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The Effect of Dry Extract of *Derris elliptica* Stem on some Enzymatic Changes in the Plasma of African Catfish *Clarias gariepinus* (Burchell, 1822).

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Abstract

The dry extract of different parts of the *Derris elliptica* is used in fish ponds to harvest and control predatory fish. However, the toxicological impact of the extract on fish has not yet been evaluated. The objective of the present study is to investigate the effects of the extract on some enzymes of plasma of the widely consumed African catfish *Clarias gariepinus*. Fish specimen were exposed to sublethal concentrations (0.00 [control], 0.75, 1.50 and 3.00g/L) of dry extract of stems of *D. elliptica* for 24, 72 and 168 h adopting the semi-static renewal bioassay technique and were then subjected to plasma analyses. The level of plasma acid phosphatase and plasma alkaline phosphatase decreased significantly ($P<0.05$) while plasma alanine aminotransferase and plasma aspartate aminotransferase increased significantly ($P<0.05$). It was concluded that the dry extract of the stem of *D. elliptica* produced disorders in liver, kidney and respiratory metabolism of *C. gariepinus*.

Keywords: *Derris elliptica*, African catfish, Enzymatic parameters.

1. Introduction

The pesticidal effect of plant extracts are of unique importance due to their chemical compositions that enhance their properties as medicinal plants, preservatives, insecticides, molluscidices, to mention a few. Thus, they have always been useful to man and aquatic animals (Akinwade *et al.,* 2007). Due to their narcotic, pesticidal and molluscidal properties, many fishermen and fish farmers indiscriminately use the extracts of different parts of these plants to weaken and kill the fishes for easy catch and clean up the aquatic systems of some pests. Some farmers use very high concentrations of these plant extracts which can lead to physiological disturbances in the aquatic organisms and ultimately to a reduction in aquatic productivity (Mondal *et al.,* 2007). Some of these used plants are non-selective in their destruction, thereby interfering with the ecological balance of the immediate environment. The usefulness of plants extracts for pesticidal and medicinal purposes was reported by Akobundu, 1987 and Adewole *et al.,* 2002.

Derris elliptica is a plant that belongs to the family Leguminosae, subfamily Papilionoideae. Over the years, *Derris* has been known as an important source for compounds with broad spectrum of insecticidal properties (Gupta, 2007). It is locally known in Southern East Asian Countries as Derris or Tuba while in Thailand as Lotin or Hang liadaeng. *D. elliptica* is a liana (woody climbing plant) which is up to 16 m long, root is reddish-brown, apical shoots often leafless for several meters and rusty pubescent, leaflets are 7-15cm long, mostly densely rusty hairy on both surfaces when young. Its extract is very poisonous and is used locally by fishermen in Nigeria for catching fish. Recently, it has also been used extensively for controlling insect pests. Different parts of the plant are also used in traditional medicine for the treatment of wounds, calculus, rheumatism and dysmenorrhoea and asthma in man. Extracts and metabolites from this plant have been found to possess significant larvicidal, pesticidal, cytotoxic, anti-fungal, anti-inflammatory, antimicrobial, nitric oxide inhibitory, and cancer chemopreventive activities (Olufayo and Akinpelumi, 2012).

The African catfish *Clarias gariepinus* is the most suitable species for aquaculture in Africa and is with a Pan-African distribution, from Nile to West Africa and from Algeria to South Africa. The African catfish has a high growth rate; the exposure of this catfish to these biocides may cause stress not necessarily leading to death. The 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

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the enzyme activities in functional organs (de la Torre *et al.,* 2000; Van Der Oost *et al.,* 2003).

Measurement of the enzymatic activities or marker enzymes in tissues plays a significant and well-known role in diagnostic, disease investigation and in the assessment of drug plant extract toxicant for the safety toxicity risk. Enzymes serve a wide variety of functions inside living organisms. They are indispensable for signal transduction and cell regulation often via kinases and phosphatases (Hunter, 1995). Enzymes are biochemical macromolecules that control the metabolic process of organisms, and a slight variation in the enzyme activities would affect the organism (Roy, 2002). The activities of alkaline phosphatase, alanine aminotransferase, aspartate and aminotransferase, are useful marker enzymes of damage to the liver and kidney (Akanji *et al.,* 1993). Changes in the activities of these enzymes may help to forecast the consequence of long-term exposure of fish to chemical pollutants (Adedeji *et al.,* 2010). Moreover, the evaluation of blood biochemistry was considered as a useful tool for the diagnosis of diseases and assessing the physiological status of fish (Stoskopf, 1993). Many studies have investigated the changes in many physiological indices of animals induced by environmental conditions and presence of contaminants (Kori-Siakpere *et al.,* 2006; Maheswaran *et al.,* 2008; Ololade and Oginni, 2010). The biochemical parameters in the fish are valid for physio-pathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant damage (Almeida *et al.,* 2002; Matos *et al.,* 2007; Osman *et al.,* 2010).

Hence, the present study is conducted to evaluate the effects of four concentrations of dry extracts of the stem of *D. elliptica* on the plasma enzymes of the African catfish *C. gariepinus* in order to evaluate its long term effect on fish..

2. Materials and Methods

2.1. Experimental Animals

Live specimens of *C. gariepinus* (mean total length 30.50 ± 0.90 cm; mean weight, 152.78 ± 8.11g) were obtained locally from a commercial fish farm. They were transferred to the Animal and Environmental Biology Research Laboratory, Delta State University, Abraka, Nigeria. The fishes were held in the laboratory in large plastic aquaria of 140L capacity with clean borehole water. They were then acclimatized for 14 days during which they were fed to satiation with commercial fish feed pellets (Coppens 4.0 mm; 35% crude protein diet) twice daily. Uneaten food and faecal matters were removed daily during the acclimation and the experimentation period. Dead fish were also promptly removed to avoid contamination. The percentage of death recorded during acclimatization was less than 2% and as such the fishes were accepted as being adapted to the laboratory conditions.

2.2. Plant Material

Fresh stems of *D. elliptica* were collected from a farm in Oleh, Isoko South Local Government Area, Delta State, Nigeria and transported to the Department of Animal and Environmental Biology Laboratory. The plant was identified as *D. elliptica* by Dr. (Mrs.) N. E. Edema of the Department of Botany, Delta State University, Abraka, Nigeria. They were air-dried for two weeks and later oven-dried for three hours at 60°C to a constant weight. The dried stems were ground into powder with an electric blender (MX - 2071, Nakai Japan), sieved and the fine powder was stored in a dry airtight container.

2.3. Toxicant Preparation

The concentrations of the dry stem extract of *D. elliptica* used for the experiment were 3.00, 1.50, 0.75 and 0.00(control) g/L which were obtained after preliminary investigation. These concentrations were introduced into four sets of tanks, each designed for one of the four mentioned-above concentrations with two replications.

2.4. Experimental Procedure

The containers used in the experiment consisted of plastic containers of 120 L capacity. The upper part of each container was covered with a lid made of fine polyethylene gauze screen of 1mm mesh size. Fifteen acclimatized specimens were stocked in a tank and were exposed to a particular concentration of the aqueous extract of *D. elliptica* for 168 h.

The toxicant and water were renewed (semi-static bioassay) after 24, 72 and 144 h of exposure to maintain the toxicant strength and the level of dissolved oxygen (D.O) as well as to minimize the level of ammonia excretion during the experiment (Kori-Siakpere, 1996). The water quality parameters of the exposure water, used in the tests and control, were temperature 28.30±1.3°C, pH 7.58±0.32, dissolved oxygen 8.32±1.04 mg L^{-1}, free carbon dioxide 4.85±0.08 mg L^{-1}, alkalinity 36.50±1.72 mg L^{-1} and hardness 134.53± 11.75 mg L^{-1}.

2.5. Sampling Procedure

At the end of the exposure periods (24, 72 and 168 hrs), the fish were taken from the control and test tanks, sacrificed and subjected to the analyses.

Five fishes were caught individually in a small hand net from the containers. After the preliminary investigation of the length and weight, the fish were then placed belly upwards and blood samples were obtained from the caudal circulation with the aid of a heparinised 2 cm^3 disposable plastic syringes with a 21 gauge disposable hypodermic needle. Plasma was obtained from blood samples by centrifugation and then drawn into a 1 cm^3 plastic syringe and transferred into a lithium heparin bottle, diluted 1:20 with deionised water. The diluted plasma was then stored in a refrigerator and later used for the analysis of plasma enzymes: acid phosphatase, alkaline phosphatase, alanine aminotransferase, aspartate and aminotransferase. All determinations were carried out in duplicates for each sample.

2.6. Enzyme Analyses

Activities of acid phosphatase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were determined spectrophotometrically using Teco Diagnostic, Anahein, USA commercial kit, following the manufacturer's instruction with the aid of spectrophotometer.

2.7. Data Analysis

The results obtained were subjected to two-way analysis of variance (ANOVA). Comparisons of the means were done using Dunnet Multiple Comparison and results were considered significant at the 95% confidence level (*P*< 0.05).

3. Results

3.1. Acid and Alkaline Phosphatase

The level of plasma acid and alkaline phosphatase significantly decreased (*P*<0.05) in the fish exposed to various concentrations of the stem powder of *D. elliptica* when compared with the control (Figures 1 and 2).

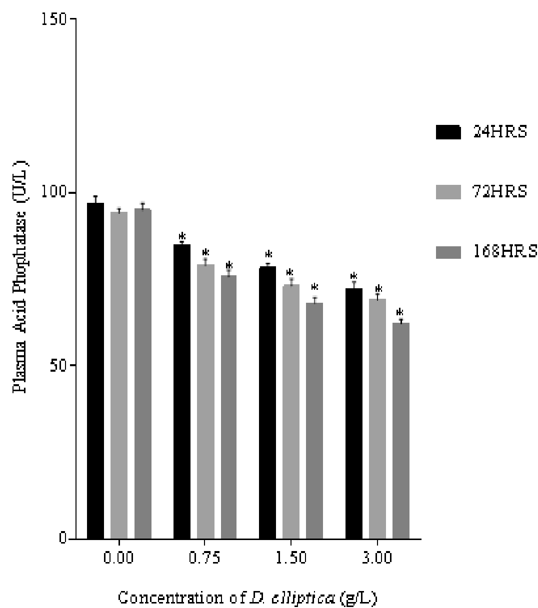

Figure 1. Mean values of acid phosphatase in the plasma of *C. gariepinus.* Each column represents the mean value and vertical bars indicate the standard error of the means. Asterisk represents significant difference between the control and experimental group at (*P<0.05*) level.

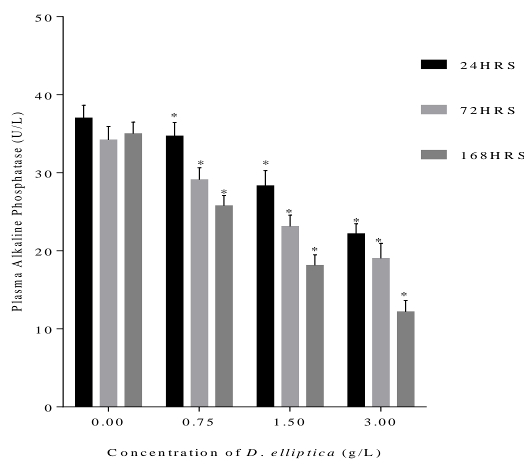

Figure 2. Mean values of alkaline phosphatase in the plasma of *C. gariepinus*

3.2 Alanine Aminotransferase

The level of plasma alanine aminotransferase in *C. gariepinus* is presented in Figure 3. The level of alanine aminotransferase showed a insignificant increase (*P*<0.05) in the fish exposed to various concentrations of the stem powder of *D. elliptica* when compared with the control.

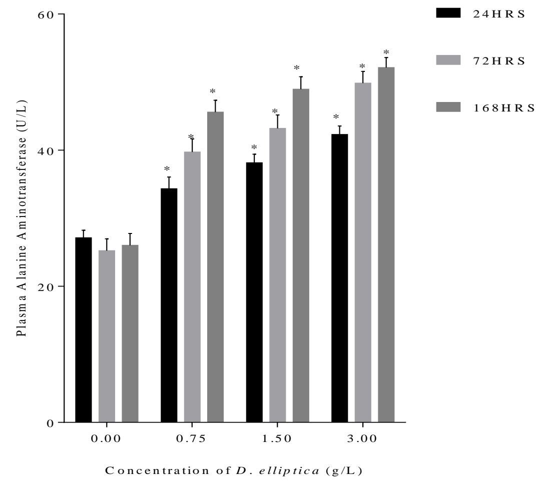

Figure 3. Mean values of alanine aminotransferase in the plasma of *C. gariepinus*.

3.2. Aspartate Aminotransferase

The level of plasma aspartate aminotransferase in *C. gariepinus* is presented in Figure 4. The level of aspartate aminotransferase showed a significant increase (*P*<0.05) in the fish exposed to various concentrations of the stem powder of *D. elliptica* when compared with the control.

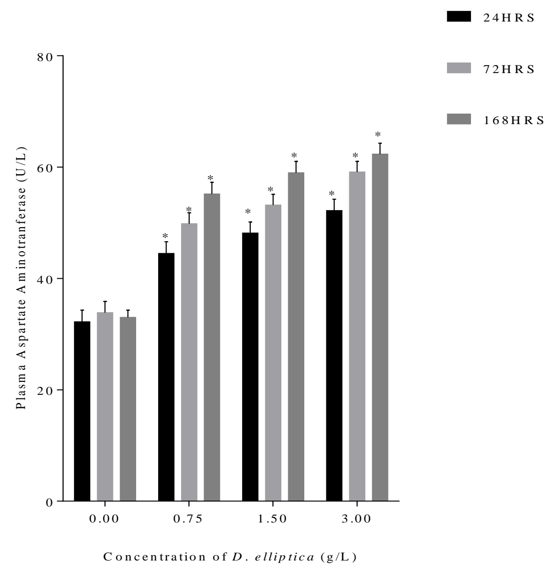

Figure 4. Mean values of aspartate aminotransferase in the plasma of *C. gariepinus*

4. Discussion

The enzymes considered in this study are useful marker enzymes which indicate the cellular damage long before the structural damage which is revealed by some other convectional techniques (Shahjahan *et al*., 2004; Akanji *et al.,* 1993).

Assay of these enzymes are parts of standard laboratory test to detect abnormalities in animals (Ayalogu *et al.,* 2001; Gabriel *et al.,* 2010). Changes in these enzymes activities resulting from toxicant or contaminant effects in various organs of fish were reported by Mgbenka *et al.,* 2005 and Oliverira *et al.,* 2006. Such alterations in fish are aimed at maintaining equilibrium in the presence of these toxicants which are known to disrupt physiological and biochemical processes (Winkler *et al.,* 2007).

Alkaline and acid phosphatase activities decreased with the increase in the concentrations of *D. elliptica* stem extract. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum (Wright, 1974). In the present study, there was a significant decrease in the plasma alkaline phosphatase and acid phosphatase activity of fish probably due to the inhibition of the enzymes by the plant extracts (Akanji, 1993). This may result in the alteration of phosphatase metabolism and is an indication of the toxic effect of the stem extract of *D. elliptica*.

The dose and time-dependent inhibition of alkaline and acid phosphatase observed in this investigation is in agreement with the reports of many authors. Adamu (2009) reported a decreased value of plasma alkaline phosphatase in *Heteroclarias* (a Hybrid of *Heterobranchus bidorsalis* and *C. gariepinus*) exposed to sublethal doses of tobacco (*Nicotiana tobaccum*) leaf dust. Ogueji and Auta (2007) reported a reduced value of plasma alkaline phosphatase in African catfish *C. gariepinus*, exposed to lambda-cyhalothrin. Sastry and Sharma (1980) reported an alkaline phosphatase inhibition after 96h exposure to diazinon. Goel *et al*. (1982) reported a plasma alkaline phosphatase inhibition by 15% in *Heteropneutes fossilis*, resulting from the effect of malathion. Similarly, Das and Mukherjee (2003) reported a depletion of alkaline phosphatase due to sublethal exposure of *Labeo rohita* fingerlings to cypermethrin. Rashatwar and Hyas (1983) reported a significant decrease in alkaline phosphatase activity in freshwater fish *Nemachelius denisonii* (day) exposed to sublethal concentrations of Basalin.

The significant ($P<0.05$) decrease in the acid phosphatase (ACP) concentration with an increase in the concentration in the plant extract in this study is similar to that observed in *C. gariepinus* adults to acute effect of diazinon on blood plasma biochemistry (Adedeji, 2010). Sastry and Sharma (1980) reported a decrease of activities in acid phosphatase in the brain of *Channa punctatus* following the effect of diazinon. Goel *et al.* (1982) reported that plasma acid phosphatase decreased by 15% in *Heteropneutes fossilis*, resulting from the effect of organophosphate malathion. The activities of acid phosphatase in blood plasma of *Cyprinus carpio* were almost identical in the control and test treatment following the exposure to acute effect of diazinon (Luskova *et al.,* 2002).

Aminotransferases are gainfully used in the diagnosis of disease and tissue damage. It functions as a link between carbohydrate and protein metabolism by catalyzing the inter conversion of strategic compounds, respectively (Martin *et al.,* 1983). They are intracellular enzymes which exist in only a small amount of the plasma. Their presence in the plasma may give information on organ dysfunction (Wells *et al.,* 1986; Gabriel and George, 2005). The aminotransferases occupy a central position in amino acid metabolism as they help in retaining amino group (to form a new amino acid) during the degradation of amino acid; they are also involved in the biochemical regulation of intracellular amino acid pool. They also help in providing necessary intermediates for gluconeogenesis. In the present study, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased significantly ($P<0.05$) in the plasma as the concentration of *D. elliptica* increased; this indicated stressed based tissue impairment (Svoboda, 2001). Under stress conditions, fish need more energy resulting from a higher demand for carbohydrate and their precursors to keep the glycolytic pathway and TCA cycles at sustained levels (Tiwari and Singh, 2004). Similarly, in other studies (Ayalogu *et al.,* 2001; Svoboda *et al.,* 2001; Tiwari and Singh, 2004) an alteration in the activities of ALT and AST was recorded, indicating that there was an increased demand for energy due to tissue impairment. Studies carried out by Das *et al.* (2004) also showed that there was an alteration in the activity level of ALT and AST of Indian major carps exposed to nitrite toxicity and suggested that the alteration of the aminotransferases is a result of the diversion of the amino acids in the TCA cycle as keto acids to argument energy production. From the pattern of the results obtained in this plasma aminotransferase, it is conceivable that the plant powder caused an increased energy demand by the exposed fish.

5. Conclusion

It was concluded from this study that the stem powder of *D. elliptica* could affect the liver function of the African catfish by decreasing its plasma ACP, ALP and by increasing the plasma ALT, AST levels. These parameters appeared to be good indicators of the deteriorating health of the fish exposed to the stem powder. Using these parameters, a presumptive prediction can be made on the health status and the possible problem (infection or toxicity) of the fish. Results of the present study provide baseline information. However, the parameter set may be, to some extent, case-dependent and requires information about the history of the fish.

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