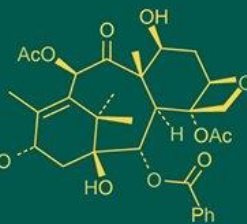
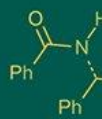


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## Evaluation of hematological, and biochemical responses in *Cyprinus carpio* following sublethal amoxicillin exposure

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### Abstract

Pharmaceutical products represent a diverse group of chemical substances extensively used in human and veterinary medicine. Among them, antibiotics are frequently detected in aquatic ecosystems due to their widespread application and improper disposal. The present study aimed to evaluate the toxic effects of amoxicillin on the freshwater fish *Cyprinus carpio* (common carp), with special emphasis on hematological and biochemical alterations as biomarkers. Fish were exposed to a sublethal concentration of 100 mg/L amoxicillin for 96 hours. Hematological, and biochemical parameters were assessed at 24, 48, 72, and 96 hours of exposure. Significant alterations were observed in the treated group compared to the control. A progressive decrease in erythrocyte (RBC) and leukocyte (WBC) counts was recorded during the exposure period, while hemoglobin (Hb) levels showed fluctuating changes. Biochemical analysis indicated a declining trend in total protein, high-density lipoprotein (HDL) cholesterol, and triglyceride levels, whereas serum glutamic-pyruvic transaminase (SGPT) levels showed a significant increase, suggesting hepatic impairment in the treated fish. The findings of the present study demonstrate that amoxicillin exposure induces marked hematological and biochemical disturbances in *C. carpio*. These parameters may serve as reliable biomarkers for monitoring pharmaceutical contamination and assessing fish health in aquatic ecosystems.

**Keywords:** Pharmaceuticals, amoxicillin, hematology, biochemical biomarkers, *Cyprinus carpio*

### Introduction

Pharmaceuticals and personal care products (PPCPs) represent a diverse group of chemical substances that include ingredients from prescription and non-prescription drugs used in human and veterinary medicine, along with industrial chemicals, disinfectants, and personal hygiene products (Kaplan, 2013) [10]. These compounds are designed to exert therapeutic effects at relatively low concentrations. However, after their use, many pharmaceuticals enter the environment through sewage discharge, agricultural runoff, and improper disposal of pharmaceutical waste. Once introduced into aquatic ecosystems, these substances may interact with non-target organisms and disrupt normal physiological processes, potentially altering ecological balance and ecosystem functioning (Kock *et al.*, 2023) [13].

Among various pharmaceuticals, antibiotics constitute one of the most extensively used classes of drugs worldwide. Their widespread use in human medicine, veterinary practices, and livestock production has led to their frequent detection in aquatic environments, where they are now considered emerging pollutants (Ramesh *et al.*, 2017) [9]. Globally, approximately 200,000 tons of antibiotics are consumed annually, with nearly 50-70% belonging to the  $\beta$ -lactam group (Moraes *et al.*, 2022) [5]. Antibiotics are widely used for the treatment and prevention of bacterial diseases in animals and are also administered in food-producing animals to enhance growth and reduce mortality (Chowdhury *et al.*, 2020). Due to their extensive use, antibiotic residues are continuously introduced into aquatic systems, exposing fish and other aquatic organisms to sub-lethal concentrations.

Amoxicillin, a  $\beta$ -lactam antibiotic, is one of the most widely prescribed drugs in both human and veterinary medicine and is listed by the World Health Organization as an essential medicine for primary health care.

Its widespread application increases the likelihood of its presence in wastewater effluents and surface waters. Although amoxicillin is considered chemically unstable due to the rapid hydrolysis of its  $\beta$ -lactam ring, its continuous input into aquatic systems may still pose ecological risks to aquatic organisms. Previous studies have shown that even low concentrations of pharmaceutical contaminants can induce physiological, biochemical, and toxicological disturbances in fish (Shaalan *et al.*, 2025) <sup>[19]</sup>.

Fish are highly sensitive to environmental contaminants because they live in direct contact with the aquatic environment. Changes in water quality are often reflected in their hematological and biochemical profiles, making these parameters useful indicators of physiological stress and toxic exposure. Hematological parameters such as hemoglobin concentration, hematocrit values, and erythrocyte and leukocyte counts provide valuable information about the health status of fish and are widely used in aquatic toxicology studies (Sahu *et al.*, 2018) <sup>[18]</sup>. Similarly, biochemical markers, including enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are commonly used to evaluate metabolic disturbances and tissue damage caused by toxic substances (Kozak *et al.*, 2025) <sup>[14]</sup>.

The common carp (*Cyprinus carpio*) is widely regarded as an important model organism in aquatic toxicology due to its ecological significance, wide distribution, and economic importance in aquaculture (Islas-Flores *et al.*, 2014) <sup>[9]</sup>. Because of its sensitivity to environmental changes, *C. carpio* serves as an effective bioindicator species for assessing the toxic effects of environmental contaminants. Therefore, evaluating hematological and biochemical alterations in *Cyprinus carpio* exposed to amoxicillin can provide important insights into the potential physiological and metabolic impacts of this antibiotic in aquatic ecosystems.

## Materials and Methods

### Chemical

Amoxicillin was purchased from a local medical store in injectable form (Zuventus Healthcare Ltd. India. For acute exposure, the sublethal concentration of amoxicillin was fixed at 100 mg L<sup>-1</sup> in water (Brito *et al.*, 2018) <sup>[3]</sup>.

### Fish Collection and Acclimatization

Healthy specimens of *Cyprinus carpio* were collected and acclimatized under laboratory conditions prior to the experiment. During the acclimatization period, fish were maintained in aerated aquaria and fed with commercially available dry food pellets. No mortality was observed during this period. The aquarium water was renewed daily by replacing three-fourths of the water while removing excess feed and fecal matter to maintain water quality. Feeding was discontinued 24 h prior to the initiation of the experiment to avoid contamination of water during exposure.

### Experimental Design

A total of 75 fish were randomly divided into five groups, consisting of one control group and four treatment groups corresponding to different exposure durations. Each group contained five fish with three replicates. The experiment was conducted in glass aquaria with a capacity of 50 L, each filled with 50 L of water.

Fish in the treatment groups were exposed to 100 mg L<sup>-1</sup> amoxicillin for 24, 48, 72, and 96 hours, while the control group was maintained under the same conditions without the addition of amoxicillin. The exposure medium was renewed every 24 h to maintain a constant concentration of the antibiotic. Water quality parameters such as temperature, pH, and dissolved oxygen were regularly monitored during the experiment. Fish were maintained under optimal environmental conditions to minimize stress and mortality during the experimental period.

### Assessment of Hematological Parameters

To evaluate the effects of amoxicillin exposure on hematological parameters, blood samples were collected from fish at 24, 48, 72, and 96 hours of exposure. Blood was drawn from the caudal vein using a heparinized syringe to prevent coagulation. Red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubauer's hemocytometer, while hemoglobin (Hb) concentration was estimated using Sahli's hemoglobinometer.

### Assessment of Plasma Biochemical Parameters

Plasma biochemical parameters including total protein, triglycerides, HDL cholesterol, and serum glutamate pyruvate transaminase (SGPT/ALT) were analyzed using commercially available diagnostic assay kits designed for serum/plasma samples. For plasma separation, blood samples were centrifuged at 10,000 rpm for 10-15 min using a Tarson Spinning MC-00 centrifuge. The separated plasma was then analyzed using a Merck Microlab 300 analyzer according to the manufacturer's instructions.

### Statistical Analysis

All experimental data were expressed as mean  $\pm$  standard error (SE). Statistical analysis was performed using SPSS software (Version 20.0). One-way analysis of variance (ANOVA) was applied to determine significant differences among the exposure durations, followed by Tukey's post hoc test to compare group means. Differences were considered statistically significant at  $p < 0.05$ .

## Results

### Hematological Parameters

Significant alterations were observed in the hematological parameters of *Cyprinus carpio* following exposure to amoxicillin over a 96-h period (Table-1). A marked decrease was observed after 24 hours, indicating an initial suppressive effect of amoxicillin on erythrocyte levels. However, RBC values increased significantly at 48 hours and reached the highest level at 72 hours before slightly declining at 96 hours though remaining higher than the control. The white blood cell (WBC) count also exhibited significant variations during the exposure period. The value decreased sharply at 24 hours when compared to control and further declined at 48 hours. Subsequently, WBC levels increased at 72 hours and reached the maximum value at 96 hours, suggesting a possible immune response to prolonged exposure to the antibiotic. The control group exhibited a hemoglobin level of  $7.30 \pm 0.115$ , which increased significantly at 24 hours and reached the highest value at 48 hours. However, Hb levels declined at 72 hours and further decreased at 96 hours, indicating potential physiological stress and impairment of oxygen-carrying capacity during prolonged exposure.

**Table 1:** Hematological parameters of *Cyprinus carpio* exposed to amoxicillin at different exposure durations

| Hematological Parameter                      | Control                       | 24 h                          | 48 h                          | 72 h                          | 96 h                         |
|----------------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| RBC ( $\times 10^6$ cells $\text{mm}^{-3}$ ) | 1.07 $\pm$ 0.02 <sup>b</sup>  | 0.43 $\pm$ 0.006 <sup>a</sup> | 1.34 $\pm$ 0.006 <sup>c</sup> | 2.81 $\pm$ 1.12 <sup>c</sup>  | 2.10 $\pm$ 0.13 <sup>d</sup> |
| WBC ( $\times 10^3$ cells $\text{mm}^{-3}$ ) | 5.08 $\pm$ 0.49 <sup>d</sup>  | 1.876 $\pm$ 1.27 <sup>b</sup> | 1.073 $\pm$ 2.03 <sup>a</sup> | 2.98 $\pm$ 1.03 <sup>c</sup>  | 5.70 $\pm$ 1.82 <sup>c</sup> |
| Hemoglobin (g $\text{dL}^{-1}$ )             | 7.30 $\pm$ 0.115 <sup>b</sup> | 8.80 $\pm$ 0.46 <sup>c</sup>  | 10.33 $\pm$ 0.08 <sup>d</sup> | 7.75 $\pm$ 1.01 <sup>bc</sup> | 6.75 $\pm$ 1.29 <sup>a</sup> |

Values are expressed as Mean  $\pm$  Standard Error (SE). Different superscript letters within the same row indicate statistically significant differences ( $p < 0.05$ ).

### Effects of amoxicillin on biochemical parameters-

Exposure to amoxicillin resulted in significant alterations in the plasma biochemical parameters of *Cyprinus carpio* during the 96-hours experimental period (Table 2). A significant increase was observed in total protein after 24 hours, representing the highest value recorded during the exposure period. Subsequently, total protein levels declined at 48 hours and 72 hours, followed by a slight increase at 96 hours. The HDL level showed a continuous decline with increasing exposure duration. The control value decreased to

1.35  $\pm$  0.057 mg  $\text{dL}^{-1}$  at 24 hours, followed by further decrease at 48 and 72 hours reaching the lowest value at 96 hours. A gradual decrease was also observed in triglyceride levels during the exposure period. The serum glutamate pyruvate transaminase (SGPT/ALT) activity also exhibited notable variations. An increase was observed at 48 hours, followed by a pronounced elevation at 72 hours representing the highest activity recorded. However, SGPT levels decreased again at 96 hours.

**Table 2:** Plasma biochemical parameters of *Cyprinus carpio* exposed to amoxicillin at different exposure durations

| Biochemical Parameter                | Control                        | 24 h                          | 48 h                          | 72 h                          | 96 h                          |
|--------------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Total Protein (g $\text{dL}^{-1}$ )  | 2.85 $\pm$ 0.005 <sup>a</sup>  | 5.63 $\pm$ 0.08 <sup>d</sup>  | 4.00 $\pm$ 0.57 <sup>c</sup>  | 3.17 $\pm$ 0.36 <sup>b</sup>  | 3.51 $\pm$ 0.86 <sup>bc</sup> |
| HDL (mg $\text{dL}^{-1}$ )           | 2.09 $\pm$ 1.11 <sup>d</sup>   | 1.35 $\pm$ 0.057 <sup>c</sup> | 1.25 $\pm$ 0.34 <sup>bc</sup> | 1.23 $\pm$ 1.10 <sup>b</sup>  | 1.11 $\pm$ 0.49 <sup>a</sup>  |
| Triglycerides (mg $\text{dL}^{-1}$ ) | 2.586 $\pm$ 0.59 <sup>cd</sup> | 2.566 $\pm$ 0.72 <sup>d</sup> | 2.563 $\pm$ 0.36 <sup>c</sup> | 2.480 $\pm$ 0.47 <sup>b</sup> | 2.420 $\pm$ 0.47 <sup>a</sup> |
| SGPT/ALT (U $\text{L}^{-1}$ )        | 42.00 $\pm$ 1.04 <sup>d</sup>  | 39.33 $\pm$ 2.40 <sup>a</sup> | 41.33 $\pm$ 2.33 <sup>c</sup> | 56.13 $\pm$ 6.19 <sup>c</sup> | 40.33 $\pm$ 5.36 <sup>b</sup> |

Values are expressed as Mean  $\pm$  Standard Error (SE). Different superscript letters within the same row indicate statistically significant differences ( $p < 0.05$ ).

### Discussion

The extensive use of antibiotics in aquaculture to control bacterial infections has raised concerns regarding their potential impact on aquatic organisms and ecosystems (Umamaheswari *et al.*, 2019) [20]. Hematological and biochemical parameters are widely recognized as reliable indicators for evaluating the physiological status of fish exposed to toxic substances (Rajabiesterabadi, 2019) [16]. Changes in blood parameters can provide important information about the health condition, stress level, and metabolic disturbances in fish (Khan *et al.*, 2018) [12].

Although amoxicillin is generally regarded as a low-toxicity antibiotic, limited studies have examined its toxicological effects on aquatic organisms. For example, the 96-h  $\text{LC}_{50}$  value of amoxicillin for *Oryzias latipes* has been reported to be 1000 mg  $\text{L}^{-1}$  (Park and Choi, 2008) [15]. However, the results of the present study indicate that short-term exposure to amoxicillin can induce significant hematological and biochemical alterations in *Cyprinus carpio*.

In the present study, RBC counts showed an initial decrease at 24 h followed by a gradual increase at 48 and 72 h, with a slight decline at 96 h. The initial reduction in RBC count may indicate stress-induced hemolysis, suppression of erythropoiesis, or damage to gill tissues affecting oxygen transport. Similar reductions in erythrocyte count have been reported in fish exposed to various toxic substances and are often associated with anemia and impaired physiological functions (Umamaheswari *et al.*, 2019; Shaalan *et al.*, 2025) [20, 19]. The subsequent increase in RBC levels during prolonged exposure may represent a compensatory physiological response to maintain oxygen transport and metabolic balance in the fish (Witeska *et al.*, 2023) [21].

Hemoglobin concentration showed a different trend, with an increase during the early exposure period followed by a decline at later stages. The initial rise in hemoglobin levels may reflect an adaptive response to stress, enhancing

oxygen-carrying capacity to meet increased metabolic demands. However, the decline observed at 72 and 96 h may indicate prolonged physiological stress or impairment of hemoglobin synthesis. Alterations in hemoglobin concentration may disrupt oxygen transport and energy metabolism, which can ultimately affect the overall health status of fish (Iftikhar and Hashmi, 2021) [8].

White blood cell (WBC) counts decreased significantly during the early stages of exposure (24 and 48 h) but increased during prolonged exposure (72 and 96 h). The initial decrease may indicate suppression of immune activity or stress-induced leukocyte depletion. In contrast, the subsequent increase in WBC counts may reflect activation of the immune defense system in response to prolonged exposure to the antibiotic. Leukocytes play a vital role in protecting organisms against infections and foreign substances, and their increase often indicates an immune response to environmental stressors (Khan *et al.*, 2018) [12].

In addition to hematological changes, exposure to amoxicillin also caused significant alterations in plasma biochemical parameters. Total protein levels increased significantly at 24 h but gradually declined during later exposure periods. The initial increase may be associated with stress-induced metabolic responses or increased synthesis of proteins involved in immune defense. However, the subsequent decline in protein levels may indicate enhanced protein catabolism or impaired protein synthesis in the liver (Kavitha *et al.*, 2010) [11]. Antibiotics such as  $\beta$ -lactams can also interact with proteins by forming hapten-protein complexes, which may influence immune responses (Ariza *et al.*, 2014) [1].

The continuous decline in HDL cholesterol and triglyceride levels observed during the exposure period suggests disturbances in lipid metabolism. Such changes may occur as a result of increased energy utilization under stress conditions or altered lipid transport mechanisms in fish

exposed to toxic substances (Iftikhar and Hashmi, 2021)<sup>[8]</sup>. Furthermore, SGPT (ALT) activity showed a pronounced increase at 72 h, indicating possible liver stress or hepatocellular damage. Transaminase enzymes such as ALT are commonly used as biomarkers of liver function, and their elevated levels in blood often result from damage to liver cell membranes, leading to leakage of intracellular enzymes into the bloodstream (Yang *et al.*, 2013)<sup>[22]</sup>. The decline in SGPT activity at 96 h may indicate partial recovery or adaptive physiological adjustments following prolonged exposure (Blahova *et al.*, 2025)<sup>[2]</sup>. Overall, the hematological and biochemical alterations observed in *Cyprinus carpio* suggest that exposure to amoxicillin can induce physiological stress, disrupt metabolic processes, and potentially affect liver function. These findings highlight the need for careful monitoring of antibiotic residues in aquatic environments and emphasize the ecological risks associated with excessive antibiotic use in aquaculture.

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