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Physiological Biomarkers of Silver Nanoparticle Toxicity in the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Despite their increasing use across various industries, concerns remain about the environmental and health risks associated with nanoparticles (NPs). Among these, silver nanoparticles (AgNPs) are widely applied for their antifungal and antibacterial properties and are incorporated into numerous consumer products. However, limited knowledge exists regarding their toxicity in aquatic environments, prompting the current study. This study investigated the effects of AgNPs on the Nile tilapia (*Oreochromis niloticus*), exposed to concentrations of 0, 1, 3, 6, and 12 µg/L of AgNPs dissolved in aquarium water for two weeks. Hematological and biochemical parameters, histological examinations, and quantitative real-time PCR (qRT-PCR) analyses were performed. Results indicated dose-dependent effects on immune and blood cell parameters. Immune cell activation—particularly increased levels of neutrophils and monocytes—was observed. At 6 µg/L, red blood cell (RBC) count and hematocrit levels increased, while hemoglobin levels remained relatively stable, with a slight increase at the same concentration. Blood glucose remained relatively stable at lower concentrations (1, 3, and 6 µg/L) but dropped significantly at 12 µg/L. Triglyceride levels increased significantly with AgNP exposure, whereas cholesterol levels decreased significantly at higher concentrations. Among liver enzymes, alanine aminotransferase (ALT) levels showed a significant increase from 3 µg/L onwards, while aspartate aminotransferase (AST) levels increased significantly even at 1 µg/L. Furthermore, a progressive increase in IL-1β gene expression was observed with increasing AgNP concentrations, peaking at 12 µg/L. In conclusion, AgNPs can be considered safe at lower concentrations, particularly up to 6 µg/L, but caution is advised at higher levels due to their adverse hematological, biochemical, and immunological effects.

INTRODUCTION

Numerous types of nanoparticles (NPs) are now extensively used worldwide **(Yan *et al.*, 2012; Yalsuyi *et al.*, 2017)**. Defined as particles with diameters ranging from 1 to 100 nanometers, NPs possess unique physicochemical properties that distinguish them from their bulk counterparts **(Blaise *et al.*, 2008)**. Despite their expanding applications

across various industries, concerns over the environmental and health-related risks associated with NPs continue to grow **(Asharani *et al.*, 2008; Yalsuyi and Vajargah, 2017)**. As NP production increases, so too does the potential for environmental exposure, particularly in aquatic ecosystems where they may cause harm to aquatic organisms **(Klaine *et al.*, 2008; Choi *et al.*, 2010)**. This has given rise to the emerging field of aquatic nanoecotoxicology, which focuses on studying the ecological impacts of nanoparticles on aquatic environments.

Among the most widely used nanoparticles are silver nanoparticles (AgNPs), which are incorporated into approximately 300 commercial products, including pharmaceuticals, textiles, and inks, due to their well-documented antibacterial and antifungal properties **(Mohsenpour *et al.*, 2020)**. The production and use of AgNPs have grown rapidly, with a projected increase of up to 63% by 2024 **(Inshakova, 2017)**. While their antimicrobial activity has been the subject of significant research **(Vali *et al.*, 2020; Mahmoudabadi *et al.*, 2021; Rashidian *et al.*, 2021)**, their release into water systems—particularly through activities such as washing—raises concerns about environmental contamination.

For over a decade, insufficient data on the toxicity of AgNPs to aquatic organisms has been a major concern **(Ale *et al.*, 2018a)**. Concentrations of AgNPs in aquatic systems have been reported to range from micrograms to milligrams per liter **(Gottschalk *et al.*, 2013)**. Their small size and surface reactivity contribute significantly to their toxicity and persistence in aquatic environments **(Ji *et al.*, 2007; Ibrahim, 2020)**. In water, silver may exist in four oxidation states—Ag^{0}, Ag^{+}, Ag^{2+}, and Ag^{3+}—but most studies attribute the toxic effects primarily to the release of Ag^{+} ions, which can be readily absorbed by aquatic organisms **(Drake & Hazelwood, 2005; Cáceres-Vélez *et al.*, 2019; Ibrahim, 2020)**. Even at low concentrations, ionic silver has been shown to harm bacteria and negatively affect freshwater and marine fish **(Waalewijn-Kool *et al.*, 2014)**.

The external fertilization mechanism of fish makes them especially vulnerable to waterborne contaminants such as AgNPs, which may interfere with reproductive success **(Johari, 2014)**. Several studies have demonstrated that metal-based NPs, including AgNPs, can accumulate in fish organs, leading to oxidative stress, impaired physiological functions, and reproductive disruption **(Savorelli *et al.*, 2017; Gobi *et al.*, 2018; Ninan *et al.*, 2020)**. Documented toxic effects of AgNPs include adverse outcomes in species such as the Nile tilapia (*Oreochromis niloticus*), rainbow trout, and common carp **(Johari, 2014; Kakavand *et al.*, 2020; Vali *et al.*, 2020)**.

Tilapia, belonging to the freshwater fish family *Cichlidae*, are native to Africa but have been introduced to many tropical, subtropical, and temperate regions since the mid-20th century **(El-Sayed & Fitzsimmons, 2023)**. Today, tilapia are cultivated in over 120

countries and represent the second most important group of farmed bony fish, surpassed only by carp. In 2022, global tilapia production reached 6.55 million metric tons, valued at over 12 billion USD, with the Nile tilapia (*O. niloticus*) accounting for 5.3 million metric tons of that total **(FAO, 2024)**. This species alone constitutes more than 80% of global farmed tilapia production.

The Nile tilapia are known for their resilience to harsh environmental conditions, including polluted waters, making them effective bioindicators for aquatic contaminants **(Ma *et al.*, 2018)**. Given their ecological and economic importance, this study aimed to investigate the chronic toxicity of AgNPs on the hematological and biochemical profiles of *O. niloticus* during a 14-day exposure period. Plasma biochemical parameters serve as reliable biomarkers for fish health **(Ramírez-García *et al.*, 2018; Burgos-Aceves *et al.*, 2019)**, while hematological analysis offers a non-lethal and efficient method for evaluating general health status in fish **(Kumar *et al.*, 2022)**.

MATERIALS AND METHODS

Characterization of colloidal AgNPs

Plasmonic silver nanoparticles (AgNPs) used in this study were obtained from NanoTech Egypt for Photo-Electronics Communication Center (Brand: NT-SNP).

- Size and Shape: Transmission electron microscopy (TEM) was conducted using a JEOL JEM-2100 high-resolution TEM operating at 200 kV to determine the size and morphology. The AgNPs had an average diameter of 45 ± 5 nm and exhibited a spherical-like shape.
- Optical Properties: UV-Vis absorption spectra were recorded using an Ocean Optics USB2000+VIS-NIR fiber optic spectrophotometer.
- General Characteristics: The AgNPs were yellow in appearance, water-soluble, and provided as a colloidal solution.

Fish and experimental facility

The experiment was conducted at the Fishery Physiology Laboratory, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Juvenile Nile tilapia (*Oreochromis niloticus*), with an average initial weight of 15.08 ± 0.88 g, were obtained from the El-Mex Experimental Station of NIOF.

Fish were randomly distributed into triplicate groups, with eight fish per 12-liter glass tank, under a 12 h light:12 h dark photoperiod. All tanks received a continuous

supply of aerated, dechlorinated tap water maintained at 25 ± 1 °C. Fish were fed a commercial diet containing 30% crude protein at 3% of body weight daily, and feeding was suspended 24 hours before and during the experiment.

Experimental treatments included exposure to 0, 1, 3, 6, and 12 µg/L of AgNPs, directly dissolved in the aquarium water. Each tank served as an independent experimental unit. Fish were fed to apparent satiation at 09:00, 12:00, and 15:00 daily. Fecal matter was removed by daily siphoning. The exposure period lasted 14 days.

All procedures were conducted in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines and approved by the Alexandria University Ethics Committee (Approval No. AU04 601 25 11 2024).

Blood and tissue sample collection

At the end of the exposure period, three groups of eight fish were sampled from each treatment. Control fish were handled identically to treated groups to ensure uniform stress conditions. Fish were captured quickly using a hand net to minimize stress. Blood samples were collected via cardiac puncture using a heparinized glass pipette.

Collected blood was divided for hemoglobin and plasma biochemical analysis. Plasma was immediately separated by centrifugation and stored at −20 °C to prevent hemolysis. Following blood collection, fish were euthanized, and liver and gill tissues were dissected and preserved for histological analysis.

Hematological and biochemical analysis

- Hemoglobin concentration was measured using a commercial Diamond Diagnostic Kit.
- Plasma glucose was measured using the GOD-PAP enzymatic method.
- Red blood cell (RBC) and white blood cell (WBC) counts were performed according to **Schaperclaus *et al.* (1991)**. For RBC, 20 µl of blood was diluted in 3980 µl of RBC diluting solution and counted using a Neubauer Hemocytometer (Rohem, India).
- Hematological indices including mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were calculated as per **Haney *et al.* (1992)**.
- Plasma cholesterol and triglyceride levels were measured using CHOD/POD and GPO/PAP method kits, respectively.

- Liver enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was assessed following the manufacturer's protocol (Biolabo, France), using a Mispa CCXL clinical chemistry analyzer (AGAPPE, Switzerland).

Histological investigation

Liver and intestinal tissues were collected from each treatment group and fixed in 10% neutral-buffered formalin. The samples were dehydrated using a graded ethanol series (70% to 100%), cleared with xylene, and embedded in paraffin. Serial sections (5 μm thick) were prepared using a Leica RM 2155 microtome (England) and stained with hematoxylin and eosin (H&E**)** according to **El-Sayed *et al.* (2014)**.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Hepatopancreas samples were excised on ice using a sterile scalpel, snap-frozen in liquid nitrogen, and stored at −80 °C for gene expression analysis.

- RNA extraction was performed using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, USA).
- RNA concentration and purity were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, USA).
- cDNA synthesis was conducted using PrimeScript RT Master Mix (Thermo Fisher, USA).
- qRT-PCR was performed in triplicate using the QuantStudio 7 Pro Real-Time PCR System (Applied Biosystems, USA) with SYBR Premix Ex Taq (Thermo Fisher, USA).

β-actin was used as the housekeeping gene and GAPDH as the internal reference. Primer sequences and NCBI GenBank accession numbers for the target gene Interleukin-1β (IL-1β) and reference genes are listed in Table (1) .

Table 1. Primer sequence and accession number used for q-PCR analysis

GENE NAME	ACCESSION NO	FORWARD SEQUENCE (5'->3')	REVERSE SEQUENCE (5'->3')	PRIMER LENGTH
INTERLEUKIN-1 BETA (IL-1B)	XM_019365844.2	GACACTGCTTCTGAACTACAAGT	TCAGCACTGGCTCTGAAGTG	209

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0 for Windows; SPSS Inc., USA). Data normality was assessed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was employed to evaluate differences among treatment groups, with statistical significance determined at $P < 0.05$ based on 95% confidence intervals. *Post hoc* comparisons were conducted using Tukey's test. Control fish were exposed to the same level of disturbance as treated fish, including transfer to a different aquarium, while remaining in their original water conditions. Results presented in tables are expressed as mean ± standard error of the mean (SEM).

RESULTS

Hematological parameters (complete blood count "CBC")

The results showed that AgNPs exposure has dose-dependent effects on immune and blood cell parameters (Table 2). At lower concentrations (1 µg/l), activation of immune cells (neutrophils and monocytes) was observed, and at higher concentrations (6 µg/l), RBC and hematocrit levels increased. Conversely, hemoglobin levels remained mostly stable, with an insignificant increase at 6 µg/l.

Table 2. Complete blood count (CBC) of the Nile tilapia (*Oreochromis niloticus*) exposed to AgNPs for 2 weeks (mean ± SE; n = 5)

PARAMETER	CONTROL	1 µG/L AGNPS	3 µG/L AGNPS	6 µG/L AGNPS	12 µG/L AGNPS
LYMPHOCYTES (%)	44 ± 2	25 ± 3 [a, c, d, e]	41 ± 2 [a, b, d, e]	39 ± 1 [a, b, c, e]	45 ± 2 [b, c, d]
NEUTROPHILS (%)	53 ± 3	65 ± 4 [a, c, d, e]	53 ± 2 [b, e]	53 ± 1 [b, e]	50 ± 2 [a, b, c, d]
MONOCYTES (%)	2 ± 1.06	8 ± 0.90 [a, c, d, e]	5 ± 0.30 [a, b]	6 ± 0.30 [a, b, e]	4 ± 0.60 [a, b, d]
EOSINOPHILS (%)	1 ± 1.17	2 ± 0.30 [a, c, e]	1 ± 0.61 [b, d]	2 ± 0.69 [a, c, e]	1 ± 0.67 [b, d]
WBCS (10^3/CMM)	92.8 ± 1.3	90.13 ± 0.5 [a, c, d, e]	115.13 ± 0.9 [a, b, d, e]	98.1 ± 2.0 [a, b, c, e]	94.2 ± 0.9 [a, b, c, d]
RBCS (10^6/CMM)	1.15 ± 0.05	1.00 ± 0.1 [a, c, d, e]	1.20 ± 0.18 [b, d]	1.41 ± 0.25 [a, b, c, e]	1.16 ± 0.20 [b, d]

HB (G DL^{-1})	5.9 ± 0.82	5.9 ± 0.05 [c, d, e]	6.1 ± 0.80 [a, b, d]	7.2 ± 0.33 [a, b, c, e]	6.0 ± 1.11 [b, d]
PLATELETS (10^9/CMM)	110 ± 0.9	110 ± 0.5 [c, d, e]	118± 0.3 [a, b, d, e]	115± 0.5 [a, b, c, e]	112± 0.4 [a, b, c, d]
MCV(%)	159± 0.6	156 ± 0.8 [c, d, e]	150± 0.9 [a, b, d, e]	158.2± 0.5 [a, b, c]	158.2± 0.4 [a, b, c]
MCH (%)	51.3± 0.6	49 ± 0.8 [a, c, d, e]	48.6± 0.6 [a, b, d, e]	51.1± 0.5 [b, c]	50.9± 0.4 [a, b, c]
HAEMATOCRIT (%)	18.3± 0.42	18.6 ± 0.81 [c, d, e]	15.8± 0.92 [a, b, d]	22.3± 0.62 [a, b, c, e]	17.4± 0.95 [b, d]

Data are expressed as mean ± SEM. n = 8 for each experimental group
[a]$P < 0.01$ vs. control group
[b]$P < 0.01$ vs. 1 µg/L of AgNPs treated group.
[c]$P < 0.01$ vs. 3 µg/L of AgNPs treated group.
[d]$P < 0.01$ vs. 6 µg/L of AgNPs treated group.
[e]$P < 0.01$ vs. 12 µg/L of AgNPs treated group.

Biochemical parameters

This study found that silver nanoparticle (AgNP) exposure significantly influenced blood glucose, triglyceride, cholesterol, and liver enzyme levels in *Oreochromis niloticus* (Table 3).

Blood glucose levels showed no significant changes in the control group or in fish exposed to lower AgNP concentrations (1, 3, and 6 µg/L). However, a marked decrease was observed at the highest concentration of 12 µg/L.

In contrast, triglyceride levels exhibited a significant, dose-dependent increase, beginning at 1 µg/L and peaking at 267.1 mg/dL at 12 µg/L.

Cholesterol levels demonstrated a reverse trend, with a significant decrease recorded at higher AgNP concentrations. Notable reductions were observed from 6 µg/L, reaching the lowest value of 212.0 mg/dL at 12 µg/L.

Regarding liver enzyme activity, alanine aminotransferase (ALT) levels showed a significant elevation starting from 3 µg/L and continued to rise with increasing AgNP concentration. Meanwhile, aspartate aminotransferase (AST) levels increased significantly from the lowest tested concentration (1 µg/L), plateaued after 3 µg/L, and remained consistently high through 12 µg/L.

Table 3. Biochemical parameters of the blood plasma (mean ± SE; n = 5) of the Nile tilapia (*Oreochromis niloticus*) exposed to AgNPs for 2 weeks

PARAMETER	CONTROL	1µG/L AGNPS	3 µG/L AGNPS	6 µG/L AGNPS	12 µG/L AGNPS
GLUCOSE (MG/DL)	34.0±15	33.4±2.9[a,c,d,e]	34.1±5.0 [a, b, d, e]	35.7±3.0 [a, b, c, e]	30.90±0.50[a,b,c,d]
TRIGLYCERIDES (MG/DL)	188.1±0.60	221.4±0.80[a]	252.9±0.70 [a]	262.1±0.60 [a]	267.1±0.50[a]
CHOLESTEROL (MG/DL)	376.8±0.70	350.5±0.50[d, e]	325.5±0.90 [e]	225.5±0.50[a, b,]	212.0±0.80 [a, b, c]
ALT	6.1±0.90	8.7±0.9 [d]	9.6±0.5 [a, d]	11.3±0.7 [a]	14.0±0.11 [a, b, e]
AST	6.1±0.7	7.0±0.9[a, c, d, e]	8.7 ± 0.8 [a, b]	8.7 ±0.7[a, b]	8.7 ±0.7[a, b]

ALT Alanine aminotransferase, AST Aspartate aminotransferase.
Data are expressed as mean ± SEM. n = 8 for each experimental group
[a]*P* < 0.01 vs. control group
[b]*P* < 0.01 vs. 1 µg/L of AgNPs treated group.
[c]*P* < 0.01 vs. 3 µg/L of AgNPs treated group.
[d]*P* < 0.01 vs. 6 µg/L of AgNPs treated group.
[e]*P* < 0.01 vs. 12 µg/L of AgNPs treated group.

Gene expression

The data in Table (4) illustrate the impact of various concentrations of AgNPs on the expression of *IL-1β* in the Nile tilapia (*Oreochromis niloticus*) as a fold-change following 30 days of exposure. The results indicate a progressive increase in *IL-1β* expression with rising AgNPs concentrations, with the highest levels observed at the 12 µg/L exposure, suggesting a heightened inflammatory response at this concentration.

Table 4. Effects of AgNPs on *IL-1β* expression of Nile tilapia (*Oreochromis niloticus*)

PARAMETER	CONTROL	1 µG/L AGNP	3 µG/L AGNP	6 µG/L AGNP	12 µG/L AGNP
IL-1B **FOLD-CHANGE**	4.25±0.01	4.35±0.02[a, c, d, e]	4.56±0.03[a, b, c, d]	4.82±0.03[a, b, c, e]	4.85±0.01[a, b, d, e]

Data are expressed as mean ± SEM. n = 8 for each experimental group
[a]*P* < 0.01 vs. control group
[b]*P* < 0.01 vs. 1 µg/L of AgNPs treated group.
[c]*P* < 0.01 vs. 3 µg/L of AgNPs treated group.
[d]*P* < 0.01 vs. 6 µg/L of AgNPs treated group.
[e]*P* < 0.01 vs. 12 µg/L of AgNPs treated group.

Histological examination

Fig. (1) presents the histological examination of liver tissues from *Oreochromis niloticus* exposed to varying concentrations of silver nanoparticles (AgNPs), revealing a dose-dependent pattern of hepatic alterations.

In the control group, liver architecture was well-preserved, with radiating cords of polygonal hepatocytes, centrally located nuclei, and clearly defined sinusoids.

At 1 and 3 µg/L of AgNP exposure, mild cytoplasmic vacuolation and scattered pyknotic nuclei were observed, indicating early signs of cellular stress.

At 6 µg/L, moderate pathological changes became evident. These included marked cytoplasmic vacuolation, nuclear degeneration (such as pyknosis and karyorrhexis), dilated hepatic sinusoids, and mild leukocytic infiltration.

At the highest concentration of 12 µg/L, the liver exhibited severe histopathological damage. This included extensive hepatocyte necrosis, intense cytoplasmic vacuolation, hemorrhagic regions, sinusoidal congestion, and dense leukocytic infiltration, resulting in near-total disruption of normal liver architecture.

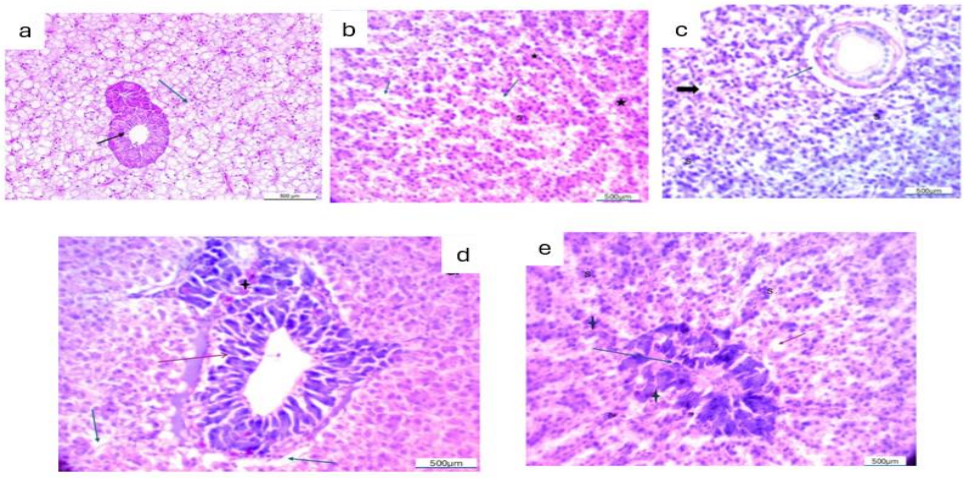

Fig. 1. Histological sections of tilapia liver from control and AgNPs exposed groups (H&E stain). (a) The control group displays normal hepatic architecture with polygonal hepatocytes, centrally located nuclei, and intact hepatic sinusoids. (b) Liver exposed to 1 µg of AgNPs shows mild cytoplasmic vacuolation (blue arrow) and early signs of nuclear pyknosis (strike). (c) Exposure to 3 µg induces hepatocellular degeneration (blue arrow), cytoplasmic vacuolization (black arrow), disorganization of hepatic cords, sinusoidal congestion (s), and prominent leukocytic infiltration. (d) At 6 µg exposure, hepatocytes exhibit moderate vacuolation, sinusoidal dilation, hemorrhage (strike), nuclear fragmentation, and inflammatory infiltration. (e) Exposure to 12 µg induces severe hepatocyte necrosis, widespread vacuolation (blue arrow), hemorrhage (strike), sinusoidal congestion (s) and complete disorganization of hepatic cords, indicating dose-dependent hepatotoxicity. Scale bar = 500 µm

Histological examination of the tilapia intestine investigated distinct dose-dependent alterations in tissue architecture in response to AgNPs exposure, compared to the control group. In Fig. (2), sections of intestinal tissue from the control group exhibited normal histological architecture. The mucosal layer displayed well-formed, tall villi lined by columnar epithelial cells with abundant goblet cells interspersed. The lamina propria contained loose connective tissue with no signs of edema or inflammation. Intestinal sections exposed to 1 and 3 µg/l of AgNPs showed slight shortening and blunting of villi, occasional epithelial lifting, and reduced goblet cells. More pronounced histological changes were observed at 6 µg/l. The intestinal villi were markedly shortened and, in some areas, fused. The epithelial lining showed focal detachment, nuclear pyknosis, and cellular necrosis. Goblet cell numbers were further reduced. The lamina propria demonstrated moderate inflammatory infiltration and dilated blood vessels. Fish exposed to 12 µg/l exhibited severe histopathological alterations. These included extensive villus atrophy, epithelial sloughing, and loss of mucosal integrity. Goblet cells were nearly depleted, compromising mucosal defense. The lamina propria showed intense leukocyte infiltration, hemorrhage, and marked tissue disorganization. The submucosa was edematous.

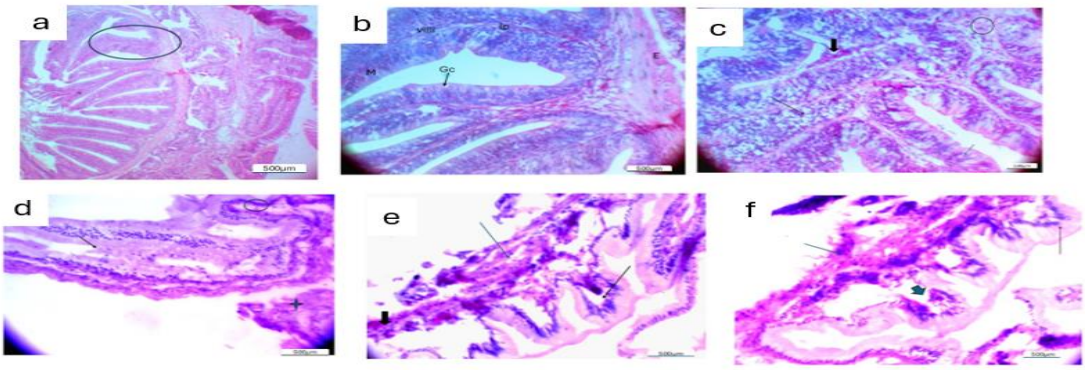

Fig. 2. Histological sections of tilapia intestine from control and AgNPs-exposed groups (H&E stain 20x & 40x). (a & b) The control group shows normal villi with intact epithelium and numerous goblet cells (Gc). (c & d) The intestines of tilapia exposed to 1 µg/l and 3 µg/l AgNPs exhibit mild villus shortening (green arrow), cytoplasmic vacuolation (thin arrow), reduced goblet cells (green arrow), submucosal fibrosis, and tissue disorganization (strike). (e) Exposure to 6 µg/L AgNPs results in villus fusion, epithelial necrosis (thick arrow), and lamina propria inflammation (black arrow). (f) Sections from intestines exposed to 12 µg/L AgNPs show severe epithelial sloughing (green arrow), villus atrophy (red arrow), submucosal fibrosis, and leukocytic infiltration (thick arrow)

DISCUSSION

The toxicity of silver nanoparticles (AgNPs) in aquatic environments is largely influenced by their bioavailability and capacity to infiltrate tissues. In this study, exposure to AgNPs in Nile tilapia (*Oreochromis niloticus*) led to several significant biochemical and physiological changes, particularly affecting lipid metabolism and liver function.

Increased triglyceride levels and decreased cholesterol levels were observed in a dose-dependent manner, highlighting disruptions in lipid homeostasis. These findings suggest an elevated energy demand in response to stress, consistent with previous research indicating that stress in fish leads to increased lipid mobilization **(Lupatsch *et al.*, 2010; Canli *et al.*, 2018)**. Liver enzyme activities—ALT and AST—also significantly increased, indicating hepatocellular damage. These enzymes are recognized biomarkers for hepatotoxicity in fish, with elevated levels often linked to cellular injury and pathological conditions **(Banaee *et al.*, 2011; Fırat *et al.*, 2011; Monfared & Soltani, 2013)**. Similar enzyme elevations have been reported following AgNP exposure in other species, including *Labeo rohita* and small mammals **(Rajkumar *et al.*, 2016; Vasanth & Kurian, 2017)**. These responses may reflect the liver's role as the primary organ for nanoparticle detoxification and accumulation **(Ale *et al.*, 2018b; Clark *et al.*, 2019)**.

Interestingly, blood glucose levels remained relatively stable at lower AgNP concentrations, suggesting that glucose metabolism was not significantly disrupted under these conditions. However, studies have shown glucose increases at higher concentrations due to nanoparticle-induced stress **(Farmen *et al.*, 2012; Valerio-García *et al.*, 2017)**. Elevated glucose is a well-established stress biomarker in fish and may result from disrupted endocrine regulation, including altered insulin secretion **(Krishnasamy *et al.*, 2022)**.

The immune response was also markedly affected. A dose-dependent increase in IL-1β expression was observed, indicating immune activation and inflammation. IL-1β is a key pro-inflammatory cytokine associated with tissue damage and cellular stress. This is consistent with findings in other species where AgNP exposure increased inflammatory markers such as TNF-α and IL-1β **(Mansour *et al.*, 2021; Seo *et al.*, 2017; Bruneau *et al.*, 2016)**. Moreover, AgNPs have been linked to oxidative stress, DNA damage, and immunosuppression across a range of aquatic organisms, including mussels and zebrafish **(Bruneau *et al.*, 2016; Bouallegui *et al.*, 2017)**.

Hematological parameters were significantly impacted by AgNP exposure. Increasing concentrations resulted in a marked reduction in RBC, WBC, and hemoglobin levels, indicating hematotoxicity. Similar outcomes have been reported in

Hypophthalmichthys molitrix and *O. niloticus* fed AgNP-contaminated diets **(Shaluei *et al.*, 2013; Rajkumar *et al.*, 2016)**. These effects are likely linked to elevated reactive oxygen species (ROS) production, impaired hematopoiesis, and cell membrane damage. DNA damage in erythrocytes and WBC necrosis through caspase activation further support these findings **(Orłowski *et al.*, 2012; Bacchetta *et al.*, 2017; Moustafa *et al.*, 2025)**. Nonetheless, exposure to lower AgNP levels (e.g., 100 µg/L) has been shown to cause no significant hematological or histological alterations in some species, highlighting a threshold effect **(Clark *et al.*, 2019)**.

Histopathological examination provided further insight into tissue-level damage. Liver tissues showed dose-dependent alterations, beginning with mild cytoplasmic vacuolation at low concentrations (1–3 µg/L), progressing to hepatocyte necrosis, hemorrhage, and architectural disruption at 12 µg/L. These observations support the liver's critical role in nanoparticle detoxification and storage **(Khalil *et al.*, 2019; Mansour *et al.*, 2021)**.

Similarly, intestinal tissues displayed a progressive pattern of damage. Mild effects such as villus blunting and epithelial lifting were seen at low concentrations, while high concentrations (12 µg/L) led to villus atrophy, goblet cell depletion, leukocytic infiltration, and submucosal edema, indicating severe mucosal disruption. Since the intestine plays a key role in nutrient and particle absorption, it is likely that AgNPs enter the bloodstream via intestinal uptake before reaching the liver **(Isani *et al.*, 2013)**. Prior studies have also confirmed the intestine as the second most affected organ following liver exposure **(Zhao & Wang, 2011; Asghari *et al.*, 2012; Moustafa *et al.*, 2025)**. Similar histological damage to liver and pancreatic tissues has been reported in fish exposed to higher AgNP doses (1–2.5 mg/L) **(Chakraborty *et al.*, 2023)**.

CONCLUSION

This study demonstrates that silver nanoparticles (AgNPs) induce a range of dose-dependent hematological, biochemical, immunological, and histopathological alterations in *Oreochromis niloticus*. While low concentrations (≤6 µg/L) may be relatively safe for short-term exposure, higher levels (12 µg/L) are clearly associated with systemic toxicity, inflammation, and organ damage.

These findings highlight the need for caution in the application of AgNPs, especially in aquatic systems. Given their widespread industrial use and potential for environmental release, further research is essential to establish safe exposure thresholds and understand long-term ecological impacts. The Nile tilapia, due to their environmental sensitivity and economic importance, serve as an effective model for assessing nanoparticle toxicity and aquatic health.

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