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Pymetrozine Causes Physical, Haematological, Blood Biochemical and Histopathological Abnormalities in Bighead Carp (*Aristichthys nobilis*)

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Key words
Pymetrozine, Bighead carp, Blood-biochemistry, Brain, Heart, Histopathology

ABSTRACT

Pymetrozine a synthetic insecticide, is widely used to control pests and insects in maize and other cereal crops. Pymetrozine may contaminate nearby aquatic ecosystems as a result of widespread and long-term use, causing harm to a variety of creatures. The residues of this pesticide can have devastating effects on various species, as well as public health, if they enter the food chain. Pymetrozine's toxicity in freshwater fish was the main focus of the current study. For this reason, 80 fish were collected and placed separately in four groups of 20 fish each. Pymetrozine was given to fish in groups B, C, and D at doses of 500, 1000, and 1500 µg/L in water. For histopathological and hematological examinations, blood and other visceral tissues were obtained on days 10, 20, and 30 of treatments. Different physical and behavioral disorders were noted in terms of time and concentration manners. In comparison to control fish, the hematological profile including lymphocyte, hemoglobin erythrocyte and monocyte counts, was considerably ($p < 0.05$) lower. The final results on the serum biochemical parameters showed significantly ($p < .05$) higher concentrations of liver biochemical profile, biomarkers of kidneys, triglyceride, glucose, and cholesterol in fish treated with pesticide. Histopathological examination showed renal tubular degeneration, widening of Bowman's space and necrosis in kidneys. Different histopathological lesions in liver of treated test specimens including edema, hemorrhages, atrophy of hepatocyte and hepatocyte having eccentric nuclei. In the brain, different histological alterations like necrosis and degeneration of neurons, and microgliosis were seen, whereas the heart of treated fish showed hemorrhages, edema, and neutrophilic myocarditis. In conclusion, Pymetrozine appears to have harmful effects on blood biochemistry and histological alterations in several tissues of bighead carp.

INTRODUCTION

Pesticides have become a severe hazard in recent years due to their widespread use in agriculture, veterinary practices, public health management and aquatic ecosystem (Jabeen *et al*., 2021; Naz *et al*., 2021). Accidental pesticide contact, such as herbicides and fungicides, kills and shortens the lifespan of a variety of target and non-target species in marine and terrestrial environments (Ghaffar *et al*., 2020). It has been proven by different studies that marine life is more sensitive to a variety of toxins than terrestrial animals, since a variety of detrimental compounds from various localities, such as, agriculture, industry, and production areas, are swiftly and easily transported toward water bodies (Warra and Prasad, 2020). It has been reported in different studies that contact to different chemicals including fluorinated substances, disinfectant by products, pesticides, herbicides and other synthetic chemicals has hazardous effects on wildlife (Adoamnei *et al*., 2018; Akram *et al*., 2020). The residues of these compounds in the food chain have negative health consequences, disturb metabolic activities, and lead to different organ dysfunctions in a number of non-target and target species (Ghaffar *et al*., 2020). Pymetrozine is related to 2nd class of hazard as its limiting indication is inhalation toxicity (Korshun, 2016). According to the World Health Organization (WHO), nearly 1,000,000 humans are affected acutely as a result of pesticide contact. Pesticides are responsible for around 0.4-1.9 percent of all deaths

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each year (Eddleston, 2020). Pymetrozine can be absorbed by marine animals, and contact with infected water through the skin produces behavioral and physical alterations like stoppage of feeding, impaired locomotion and swimming behaviors. Aquatic ecosystems are extremely sensitive and diverse. Pesticides and other contaminants may have severe implications for these ecosystems. In addition, aquatic ecosystems are frequently subjected to a variety of environmental stresses. Chemical stressors frequently interact or synergize, resulting in hazardous effects. Studies have observed that among aquatic animals, fish are the most susceptible marine species to pesticides and are important biomarkers for measuring the status of aquatic ecosystem (Faheem and Lone, 2017). There are few studies which indicate that pesticides causes molecular changes in the brain, gills, liver, kidneys, and reproductive cells and tissues of fish. Biochemical and hematological parameters are relatively well targets of toxicity in fish following exposure to pesticides and can be used as a valuable bio-indicators (Singh and Srivastava, 2010). Furthermore, the biochemical parameters changes such as glucose, protein and enzymes are frequently employed for the assessment of physiological changes in the aquatic settings and to give detailed conditions of stress. Pesticides toxicity in different organisms has been adequately reported, with effects on histology and morphology, oxidative stress and behavior. To our knowledge, however, there is little information about Pymetrozine's toxicity in freshwater fish, particularly bighead carp (*Aristhicthys nobilis*). As a result, we attempted to assess Pymetrozine toxicity in bighead carp at sublethal dosages.

MATERIALS AND METHODS

Toxicant and chemicals

Pymetrozine was purchased from M/S Ali Akbar Enterprises in Multan, Pakistan. Different chemicals (analytical grades) (USA) was purchased from Merck (Germany) and Sigma Aldrich (USA). These companies also provided additional compounds such as different reagents including commercial kits from Randox Company (Pvt.) Pakistan to estimate different serum biochemical biomarkers.

Experimental test specimens, management and acclimatization

This research trial included 80 bighead carp (*Aristichthys nobilis*) freshwater fish having same age, body mass (145-160 g), and size were obtained from local commercial fish farm in the Punjab region of Pakistan (District Bahawalnagar). The fish were transferred to the laboratory in the plastic bags which contains sufficient oxygen. Fish were kept in glass aquaria (14" length 10" width and 12" height) for 15 days to acclimatize. The fish were given commercial feed (2-3 percent of body weight) two times a day, in the morning and late evening. All aquaria were cleaned of any leftover feed or faecal contents.

Experimental treatments

Following acclimatization, all the fish were blindly divided and placed in different four groups (A-D), each contained 20 fish. A 100 liters water was added in each aquarium prior to shifting of fish. Fish kept in aquarium (group A) were considered as untreated control fish, whereas fish of groups B, C, and D were treated with pymetrozine. Pymetrozine was mixed in distilled water and given to all the fish of groups B, C, and D for a one month at a concentrations of 500, 1000 and 1500µg/L. Each concentration was renewed in the respective aquarium five days post-treatment to maintain the exact exposure concentration. The experiment was designed in triplicate. The remaining feed and fecal contents were strained and removed on daily basis throughout the trial. Fish kept in all groups were carefully observed for any obvious clinical or behavioral signs.

Hematological analyses

With the help of a 26 gauge sterile needle, blood samples (2-2.5 ml) were taken from five fish. On days 10, 20, and 30 of the study, blood was taken from the caudal vein of the fish. About 0.5 mL blood was used for estimation of different blood parameters while 2.0 mL blood was used for serum separation. Various blood parameters were measured, including total differential leukocyte count, red blood count, hematocrit and haemoglobin (Hussain *et al*., 2021). At days 10, 20, and 30 of the study, total proteins, hematocrit percent, and haemoglobin quantity were determined according to previous protocols (Hussain *et al*., 2021).

Physical and pathological parameters

Prior to collection of blood, five fish from all the treatment group were separately euthanized and weighed at different intervals (days 10, 20 and 30) of trial. After blood sampling all the fish were killed and different visceral organs such as gills, liver, heart, kidneys and brain were removed, weighed and finally fixed in 5% paraformaldehyde solution. Both the absolute and relative organ weight (% of body weight) of all the visceral tissues was recorded. All the visceral organs were processed to determine histopathological changes. Finally, by using rotary microtome nearly 5µm thick slices were cut and dipped in alcohol for dehydration purpose. After dehydration these slices cleared in xylene and stained with hematoxylin and eosin stains (Ghaffar *et al*., 2021).

Serum biochemistry

Different serum biochemical biomarkers like lactate dehydrogenase, alkaline phosphatase, renal function tests (urea and creatinine), liver function tests (ALT, AST), serum albumin, cholesterol, total proteins, triglycerides and glucose were determined (Hussain *et al*., 2021; Ghaffar *et al*., 2021) by using commercial kits (Randox company Pvt.) and using a chemistry analyzer (Randox company Pvt.).

Statistical analysis

The research data obtained from the trial are presented as mean ± S.E. For statistical analysis, the data of our trial were subjected to ANOVA using statistics (IBM SPSS version 20) software and the means in different groups were compared by post hoc Tukey's test. The level of significant difference was considered at $p < 0.05$.

RESULTS

Results obtained on physical parameters (body weight, absolute and relative weight) of various visceral organs of experimental fish exposed to different doses of pymetrozine (Table I). It was recorded that the body weight of fish of group D received higher dose of pymetrozine reduced significantly at day 30 of experiment. Results indicated that at day 30 of trial, the absolute weight of gills and brain of fish of group D was significantly increased compared to untreated group. At all experimental days, a significantly increased absolute weight of different visceral organs (liver and kidneys) of fish was determined in group D. The results on relative weight (Table II) of different visceral organs including kidneys, gills, liver and brain revealed significant difference in fish received higher doses of pymetrozine. In fish of group D, relative weight of liver, gills and kidneys was significantly higher at all experimental intervals in fish received higher concentrations of Pymetrozine while at day 30 in fish of group C. The results on various hematological parameters of bighead carp fish exposed to different concentrations of pymetrozine (Table II). When compared to untreated fish, the values of red blood cell count, hematocrit, and haemoglobin quantity were markedly reduced in fish received pymetrozine in group C at day 30 and in group D at all experimental days. The number of lymphocytes in treated fish was lower than in control fish. At higher concentrations of pymetrozine, the results revealed that the total white blood cell and neutrophil count significantly increased in treated fish. Hematological parameters in bighead carp subjected to various concentrationsof pymetrozine (Table II). Hematocrit percent, hemoglobin and erythrocyte count markedly reduced in fish kept in group C at day 30. The values of these

Table I. Body weight and absolute weight of different visceral tissues of *Labeo rohita* exposed to different pymetrozine.

Parameters/ day	Groups/ Treatments			
	A (0.0)	B (500 µg/L)	C (1000 µg/L)	D (1500 µg/L)
Body weight (g)				
10	166.25±2.07	164.3±3.79	161.1±3.42	157.51±1.84
20	167.31±4.34	162.26±3.02	162.3±3.26	155.76±2.23
30	166.5±4.21	162.4±1.97	159.0±1.67	151.2±4.23*
Absolute weight of brain(g)				
10	0.74±0.27	0.75±0.26	0.76±0.21	0.77±0.21
20	0.76±0.26	0.78±0.02	0.80±0.25	0.98±0.30*
30	0.78±0.25	0.80±0.24	0.84±0.25	0.99±0.31*
Absolute weight of gills				
10	4.68±0.48	4.85±0.53	5.08±0.46	5.19±0.40
20	5.08±0.44	5.06±0.49	5.10±0.92	7.63±0.89*
30	5.11±0.44	5.30±0.41	5.49±0.50	7.84±0.51*
Absolute weight of kidneys				
10	0.56±0.09	0.58±0.06	0.60±0.10	0.64±0.20
20	0.58±0.05	0.61±0.14	0.64±0.18	0.88±0.05*
30	0.59±0.08	0.66±0.07	0.79±0.17*	0.98±0.20*
Absolute weight of liver				
10	0.76±0.18	0.77±0.86	0.78±0.06	0.81±0.05
20	0.77±0.10	0.78±0.08	0.81±0.25	0.88±0.10*
30	0.78±0.19	0.83±0.05	0.91±0.13*	0.96±0.22*

Table II. Relative weight of different visceral tissues of *Labeo rohita* exposed to different concentration of pymetrozine.

Parameters/ days	Groups/ Treatments			
	A (0.0)	B (500 µg/L)	C (1000 µg/L)	D (1500 µg/L)
Relative weight of liver				
10	0.30±0.05	0.31±0.04	0.33±0.05	0.35±0.13
20	0.32±0.07	0.34±0.07	0.35±0.06	0.38±0.15
30	0.35±0.05	0.37±0.09	0.39±0.13	0.44±0.14*
Relative weight of gills				
10	2.79±0.28	3.17±0.25	3.19±0.30	3.26±0.33
20	3.18±0.27	3.30±0.36	3.39±0.55	3.73±0.53*
30	3.31±0.25	3.40±0.29	3.50±0.36	3.93±0.23*
Relative weight of kidneys				
10	0.37±0.07	0.39±0.05	0.40±0.07	0.42±0.14
20	0.40±0.05	0.41±0.09	0.43±0.14	0.49±0.06*
30	0.41±0.06	0.42±0.05	0.48±0.10	0.55±0.15*
Relative weight of brain				
10	0.35±0.18	0.37±0.18	0.41±0.19	0.41±0.20
20	0.39±0.16	0.40±0.03	0.54±0.17*	0.56±0.14*
30	0.40±0.17	0.43±0.15	0.56±0.16*	0.60±0.20*

Table III. Various hematological parameters of fish exposed to different concentrations of pymetrozine.

Parameters/ days	Groups/ Treatments			
	A (0.0)	B (500 µg/L)	C (1000 µg/L)	D (1500 µg/L)
Red blood cell count				
10	4.86±0.20	4.10±0.20	4.04±0.18	3.99±0.05
20	5.06±0.30	4.06±0.29	3.89±0.05*	3.36±0.13*
30	5.03±0.09	4.04±0.13	3.84±0.18*	3.28±0.20*
Hemoglobin concentration (g/dl)				
10	9.06±0.25	8.76±0.33	7.98±0.30	7.93±0.20
20	8.99±0.32	8.41±0.49	7.64±0.18*	6.85±0.28*
30	8.78±0.22	8.40±0.30	6.48±0.15*	6.33±0.23*
White blood cell counts				
10	12.58±0.23	14.09±0.44	15.47±0.53	16.70±0.52
20	13.03±0.43	14.26±0.24	16.31±0.46*	17.48±0.37*
30	14.59±0.61	14.60±0.44	17.27±0.30*	18.98±0.33*
Hematocrit				
10	34.17±2.39	33.81±0.96	32.54±0.98	32.73±0.60
20	33.21±0.43	32.75±1.03	31.55±0.97	23.46±0.27*
30	32.54±0.34	31.74±0.38	29.38±0.34	21.35±2.31*
Lymphocytes (%)				
10	20.64±0.56	18.52±0.36	17.89±0.20	14.83±0.18*
20	19.37±0.20	18.22±0.39	17.81±0.43	13.70±0.19*
30	18.93±0.38	18.17±0.37	17.13±0.31	12.80±0.19*
Neutrophils (%)				
10	15.81±0.24	16.39±0.28	17.70±0.67	21.53±0.38*
20	15.99±0.28	17.59±0.31	21.77±0.81*	22.78±0.45*
30	16.11±0.25	17.67±0.28	22.85±0.72*	23.65±0.35*

blood profile also reduced in fish placed in group D at all sampling days. Results on lymphocytes counts revealed significantly lower values in pymetrozine treated fish compared to untreated group. Results on different serum biochemical investigations revealed significantly lower concentrations of albumin and serum total proteins in fish of group C receiving pymetrozine at day 30. The concentrations of different serum tests (aspartate aminotransferase and alkaline phosphatase enzymes) were significantly increased at day 30 in fish of group C and in fish kept in group D at days 20 and 30 of research trial. The quantity of lactate dehydrogenase in treated fish were noticeably higher on days 20 and 30 of the study in group D. At days 20 and 30 of the experiment, indicators of the kidneys damage such as creatinine and urea significantly increased in fish of treated groups (C and D). In group D fish subjected to higher dosages of pymetrozine, cholesterol, glucose, and triglycerides increased noticeably increased in fish received pymetrozine (Table IV).

Table IV. Various serum biochemical parameters of fish exposed to different concentrations of pymetrozine.

Parameters/ days	Groups/ Treatments			
	A (0.0)	B (500 µg/L)	C (1000 µg/L)	D (1500 µg/L)
Albumin quantity (mg/dL)				
10	2.60±0.11	2.59±0.13	2.48±0.03	2.47±0.02
20	2.67±0.09	2.53±0.16	2.16±0.06*	2.05±0.07*
30	2.64±0.06	2.49±0.14	2.15±0.05*	2.02±0.08*
Total proteins (mg/dL)				
10	4.16±0.02	4.14±0.02	4.03±0.02	3.91±0.02
20	3.71±0.01	3.66±0.01	3.02±0.01*	2.98±0.07
30	3.75±0.01	3.39±0.01	2.97±0.01*	2.91±0.01*
Aspartate aminotransferase (U/L)				
10	15.67±1.03	15.85±1.13	16.04±1.08	16.23±1.02
20	16.53±1.06	16.87±1.03	22.22±1.06*	24.57±1.07*
30	17.97±1.03	18.15±1.01	23.34±1.03*	25.53±1.03*
Alkaline phosphatase (U/L)				
10	26.39±0.03	26.63±0.14	26.87±0.04	27.11±0.09
20	27.72±0.05	28.04±0.15	36.36±1.05*	38.68±1.02*
30	26.78±0.07	28.99±0.13	37.20±1.03*	39.42±1.01*
Alanine aminotransferase (U/L)				
10	22.88±1.03	23.09±0.03	23.30±1.03	24.54±1.06
20	23.54±1.06	23.91±0.06	24.28±1.02	28.66±0.09*
30	23.99±1.04	26.23±0.04	29.47±1.04*	30.71±1.03*
Lactate dehydrogenase (U/L)				
10	251.71±4.13	252.42±3.13	253.22±2.13	253.91±1.13
20	253.75±3.36	255.97±3.37	271.22±1.37*	275.45±2.36*
30	254.83±3.48	257.73±2.48	276.62±3.48*	283.54±2.48*
Urea (mg/dL)				
10	8.71±0.12	8.84±0.05	8.98±0.02	9.12±0.02
20	8.61±0.14	9.13±0.07	11.36±0.04*	11.59±0.04*
30	8.65±0.09	9.28±0.06	11.55±0.05*	11.82±0.04*
Creatinine (mg/dL)				
10	1.25±0.13	1.27±0.09	1.29±0.08	1.31±0.04
20	1.30±0.11	1.32±0.09	1.49±0.02*	1.53±0.02*
30	1.32±0.14	1.34±0.08	1.57±0.09*	1.59±0.01*
Cholesterol (mg/dL)				
10	155.71±1.13	156.61±1.13	157.42±2.13	158.31±2.13
20	159.71±2.17	160.71±3.17	161.72±2.17	172.73±1.17*
30	161.05±2.36	163.27±2.37	175.52±2.37*	177.75±2.36*
Glucose (mg/dL)				
10	29.55±1.16	30.52±1.15	31.47±0.15	32.45±1.16
20	31.75±1.10	32.37±1.11	37.02±1.11*	38.65±1.10*
30	32.75±1.06	33.25±1.06	39.65±1.06*	41.15±1.06*
Triglycerides (mg/dL)				
10	175.15±3.10	175.82±2.11	176.47±1.11	177.15±1.10
20	174.05±2.19	177.25±3.19	191.55±1.19*	193.75±1.19*
30	176.65±3.32	179.75±2.32	194.75±0.32*	198.85±0.32*

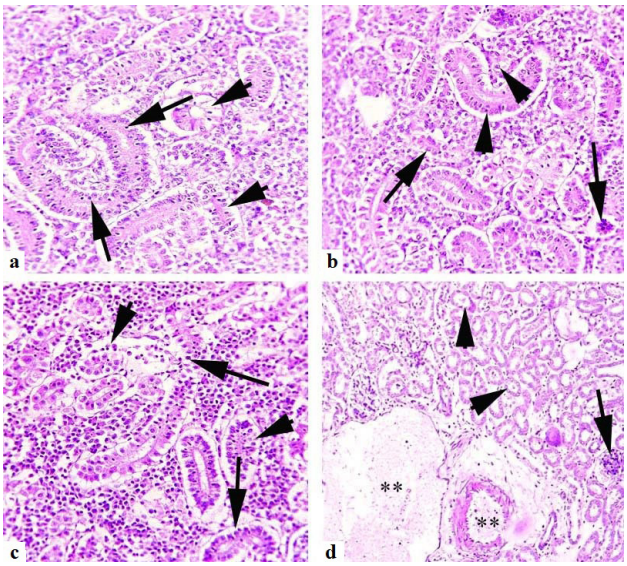

Fig. 1. Effect of Pymetrozine on histological structure kidney of fish. Photomicrograph of kidneys of treated fish showing a) necrosis of tubular epithelial cells (arrows) and necrosis of tubular epithelium (arrow heads), b) degeneration and necrosis of renal tubules (arrows) and necrotic cells (arrow heads), c) tubular necrosis (arrow heads) and necrosis of tubular cells (arrows) and d) inflammatory exudate (**), tubular necrosis (arrow) and degeneration and atrophied renal tubules (arrow heads).

After day 20 of the experiment, various microscopic abnormalities including eccentric nucleus of hepatocyte, condensation and necrosis of hepatocyte, inflammatory exudate, congestion, fatty change, edema, and enlarged sinusoidal spaces in the liver sections of different fish placed in groups (C and D) treated with pymetrozine were observed. Various histological lesions in kidneys of pymetrozine treated fish like necrosis of tubular epithelial cells, necrosis of tubular epithelium, degeneration and necrosis of renal tubules, necrotic cells, inflammatory exudate, edema, ceroid formation, infiltration of melanomacrophage, degeneration of glomeruli, increased Bowman's space, and atrophy of renal tubules were observed (Fig. 2). Different microscopic lesions in various sections of gills of fish received higher doses of pymetrozine exhibited necrosis of lamellar epithelium, aneurysm, curling of secondary lamellae, disruption and disorganization of cartilaginous core, necrosis of primary and secondary epithelial cells, lamellar fusion, degeneration of cartilaginous core and uplifting of lamellae (Fig. 3). Different histological lesions in heart of various sections of fish received pymetrozine (group C and D) included inflammatory exudate, necrosis of cardiac muscles, fatty infiltration, inflammatory exudate, neutrophilic inflammation and degeneration and disorganization of cardiac muscles (Fig. 4). Various histopathological changes in different sections of brain of pymetrozine treated fish kept in groups C and D included, inflammatory exudate, atrophy of neurons, necrosis of neurons, and microgliosis (Fig. 5).

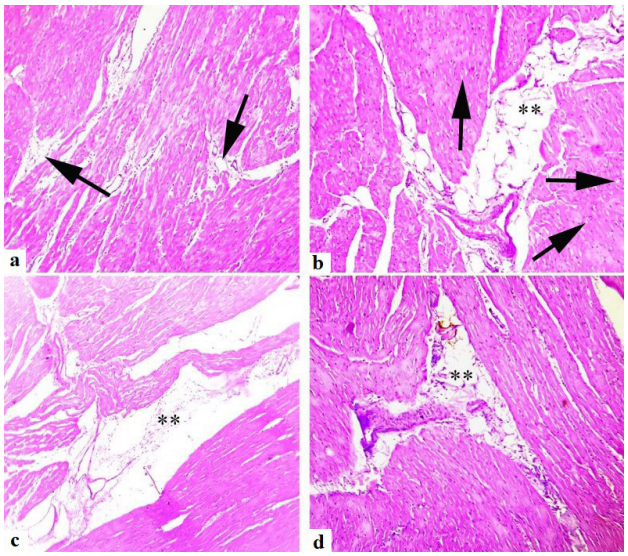

Fig. 2. Effect of pymetrozine on histological structure of heart of fish. (a) inflammatory exudate and necrosis of cardiac muscles (arrows), (b) necrosis of cardiac muscles (**) and fatty infiltration (arrows), (c) inflammatory exudate (**) and (d) necrosis of cardiac muscles and edema (**).

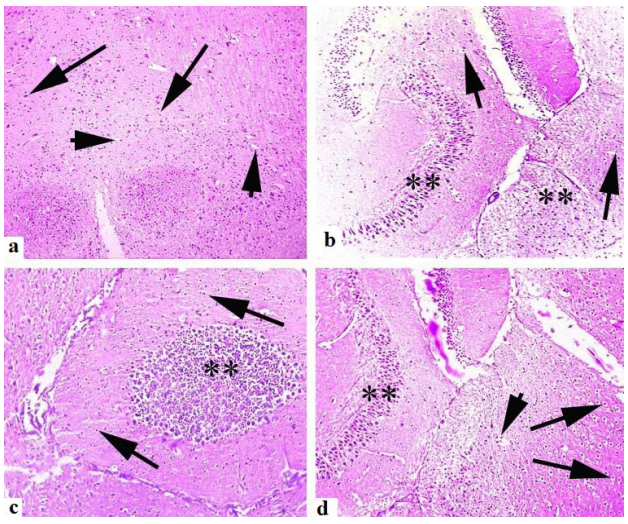

Fig. 3. Effect of pymetrozine on histological structure of brain of fish. (a) necrosis of neurons (arrows) and neurons with eccentric nucleus (arrows), (b) microgliosis (**) and atrophied and necrosis of neurons (**), (c) inflammatory exudate (**) and (d) necrosis of neurons (arrows).

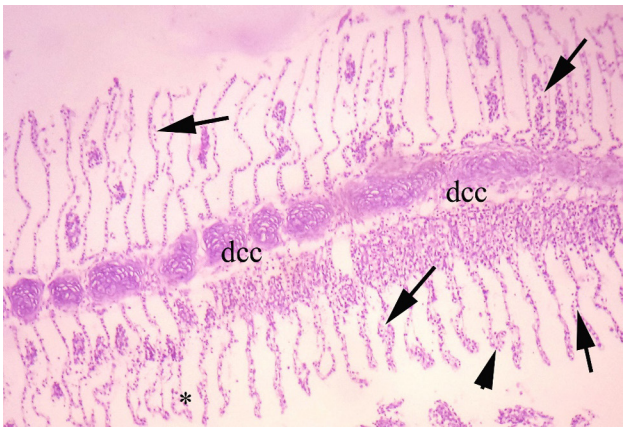

Fig. 4. Effect of pymetrozine on histological structure of gills of fish showing necrosis of lamellar epithelium (arrows), aneurysm (arrow head), curling of secondary lamellae (*) and disruption and disorganization of cartilaginous core (dcc).

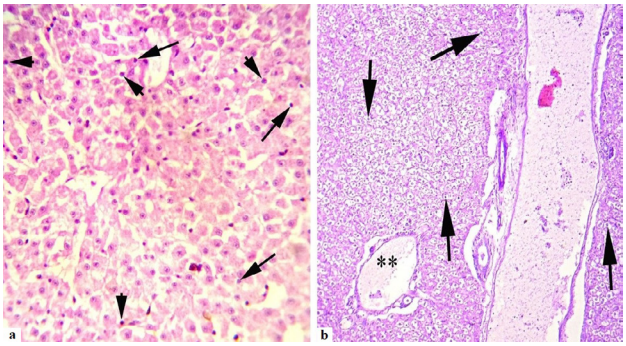

Fig. 5. Effect of pymetrozine on histological structure of gills of fish liver showing (a) eccentric nucleus of hepatocyte (arrows) and necrosis of hepatocyte (arrow head). (b) atrophied and necrotic hepatocyte (arrows) and inflammatory exudate (**).

DISCUSSION

Pymetrozine is a broadly used insecticide in the Middle East and different other parts across the globe. The widespread use of pymetrozine and other pesticides has created ecological problems to the aquatic life, soil, and the public health. Organochemical pesticides such as pymetrozine, has been shown to have a direct impact on the agro-ecosystem by affecting different biological functions in target and non-target animals and decomposition of organic matter of soil and availability of nutrients in the soil (Andrén and Lagerlöf, 1983). Pymetrozine is highly toxic to humans and other mammals, and reduces the richness and variety of non-target organisms. Aquatic species, especially fish are frequently exposed to these chemical compounds because the aquatic ecosystems serve as the final sink for all anthropogenic contaminants (Routledge *et al*., 1998). In the present experimental research, it was noted that, there was an increase in absolute weight of different organs of Pymetrozine treated fish. Previously, scanty information is available on toxic effects of pymetrozine on body weight and absolute weight of different visceral organs of fish. However, increased relative weight of liver and kidneys of albino rats (Nassar *et al*., 2020) treated with the high dose of toxicant has been reported. In various aquatic and terrestrial organisms, haematological biomarkers are considered the most important indicators for determination of physiological stress (Ghaffar *et al*., 2017; Hussain *et al*., 2021; Ghaffar *et al*., 2021; Akram *et al*., 2020). In the current study, it was noted that, fish exposed to pymetrozine had lower haemoglobin concentrations, monocytes, lymphocytes and pack cell volume. The rapid oxidation of haemoglobin, destruction of erythrocytes and hemolysis lowers parameter values (Akram *et al*., 2020). Results on different indices of blood showed increased values of total leukocyte and neutrophil counts while significantly lower of erythrocyte count in pymetrozine treated fish. Increased in neutrophils count might be due to immunological reactions associated with induction of injuries in different tissues of fish exposed to pymetrozine. Previously, increased white blood cells and neutrophil count while lower values of hematocrit, hemoglobin, lymphocytes, and erythrocyte counts have also been recorded in fish (*Oreochromis niloticus* and *Aristichthys nobilis*) exposed to various environmental toxicants (Akram *et al*., 2020). Different previous published studies have also reported lower values of hemoglobin, pack cell volume, lymphocytes, monocytes, and erythrocyte while significantly higher values of total leukocyte and neutrophil population in various species of fish like *Clarias gariepinus* (Woryi *et al*., 2020), *Cirrhinus mrigala* (Ghayyur *et al*., 2019), *Cyprinus carpio* (Vali *et al*., 2020), bighead carp (Akram *et al*., 2020), *Labeo rohita* (Ghaffart *et al*., 2021) and *Heteropneustes fossilis* (Akter *et al*., 2020) exposed to different environmental contaminants. These hematological disorders (lower values of hemoglobin, hematocrit, lymphocyte, monocyte and erythrocyte count) in our study might be due to toxic effects of pymetrozine on blood producing tissues. The increased values of total leukocyte and neutrophil counts can be related to increased and rapid generation of free radicals and oxidative stress resulting in lower supply of oxygen in pymetrozine treated fish. In this study, significantly increased white blood cells and neutrophil could also be related to inflammatory conditions induced by pymetrozine in treated fish. Serum biochemistry parameters are known as useful and reliable biomarkers and are routinely used to determine the

underlying mechanisms of toxicity of different toxicants/ environmental contaminants (Akram *et al*., 2020). It has been reported that different synthetic and environmental pollutants cause increased concentrations of serum biochemical indices in fish indicating adverse effects on different tissues (Hussain *et al*., 2021). In this study, different biochemical parameters like serum ALT, AST and ALP increased significantly while serum total protein and serum albumin quantity decreased in pymetrozine treated fish. The significantly concentrations of various serum biochemical parameter such as glucose, cholesterol, and lactate dehydrogenase might be due to toxic effects of pymetrozine in fish. The higher values of creatinine and urea in treated fish could be due to altered mechanisms of filtration in kidneys as already reported in different studies (Akram *et al*., 2020). It was also observed that urea and creatinine levels also increased in fish exposed to pymetrozine which shows damages adverse effects of kidneys. Previously, increased concentrations of LDH, ALP, ALT, AST, and cortisol in *Cirrhinus mrigala* (Ghayyur *et al*., 2020) exposed to herbicide have also been recorded. Moreover, depletion of serum total protein, increased liver enzymes in *Channa punctatus* (Sastry and Dasgupta, 1991), zebra fish, and bighead carp (Akram *et al*., 2020) exposed to toxicants have been reported. In this study, different histopathological alterations liver tissues of fish such as congestion, atrophid cytoplasm, fatty change, widening of sinusoidal space, necrosis of hepatocyte, and degeneration of hepatocyte exposed to higher concentrations of pymetrozine was observed. Previously, similar findings in liver tissue of *Clarias gariepinus* (Elias *et al*., 2020) and *Ctenopharyngodon idella* (Faheem and Lone, 2017) treated with different toxicants were observed. Different microscopic alterations like edema, ceroid formation, glomerular degeneration, increased Bowman's space, congestion, atrophy of tubules, and atrophy of lumen of renal tubules were observed in the kidneys of pymetrozine-treated fish. Earlier studies also reported similar microscopic changes including congestion, necrosis, nuclear hypertrophy, infiltrations of melanomacrophage and degeneration of renal tubules in kidneys of *Heteropneustes fossilis* (Pal and Reddy, 2018) and tilapia (Vinodhini and Narayanan, 2009) treated with toxicants. Different earlier studies have investigated that kidneys are main and primary organs which are exposed to different pollutants including herbicides, insecticides and pesticides in water bodies (Akram *et al*., 2020). It is recorded that the gills are very useful and reliable tissues for early screening of adverse effects of various toxicants due to direct contact with the water. Gills are responsible for osmoregulatory mechanisms in aquatic animals and maintain ionic balance (Ghaffar *et al*., 2018). In present trial, different histopathological changes in gills of pymetrozine-treated fish included necrosis and degeneration of primary and secondary lamellar epithelial cells, disorders in arrangements of primary and secondary lamellae, necrosis of lamellar epithelium, uplifting of primary lamellae and disorganization in cartilaginous core. Previously, similar pathological changes in gills in van fish (Oguz *et al*., 2018), *C. fluminea* (Benjamin *et al*., 2019), *Labeo rohita* (Ghaffar *et al*., 2021) and bighead carp (Akram *et al*., 2020) due various chemicals have also been observed. Limited information is available in the literature about adverse toxic effects of pymetrozine on histopathological findings in heart (neutrophilic inflammation, congestion, edema, degeneration and disorganization of cardiac muscles) and brain (microgliosis, necrosis of neurons, and atrophy of neurons). Similar histopathological changes in heart and brain of fresh water fish exposed to different insecticides (Akram *et al*., 2020) and environmental contaminants (Akram *et al*., 2020) have also been observed.

CONCLUSION

From the findings of our experimental research work it can be concluded that pymetrozine causes adverse effects on blood biochemistry and multiple tissues of bighead carp in a time and dose dependent manners.

Statement of conflict of interest

The authors have declared no conflict of interest.

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