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Research Article

Evaluation of Histopathological and Hematological Effects of Neonicotinoid (Acetamiprid 20% SP) on Grass Carp (*Ctenopharyngodon idella*)

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Pesticides are usually used as an effective tool to control pests in agriculture; however, these chemicals may be a threat to nontarget organisms, especially aquatic organisms, because aquatic environments are the last station of pollutants. The present study evaluated the toxicity of a commercial formulation of neonicotinoid (acetamiprid 20% SP) as a systematic pesticide on the survival, hematology, and histology of the grass carp (*Ctenopharyngodon idella*). For these purposes, 105 fingerlings of the grass carp with an average body weight of 30 ± 2 g were exposed to 0, 50, 100, 150, and 200 mg·L^{-1} of acetamiprid for 96 h. According to the data, a clear mortality was observed as the concentration of acetamiprid elevated ($P < 0.01$). The LC50 96 h of acetamiprid was 121.146 mg·L^{-1}. The hematological parameters in fish changed, following exposure to acetamiprid ($P < 0.05$). Also, a strong correlation was found between acetamiprid concentrations and stress bioindicators such as glucose, total protein, albumin, and cholesterol ($P < 0.01$). There was not significant tissue damage in the control group (0 mg·L^{-1} of acetamiprid); however, acetamiprid led to tissues lesions such as hypertrophy, hyperplasia, uplifting of gill filaments, necrosis of gill epithelial cells, pyknosis and karyorrhexis of liver cells, and hemorrhage and necrosis of liver cell. Finally, acetamiprid-exposed fish exhibited some clinical signs including unbalanced swimming near the water surface, increasing operculum movement, and deaths with open-mouthed. The results of the present study clearly showed the survival-reducing effects of acetamiprid in *C. Idella*, which may return to tissue damage and stress induced by the pesticide. The results of the present study can be used as a base for future studies and environmental management.

1. Introduction

Pesticides are widely used in the world to control plant pests in agriculture [1–3]. According to previous studies, only 1% of pesticides act on target pests, while the remainders (99%) are discharged into the environment, where they may be toxic for nontarget organisms [4]. This matter is so important for aquatic life, since aquatic environments are the last station of pollutants [5]. Although biological methods are developing as environmentally friendly ways to control pests, the use of chemicals is still unavoidable due to the extent of agricultural lands throughout the world [6]. Pesticides improve and stabilize agriculture by controlling of weeds, fungi, and harmful insects. Nevertheless, it is

suggested that farmers use at least pesticides with less toxicity and half-life, such as systematic pesticides [7, 8].

Acetamiprid as a systematic pesticide is a neonicotinoid insecticide which used to control a variety of pests such as rice stem borer (*Chilo suppressalis*). Acetamiprid is a chloropyridinyl neonicotinoid that is distinct from the nitro-guanidine neonicotinoid [9]. Acetamiprid is a member of a new class of insecticides, developed in the late 1980s [10, 11]. Acetamiprid is an α-chloro-N-heteroaromatic chemical compound. It has 6-chloro-3-pyridine methyl as other neonicotinoids such as imidacloprid, nitenpyram, and thiacloprid. However, their differences are in the nitro-guanidine, nitromethane, or cyanoamidine substituent on an acyclic or cyclic moiety [12]. According to the United States Environmental Protection Agency reports [13], the lowest and highest toxicity of acetamiprid is for insects and mammals, respectively. Kimura–Kuroda et al. [14] stated that bioconcentration potentials of acetamiprid is little for aquatic organisms and can be an ideal systemic pesticide with a selective toxicity effect. However, very little work has been conducted to evaluate its acute toxicity among freshwater fish [15, 16].

Toxicological studies can provide important information about the toxicity and possible effects of chemicals and their threats on nontarget organisms [17]. The acute toxicity test (the LC50 96 h test) is a base for toxicological studies [18]. This test can determine lethal concentrations of chemicals for different organisms; however, a static test has some limitations. This method cannot give clear results on the effect of sublethal concentrations, because it is limited to survival results only [3, 19]. Also, toxicity of pollutants alters depending on environmental conditions, species, age, size, and nutrition of organism, which restrict this method (as a static test) to calculate the toxicity in a static environment such as laboratory condition, resulting in different results in comparison with natural conditions [20].

Fishes are one of the main sources of protein for human and also play an important role in food chain of water bodies [5, 21]. For example, the grass carp (*Ctenopharyngodon idella*) has been introduced for the biological control of aquatic plants in many countries. Also, the grass carp is an important freshwater fish for aquaculture in Iran [22]. Grass carp have a spindle-shaped body with large scales, gray back, and light gray belly. In the larval stage, grass carp fed on zooplankton and after that it relays mainly on plants, especially plants with soft tissues [23]. Grass carp farms are usually located near agriculture farms and also there is a polyculture of plant and fish as well [24]. Due to the different sensitivity of the organism to pollutants, toxicological tests are usually performed for different organisms [25, 26].

The histopathological and hematological assays can use as a quick method to detect the direct effects of chemicals and stimulants (especially sublethal concentration) in target organs of fish on a laboratory scale. These methods can provide information about the side effects of pollutants with fish health [5, 27, 28]. Blood parameters such as red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), glucose, cholesterol, albumin, and total protein concentrations, and histology of target tissues are usually evaluated in toxicological studies [17, 29]. Owning to the importance of grass carp for consumers in northern Iran and the proximity of the fish farms to the consumption hubs of acetamiprid (rice farms), this study was conducted to evaluate the histopathological and hematological effects of neonicotinoid (acetamiprid 20% SP) on grass carp (*C. idella*).

2. Methods and Materials

2.1. Preparation. One hundred and fifty grass carp (*Ctenopharyngodon idella*) with an average body weight of 30 ± 2 g were supplied from a local fish farm (Talesh County, Guilan province, Iran) and moved to a laboratory (Tehran, Iran). Fish were kept in 4 fiberglass tanks (500 Liters dewatering volume) for adaptation to laboratory conditions for 14 days. During the adaptation step, fish were fed twice a day about 1.5% of their weight (FFC, Faradaneh Co., Shahrekord, Iran). Water physicochemical parameters were checked every day. Average concentrations and levels of these parameters were as follow: total hardness 186 ± 4 mg·L^{-1} $CaCO_3$, Oxygen concentration 8 ± 1 mg·L^{-1}, temperature $27 \pm 2^{\circ}$C, pH 7.8 ± 0.4, and NH3 less than 0.01 mg·L^{-1}. Finally, the photoperiod of 16 h light to 8 h dark was employed.

2.2. Acute Toxicity Test (The LC50 96 h Test). The grass carp were acclimated with methods are used for acute toxicity tests as suggested for fish, macroinvertebrates, and amphibians [30].

After the adaption period, 105 fingerlings of the grass carp were randomly selected and transferred to the test tanks. The number of test tanks was 15 aquariums ($100 \times 50 \times 50$ cm) containing 200 l water. Fish were divided into 5 treatments with 3 replicates. Nominal concentrations of neonicotinoid (acetamiprid 20% SP-Hisun Chemical Co., Ltd., Zhejiang, China) were 0, 50, 100, 150, and 200 mg·L^{-1} at the present test. The test time was 96 h and the fish mortality was recorded at 24, 48, 72, and 96 h post-acetamiprid exposure. All water physicochemical parameters were the same as the adaptation period and those checked every day. During the test, 50% of the water volume of tanks were replaced daily with fresh water that had the same pesticide concentrations. Fish were transferred to the test tanks 24 h before beginning of the acute toxicity test (the LC50 96 h) and did not feed during the test. To make normal distribution of the pesticide and improve dissolved oxygen content in the test tanks, water was circulated by an internal water pump (No. 118, Guangzhou Ample Technology co. ltd., Guangzhou, China) during the test.

2.3. Haematological Assays. Fish (3 samples from each group) were randomly taken after 96 h and anesthetized with 200 mg·L^{-1} ground clove (*Syzygium aromaticum*) to reduce stress during the sampling [31]. Blood was taken from the caudal vessels of live fish 96 h after exposure to acetamiprid by heparinized syringe and transferred to heparinize 1.5 mL

Eppendorf tubes and kept on ice. The whole blood was suspended in the diluent for red and white blood cell examinations using a hemocytometer [32]. Blood plasma was separated by centrifuging of blood in 4800 ×g for 10 min. Plasma was kept at −20°C until biochemical analysis. Mean red blood cell count (RBC) and mean white blood cell count (WBC) were calculated using the hemocytometer according to a Houston study [33].

Haematocrit (HCT) was determined by centrifuging whole blood in heparinized microhematocrit capillary tubes at 3500 ×g for 10 min (Deltalab, Barcelona, Spain). Hemoglobin concentration (Hb) was measured by the cyanmethemoglobin method using a commercial kit (Pars Azmun Co., Tehran, Iran) [34, 35]. Also, glucose, total protein, triglyceride, cholesterol, and albumin concentrations were calculated by auto-analyzer (Roche Cobas Mira, Basel, Switzerland) using commercial kits (Pars Azmun Co., Tehran, Iran). Total serum protein was measured by the Biuret method. The Biuret test involves the reaction of Cu ions with peptide bonds in an alkaline solution, which eventually forms a purple-blue complex; this complex stability is about 60 min. The color intensity at 520 to 560 nm is proportional to the concentration of total protein [36]. The concentrations of cholesterol, triglyceride, and glucose in serum were measured upon an enzymatic method by the calorimetric test (CHOD-PAP). Triglyceride, cholesterol, and glucose in combination with a group of enzymes produced a red solution, where the red intensity is directly related to their concentrations [37]. Finally, the albumin was measured using the bromocresol green method (BCG method), and the color change of suspension (from yellow-green to green-blue) was determined at 640 nm [38].

2.4. Histopathological Observations. The tissue sample was taken after blood sampling. For this purpose, the abdomen of the fish was cut from under the gills to the anus. The samples were taken from the second left gill arch and the middle part of the liver. The samples were fixed in Bouin's fluid for 48 h and then transferred to the buffer solution and kept for 12 h. After this step, tissues were submerged in paraffin. Slides were prepared in thickness of 5–7 μm using a microtome [29] and were examined under a light microscope and photographed by microscope tablet (scopepad-LX116V1, Labex, London, UK) after staining by hematoxylin and eosin (H&E).

2.5. Data Analyses. The lethal concentration of acetamiprid in intervals of 24, 48, 72, and 96 h (LC50 24 h, 48 h, 72 h, and 96 h of acetamiprid) was calculated by the probit test with a 95% confidence. Also, significance between the means of blood parameters in independent groups were determined by One-Way ANOVA with a 95% confidence. The authors used the Spearman test to find the correlation between nominal concentrations of neonicotinoid (Acetamiprid 20% SP) with the mortality rate, tissue damage, and change of hematological parameters (2-tail). The video data were analyzed by Adobe After Effects software (AAE CS6). The clinical signs of fish were evaluated through direct observation of recorded videos, counting the average movement of the gill operculum in 1 min, and comparing the color of the object (fish) during a period.

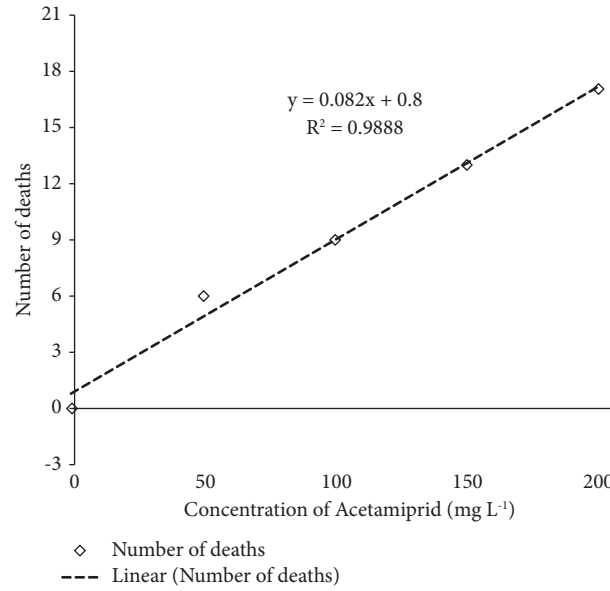


Figure 1: Correlation between the nominal concentration of neonicotinoid (acetamiprid 20% SP) and the number of deaths of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure ($P < 0.01$).

3. Results

3.1. The LC50 Test Results. The acetamiprid-exposed fingerlings of grass carp (*Ctenopharyngodon idella*) revealed some clinical signs such as increasing of operculum movement, fast swimming near the water surface, and death with open-mouthed. There was no mortality in the control group during the test. Also, there was a significant correlation between the pesticide concentration and the fish mortality (Figure 1), as the fish survival decreased significantly with increase of acetamiprid concentrations ($P < 0.01$). Also, the LC50 9 6h of acetamiprid was 121.146 mg·L^{-1} in the present study (Table 1).

3.2. Haematological Assays. There was a significant correlation between the concentration of acetamiprid with blood parameters (Figure 2, $P < 0.01$). Red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), and hemoglobin (Hb) concentrations significantly were different over 96 h exposure to acetamiprid ($P < 0.05$). Also, the concentrations of glucose, cholesterol, albumin, and total protein in the serum were significantly different (Table 2).

3.3. Pathological Assays. There were significant correlations between the concentrations of acetamiprid with the levels of liver and gill lesions (Tables 3 and 4). Acetamiprid caused hypertrophy, hyperplasia, fusing of filaments, uplifting of gill filaments, and necrosis of epithelial cells over 96 h exposure (Figure 3); The dilation of sinusoids, revealing cytoplasmic

Table 1: Lethal concentrations of neonicotinoid (acetamiprid 20% SP-Hisun Chemical Co., Ltd., Zhejiang, China) to fingerling grass carp (*Ctenopharyngodon idella*).

Point	Concentration ($mg \cdot L^{-1}$)*			
	24 h	48 h	72 h	96 h
LC10	87.135	61.531	44.710	16.722
LC20	115.741	88.296	78.079	52.568
LC30	136.368	107.596	102.141	78.416
LC40	153.993	124.087	122.702	100.503
LC50	**170.466**	**139.500**	**141.919**	**121.146**
LC60	186.940	154.914	161.136	141.789
LC70	204.564	171.405	181.696	163.875
LC80	225.191	190.704	205.758	189.723
LC90	253.797	217.470	239.138	225.570
LC95	277.420	264.450	266.685	288.491

Note. *All concentrations are nominal concentrations.

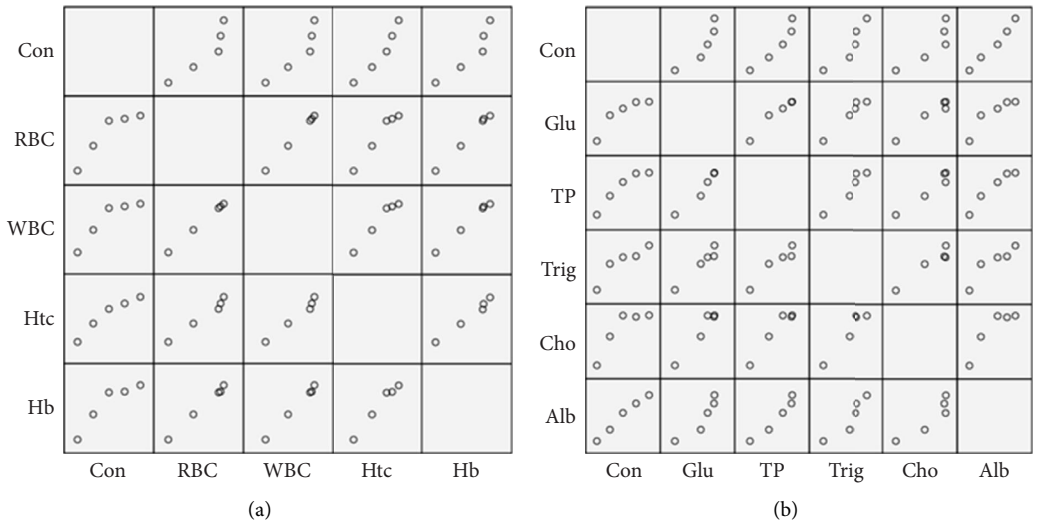


Figure 2: Correlation between blood parameters of fingerling grass carp (*Ctenopharyngodon idella*) and the nominal concentration of neonicotinoid (acetamiprid 20% SP) 96 h after exposure ($P < 0.01$); (a) The nominal concentration of acetamiprid (con); red blood cell count (RBC); white blood cell count (WBC); haematocrit (HCT); the hemoglobin concentration (Hb); (b) glucose concentration (Glu); The total protein (TP); triglyceride concentration (Trig); cholesterol concentration (Cho); albumin concentration (Alb).

Table 2: Results of blood biochemical tests (mean ± SD) of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure to neonicotinoid (acetamiprid 20% SP).

Blood parameters	Concentration of acetamiprid ($mg \cdot L^{-1}$)				
	0	50	100	150	200
RBC (10^6 μL)	1.41 ± 0.03^{d}	1.65 ± 0.06^{c}	1.89 ± 0.08^{b}	1.91 ± 0.08^{b}	1.94 ± 0.01^{a}
WBC (10^3 μL)	95.43 ± 0.38^{d}	109.83 ± 0.3^{c}	123.83 ± 0.3^{b}	124.93 ± 0.6^{b}	126.60 ± 0.4^{a}
HCT (%)	33.57 ± 0.42^{d}	37.10 ± 0.56^{c}	39.87 ± 0.72^{b}	40.92 ± 0.73^{b}	42.17 ± 0.15^{a}
Hb (g/l)	55.10 ± 0.61^{d}	65.13 ± 0.94^{c}	73.87 ± 0.77^{b}	74.18 ± 0.35^{b}	76.77 ± 0.25^{a}
Glucose (mg/dL)	112.67 ± 0.43^{d}	132.54 ± 0.35^{c}	137.77 ± 0.53^{b}	142.67 ± 0.23^{a}	142.97 ± 0.51^{a}
Total protein (g/dL)	4.73 ± 0.05^{e}	5.82 ± 0.04^{d}	6.62 ± 0.07^{c}	7.12 ± 0.03^{b}	7.16 ± 0.05^{a}
Triglyceride (mg/dL)	135.82 ± 0.11^{d}	143.42 ± 0.25^{c}	145.33 ± 0.15^{b}	145.62 ± 0.25^{b}	148.72 ± 0.13^{a}
Cholesterol (mg/dL)	118.48 ± 0.35^{c}	125.12 ± 0.09^{b}	129.92 ± 0.15^{a}	129.62 ± 0.93^{a}	129.98 ± 0.65^{a}
Albumin (mg/dL)	0.31 ± 0.02^{e}	0.42 ± 0.04^{d}	0.58 ± 0.05^{c}	0.67 ± 0.03^{b}	0.75 ± 0.05^{a}

Note; RBC: red blood cell count; WBC: white blood cell count; HCT: hematocrit; Hb: hemoglobin. Different letters (a–d) in the same rows indicate significant differences ($P < 0.05$).

Table 3: Gills damages of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure to different levels of neonicotinoid (acetamiprid 20% SP).

	Nominal concentrations (ml·L^{-1})				
Tissue damages	0	50	100	150	200
Basal hyperplasia	−	−	++	+++	+++
Epithelial hypertrophy	−	+	++	+++	+++
Uplifting of gill filaments	−	+	++	++	++
Fusing of filaments	−	−	+	+++	+++
Necrosis of epithelial cells	−	−	+	++	++++

(−) The tissue damages were not seen; (+) there was tissue slight damages; (++) there was tissue moderate damages; (+++) there was tissue intense damages; (++++) there was tissue very intense damages in the samples.

Table 4: The aggregation of erythrocytes within hepatic blood vessels (AHBVs), congestion of erythrocytes (CE), dilation of sinusoids (DSSs), revealing cytoplasmic vacuolation (CV), and necrosis (N) of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure to different levels of neonicotinoid (acetamiprid 20% SP).

	Nominal concentrations (ml·L^{-1})				
Liver damages	0	50	100	150	200
AHBV	−	+	++	+++	+++
CE	−	+	++	+++	++
CV	−	+	++	+++	++++
DSS	−	−	+	++	+++
N	−	−	+	++	+++

Note. (−) the tissue damages were not seen; (+) there was tissue slight damages; (++) there was tissue moderate damages; (+++) there was tissue intense damages; (++++) there was tissue very intense damages in the samples.

vacuolation, congestion of erythrocytes, aggregation of erythrocytes within hepatic blood vessels, and necrosis of liver cells were recognized in treatments after 96 h exposure to acetamiprid (Figure 4). There were not defining significant tissue lesions in the control group.

4. Discussion

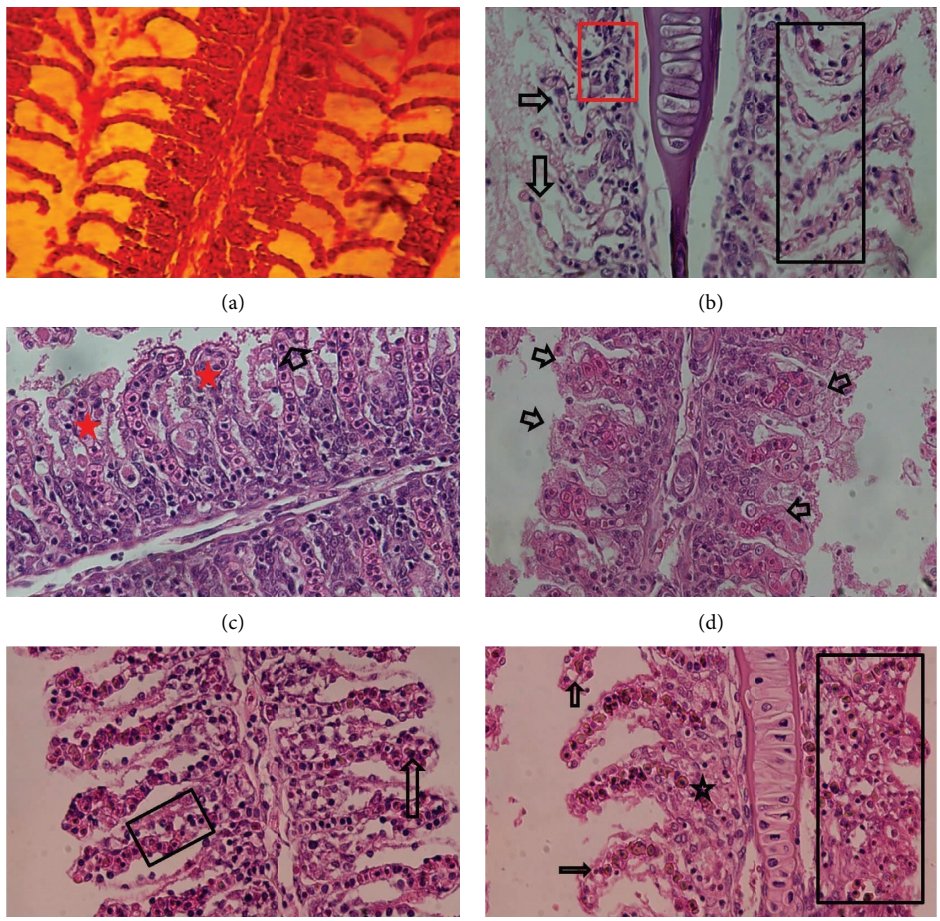
Acetamiprid is a new member of neonicotinoid insecticides, widely used in agriculture. It is accumulated in the water, thus threatening the life of nontarget organisms [39]. The results of the present study showed the survival reducing effects of acetamiprid, besides some disrupting impacts behavioral patterns and the fish health status. The swimming behavior of fish in acetamiprid exposed was significantly different from the control group, where showed clinical signs such as anxiety, fast swimming, and swimming near the surface. Yalsuyi et al. [5] attributed these clinical signs with stressful conditions.

Houndji et al. [40] reported a 96 h LC50 of 265.5 mg·L^{-1} for acetamiprid in the African catfish (*Clarias gariepinus*). The LC50 96 h of acetamiprid was 121.146 mg·L^{-1} in the present was different from those reported by Houndji et al. [40]; which may be related to differences in environmental conditions, size, age, and fish species [3].

Hematological assays are a useful method for evaluating pollutant effects on aquatic organisms [41], because blood parameters respond to low doses of pollutants [42]. In our finding, RBC, WBC, HCT, and Hb concentrations of treatments (50–200 mg·L^{-1} of acetamiprid) were significantly different upon 96 h exposure to acetamiprid. The results of previous studies showed the increasing concentration of biochemicals in serum (such as glucose, cholesterol, and total protein) in exposure to pollutants is a common response of the organism to deal with stressful conditions and oxidative stress [29, 43, 44]. In the present study, the concentrations of glucose, cholesterol, albumin, and total protein of serum as the stress biomarkers were significantly different in the treatments and were higher than in the control group (0 mg·L^{-1} of acetamiprid). These results were similar to Vali et al. [29] and Parrin et al. [44] studies.

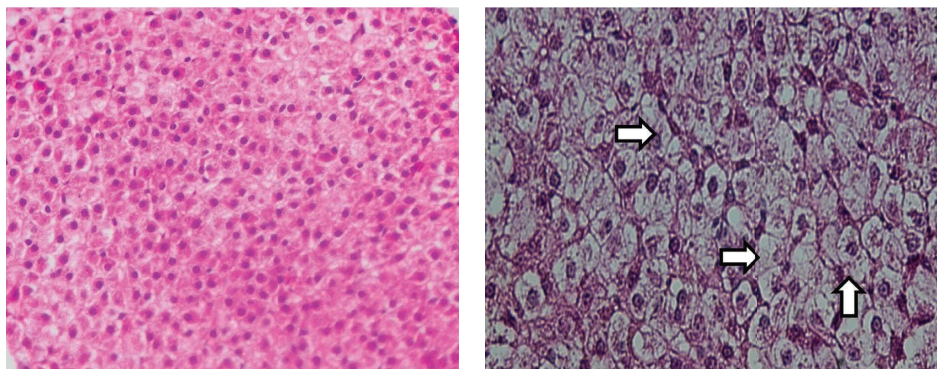
Ghayyur et al. [45] studied the toxicity of acetamiprid on hemato-biochemistry and tissue histology of the freshwater fish, *Cirrhinus mrigala*. The results of their study showed changes in blood parameters and gills and liver tissue in acetamiprid-exposed fish. Parrino et al. [46, 47] reported the toxicity effects of pesticides, their accumulations in aquatic environments and their threats for nontarget organisms, especially human. In the present study, the analyzing of liver and gills of groups (50–200 mg·L^{-1} of acetamiprid) revealed various tissue damages upon 96 h exposure to acetamiprid. These lesions included hypertrophy, hyperplasia, fusing of filaments, uplifting of gill filaments, the dilation of sinusoids, revealing cytoplasmic vacuolation, congestion of erythrocytes, the aggregation of erythrocytes within hepatic blood vessels, and necrosis of liver cells. In addition, there was a significant correlation between tissue lesions and acetamiprid concentrations. The highest level of tissue damages was related to the 200 mg·L^{-1} of acetamiprid.

Liver is an important organ for controlling many functions of the body such as homeostasis, detoxification, enzyme production, and metabolism. The results of previous studies reported that liver damages can significantly reduce the survival of the organisms and these damages may lead to disruption of enzyme functions and body



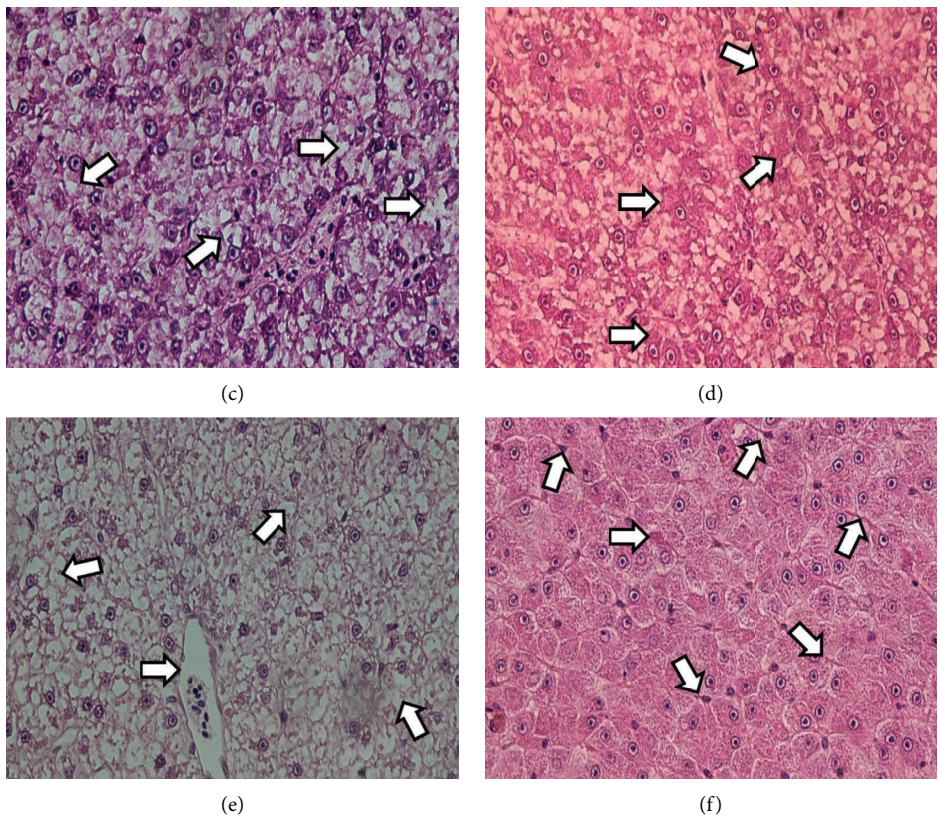
(a) (b) (c) (d) (e) (f)

Figure 3: Gills lesions of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure to lethal and sublethal concentration of neonicotinoid (acetamiprid 20% SP); (a) control group (acetamiprid was 0 mg·L^{-1}); (b) hyperplasia and uplifting of gill filaments (black rectangle) along with hypertrophy (black rows) and necrosis of epithelial cells (red rectangle); (c) necrosis of epithelial cells (red stars) and fusing of filaments (black rows); (d) necrosis of epithelial cells (black rows); (e) hypertrophy of epithelia cells (black row) and fusing of filaments (black rectangle); (f) necrosis of epithelial cells (black rectangle), hyperplasia (black star), and hypertrophy of epithelia cells (black rows). All pictures were magnified ×400.



(a) (b)

Figure 4: Continued.



(c) (d)

(e) (f)

Figure 4: Liver lesions of fingerling grass carp (*Ctenopharyngodon idella*) 96 h after exposure to lethal and sub-lethal concentration of neonicotinoid (acetamiprid 20% SP); (a) control group (acetamiprid was 0 mg·L^{-1}); (b) dilation of sinusoids; (c) revealing cytoplasmic vacuolation; (d) congestion of erythrocytes; (e) necrosis; (f) The aggregation of erythrocytes within hepatic blood vessels. All pictures were magnified ×400.

hemostasis, because there is a significant correlation between liver damage and increased oxidative stress [48–50]. Superoxide dismutase (SOD), catalase, glutathione peroxidases (GPXs), and transferases are the most important enzymes of the body's antioxidant defense system [51]. Di Giulio and Meyer [52] observed a significant correlation between liver damages and the activity of these enzymes and oxidative stress. Raibeemol and Chitra [53] observed morphological changes in gills, such as the uplifting of gill filaments and hyperplasia, followed by the dilation of blood vessels associated with changes in the space between the exterior medium and blood flow. Dilate of blood vessels can lead to disturbance of ion exchange and following disruption of homeostasis and death of organism [54]. In the present study, there were significant correlations between tissues damages, the fish mortality, and acetamiprid concentrations, which were similar to the results of previous studies.

5. Conclusion

The present studies clearly showed the adverse effects of neonicotinoids on the survival rate, histology, and blood parameters of the grass carp as an important commercial and biological freshwater fish species. The results of the present study can be used for future studies and environmental management. Also, the studied parameters have the effective potentials to use as biomarkers of the stressful condition and toxicity of pesticides.

Data Availability

The datasets in this study are available from the corresponding author on reasonable request. All data and materials are available for publication.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of Interest

The authors declare that they have no conflicts of interest for the publication of the present work.

Authors' Contributions

All the authors of this article have made important contributions to testing, collecting data, analyzing results, and writing the article.

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References

[1] M. Forouhar Vajargah and A. Hedayati, "Acute toxicity of butachlor to *Rutilus rutilus* caspicus and *Sander lucioperca* in vivo condition," *Transylvanian Review of Systematical and Ecological Research*, vol. 19, no. 3, pp. 85–92, 2017.

[2] H. Poorbagher, H. Ghaffari Farsani, and H. Farahmand, "A method to quantify genotoxicity of malathion in rainbow trout using the weighted averaging," *Toxicology Mechanisms and Methods*, vol. 28, no. 8, pp. 607–614, 2018.

[3] M. F. Vajargah, J. I. Namin, R. Mohsenpour, A. M. Yalsuyi, M. D. Prokić, and C. Faggio, "Histological effects of sublethal concentrations of insecticide lindane on intestinal tissue of grass carp (*Ctenopharyngodon idella*)," *Veterinary Research Communications*, vol. 45, no. 4, pp. 373–380, 2021.

[4] European Commission, "Acetamiprid. SANCO/1392/2001," 2004, https://pubchem.ncbi.nlm.nih.gov/compound/Acetamiprid.

[5] A. M. Yalsuyi, A. Hajimoradloo, R. Ghorbani, V. A. Jafari, M. D. Prokić, and C. Faggio, "Behavior evaluation of rainbow trout (*Oncorhynchus mykiss*) following temperature and ammonia alterations," *Environmental Toxicology and Pharmacology*, vol. 86, Article ID 103648, 2021.

[6] J. C. Akan, Z. Mohammed, L. Jafiya, and V. O. Ogugbuaja, "Organochlorine pesticide residues in fish samples from alau dam, borno state, north eastern Nigeria," *Journal of Environmental & Analytical Toxicology*, vol. 03, no. 3, p. 1, 2013.

[7] H. Kwon, T. K. Kim, S. M. Hong, E. K. Se, N. J. Cho, and K. S. Kyung, "Effect of household processing on pesticide residues in field-sprayed tomatoes," *Food Science and Biotechnology*, vol. 24, no. 1, pp. 1–6, 2015.

[8] N. H. Kim, J. S. Lee, K. A. Park et al., "Determination of matrix effects occurred during the analysis of organochlorine pesticides in agricultural products using GC-ECD," *Food Science and Biotechnology*, vol. 25, no. 1, pp. 33–40, 2016.

[9] F. Ji-chao, H.-f. Guo, X.-d. Liu, Y.-M. Qian, L.-G. Wang, and C. Hao-hai, "Mode of action and applying tactic of acetamiprid against rice stem borers," *Chinese Journal of Pesticide Science*, vol. 1, no. 3, pp. 26–32, 1999.

[10] Arla (Agence de reglementation de la lutte antiparasitaire de Sante Canada), "Registration decision acetamiprid. RD2010-06.SC pub," 2010, https://www.sciencedirect.com/topics/chemistry/acetamiprid.

[11] P. Vohra, K. S. Khera, and G. K. Sangha, "Physiological, biochemical and histological alterations induced by administration of imidacloprid in female albino rats," *Pesticide Biochemistry and Physiology*, vol. 110, pp. 50–56, 2014.

[12] K. A. Ford and J. E. Casida, "Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, Article ID 10168, 2008.

[13] Usepa, "Office of prevention, pesticides and toxic substances," *Ecological Risk Assessment for Proposed Use of Acetamiprid on Cucurbit, Stone Fruit and Tree Nut Crops*, 2005.

[14] J. Kimura-Kuroda, Y. Komuta, Y. Kuroda, M. Hayashi, and H. Kawano, "Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats," *PLoS One*, vol. 7, no. 2, Article ID 32432, 2012.

[15] B. Velmurugan, T. Ambrose, and M. Selvanayagam, "Genotoxic evaluation of lambda-cyhalothrin in mystus gulio," *Journal of Environmental Biology*, vol. 27, pp. 247–250, 2006.

[16] M. Li, J. Wang, Z. Lu, D. Wei, M. Yang, and L. Kong, "NMRBased metabolomics approach to study the toxicity of lambda-cyhalothrin to goldfish (*Carassius auratus*)," *Aquatic Toxicology*, vol. 146, pp. 82–92, 2014.

[17] R. Mohsenpour, H. Mousavi-Sabet, A. Hedayati, A. Rezaei, A. M. Yalsuyi, and C. Faggio, "In vitro effects of silver nanoparticles on gills morphology of female Guppy (Poecilia reticulate) after a short-term exposure," *Microscopy Research and Technique*, vol. 83, no. 12, pp. 1552–1557, 2020.

[18] A. Hedayati, M. F. Vajargah, A. M. Yalsuyi, S. Abarghoei, and M. Hajiahmadyan, "Acute toxicity test of pesticide abamectin on common carp (*Cyprinus carpio*)," *Journal of Coastal Life Medicine*, vol. 2, no. 11, pp. 841–844, 2014.

[19] A. M. Yalsuyi and M. F. Vajargah, "Recent advance on aspect of fisheries: a review," *Journal of Coastal Life Medicine*, vol. 5, no. 4, pp. 141–148, 2017.

[20] A. Mohamadi Yalsuyi, M. Forouhar Vajargah, A. Hajimoradloo, M. Mohammadi Galangash, M. D. Prokić, and C. Faggio, "Can Betadine (10% povidone-iodine solution) act on the survival rate and gill tissue structure of Oranda goldfish (*Carassius auratus*)?" *Veterinary Research Communications*, vol. 46, no. 2, pp. 389–396, 2022.

[21] M. Forouhar Vajargah, A. Mohamadi Yalsuyi, A. Hedayati, and C. Faggio, "Histopathological lesions and toxicity in common carp (*Cyprinus carpio* L. 1758) induced by copper nanoparticles," *Microscopy Research and Technique*, vol. 81, no. 7, pp. 724–729, 2018.

[22] M. Forouhar Vajargah and A. Hedayati, "Toxicity effects of cadmium in grass carp (*Ctenopharyngodon idella*) and big head carp (Hypophthalmichthys nobilis)," *Transylvanian Review of Systematical and Ecological Research*, vol. 19, no. 1, pp. 43–48, 2017.

[23] E. W. Chilton and M. I. Muoneke, "Biology and management of grass carp (*Ctenopharyngodon idella*, Cyprinidae) for vegetation control: a North American perspective," *Reviews in Fish Biology and Fisheries*, vol. 2, no. 4, pp. 283–320, 1992.

[24] J. Pucher, T. Gut, R. Mayrhofer et al., "Pesticide-contaminated feeds in integrated grass carp aquaculture: toxicology and bioaccumulation," *Diseases of Aquatic Organisms*, vol. 108, no. 2, pp. 137–147, 2014.

[25] J. j. Zhang, Y. Wang, H. y. Xiang et al., "Oxidative Stress: role in acetamiprid-induced impairment of the male mice reproductive system," *Agricultural Sciences in China*, vol. 10, no. 5, pp. 786–796, 2011.

[26] Phss (Public Health and Social Services), "Acetamiprid," 2015, http://www.co.thurston.wa.us/Health/ehipm/pdf-insect/insecticideactives/acetamiprid.pdf.

[27] M. Khabbazi, M. Harsij, S. A. A. Hedayati, M. H. Gerami, and H. Ghafari-Farsani, "Histopathology of rainbow trout gills after exposure to copper," *Iran. Journal. Ichthyol*, vol. 1, no. 3, pp. 191–196, 2014.

[28] A. T. Mirghaed, P. Yarahmadi, P. M. Craig, H. G. Farsani, N. Ghysvandi, and S. Eagderi, "Hemato-immunological, serum metabolite and enzymatic stress response alterations in exposed rainbow trout (*Oncorhynchus mykiss*) to nanosilver," *International Journal of Aquatic Biology*, vol. 6, no. 4, pp. 221–234, 2018.

[29] S. Vali, N. Majidiyan, A. M. Yalsuyi, M. F. Vajargah, M. D. Prokić, and C. Faggio, "Ecotoxicological effects of silver nanoparticles (Ag-NPs) on parturition time, survival rate, reproductive success and blood parameters of adult common

molly (Poecilia sphenops) and their larvae," *Water*, vol. 14, no. 2, p. 144, 2022.

[30] Us-Epa and Ers, *Methods for Acute Toxicity Tests with Fish, Macro Invertebrates and Amphibians*, USEPA ERS, Washington, DC, USA, 1975.

[31] M. Saheli, H. Rajabi Islami, M. Mohseni, and M. Soltani, "Effects of dietary vitamin E on growth performance, body composition, antioxidant capacity, and some immune responses in Caspian trout (Salmo caspius)," *Aquaculture Reports*, vol. 21, Article ID 100857, 2021.

[32] S. J. Gholami-Seyedkolaei, A. Mirvaghefi, H. Farahmand, and A. A. Kosari, "Effect of a glyphosate-based herbicide in *Cyprinus carpio*: assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters," *Ecotoxicology and Environmental Safety*, vol. 98, pp. 135–141, 2013.

[33] C. Kavitha, A. Malarvizhi, S. Senthil Kumaran, and M. Ramesh, "Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, Catla catla," *Food and Chemical Toxicology*, vol. 48, no. 10, pp. 2848–2854, 2010.

[34] T. E. Eurell, D. P. Bane, W. F. Hall, and D. J. Schaeffer, "Serum haptoglobin concentration as an indicator of weight gain in pigs," *Canadian Journal of Veterinary Research*, vol. 56, no. 1, pp. 6–9, 1992.

[35] B. Nkrumah, S. B. Nguah, N. Sarpong et al., "Hemoglobin estimation by the HemoCue® portable hemoglobin photometer in a resource poor setting," *BMC Clinical Pathology*, vol. 11, no. 1, pp. 5-6, 2011.

[36] R. M. G. Wells and N. W. Pankhurst, "Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish," *Journal of the World Aquaculture Society*, vol. 30, no. 2, pp. 276–284, 1999.

[37] R. Kopp, J. Mareš, Š. Lang, T. Brabec, and A. Ziková, "Assessment of ranges plasma indices in rainbow trout (*Oncorhynchus mykiss*) reared under conditions of intensive aquaculture," *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 59, no. 6, pp. 181–188, 2014.

[38] J. H. Jee, F. Masroor, and J. C. Kang, "Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, Sebastes schlegeli (Hilgendorf)," *Aquaculture Research*, vol. 36, no. 9, pp. 898–905, 2005.

[39] X. Ma, H. Li, J. Xiong, W. T. Mehler, and J. You, "Developmental toxicity of a neonicotinoid insecticide, acetamiprid to zebrafish embryos," *Journal of Agricultural and Food Chemistry*, vol. 67, no. 9, pp. 2429–2436, 2019.

[40] M. A. B. Houndji, I. Imorou Toko, L. Guedegba et al., "Joint toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on African catfish (Clarias gariepinus) juveniles," *Journal of Environmental Science and Health, Part B*, vol. 55, no. 7, pp. 669–676, 2020.

[41] S. A. Hedayati, H. G. Farsani, S. S. Naserabad, S. H. Hoseinifar, and H. Van Doan, "Protective effect of dietary vitamin E on immunological and biochemical induction through silver nanoparticles (AgNPs) inclusion in diet and silver salt (AgNO3) exposure on Zebrafish (Danio rerio)," *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology*, vol. 222, pp. 100–107, 2019.

[42] A. G. M. Osman, K. Y. AbouelFadl, A. E. B. M. Abd El Reheem, U. M. Mahmoud, W. Kloas, and M. A. Moustafa, "Blood biomarkers in Nile tilapia Oreochromis niloticus niloticus and African catfish Clarias gariepinus to evaluate water quality of the river Nile," *Journal of FisheriesSciences.com*, vol. 12, no. 1, Article ID 1000141, 2018.

[43] S. Ghayyur, S. Tabassum, M. S. Ahmad, N. Akhtar, and M. F. Khan, "Effect of chlorpyrifos on hematological and seral biochemical components of fish Oreochromis mossambicus," *Pakistan Journal of Zoology*, vol. 51, no. 3, p. 1047, 2019.

[44] V. Parrin, G. Tardiolo, G. De Marco et al., "The monitoring of marine ecosystems of the Milazzo gulf and the Marinello nature reserve in Sicily: the potential role of Coris julis as pesticides bioindicator," *Cahiers de Biologie Marine*, vol. 63, no. 3, pp. 189–197, 2022.

[45] S. Ghayyur, M. F. Khan, S. Tabassum et al., "A comparative study on the effects of selected pesticides on hematobiochemistry and tissue histology of freshwater fish Cirrhinus mrigala (Hamilton, 1822)," *Saudi Journal of Biological Sciences*, vol. 28, no. 1, pp. 603–611, 2021.

[46] V. Parrino, R. Minutoli, G. Lo Paro, D. Surfaro, and F. Fazio, "Environmental assessment of the pesticides in Parablennius sanguinolentus along the Western Calabrian coast (Italy)," *Regional Studies in Marine Science*, vol. 36, Article ID 101297, 2020.

[47] V. Parrino, G. De Marco, R. Minutoli et al., "Effects of pesticides on Chelon labrosus (Risso, 1827) evaluated by enzymatic activities along the north eastern Sicilian coastlines (Italy)," *The European Zoological Journal*, vol. 88, no. 1, pp. 540–548, 2021.

[48] M. F. Khan, S. Tabassum, H. Sadique et al., "Hematological, biochemical and histopathological alterations in common carp during acute toxicity of endosulfan," *International Journal of Agriculture and Biology*, vol. 22, no. 4, pp. 703–709, 2019.

[49] P. Sharma, P. Chadha, and H. S. Saini, "Tetrabromobisphenol A induced oxidative stress and genotoxicity in fish Channa punctatus," *Drug and Chemical Toxicology*, vol. 42, no. 6, pp. 559–564, 2019.

[50] C. Fouzai, W. Trabelsi, S. Bejaoui et al., "Cellular toxicity mechanisms of lambda-cyhalothrin in Venus verrucosa as revealed by fatty acid composition, redox status and histopathological changes," *Ecological Indicators*, vol. 108, Article ID 105690, 2020.

[51] J. Bhagat, B. S. Ingole, and N. Singh, "Glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation as biomarkers of oxidative stress in snails: a review," *Invertebrate Survival Journal*, vol. 13, no. 1, pp. 336–349, 2016.

[52] R. T. Di Giulio and J. N. Meyer, "Reactive oxygen species and oxidative stress. The Toxicology of Fishes," *Invertebrate Survival Journal*, pp. 273–324, 2008.

[53] K. P. Raibeemol and K. C. Chitra, "Histopathological alteration in gill of the freshwater fish Pseudetroplus maculatus (Bloch, 1795) under chlorpyrifos toxicity," *International Journal of Advanced Research in Biological Sciences (IJARBS)*, vol. 3, no. 12, pp. 141–146, 2016.

[54] R. Macirella, G. Madeo, S. Sesti et al., "Exposure and post-exposure effects of chlorpyrifos on *Carassius auratus* gills: an ultrastructural and morphofunctional investigation," *Chemosphere*, vol. 251, Article ID 126434, 2020.