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Haemogram, Organosomastic Indices and Plasma Biochemistry of C*larias gariepinus* Injected with Ethanolic Extract of *Lepidagathis alopecuroides*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors participated in the experiment. The work was supervised by author FGO, who also read the final manuscript. Author UUG designed the work, supervised the work to be sure it went according to the design, managed the statistics and read the first manuscript. Author OSE carried out the experiment and drafted the first manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Exposure of organisms to toxicants especially at high concentrations results in changes in biochemical activities of organisms in most cases. *Clarias gariepinus* were injected with concentrations of *Lepidagathis alopecuriodes* (2.00, 4.00, 6.00, 8.00 and 10.00 ppm) and a control (0.00 ppm). Precisely 2 ml of the extracts/Kg weight of the fish was used to inject the fish in each of the treatment levels. The control was injected with distilled water. They were allowed to swim freely for fourteen days. Haematological analysis of the fish showed that the parameters did not show any concentration dependent change, though they showed slight effects which were either higher or lower than those of the control value, depending on the parameter examined. Organosomatic

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indices showed a general decline in the fish condition. There was no observable change in the hepatosomatic and cardiosomatic indices of the fish except at 10.00 ppm dosage in the cardiosomatic index. Significant increase ($P<0.05$) was observed in the renatosomatic index, while there was observable significant change in the spleenosomatic index. The plasma aspartate transaminase, AST (EC 2.6.1.1) showed a concentration dependent increase in activity except at 4.00 ppm dosage. There was slight uniform (20%) decline in the activities of alanine transaminase, ALT (EC 2.6.1.2) activity in all the dosage concentrations. Alkaline phosphatase, ALP (EC 3.1.3.1) activities declined at 2.00 and 4.00 ppm dosage concentrations, while elevations were observed in all the other dosage concentrations. There was a general decline in the values of sodium ion (Na^+) observed in all the dosage concentrations, though not concentration dependent. Potassium ion (K^+) and chloride ion (Cl^-) did not show any significant change in value with either decrease or increase in their values relative to the control. There was a significant change in the values of hydrogen carbonate ion (HCO_3^-) which were not concentration dependent. The result of this work showed that the extracts from the plant (*Lepidagathis alopecuroides*) can cause deleterious effect on fish at high concentrations.

Keywords: Haemogram; Lepidagathis alopecuroides; enzymes; electrolytes; organ indices

1. INTRODUCTION

The use of synthetic pesticides and other agrochemicals has led to numerous environmental problems such as environmental contamination, persistence, water pollution and detrimental effects on non-target species [1]. These pesticide associated environmental problems has led to the search for better alternatives (botanicals). The botanicals were reported to be safer to the environment, easily biodegradable, have broader spectrum of action, non-persistent, easily processed and applicable [2,3]. Pond cleansing and fish baiting are forms of application of botanicals for the eradication of predators and fish parasites in ponds so as to increase aquaculture productivity [3,4].

However, the botanicals thus applied have also been found to possess some environmental problems associated with toxicity to aquatic species [5,6]. Different parts of toxic plants are used to catch fish. Piscicidal plants such as *Blighia sapida*, *Kigelia Africana*, *Tetrapleura tetraptera,* etc are known and have been frequently used by fish farmers. Their utility is based on potency and chemical components or phytochemicals composition [7] which act as the active components that immobilize the fish. Several plant materials are known to be toxic to zooplankton [5], shrimps [6], commercial fish species both in laboratory and field studies [3,8,9]. Different plant parts (leaves, seeds, kernels, and bark) have varying potencies and modes of actions depending on form of extract (aqueous or otherwise). Different species of plant employed as *piscicides* have different effects on the species of fish targeted [9,10]. It has been shown that these botanicals when in contact with non-target species cause various behavioural, biochemical and physiological stress responses [11,12].

Stress has been identified as the primary contributing factor of fish disease and mortality in aquaculture [13]. Any stress in fish causes hormonal changes, which decreases the effectiveness of inflammatory response of fish reared under commercial aquaculture environments which are confined to the production unit and are predisposed to and weakened by the various operational stress conditions [14]. Changes of enzymatic levels in the fish body can be induced by reactive oxygen species and they may be useful indicators of oxidative stress [15]. Furthermore, the response of aquatic organisms to pollution is given by changes through expression of several key enzymes, especially those involved in biotransformation systems [15]. When the integrity of cell is disrupted enzymes/electrolytes escape into plasma where their activity can be measured and this serves as useful indices for the determination of cell disruption or organ malfunctioning [16].

Toxicants may alter the physiological activities and biochemical parameters in the tissues and blood of exposed fish [17]. The blood of fish is sensitive to pollution-induced stress and certain serum chemistry, which may be used to identify tissue damage [18]. Such changes in the blood profile is an indication of metabolic and biochemical alteration. This gives insight into the mode of action and effect of various pollutants (toxicant) on fish [19] and the environment.

The plant *Lepidagathis alopecuroides*, found in the coastal countries of West Africa belongs to the family *Acanthaceae*. It is applied by local fishermen on the mudflats of tidal water during low tides to immobilize mudskippers and other fish species that inhabits the area in most part of Rivers and Cross River states, Nigeria [7]. This is achieved by spreading the ground leaves of the plant on the mudflats. On contact with the fish, the extract effects a quick kill. According to [20], the plant possesses some antimicrobial activity and therefore finds use by the locals for the treatment of abdominal pains and diarrhea. It also possesses larvicidal action against different mosquito species [21].

This study was carried out to investigate blood variables, organ indices and plasma chemistry of *Clarias gariepinus* injected with ethanolic extracts of *Lepidagathis alopecuroides* leaves.

2. MATERIALS AND METHODS

Fresh leaves of *L. alopecuroides* were collected from Ogbakiri, Emohua Local Government Area, Rivers State and transported to the laboratory, Department of Chemistry, Rivers State University, Port Harcourt. They were air dried for two weeks and later oven dried for three hours at 60℃ with a Gallenkamp oven to a constant weight. The dried leaves were ground into powder with an electric blender (molinex, China), sieved and the fine powder was stored in a dry air tight container. Earthen-pond raised *Clarias gariepinus* (mean total length 30.00±5.23cmSD; mean weight, 151.25±86.91g SD) were obtained from Kpite, Tai Local Government Area, Rivers State and kept individually in aerated plastic aquaria for seven days. The fish received a 35% crude protein diet produced by Coppens Nigeria Ltd at 1% biomass daily. Uneaten food and feacal deposits were removed daily during the acclimation and experimental period by washing the aquaria, and the water renewed daily both in the control and the solution of the aqueous extracts. Extraction of the plant leaves was done according to the method described in [20].

A stock solution of 100 mg/L of 99-100% ethanol extract of *L. alopecuroides* was prepared from the powder, from which five test concentrations (2.00, 4.00, 6.00, 8.00 and 10.00 ppm) were prepared by serial dilution. 2 ml extract per kg weight of the fish was injected intramuscularly above the lateral line of the fish. Fish in the control were injected with same dose of distilled water. There were four replicates in each treatment level and control.

On the fourteenth day after the injection, blood samples were collected from the fish by inserting 21G size attached to 5 ml hypodermic syringe behind the anal fin. When the needle touched the kiney of the fish, blood flowed freely into the syringe. Part (2 ml) of the blood samples were transferred into EDTA bottles for haematological analysis. The other part (3 ml) was transferred into a heparinized bottle, allowed to settle and then centrifuged at 3000 rpm for 10 minutes, for enzymatic (Aspartate transaminase AST, Alanine transaminase ALT, and Alkaline phosphatase ALP,) and electrolyte (Sodium, Na, Potassium K, Chloride Cl^- and Carbonate HCO_3^-) analysis. The fish was then killed and organs (Liver, Spleen, Heart and Kidney) were excised after dissection of the fish for organosomatic indices.

The blood parameters were determined by the method of [22]. The RBC indices (MCH, MCHC and MCV) were analyzed based on the calculation proposed by [23]. For the differential counts, thin blood films were made from drops of well mixed fish blood on clean microscope slide and allowed to dry. The slides were fixed in methanol and then stained with May Grunwall-Giemsa stain. A differential count of one to two hundred cells was done, noting the different cell types and their percentage of occurrence. The organ indices were calculated according to the method of [24]. AST and ALT were analyzed with the modified method of [25]. ALP was determined by the method of [26] with little modification as directed in the test kits. The electrolytes Na^+, K^+, Cl^- and HCO_3^- were analyzed based on the colorimetric end point techniques described by [27]. Data obtained were subjected to statistical analysis using the statistical package for social science (SPSS). One way analysis of variance at 95% confidence limit and means separated by Duncan's multiple range test [28].

3. RESULTS

The responses of the haematological variables associated with oxygen transport (PCV, HB and RBC) in the treated group were variable without ($P<0.05$) being directly related to the concentration of the extracts. The lowest value for PCV (25.50±4.20%, HB (8.50±1.30 g/dL) and RBC ($2.69\pm0.30\times10^{12}$ cell/L) were recorded in fish treated with 6.00 ppm of the extract. However, the highest value of PCV (32.00±2.42%, HB (10.69±0.83 g/dL and RBC ($3.30\pm17\times10^{12}$ cell/L) which were 1.00,0.35 and 0.11 units above the respective control values occurred at 10.00 ppm, Leucocrit values were

raised (P<0.05) 0.25 to 0.50 units above the control in all the treated groups.

Total white blood cell (WBC) was not significantly different (P<0.05) between the test solution and the control. However in some of the concentrations (6.00 and 10.00 ppm), there was a recorded decline. Platelets were the most impacted (P<0.05) of all the blood variables with a decrease in the treated groups below the control value (63.72±10.21x10^9 cell/L). Similar trends (p<0.05) was recorded in the value of neutrophil. The values of Lymphocytes in all the treated fish at 10.00 ppm were raised above the control (37.75±97%) with highest value recorded at 10.00 ppm (47.00±15.12%). Eosinophil value in the fish varied widely with both increase and decrease relative to the control value (4.50±3.11%). Monophils recorded the highest value 3.50±0.7 (6.00 ppm), followed by 1.50+0.30 (4.00 ppm) and 1.00±0.20 (8.00 ppm) as against the control value of 0.000±.00.

Red blood cell indices (MCHC, MCH and MCV) only showed slight variation from the control value. However, these variations were significant (P<0.05) in MCHC (Table 1).

There was a decline in the condition of the fish at the end of the experimental period. Besides, there was depreciation in the condition of the fish in the treated groups in comparison to the control (0.570±04). The worst condition was in the fish at 6.00 ppm and 8.00 ppm toxicant value. Hepatosomatic index slightly decreased at 2.00 ppm and 10 ppm, while size increase was recorded in the other concentrations, the highest recorded value being at 4.00 ppm. There was a slight increase in size of the heart (cardiosomatic index) in 2.00, 6.00 and 8.00 ppm, but decreased at 10.00ppm. There was a general size increase (P<0.05) in the kidney of all the exposed fish, the highest value being observed at 4.00 ppm (0.79±0.09) which was followed by that of 10.00 ppm. The spleenosomatic index in all the treated groups were similar to the control value (0.08±0.02), except at 8.00 ppm (0.14±0.04) which was about twice the other values (Table 2).

The plasma AST showed a general dose dependent rise in activity with exposure concentration above the control (11.50±5.20 IU/L). The activities at 2.00, 4.00, 6.00, 8.00 and 10.00 ppm of the extracts were 22.001±0.00, 16.50±7.90, 32.00±20.08, 89.00±0.00 and 89.00±0.00 representing 91.3, 50, 178.26, 673.91, and 673.91% rise respectively above control. ALT activity decreased in all the concentrations below the control (value, 5.00±2.00 IU/L. at (20%). ALP increased at 4.00, 6.00 and 8.00 ppm respectively which were 30.25±15.00 IU/L and 30.00±10.04 IU/L. However decrease was observed at 2.00 and 10.00ppm which were 10.39 and 43% lower than the control respectively (Table 3).

Table 1. Haematological parameters of *Clarias gariepinus* on 14th day after injection with ethanolic extracts from the leaves of *Lepidagthis alopecuroides*

Parameters	Concentration of Toxicant (PPM)					
	0.00	2.00	4.00	6.00	8.00	10.00
PCV(%)	31.00±7.35^{a}	28.75±2.99^{a}	31.50±4.20^{a}	25.50±4.20^{a}	30.50±6.61^{a}	32.00±2.45^{a}
HB(g/dl)	10.33±2.4^{a}	9.30±1.18^{a}	10.50±1.9^{a}	8.50±1.39^{a}	10.18±2.1^{a}	10.68±0.8^{a}
RBC	3.19±0.61^{a}	2.95±0.28^{a}	3.23±0.53^{a}	2.69±0.35^{bc}	3.15±0.57^{b}	3.30±0.17^{a}
Leuco	10.00±0.0^{a}	0.50±0.58^{a}	1.50±0.58^{a}	1.25±1.50^{a}	1.50±0.58^{a}	1.25±1.29^{a}
WBC	10.05±4.79^{a}	14.08±2.75^{a}	14.68±5.79^{a}	8.15±4.15^{a}	13.45±7.71^{a}	9.70±4.82^{a}
Plat.(%)	62.75±10.21^{a}	25.0±0.00^{b}	43.75±37.50^{ab}	43.75±23.94^{ab}	37.50±14.43^{ab}	43.75±23.94^{ab}
WBC counts						
Neutro(%)	63.25±6.39^{a}	54.50±12.12^{a}	61.25±21.23^{a}	60.00±18.94^{a}	57.00±11.23^{a}	51.75±16.68^{a}
Lymph(%)	31.75±7.97^{a}	38.00±8.12^{a}	44.75±19.41^{a}	32.75±19.74^{a}	37.75±11.70^{a}	47.00±15.12^{a}
Eosino(%)	4.50±3.11^{a}	7.00±5.09^{a}	5.75±8.02^{a}	1.75±3.50^{a}	4.75±4.11^{a}	1.25±2.50^{a}
Mono (%)	0.00±0.00^{a}	0.00±0.00^{a}	1.50±3.00^{a}	3.50±7.00^{a}	1.00±2.00^{a}	0.00±0.00^{a}
RBC indices						
MCHC	32.50±0.58^{a}	32.75±0.50^{a}	32.75±0.50^{a}	32.25±0.50^{bc}	31.50±0.50^{b}	32.50±0.58^{a}
MCH (pg)	32.25±0.50^{ab}	31.50±0.58^{b}	32.50±058^{ab}	31.50±0.58^{b}	32.25±0.50^{ab}	32.25±0.50^{ab}
MCV (fl)	96.25±0.50^{a}	96.50±0.58^{a}	69.75±0.50^{a}	96.00±0.82^{a}	96.25±0.96^{a}	97.00±0.00^{a}

Packed cell volume (PCV), heamoglobin (Hb), red blood cells (RBC), leucocrit (leuco.), white blood cell (WBC), platelets (plat.), neutrophils (neutro.), lymphocyte (lymph.), eosinophils (eosino), monophils (mono), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV). Means with the same superscript in the same row are not significantly different (P<0.05)

Table 2. Condition and organosomatic indices of *Clarias gariepinus* on 14th day after injection with ethanolic extracts from the leaves of *Lepidagthis alopecuroides*

Conc. of L. alopecur. (PPM)	Initial condition	Final condition	Hepatosomatic	Cardiosomatic	Renatosomatic	Spleenosomatic
0.00	0.57±0.0^{a}	0.57±0.0^{a}	1.05±0.21^{a}	0.13±0.05^{a}	0.52±0.07^{b}	0.08±0.02^{b}
2.00	0.59±0.1^{a}	0.53±0.0^{a}	1.03±0.19^{a}	0.14±0.04^{a}	0.61±0.10^{ab}	0.08±0.02^{b}
4.00	0.54±0.0^{a}	0.49±0.0^{a}	1.43±0.06^{a}	0.13±0.02^{a}	0.79±0.09^{a}	0.07±0.01^{b}
6.00	0.55±0.0^{a}	0.48±0.0^{a}	1.23±0.33^{a}	0.15±0.06^{a}	0.62±0.08^{ab}	0.08±0.01^{b}
8.00	0.54±0.0^{a}	0.48±0.0^{a}	1.21±0.26^{a}	0.14±0.04^{a}	0.60±0.07^{ab}	0.14±0.04^{a}
10.00	0.56±0.0^{a}	0.52±0.0^{a}	1.04±0.40^{a}	0.11±0.03^{ab}	0.69±0.19^{a}	0.08±0.03^{b}

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

Table 3. Enzymes in plasma of *Clarias gariepinus* after 14th day injected with ethanolic extract of *Lepidagathis alopecuroides*

Conc. in PPM	AST (IU/L)	% of Control	ALT (IU/L)	% of Control	ALP (IU/L)	% of Control
0.00	11.50±5.20^{b}	100	5.00±2.00^{a}	100	21.08±10.04^{a}	100
2.00	22.00±10.00^{b}	191.3	4.00±0.00^{a}	80.00	19.10±19.10^{a}	90.61
4.00	16.50±7.90^{b}	143.48	4.00±0.00^{a}	80.00	30.25±15.00^{a}	143.5
6.00	32.00±20.08^{b}	278.26	4.00±0.00^{a}	80.00	30.00±19.03^{a}	143.5
8.00	89.00±0.00^{a}	773.91	4.00±0.00^{a}	80.00	45.00±20.00^{a}	213.4
10.00	89.00±0.00^{a}	773.91	4.00±0.00^{a}	80.00	14.08±9.70^{a}	66.7

Means with the same superscripts in the same column are not significantly different (P<0.05)

Table 4. Electrolytes in plasma of *Clarias gariepinus* after 14th days injected with ethanolic extract of *Lepidagathis alopecuroides*

Conc. in PPM	Na^+ (mEq/L)	% of control	K^+ (mEq/L)	% of control	Cl^- (mEq/L)	% of control	$HCO3^-$ (mEq/L)	% of control
0.00	76.50±40.07a	100	6.93±1.14a	100	85.00±16.47a	100	9.13±2.32abc	100
2.00	74.25±36.78a	97.05	6.70±2.52a	96.68	90.25±15.44a	106.18	10.00±2.71a	109.53
4.00	59.75±26.59a	78.1	4.72±1.91a	68.11	106.50±26.21a	125.29	9.50±0.58ab	104.03
6.00	65.75±44.87a	85.95	4.35±1.69a	69.99	89.75±21.64a	105.59	9.25±0.96abc	101.31
8.00	71.25±44.87a	93.14	6.53±1.42a	94.23	94.25±26.96a	110.88	6.50±1.73c	71.19
10.00	50.75±29.55a	66.34	6.55±2.58a	94.52	79.00±2.71a	95.94	7.00±0.82bc	76.67

Means with the same superscripts in the same column are not significantly different (P<0.05)

The level of sodium (Na^+) generally declined (P<0.05) with the maximum, 50.75±25.37 mEq/L (33.66%) at 10.00 ppm below control (76.50±40.07 mEq/L) followed by the values at 4.00 and 6.00 ppm dosages which were 59.75±26.59 mEq/L and 65.75±26.42 mEq/L representing 40.25% and 34.25%. Potassium ion (K^+) level in the control (6.97±1.14 mEq/L) was below control was close to that at 2.00, 8.00 and 10.00 ppm. However, 31.89% and 30.01% decline was observed at 4.00 ppm (4.72±1.91 mEq/L) and 6.00 ppm (4.35±1.69 mEq/L, respectively.

Chloride ion (Cl^-) increased in all the concentrations above the control (85.00±16.47 meq/L except at 10.00 ppm. The increase ranged from 5.59% at 6.00 ppm to 25.29% at 4.00 ppm in the exposed fish. The bicarbonate ion (HCO_3^-) concentrations in the plasma of the exposed fish were either higher or lower or about the same value to that of the control (9.13±2.32 meq/L) (above Table 4).

4. DISCUSSION

Haematological, biochemical, enzymatic and genetic analysis are used to evaluate the effects of xenobiotics on organisms, the compartments affected by toxic processes and to facilitate comparison with the control and also to establish basis of the structural changes in the various parameters assessed [29].

In this study, there was a decrease as well as increase in PCV, HB and RBC. However, [30]

observed significant decrease in PCV, HB, RBC, neutrophils and the RBC counts, but increased values of leucocyte count (WBC) and lymphocytes in rats administered with chronic levels of indomethacin. In another study [31], observed decrease in PCV, HB, RBC and the RBC counts (MCH, MCHC and MCV), and a fall in the value of WBC in *Clarias gariepinus* exposed to cassava mill effluents. However, [32] noted a decrease in HB, MCH, MCHC, MCV, PCV and WBC levels in rainbow trout exposed to mancozeb concentrations. Changes (increase or decrease) in blood parameters as observed authors above were all alluded to effect of pesticides and toxicological implications on the tested animal. Decrease and increase in the blood parameters observed in this study may have resulted from the interaction of the active components of the plant extract on the cell physiology of the organ involved in erythropoiesis in the fish. Decrease in RBC and HB have resulted from breakage of blood vessels causing haemorrhagic anemia and the reduction of blood carrying capacity of the fish [30]. The decrease in HB concentration results in the impairment of oxygen supply to the various tissues which eventually lead to low metabolic rate and low energy production [33]. Fall in RBC, HB and MCH reflects an anemic state of fish which could possibly be due to iron deficiency and its consequent decreased utilization for the synthesis of haemoglobin [34]. Anemia could be due to structural changes of the haem which eventually leads to perturbations in the formation and synthesis of haemoglobin [35] and also from inhibitory active components of the plant extract. Decreased RBC may be as a result of destruction of erythrocytes and such degeneration of blood dyscrasia is a form of physiological and pathological conditions in fish exposed to toxicant [31]. The increase recorded in PCV and HB may be due to erythrocyte swelling as related to intracellular osmotic disorders and stress, while its haemolysis is associated with blood serum acidification and intracellular alkalization [36]. Decrease PCV shows the extent to which the cells may have shrunk in size and/or the decrease in the number of cells in the organism's blood [33].

Platelets declined in all the experimental doses. This may be an indication of weakness of the fish to prevent blood loss in eventual tissue damage, since platelets are involved in the clotting mechanism and blood loss prevention which is a mode to maintain the required volume of blood in the organism [37]. Change in WBC may be leucopoenia or leukocytosis, which indicate a shift in the defense mechanism of the fish due to the effect of the toxicant. Decrease and increase in WBC and Leucocrit has been observed in tench (*Tinca tinca*) exposed to mercury, lead, and cadmium [38]. This condition is probably due to the increase in lymphocytes, eosinophils and thrombocytes in the fish. Lymphopenia may be attributed to decrease in mean cellular lifespan and impaired proliferative capacity of cells [39]. Besides, it might be due to impairment of haematopoietic cells in kidney [40], necrosis of leucopoenia tissues [39] and the accumulation of lyphocytes in the lymphoid tissues or destruction of cortiscosteriod hormones [41]. Decrease in leucocrit may have resulted from haemodilution [42] and lymphocytosis may be due to injury in hematopoietic tissue or the acceleration of lymphopoiesis or the efflux of lymphocytes from the lymphoietic cell [43]. However the increase observed in some of the concentrations may be a compensatory response of the lymphoid tissues to the destruction of circulating lymphocytes.

According to [38], increase and decrease of leucocrit and WBC can be attributed to the activity of the spleen which sequesters and stores blood cells under resting conditions and releases them into circulating blood during contraction associated with various states of stress. Gradual repair of damaged blood forming tissue also contributes to the rise and fall of these parameters [44]. A change in monocytes proportion is probably due to the action of adrenal glucocorticoids, which influences mitogenesis, chemical communication and the adherence of blood cell involved in the immune response [45,46,47]. Also the activation of the catecholamines into the peripheral blood modulates the leucocytes response through receptors located on blood vessels and lymphatic organs [48].

RBC indices remained unchanged during the period of exposure. This may have resulted from the pattern or the response of the PCV and RBC to the toxicant. However, a decrease in MCH and MCV levels is attributed to a sign of hypochromic mycrocytic anemia [49].

Condition factor and organosomatic indices are forms of health assessment indices used to determine the state of well-being and the internal conditions of the fish organs [50]. In this study, a decrease in fish condition was observed similar to that reported by [51,52], who observed that reduced growth rate that was proportional to the

toxicant concentration. Reduction in growth rate has also been recorded in Nile tilapia after treatment with sublethal concentrations of lead [53]. Negative condition can be as a result of the effect of the toxicant on the normal metabolism of fish [54]. However, increased condition and decreased lipid levels have been reported in fish exposed to bleached kraft mill effluents [49]. Increased conditions may be a disruption in the metabolic capability and altered energy allocation [55]. Hepatosomatic index reflecting the relative condition or size of the liver to the whole fish quite appreciated in this study except at 10.00 ppm. The liver which is involved in ovarian development, formation of vitlelogen and protein [51] when altered will affect the reproductive endocrine system [51], and the formation of triglycerides which is suggestive of a general disorder in protein and carbohydrate metabolism. Hypertrophy may be from multiplication or increase in size of liver cells for improved performance so as to overcome the effect of the stress from increased amount of lipids stored to maintain energy at stabilized levels [56]. The increase in the heart size may be due to the effect of the toxicant in blood distribution in the fish. The kidney also showed size increase, a situation which may be due to swelling of erythrocytes [57]. The spleen remained unchanged or increased slightly in this study. Reduction in spleen weight is associated with sharp decline in the proportion of total lymphocytes and macrophages in the spleen haemopoietic tissues in the body, which helps in the production of body immune system which when altered as in this study will weaken the immune system of the organism. The increase in the size of the kidney may have been responsible for the increase in size of the heart so as to accommodate the rate of the increase of blood production from the kidney and the spleen.

The homeostatic mechanism of fish is continuously exposed to changes in their aquatic ecosystem and habitat. Both biotic and abiotic factors of the ecosystem can individually or collectively cause stress in fish [58,59]. Response of an organism to stress has a biochemical and molecular bases that can be noticed through qualitative and quantitative changes at the subcellular and extracellular levels of the organism. Biochemical and molecular indicators are ideal as biological indicators in that they react quickly to changes in the ecosystem and so provide the first warming signal of a stressed internal environment [60].

Increased activities of AST and ALT in some exposed fish and also a decrease in the activity of ALT also suggests the possibility of observed impaired liver function [61]. The authors further observed that the transaminases are good indices of the health status of fish. Liver parenchyma and tissue necrosis is considered as the main source of AST. Any increase in the plasma of fish may indicate necrotic damages. Exposure of fish to environmental pollutants may result in the stimulation or depression of the enzyme activity depending on the concentration of the pollutant and duration of exposure. These changes in the biochemical profile indicate changes in metabolism and biochemical processes of the organism [19]. Liver is the major centre for the detoxification of chemicals and its damage is confirmed by changes in the enzyme activities of AST and ALT [62]. Chronic hepatic disorders and excessive steroids result in increased plasma alkaline phosphatase (ALP) in animals, osteopathies, a situation arising from bone disease [16]. According to [63] significant elevation of ALP after exposure to toxicant is attributed to liver dysfunction. ALP is formed in the liver, membrane-bound close to biliary canaculli, secreted in the bile and its increase principally indicates cholestasis increase. ALP in the plasma may be due to increase in protein levels of the organism [64,65], since it plays an important role in protein synthesis. This leads to the augmentation of stress, a condition provided by transamination that leads to the efficient utilization of amino acids and energy [66]. The increase in AST and the decline in ALT may be a mode of mechanism in the fish to maintain the internal parenchyma of the organ cells or the mode of the interaction of the toxicant, under energy source through the oxidation of the amino acids [67]. However, decrease in activity of ALT and ALP in the plasma suggests that they offer protection by preserving the structural integrity of hepatocellular membrane [68,69].

Electrolytes are body ions which acquire the capacity to conduct electricity and their balance in the body is essential for normal function of cells and organs. Sodium and potassium are cations found in the extracellular and intracellular fluids, respectively. Sodium regulates the amount of water in the body and the transmission of fluid in and out of the cell plays a role in critical body functioning [70]. Many metabolic processes in the brain, nervous system and muscle require electrical signals or communication. Potassium is important in the regulation of heart beat, and functions of the muscle. Decrease in level of

these electrolytes as was observed in this study may be due to water imbalance and may alter rate of heart beat [71]. Decrease in sodium (Na^+) and potassium (K^+) ion will hinder the transfer of information access the synapses of cell membrane (ie the inhibition of acetylcholinesterase, a neuropatically transmitted enzyme [19]. Increase or decrease in these ions causes interference with the transmission of electrical signals across the cells causing cell malfunction, impair general co-ordination of impulses particularly and increases the chances of irregular heartbeats (arrhythmias). Increase in Na^+ and K^+ in the plasma can be attributed to kidney damage or dysfunction, since kidney is the normal path for ions [61]. Changes in Na^+ ion in fish may have resulted from the effect of toxicant on the gills.

Chloride (Cl^-) is the major anion in the extracellular fluid and blood, while the bicarbonate (HCO_3^-) is found in the intracellular fluid and blood. The Cl^- ion helps or plays a role of maintaining the normal balance of fluids in the organisms system. The bicarbonate (HCO_3^-) acts as a buffer that maintains the normal pH in blood and other fluids in the body. Increase or decrease in chloride levels is associated with stress condition [72]. Decreased concentration is associated with gill injury and/or loss of chloride across the gills [70,72]. Elevation in this study may be due to certain kidney malfunction or over reactivity of the parathyroid glands responsible for reproduction. The internal pH is often affected in the presence of toxicants which usually alter the internal homeostatic environment of the organism. The fluctuations observed in the HCO_3^- in this study may be to counter the effect of the toxicant on the kidney and the gills since these two organs are involved in the osmotic balance and respiration of the fish.

Minerals are mainly responsible for the maintenance of osmotic pressure in blood and all types of tissues, which is often compromised by stress, disease or gill lesions that increase gill permeability to ions [70,73].

5. CONCLUSION

The changes observed in the organ indices, blood variables, plasma enzymes and electrolytes of *Clarias gariepinus* injected with ethanolic extract of *L. alopecuroides* showed that the plant has negative effects on fish species and therefore its use should be controlled and restricted so as to abate its effect on non-target species in the environment. Furthermore, the locals should be properly advised on the negative impact of the use of the plant for fishing purposes and its toxicological implications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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