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Research Article

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Assessment of Metronidazole Toxicity in *Cyprinus carpio* using Biochemical Parameters

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Abstract

The present study evaluated the temporal effects of metronidazole exposure on plasma glucose and cholesterol levels in common carp (*Cyprinus carpio*). A total of 75 fish were allocated to independent experimental cohorts (one control group and four exposure groups) to evaluate their biochemical responses at 24, 48, 72, and 96 h postexposure to a sublethal metronidazole concentration of 10 mg/L. Compared with those in the control group, the plasma glucose concentrations in the treatment group increased significantly at 24 and 48 h, reaching a maximum hyperglycemic peak at 72 h. Although a decrease in glucose was observed at 96 h, the values remained significantly elevated compared with those in the control group, suggesting a sustained stress response. Conversely, plasma cholesterol exhibited a distinct, contrasting temporal profile; a marked, significant increase occurred at 24 h postexposure, followed by a progressive, stepwise decrease at 48 and 72 h, ultimately returning to near-control baseline values by 96 h. The early spike in cholesterol is likely attributable to the stress-induced mobilization of hepatic lipid reserves, while the subsequent normalization suggests the activation of compensatory regulatory mechanisms. Collectively, these findings indicate that sublethal metronidazole exposure induces acute, time-dependent perturbations in the carbohydrate and lipid metabolism of *C. carpio, which is* consistent with a generalized physiological stress response, highlighting the clear adaptive capacity of the species during prolonged exposure.

Keywords: *Cyprinus carpio*; Biochemical indicators; Metronidazole; Pharmaceuticals; Acute exposure.

1. Introduction

Antibiotics are used to treat bacterial infections in human and veterinary medicines and are also utilized as feed supplements in industrial livestock production, causing fears regarding their impact on the environment, especially on the aquatic environment.[1] Approximately 1.8 lakh tones of antibiotics were consumed globally in 2020, with China accounting for more than 55%, and 1.31 lakh tonnes used in food animals, projected to increase to 2.37 lakh tones by 2030.[2] In high-income countries, the United States of America, France, and Italy are major antibiotic consumers, whereas China, India, and Pakistan lead in low- and middle-income countries, with approximately 300 million people aged 15–64 years using antibiotics annually before COVID-19.[3] The word "antibiotics" comes from two parts: "anti", meaning against, and "biosis", meaning "life", referring to substances that act against microbes. Previously, antibiotics were defined as substances produced by one microorganism to inhibit another, but they now include both natural and synthetic compounds.[4] Hundreds of antibiotics and antimycotic substances are used in human and veterinary medicine, with more than 250 in Germany alone.[5] Uniform international data on antibiotic use are lacking, and the existing information is heterogeneous, reflecting different usage patterns worldwide.[6] Antibiotic use, whether prescribed or not, varies widely among different nations.[7] Even within a single country such as Germany, antibiotic consumption varies regionally, ranging from 9.6 to 17.3 DDDs per capita per day.[8] The antibiotics permitted for use in aquaculture include oxytetracycline, florfenicol, erythromycin, sarafloxacin, premixes, and sulfonamides enhanced with trimethoprim or orrimethoprim.[9] A study by Grave *et al*. (2008) examined the use of antimicrobial drugs in Norwegian aquaculture between 2000 and 2005.[10] The synthetic broad-spectrum antimicrobial metronidazole is widely utilized and frequently administered in veterinary medicine to treat animals of all sizes.[11] It is effective against anaerobic bacteria and protozoa responsible for diseases such as giardiasis, amoebiasis, bowel infections, and peritonitis and is widely used as a postoperative prophylactic treatment.[12] In the liver, metronidazole is metabolized by oxidation and glucuronidation and is eliminated mainly through renal excretion.[13] Metronidazole can induce neurological side effects, particularly when it is used at high doses or for extended durations.[14]

Biochemical analysis serves as an essential method for determining the physiological and metabolic responses of organisms under both standard and stress conditions.[15] It involves the comprehensive quantification of biomolecules, including carbohydrates, proteins, lipids, and enzymes, that are indispensable for cellular integrity, organization, and physiological activity.[16] Fluctuations, alterations, and variations in biochemical parameters are recognized as valuable biomarkers for detecting environmental stress, toxicity, and disease-related conditions.[17] The escalation and accumulation of environmental pollution in recent decades, particularly from pharmaceutical residues, have drawn considerable attention because of their disruptive effects on biochemical and physiological processes in living systems.[18] The tendency of aquatic ecosystems to act as final sinks makes them highly vulnerable to the accumulation of diverse anthropogenic pollutants.[19] Biochemical markers such as glucose and cholesterol are typically employed to assess metabolic disturbances induced by toxic substances.[20] The occurrence of increased glucose levels during stress conditions is commonly linked to hyperglycemia, which is regulated by endocrine responses, particularly cortisol secretion.[21] Similarly, fluctuations and changes in cholesterol levels serve as important markers of disrupted lipid metabolism and impaired membrane integrity in response to toxic conditions.[22] As a result, biochemical analysis functions as a reliable, efficient, and effective technique for determining the toxicological effects of environmental contaminants.[23] Fish are particularly vulnerable to antibiotic contamination because of their continuous exposure to waterborne pollutants, making them valuable bioindicators for ecotoxicological assessment. Among freshwater species, the common carp (*Cyprinus carpio*) is extensively used as a model organism owing to its ecological relevance, wide distribution, and sensitivity to environmental stressors.[24,25] *Cyprinus carpio* belongs to the order Cypriniformes and the family Cyprinidae - the largest family of freshwater fishes and inhabits diverse freshwater ecosystems, such as ponds, lakes, and rivers, with occasional occurrence in brackish environments.[26] Its broad geographical distribution, particularly across Asia and parts of Europe, further supports its suitability as a model species in aquatic toxicology studies.[27-29] Earlier work on metronidazole in common carp has mainly addressed hepatic oxidative stress and tissue damage. This study instead follows serum glucose and cholesterol at four consecutive time points which shows how the response develops and partly recovers rather than giving a single end-point value. This study focuses on evaluating and investigating selected biochemical parameters to determine their role as potential and reliable biomarkers of toxic stress in aquatic organisms.[30]

2. Materials and methods

The common carp (*Cyprinus carpio*) used in this study was obtained from the fish farm of the Department of Fisheries, CSK Himachal Pradesh Agricultural University, Palampur, India. The experimental animals had an average length of 18.4 ± 4.1 cm and an average weight of 88 ± 10 g. Prior to the experimental trials, the fish were preventively treated with a 0.2% potassium permanganate ($KMnO_4$) solution for 2 minutes to eliminate potential dermal ectoparasites. A total of 75 fish were transferred to the laboratory environment and acclimatized for 15 days in glass aquaria containing 50 L of dechlorinated water. Throughout the acclimation and experimental periods, the water temperature was maintained at 18–20°C, and the pH ranged between 7.6 and 7.8. Dissolved oxygen was supplied continuously via mechanical aeration pumps to maintain levels consistently above 5.0 mg/L, preventing hypoxic stress.

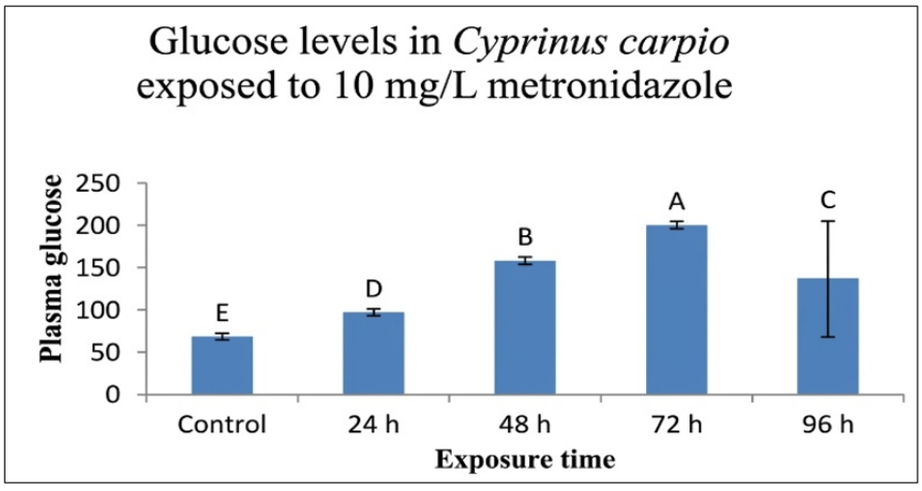


Fig. 1: Effect of 10 mg/L metronidazole exposure on plasma glucose levels in *Cyprinus carpio* at different time intervals. Data points represent the mean ± standard error (n = 3). Columns sharing distinct superscript letters indicate statistically significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD post hoc test.

The aquaria were kept under a natural seasonal photoperiod (approximately 12 h light:12 h dark cycle). The fish were fed a farm-processed pelleted diet once daily, and 100% water exchange was performed every 24 hours to eliminate metabolic excretions. The experimental architecture comprised five distinct allocations of 15 fish each (each allocation consisting of 3 replicate aquaria with 5 fish per replicate): one designated control group and four independent metronidazole exposure groups corresponding to the specific sampling intervals (24, 48, 72, and 96 hours). For the exposure cohorts, metronidazole (Sachin Parenteral Pvt. Ltd., liquid formulation, 500 mg/100 mL) was administered at a sublethal concentration of 10 mg/L, which was determined on the basis of established protocols by Çavaş and Ergene-Gözükara (2005).[31] The four exposure groups were subjected to the drug for their respective durations (24, 48, 72, or 96 h) prior to terminal sampling. To evaluate the chronological biochemical response without the confounding effects of repetitive handling stress, sampling was executed strictly without replacement. At each designated time point (24, 48, 72, and 96 h), exactly 3 fish were randomly selected from the active exposure group (1 fish per replicate aquarium (n = 3)). Simultaneously, 3 fish were sampled from the single shared control group (1 fish per replicate aquarium (n = 3)). Consequently, out of the 15 total fish in the control group, 12 unique individuals were utilized across the four time points, while the remaining 3 fish served as unsampled environmental reserve animals. No individual fish was subjected to repeated blood extraction. Animal handling, experimental design, and data reporting in this study adhered to the ARRIVE guidelines to ensure transparency and reproducibility.

2.1 Protocol for biochemical analysis

Blood samples were collected from the caudal vein of uneuthanized fish using heparinized syringes. To isolate plasma, blood samples were immediately centrifuged at 10,000 rpm for 10–15 min using a Tarson Spinning MC-00 centrifuge at room temperature. Plasma glucose and cholesterol levels were quantified using commercial diagnostic assay kits (Agappe Diagnostics Ltd., India) calibrated for serum/plasma matrices and read on a Vchem Next Biochemistry Analyzer in strict accordance with the manufacturer's operational protocols.

2.2 Statistical analysis

All data points are expressed as the mean ± standard error (SE). Variations in plasma glucose and cholesterol across the groups and intervals were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Statistical computations were performed using Minitab software, with the threshold for statistical significance set a priori at $p < 0.05$.

3. Results

Compared with the control group, exposure to 10 mg/L metronidazole resulted in significant and time-dependent alterations in the plasma biochemical parameters of *Cyprinus carpio* ($p < 0.05$). The plasma glucose levels tended to increase steadily and significantly during the initial phases of exposure. A significant increase was observed as early as 24 h postexposure compared with the control baseline, followed by a sharp and distinct increase at 48 h. The maximum hyperglycemic response was recorded at 72 h ($p < 0.05$). Although the glucose levels subsequently decreased at the 96 h interval, they remained significantly higher than those of the control group did (Fig. 1). This overall kinetic profile indicates a robust hyperglycemic response triggered by acute physiological stress during sustained chemical exposure.

Plasma cholesterol levels demonstrated a contrasting temporal pattern of metabolic disruption. A sharp, rapid increase was detected at 24 h, with the highest overall value observed across all the exposure windows ($p < 0.05$). This initial spike was followed by a progressive, stepwise decline at 48 h and 72 h; however, concentrations during these intervals remained significantly elevated relative to those in the control cohort. By the conclusion of the exposure trial at 96 h, the

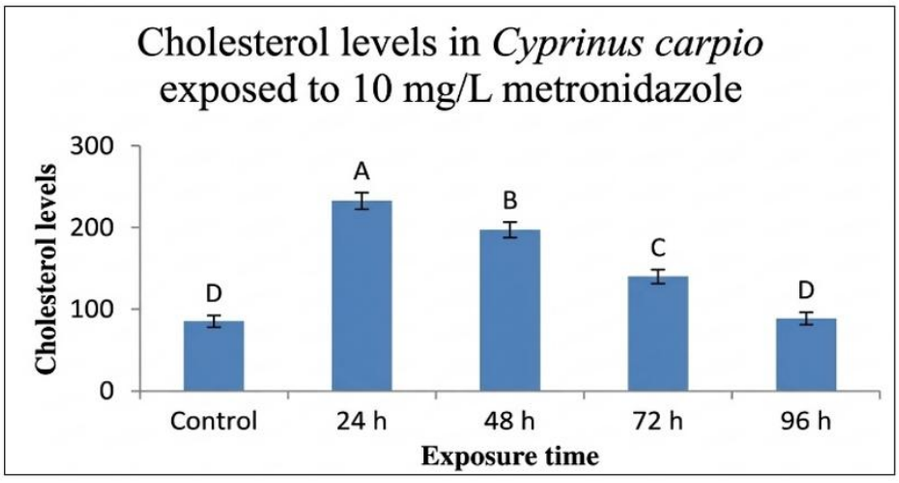


Fig. 2: Effect of 10 mg/L metronidazole exposure on plasma cholesterol levels in *Cyprinus carpio* at different time intervals. Data points represent the mean ± standard error (n = 3). Columns sharing distinct superscript letters indicate statistically significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD post hoc test.

plasma cholesterol levels had returned to baseline values, and the difference from the control group was not statistically significant (Fig. 2). This trend highlights an acute initial metabolic disturbance followed by a clear phase of partial physiological recovery.

4. Discussion

These findings confirm that sublethal exposure to metronidazole (10 mg/L) significantly alters glucose and cholesterol levels in a time-dependent manner in *Cyprinus carpio*. These alterations and variations indicate metabolic stress and adaptive, compensatory physiological responses to pharmaceutical exposure, highlighting the effectiveness of biochemical indices as reliable biomarkers in aquatic toxicology. Metronidazole toxicity is closely associated with its intracellular reductive activity, where enzymatic nitroreduction produces nitro-radical anions and other reactive intermediates.[32] The generated reactive metabolites can bind to and damage DNA or undergo redox cycling, thereby promoting the excessive formation of ROS such as superoxide radicals and hydrogen peroxide. Metronidazole increases the levels of malondialdehyde, glutathione, superoxide dismutase and catalase in the liver of common carp and induces hepatic oxidative stress and tissue damage.[33] In another study on carp, metronidazole was found to cause moderate vacuolation, cholestasis, cytoplasmic degeneration and hepatocellular necrosis.[34] The main reason for metronidazole toxicity is that it involves reductive activation, which alters the state, carbon flow and ribonucleotide reductase activity and provides a mechanistic basis for metabolic disruption.[35] The gradual increase in glucose levels over time, along with a continuous increase during prolonged exposure, suggests a stress-induced hyperglycemic response. Toxicant exposure in fish often leads to increased glucose levels as a result of HPI axis activation and elevated cortisol and catecholamine secretion.[36] The stimulation of glycogenolysis and gluconeogenesis by these hormones contributes to increased blood glucose concentrations.[37] Studies have reported similar increases in glucose levels in *Cyprinus carpio* subjected to pesticides, heavy metals, and pharmaceutical substances.[38,39] The highest glucose level at 72 h indicates a pronounced stress response, which is likely associated with enhanced mobilization and use of energy reserves to counter toxic stress.[40] Even though the levels were still significantly greater than those in the control, the observed decline at 96 h may suggest physiological adaptation or depletion of stored energy.[41] Fish subjected to prolonged toxicant exposure often display biphasic or two-phase physiological responses.[17] When fish are exposed to antibiotics, glycogen breakdown is triggered in their liver, suggesting that carbohydrate consumption meets energy demand. In a study on zebrafish, they were exposed to a rubber-derived toxicant called 6PPD-quinone. The levels of glucose and glycogen in the liver increase, whereas the levels of pyruvic acid decrease, suggesting the inhibition of glycolysis and a shift toward glycogen synthesis.[42] The expression of genes essential for glucose metabolism and insulin secretion, such as those encoding hexokinase and glucokinase, is suppressed, indicating that chemically induced metabolic disturbance and impaired insulin signaling and glucose–lipid balance. Cholesterol levels increased significantly at 24 h but gradually decreased to control levels by 96 h, indicating its involvement in membrane stability, steroidogenesis, and lipid metabolism.[43] The increase in cholesterol levels in fish under toxicant stress suggests alterations in lipid metabolism and hepatic impairment.[44] The gradual decrease in cholesterol levels during extended exposure may suggest metabolic adaptation and the use of lipids as alternative energy sources under stress.[45] The restoration of cholesterol levels toward near-control values at 96 h may indicate the activation of compensatory mechanisms and partial recovery of metabolic homeostasis.[17] These recovery trends are frequently reported in fish exposed to sublethal doses of xenobiotics, whose recovery patterns are similar under such stress conditions.[38] An environmentally detected pharmaceutical substance, metformin, disrupted the mitochondrial electron transport chain and tricarboxylic acid cycle in zebrafish, directly affecting energy

metabolism. Metformin exposure disrupts cholesterol and fatty acid biosynthesis, altering the hepatosomatic index and expression of lipid metabolism genes such as hmgcra.[46] Similarly, the triazole fungicide triflumizole upregulated the expression of genes related to lipid synthesis in zebrafish.[47] In a study on rats, metronidazole was found to affect genes that help the liver regulate cholesterol and triglycerides.[48] The observed biochemical variations may be indicative of oxidative stress, reflecting its role in the study outcomes. Like other pharmaceutical contaminants, metronidazole has been shown to stimulate the generation of reactive oxygen species (ROS), resulting in oxidative damage in aquatic organisms.[49] Disruptions in cellular metabolism, enzymatic function, and membrane stability caused by oxidative stress can significantly influence biochemical parameters.[50] The hyperglycemia and changes in lipid levels observed in this study may be linked to liver dysfunction caused by metronidazole exposure. As a crucial organ in carbohydrate and lipid metabolism, the liver can markedly alter its biochemical parameters when it is affected by toxicant-induced damage.[51] Studies in fish have demonstrated that exposure to antibiotics and pharmaceutical compounds can impair liver function, leading to alterations in glucose and cholesterol levels.[4]

5. Conclusion

Acute exposure of *Cyprinus carpio* to metronidazole at 10 mg/L produced clear, time-dependent changes in serum glucose and cholesterol over 96 h. Glucose rose progressively, peaked at 72 h, and declined slightly at 96 h while remaining significantly above control. This is consistent with a stress-induced hyperglycaemic response. Cholesterol showed a different pattern: it peaked at 24 h, fell gradually at 48 and 72 h, and returned to near-control values by 96 h, suggesting an early disturbance of lipid metabolism followed by partial compensation. Together, these results show that glucose and cholesterol respond sensitively to metronidazole and can serve as early, low-cost indicators of metabolic stress in carp. The mechanisms proposed here, including oxidative stress and hepatic involvement, are inferred from earlier studies and need to be confirmed with enzymatic, oxidative-stress and histopathological endpoints, and across a range of concentrations.

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CRediT Author Contribution Statement

Deepika Thakur: Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Madhu Sharma:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Tarang Kumar Shah:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Rijul Rana:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Yash Pal:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Deeksha Kumari:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. **Rishika Vij:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & editing. All the authors have read and approved the final version of the manuscript for publication and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding Declaration

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Institutional Review Board Statement

This study was conducted as part of a formal research project sanctioned and funded by the State Department of Fisheries Himachal Pradesh (State Adhoc Misc. 2348-46) and administratively approved by Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya. Because this project was processed as an official state agricultural/fisheries monitoring initiative, an individual, prospective protocol number from the Institutional Animal Ethics Committee (IAEC) was not obtained prior to execution. However, all experimental procedures, including 96-hour metronidazole exposure, preventive $KMnO_4$ treatments, and serial blood sampling, were conducted within university facilities in strict accordance with the baseline institutional animal welfare standards and national guidelines prescribed by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), India. The authors confirm that all efforts were made to minimize animal suffering and optimize housing conditions throughout the trial.

Data Availability Statement

The datasets generated and/or analyzed during the current study that support the findings are available from the corresponding author upon reasonable request.

Conflict of Interest

There is no conflict of interest.

Artificial Intelligence (AI) Use Disclosure

The authors declare that artificial intelligence (AI)-assisted tools were used only for language refinement, grammar improvement, and manuscript structuring purposes during the preparation of this work. All technical content, experimental implementation, results,

and interpretations were independently developed and verified by the authors.

Supporting Information

Not applicable.

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