



Effect of Imidacloprid on Glycolytic Enzymes Activity in Fish *Channa punctatus*

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Abstract

In this study the fresh water fish, *Channa punctus* (Bloch) is exposed to sublethal concentration (0.19ppm) of Imidacloprid for 24hrs, 48hrs, 72hrs and 96hrs exposure periods. The harmful toxic effect of this chemical is investigated by measuring key enzymes in carbohydrate metabolism. The investigation on substrates viz., Glycogen, Glucose, Pyruvate and Lactate is done during the exposure period. The levels of glycogen and pyruvate levels decreased while glucose and the lactate levels increased. From this data it can be concluded that Imidacloprid has more toxic effect by damaging the tissues at cellular level lead to modulation of the glycolytic enzyme. So the physiological significance of this toxicity is discussed.

Keywords: Imidacloprid, Glycolytic Enzymes, *Channa punctatus*.

INTRODUCTION

The organochloride insecticide commercially available as Imidacloprid is used as a treatment against ectoparasite and as an insecticide for crops Imidacloprid is poorly hydrolyzed and biodegrades slowly in the environment. So this compound persisted long time in the food chain and cause severe effects at the different levels of food chain. A review of the toxicological literature reveals that the exposure to toxic chemicals can produce unexpected effects in non target animals (Abdul Naveed et.al., 2004; Veronica and Collins, 2003; Gonzalez et.al., 2004). The effect of several pesticides and its by products of fish one for a long time investigated. Although many research workers reveals that these contaminates induced the different metabolism, histopathological changes and alter the haemogram of different vertebrate animals (Jeanmalie et.al., 2004; Kolbu et.al., 1997, Fair and Ricklefs, 2004; Carla Fenoglio et.al., 2005; Jen Kins et.al., 2003).

According to WHO (1983), NRC (1999), Chen et.al., (1985), chronic exposure of pesticides and other toxicants could cause anemia and cancer in the skin, bladder and lung. While OC pesticide is associated with several type of modulation in human beings and experimental animals. The purpose of present study was examined the inhibition of key enzymes of carbohydrate metabolism during the long term exposure of sublethal concentration of Imidacloprid to the fish, *Channa punctatus*.

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 . 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+1°C. The fish were fed *ad libitum* 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 LC50 of commercial grade imidacloprid (0.588 ppm) was determined for 48 hours by the method of Bayne et.al., (1977).

Batches of six fishes were exposed to 24,48,72 and 96 hours for sublethal concentration (0.19 ppm) along

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Table-1. The change in the levels of substrate under toxicity of Imidacloprid in fish, *Channa punctatus*.

	Parameters	Tissues	Control	Imidacloprid treated			
				24 hrs	48 hrs	72 hrs	96 hrs
1	Glycogen	Liver	1452±82.71	1263.11±39.80 pc= -13.008	1148.19±46.24 pc= -20.92	1058.64±28.70 pc= -27.09	859.33±39.60 pc= -40.817
		Brain	658.32±18.65	501.72±13.42 pc= -23.78	462.29±17.63 pc= -29.77	328.68±11.68 pc= -50.07	275.65±16.71 pc= -58.12
		Gill	964.84±20.85	803.61±42.65 pc= -16.71	765±40.65 pc= -20.71	633.81±17.84 pc= -34.30	526.84±17.65 pc= -45.39
		Muscle	1230.68±84.94	1126*±17.40 pc= -8.505	1086*±34.58 pc= -11.75	972.45±27.85 pc= -20.98	816.88±36.50 pc= -28.7
		Kidney	866.39±24.86	736.42*±18.72 pc= -15.00	680.11±21.65 pc= -21.50	529.32±36.82 pc= -38.90	496.20±41.86 pc= -42.72
2	Glucose	Liver	96.84±10.74	112.68±8.39 pc= 16.356	127.83±7.84 pc= 32.0	138.79±21.80 pc= 43.31	148.72±18.33 pc= 53.51
		Brain	48.34±6.82	59.30±8.84 pc= 22.67	66.35±7.65 pc= -37.25	78.49±4.54 pc= 62.37	86.15±9.81 pc= 78.21
		Gill	56.20±4.69	59.65*±7.65 pc= 6.49	72.85±8.11 pc= 29.62	81.08±3.26 pc= 44.27	88.29±6.84 pc= 57.09
		Muscle	61.89±3.21	71.82±3.69 pc= 16.04	79.86±7.42 pc= 29.03	82.84±6.82 pc= 33.85	88.39±3.44 pc= 42.81
		Kidney	33.41±2.81	36.84*±7.50 pc= 10.26	40.21±6.68 pc= 20.38	45.21±4.95 pc= 35.31	48.04±2.88 pc= 43.78
3	Pyruvate	Liver	42.83±6.34	37.80*±4.61 pc= -11.74	32.68±3.41 pc= -23.69	28.51±2.85 pc= -33.43	24.65±4.91 pc= -42.49
		Brain	27.63±6.64	24.81*±3.62 pc= -10.20	20.08±4.51 pc= -27.32	19.86±3.04 pc= -28.21	16.80±2.52 pc= -39.19
		Gill	32.45±4.63	27.03±4.61 pc= -16.70	22.38±2.75 pc= -31.03	17.33±4.05 pc= -46.59	15.80±8.61 pc= -51.3
		Muscle	37.85±2.68	35.69*±1.78 pc= -5.706	32.47*±4.69 pc= -14.21	27.49±3.82 pc= -27.37	23.64±4.85 pc= -37.54
		Kidney	18.89±3.85	15.34±2.46 pc= -18.79	12.86±3.81 pc= -31.96	8.86±2.65 pc= -53.0	7.06±2.49 pc= -62.62
4	Lactate	Liver	0.121±0.01	0.129*±0.011 pc= 6.611	0.131*±0.018 pc= 8.26	0.138*±0.016 pc= 14.04	0.143±0.010 pc= 18.18
		Brain	0.08±0.01	0.10103±0.01 pc= 26.25	0.121±0.02 pc= 51.25	0.127±0.023 pc= 358.75	0.132±0.016 pc= 65
		Gill	0.610±0.017	0.165*±0.0184 pc= 3.125	0.171*±0.009 pc= 6.875	0.178*±0.013 pc= 11.25	0.182*±0.007 pc= 13.75
		Muscle	0.143±0.012	0.147* ±0.015 pc= 2.797	0.152*±0.010 pc= 6.293	0.158*±0.013 pc= 10.489	0.161*±0.017 pc= 12.587
		Kidney	0.138±0.008	0.143*±0.010 pc= 3.623	0.147*±0.013 pc= 6.521	0.156*±0.009 pc= 13.04	0.159*±0.13 pc= 15.21

with control fish in separate tanks consisting of six liters of water at the room temperature. After stipulated time intervals the fish were removed and tissues were collected for further investigation of substrate and enzymes modulation. The physico-chemical parameters of tap water in which fish acclimatized are as follows.

Temperature 22-24°C, Hydrogen ion concentration (pH) 7.2-7.3; Electrical conductivity 0.52 milli ohms; Calcium 5mg/litre; sodium 2.1mg/lit, Bicarbonates 142mg/lit; Total alkalinity 69mg/litre; sulphates 7.1mg/litre; sulphates 7.1mg/litre; Nitrates 3.4mg/lit; Iodine 0.01mg/litre; Chlorides 37mg/lit; Dissolved oxygen

9.2mg/litre; Biological oxygen demand 1.6mg/lit; Chemical oxygen demand 0.008mg/lit; Fluoride 0.03mg/lit.

Extraction of Enzyme

Fish were killed by decapitation and liver, brain, gill, muscle and kidney tissue were taken out. These tissue were first sliced and the blood and water pressed out. A 10% homogenate (w/v) of each frozen tissue was prepared in a homogenizing buffer containing 30mM, Vernol buffer (pH6.0), 0.24M sucrose 1mM EDTA, 2 mM Mgcl₂, 1mM DTT and 3mM2 mercapto ethanol. The

homogenization was done with motor driven potter – Elve hjen type glass homogenized fitted with Teflon pestle. The homogenate was centrifuged at 750xg for 10 minutes and supernatant collected as enzyme. Extraction was done at 0 to 5°C.

Substrate Estimations

Glycogen was estimated by the method of Klicpera *et.al.*, (1957) and glucose estimated as described by Kemp *et.al.*, (1954). The levels of pyruvic acid estimated according to Friedman and Hangen (1942). The lactic acid estimated by the method of Barker and summer son (1942) modified by Huckbee (1961).

Each value in mean of 6 individuals, means were compared with Manu-Whitney is test of significance at $P < 0.05$ which is considered as statistically significant 1,2,3&4 were expressed in micrograms/100mg wet.wt of the tissue.

RESULTS AND DISCUSSION

Above table shows the levels of Glycogen, pyruvic acids decreased and increased levels of lactate, glucose in all three tissues viz., liver, brain, kidney, muscle and gill during toxic treatment of Imidacloprid. When animal needs energy during the environmental stress or other reasons, the stored glycogen is hydrolyzed into glucose, which then utilized as a source of energy. Jithender Kumar Naik *et.al.*, (2004), Nagpure and Zambore (2003), Tilak *et.al.*, (2005) reported that glycogen is used as a principle and immediate energy precursor in fish under stress condition. The levels of pyruvic acid is decreased in all the tissues of *Channa punctatus* during prolonged exposure periods of Imidacloprid. According to Harper (2005), Balaparameshwara Rao and Padmavathi (2004) the decrease in pyruvic acid may be due to increase in muscle contraction under toxicity of pesticides. Increase in blood glucose levels due to increased rate of glycogenolysis in insecticide exposed fish Prashanth (2003), Radha *et.al.*, (2005) and Subhendu Acharya *et.al.*, (2005). The levels of lactic acid were enhanced due to fish is attributed to the inadequate oxygen supply to cells to cope up with complete breakdown of carbohydrates to CO₂ and water Gopala Rao *et.al.*, (2006), Vutukeuru (2003).

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Competing interests

The authors have declared that no competing interests exist.

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