

SUB-CHRONIC DIETARY TOXICITY OF ZINC OXIDE-SILICA
NANOPARTICLES IN GRASS CARP (CTENOPHARYNGODON
IDELLA): HEMATOLOGICAL, BIOCHEMICAL, AND
HISTOPATHOLOGICAL RESPONSES

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Abstract

The rapid expansion of nanotechnology and the consequent release of engineered nanoparticles into aquatic ecosystems have raised significant concerns regarding the safety of freshwater biota. The present study evaluated the sub-chronic effects of dietary Zinc Oxide-Silica (ZnO-SiO₂) nanoparticles on hematological parameters, serum biochemistry, and tissue architecture of grass carp (*Ctenopharyngodon idella*) in a 45-day controlled trial. Four experimental groups were established: one control group receiving a basal diet, and three treatment groups (T1, T2, T3) receiving 50, 100, and 200 mg of ZnO-SiO₂ nanoparticles per 0.5 kg of feed, respectively. Blood samples collected at the end of the trial were analyzed for hematological indices (WBC, RBC, MCV, MCH, HCT, HGB) and serum biochemical parameters (urea, creatinine, total protein, globulin, albumin, cholesterol). Kidney and intestine tissues were processed for histopathological examination using hematoxylin and eosin staining. One-way ANOVA revealed significant increases in white blood cell count ($P < 0.05$), accompanied by a selective increase in mean corpuscular hemoglobin and a distinct alteration in hematocrit levels across nanoparticle-treated groups compared with the control. Serum biochemistry showed significant elevations in urea, alongside variable fluctuations in cholesterol, while creatinine and albumin remained statistically unchanged ($P > 0.05$). Notably, a robust and significant increase in globulin levels was observed across all nanoparticle-treated groups compared with the control ($P < 0.05$), accompanied by a significant decrease in total protein concentration. Histopathological evaluation demonstrated progressive degenerative and inflammatory changes in kidney (tubular degeneration, glomerular atrophy, inflammatory infiltration, hemorrhage, necrosis) and intestine (villus shortening, epithelial degeneration, inflammatory infiltration, epithelial necrosis, mucosal gland degeneration) in a clear dose-dependent response pattern. These findings indicate that dietary exposure to ZnO-SiO₂ nanoparticles induces detectable hematological imbalance, biochemical disturbance, and structural tissue damage in grass carp under sub-chronic conditions, supporting oxidative stress and inflammation as central mechanistic drivers. The results emphasize the need for ecological risk assessment frameworks of nanocomposite release into freshwater aquaculture systems.

1. Introduction

Aquaculture has emerged as one of the most rapidly expanding food production sectors worldwide, contributing substantially to food security, nutritional sustainability, and rural economies across both developing and developed regions (Zaman, 2026). With the stagnation of capture fisheries and the growing demand for high-quality animal protein, aquaculture has become an essential strategy for meeting global food requirements, particularly in low- and middle-income countries where freshwater fish provide an economical protein source (Gargotra et al., 2026; Haque & Mahmud, 2025). Among cultured freshwater species, the grass carp (*Ctenopharyngodon idella*) is of considerable economic and ecological importance. Native to East Asia, the species has been widely introduced across Asia, Europe, and other regions for both aquaculture production and aquatic weed control (Ullah, 2026). Its rapid growth rate, favorable feed conversion ratio, and herbivorous feeding habit make it a cost-efficient candidate for polyculture systems (C. Wang et al., 2026; S. Li et al., 2026). In parallel with aquaculture expansion, nanotechnology has experienced substantial growth over the past two decades, leading to large-scale manufacturing and application of engineered nanoparticles across diverse industrial sectors (Maryam et al., 2025). Metal and metal-oxide nanoparticles—including zinc oxide, titanium dioxide, silica, silver, and iron-based formulations—are among the most widely produced engineered nanomaterials and are routinely incorporated into consumer products, pharmaceuticals, coatings, and agricultural preparations (Anwer et al., 2025; Roy et al., 2025). Their small size, high surface-area-to-volume ratio, and tunable reactivity confer superior performance relative to bulk counterparts, but also elevate the risk of environmental release during manufacturing, transport, use, and disposal (Ghosh et al., 2025).

Zinc oxide-silica (ZnO-SiO_2) nanoparticles are composite nanomaterials engineered to combine the antimicrobial, photocatalytic, and UV-blocking properties of zinc oxide with the structural stability, chemical inertness, and

biocompatibility of silica (Ali et al., 2025; Bhosale et al., 2026). The silica matrix can encapsulate ZnO cores, control Zn^{2+} ion-release kinetics, reduce aggregation, and provide a versatile platform for surface functionalization (Abd Alsaheb et al., 2025; N. Singh et al., 2025). These properties make ZnO-SiO_2 nanocomposites attractive for multifunctional applications including coatings, paints, antimicrobial packaging, wastewater treatment, and agriculture as nano-fertilizers or pesticide delivery systems (Demeke et al., 2025). However, agronomic and industrial uses can result in runoff and wastewater discharge that transport nanoparticles into adjacent water bodies (Sathyamoorthy et al., 2025).

Once in the aquatic environment, ZnO-SiO_2 nanoparticles undergo complex transformations—aggregation, sedimentation, dissolution, and interaction with dissolved organic matter—that influence their bioavailability and toxicity to aquatic organisms (Harrison et al., 2023; Lu et al., 2023). Fish are exposed through multiple pathways including branchial contact, ingestion of contaminated water or feed, and dermal uptake (Hu et al., 2022). Herbivorous species such as *C. idella*, which constantly graze on aquatic flora that may adsorb nanoparticles, are particularly susceptible (Varatharajan et al., 2026). Engineered nanoparticles are well-documented inducers of oxidative stress in piscine species, generating reactive oxygen species (ROS) that overwhelm antioxidant defenses and trigger lipid peroxidation, protein oxidation, and DNA damage (Horie & Tabei, 2021; Cao et al., 2025; Xiaoting Zhou et al., 2025).

Blood biochemical and serological parameters serve as sensitive indicators of physiological stress and organ dysfunction in fish (Jia et al., 2022). Exposure to engineered nanoparticles has been shown to alter serum enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase), renal markers (urea, creatinine), and protein fractions (total protein, albumin, globulin) in exposed fish (Ale et al., 2018; Z. Chen et al., 2022; Sukhsangchan et al., 2024). Histopathological examination provides direct visual evidence of tissue damage in gill,

liver, kidney, and intestine, and offers an early-warning tool that complements biochemical indices (Yancheva et al., 2016; Egoraeva et al., 2026).

Despite the rapid growth in composite nanomaterial usage, direct toxicological data on ZnO-SiO₂ nanoparticles in freshwater fish remain scarce. Existing literature has focused predominantly on individual ZnO or silica nanoparticles, with limited investigation of hybrid nanocomposites whose altered stability, dissolution kinetics, and surface reactivity may yield toxicity profiles distinct from their single-component counterparts (Rashidian et al., 2023; Jaies et al., 2024). Furthermore, integrated studies that simultaneously examine hematological, biochemical, and histopathological endpoints in commercially important species such as *C. idella* are particularly limited (Mahmud & Haque, 2025). Considering the growing industrial and agricultural applications of ZnO-SiO₂ nanoparticles and their potential discharge into freshwater environments, a systematic assessment of their biological impact is essential. The present study therefore aimed to experimentally examine the systemic and organ-specific responses of grass carp to dietary ZnO-SiO₂ nanoparticle exposure, in order to inform environmental risk assessment and support sustainable aquaculture management. The specific objectives were: (i) to evaluate the effects of dietary ZnO-SiO₂ nanoparticles on hematological parameters of *C. idella*; (ii) to determine the impact on serum biochemical markers; and (iii) to examine histopathological alterations in kidney and intestinal tissues following dietary nanoparticle exposure.

2. Literature Review

Engineered nanoparticles are increasingly present in modern industries owing to their unique physicochemical characteristics—nanoscale size, enhanced surface reactivity, and tunable catalytic activity—which enable their incorporation into paints, cosmetics, medical devices, food packaging, and agricultural formulations (Joudeh & Linke, 2022). Aquatic environments serve as the primary sink for many engineered nanomaterials through industrial effluents, wastewater discharge, stormwater runoff, and agricultural drainage. Wastewater

treatment plants, while partially effective for particulate removal, are not designed to eliminate nanoscale substances completely, allowing residual nanoparticles to reach receiving water bodies (Verma et al., 2024). Once in aquatic systems, nanoparticles undergo aggregation, sedimentation, dissolution, and interactions with dissolved organic matter that govern their stability, mobility, and bioavailability to aquatic life (Harrison et al., 2023; Bharti et al., 2022).

The physicochemical properties of zinc oxide and silica nanoparticles determine their environmental behavior and biological interactions. ZnO nanoparticles are semiconducting, photocatalytic, and UV-absorbing, with high surface reactivity (Kareem et al., 2022). They partially dissolve in aqueous media to release Zn²⁺ ions, with dissolution kinetics governed by pH, ionic strength, and temperature—a key driver of their biological toxicity (Albarakaty et al., 2023). Silica nanoparticles, in contrast, are chemically inert, thermally stable, and possess high surface area with abundant silanol groups that enable surface functionalization (L. Wang et al., 2022). When combined as ZnO-SiO₂ nanocomposites, the silica matrix can modulate zinc ion release, enhance structural stability, and reduce aggregation, thereby altering bioavailability, cellular uptake, and overall toxicity patterns (Shaba et al., 2023; Hassan et al., 2022).

Toxicokinetic studies indicate that metal-based nanoparticles are absorbed by fish primarily through the gills, gastrointestinal tract, and to a lesser extent the integument (Naveed et al., 2025; Adilbekov et al., 2025). Following uptake, nanoparticles or released metal ions enter systemic circulation and distribute to the liver, kidney, spleen, brain, and muscle, with the liver being the principal target organ due to its central role in detoxification and biotransformation (Priyadarshi et al., 2026; Tang et al., 2025). Nanoparticles may undergo biotransformation including partial dissolution, protein corona formation, and cellular interactions that modify their biological identity and influence immune activation (Y. Li & Chen, 2025). Excretion occurs via biliary, renal, and fecal routes, with effectiveness dependent on particle size and aggregation state; partial elimination can lead to

bioaccumulation, particularly under chronic exposure (Lin et al., 2025).

Oxidative stress is widely recognized as a central mechanism of nanoparticle-induced toxicity in aquatic organisms. Metal-based nanoparticles catalyze redox reactions at their surfaces, generating superoxide radicals, hydrogen peroxide, and hydroxyl radicals that overwhelm antioxidant defenses including superoxide dismutase, catalase, and glutathione (Cao et al., 2025). ZnO nanoparticles generate ROS through photocatalytic reactions and Zn^{++} ion-mediated disruption of mitochondrial electron transport, leading to ATP depletion and impaired cellular energy metabolism (Kotb et al., 2025). Lipid peroxidation damages cell membrane integrity, while protein and DNA oxidation disrupt enzymatic activity and replication, ultimately triggering apoptosis or necrosis (Uti et al., 2025; Xiaoting Zhou et al., 2025).

Hematological and biochemical parameters are well-established indicators of physiological stress in fish exposed to environmental pollutants. Exposure to metal-based nanoparticles commonly alters erythrocyte counts, hemoglobin, hematocrit, and leukocyte numbers, indicating disrupted oxygen transport capacity and immune activation (Pitocco et al., 2025; Shyam et al., 2025). Serum biochemical markers reveal organ-specific toxicity: elevated liver enzymes signal hepatocellular damage; increased urea and creatinine indicate impaired renal filtration; altered protein fractions reflect disrupted hepatic protein synthesis or immune consumption (Kumar et al., 2026; Boima et al., 2025). In freshwater fish, where osmoregulation is critical, renal failure can have severe systemic consequences including electrolyte imbalance and nitrogenous waste accumulation (Ávila et al., 2025; Okuthe & Siguba, 2025).

The liver and kidney are principal target organs following systemic nanoparticle exposure. Hepatic manifestations include hepatocellular vacuolation, sinusoidal dilation, necrosis, and inflammatory infiltration, often accompanied by elevated serum transaminases (Shao et al., 2025; Xiaoting Zhou et al., 2025). Renal pathology encompasses tubular degeneration, glomerular shrinkage and atrophy, interstitial inflammation, and hemorrhage, which impair

filtration and nitrogen metabolism (Ugrin et al., 2025; Q. Chen et al., 2025). Comparative studies indicate that single-component nanoparticles (e.g., ZnO alone) often exhibit rapid ion release and high acute reactivity, whereas silica encapsulation in composites may attenuate dissolution but enhance dispersion, producing distinct toxicity profiles that cannot be predicted from individual component studies alone (Ashour et al., 2025; Cheepborisutikul et al., 2025; Fernández-Álvarez et al., 2025).

A substantial body of literature documents ZnO nanoparticle toxicity in freshwater fish species. Mechanistic studies consistently report ROS generation, lipid peroxidation, depleted antioxidant enzymes, hematological abnormalities (decreased erythrocytes, hemoglobin, hematocrit), elevated liver and renal biomarkers, and gill lesions including lamellar fusion and epithelial hyperplasia (Zarate-Insúa et al., 2025; Khaleda et al., 2025; Babakhani et al., 2025; Suhendra et al., 2025). Silica nanoparticles are comparatively less toxic but can still induce moderate oxidative responses, hematological shifts, and histological alterations in gill, liver, and kidney tissues at elevated concentrations or prolonged exposure (Latini et al., 2025; Hussain et al., 2025; Salaah et al., 2025). Bioaccumulation has been documented in liver, kidney, and gill tissue, with distribution influenced by particle size and dissolution characteristics (Rajeh, 2026; Ghannam et al., 2025).

Despite these advances, several critical research gaps remain. First, toxicological data on ZnO-SiO₂ nanocomposites—as opposed to single-component nanoparticles—in freshwater fish are scarce; interaction between ZnO and SiO₂ within a composite matrix can alter dissolution kinetics, surface reactivity, bioavailability, and cellular uptake, potentially producing toxicity profiles unlike those of individual constituents (Mahmud & Haque, 2025). Second, integrated studies simultaneously examining hematological, biochemical, and histopathological endpoints in economically important species such as *C. idella* are notably limited. Third, variability in experimental design—exposure duration, concentration gradients, water chemistry, and species-specific physiology—undermines inter-study

comparability. The present study addresses these gaps by evaluating sub-chronic dietary ZnO-SiO₂ nanoparticle exposure in *C. idella* using a multi-endpoint assessment framework.

3. Materials and Methods

3.1 Experimental Fish and Laboratory Conditions

Healthy fingerlings of *Ctenopharyngodon idella* were obtained from a certified fish hatchery and transported to the laboratory in aerated containers. Upon arrival, fish were visually

inspected to confirm freedom from disease and external abnormalities. Fish were acclimatized for two weeks prior to the experiment in glass aquaria containing dechlorinated tap water with continuous aeration. Water quality was maintained by replacing approximately 80% of the water daily to ensure proper hygiene and reduce accumulation of metabolic waste. Environmental conditions were kept consistent for all groups throughout the acclimatization and experimental periods (Figure 1).



Figure 1. Experimental fish (*Ctenopharyngodon idella* fingerlings) acclimatized in glass aquaria prior to dietary ZnO-SiO₂ nanoparticle exposure trial.

3.2 Experimental Design

After acclimatization, fish were randomly distributed into four experimental groups comprising one control group and three treatment groups. The experimental trial was conducted for 45 days to represent a sub-chronic exposure period allowing evaluation of cumulative physiological and histopathological effects. The control group received a basal diet without nanoparticle supplementation. Treatment groups received diets supplemented with ZnO-SiO₂ nanoparticles at graded concentrations: T1 (50 mg per 0.5 kg feed), T2 (100 mg per 0.5 kg feed), and T3 (200 mg per 0.5 kg feed). All groups were maintained under identical management and environmental conditions. The selected concentrations

represented low, moderate, and high exposure levels designed to evaluate dose-dependent responses and to simulate environmentally relevant exposure scenarios based on preliminary studies and previously reported literature.

3.3 Characterization of ZnO-SiO₂ Nanoparticles

The ZnO-SiO₂ nanoparticles used in this study were obtained from a certified commercial supplier. According to manufacturer specifications, the nanoparticles exhibited an average particle size in the nanometer range (typically < 100 nm) with predominantly spherical morphology. The composite structure consisted of zinc oxide nanoparticles embedded within or coated by a silica matrix that enhances

stability and reduces aggregation. Prior to incorporation into the experimental diet, nanoparticles were handled under controlled laboratory conditions to minimize agglomeration. Although advanced characterization techniques such as scanning electron microscopy (SEM) or transmission electron microscopy (TEM) were not performed in the present study, physicochemical properties were considered based on supplier data and previously published literature.

3.4 Preparation of Experimental Diet

Experimental diets were prepared using a premixing method to ensure uniform

incorporation of ZnO-SiO₂ nanoparticles. The required quantity of nanoparticles for each treatment group was accurately weighed and initially mixed with a small portion of basal feed to prepare a premix. This premix was thoroughly blended with the remaining feed to achieve homogeneous distribution. The feed was subsequently pelleted using a mechanical pelletizer to produce uniform pellets suitable for fish feeding. To prevent nanoparticle leaching and enhance adherence, pellets were coated by spraying a gelatin solution over their surface and allowed to dry at room temperature. The control diet was prepared identically without nanoparticle supplementation to ensure procedural uniformity (Figure 2).



Figure 2. Preparation of experimental diets for each treatment group using the premixing method with gelatin-coated pelleting to ensure uniform ZnO-SiO₂ nanoparticle distribution.

3.5 Feeding and Management During the Experimental Trial

Fish were fed once daily throughout the 45-day experimental period. All groups received pelleted feed under identical feeding conditions. During the initial three days of nanoparticle exposure, increased fecal output was observed in treated groups compared with the control, indicative of early gastrointestinal irritation or altered gut motility. Continuous aeration was provided throughout the experiment to maintain adequate dissolved oxygen levels. Approximately 80% of the water was replaced daily to maintain water quality and minimize stress on the fish.

3.6 Blood Collection and Hematological Analysis

At the end of the experimental period, fish were fasted for 24 hours prior to blood sampling to minimize dietary influence on blood parameters. Fish were anesthetized before sample collection to reduce handling stress. Blood samples were collected from the caudal vein using sterile disposable syringes (Figure 3). For hematological analysis, a portion of blood was transferred into tubes containing anticoagulant. Hematological parameters including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were

determined using standard hematological procedures and appropriate laboratory techniques (H. Chen et al., 2019).



Figure 3. Blood collection from the caudal vein of *Ctenopharyngodon idella* using sterile disposable syringes following anesthesia.

3.7 Serum Biochemical Analysis

For biochemical analysis, blood samples without anticoagulant were allowed to clot at room temperature and centrifuged to separate serum. The separated serum was carefully collected and stored under refrigerated conditions until analysis (Figure 4). Serum biochemical

parameters including urea, creatinine, total protein, albumin, globulin, and cholesterol were measured using commercially available diagnostic kits according to the manufacturer's instructions. All analyses were performed under controlled laboratory conditions to ensure accuracy and reproducibility (Sun et al., 2017).



Figure 4. Blood sample preparation workflow for hematological and serological analysis, showing anticoagulant-treated and clotting tubes used for serum separation.

3.8 Histopathological Examination

At the termination of the experiment, fish were dissected to collect kidney and intestinal tissues for histological evaluation (Figure 5).

Immediately after dissection, tissues were fixed in 10% neutral buffered formalin to preserve structural integrity. Fixed tissues were processed through graded alcohol dehydration, cleared in

xylene, and embedded in paraffin wax. Sections of approximately 4-5 μm thickness were prepared using a rotary microtome and mounted on clean glass slides. Tissue sections were stained using the hematoxylin and eosin (H&E)

method. Stained slides were examined under a light microscope at 400 $\times$ magnification to assess histopathological alterations (Pandey et al., 2021).



Figure 5. Dissection of *Ctenopharyngodon idella* for collection of kidney and intestinal tissues for histopathological examination at the termination of the 45-day experimental trial.

3.9 Statistical Analysis

All data obtained from hematological and biochemical analyses were expressed as mean $\pm$ standard deviation. Statistical evaluation was carried out using one-way Analysis of Variance (ANOVA) to determine significant differences among the control and treatment groups. Differences were considered statistically significant at $P < 0.05$. In addition, post-hoc multiple comparison analysis (Tukey's test) was applied to identify significant differences among treatment groups. Statistical analyses were

performed using standard statistical software (SPSS/GraphPad Prism) ensuring accuracy and reliability of results. Homogeneity of variance was verified using appropriate tests (Brown-Forsythe) prior to interpretation.

4. Results

4.1 Hematological Parameters

Hematological analysis of *Ctenopharyngodon idella* after 45 days of dietary ZnO-SiO₂ nanoparticle exposure revealed significant alterations in selected parameters (Table 1).

Table 1. Statistical summary (one-way ANOVA) of hematological parameters in *Ctenopharyngodon idella* exposed to dietary ZnO-SiO₂ nanoparticles for 45 days.

Parameter	F-value	P-value	Significance	Trend vs Control
WBC (White Blood Cells)	6.510	0.0154	Significant*	Increase
RBC (Red Blood Cells)	1.636	0.2567	Not significant	No change
MCV (Mean Corpuscular Volume)	0.4713	0.7106	Not significant	No change
MCH (Mean Corpuscular Hemoglobin)	6.500	0.0154	Significant*	Increase

Parameter	F-value	P-value	Significance	Trend vs Control
HCT (Hematocrit)	4.813	0.0336	Significant*	Variable
HGB (Hemoglobin)	2.319	0.1519	Not significant	No change

* $P < 0.05$ compared with control. T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed. Homogeneity of variance was confirmed ($P > 0.05$) for all parameters.

4.1.1 White Blood Cells (WBCs)

Dietary exposure to ZnO-SiO₂ nanoparticles significantly influenced white blood cell counts ($F = 6.510$, $P = 0.0154$). All nanoparticle-treated groups showed a statistically significant increase in WBC count compared with the control. The most pronounced increase was observed in the

highest concentration group (T3: 200 mg/0.5 kg feed), followed by T2 (100 mg/0.5 kg feed), with T1 (50 mg/0.5 kg feed) still showing a significant but relatively smaller increase (Figure 6).

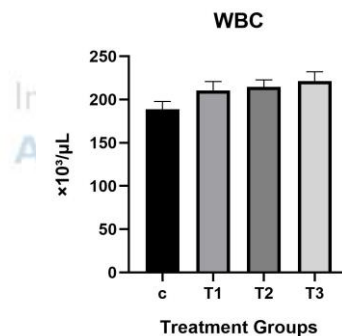


Figure 6. White blood cell (WBC) count in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 6.510$, $P = 0.0154$.

4.1.2 Red Blood Cells (RBCs)

There was no statistically significant difference in red blood cell counts between control and ZnO-SiO₂ nanoparticle-treated groups after 45 days of dietary exposure ($F = 1.636$, ($P = 0.2567$). The highest concentration group (T3) showed a slight numerical increase in RBC count, while T1 and T2 did not exhibit

significant variation and showed a slight numerical reduction compared to the control. Homogeneity of variance was confirmed ($P > 0.05$), indicating that dietary ZnO-SiO₂ nanoparticles did not produce a statistically significant change in erythrocyte number under these experimental conditions (Figure 7).

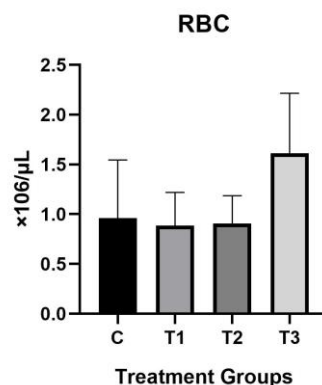


Figure 7. Red blood cell (RBC) count in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 1.636$, $P = 0.2567$ (not significant).

4.1.3 Mean Corpuscular Volume (MCV)

Mean corpuscular volume did not differ significantly between groups after 45 days of dietary exposure ($F = 0.4713$, $P = 0.7106$). Although some variations were observed in T1, T2, and T3 compared with the control, these

differences were not statistically significant. Homogeneity of variance was confirmed ($P > 0.05$), indicating that ZnO-SiO₂ nanoparticle inclusion in the feed did not cause a statistically significant change in erythrocyte volume (Figure 8).

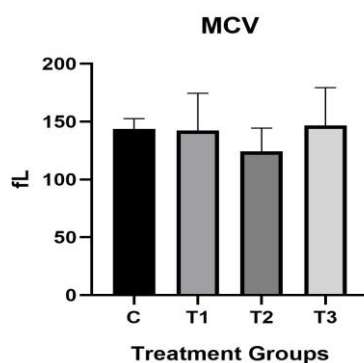


Figure 8. Mean corpuscular volume (MCV) in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 0.4713$, $P = 0.7106$ (not significant).

4.1.4 Mean Corpuscular Hemoglobin (MCH)

Mean corpuscular hemoglobin showed a statistically significant change between the control and ZnO-SiO₂ nanoparticle-treated groups ($F = 6.500$), ($P = 0.0154$). Compared with the control, treated groups exhibited a significant increase in MCH values, with the

most pronounced elevation observed in the highest nanoparticle concentration group (T3: 200 mg/0.5 kg feed), followed by T1 and T2. This increase indicates an alteration in hemoglobin incorporation in erythrocytes under nanoparticle exposure (Figure 9).

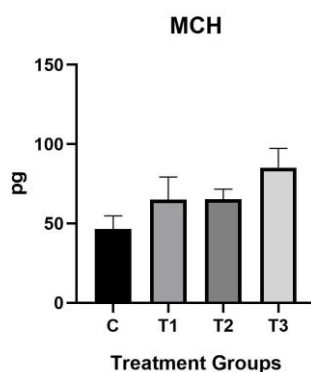


Figure 9: Mean corpuscular hemoglobin (MCH) in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 6.500$, $P = 0.0154$.

4.1.5 Hematocrit (HCT)

Hematocrit differed significantly between control and ZnO-SiO₂ nanoparticle-treated

groups at the end of the 45-day experimental period ($F = 4.813$, $P = 0.0336$). Compared with the control, treated groups showed a distinct response: a notable decrease in HCT levels was observed in the low and medium concentration groups (T1: 50 mg/0.5 kg feed and T2: 100

mg/0.5 kg feed), whereas a marked increase was recorded in the highest nanoparticle concentration group (T3: 200 mg/0.5 kg feed). The observed alterations in HCT may indicate physiological stress induced by nanoparticle exposure (Figure 10).

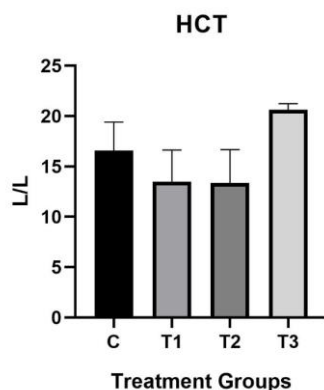


Figure 10. Hematocrit (HCT) levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 4.813$, $P = 0.0336$.

4.1.6 Hemoglobin (HGB)

Hemoglobin concentration was statistically equivalent between control and ZnO-SiO₂ nanoparticle-treated groups after 45 days of dietary exposure ($F = 2.319$), ($P = 0.1519$). Although minor numerical differences were observed in treated groups, these were not

statistically significant. The low and high concentration groups (T1 and T3) showed a slight numerical decrease in hemoglobin, while the medium concentration group (T2) showed a slight numerical increase, but these changes did not reach statistical significance. Homogeneity of variance was confirmed ($P > 0.05$) (Figure 11).

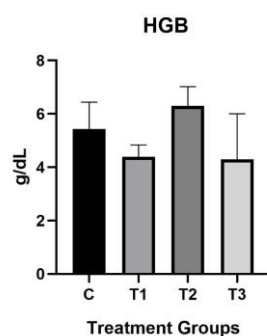


Figure 11. Hemoglobin (HGB) levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 2.319$, $P = 0.1519$ (not significant).

4.2 Serum Biochemical Parameters

Serum biochemical analysis revealed significant alterations in kidney function markers, protein fractions, and lipid metabolism following 45

days of dietary ZnO-SiO₂ nanoparticle exposure (Table 2).

Table 2. Statistical summary (one-way ANOVA) of serum biochemical parameters in *Ctenopharyngodon idella* exposed to dietary ZnO-SiO₂ nanoparticles for 45 days.

Parameter	F-value	P-value	Significance	Trend vs Control
Urea	7.318	0.0111	Significant*	Increase
Creatinine	0.6660	0.5961	Not significant	No change
Total Protein	4.116	0.0486	Significant*	Decrease
Globulin	14.27	0.0014	Significant**	Increase
Cholesterol	14.45	0.0014	Significant**	Increase
Albumin	0.7985	0.5286	Not significant	No change

* $P < 0.05$, ** $P < 0.01$ compared with control. T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed. Homogeneity of variance was confirmed ($P > 0.05$) for all parameters.

4.2.1 Urea

Serum urea levels showed significant inter-group variation ($F = 7.318$, $P = 0.0111$). All treated cohorts exhibited a significant increase in serum urea compared with the control, with T3 (200 mg/0.5 kg feed) showing the most pronounced

elevation, followed by T1 (50 mg/0.5 kg feed) and T2 (100 mg/0.5 kg feed). Homogeneity of variance was confirmed ($P > 0.05$) (Figure 12).

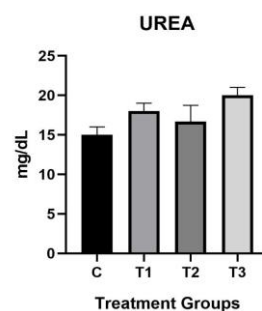


Figure 12. Serum urea levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 7.318$, $P = 0.0111$.

4.2.2 Creatinine

Serum creatinine levels did not show statistically significant changes between control and ZnO-SiO₂ nanoparticle-treated groups ($F = 0.6660$, $P = 0.5961$). Small numerical differences were

observed among treatment groups T1, T2, and T3 compared with the control, but these did not reach statistical significance. Homogeneity of variance was confirmed ($P > 0.05$) (Figure 13).

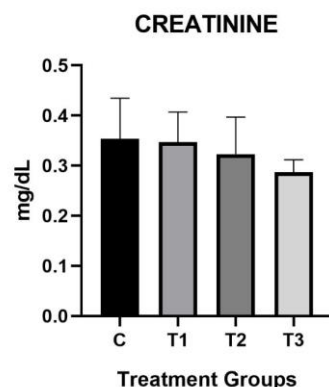


Figure 13. Serum creatinine levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA F = 0.6660, P = 0.5961 (not significant).

4.2.3 Total Protein

Serum total protein concentrations differed significantly between control and nanoparticle-treated groups (F = 4.116, P = 0.0486). Nanoparticle-treated cohorts showed a distinct response compared with the control. A statistically significant decrease in total protein concentration was observed in the low and

medium concentration groups (T1 and T2), with the most significant reduction recorded in the T2 group. Conversely, the highest concentration group (T3: 200 mg/0.5 kg feed) remained statistically equivalent to the control, exhibiting no significant reduction (Figure 14).

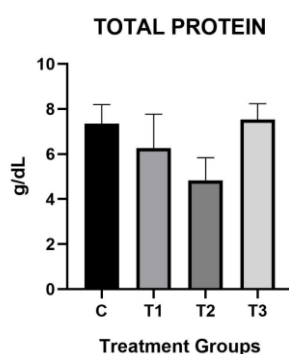


Figure 14. Serum total protein levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA F = 4.116, P = 0.0486.

4.2.4 Globulin

Serum globulin levels showed highly significant variation among groups (F = 14.27, P = 0.0014). All treated groups exhibited a statistically significant increase in globulin concentration

compared with the control. The highest concentration group (T3: 200 mg/0.5 kg feed) showed the strongest significant increase, followed by T1 and T2. Homogeneity of variance was confirmed (P > 0.05) (Figure 15).

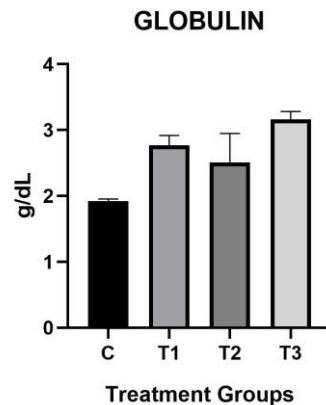


Figure 15. Serum globulin levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 14.27$, $P = 0.0014$.

4.2.5 Cholesterol

Serum cholesterol levels differed significantly between control and ZnO-SiO₂ nanoparticle-treated groups ($F = 14.45$, $P = 0.0014$). Compared with the control, the treatment groups showed a variable response: a significant

numerical decrease was observed in the lowest concentration group (T1), while the highest concentration group (T3: 200 mg/0.5 kg feed) exhibited a significant increase relative to the control. Homogeneity of variance was confirmed ($P > 0.05$) (Figure 16).

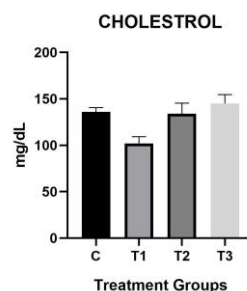


Figure 16. Serum cholesterol levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 14.45$, $P = 0.0014$.

4.2.6 Albumin

Serum albumin concentration did not significantly differ between control and ZnO-SiO₂ nanoparticle-treated groups ($F = 0.7985$, $P = 0.5286$). Minor numerical differences were observed among T1, T2, and T3 compared with

the control, but these were not statistically significant. Homogeneity of variance was confirmed ($P > 0.05$), indicating that dietary ZnO-SiO₂ nanoparticle supplementation had no significant impact on albumin levels (Figure 17).

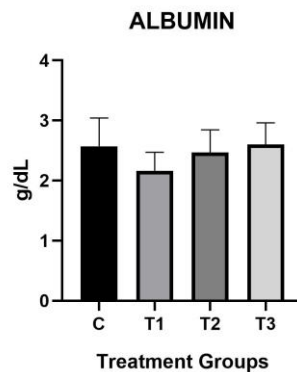


Figure 17. Serum albumin levels in *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days. Values are mean $\pm$ SD; one-way ANOVA $F = 0.7985$, $P = 0.5286$ (not significant).

4.3 Histopathological Observations

Histopathological examination of kidney and intestinal tissues provided direct visual

evidence of organ-level injury consistent with the biochemical alterations described above.

4.3.1 Kidney

Histological analysis of kidney tissue revealed significant structural changes in groups exposed to ZnO-SiO₂ nanoparticles compared with the control (Figure 18). The control group displayed normal renal architecture with intact renal tubules (RT), hematopoietic tissue (HT), and glomeruli without degeneration or congestion. T1 exhibited mild renal changes including mild tubular degeneration (TD), slight vacuolization (V), glomerular shrinkage (GS), and mild congestion (C). T2 displayed moderate renal damage with pronounced tubular degeneration (TD), epithelial degeneration (ED), vacuolization (V), and vascular congestion (C); distortion of renal tubules and moderate

alteration of glomerular structure were evident. The most severe pathological changes were observed in T3, including extensive tubular necrosis (TN), extensive tubular degeneration, extreme glomerular atrophy (GA), extreme glomerular shrinkage (GS), inflammatory cell infiltration (ICI), and hemorrhage (Hmg). Renal architecture was severely disturbed with widespread tubular epithelial degeneration and pronounced structural disarrangement. The severity of renal lesions exhibited a progressive dose-dependent pattern across groups, with the most severe lesions observed in the highest nanoparticle concentration group (T3).

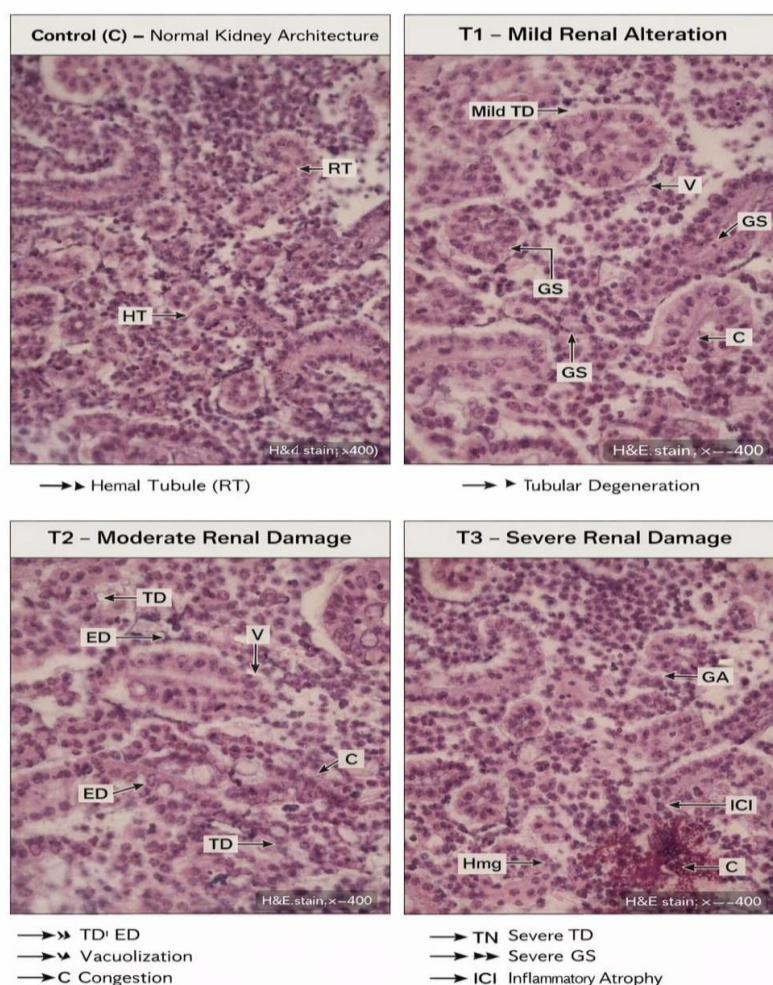


Figure 18. Histopathological alterations in kidney of *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days (H&E stain, 400×). Panels show progressive renal changes from control through T1, T2, and T3: renal tubules (RT), hematopoietic tissue (HT), tubular degeneration (TD), epithelial degeneration (ED), vacuolization (V), glomerular shrinkage (GS), congestion (C), glomerular atrophy (GA), inflammatory cell infiltration (ICI), hemorrhage (Hmg), and tubular necrosis (TN).

4.3.2 Intestine

Microscopic analysis of intestinal tissues showed progressive structural changes in groups receiving ZnO-SiO₂ nanoparticles compared with the control (Figure 19). The control group displayed normal intestinal architecture with well-structured villi, intact epithelial lining, defined mucosal folds (MF), preserved epithelial layer (EL), lamina propria (LF), and muscular layer (M). Villi were long and tightly organized without degenerative or cellular disruption. T1 exhibited mild intestinal changes including mild epithelial degeneration (MD) and slight villus shortening (VS), with early structural disturbance of the mucosal epithelium (ME) but generally preserved intestinal organization. T2

showed moderate intestinal injury with prominent villus shortening (VS), epithelial degeneration (ED), vacuolization (V), and inflammatory cell infiltration (ICI). The most severe pathological alterations were observed in T3, including extensive epithelial necrosis (EN), pronounced epithelial desquamation (E), extensive mucosal gland degeneration (MDG), and severe villus degeneration. Intestinal architecture was drastically disturbed, with shortening, distortion of villi, and widespread cellular damage. As in the kidney, lesion severity exhibited a progressive dose-response pattern, with the greatest changes observed in the highest nanoparticle concentration group (T3).

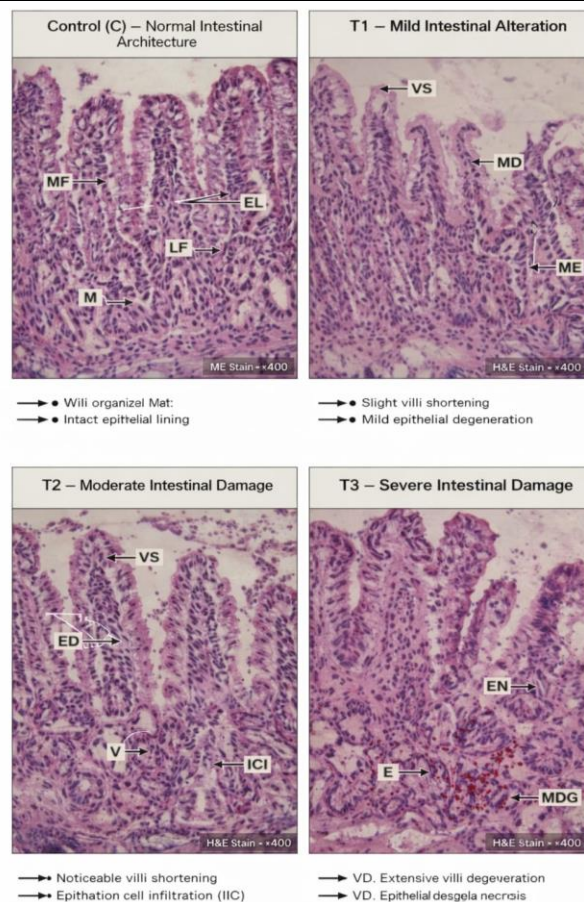


Figure 19. Histopathological alterations in intestine of *Ctenopharyngodon idella* under different dietary ZnO-SiO₂ nanoparticle treatments (Control, T1 = 50 mg/0.5 kg feed, T2 = 100 mg/0.5 kg feed, T3 = 200 mg/0.5 kg feed) after 45 days (H&E stain, 400×). Panels show progressive intestinal changes from control through T1, T2, and T3: mucosal folds (MF), epithelial lining (EL), lamina propria (LF), muscular layer (M), villi shortening (VS), mild degeneration (MD), epithelial degeneration (ED), vacuolization (V), inflammatory cell infiltration (ICI), epithelial necrosis (EN), epithelial desquamation (E), and mucosal gland degeneration (MDG).

5. Discussion

The present investigation was conducted as a controlled 45-day dietary exposure trial to evaluate the sub-chronic biological impacts of ZnO-SiO₂ nanoparticles in *C. idella* fingerlings. The experimental design used a basal diet for the control group and three graded nanoparticle-supplemented diets, with pellets prepared by premixing and surface coating using a gelatin solution to enhance nanoparticle adherence. This feeding-based exposure route was selected because ingestion is a primary pathway through which aquatic organisms encounter particulate contaminants and engineered nanoparticles in real aquatic systems (Dey et al., 2025; S. Singh et al., 2023). The daily high-rate water exchange (80%) reduced confounding stress from waste accumulation

and maintained relatively stable rearing conditions, enabling observed changes in blood indices, serum chemistry, and tissue architecture to be interpreted primarily in relation to dietary nanoparticle exposure rather than deteriorating water quality (Bathi et al., 2022). The early increase in fecal output during the first three days in treated groups is consistent with acute gastrointestinal irritation or altered gut motility typical of ingestion-based exposure to irritant particulates, likely preceding subsequent systemic effects by altering nutrient uptake, barrier integrity, and gut-organ axis immune signaling (Yassin et al., 2025).

Hematological indices are among the most sensitive physiological indicators of stress and toxicity in fish, providing an integrated measure of immune activation, oxygen-carrying capacity,

metabolism, and compensatory response to tissue damage (H. Chen & Luo, 2023). In the current study, WBC counts rose significantly in nanoparticle-treated groups relative to control. Fish leukocytosis is widely interpreted as an innate immune surveillance or inflammatory response triggered by stressors including xenobiotics, tissue damage, and infection-like stimuli (Waqar et al., 2026). Engineered nanoparticles can stimulate the immune system through several pathways: physical contact of epithelial and immune cells; ROS generation; metal ion release from metal-oxide components; and danger-associated molecular pattern signaling following cell damage (Ferdous et al., 2025). The marked increase in WBC counts observed here is consistent with inflammatory activation in the intestine and downstream kidney, supported by similar patterns of leukocyte elevation or inflammatory infiltration in fish exposed to metal-based nanoparticles (Lupu et al., 2025; Mishra et al., 2025).

In contrast to the WBC response, RBC count and hemoglobin (HGB) did not show statistically significant variation across groups, whereas hematocrit (HCT) and mean corpuscular hemoglobin (MCH) increased significantly. This pattern is biologically informative because it indicates that the oxygen-carrying system was affected through alteration of packed cell volume and erythrocyte quality rather than through a substantial decrease in RBC number or overall hemoglobin level. Decreased hematocrit in fish toxicology can result from erythrocyte membrane damage, hemodilution, impaired erythropoiesis, increased erythrocyte fragility, or plasma volume shifts secondary to osmoregulatory disturbance (Witeska et al., 2023). Nanoparticle-induced oxidative stress can cause lipid peroxidation and consequent weakening of erythrocyte membranes, decreased erythrocyte lifespan, and changes in packed cell volume without necessarily altering RBC count within a sub-chronic period (Barzegar et al., 2022). The marked elevation in MCH—the amount of hemoglobin per cell—is commonly linked to robust compensatory hemoglobin incorporation, disrupted iron handling, or altered erythrocyte maturation under stress (Obeagu et al., 2024; Makhdoumi et al., 2020).

The fact that MCV was not significantly altered suggests that erythrocyte size remained stable; the observed perturbation therefore reflects altered hemoglobinization per cell rather than macrocytic or microcytic anemia. Collectively, the hematological profile of significant leukocytosis with selective impairment of HCT and MCH suggests combined inflammatory activation and sub-lethal disruption of erythrocyte integrity and oxygen-carrying capacity (Witeska et al., 2023).

Serum biochemical markers provide additional mechanistic insight by reflecting organ function and metabolic status, often preceding or mirroring histological damage (Lucarelli et al., 2019). Serum urea increased significantly in nanoparticle-exposed groups, while creatinine did not. Elevated urea in fish is generally associated with impaired renal excretion or elevated protein catabolism under stress (Jena et al., 2025). Because urea handling is tightly linked to nitrogen metabolism and renal filtration/excretion capacity, the elevated urea can be attributed to the kidney lesions observed histologically—tubular degeneration, glomerular changes, hemorrhage, and inflammation. Tubular damage disrupts reabsorption and secretion, while glomerular injury impairs filtration, both promoting retention of nitrogenous waste and altered serum urea (Boima et al., 2025). The lack of significant change in creatinine does not exclude renal involvement; rather, it may reflect species-specific sensitivity, as creatinine in fish is often less responsive or more variable than urea, particularly under sub-chronic conditions where partial compensation may occur (Bojarski et al., 2025). Renal damage may manifest in a marker-specific manner—some toxins elevate urea earlier due to metabolic derangements and tubular dysfunction, while creatinine may remain within normal range until greater filtration loss occurs or exposure is prolonged.

The serum protein profile further supports systemic stress and active immune involvement following nanoparticle exposure. Serum globulin levels increased significantly, which reflects cellular immune activation and a robust humoral response under nanoparticle-induced stress. This significant rise in the globulin fraction aligns with the marked leukocytosis

observed in the blood, indicating a coordinated defense mechanism. However, total protein decreased, indicating that the decline was primarily driven by other protein fractions, such as albumin adjustments, or enhanced protein catabolism to meet metabolic energy demands under toxicity. This highlights a specific, demand-driven shift in protein homeostasis rather than a generalized collapse of hepatic synthesis (Samal et al., 2025).

. Serum cholesterol differed significantly in nanoparticle-treated groups, providing evidence of altered lipid metabolism and possibly membrane remodeling under stress. Hypercholesterolemia in pollutant-exposed fish can represent a metabolic adjustment associated with stress hormones, disrupted hepatic lipid regulation, or compensatory membrane stabilization responses when oxidative damage threatens cellular membranes (Cai et al., 2023). Metal oxide nanoparticles are well-documented ROS generators that increase lipid peroxidation risk; in response, organisms may alter lipid handling and cholesterol distribution as part of membrane repair and stabilization mechanisms. Cholesterol is a key structural component of membranes; altered serum cholesterol can therefore plausibly accompany tissue-level injury where membrane turnover and repair demand increase. In parallel, compromised intestinal absorption and inflammatory signaling can shift lipid metabolism, as intestinal epithelial disruption affects nutrient uptake and enterohepatic lipid handling (Desouky et al., 2025).

Histopathological findings provide direct visual confirmation of organ injury and often explain functional marker shifts. In the kidney, treated groups showed progressive lesions including tubular degeneration, glomerular shrinkage/atrophy, congestion, inflammatory infiltration, hemorrhage, and necrosis—all typical of toxicant-induced nephropathy in fish—reflecting both direct cytotoxicity to renal epithelial cells and secondary injury due to vascular disturbance and inflammation (Okuthe & Siguba, 2025). Tubular degeneration and necrosis reduce the functional capacity of the nephron to maintain ionic balance and excrete wastes, providing a plausible mechanistic basis for elevated urea. Glomerular shrinkage and

atrophy suggest impaired filtration surface and altered hemodynamics. Congestion and hemorrhage indicate vascular compromise that can exacerbate hypoxic microenvironments and amplify oxidative injury. Importantly, fish kidneys contain hematopoietic tissue, so renal injury can indirectly influence blood cell regulation and contribute to subtle erythrocyte-related changes even when RBC and HGB remain statistically stable (Mansour et al., 2025). Intestinal histopathology demonstrated clear progression from normal villus organization in control fish to villus shortening, epithelial degeneration, vacuolization, inflammatory infiltration, epithelial necrosis, desquamation, and mucosal gland degeneration in treated groups. These changes are highly consistent with dietary exposure because the intestine is the first major contact site for ingested particles; epithelial surfaces can be damaged by physical abrasion, chemical toxicity, and local oxidative stress (Xiaoxue Zhou et al., 2025). Villus shortening directly reduces absorptive surface area, potentially compromising nutrient uptake and contributing indirectly to reduced serum proteins. Epithelial degeneration and necrosis reflect loss of barrier integrity that can permit translocation of luminal contents and amplify inflammation. The presence of inflammatory infiltration aligns with elevated WBC counts in blood, and mucosal gland degeneration implies impaired mucous secretion and protective mucosal function, increasing vulnerability to further irritation.

A notable feature of the present histopathology is the apparent progressive, linear dose-response pattern, with the most severe lesions occurring in the highest concentration group (T3) rather than the lower treatments (T1 and T2). Nanoparticle toxicology frequently displays such clear responses due to aggregation behavior, altered bioavailability, and interactions with organic coatings and feed matrices (Ejazi et al., 2023). At higher inclusion levels, nanoparticles may aggregate more densely on pellet surfaces despite gelatin coating, forming larger clusters less readily internalized by enterocytes and less capable of crossing mucosal barriers, thereby reducing tissue penetration relative to a more dispersed low-dose distribution. Conversely, at lower inclusion levels, nanoparticles may

remain more evenly dispersed and bioavailable within the feed matrix, enabling greater effective exposure at the cellular interface even at nominally lower doses. Feeding behavior may also contribute: if higher nanoparticle content reduced palatability, fish in higher-dose groups might ingest less feed, inadvertently lowering the internalized dose. These explanations are consistent with established principles emphasizing that delivered and bioavailable doses can diverge substantially from nominal dose due to particle behavior and exposure dynamics (Yang et al., 2021).

Mechanistically, the collective profile of altered blood indices, serum chemistry shifts, and organ lesions is consistent with oxidative stress and inflammation as central drivers of ZnO-SiO₂ nanoparticle toxicity in *C. idella*. Metal oxide nanoparticles, including ZnO-based materials, generate ROS at particle surfaces or through released ions that attack proteins, lipids, and DNA and disrupt membrane integrity (Bezerra et al., 2023; Yosa & Wibowo, 2023). Oxidative injury triggers inflammatory cascades and leukocyte mobilization, explaining elevated WBC counts and inflammatory infiltration in tissues. Membrane damage and metabolic disruption alter lipid handling and elevate cholesterol. Protein synthesis impairment, altered nutrient absorption due to intestinal injury, and immune protein dysregulation reduce total protein concentration while significantly elevating globulin fractions. Kidney tubular and glomerular injury elevates urea even when creatinine remains stable. The observed erythrocyte-related shifts (decreased HCT and MCH without major RBC or HGB change) are compatible with oxidative stress affecting erythrocyte quality and packed cell volume rather than inducing rapid, overt anemia. These findings highlight the importance of dietary exposure models and multi-endpoint assessment approaches for understanding ecological risk in economically important freshwater fish species (Gao et al., 2025; Rudramurthy & Swamy, 2018; Xu et al., 2021).

6. Conclusion

The present study demonstrated that 45-day dietary exposure to ZnO-SiO₂ nanoparticles produces marked physiological, biochemical,

and histopathological alterations in *Ctenopharyngodon idella*. Significant elevation in white blood cell counts, together with a distinct alteration in hematocrit level and a significant increase in corpuscular hemoglobin, indicates activation of immune mechanisms and disturbance of erythrocyte-related indices under nanoparticle-induced stress. Serum biochemical changes—including significant increases in urea and cholesterol and globulin alongside significant decrease in total protein reflect impairment of renal function, metabolic imbalance, and disruption of protein homeostasis, while creatinine and albumin remained statistically unchanged. Histopathological evaluation of kidney and intestine revealed progressive degenerative changes including tubular degeneration, glomerular alterations, inflammatory infiltration, villus shortening, epithelial degeneration, and necrosis in treated groups. Collectively, these findings confirm that dietary exposure to ZnO-SiO₂ nanoparticles induces sub-chronic toxic effects in grass carp by altering hematological parameters, serum biochemistry, and tissue structure. The results highlight the potential ecological risks associated with uncontrolled release of nanoparticles into aquatic environments, particularly in aquaculture systems, and emphasize the need for risk assessment frameworks that integrate biochemical, hematological, and histopathological endpoints to evaluate nanocomposite safety in freshwater fish.

7. Limitations

The present study has several limitations that should be acknowledged. First, advanced physicochemical characterization techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and zeta potential analysis were not performed on the ZnO-SiO₂ nanoparticles; instead, physicochemical properties were based on manufacturer specifications and previously published literature, which limits the ability to confirm particle size distribution, morphology, and aggregation state in the experimental diet. Second, the trial examined a single fish species (*Ctenopharyngodon idella*) and a single sub-

chronic exposure duration (45 days), constraining generalizability to other species, life stages, and chronic exposure scenarios. Third, oxidative stress biomarkers including reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were not directly quantified, so the proposed oxidative stress mechanism is inferred from hematological, biochemical, and histopathological evidence rather than from direct molecular measurement. Fourth, tissue bioaccumulation of zinc was not quantified, which would have strengthened the link between internal dose and observed toxicity. Fifth, the observed progressive dose-dependent pattern requires further validation to completely map precise interaction kinetics, particle aggregation dynamics in the feed matrix, or potentially subtle palatability-driven differences in feed intake. Sixth, the absence of recovery/reversibility observations limits conclusions about the persistence of effects after exposure cessation.

8. Future Work

Future research should pursue several complementary directions to extend the present findings. First, molecular-level investigations are needed to quantify oxidative stress biomarkers (ROS, MDA, SOD, CAT, GSH), inflammatory cytokines, and gene expression profiles (e.g., antioxidant response genes, apoptosis markers, immune signaling genes) to confirm the mechanistic role of oxidative stress and inflammation in ZnO-SiO₂ nanoparticle toxicity. Second, long-term chronic exposure studies exceeding 45 days, combined with environmentally realistic concentration gradients, would better characterize cumulative effects, bioaccumulation kinetics, and potential ecological risks under field-relevant scenarios. Third, tissue-specific bioaccumulation of Zn and Si should be quantified across gill, liver, kidney, intestine, and muscle using inductively coupled plasma mass spectrometry (ICP-MS) to clarify dose-distribution-toxicity relationships. Fourth, direct comparative studies of single ZnO, single SiO₂, and ZnO-SiO₂ nanocomposites are needed to isolate composite-specific effects and to test whether silica encapsulation genuinely attenuates or modifies ZnO toxicity. Fifth,

mesocosm and field studies simulating complex aquatic chemistry—dissolved organic matter, ionic strength, pH variation, and trophic interactions—would improve ecological realism. Sixth, recovery and reversibility studies following exposure cessation would determine whether observed hematological, biochemical, and histopathological effects persist or reverse. Finally, screening of protective agents (e.g., dietary antioxidants, selenium nanoparticles, or plant-based supplements) alongside ZnO-SiO₂ exposure could identify mitigation strategies for aquaculture systems vulnerable to nanoparticle contamination (Adilbekov et al., 2025; Yasin et al., 2025).

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