EFFECT OF HERBICIDE (PRIMEXTRA) ON TISSUE CHOLESTEROL LEVEL IN *CLARIAS GARIEPINUS* JUVENILE

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ABSTRACT

Juvenile Clarias gariepinus were exposed to sub lethal concentrations (0.04, 0.06 and 0.10µg/L) of primextra for 21 days in a static renewal bioassay system. The changes in the tissue cholesterol concentrations were determined every seven days. The result showed that primextra had adverse effect on the tissue cholesterol levels in C. gariepinus. When compared with the control, the liver and muscle cholesterol concentrations were significantly (P>0.05) elevated due to primextra exposure. However, the kidney cholesterol levels in the primextra-exposed fish were lower (P > 0.05) than the control. The cholesterol concentrations in the treatment groups were also different (P<0.05).Generally, the liver and muscle cholesterol concentrations increased with duration of exposure. The induction of hypercholesterolemia in both the muscle and the liver and hypocholesterolemia in the kidney of the treated fish are indications of dysfunctional lipid physiological processes occurring in the fish due primextra exposure.

Keyword: Primextra, *Clarias gariepinus*, Cholesterol, Kidney, Liver

INTRODUCTION

There is a growing awareness of the effect of herbicides on aquatic organisms particularly the fish. Many studies have shown that most chemicals including agrochemicals affect several physiological and biochemical functions in an animal (Maduka, 2002). Primextra is a pre-emergent broad spectrum herbicide for weed control in maize and sorghum farmlands. Primextra can be toxic if inhaled, swallowed or absorbed through the skin. It has been found that empty containers of this herbicide retains product residue for a long time and those applied in the weed control programmes accidentally leach into the aquatic environment either through runoff and/or as aerosol carried by wind (Syngenta, 2007).

Cholesterol is a chemical that is naturally produced by the body and is a combination of lipid and steroid. It is a basic material needed in the construction of animal cell wall/ cell membrane. Tissue cholesterol is produced by the liver and is used as starting point for the synthesis of other steroid molecules. Excess cholesterol is stored in the muscle as fat and this provides energy during period of extensive exercise or during period of food deprivation. The liver manufactures and secretes LDL cholesterol into the blood. High levels of cholesterol in the blood stream, depending on how it is transported, are strongly associated with progression of arthrosclerosis in man. Biosynthesis of cholesterol is directly regulated by the cholesterol level present and

can be turned off when cholesterol level is high (Castell, 1979).

This study was therefore aimed at investigating the sublethal effect of primextra on the tissue cholesterol level of *Clarias gariepinus under* laboratory conditions.

MATERIALS AND METHODS

Eighty one (81) healthy juveniles of *Clarias gariepinus* with mean body weight 20.38 ± 1.25g and length 14.32 ± 0.50 cm were obtained from Freedom Fish Farm in Nsukka, Enugu State, Nigeria. They were transported to the Fisheries and Hydrobiology Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka in a plastic fish transport container. The fish were acclimatized for three weeks before the commencement of the study. During the period of acclimatization and the experiment, the fish was fed *ad libitum* on 45% crude protein diet.

The fish were randomly divided into three replicate groups of nine fishes per replicate. The fish in groups 1, 2 and 3 were treated with 0.04 µg/L, 0 .066 µg/L, and 0.092 µg/L of primextra respectively. The fourth group was exposed to tap water as the control experiment. The sublethal primextra concentrations were prepared from the commercial preparation containing 290g of metalachlor and 370g of atrazine as the stock. The primextra and water were changed daily in a static renewal bioassay system. The temperature and pH value of the tap water used in this study were 24.50 ± 2.0^{o}C and 7.4 pH, respectively.

The fish were killed and the tissues (kidney, liver and muscle) were extracted and rinsed with physiology saline. One gram of the excised tissues was weighed, homogenized, centrifuged and the supernatant was used for the assay of cholesterol (Zlatkis *et al.,* 1967) using Randox kit. The data obtained were analyzed statistically with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The result showed that primextra caused significant increase in the liver cholesterol concentration in *C. gariepinus* when compared with the control (Table 1). The magnitude of increase was 2.26, 2.56 and 1.79 folds in the fish exposed to 0.04, 0.06 and 0.09µg/l primextra, respectively.

The cholesterol concentration in the control group did not change throughout the study. The liver cholesterol decreased from 8.51 ± 2.1mg/g and 9.69 ± 5.23mg/g on the 7th day to 7.73 ± 3.82 mg/g and 7.19 ± 6.3 mg/g in the group treated with 0.04 and 0.06µg/l primextra, respectively at the end of the study. When the fish was exposed to 0.09µg/l primextra the liver cholesterol concentration slightly increased from 6.78 ± 4.7mg/g on the 7th to 7.03 ± 6.76mg/g liver on the last day. The percentage change in the liver cholesterol concentration is shown in Figure 1. The change was highest on day 7 being 126, 156 and 79.98 % in the fish treated with 0.04, 0.06 and 0.09 µg/l, respectively. At the end of the study the percentage change were 105.6 %, 60% and 87% in the groups treated with 0.04, 0.06 and 0.09µg/l, respectively. The observed increase in hepatic cholesterol in this study was in agreement with the work of Garthoff *et al.* (1977) that liver cholesterol level increased in rats fed with PCB. However, Maduka (2002) reported that lindane caused decreased liver cholesterol in *Clarias gariepinus* after 21 days. Similarly, Kling and Gamble (1982) had earlier reported decreased hepatic cholesterol levels in rats treated with PCB.

The muscle cholesterol concentration increased with increasing duration of exposure (Table 2). The cholesterol concentration was significantly different within the treatment groups ($P \geq 0.05$). The percentage change in the muscle cholesterol indicated that on the 7th day, the percentage increase over the control were 118, 164 and 66% in the fish exposed to 0.04, 0.06 and 0.09µg/l, respectively. By the end of the study the changes were 208, 170 and 202% increase over the control in the fish treated with 0.04, 0.06 and 0.09µg/l, respectively.

Table 1: Liver cholesterol concentration of *Clarias gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups (µg/L)	Duration of exposure (days)		
	7	14	21
Control (0.0)	3.77±0.47[a]	3.77±0.45[a]	3.76±0.56[a]
Group A (0.04)	8.51± 2.1[b]	6.98±4.29[b]	7.734±3.82[b]
Group B (0.06)	9.67± 4.56[c]	6.17±2.6[d]	7.19±6.3[d]
Group C (0.09)	6.78±4.7[d]	6.03±6.2[d]	7.03±6.76[d]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 2: Muscle cholesterol concentration of *C. gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups(µg/L)	Duration of exposure (days)		
	7	14	21
Control	1.09±1.94[a]	1.09±1.93[a]	1.09±1.94[a]
Group A (0.04)	2.38±10.77[b]	2.54±14.55[b]	3.37±25.61[b]
Group B (0.06)	2.78±1.05[c]	1.29±10.47[c]	2.95±11.14[c]
Group C (0.09)	1.82±1.09[d]	2.51±16.78[b]	3.28±8.51[b]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 3: Kidney cholesterol concentration of *C. gariepinus* exposed to sublethal concentrations of primextra for 21 days

Treatment groups (µg/L)	Duration of Exposure (days)		
	7	14	21
Control (0.0µg/L)	3.95±7.57[a]	3.95±7.6[a]	3.92±3.8[a]
Group A (0.04)	3.66±0.53[b]	3.14±2.73[b]	2.80±2.16[b]
Group B (0.06)	3.66±0.52[b]	2.22±1.89[c]	3.76±3.90[a]
Group C (0.09)	3.66±0.49[b]	3.53±2.78[d]	3.86±1.22[a]

Tabulated results are means of three determinations ± SD; Values in the column with different superscript are significantly different (p < 0.05)

Table 4: Regression functions of the percentage changes in the tissue cholesterol levels in *C. gariepinus* exposed to primextra for 21 days

Tissue	Primextra concentration(µg/l)	Regression function		
		Intercept	Slope	R^2
Liver	0.04	68.64	0.5	0.63
	0.06	125.9	-1.46	0.23
	0.09	189.2	-6.85	0.78
Kidney	0.04	2.24	-1.49	0.98
	0.06	-12.18	0.41	0.37
	0.09	-21.9	0.27	0.01
Muscle	0.04	-3.67	9.71	0.99
	0.06	112.13	0.39	0.01
	0.09	62.67	6.43	0.86

The percentage increase in the muscle cholesterol was highest on day 21 in all the treatment irrespective of the concentration. In both the liver and the muscles, it likely that primextra may have promoted the activities of HMG-CoA reductase to increase mevalonate production that favours the cholesterol biosynthesis.

The result of this study is consistent with earlier reports with mammalian subjects (Hafeiz and Bartke, 1972; Nagaoka *et al.* 1986) that xenobiotic intoxication results in elevated tissue cholesterol level.

The changes in the kidney cholesterol concentration are shown in Table 3. The cholesterol concentration in the control did not

change during the exposure period and did not differ from the values in the treatment groups on day 7 of the study (P< 0.05). Thereafter, the cholesterol concentrations in the treatment groups remained generally lower than the control (P≤0.05).

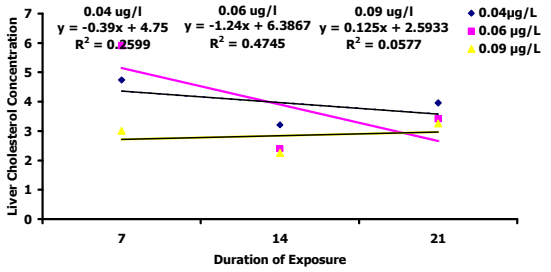

Figure 1: Percentage changes in liver cholesterol concentration of *Clarias gariepinus* exposed to sublethal concentrations of primextra for 21 days

The percentage decrease was the same on day 7 (7.2%) in all the treatment groups and thereafter, it fluctuated in the groups. The decrease was highest (43.7%) in the fish treated with 0.06 µg/l primextra on day 14. This was followed by 28% decrease observed in the fish exposed to 0.04µg/l on day 14. At the end of the study the percentage decrease in the kidney cholesterol was highest (28%) in the fish treated with 0.04µg/l and least (1.54%) in the group exposed to 0.09µg/l**.**

The observed decrease in the kidney cholesterol concentration in this study is in agreement with the report of Venkataramana *et al.* (2006) that cholesterol concentration was decreased in the heart muscles of gobiid fish *Glossogobius giuris* exposed to varying concentrations of malathion for 96h.The result of this study was consistent with the reports of Ghosh and Chatterjee (1989) and Piska *et al.* (1992), that agrochemicals induced decreased tissue cholesterol in fish. Similarly, Jyothi and Narayan (2001) reported decreased serum cholesterol level in the Indian catfish *Clarias batrachus* following exposure to pesticides carbaryl and phorate.

This trend according to Brycesmith and Waddson (1974) could be due to impaired pyruvate metabolism in the kidney, resulting in low production of acetyl-CoA. According to Kling *et al.* (1978) the observed decrease in the kidney cholesterol level could be due to inhibited conversion of acetate and mevalonate to cholesterol following the exposure to primextra. The regression functions (Table 4) showed that the percentage change was not concentration dependent in all the treatment groups. According to earlier reports (Kim *et al.,* 2004; Singh *et al.*, 2009) HMG-CoA reductase is a primary rate-determining enzyme in the tissue cholesterol biosynthesis whose inhibition would result in decreased cholesterol synthesis and *vice-versa*. Thus, it is likely that the reported decrease in the kidney cholesterol concentration may be due to inhibited HMG-CoA reductase activity in the organ. Thus, in conclusion, the induction of hypercholesterolemia in the liver and muscle and reduced renal cholesterol concentration could be part of the mechanism of primextra exerting its effect in the fish and could be of immense value in policy formulation regarding safe levels of the compound in the aquatic environment.

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