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Original Research

Evaluation of Antioxidant Response and Histopathological Alterations in *Cyprinus carpio* and *Oreochromis niloticus* Infected with Zoonotic Bacterial, *Aeromonas hydrophila*

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Abstract

Aeromonas hydrophila is one of the major virulent zoonotic bacterial diseases that adversely affect the health of farmed fish, inducing higher mortalities. The current study focused on comparing the resistance of the Common carp, *Cyprinus carpio*, and Nile tilapia, *Oreochromis niloticus,* to *Aeromonas hydrophila* infection by investigating the antioxidant responses and histopathological alterations. Juveniles of *C. carpio* and *O. niloticus* were randomly alienated into a control group (uninfected) and a challenged group infected with 100 µL of *A. hydrophila*. Samples of liver, kidney, and spleen were collected post-infection for 7 days to monitor antioxidant response, including glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT), and the assessment of the histopathological alteration in the vital organs. The antioxidant indicators showed significant alterations post-exposure to *A.*

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hydrophila. Meanwhile, *O. niloticus* has significantly regulated an active antioxidant response than *C. carpio*. Histological architecture showed that the liver is the most affected organ compared to the kidney and spleen and revealed severe aggregation of melanophores and macular degeneration. Based on the study outcomes, *A. hydrophila* is a highly virulent bacterium that induces noticeable alterations in the antioxidant mechanism and histopathological changes in the liver, kidneys, and spleen of *C. carpio* and *O. niloticus*. However, *O. niloticus* experiences an improved antioxidant response against *A. hydrophila* infection compared with *C. carpio*.

Keywords: bacterial infection, abiotic stress, Cyprinus carpio, histopathology, antioxidant enzyme activities

Introduction

Bacterial infection is one of the major stressors that threaten the fish industry. Among them, *Aeromonas sp.* is a Gram-negative bacterium that is normally established in the aquatic environments [1]. It is one of the most common bacterial pathogens in freshwater causing infections in fish which are described by sepsis with systemic hemorrhage and could induce human and fish infections [2–5]. *Aeromonas hydrophila* is a pathogenic bacterium that causes epizootic ulcerative syndrome, hepatosplenomegaly in fish, hepatic energetic metabolism, and furunculosis [6]. The liver and kidney are the main internal organs affected by *Aeromonas sp.* [7]. This bacterium is a zoonotic organism capable of infecting animals and humans through the consumption of infected fish and inducing oxidative damage that is involved in the pathogenesis of diseases [3]. Such bacterium targets the liver and causes much damage to it and oxidative stress has different antioxidant mechanisms that make them more resistant to the hosts' immune systems [8]. *A. hydrophila* infections may increase the intracellular generation of reactive oxygen species (ROS) in fish, as well as increase free radicals with changes in antioxidant defense mechanisms and infliction of oxidative stress possibly leading to the oxidative damage of cellular macromolecules [9, 10]. Many bacterial pathogens, such as *A. hydrophila*, *A. caviae*, *A. salmonicida*, and *A. veronii,* are bacterial zoonosis of fish that have received increasing focus due to the identification using molecular diagnostic techniques [11, 12]. The presence of virulence factors (aerolysin, hemolysin, gelatinase, enterotoxin cytosine, and antimicrobial peptides) in *A. hydrophila* is responsible for infecting fishes and inducing septicemia, gastroenteritis, plus causing a Motile Aeromonas Septicemia (MAS) disease [13].

Oxidative stress and oxidant status are directly related to the initiation and progression of bacterial infectious disease [14]. Catalase (CAT) and superoxide dismutase (SOD) are antioxidant enzymes that play an important role in protecting the host cell from oxidative damage induced by bacterial infection and environmental pollution [15, 16]. Exposure of fish to stress causes the release of reactive oxygen species (ROS), which includes hydrogen peroxide and other oxidant molecules, including superoxide anion, peroxyl radical, and peroxynitrite anion [17–19]. The GPx antioxidant enzyme contains selenium in its active site and eliminates inorganic and organic hydroperoxides, reducing them to water or alcohol coupled with reduced glutathione (GSH) oxidation [20]. It is clear that each fish species exhibits a different pattern of antioxidant enzymatic activity according to the type of injury and the type of bacteria [8].

The common carp (*Cyprinus carpio*) is an important fish and has a long history of cultivation, which may be followed back to 8,000 years ago [21]. *C. carpio* and *O. niloticus* are the most commonly cultivated species in Basrah, Iraq because they are resistant to aquatic stressors. Bacterial infection is a significant threat to the cultivable of *C. carpio* and *O. niloticus* causing mortality and economic loss [22, 23]. The present study is a comparative study to evaluate the response of *C. carpio* and *O. niloticus* to *A. hydrophila* infection on redux status, and histopathological alterations in different vital organs.

Materials and Methods

Preparation of Bacterial Isolation and Challenge for *A. hydrophila*.

A. hydrophila was isolated and identification of 16S rRNA using a DNA sequencer for bacterial fish diseases. The *A. hydrophila* strain 16S rRNA (OR398683.1) (BLAST) was used for the homological search. In addition, the Bioedit program was used for estimating the similarity matrix. (MEGA7) sequence analysis software with 1000 bootstrap values was utilized for constructing the phylogenetic tree. Bacteria were cultured in (Tryptic soy agar) for 24 h at 30°C, and a single colony was chosen to incubate in (Tryptic soy broth) for 24 h at 30°C, *A. hydrophila* cultured broth was centrifuged for 15 min at 3000 × g at 4°C and after that, the pellet was removed and suspended in sterile normal saline. Determination of the semi-lethal

dose (LD50) was identified as 6.9×10^7 CFU/ mL, which was used for injecting the fish intraperitoneally (IP) according to [24].

Experiment Design

Fingerlings of *C. carpio* and *O. niloticus* (n=60 for each species of fish) (average body weight of 20–25 g) were randomly caught from Karma-Ali River north of Basrah and each species of fish species was divided randomly into the control group (A) and infected groups (B) in triplicate. The experiment was carried out separately for each species of fish. Fish were injected with a semi-lethal dose (LD_{50}) of the suspension containing (0.5 ml; 6.9×10^6 CFU/ml) as an intraperitoneal injection. The control group was injected with 0.5 ml of a sterile saline solution (0.86%), and clinical signs and mortality were recorded daily according to [25].

Fish Samples

Healthy fingerlings of two species of *C. carpio* and *O. niloticus* with a body weight of 30-35g were collected live from the Al-Mas`hab River in the Basra governorate, Iraq. The healthy fish were selected for studies, which were confirmed through physical appearance, including skin luster, eyes, color, and behavior [16]. Fish were transported in a plastic container with oxygen as soon as possible to the laboratory (Department of Biological Development in Shatt Al-Arab and NW Arabian Gulf, Marine Science Center, University of Basra, Basra, Iraq), and randomly distributed in an Aquarium with a capacity of (60 × 40 × 50 cm). Fish were kept in glass tubs (10 fish/aquarium; 50 L) (All species of fish were experimentally placed separately). The fish were fed during the days of acclimatization for 7 days, and before the injection, the food was cut.

Samples Collection

Three fish were randomly selected after injection from each species of fish. The fish were killed by an overdose of anesthesia (250 mg of clove oil/liter), dissected, and their internal organs (liver, kidney, and spleen) were collected at intervals for 7 days (1st, 3rd, 5th, and 7th days) after injection and part of the internal organs was kept at a temperature of -80°C (kept in liquid nitrogen) until performing the biochemical analysis. The second part of the organs was preserved in (10%) neutral buffered formalin for histological analysis.

Protein Extraction from the Liver, Kidney, and Spleen

The protein was extracted using the Protein Extraction Buffer prepared by (the Iraqi Company for Biotechnology). The extraction buffer consists of 50 mM 4- (2 hydroxyethyle) -1-piperazineethanesulfonic acid (HEPES, pH 7.5), 150 mM NaCl, 10 mM Dithiothreitol (DTT), 1 mM polymethylsulfinyl fluoride (PMSF), 1 mM Ethylenediaminetetraacetic acid (EDTA) and 1% sodium dodecyl sulfate (SDS).

The protein was extracted with the following steps: First, 0.5 g of weight was taken from each tissue (liver, kidney, and spleen), each individually for each *C. carpio* and *O. niloticus* (three fish/replicas) and mashed well with the addition of 1 ml of Protein Extraction Buffer. Secondly, the mixture was transferred to liquid nitrogen and immersed deep 3 times for 5min. At last, it was kept at room temperature for 20 minutes, then inserted into a cooler centrifuge at 18,000 × g for 20 min.

Calculation of Protein Concentration

To calculate protein concentration, a special kit (BCA assay) from (Thermo Fisher Scientific) was used according to the following protocol of Abd-alsahb et al. (2021).

ELISA for Antioxidant Concentration in Tissues

The antioxidant concentration in *C. carpio* and *O. niloticus* in tissues (liver, kidney, and spleen), (three fish/replicas) was determined by ELISA, Elabscience (USA) following Yin et al. [26]. The plates contain the 96-well plates, the glutathione peroxidase (GPX) kit, and the absorbance of the superoxide dismutase (SOD) kit at 505 nm and using (U/mg protein). Catalase (CAT) concentration was measured as a colorimetric method, according to the following protocol kit, expressed as units (U/mg protein) and absorbance at 405 nm [27]. The protein concentration of the supernatant for the samples was homogenized using a buffer (pH 7.5) and then centrifuged for (15 min) at 5°C at 15,000 × *g*, to get the supernatant. The supernatant was centrifuged for 1 h at the same status. The final supernatant was obtained, and the pellet was collected, cleaned, and then stored in the buffer (HEPES, pH 7.5), according to [24]. Each plate contained the appropriate positive and negative controls, and the results of the test samples were calculated from standard curves.

Histopathology

Tissues from the liver, kidneys, and spleen collected from the infected fish were fixed in (10%) neutral buffered formalin and prepared for further processing, sectioning with microtome, and stained with Haematoxylin and Eosin (H & E) according to Roberts [28].

Statistical Analysis

The results were compiled by one-way ANOVA followed by All Pairwise Comparison according to the Tukey test to distinguish the difference among means. The statistical analysis was conducted using SPSS V. 21 and graphs were prepared by GraphPad Prism V7. The data were presented as mean ± standard deviation.

Fig. 1. Experimentally infected *Oreochromis niloticus* with *Aeromonas hydrophila* showing severe erythema in the skin (A) and ulceration in the skin (B). Experimentally infected *Cyprinus carpio* with *Aeromonas hydrophila* showing ulcerative spots in the skin (C) and extended areas of severe ulcerations (D).

Results

Pathological Clinical Symptoms

The experimentally infected *C. carpio* and *O. niloticus* with *A. hydrophila* showed clearly characteristic pathological symptoms of *A. hydrophila*. Fig. 1. A and B show severe hemorrhagic patches and congestion on the body of *O. niloticus*. Fig. 1. C and D show ulcers, inflammation, and falling scales on the skin of *C. carpio*.

Antioxidant Activity

The antioxidant response was determined in different tissue samples (liver, kidney, and spleen) from fish *C. carpio* and *O. niloticus* infected with *A. hydrophila after* 0, 1, 3, 5, and 7 days of infection.

Tissue Levels of GPX Activity in Fish

The activity of GPX in the liver, kidneys, and spleen of *C. carpio*, significantly increased at different sampling days post-infection and peaked on the 3rd day in all tissues (Fig. 2 A, B, & C). The levels of this enzyme decreased remarkably on the 7th day with significant differences in the control. While, the level of GPX activity was significantly ($p < 0.05$) increased in all evaluated tissues of *O. niloticus* (Fig. 3 A, B, & C). There was also an increase in the activity of the GPX on the 3rd day post-infection in all tissues, and a decrease in the kidney on the 5th and 7th.

Tissue Levels of SOD Activity for Fish

The activity of SOD in tissues of *C. carpio* was significantly increased after the 1st day of infection and continued as over the control in the kidney and spleen (Fig. 4). Meanwhile, this increase was restored on the 7th day in *C. carpio* liver (Fig. 4 A).

In *O. niloticus*, SOD was significantly increased at the 3rd and 5th days in the liver, and 1st, 3rd in the kidney, and only at the 3rd in the spleen, and the other sampling times were maintained as in the control group (Fig. 5).

Tissue Levels of CAT Activity for Fish

The effect of *A. hydrophila* infection on the activity of the CAT activity in the liver, kidney, and spleen of *C. carpio* is presented in Fig. 6 (A, B & C). In the liver and spleen, the expression of the CAT activity was found to increase significantly ($p < 0.01$) after one day post-infection, meanwhile in the kidney, increased significantly starting from day three post-infection (Fig. 6 B). *O. niloticus* experienced a significant increase in CAT activity post-infection, which peaked on day three and then declined during the sampling time.

Histopathological Changes in *C. carpio* and *O. niloticus* Post-Infection with *A. hydrophila*

Histopathological changes were revealed in the liver, kidney, and spleen of *C. carpio* and *O. niloticus*, with

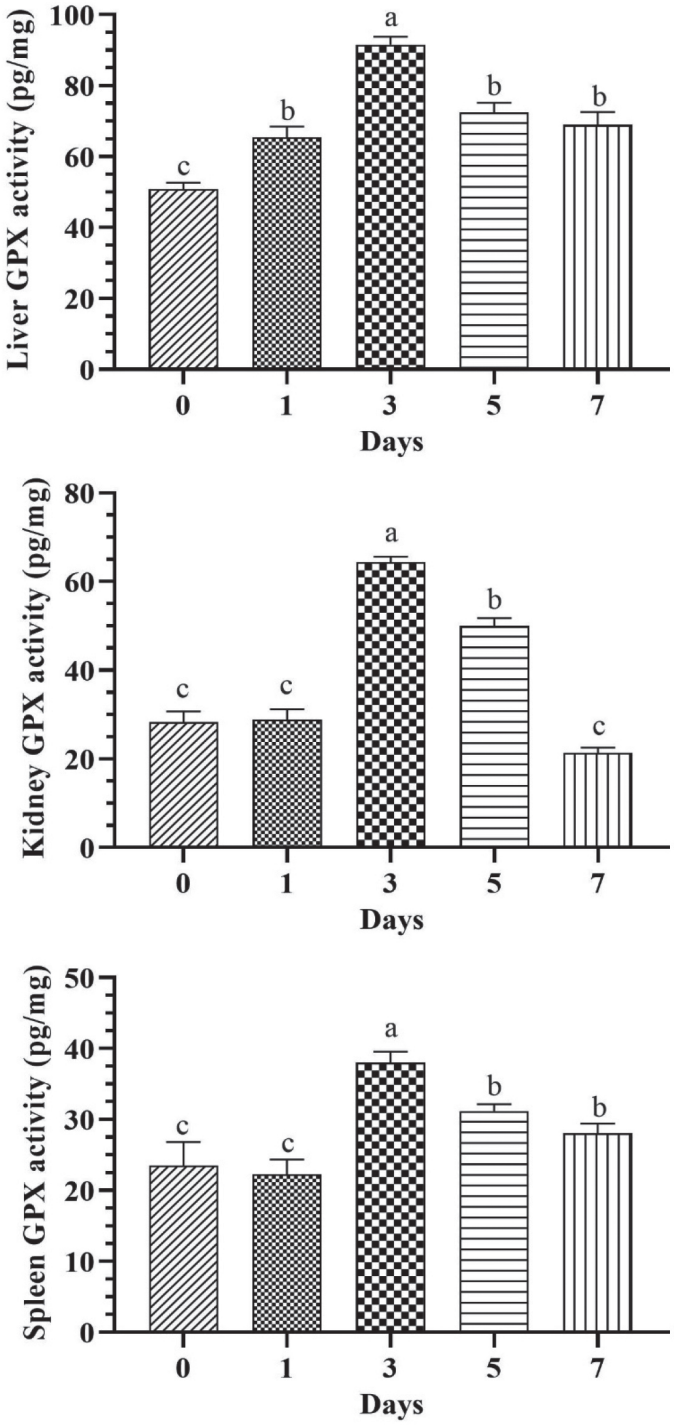

Fig. 2. The activity of glutathione peroxidase (GPx) in the liver (A), kidney (B), and spleen (C) of *Cyprinus carpio* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

the semilethal dose (LD_{50}) of a pathogenic strain of *A. hydrophila* isolated and identified bacterial by Al-Shammari et al. [29]. The pathological state of the internal organs of *C. carpio* after the experimental challenge is shown in Fig. 8. A- The liver showing necrotic and denatured cells and is congested. B-Hepatic cells showing congestion of blood vessels. C- hepatocytes showing infiltration and necrosis. D- hepatocytes showing degeneration. E- Spleen hyperplasia of white pulp and appears as round or oval nodules with few masses of hemosiderin and melanomacrophages,

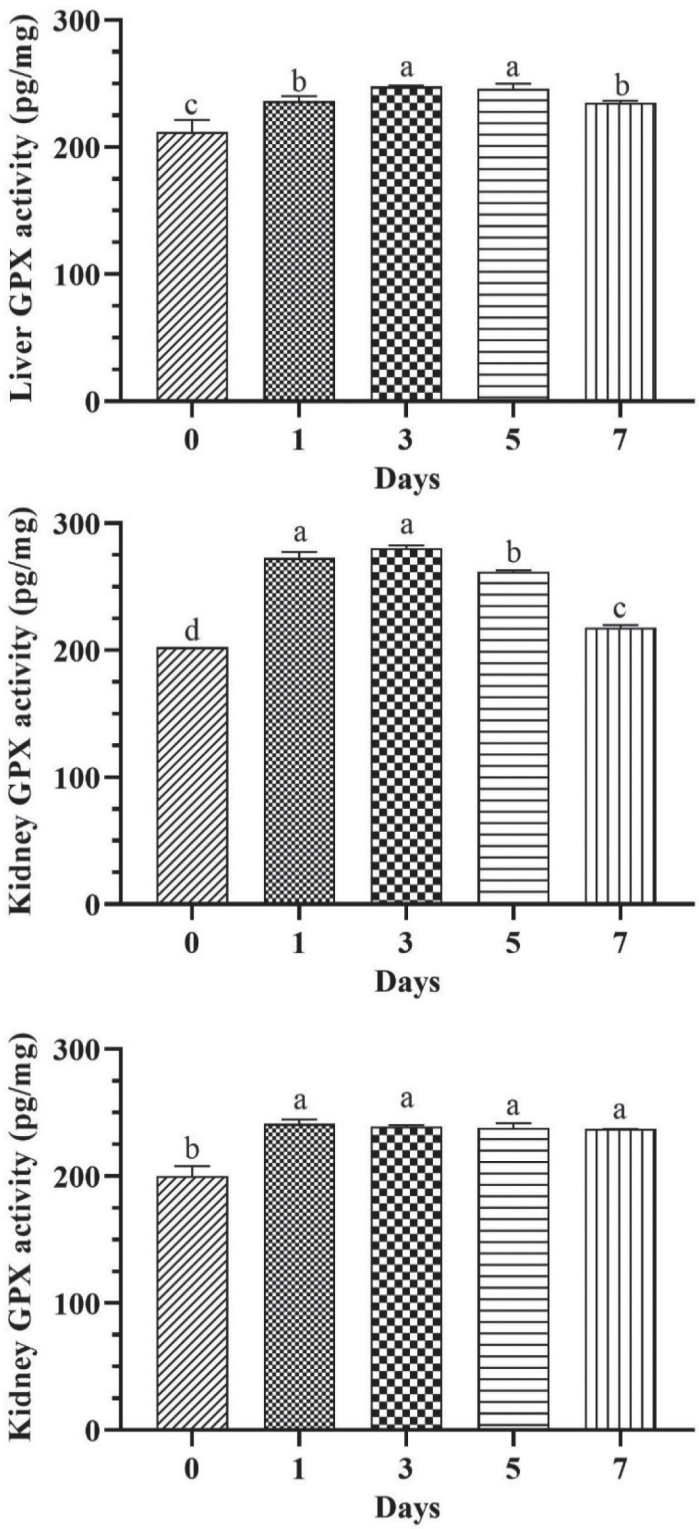

Fig. 3. The activity of glutathione peroxidase (GPx) in the liver (A), kidney (B), and spleen (C) of *Oreochromis niloticus* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

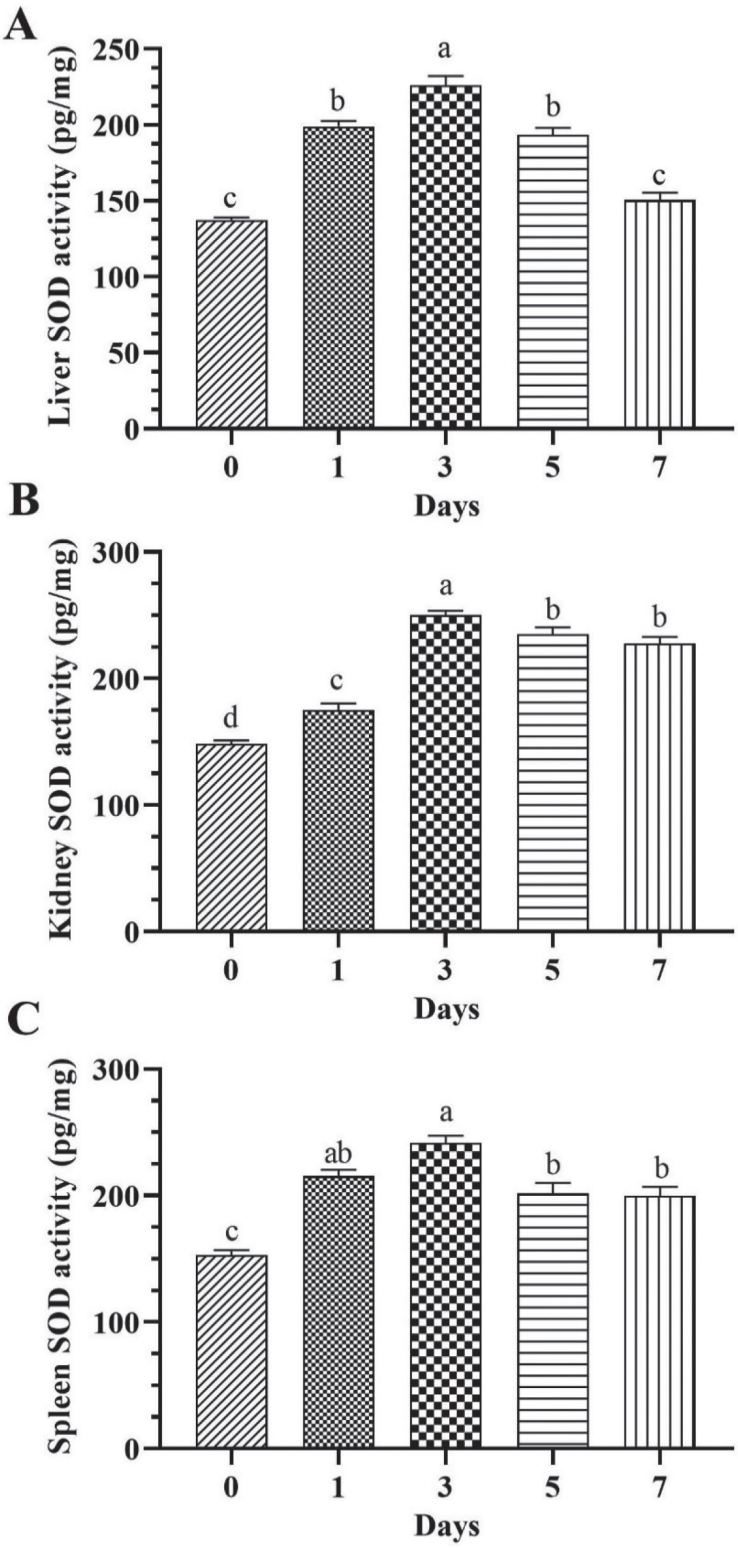

Fig. 4. The activity of superoxide dismutase (SOD) in the liver (A), kidney (B), and spleen (C) of *Cyprinus carpio* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

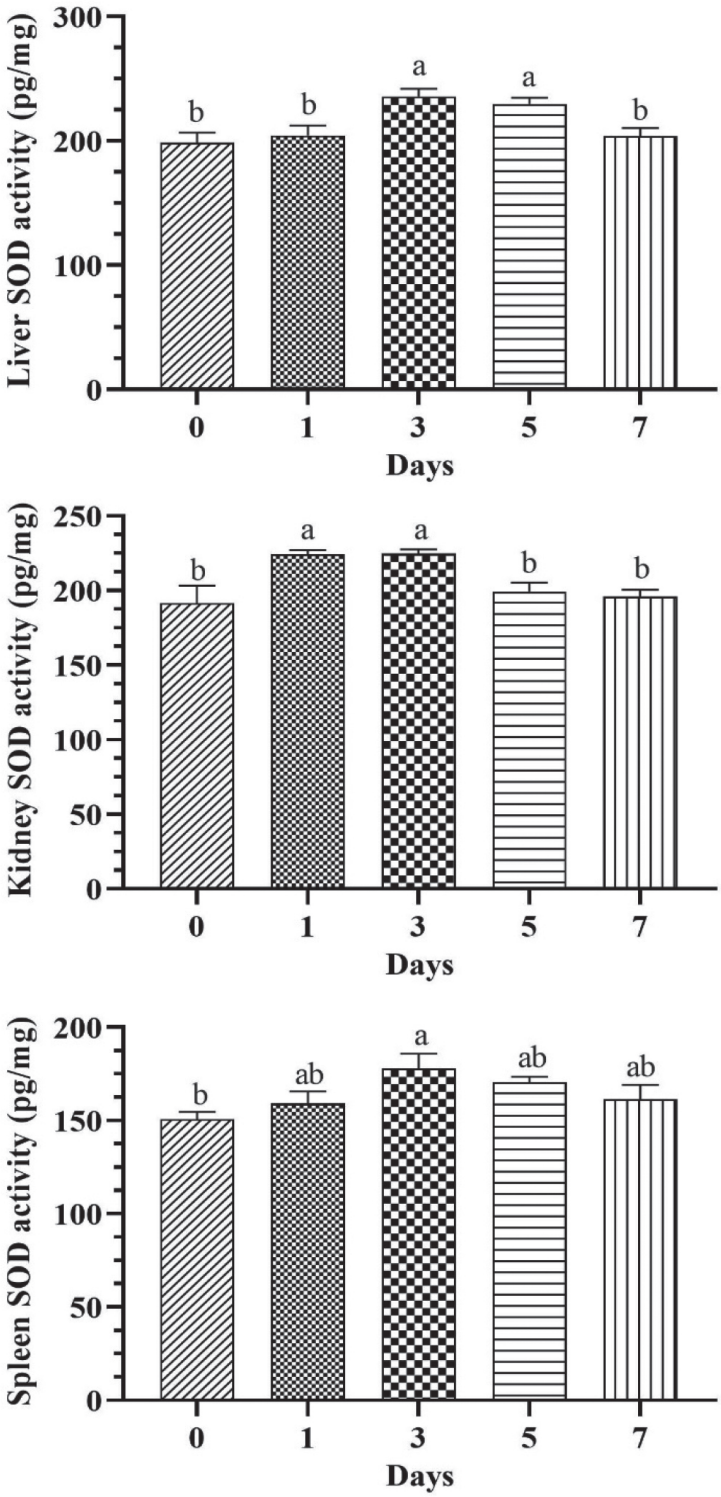

Fig. 5. The activity of superoxide dismutase (SOD) in the liver (A), kidney (B), and spleen (C) of *Oreochromis niloticus* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

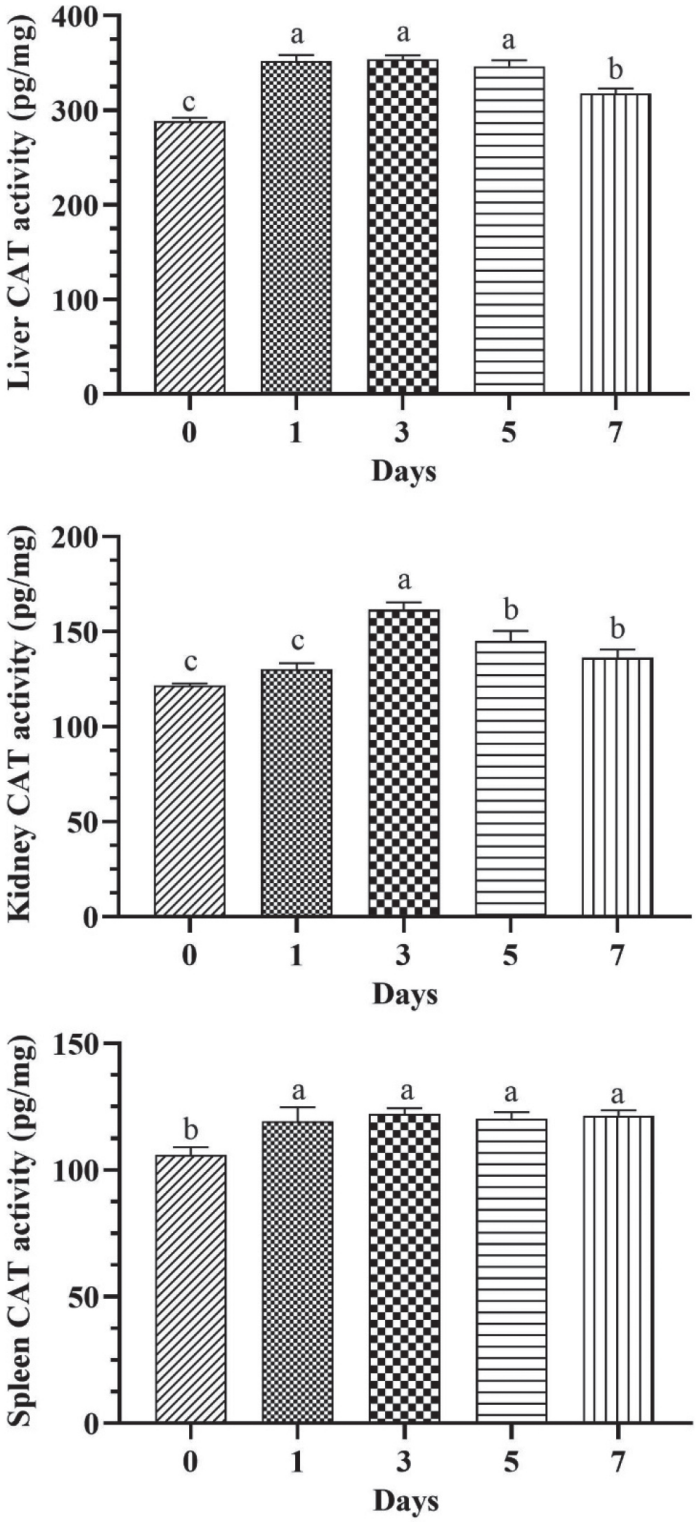

Fig. 6. The activity of catalase (CAT) in the liver (A), kidney (B), and spleen (C) of *Cyprinus carpio* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

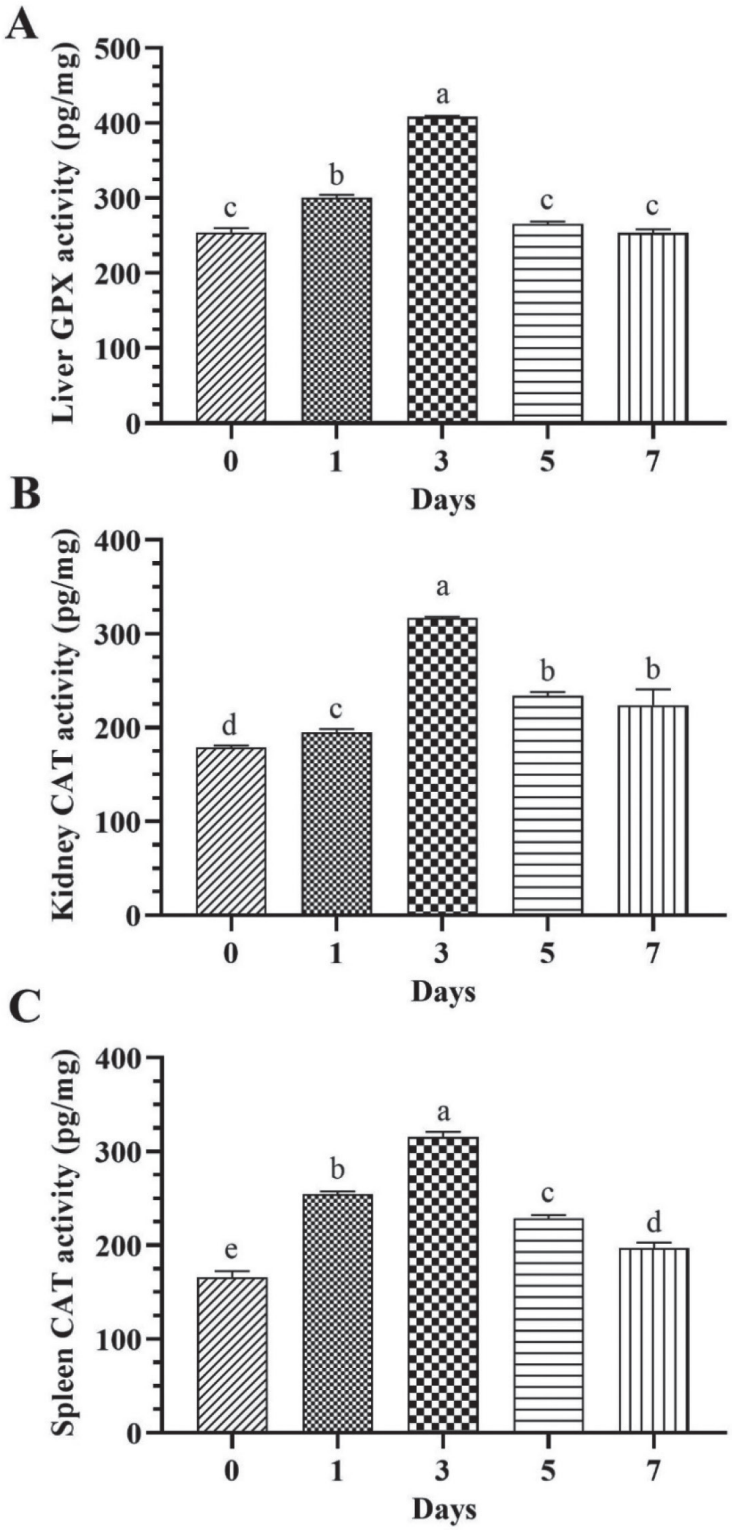

Fig. 7. The activity of catalase (CAT) in the liver (A), kidney (B), and spleen (C) of *Oreochromis niloticus* after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

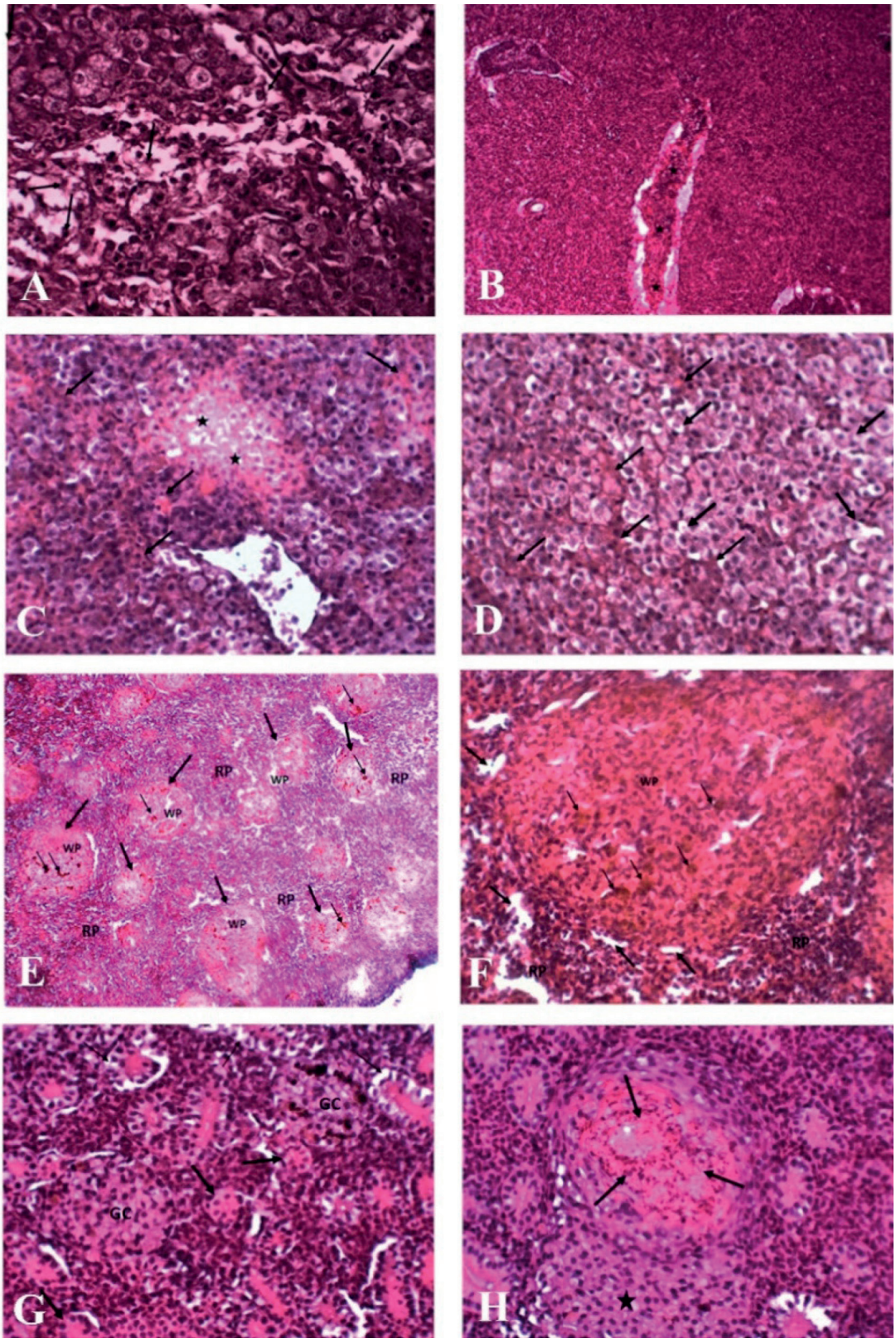

Fig. 8. The histopathological effects of *Aeromonas hydrophila* infection on the liver, kidney, and spleen of *Cyprinus carpio* after seven days of infection. A: Hepatic cells showing denaturation and necrosis; B: Hepatic cells showing congestion of blood vessels; C: Eosinophilic hepatocytes showing infiltration and necrosis; D: Eosinophilic hepatocytes showing degeneration; E: Spleen hyperplasia of white pulp (wp) and appear as round or oval nodules (thick arrows) with few masses of hemosiderin and melanomacrophages (thin arrows), degeneration of red pulp (RP); F: Spleen white pulp (wp) as a nodule with a few masses of melanomacrophages (thin arrows), the red pulp (RP), and thick arrows pointed to a sinusoid; G: Kidney degeneration (thin arrows) of renal tubules and eosinophilic (thick arrows) lining renal tubule and few melanomacrophages (head arrows) with globular cellular masses (GC) in hematopoietic tissues; H: Kidney necrotic tissue (arrows) with large globular cellular mass in hematopoietic tissue (asterisk) X40.

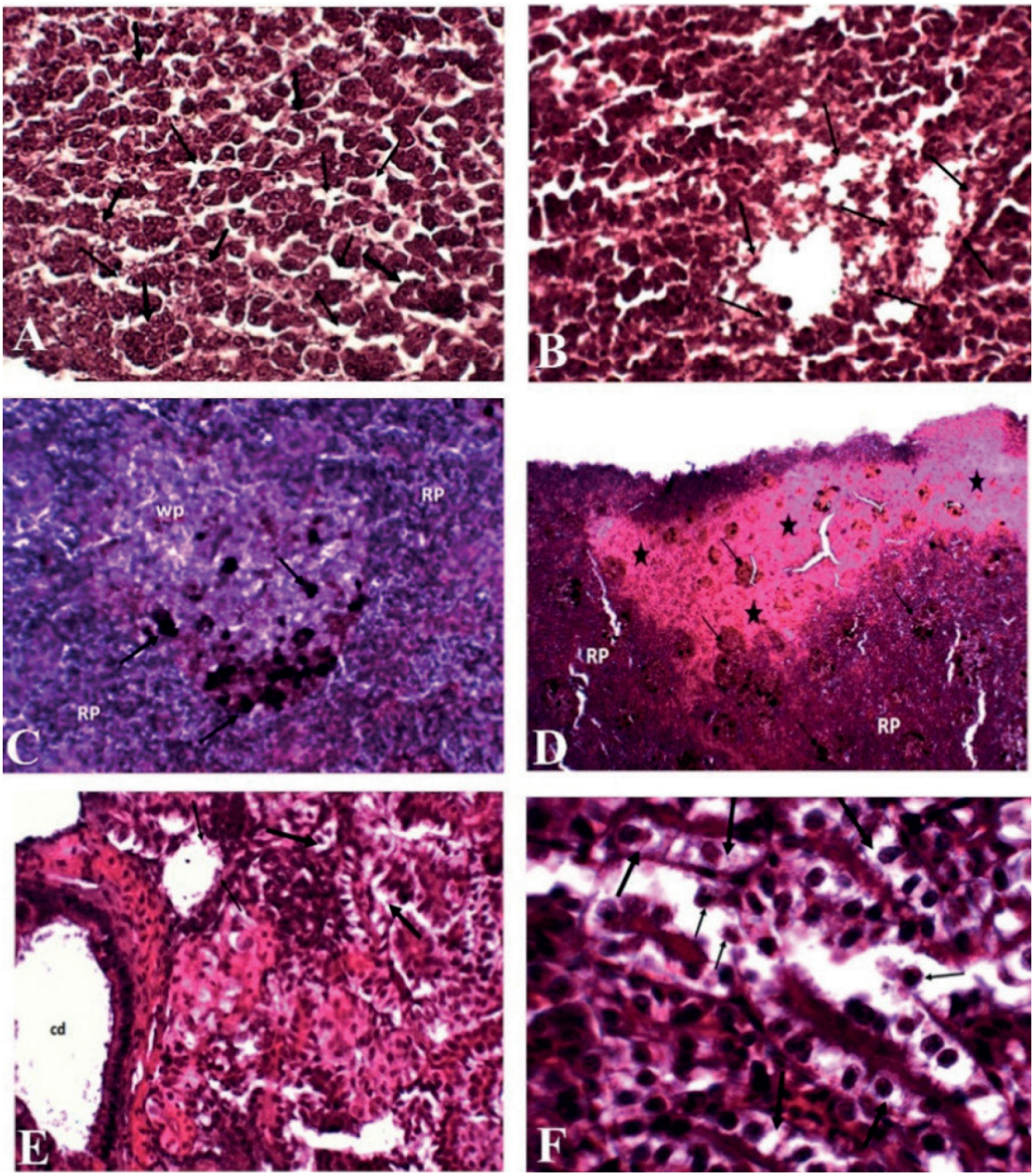

Fig. 9. The histopathological effects of *Aeromonas hydrophila* infection on the liver, kidney, and spleen of *Oreochromis niloticus* after seven days of infection. A: Hepatic cells showing dilation of the sinusoid (thin arrows) and atrophy of hepatocytes (thick arrows); B: Hepatic cells showing massive necrosis of hepatocytes; C: Spleen more masses of melanomacrophages concentrated in white pulp (arrow) X40; D: Spleen hyaline necrosis splenic tissue (asterisks) with more masses of melanomacrophages (arrows) distributed in white and red pulp; E: Kidney necrosis (thin arrows) of renal lining epithelial tubule and degeneration (thick arrows) with eosinophilic other lining epithelial cell, collecting duct (cd); F: Kidney necrosis (thin arrows) of renal lining epithelial tubule and degeneration (thick arrows) X40.

degeneration of red pulp, an example of a fish defense mechanism. Bacteria cause effects on the cytoplasm of hepatocytes containing fats and glycogen associated with normal liver metabolism. Any defect caused by bacteria between the level of synthesis of the substance in parenchymal cells and the rate of release of the systemic circulation can generally cause metabolic damage according to Dougklas et al. [30]. F - Spleen white pulp as a nodule with few masses of melanomacrophages, the red pulp, and sinusoid. G- Kidney revealing degeneration of renal tubules and has eosinophilic lining renal tubules and few melanomacrophages with globular cellular masses in hematopoietic tissues [31].

The pathological states in the internal organs of *Oreochromis niloticus* are shown in Fig. 9 A. The liver cells are dilated of the sinusoid and have atrophy of the hepatocytes

[32]. B-Hepatic cells show massive necrosis of hepatocytes. Spleen shows: C- masses of melanomacrophages concentrated in white pulp and D- hyaline necrosis splenic tissue with more masses of melanomacrophages distributed in white and red pulp. E- kidney tissue in organs shows necrosis of the epithelial tubule of the renal lining and degeneration with other eosinophilic lining epithelial cells, collecting duct [31]. F- kidney reveals necrosis of the renal lining and degeneration.

Discussion

Fisheries are an essential part of food production that assures the nation's nutritional security. In addition, it provides a major source of income for a significant portion of the population, especially the fishermen's community [33, 34]. The fish has antibacterial molecules that contribute to eliminating the bacterial infection, and post the resistance of the animal. The immune system has innate and adapted responses against pathogens, which induce hyperactivity of these components and increase the high production of reactive oxygen species (ROS) even as a weapon or waste [35, 36]. In normal conditions, there is a balance between ROS and antioxidant systems, but after infection, this balance is disrupted and induces extra cell damage [37]. Therefore, the current study aims to compare the response of the most common two fish species*, C. carpio* and *O. niloticus*, which are widespread in culture in freshwater fish. The stress caused by the *A. hydrophila* post-experimental infection negatively influenced the antioxidant defense mechanism and resulted in histopathological changes.

Motile Aeromonas is widespread in the aquatic environment, which induces serious pathological symptoms in fish and causes numerous deaths. This could be returned to the bacterial toxins products of Aeromonas, such as dermo-necrotizing factors, extracellular hemolysin, and gelatinase, which work on hemolysis and hemolytic changes in the form of intravascular blood [38].

Antioxidant enzymes, including SOD, GPx, and CAT, are the most widely used sensitive indicators for assessing oxidative stress [39], especially after *Aeromonas* spp. infections [24, 40]. The SOD is the primary enzyme that transforms superoxide radicals into hydrogen peroxide [41]. Antioxidant enzymes are excreted from the cells of fish, which actively work to lessen reactive oxygen species (ROS), while the accumulation of ROS and the elevation of lipid peroxidation usually occur in response to bacterial stress [42, 43]. In this paper, we report the appearance of oxidative stress in the liver, kidney, and spleen following bacterial infection.

Furthermore, the activity of antioxidant enzymes in general, increased in the early stages after injury, especially on the 3rd day after the experimental injury, and generally decreased on the seventh day after injury to reach a significant value ($P < 0.05$). It is assumed that the pathogenic effect post-infection by *A. hydrophila* resulted in a production of ROS in the liver, kidney, and spleen in both fish. As depicted in Fig. 3. A, B, & C, there was an increase in GPx on the 3rd day in all tissues because of the rapid stress response induced by the bacterium, and then gradually declined on the 7th day. It seems that the fish showed a transient stress response to stress, as reported by Yu et al. [44]. These outcomes were concurrent to those reported by Parida and Sahoo [10] who clarified that after 48 h of *A. hydrophila* infection, there was an elevation of liver GPx levels in *Labeo rohita*. In *C. Carpio*, it is opined that there was a formation of lipid peroxidation which was considered the first step following *A. hydrophila* infection, then; the cell membrane was damaged resulting in stimulating antioxidants in the liver, kidneys, and spleen. Meanwhile, *O. niloticus* showed a clear increase in the efficacy of GPx activity in the liver, kidneys, and spleen compared to *C. carpio*. This result could be attributed to increased GPx secretion in the early stage to compensate for cell damage as a result of exposure to infection. Furthermore, Almarri et al. [45] dominated the increased levels of antioxidants in *O. niloticus* in response to infection to displacing free radicals through their ability to activate transcription signals for antioxidants by activating the nuclear factor erythroid pathway of the protein complex. Additionally, Mahmoud et al. [46] supported our findings and found that *O. niloticus* is characterized by more tolerance to maintain its antioxidant levels despite stress and shows resistance and higher antioxidant activity when exposed to stress, compared to *C. carpio*.

Considering another antioxidant indicator, SOD activity is considered the first defense antioxidant agent and is necessary for the body to defend against reactive oxygen species by reducing the superoxide radical of hydrogen peroxide [47]. In *C. Carpio, the* value of SOD showed a smaller increase in the kidney and liver on the 3rd day following bacterial challenge compared to *O. niloticus*. The variance between the two species in the resistance against bacterium depends on the release of antioxidants at a considerable concentration, as reported by Trenzado et al. [41]. A recent study documented that *C. carpio* is more vulnerable to infection and symptoms compared to *O. niloticus* [48].

Catalase (CAT) is one of the essential enzymes for the detoxification of ROS in all organisms and it can split the level of H_2O_2 to harmless H_2O and O_2 and liver tissue may be included in energy expenditure provoked by oxidative stress [44, 49]. Tilapia has a large number of host defense peptides that work to break down bacterial cell walls [50]. In this paper, we reveal an enhanced antioxidant response in *O. niloticus* compared to *C. carpio* indicated by increased elevation in CAT level. Furthermore, the level of antioxidant capacity increased significantly on the 3rd day for all fish and clearly decreased on the day in the infected group. Wang [51] obtained similar findings after a bacterial challenge of *Aeromonas veronii* in sea bass. This could be rediscovered by the ability of *O. niloticus* to reduce cellular oxidative stress. In addition, scavengers exhibit reactive oxygen species within cells, lowering the level of cellular stress responses as clarified by Zhang et al. [52] and Sies and Jones [53]. Furthermore, a previous report by Tattiyapong et al. [54] demonstrated that *O. niloticus* has

increased antibody responses against bacterial infection after one-time exposure.

In the present perspective, *C. carpio* and *O. niloticus* were used as *in vivo* models to assess the evolution of *A. hydrophila*. It is known that this bacterium causes oxidative stress, which has a role in autophagy in the presence of stress [55]. The occurrence of the autophagy process is associated with physiological changes in the cell as a result of the presence of reactive oxygen species [56]. Interestingly, innate immunity and autophagy are two processes with a common course and common pathological signs [57, 58].

The challenge test is a verifying tool for assessing the clinical manifestations and histopathological changes [51]. Fish challenged with *A. hydrophila* showed pronounced, signs of illness including, excess mucous secretion, and hemorrhages, A Similar study by Chen, et al. [3] was conducted on *C. carpio* and *O. niloticus* and revealed signs of infection by *A. hydrophila* including enteritis, congested liver, enlarged kidney and spleen, and visceral hemorrhage. The pathogenicity of *A. hydrophila* could refer to the production of cytotoxic, spore-forming enterotoxin, which is a dangerous virulence factor [59]. Likely, our results were similar to those of Mahboub, et al. [40] who showed a pronounced enhancement in *C. carpio* resistance against *A. hydrophila*. In the present results, we report major histopathological changes in the liver and kidneys of *C. carpio* and *O. niloticus* following infection by *A. hydrophila*. This could be attributed to the occurrence of oxidative damage in the hepato-renal organs, which, in turn, resulted in alterations in tissue architectures. Concurrent with another study, Salam et al. [60] revealed that *A. hydrophila* infection resulted in histopathological changes that were prominent in the liver, spleen, and kidney of both *O. niloticus* and *C. garipinus* including perivascular melanophore aggregation and vacuolar degeneration. Numerous studies of fish species showed that the liver and kidney are the only organs in which oxidative stress occurs [61]. The decrease in the effectiveness of antioxidants in some organs is a result of various stresses, such as the liver being damaged as a result of resistance and its role in osmosis, the rest of the organs are injured, and this is called organ suffering, the rate of glomerular filtration decreases and loses its function, the permeability of blood vessels increases [62]. Oruç and Usta [63] proved that *C. carpio* is more sensitive than *O. niloticus* to the effectiveness of antioxidant enzymes.

Based on the results of the study, *O. niloticus* is distinguished by high antioxidants and high resistance to *A. hydrophila* infection, indicated by less oxidative stress and inflammation in the tissues compared to *C. carpio*.

Conclusion

The current study reports on the hazardous impact of the zoonotic pathogenic *A. hydrophila* on altering antioxidant status and inducing histopathological changes in the liver, kidneys, and spleen of *C. carpio* and *O. niloticus*. The results showed that *O. niloticus* has a higher tolerance than *C. carpio* in response to *A. hydrophila*. The activities of GPX, SOD, and CAT showed high alterations in response to the bacterial infection. The histopathological findings were prominent in the liver, kidneys, and spleen of both *C. carpio* and *O. niloticus*, particularly, hepatic tissues showed vacuolar degeneration and hemorrhagic septicemia and mainly targets the liver and tissue damage in response to *A. hydrophila.* Future researches are recommended for evaluating the adverse impact of *A. hydrophila* on other fish species.

Author Contributions

Conceptualization: N.A.H.A., A.B.A., K.S.A., and H.H.M. Methodology: N.A.H.A., A.B.A., K.S.A., and H.H.M. Software and data curation: N.A.H.A., A.B.A., K.S.A., M.S., A.T.M., S.A.A., A.M.A., H.A.H., G.F.G, H.H.M. Writing-Original draft preparation: N.A.H.A., A.B.A., K.S.A., and H.H.M. Writing- Reviewing and Editing: H.H.M.

Ethical Approval

All experimental procedures with live fish were conducted at the Department of Biological Development in Shatt Al-Arab and NW Arabian Gulf, University of Basra, Basra, Iraq, following ARRIVE Guidelines and in compliance with the ethical guidelines approved by the National Institutes of Health for Use and Treatment of Laboratory Animals at the Marine Science Center, University of Basra, Basra, Iraq.

Informed Consent Statement

Not applicable.

Data Availability

Data are available upon reasonable request.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. ZHAO Y., ALEXANDER J. Aeromonas hydrophilia infection in an immunocompromised host. Cureus, **13**, (12), **2021**.

2. YANG B., SONG H., AN D., ZHANG D., RAZA S.H.A., WANG G., SHAN X., QIAN A., KANG Y., WANG C. Functional analysis of preA in *Aeromonas veronii* TH0426 reveals a key role in the regulation of virulence and resistance to oxidative stress. International Journal of Molecular Sciences, **21** (1), 98, **2019**.
3. CHEN J., LIU N., ZHANG H., ZHAO Y., CAO X. The effects of *Aeromonas hydrophila* infection on oxidative stress, nonspecific immunity, autophagy, and apoptosis in the common carp. Developmental & Comparative Immunology, **105**, 103587, **2020**.
4. RAHMAN A.N.A., MANSOUR D.A., ABD EL-RAHMAN G.I., ELSEDDAWY N.M., ZAGLOOL A.W., KHAMIS T., MAHMOUD S.F., MAHBOUB H.H. Imidacloprid toxicity in *Clarias gariepinus*: Protective role of dietary *Hyphaene thebaica* against biochemical and histopathological disruption, oxidative stress, immune genes expressions, and *Aeromonas sobria* infection. Aquaculture, **555**, 738170, **2022**.
5. RAHMAN A.N.A., VAN DOAN H., ELSHESHTAWY H.M., DAWOOD A., SALEM S.M., SHERAIBA N.I., MASOUD S.R., ABDELNAEIM N.S., KHAMIS T., ALKAFAFY M. Dietary *Salvia officinalis* leaves enhances antioxidant-immune-capacity, resistance to *Aeromonas sobria* challenge, and growth of *Cyprinus carpio*. Fish & Shellfish Immunology, **127**, 340, **2022**.
6. IRSHATH A.A., RAJAN A.P., VIMAL S., PRABHAKARAN V.-S., GANESAN R. Bacterial pathogenesis in various fish diseases: Recent advances and specific challenges in vaccine development. Vaccines, **11** (2), 470, **2023**.
7. BALDISSERA M.D., SOUZA C.F., PARMEGGIANI B., LEIPNITZ G., VERDI C.M., SANTOS R.V., STEFANI L.M., BALDISSEROTTO B. The disturbance of antioxidant/oxidant balance in fish experimentally infected by *Aeromonas caviae*: relationship with disease pathophysiology. Microbial Pathogenesis, **122**, 53, **2018**.
8. BANDEIRA G., JUNIOR B. Fish infections associated with the genus Aeromonas: a review of the effects on oxidative status. Journal of Applied Microbiology, **131** (3), **2020**.
9. GENG R., JIA Y., CHI M., WANG Z., LIU H., WANG W. RNase1 alleviates the *Aeromonas hydrophila*-induced oxidative stress in blunt snout bream. Developmental & Comparative Immunology, **91**, 8, **2019**.
10. PARIDA S., SAHOO P.K. Antioxidant Defence in Labeo rohita to Biotic and Abiotic Stress: Insight from mRNA Expression, Molecular Characterization and Recombinant Protein-Based ELISA of Catalase, Glutathione Peroxidase, CuZn Superoxide Dismutase, and Glutathione S-Transferase. Antioxidants, **13** (1), 18, **2023**.
11. GAUTHIER D.T. Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections. The Veterinary Journal, **203** (1), 27, **2015**.
12. ZHONG Y., QI W., XU W., ZHAO L., XIAO B., YAN Q., HUANG L. Insights into mesophilic virulence, antibiotic resistant and human pathogenicity: A genomics study on the *Aeromonas salmonicida* SRW-OG1 newly isolated from the Asian fish *Epinephelus coioides*. Aquaculture, **539**, 736630, **2021**.
13. SEMWAL A., KUMAR A., KUMAR N. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. Heliyon, **9** (3), **2023**.
14. BALDISSERA M.D., SOUZA C.F., DESCOVI S.N., VERDI C.M., SANTOS R.C., DA SILVA A.S., BALDISSEROTTO B. Impairment of branchial energy transfer pathways in disease pathogenesis of *Providencia rettgeri* infection in juvenile Nile tilapia (*Oreochromis niloticus*): Remarkable involvement of creatine kinase activity. Aquaculture, **502**, 365, **2019**.
15. ALZAHRANI O.M., ELUMALAI P., NADA H.S., AHMED S.A., ZAGLOOL A.W., SHAWKY S.M., ALKAFAFY M., MAHBOUB H.H. *Pseudomonas putida*: sensitivity to various antibiotics, genetic diversity, virulence, and role of formic acid to modulate the immune-antioxidant status of the challenged nile tilapia compared to carvacrol oil. Fishes, **8** (1), 6, **2022**.
16. KAKAKHEL M.A., BIBI N., MAHBOUB H.H., WU F., SAJJAD W., DIN S. Z.U., HEFNY A.A., WANG W. Influence of biosynthesized nanoparticles exposure on mortality, residual deposition, and intestinal bacterial dysbiosis in *Cyprinus carpio*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, **263**, 109473, **2023**.
17. RASHIDIAN G., MAHBOUB H.H., HOSEINIFAR S.H., GHAFARIFARSANI H., ZARE M., PUNYATONG M., VAN DOAN H. *Allium hirtifolium* protects *Cyprinus carpio* against the detrimental responses mediated by foodborne zinc oxide nanoparticle. Aquaculture, **555**, 738252, **2022**.
18. RASHIDIAN G., MAHBOUB H.H., FAHIM A., HEFNY A.A., PROKIĆ M.D., RAINIS S., BOLDAJI J.T., FAGGIO C. Mooseer (*Allium hirtifolium*) boosts growth, general health status, and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Streptococcus iniae* infection. Fish & Shellfish Immunology, **120**, 360, **2022**.
19. MUKHERJEE S., SUNDAR A., NILADRI B., BHUNIA S., RAY M., RAY S. Immunomodulatory effects of temperature and pH of water in an Indian freshwater sponge. Journal of Thermal Biology, **59**, 1, **2016**.
20. PEI J., PAN X., WEI G., HUA Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. Frontiers in Pharmacology, **14**, 1147414, **2023**.
21. KAKAKHEL M.A., ZAHEER UD DIN S., WANG W. Evaluation of the antibacterial influence of silver nanoparticles against fish pathogenic bacterial isolates and their toxicity against common carp fish. Microscopy Research and Technique, **85** (4), 1282, **2022**.
22. WANG Y., LIU Z., LI Z., SHI H., KANG Y., WANG J., HUANG J., JIANG L. Effects of heat stress on respiratory burst, oxidative damage and SERPINH1 (HSP47) mRNA expression in rainbow trout *Oncorhynchus mykiss*. Fish Physiology and Biochemistry, **42**, 701, **2016**.
23. AL-SHAMMARI N. Opportunistic Bacteria Isolated from *Trypauchen vagina* Fish that Infected with Protozoan from Iraq Marine Water. Scientific Journal of King Faisal University: Basic and Applied sciences, **22** (1), 83, **2021**.
24. MAHBOUB H.H., FAGGIO C., HENDAM B.M., AL-GHARIB S.A., ALKAFAFY M., HASHEM M.A., MAHMOUD Y.K., KHAMIS T., ABDEL-GHANY H.M., MASOUD S.R. Immune-antioxidant trait, *Aeromonas veronii* resistance, growth, intestinal architecture, and splenic cytokines expression of *Cyprinus carpio* fed *Prunus armeniaca* kernel-enriched diets. Fish & Shellfish Immunology, **124**, 182, **2022**.
25. ABDELSALAM M., EWISS M.Z., KHALEFA H.S., MAHMOUD M.A., ELGENDY M.Y., ABDEL-MONEAM D.A. Coinfections of *Aeromonas spp.*, *Enterococcus faecalis*, and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. Microbial Pathogenesis, **160**, 105213, **2021**.

26. YIN X., MU L., FU S., WU L., HAN K., WU H., BIAN X., WEI X., GUO Z., WANG A. Expression and characterization of Nile tilapia (*Oreochromis niloticus*) secretory and membrane-bound IgM in response to bacterial infection. Aquaculture, **508**, 214, **2019**.
27. AEBI H. Catalase *in vitro,* **1984**.
28. ROBERTS R.J. Fish Pathology, Wiley, **2012**.
29. AL-SHAMMARI N.A., AL-NIAEEM K.S., AL-HAWASH A. Molecular identification of some zoonotic bacteria isolated from fishes *Cyprinus carpio* L. and *Oreochromis niloticus* (L.). J. quatic Biology & Fisheries, **27** (4), 1047 **2023**.
30. DOUGKLAS W., ALEXEY L., UUN Y., ANDAYANI H. Changes in the histopahology of the gilles and Liver of Tilapia (*O. niloticus*)infected with *Aeromonas hydrophila*. J.RJOAS. **1**, 109, **2021**.
31. FANTA E., RIOS S., ROMAO S., VIANNA A., FREIBERGER S. Histopathology of the fish Corydoras pleatus contaminated with sublethal levels of organophosphorous in waterand food. Ecotoxicology and Environmental Safety, **54**, 119, **2003**.
32. SANTOS A., VALDÉS C., GIRÁLDEZ F.J., LÓPEZ S., FRANCE J., FRUTOS J., FERNÁNDEZ M., ANDRÉS S. Feed efficiency and the liver proteome of fattening lambs are modified by feed restriction during the suckling period. Journal of Comparative Pathology, **180**, 55, **2016**.
33. KUMAR G., SHARMA J., GOSWAMI R.K., SHRIVASTAV A.K., TOCHER D.R., KUMAR N., CHAKRABARTI R. Freshwater macrophytes: A potential source of minerals and fatty acids for fish, poultry, and livestock. Frontiers in Nutrition, **9**, 869425, **2022**.
34. JANGPANGI K., KHATI A., CHAUHAN R., KUMAR N. Assessment of the Haematological and Biochemical Effects of Himalayan Herb *Urtica dioica* Leaves Diets Fed Amur Carp, *Cyprinus carpio* haematopterus. Turkish Journal of Fisheries and Aquatic Sciences, **23** (12), **2023**.
35. MANSOUR A.T., ASHOUR M., RUIZ C.E., KUMAR N., ESTEBAN M.A. Physiological performance of aquatic animals under farming-induced stress conditions. Frontiers in Physiology, **14**, 1136611, **2023**.
36. EL-HOUSEINY W., ANTER R.G., ARISHA A.H., MANSOUR A.T., SAFHI F.A., ALWUTAYD K.M., ELSHOPAKEY G.E., M ABD EL-HAKIM Y., MOHAMED E.M. Growth Retardation, Oxidative Stress, Immunosuppression, and Inflammatory Disturbances Induced by Herbicide Exposure of Catfish, *Clarias gariepinus*, and the Alleviation Effect of Dietary Wormwood, *Artemisia cina*. Fishes, **8** (6), 297, **2023**.
37. ARUN P., WILLIAM B., RITTASE D., WILDER M., WANG Y., IRENE D., JOSEPH G., LONG B. Defective methionine metabolism in the brain after repeated blast exposures might contribute to increased oxidative stress. Journal of Neurochemistry International, **112**, 234, **2018**.
38. YARDIMCI B., AYDIN Y. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). Ankara Üniversitesi Veteriner Fakültesi Dergisi, **58**, 47, **2011**.
39. ELABD H., MAHBOUB H.H., SALEM S.M., ABDELWAHAB A.M., ALWUTAYD K.M., SHAALAN M., ISMAIL S.H., ABDELFATTAH A.M., KHALID A., MANSOUR A.T. Nano-Curcumin/Chitosan Modulates Growth, Biochemical, Immune, and Antioxidative Profiles, and the Expression of Related Genes in Nile tilapia, *Oreochromis niloticus*. Fishes, **8** (7), 333, **2023**.
40. MAHBOUB H.H., NADA H.S., ABDEL-GHANY H.M., GHANEM R., AHMED ISMAIL T., ABDEL RAHMAN A.N. Detection, diagnosis, Koch's postulate, hepatorenal and antioxidant indicators for some systemic pathogenic fungi invading the liver and kidneys of African catfish (*Clarias gariepinus*) in Egypt with a histopathological approach. Aquaculture Research, **53** (7), 2670, **2022**.
41. TRENZADO C., HIDALGO M.C., GARCÍA-GALLEGO M., MORALES A.E., FURNÉ M., DOMEZAIN A., DOMEZAIN J., SANZ A. Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss*. A comparative study. Aquaculture, **254** (1-4), 758, **2006**.
42. DOGAN D., CAN C., KOCYIGIT A., DIKILITAS M., TASKIN A., BILINC H. Dimethoate-induced oxidative stress and DNA damage in *Oncorhynchus mykiss*. Chemosphere, **84** (1), 39, **2011**.
43. MANSOUR A.T., DIAB A.M., KHALIL R.H., ELDESSOUKI E.A., EL-SABBAGH N., ELSAMANNOUDY S.I., YOUNIS N.A. Physiological and immunological responses of Nile tilapia fed dietary supplementation of sweet basil ethanolic and aqueous extracts. Frontiers in Marine Science, **9**, 1064455, **2023**.
44. YU H., DENG W., ZHANG D., GAO Y., YANG Z., SHI X., SUN J., ZHOU J., JI H. Antioxidant defenses of *Onychostoma macrolepis* in response to thermal stress: insight from mRNA expression and activity of superoxide dismutase and catalase. Fish & Shellfish Immunology, **66**, 50, **2017**.
45. ALMARRI S.H., KHALIL A.A., MANSOUR A.T., EL-HOUSEINY W. Antioxidant, Immunostimulant, and Growth-Promoting Effects of Dietary *Annona squamosa* Leaf Extract on Nile Tilapia, *Oreochromis niloticus*, and Its Tolerance to Thermal Stress and *Aeromonas sobria* Infection. Animals, **13** (4), 746, **2023**.
46. MAHMOUD A.M., NOR-ELDIN E.K., ELSAYED H.M. The possible protective effect of Omega 3 fatty acids against Bisphenol A induced disruption of pituitary-testicular axis in albino rat. Biohemical, Histological and Immunohistochemical study. Egyptian Journal of Histology, **42** (1), 215, **2019**.
47. ABD EL-HAKIM Y.M., EL-HOUSEINY W., ABD ELHAKEEM E.-M., EBRAHEIM L.L., MOUSTAFA A.A., MOHAMED A.A.R. Melamine and curcumin enriched diets modulate the haemato-immune response, growth performance, oxidative stress, disease resistance, and cytokine production in *Oreochromis niloticus*. Aquatic Toxicology, **220**, 105406, **2020**.
48. ZHANG S., CHENG Y., LINHARTOVÁ Z., RODINOVÁ V., SHAZADA N. E., WU Q., LINHART O. In vivo and in vitro aging of common carp *Cyprinus carpio* sperm after multiple hormonal application and stripping of males. Fish Physiology and Biochemistry, **48** (5), 1235, **2022**.
49. SHARMA J., KUMAR N., MITTAL P., CHAKRABARTI R. Evaluation of UV–B protective properties of leaves and seeds of *Achyranthes aspera* in Asian catfish *Clarias batrachus* (Linn.). Photochemical & Photobiological Sciences, **21** (8), 1341, **2022**.
50. ELLIS A. Overcoming destructive beliefs, feelings, and behaviors: New directions for rational emotive behavior therapy. Prometheus Books, Amherst, New York, **2010**.
51. WANG B., MAO C., FENG J., LI Y., HU J., JIANG B., GU Q., SU Y. A first report of *Aeromonas veronii* infection of the sea bass, *Lateolabrax maculatus* in China. Frontiers in Veterinary Science, **7**, 600587, **2021**.
52. ZHANG L., WANG C., LIU H., FU P. The important role of phagocytosis and interleukins for nile tilapia (*Oreochromis niloticus*) to defense infection of *Aeromonas*

hydrophila based on transcriptome analysis. Fish & Shellfish Immunology, **92**, 54, **2019**.
53. SIES H., JONES D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nature Reviews Molecular Cell Biology, **21** (7), 363, **2020**.
54. TATTIYAPONG P., DECHAVICHITLEAD W., WALTZEK T.B., SURACHETPONG W. Tilapia develop protective immunity including a humoral response following exposure to tilapia lake virus. Fish & Shellfish Immunology, **106**, 666, **2020**.
55. MATSUI Y., TAKAGI H., QU X., ABDELLATIF M., SAKODA H., ASANO T., LEVINE B., SADOSHIMA J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin in mediating autophagy. National Institutes of Health, **6**, 914, **2007**.
56. LEE I.-M., SHIROMA E.J., LOBELO F., PUSKA P., BLAIR S.N., KATZMARZYK P.T. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. The Lancet, **380** (9838), 219, **2012**.
57. XU W., ZHANG G., LI X., ZOU S., LI P., HU Z., LI J. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Research, **41** (19), 4526, **2007**.
58. KUMAR N., SHARMA J., MITTAL P., CHAKRABARTI R. Effect of leaves and seeds of *Achyranthes aspera* as feed supplements on the immunological and stress parameters and related gene expressions of Asian catfish (*Clarias batrachus*). Veterinary Research Communications, **47** (1), 99, **2023**.
59. ABDELLA B., ABOZAHRA N.A., SHOKRAK N.M., MOHAMED R.A., EL-HELOW E.R. Whole spectrum of *Aeromonas hydrophila* virulence determinants and the identification of novel SNPs using comparative pathogenomics. Scientific Reports, **13** (1), 7712, **2023**.
60. SALAM M.A., ISLAM M.A., PAUL S.I., RAHMAN M.M., RAHMAN M.L., ISLAM F., RAHMAN A., SHAHA D.C., ALAM M.S., ISLAM T. Gut probiotic bacteria of *Barbonymus gonionotus* improve growth, hematological parameters and reproductive performances of the host. Scientific Reports, **11** (1), 10692, **2021**.
61. SATTIN G., BAKIU R., TOLOMEO A., CARRARO A., COPPOLA D., FERRO D., PATARNELLO T., SANTOVITO G. Characterization and expression of a new cytoplasmic glutathione peroxidase 1 gene in the Antarctic fish *Trematomus bernacchii*. Hydrobiologia, **761**, 363, **2015**.
62. LI K., LI R., ZOU P., LI L., WANG H., KONG D., ZHENG G., LI L.-L. Glycopeptide-nanotransforrs eyedrops with enhanced permeability and retention for preventing fundus neovascularization. Biomaterials, **281**, 121361, **2022**.
63. ORUÇ E.Ö., USTA D. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. Environmental Toxicology and Pharmacology, **23** (1), 48, **2007**.