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HAEMATHOLOGICAL AND HISTOLOGICAL EVALUATION OF AFRICAN CATFISH *Clarias gariepinus* FOLLOWING ACUTE EXPOSURE TO METHANOLIC EXTRACT OF *Khaya senegalensis*

Matouke Matouke Moise[1*] and Obadiah Audu Abui[2]

[1]Department of Biological Sciences, Federal University, Dutsinma, Katsina, Nigeria; [2]Department of Biological Sciences, Federal Science and Technical College, Kafanchan, Kaduna State, Nigeria

***Corresponding author:** Matouke Matouke Moise, E-mail: mosesmatouke@yahoo.fr

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ABSTRACT

Haematological and histopatholoical effects of methanolic extract of *K. senegalensis* leaves was investigated on *Clarias gariepinus* over a period of 96h exposure. The median lethal concentration of the extract was 199.69mg/L. The extract caused decreased in total erythrocytes (TEC) and Packed Cell volume (PCV) respectively and increased of total leukocytes (TLC). Histopathological lesions in the liver, cytoplasmic degeneration, less intracellular space, mild necrosis, sinusoidal blood congestion and marked blood congestion in hepathocytes were recorded. However the severity but not the type of lesions was concentration-dependent. Though, the degree of tissue change (DTC) varied with the methonolic extract used. There was significant association ($P<0.05$) between the DTC and *K. senegalensis* methanolic concentration. The cumulative DTC indicated a moderate damage in the liver. The extract was considered toxic to the exposed fish and therefore deleterious on the organs of *C. gariepinus*.

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INTRODUCTION

Khaya senegalensis is commonly used in Africa for shade, medicinal purposes and belong to the Meliacea family (Adanhounsode, 2012). Due to anthropogenic activities the plant stem and leaves might be found in the aquatic ecosystem which could be a serious threat for aquatic fauna including *Clarias gariepinus*. Exposure of aquatic organism to plant extract has been shown to have detrimental effects on fish physiology sometimes leading to mortality (Abalaka, 2015).Fish species are permanently exposed to their ecosystem and thus, exposure to a peculiar compound can act as environmental indicator. Hematology and histhopathology have been used as a biomarker for the effects of various anthropogenic pollutants on fish (Olurin *et al.,* 2006; Devi and Mishra, 2013).

Studies showed the impact of haematology and gill pathology of *Heterobranchus bidorsalis* exposed to sub lethal concentration of *Moringa oleifera* leaf extract. Erythrocytes was 2.36 ×106/mm^3, Packed Cell Volume was 20%, Haemoglobin was 6.68g/dl, mean cell volume was 85.1ft, cell haemoglobin concentration was 33.33 and mean cell concentration was 28.8pg while the mean value for white blood cell was 40.5mm^3. These results suggest a variation of values when compared to control (Olufayo and Olufunke, 2012). Many other studies showed similar variation of haematological parameters (Mammanet al., 2013; Ochang, 2007; Odeyemoet al., 2010).However, *Oreochromis niloticus* showed hispathological responses while exposed to waterborne copper, the gills were affected with edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis. In the liver, the number of hepatocytes nucleus per mm^2 of hepatic tissue decreased with the increase of copper concentration (Figueiredo-Fernandes, 2007). In india, metal uptake by *Oreochromis mossambicus* inhabiting Indus River led to histological changes in gills and liver. The abnormalities in gills were desquamation of lamellar epithelium, hypertrophy of epithelial cells, lifting up lamellar epithelium, intraepithelial oedema, aneurysm, hyperplasis and haemorrhage. Histology of liver revealed the presence of heterogenous parenchyma characterized by vacuolization, foci of necrosis, hypertrophy of nuclei and degenerated hepatocytes (Jabeen and Chaudhry, 2013).

Intensive research on the environmental impact of chemical compound in aquatic ecosystem and also the effects of some stem bark and leaves of some plant with prominent medicinal purposes including *K. senegalensis* have been documented. However, a dearth of information about the methanolic extract of the leaves on aquatic fauna including *C. gariepinus* still exists. The study therefore aimed to evaluate the haematological indices and histopathological changes in the liver of *Clarias gariepinus* exposed to acute concentration of *Khaya senegalensis*.

MATERIALS AND METHODS

Plant extraction

K. senegalensis leaves were dried in an open airy laboratory for 2weeks and later and pounded into powder using ceramic mortar and pestle and later sieved through 100 mm sieve to obtain 500g of fine powder. 200g of the powder was packed into a soxhlet extractor with 5 L of 25% v/v methanol (98% vol. Sigma-Aldrich^R Inc., St. Louis, MO 63178, USA) as the extracting solvent. The set up was placed over water bath-bath (40- 45 $^\circ$C) for 3 to 4 h for drying the substrate.

Acute fish toxicity bioassay

Three (300) Juveniles *C. gariepinus* of mean weight 18.47 ± 3.06g and standard length 10.9 ± 2.7cm. Fish were acclimatized in 500L plastic tank pond for 14 days under natural day and night photoperiods (12/12h) prior to commencement of the toxicity bioassay. Pond water was changed once every three (3) days. Fish were fed twice daily with standard feed for aquaculture.

Three hundred and sixty (360) healthy acclimatized fishes were randomly selected and distributed into 12 glass aquaria each containing 20 litres of dechlorinated water of 10 fish per aquaria, two of the aquaria served as control for acute toxicity bioassay. Twenty gram of the methanolic extract of *K. senegalensis* was obtained and dissolved in distilled water (1L) to form a stock solution of 250mg/L. 0 (control), 150, 170, 190, 210 and 230mg/L were dispersed into the experimental aquaria. The mixture was allowed to stand for about five minutes to evenly distribute via diffusion before introducing the fish. The exposure was carried out in triplicate.

At the end of the 96h exposure period, two fish were randomly sampled from the control and each treatment tanks, dissected to extract the liver.

The concentrations were selected after conducting a preliminary study, was used as an endpoint of toxicity. Probit analysis method was used to determine the median lethal concentration (LC_{50} of the extract of the exposed fish. The temperature, PH, TDS, Conductivity of fish culture water was ascertained using a Hana portable hand instrument HI 98129 and the dissolved oxygen contents were measured using Winkler-Azide method and reported elsewhere (Abui and Matouke, 2015).

Haematological analyses

Total Erythrocytes (TEC) and total leucocytes (TLC) count were evaluated using an improve Neubaeur counting chamber under microscope (Dacei and Lewis, 2001). PCV was evaluated by Wintrobe method (Blaxhall and Diasley, 1971).

Histopathological analyses

At the end of the 96h exposure period, two fish were randomly sampled from the control and each treatment tanks, dissected to extract the tissues (liver). The liver was preserved in 10% formalin, washed in running tap water to remove traces of formalin, followed by dehydration using progressive percentage of alcohol and chloroform. Samples were processed, sectioned (5µm) and stained with haematoxylin and eosin using histological techniques (Bancroft and Cook 1994). Permanent slides were prepared and photomicrographs taken, using a Carl Zeiss (Axioskope 40) Trinocular Photo micrographic microscope with digital camera for comparison with tissues obtained from those of control.

The degree of tissue change (DTC), which is based on the severity of the lesions according to the methodology described by Poleksic and Mitrovic-Tutundzik (1994). For the calculation of DTC, the alteration in the liver was classified in progressive stages of tissue damage. First stage lesions (I) are slight and would be reversible with an improvement in the environmental conditions; second-stage lesions (II) are more severe, leading to effects on tissue function; and third-stage lesions (III) are very severe, with irreparable damage. The sum of the number of lesion types within each of the three stages multiplied by the stage coefficient represents the numerical value of the DTC, based on the formula DTC = $(10^0\ \Sigma I) + (10^1\ \Sigma II) + (10^2\ \Sigma III)$, in which I, II and III correspond to the sum of the number of alterations found in stages I, II and III, respectively. The DTC was obtained for the fish of all the experimental groups and used in the statistical analysis to compare the mean degree of tissue damage between groups.

Statistical analyses

Data were analyzed using XLSTAT software version 15.5. Means (SD) were subjected to ANOVA and Chi-square for statistical significance ($P< 0.05$).

RESULTS

Quality of the water

The physicochemical parameters of water showed that EC was 79.5µs/cm, TDS 56.79 mg/L, PH 6.92 and Temperature 24.14 $^{\circ}$C (Table 1).

Table 1. Physicochemical parameters of fish culture water of *C. gariepinus* exposed to methanol extract of leaves *K. senegalensis*.

Physicochemical parameters	Value
Conductivity (5µs/cm)	79.5±3.48
Total dissolved solid (TDS) mg/L	56.79±1.81
PH	6.92±0.12
Dissolved oxygen (DO) (mgO_2/L)	6.66±0.14
Temperature (^0C)	24.14±0.18

Acute fish toxicity bioassay

The effect of *K. senegalensis* leaves on *C. gariepinus* showed a high mortality of 60, 83 and 70 at concentration 210 and 230 mg/l respectively. The mortality increased significantly with the increased in concentration ($P< 0.05$). The median lethal concentration (LC50) of leaves extract was therefore 199.69 mg/L (Table 2).

Table 2. Mortality in *C. garipinus* exposed to methanol extract of *K. senegalensis* leaves over 96h period.

Extract concentration (mg.L)	Log concentration	Total Mortality	Percentage total mortality (%)	Probit value
0.0 (control)	0.000	0.0	0.0*	2.5*
150mg.L	2.176	1	10	3.72
170mg.L	2.230	3	30	4.16
190mg.L	2.279	4	40	4.75
210 mg.L	2.322	6	60	5.25
230 mg.L	2.362	7	70	5.52

Y= 9.33x-21.47, LC_{50}= 199.69, * =corrected value

The surface of *C. gariepinus* liver in control group had a preserved architecture, normal hepatocytes and absence of blood congestion while compared to other concentration (Figure 1, 2, 3, 4, 5, 6 and 7).

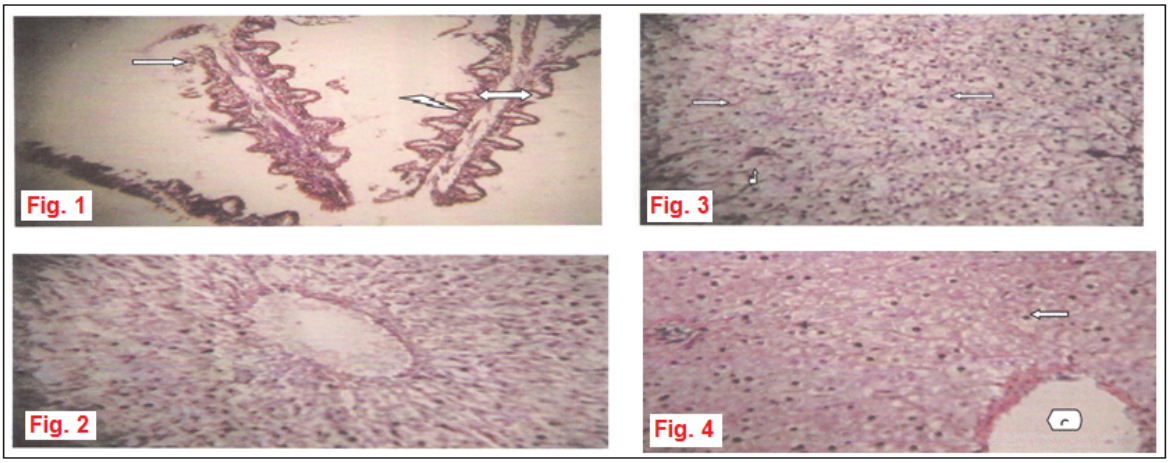

Figure 1. Photomicrograph of the liver of *C. gariepinus* showing a preserved architecture and normal hepatocytes with sinusoids intact; **Figure 2.** Photomicrograph of the liver of *C. gariepinus* showing a preserved architecture and necrosis of the hepatocytes at 170mg/L of toxicant; **Figure 3.** Photomicrograph of the liver of *C. gariepinus* showing mild necrosis of the hepatocytes at 190mg/L of toxicant; and Figure **4.** Photomicrograph of liver of *C. gariepinus* showing marked necrosis of the hepatocytes at 210mg/L of toxicant.

Haematological evaluation

The haematological evaluation showed the highest means total erythrocyte concentration (TEC) (238±3.0) at concentration 150mg/L and the lowest (122.33±4.16) at concentration 230mg/L. The total leucocytes concentration in the blood sample was highest (2888±16) and the lowest 1147.23 at control level. The pack cell volume (PCV) was the highest (39.13) during control and the lowest 13.67±1.53 at concentration 230mg/L (Table 3). There was no significant difference among means of haematological parameters.

Table 3. Means of haematological parameters on *Clarias gariepinus* to acute concentration of methanol leaf extract of *Khaya senegalensis*.

Concentration mg/L	TECx10^6	TLCx500 m^3	PCV
0	252^a	1147.23^c	39.13^a
150	238±3.0^b	1626.67±16.65^d	31±2.0^a
170	212.0±2.65^c	2072 ±8.0^c	23.67±2.51^b
190	201±3.0^d	2422.67±28.4^b	21.33±3.06^b
210	159.33±1.53^e	2870.67±22.03^a	13.33±3.06^c
230	122.33±4.16^f	2888. ±16^a	13.67±1.53^c

Means with the same letters along the columns are not significantly different (P>0.05).

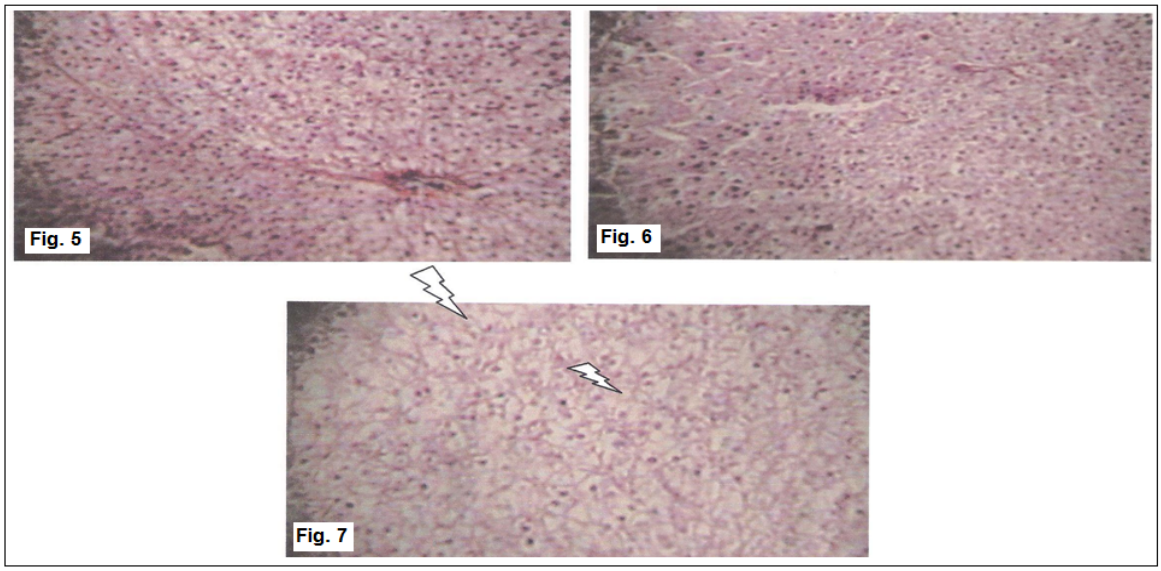

Figure 5. Photomicrograph of the liver of *C. gariepinus* showing marked necrosis of hepatocytes at 230mg/L of toxicant; **Figure 6.** Photomicrograph of the liver of *C. gariepinus* showing marked necrosis of hepathocytes at 230 mg/L of toxicant; and **Figure 7.** Photomiccrograph of the liver of *C. ganepius* showing marked necrosis of hepatocytes at 230 mg/L of toxicant.

Table 4. Type and incidence of histological lesion in the liver of *Clarias gariepinus* exposed to methanol extract of *Khayasenegalensis* leaves over 96h periods.

Stage	Degree of tissue damage in the Liver
I	Degeneration of the cytoplasm(++)
	Less intracellular space (++)
II	Mild necrosis (++)
	Sinusoidal blood congestion (+++)
III	Marked blood congestion in hepathocyte (++)

(+): low incidence (++): moderate incidence (+++): high incidence

The degree of tissue change (DTC) was highest (111.67) when the fish was exposed to 230mg/L concentration, this translate irreversible damage observed. The DTC of 72 which is moderate was observed when the fish was exposed to 210mg/L while the concentration less than 210mg/L was without danger for the liver of the fish (Table 5). The result showed significant difference when compared to the control ($P<0.05$). The cumulative degree of tissue change showed a moderate change (55.06±12.7) in the liver of fish (Table 6).

Table 5. Degree of tissue change per extract concentration in the liver of *Clarias gariepinus* exposed to methanol extract of *Khaya senegalensis* leaves over 96h period.

Concentration	00	150	170	190	210	230
Liver	12±8.5	53± 9	42.67±30.2	39±11	72± 11	111.67± 6.6

DISCUSSION

The physical and chemical parameters analyzed in this study are very relevant for the status of water quality tolerable by *C. gariepinus*. The result of the physical chemical in this study, conductivity 79.5 µs.cm, TDS 56.79mg/L, Temperature 24.14°C PH 6.92 and DO 6.66mgO_2 132mg/L corroborate with the standard of freshwater fish water quality. The physicochemical parameters of fish culture water in this study were all within acceptable limit for the survival of *C. gariepinus*. The standard as stated by Kalawole *et al*. (2011) reported a conductivity less than 135-100µs/cm, DO greater than 4 mg/L; PH between 6.5 to 9 and Temperature between 20-30 ^{0}C.

In this study the mortality increased with the increase of concentration, this increase was shown to be significant ($P<0.05$). This showed that methanolic extract of *K. senegalensis* was 138 toxic to the exposed fish. The dependence of plants extract to mortality of *C. gariepinus* was similar Abalaka *et al*. (2015). The LC_{50} value of 199.69 in this study was higher compared to Ayuba *et al.* (2012) who had a LC50 of 120.23 mg/L while studying the acute toxicity *C. gariepinus* exposed to *Daturainnoxia* leaf extract.

A decrease of total erythrocytes observed in this study when compared to the control suggests a gradual damage or inhibition of oxygen production of erythrocytes in the blood of fish.The increased and decreased values of TLC suggest some abnormalities. Leukocytes are white colored blood cells which defend the animal body against infections and diseases. They might increase in number in this study to boost the immunity of fish against certain infections due to variation of concentration extract. The PCV measures the percentage of red blood cells (RBC) to the total blood volume. These values obtained in this study decreased respectively with the increase of concentration which suggests an increase of anemia in fish. This study agreed with finding on the haematological responses of *Clarias gariepinus* exposed to sublethal concentration of *Daturainnoxia* root extract (Okomodaetal, 2013). *K. senegalesis* could be suspected to cause gradual anemia in fish through hemolysis though no significant difference was observed in the measured haematological parameters.

Using the methods described by Polekesic and Mitrovic-Tutundsie (1994) as reference, five (5) types of lesions were identified in the liver of *C. gariepinus* (Table 4). Two (2) of which were first stage (I), two (2) were second stage (II) and one (1) was stage three (III).

The liver is the organ most associated with detoxification and accumulation due to the function and blood supply, it is also affected by contaminant in the ecosystem and also play a critical role in fish physiology because acts as the main storage for many substance (Moneim-Abdel *et al*., 2008). Histopathological changes of liver in this study gave an insight on the nature of the stressor *K. senegalensis* leading to pathological changes in tissues. In this study the damage in the liver of *C. gariepinus* exposed to various concentration accounts for the response to the methanol extract *K. senegalensis* which act as a toxicant. The response in the liver tissues showed a degeneration of the cytoplasm, less intracellular, mild necrosis, sinusoidal blood congestion and marked blood congestion in hepathocytes. These changes may be attributed to direct toxic effects of pollutants on hepatocytes as found in *K. senegalensis*. The observation in this study suggests a structural damage of the hepatocytic cytoplasm with gradual increase of *K. senegalensis* concentration. Elsewhere, authors described hepathocytic alterations, hypertrophy of hepatocytes, and infiltration of leukocytes, necrosis and fibrosis in *Channapunctatus* a freshwater fish after exposure to pesticide chlorpyrifos (Devi and Mishra, 2013).

Less intracellular spaces, mild necrosis, sinusoidal congestion and marked blood congestion in this study might be the cause of the degeneration of hepathocytes with cytoplasmic vacualation and hypertrophy. These observations were reported in *Poeciliareticulate* exposed to acute concentration of textile effluent (Selvaraj *et al*., 2015). Increased vacualisation of the hepathocytes could be described as a signal of degenerative process. Cytoplasmic degeneration in this study suggests metabolic damage, probably related to exposure to acute *K. senegalensis*.

Marked blood congestion and marked necrosis lesions belonging to stage I and stage II respectively observed in this study were in agreement with many studies that examined the effect of different pollutant on fish liver, though the pollutant were metals (Olurin *et al*., 2006, Figueiredo-Fernandes *et al*., 2007; Devi and Mishra, 2013; Jabeen and Chaudhry, 2013).

The highest mean DTC was observed at concentration 230mg/L might suggest a severe damage in the hepatic cells if prolonged. But the cumulative mean of 55.06 was observed in the liver of fish exposed to acute concentration. This result agreed with findings of (Carmago and Martinez, 2007) which report showed a mean DTC of 52.34 indicating that in most cases the hepatic lesions caused moderate damage to tissues though, an increase of DTC 174 in summer was reported while studying the histopathology of liver of a Neotropical fish caged in an urban stream. Studies conducted on the gills and skin of *C. gariepinus* exposed to acute concentration of ethanol extract of *Adenium obesum*stem bark revealed a cumulative DTC of 23.12 and 1.78 in the gills and skin respectively which suggest a low damage while compared to the present study.

CONCLUSION

In the present study, haematological parameters (TEC TLC and PCV) and histopatological changes have been related to acute concentration of *K. senegalensis*. It can be concluded that haematological indices and liver alteration of fish may serve as biomarker of toxicity due to acute concentration of *K. senegalensis.* However, other studies are necessary to evaluate de degree of tissue change in other species of fish.

CONFLICT OF INTEREST

The authors have no conflicts of interest concerning the work reported in this paper.

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