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Original Article

Evaluation of Hemotoxic, Hepatotoxic and Nephrotoxic Potential of Profenofos-based Insecticide in Freshwater Labeo rohita Fish at Low Concentrations

Arva Mahmood[1], Shabbir Ahmad[1], Hasnain Akmal[1] and Khurram Shahzad[1*]

[1]Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan

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***Corresponding Author:**
Khurram Shahzad
Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan
dr.khurram@uo.edu.pk

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ABSTRACT

Profenofos, an organophosphate, is a major pollutant that pollutes freshwater bodies, causing significant impacts on fish health. **Objective:** Present study was performed to assess the toxicological impacts of pesticide profenofos on hematological, biochemical and histological alterations in different organs of Labeo rohita. **Methods:** Fish were divided in three groups. Group one was treated as control while second and third groups were exposed to 0.6 mg/L and 1.2 mg/L profenofos respectively for 28 days. **Results:** Results revealed that MCV, MCHC, MCH, RDW-SD, PCT, PDW, HGB, RBC and HCT levels were significantly reduced. WBC, RDW, PLT, MPV, neutrophils, lymphocytes, monocytes and eosinophils were increased as compared to pesticide free group. Biochemical results showed significant increase in cholesterol, triglycerides, AST, albumin, A/G ratio, HDL T3, T4, blood glucose, creatinine and urea levels were documented while levels of LDL, VLDL, ALT, total proteins, globulin, TSH and blood urea nitrogen (BUN) decreased significantly in exposed fish. Furthermore, histological changes in kidney, gills and liver of fish showed degenerative effects after exposure to profenofos in both concentrations. **Conclusions:** The present study concluded that profenofos resulted in widespread toxic effects on aquatic organisms specially fish.

INTRODUCTION

Pesticides are essential to maintain high agricultural yield. Among various types of pesticides, organophosphates are commonly used in agricultural pest management because of their insecticidal effects and low toxicity to mammals [1]. However, these pesticides are introduced into the environment through various means, such as air, soil, food chains and water [2]. Profenofos (PFF) is an insecticide that belongs to organophosphate pesticide and is frequently utilized in agriculture to manage insect population in cotton, paddy, and tobacco [3]. It is effective in controlling a broad range of sucking and chewing insects, as well as mites, on several crops, especially cotton plants [4]. It is used in agriculture in both dissolved and suspended forms. This leads to sediment residue that seeps into the groundwater and is carried into water sources through agricultural runoff that is discharged via drainage and rainfall [5]. Prolonged and continuous contact to profenofos residue from agricultural runoff can inadvertently cause toxicity in various non-target aquatic animals [6]. Profenofos can harm beneficial aquatic microorganisms and algae, leading to the death of a large number of fish due to insufficient oxygen and the production of excessive ammonia from decomposition of rotting vegetation [7]. Pesticides have the potential to

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damage the biochemical and biological processes in fish when they enter their organs [8]. The study of toxicity on Brachydanio rerio, Oreochromis mossambicus and Cyprinus carpio have been previously reported [9]. Fish have been used as bio-indicators of the effects of pollutants as they are sensitive to environmental changes. In aquatic ecosystems, fish are crucial for determining the hazards of novel chemicals [10]. Fish devour more prominent measure of green growth, phytoplankton and diverse sea-going plant tainted with pesticide, which thus lead these synthetics to bit by bit collect in tissues and organs of fish [11]. By assessing changes in enzymological, hematological, biochemical parameters, and organ damage, it is possible to investigate the potential danger of pesticides to aquatic life [12]. The toxicity of profenofos on L. rohita needs to be evaluated because it is the most important fish due to its great commercial value as its flesh is rich in minerals and proteins and fast growth rate [13]. Present study was aimed to assess the toxicity of profenofos on certain hematological, biochemical parameters and histological changes in vital organs of a freshwater teleost L. rohita. Moreover, the selected parameters can serve as excellent biomarkers in environmental monitoring of profenofos contamination in aquatic ecosystems.

METHODS

Organophosphorus pesticide profenofos easily available and purchased from a local agrochemical store. Appropriate amount of profenofos was dissolved in water to make the stock solution for the experimental use. Labeo rohita (average weight 34.4 ± 2.64 g and length 15.93± 3.22 cm) were purchased from Head Balloki fish hatchery, District Kasur, Pakistan in plastic bag with aerated water. There was no mortality found during the transportation of fish from hatchery to university laboratory and acclimatized in glass tank having freshwater for 7 days and fed twice a day. The mechanically aerated water was changed after every 24 hours during the whole experimental work. After acclimatization, fish were divided into three groups and each group contains 15 fish. Group 1 assigned as a control group which was chemical free group and groups 2 and 3 were treated groups, exposed to profenofos as 0.6 mg/L and 1.2 mg/L for 28 days. Temperature, dissolved oxygen, pH and hardness of water was maintained. At the end of experimental trial, fish (n = 10) were removed from control and treated groups. Blood collected from abdominal vein by mean of BD syringe and stored in EDTA tubes for hematology and without EDTA tubes for serology. The hematological parameters viz., total red blood cells, hematocrit, white blood cells, mean cell hemoglobin, hemoglobin, mean cell hemoglobin concentration, platelets, mean corpuscular volume, red cell distribution width, mean platelets volume, platelet distribution width, procalcitonin, neutrophil, lymphocytes, monocytes and eosinophil counts were determined using automatic hematological analyzer (Mindray BC-20). The serum samples were used to evaluate ALT, AST, creatinine, BUN and urea levels using kits of Spin react (Esteve De Bas, Girona, Spain) in granting with the earlier related methods [14]. Serum albumin, total proteins and globulin were spectrophotometrically approximate using the methods reported by Rehman et al [15]. Moreover, serum glucose, cholesterol, HDL (high density lipoprotein), LDL (low density lipoprotein), VLDL (very low-density lipoprotein) and triglycerides were determined by the spectrophotometer according to the methods suggested by Allain et al [16]. The endocrine parameters (TSH, T3 and T4) were analyzed by CE approved 4th generation immunodiagnostic kits. After blood collection, control and treated fish were dissected humanely and kidney, liver and gills were removed and fixed in 10% formalin solution and dehydrated in 70-100% alcohol. Also cut tissues in small sections (4-5µm) and stain with the help of Gymsa stain and prepared slides photographed at 40X with the help of camera fitted optical microscope (Microscope XSZ-107BN). All the data were analyzed using descriptive statistics and the level of significance was less than 0.05. The total variations in results and graphical representation were also examined by using one-way ANOVA (Analysis of variance) on IBM SPSS (Version 21.0). For graphical representation, GraphPad Prism (Version 9.5.1) was used.

RESULTS

Hematological results of control and treated fish were shown in Table 1. Results indicated that WBC, RDW, PLT, MPV values were significantly increased in profenofos exposed fish as compared to chemical free control fish. MCV, MCH, MCHC, HGB, RBC, HCT, RDW-SD, PDW and PCT concentrations were significantly decreased. Neutrophils, lymphocytes, monocytes, eosinophils were also found to be increased significantly.

Table 1: Hematological parameter of Labeo rohita after exposure to two sublethal concentrations of profenofos

Parameters	Control	Low	High
HGB (g/dl)	4.76 ± 0.35	3.40 ± 0.36*	2.73 ± 0.41*
WBC (×10^3/µL)	25.40 ± 0.45	36.43 ± 0.41*	43.77 ± 0.85*
RBC (×10^6/ µL)	1.54 ± 0.04	1.36 ± 0.03*	1.09 ± 0.04*
HCT (%)	1.54 ± 0.04	1.36 ± 0.03*	1.09 ± 0.04*
MCV (FL)	153.5 ±3.37	115.0 ± 3.95*	57.63 ± 4.16*
MCH (pg)	48.27 ± 0.75	40.80 ± 1.11*	40.07 ± 1.28*
MCHC (g/dl)	98.87 ± 1.41	62.87 ± 1.41*	31.80 ± 2.49*
RDW	98.87 ± 1.41	16.00 ± 0.50	28.13 ± 0.45*
RDW-SD (%)	65.60 ± 1.77	53.07 ± 3.04*	34.90 ± 2.57*
PLT (×10^3/µL)	199.7 ± 8.87	344.7 ± 12.85*	514.8 ± 7.97*

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MPV (fl)	5.10 ± 0.36	9.10 ± 0.45*	14.10 ± 0.55*
PDW%	14.93 ± 0.51	8.43 ± 0.55*	7.23 ± 0.61*
PCT%	0.40 ± 0.02	0.21 ± 0.03*	0.14 ± 0.01*
Neutrophils %	61.00 ± 4.77	73.27 ± 5.70*	78.07 ± 2.75*
Lymphocytes %	25.73 ± 3.65	31.97 ± 4.21NS	44.87 ± 3.08*
Monocytes %	2.10 ± 0.36	4.03 ± 0.35*	5.96 ± 0.45*
Eosinophils %	1.10 ± 0.55	3.03 ± 0.45*	5.06 ± 0.30*

The data are represented as mean ± SD. Asterisk (*) bearing values show significant difference ($p<0.05$) as compared to the profenofos free group.

Statistical values of cholesterol, triglyceride, HDL, LDL and VLDL were shown in figure 1. Results showed significant increase in cholesterol, triglyceride and HDL levels in treated groups as compared to profenofos free group. While LDL, VLDL were significantly decreased.

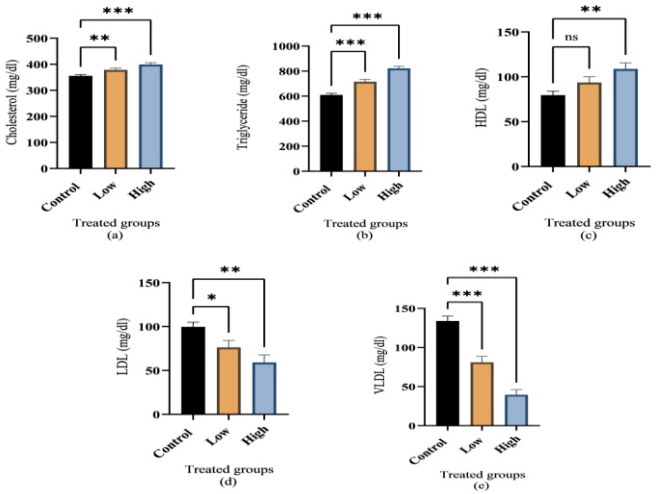

Figure 1: Variation in (a) cholesterol, (b) triglyceride, (c) HDL, (d) LDL and (e) VLDL levels of L. rohita after exposure to two profenofos amounts

The values are represented as mean ± SD at $p<0.05$ level of significance.

Levels of ALT, AST, albumin, globulin, A/G ratio and total proteins were showed in figure 2. ALT, globulin and total proteins levels were decreased significantly after the exposure of profenofos in treated group as compared to profenofos free group while AST, albumin and A/G ratio were significantly increased. The values of T3, T4 and TSH were showed in figure 3. The values of T3 and T4 were showed significant decrease and TSH was significantly elevated in treated groups as compared to profenofos free group.

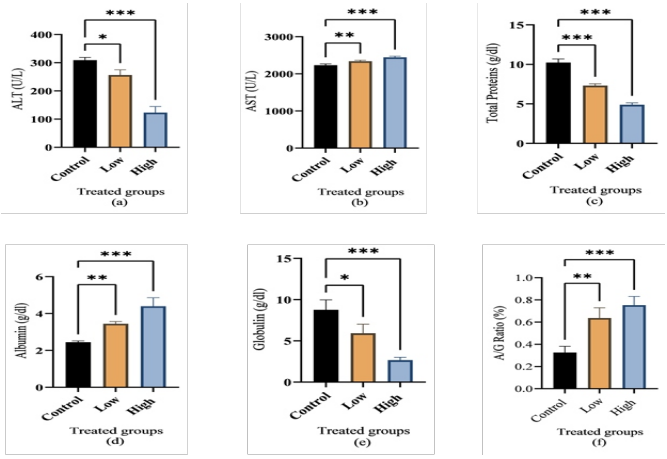

Figure 2: Variation in (a) ALT, (b) AST, (c) total Protein, (d) albumin, (e) globulin and (f) A/G ratio between control and treated groups

The values are represented as mean ± SD at $p<0.05$ level of significance.

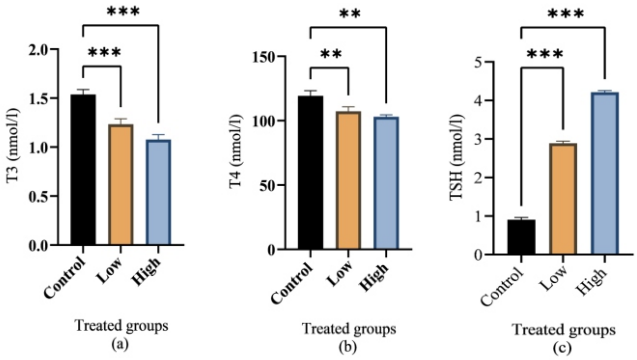

Figure 3: Variation in (a) T3, (b) T4 and (c) TSH levels between control and treated groups the values are represented as mean ± SD at $p<0.05$ level of significance.

The values of creatinine, blood glucose, urea and blood urea nitrogen were showed in figure 4. Blood glucose, creatinine and urea levels elevated significantly in treated groups as contrasted to control group while the value of blood urea nitrogen was significantly decreased.

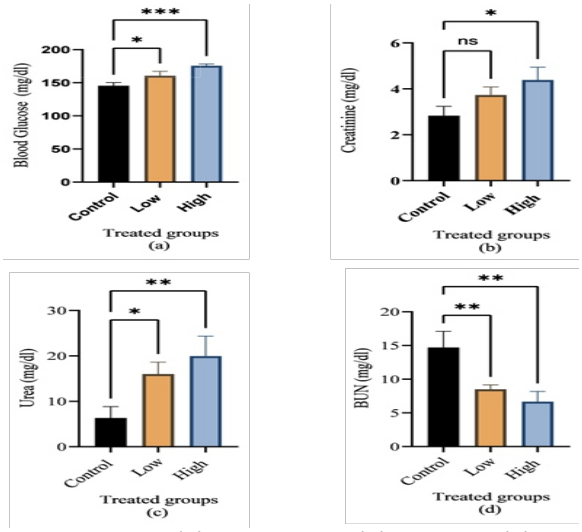

Figure 4: Variation in (a) blood glucose, (b) creatinine, (c) urea and

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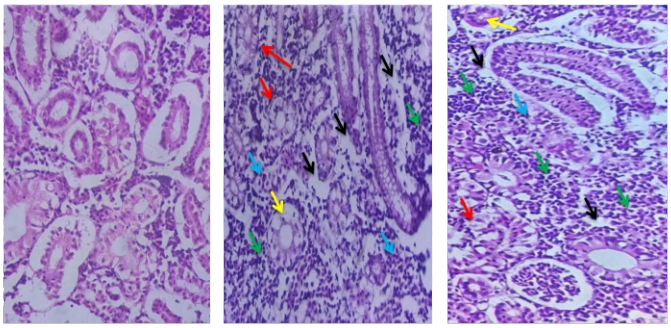
(d) BUN between control and treated groups

The values are represented as mean ± SD at $p<0.05$ level of significance.

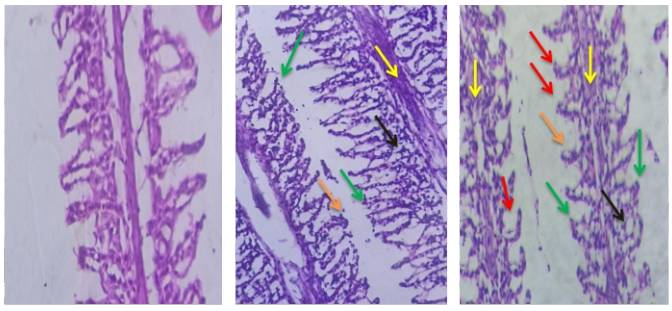
Stained slides of gills, kidney and liver were analyzed under a microscope at 40X magnification. Control fish showed normal organization and arrangement of renal cells. Tissues of exposed fish showed different histological changes such as tubular degeneration at glomerulus and necrosis area between renal tubules, tubules cells with hypertrophied cells and elongated tubules, cluster nuclei formation, the presence of damaged parenchyma, and sinusoidal space, as well as necrotic cells in soft tissues (figure 5).



(A) (B) (C)

Figure 5: Figure 5: Histological examination of profenofos on kidney of experimental fish Labeo rohita

(a) Shows the normal microscopic structure of kidney at 40X (b) shows the low dose effect and (c) shows the high dose effect on fish after 28 days of exposure to profenofos. Histological changes showing the sinusoidal spaces (black arrow), the elongated tubules (red arrow), the cluster nuclei formation (green arrow), the damaged parenchyma cells (blue arrow) and the tubules cells with hypertrophied cells in kidney (yellow arrow).

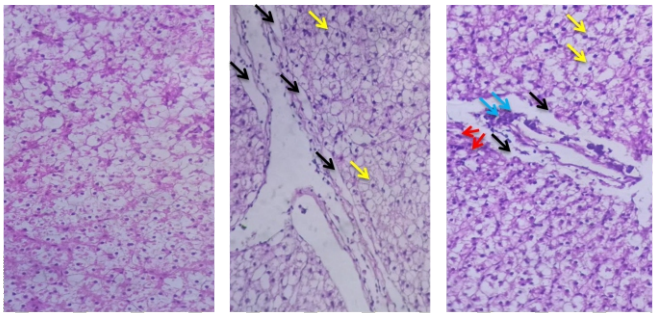


(A) (B) (C)

Figure 6: Histological examination of profenofos on gills of experimental fish Labeo rohita

(a) Shows the normal microscopic structure of gill filaments at 40X (b) shows the low dose effect and (c) shows the high dose effect on fish after 28 days of exposure to profenofos. Histological changes showing the blood congestion (orange arrow), curved lamellae (red arrow), fusion of lamellae (green arrow), bone cells deformities (yellow arrow) and damaged epithelial cells (black arrow) in gill tissues of treated fish.

The histology of control group fish showed normal arrangement of hepatic cells. Liver of exposed fish showed different histological changes including sinusoidal spaces, cluster nuclei formation, pyknotic nuclei, cellular degeneration, intravascular hemolysis in hepatic blood vessels, necrosis, cytoplasmic vacuolation in hepatocytes and dilatation-congestion in sinusoids (figure 7).



(A) (B) (C)

Figure 7: Histological examination of profenofos on liver of experimental fish Labeo rohita

(a) Shows the normal microscopic structure of liver at 40X, (b) shows the low dose effect and (c) shows the high dose effect on fish after 28 days exposure to profenofos. Liver of exposed fish showed different histological changes including sinusoidal spaces (black arrow), pyknotic nuclei in liver cells (yellow arrow), necrosis (blue arrow) and cluster nuclei formation (red arrow).

DISCUSSION

The use of pesticides in developing countries is causing water pollution and health issues. Most pesticides applied to crops do not reach their intended target, with only 0.1% reaching pests and the rest spreading throughout the environment [17]. Fish in aquatic ecosystems are frequently exposed to toxic chemicals, including pesticides [18]. The presence of profenofos in water, even at low level, is dangerous for aquatic organisms and can negatively impact the health of fish [19]. Present study was conducted to assess the toxicity of profenofos in L. rohita using multiple biomarkers such as hematology, biochemistry and histology. Hematological analysis is an important tool in monitoring the health of fish, pollutant levels in their environment, stress and diseases. Any change in blood can indicate physiological changes in the body [20]. In this study, profenofos was given to the freshwater fish, Labeo rohita. This caused various changes in a number of hematobiochemical measures as well as histological changes in the fish's important organs. In this study, the levels of HGB, HCT, RBC, MCV, MCHC, MCH, RDW-SD, PCT and PDW decreased significantly after being exposed to profenofos. Bantu et al., reported similar observations [21]. The potential of profenofos may lead to

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lower levels of hemoglobin and red blood cells (RBCs). This is because profenofos has a negative effect on the formation of these biomolecules. When hemoglobin levels decrease, oxygen supply to tissues can be disrupted, which can lead to a slower metabolic rate and less energy production [22]. A significant reduction in hemoglobin levels could indicate severe anemia due to the destruction of erythrocytes [23]. Due to hematopoietic tissue damage, destruction of RBCs and unable to synthesize new RBCs and the level of hemoglobin in the body may decrease. In the present study, significant increases in WBC, RDW, PLT, MPV, neutrophils, lymphocytes, monocytes, and eosinophil levels were reported after exposure to profenofos. WBC count can be linked to an increase in antibody production. Our results are supported by Al-Emran et al., Kesharwani et al., and Ramesh et al. [24-26]. The WBC count may increase due to toxic substances or tissue damage. The increase in WBC count of exposed fish shows toxemia and impaired defense mechanisms, leading to leukocytosis. The value of HCT (hematocrit) decreased due to the harmful effect of profenofos on the liver and kidneys of exposed fish. The value of mean cell hemoglobin (MCH) and mean corpuscular volume (MCV) decreased by exposure to pesticide due to the changes in RBC's and HB values [27, 28]. It also leads to hypochromic microcytic anemia [29] but opposite results were reported by Sharafeldin et al [30]. Ghayyur et al., also reported similar findings in previous literature [31]. They reported that pesticide profenofos causes inflammatory conditions like autoimmune diseases, trauma, iron deficiency and certain infections. Further, it also indicates inflammation and cardiovascular disease in Labeo rohita. Biochemical parameters are helpful to understand the alteration in body physiology of fish [32]. In this research, significant increase in cholesterol, triglyceride, HDL and decrease in LDL and VLDL values were reported by acute toxicity of profenofos as compared to control group. Similar findings were observed by He et al [33]. Changes in triglyceride and cholesterol levels are the main cause of alteration in total lipid profile. Pesticide toxicity in the blood and pancreatic tissues may cause insulin to be partially or fully inactivated, which could lead to an increase in total lipids and triglycerides and also cause liver dysfunction [34]. The decrease in cholesterol was attributed to the body uses stored fat as an energy source to keep up with the body's growing physiological needs [35]. One possible reason is that the high lipid group increased intake of external fats and lowered the body's internal production of fats [36]. Concentration of the aspartate aminotransferase (AST) enzyme was significantly increased, with a reduction in the alanine transaminase (ALT) value after exposure to pesticides at both low and high concentrations. Similar results were reported by Nagaraju and Rathnamma [37]. This alteration in enzymes may be a sign of damaged liver, and an increase in liver enzyme activity was also caused by liver cell damage [38], which allowed the enzymes to leak out in the blood. The concentrations of total protein were also significantly reduced because of alteration in ALT and AST enzyme concentrations and adaptation to pesticide stress in Labeo rohita because protein deficiency is also related to liver disease. The present investigation found that there was a significant decrease in serum protein levels as compared to the control group. Khan et al., and Shruti et al., reported similar findings [39, 40]. The study suggests that the low protein levels may be due to the body breaking down more protein as a way to generate energy to cope with the stress caused by exposure to toxins. After the exposure to profenofos, Albumin and the A/G ratio were significant at their maximum levels. Similar results were observed by Joseph et al [41]. It is a sign of liver and kidney damage, while a decline in globulin concentration is a sign of infection and liver diseases. A potential cause for the decline in protein synthesis is that pesticides damage the liver cells, which in turn impairs their function. significant increase in blood glucose shown by results as compared to the pesticide free group. El-Bouhy et al., reported similar findings in previous study [42]. The blood glucose is a major source of energy, and the concentration may increase because of stress and glycogenolysis, which also causes hyperglycemia [43]. Liver damage may be the reason for the reduction in protein production and be caused by the adverse impact of pesticides on liver cellulose in the blood of Labeo rohita after exposure to the organophosphate pesticide profenofos. In our study, the levels of triiodothyronine (T3) and thyroxine (T4) were low and TSH (thyroid stimulating hormone) level was significantly high in both low and high concentrations of profenofos. Similar results from a previous study indicated that profenofos causes an increase in TSH levels while decreasing T3 and T4 levels [44]. The same changes caused by exposure to pesticides in thyroid hormones were also reported by Sanna et al [45]. Low levels of T3 and T4 cause a condition called hypothyroidism. The decrease in plasma T3 levels is due to a decrease in T4 secretion or production [46]. The changes in hormones also affect all the physiological factors like growth, development, osmoregulation and behavior of exposed Labeo rohita. In the current study, concentrations of urea and creatinine were recorded as high and decreased in blood urea nitrogen levels. In the same way, Rahman et al., observed extremely similar results [15]. This could lead to azotemia, which is represented by increased blood urea and creatinine levels [47]. Fish produce urea in their livers and excrete it through their gills instead of their kidneys. The study also suggests that increased blood urea could be a result of gill

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dysfunction, liver diseases or cardiac arrest. Many authors have investigated the histological changes in fish that are caused by exposure to pesticides. Studies on fish tissues showed that exposure to toxins can lead to harmful changes in their body such as necrosis in liver cells, abnormalities in gill lamellae and tubular damage of kidney [48]. Kidney is an important organ for elimination of wastes from body [49]. The kidney can undergo structural changes when faced to eliminate toxins and these changes can be influenced by the concentration of the toxins [50]. In current research showed that PFF exposure is harmful to fish. In our investigation, no damage was observed in kidney tissues of control group. Fish in low and high concentrations groups showed various histological abnormalities such as sinusoidal spaces, elongated tubules, cluster nuclei formation, damaged parenchyma cells and tubules cells with hypertrophied cells. It indicates nephrotoxicity and severe damage in kidney after 28 days exposure time confirmed the toxicity of profenofos and it increase with increase concentrations of pesticide. Similar results were reported by Butchiram et al [51]. Gills are important and critical organs for respiration, osmoregulation and excretory function, primary targeted tissues and also take part in metabolism and perform as a barrier [52]. Gills are the primary route for pesticide entry [53]. After exposure of low and high dose, gills changed their morphology and showed different alterations such as blood congestion, bone cells deformities, damaged epithelial cells, curved lamellae and fusion of lamellae. Infected lamellae reduced the surface area and excessive accumulation of blood within vessels occurs in exposed fish. Similar results were observed by Dutta et al [54]. Liver is an important organ and indicator of chemical toxicity and main site of detoxification [55]. In the present study liver tissues of exposed fish showed different hepatic changes such as, sinusoidal spaces, pyknotic nuclei, cluster nuclei formation and necrosis. Pesticide completely damage liver tissues and regeneration of cells is not occurred as a result of exposed profenofos and irregular tubular spaces forms. The changes observed in the liver could be due to the fact that the liver is the main organ responsible for detoxification [56]. Toxicants are expected to be processed and eliminated by the liver, it is possible that they accumulate there and cause damage [57]. Similar were results reported by Boran et al., and Rahman et al [58, 59].

CONCLUSIONS

Present study suggested that Profenofos, an organophosphate is the primary factor that causes significant changes in blood and tissue of fish in aquatic ecosystem. Harmful toxicological alterations in hematological and biochemical parameters and histological changes in kidney, gills and liver were reported after 28 days of exposure in Labeo rohita. The current observation indicates that this pesticide poses a significant thread to aquatic life. To minimize the toxic effects of pesticide, their use should be reduced and more environmentally friendly organophosphate pesticide should be developed with faster degradation ability.

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Authors Contribution

Conceptualization: SA, KS
Methodology:
Formal analysis:
Writing-review and editing: AM, HK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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