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RESEARCH ARTICLE

New Insights into Hemato-Biochemical and Histopathological Effects of Acetochlor in Bighead Carp (*Aristichthys nobilis*)

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ABSTRACT

Acetochlor, an emerging herbicide is frequently used on maize and different other cereal crops to control weeds. Due to extensive and persistent applications, acetochlor may enter into nearby aquatic ecosystem and cause adverse effects on different animals. The residues of this herbicide via food chain can induce toxic impacts on different animals including public health. Therefore, it is crucial to investigate the toxic effects of acetochlor in exposed organisms. The current trial was aimed to know the deleterious effects of acetochlor in fresh water fish. A total of 80 fish were obtained and indiscriminately retained in 4 groups each containing 20 fish. Fish retained in groups (B-D) were exposed to acetochlor mixed in water @ 300, 400 and 500 µg/L respectively. Blood and different visceral tissues were collected at days 12, 24 and 36 of trial for hematological and histopathological studies. Different behavioral and physical ailments were observed in a time and concentrations manners. Results exhibited significantly ($P<0.05$) lower hematological profile including erythrocyte counts, hemoglobin, monocyte and lymphocyte count. The serum biochemistry analysis exhibited that the quantity of liver function tests, renal function tests, cholesterol, glucose, and triglyceride in treated fish increased significantly ($P<0.05$). Histopathological observation showed necrosis, increased Bowman's space and renal tubular degeneration in kidneys. Various microscopic ailments in liver like, hemorrhages, edema, atrophid hepatocyte and hepatocyte with eccentric nuclei were observed in exposed fish. Necrotic neurons, microgliosis and degeneration of neurons in brain while hemorrhages, edema, and neutrophilic myocarditis was observed in heart of treated fish. The findings of our experimental research suggested that acetochlor exerts toxic effects on fresh water fish.

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INTRODUCTION

From the last few years, pesticides are becoming serious threat because of their indiscriminate applications in agriculture, aquatic environment, public health for environmental protection and in veterinary practices (Hussain *et al*., 2011; Hussain *et al*., 2019; Ghaffar *et al*., 2021). Unintentional contact to pesticides, such as herbicides and fungicides in marine and terrestrial ecosystems causes different ailments in animals (Hussain *et al*., 2012; Ghaffar *et al*., 2019; Ghaffar *et al*., 2020). Numerous reports have shown that marine life is more vulnerable to several contaminants than terrestrial organisms because a wide range of toxic substances from different sources including industries, agriculture and production sites are quickly and readily entered into aquatic ecosystem (Sivashanmugam *et al*., 2017; Baralic *et al*., 2020; Warra and Prasad, 2020). Studies have shown that exposure to different compounds such as by-products of different disinfectants, fluorinated substance, herbicides, pesticides and other synthetic chemicals (Hussain *et al*., 2012; Hussain *et al*., 2014; Faheem and Bhandari, 2021; Akram *et al*., 2021) exert toxic effects on wildlife. The residues of these chemicals in food chain induce deleterious effects on public health, disrupt the metabolic activities and multiple organ dysfunctions in variety of exposed species (Auon *et al*., 2014; Hussain *et al*., 2019; Scarano *et al*., 2019; Zhou *et al*., 2019; Faheem

et al., 2020; Ghaffar *et al*., 2020). Acetochlor, a chlorinated herbicide is commonly used on maize, soybeans, sugar beets and various other crops to control broad leaf weeds (Gianessi and Reigner, 2007; Jia *et al*., 2020). Acetochlor can reach to marine species by absorption, and that dermal interaction with infected water causes behavioral and physical changes such as impairment of locomotion and swimming habits. The organisms present in aquatic ecosystems are very delicate (Ghaffar *et al*., 2014; Ghaffar *et al*., 2015). Contaminants, such as pesticides may have devastating consequences on these ecosystems. Furthermore, marine environments are commonly exposed to many environmental stressors. The chemical stressors often combine or synergize and induce adverse toxic effects. Fish are the most vulnerable marine animals to different synthetic chemicals and are valuable biomarkers for monitoring the quality of the aquatic environment (Ghaffar *et al*., 2016; Hussain *et al*., 2018; Sjerps *et al*., 2019; Peña *et al*., 2020). Earlier reports have shown that pesticides causes toxicity by rapid generation of free radicals and oxidative stress ultimately resulting in a variety of abnormalities in various organs (Ghaffar *et al*., 2015a; Ghaffar *et al*., 2015b; Ghaffar *et al*., 2018). Blood and biochemical profile are the main targets of toxicity in fish and can be used as valuable bio-indicators (Akram *et al*., 2021; Ghaffar *et al*., 2021; Aranha *et al*., 2021; Faheem *et al.,* 2021). Furthermore, estimations of differences in blood and serum biochemistry values including proteins and enzymes are frequently employed to determine the patho-physiological ailments in aquatic animals (Ghaffar *et al*., 2020; Yamin *et al*., 2020; Latif *et al.*, 2020; Rehma *et al*., 2021; Naz *et al*., 2021). There is little information on the toxicity of acetochlor to freshwater fish, particularly Bighead carp. Hence, we sought to assess the toxicity of acetochlor at sublethal doses in fresh water carp.

MATERIALS AND METHODS

Acetochlor and study reagents: Acetochlor was purchased from commercial market district Lodhran (M/S Ali Akbar Enterprises, Pakistan) Pakistan. Merck (Germany) and Sigma Aldrich (USA) provided several analytically graded compounds (USA). Various commercial kits were bought from Randox Company (Pvt.) Pakistan for the determination of serum biochemical parameters.

Experimental species and management: A total of 80 fresh water fish Bighead carp (*Aristichthys nobilis*) having similar body mass (140-155 g), size and age were bought from a local commercial fish farm (District Bahawal-nagar), Punjab province, Pakistan. The experimental test specimens were placed in plastic bags containing suitable oxygen and shifted to laboratory. For acclimatization, the test specimens were retained in glass aquaria for 10 days. All of the experimental fish were fed commercial feed daily, early in the morning and late in the evening. All the leftover feed and fecal constituents were removed from all aquaria.

Experimental treatments: After the fish were acclimatized, all experimental fish were classified into four groups (A-D) with 20 specimens in each group. The water carrying capacity of each aquarium was 100L. Fish retained in group A were known as untreated (control group) while fish of other groups were treated as experimental specimens. Acetochlor was dissolved in distilled water, and the experimental organisms in groups (B, C and D) were exposed to 300, 400 and 500 µg/L for 36 days. Residual feed and fecal components were strained and eliminated on regular basis during the study. All of the experimental test specimens were examined for any evident clinical or behavioral problems.

Hematological analyses: Blood sample (2.0-2.5 ml) was obtained from five fish with help of 26 gauge sterile needle. The blood was collected from caudal vein of fish on days 12, 24 and 36 of the trial. After collection, about 02 mL blood was centrifuged for serum separation, and 0.5 mL of blood was placed in anticoagulant (EDTA) coated glass test tubes for haematological analysis. Various blood parameters were examined, including red blood count, total and differential leukocyte count, haemoglobin, and hematocrit (Hussain *et al*., 2020; Ghaffar *et al*., 2021), while total proteins were measured according to earlier methods (Hussain *et al*., 2019; Ghaffar *et al*., 2020) at days 12, 24 and 36 of the trial.

Physical and Pathological Parameters: Five fish were haphazardly picked, weighed, euthanized, and necropsied from each group after blood collection. The liver, gills, brain, kidneys, and heart were separated, weighed, and fixed in a 5% paraformaldehyde solution. The visceral organs were then processed for histological examinations. Approximately 5µm thick slices were cut with a rotary microtome, dehydrated in alcohol, cleaned in xylene, and stained with Hematoxylin and Eosin (Ghaffar *et al*., 2021).

Serum Biochemistry: Various serum biochemical parameters such as liver and renal function tests, serum albumin, total proteins, alkaline phosphatase, glucose, triglycerides, cholesterol, and lactate dehydrogenase were determined using commercial kits by chemistry analyzer (Hussain *et al*., 2019; Ghaffar *et al*., 2021).

Statistical analysis: The data from our study were analyzed using ANOVA with IBM SPSS statistics (version 20) software, and the group average values were compared using a post hoc Tukey's test. The threshold for a significant difference was set at $P<0.05$.

RESULTS

Results on physical profile of fish treated with various concentrations of acetochlor like body mass, absolute and relative weight of different organs are presented in table 1. The body mass of fish of group D exposed to higher level of acetochlor significantly reduced at day 36 of the trial. Results exhibited significantly increased absolute weight of brain and gills of fish kept in group D at day 36. A significantly increased absolute weight of liver and kidneys was recorded in fish of group D at all experimental days. The results on relative weight of various visceral tissues (Table 2) like liver, gills, kidneys and brain exhibited significant changes in treated fish.

Table 1: Body weight and absolute weight of different visceral tissues of bighead carp exposed to different concentrations of Acetochlor

Parameters/ days	Groups/Treatments A (0.0)	B (300 µg/L)	C (400 µg/L)	D (500 µg/L)
Body weight (g)				
12	165.8±2.05	162.2±3.79	162.2±3.40	159.5±1.83
24	167.7±4.33	161.5±3.01	161.1±3.25	158.75±2.22
36	168.5±4.20	164.3±1.96	160.2±1.66	156.25±1.22*
Absolute weight of brain (g)				
12	0.72±0.26	0.71±0.25	0.74±0.20	0.76±0.20
24	0.73±0.25	0.72±0.01	0.75±0.24	0.78±0.29
36	0.70±0.24	0.73±0.23	0.76±0.24	0.87±0.11*
Absolute weight of gills				
12	4.66±0.47	4.83±0.52	5.06±0.45	5.17±0.39
24	5.06±0.43	5.04±0.48	5.07±0.91	5.61±0.88
36	5.07±0.43	5.28±0.39	5.58±0.49	7.83±0.50*
Absolute weight of Kidneys				
12	0.54±0.08	0.53±0.05	0.57±0.09	0.71±0.19*
24	0.56±0.04	0.63±0.13	0.58±0.17	0.78±0.04*
36	0.59±0.07	0.66±0.06	0.77±0.16	0.89±0.19*
Absolute weight of Liver				
12	0.74±0.17	0.74±0.85	0.76±0.05	0.88±0.04*
24	0.75±0.09	0.78±0.07	0.79±0.24	0.89±0.09*
36	0.77±0.19	0.81±0.04	0.89±0.12	0.99±0.21*

Values with sign (*) are exhibiting significant (P<0.05) difference from control.

Table 2: Relative weight of various organs of fresh water fish treated with different levels of Acetochlor

Parameters/ days	Groups/Treatments A (0.0)	B (300 µg/L)	C (400 µg/L)	D (500 µg/L)
Liver				
12	0.28±0.04	0.28±0.03	0.31±0.04	0.37±0.12*
24	0.31±0.06	0.29±0.06	0.33±0.05	0.38±0.14*
36	0.31±0.04	0.32±0.08	0.39±0.12*	0.42±0.13*
Gills				
12	2.97±0.27	3.15±0.24	3.16±0.29	3.84±0.32*
24	3.05±0.26	3.08±0.35	3.48±0.54	3.89±0.12*
36	3.08±0.24	3.09±0.28	3.94±0.35*	3.98±0.22*
Kidneys				
12	0.35±0.06	0.34±0.04	0.34±0.06	0.45±0.13*
24	0.37±0.04	0.39±0.08	0.35±0.13	0.46±0.05*
36	0.36±0.05	0.38±0.04	0.49±0.09*	0.49±0.14*
Brain				
12	0.33±0.17	0.35±0.17	0.37±0.18	0.38±0.19
24	0.37±0.15	0.34±0.02	0.39±0.16	0.59±0.13*
36	0.35±0.16	0.37±0.14	0.39±0.15	0.59±0.19*

Values with sign (*) are exhibiting significant (P<0.05) difference from control.

The relative weight of liver, gills and kidneys was significantly increased in fish of group D throughout the experiment exposed to higher concentrations of acetochlor while in fish of group C at day 36 of experiment. The results on different blood profile of bighead carp treated with acetochlor are shown in table 3. Red blood cell count, hematocrit and hemoglobin quantity was significantly decreased in bighead carp of group C at day 36 while in fish of group D at all experimental intervals compared to untreated fish. Results exhibited decreased values of lymphocytes in treated fish compared to control fish. The results revealed significantly increased values of total white blood cell count and neutrophil count in acetochlor treated fish at higher concentrations. Results exhibited decreased values of lymphocytes in treated fish compared to control fish. Results on serum biochemistry indicated that the quantity of serum albumin and total proteins significantly decreased in bighead carps kept in group D at day 36 of the exposure compared to control specimens. The quantity of aspartate aminotransferase and

Table 3: Blood profile of fresh water fish treated with various levels of Acetochlor

Parameters/ days	Groups/Treatments A (0.0)	B (300 µg/L)	C (400 µg/L)	D (500 µg/L)
Red blood cell count (10^9/mm^3)				
12	4.84±0.19	4.77±0.19	4.76±0.17	3.38±0.04*
24	5.06±0.29	4.74±0.28	4.66±0.04	3.34±0.12*
36	5.03±0.08	4.71±0.12	3.84±0.17*	3.29±0.19*
Hemoglobin concentration (g/dl)				
12	8.87±0.24	8.74±0.32	7.93±0.29	6.92±0.19*
24	9.04±0.31	8.39±0.48	7.89±0.17	6.82±0.27*
36	9.09±0.21	8.69±0.29	6.46±0.14*	6.31±0.22*
White blood cell counts (10^6/mm^3)				
12	12.56±0.22	15.08±0.43	15.45±0.52	16.68±0.51*
24	13.02±0.42	14.95±0.23	16.29±0.45*	18.48±0.36*
36	12.57±0.62	14.29±0.43	17.23±0.29*	18.97±0.32*
Hematocrit (%)				
12	34.15±2.38	35.79±0.95	32.9±0.97	24.71±0.59*
24	35.19±0.42	34.74±1.02	32.53±0.96*	23.49±0.26*
36	36.51±0.33	32.79±0.37	26.36±0.33*	23.31±2.30*
Lymphocytes (%)				
12	18.62±0.55	17.51±0.35	15.86±0.19	15.56±0.17
24	20.35±0.19	17.21±0.38	16.80±0.42	13.68±0.18*
36	19.98±0.37	17.16±0.36	16.32±0.30	12.76±0.18*
Neutrophils (%)				
12	15.79±0.23	17.38±0.27	19.68±0.66*	22.51±0.37*
24	15.98±0.27	17.28±0.30	19.79±0.80*	22.76±0.44*
36	15.90±0.24	17.36±0.27	19.80±0.70*	22.63±0.34*

Values with sign (*) are exhibiting significant (P<0.05) difference from control.

alkaline phosphatase enzymes were significantly increased in treated bighead carps in group C at day 36 while in group D at days 24 and 36 of experiment as compared to non-treated fish. The results showed significantly increased levels of lactate dehydrogenase in treated fish at days 24 and 36 of trial in group D. The results on biomarkers of kidneys like urea and creatinine significantly increased in groups C and D at days 24 and 36 of trial. The concentrations of serum cholesterol, glucose and triglycerides increased significantly in fish of group D exposed to higher doses of acetochlor (Table 4).

At histopathological level, different microscopic changes including increased sinusoidal spaces, congestion, edema, atrophy of cytoplasm of hepatocyte, karyolysis, pyknosis in liver of bighead carp of groups C and D (Fig. 1) treated with acetochlor at higher doses after day 24 of the experiment. Different histopathological lesions like edema, melanomacrophage accumulation, congestion, ceroid formation, increased Bowman's space, disintegration of glomeruli, atrophy of renal tubules and necrosis of renal tubular cells was examined in kidneys of fish after day 24 of exposure to herbicide (Fig. 2). Different histopathological lesions in various sections of gills of treated fish retained in groups (C-D) like congestion, disorganization of primary and secondary lamellae, degeneration of cartilaginous core, necrosis of lamellar cells, sloughing of lamellar epithelium of deformation, lamellar fusion and uplifting of lamellae were observed (Fig. 3). Microscopic examination of different sections of heart of fish in groups C and D indicated different pathological lesions like neutrophilic inflammatory response, congestion, edema and degeneration and disorganization of cardiac muscles (Fig. 4). Microscopic examination of different sections of brain of treated fish retained in groups (C-D) after day 24 of experimental trial exhibited necrosis of neurons, degeneration of neurons in cerebellum and microgliosis (Fig. 5).

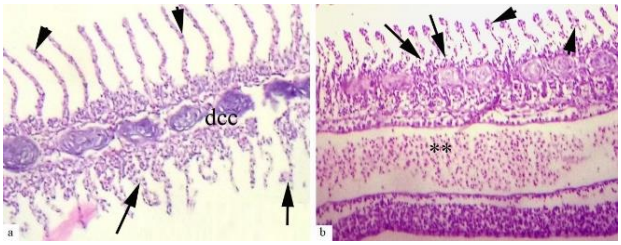

Fig. 1: Photomicrograph of gills of treated fish exhibiting a) degeneration of cartilaginous core (dcc), necrosis of secondary lamellar epithelial cells (arrows heads) and sloughing of epithelium of secondary lamellae. a) Hemorrhages (**), degeneration of primary lamellae (arrows) and necrosis of secondary lamellar epithelial cells (arrows heads) at days 36 of experiment in groups C and D respectively. (400X, H&E stain).

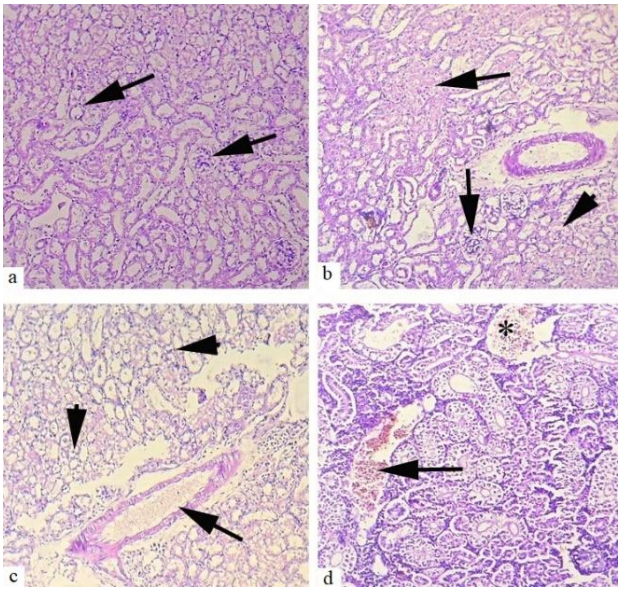

Fig. 2: Photomicrograph of kidneys of treated fish exhibiting a) degeneration of renal tubules (arrows). b) Inflammatory cells (arrows) and necrosis of tubular cells (arrow head). in group C while c) necrosis of tubular epithelial cells (arrow heads) and congestion (arrow). d) Inflammatory cells (*) and ceroid formation (arrow) in fish of group D at day 36 of experiment. (400X, H&E stain).

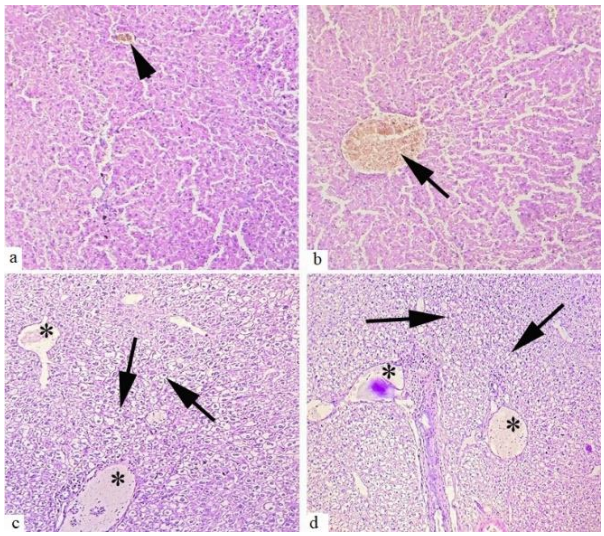

Fig. 3: Photomicrograph of liver of treated fish exhibiting a) ceroid formation (arrow head) and b) ceroid formation (arrow) at day 36 while c) edema (*) and necrosis of hepatocyte (arrows), d) necrosis of hepatocytes (arrows) and edema (*) in fish of group D at day 36. (400X, H&E stain).

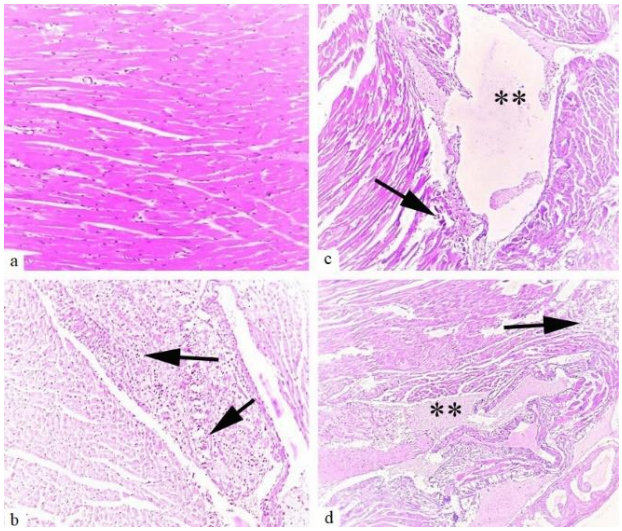

Fig. 4: Photomicrograph of heart of treated fish a) normal heart. b) showing disruption of muscles of heart of group C at day 36 while c) edema (**) and break down of cardiac muscles (arrow). d) edema and inflammatory cells) in fish of group D at day 36 of research. (400X, H&E stain).

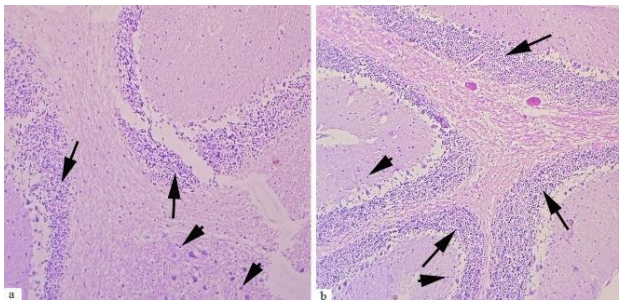

Fig. 5: Photomicrograph of brain of treated fish a) exhibiting microgliosis (arrows) and atrophy of neurons (arrow heads) in fish of group C and b) necrosis of neurons (arrow head) and microgliosis (arrows) at day 36 of research in fish of group D. (400X, H&E stain).

DISCUSSION

Acetochlor is a common herbicide which is commonly used in the Middle East and different other regions across the globe. Its widespread use has created chaos on the ecosystem, land and water. Acetochlor has a direct influence on the agro-ecosystem by altering various biological activities such as soil organic matter decomposition, soil nutrient availability and reducing the abundance and diversity of non-target organisms. Aquatic species, especially fish are frequently encountered to variety of environmental pollutants (Akram *et al*., 2021, Ghaffar *et al*., 2021; Faheem and Bhandari, 2021). In this study, the absolute weight of different tissues of fish except brain was elevated whereas the relative weight of visceral organs decreased. Previously little evidences are available about the toxic effects of acetochlor poisoning on different visceral organs. However, albino rats treated with a high dose of toxicant showed an increase in relative weight of liver and kidneys (Nassar, 2020). Hematological features are regarded as the most important biomarkers of physiological stress in a variety of animals including terrestrial and aquatic life (Ghaffar *et al*., 2017a; Ghaffar *et al*., 2017b; Akram *et al*., 2021). In the current study, lower blood values in treated fish could be related to rapid oxidation of haemoglobin and break down of red blood

Table 4: Serum biochemistry values fresh water fish treated with various doses of Acetochlor.

Parameters/ days	Groups/Treatments			
	A (0.0)	B (300 µg/L)	C (400 µg/L)	D (500 µg/L)
Albumin quantity (mg/dL)				
12	2.85±0.03	2.81±0.01	2.77±0.03	2.35±0.07*
24	2.81±0.02	2.77±0.05	2.72±0.04	2.16±0.01*
36	2.79±0.09	2.74±0.07	2.70±0.08	2.09±0.01*
Total proteins (mg/dL)				
12	3.89±0.28	3.72±0.02	3.65±0.02	3.28±0.08*
24	3.83±0.32	3.63±0.03	3.54±0.03	3.14±0.17*
36	3.95±0.13	3.49±0.04	3.44±0.04	2.98±0.04*
Aspartate aminotransferase (U/L)				
12	14.65±0.06	15.05±0.06	15.35±0.06	15.75±0.16
24	15.07±0.09	15.55±0.03	16.05±0.06	18.52±0.11*
36	15.11±0.13	16.60±0.11	18.90±0.13*	19.93±0.13*
Alkaline phosphatase (U/L)				
12	25.55±0.13	26.17±0.11	26.82±0.11	27.45±1.05
24	26.15±0.11	26.82±0.11	27.47±0.11	31.15±1.10*
36	26.41±0.03	27.23±0.13	31.10±0.13*	33.91±1.13*
Alanine aminotransferase (U/L)				
12	22.05±0.05	22.45±0.05	22.75±0.05	23.15±0.06
24	22.45±0.20	23.07±0.19	23.72±0.11	27.35±0.10*
36	23.13±0.13	23.93±0.11	28.80±0.14*	29.62±0.13*
Lactate dehydrogenase (U/L)				
12	248.35±3.19	249.55±4.19	250.75±1.19	251.95±2.19
24	251.72±5.48	254.67±4.48	257.62±2.48	267.61±3.48*
36	253.33±3.60	257.22±3.60	260.71±2.60	274.4±4.60*
Urea (mg/dL)				
12	8.32±0.07	8.57±0.02	8.81±0.08	9.06±0.04
24	8.58±0.04	8.87±0.03	10.56±0.05*	10.75±0.05*
36	8.74±0.03	9.02±0.01	10.79±0.05*	11.57±0.05*
Creatinine (mg/dL)				
12	1.16±0.01	1.18±0.02	1.20±0.03	1.22 ±0.05
24	1.20±0.04	1.22±0.03	1.54±0.06*	1.67±0.04*
36	1.27±0.03	1.29±0.01	1.57±0.08*	1.73±0.03*
Cholesterol (mg/dL)				
12	154.6±2.17	155.71±3.11	156.81±3.17	157.93±3.17
24	156.5±1.39	158.92±3.39	161.22±2.39	166.62±4.39*
36	158.91±1.49	162.05±2.35	163.15±3.49	169.25±2.29*
Glucose (mg/dL)				
12	29.25±1.16	30.17±1.13	31.125±2.15	32.05±1.15
24	29.55±1.19	30.75±1.15	31.95±2.13	35.15±1.19*
36	30.65±1.17	31.81±1.12	33.15±2.19	37.35±1.18*
Triglycerides (mg/dL)				
12	171.67±2.34	173.75±0.32	175.85±1.32	177.92±3.34
24	173.21±2.35	175.43±1.33	177.61±1.64	189.81±3.34*
36	176.25±2.41	178.75±2.21	181.25±1.91	193.75±3.41*

Values with sign (*) are exhibiting significant (P<0.05) difference from control.

cells (Gul *et al*. 2021; Akram *et al*., 2021). At increased doses of acetochlor, results exhibited a decrease in RBCs, an increase in WBCs and neutrophils counts. An increase in neutrophil count might be the result of immunological responses to damage in exposed big head carp tissues. Similar findings in *Oreochromis niloticus* (Fathy *et al*., 2019) subjected to varied doses of acetochlor included decreased red blood cells, haemoglobin, decreased lymphocytes, and increased white blood cells. *Cirrhinus mrigala* (Ghayyur *et al*., 2019), *Heteropneustes fossilis* (Akter *et al*., 2020), *Clarias gariepinus* (Woryi and al., 2020), and *Cyprinus carpio* (Vali *et al*., 2020) exposed to toxicants showed decreased haemoglobin, lymphocytes, monocytes, and pack cell volume. The decreased RBCs, increased WBCs abnormalities might be caused by damage to blood-forming cells, an increase in the generation of free radicals, or a reduction in oxygen delivery through the gills. Tissue damage induced by a higher dose of acetochlor was detected in bighead carp in this study. Stress circumstances may cause damage by inducing an inflammatory response in fish tissues resulting in an overproduction of white blood cells. Serum biochemistry serves as a clear sign of pollutant exposure, which is a clear indicator of environmental contamination and may be used to identify tissue pathology (Abdel-Tawwab and Hamed, 2018). Moreover, acetochlor has been shown to have deleterious impacts on blood biochemical profile in exposed fish leading to developmental disorders (Wang *et al*. 2016). Serum biochemical profile including ALP, AST and ALT enzymes significantly increased while serum total proteins and albumin quantity decreased in acetochlor treated fish. It has been indicated that for renal safety evaluation, kidney function tests (creatinine, urea and uric acid) are useful and reliable tools (Hamed and Tawwab, 2017; Hussain *et al*., 2020). In our experimental research, the concentrations of creatinine and urea were significantly ($p < 0.05$) elevated in treated fish indicating damage to kidneys leading to disruption of filtration processes due to acetochlor. Increased ALT, ALP, AST, cortisol, and LDH levels were previously observed in *Cirrhinus mrigala* (Ghayyur *et al*., 2019) treated with herbicide. Furthermore, depletion of serum total protein and elevated liver enzymes have been recorded in toxicant-exposed *Channa punctatus* (Sastry and Dasgupta, 1991), *Heteropneustes fossilis* (Kalita *et al*., 2003), and Zebra fish (Mahmoud *et al*., 2020). The intensity of damage produced by acetochlor to the liver of fish was determined in the current investigation by histological reactions of the fish (*A. nobilis*). Histopathological abnormalities in liver of fish included congestion, lower cytoplasmic space, vacuolar degeneration, necrosis and widening of sinusoidal spaces in hepatocytes of treated fish with acetochlor. Similar findings (necrosis of hepatocytes, fatty change and atrophy of hepatocyte) in liver tissues of *Clarias gariepinus* (Elias *et al*., 2020) have been observed. Edema, ceroid development, glomerular disintegration and congestion were also seen in the kidneys of acetochlor-treated fish. However, necrosis, vacuolation, accumulation of melano-macrophages, congestion, and different other tissue changes have also been observed in different species of fish exposed to toxicants like *Heteropneustes fossilis* (Pal and Reddy, 2018) and Tilapia (Vinodhini and Narayanan, 2009). According to earlier studies, the investigation of histopathological lesions in gill tissue is known as an excellent and reliable tool for detecting toxicant effects in aquatic life. It's because gills come into close touch with pollutants in the water, and gills are organs via which toxins enter the fish's body. Gills are in charge of the osmoregulatory system and ionic compound balancers (Gaffar *et al*., 2018). Different pathological lesions in gills due to acetochlor treatments in fish of current study have also been observed in different other organisms such as *Van fish* (Oguz *et al*., 2018) and *C. fluminea* (Benjamin *et al*., 2019), zebra fish. These histopathological lesions might be related to depletion of nutrients in the muscles and disruption of protein, carbohydrate and lipid metabolism linked with citric acid cycle (Sobha *et al*., 2007; Sulekha and Marcy, 2011; Muralidharan, 2014).

Conclusions: The findings of our experimental trial describe that fish are useful test specimens for screening of deleterious effects of synthetic compounds including

herbicides and the hemato-biochemical and histopathological being reliable parameters were adversely affected by exposure to acetochlor in bighead carp.

Authors contribution: YM, RH and AG designed the experiment. YM, RH and AG executed the research and collected the data. RH analyzed the data. YM and RH wrote the manuscript. YM and RH edited the final version of paper.

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