

Sublethal concentration of Thiamethoxam alters serum enzymes in fresh water fish, *Channa punctatus* (BLOCH)

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ABSTRACT

Sublethal concentration of thiamethoxam (organochloride), on the serum enzymes of fish, *Channa punctatus* were altered during the toxic exposure periods at different time intervals. The activities of different serum enzymes such as aldolase, lactate dehydrogenase (LDH), iso-citrate dehydrogenase (ICDH), pyruvate kinase, SGPT, SGOT, alkaline phosphatases, acid phosphatases and creatine kinase (CK) were altered under the impact of thiamethoxam. The high activities of transaminases indicated that thiamethoxam could induce malignance and hepatobiliary disease. Enhancement in creatine kinase leads to impairment of central nervous system (CNS) under toxic stress. The enhanced activities of phosphatases might be due to lysosomal mobilization. The increased serum dehydrogenases revealed the damage of kidney and liver of exposed fish

Keywords: *Thiamethoxam, OC insecticide, serum enzymes, Channa punctatus.*

INTRODUCTION

Chlorinated hydrocarbons are the most important insecticides used to control insect pests from plants. These organochloride (OC) compounds readily pass through cell membranes and alter the activities of several key enzymes (Onikiano, 1964; Gruzdev, 1983). Among the OC insecticides, thiamethoxam is one of the most toxic compounds which is used in the paddy and cotton fields to control the pests. The effects of insecticides on fish were extensively studied (Brungs *et al.*, 1977; Khalaf – Allah, 1999; Chandra, 2004; Radha *et al.*, 2005; Mishra and Bohider, 2005; Sharma and Singh, 2006). Therefore, in the present investigation the impact of commercial grade thiamethoxam on serum enzymes of fresh water edible fish, *Channa punctatus* was studied

MATERIALS AND METHODS

Channa punctatus, a fresh water edible fish, weighing average of 82-120 gms and 25.5 ± 1.21

cm in length, were procured from a local market, Warangal (A.P.). The collected fish were kept in a cement tank (6x3x3 feet) atleast for one month for acclimatization under continuous water flow. The average temperature of water was 22-24°C. The fish were fed adlibitum with ground nut-cake along with the commercial pellets (1-1.5% body weight). They were starved one day before experiment (Butlerworth, 1972). Without discrimination of sexes, both the sexes of fish were used for the experiment. The physiological parameters of water are given in Table 1. The LC₅₀ of commercial grade thiamethoxam (114.8ppm) was determined for 48 hours by the method of Bayna *et al.*, (1977).

Batches of six (6) fish were exposed to 24,48,72 and 96 hours for sublethal concentration (0.19ppm) along with control fish in separate tanks consisting of six liters of water, at the room temperature. After the stipulated time intervals, the fish were removed and the blood was collected in the tubes by caudal

puncture. For further investigations of toxic effects the following methods have been adopted. Aldolase activity is estimated by Pinto, (1969); Lactate dehydrogenase (LDH) activity is done by King and Jagtheesan (1959); and Isocitrate dehydrogenase (ICDH) is determined by Bell and Baroon (1962) and pyruvate kinase (PK) is estimated by the method of Boehringard *et al.*, (1974). The activities of SGPT and SGOT were determined by the method of Reitmann and Frankal (1957). The activities of acid and alkaline Phosphatases were estimated by the method of Kind and King (1954). Creatine Kinase (CK) was determined by Abdul Khader (2003).

RESULTS AND DISCUSSION

The activity of aldolase in the serum of *Channa punctatus* is significantly increased under thiamethoxam toxicity during different hours of exposure (Table 2). The important function of aldolase is to breakdown the Fructose-1, 6-bis phosphate into Glyceraldehyde – 3 phosphate and dihydroxy acetone phosphate, which in turn participate in further energy yielding reaction of glycolysis. And it is also an important tool to measure muscular dystrophy and impairment of liver under toxic condition. Singh and Singh (2004) observed similar results in fish *catla catla* when exposed to alphas-methrin compound.

Table 1. Physicochemical Parameters of Water

Sl.No.	Parameters	Values
1	Temperature	22-24°C
2	PH (Hydrogen ion Concentration)	7.2-7.3
3	Electrical Conductivity (Milliohms/cm)	0.52
4	Calcium (mg/l)	5.0
5	Sodium (mg/l)	2.1
6	Bicarbonate (mg/l)	142
7	Total alkalinity (mg/l) as (CaCO ₃)	69
8	Sulphate (mg/l)	7.1
9	Nitrates (mg/l)	3.4
10	Iodine (mg/l)	0.01
11	Chlorides (mg/l)	37.0

12	Dissolved Oxygen (mg/l)	9.2
13	Biological Oxygen demand (BOD)	1.6
14	Chemical Oxygen Demand (COD)	0.008
15	Free Carbon dioxide (mg/l)	10.0
16	Fluoride (mg/l)	0.03

Kuzmine *et al.*, (2002) made similar observations that insecticides could cause high mobilization of fructose 1-6 bisphosphate for the rapid production of energy under stress condition. In *C. punctatus*, the activity of LDH is reduced under thiamethoxam toxic exposure to different time hours. During hypoxic condition LDH converted into pyruvate by the utilization of NADH (Wilkie, 1976, Harper *et al.*, 1985). The inhibition of serum LDH may cause metabolic shift from aerobiosis to anaerobiosis (Abdul Naveed *et al.*, 2006).

In the present study, the activity of ICDH is significantly enhanced in the serum of *C. punctatus*, during prolonged exposure periods. The activity of this enzyme is a sensitive indicator of the animal in citric acid cycle. Singh *et al.*, (1996) reported that aldrin could cause modulation of oxidative enzymes in cat fish, *Heteropunestes fossils*. Enhanced pyruvate Kinase (PK) activity is under prolonged toxicity of thiamethoxam in *C. punctatus*, which generate ATP from ADP (Abdul Naveed *et al.*, 2006). It is further supported PK elevated levels during toxic stress condition leading to yield excessive energy (David and Michael, 2005) under toxic conditions recycling of NAD⁺ also involved in EMP Pathway. Thus, it is involved in the regulation of glycolysis and gluconeogenesis (Quayyam and Shaffi, 1997) in fresh water fish.

Activities of serum transaminases i.e; SGPT and SGOT were enhanced during the toxicity of thiamethoxam in *C. punctatus* to overcome the toxicity by excessive yield of energy for feeding the amino acid pool into keto acid in the presence of transaminases. Since the transamination is the key reaction affecting various metabolic process such as the formation of non-essential aminoacids and waste products. Hence, the measurement of transaminases is an

Table 2. Alterations in serum enzymes of fresh water fish, *Channa punctatus* exposed to thiamethoxam.

S.No.	Parameters	Control	Thiamethoxam			
			24 hrs	48 hrs	72 hrs	96 hrs
1	Aldolase (μ moles/L)	1.92 \pm 0.81	2.04* \pm 0.69 pc=6.25	2.84 \pm 0.79 pc=47.91	3.28 \pm 0.45 pc=70.83	4.08 \pm 0.47 Pc=112.5
2	LDH (mM of formazone formed/ml/hr)	0.49 \pm 0.06	0.47* \pm 0.036 pc=-4.08	0.44* \pm 0.041 pc=-10.20	0.40 \pm 0.02 pc=-18.36	0.38 \pm 0.015 pc=-22.44
3	Isocitrate dehydrogenase (μ moles/L)	0.28 \pm 0.08	0.30* \pm 0.025 pc=7-14	0.34* \pm 0.01 pc=2.14	0.37* \pm 0.05 pc=3.21	0.388* \pm 0.041 pc=3.57
4	Pyruvate kinase (PK) (μ moles/L)	12.01 \pm 0.05	13.01* \pm 0.68 pc=8.32	14.73 \pm 0.76 pc=22.64	15.84 \pm 0.78 pc=31.89	17.66 \pm 0.83 pc=47.04
5	SGPT (mM of Pyruvate formed/ml/hr)	29.14 \pm 2.34	30.09* \pm 1.17 pc=3.26	33.82 \pm 1.24 pc=16.06	36.73 \pm 1.92 pc=26.04	38.20 \pm 0.86 pc=31.09
6	SGOT (mM of pyruvate/formed/ml/hr)	20.36 \pm 1.17	21.48* \pm 1.72 pc=5.50	24.83 \pm 1.62 pc=21.95	26.04 \pm 0.86 pc=27.89	28.32 \pm 1.09 pc=39.09
7	Alkaline Phosphatases (mM of ip/ml/hr)	14.86 \pm 1.36	15.12* \pm 1.81 pc=1.74	17.20 \pm 1.24 pc=15.74	19.13 \pm 0.91 pc=28.73	21.26 \pm 0.98 pc=43.06
8	Acid Phosphatases (mM of ip/ml/hr)	8.92 \pm 1.13	9.17 \pm 0.79 pc=2.80	10.34 \pm 0.47 pc=15.19	11.86 \pm 1.01 pc=32.95	12.14 \pm 1.46 pc=36.04
9	Creatine kinase (μ moles/L)	19.21 \pm 0.84	20.42* \pm 1.36 pc=6.29	24.32 \pm 2.86 pc=26.06	27.81 \pm 1.84 pc=44.76	30.24 \pm 1.86 pc=57.41

Each value is mean \pm SD of six individuals. PC denotes Percentage Change over control. 1,3 and 4. Means were compared with Mann – Whitney μ test at $p < 0.05$ for statistical significance.

* not significant.

indicator of water pollution in fish (Vanderroost *et al.*, 2003). Abdul Naveed *et al.*, (2004; 2005) made similar observation in *C. punctatus*. The acid and alkaline phosphatases were gradually increased under the toxic effect of thiamethoxam in *C. punctatus*. During toxic stress, the fish needs excessive energy demand to overcome the toxicity, therefore, it is assumed that the hydrolytic activity and cleavage of phosphoric acids by the phosphatases might have been enhanced (Dalela *et al.*, 1980).

The serum phosphatases might suggest an increase in the lysosomal mobilization and onset of catabolism due to insecticidal toxicity (Abdul Naveed *et al.*, 2005). Satyanarayana (2005) and Power (1983) reported that phosphatases associated with elevated serum bilirubin is an indicator of biliary obstruction of cirrhosis of

liver and hepatic tumors. Creatine Kinase (CK) was gradually increased with different exposure timings in *C. punctatus*. It indicated that the fish was enhanced over exercised under toxicity in turn it required additional energy. Hence, the levels of CK for yielding of creatine phosphate. During toxic exposure periods, the fish faced the problem of hypoxia (Srinivasulu Reddy *et al.*, 1987). CK might cause various diseases, such as motor neurone disease, muscular dystrophy and hypothermia (Haerold Varley, 2005).

ACKNOWLEDGEMENTS

One of the authors V. Anil Kumar thanks to the Head, Dept. of Zoology, Kakatiya University, Warangal for providing necessary facilities and acknowledges to Dr. P. Venkateshwarlu, Reader

in Zoology, KDC, Warangal. For helping in preparation of manuscript.

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