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TOXIC EFFECT OF ENDOSULFAN ON CERTAIN SERUM BIOCHEMICAL PARAMETERS IN EXOTIC FRESHWATER FISH, *CTENOPHARYNGODON IDELLA* (CUV. AND VAL.).

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ABSTRACT
Biochemical characteristics of blood are the important indices of status of internal environment of fish organisms. The present paper deal with the variations in serum glucose, serum cholesterol and total serum protein in exotic freshwater fish, *Ctenopharyngodon idella* (Cuv. and Val.) upon exposure of two different concentrations of endosulfan (0.00075 mg/L and 0.001 mg/L) for 15, 30 and 45 days.. Serum glucose and total serum protein content have shown a declining trend. The decrease in serum glucose has been found to be highly significant ($p<0.01$) on all exposure periods except after 15 days in 0.001 mg/L concentration which has been found to be non-significant ($p>0.05$) while total serum protein decreased significantly ($p<0.05$) after 15 days as well as 30 days exposure to 0.00075 mg/L concentration, but highly significantly ($p<0.01$) in the fish exposed to 0.001 mg/L after 30 days and to both the doses after 45 days exposure. Serum total cholesterol of the test fish, *Ctenopharyngodon idella* (Cuv. and Val.) increased and increase was found to be highly significant ($p<0.01$) after 30 and 45 days exposure in both the concentrations as compared to the control groups. It increased significantly ($p<0.05$) after 15 days exposure to 0.001 mg/L as well as to 0.00075 mg/L concentrations. The variations in biochemical characteristics of blood serve as a good indicator of polluted aquatic environment.

KEY WORDS: cholesterol, endosulfan, Fish, glucose, protein, serum.

INTRODUCTION
Now-a-days, water bodies are the most exploited systems with wide variety of chemicals, the most significant ones being the pesticides and industrial wastes. Pesticides are potentially lethal to fish tissues, even at relatively low concentrations. This is because these are present all the times in water and fish is exposed to them continuously. Moreover, as a result of biomagnifications of the chemical and being at the top of food chain, the fishes have maximum concentration of toxicants and are the worst victims.

Most of the pesticides act as metabolic depressors and generally affect the activity of biologically active molecules such as proteins, carbohydrates and lipids (Agrahari and Gopal, 2009). The main route of entry of any pesticide is through the gills in fishes, from where it is transported to various parts of the body via blood. So, blood provides an ideal tool for toxicological studies. Biochemical characteristics of blood are among the important indices of status of internal environment of fish organisms (Edsall, 1999; Velisek *et al.,* 2009). A number of studies have been carried out regarding the biochemical alterations in blood induced by pesticides in general (Pant and Singh, 1983; Bhushan et *al.,* 2002, Mohamed and Gad, 2008 Jenkins *et al.,* 2003; Rajamanickam and Muthuswamy, 2008; Saravanan *et al.,* 2011 and Yekeen and Fawole, 2011).

The purpose of this study was to expand the endosulfan toxicity database of aquatic vertebrates. This was accomplished by assessing the variations in serum glucose, serum cholesterol and serum total protein in *Ctenopharyngodon idella* (Cuv. and Val.) on exposure to two different concentrations of endosulfan (0.00075 mg/L and 0.001 mg/L) for exposure periods of 15, 30 and 45 days.

MATERIALS AND METHODS
Live healthy fish (12.56 ± 1.52 cm and 25.40 ± 2.53 gm) were procured from local fish farm and acclimatized for a week to laboratory conditions. The fish were fed with grass, barseem, banana leaf etc. The water used during experiment was analyzed as per the standard methods (APHA, 2005). The physicochemical properties of water were pH 7.3-7.6, dissolved oxygen (mg/ml) 7.5 ± 0.50, conductivity (µS/cm) 308 ± 6, total hardness (mg/L) 78 ± 5, alkalinity (mg/L) 145 ± 8, salinity (gm/kg) 0.155.

Endosulfan 35 EC (commercial name), manufactured by Excel industries limited, India was used for the present investigation. Short term exposure to endosulfan gave 96 hour LC_{50} values (0.005mg/L), determined by graphic interpolation. Aliquots of stock solution (1mg/L) were added to each experimental tank (non-poisonous, aerated) so as to bring the endosulfan concentration to the desired levels of 0.00075 mg/L (20% of 96 hour LC_{50}) and 0.001 mg/L (10% of 96 hour LC_{50}). Three groups of fish were exposed to two sublethal concentrations for 15days, 30days, and 45days in plastic tanks. The tanks were cleaned and water was changed every alternate day so as to maintain the desired level of insecticidal concentration. A parallel control set was run simultaneously in toxicant free tap water.

On completion of the stipulated exposure period of 15days, 30days, and 45days free flowing blood from caudal vein was collected and serum was separated by centrifugation at 3000 rpm for 15 minute at room temperature. Prepared serum was stored at 4^0C and was used for estimation of glucose, total protein and cholesterol. Estimation of glucose has been done according to Anthrone method given by Seifter *et al.* (1950). The amount of total protein has been estimated by method of Lowry *et al.* (1951). Estimation of cholesterol has been done according to the method of Zlatkis *et al.* (1953) in the treated and control fish.

Statistical analysis: All the data were expressed as mean ± S.D. Mean value for each group of fish was tested for significance by student's t-test to establish the validity of the findings.

RESULTS

The alterations in serum glucose, serum protein and serum cholesterol in control and endosulfan exposed fish are presented in Table 1. The results of this study showed that the total serum glucose of test fish, *Ctenopharyngodon idella* (Cuv. and Val.) exposed to endosulfan has declined by 11.86 % and 10.45 % on 15th day; 28.68 % and 39.57 % on 30th day and 45.14 % and 62.18 % on 45th day as compared to control at sublethal concentrations of 0.00075 mg/L and 0.001 mg/L of endosulfan respectively (Table 1).The decrease has been found to be highly significant ($p<0.01$) on all exposure periods except after 15 days in 0.001 mg/L which has been non-significant ($p>0.05$). The decrease has been more significant in the higher concentration of toxicants. The maximum decline of 62 % in glucose level has been observed after chronic exposure of 45 days in 0.001 mg/L. The decline in glucose content resulting in hypoglycaemia is certainly due to stress induced by pesticide poisoning for longer periods.

Table 1. Endosulfan induced changes in serum glucose, serum proteins and serum cholesterol in *Ctenopharyngodon idella* (Cuv. and Val.).

Parameters	Exposure Period (days)	Control (Mean ± S. D.)	0.00075 mg/L Treated (Mean ± S. D.)	0.001mg/L Treated (Mean ± S. D.)
Serum glucose	15	120.06 ± 2.62	106.62 ± 3.84**	101.5 ± 5.90
(gm/dl))	30	119.95 ± 3.22	85.55 ± 2.66**	72.48 ± 1.46**
	45	119.82 ± 6.0 9	64.96 ± 3.45**	44.78 ± 1.08**
Serum protein	15	4.65 ± 0.250	4.15 ± 0.212*	4.19 ± 0.195
(gm/dl)	30	4.64 ± 0.187	4.02 ± I.970*	3.57 ± 0.297**
	45	4.63 ± 0.404	3.48 ± 0.317**	2.80 ± 0.240**
Serum	15	207.82 ± 5.04	223.66 ± 7.567*	231.86 ± 6.517*
Cholesterol	30	209.70 ± 7.04	241.04 ± 8.388**	253.26 ± 5.30**
(gm/dl)	45	210.03 ± 6.18	264.81 ± 6.58**	277.04 ± 7.745**

Values are mean ± S. D. of 6 observations; Level of significance *$p<0.05$; **$p<0.01$. Non significant ($p > 0.05$).

The total serum protein content of the treated fish in present investigations showed a declining trend (Table 1). It declined by 16.65% and 12.23 % on 15th day; by 18.36% and 23.84% on 30th day and by 23.96% and 39.52% on 45th day as compared to control in 0.00075 mg/L and 0.001 mg/L of endosulfan respectively. Serum protein decreased

significantly ($p<0.05$) after 15 as well as 30 days on exposure to 0.00075 mg/L, but highly significantly ($p<0.01$) in the fish exposed to 0.001 mg/L after 30 days and in both the doses after 45 days exposure.

Serum total cholesterol of the test fish *Ctenopharyngodon idella* (Cuv. and Val.) increased highly significantly ($p<0.01$) after 30 and 45 days in both the concentrations as compared to the control groups. It increased significantly ($p<0.05$) after 15 days exposure to 0.001 mg/L as well as to 0.00075 mg/L (Table 1). The control values of serum total cholesterol 207.66 ± 7.567 mg/dl of *Ctenopharyngodon idella* (Cuv. and Val.) in the present study is within the range of other freshwater fishes as reported by (Koussa, 1997; Selium and Gad, 2003). Fish exposed to endosulfan exhibited marked hypercholesterolemia. The increase has been observed to be by 8.73% and 11.37% on 15th day; 14.95% and 20.77% on 30th day while 26.08% and 31.91% with respect to control on 45th day of treatment at 0.00075 mg /L and 0.001 mg/L of endosulfan respectively.

DISCUSSION

Hypoglycaemia induced by endosulfan in *Ctenopharyngodon idella* (Cuv. and Val.) in the present study may be explained due to insecticidal respiratory stress which has been found to lead to a hypoxic / anoxic condition. As a consequence of hypoxia, the metabolic pathway is shifted from aerobiosis to anaerobiosis as pesticides are also known to inhibit energy production by suppressing aerobic oxidation of carbohydrates leading to energy crisis in animals (Kohli *et al.,* 1975). These conditions might have depleted the glucose levels in the test fish exposed to endosulfan in order to meet the increased energy demands as carbohydrates form the major source of energy under stressful conditions (Hochachka and Somero, 1984). This may explain the maximum observed depletion of the glucose levels in test fishes during the later stages of exposure as a result of increased demand of these molecules to provide energy for the cellular biochemical processes as at the end of 45 days of exposure, the percent decrease in the glucose has been found to be maximum. Saravanan *et al.* (2011) reported that endosulfan caused significant lower value of red blood corpuscles (RBC), haemoglobin, plasma glucose, and protein levels in *Labeo fimbriatus* when compared to the control groups.

The decline in protein content in the present studies can be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanisms required during stress as many organisms will mobilize proteins as an energy source via the oxidation of amino acids or induce proteolysis to meet increased energy demand under the conditions of stress. Other main reason for present study can be reduced protein synthesis which may be owing to liver damage or due to impaired incorporation of amino acids This is in agreement with reports given by many workers (Jenkins *et al.*,2003; Rajamanickam and Muthuswamy, 2008; Ramesh and Saravanan, 2008; Saravanan *et al.*, 2010 and Yekeen and Fawole, 2011). It is appropriate to mention here that the test animals in present study were observed to be restless as indicated by their constant fast movements aided by muscular action and frequent surfacing throughout the exposure period, more particularly during the initial stages of the exposure. Some energy was required to be spent for this kind of stress induced behaviour. So, extra energy was required to offset the stress.

The observed hypercholesterolemia during present investigation may be explained due to due to higher energy demands, impairment in the membrane organisation and damage to liver but main reason seems to be impairment in the membrane organisation of cells. Gad and Saad (2008) have reported that increased level of serum cholesterol in *Oreochromis niloticus* exposed to phenol may be due to impairment in the membrane organization induced by the acclimatization in acidic water or water contaminated with phenol in order to get the positive survival value under the imposed stress.

Increased production by the liver and other tissues or reduced cholesterol and triglyceride catabolism may also be the reason as it has been shown by many workers ((Pant and Singh, 1983; Bhushan *et al.,* 2002, Mohamed and Gad, 2008) that insecticides mainly affect the liver of fishes. Yekeen and Fawole (2011) have reported that in *Clarias gariepinus* exposed to endosulfan increase in serum cholesterol level which indicated retardation of fat metabolism may be due to heavy stress imposed on the exposed fish by the pesticide.

CONCLUSION

These results of present biochemical studies indicate that endosulfan intoxication demands metabolic energy and hence leads to significant changes of the metabolic profile of the fish, inducing to a certain extent a shift from carbohydrate

catabolism to protein catabolism. It causes disorganisation of cells. It can be mentioned here that exposure to endosulfan even at lower concentration is responsible for alterations in the biochemical parameters of fish, which can be useful in diagnosing the structural and functional status of fish exposed to toxicants. It could thus be suggested that indiscriminate use of endosulfan should be discouraged or banned through strict laws and legislations in order to reduce its potential risk to human health and to environment.

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REFERENCES

Agrahari S. and Gopal K. (2009). Fluctuations of certain biochemical constituents and markers enzymes as a consequence of monocrotophos toxicity in the edible freshwater fish, *C. punctatus*. *Pest. Biochem. Physiol.* 94: 5-9.
APHA, AWWA, WPCF. (2005). Standard Methods for the Examination of Water and Wastewater. 21st ed. (eds. S.C. Lenore, E.G. Arnold and D.E. Andrew). American Public Health Association, Washington, DC.
Bhushan P.B., Singh M.K. and Rani M. (2002). Dimethoate and monocil toxicity on concentration of protein and amino acid in serum and liver of *Channa punctatus*. *Nat. Environ. Poll. Technol.* 1: 147-150.
Edsall C.C. (1999). A blood chemistry profile for lake trout. *J. Aquat. Anim. Hlth.* 11: 81-86.
Gad N.S. and Saad A.S. (2008). Effect of environmental pollution by phenol on some physiological parameters of *Oreochromis niloticus*. *Glob. Veterin.* 2 (6): 312-319.
Hochachka P.W. and Somero G. (1984). Biochemical Adaptation. Princeton University Press, Princeton, New Jersey.
Jenkins F., Smith V., Rajanna B., Shameem U., Umadevi K., Sandhya V. and Madhavi R. (2003). Effect of sublethal concentrations of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*. *Bull. Environ. Contam. Toxicol.* 70 (5): 993-997.
Kohli K.K., Sharma S.C., Bhatia S.C. and Venkitasubramoniam T.A. (1975). Biochemical effects of chlorinate insecticides DDT and dieldrin. *J. Sci. Industr. Res.* 34: 462-470.
Koussa A.A. (1997). Toxicological studies on the Nile catfish *Clarias anguillaris* exposed to cadmium and lannate M.Sc. Thesis, Faculty of Science Ainshams University.
Lowry O.H., Rosenbrough N.J., Farr A.L. and Randail R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193: 265- 275.
Mohamed F.A.S., and Gad N.S. (2008). Environmental pollution-induced biochemical changes in tissues of *Tilapia zillii*, *Solea vulgaris* and *Mugil capito* from Lake Qarun, Egypt. *Glob.Veterin.* 2: 327-336.
Pant J. and Singh T. (1983). Induction of metabolic dysfunction by carbamate and organ phosphorus compounds in a fish, *Puntius conchonius*. *Pestic. Biochem. Physiol.* 20: 294-298.
Rajamanickam V. and Muthuswamy N. (2008). Effect of heavy metal induced toxicity on metbolic biomarkers in common carp (*Cyprinus carpio*). *Maejo Int. J. Sci. Technol.* 2: 192-200.
Ramesh M. and Saravanan M. (2008). Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *Intern. J. Integrat.Biol.* 3(1): 80.
Saravanan M., Vidhya A.E., Ramesh M., Malarvizhi A. and Kavitha C. (2011). Impact of endosulfan on certain hematological and biochemical parameters of catfish *Labeo fimbriatus:* Sublethal study. Toxicol. Ind. Hlth. 27(6): 555-562.
Saravanan T.S., Rajesh P. and Sundaramoorthy M. (2010). Studies on effects of chronic exposure of endosulfan to *Labeo rohita*. *J. Environ. Biol*. 31 (5): 755-758.
Seifter S., Seymour E., Novic E. and Muntwyler E. (1950). Determination of glycogen with an anthrone reagent. pp. 35-36. ***In:*** Methods in Enzymology, Vol. 273 (eds. S.P. Colowick and N.O. Kaptan). Academic Press, New York.
Selium N.A. and Gad N.S. (2003). Toxicological effect of copper on the Nile tilapia, *Oreochromis niloticus. J. Egyptian Acad. Soc. Environ.* 4: 153-166.
Velisek J., Svobodova Z. and Piackova V. (2009). Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (Oncorhynchus *mykiss). Veter. Med.* 54 (3): 131-137.
Yekeen T.A. and Fawole O.O. (2011). Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus. Afr. J. Biotechnol.* 10 (64): 14090-14096.
Zlatkis A., Zak B. and Boyle A.L. (1953). A new method for the direct determination for serum cholesterol. *J. Lab. Clin. Med.* 41: 486.

