International Journal of Animal and Veterinary Advances 1(2): 39-43, 2009
ISSN: 2041-2908

Submitted Date: July 07, 2009 Accepted Date: July 17, 2009 Published Date: October 30, 2009

Enzymes in Selected Tissues of Catfish Hybrid Exposed to Aqueous Extracts from *Lepidagathis alopecuroides* Leaves

[1]U.U . Gabriel, [2]F.G. Obomanu and [2]O.D. Oveh
[1]Department of Fisheries and Aquatic Environment,
[2]Department of Chemistry, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria

Abstract: Hybrid catfish, *Heterobranchus longifilus* ♂ x *Clarias gariepinus* ♀ (mean total length, 29.96±2.23 cm, SD; mean weight, 207.83±12.63g SD) were exposed individually in quadruplicate to 40 L of sublethal aqueous extracts of *Lepidagathis alopecuroides* (0.25, 0.50, 0.75, 1.00, 1.25 and a control, 0.0 mg L^{-1}) for 21 days in a daily static renewal bioassay. The liver, kidney, gill and muscle tissues were then assayed for enzymes activities. The activities of AST and ALT in the liver were most inhibited at 0.75 mg L^{-1}, AST activity was inhibited in all the exposure concentrations below the control value, ($p<0.05$). Generally ALT activity was inhibited. The activities of AST and ALT in the kidney were generally retarded below control values. Activities of AST and ALT declined in gill tissues below the control values. ALP activity was generally enhanced in the liver, kidney and gill of exposed fish. The activities of AST and ALP in the muscle were mostly enhanced at 1.25 mg L^{-1}, but ALP was inhibition at 0.50 and 0.75 mg L^{-1}. Although the bio-indicator enzymes could be used for monitoring the toxic effects of the plant in the fish species this will depend on the exposure levels and organs of choice.

Key words: Catfish hybrid, *Lepidagathis alopecuroides*, aspartate transaminase, alanine transaminase and alkaline phosphatase

INTRODUCTION

The use of toxic plants for catching fish is a common practice world wide. The ichtyotoxic characteristics of some of these plants make them potent tools for catching or stupefying fish all over the world. Above some forty years ago, local fishermen in Nigeria have reported used specific biocides derived from plants for fishing (Reed *et al.,* 1967). Since then, this has continued unabated in different parts of the country. Besides, a number of laboratory studies have revealed the toxicity of plant extracts to fishes (Ayuba and Ofojekwu, 2002; Ayotunde and Ofem, 2008) being used as molluscicides (Azare *et al.,* 2007; Maikai, *et al.,* 2008) in the aquatic environment where non-target fish species may suffer in various ways.

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Van Andel, 2000). The active principles in the plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action depending on whether it is applied directly and the forms of extracts, aqueous or alcohol used (Sambasivam *et al.,* 2003). The two main groups of phytochemicals that occur in most plants used for the stunning fish, the rotenones and saponins, as well as a third group of plants which liberate cynanide in water, account for nearly all varieties of fish poisons, although plants with sufficient levels of ichthyoethereol, triterpene and other ichthyotoxins are also used (Béarez, 1998). Rotenone is an alkaloid toxin and a flanoid. Bocek (1984) observed that rotenone inhibits oxidative phosphorylation, the specific site of action being the electron transport system where it blocks the mitochondrial enzyme, NADH ubiquitone reductase. It stuns fish by impairing their oxygen consumption, thereby forcing the fish to the surface. Obomanu *et al.* (2006) demonstrated the larvicidal properties of *L. alopecuroides* and *A. indica* on *A. gambiae* and *C. quinquefasciatus*. The leaves of *L. alopecuroides* at 1.0-6.0 g/l was highly toxic mudskipper, *Periophthalmus papillio* effecting death within 18-37 mins (Obomanu *et al.*, 2007). Phytochemical screening of L. alopecuroides showed the presence of alkaloids, saponins, tannins, cardiac glycosides and flavonoids (Obomanu *et al.*, 2005).

However, there is no report on the effect of *L. alopecuroides* on the various aspects of the physiology and biochemistry of the clariids. Exposure of fish to these biocides may cause stress in fish without necessarily leading to death. Stress response is characterized by biochemical and physiological changes which may be manifest in both acute and chronic toxicity tests (Singh and Singh, 2002; Tiwari and Singh, 2004). The disruption of biochemical and physiological integrity is assessable by the changes in the enzyme activities in functional organs (de la Torre *et al.,* 2000, van der Oost *et al.*, 2000). Changes in enzymes pofiles are important pollution

Corresponding Author: U.U. Gabriel, Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria

indices. Transamination is one of the principal pathways for the synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the fish under various adaptive conditions (Chetty *et al.*, 1980). Maintenance of internal homeostasis through biochemical processes in the Kreb's cycle may be reflected by variation in the levels of the enzymes AST, ALT, ALP in the serum (plasma) occasioned by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Heath, 1991). Both serum AST and ALT are raised when disease process affects cell integrity and ALT is a more liver-specific enzyme (Gabriel and George, *et al.*, 2005).

Lepidagathis alopecuroides is commonly used to catch fish in the Delta area of Nigeria. The knowledge of the dynamics of the activities of the enzymes AST, ALT and ALP in the liver, kidney, muscle and gills of catfish hybrid exposed to *L. alopecuroides* is of immense importance as it helps to determine the biochemical stability of the fish in the aquatic environment, particularly under toxicant stress. The objective of this work was to study the AST, ALT and ALP profile in the liver, kidney, muscle and gills of catfish hybrid exposed to sublethal level of *L. alopecuroides*.

MATERIALS AND METHODS

Tank-raised experimental fish, *Heterobranchus longifilus* ♂ x *Clarias gariepinus* ♀ (mean total length, 29.96±2.23 cm, SD; mean weight, 207.83±12.63 g SD) were purchased from a private farm at Abuloma, Port Harcourt, 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 plastic for seven days in rectangular aquaria in 25 L borehole water. During the period, the fish was fed a 35% crude protein feed once a day at 1% biomass. Fresh leaves of the plant, *Lepidagathis alopecuroides* were obtained from the wild, air-dried for three weeks in the laboratory to constant weight at room temperature. The dried leaves were subsequently powdered with the aid of Moulinex electric blender and stored in dry airtight containers.

Five graded concentrations (0.00, 0.50, 0.75, 1.25, 1.00 and 0.25 mg L^{-1}) of the aqueous extract were prepared in quadruplicates after range finding tests had been conducted. Experimental fish was added singly in each of the aquaria and covered with netted material which has a slit at the middle to prevent escape of the fish. The aquaria were washed daily to remove uneaten food and faecal matters. Water in the control and test solutions was renewed daily. Water analysis (dissolved oxygen, pH, hardness, conductivity and alkalinity) were done on the first, fourteenth and twenty-first day of the experimental period using standard methods in APHA (1998). The study lasted for 21 days.

At the end of the exposure the fish was killed with a blow on the head and dissected. The liver, kidney and gills and muscle tissues were carefully removed and rinsed in water. A sample, 0.5g of each of the tissues was homogenized in five millilitre physiological saline. The homogenate was centrifuged at 3000 rpm for 5minutes. The supernatant was extracted and stored for enzyme analysis. Enzyme activities (AST and ALT) were determined by colorimetric methods according to Reitman-Frankel (1957) and ALP by Bessey *et al.* (1946). The data were subjected to ANOVA and differences among means were separated by Duncan Multiple Range Test (Wahua, 1999).

RESULTS

The extracts from the plant caused an increase in the pH, conductivity and alkalinity of exposure solution, $p<0.05$ (Table 1). The activities of AST (142.75±146.83 IU/L) and ALT (39.00±34.81 IU/L) in the liver were most inhibited at 0.75 mg L^{-1}, at which concentrations the highest activity of ALP (213.75±368.12 IU/L) occurred (Table 2). AST activity was inhibited in all the exposure concentrations, below the control value, 508.25±201.62 IU/L, with marked reduction ($p<0.05$) in some of the exposure concentrations (maximum at 0.75 mg L^{-1}, 71.91% below the control value). In all the exposure concentrations except at 0.25 and 1.25 mg L^{-1}, were minimal excitation, 29.76 and 12.91% occurred in ALT activity, there was general inhibition. Excitation of ALP was recorded at 0.75 mg L^{-1} (213.75±368.12 IU/L), 388.64% above the control, (55.00±36.72 IU/L) and at 1.00 mg L^{-1}, 2.73% above the control. The activities of AST and ALT of in the kidney exposed fish were generally retarded from 0.50 to 1.00 mg L^{-1} extracts with a maximum inhibition occuring at 1.00 mg L^{-1}: AST, 114.25±77.21 IU/L, 77.73% below control; ALT, 26.50±27.92, 81.21% of the control value (Table 3). However, ALP activity was elicited at the same concentrations of extracts with maximum excitation at 0.75 mg L^{-1}, 531.75±442.55 IU/L, 242.50% above the control value (150.00±97.40 IU/L).

In the gill tissues there was a decline AST and ALT activities below that of the control (AST, 481.25±152.08 IU/L; ALT, 165.00±143.38 IU/L) except at 1.25mg/l, 205.00±154.65 IU/L in ALT (Table 4). ALP activity was enhanced in all the concentrations except at 0.25 mg L^{-1}. Higher levels were recorded at 0.50 and 0.75 mg L^{-1}, 334.75±150.59 and 168.75±60.05 IU/L, representing 268.88 and 135.54% respectively, above the control,124.50±41.52IU/L. The activities of AST (770.00±429.20 IU/L), ALT (170.00±137.17IU/L) and ALP (71.25±41.52IU/L) in the muscle of exposed fish were mostly elicited at 1.25 mg L^{-1} extracts: 93.71, 36.00, and 103.57% above their respective control values (Table 5). However, AST and ALT activities in the muscle tissues inhibited at 0.75 and 1.00mg/l with a maximum at 0.75 mg L^{-1} (AST, 76.11%; ALT, 49.00%) below their respective control values. For ALP inhibition was recorded at 0.50 and 0.75 ppm with a maximum, 57.14% at 0.50 ppm (Table 5).

Table 1: Physicochemical properties of water exposed to chronic levels of *L. alopecuroides* for 21 days (mean±SD)

Conc. of toxicant (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

Table 2: Enzyme (AST, ALT, and ALP) activities in the liver of catfish hybrid exposed to chronic levels of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	AST (IU/L)	% of control	ALT (IU/L)	% of control	ALP (IU/L)	% of control
0.00	508.25±201.62[a]	100.00	158.75±54.27[abc]	100.00	55.00±36.72[a]	100.00
0.25	493.50±33.18[a]	97.09	252.0±89.43[c]	129.76	51.75±16.68[a]	83.94
0.50	313.75±12.29[a]	61.73	51.50±11.82[ab]	32.44	41.00±26.81a	74.54
0.75	142.75±14.83[a]	28.09	39.00±34.81[a]	24.57	213.75±36.12[a]	388.63
1.00	250.25±62.59[a]	49.24	77.00±27.92[abc]	48.57	56.50±22.93[a]	31.08
1.25	473.75±31.72[a]	93.21	179.25±19.14[bc]	112.91	43.50±8.06[a]	79.09

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

Table 3: Enzymes activities (AST, ALT and ALP) in the kidney of catfish hybrid exposed to chronic levels of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	AST (IU/L)	% of control	ALT (IU/L)	% of control	ALP (IU/L)	% of control
0.00	513.00±48.81[b]	100.00	141.00±44.56[b]	100.00	150.00±97.40[a]	100.00
0.25	620.00±200.50[b]	120.85	305.50±68.61[d]	216.66	125.50±120.30[a]	83.67
0.50	261.50±106.47[a]	50.97	73.00±5.22[a]	51.77	214.25±12.87[a]	142.83
0.75	174.00±12.42[a]	33.92	65.25±34.81[a]	46.28	531.75±44.55[a]	342.50
1.00	114.25±77.21[a]	22.27	26.50±2.92[a]	18.79	303.00±119.82[a]	202.00
1.25	517.00±128.96[b]	100.77	216.00±17.28[c]	153.19	122.50±89.49[a]	81.67

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

Table 4: Enzyme activities (AST, ALT, and ALP) in the gill of catfish hybrid exposed to chronic levels of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	AST (IU/L)	% of control	ALT (IU/L)	% of control	ALP (IU/L)	% of control
0.00	481.25±15.08[a]	100.00	165.00±14.38[a]	100.00	124.50± 41.52[a]	100.00
0.25	279.00±21.78[a]	57.97	128.00±91.62[a]	77.58	114.00±44.84[a]	91.57
0.50	306.50±27.00[a]	63.69	124.75±12.54[a]	75.61	334.75±150.59[b]	268.88
0.75	209.75±53.37[a]	43.58	49.50 ± 16.92[a]	30.00	406.00±107.01[b]	326.10
1.00	251.25±14.95[a]	52.21	107.50±59.65[a]	65.15	168.75±60.05[a]	135.54
1.25	246.25±16.76[a]	51.17	205.00±15.65[a]	124.24	185.00±26.77[a]	148.59

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

Table 5: Enzyme activities (AST, ALT, and ALP) in the muscles of catfish hybrid exposed to chronic levels of *L. alopecuroides* for 21 days (mean±SD)

Conc. of *L. alopecuroides* (mg/l)	AST (IU/L)	% of control	ALT (IU/L)	% of control	ALP (IU/L)	% of control
0.00	397.50±24.08[ab]	100.00	125.00±84.75[a]	100.00	35.00±14.72[a]	100.00
0.25	406.25±227.61[ab]	102.20	131.25±12.79[a]	105.00	37.50±15.00[ab]	107.14
0.50	498.75±215.46[ab]	125.47	90.00±11..96[a]	72.00	15.00±5.77[a]	42.86
0.75	95.00± 43.20[a]	23.89	72.50± 40.31[a]	58.00	22.50±2.89[a]	64.29
1.00	147.50±19.08[a]	37.11	63.75±53.91[a]	51.00	38.750±8.54[ab]	110.71
1.25	770.00±429.20[b]	193.71	170.00±17.17[a]	136.00	71.25±49.39[b]	203.57

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

DISCUSSION

Toxicity experiments showed that aqueous extracts of *L. alopecuroides* caused significant biochemical changes in the catfish hybrid. It has been reported that alterations in enzymes activities in the serum directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture (Benjamin, 1978), a signal of underlying pathological process (Hayes *et al.*, 2002). The above observations seem to confirm the results obtained in this study that the extract had impacted on the cell integrity of the organism as reflected in marked changes in some of the enzymes. Both ALT and AST are located in the cytoplasm and mitochondria of liver cells, also in the cells of the heart, skeletal muscles, kidney and brain (Ringer and Dabieh, 1979). Stress has been reported to increase the activities of mitochondrial density in the muscle fibres of fish. (Ccarp Sänger, 1993). Stress (altered physiological conditions) in general is known to elevate aminotransferase activities (Natarajan, 1985). Transamination is one of the principal pathways for the synthesis and deamination of amino acids, thereby allowing interplay between carbohydrate and protein metabolism during inconsistent energy demands of organisms in various adaptive situations (Waarde and Henegauryen, 1982). Both the AST and ALT function as a link between carbohydrate and protein metabolism by catalyzing the interconversion of strategic compounds like

α-ketoglutarate and alanine to pyruvic acid and glutamic acid, respectively (Nelson and Cox, 2000) and in the process meeting the energy demand of the organs in crisis.

The ALT activity in all the organs appeared higher at the highest and lowest concentrations of the extracts, whereas inhibition was recorded at the other concentrations suggesting that depending on the concentration, the active ingredients in the extracts may either enhance or inhibit transamination with grave consequences to the affected organs. The degree also varied with the organs as was also recorded by a number of studies (Sharma *et al.,* 1982; Tiwari and Singh, 2004) under toxicant exposure.

ALT is present in the liver and other tissues. It is particularly useful in measuring hepatic necrosis especially in small animals (Cornelius, 1989). Since it is one of the assayable liver enzymes, its elevated level in this study may indicate hepatic damage caused by this plant extracts. Bradifield and Rees (1978) pointed out that toxicant act on the carboxyl, amino, sulfhydryl, phosphate and other similar groups of the cell components. They further summarized the mode of action as: disruption of the enzyme system by blocking active sites; immobilization of essential metabolites by formation of stable precipitates or chelates; catalytic decomposition of essential metabolites; alteration of cell membrane permeability by combining with membrane components and replacement of the structurally or electrochemically important elements in the cells, which then fail in function. In the study, the increased activitiesa of AST and ALT in some exposure levels in the muscle, kidney and liver in the catfish hybrid after exposure to different concentrations of *L. alopecuroides* may be due to changes in enzyme activity as a result of disturbances in the Kreb's cycle. Decreased activity of Kreb's cycle enzymes causes a decrease in the Kreb's cycle intermediates, and AST and ALT compensates through providing α-glutarate. This agrees with the conclusion made by Magdy and Rogers *et al*., (1993) in grass carp exposed to different concentrations of diquat, that increased level of muscle and liver AST and ALT activities was as a result of the disturbance in the Kreb's cycle.

Alkaline phosphatase, ALP, is a marker enzyme for the plasma membrane and endoplasmic recticulum. It is often used to assess the integrity of plasma membrane (Akanji *et al.*, 1993) and relates to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. Significant elevation of serum ALP is an indication of cholestasis (Van Hoof and De Broe, 1994) with no effective control of ALP activity towards improvement in the secretory function of the hepatic cell. Since the activity of the enzyme is associated with membrane permeability, it could be concluded that the extracts at some levels increased permeability in the cells of some of the organs, whereas at others it impaired it. ALP activity in albino mice and rats was not significantly affected when exposed to extracts of *Boerhavia diffusa* (Orisakwe *et al*., 2003). However, Sastry and Subhadra (1985) recorded significant reduction in ALP in the liver and kidney of catfish, *Heteropneustes fossilis* after exposure to cadmium. The general increase of ALP in the kidney, gill, and liver which peaked at 0.75mg/l of *L. alopecuroides* may indicate considerable damage have been done to the liver and kidney with increased cell membrane permeability. This confirms the findings of Wannang *et al.* (2007); in rats that increase in the serum levels of ALP indicates the extent of cellullar damage on the liver.

Results from this study revealed that elevation in all AST in the tissues of catfish hybrid is a very reliable biomarker of *L. alopecuroides* toxicosis, whereas ALT and ALP activities varied in the organs relative to level of the toxicant. Hence, ALT and ALP activities in the tissues of the catfish hybrid cannot be reliably used as bioindicators of the toxic effects of the plant extract as theyare concentration-dependent. Besides, the effects on the enzymes profile may be more severe in the field where higher levels of the extracts are used for fishing. Hence, the indiscriminate use of the ichthyotoxin for fishing should be discouraged.

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