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ALTERATIONS IN THE BIOCHEMICAL PARAMETERS OF THE AFRICAN CATFISH *CLARIAS GARIEPINUS [BURCHELL]* EXPOSED TO SUBLETHAL CONCENTRATIONS OF LAMBDA-CYHALOTHRIN

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ABSTRACT

Pesticide contamination of water bodies is increasingly becoming a threat to aquatic life with its attendant public health implications. With this in mind, this study was undertaken with a view to evaluating the effect of chronic exposure to λ-cyhalothrin on some biochemical parameters (cholesterol, glycogen, glucose and protein) in the freshwater catfish *Clarias gariepinus*. The fingerlings were exposed to sublethal concentrations (0.0, 0.01 and 0.04 mg/L) of λ-cyhalothrin for 21 days in a static renewal bioassay system. The LC_{50} value determined by probit analysis was 0.103 mg/L. The cholesterol concentration was significantly higher ($P < 0.05$) in the treatment groups when compared with the control. The increase was both concentration and duration-dependent. The protein concentration in the λ-cyhalothrin-exposed fish was significantly lower than the control ($p < 0.05$) except on day 7, when the serum protein level did not differ from the group exposed to 0.01 mg/L λ-cyhalothrin. Similarly, when compared with the control, the liver glycogen and serum glucose concentrations decreased significantly ($p < 0.05$) with increasing λ-cyhalothrin test concentration and duration of exposure. The observed hypercholesterolemia with concomitant hypoglycemia and hypoprotenemia are indications of stress due to λ-cyhalothrin exposure.

Keywords: *Clarias*, λ-cyhalothrin, glycogen, hypoglycaemia, hypoprotenemia, cholesterol.

1. INTRODUCTION

The use of pesticides has contributed significantly to increased food production in modern times through the control of pests and weeds with the attendant ecotoxicological implications. Pesticides were originally intended for use in terrestrial agriculture but some have been adopted for use in irrigated rice fields and aquaculture facilities. Generally, pesticides enter water bodies as run offs from the watersheds or as discharges from irrigated paddy rice fields and industrial effluents [1,2] thereby contaminating the water bodies and leading to the death of non-target organisms such as fish, and macro- and micro-invertebrates.

Synthetic pyrethroid insecticides are known to have high insecticidal potency [3,4] due principally to their high lipophilicity [5] that enhances their quick absorption through the gills on the one hand and its stereostructure on the other [6]. The high lipophilic property and relatively short half-life of 6-12 h in birds and mammals and 48 h in fish [7] are contributing factors to making synthetic pyrethroids occupy a commanding premium position in the pesticide market worldwide. Besides the use of pyrethroid insecticides in terrestrial agriculture and in homes, they have also gained wide acceptance in veterinary practice [5] to control ectoparasites of livestock.

Lambda cyhalothrin and other synthetic pyrethroid insecticides are neurotoxic in nature as they cause increased neuronal excitation through the inhibition of the ATPase that controls the ionic gradient, leading to the disruption of ionic balance and altered neuronal signaling [5]. Similarly, Vijverberg and Van den Bercken [8] reported that synthetic pyrethroids cause axonal damage and GABA-ergic inhibition [9]. Also, the neuronal damage due to pyrethroid insecticide exposure has been related to acethylcholinesterase activity inhibition [10,11] resulting in neurotransmission impairments.

Other studies have shown that λ-cyhalothrin and cypermethrin cause structural and chromosomal aberration, chromosomal fragmentation and DNA lesions in rats [3] and decreased DNA in *Channa striata* [12]. They also affect the reproductive capacity and development of fish [13], reduced the hatching rate as well as the fertility [14]. Furthermore, fish responses to

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pesticide xenobiotics could be behavioral [15] or changes in the histology [16] and metabolism [4,17-19]. The metabolic changes are diverse depending on the chemical species involved, the concentration and even the prevailing environmental factors. Pesticides also affect the lipid and energy metabolism in fish [20,21]. Decreased tissue levels of protein and glucose, respectively, were reported in *Labeo rohita* exposed to cypermethrin [22].

Today, the widespread and indiscriminate use of pesticides is of great public health concern as humans are constantly exposed to them either directly (during the manufacture, application or sales) or indirectly as residues in crops or animal tissues. Fish, therefore, are good animal models for the study of aquatic contaminations since altered water chemistry affects not only their behavior and histology but also their physiology and biochemical processes. Consequently, the effect of pesticides on fish can also be elucidated by analyzing the tissue biochemical parameters.

Increasing contamination of water bodies by pesticides and other xenobiotics is of great concern not only for its public health implications but also for ecosystem health concerns. The study of the effect of pesticides on non-target organisms is of paramount importance not only to ecologists but also to policy makers with a view to developing policy guidelines that will safeguard ecosystem health and aquatic living resources. This study was undertaken with a view to evaluating the sublethal general toxicity of λ-cyhalothrin on some biochemical parameters in the African Catfish *Clarias gariepinus*.

2. MATERIALS AND METHODS

2.1. Fish Collection and Experimental Design

The fish used in this study was bought at Aquafish Ltd, Awka, Anambra State, Nigeria and transported to the laboratory in a plastic fish transport container early in the morning to avoid heat-related stress on the fish during transportation. The fish were acclimatized for fourteen days in the laboratory before the experiment was started. During the period of acclimatization, the fish were fed 35% crude protein diet *ad libitum*.

Serial dilutions of λ-cyhalothrin (0.09, 0.2. 0.3, 0.4, and 0.5 mg/L) were prepared from a commercial preparation (Syngenta, Switzerland) containing 25 g/L λ-cyhalothrin as the stock solution. Thirty fish were randomly distributed into each of the test concentrations in a renewal bioassay system. Each treatment group had three replicate experiments containing 10 fish each. A parallel control experiment containing non-chlorinated borehole tap water was also set up. The water and the test solutions were changed every day in order to maintain the toxicant concentration and to avoid accumulation of wastes in the water that could affect the data. The mortality/survival data in each replicate experiment was recorded after 96 h and used to determine the median lethal concentration. The mean lethal concentration (LC_{50}) value of 0.103 mg/L was calculated by the probit method [23].

To investigate the effect of sublethal concentrations of λ-cyhalothrin on the measured parameters in *C. gariepinus,* two sublethal concentrations (0.01 and 0.04 mg/L) were prepared based on the LC_{50} value. A total of one hundred and eighty fish of mean (± SD) weight and length of 28.58 ± 1.04 g and 16.31 ± 0.62 cm, respectively, were used for the study. They were randomly divided into three treatment groups of sixty fish. Each group was further randomized into three replicate experiments containing twenty fish. One group was exposed to 0.01 mg/L λ-cyhalothrin while the second group was treated with 0.04 mg/L λ-cyhalothrin. The third group was exposed to tap water only as the control.

The qualities of the tap water (total hardness, 21 mg/L as $CaCO_3$; alkalinity 66.8 mg/L; pH 7.8; conductivity 3.85 mS/m; dissolved oxygen 6.8 mg/L; temperature 28ºC, ammonia, nil) were determined using standard methods [24].

Every seven days, tissues were taken for the analysis of cholesterol, protein, glycogen and glucose.

2.2. Biochemical Analysis

Three or four fish from each replicate experiment were killed every seven days for determination of the biochemical parameters. The blood was collected by cardiac puncture [21]. The blood samples from each replicate experiment were pooled together in eppendorf tubes before analysis.

Liver glycogen was determined by the Anthrone reagent method [25] while serum glucose was determined spectrophotometrically at 610 nm using a Boehringer-Mannheim kit. Protein was determined by the Biuret method [26] while the tissue cholesterol was determined by the method of Zlatkis et al. [27]

2.3. Statistical Analysis

The data was analyzed by one way analysis (ANOVA) of Steel, and Torrie [28] followed by a FSLD post hoc

test to determine significant differences between the means at the 5% level of significance.

3. RESULT

3.1. Effect on Cholesterol

The effects of λ-cyhalothrin on the liver and serum cholesterol levels are shown in Figure 1. The liver cholesterol concentrations increased and differed significantly ($P < 0.05$) in the treatment groups when compared with the control. There was about a 1.2-fold increase in the liver cholesterol on day 21 in the fish exposed to both test concentrations of λ-cyhalothrin representing 15% and 24% increases, respectively. When compared with the control, the liver cholesterol was significantly ($P < 0.05$) increased in the fish treated with λ-cyhalothrin.

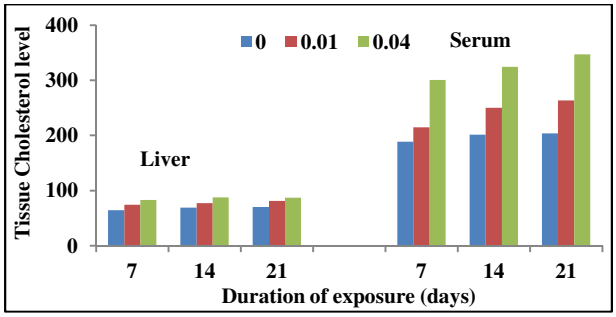

Figure 1 Changes in the tissue cholesterol in *C. gariepinus* exposed to λ-cyhalothrin

The serum cholesterol level was also significantly increased ($P < 0.05$) in the fish treated with λ-cyhalothrin. On day 21, the serum cholesterol increased by 1.3 and 1.7-fold in the fish exposed to 0.01 and 0.04 mg/L λ-cyhalothrin, respectively. On day 21, the percentage increase in the serum cholesterol levels were 29.3 and 70.5 in the fish exposed to 0.01 and 0.04 mg/L λ-cyhalothrin, respectively. The serum cholesterol level in the treatment groups differed significantly ($P < 0.05$). There were both concentration and duration dependent increase in both liver and serum cholesterol concentrations throughout the study.

3.2. Effect on Tissue Protein

The effects of λ-cyhothrin on the liver and serum protein levels are shown in Figure 2.

The protein concentration in the control group did not vary during the study but exposure to λ-cyhalothrin induced depletion of liver and serum protein concentrations in the fish. The liver protein was significantly lower ($P < 0.05$) in the treatment group than in the control and it differed in different groups ($P < 0.05$). The liver protein decreased by 2.1 and 2.7-fold in the fish exposed to 0.01 and 0.04 mg/L λ-cyhalothrin, respectively, at the end of the study.

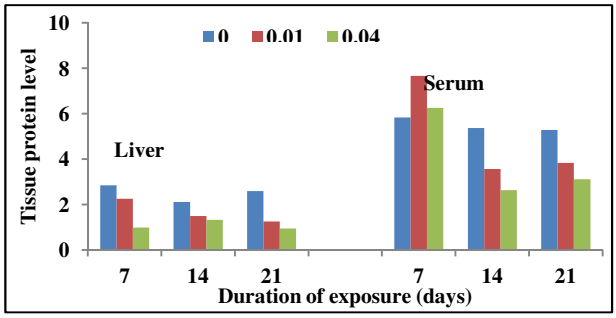

Figure 2 Changes in the liver and serum protein concentration in *C. gariepinus* exposed to λ-cyhalothrin

The serum protein concentration was also significantly ($P < 0.05$) increased on day 7 in the treated fish when compared with the control. Thereafter, it decreased significantly when compared with the control ($P < 0.05$) in the fish exposed to the test concentrations of λ-cyhalothrin. The decrease was both concentration- and duration-dependent. On day 21, the serum protein decreased by 1.4 and 1.7-fold in the fish treated with 0.01 and 0.04 mg/L λ-cyhalothrin, respectively.

The percentage decreases in the serum protein at the end of the study were 27% and 41% in the fish treated with 0.01mg/L and 0.04mg/L, λ-cyhalothrin, respectively. The magnitude of decrease was highest on day 14 when the percentage decrease was 33% and 51% in the fish exposed to 0.01 and 0.04 mg/L λ-cyhalothrin, respectively.

3.3. Effect on Liver Glycogen and Serum Glucose

The results showed that λ-cyhalothrin induced depletion of the liver glycogen concentrations (Figure 3) in *C. gariepinus*. When compared with the control, the liver glycogen concentration decreased significantly ($P < 0.05$) in the fish exposed to λ-cyhalothrin throughout the study. The decrease was both concentration- and duration-dependent. At the end of the study, the liver glycogen decreased by 2.3 and 2.6-fold, representing 48% and 61% decrease in the fish treated with 0.01 and 0.04 mg/L λ-cyhalothrin, respectively.

The serum glucose concentration (Figure 4) differed significantly ($P < 0.05$) in the treatment groups when compared with the control. It decreased significantly ($P < 0.05$) in the treatment groups. At the end of the study the percentage decreases in serum glucose were 20 and 33 in the fish treated with 0.01 and 0.04 mg/L λ-cyhalothrin, respectively, and this translated to about 1.3 and 1.5-fold decreases, respectively.

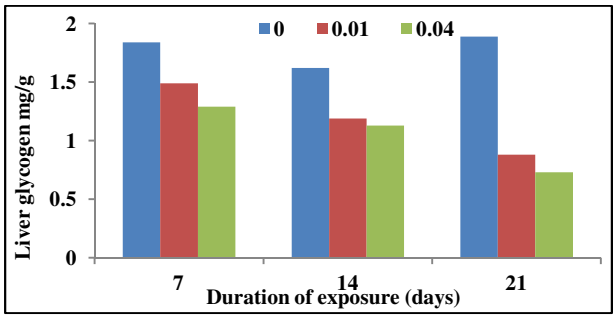

Figure 3 Changes in the liver glycogen level in *C. gariepinus* exposed to λ-cyhalothrin

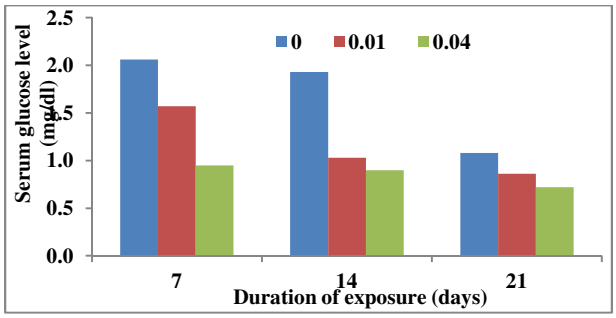

Figure 4 Changes in the serum glucose level in *C. gariepinus* exposed to λ-cyhalothrin

4. DISCUSSION

The results of this study indicate that λ-cyhalothrin has adverse effects on the assayed biochemical parameters. The observed changes in the tissue cholesterol level in *C. gariepinus* in this study are indications that the insecticide λ-cyhalothrin affects lipid metabolism in the fish. A similar result was reported in *Clarias batrachus* treated with aldrin [29]. Also, Yousafzai and Shakoori reported that aquatic pollution leads to elevated tissue cholesterol in fish [30].

On the other hand, tissue cholesterol level was reduced in *C. batrachus* [31] and in *Heteropnuestuss fossilis* [32] exposed to carbaryl. Thus, it is probable that the elevated cholesterol levels in *C. gariepnus* exposed to λ-cyhalothrin in this study could be due to increased activity of 3-hydroxy-3-methylglutaryl coenzyme reductase (HMG-COA), a key enzyme in the cholesterol biosynthetic pathway that favours the biosynthetic processes under such circumstances in the fish. By so doing, the gluconeogenetic pathway is provided with the necessary feedstock for energy production during the stressful period of exposure.

Proteins are essential biological macromolecules used in the assessment of the state of health and metabolism in fish under stress [33] and are mobilized to meet the energy needs under such situations. The data on tissue protein showed that λ-cyhalothrin induces depletion in total liver and serum protein in *C. gariepinus*. This is consistent with the reported decrease in liver protein in *A. anguilla* treated with fenitrothion [19]. Similarly, total protein and albumin was decreased in *Labeo rohita* and in *O. niloticus* treated with cypermethrin [16,22].

The result of our study is also in agreement with observed decrease in both muscle and serum protein concentrations in *Oreochromis niloticus* exposed to diazinon [21] as well as in *Channa punctatus* and in *C. gariepinus* exposed to λ-cyhalothrin [4,15]. Depletion of serum protein was reported in carp due to pyrethroid treatment [34]. Vidhi and Saxena [4] reported that λ-cyhalothrin inhibits the tissue protein content in *Channa punctatus.* Similar decrease in protein was observed in the rockfish *Sebastes schlegeli* exposed to cypermethrin [17]. Also Velisek et al. [35] reported that cypermethrin caused the lowering of total protein, albumin and globulin in both trout and carp, while deltamethrin exposure causes elevated values of these parameters in both fish. The observed decrease in tissue protein in this study is in agreement with the report of Korkmaz et al. [16] that λ-cyhalothrin induces pronounced decrease in liver and muscle protein and total lipid in *Channa punctatus*.

Garg et al. [18] attributed decrease in tissue protein in pesticide-treated fish to either reduced liver protein synthesis or impaired kidney function. This view was strengthened by the observation that pesticides impair protein synthesis [36] through the inhibition of RNA synthesis resulting in low RNA and protein levels. The hypoprotenemia observed in this study may further be explained in part on the basis that some protein could have been channeled towards tissue repair and enhanced mobilization of protein into the gluconeogenetic pathway to meet the energy needs of the fish during the exposure period. Physiologically, when glucose

replenishment from the liver is impaired or is in short supply due to malnutrition or stress, the body protein is mobilized to provide additional energy through the deamination of amino acids to form keto acids that are converted to pyruvate and utilized in the tricarboxylic acid cycle for energy generation. Generally, it has been noted that fish exposed to pesticides suffer severe metabolic interferences [36] resulting in observable decline in total plasma protein.

It has been reported that liver glycogen and serum glucose concentrations are sensitive indicators of stress in fish [33]. Our observed decrease in liver glycogen and serum glucose concentrations is in agreement with the report of some workers. Gimeno et al. [37] observed decreased tissue glycogen levels in *A. anguilla* exposed to endosulfan. Similarly, reduced liver glycogen was reported in *Cyprinus carpio* and *C. gariepinus* due to paraquat exposure [20,38]. Begum and Vijavaraghavan [39] also reported reduced muscle glycogen in *C. batrachus* treated with the pesticide rogor. In their studies on the toxic effect of three pyrethroids (cypermethrin, deltamethrin and bifenthrin) on the biochemical parameters in carp and trout, Velisek et al. [35] reported that acute exposure to deltamethrin resulted in lowering of glucose concentrations whereas bifenthrin and cypermethrin caused increased glucose levels in both species. Similar increase in serum glucose level was reported in *A. anguilla* exposed to femitrothion and endosultan [37] and in *C. batrachus* exposed to malathion [40] as well as in *C. gariepinus* treated with λ-cyhalothrin [15]. Since the activities of glycogen phosphorylase and glucose-6-phosphatase were enhanced when fish were exposed to paraquat [20], it is likely that the activities of these enzymes were enhanced on exposure to λ-cyhalothrin, resulting in depleted tissue glycogen. According to Velisek et al [41] and Jee et al [17], bifenthrin and cypermethrin caused elevated serum glucose in rockfish, rainbow trout and *S. schlegeli*. Pesticides stimulate the hypothalamus-pituitary interrenal axis [33] that increases the blood cortisol level, thereby promoting lipolysis, glycogenolysis and even gluconeogenesis to offset the energy deficit incurred during pesticide-induced stress conditions.

5. CONCLUSSIONS

Our results show that λ-cyhalothrin exposure impacts the energy and protein metabolisms in *C. gariepinus*. Measurement of these parameters could be used in biomonitoring studies and the data could provide the basis for developing a framework for establishing safe levels for the pesticide in water bodies.

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