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Comparative study of mortalities, clinical manifestations, antioxidant-associated genes, and histopathological degeneration upon experiment infection by *Aeromonas hydrophila* in *Cyprinus carpio* and *Oreochromis niloticus*

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ABSTRACT

Background: *Aeromonas hydrophila* is a pathogenic bacterial infection threatening the aquaculture industry.

Aim: The current work was performed to scrutinize the impacts of *A. hydrophila* infection on the occurrence of mortalities, altering the clinical picture of fish, antioxidants-associated gene expression, and histopathological architecture of the *Cyprinus carpio* and *Oreochromis niloticus*.

Methods: Juvenile fishes including *C. carpio*, $n = 60$, and *O. niloticus*, $n = 60$ were haphazardly alienated into the control group (uninfected) and infected group with 100 µl of *A. hydrophila*. Mortalities and clinical signs were recorded during the experiment. Samples of liver, kidney, and spleen were collected post-infection for 7 days to monitor the expression of superoxide dismutase, glutathione peroxidase, and catalase genes, plus the assessment of histopathology.

Results: The rate of mortalities was higher in *C. carpio* compared to *O. niloticus*. Additionally, infected *O. niloticus* revealed red eyes and erythema, while *C. carpio* showed exophthalmia and severe skin ulceration with exposure to viscera. The gene expression indicators showed that *A. hydrophila* significantly declined on the 1st day and the 7th day after infection, but significantly increased ($p < 0.05$) on the 3rd day compared to their respective control groups for *C. carpio*. Meanwhile, *O. niloticus* significantly regulated gene expression ($p < 0.05$) in the control groups. The histological picture indicated that the liver is the most affected organ. Moreover, *C. carpio* exhibited more disruption in histological architecture related to *O. niloticus*.

Conclusion: Overall, *A. hydrophila* is extremely virulent and results in higher mortalities, profound clinical manifestations, down-regulation in the gene expression, and histopathological alterations in the hepato-renal and splenic tissues of *C. carpio* and *O. niloticus*. *Cyprinus carpio* was more adversely affected by the infection compared to *O. niloticus*. However, *O. niloticus* revealed higher gene expression, particularly in the spleen, and the genes were more expressed in comparison to *C. carpio*.

Keywords: Gene expression, Histopathology, *Aeromonas hydrophila*, *Cyprinus carpio*, *Oreochromis niloticus*.

Introduction

Cyprinus carpio (common carp) and *Oreochromis niloticus* (Nile tilapia) are among the most economically important aquaculture species globally, with commercial cultivation established in over 100 countries, including natural populations in Iraqi waters. Common carp, renowned for its exceptional adaptability, has achieved a widespread distribution

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across diverse freshwater ecosystems and has become a cornerstone species in global aquaculture production systems (WHO, 2021; Moaheda *et al.*, 2023). However, the escalating challenges of disease resistance and the urgent need to enhance fish immunocompetence and antioxidant defense mechanisms have become critical priorities for sustainable aquaculture development (Hoseinifar *et al.*, 2021; Hendam *et al.*, 2023; Eissa *et al.*, 2024).

The pervasive presence of pathogenic bacteria in aquatic environments induces oxidative stress responses, leading to significant alterations in the histological architecture of vital organs, including the kidney, liver, and gills (Faheem *et al.*, 2016; Rashidian *et al.*, 2022; Alzahrani *et al.*, 2023). Among these pathogens, *Aeromonas* species are particularly notable for their ability to cause severe histopathological disruptions, oxidative damage, and modulation of immune-associated gene expression in both *C. carpio* and *O. niloticus* (Abdel Rahman *et al.*, 2022a,b). In *C. carpio*, *Aeromonas* infection significantly impairs immune-antioxidant functions, reduces growth performance, and alters cytokine expression profiles in splenic tissue (Mahboub *et al.*, 2022a). Bacterial pathogenesis, particularly involving *Aeromonas* species, triggers oxidative stress through increased free radical production and compromised cellular detoxification mechanisms, ultimately resulting in widespread cellular dysfunction (Li *et al.*, 2015). Fish exposed to bacterial infections exhibit significant disruptions in their immune-antioxidant defense systems (Ahmad *et al.*, 2022). Cellular responses to these challenges involve the coordinated activation of immune system components and primary enzymatic antioxidants, including catalase (CAT) and superoxide dismutases (SODs) (Hong *et al.*, 2020).

Oxidative stress induced by bacterial contamination primarily manifests through the impaired capacity of antioxidant systems to neutralize reactive oxygen species (ROS) (Pizzino *et al.*, 2017). This dysfunction alters cellular defense pathways and modulates gene expression profiles (Bayır *et al.*, 2022). Notable changes include the downregulation of key antioxidant enzymes, specifically SOD, glutathione peroxidase (GPx), and CAT (Baldissera *et al.*, 2018). While oxygen is essential for aerobic metabolism in aquatic organisms and plays a critical role in cellular functions, it also serves as a primary electron acceptor in the generation of ROS, encompassing both free-radical and non-radical species (Chowdhury and Saikia, 2020). Elevated stress conditions compromise the fish's antioxidant defense capabilities, rendering cellular components vulnerable to oxidative damage, particularly affecting lipids, proteins, and DNA integrity (Sidorczuk *et al.*, 2009).

Quantitative real-time polymerase chain reaction (QRT-PCR) has emerged as the gold standard for bacterial identification and quantification, offering superior sensitivity, rapid processing times, and an expanded dynamic quantification range. The selection of appropriate reference genes remains crucial, as their stability can vary significantly across species and experimental conditions (Liang *et al*., 2022).

This study represents the first systematic molecular and histopathological investigation in Iraq to examine the pathogenic effects of *Aeromonas* infection through multiple parameters, including mortality rates, clinical manifestations, oxidative stress markers, gene expression profiles, and cellular pathways of antioxidant enzymes in *C. carpio* and *O. niloticus*. Furthermore, this study evaluates the consequent impact on internal tissue architecture and histological alterations, particularly elucidating the genetic pathways underlying these pathological features.

Materials and Methods

Experimental design and bacteria preparation

Healthy fingerlings of *C. carpio* (common carp) and *O. niloticus* (Nile tilapia) (n = 60 per species), with an average body weight of 30–35 g, were collected from the Al-Mashab River in Basra Governorate, Iraq. Before the bacterial challenge, all fish underwent a 1-week acclimatization period in continuously aerated water maintained at 30°C ± 1°C, pH 7.5 ± 0.3, and ammonia nitrogen levels below 0.2 mg/l. The two fish species were randomly distributed into nine tanks (60 × 40 × 50 cm).

The pathogen *Aeromonas hydrophila* was previously isolated from infected carp and identified through molecular isolation of the 16S ribosomal gene (OR398683.1) using BLAST for homology search, available at NCBI (http://www.ncbi.nlm.nih.gov/). The isolate was stored as a frozen stock at −80°C in 50% (v/v) glycerol in the Central Laboratory, Department of Life Sciences, College of Qurna, University of Basrah (Al Shammari *et al.*, 2023). For the challenge trial, the frozen stock culture was resuscitated. The *A. hydrophila* strain ASS-4 was inoculated into LB Broth Medium and incubated with constant shaking at 30°C for 24 hours. The broth culture was centrifuged at 3,000 rpm for 15 minutes at 4°C to collect the bacterial pellet, which was then washed three times with sterile phosphate buffer saline (PBS). The median lethal dose was determined, and the treatment groups (six tanks) were intraperitoneally injected with 0.2 ml of bacterial suspension (1.6×10^7 CFU/ml), while control fish (three tanks) received sterile PBS. Mortalities and clinical signs were monitored throughout the experiment (Mahboub *et al.*, 2022b).

Sample preparation

Three fish from each species were randomly selected post-injection (each experiment was conducted separately). The fish were euthanized and dissected, and internal organs (liver, kidney, and spleen) were collected at intervals of 1, 3, 5, and 7 days post-injection. The organs were preserved at −80°C (in liquid nitrogen) until RNA extraction. All animal procedures

adhered to the guidelines of the Animal Experiment Ethics Committee of Basrah University and followed the ARRIVE guidelines.

Total RNA extraction from tissue

Total RNA was extracted from tissues of *C. carpio* and *O. niloticus* infected with *A. hydrophila*. Three replicates were prepared for each sample at specific time intervals (1, 3, 5, and 7 days post-injection). Fish were dissected using sterile scalpels and scissors to obtain internal organs (liver, kidney, and spleen). The tissues were homogenized and mixed with GENEzol™ Tri RNA Pure reagent according to the manufacturer's protocol (Sun *et al.*, 2019a,b).

Total RNA isolation and cDNA synthesis

Ten milligrams of each fish's internal organs (liver, kidney, and spleen) were weighed, homogenized, and preserved in liquid nitrogen. Total RNA was isolated using the GENEzol™ Total RNA Kit and quantified using a NanoDrop spectrophotometer (Thermo Scientific™ Multiskan™, USA) with absorbance measured at 260/280 nm (Okamoto and Okabe, 2000). RNA was reverse-transcribed into cDNA using the AccuPower® RocketScript™ RT PreMix Kit. The reaction mixture included: Total RNA (2 µl), Oligo (dT15) primer (1 µl), Random Hexamer primer (1 µl), and DEPC-water (16 µl), for a total reaction volume of 20 µl. The reaction served as a template for RT-qPCR (Bioneer, Korea) following the manufacturer's instructions (Cai *et al.*, 2018).

The thermal cycler conditions for cDNA synthesis consisted of a single cycle with three steps: primer annealing at 25°C for 10 minutes, cDNA synthesis at 42°C for 60 minutes, and heat inactivation at 95°C for 5 minutes. Samples were stored at −86°C until further analysis (Sun *et al.*, 2019a,b).

qPCR primers

Gene expression analysis utilized specific primers for antioxidant enzymes (SOD, GPX, and CAT) and housekeeping genes. For *C. carpio*, β-actin served as the reference gene (Sielska *et al*., 2024) with forward primer 5′-CCTGTATGCCAACACCGTGCTG -3′ and reverse primer 5′-CTTCATGGTGGAGGGAGCAAGG-3′. For *O. niloticus*, GAPDH was used as the reference gene (Jiang *et al*., 2023) with forward primer 5′-TAACTTTGCTCTTCCCCACT-3′ and reverse primer 5′-ATACCGACTTTCACCATTTTG-3′.

The antioxidant enzyme primers for *C. carpio* (Mahboub *et al.*, 2022a; Mahboub *et al.*, 2022) included: SOD (F: 5′-TGAGCTGTCGGAAGCCATCAAG-3′, R: 5′-TTGGTTCCCACATGCAGCAATCC-3′), GPX (F: 5′-CTCAACAGGAGAATGCCAAGAATG-3′, R: 5′-CCTTGAGGAACACGAACAGAGG-3′), and CAT (F: 5′-AGACGACACCATCGCTGTTCG-3′, R: 5′-AAGGTCCCAGTTGCCCTCATCG-3′).

For *O. niloticus*, the antioxidant enzyme primers (Abdelazim *et al*., 2019) were: SOD (F: 5′-CGCCTTTTACATGACCAT-3′, R: 5′-GTGTCGCTGGATGCTAAGA-3′), GPX (F: 5′-AAAATGTGGCGTCTCTCTG-3′, R: 5′-GCACACCCAAAATAACGAG-3′), and CAT (F: 5′-ATGGAAGGCGAATAGAGGCT-3′, R: 5′-AACATCTTGAACCAGCAGCG-3′).

The expression of antioxidant genes (SOD, GPX, and CAT) in two different fish (*C. carpio* and *O. niloticus*) was determined using QRT-PCR. Fish were experimentally injected with A. *hydrophila* and their expression genes in internal organs (liver, kidneys, and spleen) for each fish were assessed using housekeeping gene; (*B-actin*) for *C. carpio*; and (*GAPDH*) for *O. niloticus*.

Reaction real-time PCR qPCR

The RT-PCR reaction was carried out on (RT-Per Mix) real-time PCR system (7500 Fast System) with optimization. PCR mix in a total volume of 20 µl consisted of Go Taq ® qPCR Master Mix (2×) (20 µl), forward primer (0.5 µl), reverse primer (0.5 µl), cDNA (5 µl), and nuclease-free water (4 µl). The PCR amplification was done according to Abdelazim *et al*. (2019).

Histopathology

The hepatic, renal, and spleen tissues were washed in a solution containing 1.2% saline, stationed in a solution of 4% paraformaldehyde for 2 days, splashed in 70% ethanol solution, and lastly moved to 70% ethanol solution for packing until treated into histological slides. Embedding in paraffin and collection of images were achieved following Torrecillas *et al*. (2007). For lesion scoring, three non-repeated, randomly chosen microscopic fields (40×) were examined in one slide per group of two fish. The mean scores in the examined five microscopic fields were considered the final lesion score per fish. The reported histopathological lesions in the liver, kidney, and spleen in all groups were scored according to the following scoring system (+, ++, +++), corresponding to no change, mild change, moderate change, and severe change, respectively.

Statistical analysis

The results were compiled by one-way ANOVA followed by all pairwise comparisons 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 ± SD.

Ethical approval

All experimental trials with live fish were agreed by the animal welfare and ethical review committee of the College of Agriculture, University of Basrah, Basrah, Iraq. All experimental procedures were directed in obedience to the ethical guidelines approved by the National Institutes of Health for Use and Treatment of Laboratory Animals following the ARRIVE guidelines.

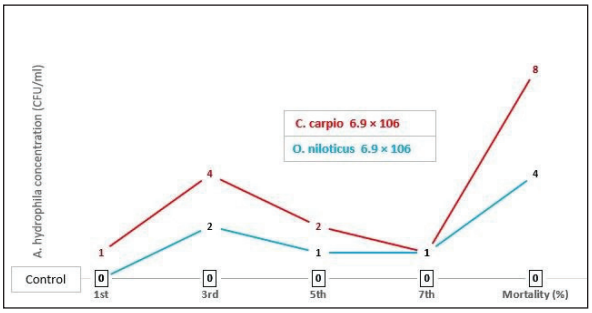


Fig. 1. Mortality of *Cyprinus carpio* and *Oreochromis niloticus* during LD 50-7th of *Aeromonas hydrophila* infection.

Results

Results of mortalities

Aeromonas infection results in the occurrence of skin ulcers and lesions, exophthalmos, inflammation of the intestines, bleeding, and eroded fins, so death is rapid when fish are infected with *A. hydrophila*. Death rates in two species of fish were high in *C. carpio* from the 1st day of bacterial injection, the fish began affected by injections, reflecting irregular swimming and interrupted feeding, then the death rates on the third day increased. The percentage of deaths increased, and *C. carpio* showed higher mortalities compared to *O. niloticus* and continued to gradually decrease by the 5th day, and then on the 7th day, there were very few deaths in two *C. carpio* and *O. niloticus* (Fig. 1).

Clinical manifestations

Infected *O. niloticus* revealed red eyes, scales loss, fin rot, and erythema (Fig. 2). However, *C. carpio* showed bilateral exophthalmia and severe skin ulceration with exposure to viscera (Fig. 3).

Quantitative real-time PCR

The primer sequences for a housekeeping gene (β_ actin) to assess relative mRNA transcript levels of gene expression for the three antioxidant enzymes (SOD, GPX, and CAT) in *C. carpio*. According to Hoseini *et al*. (2022), the housekeeping gene (On_ GAPDH) to assess relative mRNA transcript levels of gene expression for the three antioxidant enzymes (SOD, GPX, and CAT) in *O. niloticus* according to Livak and Schmittgen (2001). Relative gene expression levels were calculated using the 2−ΔΔCt method after obtaining the threshold cycle (Ct) values of each sample (Livak and Schmittgen, 2001).

The antioxidants-related gene expression in the liver, kidney, and spleen

Aeromonas hydrophila infection showed a significant effect on the expression of antioxidants-related gene expression in fish. Expression profiles of antioxidant-related genes were examined in the liver, kidney, and spleen tissues of infected *C. carpio* and *O. niloticus* (Fig. 4A and B). The *SOD*, *GPx*, and *CAT* were determined in the liver (Fig. 5A and B). The *SOD*, *GPx*, and *CAT* were assessed in the kidney (Fig. 6A and B).

Fig. 2. *Oreochromis niloticus* reveals red eye, scales loss, fin rot, and erythema in the skin.

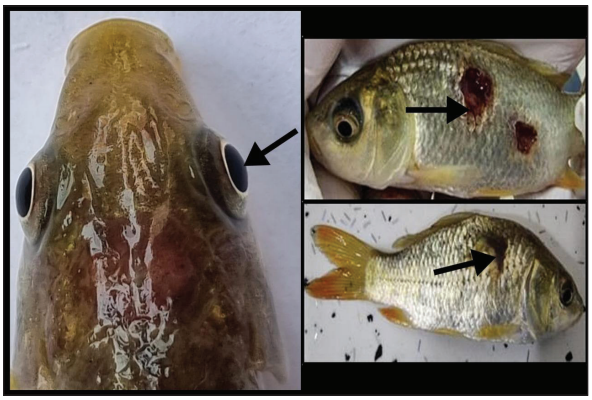


Fig. 3. *Cyprinus carpio* shows exophthalmia and severe ulceration in the skin with exposure of viscera.

Levels of *SOD*, *GPx*, and *CAT* were estimated in the *C. carpio* at 1, 3, 5, and 7th post-*A. hydrophila* infection which were largely differed from controls during the whole period, except *CAT*, at 3rd post-infection and *GPX* and *SOD*, at 3rd post-infection (Fig. 4A). In *O. niloticus*, levels of *SOD*, *GPx*, and *CAT* showed an obvious antioxidant response gene in the liver samples

Table 1. Histological changes and pathological lesions in two species of fish.

Species fish	Organ	Lesion	Negative control	A1	A2	A3
Cyprinus carpio	Liver	Vascular hyperemia	0	+++	+	++
		Abnormalities in hepatocytes		+++	+	+
		Necrosis		+	+++	+
	Spleen	Congestion of central vein and dilation of sinusoids	0	+++	+	+
		A small number of melanocytic		++	+++	+
		Phagocytic cells in the white and red pulp		+	++	+
	Kidney	Congestion and dilation of blood vessel	0	-+	+++	-+
		Dilation in Bowman space		++	+++	-+
		Exhibited necrosis of most renal tubules and detachment		+++	+	++
		of lining epithelial cells				
		Increase of Bowman space		+++	++	+
Oreochromis niloticus	Liver	Massive necrosis of hepatocytes	0	+	+	+
		Dilation of sinusoid hepatocytes		+++	+	+
	Spleen	Necrosis in red pulp	0	+	+	+
		Nodules white pulp with a lot of melanomacrophages		+	++	+++
	Kidney	Necrosis of renal lining epithelial tubule	0	+	+++	+
		Degeneration		++	+	+
		Infiltration of inflammatory cells		++	+++	+

Sample scores were calculated based on the percentage of positive cells per five non-repeated randomly selected microscopic fields (40×) as following: 0 (negative), + (weak), ++ (mild), ++++ (strong).

of *A. hydrophila* infection as compared with those of the controls (Fig. 4B).

In the kidney, the infected *C. carpio* showed that the activity of *CAT* peaked on the 3rd day post-infection. The activities of *SOD* and *GPx* peaked on the 3rd day (Fig. 5A). However, the levels of both enzymes decreased remarkably on the 7th day post-infection ($p < 0.05$). In *O. niloticus*, the controls at the first day post-infection, there was a significant difference ($p < 0.05$) and increased substantially with respect to the controls at the 3rd day post-infection. For *SOD*, *GPx*, and *CAT* ($p < 0.05$), levels in the groups peaked on the 7th day post-infection and were decreased (Fig. 5B).

In the spleen (Fig. 6A and B), the activity levels of *SOD*, *GPx*, and *CAT* fluctuated in the spleen peaked at the 3rd third day of infection and were lowest on the 7th day. Both fish markedly differed from the control on the 7th day post-infection ($p < 0.05$).

Histopathological findings post-infection by A. hydrophila

The histological changes were illustrated in the internal organs of *C. carpio* (Fig. 7). The liver showed necrosis in the hepatocytes and vacuole degeneration of most hepatocytes. The spleen showed congestion of central vein (asterisk) and dilation of sinusoids. Additionally, the spleen revealed red pulp and white pulp nodules with more pigmented masses of melanomacrophages. Kidney showed congestion and dilation of blood vessels (asterisks) and dilation in Bowman space. Kidney exhibited necrosis (thick arrows) of most renal tubules, detachment (thin arrows) of lining epithelial cells, and increase of Bowman space (asterisks).

In the case of histological changes in *O. niloticus* (Fig. 8), the liver showed massive necrosis of hepatocytes, dilatation of sinusoid, liver vacuole degeneration (arrows) of most hepatocytes and dilation of vein (V), and infiltration of inflammatory cells. The kidney showed degeneration and necrosis and, also, revealed necrosis (thin arrows) of the renal lining epithelial tubule and degeneration. Spleen revealed red pulp necrosis and nodules of white pulp with few melanomacrophages.

For lesion scoring, all lesions of the liver, kidneys, and spleen were recorded in all groups of two species of fish and are shown in Table 1.

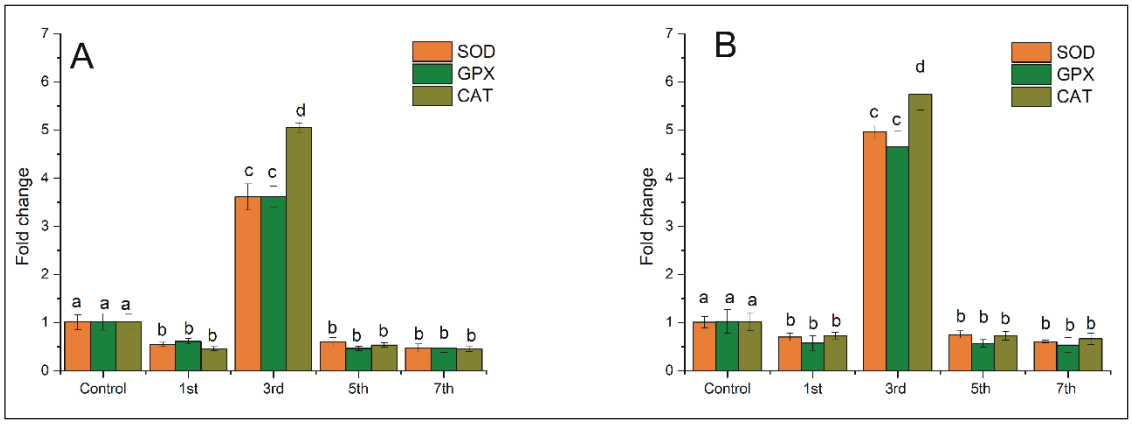


Fig. 4. Relative gene expression of superoxide dismutase (*SOD*), glutathione peroxidase (*GPx*), and catalase (*CAT*), in the liver of common carp (A) and Nile tilapia (B) after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

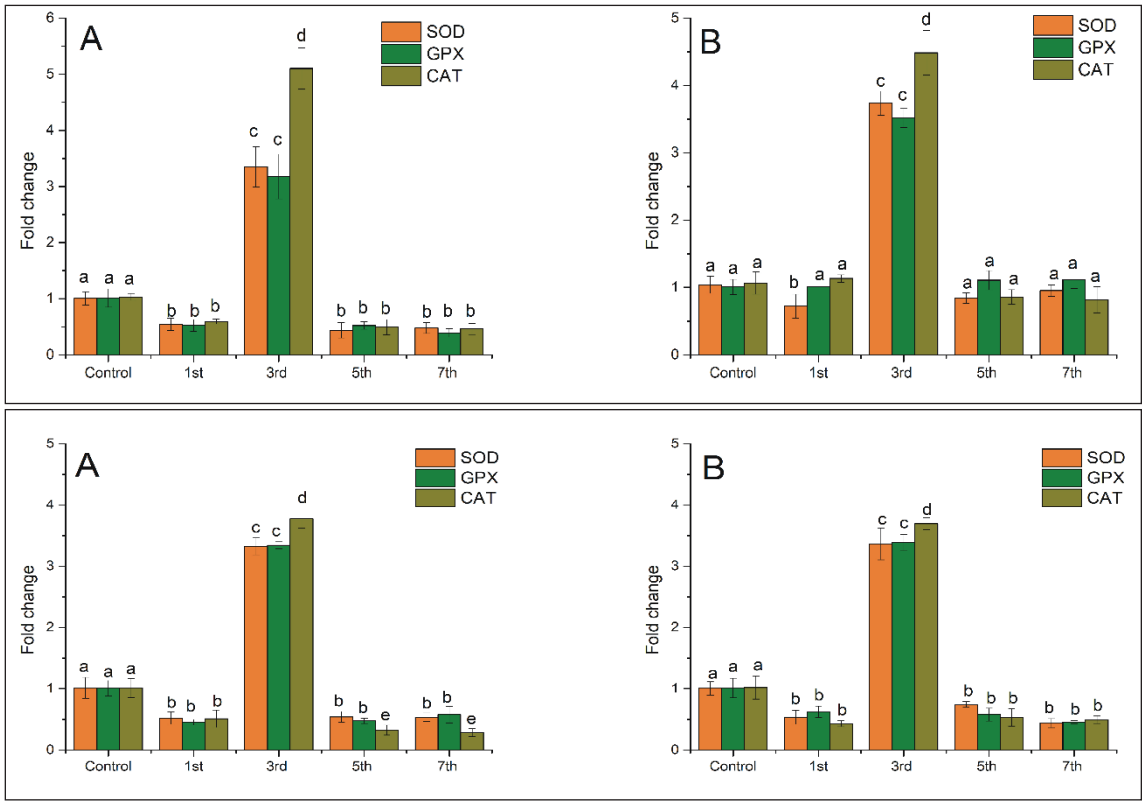


Fig. 5. Relative expression of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), in the kidney of common carp (A) and Nile tilapia (B) after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

Discussion

Bacterial infection is a life-and-death contest between the pathogen and the host, in which the host must organize all available resources to conquest (Sun *et al*., 2019a,b). Bacterial infection has a significant effect on the expression of genes in fish. *Aeromonas hydrophila* is a zoonotic virulent bacterial disease that infects both humans and animals resulting in higher mortalities (Al

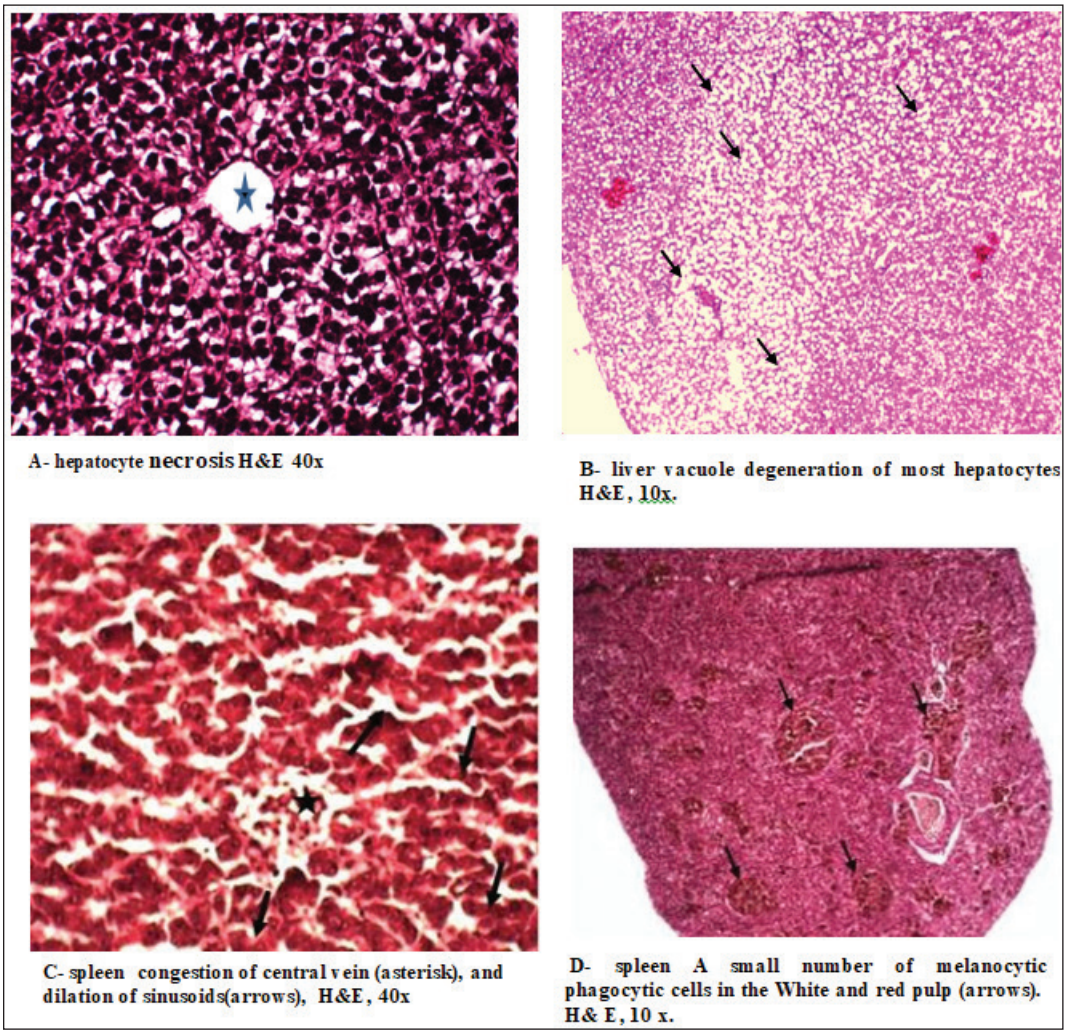


Fig. 6. Relative expression of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), in the spleen of common carp (A) and Nile tilapia (B) after 1st, 3rd, 5th, and 7th days of *Aeromonas hydrophila* infection. The experiment was performed in triplicate, and the data are shown as the mean ± SD. Values with a different letter superscript are significantly different ($p < 0.05$).

Shammari *et al*., 2023; El Gamal *et al*., 2023). To help prevent an outbreak in carp and tilapia and to improve our understanding of *Aeromonas* infection disease in fish, the current study is the first trial that aims to compare mortalities, clinical signs, antioxidant-associated genes, and histopathological changes upon experimental infection by *A. Hydrophila* in *C. carpio* and *O. niloticus*.

Monitoring mortalities is essential to indicate the pathogenicity of *A. hydrophila*. The present perspective showed that the moral level of the affected groups was significantly increased on the 3rd day compared to the control, and *C. carpio* showed higher mortalities compared to *O. niloticus*. This could be returned to the occurrence of oxidative stress, which stimulates pro-inflammatory pathways. In line with Mohammadi *et al.* (2020), this study demonstrated the beginning of mortalities after 1 day from *A. hydrophilia* injection, the cumulative mortality rate exhibited an increase in the fish population until the 10th day after in *O. niloticus*. Concurrent with Ünver and Bakıc (2021), similar signs in infected *C. carpio* were obtained, represented in small bleedings on fish skin and fin bases, swollen abdomen, and prominent eyes.

Infection by *A. hydrophila* has a varied temporal distribution in fish; it is most common in summer in fish. Infection rates were 6% in summer, 2% in spring, and 0% in autumn and winter. The water temperature changes, and unfavorable conditions in the water the majority of injuries occurred. Fish become more sensitive to stress in addition to other environmental conditions, and the prevalence of *A. hydrophila* in the current study was high and caused clinical symptoms. The incubation period of the disease depends on the

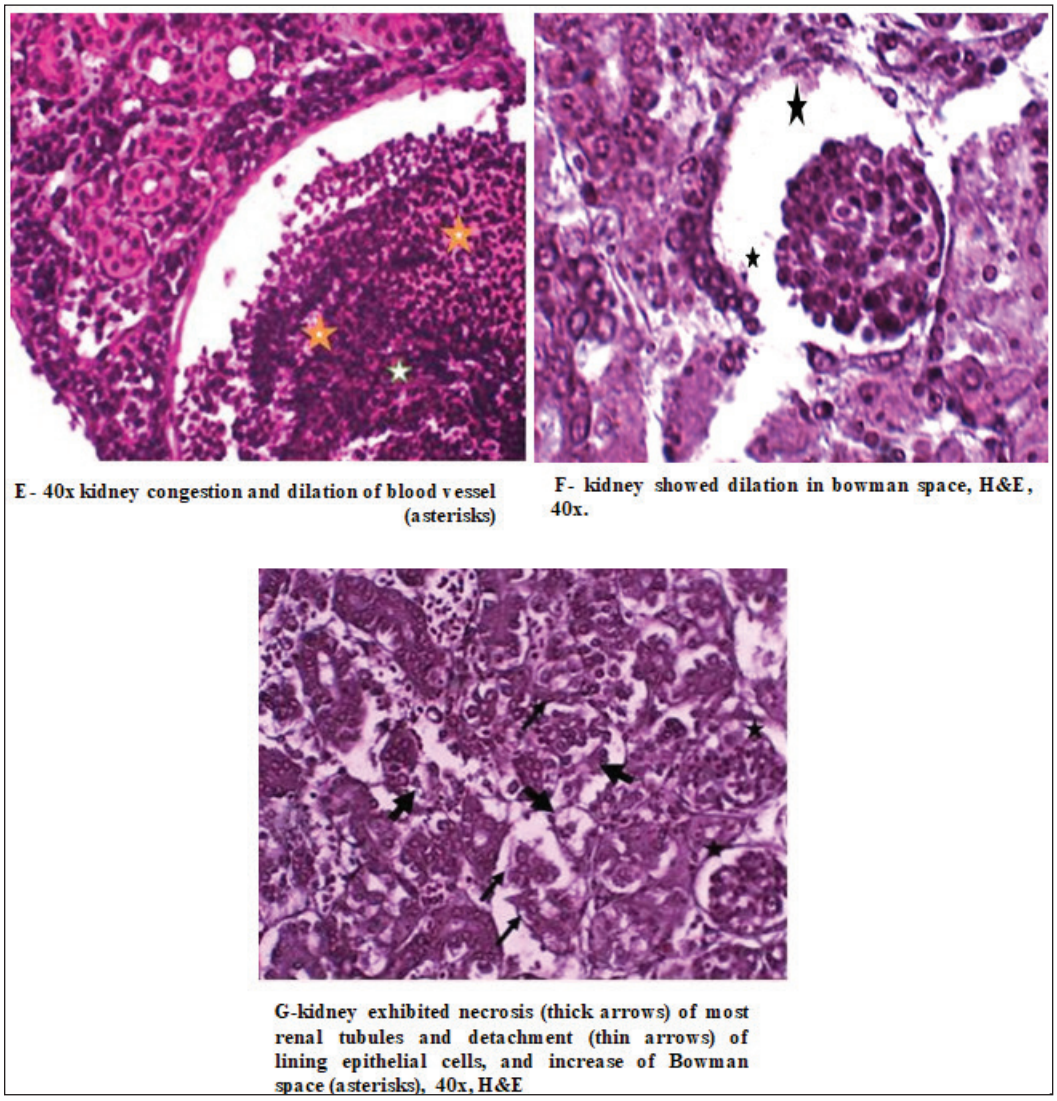
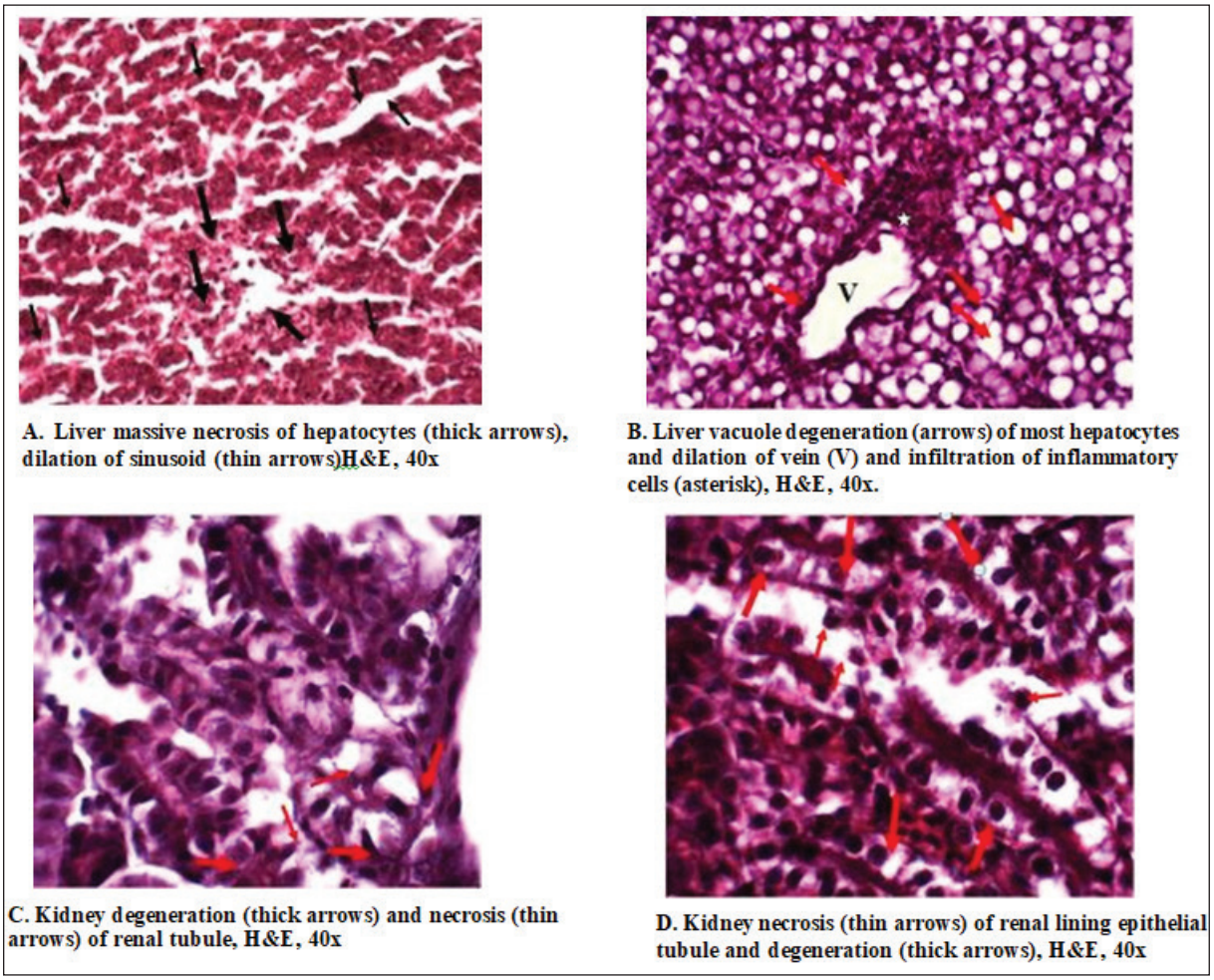


Fig. 7. Histopathological changes caused by *Aeromonas hydrophila* infection in the liver, kidney, and spleen of common carp.

species of fish and their resistance, environmental conditions, and season. This period varies from 2–4 days in natural infections and 8–48 hours in experimental infection models in the acute form of the disease (Aboelgalagel, 2015). When there are clinical signs of infection, infected fish may show exophthalmos, redness of the skin, and accumulation of fluid in the abdomen (Ammar *et al.*, 2023).

Changes in gene expression in the liver, kidneys, and spleen were also evaluated by injecting fish with pathogenic bacteria. When evaluating the results of Real time- quantitative PCR, it was noted that CAT expression in bacteria-exposed fish can be an immediate response mechanism to stress caused by bacterial contamination. The decrease in the expression of CAT indicated the involvement of oxidative stress in the mechanism of toxicity of bacteria as documented by Bayır *et al*. (2022). The disparity between (mRNA) abundance and enzyme activity should not be surprising. That is a possible reason for observed changes in the expression of the measured CAT, which is one of the stress response-related genes. As regards the observed down-regulation of CAT in fish, similar trends were obtained in a previous study (Mu *et al*., 2015). Levels of antioxidant genes, *SOD, GPX,* and *CAT* showed an obvious antioxidant response in the liver samples of *A. hydrophila*-infection in *O. niloticus* as compared with those of the controls (as illustrated in Fig. 4B). This may be due to its high tolerance and tolerability protection of Tilapia against stressful environmental conditions as recorded by El Asely *et al.* (2020). Pal *et al.* (2019) confirmed our findings and indicated that

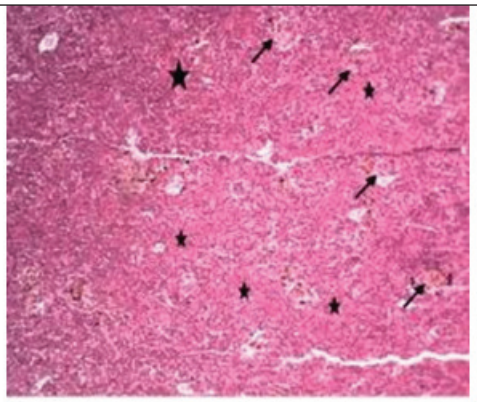


A. Liver massive necrosis of hepatocytes (thick arrows), dilation of sinusoid (thin arrows)H&E, 40x

B. Liver vacuole degeneration (arrows) of most hepatocytes and dilation of vein (V) and infiltration of inflammatory cells (asterisk), H&E, 40x.

C. Kidney degeneration (thick arrows) and necrosis (thin arrows) of renal tubule, H&E, 40x

D. Kidney necrosis (thin arrows) of renal lining epithelial tubule and degeneration (thick arrows), H&E, 40x



E. Spleen red pulp necrosis (asterisks) and with nodules of white pulp with few melanomacrophages (arrows). H& E ,40x

Fig. 8. Histopathological changes caused by *Aeromonas hydrophila* infection in the liver , kidney, and spleen of Nile tilapia.

challenged *Labeo rohita* with *A. hydrophila* resulted in a high rate of ROS production in the liver of infected fish producing oxidative stress. The expression *GPX* gene in *O. niloticus* was analyzed. In comparison to the control group, the liver had the highest level of expression of genes. In line with a new study, Yu *et al*. (2021) found that the liver showed a rapid speed of response in *GPX* expression among different tissues.

Similar outcomes by Pamplona and Costantini (2011) who measured CAT and GPx in the internal organs (liver, kidneys, and spleen) of common carp and Indigo tilapia and the levels of mRNA in the hepatic tissue were also detected. The results showed increased gene expression of CAT in the liver and kidneys, and the gene expression of antioxidants in both fish was similar during the experiment period, while the spleen had an increase in carp.

In the current study, the occurrence of oxidative damage reflects tissue architecture which exhibited necrosis of hepatocytes, kidney, and spleen red pulp necrosis. This can be explained by the increased production of ROS due to *A. hydrophila* infection. The excessive production of free radicals and lipid peroxidation result in the occurrence of necrosis, loss of epidermal, and skin epithelium as mentioned by El Gamal *et al.* (2023). Additionally, it could be attributed to the destruction of polyunsaturated fatty acids from the cell membrane lipids resulting in lipid peroxidation, which represented significant variances between the expression of genes, *GPx* and CAT in the tissues. The liver revealed the highest peak which reflects the presence of oxidative stress and cellular damage. In line with Ünver and Bakıc (2021), *A. hydrophila* induced histopathological changes in *C. carpio* such as the removal of the epithelium because of hyperplasia and hypertrophy.

Overall, this is the first report in Iraq to analyze antioxidant mRNA as a potential biomarker of oxidative stress due to the influence of the bacterium *A. hydrophila*. Analysis of important enzymatic antioxidant genes, *SOD, GPX,* and *CAT*, for defending against ROS, as a result of the attack of pathological bacteria *A. hydrophila* is crucial to study oxidative stress. Furthermore, studying the negative influence of *A. hydrophila* via recording mortalities and clinical signs is essential. Moreover, risk assessment is pathologically determined.

Conclusion

The present perspective is the first attempt in Iraq to prove the harmful influence of the virulent *A. hydrophila* on the occurrence of mortalities, markable clinical manifestations, and fluctuating relative antioxidant gene expression of *SOD*, *GPx*, and *CAT* in both *C. carpio* and *O. niloticus*. *Cyprinus carpio* demonstrated higher mortalities and major clinical picture in comparison to *O. niloticus*. Additionally, there were prominent histopathological changes in the hepato-renal tissues and spleen particularly in *C. carpio* related to *O. niloticus*. The dignified antioxidants genes displayed variations in reaction to the bacterial challenge which was more prominent in *O. niloticus* compared to *C. carpio*, and the gene expression for *CAT* was the most expressed among the genes. Nile tilapia has their gene expression augmented especially in the spleen, and the genes were more expressed compared to *C. carpio*.

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Conflict of interest

The authors declare no competing interests.

Authors' contributions

All authors contributed equally to this study

Consent for publication

All authors review and approve the manuscript for publication.

Institutional review board statement

All animal-handling protocols were performed based on the regulations of Institutional Animal Care and Use Committee (IACUC) with oversight of the Basrah University (Approval Number: BU-IACUC-2024-1-20). All experimental procedures were directed in obedience to the ethical guidelines approved by the National Institutes of Health for Use and Treatment of Laboratory Animals and following ARRIVE guidelines.

Data availability

All data regarding this study are presented in the paper.

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