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Research Article

STUDIES ON HAEMATOLOGICAL AND HISTOPATHOLOGICAL ALTERATIONS INDUCED BY SUBLETHAL CONCENTRATION OF FENOXAPROP-P-ETHYL ON FRESHWATER FISH *CYPRINUS CARPIO*

Srinivas Bharamappa Neglur[1], Rajeshwari Danappa Sanakal[1*], Muniswamy David[2], Lokeshkumar Prakash[2]

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ABSTRACT: The present study aimed to investigate the effects of sublethal concentration of herbicide Fenoxaprop-P-ethyl on freshwater edible fish *Cyprinus carpio*. Fishes exposed to a sublethal concentration of herbicide (37.5 µg L^{-1}) for 15, 30, and 45 days following the semi-static bioassay method. Haematological and histopathological studies were carried out to evaluate the effects on the vital organs, including the liver, kidney, and gills. Haematological indices such as red blood cells (RBC), haemoglobin, lipid profile, liver function tests, and kidney function tests varied with increasing exposure period. Histopathological alterations revealed hyperplasia, telangiectasia, epithelial separation, and destruction of secondary lamellae in the gills of herbicide-exposed fishes. The liver showed vacuolation, necrosis, nuclear degeneration, and blood infiltration. The kidney exhibited dilated Bowman's space, haematopoietic necrosis, tubular necrosis, pyknosis, and dilated tubules. The histo-architectural changes were directly dependent on the duration of exposure and manifested an increased detrimental effect on the physiology of the fish *Cyprinus carpio*.

Key words: *Cyprinus carpio*, Fenoxaprop-P-ethyl, Histology, Haematology, Herbicide.

INTRODUCTION

India is the second main aquaculture producer (first being China) in the world capable of producing 7.1 million tonnes of aquaculture products. A total of 54.3 million tonnes of finfish and 17.5million tonnes of molluscs were produced as the main cultured species worldwide. Among the principal species, the production of *Cyprinus carpio* was found to be 41, 89, 524 tonnes in the year 2018 (FAO 2018). The practice of integrated rice-fish farming in many countries assisted the development of new modified simple aquaculture methods (Halwart 2004, Lu 2006). However, the pesticides/herbicides used in the rice fields were found to produce many harmful effects on fishes including the bioaccumulation of pesticide residue in the muscles of *Cyprinus carpio* (Clasen 2018). Organophosphates and carbamates were well-known AChE inhibitors that significantly inhibit the acetylcholinesterase activity in pest species (Jokanovic 1997, Yanhua *et al.* 2014). Recent publications revealed the pesticide effects on non-target organisms which are contributed to the structural and physiological similarities of organisms with pest species (David and Kartheeek 2015, Clasen 2018, David and Lokeshkumar 2020).

Pyrethroids and organochlorines which were also used in the control of harmful insects in household activities as well as agricultural fields had profound detrimental effects on non-target organisms (Koprucu 2004, Karaca *et al.* 2014). Agriculture-based land and surrounding water bodies are contaminated with herbicide/ pesticide residues that had incurred serious problems worldwide (Oruc 1999, Adams *et al.* 2000). Several commercial herbicide formulations are vigorously used to control unwanted weeds which hinders the growth of main crops in the agricultural fields and aquatic ecosystems. Such unscientific use of herbicide induces pernicious effects on non-target organisms including humans (Charlene *et al*. 2012). Commercial formulations of the well-known herbicide Glyphosate (N-Phosphonomethylglycine) were

[1]*Department of Zoology, Karnatak Science College, Dharwad - 580 001, Karnataka, India.*
[2] *Department of Zoology, Karnatak University, Dharwad- 580 003, Karnataka, India.*
**Corresponding author. e-mail: sanakalraju@gmail.com*

used since the 1970s to control aquatic weeds. There were two formulations of Glyphosate that were thought to be safe in the environment but later with rigorous toxicological investigations one formulation was evaluated with potential toxic effects on aquatic organisms (Gholami-Seyedkolaei *et al.* 2013, Kondera *et al*. 2018). Recent studies revealed the effects of commercial formulations of herbicides containing Imazethapyr, Imazapic, and Penoxsulam on various biomarkers of aquatic organisms such as acetylcholinesterase activity, protein levels, and oxidative stress (Moraes *et al.* 2011, Camila *et al.* 2014). Atrazine, a type of chloro-s-triazine herbicide that inhibits photosynthesis by competing with plastoquinone II binding site in photosystem II, affects liver enzymes and lymphocyte count in *Cyprinus carpio* even in the recovery period (Jana *et al*. 2014). However, (2,4-dichlorophenoxy) acetic acid (2,4-D) was also expressed the toxic effects on the behavior of Nile tilapia which was exposed to various concentrations of herbicide (Sarikaya and Selvi 2005).

Fenoxaprop-ethyl{ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propanoate} is a widely used herbicide to control annual and perennial weeds in commercial crops including rice, wheat, and onion. The herbicide belongs to 2-(4-aryloxyphenoxy) propionic acid family that disrupts the fatty acid synthesis through the inhibition of acetyl CoA carboxylase enzyme in grasses (Cocker *et al.* 1999, Pornprom *et al.* 2006). Fenoxaprop acid persists in the soil for 21 days manifesting a half-life of 7.3 days in Indian soil. However, the degradation of Fenoxaprop herbicide was seemed to be highly pH dependant (Sing *et al.* 2013). Another study focused on the toxicity of by-products upon degradation of Fenoxaprop-P-ethyl subjected to solar irradiation. The study elucidated that the by-products formed were more toxic to *Daphnia magna* than the parent compound and suggested further detailed toxicity evaluation on aquatic animals (Jing *et al.* 2008). Exposure of Duroc boars to different concentrations of Fenoxaprop-P-ethyl had noticeable effects on the sperm motility and viability which is an ideal indication of pronounced toxic effects of the herbicide (Betancourt *et al.* 2006).

Another major study which was dealt with exposure of adult females and pre-metamorphic tadpoles (*Rana catesbeiana*) to Fenoxaprop-P-ethyl (98.0%) affected kidney, liver, skin, eggs, blood, and even on the brain. The presence of Fenoxaprop-P-ethyl in the brain of frogs indicated the penetrating ability of the herbicide via the blood-brain barrier which is a serious threat to mammalian and non-mammalian organisms (Jing *et al*. 2017). Research data indicates that the herbicides are furthermore known to damage the histoarchitecture of the fish liver, gill, and kidney. In particular, liver damage was characterized by the high vacuolization of the cytoplasm, hepatocyte hypertrophy, gills with lamellar damage, epithelial hyperplasia, and destruction of tubular epithelial cells in the kidney (Crestani *et al.* 2007, Shiogiri *et al.* 2012, Velisek *et al.* 2013). Innumerable adverse effects of Fenoxaprop-P-ethyl on various organisms indicate its toxic capacity in the environment. However, toxicity studies on aquatic fauna still lack data on pestilential information. A recent study focused on *Cyprinus carpio*, a major Indian freshwater edible fish to determine the toxic effects of Fenoxaprop-P-ethyl. The study pointed out that the herbicide was lethal at a dose of 300 µg L^{-1} and affects various behavioral parameters including lateral swimming, mucus secretion, and dyspigmentation (Neglur *et al.* 2020). The present study was carried out to evaluate the haematological and histopathological changes in *Cyprinus carpio* exposed to sublethal concentrations of commercial-grade Fenoxaprop-P-ethyl.

MATERIALS AND METHODS

Experimental animal

Healthy and active common carp (*Cyprinus carpio*) with similar sizes (Length 20±4 cm, weight 220±5 g) were collected from Bhadra fish seed farm, Shimoga, Karnataka. The fishes were maintained in large round cement tanks (Approximately 1000 liters) with sufficient aeration and pretreated with 0.05% potassium permanganate to avoid any possible dermal infections. Fishes were allowed to acclimatize for 20 days to laboratory conditions with 12-14 hours of natural photoperiod at 25°C. During acclimatization, animals were fed twice a day with balanced nutritious food pellets (Nova, Aquatic P. feed). Water was renewed daily to provide healthy environmental conditions which were free of accumulated excreta. Physicochemical analysis of water was performed according to the guidelines of the American Public Health Association (APHA 2005) and presented in Table 1.

Chemicals

Formulations of herbicide Fenoxaprop-P-ethyl (6.9% w/v EC) was purchased from Bayer House, Central Avenue, Hiranandani Estate (west), India under the marketing name "Ricestar" containing 6.70% of Fenoxaprop-P-ethyl as an active ingredient. LC_{50} of the herbicide was determined in previous studies as 300 µg L^{-1} (Neglur *et al.* 2020). For the present study, the selected

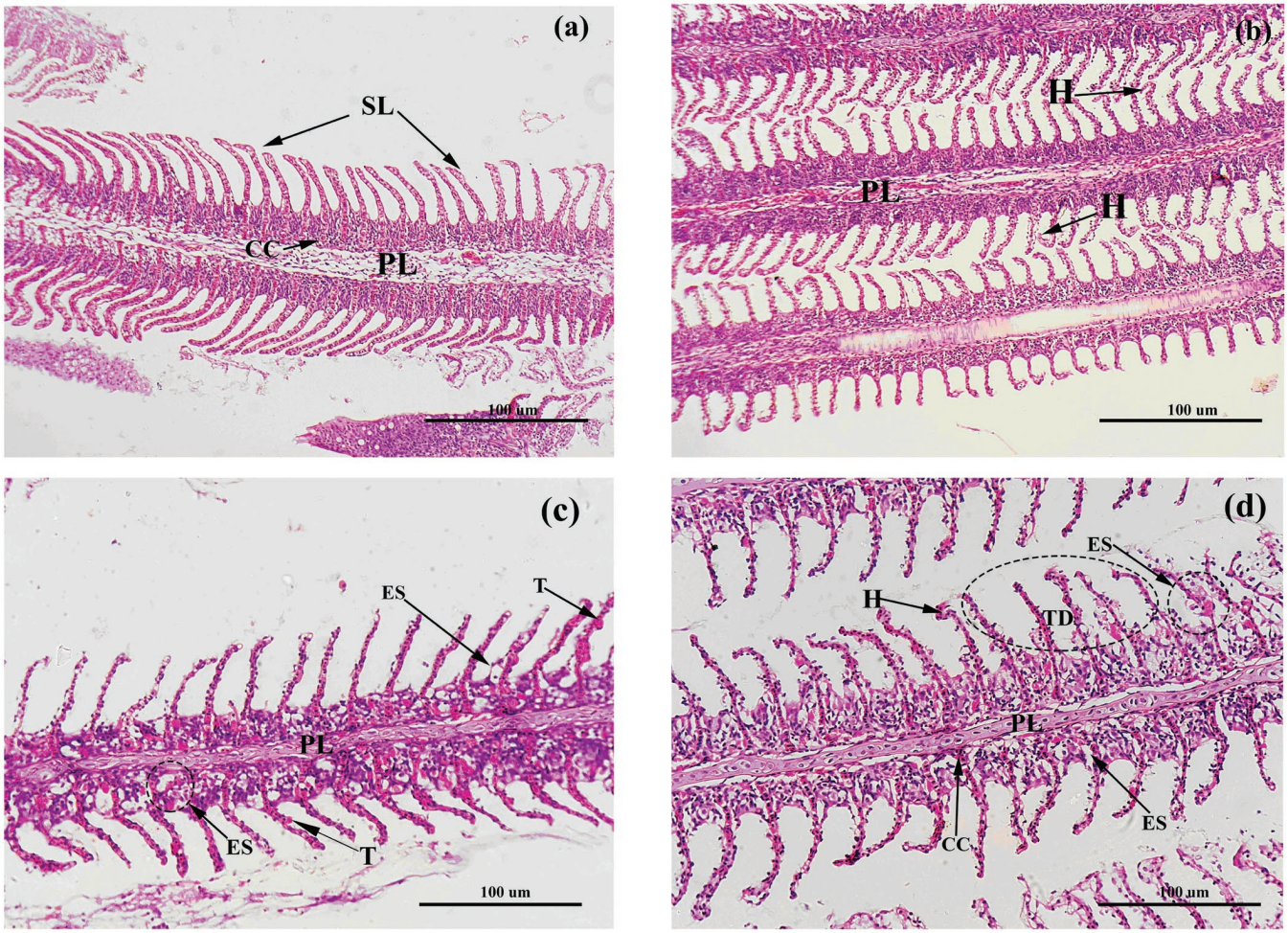

Fig. 1. Gill tissues of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

[(a) Gill structure of control fish: (PL) primary lamellae; (SL) intact secondary lamellae; (CC) chloride cell. H&E. (b) Gill tissue of *C. carpio* exposed to 37.5 µg L^{-1} of Fenoxaprop-p ethyl for 15 days: (H) hyperplasia. H&E. (c) Gill tissue of *C. carpio* exposed for 30 days: (T) telangiectasia; (ES) epithelial separation. H&E. (d) Gill tissue of *C. carpio* exposed for 45 days: (ES) epithelial separation; (H) hyperplasia; (TD) total destruction of secondary lamellae; (CC) chloride cell. H&E].

sublethal concentration of the herbicide (37.5 µg L^{-1}) was prepared by the serial dilution method from the stock solution. The herbicide concentration was freshly prepared every time before the exposure to avoid degradation in the aquatic medium. All other chemicals used in the experiment were analytical grade and purchased from SRL Chemicals Pvt. Ltd. and Sigma-Aldrich.

Experimental design

Post acclimatized 54 healthy fishes were selected and divided into 3 groups having triplicates (6 fishes/replicate) in each group. A semi-static bioassay was performed to keep the concentration of herbicide constant in all experimental days, and the experimental period was 15, 30, 45 days. Each group (G_1, G_2, G_3) was exposed to the sublethal concentration of herbicide (37.5 µg L^{-1}) for 15, 30, and 45 days, respectively. All experimental group fishes were held in glass aquaria of a capacity of 20 liters. The post-experimental fishes were carefully euthanized with MS-222 at 550 mg L^{-1}, the organs were dissected out, washed with PBS (1X), and kept in a deep freezer (-40 °C) for further analysis.

Histology

Anatomized organs (gill, liver, kidney) were washed with 1X PBS, fixed in formalin (10% formaldehyde) for 24 hours, dehydrated with graded alcohol, and embedded in paraffin (Bancroft and Gamble 2002). Thin sections of 5 µm were taken using an automated microtome (Leica RM 2255). The sections were subjected to haematoxylin and eosin staining and the images were photographed using an Olympus phase-contrast microscope (Olympus BX51, Tokyo, Japan) with an attached photographic

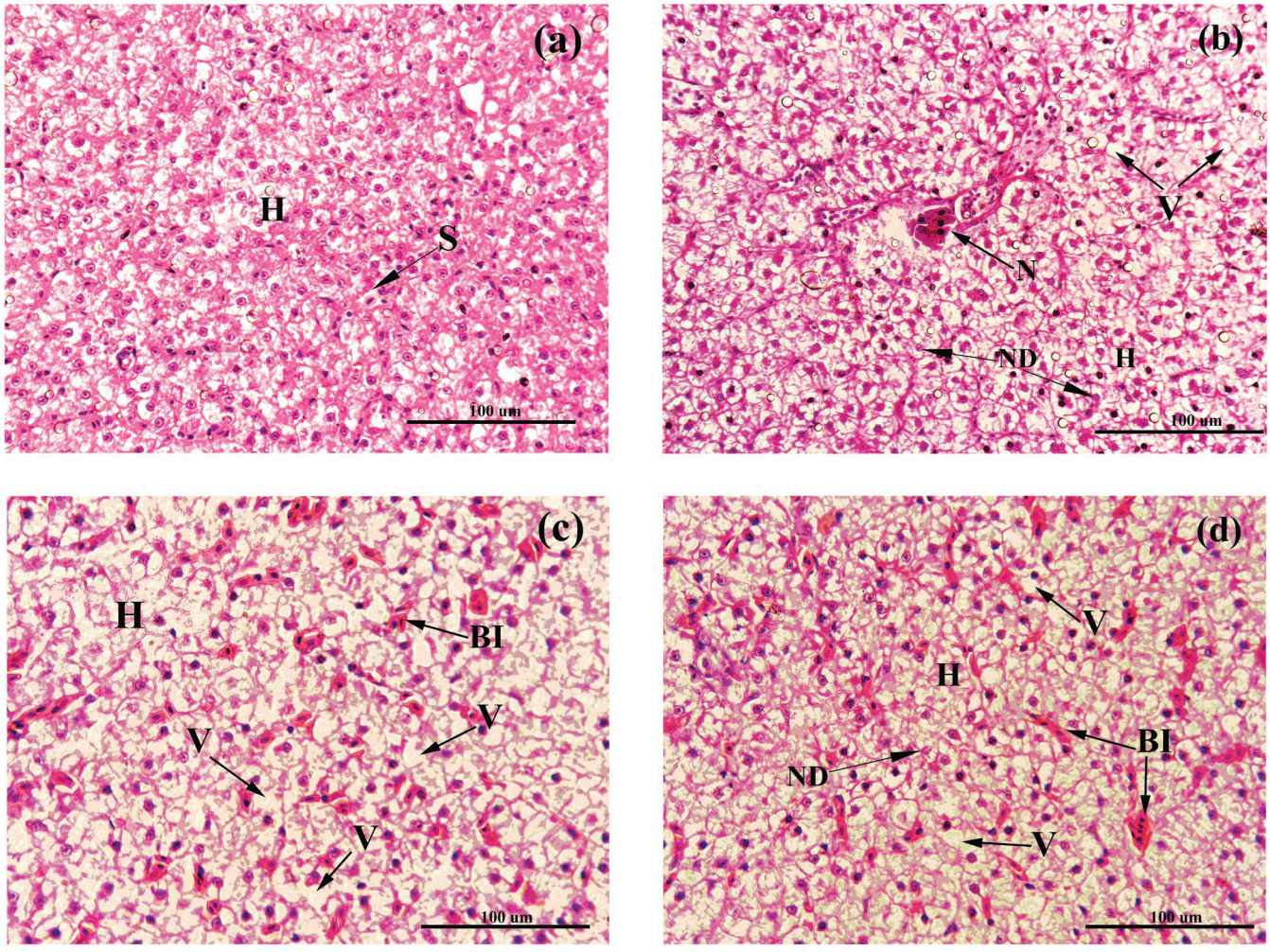

Fig. 2. Liver structure of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

[(a) Liver structure of control fish: (H) hepatocytes; (S) sinusoids. H&E. (b) Liver tissue of *C. carpio* exposed to 37.5 µg L^{-1} of Fenoxaprop-p ethyl for 15 days: (L) lipid droplets; (N) necrosis; (ND) nuclear degeneration. H&E. (c) Liver tissue of *C. carpio* exposed for 30 days: (BI) blood infiltration; (V) Vacuolation H&E. (d) Liver tissue of *C. carpio* exposed for 45 days: (L) lipid droplets; (ND) nuclear degeneration; (BI) blood infiltration. H&E].

camera (ProgRes C3, Jenoptic-Germany). Stained slides were analyzed for histopathological alterations induced by Fenoxaprop-P-ethyl.

Haematology

The blood was collected both in heparinized (50 IU sodium salt of heparin mL^{-1} of blood) and non-heparinized vacutainers by cardiac puncture. Haematological indices including erythrocyte count (RBC), leucocyte count (WBC), haemoglobin concentration (Hb), haematocrit value (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were tested. Blood samples in non-heparinized tubes were centrifuged at 500 g for 15 minutes and the serum was separated. The serum samples were analyzed for lipid profile (serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides), renal function (serum urea, uric acid, creatinine), and liver function tests such as serum bilirubin T, bilirubin D, bilirubin indirect, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein and total albumin. All parameters were analyzed using an auto haematology analyzer (UBM, Fx-19).

Ethical statement

All the experiments performed in the present study abide by the guidelines of the Institutional Animal Ethics Committee (IAEC). The experimental animals used in the study were handled with care according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals

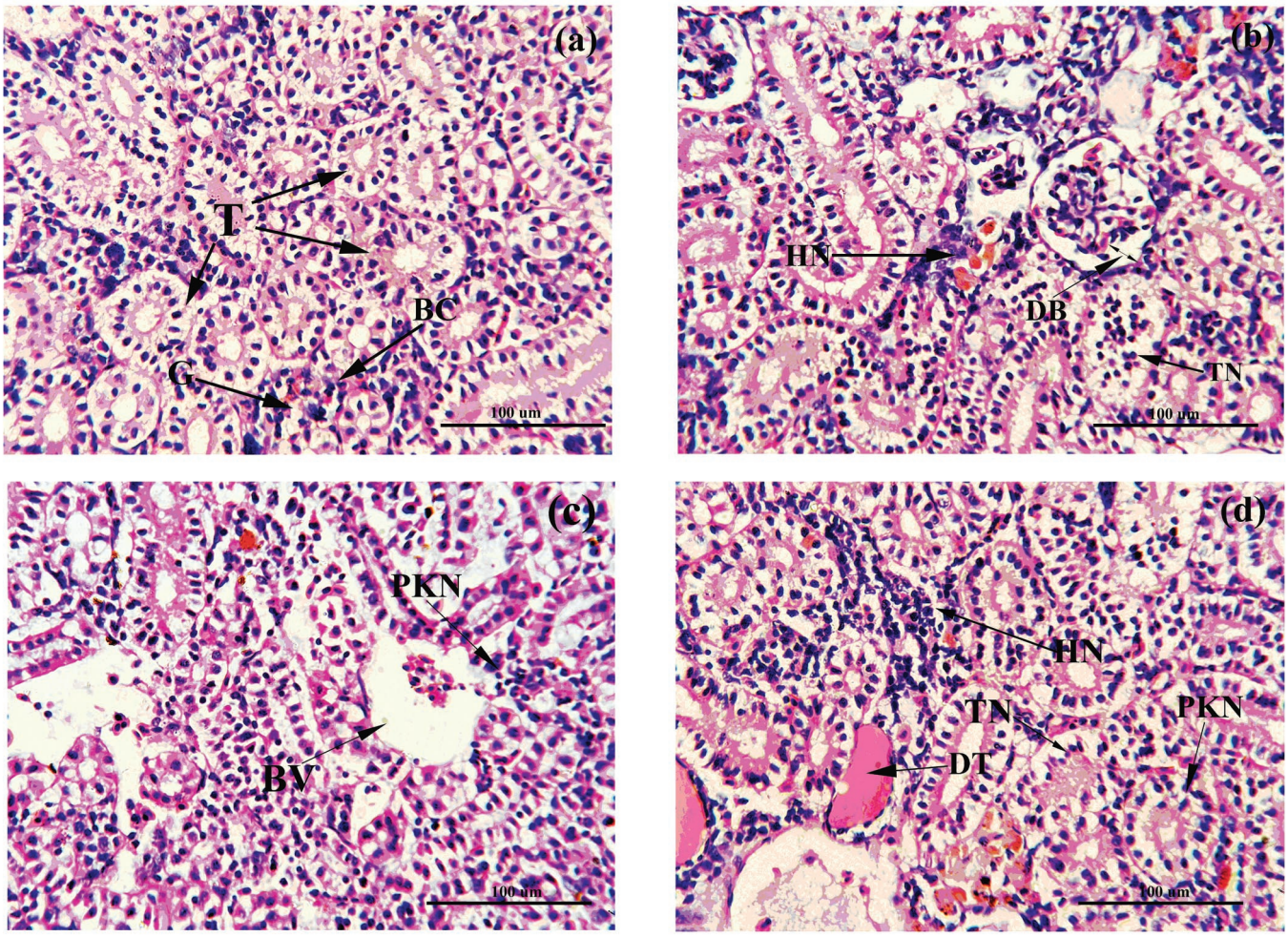

Fig. 3. Kidney structure of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

[(a) Kidney structure of control fish: (T) kidney tubules; (BC) Bowman's capsule; (G) Glomerulus. H&E. (b) Kidney tissue of *C. carpio* exposed to 37.5 µg L^{-1} of Fenoxaprop-p ethyl for 15 days: DB) Dilated Bowman's space; (HN) Hematopoietic necrosis; (TN) renal tubular necrosis. H&E. (c) Kidney tissue of *C. carpio* exposed for 30 days: (PKN) pyknosis; (BV) blood vessel. H&E. (d) Kidney tissue of *C. carpio* exposed for 45 days: (HN) Haematopoietic necrosis; (TN) tubular necrosis; (DT) dilated tubule; (PKN) pyknosis. H&E].

(CPCSEA), New Delhi, India.

Statistical analysis

All the results presented in this study were analyzed using SPSS ver. 25 and followed the one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Data were presented as Mean values ± Standard deviation (SD) with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Histology of gill structure of control fish group was observed with a typical normal gill structure including chloride cell, primary lamellae, and secondary lamellae and epithelium. The histological photographs of control and experimental group fishes were examined by using an atlas of fish histology (Takashima and Hibiya 1995) and summarized in Fig. 1. Gill structure of fishes treated for 15, 30, and 45 days was noticed with hyperplasia, telangiectasia, epithelial separation, and destruction of secondary lamellae. The histological malformations were remarkable with an increase in days of exposure. Herbicide Fenoxaprop-P-ethyl was found to be a potentially toxic drug that induced alterations in the behavior of *Cyprinus carpio* as described in a previous study (Neglur *et al.* 2020). The present study followed semi static-bioassay for fishes as described by The Organization for Economic Co-operation and Development (OECD) guidelines 203 (OECD 2019). The half-life of the herbicide Fenoxaprop-P-ethyl was found to be 7.3 days in Indian soil (Sing *et al.* 2013). However, in the present study, the experimental media was renewed every day to maintain the constant concentration of herbicide so that there was no concentration variation that might have occurred due to the half-life of the

Table 1. Physicochemical parameters of water.

Parameter	Obtained values
Temperature	25±2ºC,
pH	7.1±0.3 at 25ºC,
Dissolved oxygen	9.4±0.8 mgL^{-1}
Carbon dioxide	6.2±0.3 mgL^{-1}
Total hardness	23.8±3.1 mgL^{-1}
$CaCO_3$	nil
Phosphate	0.34±0.002 ìg L^{-1}
Salinity	nil
Specific gravity	1.001
Conductivity	less than 10 ìScm^{-1}

toxicant. Histopathological studies of gill, liver, and kidney of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-p-ethyl revealed various changes.

The gills are the main organs that directly communicate with the external environment, and any change in the environment is primarily sensible to gills. In the present study, the control group fish gill structure exhibited normal intact secondary lamellae attached with primary lamellae. However, respiratory distress is one of the initial symptoms of intoxication and could arise due to the dysfunction of respiratory organs (McDonald 1983). In sublethal exposed fishes the histopathological alterations such as curling of secondary lamellae (hyperplasia), epithelial separation/ lifting, club-shaped bulging in secondary lamellae (telangiectasia), and destruction of secondary lamellae were observed (Fig. 1). Hyperplasia significantly decreases the secondary lamellar surface area and interrupts normal respiration in the fish. Telangiectasia causes bulging of secondary lamellae which results from a disintegrated pillar cell system and is considered to be a potential injury to the gill structure (Van Dyk *et al.* 2009). The occurrence of epithelial lifting (edema) is due to the outflow of serous fluids into the interstices of the gill tissue. Similar histopathological symptoms were reported by Velmurugan *et al*. 2007, van Dyk *et al.* 2009, Ghaffar *et al*. 2018. The symptoms were progressive in both 30- and 45-day exposed gills of *Cyprinus carpio*.

Liver histology of *Cyprinus carpio* exposed to sublethal concentration was given in Fig. 2. At higher microscopic resolution control group liver revealed distinctive hepatocyte structure with sinusoids. The liver is the most important organ of various metabolic pathways and detoxification of toxic chemicals that enter the body through different forms. In our study, the liver exhibited histopathological alterations including the occurrence of vacuolation, necrosis, nuclear degeneration, and blood infiltration. Control group fish liver was observed with the normal arrangement of hepatocytes, sinusoids, and well-organized nucleus (Fig. 2). The exposure of chlorpyrifos pesticide on *Cyprinus carpio* revealed similar histological changes such as necrosis, degenerative lesions, and vacuolation (Stoyanova *et al.* 2020).

The pyknotic nucleus is a result of the condensation of chromatin which specifies the onset of cell death in liver tissue. This kind of nuclear change could result in the dysfunction of the organ and its contents including SGOT and SGPT which is true in the case of this study (Akaishi *et al.* 2004). Liver tissue vacuolation was found mainly in 30 days of exposure and indicates the sign of degradation of tissues. Ansoar-Rodriguez *et al.* (2016) obtained similar results upon exposure of *Oreochromis niloticus* to Imidacloprid. Banaee *et al.* (2013) observed nuclear atrophy and hepatocyte cloudy swelling as a prominent morphological change in liver tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to sublethal concentrations of diazinon. From the above results, it seems that the fish at a sublethal concentration of pesticide/ herbicide tried to become adapted to the toxic environment both physically and physiologically. However, further studies are required to reveal the definite pathway of adaptation of fish to the toxic environment. The current study indicates that blood infiltration was more prominent in 30 and 45 days exposed fish and suggests that the effect is exposure time dependent. Minimal histopathological observation in 15 days exposed fish could be an indication of the emergence of toxic effects.

Histopathology of control group fish kidney manifested well-organized kidney tubules, Bowman's capsule, and glomerulus. Histoarchitectural photographs of control fish and experimental fish kidneys were reported in Figure 3. The kidney is the main organ that receives a large amount of post branchial blood and helps to remove toxic materials from blood (Crestani *et al*. 2007). Any histopathological damage could cause serious problems to the fish eventually leading to death due to toxic chemical accumulation in the blood. The histological changes increased with increasing days of exposure are very prominent at 45 days of exposure. Our results were concurrent with Crestani *et al.* (2007), Poleksic *et al.* (1999) and Velisek (2010).

Table 2. Haematology of *Cyprinus carpio* exposed to sub-lethal concentration of Fenoxaprop-P-ethyl.

	Sublethal exposure			
Indices	**Control**	**15 days (G_1)**	**30 days (G_2)**	**45 days (G_3)**
Red blood cells (x 10^6 mm^{-3})	2.87 ± 0.0003[a]	2.34 ± 0.0003[b]	2.47 ± 0.0003[c]	2.66 ± 0.0003[d]
White blood cells (x 10^3 mm^{-3})	11.66 ± 0.0003[a]	12.01 ± 0.0001[b]	12.25 ± 0.0003[c]	12.79 ± 0.0003[d]
Hemoglobin (g x 10^{-2} mL^{-1})	5.99 ± 0.0003[a]	5.43 ± 0.0004[b]	5.52 ± 0.0003[c]	5.67 ± 0.0003[d]
Packed cell volume (%)	32.93 ± 0.0003[a]	48.89 ± 0.0003[b]	26.73 ± 0.0003[c]	30.50 ± 0.0003[d]
Mean corpuscular volume (%)	52.89 ± 0.0003[a]	56.32 ± 0.0003[b]	57.56 ± 0.0003[c]	53.79 ± 0.0003[d]
Mean corpuscular hemoglobin (%)	13.72 ± 0.0037[a]	20.03± 0.0003[b]	19.71 ± 0.0037[c]	15.90 ± 0.0003[d]
Mean corpuscular hemoglobin concentration (%)	16.90 ± 0.0003[a]	15.75 ± 0.0037[b]	15.00 ± 0.0003[c]	15.87 ± 0.0003[d]

*The values are given as the mean ±SD; n=6. Values in the row with different superscript are significantly different at $p<0.05$.

Table 3. Lipid profile of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

Indices ($mgdL^{-1}$)	**Control**	**15 days (G_1)**	**30 days (G_2)**	**45 days (G_3)**
Serum cholesterol	202.2 ± 0.30[a]	124.4 ± 0.31[b]	109.2 ± 0.27[c]	190.3 ± 0.29[d]
High density lipoprotein; HDL	65.2 ± 0.27[a]	31.3 ± 0.30[b]	39.4 ± 0.31[c]	54.4 ± 0.33[d]
Low density lipoprotein; LDL	121.3 ± 0.30[a]	84.3 ± 0.30[b]	115.2 ± 0.27[c]	120.4 ± 0.33[d]
Serum triglycerides	199.3 ± 0.30[a]	92.3 ± 0.30[b]	100.2 ± 0.27[c]	140.3 ± 0.29[d]

*The values are given as the mean ±SD; n=6. Values in the row with different superscript are significantly different at $p<0.05$.

Table 4: Renal function tests of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

Indices($mgdL^{-1}$)	**Control**	**15 days (G_1)**	**30 days (G_2)**	**45 days (G_3)**
Serum urea	68.8 ± 0.27[a]	30.4 ± 0.33[b]	42.4 ± 0.31[c]	56.2 ± 0.27[d]
Uric acid	7.3 ± 0.29[a]	3.4 ± 0.32[b]	5.3 ± 0.28[c]	6.2 ± 0.30[d]
Serum creatinine	1.3 ± 0.29[a]	0.42 ± 0.37[b]	0.84 ± 0.37[c]	0.25 ± 0.35[d]

*The values are given as the mean ±SD; n=6. Values in the row with different superscript are significantly different at $p<0.05$.

Analyzed haematological data were represented in Table 2. Erythrocyte count in all the experimental groups slightly varied compared to the control group. There was a significant decrease in RBC count in 15 days (2.34 x $10^6/mm^3$) exposed fish. However, in 30- and 45-days experimental groups (2.47 and 2.66 x $10^6/mm^3$ respectively) it was slightly recovered but not equal to the control group (2.87 x $10^6/mm^3$). Leucocyte count and haemoglobin concentration followed the same trend as erythrocyte with a slight decrease in 15 days exposed fish and an increase in 30 and 45 days of the experimental group. Haematocrit value (PCV) was shown to be increased in 15 days group (48.89%) compared to the control group (32.93%) that again decreased in 30 and 45 days with 26.73%, 30.50%, respectively. Blood is one of the major biological fluids that help the animal by an uninterrupted supply of oxygen to the body. Fishes being poikilothermic have varying temperatures concerning the surrounding environment. Alteration in blood parameters directly or indirectly depends on the toxicant and the exposing period of dose (Burgos *et al.* 2015). In the present study RBC, WBC, and haemoglobin concentration expressed the lowest values at 15 days of exposure and increased in the remaining days of exposure.

Table 5. Liver function tests of *Cyprinus carpio* exposed to sublethal concentration of Fenoxaprop-P-ethyl.

Indices	Control	15 days (G_1)	30 days (G_2)	45 days (G_3)
Serum bilirubin T ($mgdL^{-1}$)	1.3 ± 0.28^a	0.67 ± 0.03^b	0.90 ± 0.03^c	1.4 ± 0.32^a
Serum bilirubin D ($mgdL^{-1}$)	0.35 ± 0.37^a	0.21 ± 0.37^b	0.26 ± 0.37^c	0.34 ± 0.37^a
Serum bilirubin indirect ($mgdL^{-1}$)	0.27 ± 0.30^a	0.40 ± 0.03^a	0.41 ± 0.33^a	0.90 ± 0.03^b
SGOT (UL^{-1})	394.2 ± 0.37^a	195.9 ± 0.37^b	221.0 ± 0.18^c	294.3 ± 0.37^d
SGPT (UL^{-1})	125.1 ± 0.37^a	60.4 ± 0.25^b	76.7 ± 0.41^c	99.2 ± 0.37^d
Serum total protein (UL^{-1})	4.9 ± 0.93^a	3.2 ± 0.27^b	4.3 ± 0.03^a	4.1 ± 0.30^a
Serum albumin (UL^{-1})	1.56 ± 0.32^a	0.55 ± 0.23^b	1.28 ± 0.03^a	1.47 ± 0.03^a

*The values are given as the mean ±SD; n=6. Values in the row with different superscript are significantly different at $p<0.05$.

[SGOT = Serum glutamic oxaloacetic transaminase, SGPT= Serum glutamic pyruvic transaminase].

Decreased values of RBC and haemoglobin indicate an anemic condition that might be attributed to the destruction of haematopoietic tissues and osmoregulatory dysfunction (Jenkins *et al.* 2003, Banaee *et al.* 2008). A decrease in haemoglobin level might have occurred due to oxidation of haemoglobin to methaemoglobin or production of O_2^- radicals (Trout 2008). Alterations in haemoglobin content might also be interpreted to toxicant-induced stress on the animal (Kori-Siakpere and Ubogu 2008).

Mean corpuscular volume (MCV) was manifested decreasing trend from 15 to 45 days (56.32% and 53.79%) but observed a drastic increase in 30 days (57.56%) exposed fishes which were significantly different compared to the control group. Mean corpuscular haemoglobin (MCH) was found to rise in 15 days and decreased significantly in other groups concerning the control group. Mean corpuscular haemoglobin concentration (MCHC) was appeared to be nearly constant in all the groups. Increased levels of MCV (30 days) and MCH (15 days) might be due to the swelling of RBC or increased number of immature RBC which might have resulted from hyperplasia in erythropoietic tissues (Woo *et al.* 2018). Packed cell volume (PCV) is one of the most common diagnostic tools to assess the amount of RBC. The factors such as stress, physical characteristics, and environmental factors contribute to the variation of PCV (Grant Krystan 2015). In the present study, there was significant variation in PCV values of exposed fish groups. An increased percentage of PCV in 15-day exposed group could be due to relative polycythemia resulting from dehydration (Claus *et al,* 2008). However, in 30 and 45 days of exposure, it was found to be recovered or they might be adapted to the toxic environment. MCHC is the best indicator of RBC swelling. In our study significant but relatively less variation was observed in MCHC levels which indicates an unaltered RBC structure (Zutshi *et al.* 2010).

The lipid profile of *Cyprinus carpio* exposed to sublethal concentration is given in Table 3. Serum cholesterol levels were drastically decreased in all experimental groups compared to the control group. Serum HDL, LDL, and triglyceride exhibited the same pattern with the decrease in 15 days exposed group and increase in the 30 and 45 days of the experimental group. Decreased cholesterol, HDL, LDL levels in 15-day exposure could be a sign of the initial dynamic toxic effect of the herbicide Fenoxaprop-P-ethyl and the physiological acclimatization posed by the fish to survive within a toxic environment. This type of physiological temporary adaptation might help the animal to survive for a longer time even with toxic environments as resulted from the present study. A similar decreasing trend in HDL levels was observed in *Cyprinus carpio* exposed to organophosphorus pesticide phosalone (Kaya *et al.* 2015). Cholesterol and triglycerides are mostly dependent on the nutritional and physiological state of fish (Babin and Vernier 1989). Decreased levels of triglycerides in 15 days exposed fishes could be due to the altered physiological state of the fish (Peres *et al.* 2014). Although the fish appears to be recovered in the subsequent exposure period, it was noticeably under stress in 15 days exposed groups.

A renal function test was performed to evaluate the toxicity induced by herbicide treatment in the fish. The results of renal function tests are specified in Table 4. Serum urea and uric acid were found to be decreased in initial 15 days exposure and radically increased in 30 and 45 days of exposure. Serum creatinine was found to be highest in 30 days of exposure. Liver function test

results are presented in Table 5. The results of serum bilirubin T, bilirubin D, bilirubin indirect, SGOT, SGPT, and total albumin expressed initial decreased value in 15 days exposed fishes among other groups compared to control except total protein where the level was increased in 15- and 30-days group. The alterations in urea, uric acid, and creatinine content in the exposed groups of fishes indicate the dysfunction of the kidney. Renal dysfunction results in abnormal secretion of urea, uric acid, and creatinine.

The liver marker enzymes SGOT and SGPT showed a significant decrease in 15 days and an increase in 30- and 45-day exposure period. From the results it appears that the fish at 15 days exposure was initially suffered from a lack of energy due to low levels of enzymes and adapted with increased production of SGOT and SGPT at 30- and 45-days exposure. Increased production of liver enzymes could be attributed to meet the energy demand under stress conditions by the animal (Tilak 2005). SGOT and SGPT levels determine hepatic health, any variation in these enzymes could be a sign of hepatic injury or dysfunction (Ghorpade *et al.* 2002, Elseady and Zahran 2013). Bilirubin is one of the main bile pigments found in the fish circulatory systems derived from haemoglobin disruption (Cornelius 1991). The present study evidenced an increase in bilirubin contents (bilirubin D, bilirubin T, bilirubin indirect) from 15 to 45 days exposed groups which could be attributed to liver/ biliary tract damage (Saleh and Marie 2016). Serum protein levels were found to be low in 15 days exposed group and increased in 30 days and 45 days group. Decreased protein levels might be due to the stress condition induced by herbicide which affects the uptake of amino acids in the polypeptide chain/ inhibiting protein synthesis activity. A low level of serum protein might also indicate the utilization of protein content by the fish during stress conditions (Zaghloul *et al*. 2011). The increasing trend of albumin levels observed in the experimental groups from 15 to 45 days indicates the onset of the immune response against toxicity induced by the herbicide (Wiegertjes *et al.* 1996, Jha *et al.* 2007, Awad and Austin 2010). However, the fishes in 15 days experimental group suffered from decreased levels of albumin which might be attributed to herbicide-induced stress on the animal.

CONCLUSION

The present investigation revealed haematological and histopathological alterations in *Cyprinus carpio* exposed to Fenoxaprop-P-ethyl. Histopathological alterations in the liver, kidney, and gills evidence the penetrating ability of the herbicide. Significant alterations in the haematological parameters including the kidney function test and liver function test support the histopathological modifications in the fish. Hence, the farmers practicing integrated aquaculture were advised to take precautions during the application of herbicide and to follow the manufacturer's instructions strictly.

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