Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2022.014

RESEARCH ARTICLE

Inquisition of Toxic Effects of Pyriproxyfen on Physical, Hemato-Biochemical and Histopathological Parameters in *Labeo rohita* Fish

Sumaira Naseem[1], Abdul Ghaffar[1], Riaz Hussain[2,*] and Ahrar Khan[3,4,*]

[1]Department of Zoology, Islamia University of Bahawalpur-63100, Pakistan
[2]Department of Pathology, Faculty of Veterinary Sciences, Islamia University of Bahawalpur-63100, Pakistan
[3]Faculty of Veterinary Science, University of Agriculture, Faisalabad-38040, Pakistan
[4]Shandong Vocational Animal Science and Veterinary College, Weifang 261061, China
*Corresponding author: driazhussain@yahoo.com (RH); ahrar1122@yahoo.com (AK)

ARTICLE HISTORY (22-055)

Received: January 31, 2022
Revised: March 02, 2022
Accepted: March 05, 2022
Published online: March 07, 2022

Key words:
Blood
Histopathology
Labeo rohita
Pyriproxyfen
Serum biochemistry

A B S T R A C T

Pyriproxyfen (PPF), a well-known synthetic substance, acts as an analog of juvenile insect hormone and is persistently used as insecticides as well as larvicides across the globe. At present, meager information is available about the potential threats of PPF in aquatic species. Therefore, this study determined the toxico-pathologic effects of PPF in fish. In the current trial, 60 fish (*Labeo rohita*) of the same age, size, and body mass were randomly divided into four groups (T0-T3) and kept separate in glass aquaria with 100L water. Fish in groups T1-T3 were exposed to 300, 600, and 900µg/L of PPF concentrations for one month. On days 10, 20, and 30 of the experiment, blood and other visceral organs were collected from each fish. The findings revealed a variety of clinical ailments in treated fish and a significant ($P<0.05$) difference in the body weight of specific visceral organs. The erythrocyte counts, hematocrit, and hemoglobin reduced significantly while total leukocyte counts, and neutrophils as well as liver, kidneys, and heart biomarkers values increased significantly ($P<0.05$) compared to unexposed fish. At the microscopic level, different lesions in the liver including edema, necrosis, inflammatory exudate, and kidneys necrosis, edema, widening of Bowman's space and necrosis of tubules, brain microgliosis, hemorrhages, pyknosis of neurons, and degeneration and in heart (neutrophilic myocarditis, edema, disruption of cardiac myofibers) were observed in treated fish. The results of our experimental study shed light on blood, serum biochemistry, and the potential microscopic risks of PPF in freshwater fish.

To Cite This Article: Naseem S, Ghaffar A, Hussain R and Khan A, 2022. Inquisition of Toxic effects of Pyriproxyfen on physical, hemato-biochemical and histopathological parameters in *Labeo rohita* fish. Pak Vet J, 42(3): 308-315. http://dx.doi.org/10.29261/pakvetj/2022.014

INTRODUCTION

Insecticides and pesticides are persistently and broadly applied in agro production practices to enhance crop yield and control of various pests, animals, insects, and vector-borne infections. Frequent and continuous usage of pesticides and insecticides may induce adverse toxic impacts on both animals and public health due to their toxicity (Hussain *et al.*, 2020; Afzal *et al.*, 2022). In the published literature, studies have indicated that different types of insecticides, herbicides, and pesticides cause teratogenicity organ toxicity and classified as endocrine-disrupting substances in animals, including humans (Shahid *et al.*, 2019; Ghaffar *et al.*, 2021a).

Numerous insecticides and synthetic chemicals are not biodegraded easily and tend to remain in soil and water for years (Singh and Singh, 2019; Ahmad *et al.*, 2021; Namratha *et al.*, 2021). Accidental exposure to these chemicals in marine, freshwater, and terrestrial environments not only cause adverse effects but greatly decreases the average expectancy of life in numerous exposed organisms (Baralic *et al.*, 2020; Merdana *et al.*, 2021; Tahir *et al.*, 2021). It has been reported that various creatures in the marine environment are at risk relative to non-aquatic organisms as many synthetic substances from multiple sources like agriculture, production sites, and industries are directly introduced into nearby water sources (Amaroli *et al.*, 2018; Jabeen *et al.*, 2021).

Pyriproxyfen, (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] being a pyridine insecticide, is frequently employed in agriculture to control insects and arthropods (Ginjupalli and Baldwin, 2013). Pyriproxyfen is highly

stable in various ecosystems and causes detrimental toxic effects through the food web in non-target animals (Mehrnoush *et al.*, 2013). Various adverse effects, including neurodevelopmental toxicity, impaired reproduction, and disruption of endocrine homeostasis, have been observed in exposed animals due to continuous and persistent application of PPF (Maharajan *et al.*, 2018).

The insecticide, pyriproxyfen is a stable aromatic with an average half-life range (16-21 days) in water under an anaerobic sediment system in a lake. The recommended and maximum amount of PPF in drinking water is 0.01mg/L (Truong *et al.*, 2016). Earlier studies have indicated that PPF may induce mortality in fish and other aquatic animals during control of mosquitoes (Caixeta *et al.*, 2016). These insecticides may also cause harmful effects on non-target organisms, particularly fish. Different environmental pollutants induce adverse effects in ecosystems like changes in biodiversity, damage to the food chain, disorganization, destruction of habitat, and physiological abnormalities resulting in abnormal reproductive efficiency (Xu *et al.*, 2018; Hussain *et al.*, 2019; Taha *et al.*, 2021; Wang *et al.,* 2022).

Pyriproxyfen being a well-known synthetic insecticide inhibits the process of embryogenesis in insects and is routinely used as a registered insecticide against aphids, whitefly, jassids, bollworm, and cutworms on citrus fruit throughout the world (Dzieciolowska *et al.*, 2017; Shahid *et al.*, 2019). PPH is often used in India and Pakistan to control various vectors, including *Anopheles stephensi* and *Culex quinquefasciatus* (Jambulingam *et al.*, 2008).

Residues of PPF in soil typically join the food products and may accumulate in multiple animal organs, causing significant health hazards (mutagenic and teratogenic effects) (Muñiz *et al.*, 2017). Blood biochemistry parameters in fish are reliable and useful biomarkers and are frequently used to monitor the toxic effects (Gul *et al.*, 2017). In addition, the evaluation of alterations in serum biomarkers, including proteins and different enzymes, was carried out to assess physiological abnormalities in animals of aquatic ecosystems (Hussain *et al.*, 2020). Inaccessible data can find sufficient information regarding deleterious impacts of PPF in various invertebrate animals (Caixeta *et al.*, 2016); however, limited data is available in vertebrates (Dzieciolowska *et al.*, 2017).

Moreover, there is lack of data available on the ecotoxicological impacts of PPF in aquatic animals, especially fish. In marine vertebrates, there is no report on toxicity of PPF on various physiological, biochemical, and histopathological parameters. Hence, we executed the current experimental research to determine the adverse impacts of PPF on multiple tissues of fish.

MATERIALS AND METHODS

Animal research ethics: The experimental fish handled and treated according to the guidelines devised by the Bioethics Committee, Islamia University of Bahawalpur, regarding animal welfare and use.

Test specimens and management: Freshwater fish (*Labeo rohita*=60) with similar body mass (130-140g), age, and length were taken from a local farm (District Bahawalnagar, Punjab Province, Pakistan). All fish samples were kept in different bags with sufficient oxygen and then shifted to the laboratory. For adjustment to laboratory conditions, fishes were placed in a glass aquarium (14" L × 10" W × 12" H) for ten days. Provided commercial fish feed (2-2.5% of body weight daily to all the fish. The waste feed and fecal material were strained and discarded from all the aquariums daily.

Experimental design: After the acclimatization, the experimental fish were picked, distributed randomly, and kept into four equal groups (T0-T3) in a glass aquarium (with 100L water capacity). Fish of group T0 served as control, and fish in groups T2-T3 were exposed to PPF @ 300, 600, and 900µg/L for one month. There were three replicates in each group.

Hematological analyses: A total of five fish from each group were selected randomly for blood collection (2-2.5mL) and visceral organs at days 10, 20, and 30 of the trial. Out of 2.5mL blood, about 2mL centrifuged for separation of serum and about 0.5mL blood with EDTA to determine hematological values. Total erythrocyte and leukocyte counts were determined following the techniques already described. Briefly, blood was diluted with Natt and Herrick solution (Khan *et al*., 2017), and with the aid of Haemocytometer were counted microscopy (Gul *et al*., 2020). Hemoglobin was determined through cyanomethemoglobin method spectrophotometrically by using Drabkin's solution (Hussain *et al*., 2019; Ali *et al*., 2021). For differential leukocyte counts, four fresh blood smears were prepared from each experimental fish from each treatment groups, then these slides were fixed with methanol, and stained with Giemsa stain. A total of 100 leukocytic cells including lymphocytes, monocytes, and neutrophils were counted under microscope (Ghaffar *et al.*, 2021b), afterwards percentage ratio of each cell was calculated.

Physical and pathological parameters: For histopathological studies, tissue samples from each experimental fish were collected, washed, weighed, and then preserved in 10% paraformaldehyde solution and processed preserved liver, gills, heart, kidneys, and brain tissues for histopathological investigation. About 5µm thin slice from each tissue were cut and processed for Hematoxylin and Eosin stains (Ghaffar *et al.*, 2021a). Various serum biochemical profiles like total proteins, albumin, cholesterol, glucose, triglycerides, kidney biomarkers (urea, creatinine), and liver biomarkers (ALT, ALP, AST) were measured using commercial kits (M/S Randox Company) with the help of a spectrophotometer according to the processes described earlier (Hussain *et al.*, 2020).

Statistical analysis: Data on all the parameters were subjected to ANOVA using IBM SPSS statistics (version 20) to calculate the significant difference. Mean±SE in all the groups were subjected to post hoc Tukey's test at $P<0.05$.

RESULTS

Clinical signs and organ weight: No clinical ailments, mortality, and behavioral ailments seen in the control group (T0). All the test specimens in the control group remained healthy and active. Insignificant to moderate signs of toxicity, including erratic swimming, air gulping, tremors and darkening of fins, and mucous secretion from the mouth, were observed in experimental fish retained in group T3 after day 20 of trial.

The results obtained have been on various physical parameters (body weight and absolute weight) of some visceral tissues like gills, brain, liver, and kidneys of *Labeo rohita* treated with different doses of PPF in Table 1. The total body weight of fish in group T3 (900µg/L) decreased significantly (P<0.05) on days 20 and 30 of research work compared to normal fish. The absolute weight of the liver and kidneys of group T2 at day 30 and all sampling days in group T3. The absolute weight of gills was significantly (P<0.05) higher in PPF treated fish at days 20 and 30. Higher absolute weights of the brain were observed in group T3 fish at days 20 and 30 of the experiment compared with that of the control group (Table 1).

Hematology and biochemistry: Results have been presented on various blood values of *Labeo rohita* in control and experimental groups in Table 2. Fish treated with different concentrations of PPF exhibited significantly (P<0.05) lower-level values of RBC counts, Hb, and hematocrit in group T2 (300µg/L) at day 30 and in experimental test species retained in group T3 (900µg/L) at experimental days 20 and 30. Results exhibited significantly (P<0.05) increased neutrophils, and total leukocyte counts at 20 and 30 days of the study in fish of groups (T2 and T3) compared to control fish.

Table 1: Body weight and absolute weight of different visceral tissues of *Labeo rohita* exposed to different concentrations of pyriproxyfen

Parameters/day	Groups/Treatments			
	T0 (Control)	T1 (300µg/L)	T2 (600µg/L)	T3 (900µg/L)
Body weight (g)				
10	163.4±2.15a	162.8±3.18a	160.8±3.55a	159.91±1.93a
20	165.3±1.31a	163.5±3.11a	158.5±3.75a	151.95±1.12b
30	166.8±1.27a	161.7±1.36a	158.2±1.47a	149.52±2.02b
Absolute weight of Liver (g)				
10	0.82±0.11a	0.83±0.05a	0.86±0.02a	0.96±0.07b
20	0.86±0.15a	0.86±0.17a	0.87±0.14a	0.97±0.08b
30	0.89±0.11a	0.89±0.04a	0.98±0.12b	0.99±0.11b
Absolute weight of kidneys (g)				
10	0.65±0.08a	0.67±0.04a	0.69±0.07a	0.78±0.04b
20	0.59±0.04a	0.68±0.01a	0.70±0.05a	0.85±0.05b
30	0.64±0.06a	0.69±0.03a	0.79±0.16b	0.87±0.09b
Absolute weight of gills (g)				
10	4.69±0.41a	4.71±0.29a	4.77±0.35a	4.81±0.31a
20	4.75±0.33a	4.85±0.18a	4.93±0.55a	6.12±0.98b
30	4.83±0.35a	4.89±0.19a	4.98±0.83a	7.19±0.85b
Absolute weight of brain (g)				
10	0.63±0.16a	0.64±0.15a	0.65±0.12a	0.77±0.23b
20	0.65±0.15a	0.67±0.11a	0.69±0.14a	0.81±0.21b
30	0.67±0.14a	0.68±0.13a	0.71±0.11a	0.83±0.17b

Values (mean+SE) with different alphabets in a row differ significantly (P<0.05).

Table 2: Various hematological parameters of *Labeo rohita* exposed to different concentrations of pyriproxyfen

Parameters/days	Groups/Treatments			
	T0 (Control)	T1 (300µg/L)	T2 (600µg/L)	T3 (900µg/L)
Erythrocyte counts (10^9/mm^3)				
10	4.14±0.11a	4.10±0.09a	4.03±0.05a	4.01±0.04a
20	4.15±0.12a	4.09±0.08a	3.98±0.07a	3.61±0.11b
30	4.11±0.13a	4.07±0.02a	3.63±0.07b	3.57±0.12b
Hemoglobin (g/dL)				
10	10.11±0.14a	9.79±0.12a	9.65±0.27a	8.94±0.12a
20	10.18±0.11a	9.42±0.18a	8.83±0.11a	7.34±0.22b
30	10.17±0.13a	9.49±0.22a	7.37±0.14b	7.31±0.12b
Hematocrit (%)				
10	37.15±2.18a	36.80±1.15a	35.13±1.97a	34.22±0.51a
20	37.22±1.32a	35.74±1.02a	31.54±1.16a	28.45±0.26b
30	38.12±1.31a	34.11±1.39a	29.33±1.23b	27.34±2.30b
Leukocyte counts (10^6/mm^3)				
10	14.17±0.22a	14.28±0.41a	15.26±0.11a	15.29±0.31a
20	13.72±0.37a	14.15±0.29a	17.86±0.35b	17.97±0.46b
30	14.18±0.62a	14.39±0.23a	18.26±0.29b	19.19±0.12b
Lymphocytes (%)				
10	17.23±0.25a	17.11±0.31a	16.11±0.13a	14.81±1.07a
20	16.31±0.29a	16.22±0.18a	15.80±1.12a	12.57±0.08b
30	16.22±0.27a	15.16±0.16a	14.02±1.13a	12.19±0.03b
Neutrophils (%)				
10	20.11±0.21a	19.98±0.23a	21.55±0.66a	23.82±0.39a
20	19.38±0.37a	19.98±0.32a	21.76±0.80a	24.27±0.22a
30	19.11±0.14a	20.2±0.17a	24.89±0.63b	26.63±1.44a

Values (mean+SE) with different alphabets in a row differ significantly (P<0.05).

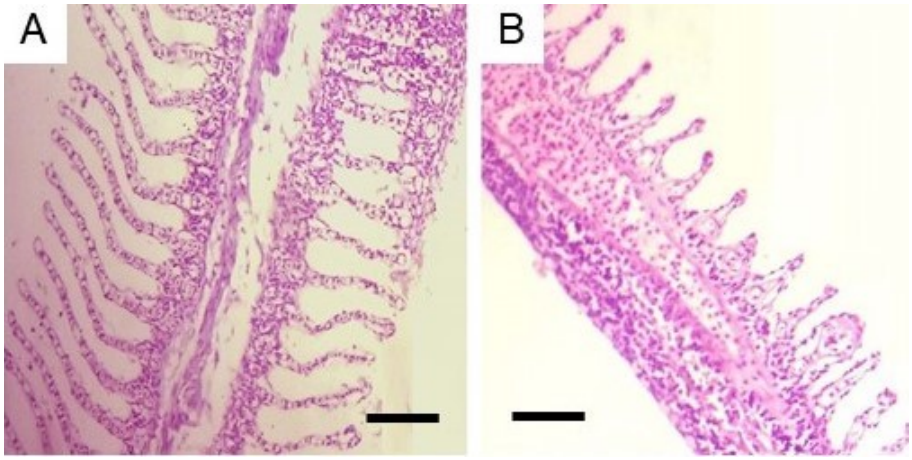

Fig. 1: Photomicrograph of *Labeo rohita* A) showing fusion of lamellae and vacuolation of gills and B) disruption of cartilaginous core, congestion, atrophy of lamellae, aneurysm, and necrosis of lamellar epithelial cells. H & E stain. 400X. Bar = 100 µm.

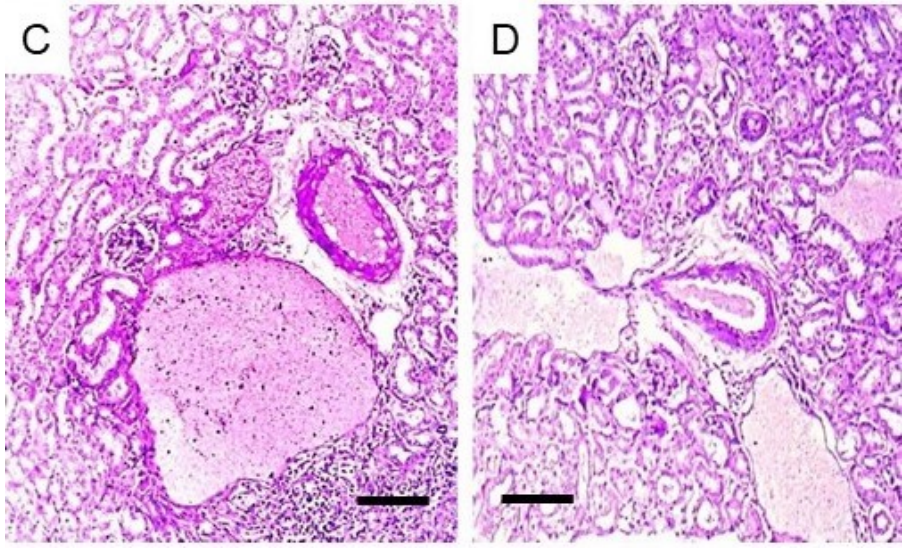

Fig. 2: Photomicrograph of kidneys of *Labeo rohita* exhibiting C) inflammatory exudate, and pyknosis of renal epithelial cells and D) showing increased urinary space, degeneration of renal tubules, edema and necrosis of tubules. H & E stain. 400X. Bar = 100 µm.

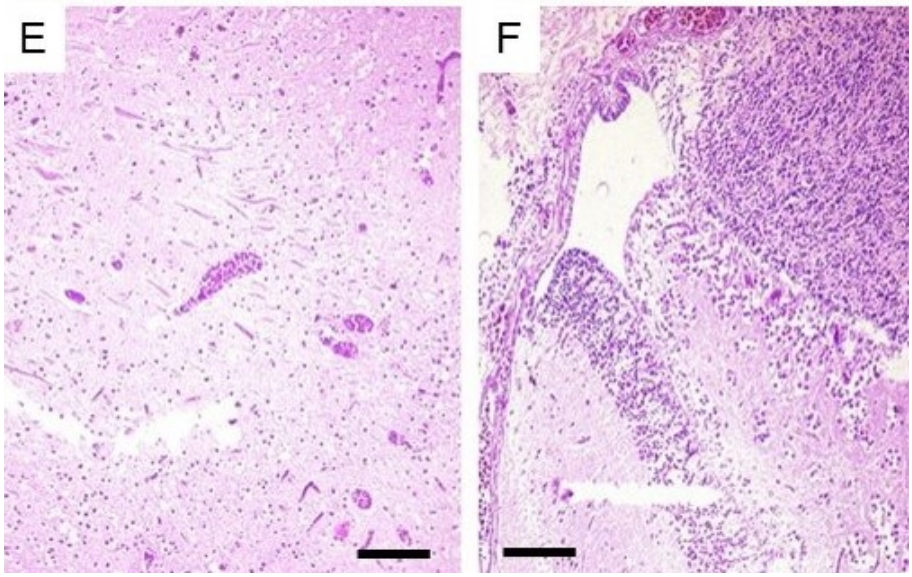

Fig. 3: Photomicrograph of brain of *Labeo rohita* showing E) congestion and necrosis of neuron and F) microgliosis, congestion, necrosis and degeneration of neurons. H & E stain. 400X. Bar = 100 µm.

Table 3: Various serum biochemical parameters of *Labeo rohita* exposed to different concentrations of pyriproxyfen

Parameters/days	Groups/Treatments			
	T0 (Control)	T1 (300µg/L)	T2 (600µg/L)	T3 (900µg/L)
Urea (mg/dL)				
10	6.12±0.17a	6.57±0.09a	7.31±0.42a	7.56±0.22a
20	6.18±0.25a	6.87±0.10a	7.36±0.55a	8.85±0.15b
30	6.24±0.35a	6.95±0.12a	8.99±0.35b	9.77±0.25b
Creatinine (mg/dL)				
10	1.36±0.05a	1.38±0.01a	1.45±0.03a	1.47±0.07a
20	1.39±0.04a	1.42±0.02a	1.54±0.05a	1.91±0.04b
30	1.37±0.03a	1.49±0.02a	1.93±0.03b	1.99±0.8b
Alanine aminotransferase (U/L)				
10	19.05±1.15a	20.45±1.05a	20.71±1.16a	24.15±1.12b
20	20.45±1.20a	21.07±1.19a	26.72±1.11b	27.55±1.10b
30	19.13±1.13a	22.13±1.11a	26.80±1.13b	28.63±1.13b
Aspartate aminotransferase (U/L)				
10	12.75±0.26a	13.05±0.36a	14.35±0.14a	17.05±0.21b
20	12.37±0.29a	13.55±0.25a	17.05±0.11b	17.52±0.19b
30	12.81±0.23a	14.61±0.17a	17.39±0.93b	19.13±0.45b
Alkaline phosphatase (U/L)				
10	23.15±1.15a	24.12±1.11a	25.52±1.01a	27.45±0.10b
20	23.21±1.19a	24.29±1.10a	28.43±1.12b	28.85±0.19b
30	23.18±1.33a	25.23±1.03a	28.63±0.18b	28.97±0.13b
Lactate dehydrogenase (U/L)				
10	228.3±2.39a	229.5±3.19a	235.7±1.39a	251.6±4.17b
20	225.7±2.43a	231.7±2.48a	237.2±2.38a	263.6±4.82b
30	227.3±2.62a	235.5±3.60a	255.7±2.65b	273.4±4.81b
Total proteins (mg/dL)				
10	4.29±0.13a	4.11±0.09a	4.05±0.03a	3.98±0.01a
20	4.33±0.23a	4.13±0.05a	3.94±0.03a	3.84±0.03a
30	4.25±0.24a	4.19±0.05a	3.44±0.04b	3.38±0.08b
Albumin quantity (mg/dL)				
10	2.45±0.18a	2.31±0.22a	2.27±0.06a	2.33±0.20a
20	2.48±0.16a	2.27±0.18a	2.22±0.12a	2.18±0.15a
30	2.49±0.14a	2.24±0.15a	1.79±0.08b	1.65±0.18b
Triglycerides (mg/dL)				
10	151.5±1.54a	157.7±1.48a	158.9±2.02a	163.9±2.39b
20	153.5±2.85a	157.4±1.45a	159.3±2.05a	166.8±3.15b
30	156.8±1.71a	158.6±1.41a	167.2±2.11b	169.3±3.45b
Glucose (mg/dL)				
10	33.2±1.16a	35.7±2.15a	36.12±1.15a	38.50±1.10a
20	34.5±1.90a	36.3±2.29a	38.90±1.19a	39.15±2.13b
30	34.6±2.90a	37.2±1.11a	41.15±1.19b	43.35±2.89b
Cholesterol (mg/dL)				
10	134.2±3.37a	135.7±3.18	137.8±2.21a	138.9±4.11a
20	135.5±3.39a	138.9±2.19	139.2±2.33a	159.6±2.89b
30	138.7±3.49a	136.5±2.39	163.2±3.92b	171.5±3.49b

Values (mean+SE) with different alphabets in a row differ significantly (P<0.05).

Different serum biochemical biomarkers (Table 3) of fish recorded at various doses of PPF exposure showed significantly elevated kidney biomarkers in fish retained in group T3 at day 20 and in groups T2-T3 at day 30 of the research trial. The levels of different liver enzymes significantly (P<0.05) increased in fish of group T3 at day 10 of trial while in groups T2-T3 at days (20 and 30) of research work. The LDH values increased significantly (P<0.05) in fish of group T3 at 10 and 20 days and in groups T2 and T3 at day 30 of the experiment. Results revealed significantly (P<0.05) reduced levels of different serum proteins (albumin and total proteins) in fish of groups T2-T3 at day 30 compared to control group fish. The results showed higher triglycerides, glucose, and cholesterol levels in fish (group T3) at days 10 and 20 while in fish of groups (T2 and T3) on the 30th day of the experiment.

Histopathological findings: Moderate necrosis of lamellar cells, twisting of secondary lamellae, degeneration, and examined disruption of the cartilaginous core of gills of fish in group T2 at day 30. In group T3, fish at days 20 and 30 severe ailments in fish gills like congestion in the cartilaginous core, necrotic cells of the lamellar epithelium, twisting lamellae, and examined disruption and degeneration of primary lamellae in fish gills (Fig. 1).

Moderate to severe histopathological lesions observed in the kidneys in groups T2 and T3 at days 20 and 30.

Lesions like degeneration and necrosis of renal tubules, the distortion of glomeruli, pyknosis, sloughing of renal tubules epithelium, and edema were observed (Fig. 2). Liver tissues of insecticide-treated groups T2 and T3 at days 20 and 30 exhibited edema, atrophy of the cytoplasm, and necrosis of hepatocytes. Histopathologically, the brain showed lesions like microgliosis, congestion, necrosis of neurons, and atrophy of neurons (Fig. 3) at days 20 and 30 in fish of group T3. Mild to moderate congestion, microgliosis, and degeneration of neurons were also observed in fish retained in group T2 on the 30th day of study and examined various microscopic cardiac ailments like inflammatory reaction, necrosis, edema, and disorder in arrangements of cardiac myofibers in treated fish (group T3) at days 20 and 30.

DISCUSSION

The persistent and inappropriate use of numerous synthetic compounds/chemicals, including pesticides and insecticides on vegetables and other cereal crops, can be a considerable risk of infertility and abnormal sperm counts in exposed animals, including humans. Moreover, many studies have investigated that exposure to pesticides and insecticides at low levels is mainly related to the induction of adverse effects like immunosuppression, cancer, endocrine disruption, and reproductive disorders.

For toxicological evaluation, various physical parameters such as clinical ailments, body weight, and weight of different visceral tissues are known as reliable toxicity biomarkers.

Furthermore, frequent use of pesticides causes the presence of residues in the food chain contamination of the environment and water resources (Hussain *et al*., 2019; Yang *et al*., 2021).

Therefore, prolonged screening and assessment of possible toxicity of PPF due to low concentrations of long-term exposure is crucial in an attempt to lessen its public health risks. In current experimental research, the weight of different visceral organs except the brain was significantly high in PPF treated fish. In previous studies, we could find little information about the harmful impacts of PPF on the weight of various visceral tissues of aquatic and non-aquatic organisms. However, previously, abnormal weight (absolute weight) of the liver, kidney, lungs, testis, ovaries, and pancreas (Badr, 2020) has been observed in mammals due to exposure to a toxicant.

The current study recorded lower hemoglobin values, monocytes, hematocrit, and lymphocytes in fish that received different doses of PPF. The lower values of this parameter could be related to induction of over-release of free radicals leading to increase oxidative stress in PPF treated fish.

The reduced blood parameters can also be related to the induction of various disorders by PPF on hematopoietic tissues and the breakdown of red blood cells (Gul *et al*., 2017). The lower value of erythrocyte counts while increased leukocyte and neutrophil counts in treated fish at higher doses of PPF in current work might be due to injury to multiple tissues. Higher values of neutrophils could be associated with the immunological responses of fish. Similar reports in *Aspidoparia morar* fish exposed to toxicants have also been recorded (Sachar and Raina, 2014). Moreover, abnormalities in hematological parameters in rats (Roshanravan *et al*., 2021), birds (Hussain *et al*., 2014), and *Labeo rohita* (Ghaffar *et al*., 2021b) exposed to different pollutants have been recorded. Limited reports are found on the hematology of aquatic organisms due to PPF.

It has been recorded that estimation and evaluation of different serum biochemical parameters provide valuable and reliable information regarding screening of toxic effects of numerous environmental pollutants and pathophysiological status of terrestrial and aquatic animals (Hussein *et al*., 2021). Previously, deleterious toxic impacts on serum biomarkers in PPF treated fish have also been reported (Wang *et al*., 2016). In the current trial, in treated fish, significantly increased serum biomarkers, including AST, ALP, and ALT could be due to exposure to PPF higher doses. Increased glucose, cholesterol, and lactate dehydrogenase levels while reduced serum total protein and albumin quantity values were recorded in exposed fish. It is reported that serum uric acid serum and creatinine are reliable and important biomarkers for monitoring metabolic status (Hamed and Osman, 2017; Akram *et al*., 2021a).

In our trial, the creatinine and urea levels also increased, reflecting disruption of filtration mechanisms and damage to the kidneys of fish exposed to PPF. Previously, recorded significantly higher levels of LDH due to PPF treatments in Zebrafish (Maharajan *et al*., 2018).

Moreover, in insecticide-exposed *Cyprinus carpio*, elevated LDH, AST, ALT, glutathione peroxidase, and catalase were reported (Stoyanova *et al*., 2020). However, assessed serum biochemistry alteration in *Channa punctatus* (Agrahari *et al*., 2007) exposed to the toxicant. In the present study, various histopathological lesions in the liver of PPF treated fish included congestion, necrosis of hepatocyte, atrophy, degeneration of hepatocyte, vacuolar deterioration, increased sinusoidal space, and karyolysis.

Previously, changes in the liver as cytoplasmic vacuolation and nuclear hypertrophy, internal hemorrhages, necrosis, and vacuolation in fish *Heteropneustes fossilis* (Akter *et al*., 2020) exposed to toxicant was reported. Cloudy swelling, hypertrophy, bile duct obstruction, disorientation in hepatic cells have been found due to toxic chemicals in *Channa Punctatus* (Mishra and Mohanty, 2008) and *Cyprinus carpio* (Sepici-Dinçel *et al*., 2009).

The current research also observed different histopathological ailments like increased Bowman's space, edema, glomerular degeneration, formation of ceroid, tubular atrophy, and degeneration of the lumen of renal tubules in different sections of PPF treated kidneys. Previously, decreased nephron count, glomerular lesions, and glomerular filtration rate were observed in the kidneys of rainbow trout exposed to toxicants (Boran *et al*., 2012). However, similar findings have been found in other fish species like bighead carp (Akram *et al*., 2021a, 2021b) due to different toxicants. Different histopathological changes in brain sections in the present study might be due to persistent exposure to PPF. Several studies reported microscopic alterations in the brains of fish exposed to various synthetic chemicals (Lakshmaiah, 2017).

No information is available regarding the toxicological effects of PPF on microscopic changes in fish brains. However, less data is available on microscopic ailments in the brain of different fish like *Cyprinus Carpio* (Lakshmanan, 2017) and *Oreochromis mossambicus* (Gobi *et al*., 2018) exposed to various environmental toxicants. Current research reports histopathological lesions in fish gills have also been reported in *Corbicula fluminea* (Benjamin *et al*., 2019). We observed various pathological lesions in the heart, including hemorrhages, edema, fibrin deposition, and neutrophilic myocarditis. Previously, changes in the extracellular matrix due to exposure to a toxicant in zebrafish's heart (Brown *et al*., 2019) leading to impaired cardiovascular functions have been observed. A Scanty of

information is available about the histopathology of the heart due to PPF.

Previously, due to PPF exposure, different microscopic ailments in different sections of the heart like altered muscular fibers, hyperemia, and pericardial edema in Zebrafish (Maharajan *et al*., 2018) were observed. However, in rats, several reports of cardiac histology are available. In previous research work, possible toxic effects of toxicants on rats (Rasdi *et al.*, 2020) have been observed in the heart in the form of myofibril disorders, hypertrophy of myocyte, myocardial fibrosis, and dilatation of intramyocardial arteriole.

Conclusions: The results on physical, blood biochemistry and microscopic parameters in our study suggest abnormal physiological conditions of fish exposed to pyriproxyfen. Furthermore, the results of our experimental study indicate that investigation and monitoring of toxic effects of various environments is of vital importance to develop control strategies regarding pollutants and to mitigate harmful effects in exposed organisms, including public health.

Author contribution: RH, SN and AG planned and designed the trial. SN and RH executed and collected the data. All the authors were involved in preparation of the manuscript. AK revised the manuscript, and all authors approved final version of the manuscript.

Funding: This study is a part of PhD thesis of first author and is not funded by any national or international funding agency.

REFERENCES

Agrahari S, Pandey KC and Gopal K, 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pest Biochem Physiol 88:268-72.

Ahmad L, Gul ST, Saleemi MK, *et al.*, 2021. The effect of different repeated doses of cypermethrin on the behavioral and histological alterations in the brain of rabbits (*Oryctolagus cuniculi*). Int J Vet Sci 10:347-54.

Akram R, Iqbal R, Riaz H, *et al.*, 2021a. Evaluation of Oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (*Aristichthys nobils*) fish at low concentrations. Environ Pollut 268:115896.

Akram R, Ghaffar A, Hussain R, *et al.*, 2021b. Hematological, serum biochemistry, histopathological and mutagenic impacts of triclosan on fish (Bighead carp). Agrobiol Rec 7:18-28.

Akter R, Pervin MA, Jahan H, *et al.*, 2020. Toxic effects of an organophosphate pesticide, envoy 50 SC on the histopathological, hematological, and brain acetylcholinesterase activities in stinging catfish (*Heteropneustes fossilis*). J Basic Appl Zool 81:47.

Ali S, Ijaz M, Ahmed A, *et al.,* 2020. Prevalence and associated risk factors of bovine babesiosis in Lahore. Pakistan. Agrobiol Rec 2:17–23.

Amaroli A, Gallus L and Ferrando S, 2018. Permethrin drastically affects the developmental cycle of the non-target slime mould *Dictyostelium discoideum*. Chemosphere 193:1–7.

Badr AM, 2020. Organophosphate toxicity:updates of malathion potential toxic effects in mammals and potential treatments. Environ Sci Pollut Res 27:26036-57.

Baralic K, Djordjevic AB, Živancevic K, *et al.*, 2020. Toxic effects of the mixture of phthalates and bisphenol A - subacute oral toxicity study in Wistar rats. Int J Environ Res Public Health 17:746.

Benjamin K, Co E, Competente J, *et al.*, 2019. Histopathological effects of bisphenol A on soft tissues of *Corbicula fluminea* Mull. Toxicol Environ Health Sci 11:36-44.

Boran H, Capkin E, Altinok I, *et al.*, 2012. Assessment of acute toxicity and histopathology of the fungicide captanin rainbow trout. Exp Toxicol Pathol 64:175-9.

Brown AR, Green JM, Moreman J, *et al.*, 2019. Cardiovascular effects and molecular mechanisms of bisphenol A and its metabolite MBP in zebrafish. Environ Sci Technol 53:463–74.

Caixeta ES, Silva CF, Santos VSV, *et al.*, 2016. Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for *Aedes aegypti* control in Brazil. J Toxicol Environ Health A 79:799–803.

Dzieciolowska S, Larroque AL, Kranjec EA, *et al.*, 2017. The larvicide pyriproxyfen blamed during the zika virus outbreak does not cause microcephaly in zebrafish embryos. Sci Rep 7:40067.

Ghaffar A, Hussain R, Abbas A, *et al.*, 2021a. Assessment of genotoxic and pathologic potentials of fipronil insecticide in *Labeo rohita* (Hamilton, 1822). Toxin Rev 40:1289-1300.

Ghaffar A, Hussain R, Ahmad N, *et al.*, 2021b. Evaluation of hemato-biochemical, antioxidant enzymes as biochemical biomarkers and genotoxic potential of glyphosate in freshwater fish (*Labeo rohita*). Chem Ecol 37:646-67.

Ginjupalli GK and Baldwin WS, 2013. The time- and age-dependent effects of the juvenile hormone analog pesticide, pyriproxyfen on Daphnia magna reproduction. Chemosphere 92:1260-66.

Gobi N, Vaseeharan B, Rekha R, *et al.*, 2018. Bioaccumulation, cytotoxicity and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*. Ecotoxicol Environ Saf 162:147-59.

Gul ST, Khan A, Farooq M, *et al.*, 2017. Effect of sub lethal doses of thiamethoxam (a pesticide) on hemato-biochemical values in cockerels. Pak Vet J 37:135-8.

Gul ST, Ahamd I, Saleemi MK, *et al.*, 2020. Toxico-pathological effects of thiamethoxam on hematobiochemical and productive performance of commercial laying hens. Pak Vet J 40:449-54.

Hamed HS and Osman AM, 2017. Modulatory effect of lycopene against carbofuran toxicity in African catfish, *Clarias gariepinus*. Fish Physiol Bichem 43:1721-31.

Hussain R, Ghaffar A, Abbas G, *et al.*, 2020. Thiamethoxam at sublethal concentrations induces histopathological, serum biochemical alterations and DNA damage in fish (*Labeo rohita*). Toxin Rev https://doi.org/10.1080/15569543.2020.1855655

Hussain R, Ali F, Rafique A, *et al.*, 2019. Exposure to sub-acute concentrations of glyphosate induces clinico-hematological, serum biochemical and genotoxic damage in adult cockerels. Pak Vet J 39:181-6.

Hussain R, Khan A, Mahmood F, *et al.*, 2014. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (*Coturnix japonica*). Pest Biochem Physiol 109:58-63.

Hussein NM, Saeed RMA, Shaheen AA, *et al.*, 2021. Ameliorative role of chitosan nanoparticles against bisphenol-A induced behavioral, biochemical changes and nephrotoxicity in the African catfish, *Clarias gariepinus*. Egypt J Aquat Biol Fish 25:493–510.

Jabeen G, Manzoor F, Arshad M *et al.*, 2021. Effect of cadmium exposure on hematological, nuclear and morphological alterations in erythrocyte of fresh water fish (*Labeo rohita*). Continental Vet J 1:20-24.

Jambulingam P, Sadanandan C, Boopathi-Doss PS, *et al.*, 2008. Field evaluation of an insect growth regulator pyriproxyfen 0.5% GR against *Culex quinquefasciatus*, the vector of *Bancroftian filariasis* in Pondicherry, India. Acta Trop 107:20–24.

Khan A, Aalim MM, Khan MZ, *et al.*, 2017. Does distillery yeast sludge ameliorate moldy feed toxic effects in white leghorn hens? Toxin Rev 36:228–35.

Lakshmaiah G, 2017. Brain histopathology of the fish *Cyprinus carpio* exposed to lethal concentrations of an organophosphate insecticide phorate. Int J Adv Res Develop 2:668-72.

Maharajan K, Sellamani M, Bojan N, *et al.*, 2018. Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos (*Danio rerio*):A multi biomarker study. Aquatic Toxicol 196:132-45.

Mehrnoush G, Mehrdad S and Saeid K, 2013. Effect of pyriproxyfen on function and tissue of testis in adult rat. Int J Curr Res Rev 5:66-74.

Merdana IM, Watiniasih NL, Sudira IW, *et al.*, 2021. The effect of ethanolic extract of *Myrmecodia pendans* on gentamicin induced nephrotoxicity in Wistar Rats. Int J Vet Sci 10:96-101.

Mishra AK and Mohanty B, 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). Environ Toxicol Pharmacol 26:136-41.

Muñiz S Gonzalvo P Valdehita A, *et al.*, 2017. Ecotoxicological assessment of soils polluted with chemical waste from lindane production:Use of bacterial communities and earthworms as bioremediation tools. Ecotoxicol Environ Saf 145:539-48.

Namratha ML, Lakshman M, Jeevanalatha M *et al.*, 2021. Hematological alterations induced by glyphosate and ameliorative effect of ascorbic acid in Wistar rats. Continental Vet J 1:32-6.

Rasdi Z, Kamaludin R, Ab Rahim S, *et al.,* 2020. The impacts of intrauterine Bisphenol A exposure on pregnancy and expression of miRNAs related to heart development and diseases in animal model. Sci Rep 10:5882.

Roshanravan B, Mehrpour O, Ashrafizadeh M, *et al.*, 2021. Age-dependent effect of chlorpyrifos on the hematological parameters in male rats. Toxin Rev 40:1035-1039.

Sachar A and Raina S, 2014. Effect of lindane on immune organs of the fish *Aspidoparia morar*. Int J Innovat Res Sci, Engg Technol 3:8510-15.

Sepici-Dinçel A, Benli AÇK, Selvi M, *et al.*, 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings:Biochemical, hematological, histopathological alterations. Ecotoxicol Environ Saf 72:1433-39.

Shahid A, Zaidi SD, Akbar H, *et al.*, 2019. An investigation on some toxic effects of pyriproxyfen in adult male mice. Iran J Basic Med Sci 22:997-1003

Singh T and Singh DK, 2019. Lindane degradation by root epiphytic bacterium *Achromobacter* sp. strain A3 from *Acorus calamus* and characterization of associated proteins. Int J Phytoremediat 21:419–24.

Afzal G, **Ahmad** HI, Hussain R, *et al.,* 2022. Bisphenol A Induces Histopathological, Hematobiochemical Alterations, Oxidative Stress, and Genotoxicity in Common Carp (Cyprinus carpio L.). Oxid Medi Cell Long https://doi.org/10.1155/2022/5450421

Stoyanova S, Georgieva E, Velcheva I, *et al.*, 2020. Multi-biomarker assessment in common carp (*Cyprinus carpio*, Linnaeus 1758) liver after acute chlorpyrifos exposure. Water 12:1837.

Taha MG, El-Hamamsy SMA, Ahmed NS, *et al.*, 2020. Amelioration effect of *Carica papaya* fruit extracts on doxorubicin – induced cardiotoxicity in rats. Int J Vet Sci 9:349-54.

Tahir R, Ghaffar A, Abbas G, *et al.*, 2021. Pesticide induced hematological, biochemical and genotoxic changes in fish:A review. Agrobiol Rec 3:41-57.

Truong L, Gonnerman G, Simonich MT, *et al.*, 2016. Assessment of the developmental and neurotoxicity of the mosquito control larvicide pyriproxyfen using embryonic zebrafish. Environ Pollut 218:1089-93.

Wang Z, Liu H and Liu S, 2016. Low-dose bisphenol A exposure:A seemingly instigating carcinogenic effect on breast cancer. Adv Sci 4:1600248.

Xu J, Zhou L, Wang S, *et al.*, 2018. Di-(2-ethylhexyl)-phthalate induces glucose metabolic disorder in adolescent rats. Environ Sci Pollut Res Int 25:3596–3607.

Yang B, Liu Y, Li Y, *et al.*, 2021. Exposure to the herbicide butachlor activates hepatic stress signals and disturbs lipid metabolism in mice. Chemosphere 283:131226.

Wang J, Hussain R, Abdul Ghaffar, *et al.,* 2022. Clinicohematological, Mutagenic, and Oxidative Stress Induced by Pendimethalin in Freshwater Fish Bighead Carp (Hypophthalmichthys nobilis). Oxid Medi Cell Long https://doi.org/10.1155/2022/2093822