Histopathological effects of ZnO nanoparticles on kidney, liver, and gills tissues of goldfish (*Carassius auratus*)

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Abstract

Nowadays, the use of nanotechnology in various industries is increasing. The application of nanoparticles in aquaculture can be investigated both to treat diseases and to control the bacterial load of water. In this research, nanoparticles were prepared by the sol-gel method. The particle size was determined by scanning electron microscope(SEM) and X-ray diffraction(XRD). To determine the possibility of using these materials directly in aquaculture, nanoparticles with concentrations of 1, 5, and 10 ppm were added to aquarium water containing redfish. 60 pieces of fish were divided into 3 treatments and 1 control group, then for 1 month, the fish were exposed to the mentioned doses was extensive in the secondary lamellae and no change was observed in the liver cells, local necrosis was observed at doses of 5 and 10, while this was not observed for doses of 1 ppm Due to the mild side effects of zinc oxide nanoparticles, it can be used as a disinfectant without side effects in fish breeding centers. The results of this research can be used in aquarium conditions.

Keywords: ZnO, Chronic dose, Histopathology, Goldfish

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Introduction

Nanotechnology has recently emerged in Iran and the world in different sciences and industries. Apart from environmental issues, such as technology is growing rapidly. By 2014, more than 15% of all products on the global market will have some kind of nanotechnology incorporated into their manufacturing process". This trend implies that the manipulation of nanotechnology is just beginning (Shahzad *et al*., 2019). The application of antibiotics in aquaculture has led to a great risk. The development of antibiotic resistance in pathogenic bacteria of fish is the most important risk, so the use of alternative antibacterial materials has been interested to researchers(Bai *et al*., 2010). In addition to the drug resistance issue in the aquaculture industry against pathogens of aquatic animals, such resistance can be transmitted to humans by consuming fish in antibiotic residue forms, and also given the use of systemic antibiotics in aquaculture, it negatively imposes environmental issues(Goda *et al*., 2023). The introduction of alternative antibiotics in aquatic animal health, and the animal industry is perhaps more urgent than others (Ahmed, 2020). considering the unique properties of various nanoparticles, ZnO nanostructures (zinc oxide nanoparticles) are widely used in electronics and optics, cosmetics, catalysts, ceramics, pigments, etc (Dube and Okuthe, 2023). ZnO is an environmental-friendly substance and it can be applied in bio-medical fields. Goldfish is used as the model in scientific research as the most popular aquarium fish in Iran (Khosravi-Katuli *et al*., 2018). To assess the toxicity of environmental pollutants such as particles of different physiological parameters in fish that are among the histopathological indicators. histopathological study of internal organ tissue would be suitable for the expression of infection or critical situations in the living environment (Etemadi Dailami *et al*., 2013). dealing with changes in fish tissues is found to be one of the most important and practical ways by which environmental (aquatic ecosystems) pollution and its adverse effects on the organisms can be evaluated. These studies serve as a valuable way to evaluate a lot of contaminants in fish (Ashokkumar *et al*., 2022). Recently, in-vivo cell experiments have shown that exposure to zinc oxide nanoparticles resulted in oxidative damage and inflammatory response of the lung vessels and endothelial cells (Lin *et al*., 2009). Animal experiments showed that the liver, spleen, heart, pancreas, bones, and organs were subjected to 20- and 120 nm ZnO (Mariam *et al*., 2023). The results show that ZnO nanoparticles have mild side effects in chronic dosing SO that can be used as a disinfectant in the absence of adverse effects in ornamental fish aquaculture.

Materials and methods

Preparation of ZnO nanoparticles and particle measurement method

About five grams of zinc acetate (Merck Ltd, Germany) with 50 mL of deionized

water was poured into the flask and mixed under heat to bring a preliminary fifth volume, then placed in the oven with a temperature of 5±100 to gradually dry up. Subsequently, it was subjected to a temperature of 300 ±20°C for 24 hours until crystals were completed, each cc-prepared nanoparticle had 123 ppm metabolite (Farbod *et al*., 2011). XRD tests were used to confirm the proper distribution of ZnO, in this case, a parallel beam of X-rays with a wavelength of 7.2 Angstrom between the sample and the sample emitted by the Bragg diffraction formula $\lambda=2$ d sin (θ) takes place, d is the atomic scale in the crystalline phase. The intensity of the diffracted x-ray diffraction as a function of angle θ2, for example, can be measured. This pattern of diffraction is used to determine the crystalline phases and measure their structural characteristics. It should be noted that the XRD method is non-destructive and requires no sample preparation (Ribeiro *et al*., 2022). SEM image showed spherical ZnO nanoparticles (Fig. 1).

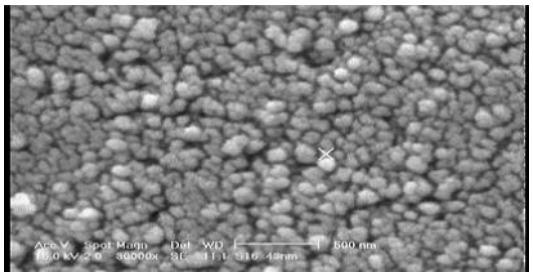

Figure 1: SEM image of ZnO nanoparticles.

Aquaculture conditions

The present research was conducted in winter 94 in Shahid Naser Fazli Barabadi Aquaculture at Gorgan University of Agricultural Sciences and Natural Resources. In this first stage, several 60 goldfish 12.05±40 g were, and fish were divided into 12 aquariums with a volume of 70-liter, 4 groups in 3 replicates so that each aquarium was randomly added with 5 fish. The fish were widely acclimatized for 2 weeks and during this period they were fed with commercial food Biomar of about 3% biomass twice a day. During the growing period the water temperature, pH, and water hardness were measured at about 16.98+0.71°C, 7,87, and 260 ppm, respectively.

Methods

The fish were transported to Shahid Naser Fazli Barabadi Aquaculture Center at Gorgan University of Agricultural Sciences and Natural Resources. All physicochemical conditions of the water and environment, such as temperature, pH, and other factors were controlled. For this purpose, 12 aquariums in size 30×40×60 were used, in which five fish were placed. 60 pieces of goldfish were randomly assigned to 3 groups with 3 replicates and controls into 12 aquariums (5 fish per tank). After calculating the LC50, 3 doses 1, 5, and 10% LC50 were calculated as the dose used in the experiment. Du performed those tests for 7-28 days. This test which was on chronic stress was conducted in 40 days using 20 percent of the total concentration of lethal toxicity. For this purpose, concentrations of 1, 5, and 10 ppm were considered.

Sampling approach

To prepare gill and liver tissue, first fish randomly were removed by the net and anesthetized within a 5-liter plastic tub with 200 ppm clove anesthetic then fish tissue samples were taken. Using the knife, the left operculum separated the fish, and the fish were removed and left the second-gill arch. The fish from the front of the ventral fins and anal fin were fully opened and then exposed to the liver which were sampled and all were removed completely. Stabilization of tissue samples in solution (10% formalin) and histology were transported to the laboratory.

Tissue sections preparation

Under an optical microscope, tissue fixed in 10% formaldehyde after 24 hours was transported to 80% alcohol. Then dehydrated with increasing ethanol series (80, 90, 97, and 100%), and the rest were poured in Xylene and paraffin. All of these steps were continued under (Tissue Processor, Triangle Biomedical Sciences USA). Then tissues were cast with paraffin (melting temperature 56-58°C) (Alberto *et al*., 2020). Paraffin casts using a microtome (Olympus CUT 4055E, USA) slices thick 5 μm and then put on slides prepared for 0.5 hours in the oven (60°C) to add paraffin to the tissue to be removed. Specimens were stained after paraffin removing and replacing it with Xylene, by decreasing ethanol series (100, 90, and 70%) in hematoxylin and eosin solutions. Tissues were taken back to the oven to dry. All materials used in the process (ethanol, Xylene, paraffin, hematoxylin, and eosin) were related to Merck's product. The plates were sealed with basalt Kanda adhesive on the slide.

Results

Behavioral and morphological changes in fish subjected to an acute dose

Compared with other groups, the control group was not affected by changes in ZnO nanoparticles. Upon a dose of 1 ppm ZnO nanoparticles, no certain symptoms were found, and in a dose of 5 ppm nano-particle deposition on the gill cover, a dose of 10 ppm of tangible lethargy was observed.

Gill tissue

Tissue complications found in microscopic specimens of gill goldfish were as follows:

No histopathological effects were found on the goldfish in 1ppm gills treatment and control groups. Details of the structure of healthy fish gills goldfish pond 1ppm treatment and control groups can be seen in Figure (2a). Also in the fish gill-dose chronic exposure dose of 5 ppm and 10 ppm zinc oxide clubbing Ross secondary lamellae, extensive hyperplasia secondary lamellae, with congestion and congestion of secondary lamellae occurred. Details of the gill structure under doses of 5 and 10 ppm can be seen in Figure 2b.

Liver

Tissue complications found in microscopic specimens of gill goldfish were as follows:

No histological complication in the liver of goldfish under 1ppm treatment and control groups was found. Details of the healthy liver in control and 1ppm treatment can be shown in Figure 3a. Fish exposure to doses 5 and 10 ppm ZnO is characterized by intact hepatocyte structure accumulated with melanomacrophage centers. Details of the structure of the liver at doses of 5 and 10 ppm are shown in Figure 3b,c.

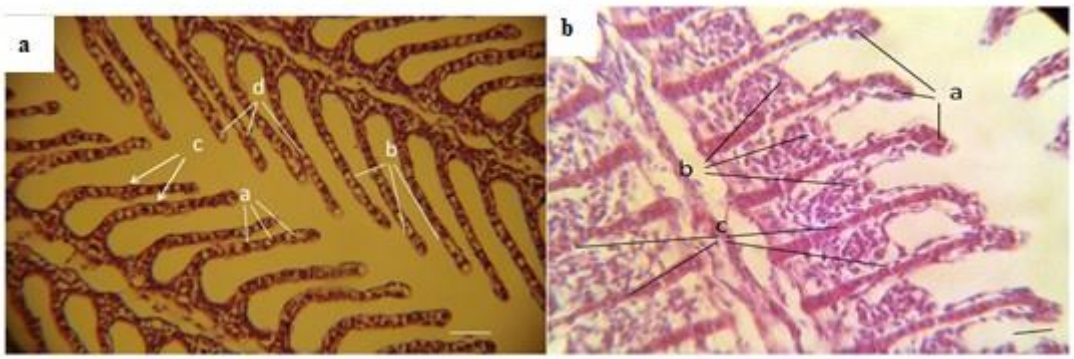

Figure 2: Sections of goldfish gill under ZnO nanoparticles.

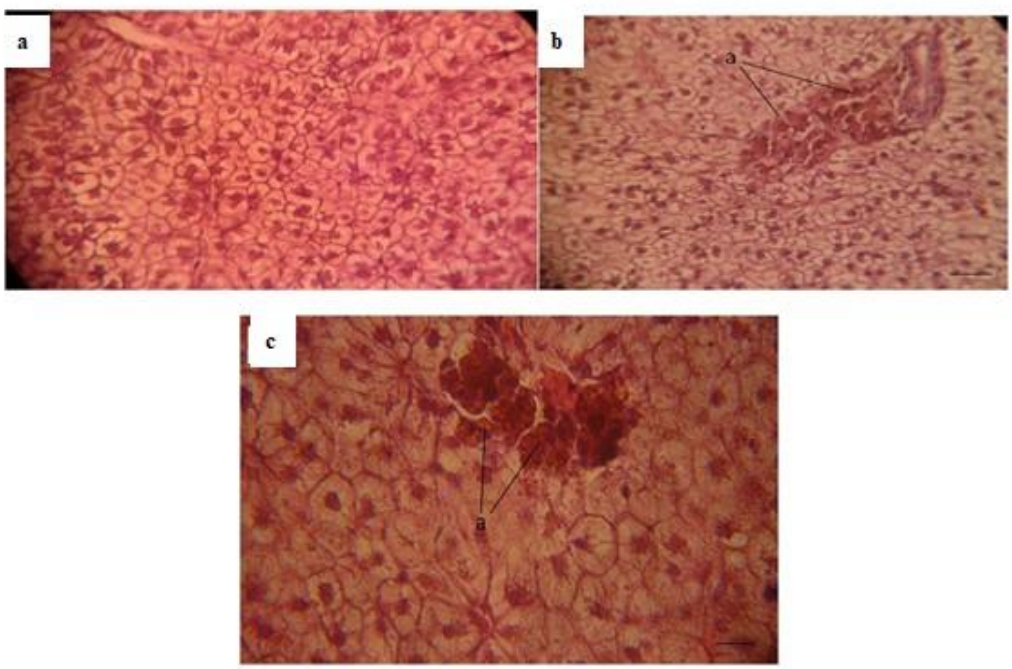

Figure 3: Goldfish liver tissue sections under ZnO nanoparticles.

Kidney

In the kidney, upon exposure chronic to doses 5 and 10 ppm ZnO, local necrosis was observed (Fig. 4).

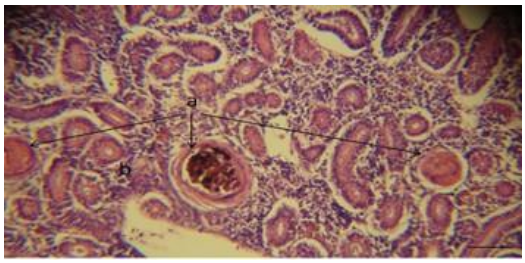

Figure 4: goldfish kidney tissue sections under ZnO nanoparticles.

Discussion and results

Nanoparticles are found to be useful materials to eliminate pollutants in water. given that the widespread use of antibiotics in treating fish diseases causes some bacterial resistance (Shahzad *et al*., 2019), today nanoparticles are common to control the bacterial load and cleaning water in fish aquaculture centers (Raissy *et al*., 2011). ZnO oxide is one of the most widely used nanoparticles followed by carbon nanotubes and nanosilver after nanotechnology and one of the reasons behind the widespread use of these particles is their anti-bacterial and antimicrobial properties (Abdel-Latif *et al*., 2021) So to deal with the possible effects of these particles in the tissue of fish as a measure of pollution is necessary. According to the report, elevated ROS generation in cells (eg, NO and O2) leads to hemodynamic changes and edema of the yolk sac in embryos and larvae of Zebra Fish subjected to dichloroacetate. Taee and Al-Hamdan (2013), while studying the effect of ZnO on Carp concentration identified ZnO lethal concentration, so that at a concentration of 50 ppm mortality was 100 percent, while at a concentration of 30 ppm, it was 50 percent(Sibiya *et al*., 2023*)*. A study in kidney tissue of chronic exposure to doses of 5 and 10 ppm nano zinc oxide represents the center's necrosis position was observed. histological study showed bleeding in the kidney, interstitial nephritic, the influence of nanoparticle oxide on the inflammatory cells, and inflation are seen in the renal tubules. this is in line with the higher trophy in gills and gill filaments secondary necrosis and necrosis of the liver and pancreas tissue (Ribeiro *et al*., 2022). Handy *et al*. (2008), found that ZnO nanoparticles can cause stress and tissue damage in the first stage, and under concentrations of 0.1-100 milligrams per liter leading to breath and swelling of the pericardium, cardiac toxicity, and pathology of the internal organs. Subashkumar and Selvanayagamr (2014) found that 50 percent of fish lethal concentration (LC50) for common carp was 4.897 mg. After 21 days of exposure to ZnO changes such as epithelial cell hyperplasia, necrosis, aneurysm, hyperinflation, lamellar fusion, and the elimination of secondary lamellae were observed. Zhou and colleagues (2009) in a similar case the study found that ZnO nanoparticles resulted in a dose-dependent toxicity applied in zebrafish embryos and larvae of fish and reduced the hatching rate and pericardial edema heart.

In the present research under chronic doses of 5 and 10 ppm ZnO, intact hepatocytes were found with liver melanomacrophage centers in line with Xiong *et al*. (2011) reported that ZnO nanoparticles affected liver and lipid peroxidation in the liver of fish exposed to 5 mm. Jalali Jafari and Aghazadeh Meshgi (2007) studying ZnO nanoparticle effects on fish found that such nanoparticles accumulate mainly in the bone and skin, although the liver, gills, and kidneys accumulate significant amounts of this element.

Ghazi *et al*. (2012) evaluated the histopathologic changes of ZnO nanoparticles on carp gills. Clinical symptoms such as edema, gill area, abdomen, and dark fins were found. Under Microscopic examination, severe edema bluegill, gill cell hyperplasia, and squamous metaplasia of the cells were observed. Vessel telangiectasia elevated mucus and the presence of mononuclear immune cells was obvious(Correia *et al*., 2020). Edema and necrosis of gill cells under the osmotic system are disturbing. In addition, the permeability of the capillary membrane filtration gills was very low cross was disrupted in the gills (Kalbasi *et al*., 2012). This is in line with the present research dealing with ZnO nanoparticles effect on fish gill-dose chronic exposure dose of 5 ppm and 10 ppm zinc oxide L Ross clubbing secondary lamellae, extensive hyperplasia secondary lamellae, with congestion and congestion in secondary lamellae. Hao *et al*., (2013) found that the gills, liver, and brain were more sensitive organs in response to the ZnO and the gut had the lowest sensitivity. More ecotoxicological evaluations cause concern for ZnO in the aquatic environment.

Conclusion

Given the increasing breakthrough in nanotechnology and its application in aquaculture, it improves water quality and prevents infectious diseases, especially in fish. Thus, the preceding particles are utilized to control bacteria and pollution. the effects ZnO nanoparticles have in chronic doses are much lower than ionic compounds with low histological effects on goldfish organs. Therefore it has a promising role in cleansing and controlling bacterial load in water aquarium fish.

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