The Effects of Sub-lethal Doses of Lambda-cyhalothrin on Some Biochemical Characteristics of the African Catfish Clarias gariepinus

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Abstract: The impact of long-term exposure to waterborne lambda-cyhalothrin on Clarias gariepinus was evaluated through changes of selected biochemical parameters. Fish was exposed to 0.0004, 0.0008 and 0.0016 mg L⁻¹ for 8 weeks. The parameters measured were serum glucose, protein, cholesterol, triglyceride, glutamic pyruvic acid transaminase (GPT), Glutamic Oxaloacetic acid Transaminase (GOT) and alkaline phosphatase (ALP). There was significant (p<0.05) alterations between the control values and the exposed groups on all parameters except GOT. The alterations in all parameters was significantly (p<0.05) dose and time dependent.

Key words: Lambda-cyhalothrin, serum, biochemical parameters, Clarias gariepinus, toxicity

INTRODUCTION

Cells contain enzymes that are necessary to their function. When the integrity of a cell is disrupted, enzymes escape into plasma/serum, where their activity can be measured as a useful index of cell integrity (Coppo et al., 2002). Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, obstruction of normal excretory route, increased cell membrane permeability, or impair circulation (Kaneko, 1989). Lambda-cyhalothrin (RS)-alpha-cyano-3-phenoxybenzyl 3-(2-chloro3, 3, 3-trifluoropropenyl)-2, 2-dimethyl cyclopropane carboxylate, is a synthetic pyrethroid insecticide and like all pyrethroids, they are neurotoxins (Beat et al., 1997). They act on the axons in the peripheral and central nervous systems. They are believed to interfere with sodium channels and the permeability of nerve cells, so affecting the transmission of nerve impulses. Charles and Hance (1968) reported the 96 h LC₅₀ for brown trout under laboratory conditions to be 0.002-0.0028 mg L⁻¹. Bradbury and Coast (1989), also reported that the 96 h LC₅₀ value of pyrethroids, determined in laboratory tests, generally lies below 10 µg L⁻¹. Due to the lipophilicity of pyrethroids, they have a high rate of gill absorption, which in turn would contribute in the sensitivity of fish to aqueous pyrethroid exposures. Fish seem to be deficient in the enzyme system that hydrolyses pyrethroid. There metabolism in fish is largely oxidative (Demoute, 1989; Rukiye et al., 2003).

Fish have an important role in the food chain; therefore, investigation of the effects of pesticides on fish has a diagnostic significance in evaluation of adverse effect of pesticides to human health (Begun and Vijayaraghavan, 1996). Clarias gariepinus is a fresh water fish and an important food supply for humans. The blood of fish is sensitive to pollution induced stress and certain serum chemistry may be used to identify tissue damage (Patil and Kulkarni, 1993). Changes in the biochemical blood profile indicate changes in metabolism and biochemical processes of the organism, resulting from the effect of various pollutants and they make it possible to study the mechanisms of the effect of various pollutants (Luskova et al., 2002). Liver is the metabolic centre for detoxification of chemicals. Liver damage was confirmed by changes in the activities of Glutamate-Oxaloacetate Transaminase (GOT) and Glutamate-Pyruvate Transaminase (GPT) activities (Asztalos and Nemesok, 1985). Chronic hepatic disorders and excessive steroids results in increase plasma alkaline phosphatase (ALP) in most animal. During normal bone growth in young animals, a large amount of ALP is found in plasma, also osteopathies result in increase plasma (ALP) (Coppo et al., 2002). Increase in blood glucose level is a general response of fish to acute pollutant effects including organophosphates and pyrethroids (Luskova et al., 2002). The quantity of protein is dependent on the rate of protein synthesis, or on the rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids in the poly peptide chains (Singh et al., 1996).

The aim of this study was to investigate the serum activities of GOT, GPT, ALP, protein and carbohydrate

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metabolisms after exposure of *Clarias gariepinus* juveniles to nominal sub-lethal concentrations of lambda-cyhalothrin (a commonly used insecticide) with a view to access the possible effects of its toxicity.

**MATERIALS AND METHODS**

Juveniles of *Clarias gariepinus* were purchased from Maigana fish farm in Zaria, Kaduna State Nigeria. The *Clarias* species averaging 14.33±0.50 cm standard length and average body weight of 20.38±1.25 g were used for the study. The fish were conveyed to fisheries laboratory in a portable well-aerated white polytherne bag containing water from the Dam. They were held in large water baths (Container) of 160 L capacity at 24.5-25.5°C and acclimatized for two weeks in dechlorinated municipal water. During this period, the fishes were fed with pelletized diet containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed every alternate day. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 h before the start of the experimental run (Reish and Oshida, 1987).

**Sub-lethal bioassay:** Based on the result of 96 h LC$_{50}$, which was estimated to be 0.008 mg L$^{-1}$ (Ogueji and Auta, 2006 unpublished) juveniles were exposed to nominal concentrations of Lambda-cyhalothrin for 8 weeks. The concentrations used for chronic study were 0.0004, 0.0008 and 0.0016 mg L$^{-1}$. Each treatment was in triplicate and there was a control in each case. With the exception of the control tanks, appropriate volumes of the toxicant were added into each tank. The fishes were randomly assigned to give a loading of 10 fish per tank. Fishes were fed to satiation twice daily. The toxicant and test water were renewed at two days intervals to maintain the toxicants strength and the level of dissolved oxygen as well as minimizing the level of ammonia during the experiment. Twelve fishes were sampled at the end of two weeks from concentrations and control and this was repeated at the end of eight weeks.

**Biochemical measurements:** For biochemical investigations, The caudal peduncle of fish was cut, blood was collected in non-heparinized tubes. The blood was immediately centrifuged at 1500 rpm for 10 min. Serum was then removed and stored at 4°C prior to immediate determination of biochemical parameters, glucose, cholesterol, triglycerides, total protein, Glutamic Pyruvic acid Transaminase (GPT), glutamic oxaloacetic acid transaminase (GOT) and alkaline phosphatase (ALP). Blood glucose was estimated using the method of Trinder (1969). Blood cholesterol was measured according to the procedure of Pearson et al. (1953). Blood triglyceride was determined using the method of Rice (1970). The method of Lowry et al. (1951) was carried out to determine the value of total protein. The activities of blood GPT and GOT were estimated according to the methods of Retiman and Frankel (1957). To determine the activity of blood ALP, Bassey et al. (1946) method was used.

**Statistical analysis:** Graph of probit kill against log concentration was used to determine the 96 h LC$_{50}$. For the various biochemical parameters, GenStat Release 4.2 programmes was used to run analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for differences between different levels of treatment and to separate means respectively, were applicable. Test of significance was at 95% probability.

**RESULTS**

Analysis of variance (ANOVA) results of sub-lethal exposure to lambda-cyhalothrin indicated significant (p<0.05) dose dependent elevations in glucose, triglyceride and GPT levels in the serum (Table 1). On the other hand there was a significant (p<0.05) dose dependent inhibition in protein and ALP. No significant difference was recorded for GOT across concentrations. Cholesterol significantly increased (p<0.05) in concentrations 0.0004 and 0.0008 mg L$^{-1}$, but was significantly inhibited in 0.0016 mg L$^{-1}$. Also GPT activity was significantly (p<0.05) elevated in 0.0004 and 0.0008 mg L$^{-1}$ exposed fish (Table 1). The control values of protein and cholesterol were significantly lower than in exposed fish (Table 1). The control values of triglyceride was significantly (p<0.05) lower than in 0.0008 and 0.0016 mg L$^{-1}$ exposed fish (Table 1) and that of ALP was significantly lower than in 0.0004 mg L$^{-1}$ but significantly elevated than in 0.0016 mg L$^{-1}$ exposed fish (Table 1). The control values for glucose was significantly lower (p<0.05) than in 0.0008 and 0.0016 mg L$^{-1}$, but significantly elevated than in 0.0004 mg L$^{-1}$ exposed fish.

There were time dependent elevations in the serum values of glucose, protein and ALP (Table 2). On the other hand and within the same time period, there was time dependent significant inhibition in cholesterol, triglyceride, GOT and GPT serum enzymes (Table 2).

**DISCUSSION**

The significant (p<0.05) increase in glucose which was dose and time dependent (Table 1 and 2) may be
considered to be manifestation of stress induced by lambda-chohalothrin. Glucose increase is a general response of fish to acute and sublethal pollutant effects (Verma et al., 1983; Ghazaly, 1994; Ceron et al., 1997; Luska et al., 2002). Wedemeyer et al. (1981) stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids (Hontela et al., 1996) and catecholamines (Nakano and Tomlinson, 1967). Both of these groups of hormones produce hyperglycemia.

The increase in serum protein was dose and time dependent (Table 1 and 2). Present findings are in agreement with that of other some other workers. For example Oruc and Uner (1999) reported increase in liver protein following exposure to 2, 4-Diamin for 30 days. Salib et al. (1984) observed that the protein content in all tissues of malathion exposed Tilapia mossambica is slightly higher. They suggested that the fish exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis.

Gill et al. (1991) found an increase in liver proteins following Endosulfan intoxication and noted that protein levels in the liver of Barbus conchonus could be due to increased protein turnover. They also concluded that compensatory production of enzymes lost as a result of tissue necrosis or to meet increased demand to detoxify the pesticides might have necessitated enhanced synthesis of enzyme proteins (Gill et al., 1990). However, the significant decrease in serum protein observed in 0.0038 and 0.0016 mg L\(^{-1}\) was in agreement with the work of Ravichandran et al. (1994). They reported depletion of protein from 7.9 to 45.0% due to proteolysis after exposing Oreochromis mossambicus to sub-lethal concentrations of phenol. The decrease in protein level observed in the two sub-lethal concentrations may be due to their degradation and also their possible utilization for metabolic purpose. Bradbury et al. (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. The quantity of protein is dependent on the rate of protein synthesis, or rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains (Ram et al., 2003). Cholesterol content in the blood is linked to lipid metabolism and depends on the calorific value of the feed. In this investigation there was a significant (p<0.05) serum cholesterol inhibition as time of exposure increased to 8 weeks. The liver is the key organ in the synthesis and excretion of cholesterol, therefore any type of obstruction in the liver either intra or extra hepatic, will cause an increase in total cholesterol levels on the serum. However in chronic conditions such as cirrhosis, that involves considerable destruction of liver cells, the cholesterol levels eventually falls below normal level since decreased synthesis is taking place (Kamath, 1972). There was also a time dependent significant (p<0.05) serum triglyceride inhibition when compared with control. Khurshid (2003) also reported that total lipid content of young chick embryo exposed to cypermethrin was increased at 200 ppm and decreased at 400 ppm and he concluded that the high dose of 400 ppm of cypermethrin seems to have caused cell death (necrosis or apoptosis or both) in the embryonic tissue. Triglyceride accumulation as observed in fatty liver due effect of toxicants is the result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation (Gabriel, 1986).

Lombardi (1966) described four general mechanisms that can account for accumulation of triglyceride: The rate of synthesis of hepatic triglyceride is normal, but the liver cell is unable to secrete the triglyceride into the plasma/serum; The secretion is normal, but rate of synthesis is increased; There is both an increase in the rate of synthesis and a block in the secretion of the synthesized triglyceride and the triglyceride synthesis takes place in a compartment of the cell other than the endoplasmic reticulum and thus these pool is not accessible to the normal secretory pathway. It appears that a combination of liver necrosis, affecting the synthesis of triglyceride and blockage of the secretion into the serum was responsible for the inhibition observed.

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### Table 1: Means for \textit{C. gariepinus} biochemical parameters after exposure to sub-lethal concentrations of lambda-chohalothrin

<table>
<thead>
<tr>
<th>Conc. (mg L(^{-1}))</th>
<th>Glucose (mg dl(^{-1}))</th>
<th>Protein (g dl(^{-1}))</th>
<th>Cholesterol (mg dl(^{-1}))</th>
<th>Triglyceride (mg dl(^{-1}))</th>
<th>GGT (IU L(^{-1}))</th>
<th>GPT (IU L(^{-1}))</th>
<th>ALP (IU L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>54.00 (^{a})</td>
<td>3.43 (^{a})</td>
<td>129.00 (^{a})</td>
<td>142.25 (^{a})</td>
<td>48.50 (^{a})</td>
<td>58.75 (^{a})</td>
<td>17.00 (^{a})</td>
</tr>
<tr>
<td>0.0004</td>
<td>52.75 (^{a})</td>
<td>3.58 (^{a})</td>
<td>145.00 (^{a})</td>
<td>138.25 (^{a})</td>
<td>47.00 (^{a})</td>
<td>46.25 (^{a})</td>
<td>20.25 (^{a})</td>
</tr>
<tr>
<td>0.0008</td>
<td>57.06 (^{b})</td>
<td>2.95 (^{b}}</td>
<td>161.50 (^{b})</td>
<td>144.50 (^{b})</td>
<td>42.50 (^{b})</td>
<td>51.75 (^{b})</td>
<td>17.50 (^{b})</td>
</tr>
<tr>
<td>0.0016</td>
<td>50.75 (^{a})</td>
<td>2.63 (^{a}}</td>
<td>139.50 (^{a})</td>
<td>150.00 (^{a})</td>
<td>47.50 (^{a})</td>
<td>59.25 (^{a})</td>
<td>15.50 (^{a})</td>
</tr>
</tbody>
</table>

Means with the same superscript along columns are not significantly different (p>0.05).

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### Table 2: Changes in some biochemical characteristics of \textit{C. gariepinus} due to lambda-chohalothrin in relation to time

<table>
<thead>
<tr>
<th>Time (Weeks)</th>
<th>Glucose (mg dl(^{-1}))</th>
<th>Protein (g dl(^{-1}))</th>
<th>Cholesterol (mg dl(^{-1}))</th>
<th>Triglyceride (mg dl(^{-1}))</th>
<th>GGT (IU L(^{-1}))</th>
<th>GPT (IU L(^{-1}))</th>
<th>ALP (IU L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>53.50 (^{a})</td>
<td>2.49 (^{a}}</td>
<td>185.50 (^{a})</td>
<td>150.50 (^{a})</td>
<td>49.00 (^{a})</td>
<td>58.75 (^{a})</td>
<td>12.75 (^{a})</td>
</tr>
<tr>
<td>8</td>
<td>58.50 (^{a})</td>
<td>4.00 (^{a}}</td>
<td>102.00 (^{a})</td>
<td>137.00 (^{a})</td>
<td>43.25 (^{a})</td>
<td>49.25 (^{a})</td>
<td>22.57 (^{a})</td>
</tr>
</tbody>
</table>

Means with the same superscript along columns are not significantly different (p>0.05).
over prolonged exposure periods of fish to lambda-cyhalothrin. There is also time dependent significant (p<0.05) serum ALP elevation due lambda-cyhalothrin exposure. Atef (2005) also reported significant elevations of ALP after exposure to cadmium and attributed the increase to liver dysfunction. ALP is made in the liver, membrane-bound close to the biliary canaliculus, secreted into the bile and its increase principally indicates cholestasis. The increase in protein level may also be due to the increase in ALP activity as it plays an important role in protein synthesis (Pilo et al., 1972). There was a significant (p<0.05) time dependent serum GOT and GPT inhibition and this finding is in agreement with that of some other workers, Oruc and Uner (1999) reported inhibition of saral GOT and GPT enzyme activities following 2 and 30 days of exposure to 2, 4-Diamin to Cyprinus carpio and Sadhu et al. (1985) reported inhibition of GOT and GPT activities in the serum of Channa stiatus following exposure to 0.1 ppm malathion for 10 days. GOT and GPT are important in the diagnosis of heart and liver damage (Dere and Polat, 2001).

The results of this study suggest that sub lethal exposure of C. garipinus to lambda-cyhalothrin could lead to alterations in carbohydrate and lipid metabolism and possible organ damage. In the light of the above observations, it is recommended that lambda-cyhalothrin should be used with caution and in a suitable manner, as it could be hazardous to aquatic biota, domestic animals and human beings as well.

REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), 1992. Standard Methods for the examination of Water and Wastewater, 18th Edn., Washington, DC.


