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Effect of Aqueous Extract of Ichthyotoxic Plant Bridelia micrantha (Hochst) (Baill) on Haematology, Histopathology and Carcass Characteristics of African Catfish (Clarias gariepinus) (BURCHELL, 1822) Juvenile

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Abstract

The Bridelia micrantha (Hochst.) (Phyllanthaceae) is an ichthyotoxic plant used in catching fish in Nigeria. The toxicity of aqueous extract of B. micrantha on Clarias gariepinus was investigated under statics bioassay conditions. The concentrations of the leaves extract used were 0.00, 0.10, 0.20, 0.30, 0.40, 0.50 and 0.0.60ml/l. The 24hrs, 36hrs, 48hrs, 72hrs, and 96hrs LC50 were 0.40mg/l, 0.30mg/l, 0.26mg/l, 0.22mg/l and 0.21mg/l respectively, with the range of maximum admissible concentration (MATC) were 0.004 – 0.04, 0.003 – 0.03, 0.0026 – 0.026, 0.0022 – 0.022, and 0.0021 – 0.021 respectively. The opercula ventilation and loss of reflex increased with an increase in the concentrations of the leaves extract at the end of 96-hour exposure period. Respiratory distress, loss of balance, settling at the bottom motionless and erratic swimming was observed before death during the exposure period. There was no significant difference in physicochemical parameters of test media ($P<0.05$) before, during and after the experimental period. Total Dissolved Solids and Conductivity increased significantly ($p<0.05$) with increase in extract concentration. Temperature had nonsignificant ($p<0.05$) reduction at all extract concentrations. Blood analysis revealed significant ($P<0.05$) reduction in the blood parameters (Haemoglobin, Packed Cell Volume, Red Blood Cells, White Blood Cells, Platelet, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin) of exposed fish.

There was severe reduction in Carcass/Proximate (Moisture, Crude Protein, Crude Lipid, Ash, NFE, and Energy) composition of Clarias gariepinus juvenile exposed to B. micrantha. Histopathological examinations of the test fish showed some pathological disruptions, such as skin layer with severe superficial spreading of melanoma, 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). The result of this study calls for the need to discourage the use of toxic plants for catching fish in Cross River and Nigeria water bodies.

Keywords: Ichthyotoxic Plant, Bridelia Micrantha, Haematology, African Catfish, Histopathology, Toxicity

1. Introduction

Aquatic pollution is a global environmental challenge which both the developing and developed countries are steadily trying to curb. Water is one of the most essential and abundant compounds of the ecosystem as all living organisms on earth including humans require water for their survival and growth [1]. Unfortunately, the availability and quality of water have been impacted upon by both natural and anthropogenic sources due to a lot of demographic factors such as increase in human population, demand for food, land conversion and use of fertilizer and fishing have led to the contamination of many river sources including Cross River [2]. Life below water is continually threatened by pollution especially those resulting from anthropogenic activities [3]. The tribal communities have been largely dependent on the wild plants for various purposes such as medicine, timber and food. Several studies have documented this traditional knowledge about wild plants, but mostly related to its dietary and medicinal aspect [4].

The ichthyotoxic plant Bridelia micrantha (Hochst.) (Phyllanthaceae) is a semi-deciduous tree up to 20m tall with a dense round-

ed crown and tall bare stem, bark on young branches grey-brown and smooth, on older branches and stems dark brown and rough, cracking into squares; branches often spiny; slash thin, fibrous, and brown to dark red. Over the last few decades, there has been an increased use of plant based ichthyotoxins (piscicidal plants) for commercial fishing activities in many parts of the world including Nigeria [4,5]. Plant-based ichthyotoxins are fish poisons derived from plants and active ingredients commonly detected in such plants include alkaloids, tannins, saponins, flavonoids, resin and phenolics [6,7]. The active ingredient in Bridelia micrantha are triterpenoid saponins and rotenoids with rotenone being the most widely used commercially. Some other poisons include sesquiterpenes, diterpenoids, including diterpenoid orthoesters, triterpenes, furanocoumarins, 2-hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone, prenyl phenyl propanoids, and sterol acylglucosides . The great variety of chemical structures presents many interesting questions regarding the relative toxicity, biochemical mechanisms, and environmental effects of fish poisons.

Fish poisons are widely used for cultural, commercial, and environmental reasons, the frequent use of Ichthyotoxic plants to catch fish is part of the traditional fishing method which is not 'environmentally friendly' to the aquatic ecosystem. Plant Ichthyotoxic are among the widely used fishing method which is biodegradable and less severe than Ichthyotoxic piscicides [8,9]. In Africa more than 325 fishing poison plant species are commonly used to catch fish [9]. The mechanism of action of ichthyotoxic plants varied from cytotoxicity (they act by causing cell death) to genotoxicity (they are able to produce their effect by altering gene expression) generally, toxicants produce their effects by interference with physiological or biochemical homeostatic (regulatory) mechanisms. Some of them can modify the regulation of cell division with harmful effects. Many toxic chemicals cause proliferative lesions on the skin of the organism.

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 [10]. 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 [11]. 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., [12]. Clarias gariepinus was 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.

2. Materials 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) (UNICROSS), Obubra Campus.

2.2 Collection and Preparation of Plant Samples

Ichthyotoxic Plants Bridelia micrantha, (leaves) was sourced around the University communities at Obubra Campus where they are 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 Bridelia micrantha, 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 [7].

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.

3. Toxicity Experiment

3.1. 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 Ezeama fish farm Ikom 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 24 h before the commencement of experiments.

3.2. 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.

3.3. 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 [13]. 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 extracts for 24 hours.

3.4. Sub-acute toxicity studies (Definitive Test)

Clarias gariepinus juveniles were exposed to sub-acute concentrations (96 h LC50) 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 LC50 with a factor 0.1 – 0.01 according to Koesomadimata [14].

3.5. 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 [15]. All haematological parameters was analysis at Haematological Unit of the University of Calabar Teaching Hospital, using automated haematology analyzer (SYSMEX KX – 21NTM).

3.6. 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, kidney 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, 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 (5-7□m), by means of a rotatory microtome and was dehydrated and stained with Harris haematoxyllin-eosin (H&E) stain, Bancroft & Cook, using a microtone and each section were cleared by placing in warm water (38oC), where it was picked with clean slide and oven-dried at 58oC for 30 minutes to melt the wax [16,17]. 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).

3.7. Carcass Composition (Proximate) Analysis

The Proximate composition of the carcass 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 [18]. 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 550oC dissolved in 10% HCl (25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

3.8 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.

4. Results

The result of Length-weight relationship and condition factors of Clarias gariepinus Juvenile exposed to ichthyotoxic plant Bridelia micrantha is presented in table 1. The weight (g) varies from (52.25 ± 1.9 - 69.15 ± 5.1) (45.88 ± 4.4 – 60.15 ± 5.1) and standard length (18.14 ± 0.9 – 19.14 ± 1.0) (16.14 ± 1.0 – 18.54 ± 0.3) with the condition factor range from (0.9 – 1.2) - (0.8 – 1.4) for range finding test and definitive test respectively, this result indicates that the experimental fish are in good conditions of health. The toxicity of ichthyotoxic plant Bridelia micrantha (Hochst.) (Phyllanthaceae) leaves extract on Clarias gariepinus are presented in Table 2. The mortality rate in the B. micrantha 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 LC50 were 0.40mg/l, 0.30mg/l, 0.26mg/l, 0.22mg/l and 0.21mg/l respectively, with the range of maximum admissible concentration (MATC)

were 0.004 – 0.04, 0.003 – 0.03, 0.0026 – 0.026, 0.0022 – 0.022, and 0.0021 – 0.021 respectively as presented in Table 2 Figure 1. The percentage cumulative mortality is presented in tables 3 and 4, mortality increases with increases in concentration and time of exposure. The 100% mortality was observed in the group fish exposed to 0.60 mg/l,

	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	55.99±4.4^{a}	18.38±0.5^{ab}	0.9	0.00	45.99±4.4^{a}	17.38±0.5^{ab}	0.9
0.15	58.65±5.6^{a}	18.52±0.3^{ab}	1.0	0.10	54.65±5.6^{a}	18.52±0.3^{ab}	0.9
0.30	58.65±11.7^{a}	19.14±1.0b	0.8	0.20	58.65±11.7^{a}	16.14±1.0b	1.4
0.60	69.15±5.1^{b}	18.14±0.9^{ab}	1.2	0.30	60.15±5.1^{b}	18.14±0.9^{ab}	1.0
1.20	59.46±5.6^{a}	18.54±0.3^{ab}	0.9	0.40	53.46±5.6^{a}	18.54±0.3^{ab}	0.8
2.40	52.25±1.9^{a}	18.22±0.7^{a}	0,9	0.50	52.25±1.9^{a}	18.22±0.7^{a}	0.9
3.60	58.23±1.6^{a}	18.24±0.5^{ab}	1.0	0.60	54.23±1.6^{a}	18.24±0.5^{ab}	0.9

Table 1: Length-weight relationship and condition factors of *Clarias gariepinus* Juvenile

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.

S/N	TIME(Hrs)	LC_{50}	MATC (mg/l)
1	24	0.40	0.040 - 0.004
2	36	0.30	0.030 - 0.003
3	48	0.26	0.026 - 0.0026
4	72	0.22	0.022 - 0.0022
5	96	0.21	0.021 - 0.0021

Table 2: The LC_{50} values of *Clarias gariepinus Adult.*

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 (Tables 5 and 6). 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. The water quality parameters during the experiment (Table 7) exhibited variation in values. No significant change in Temperature and pH (26.50 ± 0.3 – 24.83 ± 1.2) (6.45 ± 0.3 – 6.57 ±0.5) (P<0.05) was observed. Dissolve Oxygen Concentration and Conductivity (4.43 ± 0.7 – 3.66 ± 0.8) (44.00 ± 4.1 – 36.80 ± 8.3) were observed to reduce significantly (P<0.05) respectively.

There was variation in the results of blood of Catfish gariepinus Juvenile exposed to ichthyotoxic plant B. micrantha in the respective treatments as presented in table 8. During the 96 hours chronic toxicity bioassay, Results from blood analysis show that all the parameters such as the White blood cell, Red Blood cell, Haematocrit, Platelet Lymphocytes, Mean cell Volume Mean Cell Haemoglobin concentration measured, reduced in value from 1.72 x 102 ± 12.5, 2.63x 106 ± 3.1, 22.83 ± 2.5, 3.22 x 104 ± 1.3, 96.78 ± 1.0, 1.16 x 102 ± 2.3, 45.88 ±4.2mg/l increased to 1.72 x 102 ± 19.3, 1.55 x 106 ± 2.1, 2.26 x 106 ± 4.1, 9.36 ± 2.7, 2.26 ± x 104 ± 4.6, 89.96 ± 0.6, 1.08 x102 ± 7.3, 29.54 ± 5.6 mg/l when compared with the control. There was an increase in Haemoglobin and Mean Cell Haemoglobin from 11.08 ± 2.3, 41.20 ± 4.6 mg/l to 12.80 ± 2.2, 51.31 ± 7.3 mg/l, 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 *B. micrantha* Table 9, 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 102 ± 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 102 ±4.0 respectively. Histopathological examinations of the test fish showed some pathological disruptions table 10, Plate A-D (figures 2-29). The *B. micrantha* 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.

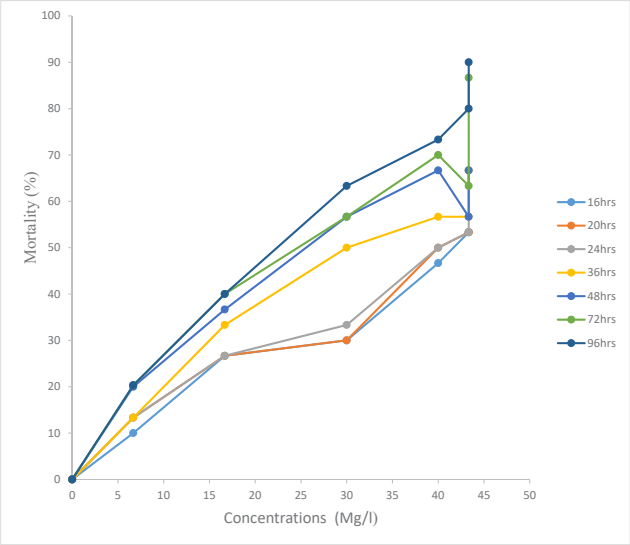
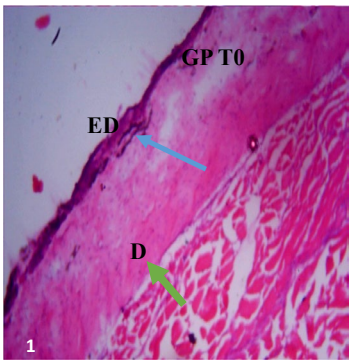
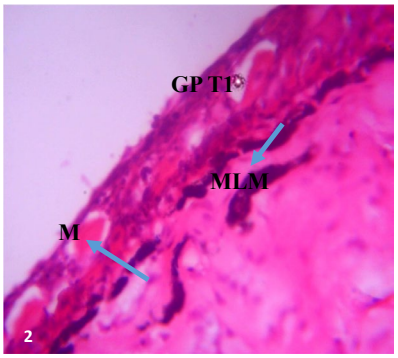


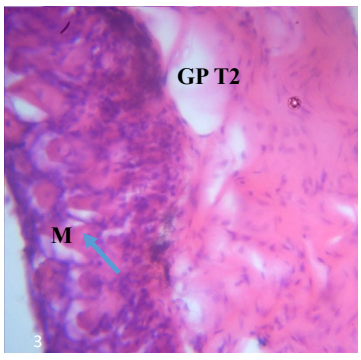
Figure 1: Determination of LC50 Using Probit (Graphical) Method (Finney 1971; USEPA 2000).



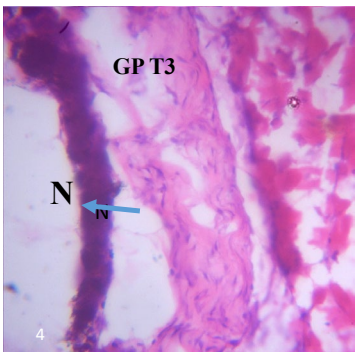
Photomicrograph of Group T0 control section of Skin (x400)(H/E) shows normal skin architecture with epidermis (E) dermis (D).



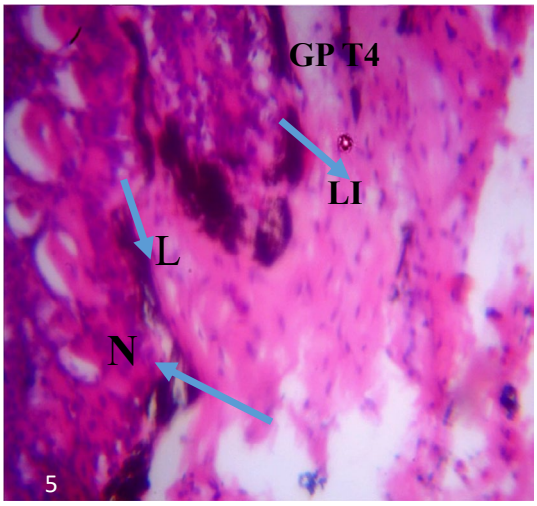
Photomicrograph of T1 section of skin (X100)(H/E) shows moderate to severe effect on the sin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain sever melanin laden macrophages(MLM) , The overall features are consistence with (MODRATE MELANOMA)



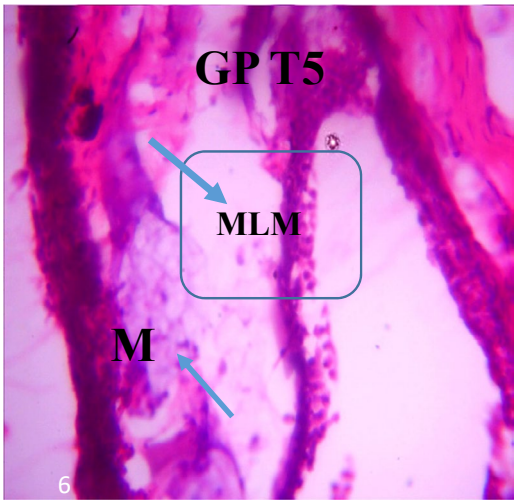
Photomicrograph of T 2 section of skin (X100)(H/E) shows moderate to severe effect on the skin layer with sever superficial spreading of melanoma (M) at the epidermis and the dermis and lymphocyte infiltration (LI) The overall features are consistence with (CHRONIC MELANOMA)



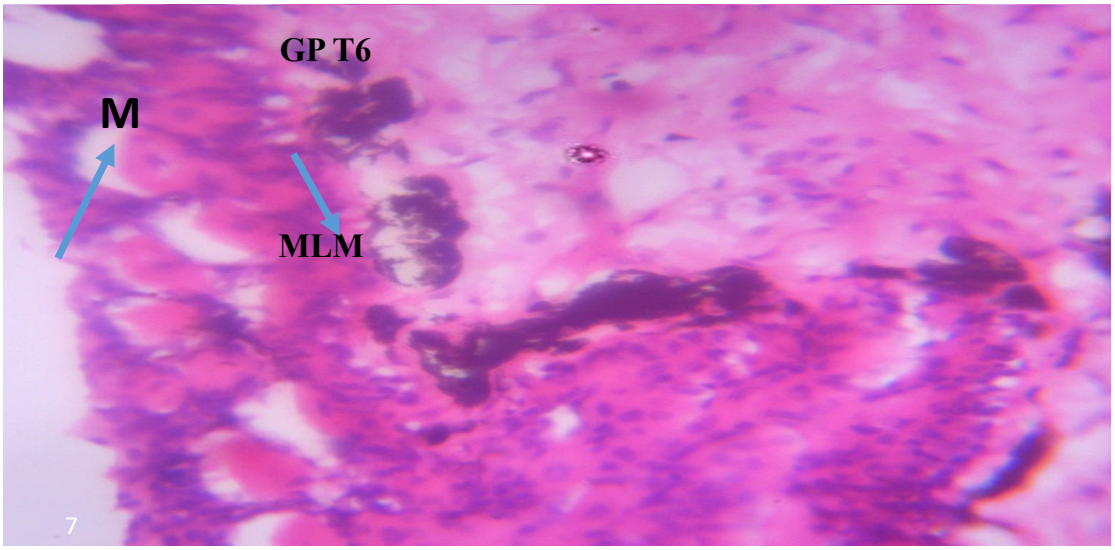
Photomicrograph of T 3 section of skin (X100)(H/E) shows moderate effect on the skin layer with necrotic epidermis and loss of dermal tissue .



Photomicrograph of T 4 section of skin (X100)(H/E) shows moderate to severe effect on the skin layer with necrotic (N)epidermis , loss (L) of tissue within the intra dermal region and and lymphocytic infiltration ,(LI) .

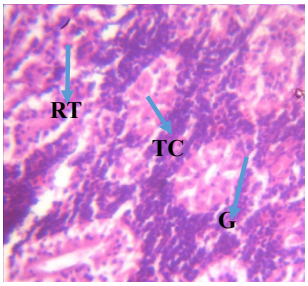


Photomicrograph of T 5 Section of skin (X100)(H/E) shows sever effect on the sKin layer with sever superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain melanin laden macrophages(MLM)

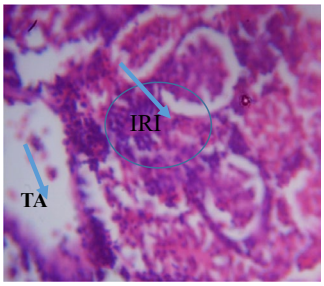


Photomicrograph of T 6 Section of skin (X100)(H/E) shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain sever melanin laden macrophages(MLM)

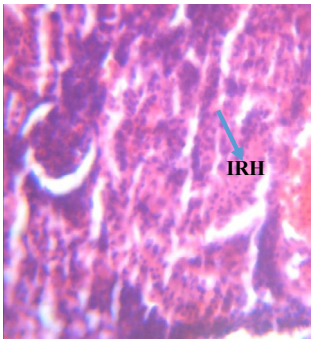
Plate A: (Fig 2-8) Histological change observed in the skin of Juvenile Catfish Clarias gariepinus treated with different concentration of ichthyotoxic plant *Bridelia micrantha.*



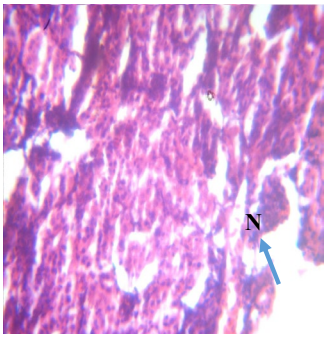
Photomicrograph of T0 section of kidney (x400) (H/E) shows normal renal tissue arragemnt.



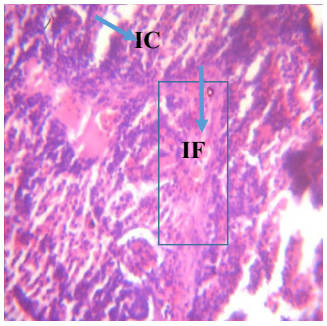
Photomicrograph of T1 section of kidney (x400) (H/E) shows moderate degeneration of the renal tissue with moderate intra renal inflammation (IRI) and moderate tubular atrophy (TA)



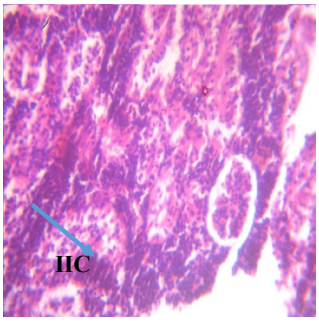
Photomicrograph of T2 section of kidney (x400) (H/E) shows mild degeneration of the renal tissue with mild intra renal hemorrhage (IRH)



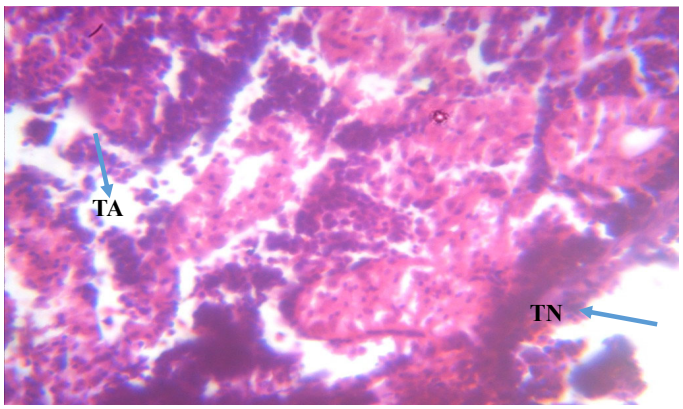
Photomicrograph of T3 section of kidney (x400) (H/E) shows moderate degeneration of the renal tissue with moderate necrotic (N) glomeruli



Photomicrograph of T4 section of kidney (x400) (H/E) shows moderate degeneration of the renal tissue with moderate intra renal fibrosis (IF) and inflammatory cell aggregate (IC)

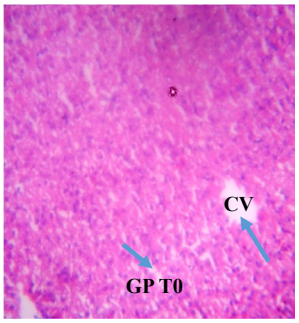


Photomicrograph of T5 section of kidney (x400) (H/E) shows moderate degeneration of the renal tissue with moderate infiltration of inflammatory cell (II)

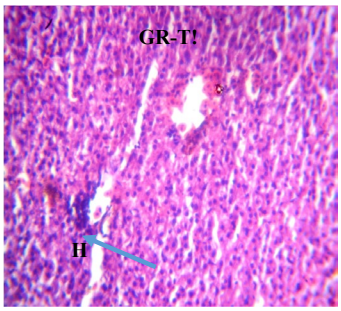


Photomicrograph of T6 section of kidney (x400) (H/E) shows severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in same areas

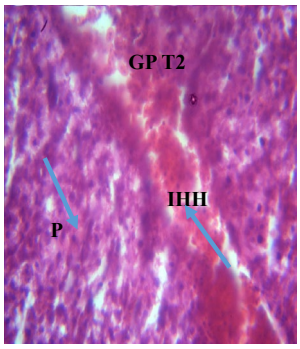
Plate B: (Figures 9-15) Histological change observed in the Kidney of Juvenile Catfish Clarias gariepinus treated with different concentration of ichthyotoxic plant *Bridelia micrantha.*



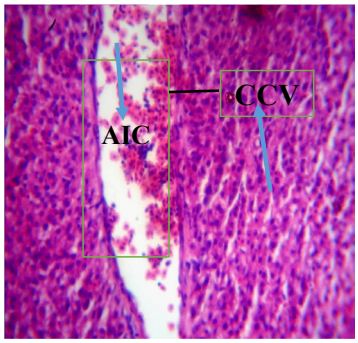
Photomicrograph of T0 control section of liver (X100)(H/E) shows normal hepatic architecture with normal hepatocyte (H) and central vain (C).



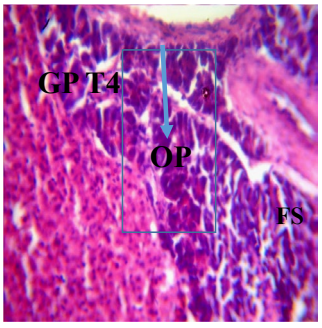
Photomicrograph of T1 section of liver (X100) (H/E) shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI)



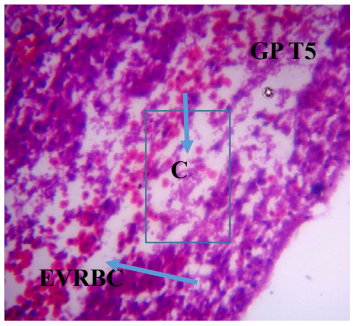
Photomicrograph of T2 section of liver (X100)(H/E) shows moderate effect on the hepatic tissue with moderate pyknotic (P) hypatocyte and intra hepatic hemorrhage (IHH)



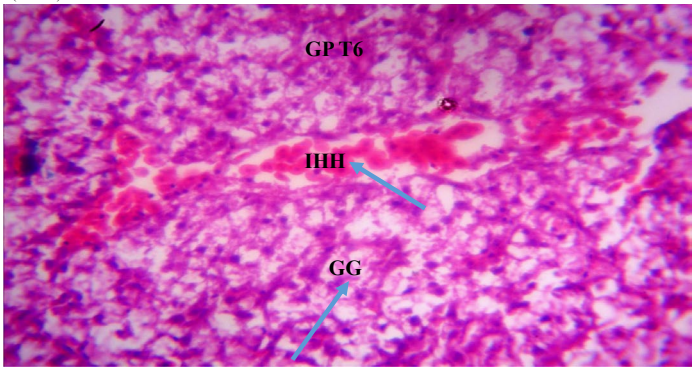
Photomicrograph of T3 section of liver (X100)(H/E) shows mild to moderate effect on the hepatic tissue with moderate congestion of the central vain (CCV)and aggregate of inflammatory cell (AIC) within the congested area focal area,



Photomicrograph of T5 section of liver (X100) (H/E) shows moderate to severe effect on the hepatic tissue with sever focal area of cirrhosis(C) and extravascular red blood cell (EVRBC)

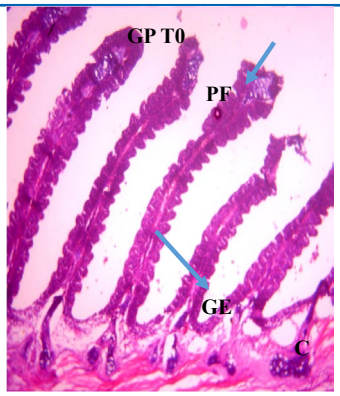


Photomicrograph of T5 section of liver (X100)(H/E) shows severe effect on the hepatic tissue with severe focal area of cirrhosis(C) and extravascular red blood cell (EVRBC)

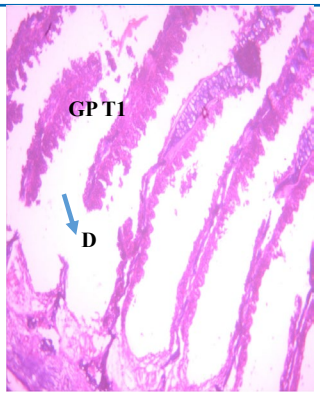


Photomicrograph of T6 section of liver (X100) (H/E) shows sever effect on the hepatic tissue with sever ground glass (GG) appearance of the cytoplasm and intra hepatic hemorrhage (IHH)

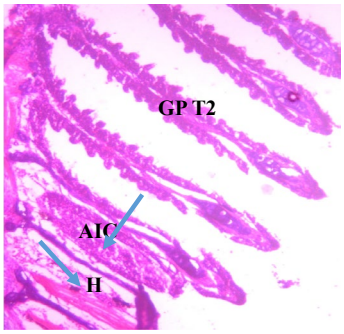
Plate C: (Figures 16-22) Histological change observed in the Liver of Juvenile Catfish Clarias gariepinus treated with different concentrations of ichthyotoxic plant *Bridelia micrantha*.



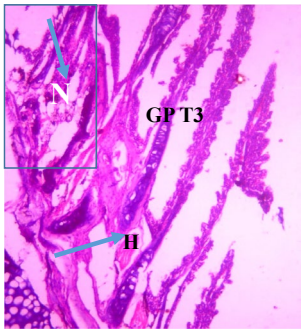
Photomicrograph of A control section of gill (X150)(H/E) shows normal gill architecture with well projected filament (PF) , gill epithelium (GE) and cartilages (C).



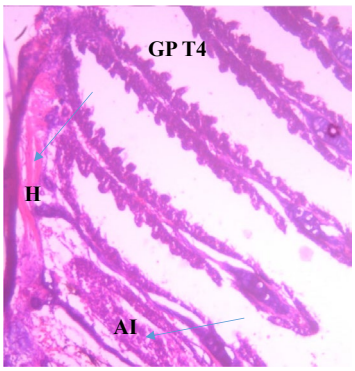
Photomicrograph of T1 section of gill (X150)(H/E) shows mild effect on the gill architecture with mild detachment (D) of the gill filament from the epithelia



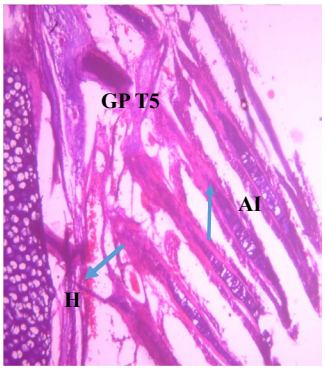
Photomicrograph of T2 section of gill (X150)(H/E) shows mild effect on the gill architecture with mild focal area of hemorrhage (H) and mild aggregate of inflammatory cell (AIC)



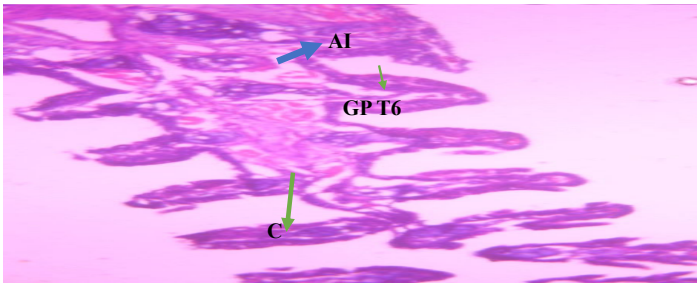
Photomicrograph of T3 section of gill (X150)(H/E) shows mild to moderate effect on the gill architecture with mild focal area of hemorrhage (H) and moderate focal area of necrosis (N)



Photomicrograph of T4 section of gill (X150)(H/E) shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) and aggregate of inflammation (AI)



Photomicrograph of T5 section of gill (X150)(H/E) shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) , necrotic (N) gill filament and aggregate of inflammation (AI)



Photomicrograph of T5 section of gill (X150)(H/E) shows severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI)

Plate D: (Figures 23-29) Histological change observed in the gill of Juvenile Catfish Clarias gariepinus treated with different concentration of ichthyotoxic plant *Bridelia micrantha.*

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
0.15	0.00	0.00	0.00	3.33	6.67	13.33	13.33	13.33	16.67	20.00	26.67	30.00
0.30	0.00	6.67	6.67	16.67	16.67	20.00	20.00	20.00	23.33	33.33	33.33	46.67
0.60	0.00	6.67	6.67	16.67	16.67	23.33	30.33	36.67	40.00	43.33	60.00	73.33
1.20	3.33	6.67	6.67	16.67	16.67	26.67	40.00	46.67	46.67	60.00	66.67	83.33
2.40	6.67	10.00	20.00	30.00	33.33	43.33	53.33	53.33	63.33	66.67	80.00	83.33

Table 3: Mean percentage cumulative mortality of *Bridelia micrantha* to C. gariepinus adult (Range Finding 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
0.10	0.00	3.33	3.33	3.33	6.67	6.67	10.00	13.33	13.33	13.33	20.00	20.33	20.33
0.20	3.33	10.00	10.00	10.00	10.00	16.67	26.67	26.67	26.67	33.33	36.67	40.00	40.00
0.30	13.33	6.67	13.33	16.67	26.67	30.00	30.00	30.00	33.33	50.00	56.67	56.67	63.33
0.40	13.33	16.67	23.33	23.33	40.00	40.00	46.67	50.00	50.00	56.67	66.67	70.00	73.33
0.50	13.33	20.00	26.00	26.64	43.33	43.33	53.33	53.33	53.33	56.67	56.67	63.33	80.00
0.60	20.00	23.33	23.33	23.33	30.00	43.33	53.33	53.33	66.67	66.67	66.67	86.67	90.00

Table 4: Mean percentage cumulative mortality of *Bridelia micrantha* to *C. gariepinus* adult (Definitive Test)

Behaviour/exposure time	12hours							16hours							20hours							24hours						
Concentration (mg/L)	0.00	0.15	0.30	0.60	1.20	2.40	3.60	0.00	0.15	0.30	0.60	1.20	2.40	3.60	0.00	0.15	0.30	0.60	1.20	2.40	3.60	0.00	0.15	0.30	0.60	1.20	2.40	3.60
Air gulping	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Loss of balance	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Excessive mucus secretion	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Operculum movement	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+
Abnormal Tail movement	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Moulting	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+
Discoloration	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+
Barbell deformation	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+
Loss of Reflex	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+

Table 5. General behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of Bridelia micrantha (range finding test)

Key

\+ = present

\- = Not present

Behaviour/exposure time	24hrs							48hrs							72hrs							96hrs						
Concentration (mg/L)	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.00	0.10	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	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+

Table 6. General behavioural changes of *Clarias gariepinus* exposed to different concentration of aqueous extract of *Bridelia micrantha* (Definitive test)

Key
+ = present
- = Not present

RANGE FINDING TEST					DEFINITIVE TEST				
Conc.	Temp(O^C)	DO_2	Conductivity	pH	Conc.	Temp(O^C)	DO_2	Conductivity	pH
0.00	25.50 ± 0.6^a	3.42 ± 0.7^a	42.00 ± 4.1^{ab}	6.95 ± 0.3^{ab}	0.00	26.50 ± 0.6^a	4.43 ± 0.7^a	44.00 ± 4.1^{ab}	6.45 ± 0.3^{ab}
0.15	25.00 ± 0.6^a	3.42 ± 0.2^a	41.00 ± 4.1^{ab}	6.97 ± 0.3^a	0.10	26.00 ± 0.6^a	3.62 ± 0.2^a	39.00 ± 4.1^{ab}	6.27 ± 0.3^a
0.30	25.17 ± 1.0^a	0.80 ± 0.6^a	36.50 ± 11.3^a	6.01 ± 1.6^b	0.20	25.17 ± 1.0^a	3.80 ± 0.6^a	36.50 ± 11.3^a	6.51 ± 1.6^b
0.60	25.00 ± 0.6^a	4.15 ± 1.1^a	33.33 ± 13.4^{ab}	8.11 ± 1.5^b	0.30	25.00 ± 0.6^a	4.15 ± 1.1^a	33.33 ± 13.4^{ab}	7.11 ± 1.5^b
1.20	25.50 ± 0.8^a	3.45 ± 0.4^a	42.33 ± 3.4^b	7.63 ± 1.0^{ab}	0.40	25.50 ± 0.8^a	4.45 ± 0.4^a	42.23 ± 3.4^b	7.63 ± 1.0^{ab}
2.40	26.00 ± 1.7^a	3.18 ± 0.7^a	47.33 ± 5.5^{ab}	6.90 ± 0.2^b	0.50	26.00 ± 1.7^a	3.18 ± 0.7^a	47.33 ± 5.5^{ab}	6.90 ± 0.2^b
3.60	25.83 ± 1.2^a	3.66 ± 0.8^a	38.80 ± 8.3^{ab}	6.97 ± 0.5^{ab}	0.60	24.83 ± 1.2^a	3.66 ± 0.8^a	36.80 ± 8.3^{ab}	6.57 ± 0.5^{ab}

Table 7: Summary of water quality parameter of *Bridelia micrantha* to *Clarias gariepinus* (mean ± SD

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.

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	$1.72 \times 10^{2} \pm 12.5^{c}$	$2.63 \times 10^{6} \pm 3.3^{b}$	11.08 ± 2.3^{b}	22.83 ± 2.5^{b}	$3.22 \times 10^{4} \pm 1.3^{a}$	96.78 ± 1.0^{b}	$1.16 \times 10^{2} \pm 2.3^{b}$	41.20 ± 4.6^{a}	45.88 ± 4.2^{a}
0.10	$1.62 \times 10^{2} \pm 10.5^{c}$	$2.13 \times 10^{6} \pm 3.1^{b}$	10.21 ± 2.2^{b}	22.34 ± 2.2^{c}	$3.21 \times 10^{4} \pm 1.2^{a}$	94.77 ± 2.2^{b}	$1.10 \times 10^{2} \pm 9.6^{b}$	39.23 ± 3.5^{ab}	43.33 ± 1.3^{a}
0.20	$1.83 \times 10^{2} \pm 14.7^{c}$	$3.25 \times 10^{6} \pm 4.3^{b}$	11.34 ± 3.6^{b}	22.85 ± 9.6^{c}	$3.50 \times 10^{4} \pm 2.2^{a}$	93.93 ± 7.9^{b}	$1.13 \times 10^{2} \pm 1.4^{b}$	41.71 ± 8.1^{a}	58.45 ± 6.5^{a}
0.30	$1.71 \times 10^{2} \pm 4.9^{c}$	$3.05 \times 10^{6} \pm 6.1^{b}$	12.52 ± 3.9^{b}	14.96 ± 6.0^{abc}	$6.30 \times 10^{4} \pm 1.7^{b}$	88.32 ± 4.6^{b}	$1.11 \times 10^{2} \pm 7.7^{b}$	46.52 ± 5.6^{a}	52.80 ± 5.1^{abc}
0.40	$1.5^{2} \times 102 \pm 8.8^{b}$	$8.23 \times 10^{6} \pm 1.0^{b}$	11.68 ± 3.6^{b}	5.73 ± 8.7^{a}	$1.26 \times 10^{4} \pm 2.0^{a}$	67.90 ± 1.5^{a}	$1.19 \times 10^{2} \pm 0.8^{b}$	52.05 ± 9.8^{b}	45.02 ± 4.6^{bc}
0.50	$1.28 \times 10^{2} \pm 10.2^{a}$	$2.10 \times 10^{6} \pm 3.1^{a}$	6.47 ± 0.9^{a}	17.13 ± 1.4^{bc}	$1.73 \times 10^{4} \pm 1.7^{a}$	93.63 ± 2.7^{b}	$1.77 \times 10^{2} \pm 0.9^{b}$	37.80 ± 4.1^{ab}	35.21 ± 3.5^{a}
0.60	$1.55 \times 10^{2} \pm 19.3^{b}$	$1.55 \times 10^{6} \pm 2.1^{b}$	12.80 ± 2.2^{b}	9.36 ± 4.1^{ab}	$2.26 \times 10^{4} \pm 2.7^{a}$	89.96 ± 4.6^{b}	$1.08 \times 10^{2} \pm 0.6^{a}$	$\mathbf{51.31} \pm 7.3^{ab}$	29.54 ± 5.6^{b}

Table 8: The summary of toxicity of aqueous extract of *Bridelia micrantha* on haematological parameters *Clarias gariepinus* adult (mean±SD)

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.

Conc. (mg/L)	Moisture (M)	Crude Protein (CP) (Mg/l)	Crude Lipid (CL)	Crude Fiber (CF)	Ash	Nitrogen Free Extract (NFE)	Energy (kcal/100g)
0.00	66.24 ± 6.9^{a}	29.71 ± 2.3^{b}	3.49 ± 0.4^{c}	0.00 ± 0.0	0.90 ± 0.0^{b}	0.38 ± 0.1^{a}	$1.37 \times 10^{2} \pm 6.6^{a}$
0.10	67.30 ± 5.5^{a}	28.66 ± 2.2^{b}	3.51 ± 0.3^{c}	0.00 ± 0.0	0.65 ± 0.3^{a}	0.40 ± 0.2^{b}	$1.32 \times 10^{2} \pm 6.2^{a}$
0.20	63.31 ± 8.7^{a}	29.23 ± 5.5^{b}	3.3 ± 0.4^{c}	0.00 ± 0.0	0.69 ± 0.3^{ab}	0.41 ± 0.1^{b}	$1.31 \times 10^{2} \pm 6.8^{a}$
0.30	69.17 ± 9.8^{a}	29.27 ± 2.0^{b}	3.48 ± 0.9^{c}	0.00 ± 0.0	0.88 ± 0.31^{a}	0.38 ± 0.0^{b}	$1.38 \times 10^{2} \pm 5.0^{a}$
0.40	63.34 ± 2.6^{a}	27.20 ± 0.4^{b}	3.10 ± 0.2^{c}	0.00 ± 0.0	0.94 ± 0.1^{ab}	0.35 ± 0.1^{b}	$1.38 \times 10^{2} \pm 2.3^{a}$
0.50	64.94 ± 9.7^{a}	26.42 ± 0.5^{b}	3.03 ± 0.5^{c}	0.00 ± 0.0	0.84 ± 0.1^{ab}	0.42 ± 0.1^{b}	$1.36 \times 10^{2} \pm 3.4^{a}$
0.60	64.57 ± 6.2^{a}	26.21 ± 1.3^{b}	3.31 ± 0.5^{c}	0.00 ± 0.0	0.83 ± 0.1^{b}	0.25 ± 0.0^{b}	$1.31 \times 10^{2} \pm 4.0^{a}$

Table 9: The summary of toxicity of aqueous extract of Bridelia micrantha on Carcass/Proximate composition of Clarias gariepinus Juvenile (mean ±SD)

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.

Conc. (mg/l)	GILLS	SKIN	LIVER	KIDNEY
0.00	Shows normal gill architecture with well projected filament (PF), gill epithelium (GE) and cartilages (C).	Shows normal skin architecture with epidermis (E) dermis (D).	Shows normal hepatic architecture with normal hepatocyte (H) and central vain (C).	Section of kidney (x400) (H/E) shows normal renal tissue arrangement.
0.10	Shows mild effect on the gill architecture with mild detachment (D) of the gill filament from the epithelia	shows moderate to severe effect on the sin layer with moderate superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain sever melanin laden macrophages(MLM). The overall features are consistence with (MODRATE MELANOMA)	shows mild effect on the hepatic tissue with mild aggregate of intra hepatic inflammation (IHI)	moderate degeneration of the renal tissue with moderate intra renal inflammation (IRI) and moderate tubular atrophy (TA)
0.20	shows mild effect on the gill architecture with mild focal area of hemorrhage (H) and mild aggregate of inflammatory cell (AIC)	shows moderate to severe effect on the skin layer with sever superficial spreading of melanoma (M) at the epidermis and the dermis and lymphocyte infiltration (LI) The overall features are consistence with (CHRONIC MELANOMA)	shows moderate effect on the hepatic tissue with moderate pyknotic (P) hypatocyte and intra hepatic hemorrhage (IHH)	shows mild degeneration of the renal tissue with mild intra renal hemorrhage (IRH)
0.30	Shows mild to moderate effect on the gill architecture with mild focal area of hemorrhage (H) and moderate focal area of necrosis (N).	shows moderate effect on the skin layer with necrotic epidermis and loss of dermal tissue	shows mild to moderate effect on the hepatic tissue with moderate congestion of the central vain (CCV)and aggregate of inflammatory cell (AIC) within the congested area focal area,	moderate degeneration of the renal tissue with moderate necrotic (N) glomeruli
0.04	shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) and aggregate of inflammation (AI)	shows moderate to severe effect on the skin layer with necrotic (N)epidermis , loss (L) of tissue within the intra dermal region and and lymphocytic infiltration ,(LI).	shows moderate to severe effect on the hepatic tissue with sever focal area of cirrhosis(C) and extravascular red blood cell (EVRBC)	moderate degeneration of the renal tissue with moderate intra renal fibrosis (IF) and inflammatory cell aggregate (IC)
0.05	shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) , necrotic (N) gill filament and aggregate of inflammation (AI)	shows sever effect on the sKin layer with sever superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain melanin laden macrophages(MLM)	shows moderate to severe effect on the hepatic tissue with sever focal area of cirrhosis(C) and extravascular red blood cell (EVRBC)	shows moderate degeneration of the renal tissue with moderate infiltration of inflammatory cell (II)
0.06	shows severe effect on the gill architecture with hypertrophy of the gill arch, gill filament and aggregate of inflammation (AI)	shows severe effect on the skin layer with sever superficial spreading of melanoma (M) restricted to the epidermis and the dermis contain sever melanin laden macrophages(MLM)	shows sever effect on the hepatic tissue with severe ground glass (GG) appearance of the cytoplasm and intra hepatic hemorrhage (IHH)	shows severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in same areas

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

5. Discussion

The toxicity of aqueous extract of *Bridelia micrantha* 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 observed restlessness and mortalities of the test fish might be due to the effect of flavonoids, alkaloids and saponin present in the extracts Obomanu *et al.,* [19]. Saponin are ichthyotoxins which destroy the erythrocytes and are assimilated directly through the gills Fry [20]. Alkaloids on the other hand inhibit oxidative phosphorylation, blocks the mitochondrial enzymes, Nicotinamide Adenine Dinucleotide (NADH) ubiquinone reductase, hence impairing their oxygen consumption Tiwari and Singh, Haffor, and Al-Ayed, [21,22]. The active ingredients such as saponin, Tannins which are directly absorbed through the gills, haemolyses erythrocytes, and the toxicity might have also been through impairment of oxygen consumption in the exposed fish as saponin is also reported to lower the surface tension of reconstituted extracts with the formation of colloidal substances within them. Tannins have been reported to have protein coagulating property on gill epithelia, causing respiratory failure or asphyxiation in the exposed fish, Mayombo *et al*. [23].

The lethal toxicity 96hrsLC_{50} of aqueous extract of *Bridelia micrantha* to the *Clarias gariepinus* Juvenile in the present study is 0.21mg/l, this value is less than the work of Olaifa *et al.* and Omitoyin *et al*. who reported a 96hrs LC_{50} of copper as 0.67 mg L^{-1} and lindane 0.38 mg L^{-1} for *C*. gariepinus, respectively stating that they are highly toxic, and is similar the work of Akpa *et al*., who reported 0.71ml/l 96hrs LC_{50} in their work to Cichlid Fish – Tilapia zilli after exposure to fish beans (*Tephrosia vogelii*) leave extract for 96 hours [24-26]. Death may also be due to disruption and failure in gill functions hence, reducing availability of surface for gaseous and ionic exchange Ayuba and Ofojekwu 2002. Ayotunde and Ofem reported that the acute toxicity of Pawpaw seed decreased with increase in time [27]. 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 [14].

Water quality parameters of experimental set-up for both treatments were similar and within the optimum range recommended for culture of *Clarias gariepinus* [28,29]. The slight fluctuations of the physio-chemical parameters in the different treatments showed no significant difference (table 7) and the effects on this study could be negligible. However, the wide fluctuations with significant difference might have been altered by the experiment and hence produced deleterious effect such as stressful conditions of abnormal behaviours prior to death and mucus secretion on the gills of the moribund fish. Konar, Steels (1983), Fafioye and Jeje (2000) and Fafioye reported that accumulation of mucus on fish gills reduces respiratory activity in fish and this might have accounted for mortality [30,31]. Tobacco had been reported to have nicotine (Hassal, 1982) which binds to acetylcholine receptors in the nervous system thus causing the excitation [32]. In this present 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 Ayotunde *et al*.,[33]. The observed behavioural abnormalities are attributed to respiratory impairment, resulting from the effects of the toxicant on the gills of the exposed fish. 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 when they exposed some species of fish to different toxicants [34-36].

The use of haematological techniques in fish culture is of growing importance to toxicological research, environmental monitoring and fish health conditions Ada et al., Olusegun and Adedayo [37,38]. Fish are so intimately associated with the aqueous environment, often physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in the fish, Olusegun and Adedayo [39]. Haematological parameters and physiological profile can be useful indicators of the physiological disturbances in animals and so can be crucial in providing vital information on the general well-being of fish, Tavares-Dias and Moraes [27,40]. Blood cell indices (RBC, WBC, Hb, PCV and ESR.) are good indicators of system response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood. Thus detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat Ojutiku *et al.,* [41]. Therefore, blood serves as a good indicator to determine the health of an organism and acts as a pathological reflector of the whole body. Hence haematological parameters are important in diagnosing the functional status of animals exposed to toxicants Olusegun and Adedayo [38].

In the present study there was reduction in the results of blood parameters of Catfish gariepinus juvenile exposed to ichthyotoxic plant *Bridelia micrantha* in the respective treatments as presented in table 8. The reductions in blood cell indices and tissue deformation observed from the chronic bioassay are in line with findings of the study on behavioural, haematological and histopathological changes in C. gariepinus exposed to Parkia biglobosa pods Bawa-Allah and Akinnuoye [42]. Similar observations have also been reported in a study on exploitation of ethanol extract of *Adenium obesum* stem bark as a potent organic piscicide, Dar and Paul [5]. Blood analysis revealed significant ($P<0.05$) reduction in the blood parameters (Heamoglobin, Packed Cell Volume, Red Blood Cells, White Blood Cells, Platelet, Mean Corpuscular Volume and Mean Corpuscular Heamoglobin) of exposed fish Ayotunde *et al.,* [33].

There is paucity of information on the effect of ichthyotoxic plants on Carcass/Proximate composition of fish. Bolu *et al.*, reported that the values of serum protein decreases with increasing level of dried pawpaw seed (DPS), in their work to determine the effect

of Graded Levels of Dried Pawpaw (Carica papaya) Seed on the Performance, Haematology, Serum Biochemistry and Carcass Evaluation of Chicken Broilers [43]. The proximate composition of carcasses in this present study reveals variations in the values obtained in the results of Carcass composition of Clarias gariepinus juvenile exposed to aqueous extract ichthyotoxic plant *Bridelia micrantha* Table 9, Crude Protein reduced from 29.71±2.3 to 26.21±1.3. Reduced carcass/proximate composition of exposed fish may have resulted from the interference of the toxicant with normal functioning of the gastrointestinal system which impaired the normal nutritional activity and coordination *C. gariepinus* [44]. Ochang *et al.,* reported Significant difference ($p<0.05$) in their work to determine the effect of diets with moringa leaf meal on growth, carcass composition and haematology of Clarias gariepinus, protein content of the carcass reduced, and fat and ash contents increased as MLM increased in the diets [45]. The results of this study show that MLM can replace up to 20% of the soybean meal in the diets of *C. gariepinus.*

Histopathology changes in the skin, gill, liver and kidney were analysed at the end of the 96hours.Normal architecture of sections of the organs were maintained in the control group. While some pathological disruptions occurred in the higher concentration, table 10, Plate A-D (figures 2-29). The *B. micrantha* 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. This present work is similar to the work of Ayotunde *et al.,* who reported that fish exposed to the high extract of pawpaw seed extract show severe gill epithelial hyperplasia, separation of the gill epithelial layers from supportive tissues [9]. These according to can lead to brachial malfunction of which may affect physiology or causes death to fish [8,46].

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. The damage done to these organs was as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. The phytochemical analysis of the leaves extract revealed the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, steroids, balsam, phenol and volatile oil Absalom et al., [36]. Jegede and Olanrewaju reported congestion of blood vessel of the gill, proliferation of mucos cells, proliferation in the epithelium of the gill filament, aggregation of inflammatory cells which are related to gill function disorders [47]. Liver shows vacuolar degeneration, diffused vacuolation in hepatocyte, inflation of the liver, vacuolation and fibrosis in the hepatocellular parenchyma and kidney shows degeneration of interstitial tissue, glomerular shrinkage, degeneration of tubular epithelia cell and severe vacuolation and lesion in kidney cell, Piscicidal effect of tobacco (*Nicotiana tobaccum*) leaf dust on african giant catfish (*Heterobranchus bidorsalis*) fingerlings [48-53].

6. Conclusion

The *Bridelia micrantha* (Hochst.) (Phyllanthaceae) is an ichthyotoxic plant used in catching fish in Nigeria. The toxicity of aqueous extract of *Bridelia micrantha* 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 102 ± 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 102 ±4.0 respectively. The B. micrantha 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 Cross River and Nigeria water bodies.

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