



Research Article

# In-vivo Toxicological (Acute) Characterization of Bio-synthesized Silver Nanoparticles in *Labeo rohita*

Jayaraju Jeyasree<sup>1</sup>, Giridharan Bupesh<sup>3,7</sup>✉, Sakthivel Vasanth<sup>3</sup>, Joseph Pinto Jasmine Beulah<sup>3</sup>, Kanniyah Pandian<sup>5</sup>, Arumugam Vijaya Anand<sup>6</sup>, Tharumasivam Shiva Vijayakumar<sup>4</sup>, Lakshmi Narayanan<sup>2</sup>

<sup>1</sup>Department of Biotechnology, Bharath College of Science and Management, Thanjavur, TN, India.

<sup>2</sup>Department of Biotechnology, Thanthai Hans Roever College, Perambalur TN, India.

<sup>3</sup>R&D wing, Sree Balaji Medical College and Hospital, Chromepet, Chennai-600044 TN, India.

<sup>4</sup>Department of Biotechnology, Srimath Andavan College, Tiruchirappalli, TN, India.

<sup>5</sup>Department of Inorganic Chemistry, University of Madras, Guindy Campus, Tamil Nadu, India.

<sup>6</sup>Department of Human Genetics and Molecular Biology, Bharathiyar University, Coimbatore, India.

<sup>7</sup>Department of Forest Science, Central University of Nagaland, Lumami, India.

✉ Corresponding author. E-mail: bupeshgiri55@gmail.com, Tel.: 8012405965

**Received:** Jan. 21, 2020; **Accepted:** Apr. 14, 2020; **Published:** Apr. 14, 2020

**Citation:** Jayaraju Jeyasree, Giridharan Bupesh, Sakthivel Vasanth, Joseph Pinto Jasmine Beulah, Kanniyah Pandian, Arumugam Vijaya Anand, Tharumasivam Shiva Vijayakumar, and Lakshmi Narayanan, In-vivo Toxicological (Acute) Characterization of Bio-synthesized Silver Nanoparticles in *Labeo rohita*. *Nano Biomed. Eng.*, 2020, 12(2): 115-123.

**DOI:** 10.5101/nbe.v12i2.p115-123.

## Abstract

This study extensively investigated to find out the in-vivo acute toxicity and lethal concentration (LC50) of biosynthesized Ag NPs in *Labeo rohita* (*L. rohita*). The haematological studies and antioxidative responses were considered in three tissues such as gill, liver and muscle of *L. rohita*. The results of this study showed that increasing the dose of Ag NPs led to bioaccumulation in the tissues. The haematological analysis showed considerable alterations in the Ag NPs-treated fish. The impact of histological changes induced by Ag NPs were confirmed by the damages in the tissues, primary lamella, blood vessels and formation of vacuolation in liver and muscle when compared with the control *L. rohita*.

**Keywords:** Nanotoxicity; Silver nanoparticles; *Labeo rohita*; Acute toxicity; Haematological; Antioxidant; Histopathology

## Introduction

Silver compounds may produce toxic effects to liver, kidney, eye, skin, respiratory, intestinal tract and untoward changes in blood cells. Since the beginning of the 21st century, nanosilver has been gaining popularity and is now being used in almost every field, most importantly the medical field [1]. However, there have been reports of how nanosilver cannot

discriminate between different strains of bacteria and can hence destroy microbes. There are only very few studies conducted to assess the toxicity of nanosilver.

The physical and chemical properties of silver nanoparticles are essential for nanomaterial characterization. The silver nanoparticles have acted as excellent candidates for a number of day-to-day activities, because they exist antimicrobial and anti-inflammatory properties [2]. However, there are studies

and reports that suggest nanosilver can allegedly cause adverse effects on humans as well as to the environment [3].

Huge quantities of silver particles are released into the environment from industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase [1]. The adverse effects of these free silver ions on humans and all living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects to liver, kidney, eye, skin, respiratory and intestinal tract irritations [4].

There are only very few studies conducted to assess the toxicity of nanosilver. In a study, toxicity assay of silver nanoparticles in rat liver cells showed that even low-level exposure to silver nanoparticles resulted in oxidative stress and impaired mitochondrial function and caused leakage through the cell membranes. Nanosilver aggregates were said to be more cytotoxic than asbestos [5]. Evaluation of silver nanoparticles toxicity on rats indicated that metabolic organs such as liver, kidney were severely affected. Moreover, histopathological studies found that there was a higher incidence of bile duct hyperplasia, with or without necrosis, fibrosis, and pigmentation in the studied animals. Therefore, in the present study the acute toxicity of biosynthesised silver nanoparticles was evaluated and characterized in *Labeo rohita*.

## Experimental

### Synthesis of AgNPs Hibiscus sabdariffa leaf

The silver nanoparticles (Ag NPs) were synthesized by biological method. 0.010 L of leaf extract was added in 200 mL of silver nitrate. The solution was mixed for a few minutes. Changes of colour were observed from light yellow to dark brown (pH 11), representing the synthesis of Ag NPs. Then, the solution was centrifuged at 5000 rpm for 20 min; the collected pellet was air dried and the precipitate was collected and made into powdered form. The characterization of synthesized silver nanoparticles were characterized with ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and dynamic light scattering (DLS).

### Experimental animal

The carp fish *Labeo rohita* was selected as the

experimental animal.

### Fish maintenance and experimental design

Rohu (*Labeo rohita*) is one of the major carps in India and was obtained from the fish farm, Thanjavur, Tamil Nadu, India. The experiment was carried out with the average fish weight of 7 to 10 g. The fish were treated in 3 different concentrations based on  $LC_{50}$  value and the same control time maintained. Each group containing 20 fish daily fed with standard pellet feed.

### Acute toxicity test of silver nanoparticles

Acute toxicity ( $LC_{50}$ ) test of silver nanoparticles was carried out on *Labeo rohita*. All experiments were carried out for a period of 3 days, with the mortality rate recorded every day. Initial  $LC_{50}$  was confirmed with each group exposed to 4 different concentrations (50, 100, 200 and 500 mg) of silver nanoparticles on 40 *Labeo rohita* individuals. Among these concentrations, the 50% mortality rate was found at 50 mg concentration. Hence, the further study was carried out using 10, 20 and 30 mg of AgNPs.

### Protein estimation

The protein concentration was estimated using the method as reported by Bradford et al. [6].

### Phosphatase assay

Acid and alkaline phosphatase activity was estimated according to Michell et al. [7] and Estiarte et al. [8]. The reaction medium for acid phosphatase containing 0.7 mL sodium acetate buffer (pH 5.0), 0.25 mL p-nitrophenyl phosphate (pNPP, 5 mM) as substrate, and 0.05 mL of enzyme, totaling to 1 mL, was incubated for 30 min at 37 °C. The reaction was stopped by adding 4 mL NaOH (0.1 N) and incubated for another 30 min at 37 °C. The reaction medium for alkaline phosphatase containing 0.5 mL glycine buffer (pH 7.8), 0.2 mL magnesium chloride (10 mM), 0.25 mL p-nitrophenyl phosphate (pNPP, 5 mM) as substrate and 0.05 mL of enzyme, totaling to 1 mL, was incubated for 30 min at 37 °C. The reaction was stopped by adding 4 mL NaOH (0.02 N) and incubated for another 30 min at 37 °C. The estimation involved measurement of yellow colour of p-nitrophenol at 420 nm (Synergy HT Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT, USA).

### Assay of catalase (CAT)

Catalase activity in gill, muscle and liver of the

fish was estimated using Claiborne's method [9].  $H_2O_2$  and phosphate buffer (3.0 mL) were taken in an experimental cuvette, followed by the rapid addition of 40  $\mu$ L of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer. The enzyme solution containing  $H_2O_2$ -free phosphate buffer served as control. Then, absorbance was taken at 1 minute interval for 4 times at 240 nm using UV-Vis spectrophotometer.

### **Assay of superoxide dismutase (SOD)**

SOD was assayed according to the method of Kakkar et al. [10]. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate (PMS), 0.3 mL of nitroblue tetrazolium chloride (NBT), 0.2 mL of the enzyme preparation and water in a total volume of 2.8 mL. The reaction was initiated by the addition of 0.2 mL of nicotinamide adenine dinucleotide (NADH). The mixture was incubated at 30 °C for 90 sec and arrested by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was then shaken with 4.0 mL of n-butanol, allowed to stand for 10 min and centrifuged. The intensity of the chromogen in the n-butanol layer was measured at 560 nm in UV-Vis spectrophotometer. 1 unit of enzyme activity was defined as the amount of enzyme that gave 50% inhibition of NBT reduction in 1 min.

### **Assay of lactase dehydrogenase (LDH)**

LDH activity in gill, liver, and muscle of the fish was estimated by Vassault's method [11]. For the estimation, LDH, gill, liver and muscle were taken in centrifuge tubes and marked as test (T1, T2 and T3) and control (C). To each tube, 2.6 mL of reagent 1 and PBS were added. Then, 0.2 mL of sodium pyruvate was added. Then, 0.1 mL of NADH was added. Then, 0.1 mL of sample from control and experimental fish was dropped in respective tube. The content in all tubes were mixed well, and absorbance was taken at 340 nm using UV-Vis spectrophotometer.

### **Assay of glutathione S-transferase (GST)**

The GST activity was determined according to Bergmeyer et al. [12].

### **Assay of glutathione reductase (GSH)**

GSH activity in gill, liver and muscle of the fish was estimated following the method by Smith et al. [13].

For the estimation of GSH, 4 centrifuge tubes were taken and marked as test (T1, T2 and T3) and control (C). To each tube, 75  $\mu$ L of GSSG was added. Then, 25  $\mu$ L of NADPH was added. Then, 50  $\mu$ L EDTA was added, Then, 1.8 mL of 0.1 M sodium phosphate buffer was added. Then, 50  $\mu$ L of sample was added and mixed well. Absorbance was taken at 340 nm using UV-Vis spectrophotometer.

### **Hematological studies**

Hematological characteristics as a tool for screening pathological status have shown to be good indicator of physiological responses. The use of hematological parameters in the assessment of fish physiology was proposed by Hesser [14]. Since then haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful conditions.

#### **Preparation of blood sample**

Blood was drawn by puncturing the cardiovascular using plastic syringes which prevented decreased coagulation time [13]. Blood from the fish was collected in the vials containing EDTA as the anticoagulant. However, blood for total RBC count, WBC count, Hb percentage and clotting time were taken directly from the fish.

#### **RBC count**

The red blood cells (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe [15].

#### **WBC count**

The total white blood cell counts (WBC) were enumerated with an improved Neubauer Hemocytometer using Shaw's diluting fluid [16]. Total number of white blood corpuscles were counted and expressed in thousand per cu.mm of blood.

#### **Hemoglobin estimation**

Hemoglobin content of the blood was estimated by Sahli's acid haematin method using Sahli's hemometer using cyanmethemoglobin method.

#### **Packed cell volume (PCV)**

Hematocrit is the volume of red cell expressed as a percentage of the volume of total blood in the sample. The Venus hematocrit is almost the same as that obtained from a skin puncture dried heparin EDTA or double oxalate or satisfactory anticoagulants.

### Determination of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. Termed red cell indices, these values are useful in elucidating the etiology of anemias. Red cell indices can be calculated if the values of hemoglobin, hematocrit (packed cell volume), and red blood cell count are known. With the general availability of electronic cell counters, red cell indices are now automatically measured in all blood count determinations. Here we identified the MCV, MCH and MCHC concentration using the method of Mari et al. [17].

### Histopathology analysis

After completion of the treatment, the fish tissues such as gill, liver and muscle were dissected and stored for histological examination.

### Statistical analysis

Values for each parameter were expressed as arithmetic mean  $\pm$  standard error (SE). The software program SPSS (Version 14.0; SPSS) for Windows was used for the analysis.

## Results and Discussion

### Hematology studies

In the present investigation, the efficacy of synthesis Ag NPs from Hibiscus sabdariffa leaf extract on the hematological changes in *Labeo rohita* was studied. Red blood cells (erythrocytes count) showed a decreasing trend ( $p < 0.05$ ) in all experimental groups when compared to the control. Groups treated with 30 mg NPs showed a decreasing trend of statistical significance ( $p < 0.05$ ) as compared to the control. The white blood cells count (leucocytes count) in treated groups (10, 20, and 30 mg) was significantly lower than those of the control group ( $p < 0.05$ ). The minimum mean value was observed in groups treated with 30 mg NPs. According to the results, Ag NPs could decrease leucocytes count of fish in experimental groups compared to the control group. The hemoglobin and hematocrit in treated groups (10, 20, and 30 mg) were significantly lower than those of the control group ( $p < 0.05$ ). The mean corpuscular volume and mean corpuscular hemoglobin in fish treated with 10, 20, and 30 mg of synthesized Ag NPs significantly increased as compared to the control group. However, there was no statistical significance ( $p > 0.05$ ) with any treated group as compared to the control (Table 1).

### Biochemical studies

Total serum protein and cholesterol level are shown in Table 2. The result indicated the total serum and cholesterol level significantly decreased gradually

**Table 1** Changes in hematological parameters when treated with synthesized Ag NPs from Hibiscus sabdariffa-leaf extract in *Labeo rohita*

Con. of NP	Hb (g/dl)	PCV (%)	MCV (cu micron)	MCH (pg)	MCHC (%)
Control	2.3 $\pm$ 0.2	21 $\pm$ 10	21 $\pm$ 10	30.4 $\pm$ 0.6	33.3 $\pm$ 11
10 mg	4.6 $\pm$ 2.3	13.7 $\pm$ 6.710	91.3 $\pm$ 0.6	33.33 $\pm$ 0.08	30.44 $\pm$ 0.9
20 mg	3.6 $\pm$ 2.5	13.3 $\pm$ 15.2	90 $\pm$ 0.5	28.6 $\pm$ 0.04	31.80 $\pm$ 0.5
30 mg	4.3 $\pm$ 2.1	13.6 $\pm$ 15.2	90 $\pm$ 0.5	32.85 $\pm$ 0.1	36.5 $\pm$ 11.2

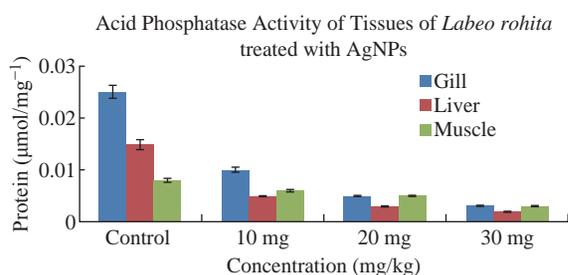
**Table 2** Changes in biochemical parameters when treated with synthesized Ag NPs in *Labeo rohita*

Concentration of Ag NPs (mg)	Protein (%)	Cholesterol (mg/dL)
Control	9.0	85.0
10	13.7	35.0
20	13.2	35.0
30	13.8	79.0

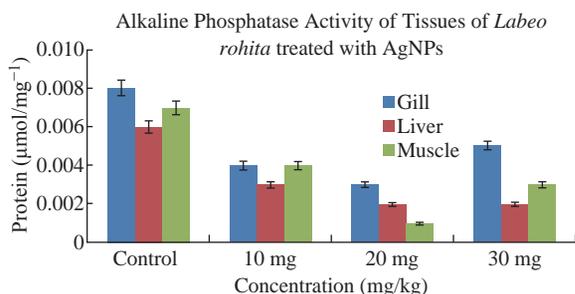
throughout the experimental period, compared to the control value. The 10, 20, and 30 mg groups showed decreases in the total protein and cholesterol levels as compared to the control, due to the addition of concentration of Hibiscus sabdariffa leaf extract (Table 2).

### Effects on antioxidant enzymes

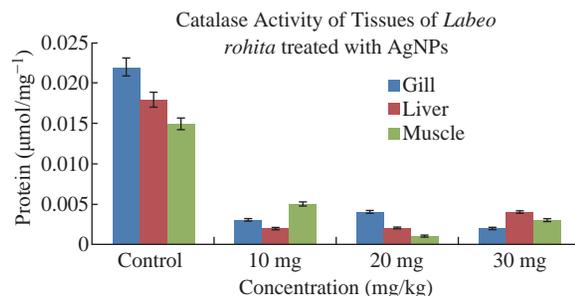
In this present investigation, toxicity of silver nanoparticles was studied by evaluating the level of antioxidant enzymes in the treated fish. The role of acid phosphatase (ACP) activity was studied, and there was an increase in the activity of ACP when compared with the control. The ACP in fish treated with 10 and 20 mg NPs was similar to the control. However, the fish treated with 30 mg significantly increased the ACP in the gills and muscle as compared to the control (Fig. 1). The activity of alkaline phosphatase (ALP) level in all the tissues exposed to 10 and 20 mg biologically synthesized silver nanoparticles was not significantly different from the control; however, it significantly increased in all tissues when exposed to 30 mg NPs (Fig. 2). The catalase (CAT) activity did not vary significantly from the control at concentration of the tissues (Fig. 3). The superoxide dismutase (SOD) activity did not increase statistically at 10 and 20 mg concentrations of NPs, whereas it significantly increased in tissues with 30 mg concentration of NPs



**Fig. 1** Acid phosphatase (ACP) activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs from Hibiscus sabdariffa leaf extract at 10, 20 and 30 mg concentrations.

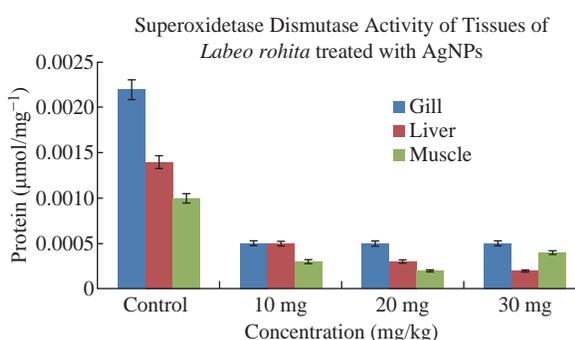


**Fig. 2** Alkaline phosphatase (ALP) activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs at 10, 20 and 30 mg concentrations.

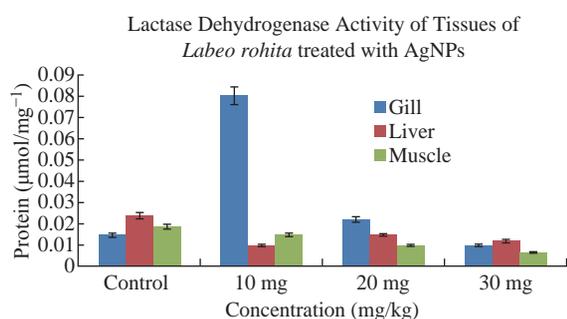


**Fig. 3** Catalase activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs at 10, 20 and 30 mg concentrations.

(Fig. 4). The lactate dehydrogenase (LDH) content did not significantly vary at any concentration in gill, liver, kidney and muscle, when compared with tissues in the control group (Fig. 5).



**Fig. 4** Superoxide dismutase activity in gill, liver, and muscle of *Labeo rohita* treated with Ag NPs from Hibiscus sabdariffa leaf extract at 10, 20 and 30 mg.

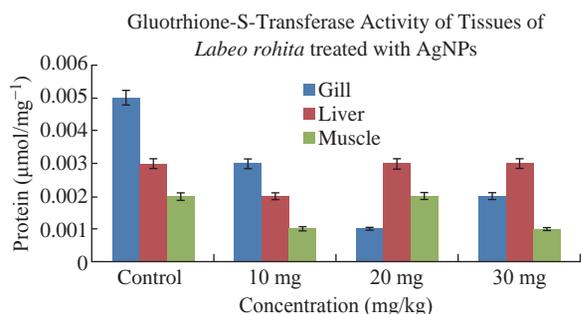


**Fig. 5** Lactate dehydrogenase activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs at 10, 20 and 30 mg.

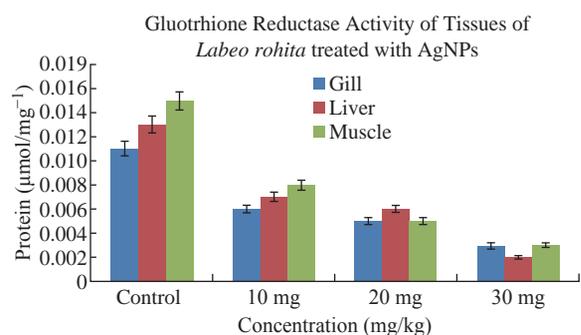
The glutathione S-transferase (GST) activity was observed in progressively decreasing trends when treated with the biologically synthesized silver nanoparticles in the tissues (Fig. 6). Glutathione reductase activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs from Hibiscus sabdariffa leaf extract is shown in Fig. 7.

### Histopathology

The histopathological changes were observed in



**Fig. 6** Glutathione S-transferase activity in gill, liver, and muscle of *Labeo rohita* treated with Ag NPs.



**Fig. 7** Glutathione reductase activity in gill, liver, and muscle of *Labeo rohita* treated with synthesized Ag NPs from Hibiscus sabdariffa leaf extract at 10, 20 and 30 mg.

Rohu (*Labeo rohita*) fish for 15 days after exposure to silver nanoparticles in selected tissues such as gill, liver and muscle (Figs. 8-10). After the treatment duration,

the tissues were dissected and subjected to investigate the histopathological effect of chemically synthesized silver nanoparticles. The histopathological images present different concentrations of silver nanoparticles.

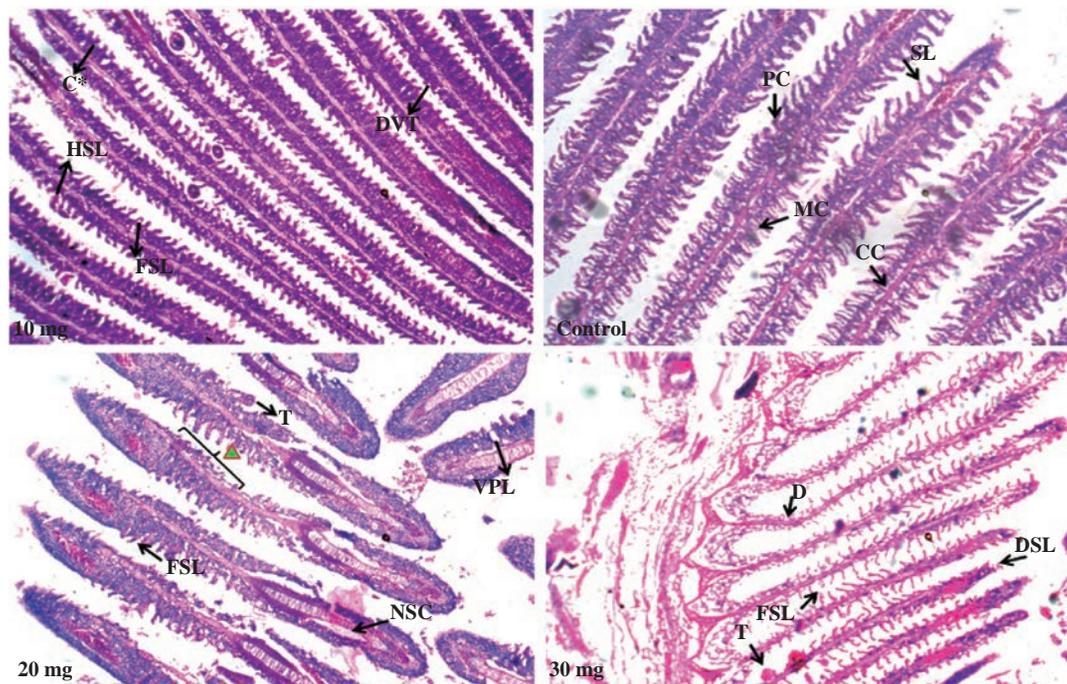
### Gill

In tissue of the control group, there was no recognizable changes observed in the gill sample during the experimental period. The control gill showed primary and secondary lamellae, and it also consisted of primary lamella and central venous sinus. The figure shows central venous sinus, chloride cells, primary and secondary lamellae with pillar cells.

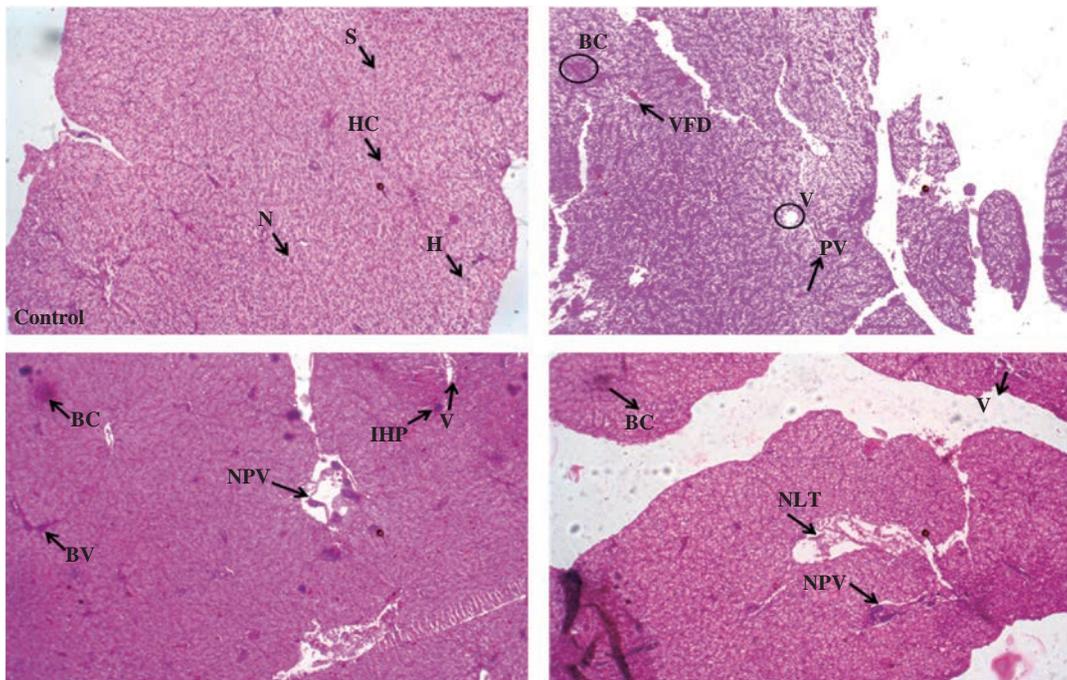
The Ag NPs-treated fish exhibited proliferation of bronchial chloride cells that led to lamellae fusion and formation of aneurism in the Ag NPs-treated group. The aneurism was localized, blood-filled balloon-like bulge of a blood vessel. It increased a significant risk of rupture, resulting in severe hemorrhage, other complications or death (Fig. 8).

### Liver

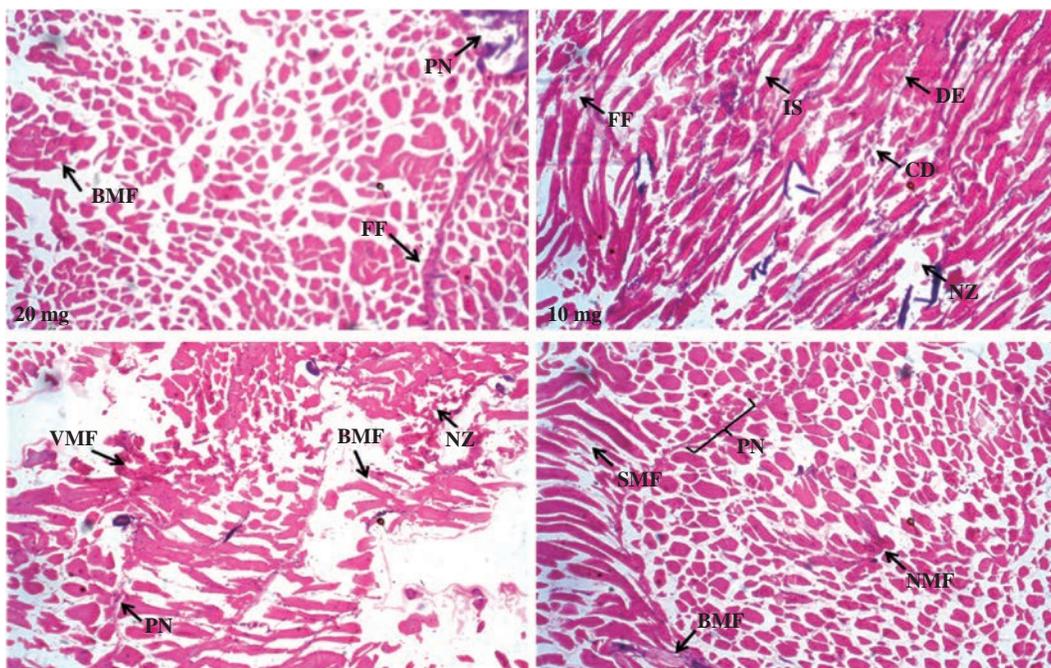
In the liver section, the control tissue showed normal hepatocytes. The Ag NPs-treated fish had congestive enlargement of liposome leading to vacuolar degenerations in liver. The necrosis was very high in liver tissues of fish exposed to Ag NPs (Fig. 9).



**Fig. 8** Histoarchitectural changes in gill tissue of *Labeo rohita* due to the upshot of synthesized Ag NPs: Histological changes of gills after exposure to Ag NPs. CC: Chloride cell; SL: Secondary lamellae; MC: Mucous cell; PC: Pillar cell; C\*: Fusion of tips on secondary lamella; D: Debris; HSL: Hypertrophy secondary lamella; FSL: Folded secondary lamella; FSL: Folded secondary lamella; T: Telangiectasia; DSL: Disintegrating secondary lamellae; T: Telangiectasia; FSL: Folded secondary lamella; VPL: Vacuolated primary. \*Degeneration and necrosis of pillar cells and dilution of capillary walls.



**Fig. 9** Histoarchitectural changes in liver tissue of *Labeo rohita* treated with Ag NPs: Histological changes of liver after exposure to AgNPs. AC: Acinar cell; HC: Hepatocytes; N: Nucleus; S: Sinusoid; H: Karyorrhexis; N: Nucleus; BC: Blood conjunction; VFD: Vacuolation and fatty degeneration; V: Vacuoles; PV: Portal vein; BV: Blood vessel; IHP: Intrahepatic pancreas; NPV: Necrotic portal vein; NLT: Necrotic livers tissue.



**Fig. 10** Histoarchitectural changes in muscle tissue of *Labeo rohita* due to the upshot of synthesized Ag NPs: Histological changes of muscle after exposure to Ag NPs. MF: Muscle fibre; SM: Striated muscle; LSM: Lesion of striated muscle; PN: Pyknotic; FF: Fragmented fiber; BMF: Bend muscle fiber; NA: Necrotic area; NM: Necrotic muscle, NZ: Necrotic zone; VMF: Vacuolated muscle fiber; SMF: Shrinkage of muscle fiber; IS: Intracellular space.

**Muscle**

The section of the muscle showed normal histological structure such as fibber bundles, connective tissue and arrangement of muscle bundles.

Fig. 10 shows the normal histological structure of the muscle of a fish in the control group. The treated fish had an abnormal arrangement of muscle bundles, and muscle fibber inflammation was seen in treated fish.

## Discussion

The present study evidenced that the tissue damaging enzymes such as ACP and ALP activities markedly increased in *Labeo rohita* after 3 days' exposure to Ag NPs. ALP enzyme was involved in the metabolisms of amino acids. Alkaline phosphatase is associated with cell membranes in multiple tissues. Evolution in the specific activity of ACP and ALP was observed in the present investigation. ACP played an important role in the detoxication process of toxic compounds entering the body Zhang et al. [18]. On the other hand, ALP has often been implicated in phosphorylative transfer of extracellular molecules against concentration gradients at cell membranes [19, 20].

In the present study, in all the analyzed tissue of *Labeo rohita*, antioxidant CAT significantly decreased when orally administered with 4 different sub-lethal concentrations of Ag NPs. Recently, it has been established that reactive oxygen species, which is produced during the toxicity of nanoparticles, can also be used as a biomarker for nano toxicity. Surface area of nanoparticles in biological samples can make the materials catalytically active and generate anions/reactive species. The treatment caused a significant elevation of enzyme level as GSH when compared to the control [21].

Recently, we have found that nano-Ag could induce cytotoxicity and protein alterations in HaCaT cells. Nanoparticle- or metal ion-induced oxidative stress leading to cell death has been previously reported at the genomic level, but has not yet been investigated at the epigenomic level [22].

A significant alteration in blood biochemistry was observed in exposure to nanosilver administration when compared with the control fish. Ag NPs treated-fish's gills showed lamellae fusion and aneurism formation, and necrosis was observed in the secondary lamellae, enlargement of liposomes leading to vascular degeneration in liver. Similar results were observed in freshwater fish *Oreochromis mossambicus* exposed to synthesized silver nanoparticles which showed changes in the histological structure of gill, liver and muscle [23].

Histopathological studies were found by different authors to be a useful tool for assessing the damage caused by nanomaterials. In the present investigation, the impacts of Ag NPs were observed in gills, muscle and liver tissue, which showed the level of injury increased with the concentration of Ag NPs

and suggested accumulation of Ag in liver. These effects and changes were in agreement with those observed in *Danio rerio*, *Drosophila melanogaster* and *Oncorhynchus mykiss* (rainbow trout) exposed to silver nanoparticles. [24-26]. Mishra and Mohanty reported histological changes in the hepatic tissue of fish after silver nanoparticle treatment. Muscle fibre inflammations were noticed in treated *Labeo rohita*. The similar change was observed in the toxic effect of pesticides on the vital organs of juvenile rainbow trout [27, 28]. An earlier report of Ostaszewska et al. [23] observed histopathological alteration in gills and liver of Siberian sturgeon when exposed to Ag NPs.

## Conclusions

Silver nanoparticles were evaluated to study the toxic nature using *Labeo rohita* as an aquaculture and environmental indicator. The enzymatic parameters viz., acid phosphatase, alkaline phosphatase, sorbitol dehydrogenase, catalase, superoxide dismutase, lactase dehydrogenase, glutathione-S-transferase and glutathione reductase were significantly elevated in gill when compared to other organs in *Labeo rohita* treated with biosynthesized silver NPs. The present study addressed the problem of environmental toxicity due to silver nanoparticles which might be toxic to aquatic ecosystem and ultimately being toxic when consumed by human. However, the optimized dosages could help in the enhancement of blood parameters and enhanced enzyme levels which acted as biomarkers to determine the toxicity of polluted environment.

## Conflict of Interests

The authors declare that no competing interest exists.

## References

- [1] N.R. Panyala, E.M. Pena-Mendez, and J. Havel, Silver or silver nanoparticles: A hazardous threat to the environment and human health? *J. Appl. Biomed.* 2008, 6: 117-129.
- [2] S. Prabhu, E.K. Poulouse, Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int Nano Lett*, 2012, 2(1): 32.
- [3] N. Panyala, E.M. Pena-Mendez, and J. Havel, Silver or silver nanoparticles: a hazardous threat to the environment and human health. *Journal of Applied Biomedicine*, 2008, 6: 117-129.
- [4] M. Allsopp, A. Walters, and D. Santillo, Nanotechnologies and nanomaterials in electrical and electronic goods: A

- review of uses and health concerns. *Greenpeace Research Laboratories*, London, 2007.
- [5] S. Hussain, K.L. Hess, J.M. Gearhart, et al., In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in Vitro*, 2005, 19: 975-983.
- [6] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, 72(1-2): 248-254.
- [7] R.H. Michell, M.J. Karnovsky, and M.L. Karnovsky, The distributions of some granule-associated enzymes in guinea-pig polymorphonuclear leucocytes. *Biochemical Journal*, 1970, 116(2): 207-216.
- [8] J. Sardans, J. Peñuelas, and M. Estiarte, Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology*. 2008, 39(2): 223-235.
- [9] A. Claiborne, Catalase activity. *Handbook of methods for oxygen radical research*. CRC Press Inc., Boca Raton, 1984: 283-284.
- [10] P. Kakkar, B. Das, and P.N. Viswanathan, A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, 1984, 21(2): 130-132.
- [11] A. Vassault, Lactate dehydrogenase, UV-method with pyruvate and NADH. *Methods in Enzymatic Analysis*, 1983, 3: 118.
- [12] H.U. Bergmeyer, E. Bernt, Glutamateoxaloacetate transaminase. *Methods of enzymatic analysis*. Academic Press, New York, 1965: 837-851.
- [13] I.K. Smith, T.L. Vierheller, and C.A. Thorne, Assay of glutathione reductase in crude tissuehomogenates using 5,5'-dithiobis (2-nitrobenzoic acid). *Analytical Biochemistry*, 1988, 175(2): 408-413.
- [14] E.F. Hesser, Methods for routine on fish haematology. *The Progressive Fish Culturist*, 1960, 22: 164-171.
- [15] M.M. Wintrobe, *Clinical haematology*. H. Kimpton, London, UK, 1978.
- [16] J.B. Miale, *Laboratory medicine haematology*, 6th edition. The CV Mosby Publishing London, 1982.
- [17] L.S. Mari, C. Jagruthi, S.M. Anbazahan, et al., Protective effect of chitin and chitosan enriched diets on immunity and disease resistance in Cirrhinamrigala against Aphanomycesinvadans. *Fish & Shellfish Immunology*, 2014, 39(2): 378-385.
- [18] F.Q. Zhang, Y.S. Wang, Z.P. Lou, et al., Effect of heavy metal stress onantioxidative enzymes and lipid peroxidation in leaves and roots of twomangrove plant seedlings (Kandeliacandel and Bruguieryagymnorrhiza). *Chemosphere*, 2007, 67: 44-50.
- [19] A.K. Mishra, B. Mohanty, Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch). *Science of the Total Environment*, 2009, 407: 5031-5038.
- [20] M.A. Monin, P.V. Rangneker, Histochemical localization of acid and alkaline phosphatases and glucose-6-phosphatase of the hepatopancreas of the crab, *Scylla serrata* (Forsk.). *Journal of Experimental Marine Biology and Ecology*, 1974, 14(1): 1-6.
- [21] A. Nel, T. Xia, L. Madler, et al., Toxic potential of materials at the nano level. *Science*, 2006, 311(5761): 622-627.
- [22] T. Ostaszewska, M. Chojnacki, M. Kamaszewski, et al., Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon. *Environmental Science and Pollution Research*, 2016, 23: 1621-1633.
- [23] R. Govindasamy, A.A. Rahuman, Histological studies and oxidative stress of synthesized silver nanoparticles in tilapia (*Oreochromis mossambicus*). *Journal of Environmental Sciences*, 2012, 24: 1091-1098.
- [24] S. Aghamirkarimi, M.A. Mashinchian, I. Sharifpour, et al., Sublethal effects of copper nanoparticles on the histology of gill, liver and kidney of the caspian roach, *rutilus rutilus caspicus*. *Global Journal of Environmental Science and Management*, 2017, 3(3): 323-332.
- [25] T.Yazdanparast, I. Sharifpour, M. Soltani, et al., Evaluation of silver retention in different organs of zebrafish (*Danio rerio*) fed diet supplemented with silver nanoparticles. *International Journal of Engineering Research*, 2016, 5(4): 269-274.
- [26] S.A. Johari, M.R. Kalbassi, I.J. Yu, et al. Chronic effect of waterborne silver nanoparticles on rainbow trout (*Oncorhynchus mykiss*): histopathology and bioaccumulation. *Comp Clin Pathol*, 2015, 24: 995-1007.
- [27] S. Vasanth, A. Ganesh, T.S. Vijayakumar, et al., Impacts and impairments of atrazine on male *Poecilia sphenops*. *Indian Journal of Advances in Chemical Science*, 2013, 2: 62-70.
- [28] X. Yang, J. Liu, H. He, et al., SiO<sub>2</sub> nanoparticles induce cytotoxicity and protein expression alteration in HaCaT cells. *Particle and Fibre Toxicology*, 2010, 7(1): 1.
- [29] T. Ostaszewska, M. Chojnacki, M. Kamaszewski, et al., Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon. *Environmental Science and Pollution Research*, 2016, 23(2): 1621-1633.

**Copyright**© Jayaraju Jeyasree, Giridharan Bupesh, Sakthivel Vasanth, Joseph Pinto Jasmine Beulah, Kanniyah Pandian, Arumugam Vijaya Anand, Tharumasivam Shiva Vijayakumar, and Lakshmi Narayanan. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.