

Hematological and Histological Study of Toxicity of Ethanol Extract of Iron Wood, *Senna siamea* Leaves to African Sharp Tooth Catfish, *Clarias gariepinus* (Burchell 1822) Fingerlings

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Abstract

This study investigates the toxic potential of Iron wood, *S. siamea* to African Sharp tooth catfish, *C. gariepinus* (Burchell 1822) fingerlings. The bioassay used 360 fingerlings of *Clarias gariepinus* (mean total length, 19.7 ± 0.76 cm and mean weight 33.8 ± 1.62 g). Randomly selected 10 fish were exposed for 21 days to 0.0 mg/L, 2.5 mg/L, 5.0 mg/L, 7.5 mg/L, 10.0 mg/L and 12.5 mg/L of leaf extract respectively each with a replicate. 96 h LC₅₀ for leaves extracts was established at 31.62 mg/L using logit technique. Maximum Allowable Toxicant Concentration, MATC of the extract was established at 1.581 mg/L. Physicochemical parameters (pH and Dissolved Oxygen) of test media showed significant (P<0.05) concentration related reduction in values (Total Dissolved Solids and Conductivity) increased significantly (p<0.05) with increase in extract concentration. Temperature had non-significant (p<0.05) reduction at all extract concentrations. Blood analysis revealed significant (P<0.05) reduction in the blood parameters (Haemoglobin, Packed Cell Volume, Red Blood Cells, White Blood Cells, Platelet, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin) of exposed fish. From Pearson correlation matrix, changes in physicochemical parameters of test media produced good statistical correlation with changes in blood parameters except for temperature. Thus changes in physicochemical parameters enhanced the toxicity of the studied plant to the test organism. Histology of tissues (gills, intestine and liver) of exposed fish revealed broad areas of inflammation, cellular proliferation, degeneration, cerebrobrachial damage etc. in exposed fish. The observed haematological and histological changes resulted in disease conditions (anaemia, leukemia, oedema, hypoxaemia etc.) in exposed fish. *S. siamea* was found to be toxic to *C. gariepinus* juveniles and is recommended as piscicidal plant for use in aquaculture.

Keywords: Bioassay; Statistical correlation; Toxicity; Aquaculture

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Introduction

Toxicity expresses the degree to which a substance is poisonous, destructive or generally harmful to life [1]. Poisons or toxicants are chemicals which have harmful or adverse effects on living organisms and the study of toxic substances is known as toxicology [2]. In a broader sense, toxicity is concerned with the chemical nature, interaction with biological systems and safety evaluation of potentially poisonous materials [2,3]. Toxicity could be acute or chronic, depending on the dosage, nature and duration of effects of the toxin [2,4]. It is acute when the effects are rapidly occurring

resulting from exposure to a relatively large quantity of the poison administered often in a single dose [2]. Chronic toxicity occurs when an organism is frequently or infrequently exposed to low levels of poison over a long period of time often years. It is characterized by long term effects which could be lethal or sub-lethal [2].

Senna siamea is an angiosperm, native of Southeast Asia and widely distributed in Africa, Latin America and in Oceania [5]. The plant is of the genus, *Senna*, specie, *siamea* and family, initially was *Caesalpiniceae*, later reclassified in Leguminosae and now in Fabaceae [6]. *S. siamea* is a tree plant, 10-12 m tall (occasionally

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reaching 20 m). It has a short bole, the crown is dense and rounded at first and later become irregular and spreading. The young bark is grey and smooth and later develops longitudinal fissures. The leaves are alternate, 15-30 cm long, with 6-14 leaflets, each ending in a tiny bristle. The flowers are bright yellow and projects up to 60 cm long upright, with pyramid-shaped pinnacles. The fruits are flat with indehiscent pad 5-30 cm long, constricted between the seeds and containing about 20 seeds per pod. The seeds are bean-shaped, greenish-brown and 8-15 mm long [7]. In Nigeria, the plant is known as Bikini raskata, odan in different localities [7].

C. gariepinus (African Sharp tooth catfish) indigenous from Africa is classified into Kingdom: Animalia, Phylum: Chordata, Class: Actinopterygii, Order: Siluriformes, Family: Clariidae, Genus: Clarias and species: *Clarias gariepinus* [8]. The African Sharp tooth catfish is a large, eel-like fish, usually of dark gray or black colouration on the back, fading to a white belly. It has an adult length of 1-1.5 m and reaches a maximum total length of 1.7 m and can weigh up to 60 kg. *C. gariepinus* is one of the most important tropical catfish species for aquaculture in spite its commanding presence in the wild [8]. In Nigeria, it is widely cultured in ponds and occurs freely in natural freshwater. The fish has hardiness with high resistance to handling and stress [9,10]. *C. gariepinus* has high adaptation for low dissolved oxygen in water especially by fishes above 14 days old with functionally developed accessory respiratory organs [11,12]. It has long tolerance for drought but cannot survive long in water temperature below 9-10°C. These qualities account for its wide application in aquaculture and increased importance in ecotoxicological studies, hence its choice as test organism for the present study [8].

Materials and Methods

Location and materials for the experiment

The study was carried out in the Department of Fisheries and Aquatic Environmental Management, University of Uyo, (latitude 50 026' N and Longitude 70 55' E), Uyo, Nigeria (**Figure 1**).

Sources of experimental materials

Plant materials: The plant materials (fresh leaves of *S. siamea*) were obtained from the arboretum of the Department of Forestry and Natural Environmental Management, University of Uyo, Nigeria. The plant was identified according to Mamadou et al. [7]. The extraction and identification of phytochemicals were carried out in the laboratory of the Department of Pharmacognosy of the University of Uyo.

Test organism: The test organisms, 400 juveniles of *C. gariepinus* were purchased from Akanse's Farms Ltd. Abak. The fish were of age ten (10) weeks, weight (33.8 ± 1.6 g) and total length (19.70 ± 0.76 cm) (total length and weight are presented in Mean \pm Standard Deviation). The organisms were transported in oxygenated bags to the Hatchery/production unit of the Department of Fisheries and Aquatic Environmental Management, University of Uyo, Nigeria, where the study was conducted.

Fish feed: The feed was 2.5 mm skretting commercial fish feed supplied by De Dino Resources, Ltd. Uyo, Nigeria. Nutritional composition of the feed is presented in Table. The feed was stored under standard conditions as prescribed by the manufacturer (**Table 1**).

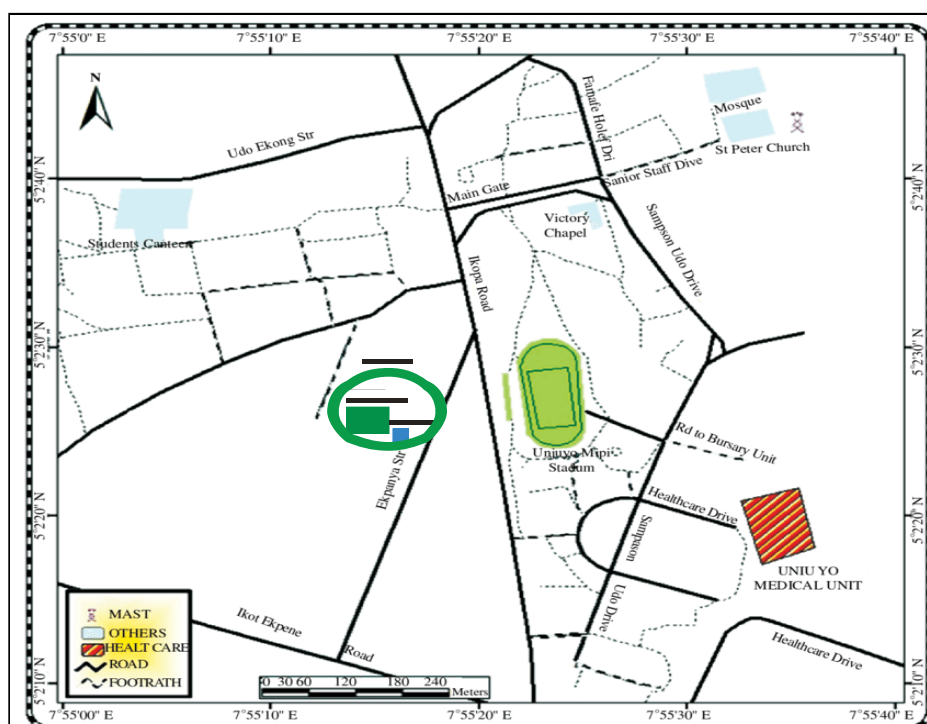


Figure 1 Map of University of Uyo showing study location.

Table 1 Nutritional composition of feed.

Macronutrient	% Composition	Micronutrient	Composition (mg/Kg)
Crude protein	43	Iron	58
Crude fat	12.5	Iodine	2.9
Crude fiber	2.5	Copper	7
Ash	7	Manganese	22
Calcium	1.5	Zinc	130
Sodium	0.3	Antioxidant	150
Phosphorus	0.9	-	-

Methods

Fish feed: Extraction was done by solvent extraction [13]. Isolation and quantification of active compounds from the extract were done by chromatography [14,15]. Collected plant specimen was cleaned, cut into smaller pieces, shade-dried to constant weight to obtain the dry matter and then pulverized to finer particles. Known weight of the pulverized dry matter was macerated in 10.0 L of 96% ethanol with intermittent agitation for 72 h to extract the active ingredients. The macerated dry matter was filtered to obtain the extract. Extract obtained was concentrated to constant weight using model HH-S stainless steel thermostatic water bath at 40 °C. Concentrated extract obtained was covered with foil paper and used as toxicant for the study.

Tannins: The sample, 500 mg of extract was dissolved in 50 ml of distilled water, homogenized in a mechanical shaker for 1 h and filtered into 50 ml volumetric flask. 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 0.1M FeCl₃ in 0.1N HCL and 0.008M Potassium Ferro cyanide. The absorbance of the resulting solution was measured at 120 nm using UV-2700 UV-VIS Spectrophotometer within 10 min to be the total tannins composition of the extract [15].

Saponins: The sample, 20 g of pulverized dry matter was macerated in 100 cm³ of 20% aqueous ethanol and heated for 4 h at 55°C (HH-S stainless steel thermostatic water bath) with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were concentrated to 40 ml at 90 °C (HH-S stainless steel thermostatic water bath). The concentrated extract was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated (HH-S stainless steel thermostatic water bath) and the resulting residue oven dried at 60 °C using Grab CAD Oven to constant weight. The saponin content was then calculated from the resulting residue [16].

Flavonoids: The method is based on the formation of flavonoid-aluminum complex which has an absorptivity maximum at 415 nm [17]. 100 ml of the plant extract in methanol (10 mg/ml) was mixed with 100 ml of 20% AlCl₃ in methanol and a drop of acetic acid and then diluted with methanol to 500 ml. The

absorption at 450 nm was read after 40 min using UV-2700 UV-VIS Spectrophotometer. Blank sample were from 100 ml of plant extracts and a drop of acetic acid and then diluted to 500 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same condition. All determinations were carried out in triplicate [18].

Alkaloids: The sample, 5 g of pulverized dry matter was macerated in 200 ml of 10% acetic acid in ethanol covered for 4 h. The mixture was filtered and concentrated (water bath) to one quarter of its original volume. Concentrated NH₄OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH₄OH. The residue was dried and weighed as the total alkaloid content of the extract [14,15].

Steroids: The sample, 1 g of pulverized dry matter was extracted by heating under reflux for 15 min with 10 ml Dichloromethane (DCM). Filtrate was evaporated to dryness (HH-S stainless steel thermostatic water bath) and residue was dissolved in 1 ml toluene. 50 µl of the toluene solution was applied to the chromatogram and was put in a pre-saturated tank containing toluene ethyl acetate and left in air-conditioned room at 18 °C for the solvent to rise to a distance of 15 cm. Reading was taken at 254 nm using UV-2700 UV-VIS Spectrophotometer, after treating the chromatogram with komarowsky reagent [14].

Glycosides: The sample, 1 g of pulverized dry matter was mixed with 5 ml of 50% methanol and 10 ml of 10% Lead (II) acetate solution, then heated (HH-S stainless steel thermostatic water bath) for 10 min and allowed to cool. Cooled filtrate was extracted with two separate 10 ml quantities of dichloromethane (DCM) and the extracts combined and completely evaporated (HH-S stainless steel thermostatic water bath). The residue was dissolved in DCM-methanol (1:1) and 100 µl of the solution was applied to the chromatogram and was put in pre-saturated tank containing ethyl acetate methanol-water (100:13.5:10) and was cooled in air conditioned room (18 °C) for the solvent to rise to a distance of 15 cm. Reading was taken at 365 nm using UV-2700 UV-VIS Spectrophotometer, after treating with Kedde reagent [19,14].

Preparation of test solutions: Test solutions were prepared by simple dilution principle and concentrations presented in milligrams per litre (mg/L). Desired weight of toxicant was accurately measured using electronic balance, spatula and 100 ml beaker of known weight. The desired weight of toxicant was dissolved in a known volume of water and the solution made up to required volume with water [6].

Acclimatization of test organisms: The juveniles of *C. gariepinus* used for this study were acclimatized for 14 days in the Hatchery/ Production unit of the Department of Fisheries and Aquatic Environmental Management of the University of Uyo. During this period, the fish were fed twice daily with 2.5 mm skretting commercial fish feed supplied by De Dino Resources, Uyo, Nigeria and water was changed after every 48 h. Feeding of organisms was ended 48 h prior to resumption of acute test and throughout the period of test to avoid interferences with stomach content [20].

Water quality tests: Water quality tests were carried out to ensure that optimum water conditions were maintained during the experiment, and that the results obtained were solely due to the effects of the toxicants on the fish samples. The physicochemical parameters of the test water with respect to temperature, dissolved oxygen (DO), pH, Total Dissolved Solids (TDS) and conductivity were measured using suitable water quality measurement instruments. Temperature was measured with HM Digital temperature thermometer, pH with ORP Pocket pH meter, TDS with ORP Pocket TDS tester, conductivity with ORP Combo-multimeter and DO with ORP Combo-multimeter. The measurements were taken before and during acclimation of the organisms, twice daily for the 96 h period of exposure of the organisms for the acute test and before and after every renewal of treatments for the chronic test.

Acute toxicity bioassay

Range finding test: Range finding test was conducted following standard procedure, to determine range of concentration for the definitive test [20]. The test was conducted in white translucent plastic aquaria (30 litres capacity) of dimension 52 x 33 x 22 cm each containing 20.0 L of standard solution of toxicant (treatment). Five (5) experimental set up of graded concentrations (0.1 mg/L, 1.0 mg/L, 10.0 mg/L, 100.0 mg/L, 1000 mg/L) of toxicant and a control, each with one replicate was considered for the test [20]. Aquaria were cleaned and sterilized using suitable reagents, sundried two days before commencement of test. Randomly selected ten (10) organisms were exposed to each aquarium containing 20.0 L of treatment. The experimental set up was covered with netting material to prevent the fish from jumping out and also prevent infestation by insects. Mortality in organisms was indicated by inability of fish to respond to external stimuli even on gentle prodding with a glass rod and no visible opercula movement [20-22]. Experimental set-up was monitored hourly in the first 4 h, four (4) hourly in the next 24 h and daily for the remaining 96 h and records of mortality and behavioural responses of organisms for the respective test solutions were recorded [19]. LC_{50} for the extract was established from mortality data generated using logit method [21,22].

Definitive test: Definitive test was conducted based on the result of the range finding test to establish LC_{50} for the extract. The test considered concentrations range of 10.0 mg/L to 100 mg/L for the extract, each with a replicate and using a spacing factor of 1.5. 10 healthy juveniles of *C. gariepinus* were randomly selected and exposed to 20 L of each treatment. The set up was observed hourly for the first four (4) h, four (4) hourly for the remaining twenty four (24) and daily for the remaining 96 h [20]. Water quality parameters were tested throughout the experiment and the Fish mortality data was generated. Inability of fish to respond to external stimuli even on gentle prodding with a glass rod and no visible opercula movement were used as indices of mortality in fish [20,22]. Confirmed death fishes were removed from the test media.

Maximum allowable toxicant concentration (MATC): MATC for the extract was determined using Boyd application factor equation, presented as eqn. (1).

$$AF = MATC / LC_{50} \quad (1)$$

AF is application factor, LC_{50} is 96 h LC_{50} . AF of 0.05 is suitable for most natural toxins, while 0.01 should be used for industrial chemicals or pesticides [23].

Chronic toxicity bioassay: Chronic toxicity bioassay was conducted to determine the toxicity of the extract on the blood parameters and tissue of sensitive organs of the exposed fish. The bioassay was designed to be intermediate termed chronic test with 21 days exposure using sub-lethal concentrations of the treatment (0.0 mg/L, 2.5 mg/L, 5.0 mg/L, 7.5 mg/L, 10.0 mg/L and 12.5 mg/L). 10 randomly selected juveniles of *C. gariepinus* were exposed to respective treatments for 21 days [20,22,24]. The treatments were renewed every 24 h and physicochemical parameters of the test media measured before and after every renewal [19]. At the end of the 21 days exposure, blood and tissue samples were collected from randomly selected fish from each treatment for further analysis.

Collection of blood and tissue samples: Blood samples were collected from randomly selected fishes from each treatment. The sample was collected from the caudal fin of the fish using hypodermic syringe and needle and preserved in EDTA treated bottles for haematological analysis. Fish tissues (gills, liver and guts) were obtained by dissecting open the lower part of the operculum to access the internal organs of fish. The organs of interest were carefully removed using suitable equipment's. Collected samples were preserved immediately in sample bottles containing 10% formalin and properly labelled against the respective organs and treatments. The samples were subsequently taken to anatomy laboratory for histological analysis.

Blood and tissue analysis: Collected blood samples were subjected to auto-haem analysis using BC 2800 auto-haem analyser in the haematology laboratory of the Department of Physiology, University of Uyo, Nigeria. Blood parameters of interest (Hb, PCV, RBC, WBC, PLT, MCV and MCH) were analyzed. Histological analysis was carried out on the tissue samples using Haematoxylin and Eosin Method [7]. The analysis involved the following histological operations/laboratory procedures, tissues were De-waxed and hydrated, stained in Ehrlich haematoxyline solution for 20 min, washed thoroughly in running tap water, differentiated in 1% acid and alcohol until only the cell nuclei retain the stain, blued in running tap water for 5 min, tissues were counter stained in 1% Eosin for 3 min, washed in water to remove excess Eosin, dehydrated in absolute alcohol, cleared in xylene, mounted in Polyesterene Dibutylephthalate xylene (DPX), examined under x 10 objective for deformities using Olympus B x 51 Microscope.

Statistical analysis: Data collected were analyzed using descriptive statistics (mean, standard deviation, frequencies and percentage). Comparison of data of physicochemical parameters and Blood parameters between the control and other treatments were carried out using Analysis of Variance (ANOVA). Duncan's statistical test was used to further analyze for significance in changes in physicochemical parameters of test media and changes in blood parameters as the concentration of treatments

increased. Statistical correlation of changes in physicochemical parameters with changes in blood parameters was tested using Pearson correlation matrix.

Ethical issues

The ethical issues of conducting toxicity test were all observed in every aspect of this research work. All physical and emotional harm that would have resulted from the conduct of this research were carefully avoided at every stage of the work. The test organisms were treated in line with professional recommendations throughout the period of the study. Experimental wastes generated in the course

of the study were properly disposed in an environmentally friendly manner. Plagiarism policies were strictly adhered to in developing the research report. Data generated from the experimental work were reported exactly as observed, devoid of data doctoring or any form distortion of experimental outcome [25]. Every illegitimate use of computer and internet were carefully avoided in the conduct of this research work. The printing materials used in printing this report were those manufactured from sustainable and environmentally friendly processes.

Results and Discussion

Results

Extraction and phytochemical screening: Extraction and concentration of the plant extract yielded 117 g and its phytochemical analysis yielded as presented in **Table 2**. The results show that *S. siamea* contains plant secondary metabolites. These metabolites have been reported to be toxic bioactive substances [22,26] (**Table 2**).

Acclimatization: Test organisms were successfully acclimatized for 14 days with less than 2.0% total mortality (3 organisms of 400 died, 0.75% mortality).

Physicochemical parameters of water of acclimatization: Changes in physicochemical parameters of water of acclimatization were observed to be within acceptable limit for survival and growth of *C. gariepinus* [26].

Range finding test: LC_{10} and LC_{50} of the extract were established as shown in **Figure 2**. Other behavioral responses to toxicity (restlessness, erratic movement, repeated attempts to jump out of culture medium, frequent opecula movements, occasional state of motionlessness, excessive mucus secretion, adoption of different postures, swirling movements, loss of balance and loss of reflex) were also observed in exposed fish (**Figure 2**).

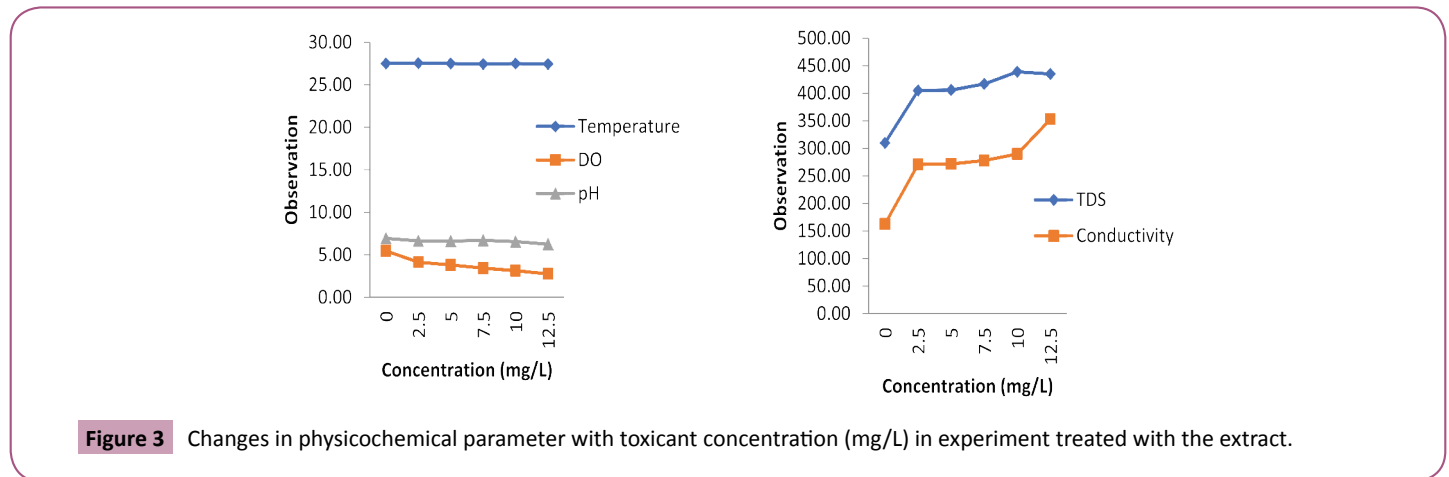
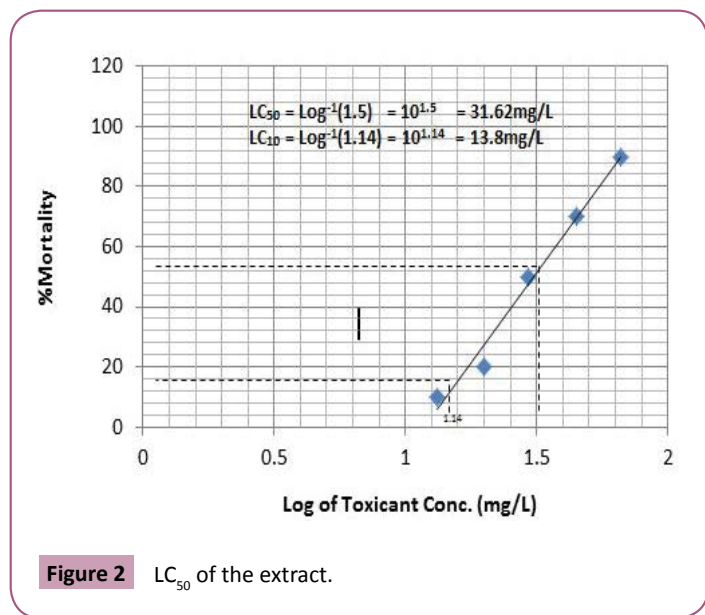
Definitive test: Results of definitive test are presented in **Figures 3 and 4**. Raw data generated from the definitive test are summarized in **Tables 3 and 4**.

Physicochemical parameters of test media: Results of physicochemical parameters of the respective test media are presented in **Figure 4**.

Table 2 Phytochemical contents of the extracts.

Phytochemical	Extract
Alkaloids	√√
Steroids	√√
Saponins	√√
Glycocides	√√
Tannins	√√
Flavonoids	√√

√√: High volume >1.0 mg



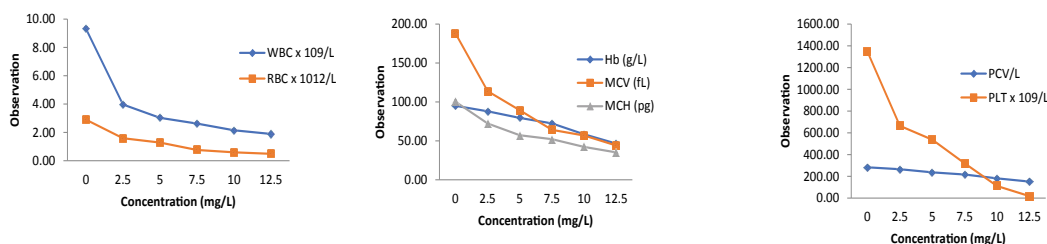


Figure 4 Changes in blood parameters with toxicant concentration in fish exposed to various concentrations of the extract.

Table 3 Physicochemical parameters with toxicant concentration in experiment treated with the extract.

	0.0 mg/l	2.5 mg/l	5.0 mg/l	7.5 mg/l	10.0 mg/l	12.5 mg/l	Total
Temperature	27.50 ± 0.50 ^a	27.55 ± 0.45 ^a	27.50 ± 0.50 ^a	27.45 ± 0.55 ^a	27.50 ± 0.50 ^a	27.45 ± 0.45 ^a	27.49 ± 0.15
DO	5.45 ± 0.12 ^a	4.15 ± 0.68 ^b	3.81 ± 0.51 ^{bc}	3.44 ± 0.25 ^{bc}	3.13 ± 0.04 ^{bc}	2.77 ± 0.16 ^c	3.79 ± 0.28
pH	6.94 ± 0.01 ^a	6.65 ± 0.05 ^{ab}	6.62 ± 0.02 ^{ab}	6.70 ± 0.20 ^{ab}	6.55 ± 0.05 ^{bc}	6.25 ± 0.05 ^c	6.62 ± 0.07
TDS	310 ± 0.00 ^a	405 ± 11.00 ^b	406 ± 10.00 ^b	417 ± 19.00 ^b	439 ± 24.00 ^b	435 ± 16.00 ^b	402 ± 13.81
Conductivity	163 ± 0.00 ^a	271 ± 46.00 ^{ab}	272 ± 35.00 ^{ab}	278 ± 32.00 ^{ab}	290 ± 20.00 ^b	353 ± 47.00 ^b	271.17 ± 19.76

Means with different superscripts along the same row are significantly different (Duncan's test) p<0.05

Table 4 Changes in blood parameters of fish treated with various concentrations of the extract.

	0.0 mg/l	2.5 mg/l	5.0 mg/l	7.5 mg/l	10.0 mg/l	12.5 mg/l	Total
Hb (g/L)	95 ± 0.00 ^a	88 ± 1.00 ^{ab}	79.5 ± 6.50 ^{bc}	72.5 ± 11.50 ^{bc}	58.5 ± 5.50 ^{cd}	46.5 ± 3.50 ^d	73.33 ± 5.34
PCV/L	283.5 ± 1.5 ^a	266 ± 1.00 ^{ab}	236.5 ± 19.50 ^{ab}	216.5 ± 27.50 ^{bc}	181.5 ± 14.50 ^{cd}	152 ± 2.00 ^d	222.67 ± 14.49
WBC x 10 ⁹ /L	9.32 ± 1.29 ^a	3.97 ± 1.14 ^b	3.03 ± 0.24 ^b	2.62 ± 0.05 ^b	2.15 ± 0.10 ^b	1.89 ± 0.03 ^b	3.83 ± 0.80
RBC x 10 ¹² /L	2.9 ± 0.81 ^a	1.59 ± 0.21 ^{ab}	1.28 ± 0.14 ^b	0.76 ± 0.27 ^b	0.59 ± 0.28 ^b	0.49 ± 0.20 ^b	1.27 ± 0.27
PLT x 10 ⁹ /L	1347 ± 402.00 ^a	664 ± 0.21 ^b	537 ± 120.00 ^{bc}	319.5 ± 29.50 ^{bc}	111 ± 76.00 ^{bc}	17.89 ± 16.31 ^c	499.4 ± 142.77
MCV (fl)	188 ± 6.00 ^a	113.85 ± 15.95 ^b	89.35 ± 5.45 ^c	64.15 ± 18.45 ^c	56.95 ± 17.35 ^c	44 ± 5.10 ^c	92.72 ± 15.07
MCH (pg)	100.25 ± 1.75 ^a	72.35 ± 13.75 ^{ab}	57.2 ± 14.70 ^b	52.2 ± 12.10 ^b	42.25 ± 7.05 ^b	35.25 ± 5.65 ^b	59.92 ± 7.18

Means with different superscripts along the same row are significantly different (Duncan's test) p<0.05

Table 5 Pearson correlation matrix for changes in physicochemical parameters with changes in blood parameters of fish treated with the extract.

Variables	Temp	DO	pH	TDS	EC	Hb	PCV	WBC	RBC	PLT	MCV	MCH
Temperature	1											
DO	0.33	1										
pH	-0.13	0.73	1									
TDS	-0.31	-0.91	-0.64	1								
EC	-0.47	-0.93	-0.71	0.9	1							
Hb	0.31	0.87	0.67	-0.79	-0.82	1						
PCV	0.28	0.87	0.66	-0.79	-0.8	1	1					
WBC	0.19	0.92	0.72	-0.92	-0.82	0.7	0.72	1				
RBC	-0.2	0.83	0.74	-0.78	-0.69	0.7	0.72	0.79	1			
PLT	0.21	0.9	0.73	-0.85	-0.78	0.81	0.83	0.93	0.72	1		
MCV	-0.1	0.89	0.82	-0.83	-0.74	0.73	0.75	0.92	0.94	0.88	1	
MCH	-0.35	0.67	0.79	-0.68	-0.52	0.66	0.69	0.77	0.83	0.79	0.9	1

Values in bold are different from 0 with a significance level alpha=0.05

Blood analysis: Results of the blood analysis of Fish from the respective treatments are presented in **Figure 4**. During the 21 days chronic toxicity bioassay, physicochemical parameters of the culture medium exhibited variation in values. No significant change (P<0.05) in Temperature was observed. DO and PH were observed to reduce significantly (P<0.05) while TDS and conductivity were observed to increase significantly (P<0.05). The variations were

all observed to be concentration related. Duncan's statistical test revealed that changes in pH of test media were significant (p<0.05) only at higher concentrations (10.0mg/L and 12.5mg/L). Results from blood analysis show that all the parameters measured, reduced in value when compared with the control [27]. The reductions were observed to be related to concentration of the extracts [28,29]. Statistical analysis of data from blood parameters

revealed that the observed reduction in the respective parameters (Hb, PCV, WBC, RBC, PLT, MCV and MCH) were all statistically significant ($P < 0.05$) [29]. However, reduction in PCV tested non-significant ($p < 0.05$) at lower concentrations (5.0 mg/L and 2.5 mg/L) and Hb, RBC and MCH all tested non-significant ($p < 0.05$) at lowest concentration (2.5 mg/L) of the extract. Reduction in WBC, PLT and MCV tested significant ($p < 0.05$) at all concentrations of the extract administered (Figure 4) (Tables 3 and 5).

Tissue analysis: Results of tissue analysis of fish from the respective treatment are presented in Figures 5-7.

Discussion

The use of haematological techniques in fish culture is of growing importance to toxicological research, environmental monitoring and fish health conditions [30]. Fish are so intimately associated with the aqueous environment, often physical and chemical

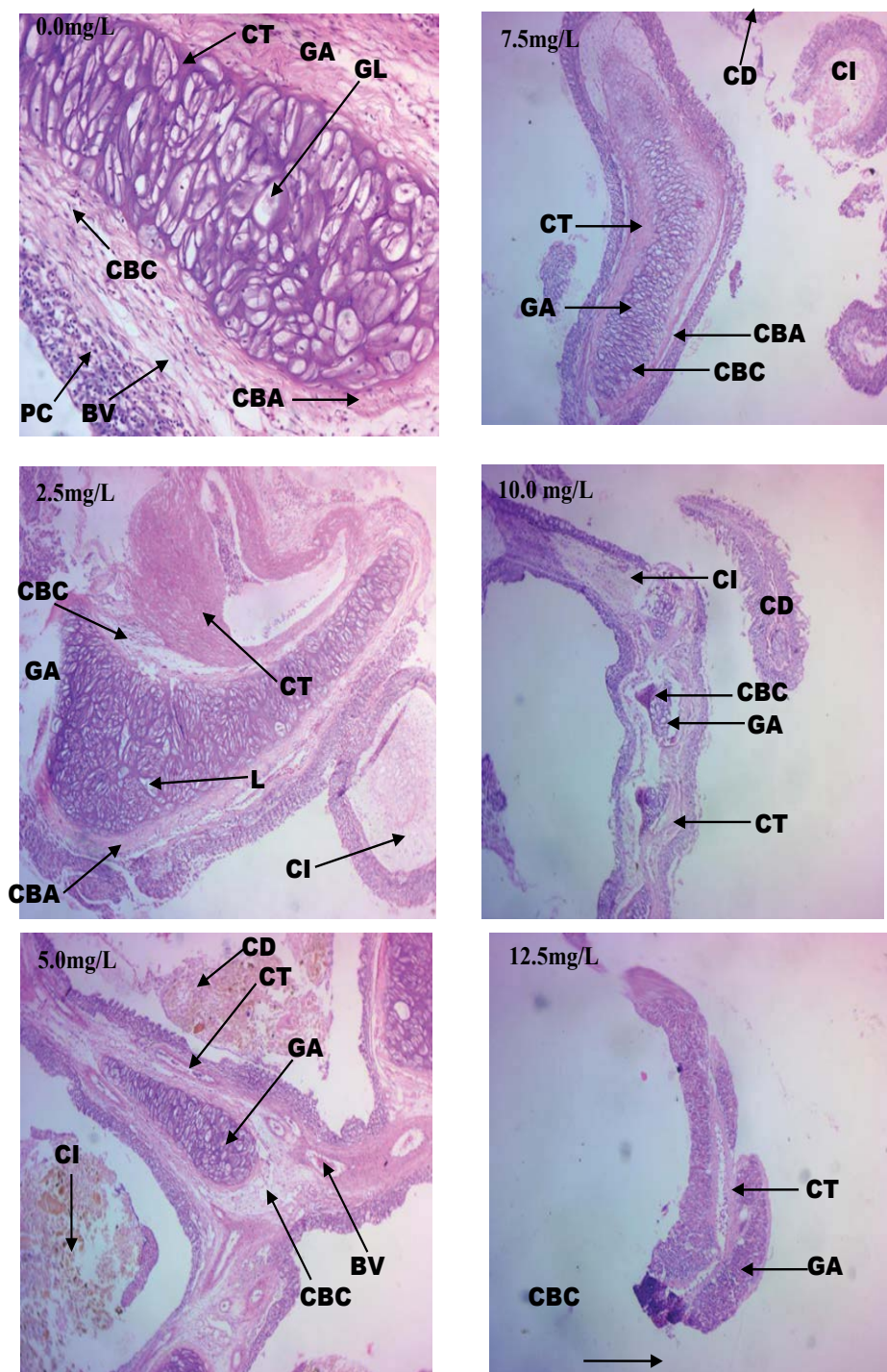


Figure 5 Photomicrograph of gill tissue of fish exposed to various concentrations of leaf extract (mg x 100).

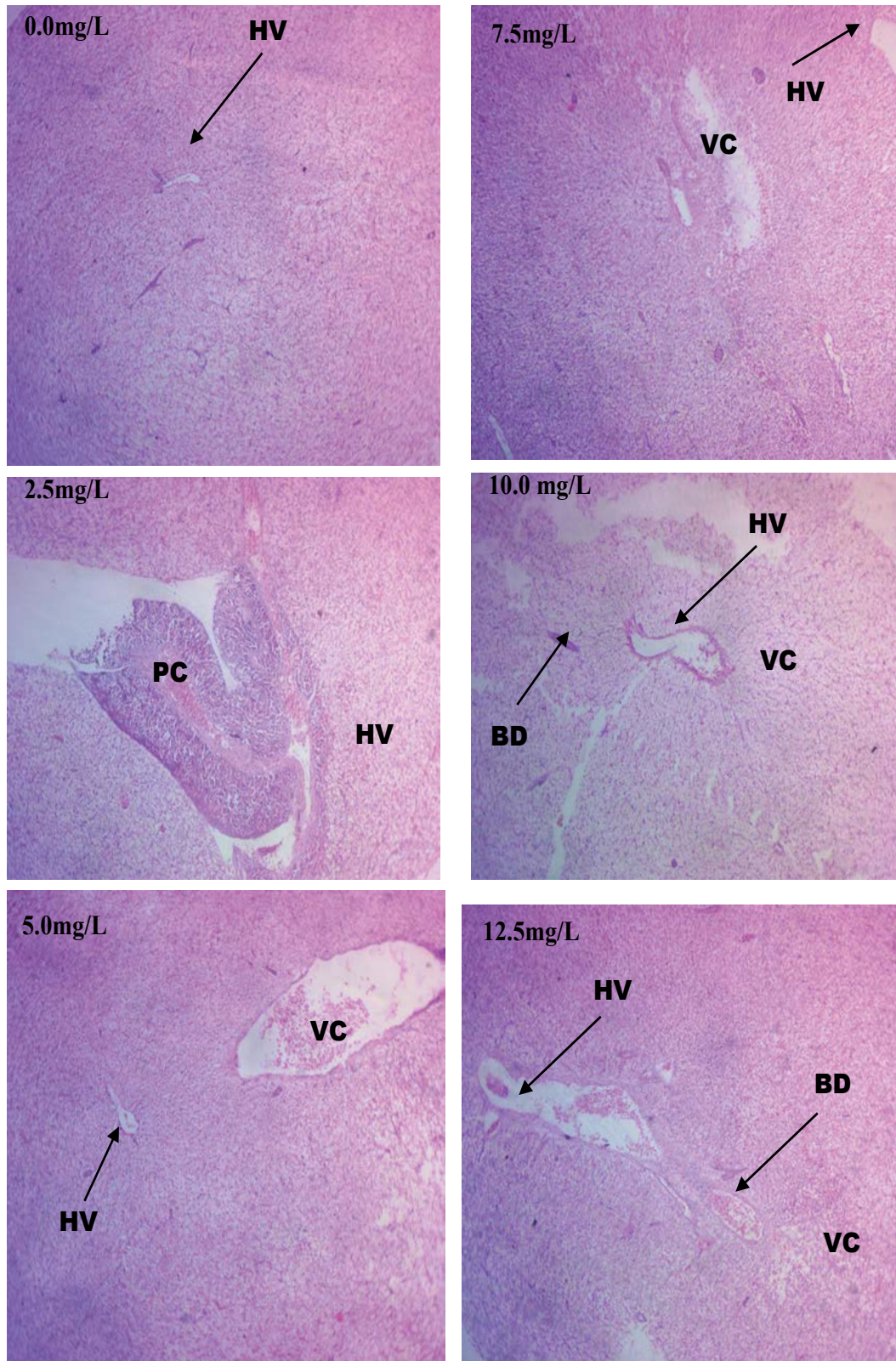
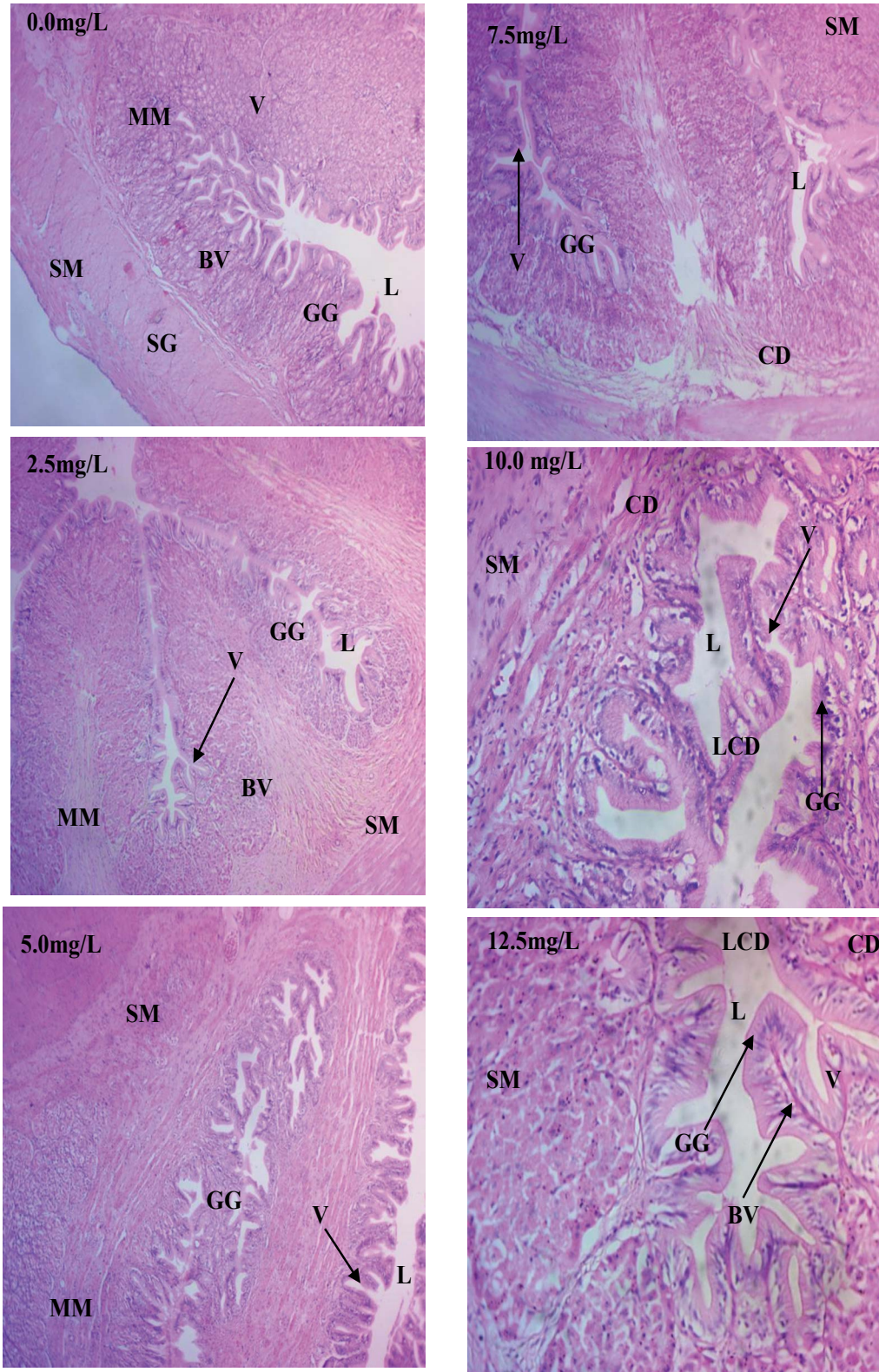


Figure 6 Photomicrograph of liver tissue of fish exposed to various concentrations of leaf extract (mg x 100).



Abbreviations: GG: Gastric Gland; SM: Smooth Muscle; L: Lumen; V: Vilus; BV: Blood Vessels; CD: Cellular Denaturation; LCD: Lining Cell Degeneration; SG: Submucous Gland

Figure 7 Photomicrograph of intestine tissue of fish exposed to various concentrations of leaf extract (mg x 100).

changes in the environment are rapidly reflected as measurable physiological changes in the fish [17,30]. Haematological parameters and physiological profile can be useful indicators of the physiological disturbances in animals and so can be crucial in providing vital information on the general well-being of fish [31]. Blood cell indices (RBC, WBC, etc.) are good indicators of system response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood. Thus detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat [32]. Therefore, blood serves as a good indicator to determine the health of an organism and acts as a pathological reflector of the whole body. Hence haematological parameters are important in diagnosing the functional status of animals exposed to toxicants [30].

In this study, behavioural responses observed in exposed fish were related to concentration of the extract as more of the responses were observed at higher concentrations of the extract [22,27,33]. The observed behavioural abnormalities are attributed to respiratory impairment, resulting from the effects of the toxicant on the gills of the exposed fish [12]. The reductions in blood cell indices and tissue deformation observed from the chronic bioassay are in line with findings of the study on behavioural, haematological and histopathological changes in *C. gariepinus* exposed to 2,4-dichlorophenoxyacetic acid [29]. Similar observations have also been reported in a study on exploitation of ethanol extract of *Adenium obesum* stem bark as a potent organic piscicide [22]. In a study conducted by Olusegun and Adadayo on Haematological Responses, Serum Biochemistry and Histology of *C. gariepinus*, exposed to sublethal concentrations of cold water fresh root bark extract of *Plumbago zeylanica* (Leadwort), it was reported that, exposure of fish to the toxicant for 21 days resulted in remarkable anaemic condition in *C. gariepinus* resulting from reductions in values of haematological parameters (RBC, Hb, PCV) in the juveniles of *C. gariepinus* exposed to *P. zeylanica* extract [30]. The study also reported histopathological alterations observed in the brain, gills, liver, intestine and muscle/flesh of the *C. gariepinus* juveniles [30].

Stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintain its homeostatic state [34]. If severe or long lasting, the response then becomes maladaptive and threatens the fish health and well-being. Therefore, in the presence of stressors (contaminants/pollutants), blood parameters and blood chemistry can be employed as standard laboratory test to determine diseased conditions and metabolic disturbances in fish [34]. Blood is a tissue fluid and serves as transport medium whose primary function is to supply oxygen and nutrients as well as constitutional elements to tissues and to remove waste products. Blood also enables hormones and other substances to be transported between tissues and organs. Blood is basically composed of the plasma, red blood cells, white blood cells and platelets, each with sub constituents which collectively contribute to the overall functioning of the blood [34,35].

The plasma is made up of 90% water, 7-8% soluble protein, 1% CO₂ and 1% plasma salt all to maintain blood osmotic integrity. RBCs contain Hb which transports oxygen from the respiratory organ to the body. Hb consists of four subunits each made up of a haem, a porphyrin ring containing iron and a polypeptide [35,36]. As a haem takes up an O₂ molecule, there is a rearrangement of the subunits that facilitates the uptake of additional O₂. The amount of O₂ in blood is determined by the amount dissolved, the Hb concentration and the affinity of Hb for O₂ [35]. WBCs (Basophils, Eosinophils, Neutrophils, Monocytes, B-and T-cell Lymphocytes) synthesize, store and release histamine which is important for allergic reactions. The WBCs help the immune system and as well remove toxins, waste, foreign bodies and abnormal or damaged cells by phagocytosis. Platelets secrete factors that increase local platelet aggregation e.g., Thromboxane A, enhance vasoconstriction e.g., serotonin and promote blood coagulation e.g., Thromboplastin [37].

These observations in blood parameters confirm concentration related toxicity of the toxicant to the test organism [28,29]. The observed reduction in the respective blood parameters results in diseased conditions such as haemolytic anaemia, leukemia and lymphopaenia in exposed fish resulting from interference with blood cells formation process (erythropoiesis) and destruction of blood cells by the toxicants [29,35]. The reduction in MCV and MCH is attributed to the reduction in cellular blood iron denoting iron deficiency in blood of exposed fish [27]. The observed reduction in blood parameters is also attributed to haemolysis resulting from haemodilution, a mechanism for diluting the concentration of pollutants in the circulatory system [28].

From Pearson correlation matrix, changes in physicochemical parameters of culture media showed good correlations with changes in blood parameters in fish treated with the extract. However, temperature showed no correlation with any of the blood parameters measured. DO and pH had positive correlation while TDS and conductivity on the other hand negative correlation with the measured blood parameters. Conductivity and pH showed poor correlation (-0.43 and 0.22 respectively) with RBC, for treatments with flower extract). These observations show that changes in the blood parameters of the exposed fish was not only due to the toxicity of the extract as a single factor but acted in synergy with the resulting changes in physicochemical parameters of test media. This is in line with the findings of the study on effect of bioactive compounds extracted from euphorbious plants on haematological and biochemical parameters of *Channa punctatus*, which reported correlation of changes in blood cell distribution with changes in environmental conditions [27].

Physicochemical factors of the extracellular environment influence the chemistry of the intracellular fluid and accounts for the osmotic integrity of the intracellular environment [10]. Reduction in factors like DO leading to reduced availability of O₂ for blood activities could result in hypoxaemic respiratory failure in exposed fish. O₂ saturation of haemoglobin has been reported to have a direct proportional relationship with the pH [35]. Thus, the observed decreased in pH of the test media inhibited the

uptake of O₂ in blood of exposed fish, hence contributed to the hypoxaemic respiratory failure in exposed fish. On the other hand histological changes caused by the toxicant in exposed fish alters the molecular configuration of Hb constituents affecting their affinity for O₂ and the overall O₂ carrying capacity of the blood [35,37]. Cellular abnormalities revealed from tissue analysis were also observed to increase in severity with concentration of the respective treatments, denoting a concentration related toxicity of the extract [29,33,37]. The above observations established disease condition in exposed fish including oedema (reported as inflammation), kanjopyknosis, cellular necrosis, cellular degeneration, vascular congestion, nuclear fragmentation, Cellular disintegration etc. The observed changes in the histology of the tissues of exposed fish are in line with the general responses of fish organs to environmental pollutants [38].

Fish gills are the prime target organ of all pollutants due to their extensive surface in contact with the external medium and the reduced distance between the external and internal medium. Gill morphology and morphometric are important biomarkers providing a rapid method for detection of the effects of pollutants [39]. In histopathological studies, gills have also been reported to act as storehouse for bio accumulating toxicants [39]. The general morphological changes in the gills recorded in this study have been reported in *Astyanax* sp. after 96 h brief exposure to water soluble fraction of crude oil and *C. gariepinus* under brief or prolonged exposure to plant extract [39]. In some of the studies brief exposure to toxicants for about 96 h have produced irreversible changes in the gills [2,40]. The changes in the gills were adaptations by the fish to cope with challenge of the toxicant [28].

Vascular changes in gills of exposed fish could be attempts by the fish to supply more blood to the gills to increase oxygen uptake and supply to the internal organs. The gill of fish is a multi-purpose organ that, in addition to providing for aquatic gaseous exchange, plays a dominant role in osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous waste. Thus, despite the fact that all fish groups have functional kidneys, the gill epithelium is the site many processes that are mediated by the renal epithelia in terrestrial vertebrates. Hence, impairment of the gill functions by the overall effect of the pathological changes in the gill of exposed fish will have grave consequences for the fish with respect to the normal functions of the gills [28]. Photomicrograph of histologic sections of the intestine from the respective treatments revealed comprehensive cellular profile of the intestine with cellular abnormalities observed. The observed abnormalities include vascular congestion, cellular inflammation, lining cells degeneration and cellular disintegration.

Results obtain for Liver tissue could be due to high detoxification activity of the organ against the toxic effects of the treatments, which could have been the reason for the slight portal constriction, vascular congestion, hepatocytosis, etc. resulting from excessive work done by the organ in detoxifying the toxicity of the toxicant. The observed deformations are attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to toxicants. Increased level of vacuolation of hepatocytes has

been reported as a signal to degenerating process that suggest metabolic damage possibly related to exposure to contaminated water [17]. The liver of freshwater fish is known to accommodate a high level of toxicants [17,32].

The toxicity of the studied plant extracts to the test organism is due to its bioactive constituents [16,41]. These bioactive constituents have various chemical properties and interfere with the physiology of the test organism. Saponins which are directly absorbed through the gills, haemolyses erythrocytes, due to their ability to lower the surface tension between the aqueous and the lipid phases of the membranes of erythrocytes resulting in the emulsification of their lipid components with Na⁺, water influx and K⁺ efflux until they rupture and release their haemoglobin content [1,2,5]. The toxicity might have also been through impairment of oxygen consumption in the exposed fish as saponins are also reported to lower the surface tension of reconstituted extracts with the formation of colloidal substances within them. Saponins are also known to cause structural damages in the respiratory and intestinal epithelia of exposed fish [22].

Tannins have been reported to have protein coagulating property on gill epithelia, causing respiratory failure or asphyxiation in the exposed fish. Observed toxicity might also be due to binding and subsequent inhibition of endogenous proteins by tannins in addition to causing intestinal damage and interference with iron absorption [2]. The toxicity could also result from impaired oxygen consumption by tannins, via its inhibition of oxidative phosphorylation involving the blockage of mitochondrial enzyme [14]. This is in agreement with the reported inhibition of pyridine-linked substrates oxidation suggestive of interference with oxidative phosphorylation in ticks exposed to aqueous extract of *Adenium obesum* stem bark [22]. The observed toxicity is also attributed to the inhibition of Na⁺/K⁺-Atpase pump caused by cardiac glycosides resulting in a variety of severe arrhythmias, subsequent blockage of cardiac activities, decreased cardiac output and death in some of the exposed fish [35,36]. Alkaloids constituent of the extracts is known to impair oxygen consumption via inhibition of the activities of NADH ubiquinone reductase and subsequent inhibition of oxidative phosphorylation in exposed fish [7].

Conclusion and Recommendations

From the present study, the levels of Ni and Fe are all lower in the fruit vegetables than those in the leafy vegetables. It is found that the Ni levels in the vegetables are highly correlated with the three geochemical and NR fractions of the habitat top soils. The Fe levels in the vegetables are highly correlated with the 'acid-reducible' fraction of the habitat top soils. The positive relationships indicated the potential of edible vegetables as good biomonitors of Ni pollution in the habitat top soils. For the health risk assessment, all the THQ values for Ni and Fe in the 18 vegetables investigated in both adult and children are all below 1.00. This indicated that there was no non-carcinogenic risk of Ni and Fe to the consumers for both adults and children. Nevertheless, regular monitoring and management of the vegetable farms is still needed.

Conclusion

From the present study, it is seen that *S. siamea* is toxic to *C. gariepinus* fingerlings. Ethanol extract of leaves of *S. siamea* influence the physicochemistry of the culture media, affect the chemistry and overall osmotic integrity of the blood and cause tissue (gill, intestine and liver) damages in *C. gariepinus* fingerlings, resulting in observable behavioural abnormalities and eventual death of exposed fish. These confirm substantial piscicidal potential of *S. siamea* to *C. gariepinus* fingerlings for use in aquaculture.

Recommendations

Based on findings from the present study, it is recommended as

follows:

- *S. siamea* has high piscicidal potential to *C. gariepinus* and as such is suitable for use by fish farmers as piscicidal plant.
- 96 h LC₅₀ of 31.62 mg/L for leaf extract of *S. siamea* is recommended for use as piscicide by fish farmers.
- MATC of 1.581 mg/L for leaf extract of *S. siamea* to *C. gariepinus*.
- Further investigation should be conducted by researchers on the ichthyotoxicity of other parts of the plants and to other aquatic species.

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