Fulton's condition, organ indices and haematological response of catfish hybrid (*Heterobranchus longifilis*, ♂ x *Clarias gariepinus*, ♀) *to* aqueous extracts of leaves of *Lepidagathis alopecuroides*

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Abstract

This study was conducted to assess the Fulton's condition, organ indices and haematological response of catfish hybrid, Heterobranchus longifilis, ♂ x Clarias gariepinus, ♀ (mean total length, 29.96±2.23cm, SD; mean weight, 207.83±12.63g,SD) exposed to sublethal concentrations of aqueous extracts (0.00, 1.25, 0.50, 0.75, 1.00, and 0.25mg/l) of leaves of Lepidagathis alopecuroides in a daily renewal bioassay for 21days. The Fulton's condition, organ indices (hepatosomatic index, HIS; cardiosomatic index, CSI; and spleenosomatic, SSI; cardiosomatic index, CSI and renatosomatic index, RSI) and haematological variables (white blood cells, WBC; red blood cell, RBC; packed cell volume, PCV; haemoglobin, Hb; thrombocytes, mean corpuscular haemoglobin, MCH; mean corpuscular volume, MCV; mean corpuscular haemoglobin concentration, MCHC) were assessed at the end of the experimental period. The toxicant caused slight increase only in the HSI ($p>0.05$), whereas for CSI, SSI and RSI in some of the concentrations the weight were same, lower or higher than the respective control values. Leucocytopaenia and thrombocyotsis, $p<0.05$ were the most pronounced haematological changes recorded in treated fish compared with the control. Differential counts (neutrophils, lymphocytes and monocytes), blood variables associated with oxygen transport (PCV, Hb and RBC) and red cell indices (MCV, MCH and MCHC) were not negatively impacted by the toxicant. Although the toxicant may not have impaired oxygen transport, it may depress the defence mechanism but enhance clotting of the event of any vascular injury to the fish. In the field where lethal concentrations of the plant material is used to catch fish more severe physiology changes may occur leading the mortality normally recorded during the application of the biocide, Hence the use the plant material for fishing should be checked by appropriate authorities.

***Key words**: Fulton's condition, organ indices, haematology, catfish hybrid, Lepidagathis alopecuroides*

Introduction

Agriculture is the major source of livelihood for the general population of the Niger Delta area before the advent of crude oil exploitation. However, a sizeable population of the communities in the Niger Delta are still involved in agricultural practices, especially fishing and fish farming. Fishing, apart from crop farming, is one of the veritable sources of livelihood most especially for the inhabitants of the coastal region. One of the methods employed in fish capture is the use of ichthyotoxins (piscicides) usually from plant sources. Varieties of chemicals found in these plants stun or stupefy fish (Kritzon, 2003). Botanicals are natural biocides that contaminate natural water in several parts of Nigeria because of widespread usage. Different parts of toxic plants are used to catch fishes. Piscicidal plants like *Blighia sapida, Kigelia africana, Tetrapleura tetraptera, Raphia vinifera, P. biglobosa* and *Tephrosia vogelii* are frequently used by fisher folks because they are highly potent due to the presence of active ingredients such as saponins and rotenone (Obomanu, 2007).

The shrub, *Lepidagathis alopecuroide*s (Vahl) belongs to the family Acanthaceae. A screening study revealed the presence of alkaloids, tannins, saponins and flavonoids (Obomanu *et al.,* 2005) with piscicidal actions on a fish like mudskipper, *Periophthalmus papillio* (Obomanu *et al.,* 2007). In the aquatic environment, ichthyotoxic botanicals can affect the physiology of fish. This may be reflected by changes in the ratios of the weight of particular organs or tissues relative to total body mass, biochemistry and haematology of exposed fish (Heath, 1995). Besides, organ indices and condition can be used as indicators of change in nutritional and energy status of fish (Adams *et al.,* 1993). Commonly used organ indices include: hepatosomatic (HSI) index, gonadosomatic index (GSI), viscerosomatic index (VSI), and spleenosomatic index (SSI). The assumption that is generally made with these indices is that lower than normal values indicate a diversion of energy away from organ or tissue growth in order to combat a stressor.

Haematological study is of immense importance when diagnostically evaluating fish health as in human health. The effects of toxicants on fish

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can be assessed by the use of haematological indices as they have been reported to be routine procedures in toxicological research, environmental monitoring and fish health conditions (Blaxhall, 1972). Also, Sampath *et al.* (1993) observed that studies on fish blood could reveal conditions within the body of fish long before an outward manifestation of disease or stress condition. Ayotunde (2006) observed that fingerlings of *Oreochromis niloticus* exposed to lethal concentration of *Moringa oleifera* showed an increase in RBC, WBC, PCV, Hb, MCH and MCHC values. But there was a reduction in the ESR and MCV values. In the adults, RBC, PCV, Hb, MCH values increased, however a reduction was recorded in the ESR, WBC and MCV while there was no change (p>0.05) in MCHC values. Adedapo *et al.* (2004) reported that poisonous plants of the genus *Euphorbia* (*E. balsamifera, E. heterophylla, E. hyssopifolia, E. lateriflora, E. hirta*) caused anaemia in the treated rats.

L. alopecuroides is one of the most commonly used biocides for fishing in the Niger Delta area where the clariids are important fish species in both the wild and culture systems, yet nothing is known about the condition, organ indices and haematological impact of exposure to the plant extracts. Therefore, the objectives of this research were to assess the impact of chronic exposure of catfish hybrid to aqueous extracts of *L. alopecuroides* on condition, organ indices and haematological responses of the hybrid catfish.

Materials and Methods

Tank-raised experimental fish, *Heterobranchus longifilis,* ♂ x *Clarias gariepinus,* ♀ (mean total length, 29.96±2.23cm, SD; mean weight, 207.83±12.63gSD) were purchased from a private farm at Abuloma, Rivers State and transported in aerated aquaria to the Research Laboratory, Department of Chemistry Rivers State University of Science and Technology, Port Harcourt. They were acclimated individually in 25*l* borehole water for seven days rectangular aquaria in. During the period the fish was fed a 35% crude protein feed once a day at 1% biomass. No mortality was recorded during the period. Fresh leaves of the plant, *L. alopecuroides* was obtained from the wild from Emohua community in Rivers State. It was air-dried for three weeks in the laboratory to constant weight at room temperature. This was subsequently powdered with the aid of Moulinex electric blender and stored in dry airtight bottles.

The trials runs and experiment were conducted according to the procedures in APHA (1998). Five graded concentrations (0.00, 0.50, 0.75, 1.25, 1.00, and 0.25mg/ l) of the aqueous extracts were prepared in quadruplicates after range finding tests had been conducted. Experimental fish was put singly in each of the aquaria with hand net and covered with netted material that had a slit at the middle to prevent escape of the fish. The aquaria were washed daily through the slit and remove uneaten food and faecal matters were removed with a hose. Water in the control and test solutions were renewed daily. Water analysis (dissolved oxygen, pH, hardness, conductivity and alkalinity) was done on the first, fourteenth and twenty-first day of the experimental period using standard methods in APHA (1998).

At the end of the study period the fish was caught individually with hand net and the weight and total length taken. Blood samples were collected from the kidney behind the anal fins with a 21G hypodermic needle and plastic syringe. The samples were preserved in EDTA bottles for analysis. Thereafter, the fish was killed with a blow on the head, the belly dissected and the organs (kidney, heart, liver and spleen) carefully removed. They were rinsed in water, dabbed dried with soft cloth and weighed. The fish condition was calculated thus:

$K = (W/L^3) \times 10^2$,

where; W = weight, L = Length (Pauly, 1983)

The organ indices were calculated as percentages of body weight (Jenkins, *et al*., 2004). The blood samples were analysed for haematocrit (PCV), haemoglobin (Hb), white blood cell (WBC), platelet and differential count (neutrophils, lymphocytes and monocytes) and red cell indices (mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH and mean corpuscular haemoglobin concentration, MCHC according to the methods in Brown (1980). Data obtained were subjected to a one-way analysis of variance (ANOVA) to test any significant difference resulting from exposure of the fish to the toxicant and difference between means was separated by Duncan Multiple Range Test, DMRT (Wahua, 1990).

Results

The extracts from the plant caused an increase in the pH, conductivity and alkalinity of exposure

solution $p<0.05$ (Table 1). Exposure of the fish to the toxicant produced somewhat slight concentration-dependent increase, $p<0.05$ in organ weight mainly in the liver, whereas the pattern in the heart and kidney was not defined, $p>0.05$ (Table 2). The weight of the other organs were not affected ($p>0.05$) by the plant extracts. The most responsive blood variables to *L. alopecuroides* were WBC and thrombocytes (Table 3) with highest value of WBC, 264.50 ± 35.70 x10^9cells/l recorded at 0.50mg/l. However, at the other concentrations the values were lower than the control value, 212.00 ± 25.87x10^9cells. The toxicant did not impact negatively ($p>0.05$) on the differential counts (neutrophils, lymphocytes and monocytes). The values of the thrombocytes in the exposure concentrations were generally higher ($p<0.05$) than that in the control, 114.50 ± 62.51x10^9cells. There was no reduction ($p>0.05$) in the values of the blood variables involved in oxygen transport (PCV, Hb and RBC) and absolute red cell indices (MCV, MCH and MCHC) in the exposed fish when compared to the control (Table 3).

Discussion

The length-weight relationship in fish is of great importance in fishery assessments (Haimovici and Velasco, 2000). Length and weight relationship in conjunction with age data can give information on the stock composite, age at maturity, life span, mortality, growth and production. The relative robustness or degree of well-being of a fish expressed as the coefficient of condition (condition factor) is an important tool for the study of fish biology, mainly when the species lies at the base of the higher food web (Diaz *et al*., 2000; Lizama and Ambrósio, 2002). Variation in fish coefficient of condition primarily reflects state of sexual maturity and degree of nourishment. Condition values may vary with fish age, and in some species, with sex (Williams, 2000). Studies on the conditions of fish under environmental contaminants (Adams *et al.,* 1996; Jenkins, 2004) and disease state such as viral haemorhagic septicaemia virus, VHSV in *Onchorhynchus mykiss (*Rehulka, 2003) were influenced negatively. In this study, the condition of fish was reduced ($p>0.05$) at all the levels of treatment except at 0.25mg/*l* similar to that observed by Rand and Cone (1990) in *O. mykiss* experimentally infested with tissue dwelling fungus, *Ichthyophonus hoferi*. The reduction in the condition of exposed fish may be accounted for by effect of the toxicant on feeding and the restricted holding conditions. However, condition of fish under toxicant exposure in laboratory experiments are sparingly reported despite the roles such could play in assessing the general health of the fish under experimental holding conditions.

Liver enlargement (raised HSI) recorded in the catfish hybrid exposed to the leaf extracts can be associated with increased detoxification enzyme activity as was reported in rainbow trout (Oikari and Nakari, 1982) and white sucker (McMaster *et al.,* 1991) exposed to bleached kraft mill effluents. Anderson *et al*. (1988) suggested that increase in hepatosomatic index resulted from increased production of endoplasmic reticulum for protein synthesis in the liver tissue under contaminant exposure. Besides, this condition has been associated with hypertrophy of liver cells and hyperplasia under contaminant condition and increased lipid storage (Elskus and Stegeman 1989). However, garlic, *Allium sativum* used as an immuostimulant and growth promoter in *O. niloticus* did not affect the HSI negativelysugesting that the active ingredients in it must have exhibited hepatoprotection. Slight reduction in the spleenosomatic index recorded in most of the exposed fish was also observed in Florida largemouth bass, *Micropterus salmoides floridanus* exposed to paper mill effluents (Sepúlveda, *et al.* 2004) and *O. mykiss* infected with VHSV in the chronic stage (Rehulka, 2003). The increase in size may result in increased in the functioning capacity of the organ, for example sequestration of RBC. However, subsequent build up of excess waste products beyond the capability of the kidney to handled may have adverse effects on the general physiology of the exposed fish.

The responses in the haematological variables in the exposed fish indicated the effect of the toxicant on the internal environment of the fish. When water quality is affected by toxicants as was observed in this study, any physiological changes resulting therefrom will be reflected in the values of one or more of the haematological parameters (Adeyemo, 2005). The altered physico-chemical variables may have acted synergistically with the extracts to effect the changes observed in the blood variables such as WBC and thrombocytes. However, Omoniyi *et al*. (2002), noted that water quality variables

produced negligible effects on the blood of *C. gariepinus* exposed to lethal and sub-lethal concentration of tobacco leaf dust extracts. This may be due to the lethality and mode of the extracts they used in their study.

Gabriel *et al. (* 2004) and Ezeri *et al*. (2004) reported significant changes in the white blood cell (WBC), neutrophils and lymphocytes of the *C. gariepinus* concerned with body defense, reduced PCV value associated with oxygen transport under the stress of acclimation. T toxicant may have impaired leucopoiesis leading to reduced WBC (leucopaenia) and possibly suppressing the immune (defense) system of the fish. In the field, under a generally more toxic levels of the plant used for fishing, the exposed fish may experience much more difficulty fighting pathogenic organisms and with less tolerance to other adverse environmental conditions, making it more susceptible (Pickering and Pottinger, 1985). Although the plasma cortisol was not measured, environmental stressors such as toxicants are known to cause elevated plasma cortisol (Pickering, 1981) probably because corticosteroids depress inflammatory response, mobilization of leucocytes and phagocytosis (Grants, 1967). This may be the case in the exposed fish. Thrombocytes are involved in blood clotting and vital to the haemostatic plug after vascular injury. The increase in the level of thrombocytes suggests the toxicant may have enhanced thrombocytopoiesis through increased rate of conversion of arachidonic acid to thromboxane B_2 (Craig *et al.,* 2002). Although the immune system may be compromised under exposure to the toxicant, in the event of any vascular injury, loss of blood will be drastically reduced through quick and efficient clotting.

Plant derived toxins are known to cause changes in the blood variables (Hb, RBC, MCV, MCH and MCHC) associated with oxygen transport in fish (Agbon *et al.* 2002; Omoniyi *et al*. 2002) and in some cases resulting in anaemia. This response could result from the destruction (lysis) of erythrocytes or inhibition of erythropoiesis by the active ingredients in the extracts, similar to the actions of other toxicants (Brown, 1980).

Notwithstanding, the exposed fish in the present study was not anaemic, suggesting that the levels of the toxicant used did not interfere with the erythropoiesis nor cause haemolysis. Similar observation was made by Zhang *et al., (*2007) in crucian carp, *Carassius auratius* intraperitoneally injected with extracted microsystins. However, the acute levels applied in the field to stupefy fish may produce effects quite different and possibly more devastating than that recorded in this study.

Although sublethal levels of the extracts did not produce anaemia in exposed fish as revealed in the minor changes in the values of PCV, RBC and Hb associated with oxygen transport, it may have interfered (depressed) with the defense mechanism of the fish. However, it may have enhanced blood clotting due to thrombocytosis. Since the effect of exposure may be worse in the field where acute doses of the extracts are used to effect death of exposed fishes, these changes may be more severe.

Results from this study suggest that although the extracts may not have impaired oxygen transport, it may have impacted negatively on the defence mechanism of exposed fish evidenced by a reduction in WBC value, but enhanced clotting time (thrombocytosis) in the event of any vascular injury to the exposed fish. In the field where lethal concentrations of the plant material is used to catch fish more severe physiology changes may occur leading to the mortality normally recorded during the application of the biocide, Hence the use of the plant material for fishing should be checked by appropriate authorities.

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Table 1. Physicochemical properties of exposure solution of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	*pH*	Dissolved oxygen (mg/l)	Conductivity (µ/cm)	Alkalinity (mg/l)	Hardness (mg/l)
0.00	*5.16±0.29*[a]	7.68±0.22[a]	47.00±12.03[a]	14.25 ±1.71[a]	5.74 ±0.00[a]
0.25	6.33±3.92[b]	7.80±0.00[a]	55.25±9.25[ab]	20.50±3.79[b]	5.04±0.47[a]
0.50	6.28±4.66[b]	7.75±0.06[a]	48.75±3.86[ab]	18.75±0.96[b]	5.27±0.54[a]
0.75	6.32±0.11[b]	7.80±0.00[a]	54.00±4.32[ab]	18.00±2.83[ab]	5.04±0.47[a]
1.00	6.51±0.36[b]	7.80±0.08[a]	51.75±6.18[ab]	19.25±0.96[b]	5.51±0.47[a]
1.25	6.47±4.79[b]	7.80±0.08[a]	61.00±10.03[b]	18.75±4.11[b]	5.31±0.96[a]

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

Table 2: Fulton's condition and organ indices of hybrid catfish exposed to chronic levels of aqueous extracts of leaves of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	Initial condition factor K_1	Final condition factor K_2	Hepato somatic (%)	Cardio-somatic (%)	Spleeno-somatic (%)	Renato-somatic (%)
0.00	0.82±0.05[a]	0.82±0.05[a]	0.54±0.04[a]	0.12±0.03[a]	0.08±0.00[a]	0.42±0.07[a]
0.25	0.85±0.07[a]	0.84±0.12[a]	0.57±0.10[a]	0.12±0.08[a]	0.05±0.03[a]	0.44±0.06[a]
0.50	0.81±0.01[a]	0.80±0.02[a]	0.69±0.14[a]	0.13±0.03[a]	0.07±0.03[a]	0.36±0.00[a]
0.75	0.74±0.04[a]	0.74±0.07[a]	0.76±0.26[a]	0.13±0.03[a]	0.05±0.03[a]	0.42±0.08[a]
1.00	0.75±0.10[a]	0.72±0.08[b]	0.75±0.26[a]	0.11±0.03[a]	0.06±0.02[a]	0.42±0.06[a]
1.25	0.81±0.07[a]	0.82±0.09[a]	0.62±0.13[a]	0.11±0.03[a]	0.08±1.80[a]	0.60±0.08[a]

Means in the same column with same superscript are not significantly different ($p>0.05$)

Table 3. Haematological responses of hybrid catfish exposed to chronic levels of aqueous extracts of leaves of *L. alopecuroides* for 21days (mean±SD), means with similar alphabets in the same column are not significantly different, p>0.05.

Conc. toxi-cant (mg/*l*)	WBC ($x10^9$cells)	Neutr (%)	Lymph (%)	Mon (%)	PCV (%)	Hb (g/dl)	RBC ($x10^6$cells/l)	MCV (fl)	MCH (pg)	MCHC (%)	Thromb ($x10^9$ cells/*l*)
0.00	212.00 ±25.87^b	21.00 ±10.89^a	77.00 ±11.94^a	2.00 ±1.63^a	33.95 ±0.82^a	11.33 ±0.29^a	3.64 ±0.09^a	93.43 ±0.05^a	31.14 ±0.02^a	33.33 ±0.00^a	114.50 ±62.51^a
0.25	69.00 ±12.27^c	35.00 ±11.37^a	64.50 ±11.70^a	0.50 ±1.00^a	34.50 ±11.37^a	11.50 ±0.44^a	3.69 ±0.14^a	93.45 ±0.04^a	31.15 ±0.02^a	33.33 ±0.00^a	185.50 ±9.71^b
0.50	264.50 ±35.70^a	20.50 ±1.00^a	79.00 ±1.15^a	0.50 ±1.00^a	32.55 ±2.11^a	10.85 ±0.70^a	3.48 ±0.23^a	93.47 ±0.01^{ab}	31.17 ±0.02^a	33.33 ±0.00^a	159.50 ±42.60^b
0.75	75.50 ±6.40^c	28.00 ±13.37^a	71.00 ±13.11^a	1.50 ±1.00^a	33.60 ±2.40^a	11.20 ±0.80^a	3.59 ±0.26^a	93.53 ±0.05^b	31.17 ±0.02^a	33.33 ±0.00^a	183.00 ±41.90^b
1.00	83.50 ±6.61^c	20.00 ±12.25^a	77.75 ±12.66^a	2.25 ±0.50^a	34.43 ±1.28^a	11.48 ±0.43^a	3.68 ±0.14^a	93.47 ±0.07^{ab}	31.16 ±0.02^a	33.33 ±0.00^a	185.00 ±33.17^b
1.25	79.00 ±6.22^c	23.75 ±11.09^a	75.75 ±12.07^a	0.50 ±1.00^a	33.75 ±1.63^a	11.25 ±0.54^a	3.61 ±0.18^a	93.49 ±0.03^{ab}	31.16 ±0.01^a	33.33 ±0.00^a	135.00 ±58.02^b

Key: WBC- white blood cells, Neutr- Neutrphils, Lymph- Lymphocytes, Mon- Monocytes, PCV-Packed cell volume, Hb-Haemoglobin, Mean corpuscular volume, MCHC- Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, Thromb- thrombocytes.
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