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Research Article

Exposure of Sublethal Concentration of Imidacloprid Alters Serum Enzymes in Fresh Water Fish, *Channa punctatus* (Bloch)

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ABSTRACT

Sublethal concentration of Imidacloprid (organochloride), on the serum enzymes of fish, *Channa punctatus* were found to be 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 imidacloprid. The high activities of transaminases indicated that imidacloprid could induce malignancy 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.

1. Introduction

Aquatic ecosystems are highly vulnerable to agrochemical contamination, with neonicotinoids such as imidacloprid posing a growing concern due to their persistence and bioactivity. Channa punctatus (Bloch), a freshwater teleost widely distributed in South Asia, serves as a sensitive bioindicator for assessing pesticide-induced stress. Sublethal exposure to imidacloprid has been shown to disrupt key biochemical and enzymatic pathways, particularly those linked to hepatic and renal function. Elevated levels of serum enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) reflect hepatocellular damage and altered metabolic activity, while oxidative stress markers indicate compromised antioxidant defense mechanisms. Recent investigations on freshwater fish exposed to agricultural and industrial pollutants demonstrate similar enzyme perturbations, reinforcing the role of biochemical biomarkers in ecotoxicological monitoring. Moreover, imidacloprid's sublethal toxicity manifests in hematological imbalances, including reduced hemoglobin and erythrocyte counts alongside elevated leukocyte levels, suggesting systemic stress responses. These findings emphasize that even non-lethal concentrations of imidacloprid can impair physiological homeostasis, threatening fish health and ecosystem stability. Therefore, evaluating serum enzyme alterations in C. punctatus under sublethal imidacloprid exposure provides critical insights into pesticide-induced biochemical disruptions and strengthens the case for stricter regulation of agrochemical use in aquatic environments.

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 and Gruzdev, 1983). Among the OC insecticides, imidacloprid 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 and Sharma and Singh, 2006). Therefore, in the present investigation the impact of commercial grade imidacloprid on serum enzymes of fresh water edible fish, Channa punctatus was studied.

2. Material 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 180 X 90 X 90 cm (6 x 3 x 3 feet) at least 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 imidacloprid (0.58ppm) 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.

Table 1. Physicochemical Parameters of Water.

No.	Parameters	Values
1	Temperature °C	22-24
2	pH	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 (CaCO3)	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 Oxy. demand (BOD)	1.6
14	Chemical Oxy. Demand (COD)	0.008
15	Free Carbon dioxide (mg/l)	10.0
16	Fluoride (mg/l)	0.03

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).

3. Results and Discussion

The activity of aldolase in the serum of Channa punctatus is significantly increased under imidacloprid toxicity during different hours of exposure (Table 2). The important function of aldolase is to breakdown the Fructose-1, 6-bi phosphate into Glyceraldehyde – 3 phosphate and dihydroxy acetone phosphate, which, in turn, participate in further energy yielding reaction of glycolysis. 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 alphamethrin compound and 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 imidacloprid 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 imidacloprid 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; Lunavath et al., 2013) in fresh water fish.

Activities of serum transaminases i.e; SGPT and SGOT were enhanced during the toxicity of imidacloprid 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 indicator of water pollution in fish (Vanderoost 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 imidacloprid 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) gradually increased with different exposure timings in C. punctatus. It indicates that the fish over exercised under toxicity, in turn, it required additional energy. Hence, the levels of CK increased for yielding of energy from creatine phosphate. During toxic exposure periods, the fish faced the problem of hypoxia (Srinivasulu Reddy et al., 1987). The CK might cause various diseases such as motor neuron disease, muscular dystrophy and state of hypothermia (Haerold Varley, 2005; Sucharitha et al., 2013).

4. Conclusion

The alterations in serum enzymes of *Channa punctatus* exposed to imidacloprid clearly demonstrate progressive biochemical disruption with increasing exposure duration. Significant elevation in aldolase, pyruvate kinase, SGPT, SGOT, alkaline and acid phosphatases, and creatine kinase indicates hepatocellular damage, metabolic stress, and impaired energy regulation. Concurrently, the decline in LDH activity reflects compromised anaerobic metabolism, while the rise in isocitrate dehydrogenase suggests altered mitochondrial function. These enzyme fluctuations collectively highlight systemic toxicity, with hepatopancreatic and muscular tissues being primary targets. The consistent pattern of enzyme elevation over 24–96 hours underscores that even sublethal concentrations of imidacloprid can induce marked physiological stress, impairing metabolic homeostasis and threatening fish health. Thus, serum enzyme biomarkers provide sensitive indicators of pesticide-

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

Sl.No	Parameters μ moles/L of blood	Control	Imidacloprid treated			
			24 hrs	48 hrs	72 hrs	96 hrs
1	Aldolase μ moles/L	1.92 ± 0.81	2.01 ± 1.13 pc=44.68	3.14 ± 0.16 pc=63.54	3.84 ± 0.72 pc=100	4.08 ± 0.85 pc=112.5
2	LDH (μ moles of formazone formed/ml/hr)	0.49 ± 0.06	0.44 ± 0.04** pc=10.20	0.44 ± 0.017 pc=18.36	0.37 ± 0.018 pc=24.48	0.34 ± 0.04 pc=30.61
3	Isocitrate dehydrogenase μ moles/L	0.28 ± 0.08	0.31 ± 0.06* pc=10.71	0.35 ± 0.07 pc=25.00	0.39 ± 0.03 pc=39.28	0.47 ± 0.02 pc=67.85
4	Pyruvate kinase (PK) μ moles/L	12.01 ± 0.05	13.46 ± 0.18 pc=12.07	15.69 ± 0.17 pc=30.64	17.89 ± 0.21 pc=48.95	21.63 ± 0.026 pc=80.09
5	SGPT (μ moles of Pyruvate formed/ml/hr)	29.14 ± 2.34	31.84 ± 3.60* pc=9.26	34.28 ± 2.86 pc=17.63	37.18 ± 1.23 pc=27.59	39.12 ± 1.82 pc=34.24
6	SGOT (μ moles of pyruvate/formed/ml/hr)	20.36 ± 1.17	22.86 ± 1.09* pc=12.27	25.08 ± 1.24 pc=23.18	27.86 ± 1.47 pc=36.83	29.81 ± 1.09 pc=46.41
7	Alkaline Phosphatases (μ moles of ip/ml/hr)	14.86 ± 1.36	16.63 ± 1.92* pc=11.91	17.84 ± 1.23 pc=20.05	21.04 ± 1.49 pc=41.58	23.14 ± 2.73 pc=55.728
8	Acid Phosphatases (μ moles of ip/ml/hr)	8.92 ± 1.13	9.66 ± 1.07 pc=8.29	11.20 ± 1.29 pc=25.56	13.49 ± 1.16 pc=51.23	15.89 ± 0.84 pc=78.13
9	Creatine kinase (μ moles/L)	19.21 ± 0.84	21.04 ± 1.23* pc=9.52	22.16 ± 1.32* pc=15.35	24.35 ± 1.15 pc=26.91	27.34 ± 1.18 pc=42.32

induced toxicity, reinforcing the ecological risk posed by imidacloprid contamination in freshwater habitats.

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Competing Interests

The authors have declared that no competing interests exist.

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