Transformative Impact of Green Synthesized Silver Nanoparticles on Immune Response and Histopathological Alterations in Muscles and Gills of *Labeo rohita*

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Abstract

This study evaluated the effects of varying concentrations of silver nanoparticles (Ag-NPs) on the histopathology and immune response of *Labeo rohita*. A total of 120 fish (average weight 30.00 ± 1.45 g) were divided into four treatment groups (N0 = 0 NPs, N1 = 5 mg/L NPs, N2 = 10 mg/L NPs, and N3 = 20 mg/L NPs), each with three replicates. Ag-NPs were synthesized from the guava plant (Psidium guajava) and confirmed using X-ray diffraction analysis. The fish were fed isonitrogenous (30.00%) diets for eight weeks, with Ag-NPs administered through the water. The results indicated that higher Ag-NP concentrations led to significant abnormalities in gill and muscle tissues. Haematological parameters were significantly lower ($P<0.05$) in nanoparticle-exposed groups, except for glucose, which increased. Enzymes such as superoxide dismutase and catalase were elevated in treated groups, while triglycerides, liver enzymes, and malondialdehyde levels were significantly higher ($P<0.05$). The study concluded that *Labeo rohita* is highly sensitive to Ag-NP toxicity, particularly at 20 mg/L, resulting in reduced erythrocytes, haematological abnormalities, leucocytosis, and stress, making it an effective model for evaluating nanoparticle-induced toxicity.

Keywords: Ag-NPs, Immune response, *Psidium guajava,* Growth rate, XRD

1. Introduction

Water pollution refers to the presence of external substances in water that reduce its quality or impair its effectiveness, rendering it unsuitable for sustaining life (Walker et al., 2019). Over recent decades, environmental and global public health concerns have escalated regarding ecological contamination by metals (Khallaf et al., 2018). Currently, substantial endeavors are being undertaken to boost the effectiveness of metals and alleviate their harmfulness by transforming them into nanoparticles (NPs) (Konate et al., 2017). In the progressive domain of nano-biotechnology, silver nanoparticles (Ag-NPs) have been created and deployed across various industries (Rajan et al., 2024). Ag-NPs are extensively utilized as antimicrobial substances to combat infectious ailments (Manjumeena et al., 2014), impede microbial proliferation on textiles (Gokarneshan et al., 2018), applied in food packaging and preservation (Fayaz et al., 2009), in catalytic oxidation for fuel and solar cell production (Ida et al., 2020), as well as in chemical sensing and imaging (Petrov et al., 2016). Furthermore, Ag-NPs have been employed in pharmaceuticals, dentistry, medicine, cosmetic products and electronic devices (Wasmuth et al., 2016).

Owing to their extensive applications, the concentration of silver nanoparticles (Ag-NPs) in aquatic environment is surging rapidly, negatively impacting aquatic ecosystems (Rajan et al.,2022). Silver exists in various forms, with ionic silver being especially harmful (Morgan et al., 2004). Ag-NPs can undergo oxidation, releasing toxic silver ions that adversely affect organisms in aquatic habitats (Zhang et al., 2015). Numerous physical transformations occur during the transit of nanoparticles (NPs), which can render them either less harmful or even more toxic. This occurs due to interactions with various environmental variables, including organic molecules, inorganic anions, and metal cations which can

modify the chemical composition and surface area of silver nanoparticles (Tortella et al., 2020). Silver nanoparticles can cluster together as a result of natural organic molecules adhering to their surface. Under similar conditions, the stability and aggregation of Ag-NPs can be affected by the ionic strength of the surrounding environment, particularly under acidic pH conditions. Given these changes in toxicity and bioavailability, it is crucial to assess the environmental risks posed by Ag-NPs (De Souza et al., 2019). Organic molecules stabilize nanoparticles through steric hindrance, electrostatic repulsion, or a combination of both mechanisms (Fabrega et al., 2011).

Modification of nanoparticles (NPs) in biological systems through mechanisms such as self-aggregation or agglomeration can lead to serious health issues (Lapresta-Fernández et al., 2012). Silver nanoparticles (Ag-NPs) can be synthesized and stabilized using various methods, including photochemical processes, ultrasound-assisted reduction, and chemical and biological procedures (Awwad and Amer, 2020). The most common chemical processes for producing Ag-NPs include chemical reduction, photochemical reduction (Yu et al., 2007), electrochemical reduction (Bandeira et al., 2020), and thermal evaporation (Smetana et al., 2005). Silver ions ($Ag^{+)}$ are typically reduced in aqueous and non-aqueous media using either inorganic or organic reducing agents, such as N-dimethylformamide, block copolymers, sodium citrate, ascorbate, and sodium borohydride. These reducing agents transform silver ions into metallic silver by inducing agglomeration and forming oligomeric clusters (Hamidi, et al., 2019). Furthermore, shape-controlled nanoparticles can be synthesized through chemical and physical methods. However, these methods have several drawbacks, including biological hazards due to the toxic chemicals used in the synthesis process, high equipment costs, and substantial energy demands (Khodashenas and Ghorbani, 2019). Several studies have shown that the chemical synthesis of nanoparticles (NPs) is both costly and environmentally damaging (Abdulazeem et al., 2023).

Consequently, there is an increasing demand for the development of cost-effective and eco-friendly methods by eliminating harmful artificial substances during the formulation stage (Tortella et al., 2020). Many researchers advocate for environmentally friendly biological processes to synthesize NPs utilizing enzymes (Iravani et al., 2016), microorganisms, plant extracts, and whole plants (Bandeira et al., 2020). Using biological resources, such as plants, is generally safe and environmentally beneficial, as they naturally contain reducing and capping agents that minimize the potential for chemical toxicity. These methods are also relatively easy to replicate, inexpensive, widely accessible, and possess therapeutic benefits (Bandeira et al., 2020).

The toxicity of chemically synthesized silver nanoparticles (Ag-NPs) has been documented by several researchers (Marin et al., 2015: Zhang et al., 2015: Kakakhel et al., 2021). However, the toxicological implications of biologically produced silver nanoparticles are still unknown. Ag-NP exposure might cause blood plasma chloride and potassium imbalance, acetylcholine esterase (AChE) inhibition, impaired T-lymphocyte recruitment, and elevated cortisol level (Bandeira et al., 2020). It can also result in histological changes in the muscles, kidneys, liver, gills, and epidermis of fish (Ostaszewska et al., 2016). *Labeo rohita*, sometimes referred to as rohu, is a common freshwater fish found across Asia. It is important for aquaculture in interior waterways, where nanoparticle contamination may often occur, from an economic standpoint. Moreover, *Labeo rohita* is constantly exposed to aquatic contaminants because of its bottom-eating and straining behaviors. Thus, the current study postulated that, in comparison to chemically created silver nanoparticles, biologically or environmentally produced silver nanoparticles (Ag-NPs) might lessen toxicity to organisms. Therefore, the present study was conducted to synthesize green Ag-NPs and evaluate their toxicity using *Labeo rohita* as a model fish species.

2. Materials and Methods

2.1 Preparation of *Psidium guajava leaf extract*

In the present investigation, healthy and fresh foliage of the guava plant (*Psidium guajava*) was collected from the University of Veterinary and Animal Sciences Lahore, Pakistan, for the preparation

of leaf extract. The leaves were meticulously washed with deionized water to eliminate surface contaminants, and consequently air-dried at 28°C for one hour. The dried leaves were finely chopped. For each batch of extract, 10 g of the finely chopped leaves were placed in a beaker with 100 mL of deionized water. The beaker was heated on a hot plate (BJPX-HPG3040, China) and stirred constantly at 400 rpm at 90°C for 25 minutes to obtain the leaf extract. The final extract was filtered twice using Whatman filter paper and stored at 30°C.

2.2 Biosynthesis of Silver Nanoparticles

To synthesize silver nanoparticles, 20 mL of $AgNO_3$ solution at different concentrations (5, 10, 15, and 20 mM) and 2 mL of *P. guajava* aqueous leaf extract were mixed together in a beaker. The mixture was heated to 60°C on a hot plate (BJPX-HPG3040, China) for 10 minutes while being continuously stirred with a magnetic stirrer (MS7-H550, USA). Afterwards, the mixture was allowed to stand at room temperature for two hours to facilitate the reduction of Ag^+ ions to metallic silver. The solution was closely observed during this time, and a gradual colour shift from light yellow to reddish-brown was noted, indicating the formation of silver nanoparticles. A small portion of the coloured solution was centrifuged for 15 minutes at 10,000 rpm using a Micro Prime Centrifuge (Pocklington, UK). After centrifugation, the material was placed on a Petri plate and dried for two hours at 60°C in an oven (Top Cloud Agri Technology, China; Model no.: GX30B). Once desiccated, the powder was annealed for two hours at 500°C and then stored in a desiccator (MERCK, Germany; Model no. BR65805) for further use. The solution hue changed from pale yellow to brown and finally to dark brown, indicating the formation of nanoparticles. Using UV-Vis spectroscopy (UV2800, China), their absorbance was measured, revealing distinct peaks.

2.3 Characterization of Silver Nanoparticles

UV-visible spectroscopy (Perkin-Elmer, MA, USA) was used to confirm the produced silver nanoparticles in the 350–750 nm range. The crystalline nature of the silver nanoparticles was confirmed using X-ray diffraction (XRD) patterns (Bruker AXS, Inc., Madison, USA).

2.4 Experimental Trial

Ethical permission from the Animal Ethics Committee (Zoo/UVAS/932) was obtained before the research began. Fish were purchased from a nearby hatchery and then moved to the fish hatchery at the University of Veterinary and Animal Sciences, Ravi Campus. The fish were allowed to acclimate in 500L aquariums for a week. The prepared diet used to feed the fish during the acclimation phase was free of nanoparticles. Following the acclimatization, the fish (initial weight = 30.00 ± 1.45 g, n = 120) were stocked in 12 glass aquaria. The trial contained four treatments, while each treatment comprises three replicates (n=10/replicate). Experiment was performed for a time period of eight weeks and silver nanoparticles were introduced to fish at four different concentrations (N= 0 NPs, N1= 5mg/L NPs, N2= 10 mg/L NPs, and T3= 20 mg/L NPs). The N0 act as control which is without nanoparticles. The fish in each treatment were fed twice daily, with nanoparticles being administered through the water after 24 hours. Each day, 10 % of the water in the tanks was exchanged. A daily ration of two percent of the biomass from all treatments was determined. Daily monitoring was conducted to assess the water quality parameters, including temperature (29.00 ± 1.00°C), pH (7.21 ± 0.41), and dissolved oxygen (DO: 7.51 ± 0.21 mg/L).

2.5 Sample Collection

The fish in the experimental investigation were deprived of food for twenty-four hours and sedated with clove oil (6 mL/L: Sigma Aldrich USA) following an eight-week period. Five fish were selected at random from each replication within each treatment group. Blood samples were drawn from the caudal vein and placed into two different tubes. For hematological analysis, one tube was treated with ethylenediamine tetraacetic acid, while the other contained a clot activator for various biochemical analyses. After spinning the blood samples at 5000 rpm for 15 minutes, the plasma was extracted and stored at -20°C. Additionally, muscle and gill samples were collected and preserved in

3 milliliters of Bouin's solution (Solarbio, Beijing, China) for histological examination.

2.6 Hematological, Biochemical and Antioxidant Analysis

Blood samples (n=15/treatment) were centrifuged (CG, 1/07; PCSIR laboratories, Pakistan) at 5,000 rpm for 20 minutes to extract serum. Aspartate aminotransferase (AST; Unit/L) and alanine aminotransferase (ALT; Unit/L) were analyzed on a clinical chemistry analyzer (Thermo Fisher Scientific, USA) using analytical kits (Thermo Fisher Scientific, USA; Catalogue # A7561-150). Triglyceride levels (mg/dl) were determined using an ELISA kit from Biocompare, USA. The absorbance was recorded at 546 nm using spectrophotometer (V-1100, USA). Fish serum total protein was determined using the Total Protein Kit (Biuret method; Sigma Aldrich, USA; TP0100). The absorbance of the samples was measured at 595 nm using a spectrophotometer (V-1100, USA). An auto-hematology blood analyzer (Sysmex-KX-21, Japan), calibrated for this study, was used to determine the counts of neutrophils (µ/L), eosinophils ((µ/L), lymphocytes (µ/L), and monocytes ((µ/L), red blood cells (RBC: µL), hematocrit (HCT: %), platelets (µL), white blood cells (WBCs) and hemoglobin (Hb: g/dl), mean corpuscular volume (MCV: femtoliters), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

The SOD activity was assessed utilizing the SOD ELISA Kit (Pars Biochem; catalog# PRS-02005 hu). The activity of catalase was determined spectrophotometrically (560nm) using catalase colorimetric activity kit (Thermo fisher scientific, USA; catalog# EIACATC), as per manufacturer instruction. Malondialdehyde (MDA) concentration was determined using ELISA Kit (catalog# PRS - 00991hu).

2.7 Histological Analysis

After the experiment, sterile tubes containing gill and muscle samples from each treatment (n = 5 per organ) were filled with 3 ml of Bouin's solution (Sunbio, Beijing, China). The samples underwent standard dehydration procedures before being embedded in paraffin. Next, each sample was sectioned into 5 µm thick slices and stained with hematoxylin and eosin.

2.8 Statistical Analysis

The data were expressed as the mean ± standard error (S.E.). Statistical analysis was performed using one-way analysis of variance (ANOVA) to detect significant difference among the treatments, with a significance threshold of $P < 0.05$. The Duncan Multiple Range Test (DMRT) was subsequently employed to examine difference between means, considering the homogeneity of variances (Levene's test) and the normality of data distribution (Kolmogorov-Smirnov test). Parameters that exhibited significant difference for each treatment are indicated with a superscript following the DMRT analysis. All statistical evaluations were carried out using SPSS version 20.

3. Results

3.1 UV-visible Spectroscopy and X-ray Diffraction Analysis

The formation of silver nanoparticles was confirmed by UV-visible spectroscopic analysis, which detected a surface plasmon resonance peak. The synthesis of Ag-NPs was provided by the observation of a broad peak at 420 nm (Fig. 1a). X The XRD spectrum displayed three main Bragg diffraction peaks at 2θ values of 26.0°, 38.1°, and 49.4° (Fig. 1b). These values correspond to the faces 101, 200, and 211 of the face-centered cubic structure of Ag-NPs.

3.2 Blood Biochemistry

There was a statistically significant variation ($P < 0.05$) in the blood biochemistry parameters between the treatments supplemented with zero nanoparticles and those fed with nanoparticles. Triglyceride, ALT, and AST levels were considerably lower with the N0 treatment than with other treatments (Table 1). At the end of the trial, compared to the other treatments, the N0 treatment showed a substantially higher neutrophil count, lymphocyte count, monocyte count, and eosinophil count. Following the trial, triglycerides, ALT, and AST levels in the N3 treatment were shown to be considerably increased (P <

0.05) in comparison to N0. The N1 treatment responded to nanoparticles noticeably better compared to the N2 and N3 treatments (Table 1).

3.3 Haematological Analysis

After experimental trial, every haematological parameter demonstrated a significant difference ($P<0.05$) between the four treatments (Table 2). The N3 treatment was found to have the lowest levels of haemoglobin, white blood cells, and haematocrit as compared to other treatments. In comparison to the N2 and N1 treatments, the glucose level was also highest in the N3 treatment. However, these values were discovered to be lowest in the N0 treatment. All haematological parameters were found to be highest in N0 treatment and lowest in the N3 treatment after eight-week of experiment (Table 2).

3.4 Antioxidants Analysis

After experimental trial, SOD and CAT were significantly increased in N0 treatment as compared to other treatments whereases, MDA level decreased in N0 treatment (Fig. 2). The brain tissues of N3 treatment were found to have the lowest levels of SOD and CAT as compared to liver tissues of other treatments. All antioxidants' biomarkers except MDA in liver and brain were found to be highest in N0 treatment and lowest in the N3 treatment after eight-week of experiment (Fig.2).

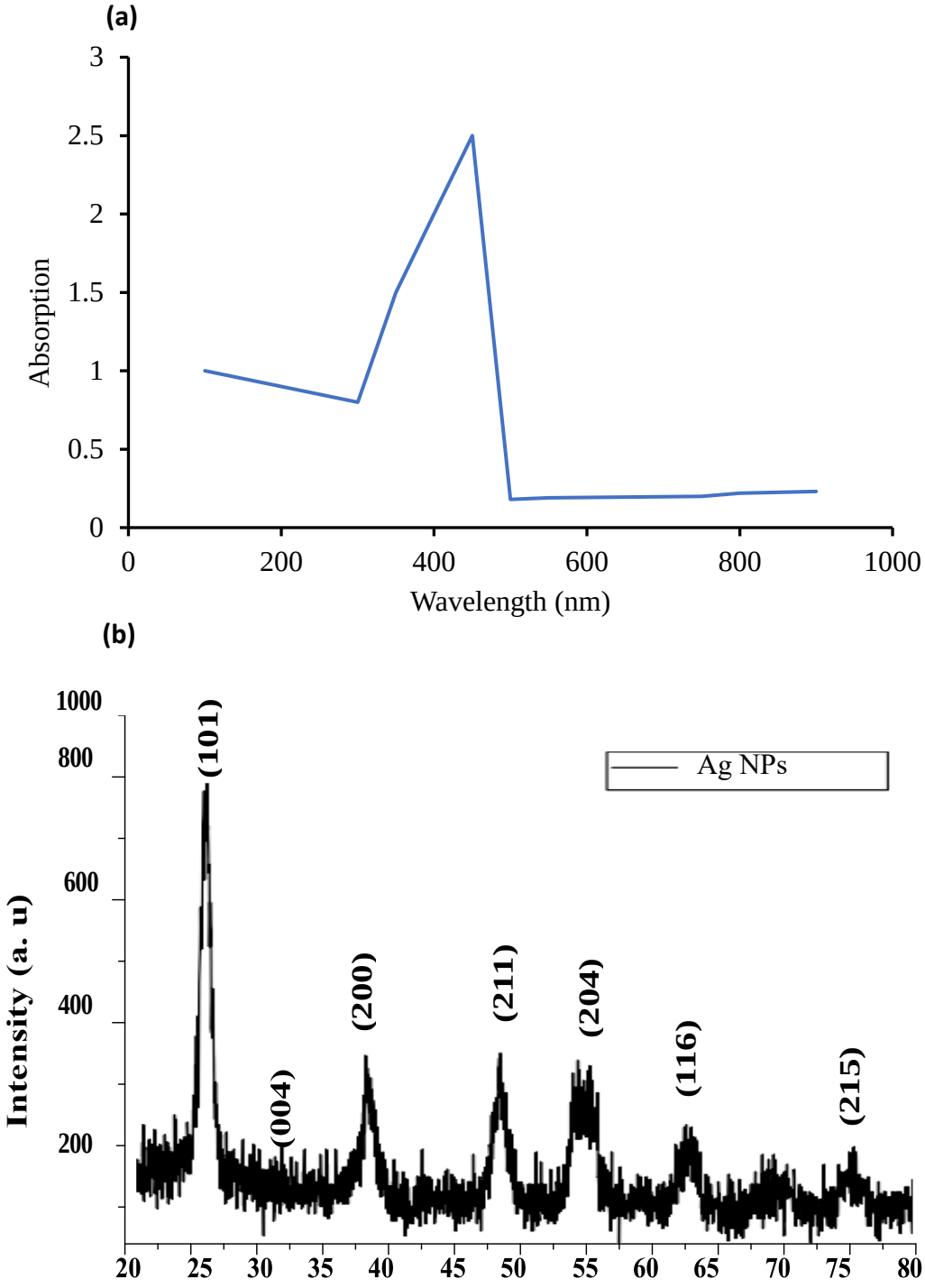

Figure 1. UV-VIS spectrum and X-ray diffraction pattern of silver nanoparticles prepared with guava leaf extract.

Table 1. Examination of the blood biochemical characteristics in four treatments followed an eight week of experimental trial. The variation across treatments is indicated by different superscripts across the rows, determined using the Duncan multirange test of one-way ANOVA at $P < 0.05$.

Parameters	N0	N1	N2	N3
Neutrophils (µ/L)	25.10 ± 0.57^{d}	24.00 ± 0.33^{c}	23.77 ± 0.33^{b}	21.23 ± 0.57^{a}
Lymphocytes (µ/L)	72.45 ± 1.15^{d}	70.22 ± 0.57^{c}	69.35 ± 0.88^{b}	64.55 ± 0.57^{a}
Monocytes (µ/L)	6.22 ± 0.33^{d}	5.67 ± 0.33^{c}	5.22 ± 0.33^{b}	4.67 ± 0.33^{a}
Eosinophils (µ/L)	5.44 ± 0.05^{d}	4.88 ± 0.05^{c}	4.11 ± 0.33^{b}	3.78 ± 0.28^{a}
Triglycerides(mg/dl)	266.21 ± 0.57^{a}	368.00 ± 0.57^{b}	384.21 ± 0.57^{c}	398.00 ± 0.57^{d}
ALT (Unit/L)	35.00 ± 0.33^{a}	46.23 ± 0.57^{b}	47.55 ± 0.57^{c}	49.22 ± 0.33^{d}
AST (Unit/L)	26.44 ± 0.57^{a}	29.34 ± 0.57^{b}	34.22 ± 0.33^{c}	40.33 ± 0.57^{d}
Total Protein (g/dl)	4.62 ± 0.03^{d}	3.84 ± 0.05^{c}	3.39 ± 0.05^{b}	3.23 ± 0.05^{a}

Table 2. Haematological markers in blood samples were examined across four treatments during an eight-week of experimental trial. Variations between treatments were determined by the Duncan multiple range test of one-way ANOVA ($P < 0.05$) and are indicated by different superscripts in each row.

Parameters	N0	N1	N2	N3
Glucose (mg/dl)	87.66 ± 0.80^{a}	88.11 ± 0.57^{b}	98.11 ± 0.57^{c}	99.66 ± 0.33^{d}
Haemoglobin (g/dl)	12.81 ± 0.05^{d}	10.41 ± 0.05^{c}	9.41 ± 0.05^{b}	7.61 ± 0.05^{a}
WBC (µL)	5.61 ± 0.05^{d}	5.76 ± 0.03^{c}	7.61 ± 0.05^{b}	5.41 ± 0.05^{a}
TOTAL RBC (µL)	3.86 ± 0.03^{d}	2.41 ± 0.05^{c}	2.11 ± 0.05^{b}	1.71 ± 0.05^{a}
MCV (fL)	175.11 ± 0.57^{d}	171.00 ± 0.57^{c}	165.33 ± 0.57^{b}	159.33 ± 0.33^{a}
HCT (%)	32.66 ± 0.31^{d}	24.83 ± 0.29^{c}	22.63 ± 0.03^{b}	19.16 ± 0.13^{a}
Platelets (µL)	227.01 ± 0.57^{d}	186.11 ± 0.57^{c}	176.11 ± 0.57^{b}	150.11 ± 0.57^{a}
MCH (%)	51.66 ± 0.88^{d}	52.01 ± 0.57^{c}	50.66 ± 0.88^{b}	48.11 ± 0.57^{a}
MCHC (%)	78.66 ± 0.33^{d}	70.33 ± 0.33^{c}	68.11 ± 0.57^{b}	66.66 ± 0.33^{a}

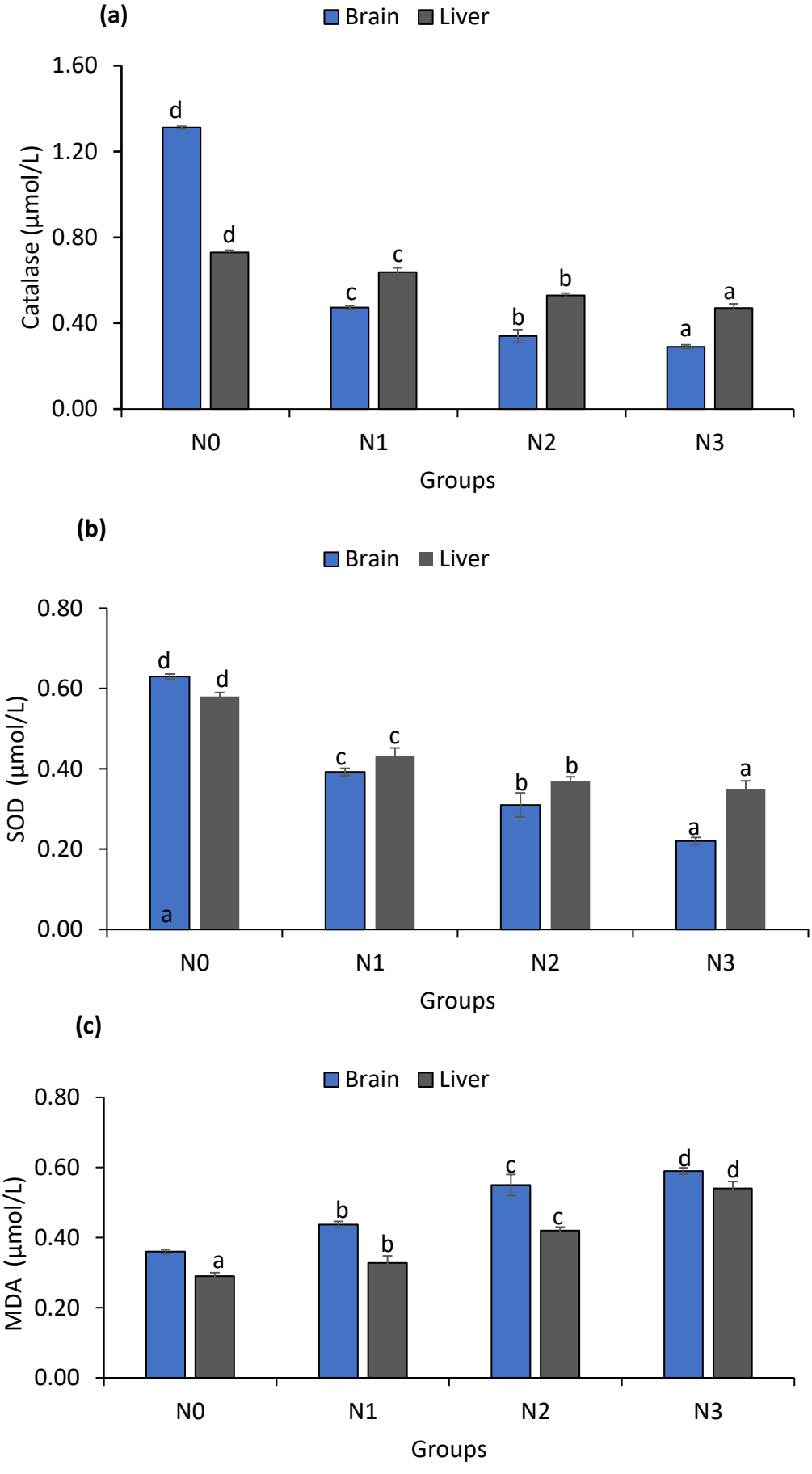

Figure 2. Antioxidants enzymes (SOD, CAT, MDA) were measured from brain and liver of four different treatments after eight weeks of experiment.

3.5 Histological analysis

In all treatments, several histological changes in the gill structure were observed (Figure 3b, 3c, 3d). The normal epithelial cell lining of lamellae was visible in the histology of the gills in the T0 treatment (Figure 3a). In contrast, treatments exposed to nanoparticles showed various structural alterations, including cellular growth in mucous membranes, disruption of the gills with noticeable hypertrophy, hemorrhage, and intracellular oedema (Figure 3b, 3c, 3d). After the four-week cytotoxic trial, the T3 treatment showed the most abnormalities as compared to the T1 and T2 treatments. Following the experiment, the muscle structures of the various treatments (Figures 4a, 4b, 4c, and 4d) displayed various abnormalities. In comparison to other treatments, the muscle structures of the T0 treatment exhibited fewer or no alterations (Figure 4a). In contrast, T1, T2 and T3 treatment revealed prominent structural alterations, such as the degeneration of muscle fibers, destabilization of vacuoles in muscle bundles, and an increase in intermyofibrillar space (IMFS) (Figures 4b, 4c, 4d). The muscles receiving T3 treatment showed the most pathological changes (Figure 4d).

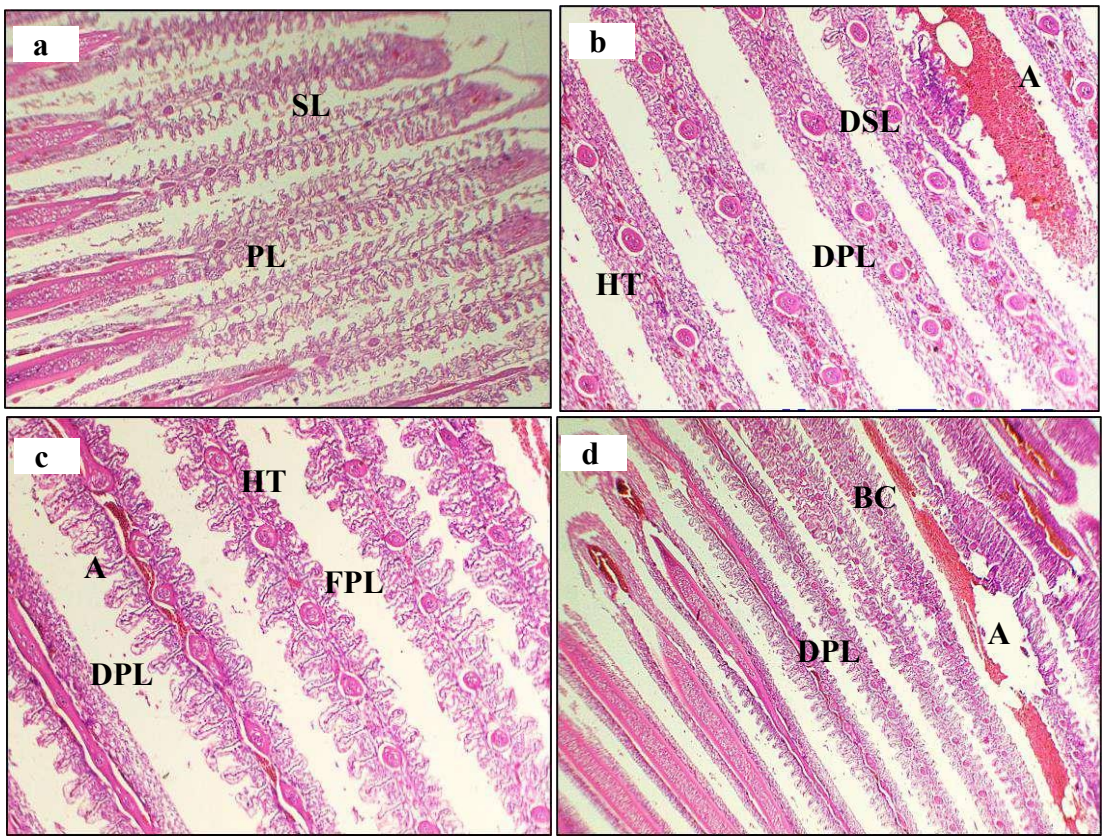

Figure 3. Histological changes in gills and muscles were examined through light micrographs of paraffin sections stained with eosin and hematoxylin (10x magnification).Gills from N0 are shown in (a), while N1, N2, and N3 are shown in (b), (c), and (d), respectively. Important characteristics observed include primary lamellae (PL), secondary lamellae (SL), fusion of secondary lamellae (FSL), degeneration of secondarylamellae (DSL), tissue debris (TD), aneurysms (A), hypertrophy (H), and blood congestion(BC).

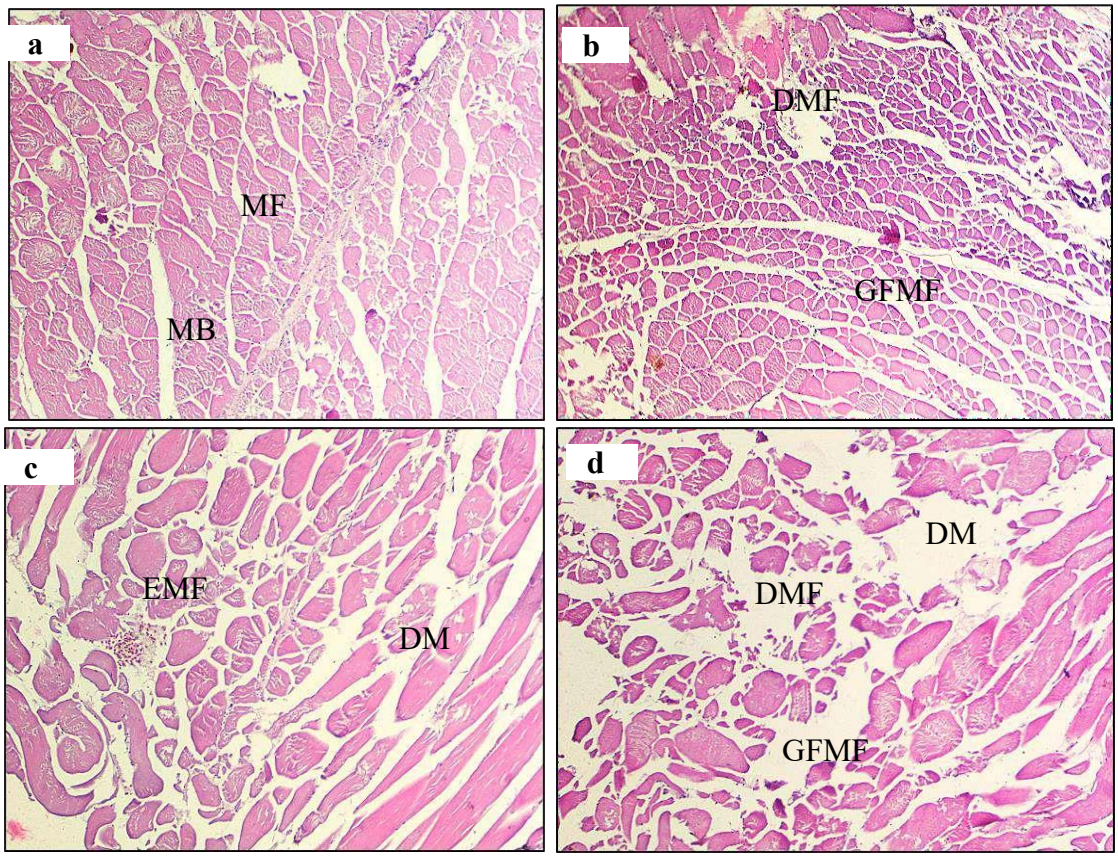

Figure 4. Histological changes in gills and muscles were examined through light micrographs of paraffin sections stained with eosin and hematoxylin (10x magnification). a: muscles of N0, b: muscles of N1, c: muscles of N2, d: muscles of N3. Identified features include myofibrils (MF), gap formation in myofibrils (GFMF), intermyofibrillar space (IMFS), disintegrated myofibrils (DMF), edema between muscle fibers (EMF), muscle degradation (MD), and muscle edema (ME).

4. Discussion

The current investigation revealed that *Labeo rohita* treated with Ag-NPs for eight weeks had higher blood glucose levels. These results are consistent with prior research that found a considerable rise in blood glucose levels in Nile Tilapia (*Oreochromis niloticus:* Haliwell, 2007), silver carp (*Hypophthalmichthys molitrix*) and juvenile Atlantic salmon (*Salmo salar*) treated with Ag-NPs (Moner et al., 2008: Wang et al., 2008). The change in glucose levels may be linked to dietary deficiencies, glucose synthesis, glycogenolysis, liver or kidney injury, and an increased amount of reactive oxygen species (ROS) in tissues, which can harm carbohydrates (Mekkawy et al., 2011). According to present results, treatments subjected to silver nanoparticles (Ag-NPs) experienced a substantial drop in blood total protein. These results align with earlier research that found exposure to zinc oxide and silver nanoparticles significantly reduced the levels of total proteins and globulin in various fish species, including *Labeo rohita* (Shalaby et al., 2001: Hori et al., 2006: Alkaladi et al., 2015). This reduction in total protein may result from the toxicological effects of silver nanoparticles, which inhibit the organism immunological response (Kunjiappan et al., 2015).

The levels of glucose, globulin, and total protein in the blood are useful indicators of the nutritional and immunological health of fish. Albumins, the most prevalent proteins in blood, regulate blood

osmotic pressure and serve as transport for external substances. Variations in blood protein concentrations may serve as a useful indicator of liver health and immunity (Monfared et al., 2013: Imani et al., 2015). The present study found that the serum enzyme activities such as aspartate aminotransferase, alanine aminotransferase (AST, ALT) in *Labeo rohita* exposed to silver nanoparticles (Ag-NPs) for eight weeks were significantly increased. These findings are consistent with previous studies that observed a significant increase in AST, and ALT, activities in rainbow trout (*Oncorhynchus mykiss*) after exposure to silver nanoparticles (Jiraungkoorskul et al., 2003). Similarly, a significant increase in these serum enzyme activities was reported in Nile Tilapia (*Oreochromis niloticus*) after exposure to copper oxide nanoparticles (Zhao et al., 2011: Johari et al., 2015).

The current study findings showed that after eight weeks of exposure, *Labeo rohita* muscle tissues and gills had accumulated a significant amount of silver. These results align with prior research indicating that rainbow trout exposed to silver nanoparticles (Ag-NPs) accumulate the highest concentrations of silver in their muscles, gills, and liver (Handy et al., 2008: Aghamirkarimi et al., 2017). This buildup in the gills suggests that fish may absorb silver nanoparticles from the water through several pathways, most notably through the gastrointestinal system and the gills during respiration. The gills serve as the primary site for nanoparticle absorption, as they are exceptionally efficient at absorbing dissolved materials from the water (Brusle, 1996: Jayaseelan et al., 2014).

The effects of Ag-NPs on muscle tissue in this study varied, consistent with findings in Mozambican tilapia exposed to nickel nanoparticles (Bhuvaneshwari et al., 2015), zebrafish exposed to heavy metals and pesticides (Yazdanparast et al., 2016), and zebrafish and rainbow trout exposed to silver nanoparticles (Farka et al., 2011). Similar results have been observed in catfish (*Mystus vittatus*) subjected to ZnS nanoparticles (Chatterjee et al., 2014), Mozambique tilapia (exposed to nickel nanoparticles: Jayaseelan et al., 2014), rohu (exposed to silver nanoparticles: Rajkumar et al., 2016), and rainbow trout and juvenile carp (*Cyprinus carpio*) treated with titanium dioxide nanoparticles (Hao et al., 2009). The damage to muscle tissues was interpreted by various other studies (Agius and Roberts, 2003: Mekkawy et al., 2013: Mekkawy et al., 2012). Numerous other investigations have provided interpretations for the injury to muscle tissues, attributing these impairments to processes such as oxidative stress, cellular damage, inflammatory responses, and disruption of ion transport, which disturb tissue structure and function, leading to impaired respiration and muscle function in the fish.

Conclusion

This investigation clearly demonstrated the adverse impacts of silver nanoparticles (Ag-NPs) at various concentrations on the immune response, as well as on the function and structure of the muscles and gills of *Labeo rohita*. The study concluded that a concentration of 20 mg/L of Ag-NPs is particularly lethal and causes high toxicity in *Labeo rohita*. Therefore, the use and application of these nanoparticles must be managed and controlled to protect the aquatic ecosystem. Furthermore, developing recovery strategies for pollutants is essential for the conservation of fish species, considering their critical position in the food web. These findings underscore the need for stringent regulations and targeted remediation efforts to mitigate the environmental and biological risks posed by nanoparticle pollution. Continued research in this area is crucial for understanding the long-term effects and for developing effective strategies to safeguard aquatic life.

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