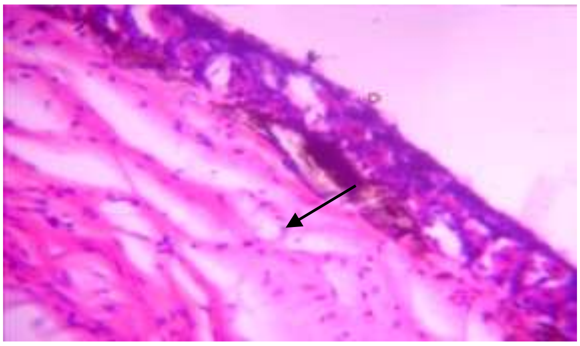


Photomicrograph of T5 section of liver (X100)(H/E) shows sever effect on the hepatic tissue with sever focal area of ovum of parasite (OP) in the liver paraenchyma and fibrous strand (FS) . The overall feature are consistence with (SCHISTOSOMIASIS OF THE LIVER)

Photomicrograph of T6 section of liver (X100)(H/E) shows sever effect on the hepatic tissue with sever ground glass (GG) appearance of the cytoplasm and moderate intra hepatic hemorrhage (IHH)

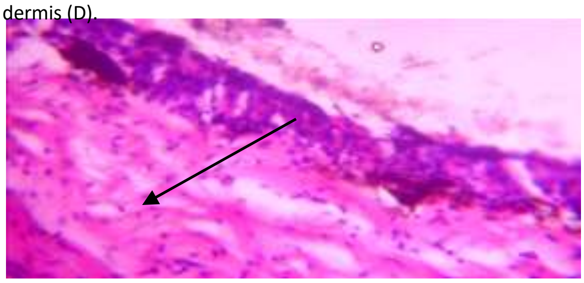
Plate D: (Fig 23-28)Histological change observed in the Skin of Juvenile Catfish *Clarias gariepinus* treated with different concentration of plant *Milletia grifoniana* .





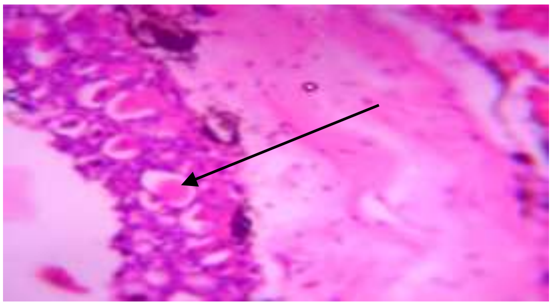
Photomicrograph of Group T0 control section of Skin (x400)(H/E) shows normal skin architecture with epidermis (E) dermis (D).

Photomicrograph of T1 section of skin (X100)(H/E) shows moderate to sever effect on the sin layer with severe aggregate of lymphocyte(L) along dermoepidermal region with moderate melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA). C

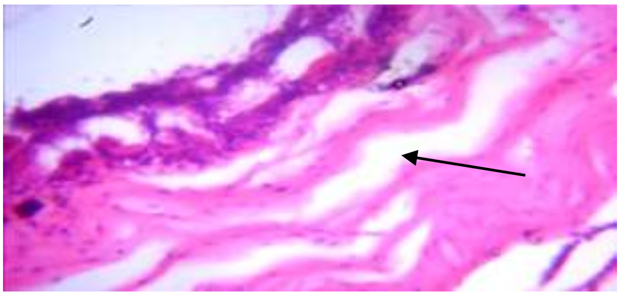


Photomicrograph of T2 section of skin (X100)(H/E) shows sever effect on the sin layer with severe aggregate of lymphocyte along dermoepidermal region with moderate melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA)

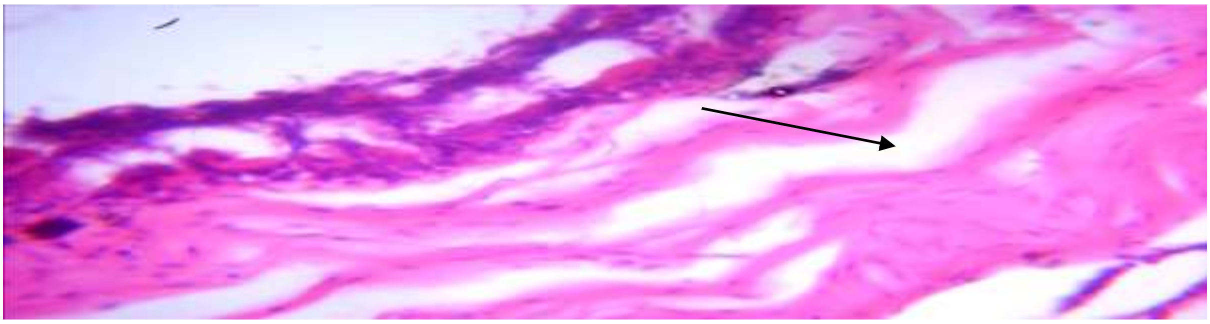
Photomicrograph of T3 section of skin (X100)(H/E) shows moderate effect on the skin layer with moderate superficial spreading of melanoma (M) at the dermoepidermal region, mild lymphocyte infilteration (LI) and mild melanin laden macrophages(MLM) , The overall



Photomicrograph of T5 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M) filled with eosinophilic material . at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)



Photomicrograph of T5 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M). at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)



Photomicrograph of T5 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M). at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)

Table 10: Histological changes observed in Juvenile Catfish *Clarias gariepinus* exposed to ichthyotoxic plant *Milletia grifoniana*.

Conc. (mg/l)	GILLS	SKIN	LIVER	KIDNEY
0.00	Normal gill architecture with well projected filament (PF) , gill epithelium (GE) and cartilages (C).	Photomicrograph of Group T0 control section of Skin (x400)(H/E) shows normal skin architecture with epidermis (E) dermis (D).	Photomicrograph of T0 control section of liver (X100)(H/E) shows normal hepatic architecture with normal hepatocyte (H) and central vain (C).	Photomicrograph of T0 control section of kidney(X150)(H/E) shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC)
0.05	shows section of gill with active filaments (F)	Photomicrograph of T1 section of skin (X100)(H/E) shows moderate to sever effect on the sin layer with severe aggregate of lymphocyte(L) along dermoepidermal region with moderate melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA). C	Photomicrograph of T1 section of liver (X100)(H/E) shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI) The hepatocyte are active.	Photomicrograph of T1 section of kidney (X150)(H/E) shows renal tissue with mild areas of inflammation (I)
0.10	shows moderate effect on the gill architecture with moderate hypertrophy (H) of the filament	Photomicrograph of T2 section of skin (X100)(H/E) shows sever effect on the sin layer with severe aggregate of lymphocyte along dermoepidermal region with moderate melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA).	Photomicrograph of T2 section of liver (X100)(H/E) shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI) and congestion of the central vain (CC) The hepatocyte are moderately active.	Photomicrograph of T2 section of kidney(X150)(H/E) shows renal tissue with severe aggregate of inflammation (AI) with eosinophilic (E) tubules .
0.15	shows mild effect on the gill architecture with mild focal area of hemorrhage (H) otherwise normal,	Photomicrograph of T3 section of skin (X100)(H/E) shows moderate effect on the skin layer with moderate superficial spreading of melanoma (M) at the dermoepidermal region,	Photomicrograph of T3 section of liver (X100)(H/E) shows moderate effect on the hepatic tissue with	Photomicrograph of T3 section of kidney(X150)(H/E) shows renal tissue with moderate aggregate

		mild lymphocyte infilteration (LI) and mild melanin laden macrophages(MLM) , The overall features are consistence with (MODERATE MELANOMA)	.moderate focal area of hemorrhage (H)	of inflammation (AI) with eosinophilic (E) tubules .
0.20	shows severe effect on the gill architecture with severe areas necrotic filament (NF)	Photomicrograph of T4 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M) filled with eosinophilic material . at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)	Photomicrograph of T4 section of liver (X100)(H/E) shows moderate effect on the hepatic tissue with .moderate focal area of hemorrhage (H)	Photomicrograph of T4 section of kidney(X150)(H/E) shows renal tissue with moderate to severe aggregate of inflammation (FA I) and areas of hemorrhage (H)c
0.25	shows severe effect on the gill architecture with severe areas necrotic filament (NF)	Photomicrograph of T5 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M). at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)	Photomicrograph of T5 section of liver (X100)(H/E) shows severe effect on the hepatic tissue with sever focal area of ovum of parasite (OP) in the liver paraenchyma and fibrous strand (FS) . The overall feature are consistence with (**SCHISTOSOMIASIS OF THE LIVER**)	Photomicrograph of T 5 section of kidney(X150)(H/E) shows renal tissue with moderate aggregate of inflammation (A I)
0.30	shows severe effect on the gill architecture with severe clumping ,necrotic (N) filament (C) and areas of hemorrhage (H)	Photomicrograph of T6 section of skin (X100)(H/E) shows severe effect on the skin layer with sever keratin microcytes (M). at the dermoepidermal region with moderate lymphocyte infilteration (LI). The overall features are consistence with (MODERATE MELANOMA)	Photomicrograph of T6 section of liver (X100)(H/E) shows sever effect on the hepatic tissue with sever ground glass (GG) appearance of the cytoplasm and moderate intra hepatic hemorrhage (IHH)	Photomicrograph of T 7 section of kidney(X100)(H/E) shows renal tissue with moderate intra renal inflammation (A I) with active tubules.

DISCUSSION

The result of Length-weight relationship and condition factors of *Clarias gariepinus* Juvenile exposed to ichthyotoxic plant *Milletia grifoniana* is presented in table 1. The weight (g) varies from (57.75±3.6 - 69.20±0.2) – (49.75±3.6 - 67.27±5.0) and standard length (18.57 ±0.5 - 20.96±1.3) – (18.57±0.4 - 20.95±1.3) with the condition factor range from (0.8 – 0.9) - (0.7 – 0.9) for range finding test and definitive test respectively, this result indicates that the experimental fish are in good conditions of health. The percentage cumulative mortality is presented in tables 2 and 3, mortality increases with increases in concentration and time of exposure. Table 2 summarizes the 96 hours LC_{50} values of Ichthyotoxic Plants *Milletia grifoniana* (leaves) of different time intervals and the mortality rate in the *Milletia grifoniana* is concentration dependent, the higher the concentration of toxicant the higher the mortality of fish as shown in tables 3 and 4. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC_{50} were 2.5mg/l, 2.3mg/l, 1.8mg/l, 1.5mg/l, 1.2mg/l respectively, with the range of maximum admissible concentration (MATC) were 0.25 – 0.025mg/l, 0.23 – 0.023mg/l, 0.18 – 0.018mg/l, 0.1 – 0.015mg/l, 0.12 – 0.012mg/l respectively as presented in Table 2 Figure 1. The 100% mortality was observed in the group fish exposed to 0.30 mg/l.No mortality was observed in the group of fish in the control experiment.

The LC_{50} value of *Milletia grifoniana* observed in this study was found to be lower than the work of Criswell *et al.*, 2014, who reported the median lethal concentration of ethanol extract of *A. obesum* stem bark to *C. gariepinus* as 7.15 mg L^{-1}. This showed that the ethanol extract of *A. obesum* stem bark was toxic to the exposed C. gariepinus. The established LC_{50} value of

7.15 mg L^{-1}showed that the ethanol extract of *A. obesum* was highly toxic to the exposed C. gariepinus over the 96-h period because the lower the LC50 of a substance the more acutely toxic is that substance (Criswell *et al.*, 2014).WHO (1992) reported 96h – LC_{50} of 0.02 – 0.2mg/L for different fish species and for daphnids (Water Fleas) exposed to rotenone. Akeredolu *et al*., (2022) reported the 96h LC_{50} values were 7.63mg/l, 0.7mg/l and 15.97mg/l for atrazine, butachlor and glyphosate in their work on Acute and sub-lethal toxicity in African mud catfish (*Clarias gariepinus*, Burchell, 1822) exposed to some pesticides, respectively. Histological sections of the liver of *C. gariepinus* exposed to the three pesticides showed mild vascular congestion but no necrosis in the tissue. There was a significant ($p<0.5$) dose-dependent increase in micronuclei and nuclear abnormalities in the erythrocytes of exposed *C. gariepinus* compared to the control by 28 days. The study confirmed that *C. gariepinus* are at risk of adverse effects from exposure to pesticides.

Guerrero *et al.,* (1986) reported 96h LC_{50} of 10 – 20ppm for *O. niloticus* fingerlings exposed to *Derris* root powder. Abalaka *et al*., 2015 worked on the histopathological effects of ethanol extract of *Adenium obesum* stem bark in the gills and skin of African sharptooth catfish, Clarias gariepinus, and reported the median lethal concentration of the extract was 7.15 mg/l. The results showed that the 24-hour LC_{50} values are within the levels used in fish ponds. Twenty four-hour (24h) LC_{50} are useful measures of relative acute lethal toxicity to organism under certain experimental conditions, but, these values do not represent safe concentration in natural habitats.

The physicochemical parameters of fish culture water were all within acceptable limit for the growth and survival of *C. gariepinus* as earlier reported by Viveen *et al.* (1985) and Bruton (1988). Table 5 summarizes the range of the physicochemical parameters of control and treated fish observed during the experimental period. There was no significant difference among the different treatments in terms of physicochemical parameters measured. Alkalinity significantly increased throughout the experiment. The result of the physicochemical parameters of the experimental media indicated a significant difference ($P<0.05$) in the values obtained in the level of dissolved oxygen, content free carbon dioxide and alkalinity with the control. Nevertheless, there were no significant difference ($P>0.05$) between the values of temperature and pH of test media with the control. The abnormal behaviour observed in fish exposed to the extract included respiratory distress, loss of balance, gulping of air, settling at the bottom motionless, and erratic swimming. The result obtained from this research from water quality of the test media is sufficient for the survival of living organism however; the mortality of the test fish could be attributed to the direct toxicity of the leaves extract of T. vogelii on Tilapia zilli. The behavioral changes which were characterized by respiratory distress, loss of balance, air-gulping, settling at the bottom motionless and erratic swimming as reported in this investigation compared favorably with the observation of (Pascual *et al*., 1994; Svecevicius, 2006; Absalom *et al*. 2009) when they exposed some species of fish to different toxicants.

Table 6 and 7 show the behavioural changes of *Clarias gariepinus* Juvenile exposed to different concentration of Ichthyotoxic Plants *Milletia grifoniana* leaves. Fish showed initial disturbed swimming movements, rapid opercular movements, loss of balance incessant gulping of air, and fish settling at the bottom motionless with slow opercular movements. There were no obvious changes in fish behaviour in the lower concentrations. The abnormal behaviors displayed by the fish increased with increasing concentrations of Ichthyotoxic Plants *Milletia grifoniana* (leaves) in water and time of exposure. Studies revealed that organisms exposed to toxicants usually exhibit changes in opercular rate, erratic, sudden jerky swimming movements and different behavioral activitiesas shown in this experiments which demonstrated to be a sensitive indicator of physiological stress in fish subjected to sub-lethal concentration of pollutants (Derris 1973). The behavioral responses obtained from the study compared favorably with the observation of Pascual *et al*., (1994) when formalin at different concentrations were used on sea bass (*Lates calcarifer*) fry. The observations in this study agreed with Lin and Liu (1990) who reported thatclinical signs such as abnormal movement and high respiration rate in hybrid tilapia (*O.mossambicus*) induced by ammonia suggested neurological dysfunction and gill damage.

Respiratory distress noticed in exposed fish could be caused by mucous precipitation and neurological dysfunction of gill epithelia in response to the toxicant which resulted in high respiratory rate as reported by (Lin and Lin, 1990; Banerjee, 2007). The opercular ventilation count which declined after each 24hours could be as a result of the inhibitory action of the toxicant on respiration as well as malfunctioning of some vital organs which may reduce the available energy for respiration. Increased ventilation rates could be as the result of the toxicant in the test media as it reduced the amount of oxygen present in the media. The fish could have increased ventilation rates in an attempt to make up for the loss in oxygen content in the gill. High opercular ventilation has been reported by (Sprague, 1973) as an index of stress when fish come in contact with an unfavourable environmental condition. The 96-hour LC_{50} which was estimated as 0.7ml/l means that the reduction of fish ability to maintain body equilibrium was recorded in concentration close to and in excess of the LC_{50} value

Table 9 present the result of toxicity of aqueous extract of *Milletia grifoniana*on haematological parameters of *Clarias gariepinus* Juvenile. There was reduction in nearly all the results of blood parameters of Sharp tooth Catfish *Clarias gariepinus* Juvenile exposed to ichthyotoxic plant *Milletia grifoniana*in the respective treatments as presented in table 8.

Variation occurred in the values obtained in the results of Proximate composition of the carcass of*Clarias gariepinus* juvenile exposed to aqueous extract of *Milletia grifoniana*Table 9, There was no significant difference ($P<0.05$) in Crude Protein (CP) (Mg/l), Energy K(cal)/mg, and Crude Fiber (CF). Ash and Nitrogen Free Extract (NFE) reduce from 3.34±0.6, 0.92±0.1 and 0.49±0.2 to 26.87±2.1, 0.16±0.2 and 0.43±0.2respectively when compared with the control, there is an increase in the value of Moisture (M) from 64.84±3.3 to 67.47±5.0when compared with the control.

Abubakar *et al.,* (2023) reported in their work that Gills and Liver Histology of *Clarias gariepinus* exposed to Aqueous Extract of *Carica papaya* Seed under Laboratory Condition. Exposed fish gill showed degeneration of the gill filament, hypertrophy, hyperchondrial of the gill arch and lesion. Normal tubules, haematopoietic structures, and a portal triad with a dilated portal vein were found in the livers of control fish. The exposed fish livers shows vacuolation, steatosis, necrosis and degeneration of hepatocyte degeneration. The findings revealed that histological studies of fish gill and liver could be useful biomarker for assessing aqueous extract of *C. papaya* dry seeds toxicosis. High mortality occurred in fish exposed to the leaves extract show severe gill epithelial hyperplasia, separation of the gill epithelial layers from supportive tissues. These according to (Omoregie and Ufodike, 1991; Banerjee and Chandra, 2005) can lead to brachial malfunction of which may affect physiology or causes death to fish. The vacuolated cells and necrosis of the liver as observed in the exposed fish are the liver lesions associated with the *T. vogelii* toxicity which could be the result of the excessive work required by the fish to get rid of the extract from its body during the process of detoxification by the liver. Similar investigation was reported by Wade *et al.* (2002) and *Akpa et al*. (2009) when cassava effluent and crude bark extract of Indian siris were exposed to *Oreochromis niloticus* respectively. The degeneration of the kidney tubule may be as a result of the toxicity effects of the extract. Rotenone as one of the active ingredients of the T. vogelii as observed in the phytochemical analysis is one of the contributing elements in fish mortality. This agreed with WHO (2007) that reported rotenone to be lethal to fish because it readily enters the blood stream of the fish through the gills. Robertson and Smith-vaniz (2008) documented that the specific site of action of rotenone is in the electron transport system where it blocks a mitochondrial enzyme called NADH ubiquinone reductase, consequently the blood oxygen content of the fish will increase because oxygen is now unavailable for respiration.

The extract caused some histopathological lesions in the gills and skin of the exposed fish. However, the severity but not the type of the lesions observed in the gills and skin of the exposed fish was concentration-dependent. Although the degree of tissue change (DTC) grading indicated moderate damage in the gills of the exposed fish, there were no significant ($p>0.05$) differences between gills DTC of the exposed and unexposed fish. Abalaka *et al*., (2015). High mortality occurred in fish showing severe gill epithelial hyperplasia, separation of the gill epithelial layers from supportive tissues, necrosis of liver hepatocytes. Gill alterations such as epithelial hyperplasia and separation of the epithelial layer from supportive tissues are usually directly related to gill function disorders, which may affect the physiology or cause the death of fish (Smart 1976). Liver parenchymal necrosis, fatty degeneration, blood cell congestion and fibroses are non-specific liver lesions associated with pesticides toxicity (Cahn 1975). The histopathological alterations found in the gills and liver of *O. niloticus* seem to be all caused by the piscicide *(Derris* root powder extracts). Based on these results, *Orechromis niloticus* can tolerate the levels of *Derris* powder being used in fishponds. However, histological analyses of gills and liver showed pathological changes even at sub-lethal levels. Thus, an application factor of 0.1 is recommended to be multiplied with the 24-hour LC50 value to estimate the safe concentration of *Derris* roots powder extract for *O. niloticus* at 186mg/L.

CONCLUSION

The *Milletia grifoniana*is an ichthyotoxic plant used in catching fish in Nigeria. The toxicity of aqueous extract of *Milletia grifoniana* to the *Clarias gariepinus* Juvenile is due to its active ingredients which have various chemical properties and interfere with the physiology of the test organism. The active ingredients in the leaves extract were alkaloid, tannin, saponin, cardiac glycoside, rotenone, and steroids. The mortality rate in the *B. micrantha* is concentration dependent, the higher the concentration of toxicant the higher the mortality of fish. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. The fish exhibited different behaviours such as higher Air gulping, Erratic swimming, Loss of balance, Excessive mucus secretion, Operculum movement, Moulting, Discoloration, Barbell deformation and Loss of Reflex to the values obtained for the control. These signs increased with increasing extract concentration and increasing exposure period. Mortality was recorded in some of the exposed fish. However, the unexposed control fish did not show any of these signs of toxicity. There was reductions in Carcass/Proximate composition of *Clarias gariepinus* juvenile exposed to aqueous extract of *B. micrantha*, Moisture, Crude Protein, Crude Lipid, Ash, NFE, and Energy reduced from 66.24±6.9, 29.71±2.3, 3.49±0.4, 0.90 ± 0.0, 0.38 ± 0.1, 1.37 x 10^2 ± 6.6 to 64.56 ± 6.2, 26.21±1.3, 3.31±0.5, 0.83±0.1, 0.25±0.0 and 1.31 x 10^2 ±4.0 respectively. The *Milletia grifoniana* shows severe effect on the skin layer with severe superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain severe melanin laden macrophages (MLM). The liver cells revealed hepatic tissue with severe ground glass (GG) appearance of the cytoplasm and intra hepatic hemorrhage (IHH). The gill showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI), while the kidney revealed severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in same areas. The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. The result of this study calls for the need to discourage the use of toxic plants for catching fish in Nigeria inland water bodies.

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