Journal of Medicinal Plants Research Vol. 3(12), pp. 995-1001, December, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Biomarker enzymes in muscle tissue and organs of *Clarias gariepinus* after intramuscular injection with aqueous extracts of *Lepidagathis alopecuroides* leaves

F. G. Obomanu[1], U. U. Gabriel[2]*,O. S. Edori[3] and J. N. Emetonjor[1]

[1]Department of Chemistry, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria.
[2]Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria.
[3]School of Remedial and General Studies, Rivers State College of Health Science and Technology, P.M.B. 5039, Port Harcourt, Nigeria.

Accepted 9 September, 2009

Tank-raised *Clarias gariepinus* (mean weight, 250.04 ± 50.22 g, SD: mean total length 30.05 ± 4.05 cm, SD) were intramuscularly injected with aqueous extracts (2 ml/kg weight of fish) of various concentrations (2.00, 4.00, 6.00, 8.00 and 10.00 ppm) of leaves *Lepidagathis alopecuroides.* The extract was administered to five replicates/treatment level and a control injected with distilled water. On the fourteenth day, samples of organs (kidney, liver and gill) and muscle tissue were analysed for alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH). ALP activity in the treated group the was inhibited below the control, 376.65 ± 222.50 IU/L, but at 87.18% elevation at 10.00ppm (705.00 ± 83.55 UI/L), AST activity was inhibited from 546.65 ± 190.70 IU/L in the control to 223.75 ± 69.80 IU/L at 8.00 ppm extract. ALT activity was inhibited with the lowest value, 30.00 ± 18.25 IU/L at 6.00 ppm, compared with the control, 53.35 ± 22.55 IU/L. LDH activity was excited 5.09, 23.54 and 5.49% at 2.00, 8.00 and 10.00 ppm extract respectively, above the control, 311.65 ± 58.35 IU/L. The activities of the respective enzyme in the muscle of treated fish had maximum inhibition below their respective control values as follows: ALP, 41.67% at 10.00 ppm; AST, 39.53% at 2.00 ppm; ALT, 83.01% at 6.00 ppm, and LDH, 45.55% at 4.00 ppm). Activities of ALP, AST and ALT in the kidney were inhibited below the control and varied very widely among the enzymes. However, LDH activity was excited in exposed fish with a peak, 592.50 ± 301.90 IU/L, 125.77% above the control, 231.65 ± 168.25 IU/L at 8.00 ppm. ALP activity in the liver was elevated at 2.00, 4.00 and 10.00 ppm; 25.00, 43.75 and 18.75%, respectively, above the control, 100.00 ± 20.00 IU/L. AST and ALT actvivties were either inhibited or excited. LDH activity was excited in the treated group (maximum, 887.50 ± 438.95 IU/L, 29.87% above control value, 683.35 ± 104.10 IU/L at 2.00 ppm. The relative activity of the ALP in the tissues was kidney>gill>liver>muscle; ALT activity was most pronounced in the kidney, but the pattern of ALT in the other tissues and that of AST varied very widely within each of the organs and among the various concentrations of the extract. LDH activity was higher in the muscle tissues and liver than the kidney and gills, however, with no defined pattern relative to the exposure concentrations. Results from this study strongly suggest that all the enzymes assayed could be good indicators of *L. alopecuroides* toxicosis in *C. gariepinus*.

Key word: Alkaline phosphatase, alanine transaminase, aspartate transaminase, lactate dehydrogenase, *Clarias gariepinus*, *Lepidagathis alopecuroides.*

INTRODUCTION

A number of ichthyotoxic plants have been known and stem, root, seeds, and bark which are toxic to aquatic organisms. *Lepidagathis alopecuroides* (Family: Acanthaceae) is one of such plants. The Efik in Cross used for fishing in many parts of the world for centuries. These

*Corresonding author. E-mail: ugwemg@yaho.com.

plants contain natural biocides in their leaves; River State uses the leaves for treating sores and fishing. In some communities in Rivers State, it is administered in alcohol as a cure for stomach disorder. The ground leaves of the plant is commonly used to immobilize and kill fish (Obomanu et al., 2007) in Rivers State and other parts of the country. Comparative assessment of the larvicidal activity of aqueous extracts of the leaves showed that *L. alopecuroides* was more toxic to *Culex quinquefasciatus* and *Anopheles gambiae* than neem, *Azardirachta indica* (Obomanu et al., 2006). The leaves also showed anti-microbial activity (Obomanu et al., 2005). The actions of the plant may be due to the presence of alkaloids, tannins, saponins and flavonoids in the leaf extracts of the plant, which have piscicidal properties, killing fish within a short exposure time depending on the concentration (Obomanu et al., 2007).

Although a number of studies have revealed the toxicity of several plant parts to fish and aquatic fauna (Onusiriuka and Ufodike, 1994; Mosta-Fa and El-Deeb, 2002, Omoniyi et al., 2002; Singh and Singh, 2002) very little is known about the internal physiological and biochemical changes leading to the mortality commonly studied. Biochemical and metabolic changes in fish as a result of aquatic contamination or toxicant induced stress from plant biocides have been reported (Tiwari and Singh, 2004; Shanmugasundaram and Venkaraman, 2005). Fishes and other aquatic organisms have been used as biomonitors or indicators of polluted or contaminated environments (Gabriel and Kparobo, 2003; Gabriel and George, 2005). This is generally achieved through the measurement of biochemical, physiological and behvaioural responses of the organisms in question (Agbon and Omoniyi, 2002; Tiwari and Singh, 2004; 2006). Interestingly, study of alterations in enzyme activities in the organs of fish is one of the most reliable means of assessing effects of toxicants (Achuba et al., 2005; Omitoyin et al., 2006).

This investigation was carried out to assess the changes in the activities of alkaline phosphatase, ALP; alanine transaminase, ALT; aspartate transaminase, AST and lactate dehydrogenase, LDH in selected organs (kidney, liver, and gill) and muscle tissues of *C. gariepinus* after intramuscular injection with crude (aqueous) extracts of the leaves of *L. alopecuroides* (Family, Acanthaceae).

MATERIALS AND METHODS

Fresh leaves of *L. alopecuroides* were collected from Ogbakiri, Ikwerre Local Government Area, Rivers State and transported to the Laboratory, Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt. They were air-dried for two weeks and later oven-dried for three hours at 60°C to a constant weight. The dried leaves were ground into powder with an electric blender, sieved and the fine powder was stored in a dry airtight container. Tank-raised *Clarias gariepinus* (mean total length 30.05 ± 4.05 cm, SD; mean weight, 250.04 ± 50.22 g SD) were obtained and transported in aerated aquaria to the same laboratory, where they were acclimated individually in plastic aquaria for seven days. The fish received a 35% crude protein diet at 1% biomass daily. Uneaten food and faecal matters were removed daily during the acclimation and experimentation period with a hose. Water in the control and solution of the extracts were renewed daily.

A stock solution of 100 mg/l of the aqueous solution of *L. alopecuroides* was prepared from the powder with distilled water from which five test concentrations (2.00, 4.00, 6.00, 8.00 and 10.00 ppm) were prepared by serial dilution for injection of the fish. 5 ml of the extract per kg weight of 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 five replicates in each treatment level and the control.

On the fourteenth day after the injection, the fish were killed by a blow on the head. Samples (0.5 g) of the gill, muscle, liver and kidney were extracted. The organ samples were homogenized and 5ml of normal saline solution (physiological saline) was added and centrifuged at 3000 rpm for 10 min. The supernatant was collected for the determination of the activities of alkaline phosphatase (ALP), aspartate transaminase, AST, alanine transaminase, ALT, and lactate dehydrogenanse (LDH). With standard kits (Randox Laboratory Ltd., Antrim, UK.) the activities ALT and AST were determined using the methods of Reitman and Frankel (1957), ALP by Hafkenscheid and Kohler (1986), and LDH by Barker and Summerson (1941) modified by Huckabee (1961). Data obtained were subjected to statistical analysis using a one way analysis of variance and means were separated by Duncan's multiple range tests at 95% probability (Wahua, 1999).

RESULTS

Generally, there was a decrease ($p > 0.05$) in the activity of all the enzymes in the gill as the amount of the plant extract injected was increased (Table 1). In the treated group the ALP activity was inhibited below the control, 376.65 ± 222.50 IU/L. However, in fish injected with 10.00 ppm, the elevation was 87.18% 705.00 ± 83.55 UI/L above the control (376.65 ± 222.50 IU/L). The extract caused a decline in ALT activity with the lowest value, 30.00 ± 18.25 IU/L recorded at 6.00ppm, compared with the control,53.35 ± 22.55 IU/L AST activity was inhibited from 546.65±190.70IU/L in the control to 223.75 ± 69.80 IU/L at 8.00ppm extract. LDH activity was excited by 5.09, 23.54 and 5.49% in fish injected with 2.00, 8.00 and 10.00 ppm extract respectively, above the control, 311.65 ± 58.35 IU/L. The activities of all the enzymes in the muscle tissues declined with increase in the amount of extract injected below the control values (Table 2). The activities of the respective enzyme in the treated group were inhibited below their respective control values with maximum inhibition recorded at follows: ALP, 35.00 ± 10.00 IU/L at 10.00 ppm, 41.67%, below the control, 60.00 ± 0.00 IU/L; AST, 681.25 ± 155.70 IU/L at 2.00 ppm, 39.53% below the control, 1126.65 ± 163.75 IU/L; ALT, 16.25 ± 6.30 at 6.00 ppm, 83.01% below the control, 33.35 ± 5.75 IU/L and LDH, 768.75 ± 85.05IU/L at 4.00 ppm, 45.55% below the control, 1113.35 ± 359.20 IU/L.

Activities of bioindicators enzymes (ALP, AST and ALT) in the kidney were inhibited below the control: ALP,

Table 1. Enzyme (AST, ALT, ALP, LDH) activity in the gill of *C. gariepinus* 14 days after intramuscular injection with crude extracts of *Lepidagathis alopecuroides* (mean ± SD). Means in the same column with same superscript are not significantly different (p > 0.05).

Conc. Of extract (ppm)	ALP (IU/l)	% Control	AST (IU/l)	% Control	ALT (IU/l)	% Control	LDH (IU/l)	% Control
0.00	376.65± 222.50[a]	100.00	546.65± 190.70[ab]	100.00	53.35± 22.55[a]	100	311.65± 58.35[a]	100
2.00	260.00± 25.15[a]	69.03	548.75± 83.25[ab]	98.55	42.50± 23.95[a]	79.66	327.50± 53.30[a]	105.09
4.00	241.25± 67.95[a]	64.05	251.25± 46.15[ab]	45.96	17.50± 8.65[a]	32.80	296.25± 66.25[a]	95.06
6.00	328.75± 166.00[a]	87.28	394.00± 163.85[ab]	71.34	30.00± 18.25[a]	56.23	281.25± 17.50[a]	90.25
8.00	291.25± 156.40[a]	77.33	223.75± 69.80[a]	40.93	21.25± 12.50[a]	39.83	385.00± 55.80[a]	123.54
10.00	705.00 ± 83.55[a]	187.18	248.75± 117.05[a]	45.50	48.75± 37.07[a]	91.38	328.75± 39.15[a]	105.49

Table 2. Enzyme (AST, ALT, ALP, LDH) activity in the muscle of *C. gariepinus* 14 days after intramuscular injection with crude extracts of *Lepidagathis alopecuroides* (mean ± SD). Means in the same column with same superscript are not significantly different (p > 0.05).

Conc. of extract (ppm)	ALP (IU/l)	% Control	AST (IU/l)	% Control	ALT (IU/l)	% Control	LDH (IU/l)	% Control
0.00	60.00± 0.00[a]	100	1126.65± 163.75[ab]	100	33.35± 5.75[ab]	100	1113.35 ± 359.20[ab]	100
2.00	50.00± 20.00[a]	83.33	681.25± 155.70[a]	60.47	7.50± 15.00[a]	22.49	725.50± 84..20[a]	67.59
4.00	40.00± 16.35[a]	66.67	843.90± 120.70[a]	74.90	13.75± 2.50[a]	41.23	606.25± 24.95[a]	54.45
6.00	55.00± 19.35[a]	91.67	707.50± 110.45[a]	62.80	5.00± 7.05[a]	14.99	756.25± 24.95[a]	65.23
8.00	40.00± 16.35[a]	66.67	718.75± 194.55[a]	64.80	7.50± 6.30[a]	22.49	768.75± 85.05[a]	69.05
10.00	35.00± 10.00[a]	58.33	792.50± 273.35[a]	70.34	16.25± 6.30[a]	48.73	668.75± 213.45[a]	66.07

Table 3. Enzyme (AST, ALT, ALP, LDH) activity in the kidney of *C. gariepinus* 14 days after intramuscular injection with crude extracts of *Lepidagathis alopecuroides* (mean ± SD). Means in the same column with same superscript are not significantly different (p > 0.05).

Conc. of extract (ppm)	ALP (IU/l)	% Control	AST (IU/l)	% Control	ALT (IU/l)	% Control	LDH (IU/l)	% Control
0.00	1980.00 ± 1497.65	100.00	776.65 ± 122.90[a]	100	81.65 ± 23.05[a]	100	231.65 ± 168.25[a]	100
2.00	1282.50 ± 453.25	64.77	603.75 ± 297.15[a]	77.74	65.00 ± 35.35[a]	79.61	297.50 ± 37.75[a]	128.43
4.00	956.25 ± 453.25	48.29	530.00 ± 275.55[a]	68.24	63.75 ± 13.15[a]	78.08	481.25 ± 123.20[ab]	207.75
6.00	793.75 ± 426.25	40.09	631.25 ± 170.80[a]	81.28	66.25 ± 27.50[a]	81.14	365.50 ± 43.30[ab]	156.49
8.00	1153.75 ± 331.35	58.27	555.00 ± 63.40[a]	71.46	58.75 ± 14.95[a]	71.95	592.50 ± 301.90[ab]	225.77
10.00	931.25 ± 750.10	47.301	483.75 ± 61.25[a]	62.29	61.26 ± 22.85[a]	75.02	311.25 ± 74.95[a]	134.36

1980.00 ± 1497.65 IU/L; AST, 776.65 ± 122.90 IU/L; ALT, 81.65 ± 23 ± 0.05 IU/L; (Table 3). The degree of inhibition varied very widely with the lowest for ALP (793.75 ± 426.25 IU/L, 59.01% of the control), AST (483.75 ± 61.25 IU/L, 37.71% of the control) and ALT (58.75 ± 14.95 IU/L, 71.95% of the control) at 8.00 ppm. However, there was excitation of LDH activity in the treated group with a peak, 592.50 ± 301.90 IU/L at 8.00 ppm which is 125.77% above the control, 231.65 ± 168.25 IU/L. ALP activity in the liver was elevated at 2.00, 4.00 and 10.00 ppm; 25.00, 43.75 and 18.75% respectively, above the control, 100.00 ± 20.00 IU/L (Table 4). However, it was inhibited by 15.00% at 8.00 ppm below the control. Inhibition was also recorded for AST (max. 25.63% of the control) at 6.00 ppm. ALT was inhibited with 41.67% excitation at 2.00 ppm above the control, 30.00 ± 0.00 IU/L. LDH was excited in the treated group (maximum, 887.50 ± 438.95 IU/L, 29.87% above control value, 683.35 ± 104.10 IU/L at 2.00 ppm except at 6.00ppm. The relative activity of the ALP in the tissues was kidney>gill>liver>muscle; ALT activity was most pronounced in the kidney, but the pattern of ALT in the other tissues and that of AST varied very widely within each of the organs and among the various concentrations of the extract (Figures 1 - 3). LDH activity was higher in the muscle tissues and liver than the kidney and gills, however with no defined pattern relative to the exposure concentrations (Figure 4).

DISCUSSION

Under stress conditions the body mechanisms are altered

Table 4. Enzyme (AST, ALT, ALP, LDH) activity in the liver of *C. gariepinus* 14 days after intramuscular injection with crude extracts of *Lepidagathis alopecuroides* (mean ± SD). Means in the same column with same superscript are not significantly different (p > 0.05).

Conc. of extract (ppm)	ALP (IU/l)	% Control	AST (IU/l)	% Control	ALT (IU/l)	% Control	LDH (IU/l)	% Control
0.00	100.00 ± 20.00[a]	100.00	793.35 ± 105.95[a]	100	30.00 ± 0.00[a]	100.00	683.35 ± 104.10[a]	100.00
2.00	125.00 ± 52.60[a]	125.00	756.25 ± 499.60[a]	95.82	42.50 ± 23.95[a]	141.67	887.50 ± 438.95[a]	129.87
4.00	143.75 ± 63.70[a]	143.75	740.00 ± 149.40[a]	93.28	21.25 ± 7.50[a]	70.83	801.25 ± 77.96[a]	117.25
6.00	100.00 ± 56.55[a]	100	590.00 ± 155[a]	74.37	27.50 ± 18.95[a]	92.38	631.25 ± 131.30[a]	92.38
8.00	85.00 ± 55.05[a]	85	751.25 ± 189.35[a]	94.69	16.25 ± 13.75[a]	54.17	733.75 ± 116.70[a]	107.38
10.00	185.00 ± 64.85[a]	118.75	701.25 ± 127.60[a]	88.39	28.75 ± 16.50[a]	95.83	740.20 ± 205.39[a]	108.32

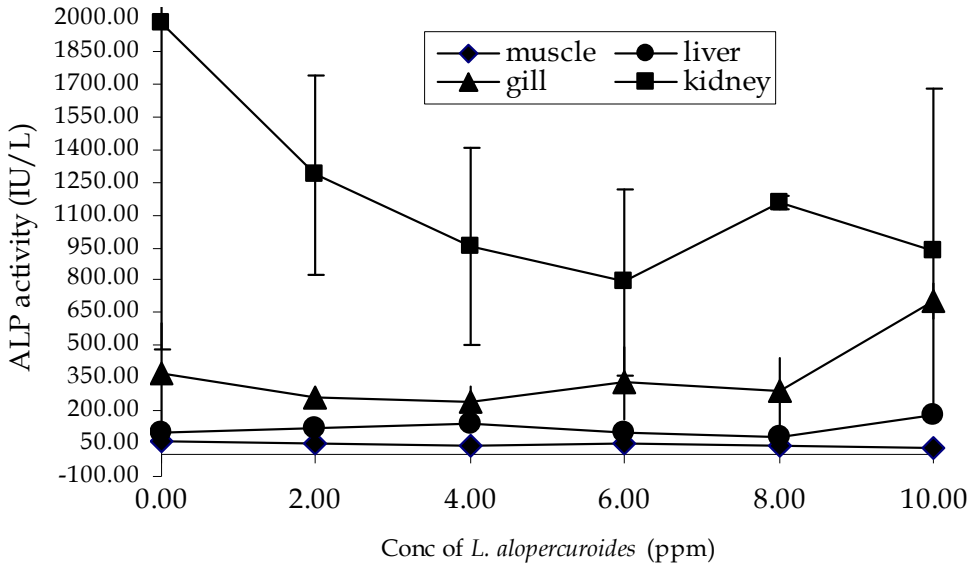

Figure 1. Relative activity of alkaline phosphatase (ALP) in the organs and muscle tissue of *Clarias gariepinus* on the 14th day from the day of intramuscular (2ml/kg) injection with aqueous extracts of L. *alopecuroides (*Bars = SD).

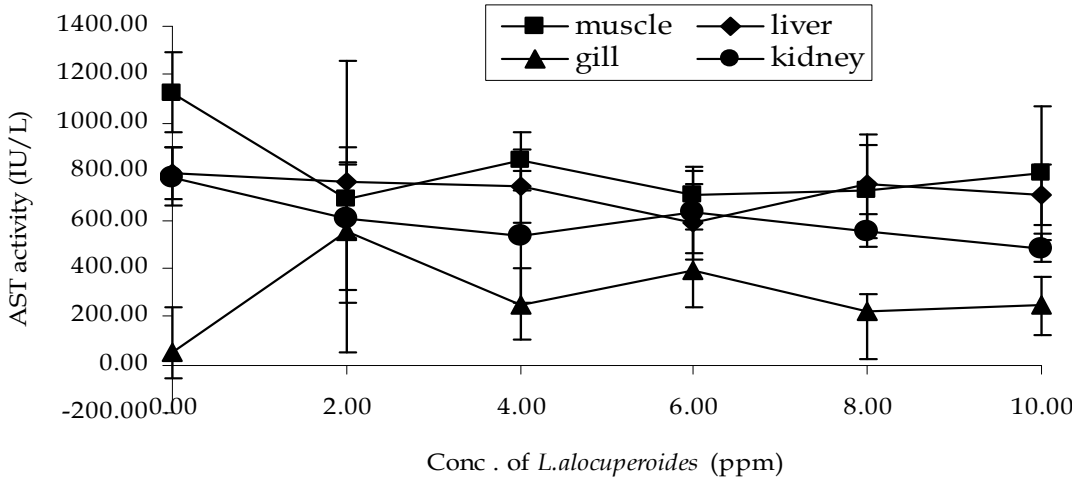

Figure 2. Relative activity of aspartate transaminase (AST) in the organs and muscle tissue of *Clarias gariepinus* on the 14th day from the day of intramuscular injection with aqueous extracts (2 ml/kg) of *L. alopecuroides (*Bars = SD)

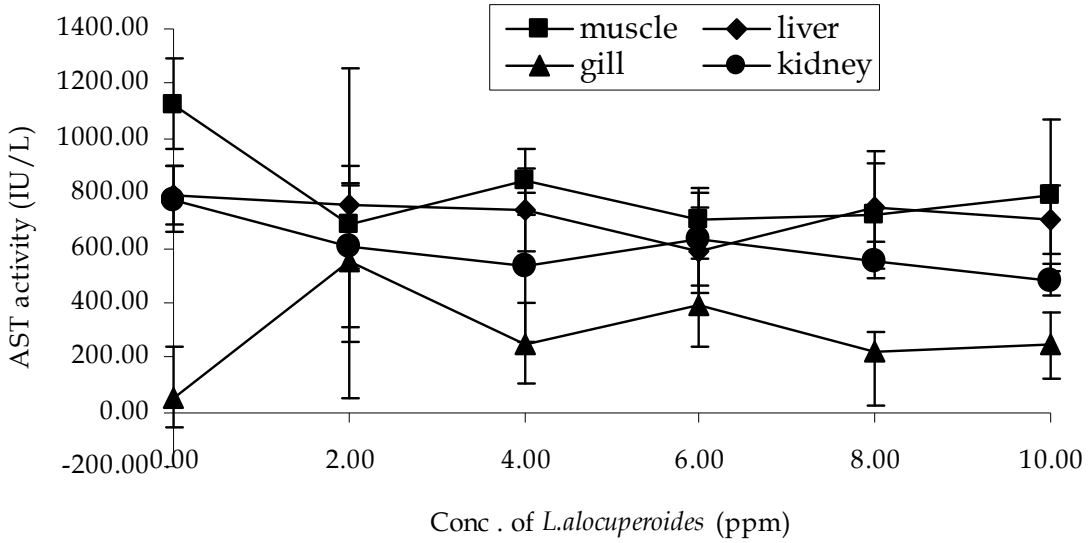

Figure 3. Relative activity of alanine transaminase (AST) in the organs and muscle tissue of *Clarias gariepinus* on the 14th day from the day of intramuscular injection with aqueous extracts (2 ml/kg) of L. *alopecuroides*.

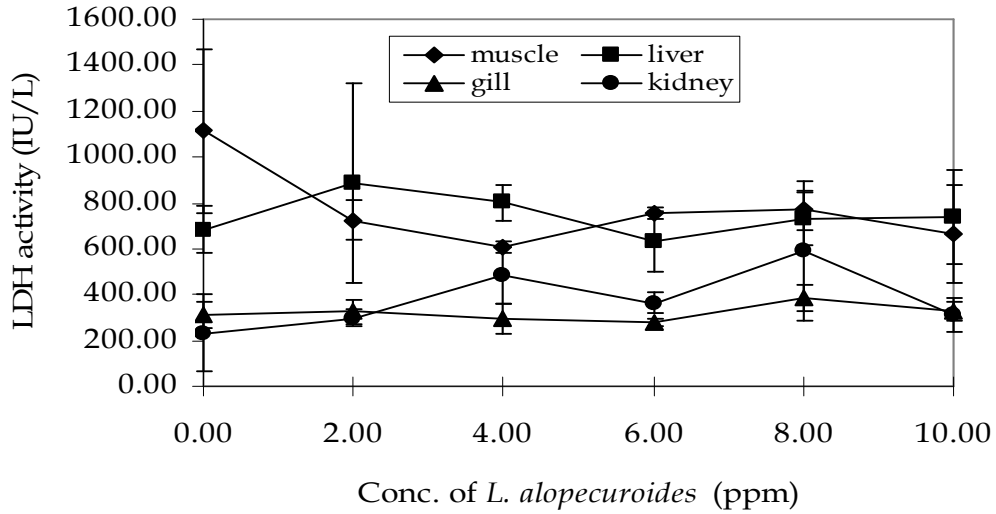

Figure 4. Relative activity of lactate dehydrogenase (LDH) in the organs and muscle tissue of *Clarias gariepinus* on the 14th day from the day of intramuscular injection with aqueous extracts (2ml/kg) of L. *alopecuroides*.

to combat the effect of the pollutants/ stressors in order to maintain equilibrium in the organism (Siva, 1980). Fish under stress mobilizes triglycerides and protein to meet an increased demand for energy resulting from increased physical activity, bio-transformation and excretion of xenobiotics (Alkahem et al., 1998). Both the amino-transferases (alanine and aspartate) function as a link between carbohydrate and protein metabolism by the interconversion of strategic compounds like α-ketoglutarate and alanine to pyruvic acid and glutamic acid, a process known as transamination (Knox and Greengard, 1965; Marking, 1992).

Aminotransferases respond to any stress or altered physiological condition (Knox and Greengard, 1965). Generally intramuscular injection of *C. gariepinus* with *L. alopecuroides* produced depressed activities of AST, ALT and ALP and elevated activity of LDH in all the organs of the fish. This suggests there was effective utilization of amino acids for metabolic processes in exposed fish. But stress generally is known to elevate amino transferase activity (Velisek et al., 2006). Under stress conditions, fish need more energy resulting in higher demand for car-

bohydrate and their precursors to keep the glycolytic pathway and TCA cycles at sustained levels (Tiwari and Singh, 2004).

Depressed AST and ALT activities suggest a decrease in energy demand, metabolic pathway and amino acids. Decrease in ALP activity in the organs (gill, kidney and liver) and muscle tissue could be attributed to a fall in the synthesis of glycogen caused by lowered metabolic demands and also due to electrolytic imbalance caused by tissue overhydration (Shaffi, 1975). However, in other studies (Ayalogu et al., 2001; Svoboda et al., 2001; Tiwari and Singh, 2004) an increase in the activities of AST and ALT was recorded indicating that there was an increased demand for energy due to tissue impairment. Contrariwise, elevation of ALP, AST and ALT reflect hepatic disease, some inflammatory disease or injury to the liver- hepatocellular damage (Ayalogu et al., 2001; Svoboda et al., 2001).

The increase in LDH activity in the liver, gill and kidney in this study suggests a shift towards anaerobiosis as a consequence of hypoxia under toxicant impact leading to respiratory distress (Siva, 1980) as the enzyme is involved in osmoregulation (Tiwari and Sigh, 2004). Depending on the level, toxicants or pesticides inhibit energy production by suppressing aerobic oxidation of carbohydrate leading to energy crisis in animals (Kohli 1995). In the liver, under the influence of the toxicant, both aerobic and anaerobic conditions are likely to operate depending on the availability of molecular oxygen and other physiological needs. In this study, the general inhibition of ALT, AST, ALP and slight to moderate increase in the LDH imply that there was no cell damage, hepatic disorders (liver disease) or renal injury but a disruption of the activity of the TCA cycle, respiratory process and glycolytic pathways.

The pattern of response of LDH, ALT, AST and ALP activities in the various organs to the toxicant seem to suggest that the excitation or inhibition of their activities is organ specific. They are variable and not concentration dependent. Besides, the trends (mostly inhibition in AST, ALT and ALP) below the control and slight to moderate increase ($p > 0.05$) in LDH above control level suggest the fish was not able to detoxify, bio-transform nor excrete the toxin within the 14 days. The enzymes assayed may not be good indicators of *L. alopecuroides* toxicosis in *C. gariepinus* especially at the level of administration assessed in this study.

REFERENCES

Achuba FI, Peretiemo-Clarke BO, Okorie TC (2005). Oxidative stress in the brain of rabbits with petroleum-induced hypoglycaemia. Biol. Lett. 421(1): 33-39.

Agbon AO, Omoniyi I (2002). Acute toxicity of tobacco (*Nicotiana tobaccum)* leaf dust on *Oreochromis niloticus* and haematological changes resulting from sublethal exposure. J. Aquat. Sci. 17(1): 3-8

Alkahem HF, Ahmed Z, Al-Akel AS, Shamsi MJK (1998). Toxicity bioassay and changes in haematological parameters of *Oreochromis niloticus* induced by trichloform. Arab Gulf. J. Sci. Res. 16: 581-593.

Ayalogu OE, Igbon NM, Dede EB (2001). Biochemical changes in the serum and liver of albino rats exposed to petroleum samples (gasoline, kerosene and crude petroleum). J. Appl. Sci. Environ. Mgt. 5(1):97-100.

Barker SB, Summerson WH (1941). The colorimetric determination of lactic acidin biological materials. J. Biological Chem.138:535-542

Gabriel UU, Kparobo SO (2003). Acute toxicity of cypermethrin (a pyrethroid pesticide) on post fingerlings of *Clarias gariepinus*. Int. J. Agric. Rural Dev. *3:*144-149

Gabriel UU, George ADI (2005). Plasma enzymes in C*larias gariepinus* exposed to chronic levels of Roundup (glyphosate). Environ. Ecol. 23 (2): 271-276.

Hafkenscheid JCM, Kohler BEM (1986). Temperature conversion factors for four enzymes in commercial control sera. Clin. Chem. 161-166.

Huckabee, W. E. (1961). Blood analysis, determination of lactic acid. In: Hawk's Physiological Chemistry, 14th edition (B.L. Oser, Ed.), New Delhi, Tata McGraw-Hill. p.1103

Knox WE, Greengard O (1965). An Introduction to enzyme physiology, pp. 257-248. *In*: Advanced Enzyme Regulation Vol. 3 (Weber, G. Ed.) Pergamon Press, New York, London.

Kohli KK (1995) Petroleum induced free radical toxicity in African catfish *Clarias gariepinus*. Indian J. Fish**.** 22 (2):97-103.

Marking LL (1992). Evaluation of toxicants for the control of carp and other nuisance fishes. Fish. 17:6-12.

Obomanu FG, Fekarurbobo GK, Howard IC (2005). Antimicrobial activity of extracts of the leaves of L*epidagathis alopecuroides* (Vahl). J. Chem. Soc. Nig. 30 (1): 33-35.

Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo SGK, Abadi SU (2007). Piscicidal Effects of *Lepidagathis alopecuroides* on Mudskipper, *Periophthalmus papillio* from the Niger Delta. Res. J. Appl. Sci. 2 (4): 382-387.

Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI (2006). Larvicidal properties of *Lepidagahtis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus*. Afr. J. Biotechnol. 5(9): 7561-765.

Omoniyi I, Agbon AO, Sodunke SA (2002). Effect of lethal and suublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf extract on weight and haematological changes in *Clarias gariepinus*. J. Appl. Sci. Environ. Manag. 6 (2): 37-41

Omitoyin BO, Ajani EK, Adesina BT, Okuagu CNF (2006). Toxicity of lindane (gamma hexachloro-cyclohexane) to *Clarias gariepinus* (Burchell 1822). World J. Zool. 1(1):57-63.

Onusiriuka BC, Ufodike EBC (1994). Acute Toxicity of water extracts of Akee Apple, *Blighia sapida* and sausage plant, *Kigelia Africana* on African catfish, *C gariepinus* (Teugels). J. Aquat. Sc. 9:35-41.

Mosta- Fa BB, El-Deeb FA (2002). Impact of certain plants and synthetic molluscicides in some fresh water snails and fish. Sci. Total Environ. 35 (3):989-1007.

Shaffi SA (1975). Effects of starvation on tissue and serum gluconeogenic enzymes, alkaline phosphatase and tissue glycogen in the freshwater catfish *Heteropneustes fossilis* (Bloch). Acta Physiol. Sci. Hung. 53 (4): 501-505.

Shanmugasundaram P, Venkataraman S (2005). Hepatoprotective and antioxidant effects of *Hygrophila auriculata* : Acanthaceae root extract. Acta Crystallographic 104(1-2): 124-128.

Reitman S, Frankel S (1957). A colorimetric method for the determination of glutamic-oxaloacetic and glutamic-pyruvic transaminases. Am. J. Clin. Pathol. 33 1-13.

Singh D, Singh A (2002). The acute toxicity of plant origin pesticides into the fresh water fish C*hanna punctatus.* Acta Hydrochim. Hydrobiol. 28(2): 92-99.

Singh SK, Yadav RP, Mishra TN, Agarwal RA (2005). Toxic effects of stem bark and leaf of *Euphorbia hirta* plant against freshwater vector snail, *Lymnea acumnita*. Indian J. Exp. Biol. 59:263-270.

Siva PRK (1980). Studies on some aspects of metabolic change with emphasis on carbohydrate utility in the cell free system of the teleost, *Tilapia mossambicus* (Peters) under methyl parathion exposure Ph.D. Thesis, S. V. Univ., Tirupti, India.

Svoboda Z (1971). Some haematological and metabolic changes in fish occurring after pesticide intoxication. *Bullet VUR* Vodnany 7: 29-36.

Svoboda M, Luskova V, Drastichova J, Llabok V (2001). The effect of diazinon on hematological indices of common carp (*Cyprinus carpio*). Acta Vet. (Brno) 70: 457-465.
Tiwari S, Singh A (2004): Piscicidal activity of alcoholic extract of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. Afri. J. Trad. CAM. 1:15-29.
Tiwari S, Singh A (2006). Biochemical stress response in fresh water *Channa punctatus* induced by aqueous extracts of *Euphorbia tircualli* plant. Afri. J. Trad. Cam. 64(1):36-42.
Velisek J, Inlason T, Gomulka P, Svoboda Z, Dobsikova R, Noyotyn L, Dudzik M (2006). Effects of cypermethin on rainbow trout (*Onchorhynchus mykiss*). Veterinani Medicina. 51 (10): 469-476.
Wahua TAT (1999). Applied statistics for scientific studies. Afrika link books. Aba, Nigeria. p.365