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Long-term exposure to high-concentration silver nanoparticles induced toxicity, fatality, bioaccumulation, and histological alteration in fish (*Cyprinus carpio*)

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Abstract

Background: Currently, nanotechnology and nanoparticles have quickly emerged and have gained the attention of scientists due to their massive applications in environmental sectors. However, these environmental applications of silver nanoparticles potentially cause serious effects on terrestrial and aquatic organisms. In the current study, freshwater fish *C. carpio* were exposed to blood-mediated silver nanoparticles for toxicity, mortality, bioaccumulation, and histological alterations. Silver nanoparticles were fabricated using animal blood serum and their toxic effect was studied against common carp fish at different concentrations levels (0.03, 0.06, and 0.09 mg/L).

Results: The findings have revealed a little influence of blood-induced silver nanoparticles on fish behavior at the highest concentration (0.09 mg/L). However, bioaccumulation of blood-mediated silver nanoparticles was reported in different organs of fish. Maximum bioaccumulation of silver nanoparticles was reported in the liver, followed by the intestine, gills, and muscles. Furthermore, the findings have shown that the bioaccumulation of silver nanoparticles led to histopathological alterations; including damaged structure of gill tissue and have caused necrosis. It is summarized that histopathological alteration in gill and intestine mostly occurred by the highest concentration of blood-induced silver nanoparticles (0.09 mg/L).

Conclusion: This study provides evidence of the silver nanoparticles influence on aquatic life; however, further systematic studies are crucial to access the effects of AgNPs on aquatic life.

Keywords: Novel Silver nanoparticles, Ecotoxicity, Bioaccumulation, Histopathological alterations, Enzymatic stress

Background

Nanotechnology (NT) and nanoscience is one of the remarkable areas of science dealing with using the structure of nanoscale [1], and tremendously developed in the last decade [2]. Due to their wide range of applications,

NT is playing a beneficial role in water treatment, food industry, household equipment, biomedical, and health care [3]. Nanoparticles (NPs) are a special kind of nano-sized materials with unique properties due to their small size and dispersal [4]. Among all the nanoparticles, silver nanoparticles (AgNPs) are well known for their commercialization worldwide [5, 6], due to their sole biological activities [7], and are being utilized widely in medicine [8]. Despite the biological activities and a wide range applications of AgNPs, there is a lack of information regarding human health and environmental toxicity [9]. The extensive use of AgNPs in the world, release into the

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aquatic environment, has raised concern [10] for a high impact on aquatic life [11].

The rare knowledge of AgNPs regarding aquatic toxicity and their linkage with living components have raised concerns in the last decade [12]. It has been hypothesized and suggested that the concentration of AgNPs in an aquatic ecosystem is in $\mu\text{g L}^{-1}$ or mg L^{-1} [13], however, these concentrations are yet toxic to any environment [14]. It has been reported that mutable properties and the small size of AgNPs are toxic and dangerous to the ecosystems [15, 16]. Silver is found in four different oxidation states (Ag , Ag^+ , Ag^{2+} , and Ag^{3+}) in the aquatic environment [15, 17], though the exact estimates of AgNPs toxicity are still hindered due to lack of acquaintance [12]. It has been accepted that the toxicity caused by AgNPs is due to the release of Ag^+ into the environment and finally absorbed by living organisms [18]. Silver nanomaterials are the most producing nanoparticles and release extensively to both terrestrial and aquatic environments [19], which is very toxic to fish fauna, and therefore it is very important to establish the toxicity of nanosilver [20].

Common carp fish (*Cyprinus carpio*) is one of the most important fish species of freshwater [21], distributed in the Asia and Europe [22]. It has been estimated that the production of common carp fish is about 4.32 million tons [21]. When fish is exposed to any kind of toxin, it could lead to changes in biochemical, hematological parameters, histological alteration, and gut microbial dysbiosis [23]. Nowadays, NT is rapidly used as targeted specific drugs to control fish diseases [24], because of its broad antibacterial activity [4] and anti-protozoal activity [25], but the exposure of AgNPs to fish led to the multiplicity of effects, even mortality [19], organ dysfunction, other lesions and gene mutation [8]. The small nanomaterials can easily penetrate inside the tissues of fish, such as the liver, gills, brain, and muscles [12]. Fish, being a healthy food source for humans, hence, nanosilver could migrate from fish to human, which might lead to silver contamination in human.

Among all aquatic organisms, fish is one of the most used aquatic organism for assessing environmental toxicity and toxic effects [12]. Therefore, this study was designed to evaluate the acute, chronic, and sublethal toxicity of three different concentrations of blood-mediated AgNPs in common carp fish for a long-time exposure of twenty days' period. In vivo, the studies regarding toxicity and toxic effects of AgNPs are principally lacking; especially in aquatic systems where ecologically significant and diverse organisms are affected. The current study was aimed to expose freshwater fish *C. carpio* to novel fabricated silver nanoparticles for toxicity,

mortality, bioaccumulation, and histological alterations. Primarily the tissues exposed to various foreign pollutants in an aquatic fish are gills, muscles, intestine, and liver, hence these tissues were studied.

Materials and methods

Nanoparticles preparation

Sheep's (*Ovis aries*) blood was collected from the slaughterhouse, Xiao Xihu Gonglin Road, Lanzhou (longitudes of $102^{\circ}30''$ to $104^{\circ}30''$ and latitudes of $35^{\circ}5''$ to 38°), Gansu, China. The blood was collected in a closed container in the presence of a buffer solution, which keeps maintaining the proteins and other biological molecules of blood serum. The blood was stored at room temperature for serum separation and then centrifuged three times at 12,000 rpm for 30 min. The serum was separated and stored at 4°C for further analysis. Pure silver nitrate (99.99%) solution (Sigma Aldrich Beijing, China) of 10 mL was mixed with 90 mL distilled water (10 mM AgNO_3 solution). Subsequently, 100 mL of serum was added to 100 mL of silver nitrate solution (10 mM) in a 500-mL Erlenmeyer flask and incubated for 24 h at 37°C at 150 rpm. The synthesized B-AgNPs were purified and separated through repeated centrifugation at 12,000 rpm for 20 min and stored for further use.

Characterization of nanoparticles

The synthesis of blood serum mediated silver nanoparticles (B-AgNPs) was indicated by the color change of the solution from reddish to brownish after incubation. However, UV-Vis spectrometry was used and plasmon bands were recorded at 422 nm that verify the B-AgNPs formation. The obtained B-AgNPs were characterized for their shape and size. The phase purity and crystalline structure of the obtained B-AgNPs were analyzed by X-ray diffraction (XRD) using Bruker D2 Phaser with graphite monochromator operated at 30 kV and 15 mA. Transmission electron microscopy (TEM) Tecnai G2 Bio-Twin and scanning electron microscope (SEM) Hitachi S-3400N was used to characterize the detailed morphology, size, and distribution of synthesized B-AgNPs. The size of the synthesized B-AgNPs was measured by ImageJ software.

Fish species and their laboratory maintenance

The juvenile common carp fish (*C. carpio*) (length: 12.3 ± 1.28 cm, weight: 9.59 ± 1.83 g) were brought from the Yantan local market, Chengguan district ($36,007^{\circ}\text{N}$ $103,082^{\circ}\text{E}$; Lanzhou, Gansu). The present study was conducted from August to October 2020. These fish were allowed to adapt in tap water under

a natural photoperiod of 14:10 h light–dark period for one week. After acclimation, the healthy fish were selected for further studies, which were confirmed through physical appearance (skin luster, eyes, color, and behavior). All fishes were kept at ambient temperature, pH, and dissolved oxygen (DO_2) given in Additional file 1: Fig. S1. Fish were fed with a commercial food diet two times a day (9:00 AM and 9:00 PM), manufactured by JK. Aquarium, China (algae powder, shrimp meal, wheat gluten, soya bean, crude protein, and fresh fishmeal). The overall experiment was conducted in 10:14 dark and light cycles. In the whole experiment, 20% of water was changed daily for avoiding bacterial or fungal infection while 100% of water was changed once a week.

Experimental setup

The current study was conducted according to the requirements of the Organization for Economic Cooperation and Development [26] for long-time exposure to B-AgNPs. There were four experimental groups as follows: a control group and three B-AgNPs (different concentrations) treatment groups. The fish in the water tanks were exposed to B-AgNPs 0.03 mg/L (LC_{50}), 0.06 mg/L (LC_{50}), and 0.09 mg/L (LC_{50}). Each group comprised 20 fish. Fish were not fed for 24 h before starting the experiment. After every 48 h, the fish were sampled from every group of studies. The collected fish were anesthetized and dissected for different organs (muscles, gills, intestine, and liver) collection. The collected organs were stored at -80°C for further studies.

Lethal concentration (LC_{50})

The mortality and acute toxicity were carried out according to the Organization for Economic Cooperation and Development [26]. The mortality rates were recorded every 24 h for 20 days.

Tissue silver contents

The collected organs were washed and properly dried. Dried organs were kept inside the muffle furnace at 450°C for 2 h for digestion. Further 10 mL HCl of 6 mM was added to 1 g of ash. After the complete absorption of ash in HCl, the solution was allowed for filtration following the protocol of [12].

Analytical detection of AgNPs in tissue

The filtered solution was analytically analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Agilent Technologies, 5100) for the bioaccumulation of AgNPs in different tissues of common carp fish.

Tissue preparation for light microscopy

Tissues were observed for histopathological alteration under a light microscope. The dissected tissues were fixed in a 9% formaldehyde solution. After the fixation, the tissues were dehydrated in gradient alcohol solutions and implanted with paraffin wax. The tissue of $4\ \mu\text{m}$ thickness was prepared by a rotary microtome Leica-2016 Germany and placed on a glass slide and stained with eosin and hematoxylin. The prepared samples were visualized with digital trinocular camera microscope BA210Digital.

Biomarker assay

Tissue extracts for antioxidant enzyme measurements were obtained from gills and liver by homogenizing the tissues using 0.1 M sodium phosphate buffer, pH 6.5 containing 20% glycerol, 1 mM EDTA, and 1.4 mM dithioerythritol. The glutathione *S*-transferase (GST) activity was conducted using 1-chloro-2,4-dinitrobenzene substrate. Glutathione reductase activity (GR) was conducted using homogenizing the tissues in 0.1 M K-phosphate, 5 mM ascorbate. Catalase assay (CAT) mixture of 3 mL consisted of 0.05 mL tissue extract, 1.6 mL phosphate buffer (100 mM buffer, pH 7.0), 0.5 mL H_2O_2 , and 0.95 mL distilled water.

Statistical analysis

In the overall experiment, the median lethal concentrations (LC_{50}) were obtained using the trimmed Spearman–Karber method. All the experimental data were tested for normality and presented as means with standard deviation. The data were tested for time (days) and treatment effects by two-way analysis (ANOVA) followed by post hoc Duncan. p value < 0.05 was considered statistically significant.

Results

Characterization of B-AgNPs

In this study, stable, spherical, small size, and well-dispersed AgNPs were obtained by using animal blood. The synthesis of B-AgNPs was initially confirmed from the color change of the solution, however, the maximum absorption of UV–Vis spectrometry at 422 nm (Fig. 1a) further confirmed the B-AgNPs synthesis. The X-ray diffraction analysis showed peaks 111, 200, 220, and 311 that indicate the crystalline structure of the obtained B-AgNPs (Fig. 1b). The other peaks lower than 30, is due to residue of organic components present in the blood serum. The size of the obtained B-AgNPs was a small-sized range from 20 to 50 nm (Fig. 2a). Moreover, the SEM analysis showed spherical and well-dispersed AgNPs at $2.0\ \mu\text{m}$ (Fig. 2b).

Toxicity of B-AgNPs against fish

The obtained B-AgNPs were applied in vitro for their mortality and toxicity in fish fauna. *C. carpio* was very prone to B-AgNPs absorption and caused damage at the tissue level, but with very little mortality. In the present study, the behavior of fish was studied and the fish group (0.09 mg/L) has shown very less abnormal behavior while exposed to B-AgNPs as given in Table 1. Therefore, B-AgNPs could be an appropriate indicator for further use in any fisheries department. The mortality potential or lethal effect of any application materials is the first parameter of any application study. The fish mortality in the lethal concentration of B-AgNPs was dose-dependent, e.g., the highest mortality was found at the highest dose (0.09 mg/L) given in (Table 2; Fig. 3). There was no mortality found in the other three groups (control, 0.03 mg/L, 0.06 mg/L) of the study. In the overall experiment, the mortality was observed on days 2, 6, 13, and 19 while no mortality has occurred on other days of the experiment. Median lethal concentration (LC_{50}) values for 2, 6, 13, and 19 days are given in Table 2.

Bioaccumulation of B-AgNPs in tissue

The result of bioaccumulation of B-AgNPs in different organs is given in Fig. 4. Overall the B-AgNPs were mostly bioaccumulated in the liver, followed by the intestine, gills, and muscles ($p < 0.05$). In all organs, the bioaccumulation of B-AgNPs was dose-dependent, e.g., the bioaccumulation of B-AgNPs was increased while the dose was increased. Comparatively the treatment groups consisted of different concentrations of B-AgNPs. The results revealed that the highest bioaccumulation of B-AgNPs was seen at the highest concentration of B-AgNPs (0.09 mg/L) while the lowest bioaccumulation was seen at the lowest concentration B-AgNPs (0.03 mg/L). The overall absorption of B-AgNPs in different organs is given in

Fig S2 while the daily basis bioaccumulation of B-AgNPs in different organs of (*Cyprinus carpio*) is given in Additional file (Table S3 = gills, S4 = intestine, S5 = muscle, and S6 = liver). The liver was the most bioaccumulated organ (Fig. 4a). The highest absorption of B-AgNPs was observed in a group (0.09 mg/L), i.e., 272.09 mg in the 20 days exposure. 299.15 mg B-AgNPs were absorbed in the intestine for 20 days exposure as shown in Fig. 4b. Gills have absorbed 225.99 mg of B-AgNPs for 20 days (Fig. 4c) while the lowest absorption of B-AgNPs was observed in the muscles of fish, i.e., 76.64 mg ($p < 0.05$) shown in Fig. 4d. However, the highest level of absorption of B-AgNPs was seen in the liver and intestine ($p < 0.05$). The bioaccumulation of B-AgNPs reported in this study was compared with previous studies (Table 3).

Histological investigation

The histological investigation showed that exposure of fish fauna to B-AgNPs led to an alteration in tissue level in targeted organs (gills, intestine). Tissue alterations in the sense of damage, atrophy, shortening of secondary lamella, degeneration, and necrosis at different concentrations of B-AgNPs have been observed. The gill tissue structure was damaged and led to atrophy and necrosis while exposed to 0.03 mg/L of AgNPs shown in Fig. 5. Figure 6 shows that necrosis has occurred, which led to vacuolation, and the tissues are arranged disorderly. The shedding and degeneration have started in the tissues of gills at 0.06 mg/L concentration of B-AgNPs. At the highest concentration of B-AgNPs (0.09 mg/L), the reduction or shortening of lamella was observed as shown in Fig. 7. Not only the gills, but the AgNPs also caused histological alterations in intestinal tissues. In the current study, some intestinal villi mucosal epithelial cells are shaded and a small number of epithelial cells on the top are missing. Figure 8 shows that there is degeneration, shedding, and necrosis have been observed. The fish

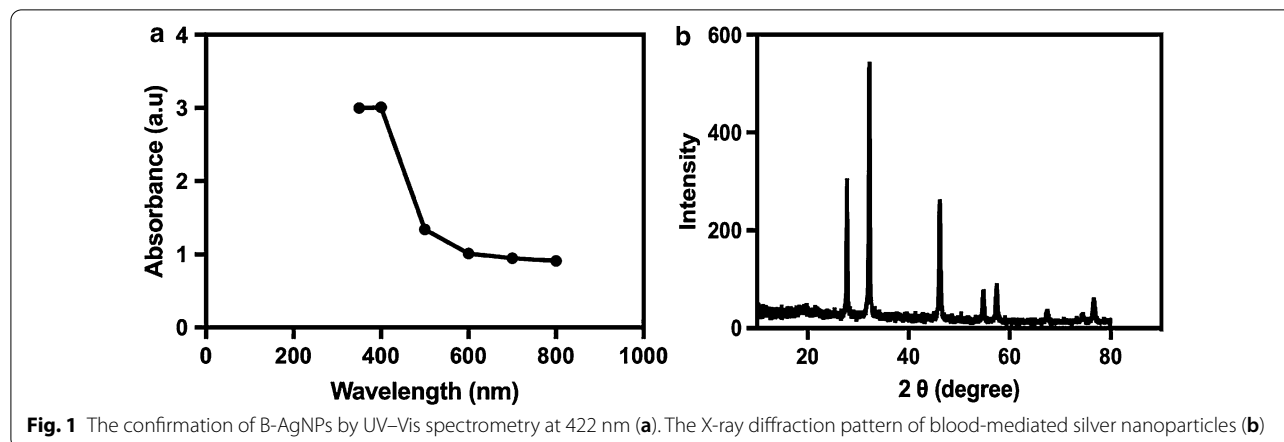


Fig. 1 The confirmation of B-AgNPs by UV-Vis spectrometry at 422 nm (a). The X-ray diffraction pattern of blood-mediated silver nanoparticles (b)

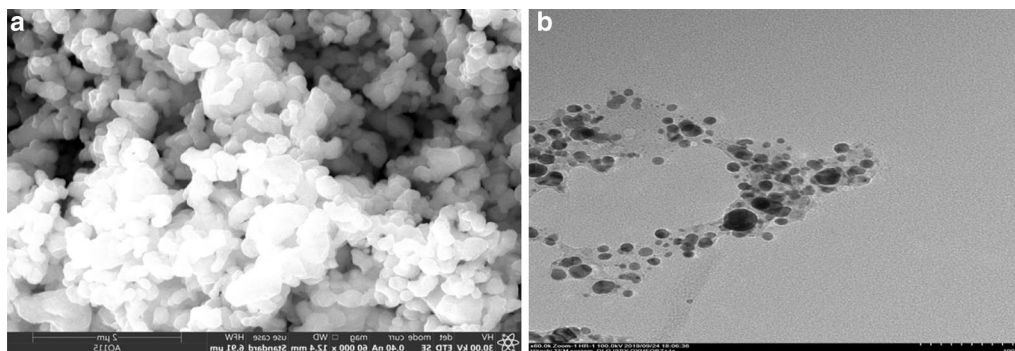


Fig. 2 The scanning electron microscopy result of blood-induced silver nanoparticles (a). The transmission electron microscope result of blood-mediated nanoparticles (b)

Table 1 The behavior study of fish during the exposure period to different concentrations of B-AgNPs

Behavior categories	Food rejection	Reduced feces excretion	Lack of swimming	Fin movement	Motionless and presence on side	Movement toward the surface
Control	Normal	Normal	Normal	Normal	Normal	Normal
B-AgNPs						
0.03 mg/L	Normal	Normal	Normal	Normal	Normal	Normal
0.06 mg/L	Normal	Normal	Normal	Normal	Normal	Less
0.09 mg/L	Less food intake comparatively other groups	Normal	Less	Normal	Less motionless	Less

exposed to 0.03 mg/L B-AgNPs concentration shows that there is shedding and a small number of epithelial cells on the top of intestinal villi are missing. The fish exposed to 0.06 mg/L concentration led to necrosis and degeneration in the mucosal cell. The exposure of fish to 0.09 mg/L led to necrosis and cell lysis in the villi mucosal epithelial cells as shown in Figure 8c. So, it was summarized that lesions, degeneration, necrosis, and shedding even cell lysis were formed mostly at the highest concentration of B-AgNPs exposure given in Additional file 1: Table S1 and S2.

Biomarker assay for enzymes

Antioxidant enzyme activities are summarized in Table 4. The different concentrations of B-AgNPs led to significant changes in the hepatic and gill enzymatic activities. With a reduction in the fish group exposed to (0.03 mg/L and 0.06 mg/L) and an increase in the highest concentration of B-AgNPs ($p < 0.05$). At the highest concentration (0.09 mg/L) of B-AgNPs, a rise in GST and decrease

Table 2 Median lethal concentration LC_{50} of B-AgNPs to common carp fish in 2, 6, 13, and 19 days

Concentration of B-AgNPs	Lethal concentration (mg/L)	Fish exposure time (days)
0.09 mg/L	0.61 ± 0.09	2
0.09 mg/L	0.49 ± 0.04	6
0.09 mg/L	0.46 ± 0.08	13
0.09 mg/L	0.39 ± 0.04	19

There was no mortality on all other days and concentrations

were noticed in GR in the case of the liver. But in the gills, GST and GR both have been decreased as compared to control. The CAT increased at 0.03 mg/L while decreased in both 0.06 mg/L and 0.09 mg/L in case of the gills and liver. The GR decreased at all the concentrations of B-AgNPs comparing to the control group gills while in the liver the GR has increased at 0.03 mg/L and 0.06 mg/L but has decreased at 0.09 mg/L.

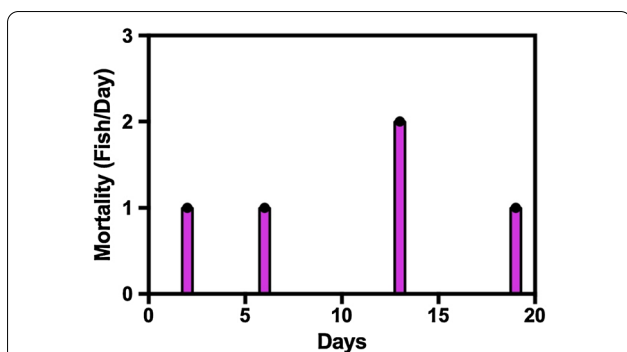


Fig. 3 The mortality of *C. carpio* during chronic exposure to B-AgNPs (0.09 mg/L). The fish were exposed to different concentrations of B-AgNPs for 20 days. No mortality has seen at 0.03 mg/L and 0.06 mg/L

Discussion

The toxicity of AgNPs in the aquatic ecosystem is affected by the capability of uptake of aquatic organisms. We herein measured and evaluated the long-time

exposure toxicity of newly synthesized B-AgNPs using animal blood in common carp fish (*C. carpio*). In the current study, the focus was on mortality, bioaccumulation in different organs, abnormal behavior, and the histopathological study of B-AgNPs. During the study period, the bioaccumulation of B-AgNPs in gills, liver, intestine, and muscles was observed. The results of the current study revealed that the B-AgNPs were mostly bioaccumulated in the liver, followed by the intestine, gills, and muscles. During the overall experiment, no abnormal behaviors were noticed. For instance, the fish properly moved toward the food. The food was not rejected by fish. The feces excretion was normal. No irregular operculum movement was found, but the fish movement toward the surface was normal. Moreover, the B-AgNPs also damage and cause necrosis, even cell lysis in intestinal villi.

It has been proved that the AgNPs are not much stable and able to be dissolved in aqueous solutions, so it releases Ag ions progressively [32]. Though the toxic effects of the silver in nano-form have been widely

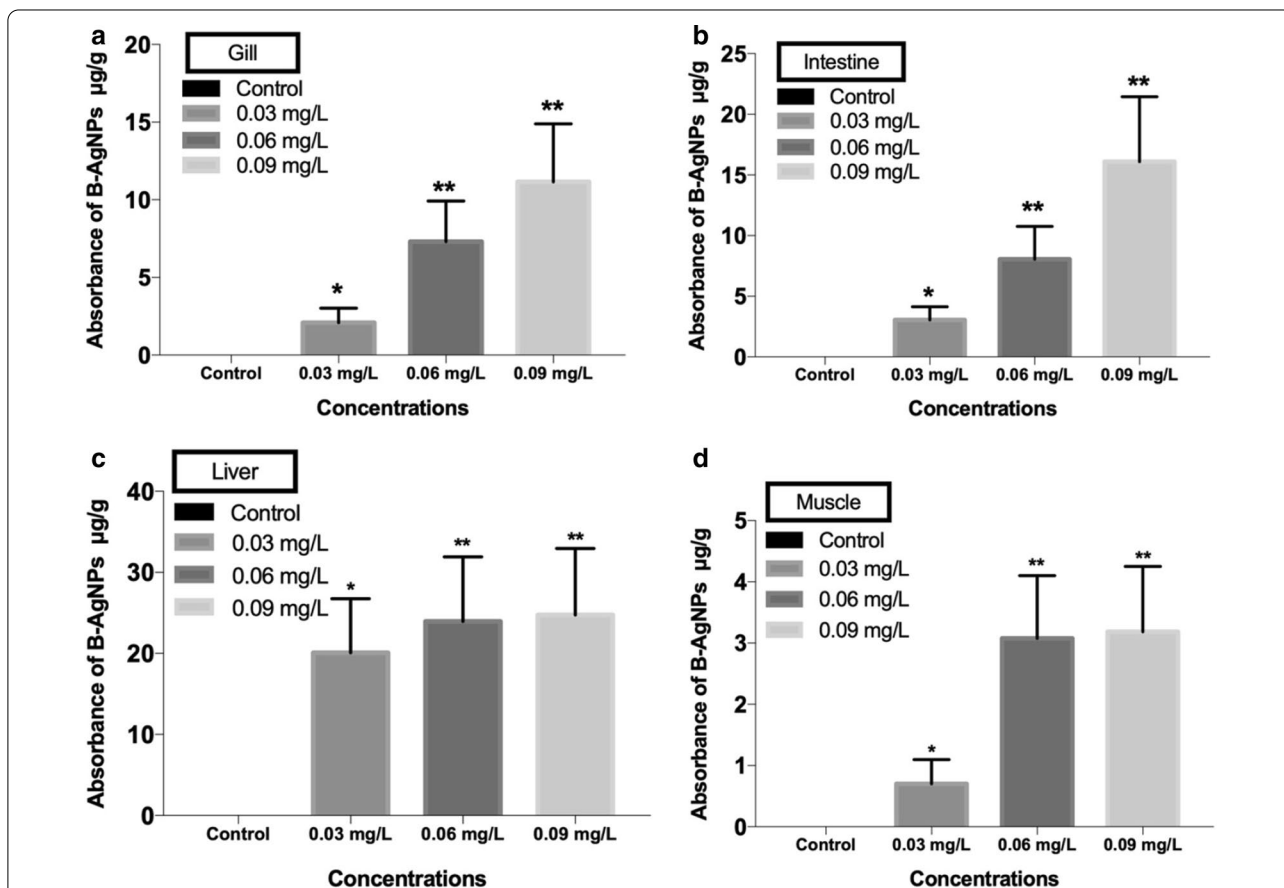
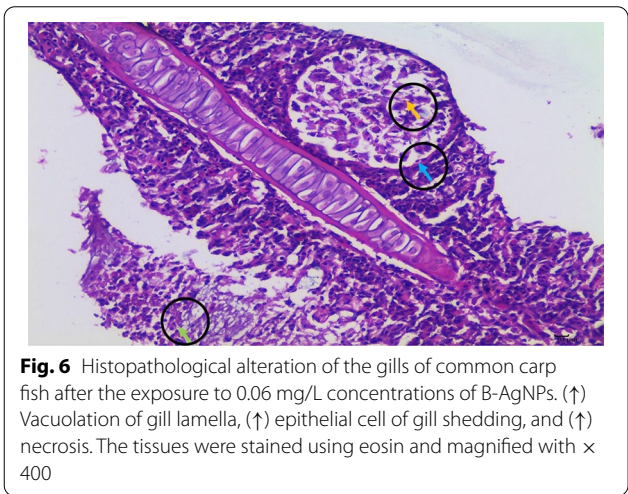
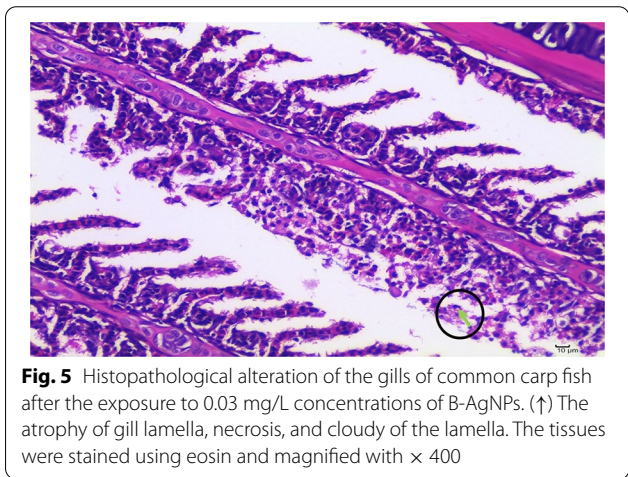


Fig. 4 The absorption and bioaccumulation of B-AgNPs in the gills (a) in the intestine (b) in the liver (c) and the muscles (d) of common carp fish (*C. carpio*) after the exposure to 0.03, 0.06, and 0.09 mg/L for 20 days ($p < 0.05$). There was no accumulation of AgNPs in control groups

Table 3 Comparison of the present study with previous studies, showing the results at the end of the experiment

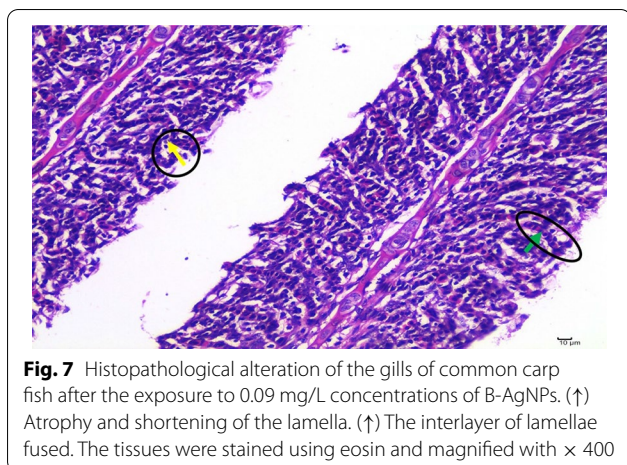
Fish	NPs type	Concentration selection method	Duration	Organs analyzed	Histological study	Bioaccumulation	Reference
Common carp fish	AgNPs	0.04, 0.08 mg/L	96 h	Gills, liver, and intestine	Epithelial lifting, telangiectasia in secondary lamellae, and epithelial necrosis	Mostly in the liver and intestine	[27]
Zebrafish, rainbow trout fish	CuNPs	1 mg/L	96 h	Gills	Damage of gill filaments	N/A	[28]
Epinephelus coioides	CuNPs	100 µg/L	25 days	Gills, intestine, liver, and muscles	Cell apoptosis in gills, liver	Liver, gills, intestine, and muscles	[29]
Common carp fish	AgNPs	0.75 mg/L	14 days	Gills	Telangiectasia and epithelial cell hyperplasia in fish gills	N/A	[5]
Common carp fish	ZnONPs	50 mg/L	30 days	Gills, liver intestine, and brain	Irregular cell outline, abnormal pyknotic nuclei, shrinkage of cell	Mostly accumulated in the liver and gills	[30]
Common carp fish	CuNPs	N/A	21 days	Gills, intestine, and liver	N/A	Mostly accumulated in the liver and intestine	[31]
Common carp fish	B-AgNPs	0.03, 0.06, 0.09 mg/L	20 days	Gills, intestine, muscles, liver	Histological alteration mostly occurred at the highest concentration of B-AgNPs in gills and intestine	Widely accumulated in the liver, followed by the intestine, gills, and muscle	Our study



reported the toxicity of AgNPs is seems to be primarily due to the Ag⁺ release [2, 33]. The high surface-to-volume ratio in metallic nanoparticles increases the probability of releasing ion form nanoparticles [2]. According to [34], the toxicity of AgNPs in fish fauna is mostly because of Ag ions instead of nanoparticles.

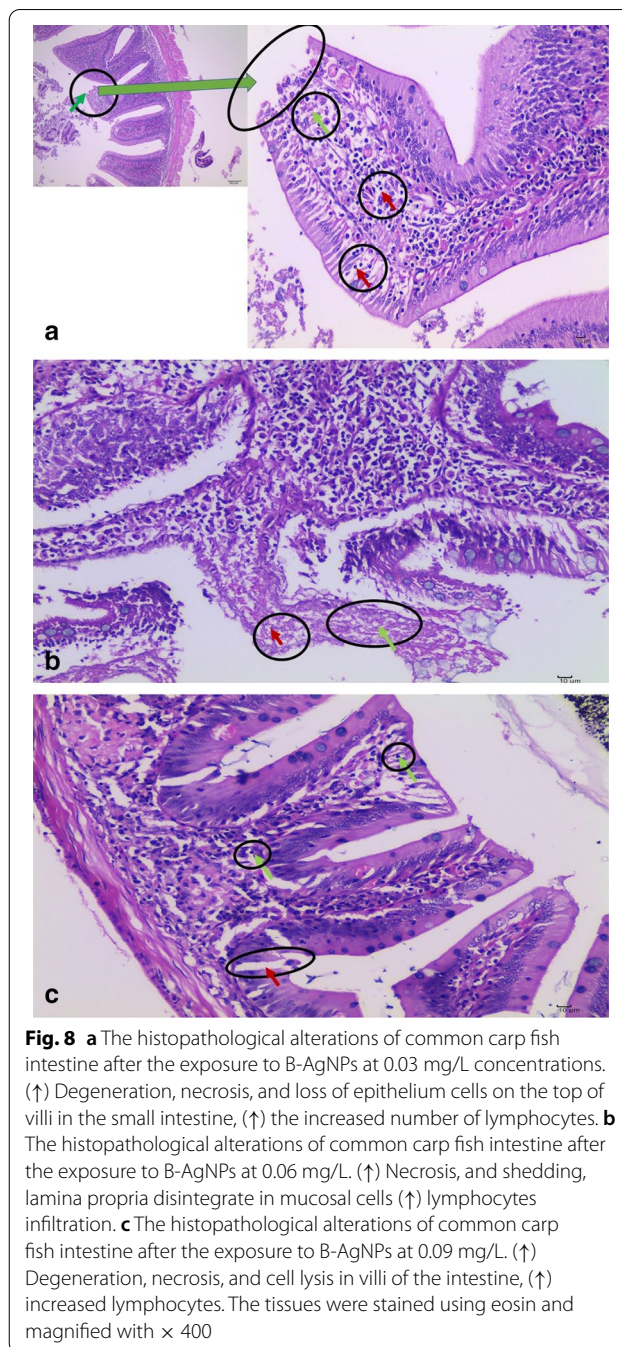
To investigate the consequences of B-AgNPs, mortality (LC₅₀) was measured in the current study. The results revealed that LC₅₀ was dose-dependent. There was no

mortality at 0.03, 0.06 mg/L concentration of B-AgNPs while 5/20 fish in the same group at the variant time died at the highest dose (0.09 mg/L) of B-AgNPs. The highest mortality (2/20) was noted on the 16th day of the experiment. In comparison, our results revealed the lowest mortality at the highest concentration of B-AgNPs. A study conducted by [27] revealed the mortality in every concentration even by the lowest concentration (0.01 mg/L) of



AgNPs. Another conducted study exhibited that 7/21 fish died at 0.03 mg/L after 96 h of exposure to AgNPs [2], but in our study, there was no mortality at the lowest concentration (0.03 mg/L) till the end of the experiment.

It has been assumed that biological synthesized AgNPs are less toxic as compared to chemically synthesized nanoparticles [35]. In the current study, the AgNPs were synthesized for the first time using animal blood serum and tested for the bioaccumulation in fish fauna. The results for the bioaccumulation revealed that the B-AgNPs were mostly accumulated in the liver, followed by the intestine, gills, and muscle (Fig. 4a–d). Similar results have been achieved by [36], in which the AgNPs were accumulated mostly in the liver and intestine. No doubt, there are few studies conducted on the fish toxicity of AgNPs. Based on the above results, it can be assumed or hypothesized that fish liver might play an important role in the metabolism of silver. Previously studies described that the silver first increase in blood when a fish is exposed to AgNPs, then the liver absorbs the silver, therefore bioaccumulation of silver NPs highly took place [37, 38]. In the current study, the fish were fed two times a day, so it can be assumed that while ingesting food particles, they also ingested silver nanoparticles, therefore the intestine is the second most bioaccumulated organ followed by the liver (Fig. 4a). Another previously conducted study also revealed that the intestine was the second most accumulated after the liver [39, 40]. The small finger-like projections (villi) present in the intestine increase the surface area for the absorption of any particles. Therefore, [41] concluded that the increased bioaccumulation of AgNPs perhaps due to the absorption from the liver by the intestine. In the present study, gills were the third most B-AgNPs bioaccumulated organ. This might be because of two reasons: (1) gills are highly exposed



to the external environment; (2) due to large surface area, the gills have the capability to absorb more and more especially small-sized materials. [36] concluded that small-sized AgNPs were mostly absorbed as compared to large-sized AgNPs.

After the long period of exposure of *C. carpio* to sub-lethal concentrations of blood-induced silver nanoparticles, different lesions and alterations were observed in the intestine and gills (Figs. 5, 6, 7, 8). In detail, it has

Table 4 Enzymatic assay of glutathione S-transferase (GST), glutathione reductase (GR), and catalase (CAT) in the tissues of liver and gills of *C. carpio* after exposure to 0.03 mg/L, 0.06 mg/L, and 0.09 mg/L for long-time exposure of B-AgNPs

Enzyme	Control	0.03 mg/L	0.06 mg/L	0.09 mg/L
Liver				
GST	753.17 ± 19.24	672.69 ± 91.83	679.22 ± 104.75	1021.32 ± 32.29
GR	56.39 ± 4.89	61.09 ± 10.42	59.91 ± 1.56	36.69 ± 2.74
CAT	109.38 ± 11.23	83.26 ± 12.28	112.32 ± 19.81	103.38 ± 6.26
Gills				
GST	212.27 ± 19.59	192.03 ± 3.11	215.47 ± 35.33	132.40 ± 16.60
GR	77.07 ± 3.86	61.79 ± 8.21	68.81 ± 20.44	53.12 ± 10.43
CAT	11.64 ± 0.83	8.35 ± 0.23	16.32 ± 3.34	7.67 ± 0.58

The values in this table are expressed as mean ± SE

been observed that there was necrosis, degeneration, and cell lysis in the gills and intestine of fish at different concentrations of B-AgNPs given in Additional file 1: Tables S1 and S2. These kinds of changes in gills are the indications of a defense mechanism, subsequently, they increase the gap between the external environment and bloodstream, and prevent the penetration of pollution [42]. In the current study, during exposure to AgNPs, the tissue damaging mostly depended on the concentration of B-AgNPs. At the highest concentration (0.09 mg/L) caused severe damage as compared to the lowest concentration (0.03 mg/L). Among all NPs, silver is the most powerful toxicant that affects the gills primarily [43]. In the current study by comparison with other previously conducted studies. It has been observed that our study showed less toxicity in the sense of mortality, bioaccumulation, and histological alterations. Therefore, blood serum-mediated silver nanoparticles might be an alternative to the other biological synthesized AgNPs.

Conclusion

The current study aimed to investigate the toxicity and toxic effects of B-AgNPs. According to the results of the present study, it might be said that after the long-time exposure, the B-AgNPs were bioaccumulated in the targeted organs while the liver was the main target for bioaccumulation. Furthermore, the bioaccumulation of B-AgNPs has led to histological alterations in the gills and intestine. The surface capping, morphology, and size of AgNPs are the major difficulties while studying the nano-ecotoxicity. These properties of AgNPs are the main factors for causing toxicity in fish fauna. Therefore, it is concluded that the focus and attention should be given to all these factors while synthesizing the green AgNPs. This might help in the future to reduce the nano-toxicity in biodiversity.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-021-00453-7>.

Additional file 1: Table S3. The daily basis bioaccumulation of B-AgNPs in gill of (*Cyprinus carpio*). **Table S4.** The daily basis bioaccumulation of B-AgNPs in intestine of (*Cyprinus carpio*). **Table S5.** The daily basis bioaccumulation of B-AgNPs in muscles of (*Cyprinus carpio*). **Table S6.** The daily basis bioaccumulation of B-AgNPs in liver of (*Cyprinus carpio*).

Abbreviations

NT: Nanotechnology; AgNPs: Silver nanoparticles; nm: Nanometer; $\mu\text{g L}^{-1}$: Microgram per liter; Ag: Silver; *C. carpio*: Common carp; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; XRD: X-ray diffraction; DO_2 : Daily oxygen; OECD: Organization for Economic Cooperation and Development; LC_{50} : Lethal concentration; HCl: Hydrochloric acid; B-AgNPs: Blood-induced silver nanoparticles.

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Authors' contributions

MAK: methodology, writing, original draft. FW: co-supervision. WW: supervision, approval of the final version. WS: critical revision and editing. IK: methodology. QZ: revision. KU: software. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The current study was ethically approved by the committee of the School of life science, Lanzhou University, Lanzhou, Gansu, China.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no conflict of interest.

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