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TOXICITY OF AQUEOUS EXTRACT OF ICHTHYOTOXIC PLANT*ADENIA LOBATA* (JACQ.) PASSIFLORACEAEON THE AFRICAN CATFISH (*CLARIAS GARIEPINUS*) (BURCHELL, 1822) JUVENILE.

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Abstract

This work investigates the toxic effects of Ichthyotoxic plant, *Adenia lobata* (Jacq.) Passifloraceae on the African Catfish (*Clarias gariepinus*) (BURCHELL, 18 22) Juvenile under statics laboratory conditions. *Adenia lobate* leaf extract was applied at concentrations of 0.50, 1.00, 1.50, 2.00, 2.50 and 2.50and 0.00mg/L as control for 96 hours. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC_{50} were 2.7mg/l, 2.0mg/l, 1.8mg/l, 1.5mg/l and 1.2mg/l respectively, with the range of maximum admissible toxicant concentration (MATC) of0.27 – 0.027mg/l, 0.20 – 0.020mg/l, 0.18 – 0.018mg/l, 0.15 – 0.015mg/l, and 0.12 – 0.012mg/l. *There was no significant difference in physicochemical parameters of test media (P<0.05) before, during and after the experimental period. F*ish exhibited different behavioural changes such as higher Air gulping, Erratic swimming, Loss of balance, Excessive mucus secretion, Operculum movement, Moulting, Discoloration, Barbell deformation and Loss of Reflex *when compared with the control*, these signs increased with increasing extract concentration and exposure period. Blood analysis revealed significant (P<0.05) reduction in the blood parameters, White blood cell, Red blood cell, Haematocrit, Lymphocytes, Mean cell volume, reduce from $1.73x10^2$±8.7, $3.40x10^6$±1.0, 23.31±2.1, 96.41±1.1, and 1.16x102±2.2 to $1.60x10^2$±22.3, $2.38x10^6$±1.0, 16.38±9.4, 88.42±1.2, and 1.09x102±1.5 respectively, *when compared with the control. There was an increase in* Haemoglobin, Platelet, Mean cell Haemoglobin, Mean cell Haemoglobin concentration increases from 9.06±0.8, 3.02x104±1.5, 47.57±3.3, and 41.06±3.0 to 9.89±4.2, 3.55x104±3.3, 49.25±1.1 and 49.10±1.3.There was significant reduction in proximate composition of the carcass of *Clarias gariepinus* juvenile exposed to aqueous extract of *Adenia lobata*, ash, Nitrogen Free Extract (NFE), and Energy reduce from 0.93±0.1, 0.51±1.2, and $1.36x10^2$±4.8 to 0.85±0.2, 0.44, 0.44±0.2 and $1.25x10^2$±3.4 respectively. There was no significant changes in the value of Crude Protein, Crude Lipid, Crude Fiber, and Moisture, their values increases slightly from 26.28±4.6, 3.34±0.1, 0.00±0.0 and 68.69±0.7 to 26.82±2.0, 3.34±0.6, 0.00±0.0 and 68.18±0.9 respectively. Histopathological examinations of the test fish showed some pathological disruptions, such as, severe superficial spreading of melanoma (M) restricted to the epidermis on the skin, the gil l showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI),the liver cells revealed moderate to severe degeneration with severe focal area of cirrhosis(C) with pale cytoplasm accumulation of fat, while the kidney revealed moderate to severe effect on the renal tissue, 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 plant sAdenia lobata for catching fish in Cross River and Nigeria water bodies, and providing alternative eco-friendly techniques for fish harvesting may possibly bring constructive outcome **i**n the near future.

Keywords: Ichthyotoxicity, Aqueous Extract, Mortality, *Adenia lobate, Clarias gariepinus*

1.0 Introduction

*Adenia lobata*belongs to the family Passifloraceae, is a large climbing shrub producing stems up to 45 metres long and up to 12cm in diameter. The stems attach themselves to other plants for support by means of tendrils. *Adenia lobata* is a medicinal plant that is widely used as traditional medicine in several countries in Africa, including Nigeria (Akendengue *et al*., 2005). It is used for the treatment of cough, respiratory disorder, syphilis, gonorrhoea and cancer of the nose (Gill, 1992; Osuagwu and Ibeabuchi, 2010).These plants used in treating human ailments and animal diseases may be considered poisonous and their beneficial effects often occur at lower doses whereas overdose can induce poisoning (Botha and Penrith, 2008). *Adenia lobata*leave when crushed is used as fish poisons.The active ingredient in *Adenia lobata* leaf are alkaloids, carbohydrate, glycosides, saponin, flavonoids and tannins, which can make the fish dizzy or kill the fish out rightly thereby making them easy to catch. Notable among them are saponins and rotenones, Bearez (1998).

Saponins are amphipathic glycosides grouped in terms of phenomenology, by the soap-like foaming they produce when shaken in aqueous solutions, Rotenone is the second major fish poison in plants. When rotenone is introduced to water by crushing or mashing of the appropriate plant parts, fish respiration is damaged and they are forced to gulp the air at the water surface where they are vulnerable it inhibits cellular processes, depriving fish of oxygen in their tissue cells. Cyanogenic glycosides, Gynocardin, flavonoids, xylosylvitexin, vitexin, violanthin, vicenin-2 and schaftoside also present are saponins and rotenones, Bearez (1998) (Katewa *et al*, 2007).However, the effects of ichthyotoxic plants on fish are varied and can be categorized as: Physical damage or irritation of the gills. Toxigenic reactions to ichthyotoxic agents,blood hypoxia from environmental oxygen depletion. The damage to fish can also be caused by a combination of these effects.

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.

2.0 Material and Methods

2.1 Location of Study: This research was carried out at the Department of Fisheries and Aquatic Science, Wet Laboratory, Cross River University of Technology (CRUTECH), Obubra Campus.

2.2 Collection and preparation of plant samples

Ichthyotoxic Plants *Adenia lobate* (leaves) was collected around the University communities at Obubra Campus where they were abundant. The plant sample was collected in the early hours of the day between 6:00 and 9:00 am. After collection, the samples were taken to the Herbarium unit of the Department of Forestry Obubra Campus, Cross River University, Nigeria for proper identification. The plant sampled was air dried in the laboratory, at room temperature for two weeks and then oven dried at 32 ˚C for 30 minutes. The leaf *Adenia lobate* 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.

2.3 Aqueous extraction of Ichthyotoxins

Ichthyotoxins from the plant sample was 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).

2.4 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.

2.5 Toxicity Experiment

Test organism/ Acclimation

Clarias gariepinus (African Catfish) was used as test organism in this study. *C. gariepinus* juveniles (4-6 weeks old) were purchased from Amazons Agro-World Venture Fish Farm Abakaliki, Ebonyi Sate. Nigeria and transported in oxygenated polythene bags, to the Fisheries wet Laboratory, Department of Fisheries and Aquatic Science, CRUTECH/UNICROSS 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 (Coppens 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 24 h before the commencement of experiments.

2.6 Stock solution of Ichthyotoxins

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

2.7 Acute toxicity studies (Range finding Test)

The acute toxicity studies was conducted 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* juveniles were exposed to 10, 20, 30, 40, 50, 60Mg/L and 0 mg/L as control, of each of the plant leaf extracts for 24 hours.

2.8 Sub-acute toxicity studies (Definitive Test)

Clarias gariepinus juveniles were exposed to sub-acute concentrations of 0.50, 1.00, 1.50, 2.00, 2.50 and 2.50and 0.00mg/L as control, of each of the plant leaf extracts for 96 hours, 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).

2.9 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^{TM}).

2.10 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 was killed with a blow on the head, using a mallet and was dissected to remove the vital organs (gill, liver and skin). The organs were fixed 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 & Cook, (1994), using a microtone and each section were cleared by placing in warm water (38^{o}C), where it was picked with clean slide and oven-dried at 58^{o}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 was observed 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).

2.11 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 (Pearson, 1976) 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^{o}C dissolved in 10% HCl (25ml) and 5% Lanthanum

Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

2.12 Statistical analysis

The dose-response data obtained from the acute toxicity study was 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.

3.0 Results

The toxicity of ichthyotoxic plant *Adenia lobata* (Jacq.) Passifloraceaeleaves extract on *Clarias gariepinus* are presented in Table 1. The mortality rate in the *A. lobata*is concentration dependent, the higher the concentration of toxicant the higher the mortality of fish as shown in tables 2 and 3. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC_{50} were 2.7mg/l, 2.0mg/l, 1.8mg/l, 1.5mg/l and 1.2mg/l respectively, with the range of maximum admissible concentration (MATC) were 0.27 – 0.027, 0.20 – 0.020, 0.18 – 0.018, 0.15 – 0.015, and 0.12 – 0.012respectively as presented in Table 1 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 3.00 mg/l. The result of Length-weight relationship and condition factors of *Clarias gariepinus* Juvenile exposed to ichthyotoxic plant *Adenia lobata* is presented in table 4. The weight (g) varies between (56.71±6.2 - 62.01±6.5) (54.71±5.2 - 60.01±6.5) and Standard length (cm) (18.68±0.5 - 19.11±1.0)-17.67±0.5) with the condition factor range from (0.8 – 0.9) - (0.8 – 1.3) for range finding test and definitive test respectively, this result indicates that the experimental fish are in good conditions of health.

The result of physiochemical parametersin the present study Table 5, exhibited variation in values, t***here was significant difference between the water quality parameters before, during the experiment and after the experiment. No significant change in Temperature (***(26.00±0.6 - 26.48±1.2) ***(P<0.05) was observed. pH increases from*** 6.01±0.4 - 7.18±1 while ***Dissolve Oxygen Concentration and Conductivity***(4.69±0.9 - 3.7±0.8) (41.57±3.9 - 35.29±9.2) ***were observed to reduce significantly (P<0.05) respectively.*** Table 6 and 7 shows the general behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Adenia lobate*, 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.

Table 1: The LC_{50} values of *Clarias gariepinus*Juvenile.

S/N	TIME(Hrs)	LC_{50}	MATC (Mg/l)
1	24	2.7	0.27 – 0.027
3	36	2.0	0.20 – 0.020

3	48	1.8	0.18 – 0.018
4	72	1.5	0.15 – 0.015
5	96	1.2	0.12 – 0.012

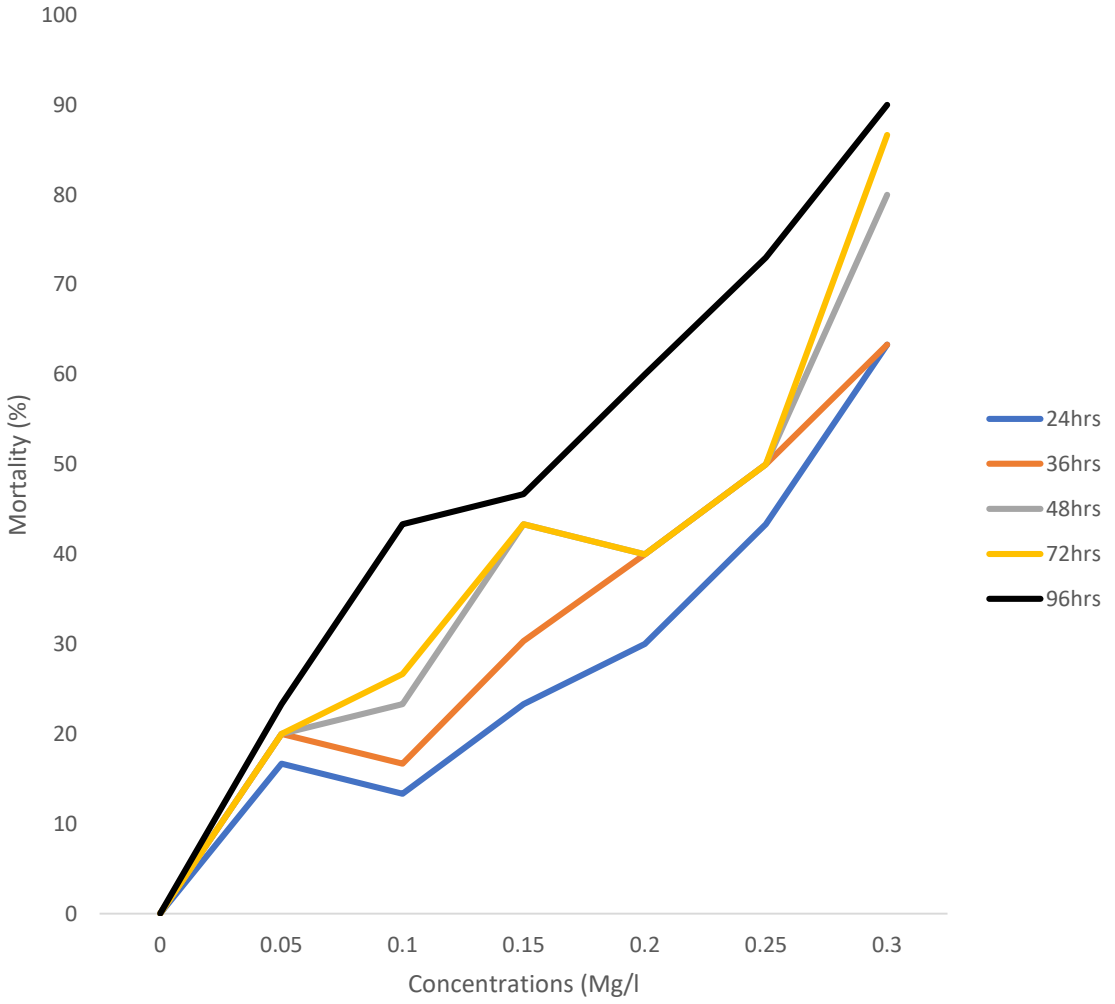


Figure 1: Determination of LC_{50} Using Probit (Graphical) Method (Finney 1971; USEPA 2000).

There was reduction in the results of blood parameters of Sharp tooth Catfish Clarias gariepinus Juvenile exposed to ichthyo toxic plant Adenia lobate 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, Red blood cell, Haematocrit ,Lymphocytes, Mean cell volume, reduce from $1.73x10^2$±8.7, $3.40x10^6$±1.0, 23.31±2.1, 96.41±1.1, and 1.16x102±2.2 to $1.60x10^2$±22.3, $2.38x10^6$±1.0, 16.38±9.4, 88.42±1.2, and 1.09x102±1.5 respectively, when compared with the control. There was an increase in Haemoglobin, Platelet, Mean cell Haemoglobin, Mean cell Haemoglobin concentration increases from 9.06±0.8, 3.02x104±1.5, 47.57±3.3, and 41.06±3.0 to 9.89±4.2, 3.55x104±3.3, 49.25±1.1 and

49.10±1.3 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 Adenia lobate Table 9,Ash, Nitrogen Free Extract (NFE), and Energy reduce from 0.93±0.1, 0.51±1.2, and $1.36x10^2$±4.8 to 0.85±0.2, 0.44, 0.44±0.2 and $1.25x10^2$±3.4 respectively, while there is no significant changes in Crude Protein, Crude Lipid, Crude Fiber, Moisture from 26.28±4.6, 3.34±0.1, 0.00±0.0 and 68.69±0.7 to 26.82±2.0, 3.34±0.6, 0.00±0.0 and 68.18±0.9 respectively.

Figures 10, Plate A-D (figures 2-29) present the results of tissue analysis of fish from the respective treatment. Histopathological examinations of the test fish showed some pathological disruptions. The Adenia lobate shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non-distinct outline. The skin shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM) The overall feature are consistence with (MCHRONIC MELOMA). The gill showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI). The liver cells revealed moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat, while the kidney revealed shows moderate to severe effect on the renal tissue with moderate focal area of intra renal inflammation (IRI) and severe tubular atrophy (TA). The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank.

Table 2: Mean percentage cumulative mortality of *Adenia lobata* 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
1.50	0.00	3.33	3.33	13.00	13.00	13.00	16.67	23.33	23.00	36.67	36.67	40.00
3.00	3.33	3.33	6.67	23.33	23.33	30.00	33.33	33.33	36.67	43.33	43.33	46.67
4.50	10.00	13.33	23.33	26.67	33.33	40.33	50.00	50.00	63.33	66.67	70.00	83.55
6.00	6.67	13.33	16.67	33.33	33.33	40.00	50.00	56.67	56.67	60.00	60.00	86.67
7.50	6.67	20.00	30.00	43.33	46.67	46.67	50.00	56.67	60.00	66.67	66.67	96.67

Table 3: Mean percentage cumulative mortality of *Adenia lobata* to *C. gariepinus* adult (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
0.50	0.00	0.00	0.00	0.00	0.00	0.00	3.33	3.33	13.33	16.67	16.67	18.67	20.00
1.00	0.00	3.33	10.00	10.00	13.33	13.33	13.33	20.00	20.00	23.00	30.00	30.00	40.00
1.50	3.33	3.33	16.67	16.67	23.33	34.33	26.67	30.00	33.33	36.67	40.00	40.00	66.67
2.00	0.00	3.33	10.00	10.00	13.33	16.67	20.00	23.33	30.00	56.67	56.67	56.67	73.33
2.50	0.00	3.33	10.00	13.33	16.67	20.00	30.00	30.00	43.33	56.67	56.67	70.00	80.00
3.00	13.33	16.67	30.00	30.00	36.67	50.00	50.00	56.67	60.00	70.00	76.67	76.67	83.33

Table 4.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	56.71 ± 6.2^{a}	18.68 ± 0.5^{a}	0.9	0.00	54.71 ± 5.2^{a}	17.67 ± 0.5^{a}	1.0
1.50	61.07 ± 5.9^{ab}	18.61 ± 0.3^{a}	0.9	0.50	60.07 ± 5.9^{ab}	16.61 ± 0.3^{a}	1.3
3.00	65.49 ± 7.2^{b}	19.35 ± 1.1^{ab}	0.9	1.00	55.49 ± 7.2^{b}	18.35 ± 1.1^{ab}	0.8
4.50	63.06 ± 8.1^{ab}	19.93 ± 1.5^{b}	8.0	1.50	53.06 ± 8.1^{ab}	18.93 ± 1.5^{b}	0.8
6.00	63.62 ± 4.3^{ab}	19.09 ± 0.6^{ab}	0.9	2.00	53.62 ± 4.3^{ab}	17.09 ± 0.6^{ab}	1.1
7.50	62.10 ± 5.1^{ab}	19.02 ± 0.9^{ab}	0.9	2.50	62.10 ± 5.1^{ab}	18.02 ± 0.9^{ab}	1.1
8.00	62.01 ± 6.5^{ab}	19.11 ± 1.0^{ab}	0.9	3.00	60.01 ± 6.5^{ab}	17.11 ± 1.0^{ab}	1.2

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 5: Summary of Water Quality Parameter of *Adenia lobata*to *Clarias gariepinus* (Mean ± SD

RANGE FINDING TEST					DEFINITIVE TEST				
Conc.	Temp(°C)	pH	Conductivity	DO (mg/L	Conc.	Temp(°C)	pH	Conductivity	DO (mg/L
0.00	25.00±0.6^{a}	7.01±0.4^{ab}	40.57±3.9^{ab}	3.69±0.9^{ab}	0.00	26.00±0.6^{a}	6.01±0.4^{ab}	41.57±3.9^{ab}	4.69±0.9^{ab}
1.50	25.14±0.9^{a}	6.21±1.6^{a}	37.57±1.0^{ab}	3.96±0.5^{ab}	0.50	26.14±0.9^{a}	6.21±1.6^{a}	37.57±1.0^{ab}	4.96±0.5^{a}
3.00	25.00±0.6^{a}	8.36±1.5^{c}	30.85±1.3^{a}	4.34±1.1^{b}	1.00	2.00±0.6^{a}	6.36±1.5^{c}	30.85±1.3^{a}	4.34±1.1^{c}
4.50	25.43±0.8^{a}	7.60±0.9^{bc}	41.57±3.7^{ab}	3.4±0.4^{a}	1.50	25.43±0.8^{a}	7.60±0.9^{bc}	41.57±3.7^{ab}	3.4±0.4^{bc}
6.00	26.14±1.4^{a}	6.94±0.3^{b}	46.43±5.6^{b}	3.2±0.6^{a}	2.00	26.14±1.4^{a}	6.94±0.3^{b}	42.43±5.6^{b}	3.2±0.6^{a}
7.50	26.14±1.3^{a}	6.97±0.5^{b}	38.71±7.6^{b}	3.6±0.7^{ab}	2.50	25.14±1.3^{a}	6.97±0.5^{b}	38.71±7.6^{b}	5.6±0.7^{ab}
8.00	25.48±1.1^{a}	7.18±1.1^{a}	39.29±9.2^{ab}	3.7±0.8^{ab}	3.00	26.48±1.2^{a}	7.18±1.1^{a}	35.29±9.2^{ab}	3.7±0.8^{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 *Adenia lobata* (Range finding test)

Behaviour/exposure time	6hrs							12hrs							24hrs							48hrs						
Concentration (mg/L)	0.00	1.50	3.00	4.50	6.00	7.50	8.00	0.00	1.50	3.00	4.50	6.00	7.50	8.00	0.00	1.50	3.00	4.50	6.00	7.50	8.00	0.00	1.50	3.00	4.50	6.00	7.50	8.00
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*Adenia lobata*(Definitive test)

Behaviour/exposure time	24hrs							48hrs							72hrs							96hrs					
Concentration (mg/L)	0.00	0.50	1.00	1.50	2.00	2.50	3.00	0.00	0.50	1.00	1.50	2.00	2.50	3.00	0.00	0.50	1.00	1.50	2.00	2.50	8.00	0.00	0.50	1.00	1.50	2.00	2.50	3.00
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 *Adenia lobata*on haematological parameters of*Clarias gariepinus* adult (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	$1.73x10^{2}±8.7^{b}$	$3.40x10^{6}±1.0^{d}$	$9.06±0.8^{a}$	$23.31±2.1^{b}$	$3.02x104±1.5^{a}$	$96.41±1.1^{b}$	$1.16x102±2.2^{b}$	$47.57±3.3^{a}$	$41.06±3.0^{a}$
0.50	$1,70x10^{2}±19.2^{ab}$	$1.25x10^{6}±1.1^{a}$	$8.44±2.9^{a}$	$17.29±2.6^{ab}$	$4.90x104±2.6^{ab}$	$88.79±1.0^{ab}$	$1.04x102±1.1^{ab}$	$43.27±1.2^{a}$	$47.07±1.0^{ab}$
1.00	$1.61x10^{2}±13.7^{ab}$	$1.93x10^{6}±5.6^{ab}$	$8.07±4.0^{a}$	$13.33±7.0^{ab}$	$7.13x104±3.6^{b}$	$88.41±4.2^{ab}$	$1.11x102±1.3^{ab}$	$55.10±1.7^{a}$	$57.72±1.5^{b}$
1.50	$1.60x10^{2}±22.9^{ab}$	$2.19x10^{6}±2.0^{bc}$	$10.07±3.4^{a}$	$15.27±1.1^{ab}$	$2.28x104±2.1^{a}$	$82.94±1.8^{ab}$	$1.13x102±8.1^{ab}$	$47.70±6.7^{a}$	$44.30±1.0^{ab}$
2.00	$1.45x10^{2}±20.4^{a}$	$2.44x106±0.9^{bc}$	$8.87±3.5^{a}$	$18.30±1.6^{ab}$	$4.62x104±4.9^{ab}$	$92.31±4.8^{ab}$	$1.15x102±6.0^{b}$	$54.64±1.6^{a}$	$48.16±1.7^{ab}$
2.50	$1.52x10^{2}±33.7^{ab}$	$3.09x10^{6}±1.0^{cd}$	$14.83±5.9^{a}$	$10.80±2.1^{ab}$	$3.95x104±3.3^{a}$	$81.84±1.8^{ab}$	$107x102±2.8a^{b}$	$47.19±8.6^{a}$	$56.26±1.5^{ab}$
3.00	$1.60x10^{2}±22.3^{ab}$	$2.38x10^{6}±1.0^{d}$	$9.89±4.2^{b}$	$16.38±9.4^{a}$	$3.55x104±3.3^{a}$	$88.42±1.2^{a}$	$1.09x102±1.5^{a}$	$49.25±1.1^{a}$	$49.10±1.3^{ab}$

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 9. The summary of toxicity of aqueous extract of *Adenia lobata* on Carcass/Proximate composition of*Clarias gariepinus* adult (mean±SD)

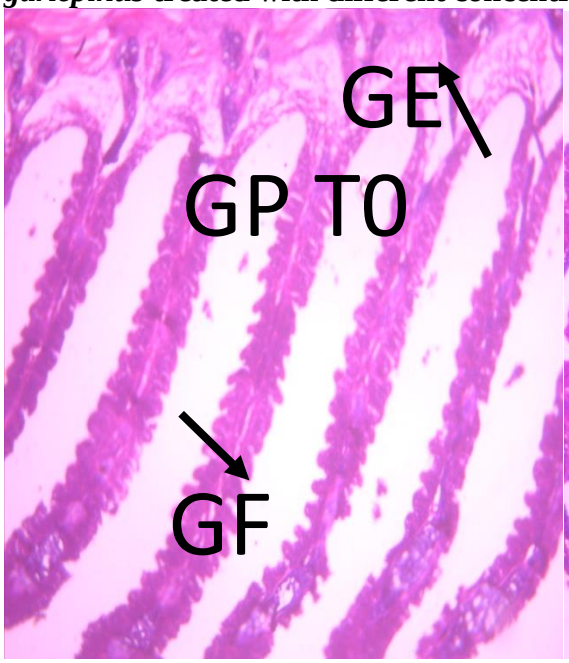
Conc. (mg/L)	Crude Protein (CP) (Mg/l)	Crude Lipid (CL)	Crude Fiber (CF)	Moisture (M)	Ash	Nitrogen Free Extract (NFE)	Energy (Kcal/100g)
0.00	26.28 ± 4.6^{a}	3.34 ± 0.1^{a}	0.00±0.0	68.69 ± 0.7^{ab}	0.93 ± 0.1^{b}	0.51 ± 1.2^{ab}	$1.36x10^{2}\pm4.8^{a}$
0.50	26.34 ± 1.2^{a}	3.28 ± 0.4^{a}	0.00±0.0	68.72 ± 0.8^{ab}	0.69 ± 0.3^{a}	0.49 ± 0.1^{ab}	$1.04x10^{2}\pm1.3^{a}$
1.00	27.13 ± 0.3^{a}	3.72 ± 1.0^{a}	0.00±0.0	71.47 ± 1.5^{b}	0.88 ± 0.0^{b}	0.54 ± 0.2^{b}	$1.37x10^{2}\pm3.0^{a}$
1.50	27.00 ± 0.5^{a}	3.13 ± 0.1^{a}	0.00±0.0	69.28 ± 1.3^{ab}	0.88 ± 0.7^{b}	0.34 ± 0.1^{a}	$1.02x10^{2}\pm9.9^{a}$
2.00	26.82 ± 0.5^{a}	3.09 ± 0.5^{a}	0.00±0.0	65.26 ± 0.5^{ab}	0.81 ± 0.1^{ab}	0.39 ± 0.1^{ab}	$1.36x10^{2}\pm3.0^{a}$
2.50	27.33 ± 1.5^{a}	3.43 ± 0.7^{a}	0.00±0.0	65.37 ± 1.2^{a}	0.90 ± 0.1^{b}	0.38 ± 0.1^{ab}	$1.35x10^{2}\pm4.2^{a}$
3.00	26.82 ± 2.0^{a}	3.34 ± 0.6^{a}	0.00±0.0	68.18 ± 0.9^{a}	0.85 ± 0.2^{b}	0.44 ± 0.2^{ab}	$1.25x10^{2}\pm3.4^{a}$

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.

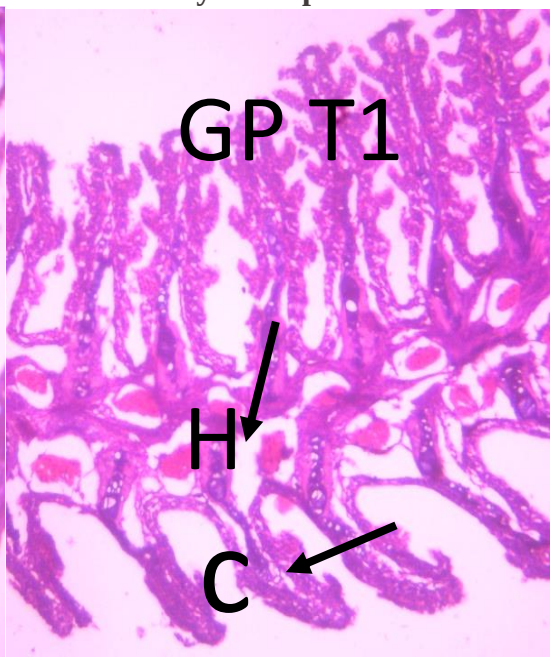
Table 10: Histological changes observed in Juvenile Catfish *Clarias gariepinus* exposed to ichthyotoxic plant *Adenia lobata*.

Conc. (mg/l)	GILLS	SKIN	LIVER	KIDNEY
0.00	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 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 kidney (X400)(H/E) shows normal renal architecture with glomeruli (G) , bowman space (BS), renal tubules (RT)and tubular cell (TC)
0.50	Photomicrograph of T1 section of gill (X150)(H/E) shows mild to moderate effect on the gill architecture with moderate focal area of hemorrhage (H) and mild clumping (C) of the filament	Photomicrograph of T 1 section of skin (X100)(H/E) shows mild effect on the sin layer with mild erosion of the epithelia lining (EEL) and intra epidermal lost (IEL) of tissue	Photomicrograph of T1 section of liver (X100)(H/E) shows moderate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) around the haemorhagic area	Photomicrograph of T1 section of kidney (x400) (H/E) shows mild effect on the renal tissue with mild intra renal inflammation (IRI)
1.00	Photomicrograph of T2 section of gill (X150)(H/E) shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia	Photomicrograph of T 2 section of skin (X100)(H/E) shows moderate effect on the sin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA)	Photomicrograph of T2 section of liver(X100)(H/E) shows moderate to severe degeneration with severe focal area of intra hepatic hemorrhage (IHH)	Photomicrograph of T2 section of kidney (x400) (H/E) shows mild effect on the renal tissue with mild intra renal inflammation (IRI)
1.50	Photomicrograph of T3 section of gill (X150)(H/E) shows moderate effect on the gill architecture with focal area of necrotic filament (NF)	Photomicrograph of T 3 section of skin (X100)(H/E) shows moderate to severe effect on the skin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM)	Photomicrograph of T3 section of liver (X100)(H/E) shows moderate degeneration with severe focal area of intra hepatic hemorrhage (IHH) and focal aggregate of intra hepatic inflammation (IHI)	Photomicrograph of T3 section of kidney (x400) (H/E) shows moderate effect on the renal tissue with moderate intra renal inflammation (IRI) and tubular necrosis (TN)
2.00	Photomicrograph of T4 section of gill (X150)(H/E) shows moderate effect on the gill architecture with focal area of necrotic filament (NF)	Photomicrograph of T 4 section of skin (X100)(H/E) shows moderate effect on the skin layer with the epidermis showing irregular layer , loss of epithelia lining (LEL) and presence of mast cell (MC) in the dermis	Photomicrograph of T5 section of liver (X100)(H/E) shows moderate to severe degeneration with sever focal area of necrosis (FAN) with fibrous strands (FS) within the necrotic area	Photomicrograph of T4 section of kidney (x400) (H/E) shows moderate effect on the renal tissue with moderate intra renal inflammation (IRI) and tubular atrophy (TA)
2.50	Photomicrograph of T5 section of gill (X150)(H/E) shows moderate to severe effect on the gill architecture with areas of necrotic filament (NF) , hypertrophy (H) and hemorrhage (H)	Photomicrograph of T 5 section of skin (X100)(H/E) shows moderate effect on the skin layer with loss of the epithelia lining (LEL) and lymphocytic infiltration ,(LI) within the intradermal region .	Photomicrograph of T5 section of liver (X100)(H/E) shows moderate to severe degeneration with sever focal area of cirrhosis (FAN) with extravassated blood (EVB)	Photomicrograph of T5 section of kidney (x400) (H/E) shows moderate to severe effect on the renal tissue with moderate intra renal inflammation (IRI) and severe tubular atrophy (TA)
3.00	Photomicrograph of T6 section of gill (X150)(H/E) shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non-distinct outline	Photomicrograph of T 6 section of skin (X100)(H/E) shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM) The overall feature are consistence with (MCHRONIC MELOMA)	Photomicrograph of T5 section of liver (X100)(H/E) shows moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat	Photomicrograph of T6 section of kidney (x400) (H/E) shows moderate to severe effect on the renal tissue with moderate focal area of intra renal inflammation (IRI) and severe tubular attrophy (TA)

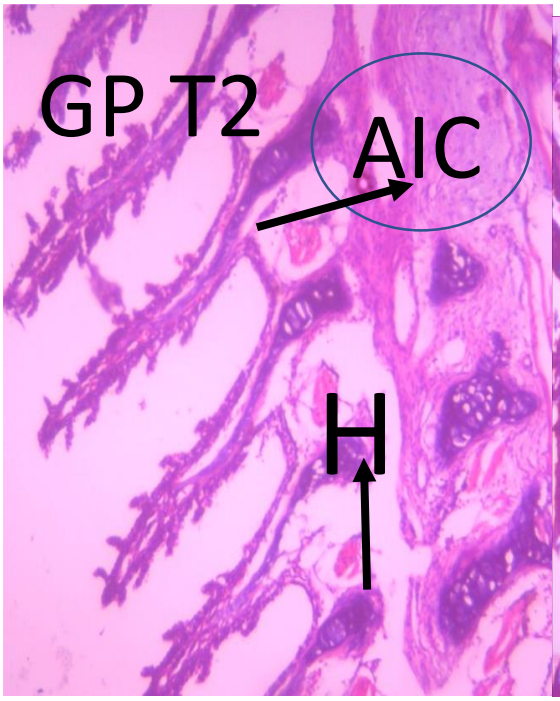
Plate A: (Fig 2-8) Histological change observed in the gill of Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Adenia lobate.*



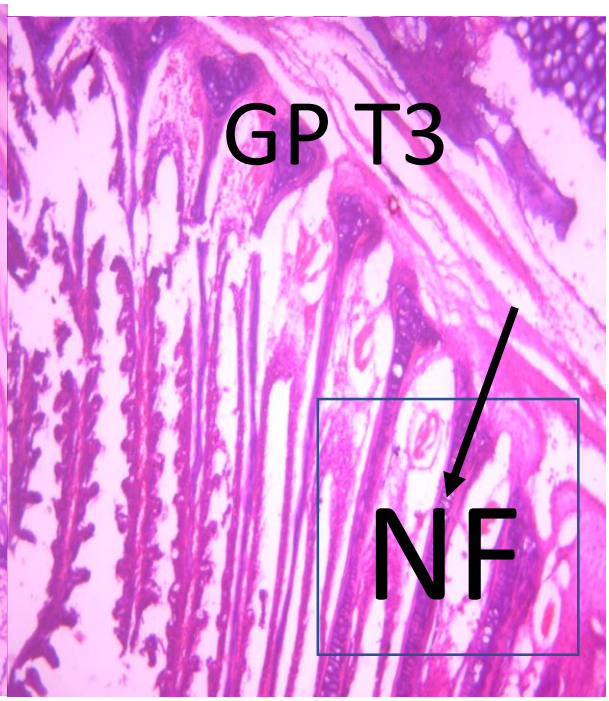
Photomicrograph of T0 control section of gill (X150)(H/E) shows normal gill architecture with well projected filament (PF) , gill epithelium (GE) and cartilages (C).



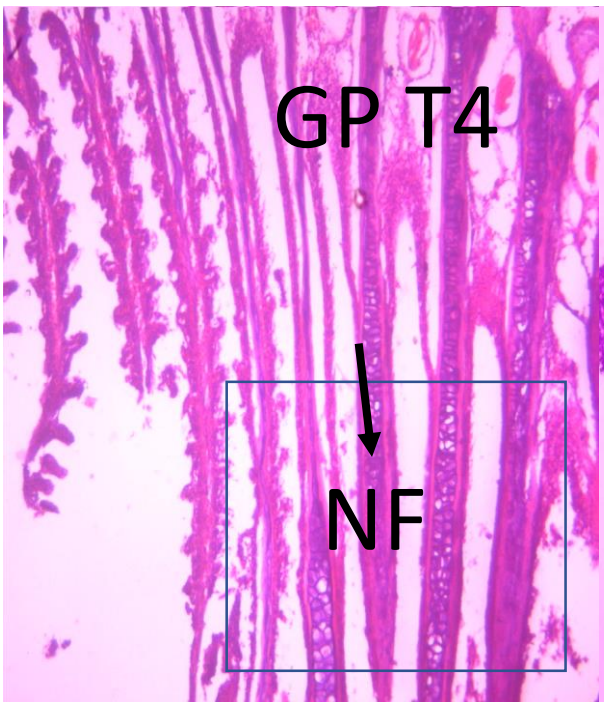
Photomicrograph of T1 section of gill (X150)(H/E) shows mild to moderate effect on the gill architecture with moderate focal area of hemorrhage (H) and mild clumping (C) of the filament



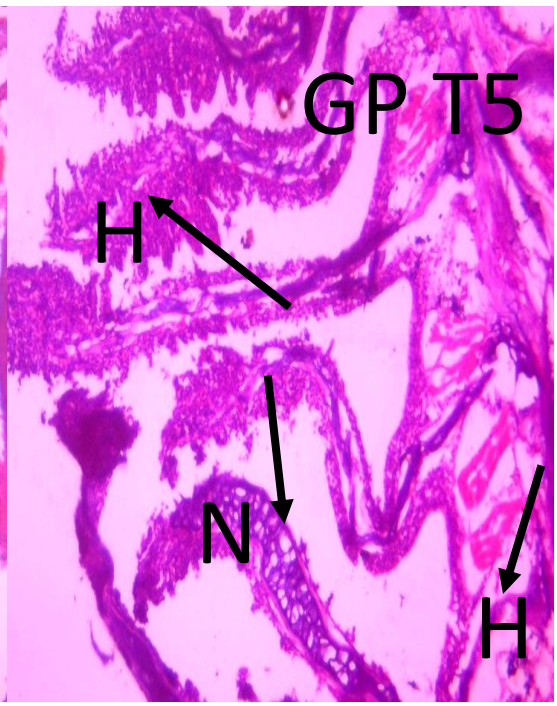
Photomicrograph of T2 section of gill (X150)(H/E) shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia



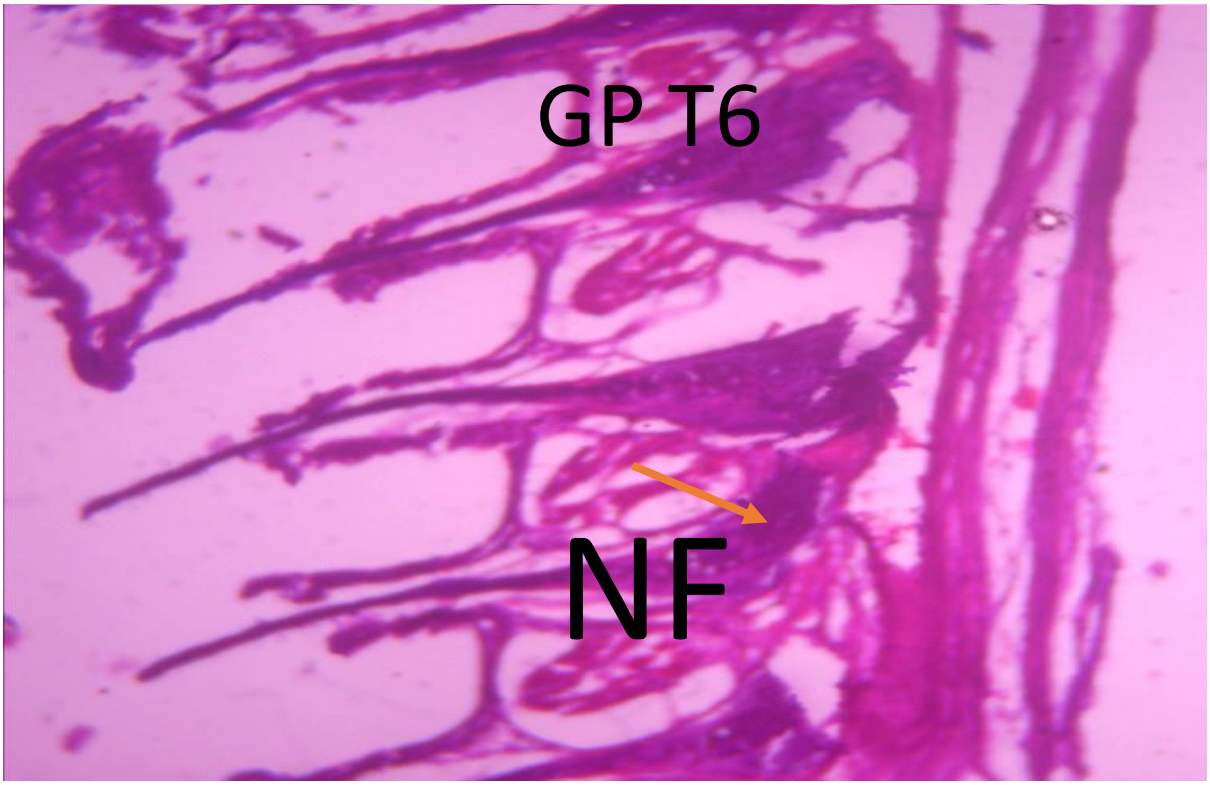
Photomicrograph of T3 section of gill (X150)(H/E) shows moderate effect on the gill architecture with focal area of necrotic filament (NF)



Photomicrograph of T4 section of gill (X150)(H/E) shows moderate effect on the gill architecture with focal area of necrotic filament (NF)

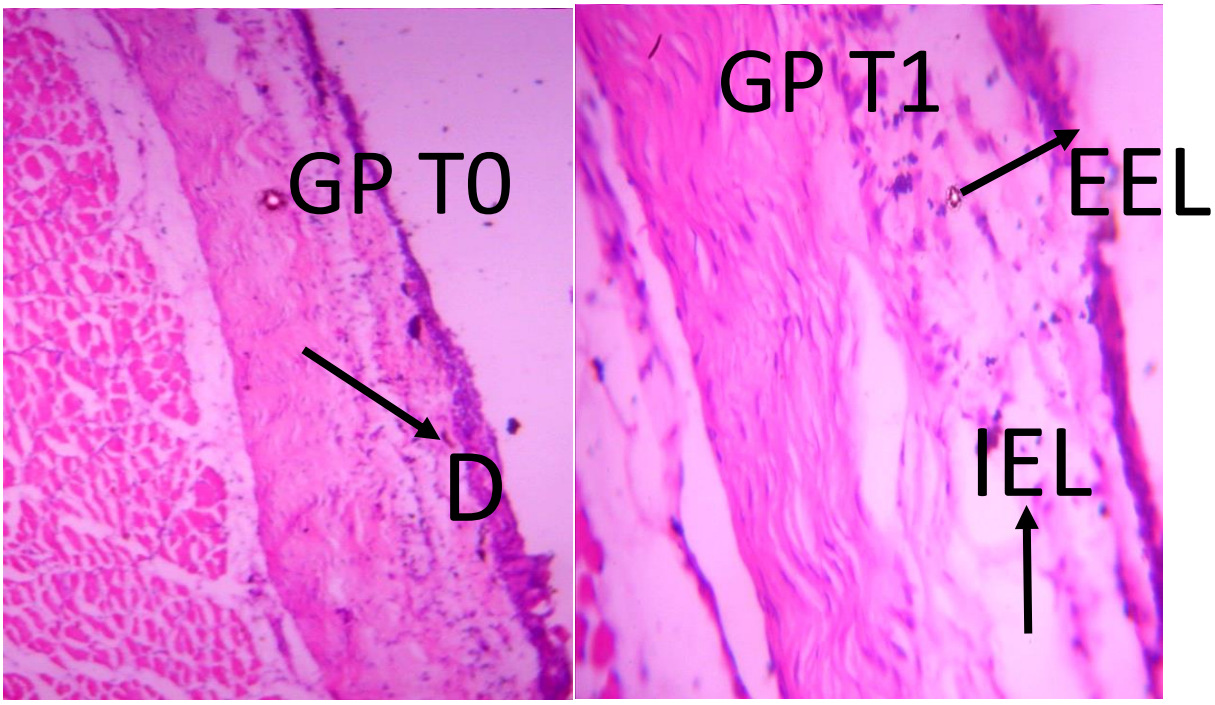


Photomicrograph of T5 section of gill (X150)(H/E) shows moderate to severe effect on the gill architecture with areas of necrotic filament (NF) , hypertophy (H) and hemorrhage (H)



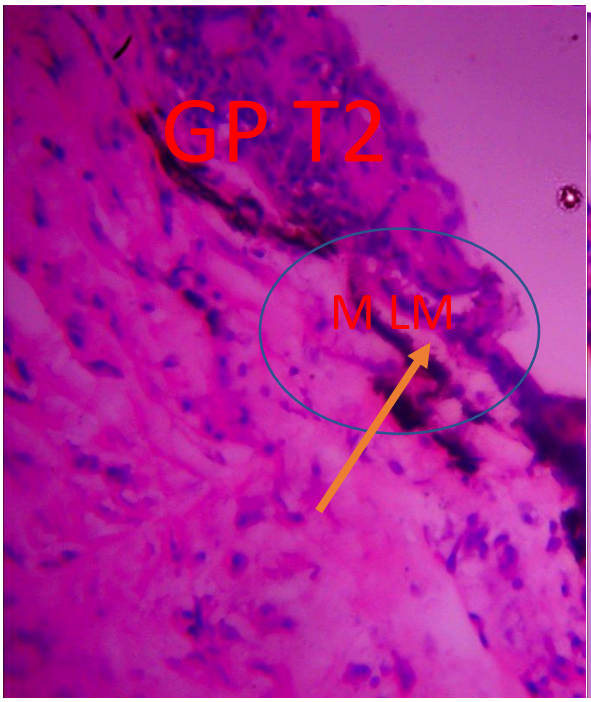
Photomicrograph of T6 section of gill (X150)(H/E) shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non-distinct outline

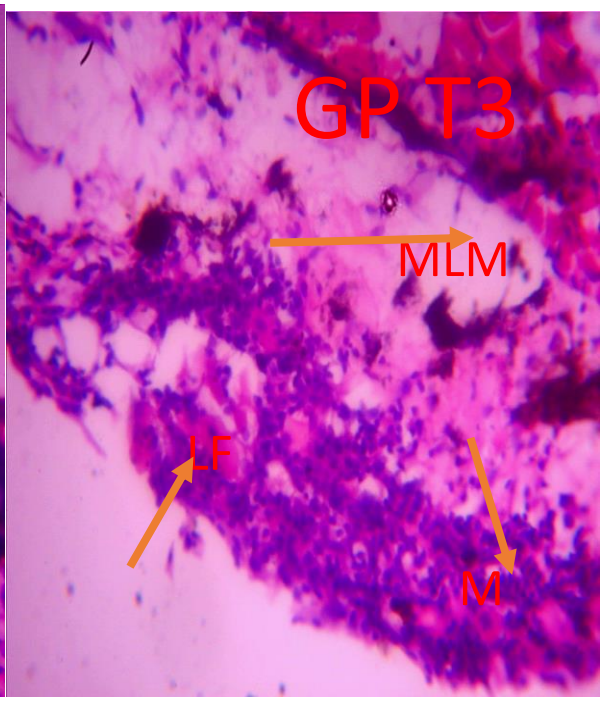
Plate B: (Fig 9-15) Histological change observed in the skinof Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Adenia lobate*.



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

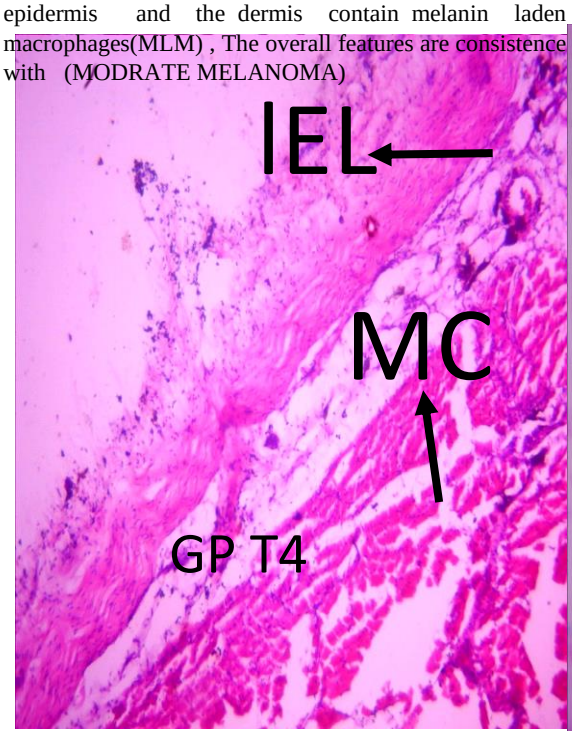
Photomicrograph of T 1 section of skin (X100)(H/E) shows mild effect on the sin layer with mild erosion of the epithelia lining (EEL) and intra epidermal lost (IEL) of tissue

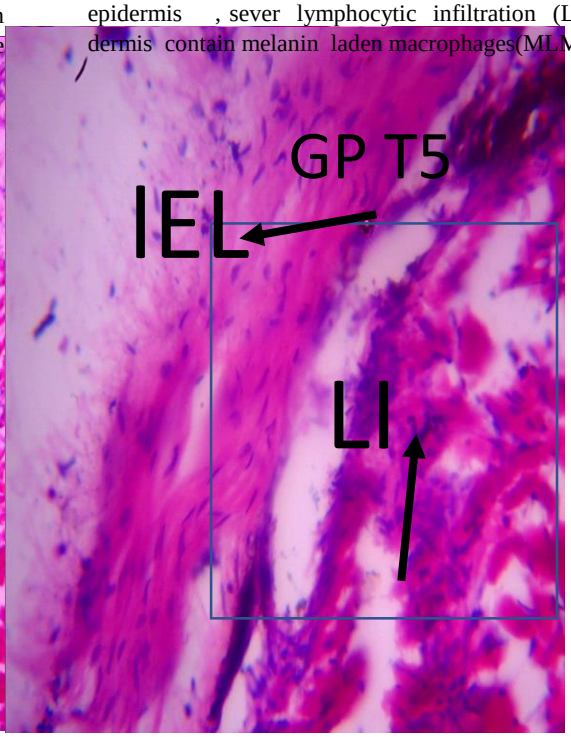




Photomicrograph of T 2 section of skin (X100)(H/E) shows moderate effect on the sin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA)

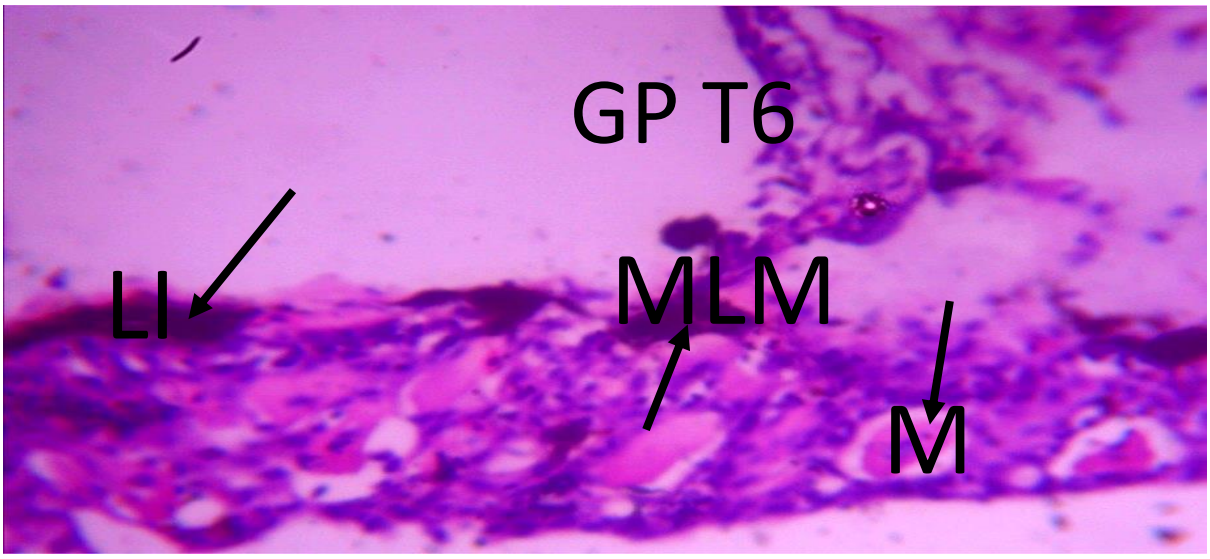
Photomicrograph of T 3 section of skin (X100)(H/E) shows moderate to severe effect on the skin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM)





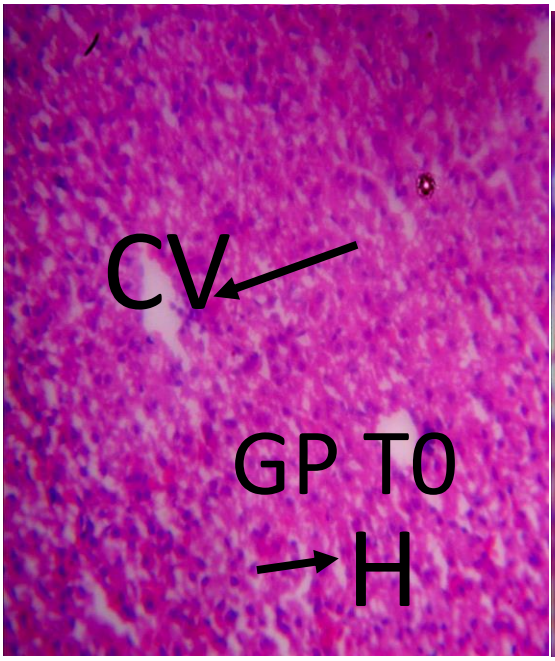
Photomicrograph of T 4 section of skin (X100)(H/E) shows moderate effect on the skin layer with the epidermis showing irregular layer , loss of epithelia lining (LEL) and presence of mast cell (MC) in the dermis

Photomicrograph of T 5 section of skin (X100)(H/E) shows moderate effect on the skin layer with loss of the epithelia lining (LEL) and lymphocytic infiltration ,(LI) within the intradermal region .

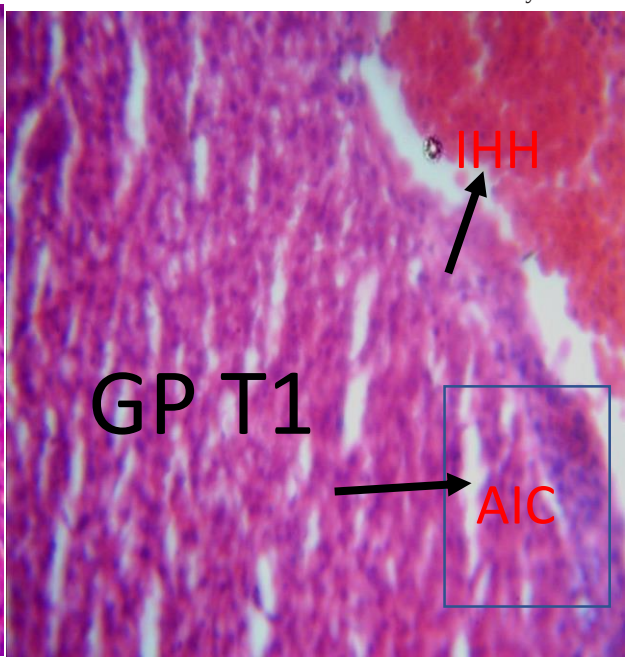


Photomicrograph of T 6 section of skin (X100)(H/E) shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM) The overall feature are consistence with (MCHRONIC MELOMA)

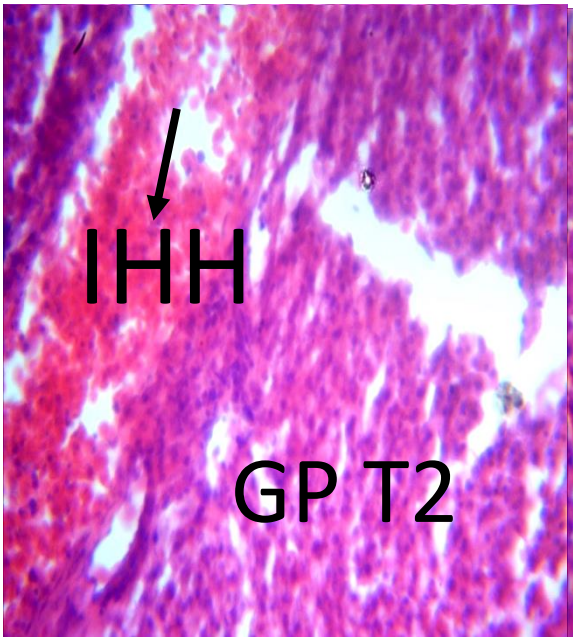
Plate C: (Fig 16-23) Histological change observed in the liver of Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Adenia lobate.*



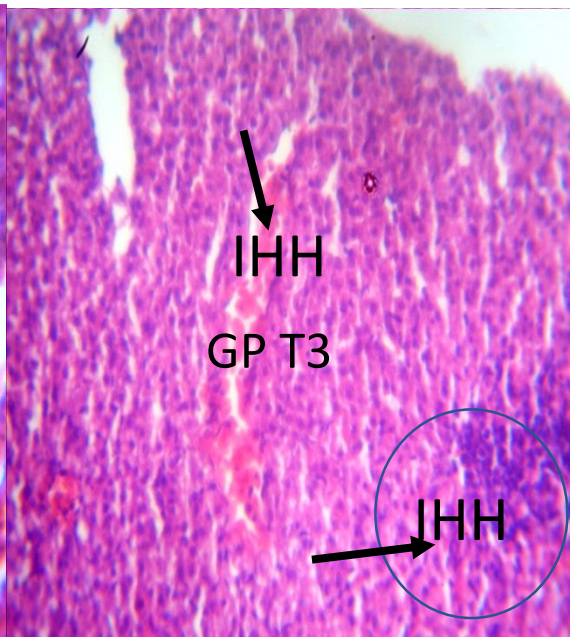
Photomicrograph of T0 control section of liver (X100)(H/E) shows normal hepatic architecture with normal hepatocyte (H) and central vain (C).



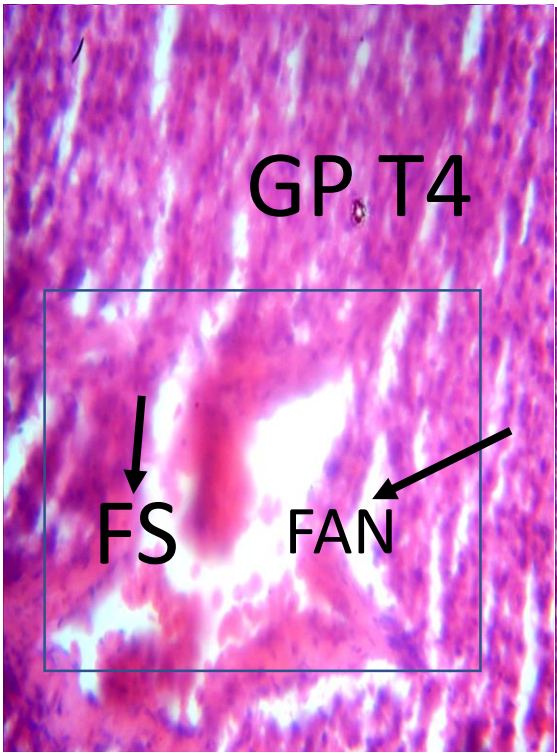
Photomicrograph of T1 section of liver (X100)(H/E) shows modrate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) arround the hemorhagic area



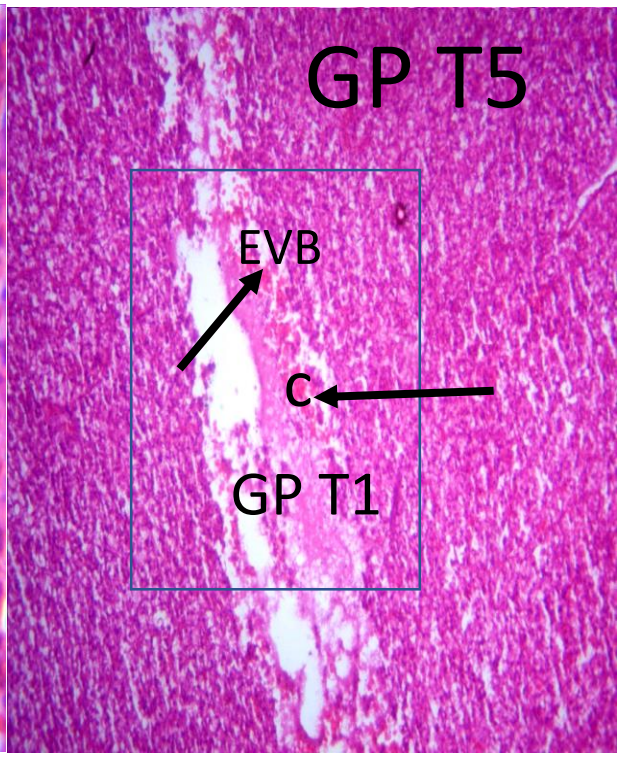
Photomicrograph of T2 section of liver(X100)(H/E) shows moderate to severe degeneration with sever focal area of intra hepatic hemorrhage (IHH)



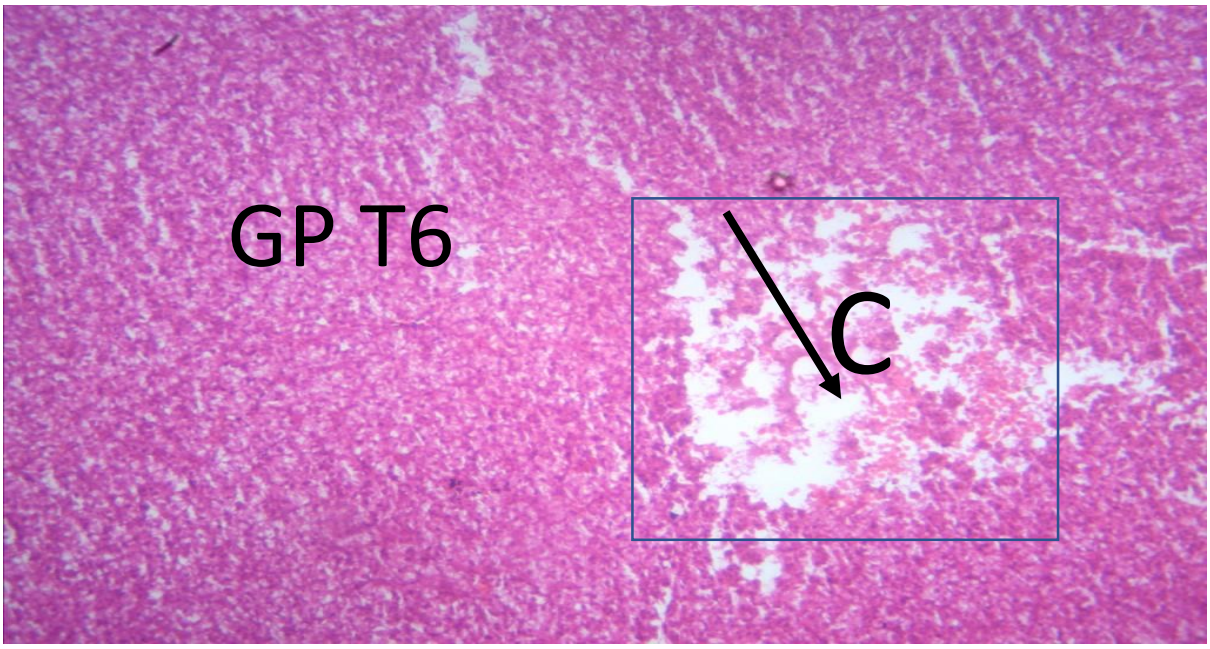
Photomicrograph of T3 section of liver (X100)(H/E) shows moderate degeneration with severe focal area of intra hepatic hemorrhage (IHH) and focal aggregate of intra hepatic inflammation (IHI)



Photomicrograph of T4 section of liver (X100)(H/E) shows moderate to sever degeneration with severe focal area of necrosis (FAN) with fibrous strands (FS) within the necrotic area

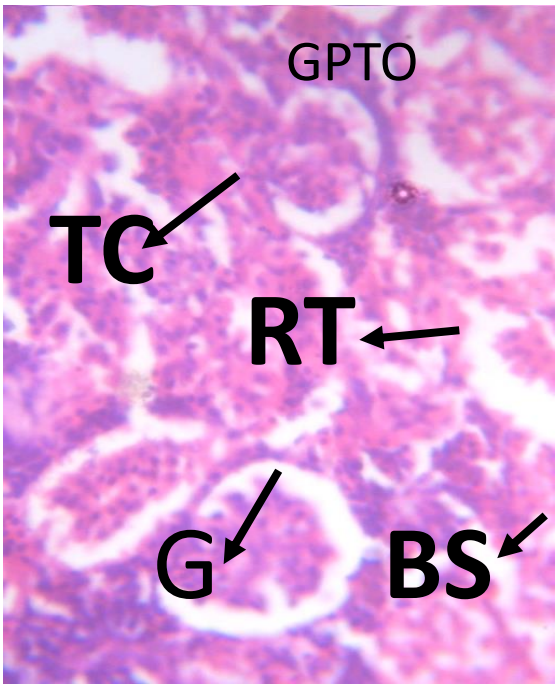


Photomicrograph of T5 section of liver (X100)(H/E) shows moderate to severe degeneration with severe focal area of cirrhosis (FAN) with extravassated blood (EVB)

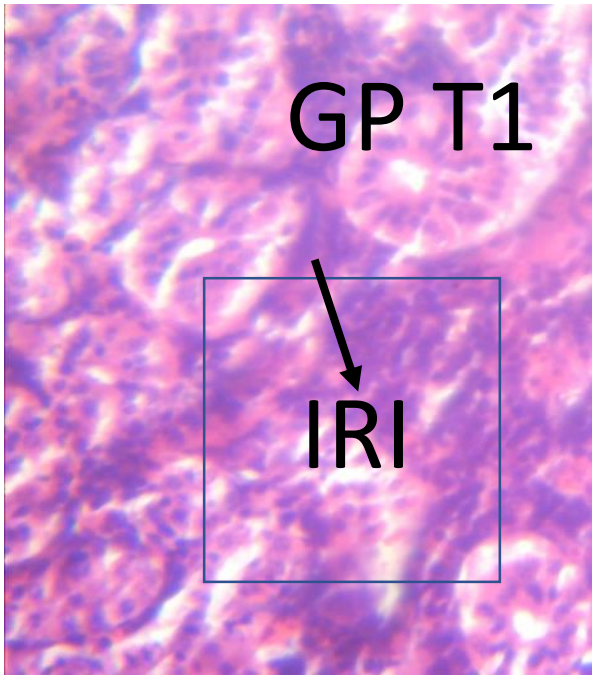


Photomicrograph of T6 section of liver (X100)(H/E) shows moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat .

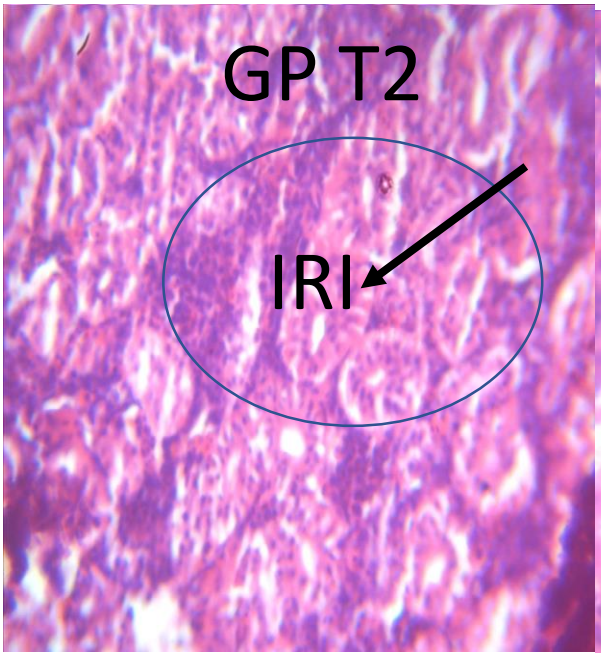
Plate D: (Fig 24-30) Histological change observed in the Kidney of Juvenile Catfish *Clarias gariepinus* treated with different concentration of ichthyotoxic plant *Adenia lobate.*

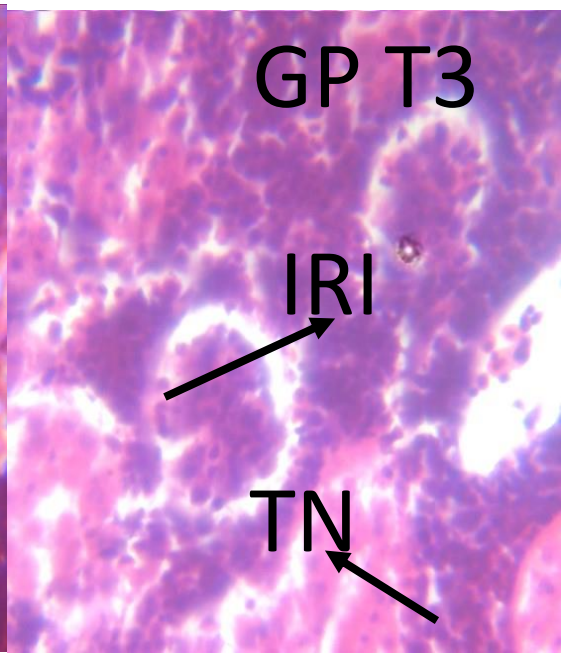


Photomicrograph of T0 control kidney (X400)(H/E) shows normal renal architecture with glomeruli (G) , bowman space (BS), renal tubules (RT)and tubular cell (TC)

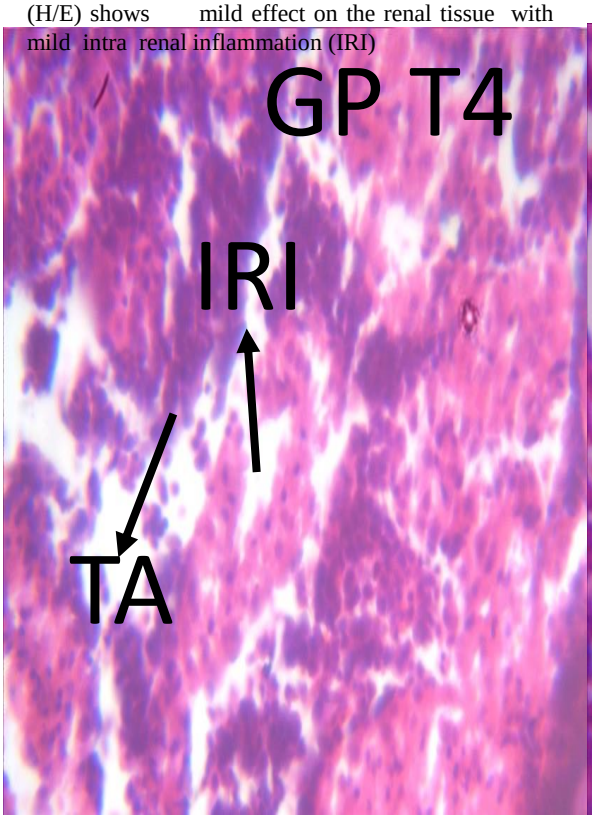


Photomicrograph of T1 section of kidney (x400) (H/E) shows mild effect on the renal tissue with mild intra renal inflammation (IRI)

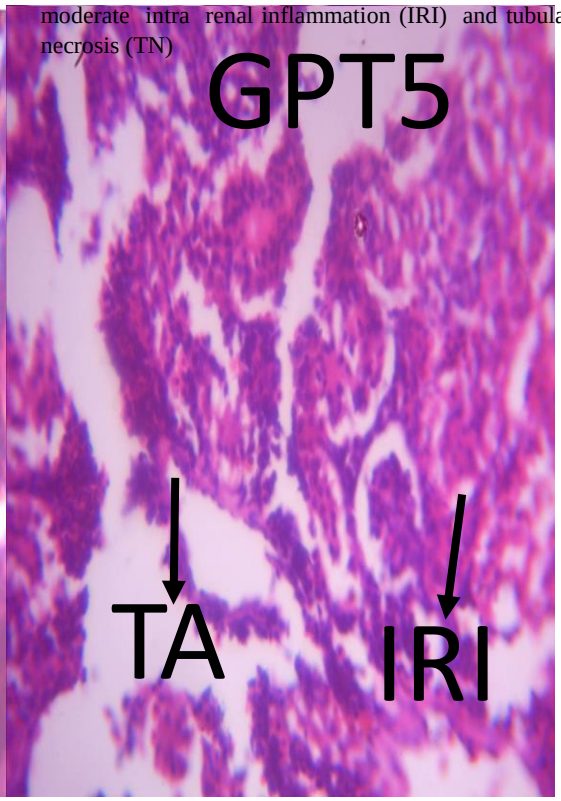




Photomicrograph of T2 section of kidney (x400) (H/E) shows mild effect on the renal tissue with mild intra renal inflammation (IRI)

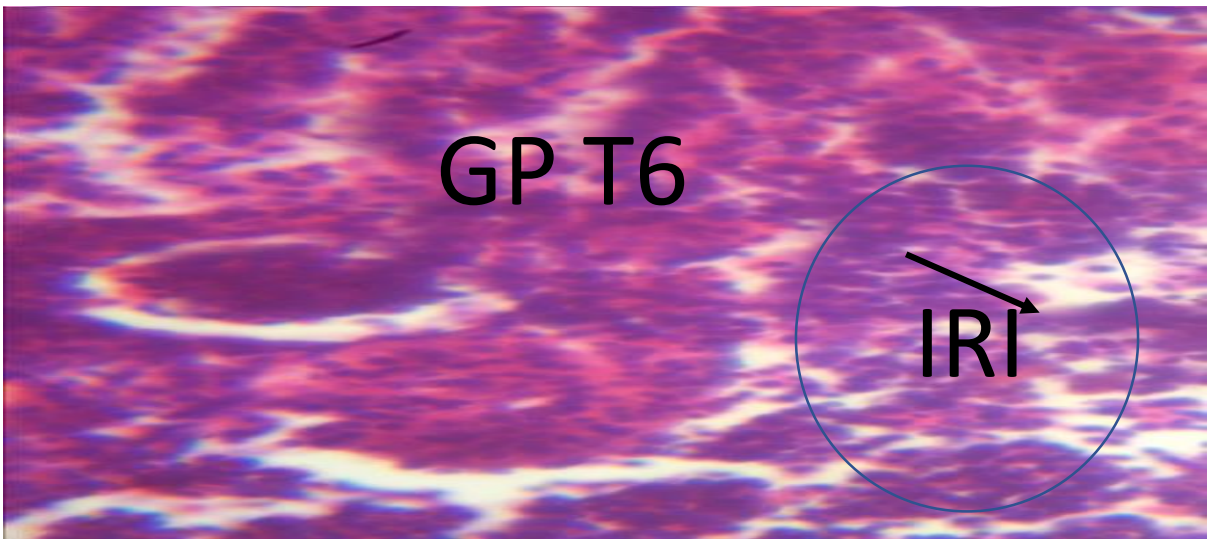


Photomicrograph of T3 section of kidney (x400) (H/E) shows moderate effect on the renal tissue with moderate intra renal inflammation (IRI) and tubular necrosis (TN)



Photomicrograph of T4 section of kidney (x400) (H/E) shows moderate effect on the renal tissue with moderate intra renal inflammation (IRI) and tubular attrophy (TA)

Photomicrograph of T5 section of kidney (x400) (H/E) shows moderate to severe effect on the renal tissue with moderate intra renal inflammation (IRI) and severe tubular attrophy (TA)



Photomicrograph of T6 section of kidney (x400) (H/E) shows moderate to severe effect on the renal tissue with moderate focal area of intra renal inflammation (IRI) and severe tubular attrophy (TA)

Discussion

Adenia lobate belongs to the family Passifloraceae is a large climbing shrub producing stems up to 45 metres long and up to 12cm in diameter. The ichthyotoxic plant *Adenia lobat a*leave when crushed is used as fish poisons. The active ingredient in *Adenia lobata* leaf are alkaloids, carbohydrate, glycosides, saponin, flavonoids and tannins, which can make the fish dizzy or kill the fish out rightly thereby making them easy to catch. Notable among them are saponins and rotenones, Bearez (1998).The lethal toxicity 96hrs$LC_{50}$of aqueous extract of *Adenia lobata*to the *Clarias gariepinus* Juvenilein the present study (Table 1, 2, 3 figure 1)is1.2mg/l, with the maximum admissible toxicant concentration of 0.12mg/l – 0.0120mg/l. which is higher than the work of Ayotunde and Igbang (2023) who reported a96hrs$LC_{50}$of aqueous extract of *Bridelia micrantha* as 0.21mg/lwith MATC of 0.0021mg/l – 0.021mg/lto the *Clarias gariepinus* Juvenile. Ayotunde and Ofem (2008) reported that the acute toxicity of Pawpaw seed decreased with increase in time. Total mortality resulted at concentration of 8mg/l and the 96hrs LC_{50} is 1.8mg/l of Pawpaw seed to fingerlings tilapia. The maximum admissible toxicant concentration of 0.018mg/l - 0.18mg/l established for fingerling tilapia was derived by multiplied a constant 0.01-0.1 by 96hours (Koesomadinata 2000)

The result of Length-weight relationship and condition factors of *Clarias gariepinus* Juvenile exposed to ichthyotoxic plant *Adenia lobata* is presented in table 4. The condition factor ranges from (0.8 – 0.9) - (0.8 – 1.3) this result indicates that the experimental fish are in good conditions of health. The abnormal responses in physio chemical parameters, such as increased ventilator rate, erratic swimming, and increased surfacing among others may increase the energy demand for metabolism beyond normal, leading to fatigue and stress (Svobodova *et al.,* 1993, Ayotunde *et al.,* 2010). In the present study Table 5, the result of physiochemical parameters, exhibited variation in values, **t***here was significant difference between the water quality parameters before, during the experiment and after the experiment. No significant change in Temperature was observed. pH increases* while *Dissolve Oxygen Concentration and Conductivitywere observed to reduce significantly (P<0.05) respectively.*

Studies have shown that fish exposed to toxicants exhibited some behavioral changes such as increased opercula beat rate, erratic swimming, mucus secretion and air gulping before death Gabriel and Okey, (2009). In the present study, Table 6 and 7 shows the general behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Adenia lobate,* 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. The pattern of behavioral changes observed in this study compared favorably with the report of Fafioye *et al*., (2004) when African catfish (*Clarias gariepinus*) was exposed to *Parkiabiglobosa* and *Raphiavinefera* extracts. Increased concentrations of *Alchornea cordifolia* leaf to erratic

swimming, air gulping, discoloration, loss of body equilibrium and mortality as was also similarly observed in *Clarias gariepinus* exposed to aqueous extracts of *Blighiasapida* and *Kigeliaafricana* Fafioye *et al*., (2004). The marked deviation in the rate of swimming, discoloration and air gulping suggests an adjustment in physical fitness as a result of the stress condition. In this study, behavioural responses observed in exposed fish were related to concentration of the extract as more of the responses were observed at higher concentrations of the extract Abalaka *et al.,* (2013), Boyd (2005) Shahi, Singh (2011) and Bobmanuel *et al*., (2006). The observed behavioural abnormalities are attributed to respiratory impairment, resulting from the effects of the toxicant on the gills of the exposed fish Ogundiran (2009).

Blood is a tissue fluid andserves as transport medium whose primary function is to supplyoxygen and nutrients as well as constitutional elements to tissuesand to remove waste productsEssien-Ibok (2019). Blood also enables hormones andother substances to be transported between tissues and organs.Blood is basically composed of the plasma, red blood cells, whiteblood cells and platelets, each with sub constituents whichcollectively contribute to the overall functioning of the bloodCelik (2004), Crook (2012).

Thereductions in blood cell indices and tissue deformation observedfrom the chronic bioassay are in line with findings of the studyon behavioural, haematological and histopathological changesin *C. gariepinus* exposed to 2,4-dichlorophenoxyacetic acidOkogwu*et al.,* (2015). Similar observations have also been reported in a studyon exploitation of ethanol extract of *Adenium obesum* stembark as a potent organic piscicide Abalaka *et al*., (2013). In the present study ***there was reduction in the results of blood parameters of Sharp tooth Catfish gariepinus Juvenile exposed to ichthyotoxic plant*** *Adenia lobata* ***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, Red blood cell, Haematocrit,Lymphocytes, Mean cell volume, reduce, while ***there was an increase in*** Haemoglobin, Platelet, Mean cell Haemoglobin, Mean cell Haemoglobin concentration increases ***when compared with the control, when compared with the control.*** This is in agreement with Joshi (2002) that reported effectsof toxicants on blood parameters in freshwater teleost fish *Clariasbatrachus*. Bhatt and Farswan (1992)also observed that Red blood cell(RBC), Total White blood cell (TWBC), Haemglobin (Hb), packed cellvolume (PCV) decreases with exposure of *Barilius bendalensis* (Ham)to plant toxicant. The abnormalities observed in the haematologicalparameters in all concentrations compared with control clearlyindicated that the haematological parameters were much lower inthe exposed fish than in control fish, thereby depicting an anaemiccondition.

Table 9 is the result of variations that occurred in the values obtained in the proximate composition of the carcass of *Clarias gariepinus* juvenile exposed to aqueous extract of *Adenia lobata*, Ash, Nitrogen Free Extract (NFE), and Energy reduced while there is no significant changes in Crude Protein, Crude Lipid, Crude Fiber, and Moisture after 96 hours expose. There were variations in all carcass biochemical parameters such astotal protein, Albumin, Globulin and Albumin/Globulin ratio when *C.gariepinus* juveniles were exposed to different concentrations of water extract of *P. zeylanica* for 21 days as comparedwith initial

and control values, respectively. A decrease in Serum totalprotein in the current study was similar to that observed by Tabassum *et al*., (2015) whenfishes were exposed to stem-bark extract of *Croton tiglium*. However,the results reported for Serum proteins are in agreement with thoseobtained by Ogueji et al 2020 who reported an oxidative stress, biochemical, lipid peroxidation and antioxidant responses in Clarias gariepinus exposed to acute concentration of Ivermectin

Figures 10, Plate A-D (figures 2-29) present the results of tissue analysis of fish from the respective treatment in the present study. Histopathological examinations of the test fish showed some pathological disruptions. The ichtyotoxicant *Adenia lobata*shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non-distinct outline.The skin shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM) The overall feature are consistence with (MCHRONIC MELOMA). The gill showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI). The liver cells revealed moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat, while the kidney revealed shows moderate to severe effect on the renal tissue with moderate focal area of intra renal inflammation (IRI) and severe tubular atrophy (TA). The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. This work is similar to the work of Adeogun *et al*., 2012, and Ayotunde and Igbang (2023) on *D. tripetala,* and*Bridelia micrantha*respectively. Toxicant introduced into aquatic systems can cause structuralchanges in tissues and organs of fish leading to obstruction ofphysiological functions. The alterations may have compromised theprocess of gaseous exchange resulting in histotoxic hypoxia.

Several reports have indicated that gill lesions do not onlyindicate possibilities of impaired respiratory functions butimpaired osmo-regulatory functions as well (Mallat, 1985;Au, 2004; Tang and Au, 2004.). Even slight structuraldamage can render a fish vulnerable to osmo-regulatory aswell as respiratory difficulties (Hughes and Morgan, 1973)thereby affecting the overall metabolism and survival of thefish.The histopathological alteration observed in the brain, gill, liver,intestine and muscle/flesh is an indication of the toxic effect of *P.zeylanica* extracts to fish. This agreed with Fafioye 2001, 2004 observationwhen *Clarias gariepinus* and *O. niloticus* were exposed to lethal andsublethal concentrations of *Parkia biglobosa* and *Raphia vinifera*respectively. The gill lamellae play a significant role in regulating theexchange of gas, water and ions in fish. The role of the gill in excretionpredisposes it in such a way that slight structural damage can render afish very vulnerable to osmoregulation as well as respiratory difficulties.

Conclusion

Adenia lobata is an ichthyotoxic plant. The active ingredient in *Adenia lobata* leaf are alkaloids, carbohydrate, glycosides, saponin, flavonoids and tannins, which can make the fish dizzy or kill the fish out rightly thereby making them easy to catch. Notable among them are saponins and rotenones, Bearez (1998). The lethal toxicity 96hrsLC_{50} of aqueous extract of *Adenia lobata*to the *Clarias gariepinus* Juvenilein the present study (Table 1, 2, 3 figure 1) is 1.2mg/l, with

the maximum admissible toxicant concentration of 0.12 mg/l – 0.0120 mg/l. The result of physiochemical parameters, exhibited variation in values, **t***here was significant difference between the water quality parameters before, during the experiment and after the experiment. No significant change in Temperature was observed. pH increases* while *Dissolve Oxygen Concentration and Conductivity were observed to reduce significantly (P<0.05) respectively*

The general behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Adenia lobate,* 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. *There was reduction in the results of blood parameters of Sharp tooth Catfish gariepinus Juvenile exposed to ichthyotoxic plant Adenia lobata 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, Red blood cell, Haematocrit, Lymphocytes, Mean cell volume, reduce, while *there was an increase in* Haemoglobin, Platelet, Mean cell Haemoglobin, Mean cell Haemoglobin concentration increases *when compared with the controlwhen compared with the control.*Variations that occurred in the values obtained in the proximate composition of the carcass of *Clarias gariepinus* juvenile exposed to aqueous extract of *Adenia lobata*, Ash, Nitrogen Free Extract (NFE), and Energy reduced while there is no significant changes in Crude Protein, Crude Lipid, Crude Fiber, and Moisture after 96 hours expose.

Histopathological examinations of the test fish showed some pathological disruptions. The ichtyotoxicant *Adenia lobata*shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non-distinct outline. The skin shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis , sever lymphocytic infiltration (LI) and the dermis contain melanin laden macrophages(MLM) The overall feature are consistence with (MCHRONIC MELOMA). The gill showed severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI). The liver cells revealed moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat, while the kidney revealed shows moderate to severe effect on the renal tissue with moderate focal area of intra renal inflammation (IRI) and severe tubular atrophy (TA).Therefore, theconcerned authorities should launch appropriateawareness campaign among the localinhabitants and fisherman about adverse effectof *Adenia lobata*leaf extract. Furthermore,providing alternative eco-friendly techniquesfor fish harvesting may possibly bring constructive outcome in the near future.

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