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Effect of Aldrin on Carbohydrate, Protein, and Ionic Metabolism of a Freshwater Catfish, *Heteropneustes fossilis*

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Organochlorine (OC) pesticides are potential environmental contaminants which create major health hazard in aquatic system. The nervous system is primary target of OC pesticides but many metabolic processes are also influenced. The consensus is that intoxication deranges intermediary metabolism primary to ATP production resulting in depletion of energy sources (Gill et al. 1991). Moreover, the divalent cations, calcium and magnesium, play a vital role in several body functions-neuromuscular excitability, enzymatic reactions, and retention of membrane permeability. The inorganic phosphate acts as a major cytoplasmic buffer and is the basis of energy exchange (Aurbach et al. 1985). The liver is a site of metabolic detoxification (Saxena et al. 1989) whereas the gills (Verbost et al. 1987) and the kidney (Rashatwar and Ilyas 1984) are involved in regulatory mechanisms. This study was undertaken to observe the effect of sublethal concentration (0.085 mg/L) of commonly used organochlorine pesticide aldrin (1,2,3,4,10,10 -hexachloro -1,4,4a,5,8,8a, hexahydro- 1,4,5,8, dimethanonaphthalene), a member of toxaphene group of insecticides, on blood glucose, plasma calcium, magnesium, and inorganic phosphate concentrations alongwith the liver and muscle glycogen and protein content of freshwater catfish, Heteropneustes fossilis. Behavior of the fish was also observed.

MATERIALS AND METHODS

Live specimens of freshwater catfish, Heteropneustes fossilis (wt 31.26 ± 6.35 g; length 14.2 ± 3.4 cm), collected locally from a large pond were brought to laboratory and acclimated in municipal tap water for 15 d during the month of September under natural

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photoperiod (l2.38-11.58 light hours) and ambient temperature (24.4 ± 1.8°C) in 50 L glass aquaria. Other physicochemical characteristics of water used were : pH 7.60 ± 0.02; electrical conductivity 278.34 ± 18.59 u mho / cm; hardness 115.60 ± 2.21 mg / L (as Calcium carbonate) ; dissolved oxygen content 6.80 _± 0.12 mg / L . Fish were fed daily with flour pellets and dried ground shrimp; aquaria were cleaned and water was changed daily. Only healthy fish of both sexes were used in the experiments.

A static acute toxicity bioassay was performed according to Standard Methods (APHA et al. 1975 1 to determine the 96 hr LC 50 value of aldrin (Sandoz India Ltd.,EC 30 %) . Stock solution (1 mg / mL) of active ingradient prepared by dissolving 0.05 mL of aldrin in 19 mL of absolute alcohol, was added to water to yield desired concentrations of the insecticide. Parallel groups of absolute alcohol control fish were also maintained under same conditions. The 96 hr LC 50 value was 0.17 mg / L . For the study of the effect of the insecticide on specified biochemical parameters, three separate groups of 36 fish each (6 fish / 20 L glass jar) were exposed to a sublethal concentration of 0.085 mg/L . Parallel groups each of 6 control fish, kept in tap water and receiving 1.7 mL of absolute alcohol as the treated fish, were sampled after 3,6, and 12 d for comparison with the exposed fish. No mortalities occurred either in treated or control fish.

The fish were anesthetized with 1 g / 3 L MS 222 (tricaine methanesulfonate) and blotted dry with absorbant paper. The caudal peduncle was cut off with sharp razor blade and free flowing blood was collected in citrated tuberculin syringes. Blood glucose was determined by deproteinizing the blood with 10% sodium tungstate and 2/3 N sulfuric acid, taking serum in Folin and Wu tubes adding alkaline copper and phosphomolybdic acid, following the method of Folin and Wu (1920) . Liver and muscle glycogen contents were estimated after precipitating the tissue with 10% trichloroacetic acid (TCA) and acid hydrolysis by 0.2% of Anthrone reagent according to the procedure of vander Vies (1954). Hepatic and muscle protein contents were assayed after extracting the tissue with 10% TCA using Folin Ciocalteus phenol reagent following the method of Lowery et al. (1953). Serum calcium was determined according to the method of Trinder (1963) using calcium reagent and Naphthalhydroxamic acid to the serum, respectively. Inorganic phosphate (Pi) level liberated in the blood was estimated after its deproteinization with 10% TCA and adding 2.5% of molybdate I and II solutions alongwith 0.25% of amino

nephthol sulfonic acid to the serum following the method of Fiske and Subbarow (1925). Serum magnesium was assayed after adding 0.05% polyvinyl alcohol, and 0.05% Titan yellow to the deproteinized blood as per technique followed by Neil and Nelley (1956). All the biochemical analyses were performed on Spectronic 20 D (Milton Roy Company, New York).The results were subjected to statistical analysis by Student's 't' test.

RESULTS AND DISCUSSION

Various biochemical parameters studied after 3,6, and 12 d exposure of the fish to 0.085 mg/L of aldrin are given in Table 1. The treated fish elicited consistent hyperglycemia throughout the exposure period, whereas liver and muscle glycogenesis occurred at 3 d.However, the fish showed a significant decline in liver and muscle glycogen content after 12 d exposure as compared with the control values. In a teleost fish, Tilapia mossambica, inhibition of acetylcholinesterase in the nerve and other tissues resulted in an increase in acetylcholine content after pesticide intoxication (Koundinya and Ramamurthy 1978). Increase in the acetylcholine content has been shown to enhance the secretion of catecholamines in the cod, Gadus morhua (Nilsson et a1.1976) which may bring about glycogenolysis and hyperglycemia through the raised levels of cyclic AMP (Terrier and Perrier 1975). The depletion in glycogen content in the test fish is probably related to increased energy demands of the nervous system in view of known neurotoxicity of OC pesticides (Anand et a1.1985). The occurrence of both liver and muscle glycogenesis at 3 d exposure shows that the pesticide either inhibited glycogenolysis or promoted gluconeogenesis. It has been shown that stressed fish secrete increased amount of cortisol which may induce synthesis of glycogen from sources other than carbohydrate precursors (Swallow and Fleming 1970). However, the muscle and liver glycogenolysis after 12 d exposure seems to be the result of increased secretion of catecholamines due to stress of pesticide treatment (Singh and Srivastava 1992).

The fish exhibited significant decrease in liver and muscle protein contents throughout the exposure period. In fact, OC insecticides have been shown to affect the liver, resulting in a reduction of total plasma and tissue protein in teleost (Saxena et al. 1989). Further, the quantity of protein is dependent on the synthesis of RNA which plays an important role in protein synthesis. The decrease in the total protein content in aldrin treated fish may be either through

Table 1. Alterations in biochemical parameters of a freshwater catfish, Heteropneustes fossilis exposed to 0.085 mg/L of aldrin for 3,6 and 12 d

Parameters	Control	Exposure period, d		
		3	6	12
Blood glucose (mg / 100 mL)	56.80 ±2.55	99.43*** ±3.43	143.56*** ±2.69	116.93*** ±5.83
Liver glycogen (mg/100 mg wet wt)	15.53 ±1.08	19.39** ±1.09	16.79 ±1.62	10.43*** ±0.51
Muscle glycogen (mg/100 mg wet wt)	1.63 ±0.05	2.50* ±0.34	1.91 ±0.15	0.49*** ±0.06
Liver protein (g / 100 g)	3.58 ±0.12	2.60*** ±0.21	1.84*** ±0.17	2.14*** ±0.12
Muscle protein (g / 100 g)	3.49 ±0.21	2.07*** ±0.10	1.55*** ±0.09	1.26*** ±0.08
Plasma calcium (mg / 100 mL)	10.49 ±0.28	11.11 ±0.55	9.40* ±0.39	7.90*** ±0.52
Plasma magnesium (mg / 100 mL)	1.78 ±0.07	2.51*** ±0.17	2.70*** ±0.25	3.75*** ±0.36
Plasma inorganic phosphate(mg/100 mL)	4.52 ±0.07	8.69*** ±0.61	9.31*** ±0.56	10.12*** ±0.39

All the values are expressed as mean ± SE ; N = 6 ; $p < 0.001$***, $p < 0.01$** $p < 0.05$* when student's 't' test was applied between treated and control groups

the inhibition of RNA synthesis at the transcriptional level or due to impaired incorporation of amino acids into polypeptide chain. Significant decline in the nucleic acid content has been reported earlier in the catfish subjected to aldrin (Singh and Srivastava 1992) .

Aldrin exposure causes significant hypocalcemia on 6 and 12 d in catfish, whereas the plasma magnesium and inorganic phosphate content increased significantly throughout the exposure period when compared with control values. We are in agreement with the earlier results showing hypocalcemia, hypermagnesemia and hyperphosphatemia in teleost exposed to sublethal concentrations of cadmium (Larsson et al. 1975).Verbost et al. (1987) have suggested that in Cd exposed fishes the hypocalcemic effect is apparently caused by inhibition of Ca uptake via gills. Pesticide exposed fishes generally exhibit damage of gill epithelium. This damage of the branchial epithelium probably inhibits the intake of calcium from the ambient water causing hypocalcemia, resulting in respiratory distress, hyperexcitability and tremors in the fish.

Magnesium is an essential element of animal cells, involved in a variety of enzymatic reactions, but there is little information about how magnesium ion is transported and regulated in fish (vander Velden et al. 1992). Although ambient freshwater represents an inexhaustable magnesium source (0.1-0.3 mMol) yet freshwater fishes normally do not rely on absorption of magnesium ions from the water via gills. The major route for magnesium uptake is via the intestine from food. Despite the fact that the fish in this study were unfed during the exposure period, they exhibited hypermagnesemia. vander Velden et al.(1992) observed that under dietary magnesium deficiency the magnesium is mobilized from internal stores, i.e., bones and scales, etc. Probably this is the reason for hypermagnesemia in the present experiment which may also appear due to cell catabolism such as lysis or ketoacidosis.

The hyperphosphatemia in the present study is in agreement with Colvin and Phillips (1968) and Gill et al. (1991) who observed a significant rise in inorganic phosphate levels in Ictalurus melas and Barbus conchonius exposed to endrin and endosulfan, respectively. The hyperphosphatemia may be related to suppressed energy metabolism due to pesticide induced branchial lesions and the subsequent lowered oxygen uptake. Colvin and Phillips (1968) concluded that since organochlorines are lipophilic in nature they can

easily gain an easy access to the mitochondrial membrane and inhibit the enzyme involved in electron transport chain and affect the phosphorylating capacity of mitochondria. This may be the reason for hyperphosphatemia during aldrin intoxication. Hypocalcemia as well as increased plasma phosphate content has also been found in Cd treated mammals (Itokawa et al. 1974) and has been interpreted as being mainly due to kidney dysfunction. Aurbach et al. (1985) suggested that increased plasma magnesium levels could also be an effect of kidney damage. Kidney damage (glomerular shrinkage, tubular dysfunction) affects the renal function in teleost exposed to pesticide (Rashatwar and Ilyas 1984). Therefore, in the present study hypermagnesemia and hyperphosphatemia are probably due to damage of the renal tubules.

In conclusion, we can say that aldrin contamination might lead to serious metabolic crisis in the catfish, which might be responsible for its highly excited state. This calls for careful application of pesticide in plant protection operations, lest it should pollute the inland waters threatening the life of fish and other aquatic organisms.

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