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Haematological Responses, Serum Biochemistry and Histology of *Clarias gariepinus* (Burchell, 1822) Exposed to Sublethal Concentrations of Cold Water Fresh root Bark Extracts of *Plumbago zeylanica* (Leadwort)

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Abstract

The effect of sub lethal concentrations of fresh root bark extracts of *Plumbago zeylanica* on blood compositions of *Clarias gariepinus* was investigated under static water renewal bioassay during a 21-day exposure period. Concentrations of *Plumbago zeylanica* extracts used were 0 (control), 26, 39 and 59 mgl^{-1}. The exposure led to anaemic response in fish, this was observed to be time and dose dependent. There were significant decreases in haematological values of PCV (20.66 ± 2.84), Haemoglobin (6.73 ± 0.87), RBC (1.70 ± 0.02), MCV (32.67 ± 0.33), MCHC (38.67 ± 4.33), and MCH (119.67 ± 14.84) at 59 mgl^{-1}. While slight increase was observed in WBC (16016.67 ± 1717. 63) at 39 mgl^{-1}. The muscle/flesh of the exposed fish showed goblet cell hyperplasia, muscle necrosis and calcification at lower concentrations of exposure for both the short term and long term exposure, but disappears at higher concentrations after one week withdrawal without bioaccumulation in the Organs and Tissues. The changes observed indicates that haematological indices could be used as risk assessment tools in aquatic environmental monitoring to assess physiological status of fish exposed to sub lethal effect of toxicants.

Keywords: Botanicals; *Clarias gariepinus*; Haematology; *Plumbago zeylanica*

Introduction

The use of haematological techniques in fish culture is of growing importance to toxicological research, environmental monitoring and fish health conditions. Fish are so intimately associated with the aqueous environment, often physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in the fish. Blood is a good indicator to determine the health of an organism. It also act as a pathological reflector of the whole body, hence haematological parameters are important in diagnosing the functional status of animals exposed to toxicants.

Application of synthetic pesticides is one of the methods used to control fish population. Due to their long-term persistence, slow degradability in water, toxicity to other organisms [1] and accumulation inside the fish body, synthetic piscicides adversely affect the aquatic environment [2]. To solve this problem, studies have been carried out on the possibility of using plants as piscicides [3] and they are considered safe for users. *Plumbago zeylanica* belong to the family Plumbaginaceae, a native of warm temperate to tropical regions of the world. *P. zeylanica* is found in southern Nigeria where it grows as ordinary shrub. It is commonly called Leadwort. The root contains a pungent, yellowish substance that is called plumbagin, sucrose, fructose and protease [4]. Lemma [5] reported that a minimum inhibitory concentration value of Plumbagin showed a comparative activity resembling the commonly used broad spectrum antibiotic, tetracycline. Currently, there is scanty information in the literature as regards the use of *Plumbago zeylanica* as a botanical piscicide in developing countries of the world. The paper present findings of an investigation on the haematological response and biochemical parameters of *Clarias gariepinus* exposed to chronic sub-lethal concentrations of fresh root bark cold water extract (FRBCWE) of *Plumbago zeylanica* under laboratory conditions.

Materials and Methods

Plant collection and processing

Plumbago zeylanica (authenticated by a forest taxonomist) was collected at the tree nursery unit of the Department of Forestry Resources Management, University of Ibadan, Nigeria. The fresh root-bark of *Plumbago zeylanica* was obtained by peeling off the root bark of the plant with a knife. These were cut into pieces and grinded in a mortal with pestle to obtain macerated samples for the cold water extraction. Ten grammes of the macerated freshly prepared samples of *Plumbago zeylanica* were placed into a conical flask and 100 ml of cold distilled water was added for ten minutes and shaken before filtration using a dry Whatman® filter paper into a measuring cylinder. The filtrates were then used for the toxicity tests.

Test fishes acclimatization for the toxicity tests

Clarias gariepinus juveniles of mean weight 22.49+2.88 g were held in translucent plastic aquaria of 55 litres capacity. These were fitted with translucent mosquito net covers as part of the safety measure to avoid fish escape from the aquaria during the experimental period. Aeration of water was constantly maintained with the use of single outlet 200V/50 Hz 3W capacity Taiyomax® aerator pumps operating at 2.5 litres/minute. The fishes were acclimatized for 14 days at water temperature of 23-25°C, 12 hr light-dark cycles and pH 7.0-7.2 before the commencement of the toxicity experiments. Fishes were hand fed twice daily with a commercial floating feed (Coppens(R)) containing 45% crude protein at 3% body weight. Uneaten feed and feacal wastes

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were siphoned out on a daily basis and the water is replaced every 24-hours to avoid pollution of culture media as recommended by [6]. Water quality parameters such as temperature, dissolved oxygen Ammonia-nitrogen, Nitrite-nitrogen, Carbon (IV) oxide and pH of the experimental setup were monitored during acclimation and the definitive chronic-sub lethal toxicity test with LaMotte aquaculture test kit in line standard methods [7]. The parameters were recorded daily during fish acclimation; before the experiment and on weekly basis for Chronic-sub lethal toxicity test.

Range finding and definitive test

A range finding concentration experiment was carried out for 24 hours following the procedure of Solbe [8]. Three *C. gariepinus* juveniles were stocked in each aquarium with the following graded concentrations of cold water extract of *P. zeylanica* fresh root-bark: 0 mg/l, 0.1 mg/l, 1 mg/l, 10 mg/l, 100 mg/l, and 1000 mg/l. Based on the result of the range finding test, a definitive test was carried out using a concentration range between 1000 mg/l and 100 mg/l of solution of water extract of *P. zeylanica* fresh root-bark. Spacing factor of 1.5 was used to determine the definitive toxicity concentration used during the 96-hrs bioassay. The spacing factor was modified to suit the environment according to Odiete [9]. The concentration used were 666 mg/l; 444 mg/l, 296 mg/l, 198 mg/l, 132 mg/l and 0 mg/l respectively. This led to ranges used for the definitive test.

The definitive test was the static renewable type (toxicity test in which no flow of test solution occurs; solutions are renewed every 24 hours throughout the duration of the test) in line with Reish and Oshida [10]. Ten juvenile of *C. gariepinus* were introduced into each aquarium containing 10 litres of solution. The aquaria were covered with netting materials to prevent the fish from jumping out throughout the duration of the experiment. The mortality of the fishes in each aquarium was monitored. The behavioural pattern of the fishes during the experiment was closely observed. The experiments were carried out using completely randomized design (CRD). The chronic-sub lethal toxicity test involved four treatments (0, 26, 39, and 59 mg/l) and three replicates each for 21 days exposure.

Haematological and biochemical assessment

Haematological and biochemical examination were carried out on the Juveniles of the test fish (*C. gariepinus*) at 0 (initial), 21 days of exposure and 1 week after withdrawal (to assess recovery of fish) from the chronic sub lethal concentration of cold water extract of fresh root bark of *P. zeylanica*. Blood samples were collected at the beginning of the experiment (day 0) and at the end of the experiment (day 21) from the caudal peduncle of both the exposed and control fish as described by Stockopf [11]. The blood samples were dispensed into tubes containing lithium heparin anticoagulant. Red blood cell (RBC) and White blood cell (WBC) were counted by Neubauers haemocytometer. Haemoglobin (Hb) was estimated by Cyanomethemoglobin method as described by Kelly [12]. Packed cell volume (PCV), Mean Corpuscular haemoglobin concentration (MCHC), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated respectively using standard formula described by Dacie and Lewis [13].

Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum biochemical parameters. Serum from the centrifuge blood were carefully siphoned out and the concentration of total proteins, Albumin, globulin were estimated. The serum total proteins and Albumin levels were determined using the methods described by [14]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by the method described by Reitman and Frankel [15].

Histopathological examination

Histopathological examinations were carried out on the brain, liver, intestine, flesh/muscle, and gills of the test fish from each treatment. These organs and tissues were preserved in a small plastic vials in line with procedure described by Rodrigues [16]. Organs and tissues were dehydrated for 1 hour in different grades of alcohol (50%, 70%, 90% and 100% respectively) to remove excessive water in the organs and tissues, and were further cleared in three changes of xylene solution to remove the alcohol in line with Ofogba [17]. Molten paraffin wax was used to impregnate the organs and tissues in a vacuum oven at 56°C and was allowed to solidify following the procedures of Luna [18]. Organs and tissues block were cut by trimming and attaching them to wood block in preparation for sectioning. Sectioning and staining of organs and tissues was done in line with Omitoyin [19,20]. A light photomicroscope attached to a 35 mm camera was used to examine the organs and tissue sections.

Statistical Analysis

Data obtained were subjected to one way analysis of variance [21] while the means were compared for significant differences using the student's t-test.

Results

The water quality parameters measured during the exposure period are presented in Table 1. The various parameters were not significantly different ($p>0.05$) from each other, however, slight variations were observed. The haematological values and changes recorded at different periods (0 day, 21 days and 1- week after withdrawal) in *C. gariepinus* Juveniles exposed to Chronic-sub lethal Fresh Root-bark Cold Water Extract (FRBCWE) of *P. zeylanica* are presented in Table 2. The result shows a decrease in the Mean RBC, PCV, and Haemoglobin compared to the initial values. Haematological indices, MCHC, MCH and MCV shows a significant differences ($p<0.05$) with a slight fluctuation in the parameters compared to the control groups. There was a slight increase in the Total WBC of the exposed fish with a slight fluctuation in the parameters compared to the control groups.

The levels of selected serum-biochemical constituents of Total protein (TP), Albumin (AL), Globulin (GL) and Albumin/globulin ratio (A/G) were presented in Table 3. The Serum-biochemical values

Conc. Mgl^{-1}	Dissolved Oxygen (ppm)	Temperature °C	pH	Ammonia nitrogen (ppm)	Nitrite nitrogen (ppm)	Carbon IV oxide (ppm)
0	5.1 ± 0.1[a]*	24.0 ± 0.2[a]	7.0 ± 0.2[a]	3.1 ± 0.1[a]	0.05 ± 0.0[a]	36.6 ± 1.3[a]
26	4.6 ± 0.3[a]	24.5 ± 0.3[a]	7.2 ± 0.3[a]	3.3 ± 0.3[a]	0.05 ± 0.1[a]	35.7 ± 1.2[a]
39	4.0 ± 0.2[a]	24.7 ± 0.3[a]	6.8 ± 0.1	3.3 ± 0.3[a]	0.07 ± 0.1[a]	35.8 ± 0.7[a]
59	3.5 ± 0.3[a]	24.9 ± 0.5[a]	6.3 ± 0.2[a]	3.5 ± 0.2[a]	0.09 ± 0.2[a]	34.5 ± 0.2[a]

*Mean with the same superscript along columns are not significantly different (P>0.05)

Table 1: Mean values of selected water quality parameters during exposure of *C. gariepinus* Juveniles to Cold Water extracts Fresh Root-bark of *P. zeylanica* for 21 days.

			Exposure Period						
Haematological			**21 days**				**1 Weeks after Withdrawal**		
Parameters	**O day**	**Conc. mgl^{-1}**	**26**	**39**	**59**	**Conc. Mgl^{-1}**	**26**	**39**	**59**
	(Initial)	**O (control)**				**O (control)**			
PCV (%)	26.00 ± 1.00^{a}	20.33 ± 4.61^{a}	23.33 ± 4.72^{a}	21.33 ± 3.21^{a}	20.66 ± 4.93^{a}	23.66 ± 3.21^{a}	22.66 ± 4.61^{b}	21.66 ± 2.51^{c}	20.00 ± 2.64^{c}
Hb (m/l)	8.40 ± 0.20^{a}	6.50 ± 1.47^{a}	7.93 ± 1.28^{a}	7.16 ± 1.09^{a}	6.73 ± 1.51^{b}	7.90 ± 0.96^{a}	7.47 ± 1.51^{b}	7.20 ± 0.85^{c}	6.43 ± 0.92^{d}
Rbc (x10^{6}/mm^{3})	1.92 ± 0.02^{a}	1.70 ± 0.07^{b}	1.90 ± 0.37^{a}	1.70 ± 0.03^{b}	1.72 ± 0.06^{b}	1.94 ± 0.19^{a}	1.47 ± 0.19^{b}	1.43 ± 0.07^{b}	1.40 ± 0.09^{b}
Wbc (x10^{3}/mm^{3})	12,533.33 ± 351.18^{a}	14,800.00 ± 2751.36^{a}	13,966.66 ± 1632.73^{c}	16,016.66 ± 2975.03^{a}	15,400.00 ± 692.82^{b}	12,533.33 ± 8,916.85^{a}	22,533.33 ± 1616.58^{b}	23,083.33 ± 3426.48^{a}	21,066.66 ± 5493.02^{b}
Platelet (x10^{3}/mm^{3})	167,666.66 ± 2,081:66^{a}	111,333.33 ± 9018.49^{a}	109,666.66 ± 12897.02^{c}	112,666.66 ± 12,220.20b	123,333.33 ± 25006.66^{a}	228,000.00 ± 72,773.62^{a}	233,000.00 ± 113,872.73^{b}	245,666.66 ± 112926.22^{a}	194,000 ± 43405.06^{c}
MCV (fl)	132.33 ± 2:51^{a}	118.00 ± 22.60^{a}	122.33 ± 13.65^{b}	124.66 ± 16.07^{a}	119.66 ± 25.69^{c}	121.00 ± 4.35^{a}	157.00 ± 49.00^{a}	151.00 ± 22.60^{a}	142.33 ± 15.27^{b}
MCHC (%)	30.00 ± 2:00^{a}	32.00 ± 1.00^{a}	34.00 ± 1.73^{a}	33.66 ± 0.57^{a}	32.66 ± 0.57^{a}	33.33 ± 0.57^{a}	33.00 ± 1.00^{a}	33.00 ± 0.00^{a}	32.00 ± 0.00^{a}
MCH (Pg)	43.00 ± 2:64^{a}	37.33 ± 7.23^{a}	41.33 ± 3.51^{a}	42.00 ± 5.29^{a}	38.66 ± 7.50^{b}	40.33 ± 1.15^{a}	45.00 ± 6.08^{b}	50.00 ± 7.21^{a}	47.33 ± 6.42^{b}
Lymphocytes (%)	61.00 ± 3:60^{a}	68.66 ± 3.21^{a}	70.66 ± 4.04^{a}	72.33 ± 0.57^{a}	69.33 ± 2.51^{b}	78.33 ± 4.73^{a}	71.33 ± 16.04^{b}	68.33 ± 4.50^{c}	71.33 ± 10.50^{b}
Heterophils (%)	34.30 ± 2:08^{a}	26.33 ± 2.51^{a}	28.00 ± 3.00^{a}	26.00 ± 1.73^{c}	27.66 ± 2.08^{b}	17.66 ± 3.78^{a}	26.00 ± 16.00^{a}	26.00 ± 3.46^{a}	25.00 + 9.53^{a}
Monocycles (%)	3.00 ± 1:00^{a}	3.33 ± 0.57^{a}	1.50 ± 0.71^{c}	2.00 ± 1.41^{b}	2.00 ± 1.00^{b}	2.66 ± 0.57^{a}	1.66 ± 1.15^{b}	2.66 ± 1.15^{a}	2.00 ± 0.00^{a}
Eosinophils (%)	2.33 ± 1:52^{a}	2.50 ± 0.71^{a}	1.00 ± -0.00^{a}	1.00 ± 0.00 ^{a}	1.50 ± 0.71^{a}	1.33 ± 0.57^{a}	1.00 ± 0.00^{b}	2.66 ± 1.15^{a}	1.00 ± 0.00^{b}

Mean with the same superscript alphabet along vertical column are not significantly different (P>0.05)

Table 2: Haematological values recorded at different periods for *C. gariepinus* Juveniles exposed to chronic-sub lethal Cold water fresh Root-bark extract of *P. zeylanica*.

				Exposure Period					
Serum Biochemical		**21 day**				**1 Weeks after Withdrawal**			
Parameters	**O day**	**Conc. Mgl^{-1}**	**26**	**39**	**59**	**Conc. Mgl^{-1}**	**26**	**39**	**59**
	(Initial)	**O (control)**				**(control)**			
Total protein (g/dl)	6.0 ± 0.49a	5.90 ± 0.36	5.43 ± 0.93	5.56 ± 0.20	5.30 ± 0.50	5.23 ± 0.40	5.30 ± 0.70	5.36 ± 0.49	5.36 ± 0.32
Albumin (Al) (mmol/l)	2.20 ± 0.26	2.36 ± 0.55^{b}	2.06 ± 0.45^{c}	2.46 ± 0.21^{a}	1.63 ± 0.31^{d}	2.83 ± 0.25	3.06 ± 0.28	2.06 ± 0.28	2.53 ± 0.76
Globulin (Gl) (mmol/L)	3.10 ± 0.35	3.53 ± 0.37	3.36 ± 0.57	3.10 ± 0.00	3.67 ± 0.74	2.36 ± 0.15	2.23 ± 0.96	3.13 ± 0.21	2.83 ± 0.45
Aspartate Aminotranseferase (AST) (IU/l)	114 ± 6.93	152.67 ± 14.04	147.00 ± 14.79	161.33 ± 8.08	141.67 ± 21.01	184.33 ± 6.11	133.66 ± 39.32	152.66 ± 38.03	166.33 ± 11.37
Alanine Aminotranseferase (ALT) (IU/l)	66.67 ± 6.11	57.33 ± 9.07	74.33 ± 9.29	62.67 ± 13.61	63.00 ± 4.58	38.66 ± 8.02	37.66 ± 5.50	47.66 ± 3.05	44.33 ± 15.04
Albumin/Globulin ratio (A/G)	0.07 ± 0.04	0.68 ± 0.23	0.61 ± 0.11	0.79 ± 0.07	0.45 ± 0.15	1.19 ± 0.08	1.65 ± 1.02	0.65 ± 0.09	0.92 ± 0.35

Mean with alphabet a,b,c,d are significantly different (p<0.05)

Table 3: Mean Serum-Biochemical values recorded at different periods for *C. gariepinus* Juveniles exposed to Chronic-sub lethal Cold Water extract Fresh Root-bark extract of *Plumbago zeylanica*.

Organs and tissues	**Histological signs**	**Exposed Concentrations (mgL^{-1})**			
		A	**B**	**C**	**D**
		0	**26**	**39**	**59**
Brain	Marked Congestion of cerebrum and cerebellum cells	-	-	++	++
Gill	Scattered gill filaments and Vascular congestion and thickening of gill filaments	-	½	++	++
Liver	Marked vascular congestion and moderate diffuse fatty degeneration of hepatocytes	-	++	++	++
Intestine	Thinning walls and shortening of villi structure	-	-	++	++
Muscle/flesh	Goblets cell hyperplasia, muscle	-	-	-	-
	Necrosis and calcification	-	-	-	-

++: = Present and distinct with morphological changes of histological signs on the organ and tissues.

½=Present but less marked (mild) than usual

- = No lesion and morphological changes in organ and tissues.

Table 4: Histopathological Changes Observed in the Organs and Tissues of *C. gariepinus* juveniles exposed to Fresh root-bark cold water extracts of *P. zeylanica* after 21 days of chronic-sub lethal exposure.

were significantly different (P<0.05) at the different periods of exposure.

The results of histological examinations of *C. gariepinus* juvenile to chronic-sub lethal concentration of 0 mgL^{-1}, 26 mgL^{-1}, 39 mgL^{-1} and 59 mgL^{-1} of fresh root-bark cold water extract (FRBCWE) of *P. zeylanica* at 21 days and 1-week after withdrawal are presented in Tables 4 and 5 respectively. At 21 days, distinct morphological changes were more pronounced in the organs and tissues. However, one week after withdrawal from FRBCWE, the brain, intestine, and muscle/flesh has no lesion and any morphological changes but the gill and liver had mild lesion and morphological changes.

Discussion

The concentration of the selected water quality of the experimental set up in all the treatments agreed with the recommended values for warm water fish culture by Viveen [22]. The exposure of fish to toxicant for 21days resulted in remarkable anaemic condition in *C. gariepinus*. Studies have revealed that when the water quality is affected by toxicants, physiological parameters will be affected, and reflected in the haematological indices [23]. Thus, water quality is one of the major factors responsible for individual variations in fish haematology, since fish live in close association with their environment and are sensitive to slight fluctuation that may occur within their internal milieu.

There were reductions in the values of haematological parameters in the juveniles of *Clarias gariepinus* exposed to *Plumbago zeylanica* extracts. This is in agreement with Joshi [24] that reported effects

Organs and tissues	Histological signs	Exposed Concentrations (mgL^{-1})			
		A	B	C	D
		0	26	39	59
Brain	Congestion of blood vessels and cerebellum cells	-	-	-	-
Gill	Scattered gill and thickening filaments but no lesions	-	½	½	½
Liver	Diffuse fatty degeneration of hepatocytes	-	½	-	-
Intestine	Necrosis of surface epithelium and shortening of villi	-	-	-	-
Muscle/flesh	Goblets cell hyperplasia and muscle necrosis and calcification	-	-	-	-

++: = Present and distinct with morphological changes of histological signs on the organ and tissues.
½ = Present but less marked (mild) than usual
- = No lesion and morphological changes in organ and tissues.

Table 5: Histopathological Changes Observed in the Organs and Tissues of *Clarias gariepinus* juveniles exposed to Fresh root-bark cold water extracts of P. *zeylanica* after 1-week withdrawal from Chronic-sub lethal Exposure.

of toxicants on blood parameters in freshwater teleost fish Clarias batrachus. Bhatt and Farswan [25] also observed that Red blood cell (RBC), Total White blood cell (TWBC), Haemglobin (Hb), Packed cell volume (PCV) decreases with exposure of Barilius bendalensis (Ham) to plant toxicant. The abnormalities observed in the haematological parameters in all concentrations compared with control clearly indicated that the haematological parameters were much lower in the exposed fish than in control fish, thereby depicting an anaemic condition.

Increase in total WBC as observed in the fishes exposed to the extracts of *Plumbago zeylanica* can be attributed to increased production of leucocytes in the haematopoietic tissues of the kidney and perhaps the spleen. Lymphocytes are the most numerous cells comprising the leucocytes, which function in the production of antibodies and chemical substances serving as defense against infection. The primary consequence of observed changes in leucocyte count in stressed fish is suppression of the immune system and increased susceptibility to diseases [26].

Decrease in RBC count, Haemoglobin and PCV values in *Clarias gariepinus* juveniles exposed to extracts of *Plumbago zeylanica* in this study is similar to the observation of Joshi [27] in *Puntiusconchonius* and *Clarias batrachuus* exposed to different toxicants. The Mean Corpuscular Volume gives an indication of the status or size of the red blood cells and reflects an abnormal or normal cell division during the production of red blood cells (erythropoiesia). The increase in MCV may be attributed to the swelling of the erythrocytes resulting in a macrocytic anaemia. Larsson [28] attributed the increase in MCV to the swelling of the red blood cell as a result of hypoxic condition or impaired water balance of somatic stress or marcocytic anaemia in fishes exposed to metal pollution.

The abnormalities observed in the haematological parameters compared with the control (0 mgl^{-1}) clearly indicated that the haematological parameters were much lower in the exposed fish compared to the control fish, thereby, depicting an anaemic condition, which could be triggered by several pathological factors.

Haematological parameters

The reduction in PCV values of all treatments for 21 days is an indication of blood loss compared to the initial; however, this may not have been severe as values remained within the range for clinically healthy rainbow trout, and *Oreochromis niloticus* [29]. The mean RBC Counts in *C. gariepinus* juveniles for all treatments is lower than the initial value. Higher values may signify some degree of haemo-concentration of mild polycythemia. According to Kelly, polycythemia is characterized by very high increase in erythrocyte numbers, such increases may result from disturbed tissue fluid balance such as occur in dehydration. Polycythemia could be attributed to hypoxia or splenic contraction in animals that are excited or have been exercised [30].

Increase in Total WBC (Leucopomia) as observed in all treatments is attributed to increase production of Leucocytes in the haematopietic tissue of the kidney and perhaps the spleen lymphocytes are the most numerous cells comprising the Leucocytes, which functions in the production of antibodies and chemical substances (toxicants) serving as defence against infection. The primary consequence of observed changes in Leucocyte count in stressed fish is suppression of the Immune system and increased susceptibility to disease [26]. However, the trend of Lymphocyte values for all the treatments indicate low Immune status of the experimental fish exposed to different concentration of the extracts of *P. zeylanica*. Low values of platelets in all treatments indicate decrease in blood clotting ability which seems to be more pronounced in all treatments.

The decrease in MCV after 21days competed with low haemoglobin content to the Fresh root-bark Cold water extracts of *P. zeylanica* that the red blood cells have shrunk, either due to hypoxia or a microcytic anaemia. At this stage, micro-cytosis may be due to the decrease in the haematocrit during exposure to the toxicant extract. Similar trend has been detected in Laveo umbratus after exposure to various pollutants [31] as well as *Oreochromis* hybrid exposed to aluminium [23]. However, MCHC in all treatments recorded higher values than the initial.

The abnormalities observed in haematological parameters in all concentrations compared with the control (0 mg/L) clearly indicated that the haematological parameters were much lower in the exposed fish than in the control fish, thereby depicting an anaemic condition. Anaemic condition could be triggered by several pathological conditions after a short exposure to the toxicant such as changes from normochronic, microlytic condition to normochronic macrolytic type after long-term exposure as observed in the current study.

The macrocytosis is an adaptive response through the influx of immature erythrocytes from haematopoietic tissues to the peripheral blood to make up the reduce red blood cell number and decreased haemoglobin concentration. However, the reversible haematological parameter observed after 1 week withdrawal is an indication of non-bioaccumulation effect of the toxicant extracts as similarly observed by Tiwari and Singh [32].

Serum biochemical parameters

There were fluctuations in all serum biochemical parameters such as total protein, Albumin, Globulin and Albumin/Globulin ratio when *C. gariepinus* juveniles were exposed to different concentrations of Fresh Root-bark Cold water extract of *P. zeylanica* for 21 days as compared with initial and control values, respectively. A decrease in Serum total protein in the current study was similar to that observed by [33] when fishes were exposed to stem-bark extract of *Croton tiglium*. However, the results reported for Serum proteins are in agreement with those obtained by [34] when Carp (*Cyprinus caprio*) were exposed to short-term and long-term effects of cypermethrin. The protein is the energy

source of spore during chronic period of stress. Animal exposed to sub lethal concentration of toxicant, experienced the greater stress during the process of detoxification of a given toxicant on the metabolic level of animal [33].

The decrease in Serum total protein level and albumin may be due to their degradation and also to their possible utilization for metabolic purpose. Bradbury [33,35] pointed out that decreased protein content and albumin might also be attributed to the destruction or necrosis of cells and consequently impairment in protein synthesis machinery.

The current study showed fluctuation in Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) of fish exposed to Fresh Root-bark Cold water extract of *P. zeylanica*. However, activities of enzymes occur during carbohydrate metabolism, broadly divided into anaerobic and aerobic segment that consist oxidation of TCA/Citric/Krebs. Both the AST and ALT function as a link between carbohydrates and protein metabolism by catalyzing the inter conversion of strategic compounds like α–ketoglutarate and alanine to pyruvic acid and glutamic acid respectively [32,36,37]. Stress in generally known to elevate aminotransferase in fish [32,38]. This also conforms to the nature of function of aminotransferases, where they respond to any stress or altered physiological condition [32,36].

Histopathological changes in organs and tissues

The histopathological alteration observed in the brain, gill, liver, intestine and muscle/flesh is an indication of the toxic effect of *P. zeylanica* extracts to fish. However, these alterations observed in the various plates are indications of toxic effect. This is in agreement with [39].

Histology of *C. gariepinus* juveniles exposure to fresh root-bark cold water extracts of *P. zeylanica* on the brain showed remarkable blood vessel congestion. This could be as a result of the toxicant on the organs and tissues. This agreed with Fafioye 2001, 2004 observation when *Clarias gariepinus* and *O. niloticus* were exposed to lethal and sublethal concentrations of *Parkia biglobosa* and *Raphia vinifera* respectively. The gill lamellae play a significant role in regulating the exchange of gas, water and ions in fish. The role of the gill in excretion predisposes it in such a way that slight structural damage can render a fish very vulnerable to osmoregulation as well as respiratory difficulties. The gill produced remarkable lesion but scattered gill filaments were observed at short term exposure and vascular congestion and thickening of gill filament were observed at long term exposure. These alterations could lead to several physiological stresses in fish and difficulty in overcoming oxygen diffusing into the fish by increasing the haemoglobin concentration [40].

The liver is considered the most important target organ from the toxicological point of view because of its role in detoxification, biotransformation and excretion of xenobiotics.

After enteric uptake of injurious chemicals, it is the first organ to be exposed to hazards via portal circulation and many xenobiotics tend to accumulate in the liver at high concentrations. Short term exposure of the liver to FRBCWE showed a mild diffuse necrosis of hepatocytes with localized calcification of liver capsule with layer of thick fibrin exudates, while at long term exposure severe diffuse fatty degeneration of hepatocytes were observed. The resultant damage of liver could be due to the sensitive nature of liver to toxicants. This agrees with works of [40-42] where livers of freshwater fishes were exposed to acute-lethal and chronic-sub lethal concentrations of different toxicants.

The intestine of the fishes exposed long term (21 days) periods showed necrosis of surface epithelium and shortening of villi with thinned wall and fibrinonecrotic exudates as histological signs. This was similar to Fafioye [41,43] respectively. The muscle/flesh of the exposed fish showed goblet cell hyperplasia, muscle necrosis and calcification at lower concentrations of exposure for both the short term and long term exposure, but disappears at higher concentrations after 1-week withdrawal without bioaccumulation in the Organs and Tissues. This agrees with [40] whereby Moringa oleifera Lam disappears in the tissues of exposed fish.

Jhingram [44,45] observed that whatever type of piscicidal plant used, they are found only to be poisonous to fish but not on the consumers. This is because the entire extracted chemical, from the plants have short life span.

It is important to quantify the effectiveness of fresh root bark cold water extract of *Plumbago zeylanica* in fresh water earthen ponds under natural conditions. This is because under natural conditions, many factors such as temperature, sunlight and adsorption by soil particles have possible influence on toxicity and toxicant degradation. Dawson [46] found that rotenone disappeared 2 or 3 times faster in earthen ponds than in concrete tanks.

The structure of the "Casual" chemicals or group of chemicals can further be elucidated and compared with already known structures of chemicals used in industrial, medical and agricultural practices to see if there are similarities in structure and function. This may open a new approach into the gradual replacement of synthetic chemicals with botanicals of plant origin.

Conclusion

The toxicity of *Plumbago zeylanica* cold water extract Fresh root bark disappeared within a short period of time; it could be an ideal plant toxin to be used for eradicating unwanted fish from nursery and rearing ponds before stocking. Thus, it is possible to use the extract in a short period of time. This study may be useful in providing information for possible further use of *Plumbago zeylanica* in aquaculture practices. More research work on *Plumbago zeylanica* extracts on other fish species apart from *Clarias* should be conducted to know their response and reaction to the plant extracts.

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References

1. Arasta T, Bais VS, Thakur P (1996) Effect of Nuvan on some biological parameters of Indian catfish, Mystus vittatus. J Environ Bio 17: 167-169.
2. Waliszewski SM, Aguirre AA, Benitez A, Infanzon RM, Infazon R, et al. (1999) Organo-pesticides residues in Human blood serum of inhabitants of Veracuz, Mexico. Bull Environ Conta Toxicol 62: 397-402.
3. Singh D, Singh A (2005) The toxicity of four native Indian plants: Effect on AChE and acid/alkaline phosphatase level in fish Channa marulius. Chemosphere 60: 135-140.
4. Ayushveda (2009) Ayushveda. com Indian's Health and Lifestyle portal.
5. Lemma H, Debella A, Addis G, Kururt O, Geyid A, et al. (2002) Anti-bacterial activity of Plumago zeylanica L. roots on some preumonia causing pathogens. Ethi J Sci 25: 285-294.
6. Oyelese OA, Faturoti EO (1995) Growth and Mortality estimates in Clarias

gariepinus fed graded levels of processed cassava peels. J Trop for Resources 11: 71-81.

7. APHA (1998) Stardand Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC, USA.
8. Solbe JF (1995) Handbook of Ecotoxicology.
9. Odiete WO (1999) Environmental Physiology of Animal and Pollution. Publ. By Diversified Resources Ltd, Lagos, Nigeria.
10. Reish DL, Oshida PS (1986) Manual of Methods in Aquatic Environment Research.
11. Stoskopf MK (1993) Clinical pathology in fish medicine.
12. Kelly WR (1979) Veterinary Clinical Diagnosis. Tindall, London.
13. Dacie SIV, Lewis SM (1991) Practical haematology.
14. Henry RJ, Sohel C, Berkman S (1957) Interferences with Biuret methods for Serum Proteins. Analytical Chemistry 29: 1491-1495.
15. Reitman S, Frankel S (1957) A Colourimetric method for the determination of Serum GOT and GPT. Am J Clin Patholy 28: 56-63.
16. Rodrigues EL, Ranzani- Pavia MJT, Pacheco FJ, Veiga ML (2007) Histopathologic lesions in the liver of Procholidatus lineatus (Pisces; Prochilodontidae) exposed to a sublethal concentration of the organophosphate insecticide Dipterex 500R (Trichlorfon). Acta Scientarium maringa 23: 503-505.
17. Ofogba CJ, Agomo FU, Abdul-kareem FB, Abaelu AM, Alatishe K (1998) Effect of Bridelia ferruginea Stem-bark on blood chemistry and Histology of some organs in rats Nigerian J Natu Prod Medi 2: 26-28
18. Luna LG (1968) Manual of histologic staining methods of the Armed Forces Institute of Pathology McGraw-Hillbook Co, New York.
19. Omitoyin BO, Ogunsanmi AO, Adesina BT (1999) Studies on Acute toxicity of piscicidal plant (Tetrapheura tetraptera) Extracts on Tilapia (Sarotherokon galilaeus) fingerlings. Trop J Anim Scie 2: 199-197.
20. Omitoyin BO, Ajani EK, Fajinmi (2006b) Toxicity of Gramoxone (Paraquat) to Juvenile African catfish, Clarias gariepinus (Burchell, 1822). Am Eur J Agri and environ scie 1: 26-30.
21. Steel RG, Torrie JH, Dickey DA (1997) Principles and Procedures of Statistics: A Biometrical approach. Mc Graw-Hill, Singapore.
22. Viveen WJAR, Richter CJJ, Van Oordt PGWJ, Janssen JAL, Huisman EA (1985) Practical manual for the culture of the African Catfish (Clarias gariepinus).Directorate General for International Technical Corporation. The Hague, Netherlands.
23. Bhagwant S, Bhikajee M (2000) Induction of hypochromic macrocytic anaemic in Oreochromis hybrid (Cichlidea) exposed to 100 mg/l (Sublethal dose) of Aluminium. J Scie Techn 5: 10-20.
24. Joshi PK, Bose M, Harish D (2002) Haematological changes in the blood of Clarias batrachus exposed to mercuric chloride. Ecotoxicological Environmental Monitoring 12: 119-122.
25. Bhatt JP, Farswam VS (1992) Haemolytic activity of Picicisidal compounds of some plants to a fresh water fish Barilius bendalensis (Ham). J EnvironI Biol 13: 333-342
26. Wedemeyer GA, Wood J (1974) Stress a predisposing factor in fish disease.
27. Joshi PK, Harish D, Bose M (2002) Effect of Lindane and Malathione exposure to certain blood parameters in a fresh water teleost fish Clarias batrachus. Pollution Resources 21: 55-57.
28. Larsson A, Haux C, Sjobeck M (1985) Fish physiology and metal pollution: results and experiences from laboratory and field studies. Ecotoxic Environ Safe 9: 250-281.
29. Wedemeyer GA, Ysutake WT (1977) Clinical methods of the assessment of the effects of environmental stress on fish health.
30. Coles EH (1986) Veterinary Clinical pathology.
31. VanVuren JHJ (1986) The effects of toxicants on the haemotology of Labeo umbratus (Teleostei, Cyprinidae). Compar Biochem and Physi Part C: Compar Pharmaco 83: 155-159.
32. Tiwari S, Singh A (2004) Piscicidal activity of alcoholic extract of Nerium indicum leaf and their biochemical stress response on fish metabolism. Afr J Trad CAM 1: 15-29.
33. Yadav RP, Singh D, Singh SK, Singh A (2003) Metabolic Changes in Freshwater fish Channa punctatus due to stem–bark extract of Croton tiglium. Pakist J Biolog Scie 6: 1223-1228.
34. Malla RP, Bashamohiden MD (1995) Alternation in protein metabolism in selected tissue of fish Cyprinus caprio during sublethal concentration of Cypermethrin. Environ Monit Assess 36: 183-190.
35. Bradbury SP, Symonic DM, Coats JR, Atchism GJ (1987) Toxicology of Fenvalerete and its constituents Isomers to the fathead minnow (Pimephales Promelos) and blue gill (Lepomis macrochirus). Bulletin of Environmental Contamination Toxicology 38: 727-735.
36. Knox WE, Greengard O (1965) In: An introduction to enzyme physiology. Pergamon Press, New York, London.
37. Watts RD, Watts DC (1974) Cyclostomes and fishes. Academic press, New York.
38. Natarajan GM (1985) Inhibition of branchial enzymes in Snakehead fish Channa striatus by oxydemeton methyl. Pes Biochem 23: 41-46.
39. Omitoyin BO, Ajani EK, Adesina BT, Okuagu CAN (2006a) Toxicity of Lindane (Gamma-Hexachlorocyclo-Hexane) to Clarias gariepinus (Burchell, 1822). Wor J Zoo l: 57-63.
40. Adesina BT (2008) Toxicity of Moringa oleifera (Lam). Extract to Oreochromis niloticus fingerlings and Juveniles.
41. Adeogun AO (2004) Effects of Methanolic extract of Raphia hookeri (Mann and Wendl 1804) on life stages of Clarias gariepinus (Burchell, 1822). Nigeria.
42. Udoh JP (2005) Pesticide-induced behavioural and Histological changes in juvenile Clarias gariepinus. Niger J Agricul Food and Environ 2: 78-87.
43. Fafioye OO, Adebisi AA, Fagede SO (2004) Toxicity of Parkia biglobosa and Raphia vinifera extracts on Clarias gariepinus juveniles. Afric J Biotechno 3: 627-630.
44. Jhingram VG (1975) Fish and Fisheries of India. Hindustan Publishing Corporation Delhi, India.
45. Chakraboty DP, Nanady AC, Philipise MT (1972) Barringtonia acutangula (Linn) Gaertn, as a fish poison. Indian Journal of Experimental Biology 10: 78-80
46. Dawson VK, Gingerich WH, Davis RA, Gilderhus PA (1991) Rotenone persistence in freshwater ponds. Effects of temperatre and sediment adsorption. North Am J Fish Manage 11: 226-231.