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Effect of Parathion on Hematological Parameters in the Serum of a Male Bagrid Catfish (*Pseudobagrus fulvidraco*)

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To evaluate the impact of parathion on aquatic organisms, a freshwater bagrid catfish (*Pseudobagrus fulvidraco*) was exposed to sublethal concentrations (63, 95, 190 and 380 μg/L) of parathion, organophosphorus pesticide for 30 days. Glucose level in the serum of the bagrid catfish was significantly increased than that of control groups in the 190 μg/L concentration at 30 days and in the 380 μg/L concentration after 10 days. Bilirubin level was significantly increased in the 190 μg/L concentration at the end of the experiment. After 10 days, a significant differences of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity increased in the ≥190 μg/L and 380 μg/L groups. Though cholesterol concentration was stable, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were significantly reduced in the 190 μg/L at 10 days and in the 380 μg/L concentration at the end of the experiment. In conclusion, if *P. fulvidraco* was chronically exposed to ≥190 μg/L concentration of parathion, the hematological changes may be induced due to the stress response.

Key words: *Pseudobagrus fulvidraco*, Parathion, Hematological parameter, Chronic exposure

Introduction

Parathion (*O,O*-diethyl-*O*-(4-nitrophenyl) phosphorothionate) is an insecticide whose main mode of action is the inhibition of acetylcholinesterase (AChE) (Gallo and Lawryk, 1991). Parathion is one of the most acutely toxic pesticides registered by the EPA. Because of its highly toxic nature, parathion is classified as a Restricted Use Pesticide (Extonet, 1993).

Extensive use of parathion has caused high mortality of farmed shrimps in the China (Li, 1999). Also, concentrations of parathion, regularly and substantially exceed the maximum permissible concentrations in the Netherlands and are, therefore indicated as 'priority pollutants' (MTPWWM, 1998). Nevertheless, it is still commonly used in some regions of South Korea to control numerous insect pests (Lee, 2000).

Toxic effects of parathion have also been investigated *in vivo* in mammals (Butler and Murray, 1997; Rojas et al., 1998) and insects (Van den Beukel et al., 1998), and in vitro in mammalian (Veronesi and Ehrich, 1993; Carlson and Ehrich, 1999) and fish (Guobaitis et al., 1986).

Although there are a number of studies on various biochmical, hematological and cellular changes due to the effect of OPs on fish (Areechon and Plumb, 1990; Asztalos and Nemcsok, 1985; Boone and Chambers, 1997; Sancho et al., 1998), the reports on the effect of parathion on hematological parameters are few, and moreover the works on the bagrid catfish (*Pseudobagrus fulvidraco*) are none. The bagrid catfish living primarily in China, Taiwan, Korea and Siberia is commercially important in South Korea (Kim and Park, 2002). Blood parameters are commonly used as indicators of the physiological or sublethal stress response to endogenous or exogenous changes in fish (Beyer et al., 1996).

The present work evaluated possible changes of hematological parameters in the serum of *P. fulvidraco* exposed to different concentrations of parathion.

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Materials and Methods

Male bagrid catfish (*P. fulvidraco*) were obtained from the Inland Fisheries Research Institute, South Korea.

Healthy fish (body weight, 57.08±5.42 g; body length, 15.53±2.05 cm) were acclimated to the laboratory condition. Temperature was kept at 23±1℃ for two weeks. Fish were fed twice a day with commercial fish feed containing 45% protein, 7.0% lipid, and 20% carbohydrate (Purina Ltd., Korea). Test chamber (glass aquaria, 300 L capacity) were filled with 20 L of freshwater. All tests were conducted with static methods in which test solutions were totally renewed at 24 hr intervals.

Physicochemical characteristics of the experiment water measured by the method described in APHA (1998) were as follows: pH was 8.2±0.4, temperature 23±1℃, dissolved oxygen, 7.4 mg/L, nitrite-nitrogen 0.01 mg/L, nitrate-nitrogen 2.10 mg/L, and ammonia-nitrogen 0.3 mg/L, phosphorus 0.2 mg/L, chemical oxygen demand (COD) 2.04±0.33 mg/L, hardness 73 mg/L as $CaCO_3$ and parathion not detectable.

Parathion-ethyl, 99.9% pure was purchased from Supelco (USA). This stock solution was appropriately diluted with the test water to achieve the desired concentrations of parathion. The 96 hr LC_{50} value (1.901 mg/L) for the bagrid catfish was studied in our preliminary examination.

Approximately, one-fifth (380 μg/L), one-tenth (190 μg/L), one-twentieth (95 μg/L) and one-thirth (63 μg/L) of the LC_{50} were taken as sublethal concentrations in which the fish can survive for 30 days.

At the end of each period, that is, 0, 10, 20 and 30 days, fish were anesthetized with MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate, Sigma). Blood was collected by caudal puncture. Immediately after blood sampling, it was transferred from the syringe to a siliconecoated tube. The needle was removed from the syringe before blood was transferred to minimize hemolysis. Blood was allowed to clot for 3-4 hr at room temperature (25℃) before centrifugation at 3,600 rpm for 30 min (Zentrifugen 201424, Hettich, Germany).

Serum was frozen (-80℃) within 5 hr of blood collection. Serum glucose (No. 315-100), bilirubin (No. 550-A) and cholesterol (No. 401-100P) were determined on serum using Sigma Diagnostic Kit. And AST and ALT (No. AM 101-K), LDH (No. AM 159-1-4) and ALP (No. 720-051) assays were performed using specific kits and reagents (Asan Pharm. Co. Ltd.). All analysis of serum samples were performed by a spectrophotometer (Hewlett Packard 8454, Germany). Statistical analysis was performed using SPSS/PC statistical package. Significant differences between groups were determined using one-way ANOVAs and Duncan's test for multiple comparisons or the Student's t-test for two groups (Duncan, 1955). Significance level was established at $P<0.05$.

Results

The mean serum glucose content in the control fish ranged between 69.41±3.48 and 75.70±5.58 μg/dL during the experiment. The level of serum glucose significantly increased in 190 μg/L concentration of parathion at 30 days, while it was significantly increased in 380 μg/L concentration after 10 days ($P<0.05$) (Table 1).

The serum bilirubin values in the control fish ranged between 0.46±0.05 and 0.61±0.08 μg/dL. The fish showed significant hyperbilirubinaemia throughout duration of the experiment to 190 and 380 μg/L concentration ($P<0.05$) (Table 1).

In the present study, serum cholesterol levels in the control fish varied between 127.78±6.48 and 134.62±7.20 mg/dL.

The concentration of cholesterol in the serum did not change significantly in all exposure concentrations and times (Table 1).

AST and ALT activities significantly decreased following 190 μg/L concentrations of parathion at 10 days and 380 μg/L after 10 days ($P<0.05$) (Table 1).

The LDH activity increased significantly in 10 days exposure to 190 and 380 μg/L concentration of the pesticide ($P<0.05$), but there was no change at the other exposure concentrations and periods (Table 1).

The ALP activity was initially higher in fish exposed to the highest concentration of parathion than the control or the fish exposed to the lower concentration of the pesticide. However, there was no significant change in the activity of this enzyme after 20 days exposure to parathion (Table 1).

Discussion

Hyperglycaemia can be viewed as a physiological response of the fish to meet the critical needs for energy under toxic stress and observed in fish exposed to a toxicant for long periods of time.

The results of the present study have demonstrated

Table 1. Changes of hematological parameters in bagrid catfish (*Pseudobagrus fulvidraco*) exposed to various concentrations of parathion for 30 days

Parameters	Days	Parathion concentrations (μg/L)				
		0	63	95	190	380
Glucose (mg/dL)	0			70.55±12.11		
	10	74.81±13.51	71.36±18.49	69.34± 2.77	80.63±3.78	121.48±10.58*
	20	69.41± 3.48	77.03± 9.87	71.39±21.86	76.84±9.27	110.50±14.26*
	30	75.70± 5.58	79.59±11.01	72.25±11.09	86.72±6.47*	82.35±13.53
Bilirubin (mg/dL)	0			0.52±0.09		
	10	0.46±0.05	0.52±0.09	0.53±0.13	0.77±0.10*	0.94±0.10*
	20	0.57±0.07	0.50±0.09	0.49±0.06	1.25±0.06*	1.04±0.07*
	30	0.61±0.08	0.60±0.12	0.56±0.17	0.77±0.14*	0.89±0.13*
Cholesterol (mg/dL)	0			130.3±3.6		
	10	132.8±4.6	127.3±6.6	135.3±8.3	126.5±3.9	131.7±7.3
	20	134.6±7.2	133.0±4.4	133.3±4.3	139.5±3.1	132.8±6.5
	30	127.8±6.5	128.9±4.2	130.6±5.0	131.1±6.0	130.0±2.2
AST	0			9.51±0.34		
	10	9.42±0.47	9.35±0.38	9.51±0.62	8.10±0.27*	8.14±0.41*
	20	9.44±0.30	9.17±0.14	9.43±0.30	8.89±0.55	7.38±0.79*
	30	9.62±0.43	9.52±0.48	9.38±0.80	9.04±0.89	8.53±0.53*
ALT	0			5.56±0.19		
	10	5.41±0.26	5.36±0.24	5.57±0.57	4.72±0.16*	4.40±0.40*
	20	5.35±0.26	5.28±0.10	5.52±0.19	5.42±0.29	4.80±0.29*
	30	5.46±0.04	5.40±0.24	5.33±0.24	5.36±0.37	4.71±0.20*
LDH	0			596.5±155.8		
	10	618.0±108.4	608.2±132.7	614.0±138.2	795.4±150.0*	787.1±144.6*
	20	591.2±134.5	622.2±111.5	587.4±157.3	626.8±107.1	662.2±189.8
	30	626.0±147.4	588.1±144.0	633.1±123.8	630.2±131.2	605.0±117.7
ALP	0			5.02±0.84		
	10	4.50±0.72	4.88±0.60	4.37±0.63	4.50±1.10	10.66±1.69*
	20	5.21±0.93	5.38±1.27	5.81±0.79	6.26±1.63	5.36±1.68
	30	5.45±0.58	5.64±1.61	5.90±1.63	5.57±2.27	6.10±1.66

AST (aspartate aminotransferase) and ALT (alanine aminotransferase) activities is expressed as Karmen Unit and LDH (lactate dehydrogenase) and ALP (alkaline phosphatase) activities is expressed as Wróblewski Unit and King-Armstrong Unit respectively. *Asterisk indicates significant difference ($p<0.05$) using a Duncan's multiple range test.

that exposure of fish to 380 μg/L of parathion concentration was evoked significant increase in glucose level after 10 days in serum. At 30 days, the level of serum glucose significantly increased in 190 μg/L concentration.

Depletion of glycogen content in liver and muscle of fish during toxic stress by pesticides was reported by several investigators. Oruç and Üner (1999) suggested that depletion of glycogen may be due to utilization of carbohydrates for energy production as a result of pesticide-induced hypoxia. This indicates pesticide-induced glycogenolysis and glycolysis in the tissue and also suggests that the fish is under stress and needs more energy.

Verma et al. (1983) observed an increase in blood glucose level and a decrease in muscle and liver glycogen levels after 30 days of pesticide exposure of fish, *Clarias batrachus*, *Saccobranchus fossilis*, and *Mystus vittatus*. Lal et al. (1986) reported that 4 and 8 days of exposure of *Heteropneustes fossilis* to malathion significantly increased the level of plasma glucose with a concomitant reduction in the level of glycogen in the muscle and liver.

These results therefore support that parathion ex-

posure could affect the carbohydrate metabolism in *P. fulvidraco*.

Hyperbilirubinaemia suggests liver damage or obstruction of the bile ducts (Leroy, 1993). *P. fulvidraco* showed significant hyperbilirubinaemia to 190 and 380 μg/L concentration throughout duration of the experiment. In support of the present results, Jayantha Rao et al. (1984) observed a rise in serum bilirubin level in *Tilapia mossambica* treated with phosphamidon. Similarly, Casillas et al. (1985) found significant increases in serum bilirubin of *Parophrys vetulus* exposed to PAHs. They ascribed elevated levels of bilirubin level to liver damage and alterations in liver function.

Hypercholesterolic effects have also been reported in the various teleosts during pesticide stress. Haux and Larsson (1984) observed hypercholesterolemia in serum of *Oncorhynchus mykiss* exposed to aldicarb.

Singh and Singh (1980) observed a rise in cholesterol level in the serum of freshwater teleosts treated with malathion. Reversely, Gluth and Hanke (1984) reported decreased plasma cholesterol levels in common carp exposed to lindane. Although some studies indicate a change in cholesterol level during aquatic pollutant exposure, no significant changes were observed in the serum of parathion-treated *P. fulvidraco* in the established exposure concentrations and times in our experiment. Transaminase activities may be used as a sensitive marker in experimental insecticide intoxication in teleosts (Sadhu et al., 1985).

A increase in both AST and ALT activity was observed in experiments in which fish were acutely exposed to domesttic waste water, while after prolonged exposure transaminase activities in blood dropped to the control level (Bucher and Hofer, 1990). These activities in *P. fulvidraco* significantly decreased following 190 μg/L concentrations of parathion at 10 days and 380 μg/L after 10 days. Oruç and Üner (1998) have reported elevation in serum AST and ALT activity of common carp which have hepatic cellular damage caused by azinphosmethyl. Conversely, Sadhu et al. (1985) have showed reduction in serum AST and ALT activities of Channa striatus following exposure to 0.1 ppm malathion for 10 days. Results for *P. fulvidraco* showed significant reduction to $\geq$190 μg/L concentration of parathion at 10 days, indicating physiological stress.

Generally serum LDH level increases in fish exposed to pesticides, carbon tetrachloride, and metals and Like ALT and AST, the source of this enzyme in the serum is release from necrotic tissue, liver, heart, and skeletal muscle (Leroy et al., 1993). Asztalos et al. (1990) also observed to increase when common carp was exposed with methidathion and Thiram® but to decrease in paraquat. Asztalos and Nemcsok (1985) also found an increase in the serum LDH activity in common carp following exposure to methidation and paraquat in combination. Similar increases in the enzyme activity were also observed by Natarajan (1984) in freshwater fish, *Channa striatus*, exposed to metasystox for 30 days. In the present tests with *P. fulvidraco*, the LDH activity increased significantly at 10 days exposure to 190 and 380 μg/L concentration of the pesticide, indicate cell damage in target tissue. ALP is mainly localized at the cell membrane. Any damage in hepatic cells may result in alteration in ALP activity. The ALP activity was initially higher in *P. fulvidraco* exposed to 380 μg/L concentration of parathion than the control or the lower concentration of the pesticide at 10 days. However, there was no significant change in the activity of this enzyme after 20 days exposure to parathion. Gupta and Dhillon (1983) studied the effects of sublethal concentrations of aldrin and Swascofix CD-38 in the serum of *Clarias batrachus* and *Cirrhina mrigala*, suggesting liver damage which might have induced production of mitochondrial enzymes such as ALP, which are subsequently released into blood. This observation was also recorded by Ram and Singh (1988), who studied the effects of carbofuran on *Channa punctatus*. Goel et al. (1984) have also observed elevated activities of ALP in Clarias batrachus exposed to Alachlor and suggested tissue damage. Hence the increased levels of ALP in the present investigation, evident with 10 days exposure to 380 μg/L of parathion may be due to cell necrosis in liver of *P. fulvidraco*.

In conclusion, if *P. fulvidraco* was chronically exposed to $\geq$190 μg/L concentration of parathion, the hematological changes may induce the stress response.

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