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Haematology, plasma enzymes and organ indices of *Clarias gariepinus* after intramuscular injection with aqueous leaves extracts of *Lepidagathis alopecuroides*

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Tank-raised *Clarias gariepinus* (mean total length, 30.05 ± 4.05cm,SD; mean weights, 250.04 ± 50.22 gSD) were injected intra-muscularly with two millilitres per kilogram of each of five concentrations (2.00, 2.00, 6.00, 8.00 and 10.00 ppm) of aqueous extracts of leaves of *Lepidagathis alopecuroides* above the lateral line. The fish in the control group were injected with same dose of distilled water. There were four fish per treatment level. On the 14^{th} day the fish was assessed for organ indices (hepatosomatic index, HIS; cardiosomatic index, CSI; renatosomatic index, RSI and spleenosomatic index, SSI) and Fulton's condition, K . Blood samples were analysed for packed cell volume, PCV; haemoglobin, Hb; red blood cell, RBC white blood cell, WBC and platelets. The plasma was analysed for the transaminases (aspartate transaminase, AST; alanine transaminase, AST), alkaline phosphostase, ALP and lactate dehydrogenase, LDH. The values of PCV ($p < 0.05$), HB and RBC ($p > 0.05$) in the control were higher than those in the treated group. However, the decline was not concentration-dependent. The increase in the number of WBC in the exposed fish was twofold and above ($p < 0.05$) that in the control, 66.67 ± 23.09x10^6cells/l with highest value (360.00 ± 33.24x10^6cells/l) recorded at 10.00 ppm. The response pattern of platelets to the extracts varied very widely among the treated group ($p < 0.05$) with fish injected with 2.00 ppm having the highest value (100.00 ± 33.20x10^9cells/l), which was about twofold that at other concentrations. Generally, the activities of AST, ALT, ALP and LDH were inhibited below the control value without any direct relationship to toxicant concentration. Never-theless, AST generally showed a decline in activity from that of the control except at 4.00 ppm (117.75 ± 9.80 IU/L); ALP declined with a slight increase at 10.00 ppm above the control. There was no difference ($p > 0.05$) between the organ indices in control and treated group except in the SSI. K and HSI showed variable responses relative to extracts concentration, while the heart showed a very slight decline in size. RSI and SSI had a reverse pattern. Exposure of *C. gariepinus* to *L. alopercuroides* in the open waters during fishing activity may impact negatively on the physiology of the fish as manifested in changes in some of the blood parameters, plasma enzymes and organ indices of the fish.

Key words: *Clarias gariepinus, Leidagagthis.alopercuroides,* plasma enzymes, blood parameters, organ indices, condition.

INTRODUCTION

In recent years there is preference for safe and environmentally friendly piscicides of plant origin than synthetic piscicides for catching fish and clearing ponds. This is because ichthyotoxins are less expensive, biodegradable, readily available, easy to handle and safe for mankind and the environment (Singh et al., 1996). Obnoxious fish-

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ing activities in the form of sublethal pollution have negative or destructive influence on the aquatic environment and may result in chronic stress conditions that may have adverse effect on aquatic species (Mason, 1991). Fish physiology (haematological and biochemical parameters) are suitable tools for assessing environmental influences and stress effects of anthropogenic origin on the condition and health of aquatic vertebrates (Cêlik, 2004). Since there is a close association between the circulatory system of fish and the external environment (Cech et al., 1996; Bonga, 1997), the effect of external stressors and toxic substances on exposed fish could be made mani-fest through clinical diagnosis of fish physiology.

Plant parts have been shown to cause death of fish and changes in biochemical responses of *Channa punctatus* (Tiwari and Singh, 2004), haematological and histopathological effects on *Clarias gariepinus* (Fafioye et al, 2004; Omoniyi et al., 2002). The leafy parts of *Lepidagathis alepecuroides* is used to immobilize and kill mudskippers (*Periophthalmus papillio*) and other fish species in Rivers and Cross River States of Nigeria (Obomanu et al., 2007) but the disruptive internal changes leading to death of the poisoned fish is yet to be examined. It has been shown that before death resulting from animal intoxication, various physiological and biochemical changes take place in the internal environment of fish (Adeyemo 2005; Fafioye et al., 2004; Obomanu et al., 2007).

The plant, *L. alopecuroides* have been in use for a long time but the effects of direct use in water and mudflats, its mode of action in fish and the resultant fish kill have not been examined. Therefore this study was carried out to assesess some haematologic, biochemical changes, condition and organ indices of the commercial fish, *Clarias gariepinus* injected intramuscularly with different sublethal concentrations of aqueous extracts of *L. alopecuroides* under laboratory conditions.

MATERIALS AND METHODS

Fresh leaves of *L. alopecuroides* were collected from the wild and air dried for three weeks in the Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt. The dried leaves were ground into power with an electric blender and sieved with a test sieve into a fine power and then stored in a dry airtight container. Tank-raised *C. gariepinus* (mean total length, 30.05 ± 4.05 cmSD; mean weights, 250.04 ± 50.22 gSD) were obtained from a private farm and transported in aerated aquaria to the same laboratory. They were acclimated individually in bore hole water (characteristics: dissolved oxygen -4.50 ± 0.05 mg/l, pH- 7.5 ± 1.3, conductivity -410 ± 20.4 S/cm, total dissolved solid- 400 ± 10.25 ppm) in circular plastic aqauarium (volume, 30*l*, effective water volume, 10*l*) for 7days and fed with a 35% crude protein diet at 1% biomass.

The aquaria were washed daily with a piece of foam to remove uneaten food and faecal matters during the accli-mation and experiment. A stock solution (100 mg/l) of the plant powder was prepared from which five concentrations (2.00, 2.00, 6.00, 8.00 and 10.00 ppm) were prepared. Each fish was injected with two millilitres per kilogram of each of the extracts concentrations above the lateral line. Fish in the control group was also injected with same dosage of distilled water. Blood was collected from each fish on the 14^{th} day after injection with a syringe and 21G hypodermic needle from the kidney between the urino-genital organ and anal fin. Blood samples for haematological studies were pre-served in EDTA embedded bottles and that for enzymes (AST, ALT, ALP and LDH) analysis in heparinised bottles. The fishes were then killed by a blown on the head and the organs (kidney, liver, heart and spleen) excised and weighed for organ indices assessment.

Packed cell volume (PCV) was determined using Hawsley micropillary tubes and centrifuged for 5 min (Abudu and Safola, 1994). Red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and platelets were analysed according to the methods of Blaxhal and Daisley (1973). The heparinised blood samples were centrifuged at 300 rpm for 10 minutes and the plasma collected for analysis. AST and ALT were analysed according to the method of Reitman and Frankel (1957); ALP according to Bessey et al. (1946) and LDH, according to the method of the Huckabee (1961). The organ indices were calculated according to the methods of (Jenkins, 2004; Adams et al., 1993). Data obtained were subjected to statistical analysis using one way analysis of variance at 95% probability and means separated by Duncan's multiple range test at 95% probability (Wahua, 1999).

RESULTS

The values of PCV ($p < 0.05$), HB and RBC ($p > 0.05$) in the control were higher than those in the treatment levels. However, the decline was not concentration dependent (Table 1). The increase in the number of WBC in the exposed fish was twofold and above ($p < 0.05$) that in the control, $66.67 \pm 23.09 \times 10^9$ cells/l with highest value ($360.00 \pm 33.24 \times 10^9$ cells/l) recorded at 10.00 ppm. The response pattern of platelets to the extracts varied very widely among the treated group ($p < 0.05$), with fish injected with 2.00 mg/l extract having the highest value ($100.00 \pm 33.20 \times 10^9$ cells/l), which was about twofold that at the other concentrations.

Generally, the activities of AST ($p > 0.05$), ALT ($p > 0.05$), ALP ($p > 0.05$) and LDH ($p < 0.05$) were inhibited below the control value without any direct relationship to toxicant concentration. Never- theless, AST generally showed a decline in activity from that of the control, 108. 6743.09IU/L except at 4.00ppm (117.75 ± 9.82 IU/L). ALT and ALP activities declined below their respective control values (ALT, 18.33 ± 10.97 IU/L; ALP, 43.33 ± 8.74 IU/L) with a slight increase at 10.00 ppm above the control. LDH appeared to be most impacted upon by extracts with a one-sixth reduction at 10.00 ppm (Table 2). There was no difference ($p > 0.05$) in all the organ indices between the control and treated group except in the spleenosomatic index (Table 3).

Condition factor (K) and hepatosomatic index (HSI) showed variable response relative to extract concentration, while the heart showed a very slight decline in size (Table 3). Renatosomatic and spleenosomatic indices (RSI and SSI) had a reverse pattern (Table 3).

Table 1. Haematological variables of *Clarias gariepinus* 14days from the day of injection with aqueous extracts of *Lepidagathis alepecuroides* (mean ± SD).

Conc. of *L. alepecuroides* (ppm)	Haematological variables				
	PVC (%)	Hb (g/dl)	RBC ($x10^6$cells/L)	WBC ($x10^9$cells/L)	Platelets ($x10^9$cells/L)
0.00	26.00 ± 14.93^{a}	8.60 ± 54.76^{a}	2.70 ± 1.56^{a}	66.67 ± 23.09^{d}	53.33 ± 23.09^{b}
2.00	8.00 ± 4.55^{b}	2.65 ± 1.49^{b}	0.90 ± 0.48^{b}	130.00 ± 38.30^{c}	100.00 ± 43.20^{a}
4.00	13.25 ± 14.66^{ab}	4.60 ± 4.85^{ab}	1.40 ± 1.49^{ab}	165.00 ± 156.95^{bc}	50.00 ± 20.00^{b}
6.00	15.50 ± 9.85^{ab}	5.10 ± 3.24^{ab}	1.50 ± 0.87^{ab}	240.00 ± 374.17^{ab}	40.00 ± 28.28^{b}
8.00	8.75 ± 5.56^{b}	3.05 ± 1.81^{b}	1.03 ± 0.56^{b}	285.00 ± 288.62^{a}	35.00 ± 19.15^{b}
10.00	13.25 ± 6.18^{ab}	4.53 ± 2.06^{ab}	1.40 ± 0.63^{ab}	360.00 ± 233.24^{a}	55.00 ± 30.00^{b}

Means with the same superscript are not significantly different ($p < 0.05$).

Table 2. AST, ALT, ALP and LDH in C. *gariepinus* 14 days from the day of injection with aqueous extracts of *L. alepecuroides* (means ± SD).

Conc of L. alepel (ppm)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)
0.00	108.67 ± 43.09^{a}	18.33 ± 10.97^{a}	43.33 ± 8.74^{a}	66.67 ± 23.09^{ad}
2.00	76.75 ± 42.77^{a}	17.25 ± 12.12^{a}	37.25 ± 6.65^{a}	125.00 ± 21.21^{a}
4.00	17.75 ± 98.02^{a}	13.50 ± 3.79^{a}	34.25 ± 4.72^{a}	57.75 ± 16.97^{c}
6.00	91.25 ± 50.99^{a}	13.25 ± 10.18^{a}	41.25 ± 6.18^{a}	81.25 ± 19.96^{bc}
8.00	88.75 ± 34.26^{a}	16.75 ± 8.26^{a}	38.50 ± 6.03^{a}	88.50 ± 11.47^{ab}
10.00	99.25 ± 42.57^{a}	22.00 ± 13.64^{a}	46.00 ± 15.14^{a}	10.00 ± 37.05^{ab}

Means with the same superscript in the same column are not significantly different ($P < 0.05$).

Table 3. Organosomatic indices of *C. gariepinus* injected with *L. alepecuroides* 14 days after injection (mean ± SD).

Conc of *L. alepecuroides* (ppm)	Fulton's Condition (K)	Hepatosomatic index (HSI)	Cardiosomatic index (CSI)	Renatosomatic index (RSI)	Splenosomatic index (SSI)
0.00	0.78 ± 0.05^{a}	0.67 ± 0.08^{a}	0.15 ± 0.01^{a}	0.59 ± 0.52^{a}	0.03 ± 0.02^{b}
2.00	0.73 ± 0.05^{a}	0.83 ± 0.035^{a}	0.15 ± 0.01^{a}	0.82 ± 0.52^{a}	0.06 ± 0.02^{ab}
4.00	0.84 ± 0.09^{a}	0.71 ± 0.21^{a}	0.15 ± 0.02^{a}	0.72 ± 0.08	0.09 ± 0.03^{a}
6.00	0.72 ± 0.09^{a}	0.59 ± 0.44^{a}	0.14 ± 0.06^{a}	0.73 ± 0.12^{a}	0.07 ± 0.03^{ab}
8.00	0.79 ± 0.05^{a}	0.71 ± 0.23^{a}	0.14 ± 0.02^{a}	0.69 ± 0.09^{a}	0.06 ± 0.02^{ab}
10.00	0.76 ± 0.03^{a}	0.60 ± 0.16^{a}	0.14 ± 0.02^{a}	0.65 ± 0.12^{a}	0.05 ± 0.05^{b}

Means with the same superscript in the same column are not significantly different ($P < 0.05$).

DISCUSSION

According to Barton (2002), stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic state. If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing. 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 (Celik, 2004).

The decrease in haemoglobin concentration with increase in the concentration of the plant extract is similar to those reported in *C. gariepinus* exposed to cassava effluents and tobacco (*Nicotiana tobaccum)* leaf extracts (Adeyemo, 2005, Omoniyi et al., 2002). This pattern of response may be attributed to haemolysis which results in haemodilution, a means of diluting the haemoconcentration of the extracts thus reducing the effect of the toxicant/pollutant in its system (Smith et al., 1979; Sampath, 1993). Besides, it may result from either an

increase in the rate of Hb destruction or decrease in its production/synthesis (Reddy and Bashamohideen, 1989). Decrease in PCV shows the extent of the shrink-age of cell size and decrease in the number of cells (Ahmad et al., 1995) . Decrease in the Hb levels may impair oxygen supply to various tissues resulting in slow metabolic rate and low energy production (Ahmad et al., 1995; Atamanalp and Yanik, 2003). Prolonged reductions also lead to blood dyscrasis and degeneration of the erythrocytes being described as a pathological condition in fishes exposed to toxicants (Buckley et al., 1976).

Adeyemo (2005) and Gabriel et al. (2007) respectively recorded significant changes in the WBC of *C. gariepinus* exposed to cassava mill effluents and refined petroleum oil (kerosene). Similar changes in was also recorded by Shakoori et al., 1996) in *Cyprinus idella* treated with fenvalerate, a pyrethroid pesticide. The above two to fivefold increase in WBC (leukocytosis) may have resulted from the excitation of the defense mechanism of the fish to counter the effect of the toxicant. Platelets showed variable responses to different levels of the toxicant which may be a mechanistic response of the cells to fight or combat the effect of the pollutant or the toxicant on the fish. Alteration in the rate of production of platelets by the head kidney and bone marrow due to toxicant effects may be responsible for the variations in the number of platelets in the exposed fish.

The general decrease in the activities of ALT and AST in the exposed fish corroborates the findings of Luskova et al. (2002) in *Cyprinus carpio* exposed to diazinon but contradicts that of Tiwari and Singh (2004) in *Channa punctatus* treated with sublethal levels of alcoholic extracts of *Nerium indicum.* ALT and AST are non plasma specific enzymes that are localized in tissues cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the blood (plasma) may give specific information about organ dysfunction (Wells, 1986; Gabriel and George, 2005) . A decrease in the transaminases suggests that there was no tissue damage (Luskova et al., 2002; Ayalogu et al., 2001), the parenchymatous tissues and skeletal muscles being intact, but rather a decrease in the rate of transfer of the amine groups which eventually affects the rate of protein and carbo-hydrate synthesis in the fish (Watts and Watts, 1974). This is a direct effect of a reduction in the conversion of α-ketoglutarate and alanine to pyruvic and glutamic acid, respectively. The decline in ALP in exposed fish in this study may be due to the fall in the rate of synthesis of glycogen resulting from the low metabolic demands (Shaffi, 1979) and a decrease in metabolic transport (Begum, 2004; Edquist et al., 1992). The decrease may also indicate that there was no kidney damage but a reduction in the hydrolytic action on a number of phosphormonoesters of organic origin such as glucose (Edquist et al., 1992). A reduction in the concentration of LDH in the plasma of the experimental fish infers a decrease in the glycolytic process due to lower metabolic rate (Luskova et al., 2002), a shift towards anaerobic respiration (Tiwari and Singh, 2004), possibly due to a hypoxic internal environment.

Reduction in organ indices and fish wellbeing (condition) depending on its severity may limit physiological systems, reduce growth rate and impair reproducetion (Adams et al., 1996; Jenkins, 2004). The minimal change ($p > 0.05$) in fish condition may be due to the fact that the concentrations of the toxicant were below threshold values or before sampling the fish was able to efficiently detoxify and excrete the wastes, thereby maintaining its physiological integrity without any negative effects on its wellbeing. A slight increase in the HSI of exposed fish indicates that the liver cells were affected possibly causing an increase in the rate of production of endoplasmic reticulum for the synthesis of protein in liver tissue (Anderson et al., 1988). The liver is responsible for enzymatic decontamination process, vitellogenin production and storage of glycogen as energy reserves. Therefore, in the presence of stressors, these qualities are altered resulting in deleterious effect on the fish (Jenkins 2004; Adams et al., 1996). Minimal changes recorded in the cardiosomatic index of fish exposed to extracts of *L. alepecuroides* suggest that the extracts did not interfere with the condition of the heart. The kidney (renatosomatic index) and spleen (spleenosomatic index) increased in this study. These organs are considered as the haematopoietic (blood producing) tissues in fish (Singh and Singh, 1982; Jenkins, 2004) . Both organs are also involved in blood filtration, the development of new blood cells and the immunological interactions. Hence, increase in their size may indicate a pathological response to combat the effect of the toxicant enhancing their ability in the destruction of blood cells (Jenkins, 2004).

Exposure of *C. gariepinus* to *L. alopercuroides* under laboratory conditions moderately affected some aspects of its physiology, a condition which may be worse in field applications where lethal doses of the plant materials are employed in stupefying and catching fish in many parts of the Niger Delta region of Nigeria. Hence, the practice should be discouraged.

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