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Effects of Sublethal Doses of Fenvalerate (A Synthetic Pyrethroid) Administered Continuously for Four Weeks on the Blood, Liver, and Muscles of a Freshwater Fish, *Ctenopharyngodon idella*

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A new class of agricultural insecticides, the synthetic pyrethroids, have emerged as a complement to the organochlorines, organophosphates and carbamates and are more popular for their high toxicity to wide range of insects, including resistant strains (Elliott *et al.* 1978), low toxicity to mammals and birds (Parker *et al.* 1984) and rapid biodegradibility (Leahey 1979). Fenvalerate synthesized by Ohno *et al.* (1976) using 3-phenoxy benzaldehyde cyanohydrin and 2-(4-chlorophenyl)-3-methylbutyric acid, is one of the recent pyrethroids being used for cotton pest control and wide variety of other crops in Pakistan. Relatively short half life and rapid degradation (Ohkawa *et al.* 1978) have also made Fenvalerate a much more effective insecticide. Another aspect of Fenvalerate metabolism which is not found in other pyrethroids is a unique path of degradation, i.e., ether cleavage which retains the ester intact.

Bioaccumulation of xenobiotics including insecticides in aquatic species is increasing alarmingly, thus posing a threat to aquatic life. A variety of fish species show uptake and accumulation of many contaminants such as pesticides (Heger *et al.* 1995), polychlorinated biphenyls (Guiney and Peterson 1980), polycyclic aromatic hydrocarbons (Tuvikene 1995) and heavy metals (Rajan *et al.* 1995). Pesticides have been found to be highly toxic not only to fishes, but also to fish food organisms, thus threatening the life of fish (Grande *et al.* 1994).. It is important therefore, to evaluate the toxicity of newly marketed pesticides on fish, as they form an important part of human food.

The present report describes toxic effects of Fenvalerate on blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella* which has recently been introduced in Pakistan and has gained popularity for its effectiveness in weed control and high growth rate.

MATERIALS AND METHODS

Chinese grass carp, *Ctenopharyngodon idella,* obtained from Hatchery of Department of Fisheries, Punjab, at Manawan, Lahore, were maintained and acclimatized at 29±2 °C for 15 d in the fish ponds of

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A group of 20 fish, average size 7 cm, was exposed to a sublethal dose (5 µg/L of water) of a synthetic pyrethroid insecticide, Sumicidin 20 EC [= Fenvalerate; α-Cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methyl-butyrate] obtained from Granulars (Pak) Ltd., 1-Shadman, Lahore, every 3rd d, for 4 wk in aquaria with 40 L of water. Fish were transferred to fresh water every time new insecticide dose was administered. A control group of 20 fish was also maintained likewise but without treatment with insecticide. Four fish were taken out every week from both the control and treated tanks. The fish were stunned by gently striking their heads on the table and blood was collected from the caudal vein in the presence of EDTA (an anticoagulant). The fishes were immediately dissected after that and pieces of liver and pectoral muscles were frozen in liquid nitrogen and stored at -80°C for 24 hr for biochemical analysis.

The blood samples were used for the estimation of haemoglobin (Hb) content according to Van Kampen and Zijlstra (1961), packed cell volume (PCV) according to microhaematocrit method of Strumia *et al.* (1954), and red blood cell (RBC) and white blood cell (WBC) counts by routine clinical methods (Chanarin 1989). These values were then utilized for calculating erythrocyte indices, viz., the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Saline extracts (0.89%) of liver and muscles were prepared in teflon-glass homogenizer. For the estimation of glycogen and total proteins, 1 ml and 0.1 ml of the homogenate were pipetted out, respectively, while the remainder of the homogenate was centrifuged at 3500xg for 20 min at 5 °C and supernatant was used for the estimation of activities of alkaline phosphatases (AkP; orthophosphoric monoesterphosphohydrolase; EC 3.1.3.1) according to the method of Bessey *et al.* (1946), acid phosphatase (AcP; orthophosphoric monoesterphosphohydrolase; EC 3.1.3.2) according to Andersech and Szcypinski (1947), glutamate oxaloacetate transaminase (GOT; L-aspartate, 2-oxoglutarate aminotransferase; EC 2.6.1.1) and glutamate pyruvate transaminase (GPT; L-alanine 2-oxoglutarate aminotransferase; EC 2.6.1.2) according to Reitman and Frankel (1957), lactate dehydrogenase (LDH; EC 1.1.1.27) according to Cabaud and Wroblewski (1958) and creatine phosphokinase (CPK, adenosine triphosphate creatine N-phosphotransferase; EC 2.7.3.2) according to Hughes (1962), and concentrations of glucose according to O-toluidine method of Hartel *et al.* (1969), soluble and total proteins according to Lowry *et al.* (1951), and free amino acids according to Stein and Moore (1954). For total protein estimation the liver and muscle homogenates were disgested

with 4N KOH and then used for calorimetric reaction of Lowry *et al.* (1951). The glycogen content was extracted and estimated according to the method of Shibko *et al.* (1967). Another piece of liver and muscle were homogenized for 15 min in 2 ml absolute ethanol, incubated overnight at 37 °C and then centrifuged at 2000xg for 15 min at 5° C after mixing. Supernatant was used for cholesterol estimation according to Liebermann and Burchard reaction described by Henry and Henry (1974), while the pellet was used for the extraction and estimation of RNA and DNA contents according to Schmidt and Thannhauser described by Schneider (1957).

RESULTS AND DISCUSSION

Hb content and RBC count increased, whereas PCV decreased after Fenvalerate administration (Table 1). The MCV and MCH showed a continuous decrease and MCHC continuous increase during insecticide administration. The increased Hb content may be attributed to increased erythropoiesis and haemoglobin synthesis, which in turn justifies the increased MCHC. The decreasd MCV and MCH alongwith increased MCHC is indicative of hypochromic microcytic anemia. PCV decreased inspite of increase in the RBC count, which shows the magnitude of shrinking of cell size due to insecticide intoxication. The insecticidal stress causes electrolyte imbalance resulting in RBC exosmosis and decreased cellular size (Soivio and Oikari 1976).

The RBC/WBC ratio increased over a period of 4 wk after insecticide intoxication. This increase is obviously reminder of increased RBC count after insecticide treatment, as the WBC count remained unaffecfed during this treatment.

Table 1. Effect of Fenvalerate, administered at a dose of 5µg/L every 3rd d for a total period of 4 wk on the various haematological parameters of *Ctenopharyngodon idella*

Parameters[a]	Control (n=4)	Fenvalerate-treated			
		1 wk (n=4)	2 wk (n=4)	3 wk (n=4)	4 wk (n=4)
Hb (g/dL)	4.33±0.18[b]	5.45±0.15**	4.94±0.17*	4.92±0.13*	5.0±0.15*
PCV (%)	17.6±0.4	15.7±0.3**	15.2±0.1**	15.3±0.4**	15.7±3.9*
RBC (x10^6/µL)	1.16±0.08	1.64±0.06**	1.56±0.04**	1.51±0.06*	1.48±0.05*
WBC (x10^3/µL)	20.6±2.4	25.0±2.9	25.0±2.9	15.0±2.9	12.5±2.5
MCV (fL)	153±6	96±4.5***	97±3***	102±2***	106±1***
MCH (pg)	38.1±0.7	33.2±0.9**	31.7±1.6**	32.8±1.9*	28.5±0.2***
MCHC (%)	25.1±0.9	34.6±1.3***	32.5±1.3**	32.3±2.2*	26.9±0.4

[a]Abbreviations used; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell.

[b]Mean±SEM; Student's 't' test; *P<0.05, **P<0.001, ***P<0.0001.

Table 2 shows the effect of Fenvalerate on the hepatic enzymes and other biochemical components of liver. During the first wk of insecticide administration the hepatic AcP activity decreased 67%, while GPT activity increased 167%. The AkP, GOT and LDH activities did not show any significant change. At the end of 4 wk of treatment, the AcP, GOT and LDH activities showed distinct decrease (61%, 68% and 65%, respectively), whereas AkP activity increased 52%. The AkP, GOT and LDH activities were first significantly affected after 3 wk of insecticide exposure.

Table 2. Effect of Fenvalerate, administered at a dose of 5µg/L every 3rd d for a total period of 4 wk on the activities of enzymes and concentrations of some biochemical components of liver of ***Ctenopharyngodon idella.***

Parameters[a]	Control (n=4)	Fenvalerate-treated 1 wk (n=4)	2 wk (n=4)	3 wk (n=4)	4 wk (n=4)
AcP (IU)[b]	1.34±0.12[c]	0.44±0.06***	0.37±0.05***	0.95±0.06*	0.58±0.06**
AkP (IU)	1.10±0.10	0.94±0.05	1.04±0.14	1.59±0.16*	1.67±0.03**
GOT (IU)	4.63±0.32	3.94±0.31	4.61±0.65	2.77±0.66**	1.46±0.08***
GPT (IU)	0.94±0.12	2.51±0.29**	1.49±0.09*	1.32±0.12	0.65±0.10
LDH (IU)	71.0±6.9	86.6±9.0	59.2±1.9	40.7±3.9**	25.0±4.5***
Glucose (mg)	15.1±0.7	19.6±0.9**	18.5±0.9*	20.3±0.6***	20.9±1**
Glycogen (mg)	62.2±5.8	57.0±1.9*	36.8±4.6*	39.9±2.9*	39.7±2.1*
Total proteins (mg)	217±16	184±13	164±7*	159±9*	138±28*
Sol. proteins (mg)	100±11	68±9	88±8	73±3	63±10*
FAA (mg)	0.59±0.07	0.79±0.06	0.93±0.12*	1.38±0.15**	18.55±0.07***
Cholesterol (mg)	8.13±0.54	6.10±0.52*	5.98±0.26*	2.83±0.31***	4.24±0.74**
DNA (mg)	1.73±0.13	1.44±0.09	1.55±0.11	1.32±0.10*	1.48±0.10
RNA (mg)	7.30±0.64	7.79±0.33	5.81±0.60	5.04±0.38*	4.38±0.23**

[a]Abbreviations used: AcP, acid phosphatase; AkP, alkaline phosphatase; DNA, deoxyribonucleic acid; FAA, free amino acids; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; IU, international units, the amount of enzyme, that under defined assay conditions, will catalyze the conversion of 1 µmol of substrate per min; LDH, lactate dehydrogenase; RNA, ribonucleic acid.
[b]All enzymatic values and other biochemical componepts are in terms of IU or mg/g wet wt of liver.
[c]Mean±SEM; Student's 't' test; *P < 0.05, P<0.0l, * *P<0.001.

Decreased activity of AcP could be due to inhibition of enzyme (Roux *et al.* 1976). Parker *et al.* (1984) reported increase in hepatic AkP activity in dog exposed to Fenvalerate, which may be due to toxic effect on the liver function. GOT inhibition shows that oxaloacetate and glutamate are not available to Kreb's cycle through this route of transamination but through GPT, which accounts for its increased activity.

Almost all the metabolites studied decreased significantly after insecticide treatment (Table 2). At the end of first week of treatment the glycogen and cholesterol contents decreased 8% and 25%, respectively, but at the end of 4 weeks of insecticide administration the glycogen, total proteins, soluble proteins, FAA, cholesterol, and RNA decreased (36%, 37%, 38%, 163%, 48%, and 40%, respectively). Glucose was the only metabolite which increased throughout the experimental

period, whereas the first significant change in total proteins, soluble proteins, FAA and RNA occurred 2, 4, 2 and 3 weeks, respectively, after insecticide exposure (Table 2).

The enhanced hepatic glucose content and decreased glycogen content after insecticide treatment suggest enhanced glycogenolysis and inhibited glycolytic pathway. Kacew and Singhal (1973) have also correlated hyperglycemia and depletion of glycogen with gluconeogenesis. The decreased hepatic LDH activity which could be due to enzyme inhibition, entails gearing up of Kreb's cycle and/or diversion towards gluconeogenesis, leading to hyperglycemic condition. A decrease in LDH activity has also been reported by Dragomirescu *et al.* (1979) in the liver of *Cyprinus carpio* exposed to organophosphorus pesticide.

In the absence of carbohydrate based energy source, unlike mammals the fish can utilize proteins directly even under normal nutritional conditions. Decrease in hepatic proteins, lipid and glycogen contents is also reported by Murty and Priyamvada-Devi (1982) in *Channa punctatus* treated with endosulfan, and in muscle and liver glycogen by Gluth and Hanke (1985) in *Cyprinus carpio,* after treatment with different insecticides. Sastry and Siddiqui (1984) reported increase in the glycogen content of liver and muscle of *Channa punctutus* after exposure to quinalphos. Contrary to that and in agreement with the present results Koundinya and Ramamurthi (1979) observed hyperglycemia accompanied by decrease in the glycogen content of liver and muscle of *Sarotherodon mossambica* after treatment with fenitrothion.

Lipid oxidation supplies the major part of energy requirement during slow swimming of fish (Bilinski and Beis 1975). Cholesterol decrease may be due to utilization of fatty deposits, instead of glucose, for energy purposes.

Table 3 shows the effect of Fenvalerate on the various biochemical components of fish muscle. The muscle LDH activity shows a very singnificant decrease throughout the experimental period (58% after 1 wk and 86% after 4 wk), whereas CPK activity is not changed significantly. The total and soluble proteins and FAA content increased 19%, 81%, and 157%, respectively during the first wk of insecticide administration. At the end of 4 wk, however, the total proteins decreased (25%), whereas soluble proteins and FAA contents maintained their increasing trend (178% and 110%, respectively). The glycogen content, on the other hand, decreased throughout the experimental period, while RNA increased first after 3 wk of insecticide exposure.

The decreased muscle LDH activity may be due to its inhibition, or

decreased synthesis. LDH activity is decreased due to tissue damage (Kristoffersson *et al.* 1974). The fish use proteins direclty for muscle energy under normal conditions. So the decrease in total proteins content may be due to its enhanced direct use for muscle energy. High concentration of FAA may be due to degradation of proteins and tissue breakdown as well or due to inibition of protein synthesis.

Table 3. Effect of Fenvalerate, administered at a dose of 5µg/L every 3rd d for a total period of 4 wk on various enzymatic activities and some biochemical components of muscles of ***Ctenopharyngodon idella***

Parameters[a]	Control (n=4)	Fenvalerate-treated 1 wk (n=4)	2 wk (n=4)	3 wk (n=4)	4 wk (n=4)
CPK ($X10^3$SU)[b]	19.3±1.5[c]	16.6±1.7	26.0±2.4	20.2±0.8	25.2±2.1
LDH ($X10^4$IU)	83.5±5.6	35.3±2.7***	24.1±2.1***	24.1±2.4***	12.0±0.9***
Total proteins (mg)	160±9	190±8*	144±4	186±12	120±4**
Sol. proteins (mg)	19.7±1.6	35.5±2.1***	45.5±4.3***	54.8±3.8***	54.6±2.2***
FAA (mg)	0.21±0.02	0.54±0.04***	0.48±0.04***	0.53±0.13***	0.44±0.03**
Glycogen (mg)	32.9±2.9	12.3±1.0***	10.7±1.3***	13.5±3.4***	13.0±2.4**
DNA (mg)	1.38±0.16	1.28±0.17	1.15±0.13	1.30±0.17	1.44±0.13
RNA (mg)	2.88±0.34	3.44±0.26	3.88±0.55	4.85±0.33**	5.88±0.37***

[a]Abbreviations used: CPK, creatine phosphokinase; SU, Sigma unit, one Sigma unit of enzyme will phospholylate 1 nmole of creatine per min at 25°C, pH 7.5, under the conditions of the test. For other abbreviations, see Table 2.
[b]All enzymatic values and other biochemical components are in terms of IU or mg/g wet wt of muscle.
[c]Mean±SEM; students 't' test; *P < 0.05, **P< 0.01, ***P<0.001.

Decrease in muscle glycogen content may account for enhanced muscular activity to meet high energy demand under stress given by fenvalerate treatment (Black *et al.* 1962). It is well known that fish under stress secrete high amounts of catecholamine which deplete glycogen reserves (Pickering 1981). Studies on fish show that epinephrine induction reduces hepatic glycogen by increasing glycogen phosphorylase (Umminger and Benziger 1975). It is known from a general physiological view that muscle glycogen content depends on both the blood glucose concentration and the capacity for glycogen synthesis. This is somewhat different from liver glycogen concentration, which mainly depends on the synthetic capacity.

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