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Minute Concentrations of Imidacloprid Pesticide under Short Term Experimental Conditions to *Oreochromis niloticus* (Gift Tilapia) Significantly Alters Physiological and Histogical Parameters

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ABSTRACT

Imidacloprid is the most extensively used insecticide against agricultural insect pests in recent times; unfortunately, their excessive use has some detrimental effect to non-target organism such as fish and ultimately humans. In this research work *Oreochromis niloticus* (Gift Tilapia) was exposed to very low sublethal concentration of imidacloprid for 2,4, 8 and 16-days experimental conditions. Exposure of 6.5mm/L a low dose of imidacloprid can also have the ability to elicit some serious impairment in metabolism, morphological, Hematological, physiological, structural, biochemical and abnormal behavioral responses. Hematological changes lead to alteration in the normal hematological profile with a significant reduction in the level of erythrocytes, Hemoglobin, mean cell Hemoglobin concentration, leucocytes, Packed cell volume value and platelets moreover increase in Mean cell Volume (MCV) and MCH in experimental group. Biochemical analysis showed the increase in serum aminotransferase, alanine aminotransferase and hypercholesterolemia. The findings suggested that the prolonged exposure of very low dose of imidacloprid also have the potential to alter the histology of liver and heart, and blood serum biochemical parameters by exerting some serious metabolic distress to fish.

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INTRODUCTION

One of the best ways discovered by human beings to reduce the attack of pests on agricultural products is chemical control (Prashanth, 2011; Reethamma and Joseph, 2016). Chemical control has some adverse effect on the environment and human health and other non-targeted organisms (RS and Shaikh, 2013). The excessive use of pesticides resulted in global contamination. Only 0.1% of the applied pesticides reach the targeted pests but the remaining 99.9% become a part of different components of environment (Rudragouda Marigoudar *et al*., 2009). The devastating effects of pesticides are mainly due to the biomagnifications in the body of fish and other aquatic organisms; therefore, fish are widely used to evaluate the quality of ecosystem.

Oreochromis niloticus, Nile Tilapia is considered as one of the most common freshwater specie for toxicological studies (Abarike *et al*., 2019; Pauly and Cheung, 2018).

Pesticides enter into the body of fish directly from contaminated water through gills and reach to the circulatory system via blood capillaries. Persistence of these substances in the body induces many behavioral and histopathalogical changes (Chaudhry and Jabeen, 2011; Dutta and Dalal, 2008). Fish being a biological indicator of water pollution having the ability to bioaccumulate and retain these toxic pesticides. This can lead to some serious impairment in metabolism, physiological and structural changes in different organs. Accumulation of pesticides into the tissues depends upon exposure time and concentration (Napit, 2013). Therefore, the present study designed to evaluate the intoxication of imidacloprid on blood hematological, histopathological and biochemical parameters of *Oreochrimis niloticus* during short term exposure treatments.

MATERIAL AND METHODS

Sample collection

A random sample of 400 fry of *Oreochromis niloticus* with body length 12.7- 15.7cm and body weight of 39-57.79g were purchased from the fish hatchery Mianchanu and transported in aerated containers to the fish laboratory, University of Education Faisalabad. The collected fish samples after transportation were acclimated under laboratory conditions for two weeks within fiberglass containers and recirculation aerated system. Other experimental conditions maintained 24.6±2.5^{o}C temperature, pH 7.2 ±0.31 and oxygen concentration 7.3±0.45 mgL^{-1} and water renewal performed after every 24h as described (Umer *et al*., 2011).

Experimental design

For intoxication assessment *Oreochromis niloticus* exposed to imidacloprid for short term exposure. During short term exposure three treatment groups (each consisted of 30 fishes) were exposed to sub-lethal concentration (1/20th of LC50) of 6.5mgL^{-1} imidacloprid for 2 (48h), 4 (96h), 8 and 16-days duration along with the separate control group for each treatment group. All the experimental animals were fed with the ordinary fish diet commonly used in fish farms consisting of 24% protein. All experimental protocols and handling procedures were also approved by ethical committee of Education Lahore via application no UE/AR/2017/4761.

Blood and serum collection

Haematological and biochemical examination were performed after exposure treatments of 2-, 4-, 8- and 16-days duration. Blood was collected through direct cardiac puncturing by sterile syringe and was alienated into two parts, one for Haematological studies in EDTA vial and other for serum biochemical profiling.

Haematological and serum analysis

Haematological analysis includes the estimation of White Blood cells (WBC), Red Blood cells (RBC), Haemoglobin, Packed cell Volume (PCV), Mean cell Volume (MCV), Mean cell Haemoglobin (MCH) Mean cell Haemoglobin concentration (MCHC) and platelets counting were performed by using haematological analyser (CBC) Analyser, HORIBA Abx SAS France). Serum biochemical analysis was performed by separating serum for that purpose blood was centrifuged at 13000rpm for 10 minutes. After separation level of serum biochemical parameters, Aspartate transaminase (AST), Alanine transaminase (ALT), cholesterol, and Creatinine were estimated by using SELECTRA PRO M (Dieren, Netherlands) using diagnostic kit manufactured by Egy chem (Egypt).

Histopathology analysis

At the end of short-term experimental phase heart and liver were surgically removed from each treated and untreated fish for histopathological study. Tissues were sliced and fixed following the method of Shazia Qadir and Furhan Iqbal 2016.

Statistical analysis

Statistical package Minitab (version18, Pensylvania) was used to calculate two sample t-test to compare various parameter of Haematology and serum biochemical profile between imidacloprid treated and untreated fish group for short term experimental conditions.

RESULTS

Behavioral responses

The behavioral responses of *O. niloticus* were noticed daily and compared with the control group. Normal behavioral responses were observed for the control group but treated (by Imidacloprid) groups showed abnormal behavioral responses such as casual jumping and striking to the walls of containers (erratic and hysteric swimming) and rapid operculum movements. Clinical toxic signs of imidacloprid exposure in treated group included changed body colour e.g., light brown which was amazingly dissimilar from the control group. *O. niloticus* exposed to imidacloprid showed fast scales loss and continuous mucoid body secretions. The 96H

LC_{50} value for *O. niloticus* treated with Imidacloprid was 130mg/L. All fishes survived at 70mg/L, while 100% mortality was observed at 190mg/L imidacloprid concentration. When *O. niloticus* was exposed to sublethal (6.5 mg/L) concentration of imidacloprid for the short duration of time (2, 4, 8 and 16 days), significant effect was observed on hematological and serum biochemical parameters.

Haematological findings

Experimental findings of short-term exposure (48h) to 6.5mg/L of imidacloprid to *Oreochromis niloticus* indicated significantly increased values of platelets ($p = 0.04$), haemoglobin ($p < 0.002$) and mean cell hemoglobin (MCH) ($p < 0.001$). However, the level of white blood cells (WBCs) ($p = 0.001$), Mean Cell Volume (MCV) ($p = 0.005$) were significantly decreased in comparison to the control group of *O. niloticus*. However, values of red blood cells (RBCs), packed cell volume (PCV) and mean corpuscular heamoglobin concentration (MCHC) varied non-significantly in comparison to control group of *O. niloticus* (Table1). Analysis of results indicated that 96h exposure had significantly elevated level of Haemoglobin ($p < 0.001$), RBCs ($p < 0.001$), platelets ($p < 0.001$) and MCH ($p < 0.001$). However, level of WBCs ($p < 0.001$) was significantly reduced. But MCV, MCHC and PCV varied non-significantly ($p > 0.05$) (Table.1).

Results obtained from 8days exposure indicated that the values of RBCs ($p = 0.02$), platelets ($p < 0.01$), Haemoglobin ($p < 0.001$) and MCHC ($p = 0.002$) were significantly raised in treated group as compared to the control group. While a significantly declined WBCs ($p < 0.001$) and MCV ($P=0.003$) were recorded. Values of PCV and, MCH altered non-significantly ($p > 0.05$) when compared with control group (Table.1).

Under 16 days imidacloprid exposure, there was significant effect of Imidacloprid on all the studied hematological parameters as compared to control group. There was significant increase in RBCs ($p < 0.001$), WBCs ($I<0.001$), Heamoglobin ($p = 0.002$), MCV ($p = 0.020$), PCV ($p = 0.007$) and MCH ($p = 0.001$) in treated group as compared to control group while the level of MCHC decreased significantly in experimental group (Table 1).

Serum biochemistry

Results obtained from the sub-lethal concentrations of imidacloprid exposure showed pronounced effects on the serum biochemistry of *O. niloticus*. A treatment group of 48h exposure to 6.5mg/L imidacloprid showed significantly decreased Creatinine ($p = 0.001$) than untreated group. While the values of Alanine aminotrasferase (ALT), Asparate aminotransferase (AST) and Cholesterol varied non-significantly ($p>0.05$) when compared with control group (Table 3). Whereas 96h exposure to subleathel concentration of imidacloprid showed significantly increased Creatinine ($p = 0.003$) and cholesterol level ($p = 0.005$) than control group. Other serum parameters ALT and AST varied ($p > 0.05$) non-significantly (Table 2).

Similarly, 8 days exposure to sublethal concentration of imidacloprid showed significantly increased Creatinine ($p = 0.003$), ALT ($p < 0.001$) and AST level ($p = 0.008$) than control group, while the level of Cholesterol varied non-significantly, interestingly 16 days exposure to sublethal concentration of imidacloprid showed non-significant variation in all biochemical parameters (Table 2).

Histopathological findings

Histology of liver

The comparison between treated and control group showed no variation in the structure of nucleus, central vein or blood vessels of liver tissue that's why there is no congestion and hemorrhage: necrosis and dilations of central vein were noticed in control group (Figure 1A). Initially major histopathological changes were evaluated in liver such as wrinkling of hepatocyte cell membrane, necrosis and degeneration of hepatocyte, dilation of blood sinusoid, dislocation of nucleus and pyknosis of hepatic nuclei. Karryorhexis (destructive fragmentation of the nucleus of a dying cell) of hepatocytic cells was observed (Figure 1B). The control group showed normal arrangement of hepatocyte and blood sinousoid shape. The number and position of nucleus is also normal (Figure 1A). The treatment of 4 ,8 and 16 days exposure of imidacloprid on liver of *O. niloticus* during short term condition (B, C and D) section of liver tissue was not normal and abrupt change were observed due to chemical exposure of sub lethal concentration of imidacloprid, there was an increased degenerative and necrotic changes in the smooth muscles' fibers along with an increased number of infiltrating cells. Congestion or mean accumulation of blood and hemorrhage represents the rupturing and necrosis of blood vessels. The liver parenchyma exhibited an increase amount of infiltrating cellular population, increased individual hepatocytes necrosis and cytoplasmic vacuolation. The sinusoidal spaces were absent the population of Kupffer cells was also increased.

Table 1. Effect of sublethal-dose of imidacloprid (6.5mg/L) on hematological parameters of *O. niloticus* under short term experimental conditions.

Parameters	48h			96h			8day			16days		
	Control	Treatment	p-value	Control	Treatment	p-value	Control	Treatment	p-value	Control	Treatment	p-value
RBC (×1012/L)	1.144±0.098	1.340±0.12	0.3	1.050±0.042	1.722±0.054	0.001*	1.116±0.099	1.526±0.029	0.02*	1.048±0.042	1.764±0.028	0.001*
WBC(×109/L)	139.30±9.4	43.02±2.7	.001*	136.48±3.6	55.93±0.68	0.001*	136.80±8.7	53.47±0.71	0.001*	134.24±4.3	197.47±2.0	0.001*
Platelets(×109/L)	66.0±11	302.0±38	0.004*	79.2±20	247.60±0.55	0.001*	76.8±22	167.8±16	0.01*	96.6±16	134.9±14	0.123
Haemoglobin (g/dl)	5.000±0.40	8.28±0.52	0.002*	4.800±0.35	11.586±0.21	0.001*	4.840±0.30	11.382±0.25	0.001*	5.560±0.32	7.416±0.23	0.002*
MCV (fl)	158.92±2.7	84.6±13	0.005*	156.82±3.5	157.05±0.78	0.9	155.00±2.6	137.97±0.74	0.003*	154.82±1.6	160.51±0.66	0.020*
MCHC (g/dl)	43.76±0.58	28.5±6.0	0.06	42.82±1.5	41.85±0.75	0.6	41.90±1.5	52.55±0.31	0.002*	43.14±0.71	26.622±0.45	0.001*
PCV (%)	18.20±1.7	13.87±1.6	0.1	20.62±2.6	27.114±0.35	0.07	17.6±1.5	20.866±0.37	0.1	18.40±1.8	27.612±0.28	0.007*
MCH (pg)	43.76±0.58	71.34±3.2	0.001*	46.14±1.2	68.50±0.57	0.001*	46.38±0.63	47.33±0.57	0.2	46.36±0.45	42.15±0.50	0.001*

$p \leq 0.05$ = Significant; Data are presented in terms of mean ± S.E; RBC = Red Blood Cells, WBC = White Blood Cells (× 10^9/L) MCV = Mean Cell Volume (fl), PCV = Packed Cell Volume (%), MCHC= Mean Cell Haemoglobin Concentration (g/dl), MCH= Mean Cell Haemoglobin (pg).

Table 2. Effect of sublethal dose of imidacloprid (6.5 mg/l) on serum biochemical parameters of *O. niloticus* under short term.

Parameters	48h			96h			8days			16 days		
	Control	Treatment	p-value	Control	Treatment	p-value	Control	Treatment	p-value	Control	Treatment	p-value
ALT (IU/L)	25.00±2.6	32.88±2.9	0.058	26.40±2.9	20.40.00±1.7	0.8	31.40±2.6	80.90±0.62	p < 0.001	30.8±4.9	21.42±0.51	0.10
AST (IU/L)	26.20±2.4	22.84±0.39	0.38	28.00±2.1	23.60±0.75	0.2	25.60±2.3	39.14±0.39	0.008	26.40±4.1	77.20±37	0.25
Cholesterol (mg/dl)	178.60±3.3	182.02±2.1	0.55	172.4±4.8	186.20±0.73	0.05	164.60±4.9	462±300	0.38	165.6±6.2	167.77±3.3	0.87
Creatinine (mg/d)	5.20±0.40	0.552±0.088	p <0.001	0.250±0.068	0.480±0.037	0.03	0.240±0.051	0.594±0.018	0.003	0.230±0.051	0.25±0.017	0.9

$p \leq 0.05$ = Significant

Data are presented in terms of mean ± S.E

ALT=Alanine aminotrasferase, AST=Asparate aminotransferase

Histology of heart

Heart tissue of control compared with experimental group of sublethal exposure of imidacloprid for 4, 8 and 16 days. Control group samples have no abnormalities, but experimental groups were entirely damaged with the passage of time. At day 4 and 8 there was an increase in degenerative and necrotic changes in the smooth muscles' fibers of the myocardium along with an increase in the number of infiltrating cells. The individual muscle fibers were separated from each other and there was an increased vacuolation in the myocardial parenchyma along with dilation of central vein also take place (Figure 2 A, B).

At day 16 the changes were severe compared to control and at day 4 and 8. There was an excessive cellular infiltration in the myocardial parenchyma. Smooth muscle fibers showed necrotic changes and were detached from each other. At places vacuolar degeneration of the muscle fiber, muscle atrophy and cardiac muscle thinning was observed (Figure 2 C, D).

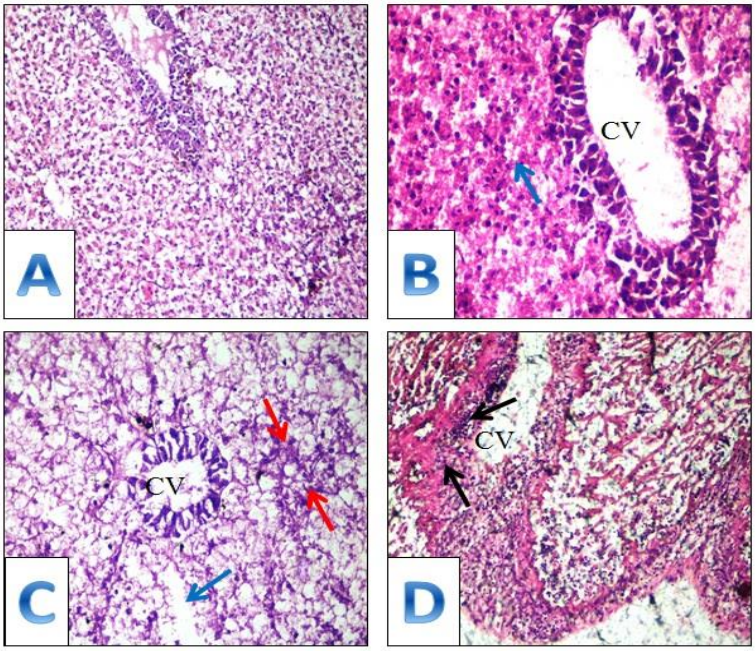

Figure 1. Transverse sections of liver stained with H and E.
A; control Hepatocyte is normal with minor changes B; 96 hours exposure: only dilation of central vein noted C; 8 days exposure: necrosis and dilation of central vein. D; 16 days karyorhexis of hepatocytes, degeneration of cell nucleus. Fusion of cell borders loss of cord like pattern of hepatocyte.

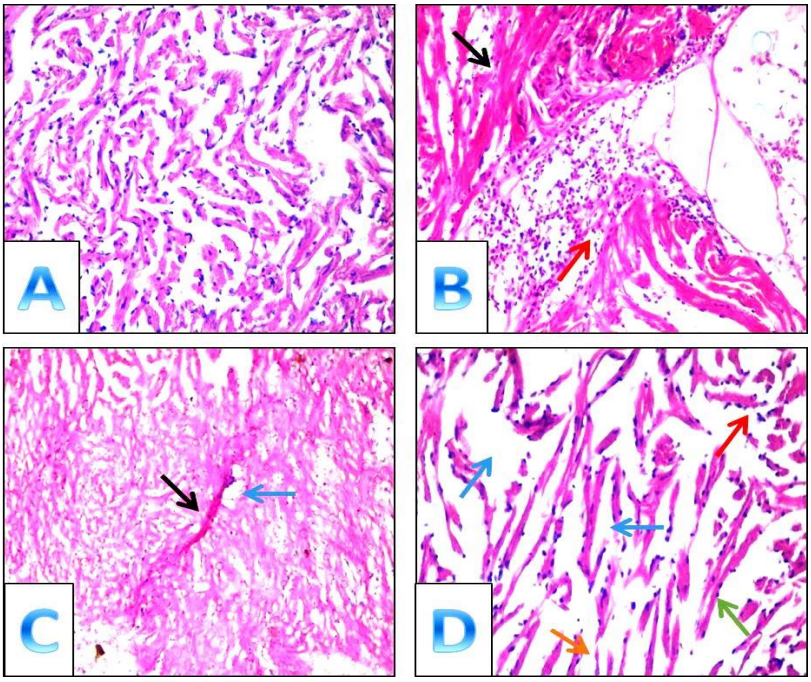

Figure.2. Transverse sections of heart stained with H and E.
A; Section of a control group heart and show normal arrangement of cell and tissue; B tissue section after 4 days of exposure shows necrosis along with Congestion and hemorrhage C; 8 days exposed tissue have severe stages of congestion and hemorrhage and dilation; D; slide of 16 days exposure of imidacloprid cause congestion and hemorrhage, necrosis, dilations, muscle atrophy and cardiac muscle thinning. Arrows represents abnormalities (Black), Congestion and hemorrhage: (Red), Necrosis; (Blue), Dilations; (Green), Muscle Atrophy; (Orange), Cardiac muscle thinning.

DISCUSSION

Extensive use of pesticides to eradicate the pests in agriculture has become the need of the time, unfortunately their indiscriminate use has resulted in detrimental effects on non-target species such as aquatic animals, fishes and ultimately humans. One of the most commonly used pesticides, imidacloprid is used extensively and have the ability to bind the acetylcholine receptors, ultimately causes the hindrance in the passage of nerve impulse which results in behavioral disorders of fish responses to the environmental stimuli. The activity of acetylcholine changes in response to imidacloprid exposure so it can be used as a biomarker to measure imidacloprid induced neurotoxicity (Gholami-Seyedkolaei *et al*., 2013). Acetylcholine level imbalance can be dangerous to fish by influencing different activities such as feeding capabilities, swimming activity, and spatial orientation (Banaee *et al*., 2011). Therefore, present observations of disturbed fish's behavioral responses are due to disturbed acetylcholine level in response to imidacloprid as described by the (Banaee *et al*., 2011).

Fish have the ability to bioaccumulate various toxicants therefore they can be used as a bioindicator of water pollution (Chaudhry & Jabeen, 2011; Dutta & Dalal, 2008). Accumulation of pesticides in vital organs of the fish results in dysfunctions and mortality of fishes (Srivastava & Kaushik, 2001). The stressed condition of fishes can be measured by assessing their behavioral responses, biochemical and histological parameters. Blood is an ideal indicator of toxic stress condition in fishes therefore various haematological parameters were measured to determine the impact of sub-lethal concentration of imidacloprid. Exposure of *O. niloticus* to sub-lethal concentration of imidacloprid caused significant alterations in blood hematological parameters such as overall increase in the level of RBCs, Heamaglobin MCV, PCV, platelets and MCH while WBCs showed differential level among all experimental groups.

The increased RBC numbers in blood of treated fish may be associated with their release from blood depots and activation of erythropoiesis in blood-forming tissues (Soldatov, 1996). Increase in the level of RBCs at early time of exposure may be an adaptaion through which fish might compensate for poor oxygen uptake in prevailing hypoxic condition (Varadarajan *et al*., 2013). Another adaptation for the survival in hypoxic condition is via the release of large number of RBC in general circulation it is thought to be stimulated by b- adrenergic action on the haemopoetic tissues (Gholami-Seyedkolaei *et al*., 2013). In acute stress condition, androgenic stimulus triggers splenic contraction and release of large number of RBC in the blood (Heath, 1995). packed cell volume (PCV) is the percentage of RBCs in the blood which depends upon the number as well as size of RBCs. Therefore, any change in the size and number of RBCs or Hb changes the PCV percentage. The hematological alterations in mean cell hemoglobin (MCH), mean cell volume (MCV), and mean cell hemoglobin concentration MCHC values also change because the calculation of these parameters depend on RBCs, Hb and PCV values. The group treated with imidacloprid (16 days) shows significant variation in the haemoglobin, MCV, MCHC, PCV and MCH.

In the present investigation significant elevation in total WBC & platelets count was noted in all the treated groups after 8h, 96h and 8days and 16days but maximum elevation was observed in 96h treated group. A similar trend in WBCs & platelets count was reported when *O. mossambicus* were treated with different concentrations of pesticide (Ghayyur *et al*., 2019). Our results are supported by similar investigations in *Ctenopharyngodon idella* exposed to Endosulfan during acute toxicity (Hasan *et al*., 2015). WBCs have a key role in the regulation of immunological function; the alteration in WBCs to pollutant shows a decline in nonspecific immunity of the fish. Monocytes and granulocytes play function in the removal of injured cell debris, while lymphocytes related to the production of antibodies. A higher percentage of WBCs is the normal response of fish when exposed to toxicants due to tissue damage and compensatory response of lymphoid tissue to circulating lymphocytes (Kavitha *et al*., 2010). It was observed that the counted value of WBCs of gift tilapia significantly increased after exposed to acute concentration (Table 1). In the treated group, WBCs count showed positive relation with time and reached maximum value at 16days. Rafiq *et al*., 2015 also observed a significant increase in WBCs in relation to time with cypermethrin (Ullah *et al*., 2015). Like our results, other scientist also observed increased in WBCs counts in response to other pesticides and other pollutants like methyl mercury (Adhikari *et al*., 2004). In the present study, WBCs level reduced during initial exposure of 48h, 96h and 8 days of exposure of imidacloprid this decrease may be due to decrease in the number of cells in the coelomic cavity or the haematopoisis provoking immunosuppressant (Kavitha

et al., 2010).

In vertebrates, including fish, liver is the main organ that plays an important role in detoxification of pesticides. During metabolism, liver has the ability to break down these harmful substances, but beyond a certain limit these toxic compounds disturb the regulating mechanism of the liver and cause morphological alteration (Brusle & Anadon, 1996). In environmental studies also blood and tissues level of AST and ALT have been measured to assess the toxic impact of pesticides. Hence increased and decreased AST and ALT concentrations in blood are considered as indicators of abnormal physiology (Ellakany and Gaafar, 2002).

In the present study decreased levels of AST and ALT concentration in blood are considered as an indicator of abnormal liver function. The decrease in the level of both enzymes in 8 days of treatment is probably due to decrease production of this enzyme. Other biochemical parameters such as cholesterol and creatinine levels are also directly influenced by environmental toxicants. These parameters can be used as biomarkers to determine stress condition in fishes as well as aquatic pollution. Imidacloprid causes alteration in the permeability of hepatic cells that can be interrelated with the altered cholesterol level and leads to the abnormal physiology of cells (Eraslan *et al*., 2007). ALT and AST enzymes are found in the different tissues of liver, heart, kidney, pancreas, muscles and spleen, erythrocytes, brain and gills. Their main function is the metabolism of carbohydrates and proteins and act as an indicator of cellular damage (Adhikari *et al*., 2004).

Physiological and metabolic activities of the fish are damaged by the toxicants. Physiological studies are not enough to satisfy the pathological conditions of tissue under chemical stress. Hence, it is important to have insight into histological analysis as they as a biological marker to assess the toxicity impact of the treatment (Srivastava & Shivanandappa, 2010). The intensity of tissue damage depends upon the length of period exposed to toxin (Fanta *et al*., 2003). Prolonged insecticide exposure has pronounced effects on histopathological profile of many fishes. The current study showed histology of liver and heart changed after prolonged exposure to of the sub-lethal concentration of imidacloprid. The necrosis in different parts of liver is due to extra workload on hepatocytes during detoxification of the imidacloprid. Current investigation showed a significant increased level of ALT, AST and creatinine in response to the sub-lethal concentration of imidacloprid after 16 days of exposure. This increased level may be attributed to the gills, liver or kidney damage. Similar findings are also reported by Gholami *et al*., (2013) who estimated increased ALT, AST ALP levels in common carp blood exposed to sublethal concentration of herbicide (Gholami-Seyedkolaei *et al*., 2013).

Initially major histopathological changes observed in liver includes wrinkling of hepatocyte cell membrane, necrosis and degeneration of hepatocyte, dilation of blood sinusoid, dislocation of nucleus and pyknosis of hepatic nuclei. Abrupt changes observed at 8- and 16-days exposure in response to exposure of imidacloprid; includes increase in degenerative and necrotic changes in the smooth muscles' fibers, the liver parenchyma exhibited an increase amount of infiltrating cellular population. There was an increase in individual hepatocytes necrosis and cytoplasmic vacuolation. The sinusoidal spaces were absent and the population of kupffer cells also increased. The control group showed normal arrangement of hepatocyte and blood sinousoid shape. liver after exposure to different conc. In relation to time our results are in accordance with the findings of (Kumar Maurya *et al*., 2019).

Histology of heart tissues for control group has no abnormalities but experimental groups show increased damage in relation to time. Initial exposure showed an increase in degenerative and necrotic changes in the smooth muscles' fibers of the myocardium along with an increase in the number of infiltrating cells. The individual muscle fibers were separated from each other and there was an increased vacuolation in the myocardial parenchyma along with dilation of central vein also take place. Long term exposure caused severe changes compared to control group; an excessive cellular infiltration in the myocardial parenchyma, Smooth muscle fibers were separated and showed necrotic changes, vacuolar degeneration of the muscle fiber, muscle atrophy and cardiac muscle thinning were also observed. Similar results were also recorded by Maurya *et al*. (2018) who investigated the effects of industrial wastewater containing pesticide on the liver, heart and muscle's histology of fish *Heteropneustes fossilis* (Maurya *et al*., 2019). The liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to pesticides (Rodrigues & Fanta, 1998). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. In

another study, cloudy swelling, bile stagnation, focal necrosis, atropy and vacuolization have been reported in the *Corydoras paleatus* exposed to methyl parathion (Fanta *et al*., 2003). Hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocytes, focal coagulative necrosis, disorganized hepatic canaliculi were observed by Sarkar *et al*. (2005) in *Labeo rohita* exposed to cypermethrin (Fanta *et al*., 2003; Sarkar *et al*., 2005). Hepatic lesions in the liver tissues of fish *Gambusia affinis* exposed to deltamethrin were reported such as hypertrophy of hepatocytes, increase of Kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis, narrowing of sinusoids (Gholami-Seyedkolaei *et al*., 2013).

CONCLUSION

The excessive use of pesticides such as imidacloprid has become the major cause of aquatic pollution. Fish being a biological indicator of water pollution and have the ability to bioaccumulate and retain these toxic pesticides. This can lead to some serious impairment in metabolism, physiological and structural changes in different organs. Accumulation of pesticides into the tissues depends upon exposure time and concentration. In this study it is reported that the exposure of *Oreochromis niloticus* to the very low-level concentration of imidacloprid for short-term exposure treatment can also cause some major alteration into the behavioral, Hematological, Biochemical and Histological changes. Therefore, it is recommended that these types of chemical pesticides should be used with specific and defined quantity under strict measures.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTION

N.M, M.A.A. and M.L. designed and conducted the research. A. U. and MSK wrote the first draft while H.U, R.I. and M.S revised the manuscript.

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