Effect of Dimethoate on the Acid Phosphatase Activity and Protein Metabolism in the Fresh Water Fish *Anabas Testudineus*

M R RENUKA
Department of Zoology, N. S. S. Hindu College, Changanacherry, 686102, Kerala, India.

DOI: 10.29322/IJSRP.9.04.2019.p8810
http://dx.doi.org/10.29322/IJSRP.9.04.2019.p8810

ABSTRACT

The protein level of blood, liver and muscle in *Anabas testudineus* exposed to sublethal and median lethal concentrations of pesticide, dimethoate showed gradual decrease, which was statistically significant. The acid phosphatase activity in the blood, liver and muscle of Anabas testudineus was found to increase on exposure to sublethal and median lethal concentrations of dimethoate. The changes were concentration and duration dependent. Rise in the activities of acid phosphatase along with a reduction in protein content in the different tissues suggests enhanced protein catabolism to meet energy demands.

Key words: Protein, Acid phosphatase, Dimethoate, *Anabas*

INTRODUCTION

Stress due to anthropogenic contaminants such as pesticides and fertilizers could affect the health and performance of non - target species. Animals in the natural environment are usually exposed to low concentrations of these contaminants, which are sublethal. Fishes are important component of the food chain and are sensitive to a great number of contaminants such as pesticides, heavy metals, PCBs etc. Pesticides are receiving increased attention as a potential cause of biodiversity declines. Surveys on natural populations of many animals have shown correlations between population declines and proximity to agricultural lands (Birge *et al* 2000).

MATERIALS AND METHODS

Healthy, adult individuals of *Anabas testudineus* were collected from their habitats, brought to the laboratory and acclimatized to laboratory conditions for a week. Specimens weighing 40 to 50 g were used for the experiment. Commercial formulation of dimethoate, an organophosphate pesticide (30% EC) was used for toxicity studies. The 96 hour LC_{50} value for the pesticide was determined using bioassay methods (Doudoroff *et al* 1951) and Probit analysis (Finny 1971). From LC_{50} value a sublethal concentration (1/10th of LC_{50}) and a median lethal concentration (1/2 of LC_{50}) were calculated and fixed as the experimental concentrations. The experiment was maintained up to 30 days along with a control. *Anabas testudineus* were force fed with pieces of earthworms to ensure equal availability of food before and during the course of the experiment. Six individuals from each concentration were sacrificed on 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} days for collecting blood, liver and muscle. Total protein was estimated in blood, liver and muscle using Biuret method (Gornall *et al* 1949). Acid phosphatase activity was estimated in these tissues by Kind and King method (1954). Data obtained were subjected to statistical analysis using analysis of variance (ANOVA).

RESULTS

The 96 hour LC_{50} value for dimethoate for *Anabas testudineus* was found as 0 .1 ppm and the experimental concentrations were fixed as 0.01 ppm (sublethal) and 0.05 ppm (median lethal). In experimental fishes control values showed no significant variations for blood protein. Control values ranged from 8.38 to 8.63mg /100ml blood. In sublethal concentrations of dimethoate lowest value recorded was 2.7 mg /100ml on the thirtieth day of exposure (Table 1). Similarly in median lethal concentration this was 1.11mg / 100 ml.(Table 2). Liver protein showed same trend as that of blood protein. Control values ranged between 11.50 and 11.75 mg / g wt. of tissue. In sublethal concentrations of nuvacron the lowest value recorded was 4.58 mg / g wt. of tissue (Table 1) and in median lethal concentrations it was 1.97 mg / g wt. of tissue (Table 2). Muscle protein also showed declining trend. Control values ranged between 10.5 and 12.16 mg / g wt. of tissue. In sublethal concentrations the muscle protein recorded the lowest value 1.53 mg / g wt. of tissue on the thirtieth day. In median lethal concentration, this was 1.03 mg / g wt. of tissue (Table 2). Statistical analysis of the data showed significant difference between control and treated values in all the treatments of sublethal and median lethal concentrations of the pesticide.

The acid phosphatase activity in the blood, liver and muscle of *Anabas testudineus* was found to increase on exposure to sublethal and median lethal concentrations of dimethoate. In blood the control values ranged between 37.93 and 40.12 micromol phenol produced/min/l. In sublethal concentration there was a significant elevation of value, reaching up to 73.65 micromol phenol produced/min/l(Table 3). Median lethal concentration elicited more sharp increase in value, reaching up to 79.82 micromol phenol produced/min/l.(Table 4). Similar results were obtained in liver and muscle acid phosphatase values. In liver the control values were between 7.42 and 8.07 micromol phenol produced/min/l., whereas the sublethal concentrations induced an increase up to 14.32 micromol phenol produced/min/l(Table 3). Median lethal concentration raised the values, up to 23.4 micromol phenol produced/min/l. (Table 4). In muscle the control values were 12.3 and 12.8 micromol phenol produced/min/l. and the sublethal value reached up to 24.0. In median lethal concentration the value reached up to 28.4 micromol phenol produced/min/l. (Table 4).Throughout the experiment, control values showed no significant differences. But in the treatment with the pesticide, the values showed statistically significant differences from control even on the fifth day. This difference was time and duration dependent. These enzymatic changes are indicative of the cellular toxicity and tissue damage induced by the pesticide in fish, probably by imposing an alteration in the specific metabolic pathways. It is well known that acid and alkaline phosphatases play a significant part in various metabolic processes, especially protein and carbohydrate metabolism.

DISCUSSION

The protein level of blood, liver and muscle in *Anabas testudineus* exposed to sublethal and median lethal concentrations of pesticide, dimethoate showed gradual decrease, which was statistically significant. The decline was more pronounced in median lethal treatments in all the tissues indicating a dose dependent effect. The decrease in protein content in the various tissues of the treated *Anabas testudineus* indicates the physiological adaptability of the animal to compensate for pesticide stress. This energy demand might have led to stimulation of protein catabolism. Protein content in tissue is dependent on the dynamic equilibrium between the rates of its synthesis and degradation. The pesticide exposure might have accelerated proteolytic process to liberate aminoacids to overcome the metabolic stress. Pesticide induced reduction in protein content in the tissues of different animals were reported by a number of workers (Pan and Dutta, 2000, Das and Mukherjee, 2001 Luskova *et al*., 2002, Naveed *et al.* 2004).

Rise in the activities of this enzyme along with a reduction in protein content in the different tissues suggests enhanced protein catabolism to meet energy demands. This inference is further supported by the findings of Das and Mukherjee (2000) who reported an increase in acid phosphatase activity and a decrease in protein and RNA levels in *Labeo rohita* fingerlings exposed to quinalphos. Hence, the increased level of acid phosphatase in blood, liver and muscle of treated frogs indicates enhanced metabolic activity, perhaps to meet the stress induced by the pesticides.

Table 1. Effects of sublethal concentration of dimethoate on the blood, liver and muscle protein of *Anabas testudineus*.

Tissue	5th Day		10th Day		15th Day		20th Day		25th Day		30th Day	
	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt
Blood	8.38	4.57	8.58	4.52	8.63	4.10	8.40	3.65	8.50	3.21	8.45	2.7
Liver	11.75	11.27	11.52	10.22	11.67	9.15	11.50	7.22	11.72	5.73	11.78	4.58
Muscle	10.67	5.00	10.83	4.23	10.50	3.31	12.83	2.62	12.12	2.01	12.16	1.53

VR**
CD(5%)=0.30

Table 2. Effects of median lethal concentration of dimethoate on the blood, liver and muscle protein of *Anabas testudineus*.

Tissue	5th Day		10th Day		15th Day		20th Day		25th Day		30th Day	
	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt
Blood	8.38	3.70	8.58	3.25	8.63	2.82	8.40	2.18	8.50	1.55	8.45	1.11
Liver	11.75	9.82	11.52	8.05	11.67	6.15	11.50	3.90	11.72	2.68	11.78	1.97
Muscle	10.67	5.03	10.83	4.40	10.50	3.60	12.83	2.20	12.12	1.32	12.16	1.03

VR**
CD(5%) =1.89
Table 3.Effects of sublethal concentration of dimethoate on the acidphosphatase concentration in the blood, liver and muscle of *Anabas testudineus*.

Tissue	5th Day		10th Day		15th Day		20th Day		25th Day		30th Day	
	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt
Blood	39.2	43.50	38.12	45.10	40.12	47.63	39.68	53.75	37.93	61.22	39.83	73.65
Liver	7.62	8.57	7.52	9.12	8.07	9.68	7.42	10.68	7.68	12.40	7.62	14.32
Muscle	12.33	13.48	12.47	14.35	12.82	15.62	12.5	17.58	12.45	20.18	12.43	24.03

VR **
CD (5%) = 2.73

Table 4.Effects of median lethal concentration of dimethoate on the acidphosphatase concentration in the blood, liver and muscle of *Anabas testudineus*

Tissue	5th Day		10th Day		15th Day		20th Day		25th Day		30th Day	
	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt
Blood	39.2	47.83	38.12	51.53	40.12	55.18	39.68	60.98	37.93	67.83	39.83	79.82
Liver	7.62	9.15	7.52	10.45	8.07	12.13	7.42	14.30	7.68	18.12	7.62	23.42
Muscle	12.33	15.32	12.47	16.27	12.82	18.40	12.5	21.47	12.45	24.60	12.43	28.35

VR **
CD (5%) = 0.62

REFERENCES

Birge, W. J., Westerman, A. G. and Spromberg, J. A. 2000. Comparative toxicology and risk assessment of amphibians, In: Ecotoxicology of amphibians and reptiles. Sparling, D. W., Linder, G. and Bishop, C. A. Eds, SETAC, Pensacola, FL, 727.

Das , B. K. and Mukherjee, S.C. 2000. Sublethal effect of quinalphos on selected blood parameters of Labeo rohita (Ham.) fingerlings. Asian. Fisheries Science, 13: 225 – 233.

Das , B. K. and Mukherjee, S.C. 2001. Effect of nuvan on hematology of rohu Labeo rohita (Ham.). Indian. J. Fish., 48 (1): 85 – 92.

Doudoroff, P., Anderson, B.G., Burdick, G.E., Hart, W. B., Patrick, R., Strong, E. R., Surber, R. and Van Horn, W.M. 1951. Bioassay methods for the evaluation of acute toxicity in industrial wastes to fish. Sew. Ind.Wastes., 23: 1380 – 1397.

Finney, D. J. 1971. Probit analysis. Cambridge University Press.

Gornall, A. G., Bardawil, C.J. and David, M.M. 1949. Determination of serum proteins by means of the Biuret reagent. J. Biol. Chem., 177: 751-756.

Kind, P. R. N. and King, E. J. 1954. Estimation of plasma phosphatase by determination of hydrolised phenol with amino-antipyrine. J.Clin. Pathol., 7 (4): 322 – 326.

Luskova, V., Svoboda, M. and Kolarova, J. 2002. The effect of diazinon on blood plasma biochemistry in carp (Cyprinus carpio). Acta Vet. Brno., 71: 117 – 123.

Pan, D. and Dutta, H. 2000. Dazinon induced changes in the serum proteins of large mouth bass, Micropterus salmiodes. Bull. Environ.Contam. Toxicol., 64: 287 – 293.

Naveed, A.P.,Venketeswaralu, P. and Janaiah,C. 2004. The action of sublethal concentration of endosulfan and kelthane on regulation of protein metabolism in the fish Clarias batrachus (L.). Nature. Environ. Poll. Technol., 3 (4): 530 – 544.