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Research Article

Some Pathological Effects of Sub-lethal concentrations of the Methanolic Extracts of *Raphia hookeri* on *Clarias gariepinus*

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ABSTRACT: *Raphia hookeri* is a piscicidal plant introduced into inland waterways by artisanal fishermen in Nigeria to stupefy fish for easy catch. This study was aimed at evaluating the sub-lethal toxicity of methanol extracts of *Raphia hookeri* (MERH) based on histopathological alterations in the gills, liver, kidney, brain and heart of the African catfish (*Clarias gariepinus*) over a 56-day exposure and 56-day post-exposure period respectively. Prolonged exposure resulted in pathological changes like erosion of gill filament, thinning of gill lamellae, swollen and oedematous gill filament in the gills and centrilobular vacuolar degeneration, kupffer cell hyperplasia and focal necrosis in the liver. Severe widespread tubular degeneration, sub-capsular congestion and haemorrhage were also observed in the kidney. Pathological changes like meningeal congestion; neuronal degeneration and satellitosis were observed in the brain while hyaline degeneration, epicardial congestion and haemorrhage were some of the changes observed in the heart. In view of these findings, the use of *R. hookeri* extracts in harvesting fish by local fishermen in Nigeria should be discouraged until information that aid its application in a sustainable manner is made available by further studies.

Keywords: methanolic extract, *Raphia hookeri*, sub-lethal toxicity, fish pathology, *Clarias gariepinus*

INTRODUCTION

The use of piscicidal plants by African fishermen to enhance catches has been largely reported (Neuwinger, 1994, 2004; Adeogun *et al.*, 1995; Woldemichael and Wink, 2001; Fai and Fagade, 2005). A wide range of active compounds (alkaloids, flavonoids, sugars, glycosides) are present in these plants (Seigler, 2002; Neuwinger, 2004) leading to a growing concern on their potential to cause adverse effects on the health of aquatic organisms particularly non- target species (Woldemichael and Wink, 2001; Sparg *et al*., 2004; Sun *et al*., 2005)

A number of plants have been identified for their ichthyotoxic properties (Tiwari and Singh, 2003; Sparg *et al*, 2004) including the pounded and fermented fruits of *Raphia hookeri* (Neuwinger, 2004). Obuotor *et al.*, (2006) isolated and characterized a spirostenol saponin - Raphiasaponin1 as the major ichthyotoxic compound in the fruit of *R. hookeri*. Raphiasaponin1 belong to a group of triterpenoid glycoside saponins with a distinctive foaming characteristic due to the combination of the non-polar sapogenin and their water soluble side chain (Sparg *et al*., 2004). Ahn *et al.*, (1998) reported that saponins exhibit potent anti-cell adhesive activity. Due to its widespread use by artisanal fisherfolks (Sowunmi and Adeogun, 2001), investigations into its toxic effects on commercially important fish species especially under chronic exposures is not only necessary but appropriate (Singh and Singh, 2002). Preliminary investigations had indicated that aqueous extracts of *R. hookeri* caused significant mortality to fingerlings of *Oreochromis niloticus* and *Clarias gariepinus* (Adeogun, 1994;

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Adeogun *et al.*, 1995). While some authors (Pacheco and Santos, 2002; Thophon *et al*., 2003; Au, 2004; Costa *et al*., 2009; Velisek *et al*, 2009; Pathan *et al*., 2010) have reported pathological lesions in different organs of fish as a result of exposure to synthetic chemicals and heavy metals, very little information exist on the effect of plant piscicides on pathological alterations in fish organs.

Considering the reliability of histopathological lesions as biomarkers of stress in fish under toxic conditions (Costa *et al*., 2009), this research work was carried out to establish the incidence of pathological changes in key organs like the gills, liver, kidney, brain and the heart as a result of long term exposure of fish to methanolic extracts of *R. hookeri* (MERH). This study also reports for the first time post-exposure responses in tissue morphology. The African catfish (*Clarias gariepinus*) was the test organism of choice due to its relevance to aquaculture in Africa.

MATERIALS AND METHODS

Plant Material

Ripe fruits of *R. hookeri* were collected from the swamps of Olosoko village, Moniya, Ibadan. They were authenticated at the Department of Botany and Microbiology and a voucher specimen (Voucher no: 13451) was deposited at the Herbarium. The fruit were air-dried until a constant weight was achieved and grinded into powdery form for extraction.

Extraction Procedure

Air-dried and grounded plant material (17.05kg) was extracted by percolation with hexane (1.82% yield) and then through soxhlet extraction (90^0C in a steam bath) with methanol (95%MeOH; 21.25%yield). The percolates were evaporated to dryness on a rotary evaporator at 40^oC and a low vacuum pump for complete removal of solvent and water. Extracts were then kept in airtight desiccators containing calcium chloride salts to absorb moisture.

Collection of Test Organisms

Two size classes (fingerlings: wet body mass 4.80±2.09g and sub adults: wet body mass 89.06±28.03g) of the African clariid catfish (*C. gariepinus*), were obtained from a private fish farm in Ibadan, transported in oxygenated waterproof bags and acclimatized to laboratory conditions for a period of two weeks in large glass aquaria (80L, 150L and 250L capacity). Fish were fed daily with pelleted diet (40% crude protein) at a rate of 5% body weight/ day for fingerlings and 3% body weight for sub-adult fish. Uneaten food and waste were siphoned out regularly and holding medium were changed every three days to prevent accumulation of metabolites. Well aerated dechlorinated municipal tap water was used as the holding medium (temperature, 28.5±2.5^{o}C; pH, 7.1 ±0.3; DO 6.3±0.2mgL^{-1}).

Bioassay Procedure

Standard procedures for bioassay as described by Reish and Oshida (1986) were used with slight modifications (12g of fish to 5L of water for fingerlings, 12g of fish to 2L of water for sub-adults) and 50g of methanolic extracts of *R. hookeri* (MERH) was diluted in 5L of water to give a 10,000mgL^{-1} stock solution. Nominal fractions of a predetermined 96hr LC_{50} values (80.00mgL^{-1} and 225.21mgL^{-1}) which gave concentrations of 5.30mgL^{-1}, 8.0mgL^{-1}, 16.0mgL^{-1} and an unexposed control (0.0mgL^{-1}) series; and 15.0mgL^{-1}, 22.5mgL^{-1}, 45.0mgL^{-1} and untreated control (0.00mgL^{-1}) exposures were used in a 112 day experimental period comprising of a 56 day exposure/56 day post-exposure period for fingerlings and sub-adult fish respectively. Twenty fingerlings were placed in each exposure concentration and ten sub-adult fish were in each exposure concentration respectively. All exposure concentrations were in triplicate and test medium was renewed every 72hr with fresh preparations of the toxicant to maintain a continuous concentration using a static/renewal protocol.

Histological preparations

Ninety six fishes in each size class were selected for histopathological evaluation. For each sampling period, twelve fishes were randomly selected in all exposure concentrations on the 7, 14, 28 and 56 days exposure and post-exposure periods respectively and fish were sacrificed by medullar transection according to the methods described by Lucky (1977). Dissection was performed immediately and samples were prepared for histological analyses. Gills (3rd gill arch), liver, kidney, heart and brain tissues were excised and fixed in Bouin's fluid for 6 hrs. They were then transferred into 10% phosphate buffered formalin. Samples were dehydrated in a progressive series of ethanol dilutions, cleared in xylene and embedded in paraffin. The blocks of the tissues were sectioned at 3-5µm thickness, stained with Delafield's Haematoxylin and Eosin and mounted on a DPX mountant for structural analysis. Standard histopathological procedures (Roberts, 1989) were followed for histopathological investigations. Tissues were examined with an Olympus Micronal microscope and histopathological changes with striking features were photographed with an attached video camera (Authotek camera, Leith GmBH, Germany) and selected for presentation.

RESULTS

General Observations

Fish had abdominal swellings (ascites) by 14 days exposure period. This was more pronounced in higher concentrations than lower concentrations with very sluggish movements. These behavioral deviations from the norm were highly reduced during post-exposure periods becoming less apparent with length of time such that by 56 days post-exposure period, test organisms seemed to have regained, to a larger extent, their normal behaviour especially in lower concentrations except for the slightly swollen abdominal regions and slight sluggish movement that were still persistent in the highest concentration for sub-adult fish. Fish were also observed to cough more often in an attempt to clear the gills of excess mucus from 7 days post-exposure period until 35 days post-exposure period.

Gross Examination of Organs

Gills: The gills of exposed fish were pale compared to unexposed (control) fish and this pale colouration increased with increasing concentrations in the gills. There was also accumulation of mucus on the gills filaments of exposed fish compared with control. Though the gills were still pale in the highest concentrations at 56 days post-exposure period, slime production was highly reduced compared to exposure periods.

Liver: The liver was observed to be pale in specimens exposed to the highest concentrations of 16.0 and 45.0mgL^{-1} at 7 days in both fingerling and sub-adult fish. There were focal white spots (appearing as blobs of fat) on the liver in those exposed to 8.0mgL^{-1}, 16.0mgL^{-1} and 22.5mgL^{-1} and 45.0mgL^{-1} of both fingerlings and adults increasing with increasing concentrations. By 28days exposure period, the liver had assumed a dark greenish colouration and at 56 days exposure, the entire liver surface in the highest concentrations, that is, 16.0mgL^{-1} and 45.0mgL^{-1} were a very dark green colour and had become highly shrunken in size (about half the size of the liver in the control). A gradual return to normal colouration was however observed from 14days post-exposure in the lowest concentrations and by 56 days post-exposure period, the liver in the highest concentration had regained about 50% of its normal colouration.

Kidney: The kidney was dark and this colouration increased with increasing concentrations at 14 days until 56 days exposure periods when it was very dark in colour and shrunken. These colourations became less pronounced by 56 days post-exposure periods.

No observable lesions were found on the hearts and brains of both fish in exposure concentrations and unexposed (control) fish throughout the experimental period.

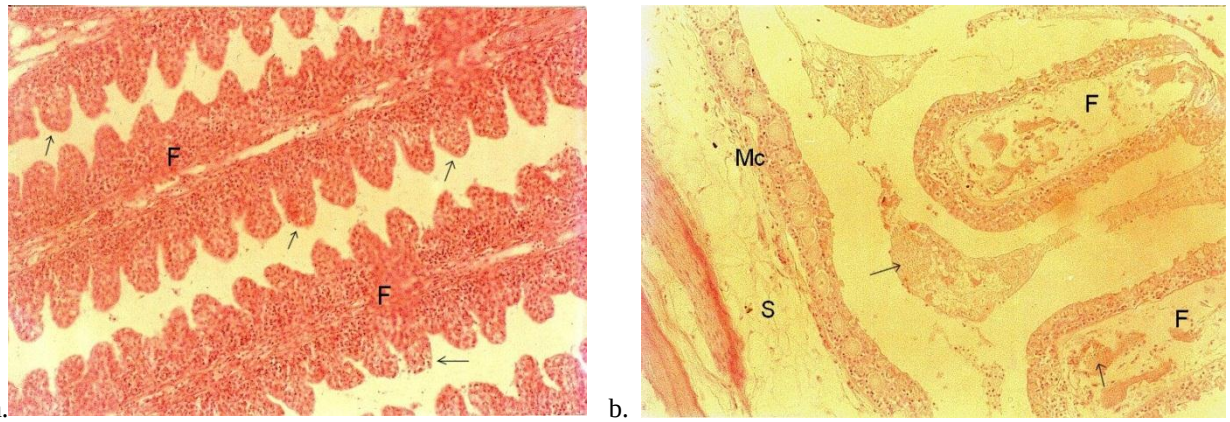
Histopathological changes in Fingerlings

Erosion of gill filaments, swelling of chondrocytes and thinning of lamellae with swollen and oedematous gill filaments (Table 1; Fig. 1b) were some of the changes observed in the gills of fingerlings exposed to different sub-lethal concentrations of MERH. These changes were highly pronounced during the first 28 days of exposure and 28 days post exposure periods in the highest concentrations but recovery was observed at 56 days post-exposure period with no pathological changes in the gills.

Table 1: Pathological changes in the gills of fingerling and sub-adult *C. gariepinus* after MERH exposure (56 day exposure/recovery period)

Day	FINGERLING				SUB-ADULT			
	Control (0.0mg/L)	**5.3mg/L**	**8.0mg/L**	**16.0mg/L**	**Control (0.0mg/L)**	**15.0mg/L**	**22.5mg/L**	**45.0mg/L**
07	-	-	-	-	-	-	A, B (+)	A, B, C (++)
14	-	-	-	B (+)	-	-	-	B (+)
28	-	-	A, B (+++)	B, C, D (++)	-	B (+)	A, B, D (+++)	B, C, D (+++)
56	-	-	-	B, D (++)	-	-	-	-
	POST-EXPOSURE							
07	-	-	-	-	-		-	-
14	-	-	-	A (++)	-	-	-.	-
28	-	A (+)	B, C, D (+++)	A, C, D, E (++)	-	-	-	A, C, E (+++)
56	-	-	-	B (+)	-	-	-	

Key to lesions: -= no lesions observed; A=congestion and oedema of gill filaments; B= stunting, matting/clubbing/erosion of villi; C=cellular infiltration; D=rarefication of cartilage; E=thrombosis.
Severity of lesions: -= absent; +=mild; ++=moderate; +++=severe.



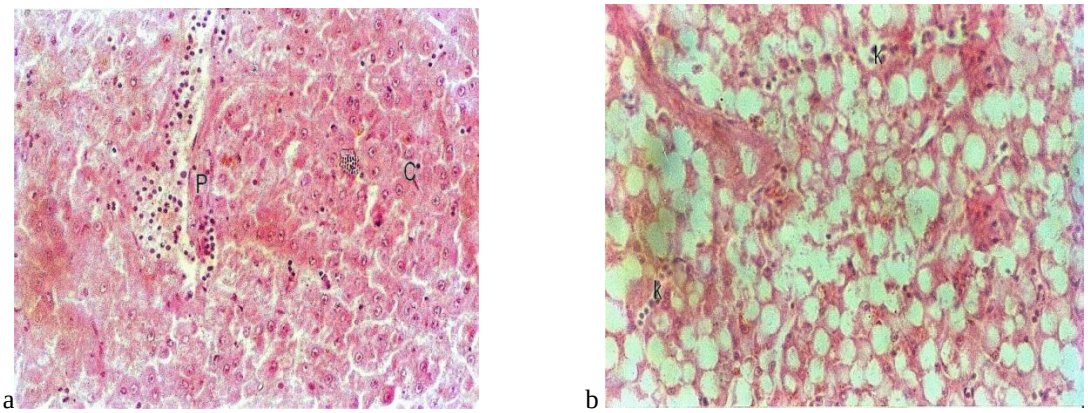
a. b.

Figure1a-b: Light photomicrograph of the gill of *Clarias gariepinus* fingerlings (a) control (unexposed) fish gill showing filaments (F) and villi (arrows). (b) 28days post-exposure/recovery period after exposure to 16mgL^{-1} of MERH showing diffuse and severely swollen and oedematous filaments (F) and submucosa (S). Note the presence of fibrin thrombi (arrow) in submucosal capillaries and mononuclear cellular infiltration (Mc) in the propria of primary lamella. H & E (x300)

Table 2:
Pathological changes in the liver of *C. gariepinus* fingerling and sub-adult after MERH exposure (56 day exposure/recovery period).

Day	FINGERLING				SUB-ADULT			
	Control (0.0mg/L)	**5.3mg/L**	**8.0mg/L**	**16.0mg/L**	**Control (0.0mg/L)**	**15.0mg/L**	**22.5mg/L**	**45.0mg/L**
07	-	-	-	B, C, F (+++)	-	-	B (++)	B (+)
14	-	-	B (+)	B (+++), E (+)	-	B (++)	B (+++), E (+)	B (+++), F (+)
28	-	-	B (+++)	A, B (+++)	-	B (+)	B (+++)	B (++)
56	-	-	-	A, B, C, D, E (+++)	-	B, D (++)	B (+++)	B (+++)
	POST-EXPOSURE							
07	-	-	B (+)	B (++)	-	-	G (++)	B (+++)
14	-	-	D (+)	A, C (++)	-	-	.-	B (+++)
28	-	-	-	E (++)	-	-	B (+)	B (+)
56	-	-	-	B (++)	-	-	-	C, B (++)

Key to lesions: *-= no lesions observed; A= individualisation of hepatocytes; B= vacuolar degeneration of hepatocytes; C=hepatic necrosis; D=megalocytosis; E=kuppfer cell hyperplasia; F=cellular infiltration; G=haemosiderosis.*
Severity of lesions: -= absent; +=mild; ++=moderate; +++=severe.



a b

Figure 2a-b: Light photomicrograph of the liver of *Clarias gariepinus* fingerlings (a) unexposed fish showing normal hepatocytes at the periportal (P) and centrilobular (C) regions. (b) 14 days exposure to 16 mgL^{-1} of MERH showing severe widespread vacuolar degeneration of hepatocytes and moderate kupffer cell hyperplasia H & E (x 750).

Varying degrees of degeneration had taken place in the liver of exposed fish. These ranged from widespread vacuolar degeneration of hepatocytes through necrosis to periportal fibrosis (Table 2; Fig.2b). Degenerative changes were however more pronounced in the highest concentration (16.0mgL^{-1}) while no pathological changes were recorded for the lowest concentration (5.3mgL^{-1}).

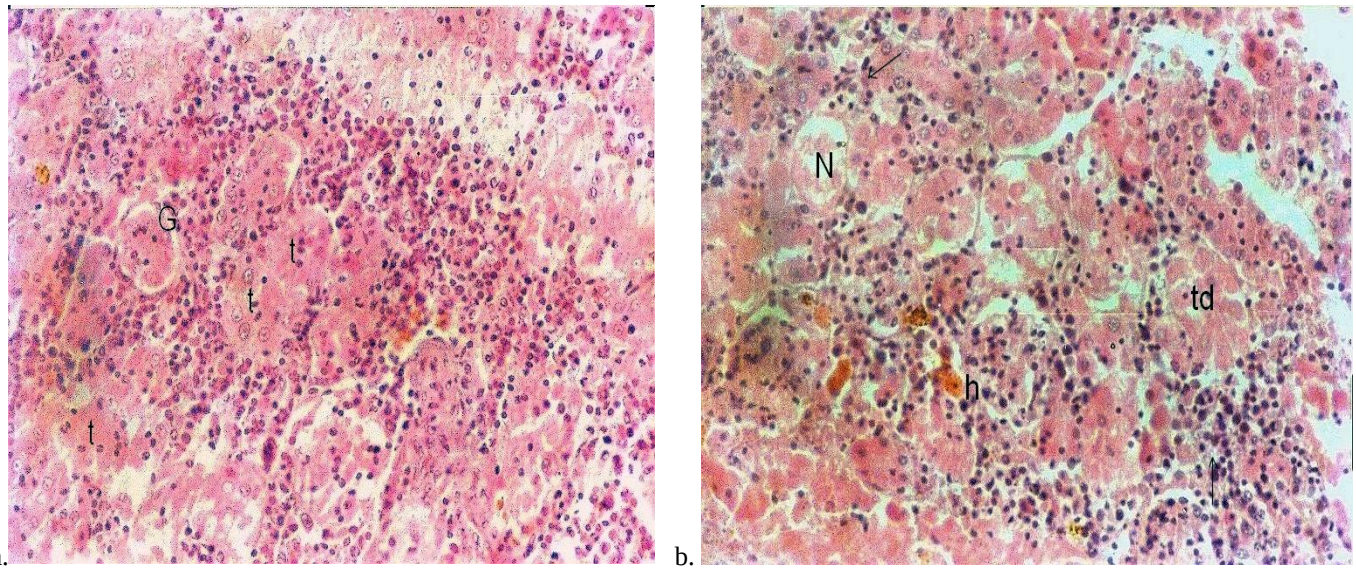
Widespread tubular degeneration and necrosis, widespread heterophilic (neutrophilic) and mononuclear cellular infiltration (55:45; lymphocytes: MQ) in the interstitium were observed in the kidney at 28 days exposure in the highest concentration (16mgL^{-1}). By 56 days post-exposure period, no pathological changes were observed in the highest concentration (Table 3; Fig.3b).

Unexposed fingerlings had normal neurons arranged in a regular pattern with nerve trunks and few glia cells. Meningeal congestion, neuronal degeneration, satellitosis were among some of the pathological changes observed in the brain (Table 4).

Table 3:
Pathological changes in the kidney of *C. gariepinus* fingerling and sub-adult after MERH exposure (56day exposure/recovery period)

Day	FINGERLING				SUB-ADULT			
	Control (0.0mg/L)	**5.3mg/L**	**8.0mg/L**	**16.0mg/L**	**Control (0.0mg/L)**	**15.0mg/L**	**22.5mg/L**	**45.0mg/L**
07	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
28	-	-	-	A, B, C (+++)	-	A, B, C (++)	B, C (+++)	A, B, C (++)
56		-	-	-	-	-	-	A (++)
				POST-EXPOSURE				
07	-	-	-	-	-	-	-	A, D (++)
14	-	-	-	-	-	-	.-	A, D (++)
28	-	C (+)	A, B, C (+++)	A, B, C (+++)	-	-	C (+)	A, D (++)
56	-	-	-	-	-	-	-	D (+)

Key to lesions: -= no lesions observed; A= congestion, oedema and haemorrhage; B= tubular degeneration and necrosis; C=cellular infiltration; D=haemosiderosis. Severity of lesions: -= absent; +=mild; ++=moderate; +++=severe.



a. b.

Figure 3a-b:
Light photomicrograph of the kidney of *Clarias gariepinus* fingerling (a) unexposed (control) fish showing normal glomerulus (G) and tubules (t). (b) 28 days after exposure to 16.0mgL^{-1} of MERH. Note severe widespread tubular degeneration (td) and necrosis (N), interstitial heterophilic and mononuclear cellular infiltration (arrow) and haemosiderosis (h). H & E (x 750)

Hyaline degeneration, necrosis of myofibrils, epicardiac congestion, mononuclear cellular aggregation in affected muscle bundles and haemorrhage were some of the pathological changes observed in the heart of fish exposed to the highest concentration (16.0mgL^{-1}). No pathological changes were observed in the lowest concentration (5.3mgL^{-1}) (Table 5).

Histopathological changes in sub-adult *Clarias gariepinus*

Gills: Pathological changes in the gills of sub-adult *C. gariepinus* included severe stunting, clubbing and erosion of gill filaments and their villi, swollen and oedematous gill filaments and rarefication of cartilage (Table 1; Fig. 4).

Liver: Very severe widespread vacuolar degeneration of hepatocytes, sinusoidal and vascular congestion and mononuclear cell aggregations were observed in the highest concentration (45.0mgL^{-1}). Periportal fibrosis (Table 2; Fig. 5) (indicating a healing process) and the presence of megalocytes (indicative of regenerative change) were observed in fish exposed to the lowest concentration (15.0mgL^{-1}) at 56days exposure period

Kidney: Changes observed in the kidney of exposed fish included haemorrhage and subcapsular congestion, degenerate glomeruli and lymphocytic cellular

Table 4:
Pathological changes in the brain of fingerling and sub-adult *C. gariepinus* after MERH exposure (56day exposure/recovery period)

Days	FINGERLING				SUB-ADULT			
	Control (0.0mg/L)	**5.3mg/L**	**8.0mg/L**	**16.0mg/L**	**Control (0.0mg/L)**	**15.0mg/L**	**22.5mg/L**	**45.0mg/L**
07	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	B, C, D, E (+++)
28	-	-	-	A, B, C (++)		A, D (+)	A (+),B,C	A, B, D (+++)
56	-	-	A (+)	A (+)	-	-	A (+)	A, B, C (++)
				POST-EXPOSURE				
07	-	-	A (+)	A (+)	-	-	-	-
14	-	-	-	A (+)	-	-	.-	A, B, C (+)
28	-	C (+)	A, C, D (++)	A, C, (++)	-	-	-	A, B, F (+)
56	-	-	-	-	-	-	-	-

Key to lesions: -= no lesions observed; A= meningeal congestion; B= neuronal degeneration; C=neuronophagia/gliosis; D=spongiosis/malacia; E=demyelination; F=mononuclear perivascular cuffing. Severity of lesions: -= absent; += mild; ++=moderate; +++=severe.

Table 5:
Pathological changes in the heart of fingerling and sub-adult *C. gariepinus* after MERH exposure (56 day exposure/recovery period)

Day	FINGERLING				SUB-ADULT			
	Control (0.0mg/L)	**5.3mg/L**	**8.0mg/L**	**16.0mg/L**	**Control (0.0mg/L)**	**15.0mg/L**	**22.5mg/L**	**45.0mg/L**
07	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
28	-	-	C (+)	A, C (++)	-	B, C (++)	A, B, C (++)	A, B, C (++)
56	-	-	-	A,B, C (++)	-	-	B (++)	B, C (+++)
				POST-EXPOSURE				
07	-	-	-	-	-	-	B (+)	A, C (++)
14	-	-	-	-	-	-	-.	-
28	-	-	-	A (+)	-	-	A (+)	B (+)
56	-	-	-	-	-	-	-	-

Key to lesions: -= no lesions observed; A= hyaline degeneration; B=congestion and haemorrhage; C=cellular infiltration; Severity of lesions: -= absent; += mild; ++=moderate; +++=severe.

infiltration at 28days exposure period in the highest concentration (Table 3; Fig. 6). Haemorrhage and extensive degeneration of renal tubules were also observed at 7 and 14 days post-exposure periods (45.0mgL^{-1}), but by 56days post exposure period, the kidney architecture had recovered to renal normal, but with multiple focal areas of haemosiderosis.

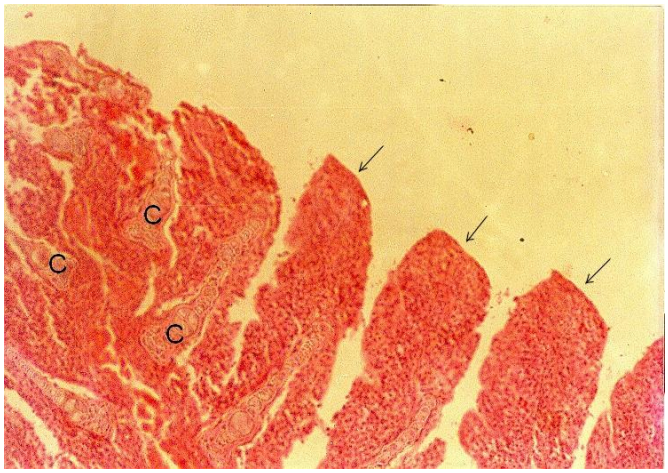


Figure 4:
Light photomicrograph of the gill of sub-adult *Clarias gariepinus* 28 days after exposure to 45mgL^{-1} of MERH showing severe clubbing, stunting, erosion of gill filaments (arrows), loss of villi and fusion of gill filaments (left top corner). Note swelling and rarefication of filament cartilages (C). H & E (x450).

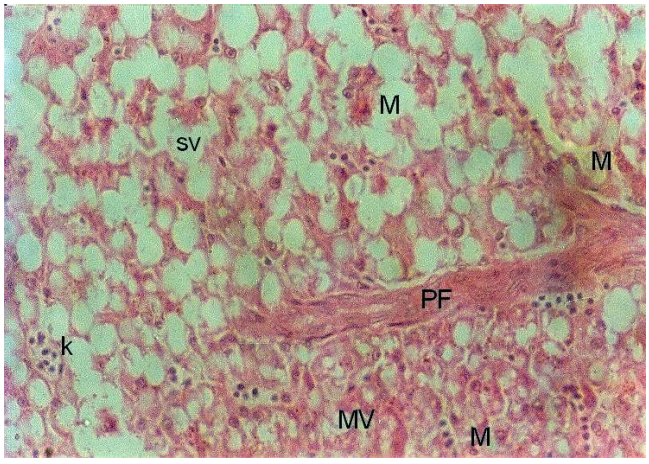


Figure 5:
Light photomicrograph of the liver of *Clarias gariepinus* sub-adult 56 days after exposure to 45mgL^{-1} of MERH. Note areas of severe (sv) and moderate (MV) vacuolar degeneration of hepatocytes, periportal fibrosis (PF), kupffer cell (k) proliferation and presence of megalocytes (M). H & E (x750)

Brain: Pathological changes in the brain were mainly meningeal congestion, neuronal degeneration, satellitosis, neuronophagia and spongiosis in fish exposed to the highest concentration (45.0mgL^{-1}). These changes were however no longer apparent by 56days post-exposure period (Table 4).

Heart: The heart of fish exposed to 22.5mgL^{-1} had focal areas of hyaline degeneration, severe epicardiac congestion and endocardial haemorrhages were observed in the heart of fish exposed to the highest concentration (45.0mgL^{-1}). No pathological changes were however visible at the end of post-exposure period (Table 5).

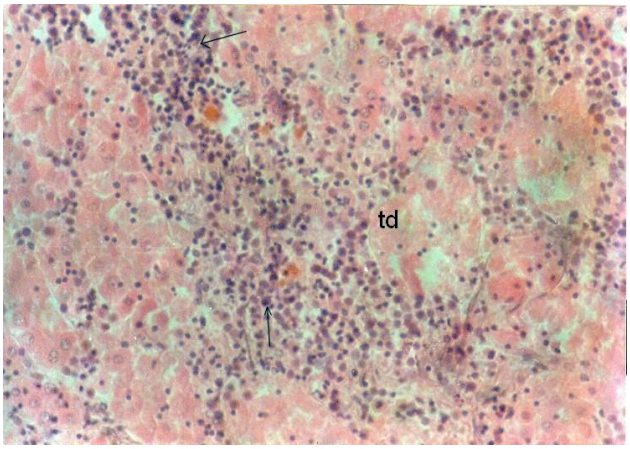


Figure 6:
Light photomicrograph of the kidney of sub-adult *Clarias gariepinus* 28 days after exposure to 22.5mgL^{-1} of MERH showing severe widespread tubular degeneration (td) and necrosis. Note interstitial mononuclear cellular infiltration (arrows). H & E (x750)

DISCUSSION

Toxicants introduced into aquatic systems can cause changes in tissues and organs of organisms leading to alterations of physiological functions. The observations in this study show that following the exposure of *C. gariepinus* to MERH, the expression of toxicity differed considerably across the organs studied. This concurs with studies which proposed that toxicants are most times organ specific in reaction (Hassanein *et al.*, 1999, Harper and Wolf, 2009; Pathan *et al.*, 2010).

The pathological changes in the gills and liver were very prominent and pronounced and strongly implicate *R. hookeri* extracts as a toxicant because these organs are important for diffusion of oxygen and detoxification of xenobiotics. Significant pathological changes like erosion of gill filament, rarefication of cartilage,

presence of fibrin thrombi (vascular changes resulting in blockage/clotting in blood vessels) in capillaries and lymphatics, oedema and clubbing of gill filament tips will lead to an overall reduction in the efficiency of gill filaments to aid in diffusion of oxygen across the gill lamellae resulting in the development of a hypoxic condition within the fish (Elahee and Bhagwant, 2007). This is as a result of loss of epithelial cells in the gill hence normal gaseous exchange across these cells would be compromised and this may have resulted in hypoxia. Some authors have indicated that gill lesions do not only indicate possibilities of impaired respiratory functions but impaired osmo-regulatory functions too (Mallat, 1985; Au, 2004; Tang and Au, 2004). Heath (1987) was of the opinion that all these changes would reduce diffusion of oxygen across the epithelium leading to blood hypoxia. The gill being a very simple structure can only manifest a limited number of reactions in adverse environmental conditions. Mallat (1985) reviewed 133 published studies on fish gill pathology and concluded that the structural alterations in fish gill are a stereotyped physiological reaction to environmental stressor.

Increased mucus secretion observed in the gills of exposed fish with increase in concentration is a protective feature aimed at preventing further damage to the gill lamellae. Playle and Wood (1989) observed that a thickened mucous layer might act as a filter minimizing injury to the gaseous exchange part of the gill (lamellae) since the raker is the first area of contact with polluted water. Hyper secretion of mucus may however, impede gaseous exchange and further predispose fish to hypoxia. The inability of the fish gill to recover fully was evident in the persistence of oedematous conditions in the 28 day post-exposure period for both fingerling and sub-adult fish; however adaptational/compensatory responses were evident by 56 day post-exposure period as mild congestion and slightly stunted and clubbed villi were observed. This suggests that an on-going process of repair to maintain a new homeostatic balance with what was left of the gill structure was taking place in exposed fish.

Pathological changes observed in liver tissues indicate that MERH is both directly hepatotoxic (vacuolar degeneration of hepatocytes around the periportal region) and indirectly hepatotoxic (degeneration around the centrilobular region) (Adeogun, 2004; Velisek *et al.,* 2009). Witthawaskul *et al.,* (2003) posited that saponin mixtures directly impact liver and kidney functions and similar responses due to Raphasaponin1 contained in MERH were observed in this study. Studies have shown that vacuoles in the cytoplasm of the hepatocytes contain lipids and glycogen, which is related to the normal metabolic function of the liver thus vacuolar degeneration will result in a depletion of the glycogen reserves in the hepatocytes (Hinton and Laurén, 1990; Wilhelm -Filho *et al.,* 2001). Vacuolar degeneration will result in stress to fish because glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations (Panepucci *et al.,* 2001). Furthermore Pacheco and Santos (2002) reported that increased vacuolisation of hepatocytes in fish exposed to contaminated water was a sign of degenerative process which itself suggests metabolic damage. Lesions such as focal necrosis have been reported to imply metabolic impairment in hepatocytes (Camargo and Martinez, 2007). Adeogun (1994) observed similar pathological changes in fingerlings of *C. gariepinus* exposed to sub-lethal concentrations of the different parts of the fruit of *R. hookeri* aqueous extract. The liver is considered the most important target organ from a toxicological point of view because of its role in detoxification, biotransformation and excretion of xenobiotics (Hassanein *et al.,* 1999). The regenerative changes in liver tissues as inferred from the presence of megalocytes and fibrosis in both fingerling and adult *C. gariepinus* suggests that the toxicity of MERH may not be beyond the physiological coping mechanism of the fish (van der Oost *et al.,* 2003, Adeogun, 2004, Costa *et al.,* 2009) irrespective of life stages but this remains to be established.

The teleostean kidney is one of the first organs to be affected by contaminants in water (Thophon *et al.,* 2003) and appears to be particularly sensitive to a variety of toxins due to the high renal blood flow, the ability to concentrate substances, and the biotransformation of the parent compound to a toxic metabolite (Mohssen, 2001). Observations made in the kidney of exposed fish implicates MERH in renal toxicity. Previous studies have shown that the most common alterations found in the kidney of fish exposed to water contamination are tubular degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space (Pacheco and Santos, 2002; Carmago and Martinez, 2007). Exposure to metals frequently cause alterations in the tubules and glomerulus, such as was described by Thophon *et al.* (2003) for the perch (*Lates calcarifer)* exposed to cadmium; Handy and Penrice (1993) found swollen Bowman´s capsule cells and melanomacrophages in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to mercuric chloride. In this study the presence of large numbers of melanomacrophages and haemosiderosis in post exposure period is indicative of a healing process. This is consistent with reports that the development of

new nephrons in fish continues throughout life, and is more frequent in young and fast-growing (sub-adult) fish (Reimschuessel, 2001) as was in this study. Hinton and Laurén (1990) and Cormier *et al.* (1995) have reported an increase in the frequency of new nephrons and regenerated tubules, during the process of the recovery of damaged kidney in fish. Similar observations have also been made in the siluriform *Ameiurus nebulosus* and in cod (*Microgadus tomcod*) collected from contaminated streams (Cormier *et al.*, 1995), and in goldfish (*Carassius auratus*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Brachidario rerio*) and tilapia (*Oreochromis mossambicus*) exposed to contaminants such as mercury, antibiotics and solvents, that are known to cause necrosis and vacuolisation (Reimschuessel, 2001). The kidney plays the major role in this fight, producing large quantities of diluted urine. Although the kidney does not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in the liver have been shown to occur in the kidney (Mohssen, 2001). It receives the bulk of the post branchial blood flow and kidney tissue is of importance in the detoxification and elimination of aquatic contaminants in fish (Durmaz *et al.*, 2006).

The pathological changes observed in the brain of exposed fish may be associated with the hypoxic condition developed within the fish as a result of the inability of the gill to aid sufficient oxygen diffusion across the gill epithelium due to degenerative changes in gill structure. Brain cells are very sensitive to hypoxia and neuronal degeneration may occur in the absence of oxygen leading to changes in behavioural patterns as evidenced in jerky movement, hyperventilation and spiral movement observed in exposed fish and this implies loss of nervous coordination. Roberts (1989) observed that very limited information exist on the pathology of nervous tissue but inflammatory conditions in other parts of the body could result in focal glial change as observed in this study. Evidence of acclimation was however observed in post-exposure responses as no pathological changes were observed by 56 days post-exposure period for both fingerling and adult fish. This suggests that if the hypoxic condition due to toxicant exposure could be reversed, brain cells may recover to a large extent, hence the gradual return to normal behaviour observed at the end of post-exposure periods.

The severe epicardial congestion observed in the heart may be due to haemoconcentration. In a complimentary evaluation of haematological indices of fish exposed to MERH, Adeogun (2004) reported significantly higher values of RBC, Hb and PCV and concluded that such elevations were due to haemoconcentration. This made the heart come under more pressure to pump blood and oxygen carried by blood may then become highly reduced leading to necrosis of myofibrils. The movement of fluid from interstitial spaces and ingestion of more water by fish to compensate for dehydration and loss of fluid from blood vessels may result in dilation of blood vascular channels due to increase in blood supply per surface area. Blood vascular channels which may have previously been blocked as a result of increased efflux of cells may become damaged leading to haemorrhage observed in sections of the heart at 56 days exposure for sub-adult fish and 28 days post-exposure periods for both fingerling and sub-adult fish species respectively. Inflammation will result in mononuclear cellular infiltration and this is a typical inflammatory and basic protective responses to tissue damage in all vertebrates including fish. Roberts (1989) observed that inflammatory responses develop to maintain both the structural integrity of the tissue concerned despite the injury or insult and maintain a functional blood supply. These responses follow traumatic wounds, bacterial or viral mediated injury and chemical or toxic damage, as was the case in this study. No pathological changes were however recorded by 56 days post-exposure period and this may be due to the fact that the heart is a very resilient organ and is highly regenerative in nature such that the process of acclimation was more rapid in this organ than all the other organs. Oyelese *et al.*, (1999) reported cardiac lesions like subepicardial and endocardial haemorrhages and hyaline degeneration of myocardial fibres in *C. gariepinus* fingerlings fed with varying inclusion levels of processed cassava peels containing varying levels of cyanide, a hypoxia inducing agent.

In conclusion, the pathological alterations reported in this study proved to be a semi-quantitative response of critical organs in fingerling and sub-adult *C.gariepinus* to the action of MERH. The trend in pathological alterations showed organ specific responses and seemed to be dependent on observed gill damage and subsequent hypoxia coupled with metabolic damage in the liver due to the effect of toxicant on fish organs.

The results also indicate that MERH induced dose-dependent histopathological changes in the gill, liver, kidney, heart and brain of exposed *C. gariepinus* and although the toxic nature of MERH to aquatic species was demonstrated the toxic responses showed reversible trends in some organs during the post - exposure period.

Consequences of extracts of *Raphia hookeri* exposure in natural environments might include hazardous effects at various levels to non-target

organisms. Therefore the use of *R. hookeri* extracts in harvesting fish by artisanal fishermen in Nigeria should be discouraged or regulated to avoid toxic effects on fish of commercial importance and other non-target biota thus protecting the integrity of aquatic communities. The reversibility of the action of the extract despite prolonged exposure could be an added advantage if regulated use is enforced.

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