Journal of Wasit for Science and Medicine 2023: 16 (2), 26-39

Study The Acute Toxicity of Zinc Oxide Nanoparticles on *Cyprinus carpio*'s Tissues.

Ayad Ghazi Hashim, and Yaaroub Faleh Khalaf Al Fatlawy

Biology Department, College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

Despite nanotechnology's rapid growth and early acceptance, the health risks of prolonged exposure at varied concentrations in individuals and the environment have yet to be investigated. Due to nanomaterial use, many nanoscale products enter aquatic habitats, affecting ecosystems and human health. Nanomaterials' environmental impacts will increase. Ceramics, sunscreens, and hair care products use ZnO nanoparticles. Zinc oxide nanoparticles (ZnO NPs) may enter aquatic ecosystems, generating concerns regarding their effects on aquatic animals. This study examined how ZnO NPs affected common carp (*Cyprinus carpio*) diets. Toxicological studies reveal ZnO NPs harm humans and the environment. Thus, ZnO nanoparticles must be characterized for consumer and environmental health and safety. This study aims to determine ZnO nanoparticles' acute toxicity (LC50) and gill histopathology in *Cyprinus carpio*. 3500 mg/L ZnO nanoparticles kill *C. carpio* at 50%. In this work, sub-lethal Zinc Oxide nanoparticle exposure for 21 days affects the ovary, liver, and kidneys.

Keywords: ZnO nanoparticles, acute toxicity, common carp (*Cyprinus carpio*).

1. Introduction

The study of materials at the atomic and molecular level is known as nanotechnology [1]. The purpose of creating nanoparticles is to create a material with unique features [2]. Professor Norio at Tokyo University defined "nanotechnology" in 1974 as an atom-by-atom and molecule-by-molecule separating or incorporating procedure for materials [3]. Nanotechnology (NT) is being used in a variety of fields of sciences to produce materials and electronics using nanometer-scale processes. Nanoparticles are single particles with a diameter of 1–100 nm that are part of nanomaterials [4, 5].

Hydrolysis of transition metal ions produces engineered metal oxide

nanoparticles like zinc oxide (ZnO) [6]. It is already present in many consumption products, most importantly sunscreens and cosmetics [7, 8].

Nanomaterials have been increasingly developed in recent years for these qualities of nanoparticles because they have applications in industry, medicine, and industrial biomaterials, since many of these materials have biocompatibility with human tissues [9]. Because the properties of zinc oxide nanoparticles (ZnO-NPs), such as clarity, high surface charge point, bioactivity, and photo catalysis efficiency, are useful in medicine and industry, the synthesis of ZnO-NPs has occupied a large area of research [10], and they have been used in many fields such as cosmetic products, toothpaste, skincare products, dental work through medicine materials, textile industries, and construction materials. The toxicity mechanisms as well as environmental implications of ZnO-NPs, on the other hand, are unknown. In general, aquatic invertebrates and fish are more vulnerable to toxic insults than mammals [11, 12].

As a result, low nanoparticle concentrations may pose a threat to aquatic animals. The toxicity of ZnO-NPs has been linked to the dissolution of Zn^{2+} ions in Daphnia magna studies [13]. In particular, the aquatic milieu is susceptible to the buildup of a wide range of industrial substances; also, the nanotechnology industry's continued expansion will almost certainly result in the discharge of nanomaterials into the aquatic surroundings. The aquatic environment is particularly sensitive to contamination with artificial nanoparticles because it works as a sink for almost all toxins.

Generally, common carp is a type of fish (*Cyprinus carpio* L.) is a widely distributed fishes that is popular in fish hatcheries and sport fishing because its rapid rate of development, toughness as well as abundant reproduction within restricted water. Due to its relative insensitivity, carp has also been suggested as a research organism for several toxicological studies and hence persists and accumulates poisons even in extensively contaminated environments [14, 15].

Concern has grown over the past few decades regarding the contamination of freshwaters by a variety of heavy metals produced by agricultural, industrial, and other artificial activities, with potentially disastrous consequences for the

recipient environment's ecological balance and a diverse range of aquatic organisms [16]. *Cyprinus carpio* is a globally significant ecological and economic fish that can withstand moderate levels of hypoxia.

As a result, the response of this species to various environmental difficulties was chosen to be studied. When exposed to normoxic or hypoxic settings, carp were either fed (2% of body weight) or fasted. Up to comparatively low levels of hypoxia, C. Carpio is presently hyperventilating (50-60 percent saturation) [17].

In a previous study fullerene solution made without solvents at 4.5 mg L-1 was administered to the *Cyprinus carpio* in vivo and carp brain homogenates in vitro for 48 hours. The fullerene hadn't reached the brain tissue due to the exposure, and there was peroxidation of lipid in the brain homogenate [18].

Fish generated NPs' uptake, dispersion, digestion, and elimination are examined by Handy *et al*. [19]. (A. D. M. E.). According to this review, the gastrointestinal tract, fins, brain, and liver are identified as possible receptor sites for the harmful effects of some synthetic NPs on carp, along with variety of harmless effects like cellular diseases such as the development of liver tumors, oxidative, certain organ dysfunction ion concentration disorders, besides damage to blood vessels.

Efendic *et al.* [20] revealed that ZnO NP can enter and accumulate in a wide range of tissues, such as those located in the ovaries. Zinc Oxide NP causes peroxidation, cytotoxicity, and mutagenicity in ovarian cells, ZnO NPs decrease follicular growth and follicle atresia while enhancing ovarian damage, and intrinsic apoptotic activation. Regular use of ZnO may reduce the number of follicles in the follicular pool, the odds of ovulation, and result in infertility.

According to Shaw and Handy [21], the accumulation of ZnO NPs in fish may enhance the probability for exposure to either through sediments or organic materials containing NPs, dietary routes, or through the nourishment on species carrying accumulated NPs. Metal dietary exposure, on the other hand, is widely accepted as the primary method of chronic metal exposure to fish in aquatic environment.

According to a study by Johnston *et al.* [22], metallic nanoparticles - treated fish through their habitat don't aggregate the nanoparticles in the body tissue due to

the exposure. *Danio rerio* were treated with CeO_2 in addition to ZnO NPs through the surrounding aquatic habitat, and *Oncorhynchus mykiss* were revealed to titanium oxide nanoparticle and large particles by surrounding aquatic habitat also Different studies sought to identify target organs, evaluate the relative importance of different exposure routes, and determine the digestibility and ingestion of nanoparticles through fish bodies.

Numerous research studies [23-26] have assessed the toxicity of Zinc Oxide NPs on various aquatic environment species. Acute treated with ZnO NPs causes developing toxicity and DNA damage in zebrafish, according to Zhao *et al.* [27].

2. Materials and methods

2.1 Zinc oxide (ZnO) nanoparticles

SIGMA-Aldrich produced the ZnO nanoparticles, which are pure (99.8%) and have a small particle size (10-30nm). Before exposing fish to Nanoscale ZnO suspensions are created using a bath sonicator at 33 kHz for one hour at a time, and aerated, single-distilled water [28].

2.2 Experimental design

seventy *C. carpio* fish (weight 120±5 g) 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 [29], 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.

2.3 Acute toxicity

Common carp *(C. carpio)* was treated with 6 concentrations from the ZnO nanoparticles. Test concentrations for lethality were 2000, 2500, 3000, 3500, 4000 and 4500 mg/L for ZnO nanoparticles. An acute toxicity test of C. carpio was undertaken and carried out for a period of 96 hours, based on the (OECD) [30]. At the conclusion of (24, 48, 72, and 96) hours, the mortality rate was calculated, and dead fish were removed when they were observed. The fish were separated into groups, each with ten fish. They were placed in a 45L water-containing glass aquarium without food for the duration of the acute toxicity test,

which lasted 96 hours. Seven groups of treated fish samples were created. The first served as a control group and the other six were exposed to varied concentrations. The LC50 values for ZnO nanoparticles were calculated using Finney's [31]. Probit analysis method, and MS Excel 2007 was used to determine the linear relationship (Y=mortality; X=concentrations). The LC50 was then generated from the best-fit line discovered.

2.4 Sub chronic toxicity test for ZnO nanoparticles

The fish were exposed to three concentrations of ZnO nanoparticles which calculated by 1/15th, and 1/7th of the 96 LC50 value which calculated in this study [32] which were = 250 mg/L and 500 mg/L correspondingly.

2.5 Histological analysis

Fish dissection was performed in conformity with the instructions of the EMERGE Protocol [33]. All the samples were placed in vials with 10% neutral buffer formaldehyde solution (pH 7.0). Flushing in distilled water, dehydration in a graduated sequence of ethanol concentrations, having cleared in xylene, embedding in paraffin wax with a melting of 54–56ºC, separating to a diameter of 5-7 μm that used a semi-automated rotating microtome (Leica RM 2245), and going to mount on sterile glass slides were all procedures that were performed on the samples. Sections were created after deparaffinization for histological analysis, light microscopy examination, and hematoxylin and eosin (H&E, 100x and 400x) staining [34].

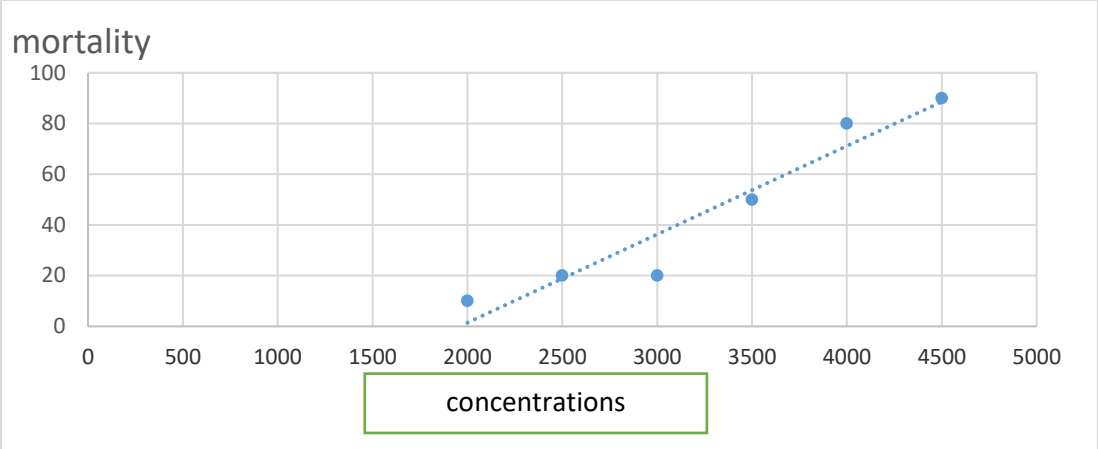
3. Results and discussion

3.1 Acute toxicity

Throughout the exposure duration, the water's temperature was maintained between 24°C and 2°C. pH and dissolved oxygen levels (DO) of the water were examined (6.7–7.7 and DO > 5.10 mg/L, correspondingly). The toxicity of ZnO nanoparticles to common carp increases with particle concentrations, demonstrating that the toxic effects are dose dependent. The LC50 in this investigation was measured at 3500 mg/L at 95% confidence limits and an expected regression line. A concentration of 4000 and 4500 mg/L Nano particles solution cause 100% death with a predicted 96 hour. As seen in Graph 1. Subashkumar and Selvanayagam's research [35]. The 50% of lethal concentration (LC50) of Zinc Oxide nanoparticle for *C. carpio* was discovered to be 4.897 mg/L. The main harmful mechanisms were most likely

linked to the chemical and physical properties of Zinc Oxide nanoparticles, and it was also found that nanoparticles have a toxic effect [36].

Graph 1: LC50 (96 hr.) of ZnO nanoparticle for *C. carpio*



3.2 Histopathology

3.2.1 Ovary

When treated with 250 mg/l of Zinc Oxide nanoparticles for three weeks the section showed normal appearance of ovary contain primary oocytes at stage two which were located in the center of ovary, whereas the primary oocytes at stage one and oogonia were located in the periphery of the ovary (fig. 1, a). And when treated with 500 mg/l of ZnO NP for three weeks the section showed normal appearance of ovarian primary oocytes, oogonia and secondary oocytes (fig. 2, b). Efendic *et al*. (20) revealed that ovarian cells are among the many cells that ZnO NP can penetrate and build up in. In ovarian tissues, zinc oxide nanoparticles produce toxicity, oxidative stress, and genotoxicity, ZnO NPs decrease follicular growth and follicle atresia while enhancing ovarian damage, apoptosis and apoptotic signaling activation.

3.2.2 Liver

The liver is the principal organ for metabolism, detoxification of pollutant, and toxic material release (37). When treated with 250 mg/l of Zinc Oxide nanoparticles for three weeks The sections of liver showed normal appearance of hepatic tissue involved bile ductules, exocrine pancreatic acini and pancreatic ductules (fig.3, c) and the magnified section of liver showed normal appearance of hepatocytes, epithelial cells of bile ductules and pancreatic acini filled with eosinophilc serous zymogen granules (fig.3, d). and When

treated with 500 mg/l of Zinc Oxide nanoparticle for three weeks The sections of liver showed normal appearance of hepatic tissue involved bile ductules, exocrine and pancreatic acini (fig.4, e) and the magnified section of liver showed normal appearance of hepatocytes showed exhausted of glycogen granules (fig.4, f).

3.3.3 Kidney

The kidney is responsible for a variety of functions in the body, including hormone activation, maintaining constant levels of essential molecules in the blood, and toxin excretion (38). When treated with 250 mg/l of Zinc Oxide nanoparticle for three weeks the section of anterior kidney showed mild focal vacular degeneration and necrosis of collecting and proximal tubules (fig.5, g) and the magnified sections showed epithelial desquamation of these tubules with normal cytoarchetecture of renal glomeruli (fig.5, h). And when treated with 500 mg/l Zinc Oxide nanoparticle for three weeks a section of head kidney showed moderate focal vacular degeneration and desquamation of epithelial cells of collecting and proximal tubules with marked tissue depletion (fig.6, i), the magnified section showed mild deposition of hemosiderin pigments (fig.6, j).

4. Conclusion

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

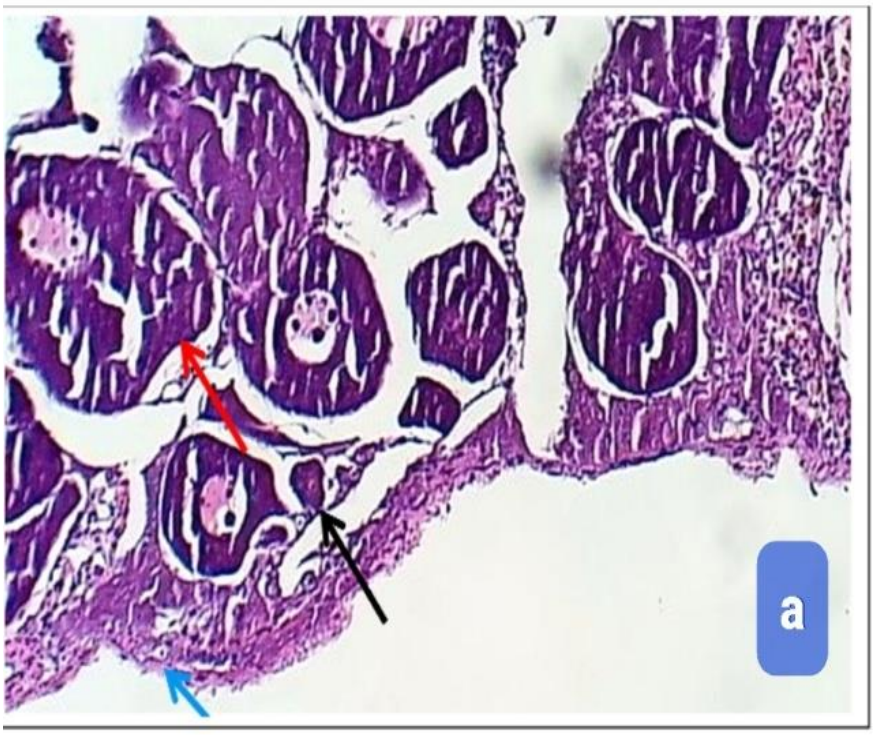


Figure 1: Histological changes in the ovary (H and E stain100x and 400x).

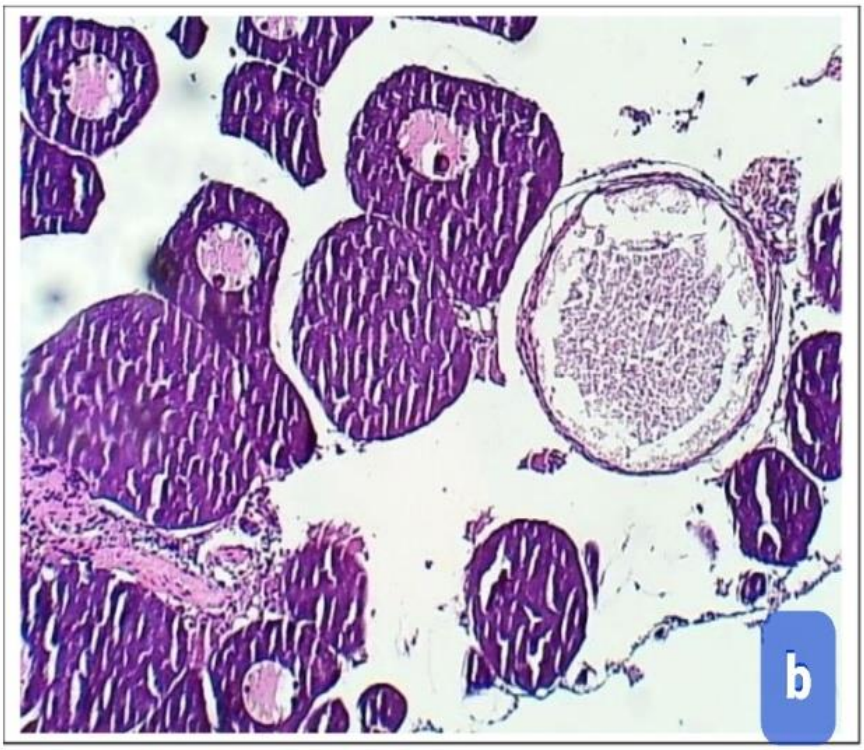


Figure 2: Histological changes in the ovary (H and E stain100x and 400x).

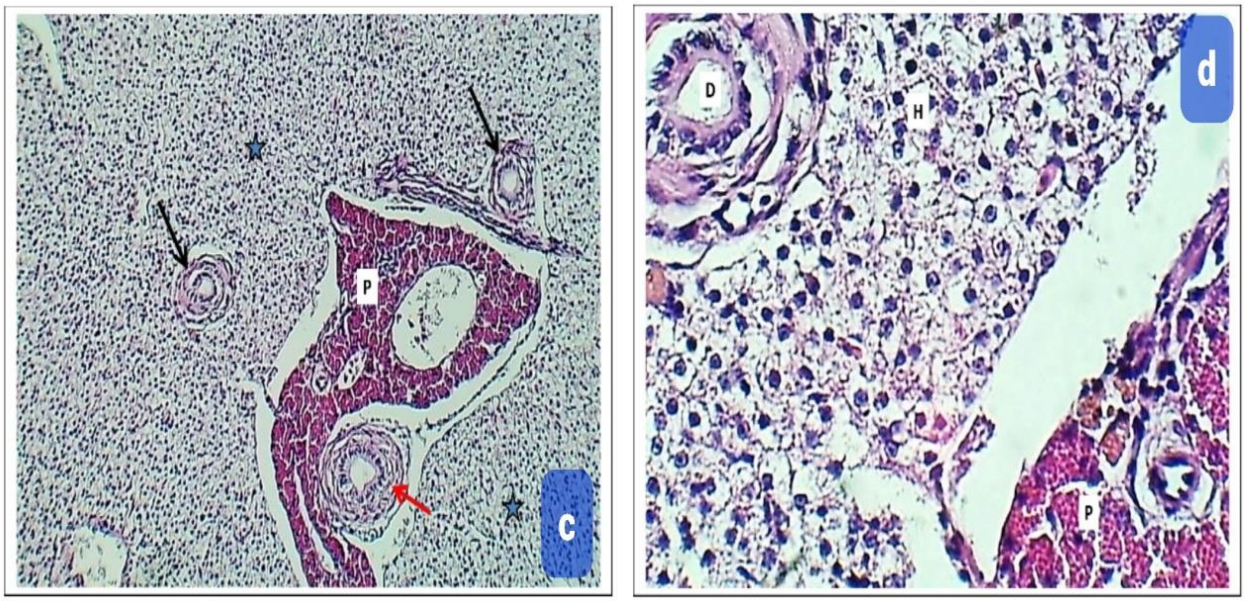


Figure 3: Histological changes in the Liver (H and E stain100x and 400x).

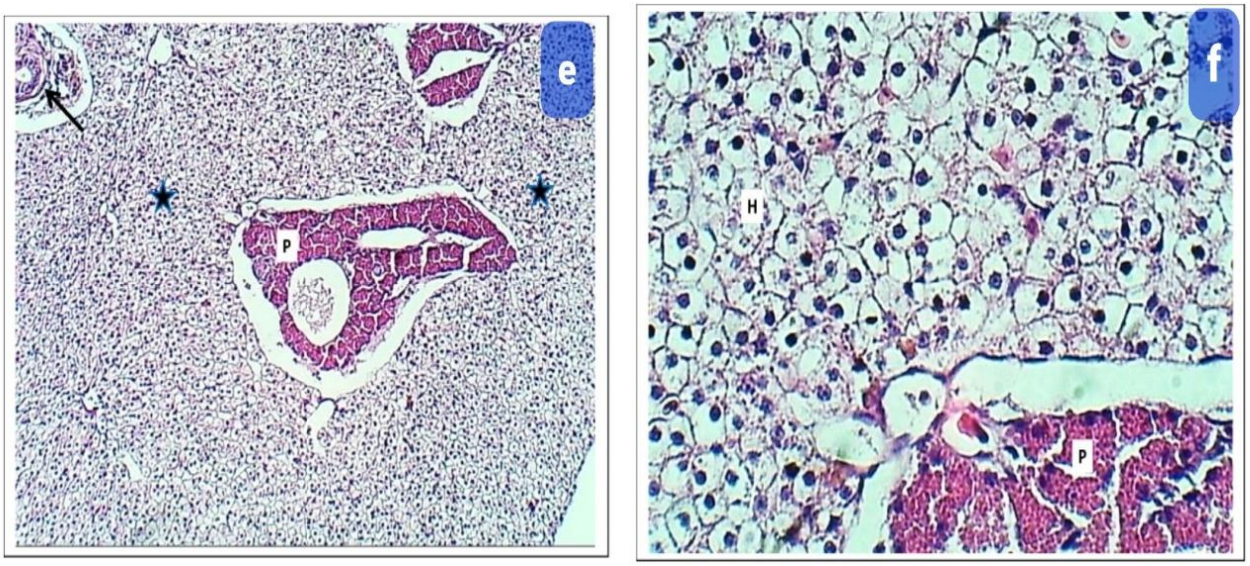


Figure 4: Histological changes in the Liver (H and E stain100x and 400x).

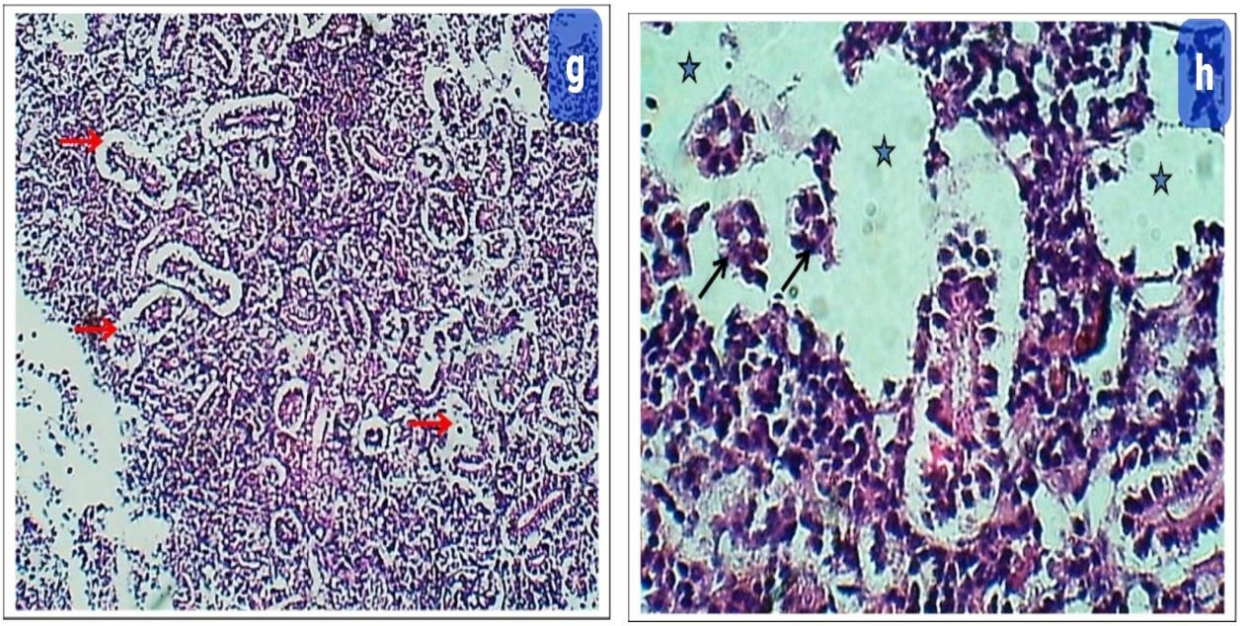


Figure 5: Histological changes in the Kidney (H and E stain100x and 400x).

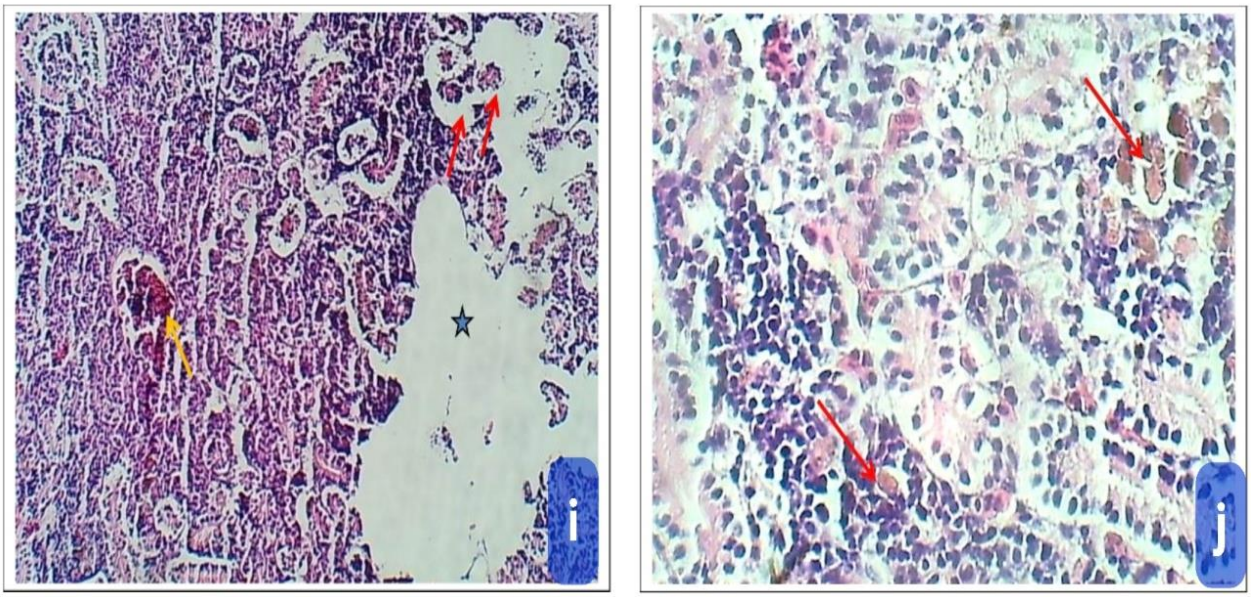


Figure 5: Histological changes in the Kidney (H and E stain100x and 400x).

5. References

1. Bhushan, B., Luo, D., Schricker, S. R., Sigmund, W., and Zauscher, S. (2014). Handbook of nanomaterials properties. Springer Science and Business Media.
2. Nasir, A. (2010). Nanodermatology: a glimpse of caution just beyond the horizon-part II. Skin therapy letter, 15, 9, 4-7.

3. Hall, J. S. (2005). Nanofuture What`s next for Nanotechnology. Prometheus Books, New York, USA.
4. Zheng, M., Davidson, F., and Huang, X. (2003). Ethylene glycol monolayer protected nanoparticles for eliminating nonspecific binding with biological molecules. Journal of the American Chemical Society, 125, 26, 7790-7791.
5. Hussein, N. A., and Ali, N. A. (2022). Synthesis and Characterization of Ni_2O3 as a Phase of Nickel Oxide Nanomaterial. Iraqi Journal of Science, 4733-4739.
6. Masala, V., and Seshadri, R. (2004). Synthesis routes for large volumes of nanoparticles. Annu. Rev. Mater. Res., 34, 41-81.
7. Brezová, V., Gabčová, S., Dvoranová, D. and Staško, A. (2005). Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study). Journal of Photochemistry and Photobiology B: Biology, 79, 2, 121-134.
8. Lademann, J., Weigmann, H. J., Schaefer, H., Mueller, G., and Sterry, W. (2000). Investigation of the stability of coated titanium microparticles used in sunscreens. Skin Pharmacology and Physiology, 13, 5, 258-264.
9. Darroudi, M., Ahmad, M. B., Zak, A. K., Zamiri, R., and Hakimi, M. (2011). Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light. International journal of molecular sciences, 12, 9, 6346-6356.
10. Jing, L., Xu, Z., Sun, X., Shang, J. and Cai, W. (2001). The surface properties and photocatalytic activities of ZnO ultrafine particles. Applied Surface Science, 180, 3-4, 308-314.
11. Al-Khafaji, B. Y., Mohammed, A. B., and Maqtoof, A. A. (2011). Distribution of some heavy metals in water, sediment, and fish Cyprinus carpio in Euphrates River near Al-Nassiriya city center south Iraq. Baghdad Science Journal, 8, 1, 552-560.
12. Delistraty, D., Taylor, B., and Anderson, R. (1998). Comparisons of acute toxicity of selected chemicals to rainbow trout and rats. Ecotoxicology and environmental safety, 39, 3, 195-200.
13. Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H. C., and Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to

bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. Chemosphere, 71, 7, 1308-1316.

14. Reynders, H., Bervoets, L., Gelders, M., De Coen, W. M., and Blust, R. (2008). Accumulation and effects of metals in caged carp and resident roach along a metal pollution gradient. Science of the Total Environment, 391, 1, 82-95.
15. Al-Rudainy, A. J., and Khalel, H. A. (2019). Histopathological changes (gills and liver) and clinical signs of common carp, Cyprinus carpio L. exposed to graphene nanoparticles. Iraqi J. Agric. Sci, 50, 3, 901-908.
16. Farombi, E. O., Adelowo, O. A., and Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (Clarias gariepinus) from Nigeria Ogun River. International journal of Environmental research and public health, 4, 2, 158-165.
17. Soncini, R., and Glass, M. L. (2000). Oxygen and acid-base status related drives to gill ventilation in carp. Journal of fish biology, 56, 3, 528-541.
18. Shinohara, N., Matsumoto, T., Gamo, M., Miyauchi, A., Endo, S., Yonezawa, Y., and Nakanishi, J. (2009). Is lipid peroxidation induced by the aqueous suspension of fullerene C60 nanoparticles in the brains of Cyprinus carpio. Environmental science and technology, 43, 3, 948-953.
19. Handy, R. D., Henry, T. B., Scown, T. M., Johnston, B. D., and Tyler, C. R. (2008). Manufactured nanoparticles: their uptake and effects on fish a mechanistic analysis. Ecotoxicology, 17, 5, 396-409.
20. Efendic, F., Sapmaz, T., Canbaz, H. T., Pence, H. H., and Irkorucu, O. (2022). Histological and biochemical apoptosis changes of female rats′ ovary by Zinc oxide nanoparticles and potential protective effects of l-arginine: An experimental study. Annals of Medicine and Surgery, 74, 103290.
21. Shaw, B. J., and Handy, R. D. (2011). Physiological effects of nanoparticles on fish: a comparison of nanometals versus metal ions. Environment international, 37, 6, 1083-1097.
22. Johnston, B. D., Scown, T. M., Moger, J., Cumberland, S. A., Baalousha, M., Linge, K., and Tyler, C. R. (2010). Bioavailability of nanoscale metal oxides TiO_2,

CeO2, and ZnO to fish. Environmental science and technology, 44, 3, 1144-1151.

23. Hao, L., and Chen, L. (2012). Oxidative stress responses in different organs of carp (Cyprinus carpio) with exposure to ZnO nanoparticles. Ecotoxicology and environmental safety, 80, 103-110.
24. Adam, N., Vergauwen, L., Blust, R. and Knapen, D. (2015). Gene transcription patterns and energy reserves in Daphnia magna show no nanoparticle specific toxicity when exposed to ZnO and CuO nanoparticles. Environmental research, 138, 82-92.
25. Falfushynska, H., Gnatyshyna, L., Fedoruk, O., Mitina, N., Zaichenko, A., Stoliar, O., and Stoika, R. (2015). Hepatic metallothioneins in molecular responses to cobalt, zinc, and their nanoscale polymeric composites in frog Rana ridibunda. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 172, 45-56.
26. Mustafa, S. A., and Ashour, A. A. (2021). Effect of chronic exposure to sliver nanoparticles on histopathological manifestations of common carp, Cyprinus carpio L. Biochem. Cell. Arch. 21, 2719-2725.
27. Zhao, X., Ren, X., Zhu, R., Luo, Z. and Ren, B. (2016). Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria-mediated apoptosis in zebrafish embryos. Aquatic Toxicology, 180, 56-70.
28. Taurozzi, J. S., Hackley, V. A. and Wiesner, M. R. (2011). Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment–issues and recommendations. Nanotoxicology, 5, 4, 711-729.
29. USEPA (United States Environmental Protection Agency) (1975). Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.
30. (OECD) Organization for Economic Co-Operation and Development. (1993). Guidelines for Testing of Chemicals. Test No. 203: Fish Acute Toxicity Test, OECD, Paris, France.
31. Finney, D. J. (1971). Probit Analysis. 3rd Edition, Cambridge University, Press, London. 333. Aquaculture. 28(1–2):39–48.

32. Al-Sawafi, A. G. A. and Yan, Y. (2013). Bioconcentration and antioxidant status responses in zebrafish (Danio rerio) under atrazine exposure. International Journal of Chemical Engineering and Applications, 4, 4, 204.
33. Rosseland, B. O., Massabuau, J. C., Grimalt, J., Rognerud, S., Hofer, R., Lackner, R. and Trichkova, T. (2000). Fish ecotoxicology. EMERGE Final Report, 41-50.
34. Romeis B. (1989). Microscopic technology. München: Urban und Schwarzenberg, 697.
35. Subashkumar, S. and Selvanayagam, M. (2014). First report on: Acute toxicity and gill histopathology of freshwater fish Cyprinus carpio exposed to Zinc oxide (ZnO) nanoparticles. International Journal of Scientific and Research Publications, 4, 3, 1-4.
36. Xiong, D., Fang, T., Yu, L., Sima, X. and Zhu, W. (2011). Effects of nanoscale TiO_2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Science of the Total environment, 409, 8, 1444-1452.
37. Capkin, E., Terzi, E., Boran, H., Yandi, I. and Altinok, I. (2010). Effects of some pesticides on the vital organs of juvenile rainbow trout (Oncorhynchus mykiss). Tissue and Cell, 42, 6, 376-382.
38. Pizzorno, J. (2015). The Kidney Dysfunction Epidemic, Part 1: Causes. Integrative medicine (Encinitas, Calif.), 14, 6, 8–13.