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Effects of Imidacloprid on the Hematological and Serum Biochemical Profile of *Labeo rohita*

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Abstract.- The present study was designed to assess the effect of chloro-nicotinyl insecticide, Imidacloprid, on the hematological and serum biochemical profile of fresh water fish *Labeo rohita.* A sub lethal dose of 120 mgL^{1} was applied under short (2, 4 and 8) and long term (16, 32 and 64 days) experimental condition. Pesticide effect was more pronounced in the short term experiments. Hemoglobin, RBC, PCV, MCV and platelet values reduced significantly in Imidacloprid exposed fish indicating severe anemic condition. Hyperglycemia, hypercalcemia and hypoprotonemia was observed in insecticide treatments indicating high energy demand of fish to neutralize the effect of toxicant. Significant increase in serum ALT, AST and lactate dehydrogenase shows the disturbance of liver physiology in *Labeo rohita* upon pesticide exposure. Observed effects were less severe in long term experiment indicating an inverse correlation between change in physiology and duration of Imidacloprid exposure.

Key words: *Labeo rohita*, Imidacloprid, hematological profile, serum biochemistry.

INTRODUCTION

In order to meet the growing food demand, use of pesticides is a common feature in agriculture now a days but extensive use of pesticide has resulted in global contamination of the environment and only 0.1 % of the applied pesticides reach the pests and remaining 99.9 % find their way to different components of environment (Marigoudar *et al*., 2009). 1-(6-chloro-3-pyridylmethyl)-*N*-nitro-imidazolidin-2-ylideneamine, Imidacloprid is a systemic chloro-nicitnyl and is relatively new pesticide (Tomizawa and Casida, 2005). It belongs to group called as neonicotinoids, which acts as agonist of the postsynaptic nicotinic acetylcholine receptors (nAChRs) which impairment of normal nerve function in organisms (Matsuda *et al*., 2001).

A hematological analysis is routinely used in determining the physiological state of animals which is known to be affected by different environmental factors and is used as a guide in the diagnosis of many diseases in both animals and humans (Solomon and Okomoda, 2012). Present study was designed to determine the LC_{50} value of Imidacloprid and to demonstrate the effect of sub lethal concentration of this most extensively used insecticide in Pakistan on selected hematological and serum biochemical parameters of *L. rohita.*

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MATERIALS AND METHODS

Specimen collection

Randomly selected 500 fingerlings of freshwater cyprinid fish Rahu (*Labeo rohita*) of both sex (body length 4.2-16.1 cm and body weight 8.83-57.79 g) were acclimated to laboratory conditions for two weeks in fiberglass containers with recirculation aerated system (RAS). All experiments were carried out in semi-static conditions with water renewal after every 24h. In treated groups, all the applied doses were maintained following every renewal. Temperature (25.6±2.5°C), pH (7.2±0.31) and oxygen concentration (7.8±0.45) mgL^{-1} in water were maintained throughout the experimental duration following Iqbal *et al.* (2005).

LC_{50} determination

For the determination of 96h Imidacloprid LC_{50} values, group of 10 juveniles of *L. rohita* were exposed to one of the seven concentrations *viz.*, 100, 200, 300, 400, 500, 600 and 700 mgL^{-1} of Imidacloprid. An untreated control group was

maintained in parallel. Fish mortality was observed after 24, 48, 72 and 96 h. LC_{50} values were calculated following Iqbal *et al.* (2005).

Experimental design

Experiment had short and long term phases. During short term phase, 3 treatment groups (each having 30 fish) were exposed to sub lethal concentration of 120 mgL^{-1} Imidacloprid for 2, 4 and 8 days, respectively, while in long term phase, fish were exposed to same dose for 16, 32 and 64 days. Separate control groups were used for each treatment. Each experimental treatment had a separate replicate. All fish were fed with ordinary fish diet used in fish farms (24% protein). All the experimental procedure and fish handling protocols were approved by ethical committee of Zoology Department at Bahauddin Zakariya University, Multan.

Blood sample collection

At the end of each experiment, blood sample was collected from each fish by making a cardiac puncture. Part of the blood was directly used to study hematological parameters hemoglobin (Hb), pack cell volume (PCV), red blood cells (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet counting by using hematology analyzer Sysmex-21 (Japan), while remaining blood was centrifuged at 13,000 RPM for ten minutes for separation of serum which was used for the determination of serum biochemical parameters *viz.*, glucose, total proteins, albumins, triacylglycerols, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), alkaline phosphatase (ALP), calcium (Ca^{2}), magnesium (Mg^{2}), inorganic phosphate, uric acid, cholesterol, urea, creatinine, total bilirubin and lactate dehydrogenase (LDH) by using biochemical analyzers MICRO-LAB 300 (Merck, Germany).

Statistical analysis

All data is expressed as mean ± standard error of mean (SEM). Statistical package Mini Tab (Minitab, USA) was used to calculate two sample t-test to compare various parameters between control and treated groups.

RESULTS

Exposure of *L. rohita* to 120 mgL^{-1} of Imidacloprid divulged influential changes in fish behavior. An avoidance response was observed in Imidacloprid treated groups and fish showed abrupt and sluggish swimming movements in various directions. Occasional jumping and hitting against the walls of tanks was also observed. *L. rohita* experienced rapid scale loss and mucous secretion. Due to pesticide, body colour changed to light brown in treated groups and it was remarkably different from control group. As the Imidacloprid exposure time increased, fish tend to recover from disturbed condition and frequency of abnormal behavior decreased but swimming speed remained slow as compared to control and it was easy to catch treated fish with hand nets as compared to the untreated *L. rohita*.

The 96 h LC_{50} value for *L. rohita* treated with Imidacloprid was $550mgL^{-1}$. All fishes survived at $200mgL^{-1}$, while 100% mortality was observed at 900 mgL^{-1}.

Significant effect of Imidacloprid was observed on the hematological profile of *L. rohita*. The effects were more pronounced in 8 days Imidacloprid exposed group where Hb, PCV, RBC, MCV and platelet count was significantly higher in control group compared to fish exposed to $120mgL^{-1}$Imidacloprid for 8 days. All the hematological parameters remained unaffected when the fish was exposed to Imidacloprid for 2 and 4 days (Table I).

Effect of Imidacloprid was more pronounced on serum biochemical profile of *L. rohita* exposed for 2 and 4 days when compared with their respective control groups. In 2 days treated group, albumin, total protein, AST, inorganic phosphate, uric acid and urea were significantly decreased, whereas glucose, triglyceride, ALT, ALP, Mg^{++}, cholesterol, total bilirubin and LDH were significantly increased (Table II).

After 4 day treatment albumin, ALP, inorganic phosphate, triglyceride and LDH were significantly decreased, whereas glucose, total protein, AST, ALT, CK, Ca^{++}, uric acid and total bilirubin were significantly increased (Table II). In 8 days pesticide treated group all studied parameters remained unaffected when compared with their

Table I.- Effect of Imidacloprid exposure on hematological parameters of *Labeo rohita* under short term experimental conditions.

Parameters	2 day treatment		4 day treatment		8 day treatment	
	Control	Imidacloprid-treated	Control	Imidacloprid-treated	Control	Imidacloprid-treated
HB (gdl^{-1})	4.5 2 ± 1.6	6.9 ±2.09 ^{NS}	4.4±2.40	4 ± 1.2 ^{NS}	5.03 ± 1.07	1.4 ± 0.1***
PCV (%)	12.23 ± 6.3	21.9±8.71 ^{NS}	10.1±4.67	10.1 ± 3.6 ^{NS}	16.7 ± 3.65	4 ± 0.1***
RBC (%)	1.36 ± 0.67	1.82±0.44 ^{NS}	1.22±0.45	0.9 ± 0.54 ^{NS}	1.797 ± 0.38	0.5 ± 0.1***
MCH (pg)	34.61 ± 2.98	37.55±2.96 ^{NS}	34.8±6.79	36.43 ± 1.72 ^{NS}	27.9 ± 0.035	28.0± 1.0
MCHC (gdl^{-1})	38.31 ± 5.8	32.45±3.86 ^{NS}	37.6±2.97	37.1 ± 1.54 ^{NS}	30.2 ± 0.49	35.0± 1.0
MCV (f l)	89.44 ± 7.8	117.3 ±20.79 ^{NS}	81.3±8.06	98.2 ± 3.31 ^{NS}	92.81 ± 1.43	80.0± 1.0***
Platelet (/μL)	79584.4±94936	97000±132063.1 ^{NS}	188000±118793.9	46320±2730.9 ^{NS}	12575 ±2667	3500 ± 1.0***

Mean±SD; Student's t test: ***P<0.01; NS, non significant.

Table II.- Effect of Imidacloprid exposure on serum biochemical profile of *Labeo rohita* under short term experimental conditions.

Parameters	2 day treatment		4 day treatment		8 day treatment	
	Control	Imidacloprid-treated	Control	Imidacloprid-treated	Control	Imidacloprid-treated
Glucose ($mgdl^{-1}$)	71.3±37.5	124.4±54.4 ^{NS}	71.3±28.4	124.4±35.6***	83.0±17.0	94.0±.07 ^{NS}
Total protein ($mgdl^{-1}$)	2.85±0.90	0.85±0.08**	2.85±0.68	3.83±0.58***	1.95± 0.07	1.65±0.21 ^{NS}
Albumin (gdl^{-1})	2.58±0.42	1.66±0.67 ^{NS}	1.75±0.45	1.66±0.43 ^{NS}	0.80±0.14	0.75±0.07 ^{NS}
Triglyceride ($mgdl^{-1}$)	234.0±0.70	313.0±35.9***	146.7±46.1	134.9±24.3 ^{NS}	38.5±0.70	57.0±7.07 ^{NS}
Asparate aminotransferase (AST) (IU/L)	1040.0 ±0.70	1030.5±11.3 ^{NS}	900.0±80.2	1021.0±0.37***	253.5±58.7	87.5±89.8 ^{NS}
Alanine aminotrasferase (ALT) (IU/L)	767.0±0.70	1216.5±3.75***	687.5±52.1	1617±475***	32.0±2.83	27.0±2.83 ^{NS}
Creatin kinase (CK) (IU/L)	-	-	1045.0±0.48	4888±1077***	629.5±14.8	945±310 ^{NS}
Alkaline phosphatase (ALP) (IU/L)	242.0±0.70	288.5±0.28***	405.0±88.2	182.5±40.6***	61.5±3.54	137.0±17 ^{NS}
Calcium (Ca) ($mgdl^{-1}$)	-	-	4.70±0.05	5.85±0.13***	3.0±0.14	2.6±0.14 ^{NS}
Magnesium (Mg) ($mgdl^{-1}$)	2.0±0.07	5.85±0.14***	2.10±0.04	2.03±0.14 ^{NS}	1.69±0.007	2.0±0.14 ^{NS}
Inorganic phosphate ($mgdl^{-1}$)	7.4±0.07	2.40±0.173***	8.15±0.50	7.0±0.71***	2.20±0.14	2.3±0.28 ^{NS}
Uric acid ($mgdl^{-1}$)	6.64±4.18	5.05±0.028 ^{NS}	2.0±0.21	3.56±0.28***	0.60±0.14	0.45±0.071 ^{NS}
Cholesterol ($mgdl^{-1}$)	115±0.70	466±69.9***	131 ±75.6	172.7±45.7 ^{NS}	67±5.66	89.5±7.78 ^{NS}
Urea ($mgdl^{-1}$)	10.0±0.70	9.0± 0.816*	12.0±3.3	16.0±3.61 ^{NS}	22.0±4.24	13.0±2.83 ^{NS}
Creatinine ($mgdl^{-1}$)	0.5±0.12	0.65±0.028 ^{NS}	0.83±0.18	0.625±0.04 ^{NS}	0.20±0.14	0.15±0.07 ^{NS}
Total bilirubin ($mgdl^{-1}$)	0.10±0.007	0.55±0.02***	0.09± 0.0005	0.60±0.037***	0.65±0.49	0.65± 0.21 ^{NS}
Lactate dehydrogenase (IU/L)	575.0±0.70	628.5±13.0***	520.0±37.4	488.5±59.5 ^{NS}	707.0±11.3	631 ±50.9 ^{NS}

Mean±SD; Student's t test: NS, non significant; *P>0.05 or; **P<0.01, ***P<0.001

respective untreated control group (Table II). Our results are clearly indicating the stress in *L. rohita* following Imidacloprid exposure. Liver and kidney were the major affected organs as their marker parameters were severely affected in treated groups.

No significant effect of Imidacloprid on all the studied hematological parameters was observed when treated fish was compared with untreated *L. rohita* under long term experimental conditions (Table III). All studied serum biochemical parameters remained unaffected in 16 days pesticide treated group (Table IV). Result indicated that CK (P<0.000) was the only parameter found to be significantly decreased after 32 days of Imidacloprid treatment (Table IV). Effect of Imidacloprid was more pronounced in fish treated with pesticide for 64 days. In this group, glucose, triglyceride and cholesterol were significantly decreased, whereas Ca^{++} and inorganic phosphate significantly increased (Table IV). Our result indicated that *L. rohita* has a tendency to neutralize the effect of Imidacloprid over the period of time as majority of the parameters did not reach the statistical significance when pesticide treated group was

Table III.- Effect of Imidacloprid exposure on hematological parameters of *Labeo rohita* under long term experimental conditions.

Parameters	16 day treatment		32 day treatment		64 day treatment	
	Control	Imidacloprid-Treated	Control	Imidacloprid-treated	Control	Imidacloprid-treated
HB (gdl^{-1})	4.8 ± 1.04	4.40 ± 0 .62 ^{NS}	5.3 0 ± 1.78	6.65 ± 1.69 ^{NS}	5.39 ±1.42	6.08±1.56 ^{NS}
PCV (%)	15.8 ± 3.56	14.33 ± 2.08 ^{NS}	17.5 ± 5.80	22.0 ± 5.35 ^{NS}	17.77 ± 4.67	20.0±5.14 ^{NS}
RBC (%)	1.71 ± 0.37	1.57 ± 0.22 ^{NS}	1.89 ± 0.63	2.4 ± 0.60 ^{NS}	1.93 ± 0.50	2.16±0.55 ^{NS}
MCH (pg)	28.01 ± 0.04	28.03 ± 0.02 ^{NS}	28.00 ± 0.04	28.01 ± 0.04 ^{NS}	27.9 ± 0.26	28.01±0.04 ^{NS}
MCHC (gdl^{-1})	30.4 ± 0.41	30.71 ± 0.11 ^{NS}	30.3 ± 0.92	30.2 ± 0.35 ^{NS}	30.32 ± 0.71	30.4 ± 0.40 ^{NS}
MCV (f l)	92.0 ± 1.19	91.27 ± 0.30 ^{NS}	92.64 ± 2.81	92.84 ± 1.11 ^{NS}	91.62 ± 1.89	92.24 ± 1.16 ^{NS}
Platelet (/µL)	12000 ± 2598	11000 ± 1561 ^{NS}	13250 ± 4449	16625 ± 4235 ^{NS}	13469 ± 3547	15188 ± 389 ^{NS}

p>0.05 NS or non significant.

Table IV.- Effect of Imidacloprid exposure on serum biochemical profile of *Labeo rohita* under long term experimental conditions.

Parameters	16 day treatment		32 day treatment		64 day treatment	
	Control	Imidacloprid-treated	Control	Imidacloprid-treated	Control	Imidacloprid-treated
Glucose ($mgdl^{-1}$)	70.3 ± 25	102.7 ± 15.8 ^{NS}	54.5 ± 35.5	113.5 ± 35.5 ^{NS}	30.0 ± 0.1	25.35 ± 1.9**
Total protein ($mgdl^{-1}$)	3.13 ± 0.94	2.56 ± 0.25 ^{NS}	2.4 ± 0.65	2.03 ± 0.30 ^{NS}	2.67 ± 1.0	1.65 ± 0.19 ^{NS}
Albumin (gdl^{-1})	1.06 ± 0.47	0.73 ± 0.05 ^{NS}	0.70 ± 0.78	0.33 ± 0.15 ^{NS}	1.07 ± 0.47	0.38 ± 0.12 ^{NS}
Triglyceride ($mgdl^{-1}$)	144.3 ± 22.1	122.3 ± 29.2 ^{NS}	138 ± 115	127 ± 0.9 ^{NS}	122.0 ± 5.20	83.5 ± 5.79**
Asparate aminotransferase (AST) (IU/L)	280 ± 119	120.3 ± 68.5 ^{NS}	638 ± 600	104.0 ± 18.0 ^{NS}	243 ± 119	132.0 ± 37.9 ^{NS}
Alanine aminotrasferase (ALT) (IU/L)	40.0 ± 19.1	23.0 ± 12.1 ^{NS}	75 ± 46.0	22.0 ± 9.85 ^{NS}	21.0 ± 4.0	20.8 ± 3.31 ^{NS}
Creatine kinase (CK) (IU/L)	-	-	4729 ± 0.9	967.0 ± 0.9***	-	-
Alkaline phosphate (ALP) (IU/L)	41.7 ± 4.4	55.7 ± 8.50 ^{NS}	59.7 ± 54.2	73.7 ± 40.1 ^{NS}	55.7 ± 13.7	57.5 ± 8.12 ^{NS}
Calcium (Ca) ($mgdl^{-1}$)	12.03 ± 1.37	9.67 ± 0.76 ^{NS}	5.77 ± 1.44	9.23 ± 0.61 ^{NS}	4.57 ± 0.75	9.67 ± 0.56**
Magnesium (Mg) ($mgdl^{-1}$)	2.93 ± 0.66	1.33 ± 0.94 ^{NS}	2.0 ± 0.79	2.97 ± 0.60 ^{NS}	5.27 ± 0.66	4.18 ± 0.42 ^{NS}
Inorganic phosphate ($mgdl^{-1}$)	2.07 ± 2.45	1.87 ± 1.21 ^{NS}	4.70 ± 0.01	6.20 ± 0.60 ^{NS}	2.93 ± 0.85	13.2 ± 2.39***
Uric acid ($mgdl^{-1}$)	2.03 ± 0.55	0.70 ± 0.26 ^{NS}	1.03 ± 0.90	0.50 ± 0.01 ^{NS}	1.20 ± 0.30	1.65 ± 0.30 ^{NS}
Cholesterol ($mgdl^{-1}$)	131.0 ± 55.0	141.0 ± 67.8 ^{NS}	135 ± 132	106.0 ± 0.8 ^{NS}	173.7 ± 15.2	60.3 ± 7.61**
Urea ($mgdl^{-1}$)	6.0 ± 2.00	4.0 ± 1.10 ^{NS}	3.0 ± 1.00	2.50 ± 0.5 ^{NS}	8.33 ± 1.53	10.0 ± 1.79 ^{NS}
Creatinine ($mgdl^{-1}$)	0.13 ± 0.05	0.13 ± 0.06 ^{NS}	0.20 ± 0.1	0.15 ± 0.05 ^{NS}	0.13 ± 0.05	0.20 ± 0.08 ^{NS}
Total billirubin ($mgdl^{-1}$)	0.43 ± 0.49	0.09 ± 0.005 ^{NS}	3.05 ± 2.25	0.099±0.0006 ^{NS}	0.66 ± 0.58	0.11± 0.04 ^{NS}
Lactate dehydrogenase (IU/L)	425 ± 250	345 ± 165 ^{NS}	1517 ± 1485	263.3 ± 74.1 ^{NS}	761 ± 266	1101±1532 ^{NS}

NS P>0.05 or non significant, ** p<0.01, ***p<0.001

compared with the untreated group under long term experimental conditions.

DISCUSSION

Most insecticides affect the behavioral patterns of fish by interfering with the nervous systems [the activity of acetylcholinestrase (AChE)] and consequently lead to disorders in the fish response to environmental stimuli and mobility. Inhibition of AChE in fish is reported to be accompanied by an increase in acetylcholine (ACh) levels that can be dangerous since it will impact feeding capability, swimming activity, identification and spatial orientation of the fish species (Banaee *et al*., 2008). Irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom was reported in *L. rohita* upon Malathion and Cypermethrine exposure (Marigoudar *et al*., 2009) indicating that pesticides can affect nervous system of *L. rohita*. These observations justify the behavioral changes

observed in present study as Imidacloprid has reported effect on ACh receptors and might be involved in disturbed fish behavior.

In our study, the 96h LC_{50} of Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine] was found to be 550mgL^{-1}. Although the effects of various pesticides on *L. rohita* has been investigated in Pakistan but virtually there is no scientific documentation regarding effect of Imidacloprid on the mortality of *L. rohita*.

In present study, 8 days Imidacloprid exposed group showed significant variability in Hb, PCV, and RBC, MCV and platelet counting between pesticide treated and untreated control *L. rohita*. Reduction in hematological values was observed indicating anemia in the pesticide exposed fish which may be due to erythrpoiesis, haemosynthesis and osmoregulatory dysfunction or due to increase in the rate of erythrocyte destruction in haematopooetic organ (Seth and Sexena, 2003). Decreases in the number or size of red blood cells also decrease the amount of space they occupy, resulting in a lower hematocrit. Prusty *et al*. (2011) and Kumar *et al*. (2012) who had also reported reduction in RBC count, hemoglobin concentration and WBC, HCT in *L. rohita* upon chlorpyriphos and pyrethroid and λ-cyhalothrin exposure respectively.

Data analysis revealed a significant increase in blood glucose concentration in all *L. rohita* exposed to Imidacloprid as compared to their untreated control groups (Table II). Our results are in agreement with those of Das and Mukherjee (2003), Saravanan *et al*. (2010), Prusty *et al*. (2011) and Kumar *et al*. (2012) who had also reported hyperglycemia in *L. rohita* upon cypermethrin, endosulfan, fenvalerate and λ-cyhalothrin exposure. This increase in blood glucose concentrations is known as a general secondary response to stress of fish to acute toxic effects and is considered as a reliable indicator of environmental stress. It helps the animal by providing energy substrates to vital organs to cope with the increased energy demand (Saravanan *et al.*, 2010).

AST and ALT are found in the liver and due to diseases or injuries, when the cells are destroyed, these enzymes are released into plasma and their high concentration in plasma are considered as indicator of abnormal physiology. We have observed increased AST and ALT concentrations in various Imidacloprid exposed fish treatments. Similar increased activities of AST and ALT were reported in plasma of *L, rohita* exposed to Endosulfan and Fenvalerate by Saravanan *et al*. (2010) and Prusty *et al.* (2011).

ALP and LDH play a significant role in phosphate hydrolysis, membrane transport and considered a good bio-indicator of stress in biological systems especially in liver (Banaee *et al*., 2011). Our results are indicating that Imidacloprid is a serious toxicant for *L. rohita* on the basis of ALP and LDH fluctuation. A similar trend in *L. rohita* was reported by Das and Mukherjee (2003) upon Cypermethrin exposure.

Serum triglycerides under pesticide exposure was significantly higher than fish of control group suggesting hepatopathy which could be due to free radical induced oxidative stress. Similar results were observed after Fenvalerate exposure in *L. rohita* fingerlings (Prusty *et al*., 2011).

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