HISTOPATHOLOGICAL CHANGES IN THE GILL AND LIVER OF *CLARIAS GARIEPINUS* EXPOSED TO ACUTE CONCENTRATIONS OF *VERNONIA AMYGDALINA*

[1]AUDU, Bala Sambo, [2]OMIRINDE, Jamiu Oyewole, [2]GOSOMJI Innocent Jonah and [1]WAZHI, Ponnak Ezekiel

[1]Applied Hydrobiology and Fisheries Unit, Department of Zoology, Faculty of Natural Sciences, University of Jos, Jos, Plateau State, Nigeria.

[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Plateau State, Nigeria.

Corresponding Author: Omirinde, J. O. Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Plateau State, Nigeria. **Email:** omirindejamiu@yahoo.com **Phone:** +234 8069735125

ABSTRACT

Vernonia amygdalina is a tropical African woody shrub with diverse phytochemical constituents recently linked with insecticidal properties that could replace the harmful agrochemical pesticide usage around aquatic environment. This study investigates the histopathological changes in the liver and gills of Clarias gariepinus exposed to acute toxic concentrations of V. amygdalina. C. gariepinus juveniles of varied weight (7.28 ± 0.03 g) and length (4.82 ± 0.06 cm) were exposed to graded aqueous concentrations (0.188, 0.375, 0.75, 1.50 and 3.00 g/l) of V. amygdalina. The varied concentrations of V. amygdalina precipitated varied dose-dependent histopathological distortions in the hepatic (central venous congestion and hepatocellular degeneration) and gill parenchyma (lamellar hyperplasia, clubbing and occluded inter-lamellar space) of exposed C. gariepinus. The liver (hepatocyte nuclear diameter and surface area) and gill (secondary lamellar length, width, interlamellar distance and surface area) morphometrics were strikingly altered varied concentrations of V. amygdalina. V. amygdalina seems to be toxic to fish and therefore has to be cautiously applied when used as insecticides to control unwanted organisms around the fish habitats.

Keywords: *Vernonia amygdalina*, *Clarias gariepinus*, Histopathology, Liver, Gill

INTRODUCTION

Vernonia amygdalina is a predominantly tropical African small soft woody shrub with elliptical petiolate leaf of about 6 mm width (Akah *et al*., 2009; Nabukenya *et al.,* 2014). The plant is commonly called bitter leaf due to its bitter taste which can be subsided by boiling or soaking the leaves in several changes of clean water (Onabanjo and Oguntona, 2003). *V. amygdalina* is a famous vegetable used for making soup in the tropics especially in Nigeria and has been reported to contain some nutritional needs of man and animals (Aregheoere *et al.,* 1998; Eleyinmi *et al.,* 2005). The popularity of the leaves and roots of *V. amygdalina* in folkloric medicine in Nigerian communities has been recognized in the treatment of ailments like fever, stomach discomfort, kidney and heart disease. The efficacy of this plant against diseases has been proven in plethora of studies in laboratory mammals to potentially have hepatoprotective, anti-inflammatory, anti-cancer, anti-oxidant, antihelminthic, antidiabetic and hypolipidaemic properties (Huffman, 2003; Ebong *et al.,* 2008; Omolola and Olatunde, 2009; Adaramoye *et al.,*

2010; Adesanoye and Farombi, 2010; Eyo *et al.,* 2013 a, b).

Generally, plant is a store of wide range of structurally diverse bioactive substances (Chikezie *et al.,* 2015). Recently, advanced phytochemical analyses of *V. amygdalina* have revealed the presence of some bioactive substances (sesquiterpene lactones, terpenoids, flavonoids like luteolin, luteolin 7-O-glucosides and luteolin 7-O-glucuronide, steroid glycosides, saponin, terpenoids and vernonioside A, B, A1, A2, A3, B2, B3 and A4 eucalyptol, beta pinene and myrtenal alpha-muurolo) with insecticidal properties against the common bean aphids and maize weevils (Mwanauta *et al.,* 2014). This finding has placed it in the group of *Azadiracta indica* and host of others plant based insecticide (Suresh Babu *et al.,* 2013; Mwanauta *et al.,* 2014) and as such making it a good candidate for replacing the synthetic agrochemical pesticides that are popularly used for controlling pests.

Agrochemical pesticides when used close to waterways are usually washed into the aquatic environment where they hamper aquatic life. The latter is harmful to fish health because of their non-biodegradability with tendency of high residues formation in both fish tissues and their immediate aquatic environment (Ojutiku *et al.,* 2012; Gill and Garg, 2014). However, plant based insecticides can suitably edge out their synthetic counterpart due to their biodegrability in water bodies (Ajani and Ayoola, 2010).

Fish are excellent model for determining aquatic ecosystem health (Min and Kang, 2008). *Clarias gariepinus* is a large, eel-like fish that belongs to the family Clariidae (Bruton, 1979). The catfish is widely cultured throughout Africa in both natural and artificial habitats (Nguyen and Janssen, 2002; Adeyemo, 2008). Owing to its fast growth rate and superior tolerance to deranged water quality, it remains the choice fish for research on aquatic ecotoxicity (Mahmoud *et al.,* 2009). There is paucity of information on the histopathological and histomorphometrical changes in the gills and liver of *C. gariepinus* fish acutely exposed to graded concentrations of *V. amygdalina.* Thus, this study seeks to investigate the possibility.

MATERIALS AND METHODS

Aqueous Extract: *Vernonia amygdalina* leaves (1000 g) were collected from Jos, Plateau State, Nigeria and deposited in the Federal College of Forestry Herbarium, Jos, for identification.

The leaves were air dried at room temperature (27 ± 2°C) in the laboratory of Zoology Department, Faculty of Natural Science, University of Jos. The dried leaves (400 g) were pounded with laboratory mortar and pestle into powder, sieved with a 30 µm mesh size sieve and the filtrate powder (300 g) decocted in one litre of distilled water.

Phytochemicals: The standard procedures described by Evans (2002) were adopted for the screening of plant extract for the presence of alkaloids, flavanoids, tannins, saponins and phenols.

Fish: *Clarias gariepinus* juveniles with average weight (7.28 ± 0.03 g) and length (4.82 ± 0.06 cm) were purchased from Global Aquaculture and Allied Ventures fish farm in Jos, Plateau State, Nigeria. The fish were acclimatized in the Fishery and Hydrobiology Laboratory of the Zoology Department, University of Jos, Jos, Nigeria for a period of 1 week in two plastic containers of 50 litres volume capacity containing clean chlorine free water at pH 7.1; temperature 27 – 28 ºC; carbon dioxide concentration 6 – 8 mg/l, oxygen 88 – 95 % saturation; total alkalinity 85 - 95 mg/l and photoperiod 12: 12 light: dark. The fish were fed on commercially prepared fish feed (Vital Fish Feed, Nigeria) twice in a day during acclimatization phase prior to the commencement of experiment.

Experimental Design: The static renewal bioassay technique of EPA (1985) was adopted for this experiment. The toxicant concentration of 3 g/l was carefully chosen for serial dilution as the least tolerance test concentrations (LC_{50}) after pilot tests. Subsequent concentrations of 1.5, 0.75, 0.375 and 0.188 g/l of the extract were obtained through serial dilutions of the toxicant concentration.

The *C. gariepinus* juveniles are then randomly divided into six groups of two replicates each with each replicate having five catfishes as follows; Group 1 (control): *C. gariepinus* received 0.00 g/l of the aqueous extract of *V. amydalina*. Groups 2 – 6 (*V. amydalina exposed*): received graded concentrations (0.188, 0.375, 0.75, 1.5 and 3 g/l) of *V. amydalina* respectively. *C. gariepinus* were acutely exposed to *V. amydalina* for a specific period of 96 hours.

Behavioural Signs and Mortality: *C. gariepinus* exposed to grades of *V. amygdalina* were keenly monitored during the 96 hours of acute toxicity test for the following behavioural changes; air gulping, stunned positioning, skin peeling, aggression and erratic swimming (fast and spiral movement). The changes were ranked as weak, moderate and high. The duration and pattern of mortality were equally observed during the acute toxicity test. Fish were considered dead when they fail to respond to touch from a glass probe with floating belly. Also they may sink to the bottom of the container.

Histopathology: *C. gariepinus* were anesthetized after the experiment using benzocaine (0.1 g/l) and then sacrificed by cervical section. The liver and gills tissues were dissected out, rinsed in physiological saline and fixed in aqueous Bouin's fluid. The two fixed tissues were subjected to dehydration in grades of ascending alcohol concentrations. Subsequently cleared in xylene, embedded in paraffin and sectioned at 15 µm. Sections of liver and gills were further stained with Haematoxylin-Eosin (HE) and examined with light microscope (Olympus, China) at x40 magnification.

Histomorphometrics: Gill histomorphometrical measurements like secondary lamellar width - SLW (distance between two edges of a lamellar base), secondary lamellar length - SLL (distance between the tip and the most distal point of the lamellae from the filament), inter-lamellar distance - ILD (distance between two adjacent secondary lamellae) and secondary lamellar surface area - SLSA (the entire outer part of secondary lamella) were determined by using Motic image plus 2.0 (Motic Asia, Hong Kong) software. Ten randomly selected sections of 10 fish from each experimental group were used. In addition, hepatocyte histomorpometrical measurements such as hepatocyte nuclear diameter and hepatocytes surface area were determined.

Data Analysis: Data collected were subjected to analysis of variance (ANOVA) using SPSS version 16. Significant means were set at $p<0.05$ and separated using Duncan new multiple range test. Results were presented as means ± standard errors of mean.

RESULTS

Phytochemicals: The result on phytochemical analysis of aqueous extract of *V. amygdalina* leaf showed that the leaf of the plant contained varied proportions of some bioactive substances that include alkaloid, flavanoids, tannins, saponins, and phenol (Table 1).

Table 1: Qualitative phytochemical constituents of aqueous extracts of *Vernonia amygdalina* leaf

Bioactive substances	Colour	Remarks
Alkanoid	Orange	+
Flavanoids	Yellow	+
Tannins	Green/blue	++
Saponins	Froth	++
Phenol	Deep blue	++

In respect to this, alkaloid and flavanoids was weakly present in the leaf extract of *V. Amygdalina*. However, tannins, saponins and phenol showed strong presence in the aqueous leaf extract of *V. amygdalina*.

Behavioural Signs: The result in Table 2 showed the various behavioural signs displayed by *Clarias gariepinus* at regular interval of the toxicity test. There was no obvious deviation from the normal behavioural patterns in the fish exposed to the lowest concentration (0.186 g/l) of *V. amygdalina* in all the durations of the acute toxicity. With the exception of skin peeling, the fish exposed to 0.375 g/l weakly

displayed virtually all the clinical signs in all the durations of the toxicity. The *C. gariepinus* given 0.75g/l of the extract showed weak clinical signs of the abnormalities with no evidence of skin peeling during the early hours of the toxicity test. However, during the late hours of the toxicity especially during 96 hours, *C. gariepinus* displayed a moderately abnormal clinical signs. In addition, fish exposed to 1.5 and 3.0 g/l of the extract respectively shown markedly progressive severity in clinical signs more particularly during the late hours of the test. However, signs like spiral movement and aggression were weakly shown at 96 hour with no visible sign of fast movement in fish exposed to 3 g/l of the extract.

Mortality: The mortality profile (Table 3) showed by *C. gariepinus* acutely exposed to a *V. amygdalina* appeared to be duration and concentration dependent. The lower concentration groups had markedly reduced mortality especially at the later phases of the test.

However, in the groups given higher concentration of the extract, conspicuous death was recorded mainly at the early phases of the test.

Histopathology: The effect of the exposure of the *C. gariepinus* to grades of *V. amygdalina* extract on the parenchyma of the liver and gill are shown in Figures 1 and 2 respectively.

The hepatic parenchyma of the control group of *C. gariepinus* showed that the architecture of the liver is characterized by the presence of normal nuclei within the hepatocytes cytoplasm, abundant sinusoids between the liver cells and the presence of central vein (Figure 1A). Similarly, hepatic parenchyma seemed to be normal in the group exposed to 0.1875 g/l relative to the control group histoarchitecture (Figure 1B). However, exposure to grades of *V. amygdalina* (0.375 – 3 g/l) precipitated varying degrees of moderate distortions to the liver parenchyma especially at lower concentrations (0.375 and 0.75 g/l) of the extract to severe hepatocellular damage at higher concentrations (Figures 1 C – F).

The gill histoarchitectural integrity of the control group is characterized by the presence of a primary filament, a centrally placed rod-like supporting axis, with several rows of secondary gill lamellae laterally radiating from each side of the interbranchial septum of the filament (Figure 2A). The secondary lamellae are covered by epithelial cells. In addition, one of the prominent features of the gill of the control group is the interlamellar space (water channel) separating two adjacent secondary lamellae on the filament. On the contrary, *C. gariepinus* exposed to grades of *V. amygdalina* showed varying degrees of moderate (lamellar fusion and clubbing) to severe (lamellar hyperplasia and total water channel occlusion) lesions in their gill parenchyma (Figures 2C – F). It is important to mention that the parenchyma of the gill of *C. gariepinus* exposed to the 0.188 g/l of the extract had no visible lesion (Figure 2B).

Histomorphometry: The histomorphometry of liver and gill of *C. gariepinus* fingerlings exposed to concentrated grades of *V. amygdalina* is shown in Tables 4 and 5 respectively. There was a significant progressive concentration dependent reduction ($p<0.05$) in the hepatocyte nuclear diameter (HND) values of the *C. gariepinus* exposed to grades of *V. amygdalina* extract relative to the control group.

However, the HND values of *C. gariepinus* exposed to the lowest concentration of the extract (0.188 g/l) was not significantly different ($p>0.05$) from the control.

The hepatocyte surface area (HSA) values of *V. amygdalina* intoxicated fish decreased significantly with the increasing extract concentrations when compared with the control fish. However, there were no significant changes in HSA values between the lowest concentration (0.188 g/l) of the extract and the control. Also, the higher concentrations (0.75 – 3.0 g/l) of *V. amygdalina* exposed fish showed an insignificant difference ($p>0.05$) in HSA values. The inter lamellar distance (ILD) values of the gill of *C. gariepinus* exposed to grades of *V. amygdalina* extract significantly decreased ($p<0.05$) with the increasing concentrations of the extract.

Table 2: The various behavioural signs displayed by *Clarias gariepinus* exposed to graded concentrations of *Vernonia amygdalina* leaf aqueous extract

Conc. (g/l)	Clinical signs	Duration of exposure (Hours)						
		8	16	24	36	48	72	96
0.00	Surface air gulping	-	-	-	-	-	-	-
	stunned position	-	-	-	-	-	-	-
	skin peeling	-	-	-	-	-	-	-
	aggression	-	-	-	-	-	-	-
	spiral movement	-	-	-	-	-	-	-
	fast swimming	-	-	-	-	-	-	-
0.186	Surface air gulping	-	-	-	-	-	-	-
	stunned position	-	-	-	-	-	-	-
	skin peeling	-	-	-	-	-	-	-
	aggression	-	-	-	-	-	-	-
	spiral movement	-	-	-	-	-	-	-
	fast swimming	-	-	-	-	-	-	-
0.375	Surface air gulping	-	+	+	+	+	+	+
	stunned position	-	+	+	+	+	+	++
	skin peeling	-	-	-	-	-	-	-
	aggression	-	+	+	+	+	+	++
	spiral movement	-	+	+	+	+	+	++
	fast swimming	-	+	+	+	+	+	++
0.75	Surface air gulping	+	+	+	+	+	+	++
	stunned position	+	+	+	+	+	++	++
	skin peeling	-	-	-	-	-	-	-
	aggression	+	+	+	+	+	+	++
	spiral movement	+	+	+	+	+	+	++
	fast swimming	+	+	+	+	+	+	++
1.50	Surface air gulping	+	++	++	++	++	++	++
	stunned position	-	+	+	++	++	++	+++
	skin peeling	+	++	++	++	++	+++	+
	Aggression	+	++	++	++	++	+	+
	spiral movement	+	++	++	++	++	++	+++
	fast swimming	+	++	++	++	+++	++	+
3.00	Surface air gulping	+	++	++	++	+++	+++	++
	stunned position	+	++	++	++	+++	+++	+++
	skin peeling	+	++	++	++	+++	+++	++
	Aggression	+	++	++	++	+++	++	+
	spiral movement	+	++	++	+++	++	+	+
	fast swimming	**+**	**++**	**++**	**++**	**++**	**+**	**-**

no visible sign (-), weak (+), moderate (++), high (+++)

Table 3: Mortality of *Clarias gariepinus* juveniles exposed to various concentrations of *Vernonia amygdalina* aqueous leaf extracts

Conc. (g/l)	Mortalities /Time							Total Mortality	Percentage Mortality
	8	16	24	36	48	72	96		
0.00	0	0	0	0	0	0	0	0	8.7190
0.186	0	0	0	0.5	0	0	0.5	1	5.5244
0.375	0	0	0	0	0.5	1.5	0	2	5.0000
0.75	0	0.5	0	0	3.5	1.0	0	5	4.1584
1.50	0	2.5	3.0	1.5	0	0	0	7	3.1784
3.00	1.5	1.5	4.5	2.5	-	-	-	10	-

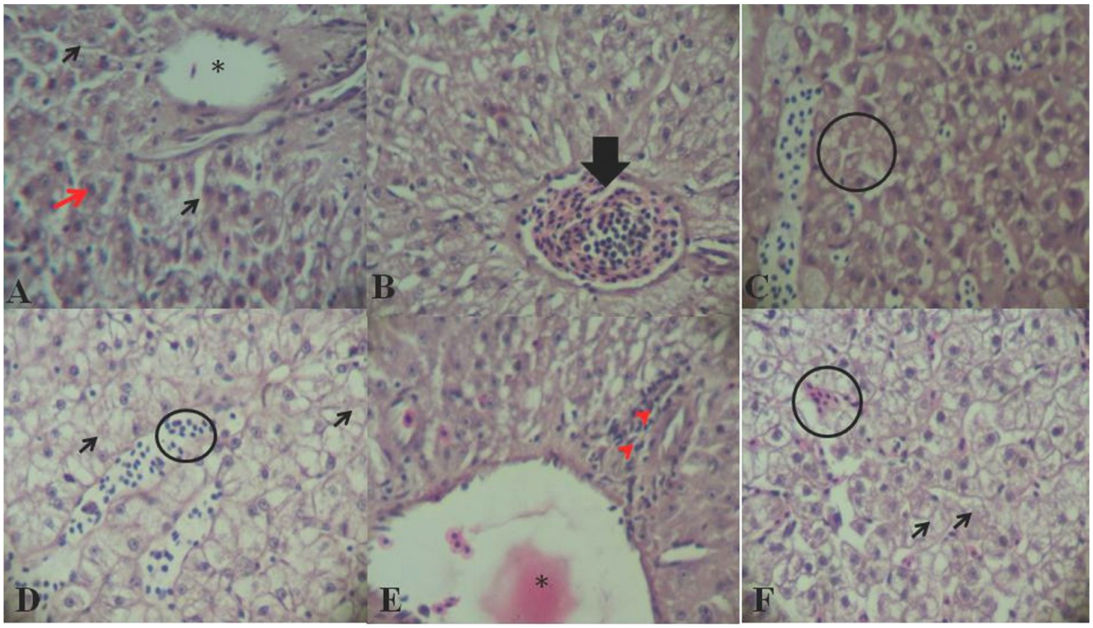

Figure 1: Photomicrographs of the liver of *Clarias gariepinus* exposed to grades of *Vernonia amygdalina* concentrations. (A) 0.00 g/l of VA: Normal liver parenchyma architecture with normal nuclei (red arrow), sinusoids (black arrow) and central vein (asterisk) morphologies. (B) 0.1875g/l: Normal hepatic parenchyma architecture but with markedly congested central vein (black arrow) (C) 0.375g/l: showed moderate hepatic necrosis (oval). (D) 0.75g/l: showed marked hepatocyte nuclei and cytoplasmic degeneration (black arrow) with intravascular aggregation of blood cells (oval). (E) 1.5g/l: Hepatic parenchyma showed moderate central venous congestion (asterisk) and mild perivascular inflammation as evident by inflammatory cell infiltrations (red arrowheads). (F) 3g/l: Marked hepatocyte degeneration (black arrow) and mild sinusoidal congestion (oval). H&E; Magnification: X400

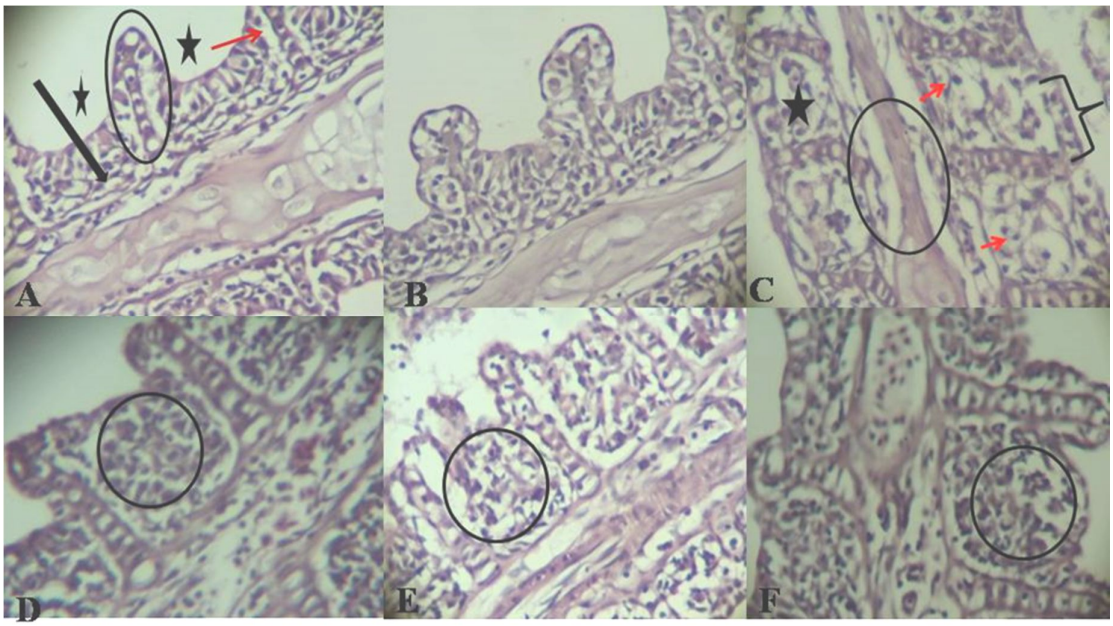

Figure 2: Photomicrographs of the gill of Clarias *gariepinus* exposed to graded concentrations of *Vernonia amygdalina*. (A). 0.00 g/l of VA: The gill parenchyma architecture are normal and characterized by the presence of primary gill filament (black arrow), secondary lamellae (oval), epithelial cells of the secondary lamellae (black arrow), the inter-lamellae space or water channel (star) (B) 0.1875 g/l. No visible lesion (C) 0.375 g/l: There is marked fusion (star) and apical clubbing of secondary lamellae (brace), mild inflammation of the lamellae (red arrow) and distorted primary lamella architecture (oval). D (0.75g/l), E (1.5 g/l) and F (3 g/l): are typified by severe lamellar hyperplasia with complete occlusion of the inter-lamellar distance (circle), H&E; Magnification x400

Table 4: The hepatocyte histomorphometrics of *Clarias gariepinus* fingerlings exposed to concentrated grades of *Vernonia amygdalina*

***V. amygdalina* concentration (g/l)**	**Liver morphometrics**	
	HND (µm)	**HSA (µm)^2**
0.000	2.44 ± 0.14^a	20.18 ± 0.49^a
0.188	2.21 ± 0.08^a	19.51 ± 0.41^a
0.375	1.52 ± 0.05^b	13.36 ± 0.55^b
0.750	1.35 ± 0.25^b	10.59 ± 0.24^c
1.500	0.96 ± 0.06^c	9.13 ± 0.26^c
3.000	0.92 ± 0.08^c	9.01 ± 0.26^c

Values with different superscript are significantly different HND (hepatocyte nuclear diameter), HSA (hepatocyte surface area)

Table 5: The histomorphometrics of the gills of *Clarias gariepinus* fingerlings exposed to concentrated grades of *Vernonia amygdalina*

***V. amygdalina* concentration (g/l)**	**Gill histomorphometrics**			
	ILD (µm)	**SLL (µm)**	**SLW (µm)**	**SLSA (µm)2**
0.000	24.61 ± 0.63a	33.21 ± 0.53a	15.86 ± 0.21a	97.09 ± 1.67a
0.188	21.62 ± 0.46a	32.99 ± 0.37a	13.87 ± 0.66a	90.83 ± 1.33a
0.375	13.89 ± 0.56b	31.99 ± 0.44a	9.01 ± 0.17b	80.05 ± 0.91b
0.750	8.67 ± 0.61c	29.55 ± 0.36b	8.46 ± 0.23b	74.90 ± 2.06b
1.500	4.13 ± 0.23d	22.88 ± 0.68c	8.39 ± 0.49b	67.73 ± 2.11c
3.000	2.92 ± 0.63d	22.02 ± 0.46c	4.76 ± 0.15c	57.26 ± 0.27c

Values with different superscript are significantly different, ILD (interlamellar distance), SLL (secondary lamellar length), SLW (secondary lamellar width), SLSA (secondary lamellar surface area)

Interestingly, the ILD values remained insignificantly different ($p>0.05$) between fish exposed to 0.188 and the control fish.

The values of the secondary lamellar length (SLL) especially in fish intoxicated with the higher concentrations of the extract (0.75, 1.5 and 3.0 g/l) decreased significantly ($p<0.05$) relative to the SLL values of others.

However, an insignificant difference ($p>0.05$) was noticed between the SLL values of fish exposed to 0.188 and 0.375 g/l. Similar trend equally exist between SLL values of fish given 1.5 and 3.0 g/l of the extract.

The secondary lamellar width (SLW) values decreased significantly ($p<0.05$) with the increasing grades of the concentration of the extract. The SLW was markedly reduced ($p<0.05$) in the fish exposed to the highest concentration of the extract compared to others. However, SLW values were insignificantly different ($p>0.05$) among the *Clarias gariepinus* exposed to 0.188 g/l of the extract and the control fish.

The values of the secondary lamellar surface area (SLSA) more particularly fish intoxicated with the higher concentrations of the extract (1.5 and 3.0 g/l) decreased significantly ($p<0.05$) relative to the SLSA values of others.

Conversely, an insignificant difference ($p>0.05$) was noticed between the SLSA values of fish exposed to 0.188 g/l of the extract and the control group. Similarly, there was no significant difference ($p>0.05$) between the SLL values of fish given 1.5 and 3.0 g/l of the extract.

DISCUSSION

This study has shown that some bioactive substances such as alkanoid, flavanoids, tannins, saponins and phenol to be present in the aqueous leaf extract of *V. amygdalina* in definite quantities. The acute exposure of *C. gariepinus* to concentrated grades of *V. amygdalina* induced progressive striking histological alterations in the liver (hepatocellular degeneration, central and sinusoidal congestions) and the gills (secondary lamellae apical clubbing and fusion, hyperplasic lamellar epithelial cells and total water channel occlusion) parenchyma with accompany histomorphometric alterations in both gills (secondary lamellar width, length surface area and interlamelar distance) and hepatic (hepatocyte nuclear diameter and surface area) tissues. The findings from this study are related to previous reports on fish exposure to plant

extracts; *Adenia cissampeloides* (Ajani and Ayoola, 2010), *Parkia biglobosa* (Abalaka *et al.,* 2010; Ojutiku *et al.,* 2012), *Pangasius hypophthalmus* (Suresh Babu *et al.,* 2013) and *Adenium obesum* (Abalaka *et al.,* 2015).

Potent bioactive substances in plants are numerous and diverse in structural compositions (Chikezie *et al.,* 2015). The presence of bioactive substances like alkanoid, flavanoids, tannins, saponins and phenol in the preliminary phytochemical screening of the leaves of this plant further confirmed the previously documentations on its phytochemical constituents (Gabriel *et al.,* 2015; Yusmazura *et al.,* 2016). Tannins, phenols and saponins in *V. amygdalina* aqueous extract have been associated with anti-viral, anti-cancer and anti-lipidaemic properties (Narayanan *et al.,* 1999; Cheng *et al.,* 2002; Ghasemzadeh and Ghasemzadeh, 2011), while microbial and insect repellent properties have been attributed to alkaloids and anti-oxidative potential to flavonoid (Erdman, 2007; Mwanauta *et al.,* 2014). It is important to mention that phytochemical constituent analyses vary with the geographical location (Wadood *et al.,* 2013). On the impact of bioactive substance on fish health, studies have incriminated saponin and alkaloid in fish intoxication (Chuck, 2003; Tiwari and Singh, 2003). Saponins are recognized ichthyotoxins that are capable of inducing respiratory asphyxiation and erythrocytes damage (Chuck, 2003), while alkaloids can partake in the inhibition of oxidative phosphorylation thereby blocking the mitochondria enzyme, NADH ubiquinone reductase and could culminate in oxygen consumption compromise (Tiwari and Singh, 2003).

The behavioural signs (air gulping, stunned position, skin peeling, aggression, spiral movement, fast and spiral movements) displayed by fish exposed to *V. amygdalina* largely seem to be due to the disruption of nervous system activity and biochemical derangements (Fadina *et al.,* 1991; Fafioye *et al.,* 2005). Also, the mortality rate and trend appear to be dose dependently related as evidenced by increased mortality especially in the fish that received the high concentrations of the plant extract. The findings on the behavioural signs and the mortality pattern corroborates the previous reports from exposure to similar plants extracts; *Nicotiana tobaccum* (Kori-Siakpere and Oviroh, 2011), *Caraca papaya* (Ayotunde *et al.,* 2011; Eyo *et al.,* 2013a), *Parkia biglobosa* (Ojutiku *et al.,* 2012) and *Trephosia vogelii* (Adewoye, 2010).

The liver is the recognized primary organ of detoxification and biotransformation by virtue of its position and blood supply (Van Der Oost *et al.,* 2003). It also remains one of the organs that are sensitive to pollutants in aquatic environment (Rodrigues and Fantail, 1998). Therefore, the histo-architectural distortions observed in the liver (moderate to severe hepatocellular degeneration, central and sinusoidal congestions) tissues of *C. gariepinus* exposed to grades of *V. amygdalina* further substantiate the toxic potential of this plant. With the exception of the low concentration of this extract every other concentration appeared to be toxic and run a concentration dependent histological disruption. The histoarchitectural damages seen in the liver of this fish could be linked to high presence of some bioactive substances like alkaloids, which is well recognized for inducing hepatic lesions (Ayoola, 2011; Wikipedia, 2016). The liver morphological alterations provoked by higher concentrations of *V. amygadalina* in this study could have serious functional consequences especially on the detoxification and biotransformation roles of the former. The hepatic histological lesions elicited by concentrated grades of *V. amygdalina* are similar to those reported for *Adenia cissampeloides* (Ajani and Ayoola, 2010) and *Parkia biglobosa* (Abalaka *et al.,* 2010).

The marked reduction in the hepatocyte morphometrics (nuclear diameter and surface area) showed by *V. amydalina* exposed fish appear to be dose related with pronounced decrease nuclear diameter and surface area in the liver cells of the higher toxicant groups. These morphometrics findings further aligned with the result on the histopathology of the liver parenchyma stated earlier in this study.

The gill of fish is a multifunctional organ that partakes in several essential roles that include respiration, osmoregulation and

excretion. Due to its proximity with the immediate external environment and the presence of an extensive respiratory epithelia surface area, it constitutes the prime organ that is usually susceptible to deranged water quality that may ensue from the presence of contaminants or pollutants in aquatic environment (Mazon *et al.,* 2002; Fernandes and Mazon, 2003; Au, 2004; Reddy and Waskale, 2013). The progressive moderate to severe histo-architectural changes (lamellar hyperplasia and occluded water channels) observed in the gills of *C. gariepinus* exposed to concentrated grades of *V. amydalina* depicts a dose-dependent distortion especially with marked severity in those given the higher concentration of the extract. The functional implication of these lesions portend serious threat to respiration more specifically the obstruction of the interlamellar space (water channel) which has a direct effect on gaseous exchange across the lamellar epithelium of the gill (Camargo and Martinez, 2007; Kumar *et al.,* 2010). The gill histo-architectural alterations precipitated by *V. amygdalina* are comparable to gill lesions documented in *A. cissampeloides* effect on the gill of tilapia (Ajani and Ayoola, 2010) and *P. biglobosa* in cat fish (Abalaka *et al.,* 2010).

Both the gill histology and morphometry collaborate to provide information about the mode of life and metabolic requirements of fish (Satora and Romek, 2010). Wegner *et al.* (2010) observed that the disruption of the oxygen uptake ability of gill surface epithelial covering can be assessed by some gill measurements that include the respiratory lamellae number on the filaments, the size and length of gill filaments and gills' lamellar bilateral surface area. Hence, the progressive decline noticed in the histomorphometric measurements on the gills (secondary lamellar width, length, interlamellar distance and lamellar surface area) of *C. gariepinus* exposed to *V. amygdalina* seem to be dose dependent with marked alterations displayed by groups exposed to the higher concentrations of the extract. The histomorphometric profile shown by *C. gariepinus* further lend credence to the earlier stated histological findings to be specific the lamellar epithelial fusion that eventually resulted in marked interlamellar distance reduction. However, the lower concentrations of the extract most especially the 0.188 g/l appear to be non-toxic as evidenced from its morphometrics which are closely related to the control group.

Conclusion: This study has demonstrated that *V. amygdalina* seems to be toxic to fish and therefore has to be cautiously applied when used as insecticides to control unwanted organisms around the fish pond.

REFERENCES

ABALAKA, S. E., FATIHU, M. Y., IBRAHIM, N. D. G. and AMBALI, S. F. (2015). Liver histopathological changes in *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. *Journal of Morphological Science,* 32(1): 22 – 28.

ABALAKA, S. E., FATIHU, M. Y., IBRAHIM, N. D. G. and KAZEEM, H. M. (2010). Histopathologic changes in the gills and skin of adult *Clarias gariepinus* exposed to ethanolic extract of *Parkia biglobosa* pods. *Basic and Applied Pathology,* 3: 109 – 114.

ADARAMOYE, O., ACHEM, J., AKINTAYO, O. and FAFUNSO, M. A. (2010). Lipid lowering effect of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol. *Atherosclerosis,* 2: 17 – 108.

ADESANOYE, O. A. and FAROMBI, E. O. (2010). Hepatoprotective effects of *Vernonia amygdalina* (Astereaceae) in rats treated with carbon tetrachloride. *Journal of Experimental Toxicology and Pathology,* 62: 197 – 206.

ADEWOYE S. O. (2010). Haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract. *Advances in Applied Science Research*, 1(1): 74 – 79.

ADEYEMO, O. K. (2008). Histological alterations observed in the gills and ovaries of *Clarias gariepinus* exposed to environmentally

relevant lead concentrations. *Journal of Environmental Health,* 70(9): 48 – 51.

AJANI, A. A. and AYOOLA, S. O. (2010). Acute toxicity of piscicidal plant extracts (*Adenia cissampeloides*) on tilapia (*Sarotherodon galilaeus*) juveniles. *Iranian Journal of Energy and Environment*, 1(3): 246 – 254.

AKAH, P. A., ALEMJI, J. A., SALAWU, O. A., OKOYE, T. C and OFFIAH, N. V. (2009). Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. *Asian Journal of Medical Sciences,* 1: 108 – 113.

AREGHEOERE, E. M. K., MAKKAR, H. P. S. and BECKER, K. (1998). Feed value of some browse plants from the central zone of Delta State, Nigeria. *Tropical Science,* 38(2): 97 – 104.

AU, D. W. T. (2004). The application of histocytopathological biomarkers in marine pollution monitoring: A review. *Marine Pollution Bulletin,* 48: 821.

AYOOLA, S. O. (2011). Histology of Nile tilapia (*Oreochromis niloticus*) juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf. *International Journal of Fisheries and Aquaculture,* 3(4): 244 – 259.

AYOTUNDE, E. O., OFFEM, B. O. and BEKEH, A. F. (2011). Toxicity of *Caraca papaya* Linn: Haematological and piscicidal effect on adult catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Sciences*, 6(3): 291 – 308.

BRUTON, M. N. (1979). The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias (Clarias)*. *The Transactions of the Zoological Society of London,* 35: 1– 45.

CAMARGO, M. M. P. and MARTINEZ, C. B. R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream *Neotropical Ichthyology*, 5(3): 327 – 336.

CHENG, H. Y., LIN, C. C. and LIN, T. C. (2002). Anti-herpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Research,* 55: 447 – 455.

CHIKEZIE, P. C., IBEGBULEM, C. O. and MBAGWU, F. N. (2015). Bioactive principles from medicinal plants. *Research Journal of Phytochemistry,* 9: 88 – 115.

CHUCK, K. (2003). *Fishing with Poisons. The Bulletin of Primitive Technology.* www.primitiveways.com. Accessed 3rd December 2016.

EBONG, P. E., ATANGWHO, I. J., EYONG, E. U. and EGBUNG, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African bitter leaf). *American Journal of Biochemistry and Biotechnology,* 4(3): 239 – 244.

ELEYINMI, A. F., FASASI, O. S. and OYAREKUA, M. A. (2005). Effect of some traditional processing operations on the functional properties of African breadfruit (*Treculia africana*) seed. *LWT-Food Science Technology,* 40: 513 – 519.

EPA (1985). *Disciplinary Review Ecological Effects Profile*. US Environmental Protection Agency, Office of Pesticide Programs, 401 M St. S.W., Washington DC 20460, USA.

ERDMAN, J. W. (2007). Flavonoid and heart health. *Proceedings of the ILSI North America Flavonoid Workshop.* May 31 – June 1 2005. *Journal of Nutrition,* 137(3): 718s – 737s.

EVANS, W. C. (2002). *Trease and Evans Pharmacognosy.* 15th Edition, WB Saunders Company, Bailliere Tindall, USA.

EYO, J. E., LEVI, C. A., ASOGWA, C. N., ODII, E. C., CHUKWUKA, C. O., IVOKE, N., ONOJA, U. S. and ONYEKE, C. C. (2013). Toxicity and effect of *Carica papaya* seed aqueous extract on liver biomarkers of *Clarias gariepinus. International Journal of Indigenous Medicinal Plants*, 46(3): 1301 – 1307.

EYO, J. E., NWACHUKWU, L. O., ONAH, I. E., ATAMA, C. I., EKEH, F. N., EZENWAJI,

N. E. and IVOKE, N. (2013). Effects of the aqueous root extract of *Vernonia amygdalina* on the haematological profile of *Rattus norvegicus*. *Advances in Life Science and Technology*, 11: 19 – 24.

FADINA, O. O., TAIWO, V. O. and OGUNSANMI, A. O. (1991). The effects of single and repetitive oral administration of common pesticides and alcohol on rabbits. *Tropical Veterinarian,* 17: 97 – 106.

FAFIOYE, O. O., FAGADE, S. O. and ADEBISI, A. A. (2005). Toxicity of *Raphia vinifera*, *P. beauv* fruit extracts on biochemical composition of Nile tilapia (*Oreochromis niloticus*, Trewavas). *Biokemistri,* 17: 137 – 142.

FERNANDES, M. N. and MAZON, A. F. (2003). Environmental pollution and fish gill morphology. Pages 203 – 231. *In:* VAL, A. L. and KAPOOR, B. G. (Eds.). *Fish Adaptations.* Enfield Science Publishers, Enfield, New Hampshire 03748, USA.

GABRIEL, A. A., LEE, L. Y. M. and JIN, H. C. (2015). Toxicological evaluation of *Vernonia amygdalina* methanol leaves extract in rats. *Oriental Pharmacy and Experimental Medicine*, 15(4): 365 – 369.

GHASEMZADEH, A. and GHASEMZADEH, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, 5(31): 6697 – 6703.

GILL, H. K. and GARG, H. (2014). Pesticides: environmental impacts and management strategies. *Pesticides – Toxic Aspects, InTech,* 2014: 188 – 230.

HUFFMAN, M. A. (2003). Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proceedings of the Nutrition Society,* 62(2): 371 – 381.

KORI-SIAKPERE, O. and OVIROH, E. O. (2011). Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on the African catfish: *Clarias gariepinus* (Burchell, 1822). *Archives of Applied Science Research*, 3(2): 1 – 7.

KUMAR, M., PRASAD, M. R., SRIVASTVA, K., TRIPATHI, S., and SRIVASTVA, A. K (2010). Branchial histopathological study of catfish *Heteropneustes fossilis* following exposure to purified neem extract, azadirachtin. *World Journal of Zoology*, 5(4): 239 – 243.

MAHMOUD, U. M., MEKKAWY, I. A. A. and SAYED, A. H. (2009). Ultraviolet radiation-A (366 nm) induced morphological and histological malformations during embryogenesis of *Clarias gariepinus* (Burchell, 1822). *Journal of Photochemistry and Photobiology B: Biology*, 95: 117 – 128.

MAZON, A. F., PINHEIRO, G. H. D. and FERNANDES, M. N. (2002). Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Brazilian Journal of Biology*, 62 (4A): 621-631.

MIN, E. Y. and KANG, J. C. (2008). Effect of waterborne benomyl on the hematological and antioxidant parameters of the Nile tilapia, *Oreochromis niloticus*. *Pesticide Biochemistry and Physiol*ogy, 92: 138 – 143.

MWANAUTA, R., MTEI, K. and NDAKIDEMI, P. (2014). Prospective bioactive compounds from *Vernonia amygdalina, Lippia javanica, Dysphania ambrosioides* and *Tithonia diversifolia* in controlling legume insect pests. *Agricultural Sciences*, 5: 1129 – 1139.

NABUKENYA, I., RUBAIRE-AKIIKI, C., MUGIZI, D., KATEREGGA, J., OLILA, D. and HÖGLUND, J. (2014). Sub-acute toxicity of aqueous extracts of *Tephrosia vogelii, Vernonia amygdalina* and *Senna occidentalis* in rats. *Natural Products Chemistry and Research,* 2(5): 143.

NARAYANAN, B. A., GEOFFREY, O., WILLINGHAM, M. C. and NIXON, D. W. (1999). Expression and its possible role in GI arrest and apoptosis in allergic acid treated cancer cells. *Cancer Letters,* 136(2): 215 – 221.

NGUYEN, L. T. H. and JANSSEN, C. R. (2002). Embryo-larval toxicity tests with the

African catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints. *Archives of Environmental Contamination and Toxicology*, 42: 256 – 262.

OJUTIKU, R. O., AVBAREFE, E. P., KOLO, R. J. and ASUWAJU, F. P. (2012). Toxicity of *Parkia biglobosa* pod extract on *Clarias gariepinus* juveniles. *International Journal of Fisheries and Aquaculture*, 4(7): 133 – 138.

OMOLOLA, A. and OLATUNDE, F. (2009). Hepatoprotective effects of *Vernonia amygdalina* (Astereaceae) in rats treated with carbon tetrachloride. *Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft fur Toxikologische Pathologie*, 62(2): 197 – 206.

ONABANJO, O. and OGUNTONA, C. R. (2003). Iron, zinc, copper and phytate content of standardized Nigerian dishes. *Journal of Food Composition and Analysis*, 16: 669 – 676.

REDDY, P. B. and WASKALE, K. (2013). Using histopathology of fish as a protocol in the assessment of aquatic pollution. *Journal of Environmental Research and Development*, 8: 372.

RODRIGUES, E. L. and FANTAIL, E. (1998). Liver histopathology of the fish *Brachydanio rerio* after acute exposure to sublethal levels of the organophosphate Dimetoato 500. *Revista Brasileira de Zoologia,* 15: 441 – 450.

SATORA, L. and ROMEK, M. (2010). Morphometry of the gill respiratory area in ruffe, *Gymnocephalus cernuus* (L.). *Archives of Polish Fisheries,* 18: 59 – 63.

SURESH BABU, C. H., SHAILENDER, M., RAJAGOPAL, R. S and KRISHNA, P. V. (2013). Effect of acute toxicity of azadirachtin on the survival of freshwater catfish, *Pangasius hypophthalmus*. *International Journal of Research in Biological Sciences*, 3(3): 112 – 115.

TIWARI, S. and SINGH, A. (2003). Control of common freshwater predatory fish, *Channa punctatus*, through *Nerium indicum* leaf extracts. *Chemosphere*, 53(8): 865 – 875.

VAN DER OOST, R., BEBER, J. and VERMEULEN, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology,* 13: 57 – 149.

WADOOD, A., GHUFRAN, M., JAMAL, S. B, NAEEM, M., KHAN, A., GHAFFAR, R. and ASNAD (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, 2(4): 144 – 148.

WEGNER, N. C. Gill (2011). Respiratory morphometrics. Pages 803 – 811. *In:* FARRELL, A. P. (Ed.), *Encyclopedia of Fish Physiology: From Genome to Environment,* Volume 2, Academic Press, San Diego, USA.

WIKIPEDIA (2016). *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: Pyrroolizidine Alkaloids.* Bad Bug Book, United States Food and Drug Administration. Accessed December 4, 2016.

YUSMAZURA, Z., NURHAZIRAH, Z. A., NIK FAKHURUDDIN, N. H. and HUSSIN, M. (2016). Phytochemicals and acute oral toxicity studies of the aqueous extract of *Vernonia amygdalina* from state of Malaysia. *Journal of Medicinal Plant Science*, 4(3): 01 – 05.