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RESEARCH ARTICLE

Toxic effect of Phorate on the Serum Biochemical Parameters of Snake Headed Fish *Channa punctatus* (Bloch)

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ABSTRACT
Environmental pollution is a burning issue at the moment for whole world. This type of pollution has many categories, classes, types. Many factors are behind these various types of pollution and varied effects revealed by the pollutants. Aquatic pollution has its own importance and concern with human life, as water is the main component of living organisms and is in bottom of our every day life activities either cleaning, drinking, washing, agricultural purpose, sea food, fish food. So that it is easily reflected that the water is to be clean and pollution free for safe food and drinking water. The harmful effect of any toxicant which was moved to water bodies can be assessed by investigating health of aquatic fauna. In the present study, an attempt has been made to examine the sub-lethal toxic effect of phorate, an organophosphate pesticide on biochemical parameters in fish Channa punctatus. The serum protein level has been observed to be increased significantly, while serum cholesterol and glucose level has been observed to be decreased after phorate treatment for different time intervals (24hrs, 48hrs, 72hrs and 96hrs) and for all experimental concentrations such as 0.03mg/L, 0.04mg/L, 0.05mg/L and 0.06mg/L.
KEY WORDS: serum protein, serum cholesterol, *Channa punctatus*, phorate

INTRODUCTION

Water is most abundant on our earth. Water pollution is a serious problem in world and in developing countries especially in India on account of huge population. The contaminant are entered in body of organisms via absorption and other routes, goes deep in tissues and affect physiology, biochemistry and metabolism of the organism. Various fertilizers and pesticides with different chemical composition have come in use to achieve this target, which have directly or indirectly certain lethal effects on various animals. Diffuse sources include run off and leaching from agricultural land after pesticide application, spray drift during application and discharge of wastes from the domestic sector. Pesticides are related chemicals destroy the delicate balance between species that characterize a functioning ecosystem. Pesticides include a wide variety of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to target and non-target organisms. Poisoning risks depend on dose, toxicity, duration of exposure and sensitivity. Organophosphorous are some of the most widely used pesticides in the world. They used in agriculture, homes, gardens and veterinary practices. Sastry *et al*. [1] noted the alteration in biochemical parameter treated with organophosphorous. Phorate is of moderate persistent in the soil environment with reported field half lives of 2 to 173 days. Phorate binds moderately well to most soils and is slightly soluble in water. Phorate itself is not persistent in plants but plants metabolize phorate to very potent anticholinesterase agents such as the sulfoxide and sulfone derivatives of the compound. Phorate and its metabolites are absorbed from the soil by plant roots and are translocated to above-ground portions of the plant. Phorate is toxic to fishes and other animals [2,3]. The fishes, best indicator of water body pollution, are the most sensitive of all the aquatic animals, towards the pollutant poisoning through the river water from adjoining settlement and industries. The accumulation of effluents becomes hazardous to the aquatic organisms and to surrounding human population because the fishes are the most important factors of food chain which have great nutritive value in the environment. Biochemicals are the most assessable body contents for checking the toxicity of any chemicals. Any alteration in biochemical parameters can result in serious outcomes in the form of various

diseases in both the animal and its consumers. *Channa punctatus* (Bloch.) is selected for present study due to easy availability and handling. The aim of this study to determine
toxic effects of phorate on the total protein, total cholesterol and glucose level in fish *Channa punctatus* and determine the harmful effect on other animals that intake fishes in the route of food chain.

MATERIALS AND METHODS

The fresh water an air breathing fish *Channa punctatus* which is also known as snake headed fish, ranging from 8 to 15cm in length and 45 to 65g in weight were collected from the local fish market. The fishes were carefully examined for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid off dermal infection. Finally they were stored in large glass aquaria in measuring 75cmx37.5cmx37.5cm and fed on boiled egg yolk and commercial fish food. Bioassays were conducted to determine acute toxicity (LC_{50}) employing the technique under standard laboratory condition [4]. Fishes were divided into 4 groups (A, B, C, D). Each group consisting of six individuals with standard solution of experimental test compound phorate, was given to each set of fishes kept in 25 litre of water in aquaria by weighing. The survival number of fishes was recorded for each concentration after 24hours, 48hours, 72hours and 96hours. The data was analyzed statistically by log-dose/probit analysis method [5]. Regression line was drawn on the basis of two variables. Log dose and empirical probit, on a simple graph paper and was used to determine the expected probit necessary for LC_{50} determination. The concentration of phorate at which 50% of the fishes died is taken as the lethal concentration. The mortality percentage was calculated and the graph was plotted between the mortality % and the concentration of phorate. LC_{50} value was obtained by the straight line graphical interpolation. In the present study, calculated LC_{50} of organophosphate phorate is 0.3mg/L. The selected concentrations were 0.03mg/L, 0.04mg/L, 0.05mg/L and 0.06mg/L for 24hours, 48hours, 72hours and 96hours respectively. Five fishes from each set were sacrificed for the collection of blood. The blood was collected after severing the caudal peduncle of the living fish by a scissor. The collected blood was allow to centrifugal tubes, stored in a slanting position to clot for 3 minutes. It was then centrifuged at 3000rpm for 15 minutes. The supernatant serum was separated by a fine rubber bulb pipette in separate tube. The serum samples were used for the estimation of biochemical parameters i.e. serum total proteins and serum cholesterol. The serum total protein was estimated by Dumas [6] method, while serum cholesterol was estimated by the method of Warnick [7] and glucose by Mendel *et al*. [8]. The experimental data were analyzed by student's 't' test for determine the significance of the changes from controls[9].

RESULTS AND DISCUSSION

In the present study, an attempt was made to examine the sub-lethal toxic effect of phorate, an organophosphate on the lipid and protein metabolism in the terms of serum proteins and cholesterol level in fish. The calculated LC_{50} of organophosphate phorate is 0.3mg/L in the present study (Table-1, Fig. 1 and 2). This value differs from LC_{50} values calculated by other scientists for different species of fishes and against various compounds. Auta *et al*. [10] estimated LC_{50} value of dimethoate for *Clarias gariepinus* as 39.40mg/l. Sulodia and Singh [11] calculated LC_{50} of organophosphate folidol to the fish as 500ppm, while Babu *et al*. [12] reported as 0.36 mg/l while observing the effects of fenvalerate on different tissues of fresh water fish *Cirrhinus mrigala*. The serum total protein level has been observed to be increased after phorate treatment for different time intervals, while a decrease in cholesterol level and glucose content (Table-2). The toxic stress may decrease protein content in tissues which is supported by Singh and Khare [13], Desai [14]. Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein to meet the increased energy demand. The depletion of protein fraction in liver may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Further, due to this degradation of protein in liver, the serum protein level has been increased which was released. Reddy *et al.* [15]; Singh and Sharma [16] have also reported decline in protein constituent in different fish tissues exposed to sub-lethal concentration of insecticides in liver and increase in serum.he decline in cholesterol level is due to utilization of stored and circulatory cholesterol and other lipid fractions in phorate treated fish to counteract toxic effect produced and further stabilization of the toxic phorate molecules to prevent harm caused by them. Ghosh [17] in blood of *Channa punctatus* who observed reduction of cholesterol after chromium intoxication. Shivaramakrishna and Radhakrishna

Experimental animal	Compound	Regression equation	LC_{50} mg/L	Variance	Fiducial limits
Channa punctatus (Bloch.)	Phorate	Y = 5.50±7.96 (X-1.50)	0.30	0.001	m_1 = (±) 1.5126 m_2 = (-) 1.4194

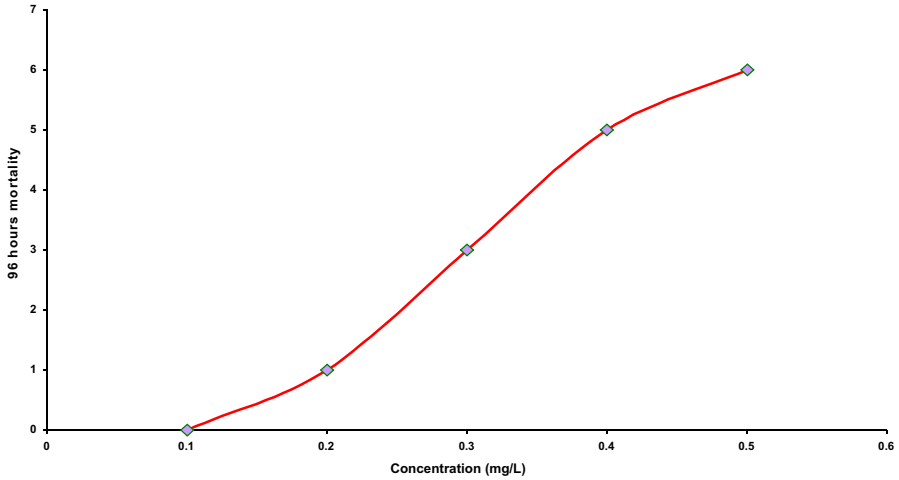

Fig. 1: Graph showing mortality of *Channa punctatus* (Bloch.) at 96 hours after treatment of phorate

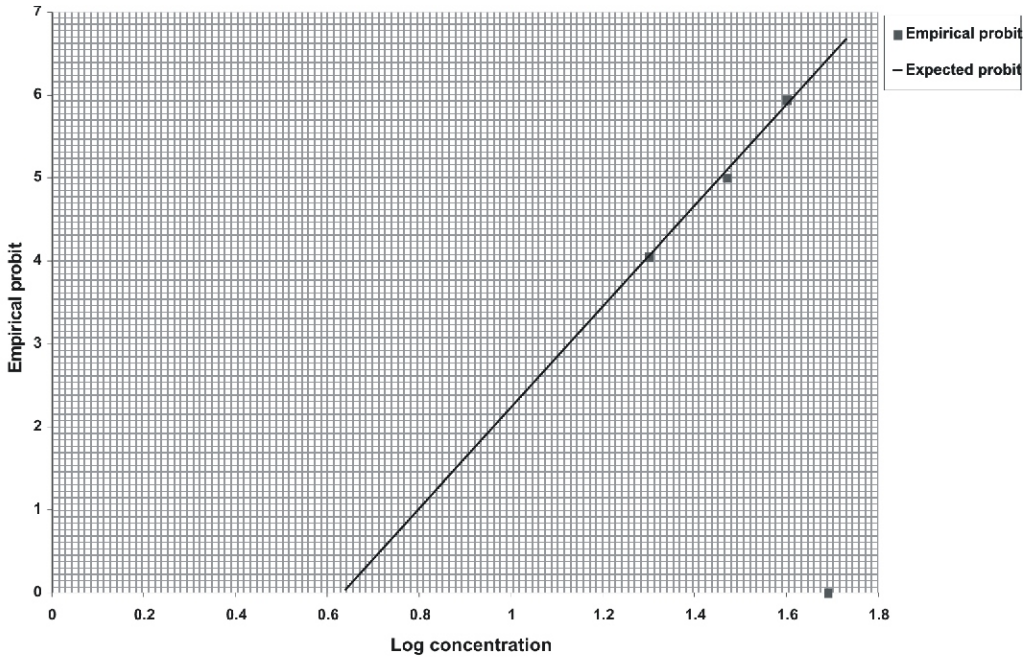

Fig. 2: Regression line for LC_{50} determination of phorate against *Channa punctatus* (Bloch.)

TABLE-2 .Serum biochemistry of *Channa punctatus* (Bloch.) in control and after phorate treatment

Exp. set	24hrs	48hrs	72hrs	96hrs
	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
SERUM PROTEINS (g/dl)				
Control	2.50±0.50	2.55±0.50	2.70±0.53	2.68±0.52
0.03mg/L	2.59±0.10*	2.60±0.25*	2.74±0.11*	2.75±0.11*
0.04mg/L	2.62±0.20*	2.68±0.50*	2.78±0.80*	2.81±0.50**
0.05mg/L	2.68±0.20**	2.73±0.55**	2.83±0.80**	2.89±0.50***
0.06mg/L	2.75±0.25**	2.79±0.80**	2.90±0.11**	2.97±0.10****
SERUM CHOLESTEROL (mg/dl)				
Control	128.0±0.52	132.9±0.30	131.5±0.66	135.8±0.30
0.03mg/L	119.5±0.55*	129.3±0.35*	125.2±0.30*	129.8±0.66*
0.04mg/L	112.6±0.50*	119.0±0.80*	115.5±0.33*	120.3±0.66**
0.05mg/L	107.5±0.50**	114.8±0.52**	108.6±0.33**	110.5±0.10***
0.06mg/L	100.1±0.10***	110.1±0.20***	101.5±0.55****	100.0±0.55****
SERUM GLUCOSE (mg/dl)				
Control	11.20±0.10	11.00±0.33	11.40±0.33	11.10±0.20
0.03mg/L	10.00±0.50*	9.59±0.20*	9.50±0.10*	9.33±0.09*
0.04mg/L	9.95±0.09*	9.90±0.50*	9.45±0.60*	9.10±0.63*
0.05mg/L	9.50±0.19*	9.25±1.00*	9.05±0.92**	9.00±0.08**
0.06mg/L	9.00±0.82**	8.68±0.20***	8.25±0.10***	7.62±0.09***

*Non-significant (P>0.05);**Significant (P<0.05); ***Highly-significant (P<0.01); ****Very highly significant (P<0.001)

18] and Rani *et al*. [19] found same results. Shankar and Kulkarni [20] also observed the same trend in *Notopterus notopterus* during stress. This is mainly due to altered lipid metabolism and energy demand. The fall in the glucose content in the serum indicate its rapid utilization by the organism's body as a consequence of metabolic toxic stress. Similar decrease has also been reported by Sastry *et al*. [21] due to sublethal effects of Sevin on *Channa punctatus*. Devi [22] recorded that after 96 hours exposure in *Channa punctatus* the endosulfan significantly decrease protein, glucose and lipid concentration. Also Saxena and Chauhan [23] reported similar results while studying copper sulphate induced haematological and biochemical anomalies in the Indian catfish, *Heteropneustes fossilis* (Bloch.). Overall depiction is that the experimental compound pose toxic metabolic stress hence altered the serum biochemistry of *Channa punctatus* (Bloch.).

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