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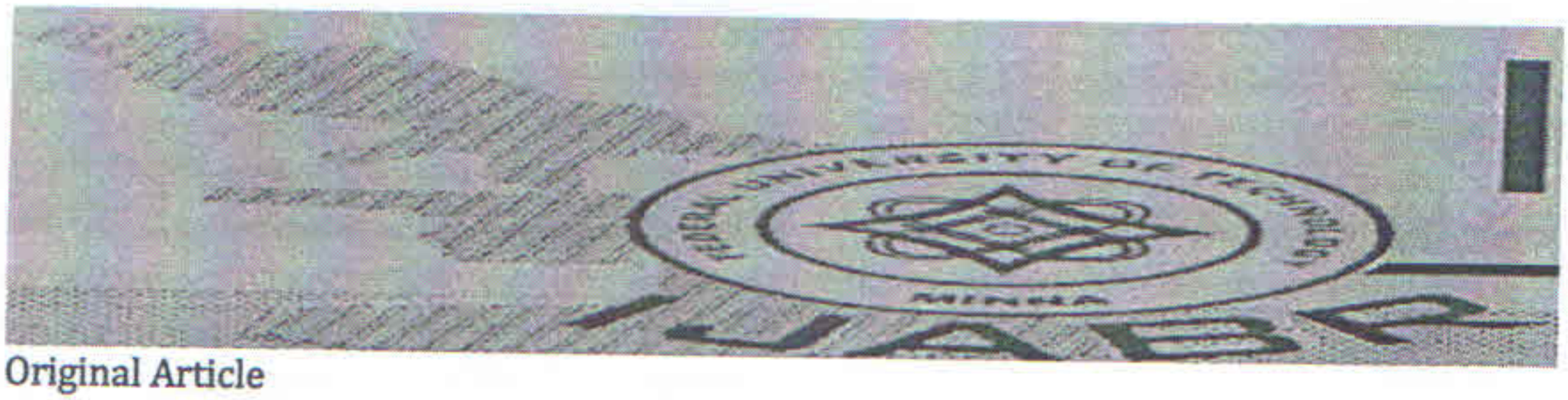

Original Article

HAEMATOLOGICAL EFFECTS OF DIFFERENT SUBLETHAL CONCENTRATIONS OF THE AFRICAN MAHOGANY (*Khaya senegalensis*) LEAF EXTRACT ON THE AFRICAN CATFISH (*Clarias gariepinus* BURCHELL 1822) FINGERLINGS

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ABSTRACT

The sublethal effects of the leaf extracts of *Khaya senegalensis* was evaluated using the haematological parameters of the fingerlings of the African catfish (*Clarias gariepinus*) with a mean weight of 1.90g(20 fingerlngs per tank) in a static renewal bioassay system during 84-day bioassay exposure period. Water quality parameters such as pH, dissolved oxygen, conductivity and alkalinity did not differ significantly ($P>0.05$) from the control test. There were however significant reductions ($P<0.05$) in blood parameters associated with oxygen transport; Packed Cell Volume (PCV), Red blood counts (RBC), Mean corpuscular volume (MCV), Mean Corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The concentration of haemoglobin (HB) was much lower in the exposed fish compared to the control depicting an anaemic condition. There were significant increase ($P<0.05$) in the values of White Blood cells (WBC) from a mean of 3.23 in the control to 4.53 ($10^3/mm^3$) in the exposed fish to concentration of 1.00g/l of the extract. In addition, it was revealed that the variation in the different haematological indices were proportional to the concentration of the toxicant used. It is therefore recommended that haematological indices assessment of possible impact of riparian plant used for erosion control be conducted at intervals to ascertain the impact status on aquatic fauna.

Keywords: Haematology, *Khaya senegalensis*, *Clarias gariepinus*, PCV, HB, RBC, WBC, MCH, MCV, MCHC

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INTRODUCTION

Plants overtime have been regarded as one of the valued components of the biosphere due to their use as medicine, food and chemical constituents. Plant materials (leaves, fruit and flowers) play an important role in aquatic ecosystem by serving as food source for aquatic organisms. However, some of these materials have the potential of inducing environmental stress by the disruption of the water quality, increased biological activities and changes in the normal physiological activities of the aquatic biota (Ayuba and Ofojekwu, 2005).

Khaya senegalensis or Africa Mahogany known as Ono in Igbo, Madaci in Hausa, and Wuchi in Nupe language, belongs to the family Meliaceae. The tree has been reported to be a dominant tree species in riverine forest and scattered within higher rainfall savannah woodlands. It is found on uplands in moist areas but is restricted to riparian habitats (Jibrin, 2013). It is drought-tolerant and the timber is rated as one of the best African mahogany woods. The bark is used to treat a number of diseases and also used as arrow poison. The leaves have low forage value and the seed oil is used for cooking in West Africa (Joker *et al.*, 2003, ICRAF, 2004).

Phytochemical screening of the leaves of *Khaya senegalensis* has revealed the presence of alkaloids, saponins, tannins, resins, glycosides, terpenes, anthroquinoines and limonoids as active constituents (Wakirwa *et al.*, 2013; Takin *et al.*, 2013). Shedding of leaves into water bodies during wet and dry seasons increases biological activities, which is capable of inducing stressful conditions in fish.

Clarias gariepinus is considered to be an ecologically important fish species in the inland waters of Nigeria. It is hardy and amenable to laboratory condition. Because Fish are closely associated with the aquatic environment, physical and chemical alterations in the environment are often reflected as physiological changes in fish.

Haematological indices are growing in importance for toxicological research, environmental monitoring and assessment of fish health conditions. Haematology has been used for assessment of fish health in a number of fish species to detect physiological changes caused by exposure to different pollutants, acclimation and anaesthesia (Adeyemo 2005; Dada and Ikuerowo 2009; Akinrotimi *et al.*, 2010; Ayuba and Ofojekwu 2012a). It is assumed that the leaf runoff into the riparian waters might affect the early life stage of fish. This study was conducted to ascertain the effect of the methanol leaf extract of *Khaya senegalensis* on the haematological indices of the fingerlings of the Africa catfish (*C. gariepinus*).

MATERIALS AND METHODS

Healthy fingerlings of *Clarias gariepinus* of the same age, mixed sexes, mean weight (1.90±0.06g) and mean length (6.30±0.23cm) were procured from IRU Tropical Fish farm, Keffi, Nasarawa state, Nigeria. The fish were transported in large plastic containers in the early hours of the morning to the laboratory of the Department of Biological Sciences, Federal University of Technology, Minna. The fish were allowed to acclimatise to laboratory condition for 14 days during which they were fed commercial feed pellets (Coppens 2.0mm) twice daily. Feeding was stopped 24 hours before

commencement of the experiment as recommended by Reish and Oshida, (1987).

Fresh leaves of *Khaya senegalensis* were collected from the field around Maryam Babagida Girls Science secondary school, Minna, air dried and pulverized into powder with a Philips electric blender. Soxhlet method was used to distil the leaves using methanol as solvent. The extract was dried over a water bath between 40°C - 45°C. The experimental set up consists of four treatments (0.25g/l, 0.50g/l, 0.75g/l and 1.0g/l) and one control (0.0g/l) in three replicates each containing 10 fish per aquaria. Concentrations were taken from stock solution of 25g/l according to Fafioye (2012). Water quality parameters were monitored weekly. Water samples were collected according to APHA (1999), temperature was monitored with mercury in glass thermometer, Dissolved oxygen, pH and conductivity were monitored with a WTW 3320 automated multiparameter. Alkalinity and Total dissolved solids measurements were carried out according to APHA (1999). The exposure period lasted for 84 days. At the end of the experiment, five fish from each group were subjected to pool blood samples. Blood samples were collected through caudal puncture using hypodermic needle and 2ml heparinised syringe into EDTA vials. Haematological parameters of Packed cell volume, Red blood count, white blood count, Haemoglobin, white blood count, and haematological indices of MCV, MCH and MCHC were determined according to Jain (1986).

$$MCV = \frac{\text{Hematocrit(\%)} \times 10}{\text{RBC} (\times 10^{12}/\text{L})}$$

Femtoliters (fL) or 10-15Liter

$$MCH = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC} (\times 10^{12}/\text{L})}$$

Picograms (pg) or 10-12 grams

$$MCHC = \frac{\text{Hb (g/dL)} \times 100}{\text{Hematocrit (\%)}}$$

Grams/deciliter (g/dL)

Data analysis

The data of water quality parameters and changes in haematological parameters in the test and experimental tanks were collated and analyzed using PAST software for windows (Hammer *et al.* 2001). Data were first tested for normality (Kolmogorov - Smirnov test) and homosesdasticity of variance (Bartetts test). When these conditions were satisfied, a one way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Turkey's multiple comparison test was applied to identify which treatments were significantly different.

RESULTS

The mean values of water quality parameters (Table 1) showed no significant difference ($P>0.05$) in temperature, Dissolved oxygen, pH, total alkalinity, conductivity and total dissolved solids (TDS) with the control. Temperature varied from a mean of 26.3°C in the control to 27.00°C in the 1.00g/l concentration. Conductivity fluctuated between 122.33 to 135,00□S/cm. Hydrogen ion concentration (pH) fluctuated from 6.1 to 6.4, The haematological response of the fish showed a significant reduction ($P<0.05$) in the PCV from a mean of 14.06 in the control to 8.69 in the 1.00g/l test tank. Similarly Red Blood

cells count (RBC) decreased from a mean of 1.39 x 10^6/mm^3 in the control to 1.12 x10^6/mm^3 in the tank with the highest concentration. Haemoglobin (HB) decreased from 4.38g/dl in the control to 2.25 g/dl in the tank with the highest concentration. Similarly MCV, MCH and MCHC all showed progressive decrease with increase in the concentration of the plant extract (Table 2) while the values of WBC increased significantly ($P<0.05$) from a mean of 3.23x10^3/mm^3 in the control to 4.53 x10^3/mm^3 in the plant extract with the highest concentration. Percentage PCV decreased significantly from a mean of 14.06 to a mean of 8.69%.

Table 1: Mean values of physicochemical parameters

Parameters	Control (0.00g/l)	0.25g/l	5.00g/l	0.75g/l	1.00g/l
Temperature (°C)	26.83±0.20[a]	26.60±0.26[a]	26.93±0.30[a]	26.90±0.23[a]	27.00±0.09[a]
Dissolved oxygen (mg/l)	6.30±0.10[a]	6.50±0.15[a]	6.53±0.15[a]	6.47±0.15[a]	6.57±0.12a
pH	6.20±0.03[a]	6.33±0.03[a]	6.43±0.07[a]	6.47±0.15[a]	6.33±0.01[a]
Total Alkalinity (mg/l)	30.93±0.23[a]	29.70±0.24[a]	28.30±0.90[a]	26.47±0.71[a]	26.50±1.81[a]
Conductivity (µS/cm)	122.33±1.45[a]	126.00±3.06[a]	130.33±2.33[a]	135.00±1.73[a]	135±1.53[a]
Total Dissolved Solids (mg/l)	75.85±0.90[a]	78.12±1.89[a]	80.60±1.64[a]	83.7±1.07[a]	83.7±0.95[a]

Means with same superscript across rows are not significantly different using.....?????????

Table 2: Mean values of Haematological for the 84 days sublethal exposure

Haematological Parameters	Control (0.00g/l)	0.25g/l	0.50g/l	0.75g/l	1.00g/l
PCV (%)	14.06±0.15[a]	13.00±0.10[a]	11.23±0.35[b]	10.90±0.06[b]	8.69±0.41[c]
RBC(10^6/mm^3)	1.39±0.03[a]	1.34±0.02[ab]	1.30±0.01[ac]	1.27±0.01[bc]	1.12±0.04[d]
HB(g/dl)	4.38±0.28[a]	3.84±0.03[ab]	3.23±0.09[bc]	2.97±0.04[c]	2.25±0.09[d]
WBC (10^3/mm^3)	3.23±0.15[b]	3.93±0.09[d]	4.13±0.09[ac]	4.37±0.07[ad]	4.53±0.07[a]
MCV (fl/cell)	101.03±1.56[a]	97.04±0.82[a]	89.44±3.03[b]	85.83±0.33[b]	77.26±0.62[c]
MCH (Pg)	31.55±2.50[a]	28.7±0.70[ab]	24.82±0.72[bc]	23.39±0.36[b]	20.03±0.55[bc]
MCHC (%)	31.20±2.28[a]	29.53±0.49[a]	28.18±0.74[a]	27.25±0.36[a]	25.91±0.83[a]

Means with same superscript along rows are not significantly different using Tukey post hoc test

DISCUSSION

Studies have shown that haematological changes are associated with exposure (Ezemonye and Tongo, 2010) and ichthyo-plant extract, (Omoniyi *et al.*, 2002; Ayuba *et al.*, 2012b). In this investigation PCV, HB, and RBC increased significantly with increasing concentration and exposure to the toxicant compared to the control. This is in consonance with earlier studies (Akinrotimi *et al.*, 2010; Ayuba *et al.*, 2012b). The decrease in the PCV may be due to an increase in respiratory demand and receding of life processes.

Akinrotimi *et al.* (2010) reported that increase in the blood oxygen carrying capacity is attributed to increase in fish osmoregulatory activities. Haemoglobin values significantly decreased as concentration increased, this may have caused a deprivation of a reserve of oxygen capacity therefore preventing it from potentially higher arterial oxygen content. In addition, the haemoglobin and oxygen relationship shows adaptations not only to environmental conditions but also to metabolic requirements both of which govern oxygen availability and transport to the tissues (Wells, 1999). The RBC observed in the present study decreased significantly when compared with the control. This may be due to swelling and subsequent destruction of the erythrocytes. Earlier researches have reported haemolysis of erythrocytes (Alwan *et al.*, 2009; Akinrotimi *et al.*, 2010; Fafioye, 2012). Bananee *et al.* (2011) has related the decrease in the number of RBC, haemoglobin and haematocrit values of fish exposed to diazon to the destruction and or decrease in size of the Red blood cells due to the adverse effects of pesticide. The observations made in this study indicate similar condition which may probably be due to toxicity of the leaf extract of *K. senegalensis*.

The WBC increase with increase in toxicant concentration may be due to malfunctioning of the haematopoietic system caused by exposure to the methanol leaf extracts of *Khaya senegalensis*. The values for WBC noticed in this study agree with the findings of Ayuba *et al.* (2012b). High WBC is an indication of inflammation, hypersplenism trauma and stress (Hetchman 2011). The increase in the WBC values noticed in this study may be due to the immune response of *C. gariepinus* to the methanol leaf extracts of *K. senegalensis*.

Fluctuations in the values of MCV, MCH and MCHC correspond with the values of PCV, HB and RBC. In the present study, the value of MCV, MCH and MCHC increased as toxicant concentration increased. Similar observations were reported by Omoniyi *et al.* (2002) and Fafioye (2012). The reduction in blood metabolites of PCV, HB, RBC, MCV, MCH, MCHC is an indication of anaemia which might be attributed to destruction of erythrocytes, inhibition of erythrocyte production, haemodilution or stress induced by the leaf extract of *K. senegalensis*.

In conclusion, the exposure of *C. gariepinus* fingerlings to sub-lethal concentration of leaf extract of African Mahogany for 84 days did not cause outright mortality but had effect on the physiology of the fish by altering negatively the haematological parameters.in the fish (*Clarias gariepinus*). It is recommended that haematological indices assessment of possible impact of riparian plant used for erosion control and river bank protection be conducted at intervals to know the impact status of the fallen leaves on aquatic fauna.

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REFERENCES

Adeyemo, O. K. (2005). Haematological and Histopathological effects of cassava mill effluent in *Clarias gariepinus*.

African Journal of Biomedical Research, 8: 179 - 183.

Akinrotimi, O. A., Uedememe-Naa, B. and Agokei, E. O. (2010). Effects of Acclimation on Haematological parameters of Tilapia guineensis (Bleeker, 1862). *Science World Journal,* 5(4): 1 - 4.

Alwan, S. F., Hadi, A. A. and Shokr, A. E. (2009). Alterations in haematological parameter of fresh water fish, *Tillapia zilli* exposed to Aluminium. *Journal of Science and its Applications,* 3(1): 12 – 19.

APHA. (1999). American Public Health Association. Standard methods for the examination of water and waste water, 20th Ed. APHA, Washington DC.

Ayuba, V. O. and Ofojekwu, P. C. (2005). Effects of sublethal concentrations of *Datura innoxia* leaf on weight gain in the African catfish, *Clarias gariepinus* *Journal of Aquatic Sciences,* 20(2): 113 – 116.

Ayuba, V. O., Ofojekwu, P. C. and Musa, S. O. (2012a). Haematological response and weight changes of the African catfish *Clarias gariepinus* exposed to sublethal concentration of *Datura innoxia* root extract. *Production Agriculture and Technology,* 8(2): 134 - 143.

Ayuba, V. O., Ofojekwu, P. C. and Musa S. O. (2012b). Acute toxicity of *Clarias gariepinus* exposed to Datura innoxia leaf extract. *Journal of Medicinal Plants Research,* 6(12): 2453 – 2457.

Bananee, M., Sureda, A., Mirvaghafi, A. R. and Ahamadi, K. (2011). Effects of Diazon on Biochemical Parameters of Blood in Rainbow trout (*Oncorhynchus mykiss*). *Pest. Biochem. Physiol.*, 99: 1 – 6.

Bat, L. and Akubulut, M. (2001). Studies on sediment toxicity bioassays using *Chironomus Thummi* K., 1911 larvae. *Turkish Journal of Zoology,* 25: 87 – 93.

Dada, A. A. and Ikuerowo, M. (2009). Effects of ethanolic extracts of Garcinia kola seeds on growth and haematology of catfish (*Clarias gariepinus)* broodstock. *African Journal of Agricultural Reseaerch,* 4(4): 344 - 347.

Ezemonye, L., and Tongo, I. (2010). Sublethal effects of endosulfan and diazinon pesticides on glutathione-S-transferase (GST) in various tissues of adult amphibians (Bufo regularis). Chemosphere, 81(2): 214 - 217.

Fafioye, O. O. (2012). Acute and sub-acute toxicities of five plants extracts on white tilapia, Oreochromis niloticus (Trewavas). *International Research Journal of Agricultural Science and Soil Science,* 2(13): 525 - 530.

Hammer, O., Harper, D. A. T. and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1): 9. *http://palaeo-electronica.org/2001_1/past/issue1_01.htmm.*

Hetchman, L. (2011). Clinical Naturopathic medicine. Churchhill Livingstone/ Elsevier Australia Publishers. Sydney Australia. 2nd edition 15-96pp

ICRAF. (2004). Agroforestry tree database: A tree species references and selection guide. pp 1 - 2.

Jain, N. C. (1986). Schalm's veterinary hematology (No. Edition 4). Lea & Febiger

Jibrin, A. (2013). A study of variation in physiognomic Characteristics of Guinea Savanna vegetation. *Environment and Natural Resources Research*, 3(2): 52 - 60.

Joker, D., DFEC. (Danida Forest Seed Centre), Graméné, S. & CNSF (Centre National De Semences Forestieres Burkina Faso) (2003). Seed Leaflet, *Khaya senegalensis* (Desr.) A. Juss. N0. 66.

Omoniyi, I., Agbon, A. O. and Sodunke, S. A. (2002): Effect of Lethal and Sub-Lethal Concentrations of Tobacco (*Nicotianna tobaccum*) Leaf Dust Extract on Weight and Haematological Changes in *Clarias gariepinus*(Burchell). *Journal of Applied Science and Environmental Management*, 6(2): 37 - 41.

Reish, D. L. and Oshida, P. S. (1987). Manual of methods in aquatic environmental research - part 10-short term static bioassay. F.A.O. Fish Tech. Paper, 247: 62.

Takin, M. C., Attindehou, S., Sezan A., Attakpa, S. E. and Baba-Moussa, L. (2013). Bioactivity, therapeutic utilility and Toxicological risks of *Khaya senegalensis*. *Indian Journal of Pharmaceutical and Biological Research*, 1(4): 122 - 129.

Wakirwa, J. H., Idris, S., Madu, S. J, Dibal, M. and Malgwi, T. (2013). Assessment of the in-vitro antimicrobial potential of *Khaya senegalensis* leaf extract. *Journal of Chemical & Pharmaceutical Research*, 5(7): 182 - 186.

Wedemeyer, G. A., Gould, R. W. and Yatsutake, W. (1983). Some potentials and limits of the leucocrit test as fish health assessment method. *Journal of Fish Biology*, 23: 711 - 716.

Wells, G. M. G. (1999). Haemoglobin function in aquatic animals: Molecular adaptations to environmental challenge. *Mar Freshwater Resource*, 50: 933 - 936.

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