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First report on: Acute toxicity and gill histopathology of fresh water fish *Cyprinus carpio* exposed to Zinc oxide (ZnO) nanoparticles

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Abstract – Nanotechnology is an advancing field of research which has revolutionized all industrial needs, such as medical, environmental and other industrial applications. Despite the rapid progress and early acceptance of nanotechnology, the potential for adverse health effects due to prolonged exposure at various concentration levels in humans and the environment has not yet been studied. With the widespread application of nanomaterials, numerous nanoscale products might consequently be released into aquatic environments and elicit an impact not only on one particular ecosystem but also on human health. The environmental impact of nanomaterials is expected to increase dramatically in near future. Zinc oxide (ZnO) nanoparticles have wide ranging applications in a diverse array of industrial and consumer products, ceramic manufacturing, paint formulation, sunscreen and hair care products. Toxicological studies indicate that ZnO nanoparticles have adverse impacts on human health and environmental species. Hence it is due process to characterize the health and safety aspects of ZnO nanoparticles to humans and environment. The present study has been conducted to develop first hand information on the acute toxicity (LC_{50}) and gill histopathology of ZnO nanoparticles in fresh water fish, *Cyprinus carpio*. It was found that the 50% lethal concentration (LC_{50}) of ZnO nanoparticle for *C. carpio* is 4.897 mg/L. Gill histopathological damage exposed to sub-lethal concentration of ZnO nanoparticles for 21 d are also discussed in this paper.

Key words: Nanotoxicology, Ecotoxicology, Zinc oxide (ZnO) nanoparticles, Common carp (*Cyprinus carpio*), Acute toxicity, Gill histopathology.

I. Introduction

In recent decades, nanoparticles have been increasingly manufactured and used in daily consumer products, such as textiles, pharmaceuticals, and cosmetics, as well as in pollution treatment and remediation processes [1, 2]. Among numerous nanomaterials, Zinc oxide (ZnO) nanoparticles have attracted special attention worldwide due to their excellent properties in applications such as cosmetics, sunscreens, paints, ceramics, photocatalysis, UV filters and biosensors [3-8]. The potential adverse effects of few nanoparticles are widely studied in organisms, including humans and mammals [9-13]. However, most of the data has been obtained on limited types of particles and mostly *in vitro* cell cultures or *in vivo* respiratory exposures on rodents [14]. Therefore, it is important to develop an understanding of the effects of nanoparticles. Hence it is due process to characterize the health and safety aspects of ZnO nanoparticles to humans and environment. The present study has been conducted to develop information on the acute toxicity (LC_{50}) and gill histopathology of ZnO nanoparticles in fresh water fish, *Cyprinus carpio* and the result will be first hand information in the field of aquatic ecotoxicology and nano-ecotoxicology.

II. Materials and methods

Zinc oxide (ZnO) nanoparticles

ZnO nanoparticles (<100 nm) were purchased from SIGMA-Aldrich (Product Number-544906). Suspensions of nano-scale ZnO are prepared with aerated single-distilled water and dispersed with a bath sonicator (33 kHz) for 1 h every time before exposure to fish [15].

Experimental animals

Common carp, (*C. carpio*) with a mean age of 120 d, mean length of 3.02± 0.33 cm, and mean weight of 0.22±0.05 g were obtained from the Tamil Nadu fish farm, Thiruvallur District. Animal experiments were performed in the laboratory, fulfilling the criteria of Good Laboratory Practice. Selection of fish and experiments were performed according to OECD guideline [16]. Distilled water, rather than standard laboratory water was used to prepare the ZnO nanoparticle suspension, so as to avoid the influence of other environmental factors on toxicity of ZnO nanoparticles [8, 17]. Fish were stocked in standard laboratory water and acclimatized in distilled water for 10 d with a natural light-dark cycle (12 h light /12 h dark) and fed twice daily. During this period, the water temperature was maintained at 23±2 °C, and no fish died.

Acute Toxicity (LC_{50})

In median lethal toxicity study, lethality was the endpoints. Test concentrations for lethality were 0, 2, 4, 8, and 16 mg/L for ZnO nanoparticles. Test suspensions were prepared and dispersed using bath sonicator for 1 h immediately prior to use without the addition of any stabilizing agents. Ten fish were randomly exposed to each concentration for 96 h in 2.5 L container with 1.5 L of the test solution. To ensure a constant concentration, all the test solutions were changed every 24 h (semi-static method). The control group was provided with distilled water without nanoparticles. Each treatment was run in triplicate and placed under the same conditions. In order to maintain water quality, fish were not fed on the day before or during the experimental period to minimize the absorption of the nanoparticles in food and the production of faeces. The 96 h LC_{50} were calculated according to the EPA probit analysis.

Histopathology of gill

Four batches of ten fish each were exposed to three sub-lethal concentrations fixed via 5, 10 and 20 % from the calculated

LC_{50} value and one batch was maintained as control for 21 d. Fish were anesthetized, and they were dissected. Gill arches (first and second from the eye) were excised and fixed in Bouin's fluid for 48 h, dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin wax. Sagittal sections of 5 micron thick were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with haematoxyline-eosin (H&E) for structural analysis of tissue. Histological alterations were examined and micrographs were taken in Carl ZEIS AxioCam MRC microscope.

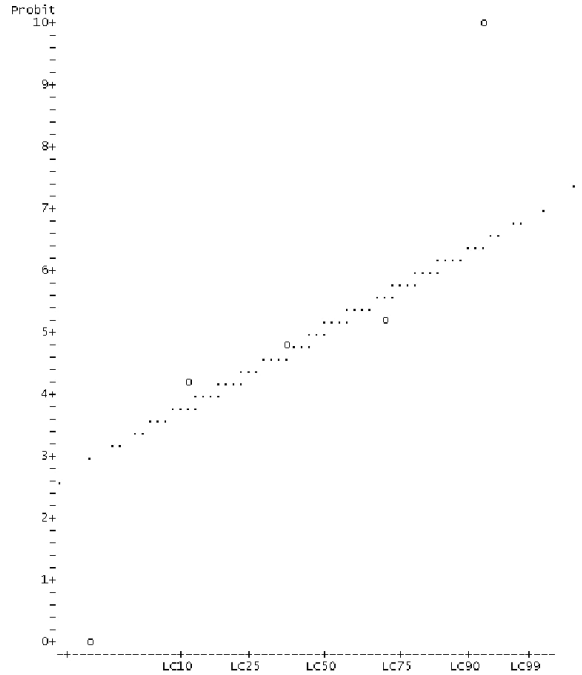
III. Result

Acute toxicity

During the exposure period, the water temperature was maintained at 23±2°C. The pH and dissolved oxygen content (DO) of the water were monitored (6.7–7.2 and DO no less than 5.10 mg/L respectively). The number of dead fish was recorded every 12 h, and they were removed immediately to avoid contamination of the exposure solutions.

The toxicity of ZnO nanoparticles to common carp was increased with particle concentration, demonstrating a dose dependency. Although ZnO nanoparticles at a concentration of 1 mg/L produced no mortality in common carp (*C. carpio*), as in the control group, the 16 mg/L ZnO nanoparticles suspension caused 100% mortality with a calculated 96 h LC_{50} of 4.897 mg/L at 95% confidence limits and predicted regression line as shown in Graph 1.

Graph 1. Plot of adjusted probits and predicted regression line



Gill

Gill of common carp (*C. carpio*) was composed of filament, lamellae, pillar cell, epithelial cell, secondary lamellae, water cannel and mucous cell. Primary gill lamellae consisted of cartilaginous skeletal structure, multilayered epithelium and vascular system. Numerous secondary lamellae were lined up along both sides of primary lamella. Secondary gill lamella was constituted of epithelial cells supported by pillar cells. Histological observations showed normal structure of gill in control fish through out the duration of the experiment (Fig.1A). Pathological lesions after 21 d exposure at three different sub-lethal test concentrations are shown in Fig.1(B-F). In 5% exposure group, lamellae with marginal channel dilation, epithelial lifting, desquamation and necrosis, alteration in secondary structure and loss of secondary lamellae were of serious concern (Fig.1B&C). In 10 % exposure group, acute cellular swelling and blood congestion were observed (Fig.1D). In 20 % exposure group, hyperplasia of epithelial cells, lamellar fusion, aneurism, lamellar disorganization and curling were newly recorded (Fig.1E&F).

IV. Discussion

Acute toxicity

Relatively small amount of work is available in the literature that discusses the toxicity of ZnO nanoparticles in fish [18]. As stated earlier most studies have focused on *in vitro* analysis. Present study shows acute toxicity of ZnO nanoparticles for common carp (*C. carpio*) as 4.897 mg/L and this is well in accordance with the acute toxicity of ZnO nanoparticle in zebra fish (96 h LC_{50} of 4.92 mg/L) and principal toxic mechanisms were probably associated with the physical and chemical characteristics of ZnO nanoparticle and also reported as nanoparticles will cause toxic effect [19].

Gill

Fish gill is a crucial organ for respiration [20], osmoregulation and there is close relationship between gill morphology and stress [21]. Histological study of the gills shows a typical structural organization of the lamella in the control group (Fig.1A). In this study, the treated group (Fig.1B-F) shows progressive architectural distortions like alteration in secondary structure, blood congestion, lamellae with marginal channel dilation, hyperplasia of epithelial cells, epithelial lifting, lamellar fusion, desquamation and necrosis, aneurism, acute cellular swelling, lamellar disorganization, curling, and loss of secondary lamellae at 21 d exposures were observed due to ZnO nanoparticle toxicosis. Pathological alterations like hyperplasia of epithelial cells, epithelial lifting and lamellar fusion may increase the space of contact of toxicants with the vascular system of the gill, resulting in impairment of respiration as well as fish health. Aneurism was observed due to collapse of pillar cells in the secondary lamellae and rupture of blood vessels, releasing large quantities of blood resulting in the lamellar disorganization. Desquamation and necrosis are the direct deleterious effects induced by ZnO

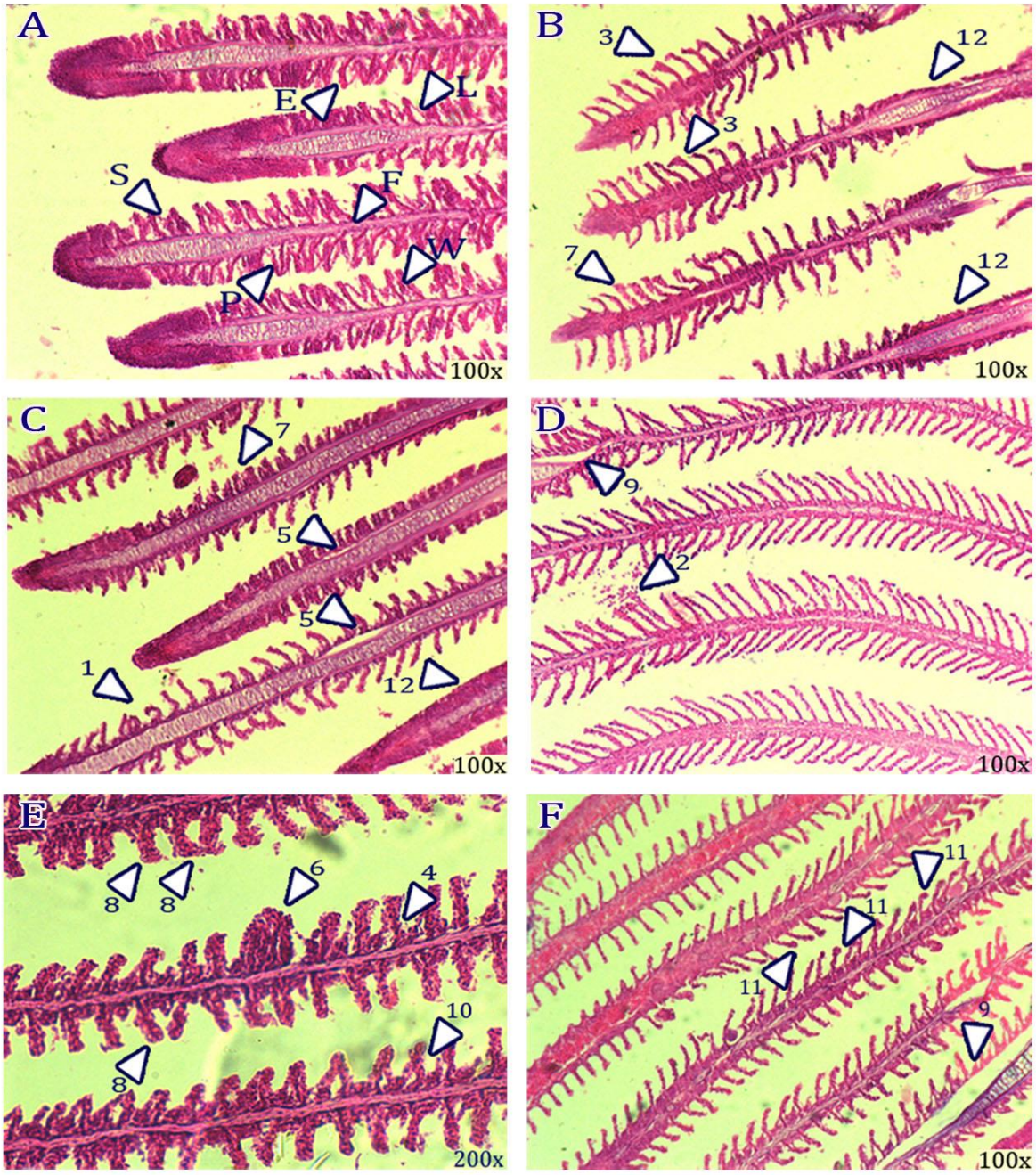


Fig. 1 Histology of gill tissue. A. Gill tissue of Control fish, B&C. Gill tissue exposed to 5% of test solution, D Gill tissue exposed to 10% of test solution, E&F. Gill tissue exposed to 20% of test solution.

F-Filament, L-Lamellae, P-Pillar Cell, E-Epithelial Cell, S-Secondary lamellae, W-Water cannel, M-Mucous cell, 1.Alteration in secondary structure, 2.Blood congestion, 3.Lamellae with marginal channel dilation, 4.Hyperplasia of Epithelial cells, 5.Epithelial lifting, 6.Lamellar fusion, 7.Desquamation and necrosis, 8.Aneurism, 9.Acute cellular swelling, 10.Lamellar disorganization, 11.Curling, 12.Loss of secondary lamellae.

nanoparticles exposure. Similar histological lesions were reported for different xenobiotics including heavy metals [22], pesticides [23,24] and few metal and metal oxide nanoparticles [25] and other abiotic stress by many researchers. The findings of this study are the first of its kind with regard to a detailed survey of gill histopathology.

V. Conclusions

ZnO nanoparticles suspension were prepared using distilled water, rather than standard laboratory water and dispersed using sonicator for 1 h. Acute toxicity of ZnO nanoparticles to common carp (*C.carpio*) were determined at 96 h (LC_{50}) as 4.897 mg/L. The histopathologal alterations in gill indicated that sub-lethal concentration of ZnO nanoparticles may cause severe damage resulting in dysfunction and ultimately the death of fish at higher concentrations. The histopathological changes in the cellular level in gill of common carp and their direct correlation with the concentration and exposure period of ZnO nanoparticles indicated that gill histopathological changes can be considered as a biomarker for the ZnO nanoparticles induced toxicity. According to the results, gill can be good target organs, and common carp can be used as a test fish for ZnO nanoparticles toxicity in freshwater.

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