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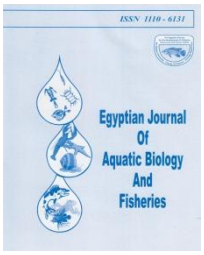

Hematological variations, histopathology and reversibility of liver function enzymes in post juvenile *Clarias gariepinus* exposed singly to five botanical piscicides.

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ABSTRACT

This study assesses the hematological and histopathological effects of some identified piscicidal plants on post-juvenile *Clarias gariepinus*. $^{1/}10LC_{50}$ values of each of the five aqueous extracts and ammonium solution were used for the 28-day sub-acute test. The fishes were exposed for 14 and 28 days and on the 28th day, the group was allowed to recover. RBC, WBC, and PCV were estimated using electronic counters. The activities of the liver enzymes; ALT, AST, and ALP were determined for exposure and recovery. Gill and liver alterations after exposure were estimated for each of the extracts. There was a decrease in RBC and PVC from the 14th day and 28th day exposure in all the extracts. *A. occidentale* had the lowest RBC level after 28 days of exposure. ALT increased after recovery for all the extract except for *N. tabacum*. ALP increased after recovery only for *A. occidentale*. *A. occidentale* and *N. tabacum* showed the highest liver and gill alterations with high frequencies of severe vacuolization, inflammation, degeneration, and necrosis with alteration index of 2.44 and 3.33 respectively.

INTRODUCTION

Over last several decades, botanical extracts have been shown much interest in aquaculture to control fish parasites, fish fry predators and unwanted fishes from aquaculture ponds as attempts to replace chemical pesticides and piscicides, since extensive and indiscriminate use of these non-biodegradable synthetic chemicals results in harmful impact on aquatic environment and presents high risk to the non-targeted organisms (Das, 2013). Plant extracts are considered promising agents because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability and reduced toxicity to non-targeted animals (Yunis Aguinaga *et al*, 2014). To date, a good number of plants have been investigated in different countries to evaluate their pesticidal (Miresmailli and Isman, 2014) and piscicidal activities (Murthy *et al*, 2010; Akinsanya, 2016a; Akinsanya, 2016b). However, commercially available plant products are still limited, and hence, efforts should be made to find out new sources of botanical pesticides and piscicides for rapidly growing pisciculture (Ramanujam and Ratha, 2008). Plant extracts are referred to piscicides if they exert toxicological effects on fishes and cause death to these aquatic animals (Burkill, 1995). Plant piscicides are obtained from a variety of plants belonging to different families and species that may vary considerably not only for their taxonomic variations but also for the plant parts used (leaves,

barks, fruits and seeds), mode of use, mode of extraction and species of target fishes (Neuwinger, 2004).

Plant extracts used as piscicides in fisheries are considered advantageous when viewed against the backdrop of using persistent chemicals (Gabriel *et al*., 2009). Ichthyotoxic plants used for baiting or stupefying fish are often crushed and cast into stagnant, slow moving water or spread on mud flats to poison fish (Gabriel *et al*., 2009). The leaves are used to immobilized or kill fish in many communities of Cross River and Rivers States of Nigeria. In Rivers State it is used for quick kill of hardy fishes like mudskippers (Obomanu *et al*, 2007) and the clariids and in Cross River it is normally applied in pools to kill mostly tilapias and catfishes constituting the major source of dietary protein (Ekanem *et al*., 2003). The phytochemistry of the plant revealed it contains flavinoids, saponins tannins, glycosides and alkaloids (Akinsanya *et al*., 2016b). Botanical materials contain a number of bioactive compounds that work either individually or synergistically as piscicides (Obomanu *et al*, 2007). Akinsanya *et al.* (2016a) and Akinsanya, *et al.* (2016b) confirmed the presence of these phytochemical constituents in alcohol extracts of selected tropical plants. These extracts had significant haematological and histopathological effects on *Clarias gariepinus* (Akinsanya *et al.*, 2016a&b).

The aim of this study is to investigate the hematological and pathological effects of some identified piscicidal plants on *Clarias gariepinus* as well as the reversibility of the liver function enzymes.

MATERIALS AND METHODS

Each of the plants (botanical) samples collected was air dried at room temperature for seven days and oven dried at 32^0C for 3 hours to make it more bristle before pounding into powder with clean mortar. The powder of each of the botanical samples was sieved with 100 micron sieve to obtain fine powder which was stored separately in dry airtight bottle containers. The stock solution of each of the botanicals was prepared by dissolving 50.00g of the fine powder of each in 1 liter of water in 5 liters transparent jerry-can and kept for 3 days to ferment before filtering to obtain their aqueous extracts.

Total of eighty post juvenile *Clarias gariepinus* (average weight, 150.00±15.00g, average length, 22.00±5.00cm) were obtain from local fish farm. The acute toxicity bioassay experiment for each of the extracts has been reported by Ekpendu *et al.* (2016). One tenth of the LC_{50} values of each of the botanicals including the ammonium solution were used for the 28 day sub-acute test, which include twelve experimental fishes and eight controls. For each extract and ammonium solution sub-acute test, blood was collected from the central vein of four randomly selected fishes with heparinized 2ml disposable syringes, every 14 days for a period of 28 days and the remaining four fishes for the 28 day exposure group were allowed 7 days to recover. The blood was transferred to sampling bottles containing EDTA anti-coagulant (Okomoda *et al.*, 2010). The Full Blood Count was done in the Department of Hematology, College of Medicine University of Lagos, using Swelab Alpha automatic hematology analyzer (Buttarello and Plebani, 2008). Red blood cell (RBC), White blood cell (WBC) and Packed cell volume (PCV) indices were estimated using electronic counters. Blood-filled heparinized micro-haematocrit capillary tubes were centrifuged at 12000 for 5 min using a micro-haematocrit centrifuge and the haematocrit (Hct) values were read directly. The haemoglobin concentration was measured by the cyan-methaemoglobin method at a wavelength of 540nm. Concurrently, the Total Red Blood Cell (RBC) was obtained by employing the methods described by Dacie & Lewis, (1984). The activities of the liver enzymes; Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were determined using the blood sample. The blood was centrifuged at

5000 rpm for 5 minutes; the plasma sample was then collected and kept at 20^{o}C. The enzyme activities were determined by colorimetric method. The enzymes were then assayed using reagents obtained from enzyme assay kits.

The gill and liver samples were fixed in 10% formalin, dehydrated in graded ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 5 μm on a rotary microtome. Slides were stained using the haematoxylin and eosin technique for light microscopy. The histopathological changes were evaluated according to Saliu et al, (2016), which include the calculation of the Histopathological Alteration Index (HAI) for each fish using the formula:

$$HAI = 1 \sum I + \sum II + \sum III$$

Because I, II and III correspond to the number of stages of change, the mean HAI was scored on five scale: 0 = normal tissue; 2.0 = mild damage to the tissue, 4.0 = moderate damage to the tissue, 6.0 = partially severe damage to the tissue, 8.0 = severe damage and irreparable damage to the tissue. This stages of change and scoring system or scale is given in Table 1 below;

Table 1: Stages of change and histological alterations of the gill and liver

Alteration Score	Scope Description	Histological Alteration
0.0	Normal tissue	No lesion or any alteration (NT)
2.0	Mild damage	Hepatocytic Hypertrophy (HH), Epithelial Hypertrophy (EH), Degeneration of Primary Lamellae (DPL).
4.0	Moderate damage	Connective Tissue Degeneration (CTD), Inflammation (I), Filament Degeneration (FD), Artifact (A), Tissue Degeneration (TD).
6.0	Partially Severe damage	Severe Degeneration (SD), Fat Accumulation (FA), Vacuolization (V), Severe Inflammation (SI)
8.0	Severe/Irreversible damage	Severe Vacuolization (SV), Necrosis (N)

Data were analyzed using one sample T-test, SPSS 16.0 version.

RESULTS

Hematological Parameters of *Clarias gariepinus* after 14th and 28th Day Exposure.

Table 2 shows the hematological parameters of *Clarias gariepinus* after 14th and 28th sub-lethal exposure to the various plant extracts. There were decrease in red blood cell counts from the 14th day and 28th day exposure in all the extracts, Mean±SD (x 10^{12}/L); *A. occidentale*, 12.87±1.20; 7.00±3.36, *C. papaya*, 12.58±3.38; 8.79±1.20, *L. cylindrica*, 11.55±1.20; 8.37±3.69, *S. occidentalis*, 11.33±1.03; 8.56±3.69, *N. tabacum*, 9.21±2.40; 8.03±3.03. *A. occidentale* had the lowest RBC level after 28 days of exposure. The 14th day exposure concentrations were higher than the control for *A. occidentale* and *C. papaya*, while the other extracts had lower RBC level. The white blood cell counts from the 14th and 28th day exposure to *L. cylindrica*, *S. occidentalis* and *N. tabacum* increased, Mean±SD (x 10^{9}/L); *L. cylindrica*, 2.49±2.51; 4.34±3.52, *S. occidentalis*, 1.00±0.44; 3.28±3.45, *N. tabacum*, 2.31±3.88; 2.62±2.88, and decreased on exposure to *A. occidentale* and *C. papaya*, Mean±SD (x 10^{9}/L); *A. occidentale*, 3.07±1.89; 1.74±9.30, $p<0.05$, *C. papaya*, 7.32±7.57; 2.06±1.03. The 14th day exposure concentrations were higher than the control for all extracts except for *S. occidentalis*. There were decrease in the hemoglobin (HGB) level from the 14th day and 28th day exposure in *A. occidentale* and *N. tabacum*, Mean±SD (mg/dL); *A. occidentale*, 3.84±8.23; 3.43±7.43, *N. tabacum*, 10.78±1.83; 5.16±8.34, but the HGB level

increased in *C. papaya*, *L. cylindrica* and *S. occidentalis*, Mean±SD (mg/dL); *C. papaya*, 1.03±11.70; 3.48±9.00, *L. cylindrica*, 1.50±11.70; 4.43±9.62 and *S. occidentalis*, 0.99±12.47; 3.46±9.62. The 14th day exposure concentrations were higher than the control for all extracts. The packed cell volume (PVC) from the 14th and 28th day exposure increased in all the extracts except for *N. tabacum*, Mean±SD (mg/dL); *A. occidentale*, 3.24±6.49; 2.16±10.94, p<0.05, *C. papaya*, 1.77±4.79; 7.58±3.47, *L. cylindrica*, 1.99±4.62; 4.29±6.67, *S. occidentalis*, 5.99±1.69; 5.74±2.85, *N. tabacum*, 11.38±2.51, p<0.05; 12.05±2.85, p<0.05. The 14th day exposure concentrations were higher than the control for all extracts except for *C. papaya.*

Table 2: Hematological Parameters of *Clarias gariepinus* after 14th and 28th Day Exposure to the Sub-Lethal Concentrations of the Plant Extracts.

S/N	Botanical Piscicides	HBC ($X10^{12}$/L) Ctrl, 11.56±1.20		WBC ($X10^{9}$/L) Ctrl, 1.56±0.85		HGB (mg/dL) Ctrl, 0.88±12.33		PCV (mg/dL) Ctrl, 1.99±4.63	
		14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
1	*A.occidenale*	12.9±1.2	7.0±3.4	3.1±1.9	1.7±0.3*	3.8±3.2	3.4±7.4	3.2±6.5	2.2±0.9*
2	*C.papaya*	12.6±3.4	8.8±1.2	7.3±7.6	2.1±4.0	1.03±11.6	3.5±9.0	1.8±4.8	7.6±3.5
3	*L.cylindraca*	11.6±1.2	8.4±3.7	2.5±2..5	4.3±3.5	1.5±11.7	4.4±9.6	2.0±4.6	4.3±6.7
4	*S.occidentalis*	11.3±1.0	8.6±3.7	1.0±0.4.	3.3±3.5	0.99±12.5	3.5±9.6	6.0±1.7	5.7±2.9
5	*N.tobacum*	9.2±2.4	8.0±3.0	2.3±3.9	2.6±2.9	10.8±1.8*	5.2±8.3	11.4±2.5*	12.0±2.9*

*Signifies mean value is significant at p<0.05 level. Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin (HGB), Packed Cell Volume (PVC)

Liver Function Parameters in *Clarias gariepinus* after 28 Day Exposure and Recovery

Table 3 shows the liver function parameters in *Clarias gariepinus* after 28 day exposure to sub-lethal concentrations of the extracts and its recovery. Aspartate Aminotransferase (AST) increased after recovery for *A. occidentale, S. occidentalis* and Ammonium solution; Mean±SD (U/L); *A. occidentale,* 37.11±5.11, p<0.05; 15.75±12.50, *S. occidentalis*, 17.00±10.31; 23.00±11.50, Ammonium solution, 32.50±3.61, p<0.05; 47.75±4.61 and decreased for *C.papaya*, *L. cylindrica* and *N. tabacum*; Mean±SD (U/L); *C. papaya,* 41.75±10.31, p<0.05; 12.75±7.56, *L. cylindrica* 42.75±9.16, p<0.05; 16.75±8.11, *N. tabacum,* 51.75±5.61, p<0.05; 24.25±13.61. The 28th day exposure concentrations were higher than the control for all extracts. Alanine Aminotransferase (ALT) increased after recovery for all the extract except for *N.tabacum*; Mean±SD (U/L); *A. occidentale, S. occidentalis* and Ammonium solution; Mean±SD (U/L); *A. occidentale,* 13.35±9.61, p<0.05; 9.00±6.17, *S. occidentalis*, 39.50±6.20; 6.20±18.75, Ammonium solution, 16.75±11.50, p<0.05; 40.75±6.11, *C. papaya,* 22.56±4.61, p<0.05; 19.50±11.96, *L. cylindrica* 11.85±6.91; 7.75±5.89, *N. tabacum,* 26.25±18.67; 30.00±19.91. The 28th day exposure concentrations were higher than the control for all extracts. Alkaline Phosphatase (ALP) increased after recovery only for *A. occidentale,* Mean±SD (U/L); 12.75±9.64; 16.75±3.25, p<0.05, but for the other extracts, ALP decreased; Mean±SD (U/L); *S. occidentalis,* 11.53±10.01; 11.53±9.95, Ammonium solution, 16.25±13.91; 16.03±14.01, *C. papaya,* 19.03±4.56, p<0.05; 11.55±9.01, *L. cylindrica* 10.75±7.75; 9.75±6.12, *N. tabacum,* 19.38±15.01; 18.15±10.56. The 28th day exposure concentrations were higher than the control for all extracts.

Gill Alteration Index of *Clarias gariepinus* after 28th Day

Table 4 and Plates 1a to 1g show the gill pathological alterations in *Clarias gariepinus* after 28 day exposure to sub-lethal concentrations of the extracts. Nine key alterations were identified; Connective Tissues Degeneration (CTD), Filament Degeneration (FD),

Degeneration of Primary Lamellae (DPL), Separation of Filaments (SF), Severe Degeneration (SD), Fat Accumulation (FA), Vacuolization (V), Inflammation (I) and Necrosis (N). Alterations ranged from mild to severe. *S. occidentalis* had no alteration. *C. papaya,* and *L. cylindrica* produced mild alterations such as CTD, FD and Inflammation in the gills with alteration index of 1.78 respectively. *N. tabacum* and Ammonium solution had severe alterations such as vacuolization, severe inflammation and degeneration and necrosis with alteration index of 3.11 respectively. *A. occidentale* showed the highest gill alteration with high frequencies of severe inflammation, vacuolization and necrosis with alteration index of 3.33.

Table 3: Liver Function Parameters in *Clarias gariepinus* after 28 Day Exposure and Recovery to the Sub-Lethal Concentrations of the Plant Extracts.

S/N	Botanical Piscicides	AST (U/L) Ctrl, 115.6±1.20		ALT (U/L) Ctrl, 1.56±0.85		ALP (U/L) Ctrl, 0.88.6±12.33	
		Treatment	Recovery	Treatment	Recovery	Treatment	Recovery
1	*A.occidenale*	37.11*±5.11	15.75±12.50	13.35±9.61	9.00±6.17	12.75±9.64	16.75*±3.25
2	*C.papaya*	41.75*±6.79	12.75±7.56	22.56±4.61	19.50±11.96	19.03*±4.58	11.55±9.01
3	*L.cylindraca*	42.75*±9.16	16.75±8.11	11.85±6.91	7.75±5.89	10.75±7.75	9.75±6.12
4	*S.occidentalis*	17.00±10.31	23.00±11.50	39.50±6.21	18.75±10.91	11.53±10.01	11.53±9.95
5	*N.tobacum*	51.75±5.61	24.25±13.61	26.25±18.67	30.00±19.38	19.38±15.01	18.15±10.56
6	*Ammonium Solution*	51.75±5.61	47.75±4.61	16.75±11.50	40.75±6.11	16.25±13.91	16.03±14.01

*Signifies mean value is significant at $p<0.05$ level. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP)

Table 4: Gill Alteration Index of *Clarias gariepinus* after 28th Day Exposure to the Sub-Lethal Concentrations of the Plant Extracts.

S/N	Botanical	CTD	FD	I	V	SD	SF	FA	DPL	N	GAI	RGAI
1	*A.occidenale*	0.0	0.0	4.0	6.0	6.0	0.0	6.0	0.0	8.0	3.33	1.87
2	*C.papaya*	4.0	0.0	0.0	0.0	0.0	6.0	0.0	6.0	0.0	1.78	1.00
3	*L.cylindraca*	4.0	4.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0	1.78	1.00
4	*S.occidentalis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
5	*N.tobacum*	4.0	0.0	4.0	6.0	6.0	0.0	0.0	0.0	8.0	3.11	1.75
6	Ammonium Solution	4.0	0.0	4.0	6.0	0.0	0.0	6.0	0.0	8.0	3.11	1.75

Connective Tissues Degeneration (CTD), Filament Degeneration (FD), Degeneration of Primary Lamellae (DPL), Separation of Filaments (SF), Severe Degeneration (SD), Fat Accumulation (FA), Vacuolization (V), Inflammation (I), Necrosis (N), Gill Alteration Index (GAI), Relative Gill Alteration Index (RGAI).

Hepatic Alteration Index of *Clarias gariepinus* after 28th Day Exposure

Table 5 and Plates 2a to 2g show the liver pathological alterations in *Clarias gariepinus* after 28 day exposure to sub-lethal concentrations of the extracts. Nine key alterations were identified; Hepatocytic Hypertrophy (HH), Severe Degeneration (SD), Vacuolization (V), Inflammation (I), Severe Inflammation (SI), Necrosis (N), Artifact (A), Tissue Degeneration (TD), Severe Vacuolization (SV). Alterations ranged from mild to severe. *S. occidentalis* had no alteration. *C. papaya,* and *L. cylindrica* produced mild alterations such as Artifact, Tissue Degeneration with alteration index of 1.56 and 1.33 respectively. Ammonium solution had alterations such as inflammation, degeneration and necrosis with alteration index of 2.00. *A. occidentale* and *N. tabacum* showed the highest liver alteration with high frequencies of

severe vacuolization, inflammation and degeneration with alteration index of 2.44 respectively.

Table 5: Hepatic Alteration Index of *Clarias gariepinus* after 28th Day Exposure to the Sub-Lethal Concentrations of the Plant Extracts.

S/N	Botanical	HH	I	A	TD	V	SD	SI	SV	N	HAI	RHAI
1	*A.occidenale*	0.0	0.0	4.0	0.0	6.0	6.0	6.0	0.0	0.0	2.44	1.83
2	*C.papaya*	0.0	0.0	4.0	4.0	6.0	0.0	0.0	0.0	0.0	1.56	1.17
3	*L.cylindraca*	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	8.0	1.33	1.00
4	*S.occidentalis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
5	*N.tobacum*	0.0	0.0	4.0	0.0	6.0	6.0	6.0	0.0	0.0	2.44	1.83
6	Ammonium Solution	2.0	4.0	0.0	4.0	0.0	0.0	0.0	0.0	8.0	2.00	1.50

Hepatocytic Hypertrophy (HH), Severe Degeneration (SD), Vacuolization (V), Inflammation (I), Severe Inflammation (SI), Necrosis (N), Artifact (A), Tissue Degeneration (TD), Severe Vacuolization (SV), Hepatic Alteration Index (HAI), Relative Hepatic Alteration Index (RHAI).

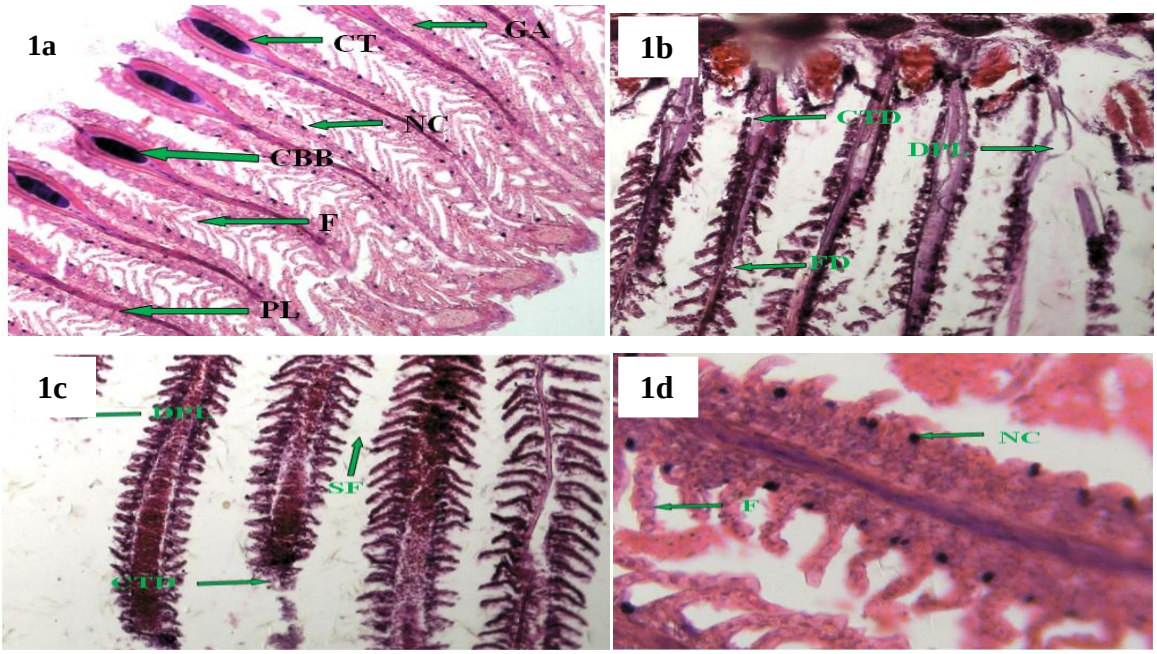
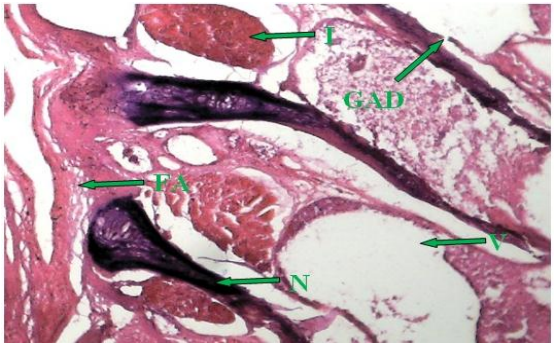
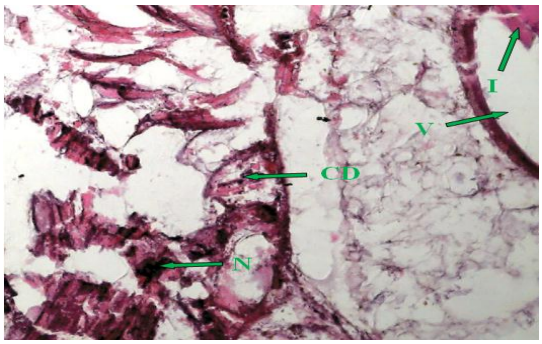


Plate 1(a-d): Magnification (x400) after 28 days Gill of post juvenile *Clarias gariepinus* exposure for all figures (1a to 1d) **1a:** Control: Transverse Section (TS) of gill at showing normal cellular pattern; **1b:** Exposure to *Luffa cylindrica*, **1c:** Exposure to *Carica papaya*, **1d:** Exposure to *Senna occidentalis*, **Acronyms:** Ceratobrachial Bone (CBB), Connective Tissue (CT), Prominent Nucleus (NC), Distinct Primary Lamella (PL), Gill Arch (GA), and Filament (F), Filament (F) with no morphological impacts or tissue damage, Connective Tissue Degeneration (CTD), Filament Degeneration (FD), Degeneration of Primary Lamella (DPL), Separation of Filament (SF).





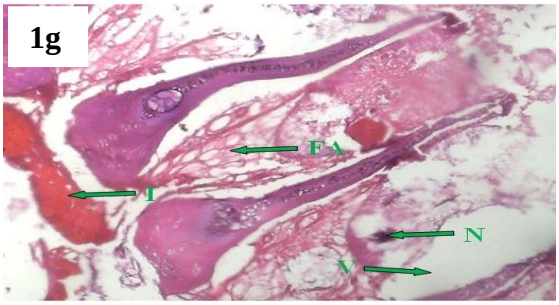
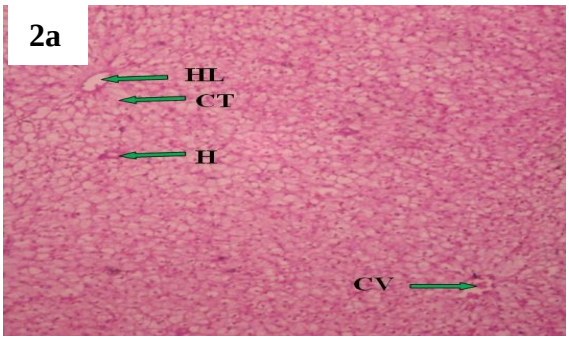
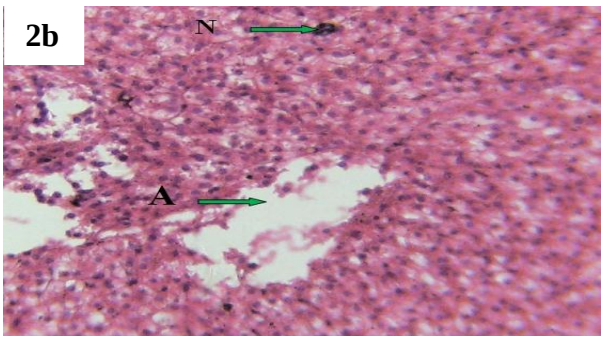
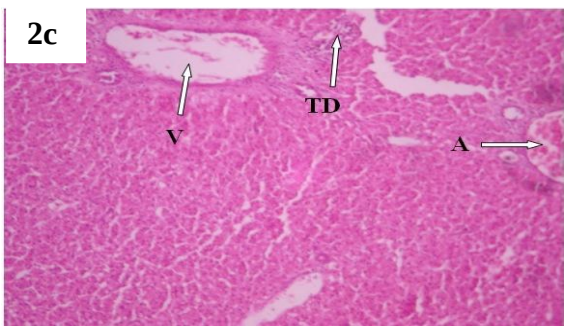


Plate 1(e-g): Magnification (x400) after 28 days Gill of post juvenile *Clarias gariepinus* exposure for all figures (1e to 1g) **1e:** Exposure to *Anacardium occidentale*, **1f:** Exposure to *Nicotiana tabacum*, **1g:** Exposure to Ammonia solution, **Acronyms:** severe Fat Accumulation (SFA), Vacuolization (V), Inflammation (I) and Necrosis (N), severe Gill Arch Degeneration (GAD), Fat Accumulation (FA), Vacuolization (V), Inflammation (I) and Necrosis (N), severe Cellular Degeneration (CD).







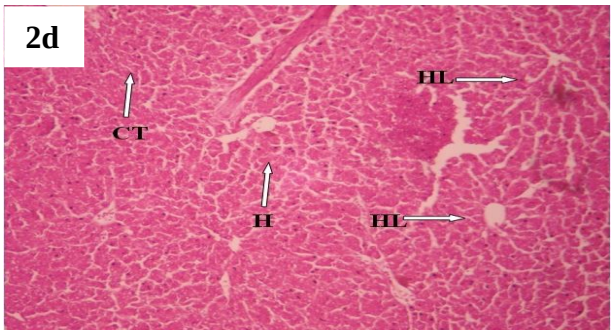


Plate 2(a-d): Magnification (x400) after 28 days Liver of post juvenile *Clarias gariepinus* exposure for all figures (2a to 2d) **2a:** Control: Transverse Section (TS) normal cellular pattern, **2b:** Exposure to *Luffa cylindrica*, **2c:** Exposure to *Carica papaya*, **2d:** Exposure to *Senna occidentalis*, Acronyms: Nucleus (NC), Filament (F), Hepatic Lobule (HL), Central Vein (CV), Hepatocytes (H), Connective Tissue (CT), Tissue Degeneration (TD), Vacuolization (V), Artifact (A), Necrosis (N).

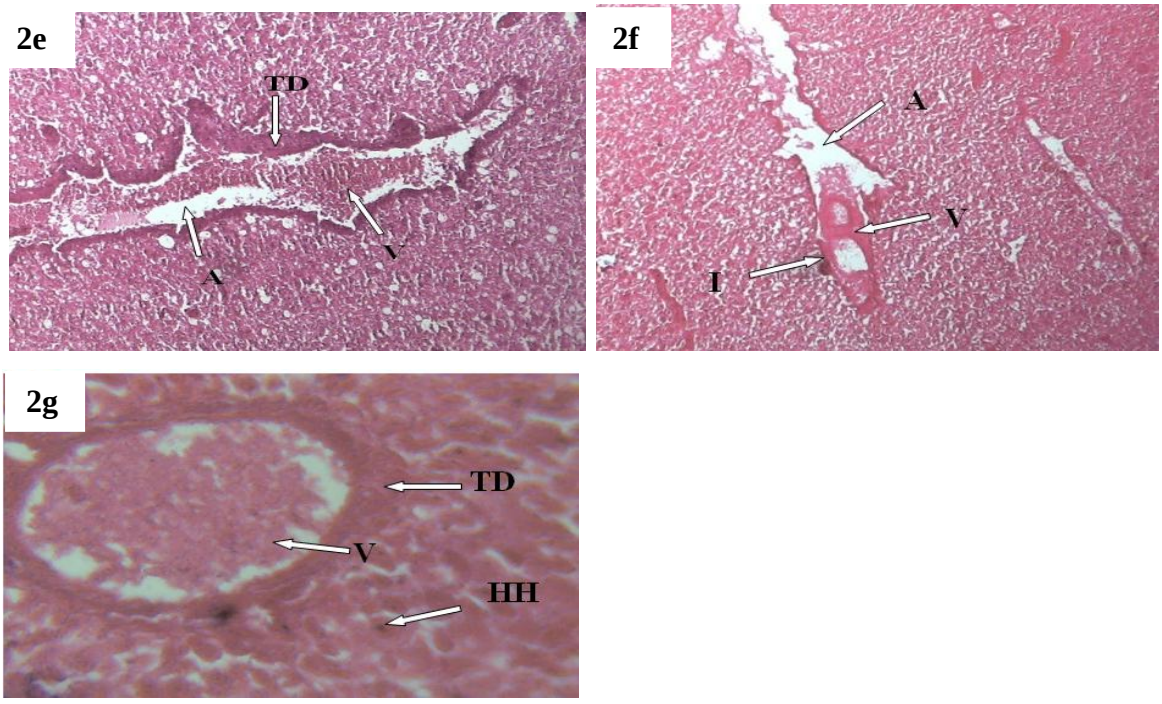


Plate 2(e-g): Magnification (x400) after 28 days Liver of post juvenile *Clarias gariepinus* exposure for all figures (2e to 2g) **2e:** Exposure to *Anacardium occidentale*, **2f:** Exposure to *Nicotiana tabacum*, **2g:** Exposure to Ammonia solution, **Acronyms:** Tissue Degeneration (TD), Vacuolization (V) and Artifact (A), Vacuolization (V), Inflammation (I), Tissue Degeneration and Hepatocytic Hypertrophy (HH), Inflammation (I), Artifact (A) and Vacuolization (V).

DISCUSSION

Evaluation of blood parameters can be used to determine the level of negative effect of foreign compounds, including medicinal plants (Ibrahim *et al*., 2016). This method can also be used to explain haematological relating functions of plant products (Yakubu *et al*., 2007). In this study, there were decrease in red blood cell counts from the 14th day and 28th day exposure in all the extracts; *A. occidentale* had the lowest RBC level after 28 days of exposure. The 14th day exposure concentrations were higher than the control for *A. occidentale* and *C. papaya*, while the other extracts had lower RBC level. This is in agreement with the research by Asgary *et al.* (2005), who reported that some extracts cause decrease in the RBC through hemolysis. Fluctuation in different hematological parameters in treatment fishes can be described as either increase or decrease from the respective values of the control fishes. The total RBCs count, PVC and Haemoglobin concentration decreased significantly in the treatments over time than those of control fishes. Similar findings have also been reported in several studies with different fish species being exposed to either botanical extracts (Kavitha *et al*, 2012) or chemical toxicant (Deka and Dutta, 2012). Fishes could meet stress conditions during exposure to any toxicant that may decrease in haemoglobin synthesis rate, resulting in an interruption of oxygen supply to the tissues and thus creating an anaemic condition (Witeska, 2015). Defining anemia in fish is, however, difficult due to a lack of clear reference values of red blood parameters. The mechanism of anemia are different; impaired erythropoiesis, accelerated hemolysis, hamorrahage or a combination of these factors but they all produce similar effects; a decrease in haemoglobin concentration and erythrocyte count which results in reduced oxygen supply to the tissues and thus impairment of growth and health status of fish (Witeska, 2015). In this study, there were decrease in the hemoglobin (HGB) level from the 14th day and 28th day exposure in *A. occidentale* and *N. tabacum*, but the HGB level increased in *C. papaya*, *L. cylindrica* and *S.*

occidentalis, while the packed cell volume (PVC) from the 14th and 28th day exposure increased in all the extracts except for *N. tabacum*, The 14th day exposure concentrations were higher than the control for all extracts for HGB and PVC.

The white blood cells (WBC) are the first line of defence which responds to infectious agents, inflammatory process or tissue injury. The WBCs count in the treatment fishes significantly increased in response to the toxic condition, probably for a stimulation in lymphopoiesis by the extract or an increase in the release of lymphocytes from lymphomyeloid tissue as defence mechanisms to tolerate and overcome the situation that eventually increased WBCs count (Kavitha *et al*, 2012; Harabawy and Ibrahim, 2014). In this study, the white blood cell counts from the 14th and 28th day exposure to *L. cylindrica, S. occidentalis* and *N. tabacum* increased and decreased on exposure to *A. occidentale* and *C. papaya.* The 14th day exposure concentrations were higher than the control for all extracts except for *S. occidentalis*.

AST, ALT and ALP are enzymes which are predominantly found in the liver cells and their high presence in the blood plasma will indicate problems with the liver cells (Hayashi *et al*, 2003, Wang *et al*, 2012). The tests for these enzymes are means of evaluating liver function. This gives an evaluation of the health condition of the liver in terms of performance. AST, ALT and ALP are enzymes associated with liver parenchymal cells. When body tissues or an organ such as the heart or liver is damaged, their additional levels are released into the bloodstream. The amount of each in the blood is directly related to the extent of the tissue damage (Tolman and Keji, 1999). In this study, AST increased after recovery for *A. occidentale, S. occidentalis* and Ammonium solution and decreased for *C. papaya, L. cylindrica* and *N. tabacum,* while ALT increased after recovery for all the extract except for *N. tabacum.* In addition, there were increase in ALP after recovery, this was only for *A. occidentale* extract, ALP decreased in other extracts. The 14th day exposure concentrations had higher enzyme levels than the control for all extracts.

The pattern of reversibility of the liver function enzymes levels in post juvenile *Clarias gariepinus* exposed singly to aqueous extract of each of the botanicals showed that there was increase in the levels of AST, ALT and ALP with increase in the duration of exposure to the botanical extracts and decrease in their levels when transferred to fresh water as the duration of exposure to fresh water was increased, except those exposed to ammonia solution. This means that the effects of the botanicals on the liver of the fishes are reversible and the affected fishes can return to their pre- effect state. As such, the use of botanical piscicides should be encouraged.

The effects of toxicants that cannot be quantitatively measured may be described. For instance, the general effects of toxicants on the tissues of organisms can be described by carrying out histopathological analysis of the suspected tissues of a particular organ. In this study, the effects of each of the botanical piscicides on post juvenile *Clarias gariepinus* were described. Alterations ranged from mild to severe alterations. *S. occidentalis* had no alteration on tissues. *C. papaya,* and *L. cylindrica* produced mild alterations such as CTD, FD and Inflammation in the gills with alteration index of 1.78 respectively. *N. tabacum* and Ammonium solution had severe alterations such as vacuolization, severe inflammation and degeneration and necrosis with alteration index of 3.11 respectively. *A. occidentale* showed the highest gill alteration with high frequencies of severe inflammation, vacuolization and necrosis with alteration index of 3.33. *C. papaya*, and *L. cylindrica* produced mild alterations on the liver such as Artifact, Tissue Degeneration with alteration index of 1.56 and 1.33 respectively. Ammonium solution had alterations such as inflammation, degeneration and necrosis with alteration index of 2.00. *A. occidentale* and *N. tabacum* showed the highest

liver alteration with high frequencies of severe vacuolization, inflammation and degeneration with alteration index of 2.44 respectively.

CONCLUSION

This study has revealed that aqueous extract of *A. occidentale* and *N. tabacum* are the most toxic extracts to *Clarias garipinus* at low concentration. Sub-lethal toxicity on the fish specimens showed that they had brought about significant alteration in both hematological (RBCs, WBCs, HGB and PCV) and liver function enzymes (AST, ALP, ALT) parameters of *C.garipinus* including its histological alterations. Fluctuations in these essential attributes of fishes due to exposure to the extracts indicate their mode of action as piscicide. Therefore, *A. occidentale* and *N. tabacum* extracts could be a potential plant-derived fish poison to catch fishes or control unwanted species from aquaculture ponds.

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