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RESEARCH ARTICLE

EFFECTS OF GREEN AND CHEMICAL CERIUM OXIDES NANOPARTICLES ON THE BLOOD PROFILE AND LIVER ENZYMES OF *Cirrhinus mrigala*

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Abstract As nanotechnology advances, minute contaminants are increasingly found in both the terrestrial and aquatic environments, raising concerns about nanoparticle pollution such as cerium oxide. They could be consumed by fish and other aquatic life, leading to poisoning, illness, and even death. The goal of the current study was to investigate how cerium oxide nanoparticles physically affected the freshwater *Cirrhinus mrigala's* liver and hematological markers. Fish for 28 days were exposed to two dosages of each type: 50 mg and 100 mg. The fingerlings in each experimental aquarium were twelve. Following CRD, hepatotoxicity was measured in a spectrophotometer Microlab 300® using the QCA diagnostic kit method for markers such as ALT and AST, alkaline phosphatase (ALP), and total bilirubin. Hematological parameters were evaluated by CBC tests, which included hemoglobin, hematocrit, differential RBCs, WBCs, and platelets. One-way ANOVA was used to statistically examine the data. This study showed that both chemically produced cerium oxide nanoparticles and green cerium oxide had an effect on the liver and blood profile of *Cirrhinus mrigala*. Histopathological analysis revealed cellular deterioration and necrosis in the liver tissue. Hematological markers like white blood cell counts and hemoglobin levels showed notable variability in the blood profile. These results suggested that both kinds of nanoparticles may have toxicological consequences on fish.

Keywords Chemical cerium oxide, green cerium oxide, hematology, liver, nanoparticles, *Cirrhinus mrigala,* toxicity

Introduction

Water contamination is a global concern that threatens both ecological systems and human health. Pollutants biological, molecular, and material introduced through human activity adversely affect aquatic ecosystems (Saxena et al., 2025). Among these, cerium oxide nanoparticles (CeO_2 NPs) are especially hazardous due to their industrial use and persistence in water bodies (Senthil et al., 2024). Studies confirm that CeO_2 NPs negatively impact aquatic life via multiple exposure pathways (Raj et al., 2023). These nanoparticles, though valued for applications in drug delivery and catalysis, are often produced via environmentally harmful methods (Wang et al., 2022). Green synthesis using biological materials offers a safer alternative. Fish, particularly Cirrhinus mrigala are widely used as biological indicators of nanoparticle toxicity in aquatic environments due to their sensitivity to pollutants (Lv

et al., 2022). Contamination affects their behavior, metabolism, and health. Hematological assessments such as erythrocyte and leukocyte counts provide reliable insights into fish health and are key in aquaculture diagnostics (Witeska et al., 2023; Nabi et al., 2022; Chen et al., 2023). Research on cerium oxide nanoparticles (CeO_2 NPs) has expanded rapidly, focusing on their synthesis, applications, and environmental impacts, particularly in aquaculture and biomedicine. CeO_2 NPs are widely used due to their stability and reactivity, but conventional synthesis methods pose environmental risks (Güngör et al., 2025; Adesibikan et al., 2024). Green synthesis, using biological sources, offers a safer alternative (Mustafa et al., 2025). The ecological consequences of nanoparticle contamination in water bodies are significant, affecting aquatic organisms, food chains, and ecosystems (Albou et al., 2024; Meng et al., 2025).

Studies on species like *CIRRHINUS MRIGALA* show these particles can damage vital organs and alter physiology (Swamy et al., 2021; Khan et al., 2024). Hematological assessments and biomarker analysis help gauge nanoparticle toxicity (Witeska et al., 2023; Hamed et al., 2022). While some studies report limited toxicity in other species, others highlight the threat to biodiversity and food security due to heavy metals and engineered nanoparticles (Romero et al., 2024; Othman et al., 2024). Water pollution, caused by industrial, agricultural, and urban waste, alters physical and chemical water properties, posing risks to aquatic life and human health (Mishra et al., 2023). Pollutants such as heavy metals and pesticides bioaccumulate in organisms and disrupt ecosystems (Tudi et al., 2022). Globally, waterborne diseases account for hundreds of thousands of deaths annually (Mustafa et al., 2024).

Nanotechnology manipulates matter at the atomic and molecular levels, producing materials with novel properties. Its applications span medicine, agriculture, and energy. However, the rise in nanoparticle use raises environmental safety concerns (Fuhrmann et al., 2024; Singh et al., 2024). Due to their small size, nanoparticles penetrate biological membranes and are difficult to eliminate from ecosystems. Their high reactivity makes them effective but also potentially harmful to aquatic species and human health (Joseph et al., 2023; Wang et al., 2021). Metal oxide nanoparticles like CeO_2 are used in filtration, but their environmental behavior remains poorly understood (Skar et al., 2025).

CeO_2 NPs have diverse uses in pharmaceuticals and catalysis due to their stability and biocompatibility. Conventional synthesis involves toxic chemicals, while green synthesis using plant extracts provides a safer, cost-effective method (Pundir et al., 2023; Borah et al., 2023). Aquatic habitats are affected by pollutants including nanoparticles, which disrupt cell function and metabolic processes in fish. Though some nanoparticles improve aquaculture feed efficiency, their unchecked release into the environment threatens aquatic biodiversity (Turan et al., 2019; Xin et al., 2023).

Fish are crucial to global nutrition and aquaculture. Species like *CIRRHINUS MRIGALA* are vital to food security due to their nutritional profile, adaptability, and commercial viability. However, pollution, including nanoparticle exposure, poses challenges to sustainable production (Boyd et al., 2023; Naylor et al., 2021). It is widely farmed for its rapid growth and resistance to disease. It has been introduced in multiple countries and thrives in varied environments. Despite its aquaculture potential, challenges like salinity shifts and water contamination affect its health and productivity (Mohsin et al., 2021; Rossignoli et al., 2023).

Nanoparticles ingested by aquatic organisms accumulate in tissues, causing cellular disruption. CeO_2 NPs have shown both toxic and therapeutic effects, depending on their properties and concentrations. Their environmental fate is influenced by water chemistry and nanoparticle characteristics (Nguyen et al., 2024; Lu et al., 2023). Blood is a key indicator of fish health. Hematological parameters change in response to environmental stressors, making them useful in monitoring health in aquaculture systems. Regular blood analysis helps detect disease early and improve fish welfare (Thiagarajan et al., 2021; Witeska et al., 2022).

The liver plays a central role in filtering toxins, including nanoparticles. It is a primary site for their accumulation and elimination, but this function can limit therapeutic nanoparticle delivery and increase toxicity risks. Advances in surface engineering aim to reduce hepatic uptake in medical applications (Milligan et al., 2022; Guerrera et al., 2021).n Despite extensive research into CeO_2 NPs toxicity, there is a lack of comparative studies on the effects of green versus chemically synthesized CeO_2 NPs in *Cirrhinus mrigala*. This study addresses that gap by assessing liver function and hematological changes under both nanoparticle types (Jampílek et al., 2025). The aim of this study is to investigate the toxicological effects of green-synthesized and chemically synthesized CeO_2 nanoparticles (NPs) on the liver and blood of Cirrhinus mrigala, and to evaluate the impact of varying nanoparticle concentrations on hematological parameters. Additionally, the study seeks to compare the overall toxicity between green-synthesized and chemically synthesized CeO_2 NPs.

Research Methodology

A 4-week toxicological study was conducted at Riphah International University, Faisalabad, Pakistan, to evaluate the hematological effects of nanoparticle ingestion in Cirrhinus mrigala. Fish were maintained in customized aquariums. Blood parameters such as RBCs, WBCs, hemoglobin (Hb), and hematocrit (HCT/PCV) were assessed using a Neubauer hemocytometer and Drabkin's reagent. Liver function

tests (LFTs) were performed using QCA diagnostic kits and a Microlab300® spectrophotometer. The framework involves three key phases: fish collection, nanoparticle exposure, and profiling of blood and liver parameters. Collected data filled in the framework for evaluating toxicity.

Data Collection & Sample Preparation

Juvenile Cirrhinus mrigala were obtained from the Government Breeding Center in Faisalabad and acclimatized in laboratory conditions. Both green-synthesized and chemically synthesized cerium oxide nanoparticles were procured from local sources. A stock solution (2.5 g/L) was prepared, stirred thoroughly, and diluted accordingly for experimental treatments (Qu et al., 2025).

Research Methodology

Five groups of *C. MRIGALA* (12 fish each) were studied: a control group (T0), two green CeO_2 groups (T1: 50 mg/L, T2: 100 mg/L), and two chemically synthesized CeO_2 groups (T3: 50 mg/L, T4: 100 mg/L). Exposure lasted 28 days via feed. On day 28, samples were collected from five randomly selected fish per group (Scheijmans et al., 2025). Fish were anesthetized using a 100–200 mg/L solution of anesthetic 122 2248, buffered for pH stability. Once sedated, fish were chilled on ice to preserve tissue integrity. Blood was drawn from the caudal vein using sterile syringes and stored in EDTA-coated tubes. Aseptic techniques were followed throughout the procedure.

Hematological and Biochemical Assessment

MCH, MCHC, MCV were calculated using standard formulas. Platelets, WBCs, RBCs, differential leukocyte counts, HCT, and Hb levels were determined using CBC. RBCs were counted using erythrocyte pipettes diluted with Hayem's solution and analyzed microscopically (250×) with a standard formula. WBCs were measured by diluting blood with a specific solution, loaded into a counting chamber, and counted in the large squares under a microscope. Hb was quantified using Drabkin's reagent and a spectrophotometer (530–550 nm), compared against a reference reagent. Blood in heparinized tubes was centrifuged to determine HCT. MCH, MCHC, and MCV were then derived using standard formulas. Fish were descaled and dissected from the ventral side. Livers were excised and preserved in 10% formalin for histological analysis. Details like fish size, gender, and abnormalities were recorded. Liver tissues (3–5 mm) were fixed in Bouin's solution or 10% NBF in labeled vials for 4–6 hours. Sterile tools were used to ensure integrity for histological and biochemical tests. Liver tissues were washed, homogenized, and centrifuged. Supernatants were aliquoted and stored. Biochemical tests followed QCA kit protocols using a spectrophotometer. Liver function was assessed through levels of ALT, AST, ALP, bilirubin, albumin, and total protein. Elevated values indicated possible liver damage due to nanoparticle exposure.

Statistical Analysis

A Completely Randomized Design (CRD) was used. Data were analyzed through one-way ANOVA to assess the effect of nanoparticle concentration on hematological and liver function parameters.

Results

This study aimed to evaluate the physiological and toxicological effects of green- and chemically synthesized cerium oxide (CeO_2) nanoparticles on the hematological and liver health of *CIRRHINUS MRIGALA*. Sixty healthy fish were randomly assigned to five groups: a control (T0) and four treatment groups exposed to green- or chemically synthesized CeO_2 at 50 mg/L and 100 mg/L concentrations over a four-week period. All groups were maintained under identical environmental and feeding conditions. Water quality was monitored weekly to ensure consistency and eliminate external variables.

At the end of the trial, blood samples were collected to assess hematological parameters through complete blood count (CBC), including RBC, WBC, Hb, HCT, MCV, MCH, MCHC, and platelet levels. Liver function was evaluated by analyzing plasma levels of ALT, AST, ALP, total protein, albumin, and bilirubin. Results indicated that chemically synthesized nanoparticles caused more pronounced hematological disruptions and elevated liver enzyme levels, alongside histopathological liver damage. Green-synthesized nanoparticles induced comparatively milder effects, suggesting reduced toxicity potentially due to plant-based surface modifications.

Exposure of Cirrhinus mrigala to varying concentrations of polyethylene glycol (PEG) microplastics for 28 days produced notable changes in several hematological parameters compared with the control group. A general decline in red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (HCT) values was observed at lower concentrations (T_1 and T_2), suggesting initial stress and possible anemia due to microplastic-induced physiological disruption. However, at higher concentrations, particularly T_4 (100 mg/L chemically treated), these parameters showed a gradual recovery or elevation, which may indicate compensatory erythropoietic activity or adaptation to prolonged exposure.

Indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)

followed a similar trend, with lower values at T_1 indicating hypochromic and microcytic tendencies, while higher doses showed normalization toward control levels. White blood cell (WBC) counts and lymphocyte percentages increased significantly in higher treatment groups (T_3 and T_4), reflecting an activated immune response to microplastic exposure. The rise in monocytes and neutrophils further supports the occurrence of mild inflammatory or immune-modulated reactions. Platelet counts remained relatively stable across treatments, though a slight reduction was observed at T_1, which could be linked to transient hematopoietic stress.

Overall, the results suggest that exposure to PEG microplastics alters hematological balance in Cirrhinus mrigala, particularly at lower concentrations, where stress responses are more evident. The partial recovery of parameters at higher concentrations may indicate physiological adaptation, though sustained exposure still poses potential risks to hematopoietic and immune functions.

Table 1: Hematological parameters of Cirrhinus mrigalaafter 28-day exposure to different concentrations of polyethylene glycol microplastics.

Parameters	Treatments	RBCs (10/mm³)	Hb (g/dl)	Hematocrite (%)	MCV (mm3)	MCH (pg)	MCHC(g/dl)	Platelets (10³/mm³)	White blood cells (103/mm3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
	T0 Control	3.133+0.008	7.29 +0.005	21.33+0.881	132.53+0.706	46.76+0.307	46.45+0.3067	314.21+0.254	13.51+0.008	81.33+0.88	3.07+0.02	7.15+0.01	2.417+0.009
	T1 50mg/L	2.06+0.005	5.493+1.72	9.683+3.828	89.197+43.58	31.47+14.72	30.75+14.35	209.94+03.95	9.65+3.80	60.0+28.99	2.717+0.333	5.407+1.678	2.113+0.0328
	T2 100mg/L	2.53+0.016	7.315+0.012	16.67+0.667	133.6+0.082	45.27+0.009	46.69+0.167	310.96+0.177	14.76+0.009	92.17+0.441	3.083+0.003	7.247+0.009	2.207+0.012
	T3 50mg/L	2.873+0.008	7.35+0.005	19+0.577	134.03+0.213	47.26+0.012	46.896+0.34	311.6+0.603	16.65+0.006	91.87+0.935	3.61+0.006	7.273+0.015	2.213+0.003
	T4 100mg/L	3.35+0.005	8.727+0.008	24.67+0.882	135.01+0.03	48.05+0.013	48.03+0.008	290.93+0.277	18.16+0.009	94.81+0.1	4.52+0.017	7.5+0.012	2.81+0.012

Exposure of Cirrhinus mrigala to both green-synthesized and chemically synthesized cerium oxide nanoparticles (CeO_2 NPs) produced marked alterations in liver function biomarkers. A consistent, concentration-dependent pattern was observed across most parameters.

Fish treated with chemically synthesized CeO_2 NPs, particularly at 100 mg/L (T_4), exhibited the highest activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), indicating pronounced hepatocellular damage and membrane leakage. In contrast, minimal enzyme

activity was recorded in the control (T_0) and in fish exposed to the lower concentration of green-synthesized NPs (T_1), suggesting comparatively reduced hepatic stress under these conditions.

Albumin and total protein levels declined markedly in fish exposed to higher concentrations of chemically synthesized nanoparticles, reflecting possible impairment of hepatic synthetic capacity and protein metabolism. However, a moderate increase was evident in fish treated with green-synthesized CeO_2 NPs at lower concentrations, implying a milder physiological response.

Bilirubin levels remained within or slightly above the normal range, showing minor fluctuations among treatments; this parameter appeared less sensitive to nanoparticle exposure than the transaminases. Overall, the results demonstrate that chemically synthesized CeO_2 NPs exert stronger hepatotoxic effects than their green-synthesized counterparts, with severity increasing at higher exposure levels. These findings indicate that green synthesis may confer reduced toxicity, possibly due to surface modification or biological capping agents that mitigate oxidative stress in Cirrhinus mrigala.

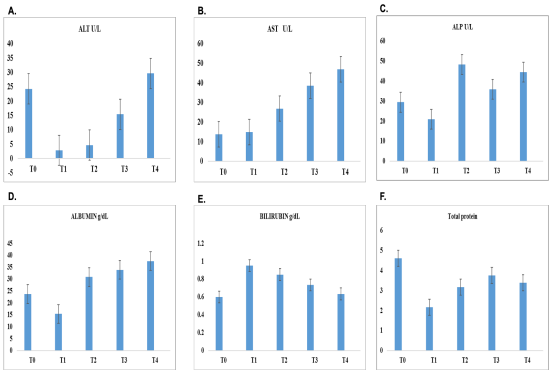

Figure 1: Mean values of (A) ALT (U/L), (B) AST (U/L), (C) ALP (U/L), (D) albumin (g/dL), (E) bilirubin (g/dL), and (F) total protein (g/dL) in Cirrhinus mrigala exposed to different concentrations (50 and 100 mg/L) of green-synthesized and chemically synthesized cerium oxide nanoparticles (CeO_2 NPs).

Discussion

Water pollution remains a major global issue, with emerging contaminants like engineered nanoparticles (ENPs) adding new complexity. Among these, cerium oxide (CeO_2) nanoparticles stand out due to their redox activity and widespread industrial use. While beneficial in many applications, their release into aquatic systems through industrial discharge, runoff, and wastewater can disrupt ecosystems, accumulate in organisms, and alter water chemistry (Saxena et al., 2025; Atanda et al., 2025). In aquatic environments, nanoparticles interact with sediments, organic matter, and microbes, affecting their behavior and toxicity. Metal oxide NPs, including CeO_2, TiO_2, and ZnO, can generate reactive oxygen species (ROS), damage membranes and DNA, and trigger inflammation in aquatic organisms (Gigl et al., 2024; Singh et al., 2023). While CeO_2's redox properties may offer antioxidant benefits in medicine, they can destabilize oxidative balance in natural systems.

Synthesis method significantly affects nanoparticle toxicity. Chemically synthesized CeO_2 NPs often contain toxic residues, while green-synthesized variants, produced using plants or microbes, are considered more sustainable. However, green NPs are not inherently non-toxic and still require rigorous testing (Shcherbakov et al., 2021; Pansambal et al., 2023). Cirrhinus mrigala, a freshwater carp, serves as an effective bioindicator for nanoparticle toxicity. In this study, exposure to both green and chemically synthesized CeO_2 NPs led to reduced RBCs, hemoglobin, and hematocrit, indicating anemia. Increased WBCs and immune cell counts pointed to systemic inflammation (Ryan et al., 2022). Liver tissue showed signs of damage, including cell swelling, vacuolization, and disrupted structure. Biochemical markers (ALT, AST, ALP, albumin, bilirubin) were elevated, confirming liver stress and systemic toxicity (Li et al., 2022; Nagube et al., 2020).

These findings highlight the environmental risks posed by CeO_2 NPs, particularly at realistic exposure levels. Differences between green and chemical synthesis underline the need to assess nanoparticle behavior, not just composition. Regulatory efforts should focus on nanoparticle-specific properties and environmental conditions. Continued research is essential for developing safe, sustainable nanotechnology (Jampílek et al., 2025).

Conclusion

The study demonstrated that chemically synthesized CeO_2 NPs caused significant liver damage and blood abnormalities in *CIRRHINUS MRIGALA*, while green-synthesized NPs showed comparatively milder effects. Histological liver changes included necrosis and vacuolation, while hematological disruptions affected oxygen transport and immune competence. The findings suggest that green synthesis, using biological agents, may offer safer alternatives. These results highlight the influence of nanoparticle production methods on biological outcomes and call for more targeted toxicity evaluations. Exposure to both green and chemically synthesized CeO_2 NPs induced marked liver and blood abnormalities in *CIRRHINUS MRIGALA*, with the chemically synthesized variants causing more pronounced effects. Histological liver damage and hematological shifts point to systemic toxicity, physiological stress, and impaired detoxification. These disruptions could reduce the fish's ability to survive and reproduce, raising concerns about nanoparticle accumulation and

ecosystem health. The study calls for integrating synthesis methods into nanotoxicity assessments and urges further research into long-term and ecological impacts of nanoparticle exposure in aquatic environments.

Author Contributions

Iqra Akram, and Maryam Riasat contributed equally to this work and were primarily responsible for the experimental design, data collection, and original draft preparation. Rida Younas and Naureen Rana assisted in laboratory analyses, data organization, and figure preparation. Nawaz Haider Bashir contributed to data interpretation and manuscript refinement. Muhammad Naeem and Huanhuan Chen supervised the overall research, provided resources, guided data validation, and contributed to manuscript review and editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Data Availability

Data will be made available on request

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