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Haematological Studies of *Clarias gariepinus* Exposed to Ethanolic Stem Bark Extracts of Ackee, *Blighia sapida*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MUE designed the study, managed the literature searches and analyses of the study and wrote the protocol of the manuscript. Author EAS wrote the first draft of the manuscript. Author FAE performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated effects of sub-lethal concentrations (0.00, 1.6, 3.2, 6.4, 12.8 g/l) of ethanolic stem bark extracts of ackee, *Blighia sapida* on haematological profile of *Clarias gariepinus* (mean weight, 6.95± 1.02 g; total length 11.7±1.32 cm). Each concentration was treated in triplicate using a static bioassay system. The exposure period was 21 days. Physico-chemical parameters of water were monitored throughout the study period. Results revealed that haematocrit, red blood cell, haemoglobin and mean corpuscular haemoglobin concentration counts were significantly ($p<0.05$) depleted with increase in extract concentration while white blood cell counts increased ($p<0.05$) linearly from 198.2, 198.5, 208.8, 219.4 and 241.53 x 10^3 cells/µl in 0.00, 1.6, 3.2, 6.4 and 12.8 g/l respectively. The levels of water temperature increased with extract concentrations while dissolved oxygen and pH contents decreased. The implications of these results showed that stem bark extract of ackee had negative effects on the blood of treated fish. This may further explain the decline in wild fishery resources in the locality where the plant parts were collected. In view of this,

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the direct use of this plant in harvesting fish by local fisher folks in Southern Nigeria should be discouraged until information that aid its application in a sustainable manner is made available through further studies.

Keywords: Catfish; ackee apple; ichtyotoxic; stem bark extract; haematological profile.

1. INTRODUTION

The catfish (*Clarias gariepinus*) is well known as being both commercially and ecologically important culture fish in the Nigerian fishery industry [1]. The high nutritional food value makes it the most preferred by fish famers. This had led to it high demand in the Nigerian fish markets. Thus, prompting the use of some obnoxious methods for ease and possible surplus catch. The use of ichtyotoxic plants by local fisher folks to stun or kill fish is a common practice worldwide [2]. The method is cheap and had been used by fishermen in rural areas of developing countries [3]. A wide range of active compounds such as alkaloids, flavonoids, sugars and glycosides are present in these plants [4]. Some of these plants are even harmful not only to the fish but also to the consumers of these fish. For instance, [5] reported *Cleistanthus collinus* to be a highly toxic piscicidal plant which is commonly used in suicidal and homicidal poisoning in certain rural areas of Southern India. Despite the potential harmful effects of these plants, they are indiscriminately being used in Nigerian freshwater bodies by local fisher folks [1]. Of particular interest is the ackee apple, *Blighia sapida.* This leads to growing concern on the possible negative effects the use of these plants may pose on the health of aquatic organisms particularly non-target species [6].

In developed nations, fisheries laws prohibiting the use of the **ichtyotoxic** plants to catch fish had been enacted. But in Nigeria, obnoxious fishing methods include the use of these plants to capture fish. This usually results into mass mortality of fish, other non-target organisms as well as the contamination of freshwater environment [3]. The physical and chemical alteration of the aquatic environment often cause some physiological changes in fish leading to low productivity of commercially importance species. Catfish in it natural habitat, lives in direct contact with the aquatic environment where these changes occur. Therefore, detailed information can be obtained on the rate of metabolism, physiological status, state of health, survival, reproduction and growth through the study of the blood [7]. Haematological profile had been used as important index in monitoring pathological changes in fish. They are also considered important in evaluating the physiological status of organisms. Other studies noted that haematological indices could provide reliable information on metabolic disorders, deficiencies and chronic stress of an organism [8,9]. The count of blood cells is a stable index and fish tries to maintain this count within the limits of physiological standards using various physiological mechanisms. Therefore, exposure to any pollutant can either induce increase or decrease in haematological levels causing inadequacies in the functioning of blood cells [7]. Thus, hematological studies on fishes could be used to determine sub-lethal effects of toxicants [9]. Presently there had been paucity of research on the use of piscicidal plants in fish capturing. The present study was designed to assess acute effects of ethanolic stem bark extracts of ackee on the haematological profile of the catfish.

2. MATERIALS AND METHODS

2.1 Experimental Site and Design

The work was carried out at the United Nations Development Programme (UNDP) pilot fish farm located at No. 6A Phenson Street, Federal Housing Estate, Uyo, Akwa Ibom State. The experimental design consisted of a static system made up of 15 plastic tanks of 30litre capacity. Each of them containing clean and untreated borehole water.

2.2 Sample Collection, Authentication and Extraction

Ackee (*Blighia sapida*) stem bark used for the study was obtained from Ikot Akap village in Ikono Local Government Area of Akwa Ibom State. Identification was done at the Department of Botany and Ecological Studies, University of Uyo, Nigeria while extraction was carried out at Pharmacognosy Laboratory, Department of Pharmacognosy and Natural Medicine, University of Uyo. The stem bark was washed and sorted to remove unwanted materials, chopped into small pieces and air dried at room temperature for five days. This was thereafter pulverized using mortar and pestle to obtain fine powder which was thereafter macerated in 70% ethanol and distilled water for 72 hours with periodic stirring [10]. The extracts were thereafter stored in a refrigerator at a temperature of 4°C prior to use. The method of [11] was employed in the estimation of acute median concentration (LC_{50}). Catfish (mean weight, 6.95 $\pm$ 1.02 g and length 11.7 $\pm$ 1.32 cm) were fasted for 24 hours before commencement of experiment. They were thereafter randomized to 5 groups of 10 fish each. The extract was administered at a rate of 1.6 g/l, 3.2 g/l, 6.4 g/l and 12.8 g/l to groups T2 to T5 respectively while group T1(0.00 g/l) was kept as control. Each treatment had three replicates. The fish were observed closely for the first 24 hours and then every 6 hours for 21 days after which the experiment was terminated. Fish were fed maintenance ration (minimally) during the exposure period.

2.3 Haematological Analysis

Five specimens were randomly collected from each tank for blood analysis. The method of blood collection and analysis followed that described by [12]. All haematological parameters were estimated at Haematological Unit of the University of Uyo Teaching Hospital using automated haematology analyzer (SYSMEX, model: KX-21N, USA, 2012) based on the reference method described in international Federation of Clinical Chemist [13]. All haematological analyses were done within two (2) hours of blood collection.

2.4 Statistical Analysis

Data collected were subjected to descriptive statistics involving mean and standard error and the New Duncan Multiple Range Test was used to rank the means using Statistical Package for Social Sciences (SPSS) 19.0, 2010 version.

3. RESULTS

The results of the haematological variables of catfish exposed to ethanolic stem bark extract of *Blighia sapida* for 21 days are presented in Table 1. The mean value of white blood cell (WBC) was 198.2 $x10^3$ cell/µl blood in the control group, which increased progressively (198.5, 208.8, 219.4 and 241.53 $x10^3$ cell/µl blood) in groups T2, T3, T4 and T5 respectively. The results showed that WBC counts increased linearly with concentration of the toxicant. Statistically similar red blood cell (RBC) counts ($p > 0.05$) were obtained between control and T2 as well as between T4 and T5. The erythrocyte indices (MCV, MCH and MCHC) decreased significantly ($p < 0.05$) in all treatments when compared with the control. A gradual decrease in the mean levels of haemoglobin was recorded thus: 7.47 g/dL in the control, 7.17 g/dL in T2, 7.0 g/dL in T3, 6.43 g/dL in T4 and 6.03 g/dL in the T5 groups of the experimental fish. The haematocrit counts decreased ($p < 0.05$) with increase in concentration of toxicants in all treated groups. Leucocyte components: lymphocyte, monocytes, eosinophils, basophils and neutrophil followed similar trend as the WBC. The values all increased considerably in all treatments compared with the control (Table 2).

A further comparison using Pearson correlation (bivariate) analysis (Table 3) indicated a strong positive correlation in WBC ($r = 0.948$; $p < 0.05$), thrombocyte ($r = 0.937$; $p < 0.05$), mixed leucocyte components ($r = 0.991$; $p < 0.05$), lymphocyte ($r = 0.947$; $p < 0.05$) and neutrophil ($r = 0.924$; $p < 0.05$) in all tested extract concentrations. This signified that there was a positive influence of ethanolic stem bark extract of *Blighia sapida* on the white blood components the catfish. The results in Table 4 revealed that water temperature increased linearly with extract concentrations while the reverse was the case for dissolve oxygen and pH levels.

4. DISCUSSION

The sub-lethal toxicity test carried out showed that ethanolic stem bark extract of *Blighia sapida* caused significant changes in the haematological indices of *C. gariepinus* fingerlings. The changes in the values of the haematological indices of the test fish were similar to other studies [12,14]. The haemoglobin, haematocrit and red blood cell counts are good indicators of oxygen transport capacity of organisms. This makes it possible to establish a relationship between the oxygen concentration available in the habitat and that in the fish blood [15]. The observed decrease in the haemoglobin counts in this study was in agreement with the studies of [14] who reported that the century plant, *Agave Americana* leaf dust caused decreased haemoglobin concentration in *C. gariepinus* while [16] also noted a decrease in haemoglobin count of *C. gariepinus* exposed to diethyl phthalate. Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration had been used as a good indicator of anaemia [12]. Thus, the decrease in haemoglobin counts corresponds with the

Table 1. Haematological variables of *C. gariepinus* exposed to stem bark extracts of ackee

Indices	Control (T1)	T2 (1.6 g/l)	T3 (3.2 g/l)	T4 (6.4 g/l)	T5 (12.8 g/l)
WBC (x10^3cell/µl)	198.2±9.6^a	198.5±3.7^a	208.8±3.6^b	219.4±7.9^c	241.53±9.6^d
RBC (x10^6 cell/µl)	1.95±0.21^c	1.91±0.05^c	0.94±0.07^b	0.81±0.28^a	0.80±0.18^a
HGB (g/dl)	7.47±0.76^c	7.17±0.03^c	7.0±0.23^b	6.43±1.3^a	6.03±0.60^a
HCT (%)	23.3±2.1^d	21.67±0.5^c	21.1±0.89^b	20.3±2.4^a	20.0±1.35^a
MCV (fL)	123.3±2.3^e	119.5±7.8^d	118.1±4.6^c	101.2±6.6^b	94.97±2.3^a
MCH (pg)	38.3±0.5^e	37.3±0.65^d	36.57±0.43^c	33.93±1.2^b	29.63±7.1^a
MCHC (g/dl)	31.78±0.41^e	31.10±0.70^d	30.8±0.46^c	29.1±2.0^b	24.5±0.4^a
THROM(10^3 cell/µl)	163.67±1.6^e	151.33±3.8^d	108.5±8.2^c	87.8±5.7^b	46.33±3.7^a

Data are mean ± standard error: means with different superscript within a row are significantly different (p<0.05). Where: WBC=white blood cell; RBC=red blood cell; HGB=heamoglobin; HCT=heamatocrit; MCV=mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration; THROM=thrombocyte

Table 2. Leucocyte population of *Clarias gariepinus* exposed to stem bark extracts of ackee

Indices	Control(0.0 g/l)	T2 (1.6 g/l)	T3 (3.2 g/l)	T4 (6.4 g/l)	T5 (12.8 g/l)
Lymphocyte (%)	178.7±7.0^a	189.1±2.4^b	195.9±3.4^c	200.9±9.0^d	204.9±9.9^e
Monocytes (%)	1.0±0.23^a	1.1±0.08^b	1.32±0.12^c	1.8±0.3^d	2.33±0.6^e
Eosinophils (%)	0.9±0.02^a	0.98±0.03^b	1.08±0.04^c	1.19±0.3^d	2.47±0.37^e
Basophils (%)	1.01±0.23^a	1.14±0.08^b	1.22±0.12^c	1.38±0.3^d	2.03±0.6^e
Neutrophil (%)	0.8±0.02^a	0.9±0.03^b	1.48±0.04^c	1.9±0.3^d	2.67±0.37^e

Data are mean ± standard error: means with different superscript within a row are significantly different (p<0.05)

decrease in dissolved oxygen in the tank water as observed in the present study. This indicates that the decrease in haemoglobin resulted in haemodilution which is an indication of impaired oxygen delivery to the tissue.

On the other hand, the white blood cells confer protection against infectious agent caused by microbial and chemical factors [17]. The increase in the counts of white blood cell observed in this study may have been induced as a protection against disease, thus, improving the health mechanism of the fish in the stressed condition. The mechanism commonly known as 'leucocytosis' is a usual response of vertebrates to conditions or substances that attempt to change their normal physiology [16]. Thus, leucocytosis experienced by the test fish in this study showed that *B. sapida* stem bark extracts elicited the stimulation of the immune system of catfish to protect it against possible infection or secondary effects of the toxicant. Similar leucocytosis was reported in *Heteropneustes fosilis* treated with sewage, fertilizer and insecticides [18]; *Clarias batrachus* exposed to fertilizers [19]; *Anabas testudinus* exposed to monocrotophos [20] and *Clarias albopunctatus* exposed to Gammalin-20 [21]. However, the significant reduction in the erythrocyte components could be an indication of anaemia caused by the destruction of the red cells [22] or haemodilution resulting from impaired osmoregulation across the gill epithelium [23]. Acute concentrations of extract had lethal effects on the exposed fish and the results of phytochemical screening revealed a wide range of active compounds like saponins, alkaloids and flavonoids. Saponins had been reported to have some lytic effects on erythrocyte membrane [24]. This, may have caused the destruction of the red blood cells leading to reduction in the levels of red blood cell, haematocrit and haemoglobin recorded in this study. Since haematocrit and haemoglobin concentrations are directly correlated to erythrocyte counts and are useful in determining the ratio of plasma to corpuscles in the blood [25].

Physico-chemical parameters such as temperature, dissolved oxygen and pH are paramount to the many factors which affect fish health, growth and reproduction [26]. In this study, water parameters were significantly different (p<0.05) from that in the control group. Temperature levels increased with extract concentrations while DO and pH contents decreased. The decline in the pH levels may have been caused by production of acidic metabolites in plant extracts present in the treated water. A similar report was earlier given by [27]. Also, [28] recommended pH range of 6.5 to 8.5 for fresh water fishes, the value of pH in 6.4 g/l and 12.8 g/l of plant stem bark extract were found to be lower than the recommended value.

Table 3. Correlation matrix of haematological variables of *C. gariepinus* exposed to stem bark extracts of ackee for 21 days

	Conc.	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	THROM	LYM	MXD	NEUT
Conc.	1											
WBC	0.948*	1										
RBC	0.738	0.806	1									
HGB	0.478	0.953	0.992*	1								
HCT	0.732	0.872	0.965*	0.955**	1							
MCV	0.619	0.107	0.935	0.023	0.293	1						
MCH	0.352	0.491	0.899	0.316	0.179	0.061	1					
MCHC	0.175	0.809	0.784	0.150	0.146	0.877**	0.821*	1				
THROM	0.937*	0.722	0.638	0.898	0.974*	0.889*	0.262	0.629**	1			
LYM	0.947*	0.810	0.841	0.943	0.854	0.710	0.882	0.841	0.951*	1		
MXD	0.991*	0.733**	0.953	0.411	0.345	0.830	0.565**	0.260	0.315	1.000*	1	
NEUT	0.924*	0.406	0.427	0.773	0.622	0.340	0.914	0.807	0.933	0.941*	0.909*	1

***Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). Where: WBC=white blood cell; RBC=red blood cell; HGB=heamoglobin; HCT=heamatocrit; MCV=mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration; THROM=thrombocyte; LYM=lymphocyte; MXD=(monocytes+eosinophils + basophils); NEUT=neutrophil*

Table 4. Physico-chemical parameters of the test water

Variables	Control	T2(1.6 g/l)	T3(3.2 g/l)	T4(6.4 g/l)	T5(12.8 g/l)
Temperature (°C)	24.40 ± 0.03^a	24.60 ± 0.2^a	25.20 ± 0.02^b	25.43 ± 0.2^a	25.61 ± 0.13^c
Dissolved oxygen (mg/l)	6.55 ± 0.21^c	6.5 ± 0.20^c	4.7 ± 0.03^b	4.3 ± 0.20^a	4.0 ± 0.10^a
pH	6.81 ± 0.01^c	6.65 ± 0.14^c	6.31 ± 0.01^b	5.65 ± 0.31^b	5.04 ± 0.06^a

Data are mean ± standard error: means with different superscript within a row are significantly different (p<0.05)

5. CONCLUSION

The study revealed that sub-lethal concentrations of *Blighia sapida* stem bark extracts had debilitating effects on the haematological indices of *Clarias gariepinus* fingerlings. The extracts also interfered with the water quality. Thus, the introduction of the plant parts in natural environments may cause hazardous effects at various higher concentrations even to non-target organisms.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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