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Biochemical and Pathophysiological Responses in *Capoeta capoeta* under Lethal and Sub-Lethal Exposures of Silver Nanoparticles

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Abstract: The increasing use of nano-based products raises concerns regarding potential risks related to their manufacturing, transportation, waste disposal, and management operations. We used the riverine carp, *Capoeta capoeta*, as an aquatic animal model to demonstrate the acute toxicity of silver nanoparticles (Ag-NPs). This study focuses on acute toxicity first, and then integrates the findings through histopathology, hematological, and biochemical testing of lethal and sub-lethal Ag-NPs exposures. Red blood corpuscles (RBC), white blood corpuscles (WBC), hematocrit, and total serum glucose levels were significantly lower in Ag-NPs-exposed fish than in control fish. Total serum protein, triglycerides, cholesterol, and albumin were all significantly greater in exposed fish. This research focused on the impacts of Ag-NPs on gills and liver tissue, and it was found that the level of injury escalated as the concentration of Ag NPs increased. Epithelial lifting of secondary lamellas (ELSL), epithelial hypertrophy (EH) of secondary lamellae (SL), leukocyte infiltration (LI), and bottom hyperplasia (BH) were all detected in Ag-NPs-exposed fish. In Ag-NPs-treated liver cross-sections of *Capoeta capoeta*, macrophage aggregates (MA), fatty liver (FL), sinusoid dilatation (SD), and necrosis (N) were identified. Ag-NPs dosages, according to biomarker representations, elicit stress-specific biochemical and physiological effects, compromising the general overall health status of aquatic animals. The gradients of toxic responses across exposure concentrations and portrayals of disrupted fish health with increasing silver nanoparticle exposure time indicate a reduced physiological ability for surviving in the wild.

Keywords: silver nanoparticles (Ag-NPs); *Capoeta capoeta*; acute toxicity test; hematological alterations; biochemical endpoints; histopathological biomarkers

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1. Introduction

Nanoparticles have been in high demand in the metal industry, biological science, and other fields in recent years [1–5]. Nanoparticles are now utilized in daily household appliances [6]. They are widely manufactured, with an annual production of 60,000 tons [7]. Six-hundred and twenty-two businesses from 30 countries develop 1814 nanoproducts for a variety of uses [8]. Only 435 nanoproducts are based on silver, accounting for 34% of overall output, with 320 tons annually [7,8]. The study of the effects of produced nanoparticles on living beings and the environment is known as nanotoxicology [9–13]. It also covers the quantitative assessment of the severity and frequency of nano-toxic effects in proportion to

organism exposure [14]. Metal nanoparticles have been used in a variety of fields, including consumer products, industrial applications, and health care technology, and are likely to enter the environment [6,12,14–16].

Silver nanoparticles (Ag-NPs) are widely used, which increases the number of pollutants released into aquatic and terrestrial habitats [6,8,17,18]. Cement manufacturing, rock weathering, fossil fuel burning, mineral processing, leaching, and anthropogenic activities all contributed to additional contamination of the environment [19,20]. Textiles frequently contain Ag-NPs, and these nanoparticles may be discharged into the atmosphere [21,22]. The wide range of applications for Ag-NPs necessitates large-scale, cost-effective synthesis. Chemical, physical, and biological methods have all been used to create these particles in recent decades. Chemical reduction is the most acceptable method due to lower chemical costs, simplicity of control, and fewer by-products [23]. Currently, formaldehyde is used as a reducing agent, whereas triethylamine serves as both a promoter and a stabilizer [8].

Ag-NPs' toxicological evidence is currently inadequate, and safety standards for these nanoparticles must be established. Engineered nanomaterials that have harmful impacts on fish include carbon nanotubes, carbon spheres termed fullerenes [24], metal nanoparticles [25,26], metal oxides [27], and composites consisting of various metals [28]. There is less information about Ag-NPs toxicity, particularly genotoxicity and cytotoxicity [29,30]. Size and surface area are important toxicity factors [31]. According to the research, Ag-NPs are more harmful than other types because they are more easily absorbed. When Ag-NPs reach the aquatic environment, they cause a physiological reaction and genotoxicity in animals [32,33]. Ag-NPs cause oxidative stress in aquatic species, according to further studies [34,35]. By increasing the production of reactive oxygen species, they promote lipid peroxidation, interaction with nucleic acid, lipid and protein, loss of membrane integrity, functional changes, and mutation (ROS). Nanoparticles have also been discovered to cause toxicity by raising intracellular ROS levels and lowering antioxidant levels [35,36]. Increased ROS levels are also an indication of acute toxicity's main mechanism [37,38]. In reaction to Ag-NPs, mitochondrial function was recently discovered to be impaired [39]. Miao, et al. [40] showed that Ag-NPs are harmful to the marine diatom, *Thalassiosira weissflogii*. Furthermore, Ag-NPs toxicity in a freshwater alga (Chlamydomonas reinhardtii) has been reported [41]. Kakakhel et al. [42] tested freshwater fish, *Cyprinus carpio*, for toxicity, mortality, bioaccumulation, and histological changes after exposing them to silver nanoparticles for longer term. The findings indicate the bioaccumulation of silver nanoparticles in many organs of fish. The liver had the highest bioaccumulation of silver nanoparticles, followed by the intestine, gills, and muscles. Furthermore, the study revealed that silver nanoparticle bioaccumulation resulted in histopathological changes, including damaged gill tissue and intestinal structure.

Fish species have been commonly utilized as a pollution indicator in studies of aquatic toxicity, and they respond significantly to stress conditions [1,43–45]. The acute toxicity effects of Ag-NPs were investigated in this study utilizing *Capoeta capoeta* as an in vivo model. Hematological, biochemical, and histopathological characteristics have long been regarded as useful indicators of fish health. The effects of Ag-NPs on these parameters of *Capoeta capoeta* were examined in this work. These results would be crucial in determining the possible toxicity and bio-distribution of Ag-NPs in the piscine model.

2. Materials and Methods

2.1. Study Site and Fish Maintenance

The ZarrinGol River is one of the main tributaries of the Gorgan River which is located in Golestan Province and the geographical location of the river (longitude: ″40′43°54 to 36′11°55 East and latitude: ″30′43°36 Up to 44′08°37 North). Due to the particle size of the bed particles, this river is one of the rivers with a coarse bed (rocky–sandy). One-hundred and seventy pieces of Capoeta were caught from the ZarrinGol River in Golestan Province using an electroshock device. The average weight of fish was 15 ± 3.5 g. The fish were transferred to the aquaculture Hall of Gorgan University of Agricultural Sciences and

Natural Resources using plastic containers, one-third of which were air. The fish were adapted to the new conditions for 4 weeks before the start of the experiment. During the adaptation period, the water in the tanks was aerated, the feeding was done twice a day, and the feeding was stopped during the experimental period. During the experimental period, the amount of aeration, feeding, and physicochemical conditions of water (temperature, pH, dissolved oxygen) were controlled. No mortality was observed during the experimental period. After feeding, uneaten food was siphoned off the bottom of the tanks to prevent contamination of the aquarium water. Throughout the experimental period, all ethical concerns were met as per the regulation provided by the Institutional Biosafety Committee-Gorgan University of Agricultural Science and Natural Resources (9523024101e.gau).

2.2. Test Chemical

This study was performed using a standardized composition of silver nanoparticles (Ag-NPs) (trade name-Nanocid L-2000) purchased from Nanonasb Pars Co., Tehran, Iran. The NPs were characterized in the MilliQ water. The surface plasmon resonance analysis of Ag-NPs was carried out in a quartz cuvette with a path length of 1 cm using a Varian Cary 50 UV-visible spectrophotometer [46]. Drop-casting the NP solution on carbon-coated copper grids measured the NP size using transmission electron microscopy at 100 kV. (JEOL 1010) [46]. The zeta potential and hydrodynamic diameter (HDD) of the nanoparticles were measured on a Zetasizer using a folded capillary cell and a glass cuvette, respectively (dynamic light scattering; Malvern Zetasizer Nano series, NanoZS).

2.3. Experimental Setup

2.3.1. Acute Toxicity Assay

Following the adaption phase, 105 fish were separated into five groups (average weight: 153.5 g) with three replicates (12 aquariums: 1,506,565 cm), and treated with four different Ag-NP concentrations. Acute toxicity of Ag-NPs to fish was assessed by exposing them to four concentrations of Ag-NPs plus a blank control (0 mL/L). The testing time was 96 h and the nominal Ag-NPs concentrations were 0, 5, 10, 15, and 20 mL/L; these concentrations were selected according to Mohsenpour et al.'s [17] study and the laboratory facilities. The number of fish deaths was recorded 24, 48, 72, and 96 h after exposure of the fish to Ag-NPs [5]. The fish were moved into the aquarium or test tank 48 h before the start of the test and did not eat during the toxicity test. pH 7.9–8.6, DO 7.9–8.6 mgL^{-1}, NH3 0.02 mgL^{-1}, temperature 24 $\pm$ 2 °C, and total hardness 210 mgL^{-1} $CaCO_3$ were maintained during the adaption periods. The LC_{50} 96 h test was conducted in a static environment. Finally, the concentrations of Ag-NPs were manually added. During the LC_{50} 96 h test, a camera (Canon, SX230 Hs, 5.0–70 mm) was used to record fish swimming in front of the aquarium.

2.3.2. Blood Collection and Assay of Hematological Endpoints

Blood was drawn from the caudal vein. Using this method, the sample was taken from the midline, somewhat posterior to the anal fin. Before being delivered to the lab for hematological analysis, blood samples were spun at 2500 rpm for 10 min. Blood samples were taken from each tank's fish and 12 blood samples were analyzed. Total protein, albumin, triglyceride, cholesterol, and glucose levels in serum from each sample were measured using an automated biochemical analyzer (Roche Hitachi 911 Chemistry Analyzer, Tokyo, Japan) and accompanying kits (Pars Azmoon Inc., Tehran, Iran) [47]. The samples were collected from live fish subjected to sub-lethal levels of Ag-NPs for 96 h.

2.3.3. Histopathological Analysis

Fish gill specimens were collected 96 h after Ag-NPs exposure (4 samples per treatment) and treated with a diluted Formalin solution (formaldehyde 10% v/v, Sigma®, Missouri, CA, USA). After 24 h, the gill and liver samples were replaced with formalin. The center parts of the liver and the second-gill arch from the fish's left side were sampled.

The samples were submerged in a series of alcohols for 30 min (50, 70, 80, and 96 percent). The gill sample was then cleaned for two hours in 1-butanol alcohol before being immersed in chloroform for one hour of clarity. The gill samples were then paraffinized and softened in an incubator at 37 °C using a chloroform and paraffin solution (1:1). The tubes were then kept in pure paraffin for 24 h at 54 °C before being prepared for tissue incisions. The tissue incisions were 6 m thick and were created with tissue processor equipment (TP1020, Leica Microsystems Inc., Buffalo Grove, IL, USA). The tissue incisions were stained with hematoxylin and eosin. To monitor and analyze tissue damage, light microscopy (Model RH-85 UXL, UNILAB®, Delhi. India) was utilized. Alive fish were used to collect treatment tissue samples.

2.3.4. Semi-Quantitative Scoring

In replicates of the different exposure concentrations and the control, histopathological alterations in gill and liver tissue were investigated. The severity of lesions was assessed using a slightly modified version of Rajkumar et al.'s [1] semi-quantitative grading approach. No histological alterations; (+) mild histopathological changes; (++) moderate histopathological changes; or (+++) severe histopathology changes were assigned to each lesion. Each modification was given a significance factor (1–3) depending on the implications for the organism's survival, according to Bernet, et al. [48]. As a result, the significance factor shows the likelihood of the change being reversed once the stressor or the organism's physiologic handling capacity has been removed.

2.4. Statistical Analyses

A probit test was used to calculate the fatal concentration of Ag-NPs in intervals of 24, 48, 72, and 96 h (LC50 24 h, 48 h, 72 h, and 96 h of Ag-NPs). To evaluate the connection between different nominal concentrations of commercial Ag-NP compositions and mortality, the Spearman test was used (2-tail). Finally, using SPSS software's two-tailed significant Spearman testing, the relationship between the fish mortality rate and Ag-NP concentration was investigated (IBM SPSS Statistics 20). On a Windows platform, Adobe After Effects software (AAE CS6) was utilized to analyze the video data (Windows 7 Ultimate, Microsoft Corporation). Fish clinical symptoms were reported using video monitoring, average gill operculum activity in 1 min, and AAE CS6 color comparison of the item (fish) over time.

3. Results and Discussion

3.1. Acute Toxicity

The UV-visible absorbance spectra of Ag-NPs exhibit an SPR (surface plasmon resonance) band at 406 nm, which is typical of Ag-NPs, and a peak at 274 nm [46,49]. The HDD (hydrodynamic diameter) was 86.6 nm, whereas core size determined by TEM was ∼30 nm in diameter. The negative charge of Ag-NPs, as determined by zeta potential, is −42.4 mV. [49]

The experimental fish (*Capoeta capoeta*) was administered various exposure concentrations of Ag-NPs (5, 10, 15, and 20 mgL^{-1}) to determine the LC_{50} value. Syafiuddin, et al. [50] recorded AgNP values in rivers and sewage treatment plants ranging from 0.13 to 10.16 mgL^{-1} and 0.13 to 20.02 mgL^{-1}, respectively, in Malaysia. Thus, we selected these concentrations of Ag-NPs for toxicity testing on fish hematological and biochemical endpoints. Based on the standard protocols of 96-h toxicity tests, we chose the durations of experiments [51–53].

The mortality in each group was thoroughly investigated and documented after the 96-h treatment period. The mortality of exposed C. capoeta to Ag-NPs is presented in Table 1. The LC_{50} values of Ag-NPs to *C. capoeta* were 17.213, 14.038, 11.574, and 9.619 mgL^{-1} after 24, 48, 72, and 96 h of exposure (with 95% fiducial intervals) (Table 2). The correlation between nominal lethal concentrations of Ag-NPs with the mortality rate of *C. capoeta* ($p < 0.01$) is shown in Figure 1. During the entire study period, there was no death of fish

in the control group. The effectiveness of chemically manufactured Ag-NPs was dose-dependent, and mortality increased as Ag-NPs exposure concentrations increased. At an exposure concentration of 20 mg kg^{-1}, 100% death was recorded, whereas the lowest concentrations of Ag-NPs, such as 5 mg kg^{-1}, had the lowest mortality rate. In the test animals, a significant relationship between mortality rate and exposure times was observed ($p < 0.05$). The correlation between nominal lethal concentrations of Ag-NPs with the mortality rate of *C. capoeta* is summarized in the Supplementary Materials.

Table 1. Mortality of exposed *C. capoeta* to Ag-NPs.

Concentration (mgL^{-1}) *	Number	No. of Mortality			
		24 h	48 h	72 h	96 h
0	21	0	0	0	0
5	21	1	3	4	5
10	21	3	6	8	10
15	21	9	11	14	18
20	21	13	17	20	21

Note: * All concentrations are nominal concentrations.

Table 2. Lethal concentrations of Ag-NPs to *C. capoeta*.

Point	Concentration (mgL^{-1})			
	24 h	48 h	72 h	96 h
LC_{10}	8.161	5.049	4.164	3.609
LC_{20}	11.268	8.135	6.708	5.672
LC_{30}	13.509	10.360	8.542	7.160
LC_{40}	15.424	12.261	10.109	8.431
LC_{50}	17.213	14.038	11.574	9.619
LC_{60}	19.003	15.815	13.039	10.808
LC_{70}	20.918	17.716	14.606	12.079
LC_{80}	23.158	19.941	16.440	13.567
LC_{90}	26.266	23.027	18.984	15.630
LC_{95}	28.832	25.575	21.084	17.334

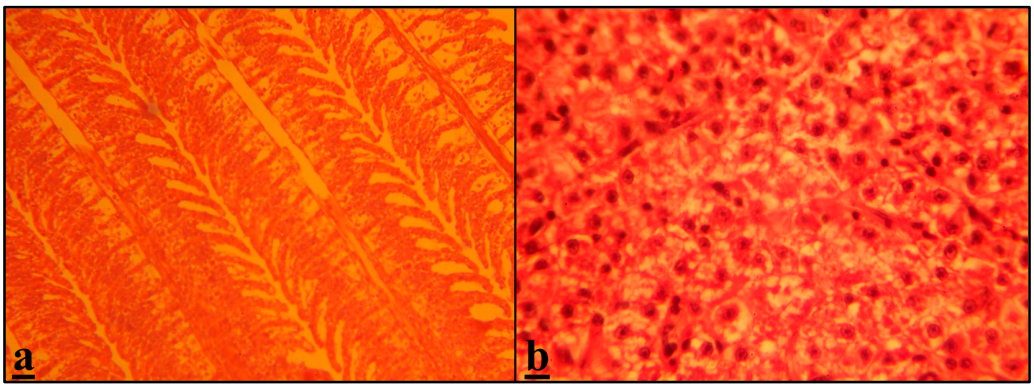

Figure 1. (**a**,**b**) Tissue sample of Capoeta *(Capoeta capoeta)* in the control group (0 mgL^{-1} of Ag-NPs); (**a**) gills of fish (magnified ×40); (**b**) liver of fish (magnified ×40).

Xue, et al. [54] conducted a study in which mice were given different doses of Ag-NPs (7.5, 30, or 120 mg/kg). The cells in the lung and liver of mice were induced to become inflamed at a dose of 120 mg/kg [54]. In TiO2-treated Danio rerio, the LC_{50} value was estimated to be 100 mgL^{-1} [55,56]. Ag-NPs were used to generate the standard Drosophila culture medium at silver concentrations ranging from 10 mgL^{-1} to 100 mgL^{-1}, according to Panacek, et al. [57]. For a silver concentration of 20 mgL^{-1}, Ag-NPs had an acute toxic effect on Drosophila melanogaster [57]. At this level of silver, 50% of the tested flies were

unable to escape the pupae and did not complete their developmental cycle [57]. In the instance of Daphnia magna, García, et al. [58] found that cerium oxide NPs are exceedingly toxic, with an LC_{50} of 0.012 mg/mL. In Pangasius hypophthalmus, Kumar, et al. [59] found that the lethal concentration of Se-NPs was 3.97 mgL^{-1} after 96 h.

3.2. Hematological and Biochemical Endpoints

Hematology and biochemistry are important tools for monitoring health, diagnosing illness, and tracking disease progression and treatment response [47,60–71]. The effects of Ag-NPs on the hematological and biochemical parameters of the Caucasian scraper, *C. capoeta*, were investigated in this study.

The changes in RBC, WBC, and Hematocrit levels were investigated. When compared to other exposure concentrations and control fish samples, the levels of all the hematological parameters specified above were reduced at 5, 10, and 15 mg kg^{-1} concentrations, according to the hematological investigations (Table 3). Stressful situations that alter the metabolism and regular functioning of the fish's physiology could cause changes in hematological markers [72–74]. The percentage of erythrocytes in blood smears also reduced (Table 3), indicating that the spleen was not replacing osmotically-damaged red cells in the circulation. Clark, et al. [75] found similar results when looking at the effects of Ag-NPs on the blood profile of rainbow trout. The treatment groups (5, 10, and 15 mgL^{-1}) had significantly lower white blood cell counts (WBC) than the control group ($p < 0.05$). The lowest overall value was recorded in groups administered 15 mgL^{-1}. According to the findings, Ag-NPs can decrease the number of leucocytes in fish in experimental groups as compared to control groups. Despite minor statistical discrepancies, the Hct levels presented here are consistently falling with values for the normal range in Caucasian scraper, *C. capoeta* [76,77]. Furthermore, because the Hct values dropped at all exposure concentrations, the hematology is unlikely to be an artifact of gill damage-induced hypoxia. RBC, WBC, and Hct levels were lower in the treatment group than in the control group post-exposure to Ag-NPs. This indicated that higher levels of Ag-NPs can reduce the oxygen-carrying capacity of C. capoeta blood [78]. These negative effects could include Hb and RBC degeneration [79,80], hematological tissue damage [81], and the inhibition of aerobic glycolysis, resulting in a lack of energy for Hb synthesis [82,83].

Table 3. Hematological and biochemical endpoints (mean ± SD) of *C. capoeta* after exposure to different levels of Ag-NPs at 96 h.

Hematological and Biochemical Indices	Control (0 mgL^{-1})	5 mgL^{-1}	10 mgL^{-1}	15 mgL^{-1}
RBC (10^6 µL)	2.43 ± 0.06^{a}	1.97 ± 0.3^{b}	1.63 ± 0.12^{c}	1.28 ± 0.24^{d}
WBC (10^4 µL)	2.35 ± 0.68^{a}	1.94 ± 0.5^{b}	1.90 ± 0.62^{b}	1.51 ± 0.50^{c}
Hematocrit (%)	46.63 ± 0.3^{a}	35.3 ± 0.5^{b}	35.3 ± 0.22^{b}	29.8 ± 0.38^{c}
Total serum glucose (mg/dL)	4.24 ± 0.07^{a}	4.19 ± 0.11^{a}	3.15 ± 0.17^{b}	2.02 ± 0.05^{c}
Total serum protein (mg/dL)	1.92 ± 0.31^{d}	2.98 ± 0.25^{b}	2.96 ± 0.13^{b}	3.78 ± 0.11^{a}
Triglyceride (mg/dL)	1.90 ± 0.09^{d}	2.23 ± 0.08^{b}	2.23 ± 0.03^{b}	2.98 ± 0.27^{a}
Cholesterol (mg/dL)	3.97 ± 0.21^{c}	3.98 ± 0.30^{c}	4.14 ± 0.14^{b}	4.12 ± 0.16^{b}
Albumin (mg/dL)	0.49 ± 0.13^{c}	0.63 ± 0.16^{b}	0.62 ± 0.11^{b}	0.78 ± 0.22^{a}

Note: Different letters (a–d) in the same columns indicate significant differences ($p < 0.05$).

Exposed fish showed a significant dose- and time-dependent reduction in total serum glucose values in groups with higher exposure concentrations, but a significant increase in total serum protein, cholesterol, triglyceride, and albumin values in groups with increasing dose and exposure time when compared to control fish (Table 3). The raised triglyceride and cholesterol levels reported in the blood, muscle, and liver tissues of other fish exposed to various toxicants in the current study were also corroborated in the blood, muscle, and liver tissues of other fish [84–86]. This study's hypercholesterolemia could be related to a disrupted cholesterol transformation pathway, i.e., decreased enzyme activity in the conversion of cholesterol to bile acid [87]. The current rise in serum cholesterol levels in

exposed fish may be due to the damaged liver parenchyma releasing volatile fatty acids into the circulation, or to decreased cholesterol excretion by the wounded liver in stressed fish [88]. Albumin is a high-molecular-weight serum protein that helps lipids, hormones, and inorganic ions flow about the body. Albumin helps the body filter fluids and maintain colloidal blood osmotic pressure [89]. Hyperalbuminemia was discovered in the current investigation as a result of exposure to Ag-NPs. When exposed to microplastic particles, *Cyprinus carpio* showed a similar trend of increased albumin levels [90].

3.3. Histopathological Assessment

Several researchers have indicated histopathological investigations to be an effective method for measuring nanomaterial damage [91–96]. The effects of Ag-NPs on gills and liver tissue were studied in this study and it was observed that the level of harm increased as the concentration of Ag-NPs raised; however, no tissue lesions were recognized in tissue samples of the control group (Figure 1). The gills of *C. capoeta* represent a typical histological structure, with numerous secondary lamellae (SL) developing perpendicularly to the primary lamellae (PL) and a regular gill arch (Figure 2a–d). The SL had a typical primary lamellar architecture, intact epithelial lining, and normal inter-lamellar space. The Ag-NPs-exposed fish, on the other hand, demonstrated epithelial lifting of secondary lamellas (ELSL), epithelial hypertrophy (EH) of SL, leukocyte infiltration (LI), and bottom hyperplasia (BH). Remnants of a secondary lamellar tip separated from the primary lamella were also seen in numerous focus fields. Similar changes in the histological structure of the gill, liver, and muscle were seen in freshwater fish, *Oreochromis mossambicus* and *Labeo rohita*, subjected to manufactured silver nanoparticles [97,98]. The gills are the most important organ in fish, with breathing and osmoregulation as their principal roles [99–103]. Because of their large surface area and short diffusion distance, they are vulnerable to xenobiotic-induced water changes [104,105]. Changes in vascular regulation, sudden gaseous exchange, and, ultimately, homeostatic disturbance in fish result from these diverse histopathological lesions [106,107]. Kakakhel et al. observed the bioaccumulation of Ag-NPs in gills, liver, intestine, and muscles in *Cyprinus carpio* [42]. The results of their study revealed that the Ag-NPs were mostly bio-accumulated in the liver, followed by the intestine, gills, and muscles [42]. The gill tissue structure of *Cyprinus carpio* was damaged and led to atrophy and necrosis while exposed to Ag-NPs [42]. The gills of metallic nanoparticles-treated fish displayed lamellae fusion, aneurism formation, necrosis in secondary lamellae, and liposome expansion in zebrafish [108]. The gills of zebrafish exposed to copper nano-particulates were substantially broader than those of zebrafish exposed to soluble copper [108,109]. Griffitt, Hyndman, Denslow and Barber [108] found that Ag-NPs-exposed zebra fish had wider gill filaments.

The regular orientation of cord-like compact hepatocytes spreading out from the central vein to the margin of the hepatic lobules was seen in a histological cross-section of *C. capoeta* liver tissue. Nonetheless, macrophage aggregates (MA), fatty liver (FL), sinusoid dilation (DS), and necrosis (N) were seen in Ag-NPs-treated liver cross-sections (Figure 3a–d). The liver is the primary fish organ responsible for xenobiotic metabolism, detoxification, and excretion [110]. An excess of dangerous substances impairs the body's ability to break them down, resulting in a variety of histopathological alterations [111,112]. The activation of aberrant and physiologically nonfunctional proteins, which results in mitochondrion dysfunction and nuclear protein breakdown, could explain the histopathological abnormalities seen in the liver tissues of present Ag-NPs-treated *C. capoeta* [113,114]. Hypertrophy in the hepatocytes suggests an increase in the organelles involved in metabolism, as demonstrated in *Piaractus mesopotamicus* treated with veterinary pharmaceutical drugs [104]. Furthermore, all of the aforementioned diseases are most likely caused by the suppression of hepatic kinase enzymes, which are necessary to catalyze the phosphotransferase network, resulting in a reduction in hepatic energy homeostasis and ATP availability [115,116]. Ag-NPs accumulation in the liver has also been reported by previous studies [117,118]. These impacts and alterations were similar to those seen in silver nanoparticle-exposed *Oncorhynchus*

mykiss (rainbow trout) [91,119–122]. When Siberian sturgeon were exposed to Ag-NPs, Ostaszewska, et al. [123] detected histological changes in the gills and liver.

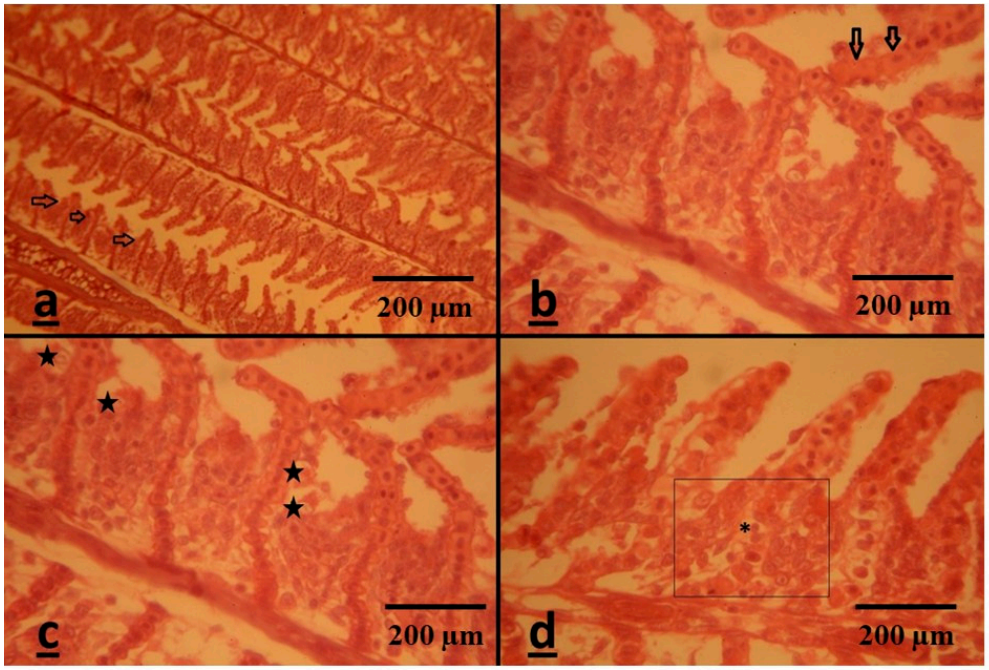

Figure 2. (**a**–**d**) Gill lesions of Capoeta (*Capoeta capoeta*) 96 h after exposure to different levels of Ag-NPs; (**a**) epithelial lifting of secondary lamellas (Ag-NPs were 5 mgL^{-1}—magnified ×10); (**b**) epithelial hypertrophy (Ag-NPs were 5 mgL^{-1}—magnified ×40); (**c**) leukocyte infiltration (Ag-NPs were 10 mgL^{-1}—magnified ×40; (**d**) bottom hyperplasia (Ag-NPs were 15 mgL^{-1}—magnified ×40).

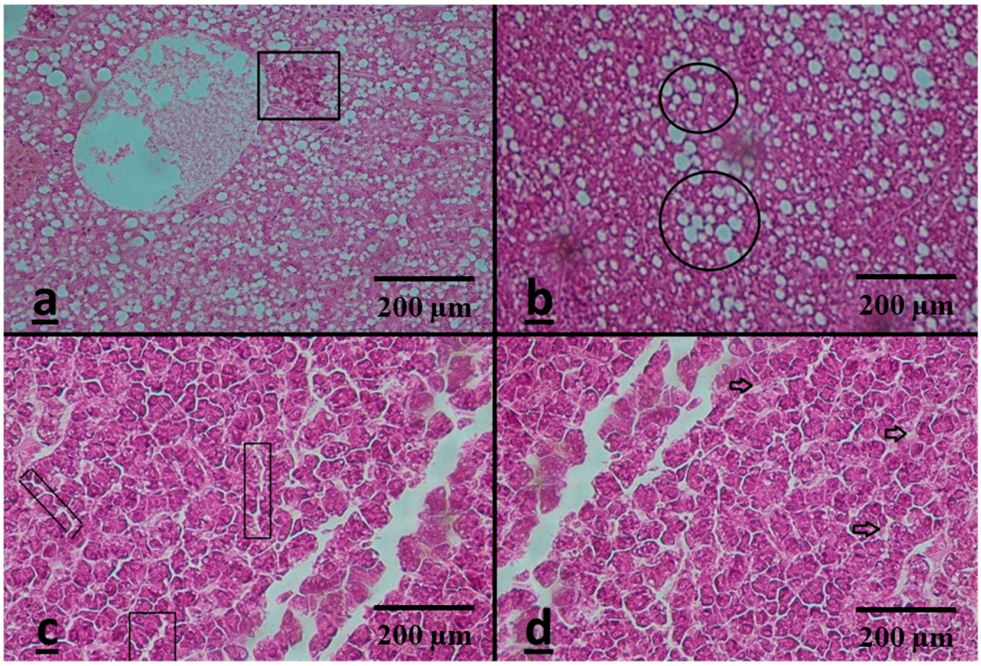

Figure 3. (**a**–**d**) Liver lesions of Capoeta (*Capoeta capoeta*) 96 h after exposure to different levels of Ag-NPs; (**a**) macrophage aggregates (Ag-NPs were 5 mgL^{-1}—magnified ×40); (**b**) fatty liver (Ag-NPs were 10 mgL^{-1}—magnified ×40); (**c**) dilation of sinusoid (Ag-NPs were 15 mgL^{-1}—magnified ×40); (**d**) necrosis (Ag-NPs were 15 mgL^{-1}—magnified ×40).

Semi-Quantitative Analysis

Gills

Epithelial lifting of secondary lamellae, epithelial hypertrophy, bottom hyperplasia, and leukocyte infiltration have all been seen in exposed fish gills. Except for epithelial hypertrophy, which demonstrated progressive histological alterations, all changes in gill tissue were regression-type responses. Although all exposure groups had gill lesions, the degree of the lesions differed (Table 4). The fish exposed to 15 mgL^{-1} Ag-NPs had moderate severity for all regression-type lesions and severe severity for progressive-type lesions, whereas the fish exposed to 5 and 10 mgL^{-1} Ag-NPs had mild-to-moderate severity for regression-type lesions and moderate severity for progressive-type lesions. Regressive alterations have been linked to oxidative stress [124]; contaminant-induced enzyme inhibitions frequently cause oxidative stress, resulting in a loss of cellular membrane integrity and ultimate cell death [125–127].

Table 4. Gills damages of Capoeta (*Capoeta capoeta*) 96 h after exposure to different levels of Ag-NPs.

Tissue Damages	Reaction Pattern	Nominal Concentrations (mgL^{-1})			
		0	5	10	15
Epithelial lifting of secondary lamellae	R	−	++	++++	+++
Epithelial hypertrophy	P	−	+++	++++	+++
Bottom hyperplasia	R	−	++	++++	++++
Leukocyte infiltration	R	−	++	+++	++++

Notes: (−) No tissue damages observed; (+) there were tissue damages from 1 to 3; (++) there were tissue damages from 3 to 5; (+++) there were tissue damages from 5 to 9; (++++) there were tissue damages from 9 to 15. Abbreviations: (R) regressive changes, (P) progressive changes.

Liver

Histopathological changes in the liver of exposed fish demonstrated a variety of lesions across exposure concentrations, including macrophage aggregates, fatty liver, dilation of sinusoids, and necrosis. Except for the increased sinusoidal space and macrophage aggregation, which are circulatory-type lesions, all of these lesions are regressive. The largest proportion of lesion severity was seen in the highest exposure group (15 mgL^{-1} Ag-NPs) (Table 5). Furthermore, the uptake of the toxicant can be responsible for the increased severity of observed lesions in the liver tissue of the maximum concentration group [128].

Table 5. Liver damages of Capoeta (*Capoeta capoeta*) 96 h after exposure to different levels of Ag-NPs.

Tissue Damages	Reaction Pattern	Nominal Concentrations (mgL^{-1})			
		0	5	10	15
Macrophage aggregates	C	−	+++	+++	+++
fatty liver	R	−	+++	+++	++++
Dilation of sinusoid	C	−	++	+++	++++
necrosis	R	−	+	+++	++++

Notes: (−) No tissue damages observed; (+) there were tissue damages from 1 to 3; (++) there were tissue damages from 3 to 5; (+++) there were tissue damages from 5 to 9; (++++) there were tissue damages from 9 to 15. Abbreviations: (R) regressive changes, (C) circulatory disturbances.

4. Conclusions

The current study aimed to investigate the toxicity and toxic effects of Ag-NPs on *Capoeta capoeta.* According to the results of the present study, it can be concluded that the Ag-NPs have led to alterations of hematological and biochemical endpoints, as well as histological changes in the gills and liver. The hazardous potential of Ag-NPs in various industrial sectors should be carefully analyzed and effluents should be processed before being released into the environment to preserve aquatic eco-systems and human life, according to the findings of this experimental investigation. To protect aquatic organisms

against Ag-NPs exposure, water quality guidelines must be established based on data from toxicity studies relevant to diverse species in such environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15030585/s1, Figure S1. correlation between mortality rate of *C. capoeta* and silver nanoparticles (Ag-NPs).

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