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Histopathological and Ultrastructure Alterations in Gills of Common Carp (*Cyprinus carpio*) after Long Time Exposure to Zinc Oxide Nanoparticles

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ABSTRACT

This study aimed to assess the toxicity and severity of zinc oxide nanoparticle (ZnO-NPs) in the tissues of gills. Therefore, carp fish (*Cyprinus carpio*) were subjected to sub-lethal concentration of (ZnO-NPs) 9mg/l for periods of (7, 14, 21, 28, 35, 42) days that led to a histopathological and ultrastructural alteration in gills tissue. Microscopic examination showed the occurrence of bleeding and the pyramidal form (clump shape) of the epithelial cells lining the secondary gill filament after the lapse of 7 and 14 days after the treatment, and hyperplasia in mucus cells with hypertrophy of pillar cells at 28 days of treatment. At day 42, the lesions were characterized by basement thickening in the secondary gill filament with edema and vacuolar degeneration of pillar cells. While ultrastructural examination showed the presence of cloudy swelling of chloride cells and condensation of micro organelles at seven days of exposure increased with increasing duration of exposure and, therefore, at day 14 of exposure to the (ZnO-NPs). The electron microscope examination showed a thickening of blood vessels wall, hyperplasia of mucous cells with vacuolar degeneration of chloride cells. Ultra-structural changes in gills tissue of fish that exposed to N-ZnO for 42 days revealed microvilli and adhesion of micro ridge with degeneration of epithelial cells lining the secondary gill filaments. In conclusion, ZnO-NPs are toxic in a concentration of 9mg/l in a common carp (*Cyprinus carpio*), that lead to histopathological and ultra-structure alteration in gills and there was a positive relationship between the severity of lesions and time of exposure (7, 14, 21, 28,35 and42) days.

Keywords: Zinc Oxide nanoparticles, Gill, ultrastructure, *Cyprinus carpio*.

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INTRODUCTION

Nanoparticles (NPs) are several small-scale substances (usually < 100 nm) that must attain specific optical, mechanical, electrical and magnetic properties for a wide range of applications. Nanoparticles are increasingly being developed and applied because of their physicochemical properties **(Ramesh *et al.*, 2013),** so it may have risks for the environment and human health. Nanoparticles(NPs) vary in origin, size and content which lead to changes in biological function as altered in respiratory rate, mucus secretion, and toxic effects in gills and internal organs may involve oxidative stress and ion regulatory disturbances **(Handy *et al.*, 2011)**.ZnO-NPs are one of the most common nanomaterials which is widely produced and applied in many products mainly in cosmetic product and wastewater treatment **(Chen *et al.*,2004; Handy and Shaw,2007**). Through bathing or sewage or effluent, NPs enter the aquatic environment and can cause problems to aquatic animals and plants then may affected humans **(Handy *et al.*,2008 and Danovaro *et al.*,2008).**

Many studies have shown the side effects of ZnONPs on aquatic organisms **(Xiong *et al.*, 2011 and Kim *et al.*, 2017)**. The researchers **Jin–Ling *et al.*, (2011)** wrote about the toxic effect of ZnO-NPs on Acanthopagrus *schlegelii* that cause ultrastructure changes in gills of common carp with hemorrhage and vacuolar degeneration in the kidney **(Chupani *et al.*, 2018).** There are many studies about the effect of ZnO NPs on human and other vertebrates. However, a study of the toxic effect of ZnO NPs on fish has been few. The present research was conducted to estimate the possible impacts of ZnONPs on *Cyprinus carpio* based

on evaluating the alteration in histopathology and ultrastructure in the gill tissues.

MATERIALS AND METHODS

Fish

Thirty-five fish of *C. carpio* weight (150±10 g) were obtained from livestock, Faculty of Agriculture , University of Mosul and kept in glass aquarium (40*40*80 cm) supplied with dechlorinated water with 7.5 pH and 23±2 °C with continuous oxygen supply and feeding for seven days which was stopped at 24h before starting of the experiment.

Preparation and characterization of ZnO-NPs.

ZnO-NPs were purchased from the Shijiazhuang Sun power Technology CO., which has purity (95%) and average particle size (50nm). The characterization of ZnO NPs was described in table (1).

Table 1: Shows the characterization of ZnO NPs

Specification	Content Zn O %	Metallic Zinc	Pb %	Mn %	Cu %	AS %	Cd %
95 %	95.2	—	0.03	0.005	0.003	—	—
	Hg %	Water-soluble matter%	HCl insoluble matter%	Specific surface area	Particle Size/nm	105C° volatile matter %	Ignition Loss%
		0.7	**0.05**	**3.6**	**50**	**0.7**	**4.0**

Suspension of stock ZnO NPs was prepared with aerated single –distilled water and dispersed with a magnetic stirrer for 20 min instead stabilizing agent.

Experimental Design

The fish (No.=35) were divided randomly into two groups as the following:

Group 1: Five fish kept in dechlorinated water only as a control group.

Group 2: Thirty fish Treated with ZnONPs in sub-lethal concentration (9 mg/l)for 7, 14,21,28,35 and 42 days and each period have five fish.

The gills were collected after 7,14,21,28,35 and 42 days the treatment with ZnONPs.

Light and Transmission electron microscope examination

Samples from the gills were divided into two parts one of them fixed in 10% neutral buffered formalin for 72 days, dehydrated in an increasing concentration of ethyl alcohol, cleared by Xylene, infiltrated and embedded in paraffin wax, then sectioned at 5 microns by using a rotary microtome. Then slides were stained by Eosin and Hematoxylin (Luna,1968). The other part was fixed in 2% buffer glutaraldehyde then dehydration by 1% osmium tetraoxide. The specimens after dehydration are hardened by embedding them in the embedding mixture (Epon mixture and hardener). After that sections should be prepared as ultra-thin and stained by writing blue stain (Yacob, 1978).

RESULTS

Gills of fish which exposed to sublethal concentration of ZnONPs (9mg\l) for 7 and 14 days exhibit revealed hemorrhage and clup shape in the lining of epithelial cells of secondary gill filaments (Fig. 1), epithelial cell hyperplasia in the primary gill filaments with hypertrophy of pillar cells at 28 days from treatment (Fig. 2), when the fish continues exposure to ZnONPs for 42 days the lesions characterized by thickening of basement secondary gill filament with edema and vacuolar degeneration of pillar cells (Fig. 3).

The ultrastructure examination revealed vacuolar degeneration in the chloride cells of primary gill filaments and condensation of cell organelles (Fig. 4). When fish continues exposure to ZnONPs for 14 days, the electron microscope examination showed congested blood vessels and hyperplasia of mucous cells (Fig. 5). Furthermore, presence of microvilli and epithelial cells hypertrophy in the secondary gill filaments, hyper atrophy of Pillar cells, aneurism of blood vessels after continuous exposure to ZnO NPs for 28 days (Fig. 6). The severity of pathological lesions was increased with prolonged exposure to ZnONPs, so the lesions at the 42 days of exposure to ZnONPs were raised in the numbers of microvilli, adhesion of micro ridge and vacuolar degeneration, as well as there was aneurism (Fig.7).

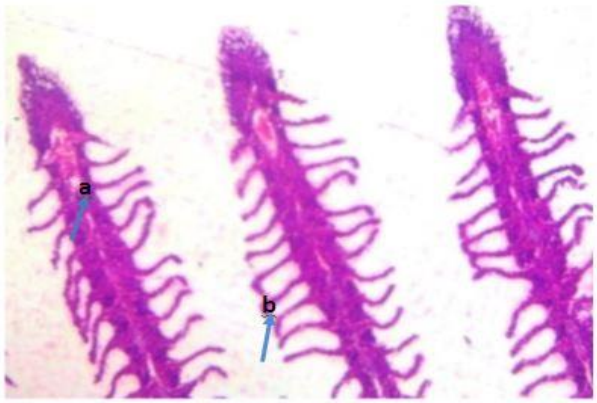

Fig. 1: Gills of fish exposed to sublethal concentration of ZnONPs 9mg/L for (7and 14) day exhibited hemorrhage (a) and clup shape of secondary gill filament (b), H&E ، 350X.

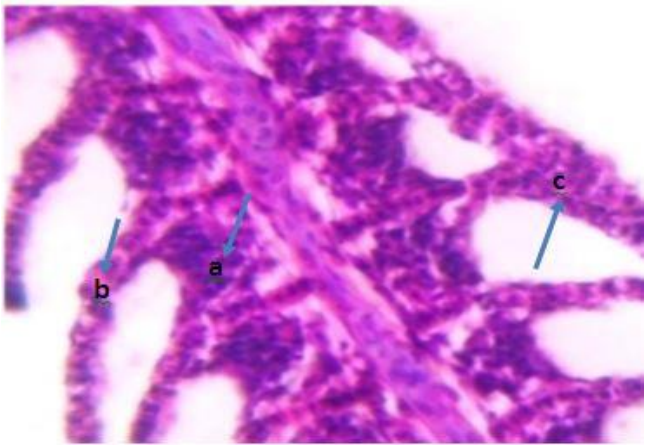

Fig. 2: Gills of fish exposed to sublethal concentration of ZnONPs 9mg/L for (28) days exhibited epithelial cell hyperplasia in the primary filament (a) hypertrophy of pillar cells (b) with the adhesion of secondary gill filament (c), H&E ، 420X.

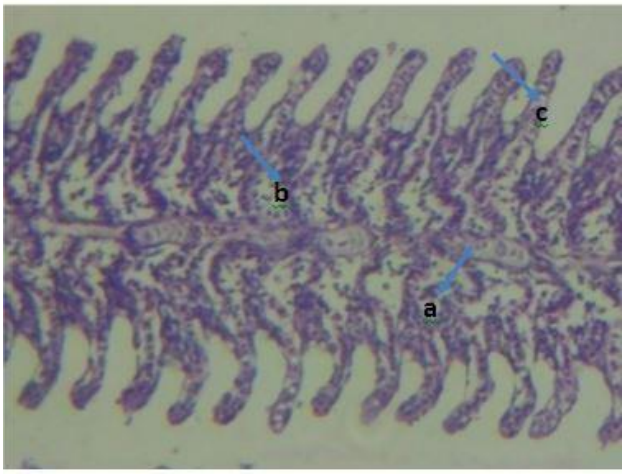

Fig. 3: Gills of fish exposed to sublethal concentration of ZnONPs 9mg/L for (42) days showed thickening of basement secondary gill filament(a) with edema (b)and vacuolar degeneration of pillar cells(c), H&E ، 400X.

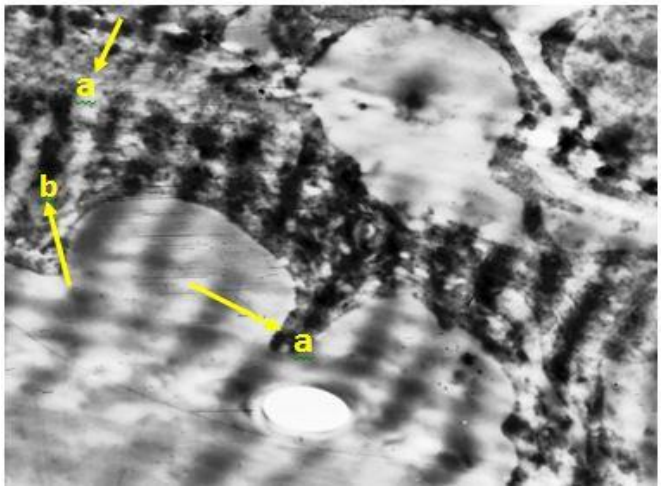

Fig.4: Gills microstructure in fish exposed to sublethal concentration of ZnONPs 9mg/L for 7day exhibited vacuolar degeneration of chloride cells(a) and condensation of microorganells(b),13500 X TEM.

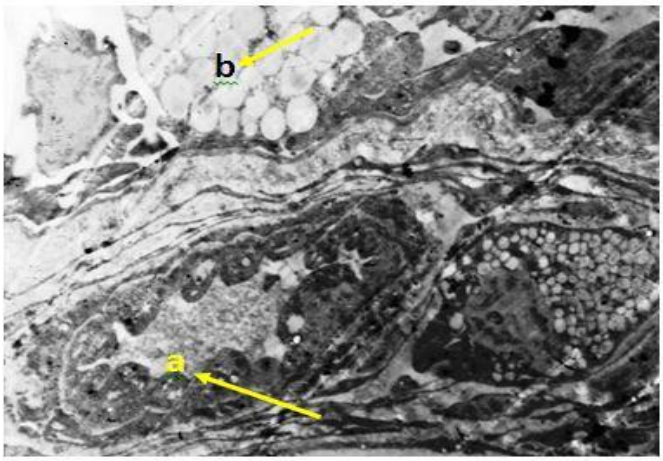

Fig.5: Gills microstructure in fish exposed to sublethal concentration of ZnONPs 9mg/L for 14days exhibited thickening of blood vessels (a) hyperplasia of mucus cells(b), 4600X.TEM

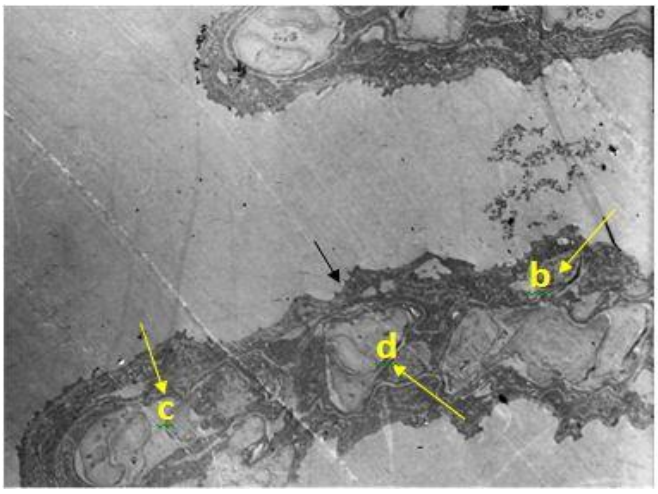

Fig. 6: Gills microstructure in fish exposed to sublethal concentration of ZnONPs 9mg/L for 28 days exhibited presences of microvilli (⟵) hyper atrophy of epithelial cells lining secondary gill filaments(b) aneurism (c) hyper atrophy of pillar cells. 1905X.TEM

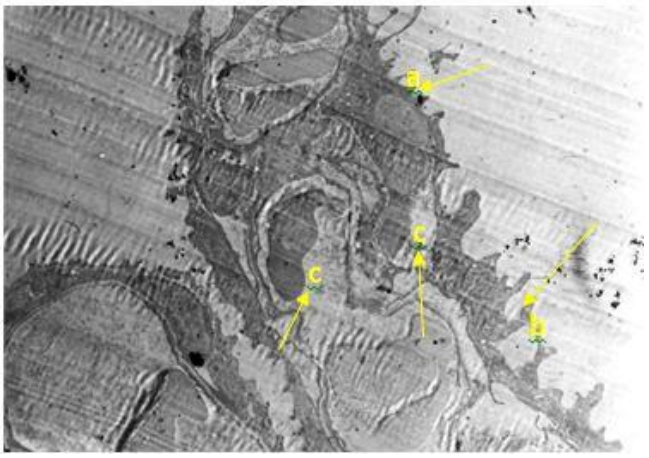

Fig.7: Gilla microstructure in fish exposed to sublethal concentration of ZnONPs 9mg/L for 42days exhibited presences of aneurism (a) forming microvilli (b) adhesion of micro ridge (c) degeneration in epithelial cells lining secondary gill filaments. 2600X.TEM

DISCUSSION

Gills are considered as indicator organs for estimation of the pollution and metal ion toxicity in the aquatic environment (**Miron *et al.*, 2008 and Nwani, *et al.*, 2010)**, which cause gills histological and ultrastructural changes. Gills pathology in our study agreements with the result of **Subashkumar and Selvanayagam (2014**), they reported the pathological changes in gills of *Cyprinus carpio* exposed to different sublethal concentration of ZnONPs for 21 days.

Electron microscopy analyzes considered as a tool in the monitoring of fish exposed to contaminants and biological indicators for the initial signs of alterations tissue that difficult to detect in morphologically or macroscopic examination. In our research , fish were exposed to ZnONPs 9 mg/l for(7,14, 21,28,35,42) day and cause ultrastructural changes in gills, that characterized by mitochondrial condensation and blood vessels congestion and cloudy swelling of chloride cells when fish treated for seven days these result agreements with **Lee, *et al.*, (2014)** who refer to changes in gill when *Cyprinus carpio* exposed to ZnONPs at different concentration for 12 weeks that characterized by degeneration of chloride cell and sloughing of lining epithelial cell in the secondary gill filaments.

The lesions were increased in severity when prolonged exposure to ZnONPs, so the lesion in day s 42 of exposure was more severe than other periods of exposure by forming the micro ridge. These results resemble the result of **Jin-Ling *et al.*, (2011),** the micro ridge may be playing a role in osmoregulation as a compensatory mechanism. **Jinyuan et al., (2011)** classified the types of gill injuries as two types: the first type of injury including hyperplasia of the gill filaments epithelium, edema of gill lamellae these are considered defense mechanism while direct injury as sloughing of gill epithelium and necrosis consider the second type of injury.

Because of gills' unique properties which include a specific histological structure with large surface area and direct contact with the aquatic environment as well as play essential roles in osmoregulation and acid-base balance, for these reasons, it considered main target organ for aquatic toxicant. ZnONPs enter the body through ingestion or by penetration across gill membrane or adsorption directly on the gill **surface (Shaw *et al.*,2012; Linhua *et al.*, 2013).**

CONCUSION

This study concluded that ZnONPs cause ultrastructural and histological alteration in gill tissue.

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