Sub-chronic Effects of Zinc oxide Nanoparticles on Ovary, Gills, Kidney and Liver of Fresh Water Fish (*Cyprinus carpio*): histopathological Study

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Abstract

Concerns have been raised about the potential environmental dangers connected with ZnO nanoparticles (ZnO NPs) entering aquatic systems due to the growing use of ZnO NPs in various fields. This study looked about how freshwater fish (*Cyprinus carpio*) respond to sub-chronic exposure to ZnO NPs through food. Common carp (*C. carpio*) from nearby fish hatcheries were employed in this investigation (city of Madaan, south of Baghdad). These fish had an approximate length and weight of 15-18 cm and 120-5 gram, respectively. Once every day, fish fed commercial fish meal. They were acclimated in 40 L aquariums (60 x 40 x 50 cm) with continuous aeration and 16- and 8-hours photoperiod for 12 days prior to the commencement of the experiment. For six weeks, two groups of fishes were treated to doses of 250 and 500 mg/l. Samples were taken six weeks after the ZnO NPs exposure. Five fish from every ZnO NPs treated group as well as the associated controls were taken out, killed, and preserved in formalin after necessary exposure times for tissue samples (liver, gills, kidney, and ovaries). The tissues samples exhibit pathological changes as a result, particularly at concentrations of 500 mg/l of Zinc oxide nanoparticles.

Keywords: ZnO NPs, Sub-chronic toxicity, common carp (Cyprinus *carpio*).

Introduction

With an annual production of 10^5 t, zinc oxide (ZnO) one of the most widely utilized nanomaterials in the world [1]. ZnO is widely employed in both medicine and cosmetics, in addition to many industry applications, as a result of its unique physico-chemical characteristics. Even though ZnO toxicity has been the subject of a considerable quantity of literature [2]

the matter of how much the dissolved ions and metal-based nanoparticles (NPs) contribute to overall toxicity is still up for debate. Some writers claimed that the solubilized zinc ions were to blame for the toxic effects of Zinc Oxide in aqueous system [3, 4]. Others demonstrated that these latter substances only marginally related to the toxicity [5] and that their total contribution was minimal with effects distinct from those brought on by NPs [6].

Recently, zinc oxide has drawn a lot of attention among metal oxide NPs because it has unique chemical, optic, magnetic, and mechanical characteristics that set them apart from related bulk materials [7].

Fish can serve as a bioindicator of pollution because they are a component of the aquatic ecosystem and are possibly harmed by anything that harms the environment [8].

Some of heavy metals (Cu, Cd, pb, Fe and Zn) were identified as in suspended particle components in the aquatic environment, as well as transferable and residue aspects of a sediments as well as through the chosen tissues in the *Cyprinus carpio* in a study by Al-Khaffaji *et al.* [9]. The findings demonstrated that the concentration of metals in the particulate aspects was larger compared to the dissolved aspect. Metal concentration in the fish organs varied, as well as the muscle organs had a lower concentration of metals than other organs.

To ascertain how graphene NPs affect histological alterations, Al-Rudainy and Khalel [10] exposed common carp *Cyprinus carpio* to graphene nanoparticles. The results revealed that secondary lamellae fused with shortening, lifted secondary lamellae with necrosis, and had hyperplasia of their endothelial cells in the gills. As opposed to that, the liver displayed hypertrophy, hydrobic degeneration, hepatocellular lysosomes, necrosis of hepatocytes, dilatation of sinusoids, and hydrobic degeneration. To be able to provide clear comprehension of the effects of grapheme nanoparticles upon fish and the dangers associated with their accumulation in aquatic environments, it was suggested that the study investigate these effects on histological changes and clinical signs.

Among many prepared Nanoparticles (TiO_2, Ag, carbon nanotubes [CNT-NPs] and Zinc oxide [ZnO]) only a little are identified to be very efficient to the surroundings. The ZnO Nanoparticles with the addition of TiO_2 Nanoparticles be there particularly

susceptible to environment threat analysis, and study of these combinations should be given top importance [11].

Furthermore, it was discovered by [6] and [12] those epitheliums of the gills of rainbow trout also carp are possible pathways for ZnO NP uptake.

In a study by [13], the acute toxicity of Zinc Oxide nanoparticles (96 hours LC50) to adult zebrafish was examined. According to this study, 3.97 mg / L of ZnO nanoparticles with a primary particle diameter of 30 nm killed half the fish within 96 hours.

ZnO exposure resulted in progressive architectural distortions in the treated common carp (*Cyprinus carpio*), including changes to secondary structure, blood clotting, lamellae's marginal channels enlarging, epithelial hyperplasia, Lamellar integration, epithelium elevation, erythema, necrotic, aneurism, severe cellular swelling, lamellar rupture, curling, and absence of secondary lamellae are some of the lamellar changes that can occur [14].

Introducing ZnO-NPs for into aquatic habitat has a significant impact on the ecosystem and other living things, such as fish, according to research by Asghar *et al.* [15] on the toxic effects of zinc nanomaterials in fish (*Cyprinus carpio*), Zebrafish (*Danio rerio*), and Nile Tilapia fish (*Oreochromis niloticus*).

Silver nanoparticles have potential consequences on the morphological of the livers, gills, and kidneys of C. carpio were established by Mustafa and Ashour's research [16]. The toxic impacts of Zinc Oxide nanoparticles on fish have been evaluated by additional researches [17].

Materials and methods

Zinc oxide (ZnO) nanoparticles

The ZnO nanoparticles were created by SIGMA-Aldrich and are extremely pure (99.8 %). (10-30 nm). Before exposure fish to Nanoparticle suspensions, aerated, single-distilled water is sonicated in a bath at a frequency of 33 KHz for one hour at a time [18].

Experimental design

C. carpio fish (weight 120 ± 5 g) These fish, with an average length of 15-18 cm, were obtained from local fish hatchery incubators (the city of Madaan, south of Baghdad) and kept in a glass aquarium (60 × 40 × 50 cm) provided with de-chlorinated water with 7.5 pH and 23 ± 2 °C as well as with feeding and constant oxygen

delivery, for one week, which was stopped at 24 hours before the experiment began. Every 24 hours, tank water was replaced and a fresh solution of ZnO nanoparticles was added [19], as well as the use of an air pump to keep the dissolved oxygen concentration at an optimal level and the concentration of ZnO nanoparticles constant.

Sub chronic toxicity test for ZnO nanoparticles

The fish were exposed to three concentrations of ZnO nanoparticles which computed using $1/15^{th}$, and $1/7^{th}$ of the 96 LC50 value which determined in this study [20] which were = 250 mg / L and 500 mg / L correspondingly.

In each aquarium, two groups of fish were exposed to 250 and 500 mg / L of the selected concentrations for three and six weeks. Every 48 hours, the water was changed to remove any waste and supply oxygen. After three and six weeks of exposure to ZnO NPs, samples were taken. 5 fish from each ZnO NPs-treated group and the related controls were removed and slaughtered for tissue sample (liver, gill, kidney, and ovaries) and maintained in formalin after the required exposure times. Fish were fed once a day [21], indicating that the importance of water has changed due to decreased concentrations due to adsorption on glass aquaria, evaporation, and absorption by animal tissues and fecal deposit accumulation in the aquarium.

Histological examination

Fish dissection was done in accordance with the emerge protocol's instructions [22]. In vials containing 10 % neutral buffered formaldehyde solution, all the samples were put (pH 7.0). They underwent rinsing in tap water, dehydration in a graduated series of ethanol concentrations, clearing in xylene, embedding in paraffin wax with a melting point of 54-56 C°, sectioning to a thickness of 5-7 m using a semi-automated rotary microtome (Leica RM 2245) and mounting on sterile glass slides. Following deparaffinization, sections were produced for light microscopy inspection, histological investigations, and staining with hematoxylin and eosin (H&E, 100x and 400x) [23].

Results and discussion

Gills

In control samples, the gills showed normal appearance of primary lamellae, normal appearance of epithelial secondary lamellae and normal pillar cells with encourage blood capillaries with RBC (fig. 1, a).

after exposure to 250 mg / L for six weeks. The sections of gills showed normal appearance of gills primary and

secondary lamella which covered by normal epithelial cells and supported by pillars cells (fig. 1, b). And after exposure to 500 mg / L for six weeks the sections of gills showed moderate gill hyperplasia of primary lamellar cells associated with disappearance of secondary lamellar (fig. 2, c). These results agreed with AL-Taee and AL-Hamdani [24].

Similar results by Chupani, *et al.* [25] revealed the histopathology study revealed certain background lesions, primarily in the gills of control fish, such as mild clubbing of lamellae ends. Increased concentrations of gill destruction that results from an inability to maintain homeostatic and that may be linked to hematopoietic tissue damage from lower oxygen absorption brought on by the Zinc oxide Nanoparticles impact, particularly during sub-chronic treatment [26]. Additionally, these gill tissues alterations are brought on by the fact that dissolved ZnO enters the fish's tissues mostly through the gills.

Liver

A control section of the liver showed normal appearance of hepatic tissue, bile ductile, exocrine pancreatic acini. and the liver's magnified segment displays a normal look of hepatocyte (fig. 3, d). After exposure to 250 mg / L of ZnO NP for six weeks the sections of liver showed normal appearance of hepatic tissue involved exocrine pancreatic acini, hepatocytes, and normal sinusoids with kupffer cells (fig. 3, e). But After exposure to 500 mg / L of ZnO NP for six weeks the sections of liver showed normal appearance of hepatic tissue involved exocrine pancreatic acini and hepatocytes which revealed filled with cytoplasmic glycogen granules (fig. 3, f). Changes like these were also noticed by Georgieva *et al.* [27] and Chupani *et al.* [25].

Kidney

The control section of the kidney showing normal appearance of renal tubules (Red arrows) and glomerulus (Black arrows), Magnified section of kidney shows renal tubule (R) and hemopoietic tissue (fig. 4, g).

After exposure to 250 mg / L of ZnO NP for six weeks the section of anterior kidney showed multiple focal cast tubular cast formation and mild vacular degeneration of collecting associated with epithelial desquamation (fig. 4, h). But After exposure to 500 mg / L of ZnO NP for six weeks the section of head kidney showed moderate vacular degeneration and necrosis of

collecting and proximal tubules with tubular cast formation (fig. 4, i).

Ovary

The control section of the Ovary showing normal appearance of ovary showed primary oocytes (Black arrow) and secondary oocyte (Red arrow) and mature (Asterisks) (fig. 5, j).

After exposure to 250 mg / L of ZnO NP for six weeks the section showed normal appearance of ovary with primary oocytes at different stages of development in addition for secondary oocytes and mature oocytes (fig. 5, k).

In conclusion, the findings of this study reveal that ZnO NP produced histological changes in the Gills, ovary, liver, and kidney, and that there is a clear link between these changes and an increase in ZnO NP concentration.

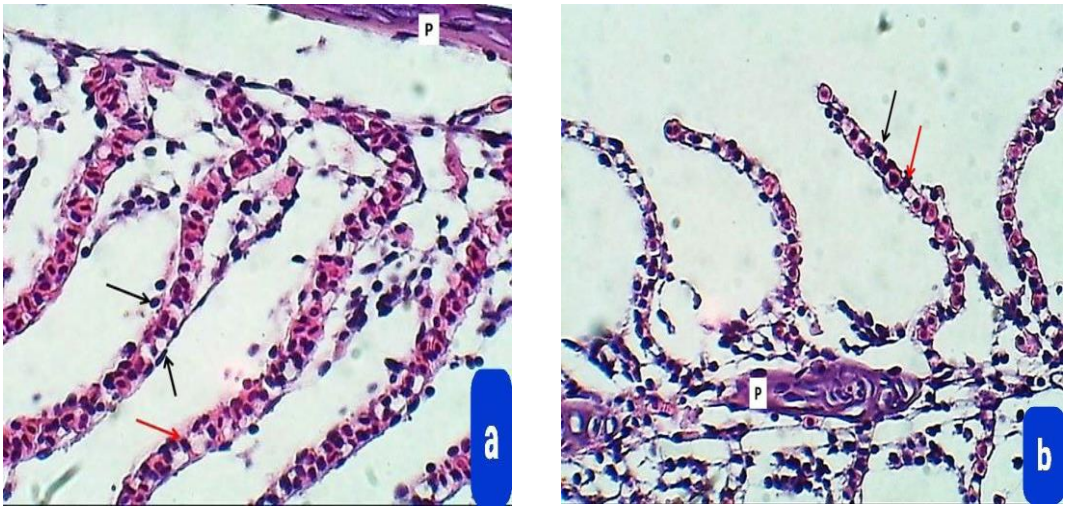

Figure 1: Histological changes in the Gills (H and E stain100x and 400x).

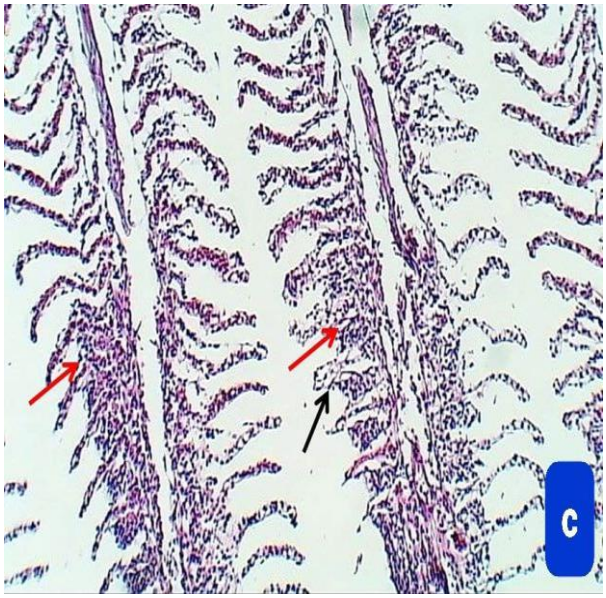

Figure 2: Histological changes in the gills (H and E stain100x and 400x).

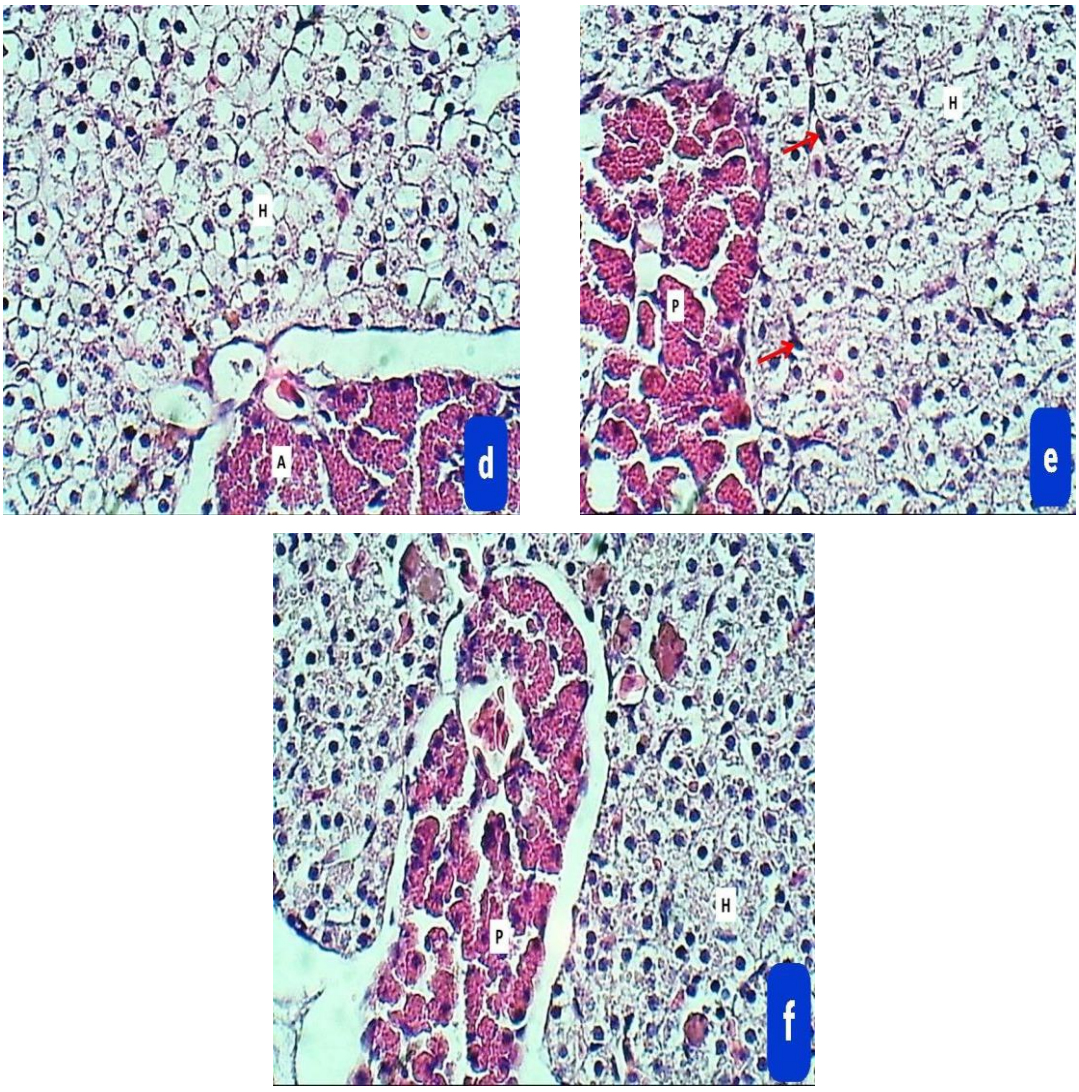

Figure 3: Histological changes in the liver (H and E stain100x and 400x).

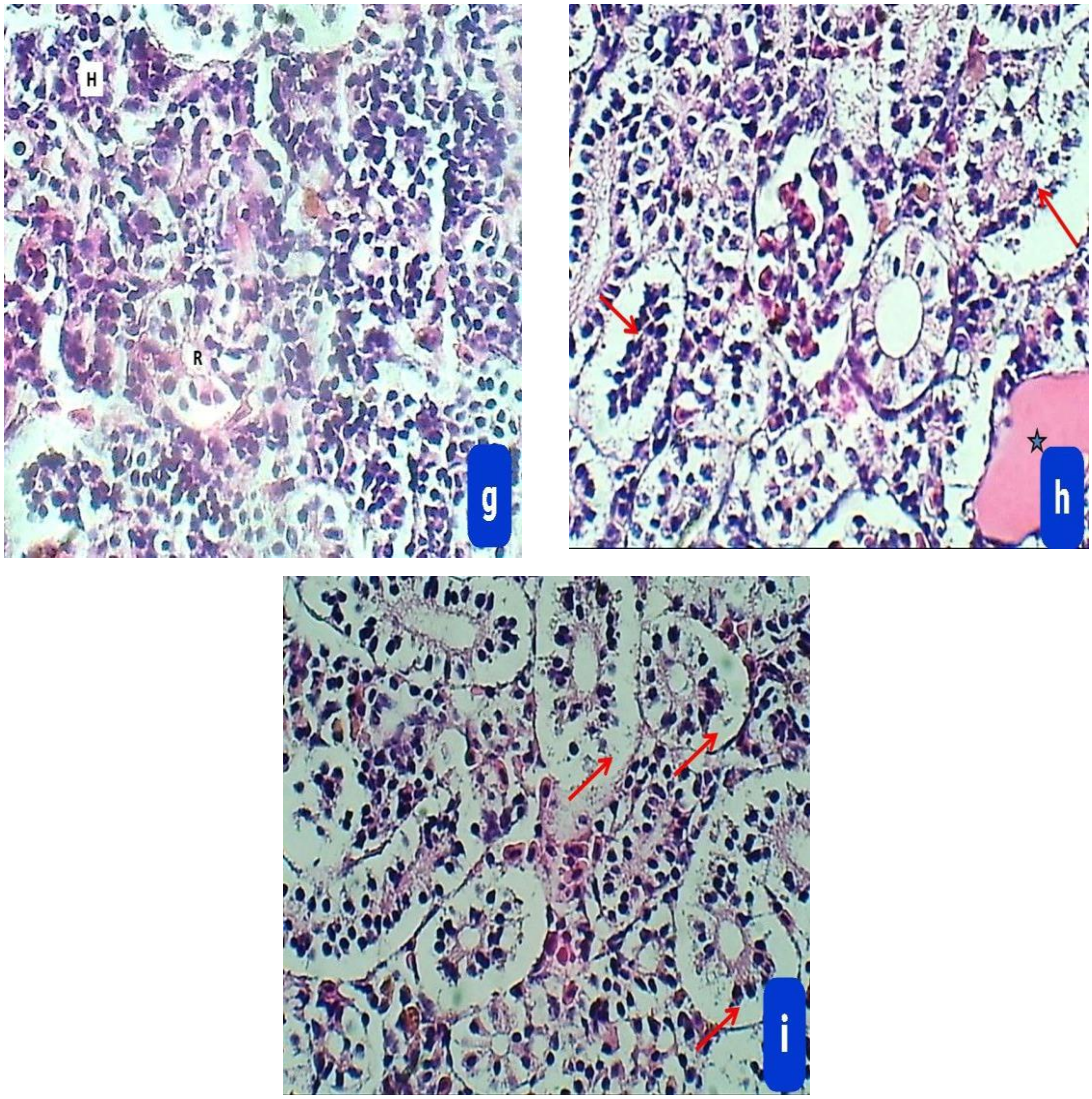

Figure 4: Histological changes in the kidney. (H and E stain100x and 400x).

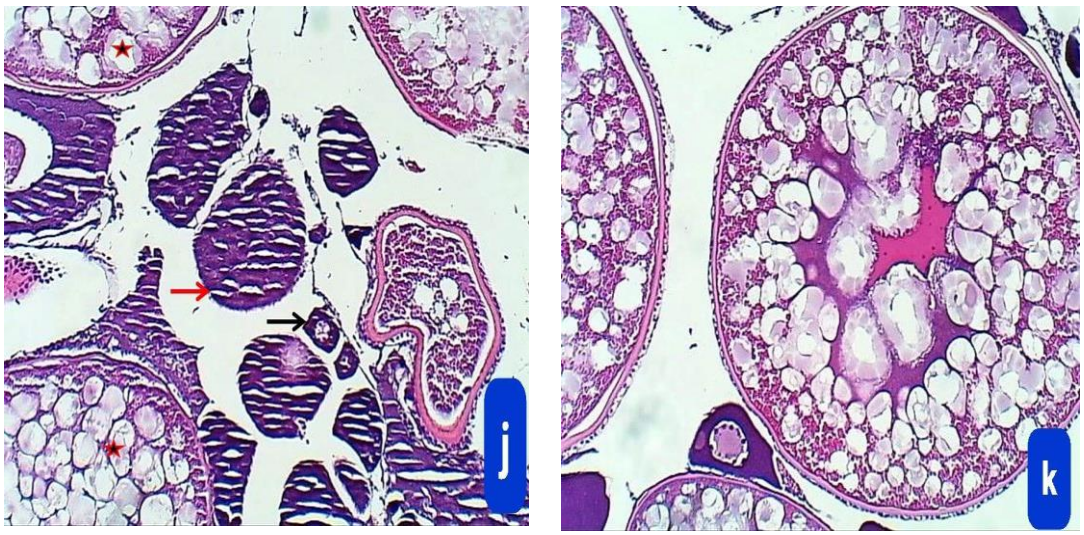

Figure 5: Histological changes in the ovary (H and E stain100x and 400x).

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