

Ascorbic Acid Mediated Amelioration of *Bridelia micrantha* Induced Alterations in Haematological, Histological and Proximate Composition of the Carcass of African Catfish, *Clarias gariepinus* (Burchell, 1822)

Abstract

Acute and sub-acute exposure of piscicidal plant *Bridelia micrantha* (BM) during fishing of Sharptooth Catfish *Clarias gariepinus* juvenile causes alterations in haematological, histological and the carcass analysis. The result of water quality shows the normal value for fish culture in the tropics, the DO₂ level of the water varies slightly such that the T1, T2, T3, T5 and T0 were in the normal range, conductivity followed by T2 whereas T1 had the least value for conductivity, which the mean differences were statistically not significant ($p > 0.05$) among the individual treatments. There was severe alterations in haematology, of *Clarias gariepinus* exposed to *Brandelia micrantha*, at 0.00, 0.042, 0.084, 0.126, 0.168 and 0.21mg/l during 60days of exposure periods, blood analysis show a reduction in white blood cell (mg/l), Haemoglobin, Red blood cell (mg/l) Haemoglobin (g/dl), Lymphocytes (mg/l), Mean cell volume (fl), Mean cell concentration, tissue analysis indicated an alterations in the gill, liver and kidney while tissue analysis reveals severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in some areas and carcass analysis show severe reduction in Moisture (M), Crude Protein (CP) (Mg/l), Crude Lipid (CL), Ash, Nitrogen Free Extract (NFE), and Energy (kcal/100g). The recovery of *C. gariepinus* from *Bridelia micrantha* (BM) was evaluated by exposing *C. gariepinus* to a 60-days depuration, on ascorbic acid (AA) supplemented diet at concentrations of 0.00, 25, 50, 75, 100, 125ml/l. After 60-days depuration some of the lesions in histological, haematological and proximate composition of the carcass attained different stages of recovery as compared to the control.

KEY WORD: - Ameliorative Effect, Ascorbic acid, *Bridelia micrantha*, Haematology, Histopathology, Proximate composition, Catfish *Clarias gariepinus*

1.0 Introduction

Coastal golden leaf Plant *Bridelia micrantha*, have been used as a biocide to control unwanted aquatic animals during pond preparation because of its inexpensiveness, local availability and easy degradability Audu *et al.* (2017). Plant extracts used as piscicides in fisheries are considered advantageous when viewed against the backdrop of using persistent chemicals (Ekpo *et al.* (2019). In addition, it was discovered to have the potential of killing aquatic lives such fishes, hence it has been used for commercial fishing activity such as harvesting of fishes. The toxic parts of plants *M grifoniana* employed as fish poisons include the roots, seeds, fruits, barks or leaves. *Bridelia micrantha* (Hochst.) (Phyllanthaceae) is a fish poisons use to catch fish is part of the traditional fishing method which is not 'environmentally friendly' to the aquatic ecosystem (Connell 1999, Ayotunde and Igbang 2023). *Bridelia micrantha* are widely available in the tropics and their leaves have been used as natural piscicides in artisanal fisheries and aquaculture ponds in Nigeria. The active substance of the majority of ichthyotoxic Plant are resin, tannin, rotenone, saponin alkaloid, diterpenes, cynogenic/cynogenetic glycosides, nitro containing compounds, oxalates, resins

and certain proteins and or amino acids (Biswas, *et al.*, 2002). The toxic parts of plants *Bridelia micrantha* employed as fish poisons include the roots, seeds, fruits, barks or leaves. *Bridelia micrantha* contains active ingredients; alkaloids such as triterpenoid saponins nicotine, pyrethrum, ryania, rotenone, coumerin, resin, akuammine, tannins, and diosgenin and rotenoids, with rotenone being the most widely used commercially (Dar and Paul. 2018). Other toxic compounds found in the plant include sesquiterpenes, diterpenoids (including diterpenoidorthoesters), triterpenes, furanocoumarins, 2-hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone, prenyl phenyl propanoids, and sterol acylglucosides often called fish poison (Ayotunde, 2019).

Ekpo *et al.* (2019) identified these active ingredients of *Bridelia micrantha* as plant poison; hence a poisonous plant is defined as one containing toxic compounds that can be harmful when consumed by humans or animals. These plants possess special structures that cause mechanical injuries upon contact, releasing chemicals that trigger systemic reactions even without ingestion. Fish poisons derived from plants are used for cultural, commercial and environmental purposes. Traditional fishing methods involving ichthyotoxic plants are not considered environmentally friendly due to its adverse effect on fish's health, reproduction and metabolism processes. The diverse chemical structures of these compounds raise questions about their relative toxicity, biochemical mechanisms, and environmental impact on fish. The mechanism of action of ichthyotoxic plants *Bridelia micrantha* varies from cytotoxicity to genotoxicity, affecting cellular processes and gene expression (Aafreen *et al.*, 2021). Toxicants generally interfere with physiological or biochemical regulatory mechanisms, sometimes altering cell division regulation and causing skin lesions. Work has been done on dietary efficacy of vitamin C in ameliorating pollutants toxicity to fish (El-Keredy *et al.*, 2017). During laboratory studies, fish are usually returned to toxicant-free water after an exposure to initiate 'self' detoxification process sufficient to reverse any morphological, biochemical or physiological changes arising from such exposure. Earlier studies have shown that, after exposure, the normal physiology of fish could either be moderately restored (Adhikari *et al.*, 2004; Ramesh *et al.*, 2015, Ayotunde 2019,) or fully restored (Deshmukh and Waghmode 2011)) when relocated to water free of toxicant.

Clarias gariepinus is an eel-like fish, belonging to the Clariidae family, known as air-breathing catfishes that respire bimodally (Piria *et al.*, 2020). It has a dark gray or black coloration on its back transitioning to a white belly, with length of 1 to 1.5m and a maximum total length of 1.7m, weighing up to 60kg (Abalaka *et al.* 2015; Ogueji *et al.*, 2017). It inhabits freshwater lakes, rivers, swamps, streams and floodplains and Middle East (Idowu *et al.*, 2019; Ibiam *et al.*, 2015). *C. gariepinus* stands out as one of the most commonly utilized fish species globally whose aquaculture potential have been documented (Abalaka *et al.* 2015). This fish was chosen for this study due to its availability, frequent cultivation for commercial purposes, and easy maintenance in laboratory settings. While it tolerates drought well, it struggles in water temperatures below 9-10°C. Additionally, *C. gariepinus* is omnivorous and known to cannibalize fish half its size or 10% of its body weight. It's also capable of cannibalizing its own species in specific pond conditions until reaching a size of 50 to 100 g, particularly when proper feeding isn't assured. Despite its presence in the wild, *C. gariepinus* holds a crucial role in tropical catfish aquaculture. *C. gariepinus* has been proven to be a suitable bioindicator species for use in ecotoxicological studies involving synthetic piscicides (chemicals) and plant derived piscicides due to its ability to tolerate a wide range of environmental conditions Ayotunde *et al.* (2010), high stocking densities under culture conditions Ayotunde *et al.* (2011), ease of maintenance in laboratory conditions Kachi *et al.* (2019), fast growth rate Idowu *et al.* (2019), high yield potential Ogueji *et al.*, 2017, high fecundity Ibiam *et al.* (2015), air breathing characteristics and high market value Ogueji *et al.*, 2017; and Jamabo *et al.*, 2017).

2.0 Materials and Methods

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

2.2 Collection and Identification of the Plant Samples

The coastal golden leaf plants *Bridelia micrantha*, specifically its leaves, were collected from the abundant plant populations around the communities at Obubra Local Government Area of Cross River state e, Nigeria. The collection exercise was done during the early hours of the day, between 6:00am and 9:00am. Subsequently, the collected plants were transported to the herbarium unit of the Department of Forestry Obubra Campus, University of Cross River State for proper and accurate identification.

2.3 Preparation of the Identified coastal golden leaf plants *Bridelia micrantha* Samples

The identified coastal golden leaf plants *Bridelia micrantha* plant samples were air-dried at room temperature for duration of two weeks. Afterward, they have undergone oven- drying at 32⁰C for 30 minutes. Then, the dried leaves of *Bridelia micrantha* was then be pulverized into powder using electric blender. The resulting *Bridelia micrantha* powder was passed through a 100 μ m sieve to obtain a fine powder and was transfer into air-tight sterile bottles, labelled, and stored at 4⁰C for the extraction processes. The solutions was left for 72 hours 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. The stock solutions was serially diluted 1:100(water content: toxicant) depending on required concentrations, for use in toxicity testing studies.

2.4 Toxicity Experiment

2.4.1 Test organism/ Acclimation

Five hundred and fifty (550) apparently healthy *Clarias gariepinus* juveniles (4-6 weeks old) was procured from Amazons Fish Farms hatcheries Abakaliki, Ebonyi State, Nigeria with an average body weight (66.38 \pm 6.3g) and length (23.27 \pm 3.5cm) Table 1 and transported in oxygenated polythene bags, to the Fisheries wet Laboratory, Department of Fisheries and Aquatic Science, University of Cross River State (UNICROSS) Calabar, Obubra Campus. Fish acclimatization lasted for a period of 14 days (2 weeks) under natural day and night photo-periods in 150litres capacity tanks containing 100litres de-chlorinated water at room temperature. Netted materials with central slits were tied to the top of the tanks to prevent fishes from jumping out and other organisms from entering. The fishes was nourished with commercially prepared fish feeds (ad libitum) twice daily with 2 mm Coppens fish feed for aquaculture (Coppens[®] International by Helmond, Holland) with 40% crude protein. During this acclimatization period, Water renewal occurred every two days to prevent accumulation of fish metabolic wastes and leftover foods. The feeding was stopped to minimize waste production and ammonia build-up in the tanks at 24 hrs prior to the commencement of the experiments.

2.4.2 Experimental Design

Complete Randomized Design (CRD) was used as experimental design. The experiment consisted of five treatments (0.00 , 0.42, 0.084, 0.126, 0.168 and 0.21ml/l of which treatment one served as control and was further replicated three times each. Each replicate will contain ten (10) juveniles, making a total of 180 juveniles for the research. The fish in the experimental tanks was fed twice daily between 8-8:30am and 5-5:30pm at 5% body weight.

2.5 Ameliorative experiment

2.5.1 Preparation of Vitamin C stock solution

Twenty (20 packets of Ascorbic acid tablets (100 mg/tablet), MedVit C1 (Dol-Med Laboratories Limited, Lagos, Nigeria) was obtained from a Pharmaceutical Store in Calabar. In the laboratory the Vitamin C tablets were ground and a stock solution was prepared by dissolving a known weight of the powder in 900mls of distilled water. The volume was now made up to the 1 L mark using distilled water. The concentration of the solution was calculated from the formula: milligram of powder/ volume of distilled water. This was shook until a homogenous mixture is formed and all the Vitamin C granules have completely dissolved. This was labelled and preserved in screw capped container in a cool place in the laboratory to be used later in formulating fish diet (Ambali *et al.*, 2011). Ameliorative effect of ascorbic acid (vitamin C) on *Bridelia micrantha* exposed fish was evaluated by placing the fish on ascorbic acid supplemented diet of 45% protein level. Twenty one (21) (75cm x 45cm x 45cm) glass tanks of 121.5 litres capacity each were filled with 50 litres aerated unchlorinated rain water. Ten juvenile of the test organism was batch-weighed with a top-loading mettler balance (Mettler Toledo (K), and distributed randomly in triplicate per treatment. Five treatments and one control labelled were used for the ameliorative experiment. The concentrations of the ascorbic acid (vitamin C) used for the experiment were 0.00, 25, 50, 75, 100, and 1501ml/l.

2.6 Haematological Examinations

One test organism was removed, from each tank for blood analysis. 2 – 5 ml blood per fish was collected from vertebral blood vessel in juvenile using 2ml disposable heparinized syringes treated with tetra acetic acid (EDTA) as anti-coagulant. The blood was stored at -4°C in deep freezer prior to analysis. The blood analysis was followed by the method described by Svobodova *et al.*, (1991). Haemocytometer was used in blood cell counts.

$$\text{RBC} = \text{No of cells counted} \times 5 \times 10 \times 200 \text{ (} 10^6 \text{mm}^3 \text{)}$$

$$\text{WBC} = \text{No of cells counted} \times 0.25 \times 10 \times 20 \text{ (} 10^4 \text{mm}^3 \text{)}$$

2.6.1 HAEMOGLOBIN ESTIMATION

Haemoglobinometer will be used for haemoglobin estimation. The apparatus and the reagents used include, Shali graduated tube, standard (SHILONOMETER), N/10 HCL, 0.02 ml pipette. The graduated tube will be filled to 20 ml marked with N/10 HCL; 0.02 ml blood will be added and mixed thoroughly. It will be allowed to stay for 5 minutes, and distilled water will be added drop by drop and each addition was mixed thoroughly, until colour matches the standard. The amount of solution in the graduated tube gives the haemoglobin concentration as percentage.

$$\text{Haemoglobin} = \text{Value obtained} / 100 \times 17.2 \text{ (gm/100ml)}$$

2.6.2 PACKED CELLS VOLUME (PCV)

Non-clotted blood was drawn by capillary action into microhaematocrit tubes; one end of the tubes were sealed with synthetic sealant. The sealed tube was centrifuged in a microhaematocrit centrifuge. Centrifugation lasted for 5 minutes at 10500 rpm. The packed cell volume was measured using microhaematocrit reader and expressed as percentage.

2.6.3 ERYTHROCYTE SEDIMENTATION RATE (ESR)

Non-clotted blood was drawn by capillary action into microhaematocrit tube, one end of the tube was sealed, by a synthetic sealant, and placed, onto a special metal holder consisting of two plates slanting at an angle of 45°. The rate at which erythrocytes spontaneously settled, was determined the erythrocyte sedimentation rate. It was allowed to stand for 40 minutes. The value of erythrocyte sedimentation rate was determined by means of the haematocrit reader. The erythrocytes column was determined as percentage of the total column of the blood. The value of erythrocyte sedimentation rate with the given time interval is the difference 100% and the percentage part represented by the corpuscle column (Svobodova *et al.*, 1991).

2.6.4 MEAN CELL HAEMOGLOBIN CONCENTRATION

This refers to the percentage of haemoglobin in 100ml of red blood cell. This was calculated by dividing the haemoglobin content in g/100ml by the pcv/100ml of red blood according to the formulae: -

$$\text{MCHC} = \text{HB/PCV} \times 1000 \text{ T/l}^{-1}$$

2.6.5 MEAN CORPUSCULAR VOLUME (MCV)

The value of the mean corpuscular volume was calculated from the haematocrit value (pcv) (%) and the erythrocyte count (Er.) ($10^6/\text{mm}^3$), according to the following formula (Svobodova *et al.*, 1991).

$$\text{MCV} = \text{PCV} \times 1000 / \text{Er} (\mu^3)$$

2.6.6 MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

Mean corpuscular haemoglobin concentration, expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemoglobin value (Hb) and from the erythrocyte count (Er) according to the following formula.

$$\text{MCH} = \text{Hb/Er} (\text{Picogramme})(\text{pg})$$

2.7 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 will be determined by the micro-kjedahl method and the crude protein will be taken as $\text{N}\% \times 6.25$ (constant factor) where N is equal to Nitrogen content per 100g sample. Total carbohydrate will be determined using the phenol-sulphuric acid method. The crude fibre was obtained by dry ashing of the sample at 550°C dissolved in 10% HCl (25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

2.8 Histological Examination of Test Organ

The tissues were processed in an automatic tissue processor (Model-Leica AP). The tissues were first processed by putting it in tissue cassette and dehydrated in graded, alcohol of 70-100% and the alcohol was removed with xylene (Limbu, 2020). The clearing agents were replaced by passing the tissues through molten paraffin wax maintained at temperature higher than the melting point of wax, to fill the intercellular spaces for easy microtome. The tissues were embedded in the molten paraffin wax poured inside the Lockhart embedding metal box and allowed for some minutes to solidify before it was detached. The detached paraffin blocks were mounted on the wooden block with the aid of hot spatula, which melted the portion of the paraffin to be placed on wooden block. The melted part quickly solidified to the wooden block on removing the spatula. The wooden blocks were screwed to the microtome chuck and paraffin block trimmed to the embedded tissues and then sectioned at 6µm with a rotator microtome (Limbu, 2020). Sectioned tissues were stained with

hematoxylin and eosin using the method of SRAC (2010). Microphotography of the sectioned tissues produced: micrograph of cells of CAP exposed tissues with control with the use of microscope; procedure was conducted at Federal Teaching Hospital Calabar, Nigeria.

2.9 Statistical analysis

The data was collated and analysed using descriptive statistics, one way analysis of variance and Pearson's correlation. The differences in the means between both values was assessed with Duncan multiple range test Using for means was use to compare differences between the various means using SPSS version 23.0 (SPSS, 2023).

3.0 Results

The result of Length-weight relationship of *Clarias gariepinus* Juvenile exposed to piscicidal plant *B. micranthan* and Ascorbic acid is presented in table 1 shows that the weight (g) varies from (55.18±6.1 - 81.25±8.3) – (55.83±5.4 - 79.22±8.3) and standard length (22.52±3.0 - 27.47±4.3) – (22.52±3.0 - 30.90±2.8) for *Bridellia michranta* and Ascobic acid tests respectively, this result indicates that the experimental fish are in good conditions of health.

Table 1: Length and Weight relationship of Catfish *Clarias gariepinus* Juveniles used for the Experiments (Mean±SD).

Treatment	L/W	Control	1	2	3	4	5
Treated with <i>Bridellia micranthan</i>	Standard Length (cm)	21.62±3.0 ^a	20.60±2.8 ^a	16.82±7.9 ^a	27.47±4.3 ^a	18.24±6.6 ^a	23.27±3.5 ^a
	Weight (g)	81.25±8.3 ^b	55.18±6.1 ^{ab}	65.02±8.1 ^{ab}	70.83±5.4 ^a	68.73±9.4 ^{ab}	66.38±6.3 ^{ab}
Treated with Ascobic Acid	Standard Length (cm)	22.52±3.0 ^a	30.90±2.8 ^a	27.82±7.9 ^a	25.47±4.3 ^a	26.24±6.6 ^a	23.27±3.5 ^a
	Weight (g)	79.22±8.3 ^b	61.18±6.1 ^{ab}	61.02±8.1 ^{ab}	55.83±5.4 ^a	74.73±9.4 ^{ab}	64.38±6.3 ^{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

Table 2 summarizes the range of the water quality parameters of control and treated fish observed during the experimental period. There was no significant difference between the different treatments in terms of water quality parameters measured. Alkalinity significantly increased throughout the experiment. The result of the water quality parameters of the experimental media reveal a significant difference (P<0.05) in the values obtained in the level of dissolved oxygen, content free carbon dioxide and alkalinity with the control. Nevertheless, there were no significant difference (P>0.05) between the values of temperature and pH of test media with the control. The abnormal behaviour observed in fish exposed to the extract included abnormal swimming method, respiratory distress, loss of balance, gulping of air, settling at the bottom motionless, erratic swimming, loss of reflex.

Table 2: - Effect of *Bridellia michranta* and Ascorbic acid on water quality during the experiment

Conc	<i>Brandellia micrantha</i>				Conc. mg/l	Ascorbic Acid			
	Temp(O ^c)	DO ₂	Conductivity	pH		Temp(O ^c)	DO ₂	Conductivity	pH

mg/l.									
0.00	25.50±0.6 ^a	4.42±0.7 ^a	42.00±4.1 ^{ab}	6.95±0.3 ^{ab}	0	25.50±0.6 ^a	4.42±0.7 ^a	42.00±4.1 ^{ab}	6.95±0.3 ^{ab}
0.042	25.00±0.6 ^a	3.42±0.2 ^a	41.00±4.1 ^{ab}	6.97±0.3 ^a	25	25.00±0.6 ^a	3.42±0.2 ^a	41.00±4.1 ^{ab}	6.97±0.3 ^a
0.084	25.17±1.0 ^a	4.80±0.6 ^a	36.50±11.3 ^a	6.01±1.6 ^b	50	25.17±1.0 ^a	3.80±0.6 ^a	36.50±11.3 ^a	6.01±1.6 ^b
0.126	25.00±0.6 ^a	4.15±1.1 ^a	33.33±13.4 ^{ab}	7.11±1.5 ^b	75	25.00±0.6 ^a	4.15±1.1 ^a	33.33±13.4 ^{ab}	8.11±1.5 ^b
0.168	26.00±1.7 ^a	3.18±0.7 ^a	47.33±5.5 ^{ab}	6.90±0.2 ^b	100	26.00±1.7 ^a	3.18±0.7 ^a	47.33±5.5 ^{ab}	6.90±0.2 ^b
0.21	25.83±1.2 ^a	3.66±0.8 ^a	38.80±8.3 ^{ab}	6.97±0.5 ^{ab}	125	25.83±1.2 ^a	3.66±0.8 ^a	38.80±8.3 ^{ab}	6.97±0.5 ^{ab}

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

3.1 Histological alterations in *Clarias gariepinus* exposed to *Brandelia micrantha*)

The results of histological alterations in the liver of *Clarias gariepinus* exposed to *Brandelia micrantha*) is presented in Plate A Figure 1-24, at 15 days treatment 0.00mg/l control section of liver (X100)(H/E) shows normal moderate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) around the hemorrhagic area, the kidney of *Clarias gariepinus* at 60 days treatment 0.21 figs. 30, 36, 42 and 48 section of kidney shows normal renal architecture with glomeruli (G), Bowman space (BS), renal tubules (RT) and tubular cell (TC) in some areas. The gill of *Clarias gariepinus* at 15 days in treatment 0.00 Figures 49, 55, 61 and 67 control section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia. moderate effect on the gill architecture with focal area of necrotic filament (NF) At 30 days in treatment 0.042 figs 50, 56, 62, and 68 section of gill shows moderate effect on the gill architecture with focal area of necrotic filament (NF) at treatment 0.084 Figs 51, 57, 63 and 69 section of gill shows mild effect on the gill architecture with mild focal area of hemorrhage (H) and mild aggregate of inflammatory cell (AIC) at 45 days treatment 0.126 figs 52, 58, 64 and 70 section of gill 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 T0.168 figs 53, 59 65 and 71 section of gill shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) and aggregate of inflammation (AI). The result at 60 days of treatment 0.21 figs 54, 60, 66 and 72 section of gill shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non distinct outline

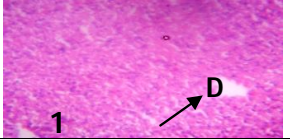
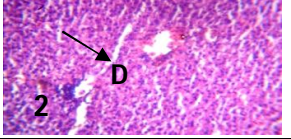
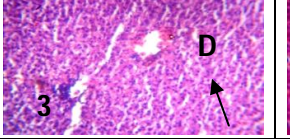
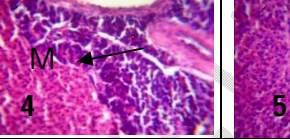
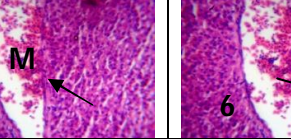
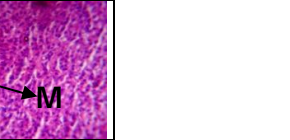
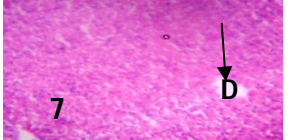
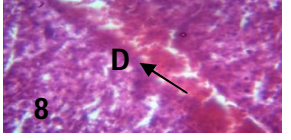
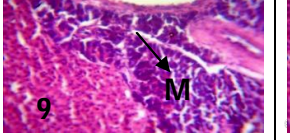
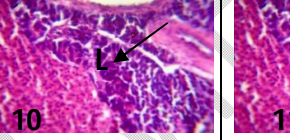
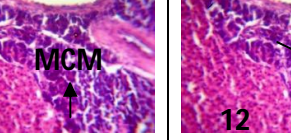

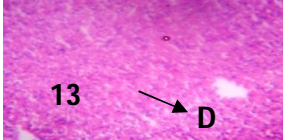
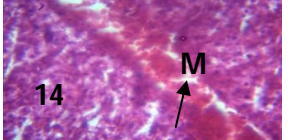
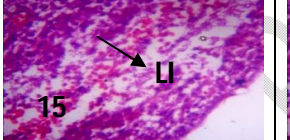
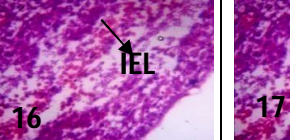
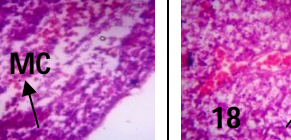
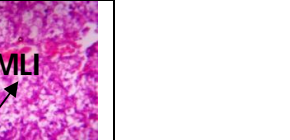
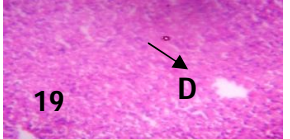
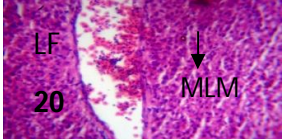

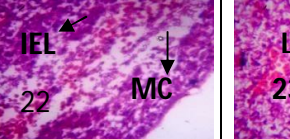
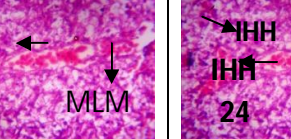
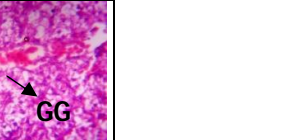
Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	0.042	0.084	0.126	0.168	0.21
15						
30						
45						
60						

Plate A Figure 1:- 24 - Histological alterations in the liver of *Clarias gariepinus* exposed to *Brandelia micrantha*)

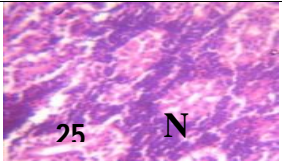
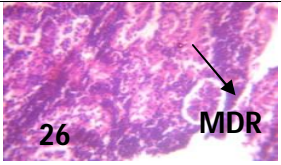
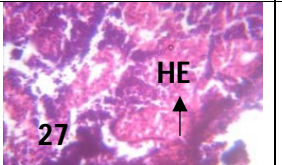
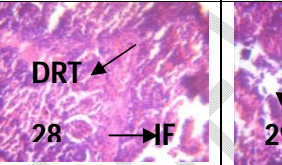
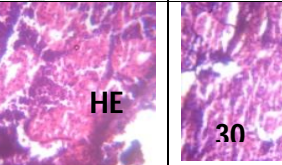
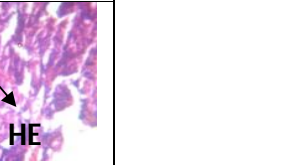
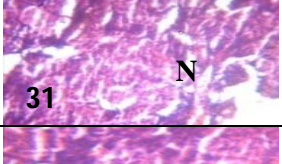
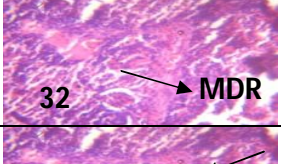
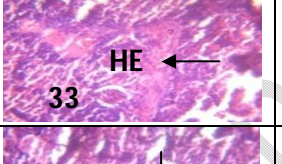
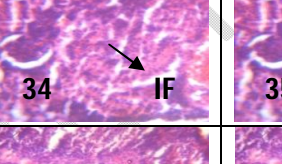
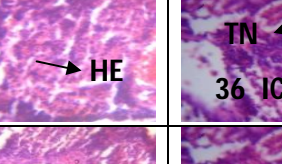
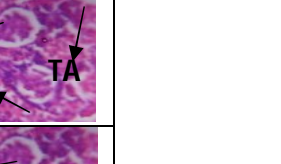
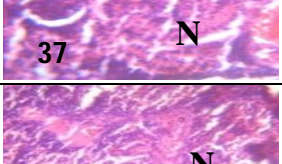
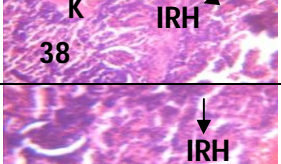
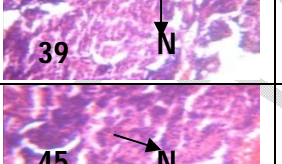
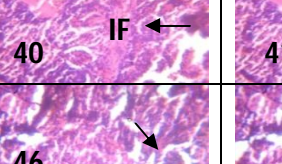
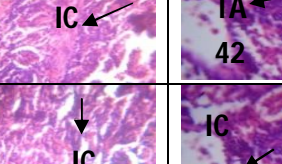
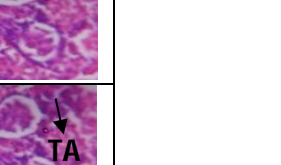
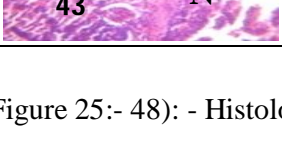
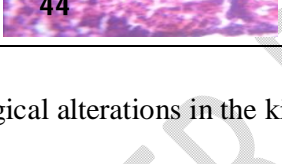
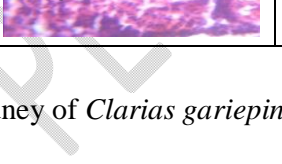
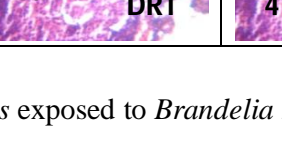
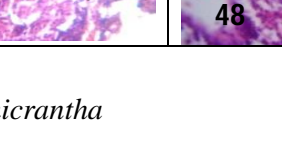

Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	0.042	0.084	0.126	0.168	0.21
15	 25 N	 26 MDR	 27 HE	 28 DRT IF	 29 HE	 30 HE
30	 31 N	 32 MDR	 33 HE	 34 IF	 35 HE	 36 TN IC TA
45	 37 N	 38 K IRH	 39 N	 40 IF	 41 IC	 42 TA
60	 43 N	 44 IRH	 45 N	 46 DRT	 47 IC	 48 IC TA

Plate B (Figure 25:- 48): - Histological alterations in the kidney of *Clarias gariepinus* exposed to *Brandelia micrantha*

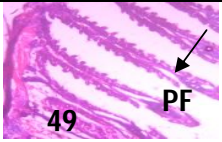


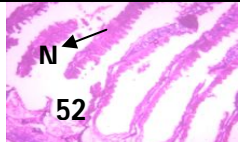
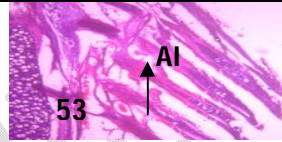
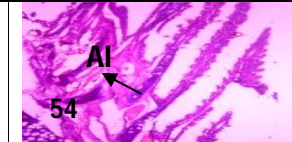
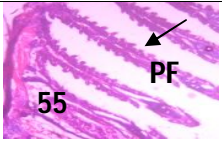
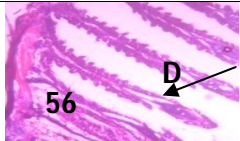
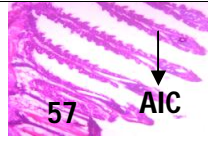
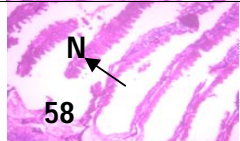
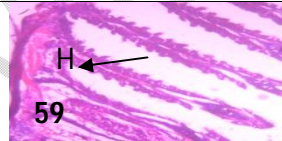
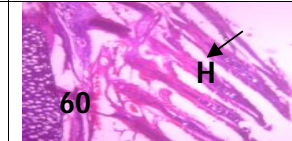
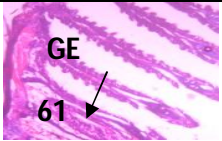

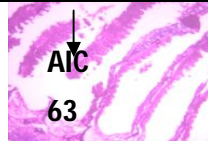
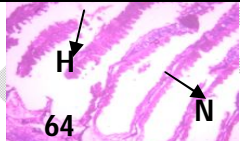
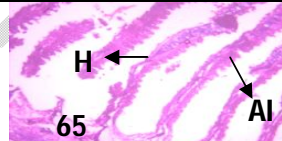

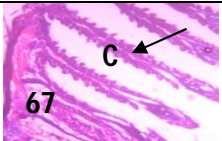
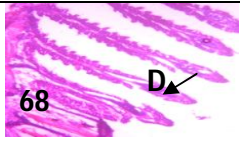
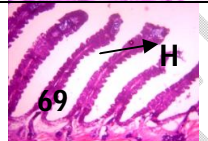
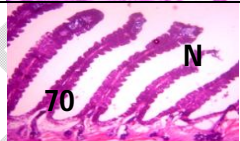
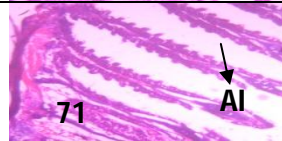
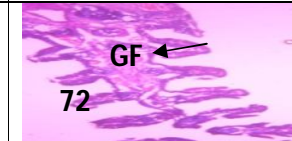
Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	0.042	0.084	0.126	0.168	0.21
15						
30						
45						
60						

Plate C (Figure 49:- 72): - Histological alterations in the gill of *Clarias gariepinus* exposed to *Brandelia micrantha*

3.2 Ameliorative effects of Ascorbic Acid on structural changes in the histology of African Sharptooth Catfish *Clarias gariepinus*

The Photomicrograph (X150)(H/E) of ameliorative effects of ascorbic acid(AA) on structural changes in the gill of African Sharptooth Catfish *Clarias gariepinus* at 15 days treatment at 0.00mg/l control figures 73 , 79, 85 and 91 control section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia. At Treatment 25mg/l, figs 74, 80 and 86 section of gill shows moderate effect on the gill architecture with focal area of necrotic filament (NF). At treatment 50mg/l figures 75, 81, and 87, section of gill shows severe effect on the gill architecture with severe areas necrotic filament (NF); at Treatment 75mg/l figures. 76, 82, and 88 section of gill shows moderate effect on the gill arrangement with focal area of necrotic filament (NF) at treatment 100mg/l figures. 77, 83, and 89 section of gill shows mild effect on the gill architecture with mild focal area of haemorrhage (H) otherwise normal; at treatment 125mg/l figures 78,84, and 90 section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia, in treatments 25, 50, 75, 100 and 125mg/l at 60 days Fig 91, 92, 93, 94, 95 and 96 at 60 days section of gill show mild effect on the gill architecture with severe areas of necrotic filaments (NF) with non distinct outline.

Ameliorative effects of vitamin C on structural changes in the liver of African Sharptooth Catfish *Clarias gariepinus* at treatment 0.00mg/l figures 97, 103, 109 and 115 control section of liver at 15days shows normal shows normal hepatic architecture with normal hepatocyte (H) and central vein (C). In the treatment 25mg/l figures 98, 104 and 110 at 15 to 45 days section of liver shows moderate to severe degeneration with sever focal area of cirrhosis (FAN) with extravassated blood (EVB). Treatment 50mg/l, figures. 99, 105, and 111 section of liver show moderate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) around the hemorrhagic area. Treatment 75mg/l, Figures 100, 106, and 112 section of liver shows moderate effect on the hepatic tissue with moderate focal area of haemorrhage (H). Treatments 75mg/l figures 101, 107, 113 section of liver shows moderate effect on the hepatic tissue with moderate focal area of hemorrhage (H) , with mild aggregate of intra hepatic inflammation (IHI) and congestion of the central vein (CC). The hepatocyte are moderately active at treatment 100mg/l figures 102, 108, and 114 section of liver shows moderate to severe degeneration with sever focal area of cirrhosis(C) with pale cytoplasm accumulation of fat. At treatment 125mg/l figures 115, 116, 117, 118, 119 and 120 section of liver at 60 days shows normal hepatic architecture with normal hepatocyte (H) and central vein (C).

Ameliorative effects of vitamin C on structural changes in the kidney of African Sharptooth Catfish *Clarias gariepinus* of Treatment 0.00mg/l, figures 121, 127, and 133 section of kidney control section at 15 days shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC) tubules at treatment 25mg/l figures 122, 128, 134 section of kidney shows renal tissue with mild areas of inflammation (I). At Treatment 50mg/l figures 124, 130, and 137 section of kidney shows renal shows renal tissue with severe aggregate of inflammation (AI) with eosinophilic (E) tubules . and areas of hemorrhage (H), at treatment 50mg/l figures 125, 131, 135 section of kidney shows renal tissue with moderate aggregate of inflammation (AI) with eosinophilic (E) tubules. At 60 days treatment 135 mg/l figures 126, 132, and 138 of kidney shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC), at 125mg/l figures 139, 140, 141, 142, 143 and 144, section of kidney at 60 days of the experiment with ascorbic acid shows normal renal tissue with moderate intra renal inflammation (A I) with active tubules.

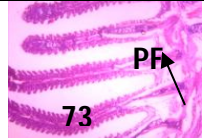
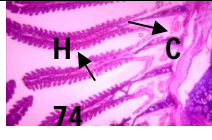
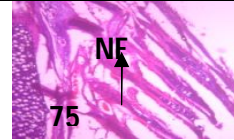
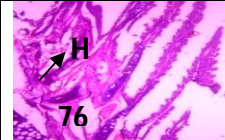


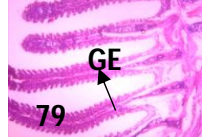
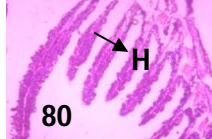
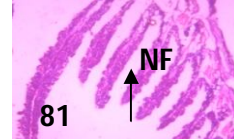

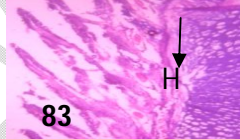
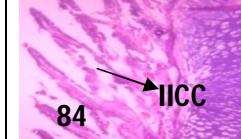
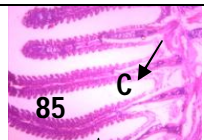
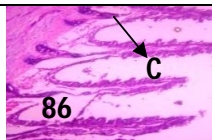
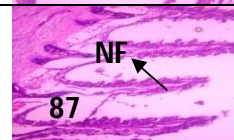
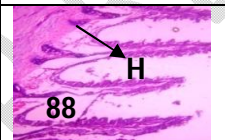
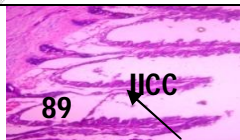
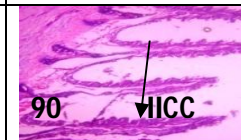

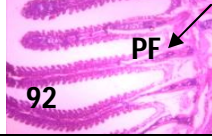


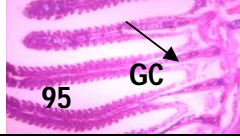
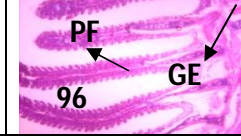
Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	25	50	75	100	125
15	 73	 74	 75	 76	 77	 78
30	 79	 80	 81	 82	 83	 84
45	 85	 86	 87	 88	 89	 90
60	 91	 92	 93	 94	 95	 96

Plate D (Figure 73:- 96): Ameliorative effects of vitamin C on structural changes in the gill of the African Sharptooth Catfish *Clarias gariepinus*

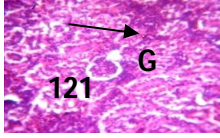
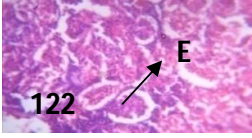
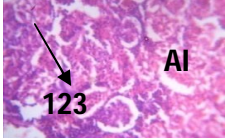
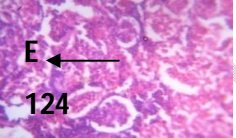
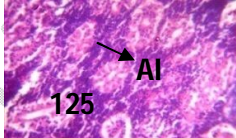
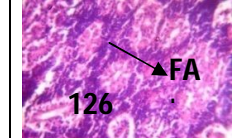
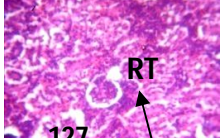
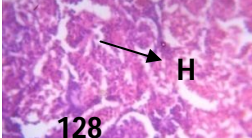
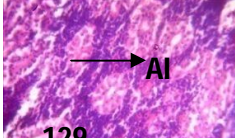
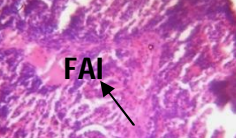
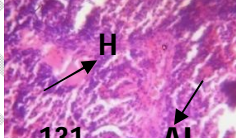

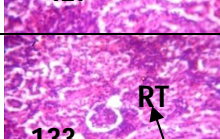
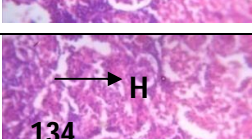

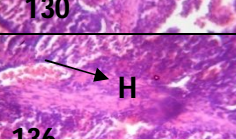
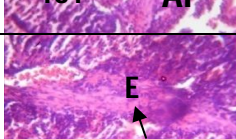
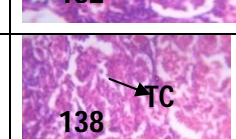
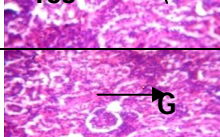
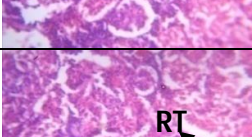
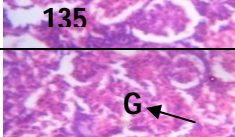
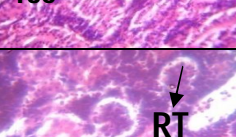
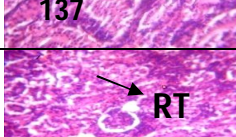
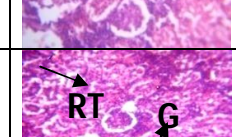
Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	25	50	75	100	125
15	 121 G	 122 E	 123 AI	 124 E	 125 AI	 126 FA
30	 127 RT	 128 H	 129 AI	 130 FAI	 131 H AI	 132 AI
45	 133 RT	 134 H	 135 H	 136 H	 137 E	 138 TC
60	 139 G	 140 RT	 141 G	 142 RT	 143 RT	 144 RT G

PLATE F: (Figures 121-144): Ameliorative effects of vitamin C on structural changes in the Kidney of the African Sharptooth Catfish *Clarias gariepinus*

Exposure Period (Days)	Concentration (mg/l)					
	Control (0.00)	25	50	75	100	125
15						
30						
45						
60						

Plate E (Figure 97:- 120): Ameliorative effects of vitamin C on structural changes in the Liver of the African Sharptooth Catfish *Clarias gariepinus*

3.3 The ameliorative effect of ascorbic acid on haematological parameters of *Clarias gariepinus* Juvenile

Haematological alterations in the *Clarias gariepinus* exposed to *Brandelia micrantha*, at 0.21mg/l during 60 days of exposure periods show reduction in white blood cell (ul), Red blood cell (ul), Haemoglobin (g/dl), Lymphocytes (ul), Mean cell volume (fl), Mean cell Haemoglobin concentration in the control section from $1.72 \times 10^2 \pm 12.5$, $2.63 \times 10^6 \pm 3.3$, 11.08 ± 2.3 , 96.78 ± 1.0 , 41.20 ± 4.6 , and 35.88 ± 4.2 to $2.55 \times 10^2 \pm 19.3$, 12.80 ± 2.2 , $3.26 \times 10^4 \pm 2.7$, 99.96 ± 4.6 , $1.98 \times 10^2 \pm 0.6$, and 45.54 ± 5.6 at 125mg/l in 60 days respectively, there was a decrease in the value of, Haematocrit (%), from 22.83 ± 2.5 to 10.36 ± 4.1 as presented in Table 3. Ameliorative effects of vitamin C on structural changes in the African Sharptooth Catfish *Clarias gariepinus*, presented in Table 4 at 125mg/l 60 days, the 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 normalizes with the control $1.61 \times 10^2 \pm 22.9$, $2.41 \times 10^6 \pm 7.5$, 6.53 ± 4.2 , 16.94 ± 8.7 , $2.83 \times 10^4 \pm 2.0$, 95.43 ± 1.0 , $2.11 \times 10^2 \pm 5.6$, 48.98 ± 9.5 and 46.07 ± 12.9 at 125mg/l at 60 days of exposure period as compare to (0.00mg/l) control.

3.4 The ameliorative effect of ascorbic acid on proximate composition of the Carcass of *Clarias gariepinus* Juvenile

The alterations of the proximate composition of the Carcass in *Clarias gariepinus* exposed to *Brandelia micrantha* at 0.21mg/l at 60 days of exposure period is presented in table 5 there was a significant reduction in the value of proximate composition of the carcass after the exposure of *Clarias gariepinus* to *B. Micranthan* the Moisture (M), Crude Protein (CP) (Mg/l), Crude Lipid (CL), Ash, Nitrogen Free Extract (NFE), and Energy (kcal/100g) reduce from 66.24 ± 6.9 , 29.71 ± 2.3 , 3.49 ± 0.4 , 0.90 ± 0.0 , 0.38 ± 0.1 , and $1.37 \times 10^2 \pm 6.6$ to 64.57 ± 6.2 , 26.21 ± 1.3 , 3.31 ± 0.5 , 0.83 ± 0.1 , 0.25 ± 0.0 , and $1.31 \times 10^2 \pm 4.0$ respectively. The ameliorative effect of ascorbic acid on Carcass/Proximate composition of *Clarias gariepinus* juvenile is presented in table 6 at 125mg/l during 60 day exposure period the values of proximate composition of the carcass after exposure of *Clarias gariepinus* to Vitamin C Ascorbic acid (AA) increases from Crude Protein (CP), Crude Lipid (CL), Energy K(cal)/mg, Moisture (M), Ash, and Nitrogen Free Extract (NFE) were 26.25 ± 5.0 , 3.34 ± 0.6 , $1.36 \times 10^2 \pm 5.3$, 64.84 ± 3.3 , 0.92 ± 0.1 to 0.49 ± 0.21 to 27.87 ± 2.1 , 3.59 ± 0.6 , $1.38 \times 10^2 \pm 9.4$, 67.47 ± 5.0 , 1.05 ± 0.2 , and 0.56 ± 0.2 respectively, while the value of Crude Fiber (CF) 0.00 remains constant throughout.

Table 3: - The summary of alterations of aqueous extract of *Bridelia micrantha* on haematological parameters *Clarias gariepinus* Juvenile (mean±SD)

Conc. (mg/L)	White blood cell (ul)	Red blood cell (ul)	Haemoglobin (g/dl)	Haematocrit (%)	Platelet (ul)	Lymphocytes (ul)	Mean cell volume (fl)	Mean cell Haemoglobin (pg)	Mean cell Haemoglobin concentration.
0.00	1.72 x 10 ² ±12.5 ^c	2.63x10 ⁶ ±3.3 ^b	11.08 ±2.3 ^b	22.83 ± 2.5 ^b	3.22x10 ⁴ ±1.3 ^a	96.78±1.0 ^b	1.16x10 ² ± 2.3 ^b	41.20±4.6 ^a	35.88±4.2 ^a
0.042	1.62 x 10 ² ±10.5 ^c	2.13x10 ⁶ ±3.1 ^b	10.21 ±2.2 ^b	22.34 ± 2.2 ^c	3.21x10 ⁴ ±1.2 ^a	94.77±2.2 ^b	1.10x10 ² ±9.6 ^b	39.23±3.5 ^{ab}	43.33±1.3 ^a
0.084	1.83 x 10 ² ±14.7 ^c	3.25x10 ⁶ ±4.3 ^b	11.34 ± 3.6 ^b	22.85 ± 9.6 ^c	3.50x10 ⁴ ±2.2 ^a	93.93±7.9 ^b	1.13x10 ² ±1.4 ^b	41.71±8.1 ^a	58.45±6.5 ^a
0.126	1.91 x 10 ² ±4.9 ^c	3.05 x 10 ⁶ ±6.1 ^b	12.52 ± 3.9 ^b	14.96 ± 6.0 ^{abc}	3.30x10 ⁴ ±1.7 ^b	88.32±4.6 ^b	1.11x10 ² ± 7.7 ^b	46.52±5.6 ^a	52.80±5.1 ^{abc}
0.168	1.88 x 10 ² ±10.2 ^a	2.10 x 10 ⁶ ± 3.1 ^a	6.47 ± 0.9 ^a	17.13 ± 1.4 ^{bc}	2.73x10 ⁴ ±1.7 ^a	93.63±2.7 ^b	1.77x10 ² ±0.9 ^b	37.80±4.1 ^{ab}	35.21±3.5 ^a
0.21	2.55 x 10 ² ±19.3 ^b	2.85 x 10 ⁶ ± 2.1 ^b	12.80 ± 2.2 ^b	10.36 ± 4.1 ^{ab}	3.26x10 ⁴ ± 2.7 ^a	99.96±4.6 ^b	1.98x10 ² ±0.6 ^a	51.31±7.3 ^{ab}	45.54±5.6 ^b

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

Table 4: - The ameliorative effect of ascorbic acid on haematological parameters of *Claria sgariepinus* Juvenile (mean±SD)

Conc. (mg/L)	White Blood Cell (ul)	Red Blood Cell (ul)	Haemoglobin in (g/dl)	Haematocrit (%)	Platelet (ul)	Lymphocytes (ul)	Mean Cell Volume (fl)	Mean Cell Haemoglobin (pg)	Mean Cell Haemoglobin Concentration
0.00	1.65x10 ² ±21.9 ^b	2.37x10 ⁶ ±1.0 ^a	5.07±0.9 ^a	15.25±2.3 ^b	2.64x10 ⁴ ±1.5 ^a	95.60±1.1 ^b	1.16x10 ² ±2.3 ^{ab}	48.28±3.5 ^a	45.40±10.4 ^a
25	159x10 ² ±12.3 ^{ab}	1.93x10 ⁶ ±7.7 ^a	8.93±2.8 ^a	19.52±2.2 ^{ab}	3.49x10 ⁴ ±1.7 ^a	90.58±9.8 ^{ab}	1.06x10 ² ±10.8 ^{ab}	43.31±1.4 ^a	47.75±10.1 ^a
50	1.53x10 ² ±14.6 ^{ab}	2.12x10 ⁶ ±1.1 ^a	8.48±4.2 ^a	13.12±7.6 ^{ab}	3.84x10 ⁴ ±2.8 ^a	88.98±4.3 ^a	1.10x10 ² ±13.6 ^{ab}	54.95±1.9 ^a	62.85±11.6 ^b
75	1.42x10 ² ±12.5 ^{ab}	1.73x10 ⁶ ±4.9 ^a	9.37±3.7 ^a	11.37±8.7 ^a	2.23x10 ⁴ ±2.4 ^a	79.80±1.8 ^a	1.16x10 ² ±7.8 ^{ab}	50.60±6.4 ^a	46.10±10.5 ^a
100	1.53x10 ² ±41.2 ^{ab}	1.90x10 ⁶ ±5.1 ^a	6.30±0.9 ^b	16.53±1.1 ^{ab}	2.29x10 ⁴ ±1.2 ^a	92.93±4.6 ^b	2.78x10 ² ±3.8 ^{ab}	44.77±7.7 ^a	53.30±7.5 ^b
125	1.61x10 ² ±22.9 ^{ab}	2.41x10 ⁶ ±7.5 ^a	6.53±4.2 ^a	16.94±8.7 ^{ab}	2.83x10 ⁴ ±2.0 ^a	95.43±1.0 ^b	2.11x10 ² ±5.6 ^{ab}	48.98±9.5 ^a	46.07±12.9 ^b

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

Table 5: -The alterations of the proximate composition of the Carcass in *Clarias gariepinus* exposed to *Brandelia micrantha*)

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	1.37x10 ² ±6.6 ^a
0.042	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	1.32x10 ² ±6.2 ^a
0.084	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	1.31x10 ² ±6.8 ^a
0.126	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	1.38x10 ² ±5.0 ^a
0.168	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	1.36x10 ² ±3.4 ^a
0.21	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	1.31x10 ² ±4.0 ^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 6: - The ameliorative effect of ascorbic acid on Carcass/Proximate composition of *Clarias gariepinus* Juvenile (Mean±SD)

Conc. (mg/L)	Crude Protein (CP) (Mg/l)	Crude Lipid (CL)	Energy K(cal)/mg	Crude Fiber (CF)	Moisture (M)	Ash	Nitrogen Free Extract (NFE)
0.00	26.25±5.0 ^a	3.34±0.6 ^a	1.36x10 ² ±5.3 ^a	0.00±0.0	64.84±3.3 ^{ab}	0.92±0.1 ^b	0.49±0.2 ^a
25	26.54±1.2 ^a	3.31±0.4 ^a	1.42x10 ² ±1.3 ^a	0.00±0.0	67.08±3.1 ^{abc}	0.67±0.3 ^a	0.48±0.1 ^{ab}
50	27.16±0.3 ^a	3.49±0.8 ^a	1.42x10 ² ±3.2 ^a	0.00±0.0	71.11±7.8 ^c	0.88±0.3 ^b	0.57±0.3 ^b
75	26.98±0.5 ^a	3.16±0.2 ^a	1.34x10 ² ±8.5 ^a	0.00±0.0	69.49±1.5 ^{bc}	0.91±0.1 ^b	0.29±0.1 ^b
100	27.10±1.8 ^a	3.47±0.7 ^a	1.33x10 ² ±3.4 ^a	0.00±0.0	63.73±5.4 ^a	1.27±0.7 ^b	0.52±0.1 ^{ab}
125	27.87±2.1 ^a	3.59±0.6 ^a	1.38x10 ² ±9.4 ^a	0.00±0.0	67.47±5.0 ^a	1.05±0.2 ^b	0.56±0.2 ^b

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

4.0 Discussion

Aquatic environments are generally known to be altered from their pristine state due to anthropogenic activities carried out in nearby areas. The *Bridelia micrantha* (Hochst.) (Phyllanthaceae) is an ichthyotoxic plant used in catching fish in Nigeria, the 96hrs LC₅₀ of *Bridelia micrantha* to Catfish *Clarias gariepinus* was 0.21mg/l (Ayotunde and Igbang 2023) the active ingredient in *Bridelia micrantha* are triterpenoid saponins and rotenoids with rotenone being the most widely used commercially. The result of Length-weight relationship of *Clarias gariepinus* juvenile exposed to ichthyotoxic plant *B.micranthan* and ameliorative activity of ascorbic acid as presented in table 1 shows that the experimental fish are in good conditions of health. Table 2 summarizes the range of the physicochemical parameters of control and treated fish observed during the experimental period. There was no significant difference among the different treatments in terms of physicochemical parameters measured. The result of water quality shows the normal value for fish culture in the tropics, the DO₂ level of the water varies slightly such that the T1 , T2 , T3, T5and T0 were in the normal range, conductivity followed by T2 whereas T1 had the least value for conductivity, which the mean differences were statistically not significant (p>0.05) among the individual treatments. There is no significant (p>0.05) value in the value of pH and temperature through the alteration and ameliorative experiments, this result is similar to the works of El-Keredy *et al.*, 2017, Abdallah (2022) and Deborah *et al.*, (2023).

The result of the present haematological alterations in the *Clarias gariepinus* exposed to *Brandelia micrantha* is presented in table 3, the White blood cell (mg/l), Red blood cell (mg/l) Haemoglobin (g/dl), Lymphocytes (mg/l), Mean cell volume (fl), Mean cell Haemoglobin concentration increases from $1.72 \times 10^2 \pm 12.5$, $2.63 \times 10^6 \pm 3.3$, 11.08 ± 2.3 , 96.78 ± 1.0 , 41.20 ± 4.6 , and 35.88 ± 4.2 to $2.55 \times 10^2 \pm 19.3$, 12.80 ± 2.2 , $3.26 \times 10^4 \pm 2.7$, 99.96 ± 4.6 , $1.98 \times 10^2 \pm 0.6$, and 45.54 ± 5.6 respectively there was a decrease in the value of, Haematocrit (%), from 22.83 ± 2.5 to 10.36 ± 4.1 as presented in Table 3. Ameliorative effects of vitamin C on structural changes in the African Sharptooth Catfish *Claria sgariepinus*, Table 4 shows the White blood cell (mg/l), Red blood cell (mg/l), Haemoglobin (g/dl), Haematocrit (%), Platelet (mg/l), Lymphocytes (mg/l), Mean Cell Volume (fl), Mean Cell Haemoglobin (pg), Mean Cell Haemoglobin Concentration normalizes with the control $1.61 \times 10^2 \pm 22.9$, $2.41 \times 10^6 \pm 7.5$, 6.53 ± 4.2 , 16.94 ± 8.7 , $2.83 \times 10^4 \pm 2.0$, 95.43 ± 1.0 , $2.11 \times 10^2 \pm 5.6$, 48.98 ± 9.5 and 46.07 ± 12.9 as compare to control. This work is similar to the work of Al-Agidi *et al.*, (2019) they eported the results of Ameliorative Effect of Ascorbic Acid on haematological Parameters of *Cyprinus carpio* due to cadmium toxicity and showed significant differences at $P \leq 0.05$ between treatments with cadmium and control this is similar to the work of Owolabi and Abdulkareem (2021) who work on the effects of vitamin C on structural changes in the tissues of the giant African catfish, *Heterobranchus longifilis* exposed to Zinc Oxide nanoparticle.

The result of the alterations of the proximate composition of the Carcass in *Clarias gariepinus* exposed to *Brandelia micrantha*) is presented in table 5. There was a significant (p<0.05) reduction in the results of the carcass after the exposure of *Clarias gariepinus* to *B. micranthan* the Moisture (M), Crude Protein (CP) (Mg/l), Crude Lipid (CL), Ash, Nitrogen Free Extract (NFE), and Energy (kcal/100g) in their experiment to determine the ameliorative effect of quercetin against abamectin-induced hemato-biochemical alterations and haepatorenal oxidative damage in Nile Tilapia, *Oreochromis niloticus*. The ameliorative effect of ascorbic acid on Carcass/Proximate composition of *Clarias gariepinus* juvenile is presented in table 6 at 125mg/l during 60day exposure period the values of proximate composition of the carcass after exposure of *Clarias gariepinus* to

Vitamin C Ascorbic acid (AA) increases from Crude Protein (CP), Crude Lipid (CL), Energy K(cal)/mg, Moisture (M), Ash, and Nitrogen Free Extract (NFE) while the value of Crude Fiber (CF) 0.00 remains constant throughout.

The results of histological alterations in *Clarias gariepinus* exposed to *Brandelia micrantha* is similar to the work Ekpo *et al.*, (2019) who work on ichthyotoxic Effect of *Dracaena arborea* bark and root Extract on *Clarias gariepinus* Post Fingerlings. The results of the present study is presented in Plate A Figure 1 - 24, at 15 days treatment 0.00mg/l. The results of histological alterations in the liver of *Clarias gariepinus* exposed to *Brandelia micrantha* is presented in Plate A Figure 1-24, at 15 days treatment 0.00mg/l control section of liver (X100)(H/E) shows normal moderate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) around the hemorrhagic area, the kidney of *Clarias gariepinus* at 60 days treatment 0.21 figs. 30, 36, 42 and 48 section of kidney shows normal renal architecture with glomeruli (G), Bowman space (BS), renal tubules (RT) and tubular cell (TC) in some areas. The gill of *Clarias gariepinus* at 15 days in treatment 0.00 Figures 49, 55, 61 and 67 control section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia. moderate effect on the gill architecture with focal area of necrotic filament (NF) At 30 days in treatment 0.042 figs 50, 56, 62, and 68 section of gill shows moderate effect on the gill architecture with focal area of necrotic filament (NF) at treatment 0.084 Figs 51, 57, 63 and 69 section of gill shows mild effect on the gill architecture with mild focal area of hemorrhage (H) and mild aggregate of inflammatory cell (AIC) at 45 days treatment 0.126 figs 52, 58, 64 and 70 section of gill 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 T0.168 figs 53, 59 65 and 71 section of gill shows moderate effect on the gill architecture with mild focal area of hemorrhage (H) and aggregate of inflammation (AI). The result at 60 days of treatment 0.21 figs 54, 60, 66 and 72 section of gill shows severe effect on the gill architecture with severe areas of necrotic filaments (NF) with non distinct outline is similar to the work of El-Keredy *et al.*, (2017) who work on the effect of dietary vitamin C and β -Glucan to alleviate the toxic effect of copper sulphate in Tilapia fish.

The results of ameliorative effects of ascorbic acid(AA) on structural changes in the gill of African Sharptooth Catfish *Clarias gariepinus* at 15 days treatment at 0.00mg/l control figures 73, 79, 85 and 91 control section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia At Treatment 25mg/l, figs 74, 80 and 86 section of gill shows moderate effect on the gill architecture with focal area of necrotic filament (NF). At treatment 50mg/l figures 75, 81, and 87, section of gill shows severe effect on the gill architecture with severe areas necrotic filament (NF); at Treatment 75mg/l figures. 76, 82, and 88 section of gill shows moderate effect on the gill arrangement with focal area of necrotic filament (NF) at treatment 100mg/l figures. 77, 83, and 89 section of gill shows mild effect on the gill architecture with mild focal area of haemorrhage (H) otherwise normal; at treatment 125mg/l figures 78,84, and 90 section of gill shows mild effect on the gill architecture with focal area of hemorrhage (H) and aggregate of inflammatory cell (AIC) within the epithelia, in treatments 25, 50, 75, 100 and 125mg/l at 60 days Fig 91, 92, 93, 94, 95 and 96 at 60 days section of gill show mild effect on the gill architecture with severe areas of necrotic filaments (NF) with non distinct outline.

Ameliorative effects of vitamin C on structural changes in the liver of African Sharptooth Catfish *Clarias gariepinus* at treatment 0.00mg/l figures 97, 103, 109 and 115 control section of liver at 15 days shows normal hepatic architecture with normal hepatocyte (H) and central vein (C). In the treatment 25mg/l figures 98, 104 and 110 at 15 to 45 days section of liver shows

moderate to severe degeneration with severe focal area of cirrhosis (FAN) with extravasated blood (EVB). Treatment 50mg/l, figures. 99, 105, and 111 section of liver show moderate degeneration with focal area of intra hepatic hemorrhage (IHH) and aggregate of inflammatory cell (AIC) around the hemorrhagic area. Treatment 75mg/l, Figures 100, 106, and 112 section of liver shows moderate effect on the hepatic tissue with moderate focal area of haemorrhage (H). Treatments 75mg/l figures 101, 107, 113 section of liver shows moderate effect on the hepatic tissue with moderate focal area of hemorrhage (H), with mild aggregate of intra hepatic inflammation (IHI) and congestion of the central vein (CC). The hepatocyte are moderately active at treatment 100mg/l figures 102, 108, and 114 section of liver shows moderate to severe degeneration with severe focal area of cirrhosis (C) with pale cytoplasm accumulation of fat. At treatment 125mg/l figures 115, 116, 117, 118, 119 and 120 section of liver at 60 days shows normal hepatic architecture with normal hepatocyte (H) and central vein (C).

Ameliorative effects of vitamin C on structural changes in the kidney of African Sharptooth Catfish *Clarias gariepinus* of Treatment 0.00mg/l, figures 121, 127, and 133 section of kidney control section at 15 days shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC) tubules at treatment 25mg/l figures 122, 128, 134 section of kidney shows renal tissue with mild areas of inflammation (I). At Treatment 50mg/l figures 124, 130, and 137 section of kidney shows renal shows renal tissue with severe aggregate of inflammation (AI) with eosinophilic (E) tubules . and areas of hemorrhage (H), at treatment 50mg/l figures 125, 131, 135 section of kidney shows renal tissue with moderate aggregate of inflammation (AI) with eosinophilic (E) tubules. At 60 days treatment 135 mg/l figures 126, 132, and 138 of kidney shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC), at 125mg/l figures 139, 140, 141, 142, 143 and 144, section of kidney at 60 days of the experiment with ascorbic acid shows normal renal tissue with moderate intra renal inflammation (A I) with active tubules, this work is positively related to the work of (Deborah *et al.*, 2023) who work on the ameliorative effects of ascorbic acid on haematological and water quality parameters following a 100 km transportation of adult *Clarias gariepinus*.

5.0 Conclusion

Ameliorative effects of ascorbic acid on ichthyotoxic plant *Bridelia micrantha* induced haematological, histological and plasma biochemical alterations in African Catfish *Clarias gariepinus* juvenile shows partial and full recovery at 125mg/l of ascorbic acid after 60 days exposure period. Chronic exposure during fishing of African catfish *Clarias gariepinus* to ichthyotoxic plant *Bridelia micrantha* (BM) causes increased haematological, histological and plasma biochemical alterations. The results of histological alterations in *Clarias gariepinus* exposed to *Brandelia micrantha*) at 60 days of treatment shows severe effect on the gill architecture with hypertrophy of the gill arch, gill filament degeneration and aggregate of inflammation (AI), the liver shows moderate to severe effect on the hepatic tissue with severe effect on the hepatic tissue with severe ground glass (GG) appearance of the cytoplasm and intra hepatic hemorrhage (IHH) while the kidney shows severe degeneration of the renal tissue with severe intra renal necrosis (TN) and inflammatory cell aggregate (IC) with tubular atrophy (TA) in some areas.

The mean level tissue change in both organs of *B. micrantha* (BM) exposed fish gradually increased with increase in *Bridelia micrantha* (BM)-concentration and exposure period. The recovery of fish from *Bridelia micrantha* (BM) was evaluated by exposing fish to a 60-days depuration. Ascorbic acid (AA) ameliorative potential against *Bridelia micrantha* (BM) toxicity was assessed by placing the fish on ascorbic acid (AA)-supplemented diet at 0.00, 25, 50, 75, 100, and 125mg/l levels. After 60-day depuration, some of the lesions in tested organs attained partial and full recovery. Ascorbic

Acid administration to *Bridelia micrantha* (BM)-exposed fish at 125mg/l indicated partially and completely reversed the lesions, the gill shows normal gill architecture with well projected filament (PF), gill epithelium (GE) and cartilages (C), liver shows normal hepatic architecture with normal hepatocyte (H) and central vein (C) while the kidney with ascorbic acid shows normal renal architecture with active glomeruli (G) renal tubules (RT) and tubular cell (TC) after 60-days depuration, some of the lesions in both haematological and proximate composition of the carcass attained recovery. Ascorbic acid administration to *Brandelia micrantha* exposed fish at 125 mg/l completely reversed the lesions in both blood and nutrient composition compared to the in the control

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

6.0 References

- Aafreen, A. H., Jayashree, D., Aqib, H. L., Syed, D. and Irfan, A. P. (2021). Study of some plant extracts on the histology and toxicity in few freshwater fishes. *International Journal of Recent Scientific Research*, **12**(60):42081-42082
- Abalaka S E (2013) Evaluation of the haematology and biochemistry of *Clarias gariepinus* as biomarkers of environmental pollution in Tiga dam, Nigeria. *Braz Arch Biol Technol* 56: 1-7.
- Abalaka, Samson Enejo Abalaka, Muhammad Yakasai Fatihu, Najume Doguwar Giginya Ibrahim, Suleiman Folorunsho Ambali (2015) Gills and skin histopathological evaluation in African sharptooth catfish, *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark, *Egyptian Journal of Aquatic Research* 41, 119–127
- Audu, B.S., Omirinde, J.O., Gosomji, I.J. and Wazhi, P.E. (2017). Histopathological changes in the gill and liver of *Clarias gariepinus* exposed to acute concentrations of *Vernonia amygdalina*. *Animal Research International*, **14**(1): 2576–2587.
- Abdallah Tageldein Mansour, Heba H. Mahboub, Rehab M. Amen, Marwa A. El-Betagy, Amany Ramah, Abdefattah M. Abdelfattah, Hossam S El-Beltagi, Tarek A Shalaby, Hesham S Ghazzawy, Khaled M, A. Ramadam, Adnan H M. Alhaji and Heba S Hamed (2022) Ameliorative Effect of Quercetin against Abamectin-Induced Hemato-Biochemical Alterations and Hepatorenal Oxidative Damage in Nile Tilapia, *Oreochromis niloticus*, *Animals (Basel)* 2022 Dec; 12(23): 3429. Published online 2022 Dec 5. doi: 10.3390/ani12233429.
- Adewolu, M. A., Adeniji, C. A., & Adejobi, A. B. (2008). Feed utilization, growth and survival of *Clarias gariepinus* (Burchell, 1822) fingerlings cultured under different photoperiods. *Aquaculture*, 283, 64-67.
- Ambali Suleiman F, Joseph O Ayo, Samuel A Ojo and King AN Esievo (2011) Ameliorative effect of vitamin C on chronic chlorpyrifos-induced erythrocyte osmotic fragility in Wistar rats. DOI: 10.1177/09603271110368415 *Human and Experimental Toxicology* 30(1) 19–24

- Amoatey P and Baawain M S (2019) Effects of pollution on freshwater aquatic organisms. *Water Environment Research*. 91: 1272-1287.
- Adhikari, S.; Sarkar, B.; Chatterjee, A.; Mahapatra, C.T. and Ayyapan, S. (2004). Effects of cypermethrin and carbofuran on certain haematological parameters and prediction of their recovery in a freshwater teleost, *Labeorohita*(Hamilton). *Ecotoxicol. Environ. Saf.*, 58 (2): 220-226. Doi: 10.1016/j.ecoenv.2003.12.003.
- AyotundeEO, and Ofem BO. (2008) Acute and chronic toxicity of pawpaw to adult Nile tilapia (*Oreochromisniloticus*Linne 1757) fingerlings. *African Journal of Biotechnology*. 7(13):2265–2274.
- Ayotunde, E. O., Offem, E. O. and Bakeh A. F. (2011). Toxicity of *Carica papaya* Linn: haematological and piscicidal effect on adult catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Sciences*, 6(23): 291–308.
- Ayotunde, Ezekiel Olatunji (2019) Fish Habitat Health and Integrity: The Key to Sustainable Fisheries and Protein Malnutrition, the 3rd Inaugural Lecture series, Cross River University of Technology (CRUTECH) Calabar on 31st October 2019. Pp134.
- Ayotunde, E. Olatunji, Igbang Sunday K. (2023). 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. *J Mari Scie Res Ocean*, 6(3), 106-122.
- Al-Agidi, Al-AgidiHamza G., Kadhim O.M. AL-Humairi and Sadeq M. Al-Haider (2019) Ameliorative Effect of Ascorbic Acid on Hematological Parameters Due to Cadmium Toxicity in *Cyprinus carpio*. *Indian Journal of Ecology* 46 Special Issue (8): 36-39.
- American Public Health Association (APHA). 1988 Standard method for the examination of water and waste. New York: APHA Press.
- AOAC. (1990) Official Methods of Analysis, 15th edn. Washington, DC USA: Association of Official Analytical Chemists, pp. 69 – 88.
- Biswas, K., Chattopadhyay, I. Banerjee, R. K. and Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*), *Current Science*, 82(11): 1336-1345.
- Bielski, B.H. (1982). Chemistry of ascorbic acid radicals, ascorbic acid: chemistry, metabolism and uses. *Advanced Chemistry, Series*, 200: 81-100.
- Cannon Ak, J. G., Burton, R. A., Wood, S. H. and Owen, N. L (2004). "Naturally occurring fish poisons from plants". *Journal of Chemical Education* 81(10):1457-1461.
- Connell, D., P. Lam, B. Richardson and R. Wu. (1999) Introduction to Ecotoxicology, Oxford, England, Blackwell Science.

- Dar, U. Q. and Paul, V. I. (2018). Impact of ethanol extract of *Anamirtacocculus*(Linn.) seeds on tissue damage biomarkers of the predatory catfish *Heteropneustes fossilis*(Bloch.).*Journal of Taibah University for Science*, **12**(2): 357-370.
- Deborah Adah Arimie, Adah Sylvanus Adakole, Nwonuma Charlse Obiora, Olaosebikan Boluwaji, and Oyekunle Taiwo (2023) Ameliorative effects of ascorbic acid on haematological and water quality parameters following a 100 km transportation of adult *Clarias gariepinus*. *Media Kedokteran Hewan* 34(1):13-26, DOI:10.20473/mkh.v34i1.2023.13-26.
- DeSilva, S.S. (1991). Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. 217 pp.
- Deshmukh B. and Waghmode A. (2011). Role of wild edible fruits as a food resource: Traditional knowledge; *International Journal of Pharmaceutical & Life Sciences*, 2 (7) 919-924.
- Ekpo, I. E., Akpabio, I. G. and Omini D. E. (2019) Ichthyotoxic Effect of *Draceana arborea* Back and root Extract on *Clarias gariepinus* Post Fingerlings. *Trends in Applied Sciences Research*, 14(3): 170-177.
- El-Keredy, M.S.; Abeer, M-S.; Amany, M.D.; Gehan, I.E.A. and Walid, S.K. (2017). Effect of dietary vitamin C and β -Glucan to alleviate the toxic effect of copper sulphate in Tilapia fish. *Alex. J. Vet. Sci.*, 55 (2): 36-49. Doi:10.5455/ajvs.276295.
- Fafioye O (2005). Plants with piscicidal activities in south western Nigeria. *Turkish Journal of Fisheries and Aquatic Science*. 5: 91-97.
- Flores-Lopes, F. and Thomaz, A. (2011). Histopathologic alterations observed in fish gills as a tool in environmental monitoring. *Braz. J. Biol.*, 71 (1): 179-188.
- Gill, L.S. (1992). Ethnomedical uses of Plants in Nigeria. University of Benin Press. Benin, Nigeria. P.276.
- Gupta P.K. (1977) *Haematological Techniques*. 4th ed. Syndicate; London, UK: p. 231.
- Ibiam, U. A., Obasi, C. D., and Obasi, U. O. (2015). GC- MS Analysis, Acute Toxicity and Oxidative Stress Potentials (Effects) of *Albizia Chevalieri* Extract on Juvenile African Catfish (*Clarias gariepinus*). *Middle- East Journal of Scientific Research*, **23**(2): 192-199.
- Idowu, A. A., Soetan, M. O., Akinde, A. and Popoola, O. C. (2019). Effect of *Euphorbia hirtaleaf* extracts on histopathology of juvenile *Clarias gariepinus*. *Nigerian Journal of Animal Science*, **21** (1): 96-109
- Karami, A., Christianus, A., Ishak, Z., Courtenay, S. C., Sayed, M. A., Noor, A. M., & Noor, S. H. (2010). Effect of triploidization on juvenile African catfish (*Clarias gariepinus*). *Aquaculture International*, 18, 851-858.
- Limbu, S.M. (2020). The effects of on-farm produced feeds on growth, survival, yield and feed cost of juvenile African sharp-tooth catfish (*Clarias gariepinus*), *Aquaculture and Fisheries* 5(1), 58-64

- Maltez, L.C.; Stringhetta, G.R.; Enamorado, A.D.; Okamoto, M.H.; Romano, L.A.; Monserrat, J.M.; Sampaio, L.A. and Garcia, L. (2017). Ammonia exposure and subsequent recovery trigger oxidative stress responses in juveniles of Brazilian flounder *Paralichthys sorbignyanus*. *Fish Physiol. Biochem.*, 43 (6): 1747-1759. <https://doi.org/10.1007/s10695-017-0406-8>
- Neuwinger HD (2004) Plants used for poison fishing in tropical Africa. *Toxicon* 44(4):417-430.
- Ramesh, M.; Narmadha, S. and Poopal, R.S. (2015). Toxicity of furadan (carbofuran 3% g) in *Cyprinus carpio*: Haematological, biochemical and enzymological alterations and recovery response. *Beni-Suef Uni. J. Basic Appl. Sci.*, 4: 314-326.
- Southern Regional Aquaculture Centre (SRAC) (2010). Principle of Fish Nutrition. SRAC Publication No 5003, 1-7.
- Ogueji, O., Nwani, C. D., Iheanacho, S. C., Mbah, C. E. and Ibrahim, B. U. (2017). Hematological alterations in the African catfish (*Clarias gariepinus*) juveniles exposed to sub- chronic concentrations of diazepam. *Nigeria Journal of Fisheries*, 14(2017):1-10.
- Osuagwu, G.G.E. and Ibeabuchi, I.C. (2010). The influence of aqueous leaf and stem extracts of *Adenia lobata* (Jacq) on the flowering and fruiting of okra (*Abelmoschus esculenta*) and groundnut (*Arachis hypogea*) *African Journal of Biotechnology* 9: 3260-3263.
- Owolabi, Olufemi D. and Abdulkareem, Saratu I. (2021) Effects of vitamin C on structural changes in the tissues of the giant African catfish, *Heterobranchus longifilis* exposed to Zinc Oxide nanoparticles *Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 25(5): 103 – 129* www.ejabf.journals.ekb.eg.
- Piria, M., Jelkić, D., Gavrilović, A., Balogh, Špelić, I., Radočaj, T., Vilizzi, L., Ozimec, S. and Opačak, A. (2022). Finding of hybrid African catfish “*Clariobranchus*” in the River Danube, *Journal of Vertebrate Biology*, 6(5):1-10.
- Vimal J B and Das S M (2015). Toxicity of *Euphorbia antiquorum* latex extracts to freshwater fish *Poeciliareticulata*. *International Journal of Fisheries and Aquatic Studies*. 2: 214-216.
- Viveen, W.J.A.R., Rither, C.J.J., van Oordt, P.G.J., Janssen, J.A.L., Huisman, E.A., (1985). Practical Manual for the Culture of the African catfish (*Clarias gariepinus*). Directorate General for International Technical Cooperation, The Hague, Netherlands, p. 93.
- Suleiman, B.; Maruff, L. and Oniye, S.J. (2015). Radiographic studies on morphological anomalies in artificially spawned *Heterobranchus longifilis* Valenciennes, 1840 F1 generation. *Sokoto J. Vet. Sci.*, 13 (1): 9-16.
- Svobodova D. Ravds, J. &Palackova, (1991): Unified method of Haematological examination of fish. Research Inst. of Fish Culture and Hydrobiology. Vonnony Czechoslovakia.
- Tingman, W., Jian, Z., & Xiaoshuan, Z. (2010). Fish product quality evaluation based on temperature monitoring in cold chain. *African Journal of Biotechnology*, 9, 6146-6151

UNDER PEER REVIEW