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In Vivo Toxicity of Dimethoate on Proteins and Transaminases in the Liver Tissue of Fresh Water Fish *Clarias batrachus* (Linn)

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Environmental pollution due to extensive usage of the pesticides to enhance farm productivity and inexpensive control of vector-borne diseases without proper management has far reaching effect on the survival potential of aquatic animals. Some of these toxic chemicals may persist in the environment for long periods, often unchanged. These chemicals, apart from affecting the target pests also affects the nontarget organisms present in that media. Fish form one of the important nontarget organisms in any aquatic systems, and are one of the major sources of cheap protein for human beings in India.

Results of controlled laboratory exposures of fishes to pesticides and related chemicals have revealed that liver is often the organ with highest pesticides concentration (Duke and Wilson 1971). Although more than 900 commercial pesticides are in general use, fewer than 30 have been examined for their adverse effects on fish liver (Pimentel 1971; Gupta 1986). Therefore in the present investigation, the effect of commercial Dimethoate EC 30 % (O, O-dimethyl S - (N - methylcarbamoylmethyl) Phosphorodithioate), and organophosphorus (OP) insecticide on the liver of Clarias batrachus (Linn) was studied.

MATERIALS AND METHODS

C. batrachus in the weight range of 35 - 40 g and length 20 - 22 cm were collected locally and acclimatized to laboratory conditions for 2 wk, fed with commercial balanced fish feed and starved for 1 d prior to the day of experimentation. The physicochemical characteristics of the laboratory water were in the following range: pH 7.3 - 7.5; dissolved oxygen 7.5 - 8 mg/L; temperature 27°C - 29°C; hardness 98 - 100 mg/L as $CaCO_3$; and alkalinity 85 - 90 mg/L as $CaCO_3$. The dimethoate EC 30% was obtained from the local market (Rallis India Ltd., Bombay, India). The other 70% were the solvents and emulsifiers. The control fish were maintained with 0.1 mL/L alcohol as it was used for dissolving dimethoate. To determine the LC_{50} value, ten fish per 10 L of water were exposed to six serial concentrations of dimethoate. The bioassay experiment of each concentration was repeated six times

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with parallel controls, and mortality was noted in each concentration at the end of 96 hr. The LC_{50} value for 96 hr was determined by the method of Finney (1964). The LC_{50} value was found to be 65 μg/mL. All the chemicals and reagents used were Analar grade (BDH, Bombay, India).

The fish were divided into two groups of 24 each. Group one without toxicant served as control and group two were exposed to sublethal concentration of dimethoate (1/3 of LC_{50}, i.e., 21.66 μg/mL) for a period of 8 d served as experimental. The toxicant water and control water were renewed every 24 hr after feeding. Six fish each from experimental and control groups were killed by severe blow on the head on 1st, 2nd, 4th and 8th day. Liver was isolated and used to investigate total proteins, soluble and structural proteins and transaminases, aspartate aminotransferase and alanine aminotransferase. Total, soluble and structural proteins were estimated by the method of Lowry et al (1951) and transaminases by the method of Rietman and Frankel as described by Bergmeyer (1965). The procedure of soluble and structural proteins is as follows :1% liver homogenate was prepared in 0.25 M ice-cold sucrose solution and centrifuged at 3000 X g for 15 min. The residue was set aside for estimation of structural protein. To the supernatant an equal volume of 10% trichloroacetic acid (TCA) was added to precipitate the soluble proteins, and centrifuged at 1000 X g for 10 min. The supernatant was discarded and the residue was dissolved in known volume of 0.1N NaOH. To 1 mL of NaOH aliquot 4 mL of reagent containing copper sulfate, sodium carbonate, sodium potassium tartarate and sodium hydroxide was added. The tubes were kept at room temperature for 10 min. Then 0.4 mL of diluted Folin-phenol reagent was added and color developed was read at 620 nm in spectrophotometer (Carlzeiss Jena) aganist a reagent blank. The residue of sucrose homogenate was dissolved in known volume of 0.1 N NaOH. I mL of this solution was processed in a similar way as was done for soluble proteins. Bovine serum albumin was used to construct the standard curve.

For the estimation of transaminases 5% liver homogenate was prepared in 0.25 M ice-cold sucrose solution in an ice-jacketed homogenizer with a motor-driven pestle. The homogenate was centrifuged in cold centrifuge machine at 3000 X g for 20 min to remove nuclei and cell debris. A clear cell-free extract was used for the estimation of transaminases. For aspartate aminotransferase (EC 2.6.1.1) the reaction mixture contained in 1 mL volume: 100 μmoles of L-aspartic acid (pH 7.4), 2 μmoles of L-ketoglutaric acid (pH 7.4), 100 μmoles of phosphate buffer (pH 7.4), 0.2 mL of freshly prepared homogenate. The reaction mixture was incubated for 1 hr at 37°C. The reaction was stopped by the addition of 1 mL of 2,4-dinitrophenylhydrazine and allowed to stand at room temperature for 20 min. The color was extracted by the addition of NaOH (0.4 N) and read at 546 nm in spectrophotometer against a reagent blank.

The incubation mixture for alanine aminotransferase (EC 2.6.1.2) contained in 1 mL of volume: 100 μmoles of DL-alanine (pH 7.4), 2 μmoles of L-ketoglutaric

acid (pH 7.4), 100 µmoles of phosphate buffer (pH 7.4), 0.2 mL of homogenate. The mixture was incubated for 30 min. The remaining procedure followed as mentioned for aspartate aminotransferase. Data are presented as mean of six samples ± standard error. Student's 't' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

The results obtained in the present investigation are summarized in Table 1. Depletion in total proteins, soluble and structural protein content was observed throughout the exposure periods. Depletion was maximum on 2nd day of exposure; thereafter a slight decrease in depletion was observed. The transaminases activity levels were enhanced throughout the investigation. The enhancement was maximum on 4th day followed by slight decrease in enhancement.

The effect of toxicants on the vertebrate liver metabolism is diverse. The liver, which is the main detoxifying tissue, reacts primarily to the stress of the toxicant. Tissue protein content depends on the dynamic equilibrium between the rates of its synthesis and degradation.

Casida et al (1983) reported that there is an increased evidence of pesticide protein interaction which is relevant to the mode of action of insecticide. The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes as reported by Ravinder et al (1988). The depletion in total proteins observed during present investigation might also be due to the inhibition of nucleic acid synthesis, which in turn suppressed protein synthesis. Rate of protein synthesis depends on RNA content and RNA/DNA ratio of the tissue. Rath and Misra (1980) reported decrease in DNA, RNA and protein content in Tilapia mossambica exposed to sublethal concentration of dichlorvos. Devi (1981) reported that the reduction in total protein may be related to the action of chemical on nucleic acids. These findings support the results of present study.

The depletion in soluble and structural proteins was observed in liver. A similar decrease in soluble and structural proteins in branchial tissue of Cyprinus carpio exposed to fenvalerate was observed by Malla Reddy and Bashamohideen (1988). Decrease in sucrose soluble and insoluble proteins in Tilapia mossambica exposed to benthiocarp was observed by Seshagiri et al (1987). They suggested possible degradation by increased proteolysis. The maximum depletion in structural protein might be due to the increased neutral and alkaline proteases, which act preferentially on the structural proteins of the tissue (Kabeer Ahmed 1979). Proteases were not estimated in the present study but the results of trichlorfon, an OP pesticide, showed decreased protein content with increased proteases (Joyce et al 1991). The decrease in protein content of dimethoate intoxicated fish also indicated the physiological adaptability of the fish to compensate for pesticide

stress. To overcome the stress the animals require high energy. This energy demand might have led to the stimulation of protein catabolism.

Table 1. Levels of total, soluble and structural proteins. (mg/gm wet wt) aspartate and alanine aminotransferase activity levels (μmoles of pyruvate formed/mg protein/ hr) in liver of Control and Experimental fish.

Parameters		Exposure periods (d)			
		1	2	4	8
Total Proteins	Control, X	77.30	77.90	78.38	78.82
	SE	±1.05	±1.91	±1.10	±2.03
	Experimental, X	67.58***	55.06***	61.38***	70.63*
	SE	±1.23	±1.30	±0.93	±2.33
	% variance	(-12.57)	(-29.32)	(-21.70)	(-10.40)
Soluble Proteins	Control	55.13	55.40	55.00	55.59
	SE	±1.09	±0.64	±0.64	±0.47
	Experimental	40.52***	35.03***	39.17***	42.20***
	SE	±2.25	±0.23	±0.73	±2.08
	% variance	(-26.51)	(-36.76)	(-28.78)	(-24.07)
Structural Proteins	Control	34.93	34.95	34.03	34.40
	SE	±0.68	±0.35	±0.25	±1.01
	Experimental	27.37***	21.95***	23.34***	29.71^{NS}
	SE	±1.25	±1.10	±0.40	±2.25
	% variance	(-21.66)	(-37.19)	(-31.42)	(-13.62)
Aspartate amino-transferase	Control	0.66	0.67	0.64	0.70
	SE	±0.04	±0.01	±0.02	±0.03
	Experimental	0.83***	1.00***	1.08***	0.91***
	SE	±0.02	±0.01	±0.06	±0.01
	% variance	(+26.49)	(+48.96)	(+70.28)	(+30.33)
Alanine amino-transferase	Control	1.84	1.90	1.86	1.83
	SE	±0.03	±0.06	±0.02	±0.04
	Experimental	2.28***	2.88***	2.98**	2.35**
	SE	±0.03	±0.13	±0.20	±0.09
	% variance	(+24.22)	(+51.77)	(+57.27)	(+28.15)

Values are mean ± SE of six observations. Values in parenthesis are % decrease (-) or increase (+) over control, significant at $p<0.05$*, $p<0.01$**, $P<0.001$***, NS not significant.

The free amino acid content of the liver tissue decreased after dimethoate treatment in C. batrachus (Ghousia 1993). It is likely that amino acids might have been converted to ketoacid by transaminases which in turn fed into TCA cycle. Hence there was an increase in the activity of transaminases. Similar increase in aspartate and alanine aminotransferase activity was observed in exposed fish (Bakthavatsalam et al 1982). The elevation in transaminases suggests the existence of heavy drain on metabolites during dimethoate stress, since stress is known to induce elevation of aminotransferases (Kulkarni and Mehrotra 1973).

The liver has vital physiological role to perform under any stress condition. Firstly, the toxicant in the system should be metabolized, degraded, and eliminated from the organism. Secondly, there is a necessity for increased energy production for the physical activities manifested under stress effect. The processes of glycogenolysis and glyconeogenesis are utilized. In the latter process, amino acids form the precursors in fish liver (Premakumari 1988). Therefore the role of liver is important in this process. The liver tissue possesses enzymatic machinery to carry out the energy production and detoxification. The data obtained in this investigation clearly shows that liver plays a major role in the physiological reorganization under the pesticide impact.

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