Toxicological impact of Zinc Nano Particles on tilapia fish (*Oreochromis mossambicus*)

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**Abstract**

In this study we investigated the acute toxicity of Zinc Nano Particles (ZnO NPs) and bulk ZnO on tilapia fish (*Oreochromis mossambicus*). *Oreochromis mossambicus* was exposed to the different concentration of ZnO NPs, ZnO and mixed solution of both ZnO NPs and ZnO (20 ppb, 20 ppb, 20 ppb) respectively for 96 h. A very high impact was recorded in hematological parameters which shows significant increased (p < 0.05) in count of white WBCs and platelets in all the experimental groups compared to control group. The count of RBCs, Hb, hematocrit and MCHC were significantly decreased. The remarkable changes which were recorded during this study were histopathological lesions in the gills of exposed fish including, disorganization of gill lamella, cartilaginous core disruption, lifting of epithelium, loss of secondary gill lamellae, blood congestion, fusion of secondary gills lamellae, shortening of secondary gills lamellae, atrophy and curling. Disassembly were seen in plasma membrane of liver along with blood congestion, pyknosis, necrosis, hyperplasia and formation of vacuoles. Intestinal alterations which were observed include shortening of villi, necrosis, detachment and fusion of villi and extreme goblet cells formation. It is concluded from the present study that high level of ZnO NPs, ZnO and mixed solution has a strong tendency to alter hematological parameters, histological architecture, therefore, the indiscriminate use of ZnO NPs and ZnO can subsidize in reducing the population of *Oreochromis mossambicus* in natural water bodies.

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1. Introduction

*Oreochromis mossambicus* is the inhabitant of both fresh and marine water, mostly found in fast flowing rivers. Development of this fish occurs in standing water bodies. Commonly it is found in blind estuaries and coastal lakes. This fish feed on Algae, diatoms, detritus and sometimes invertebrate and insect are the prefer food of *O. mossambicus* and economically tilapia fish are the most important group of wild captured fish and become famous seafood throughout the world due to its nutritional value (Mjoun et al., 2010).

Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter. The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (Rajput et al., 2017). Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts. Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behavior. These properties make them of potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value (Hasan et al., 2015). The ZnO NPs is absorbed 15–20 times more than their bulk form. Due to accumulation of the nanoparticles ZnO NPs different effects has been reported. When the zebra fish were exposed to the ZnO NPs oxidative stress and late hatching of the embryo were recorded (Rajput et al., 2017). ZnO NPs also

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Effect the hematological parameters. Due to exposure of ZnO NPs the erythrocyte elevation is also reported in O. niloticus. It has been noted that due to increase in concentration of ZnO causes negative impact on liver and gills (Rajput et al., 2017).

In Pakistan, zinc nano and bulk ZnO are still popular and are used in cosmetics, rubber, plastic and cement industries Hasan et al., (2015). The current study was conducted with the aim to evaluate the toxic effect of zinc nano and bulk ZnO on hematology and histopathology of tilapia fish (Oreochromis mossambicus).

2. Material and methods

2.1. Sample collection

Total of 20 fish (O. mossambicus) were collected from the Tarbella dam and were transported to Zoology lab of Govt Post Graduate College Haripur in polythene bags which were provided with water and sufficient oxygen. First the mean weight of the fishes was measured in gram which were recorded ± SD = 82.09 ± 8.6; the mean length was measured in cm which were 20.64 ± 0.8; mean standard length were 16.98 ± 0.91 and mean body depth were recorded 6.1 ± 0.16.

2.2. Fish acclimatization

Before starting the experiment the collected fishes were acclimatized for two weeks so that they adopt with the new environment. During the period of acclimatization the water was renewed on a daily basis.

2.3. Experimental design

In this study Zinc oxide and zinc oxide nanoparticle were used, which was obtained from the department of chemistry, Government post graduate college Haripur, KP, Pakistan. The solution of 20 ppb of ZnO NPs, 20 ppb ZnO and mixed solution of 20 ppb ZnO NPs and ZnO were prepared. Four different groups of fishes were arranged and five fishes per group were randomly kept in the glass aquarium containing 40 L water. These groups were labelled as control E0, and the three remaining experimental groups containing ZnO (20 ppb), ZnO NPs (20 ppb) and combined solution of both ZnO and ZnO NPs (20 ppb) were labelled as E1, E2 and E3 respectively.

2.4. Blood collection and hematology

To study hematological parameters of Oreochromis mossambicus, the blood was drawn from the caudal fin of the fish and was stored in EDTA tubes. Hemoglobin (Hb) amount was accomplished by following cyano-methemoglobin technique having spectrophotometer of 540 nm absorbance (spectrophotometric technique). To determine packed cell volume (PCV), the technique of micro hematocrit was used. Using dacie’s solution, red blood cells (RBC) and white blood cells (WBC) were determined (Blaxhall and Daisley, 2013). Documentation of erythrocyte catalogs containing mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were resolute by (Haney et al., 1992) process. Furthermore, variance total was executed for leukocyte proportion documentation. Blood smears were marked using 5% Giemsa solution and description of every cell proportion was calculated in one hundred cells.

2.5. Histopathology

To study histopathology, fish was sacrificed to obtain specific biomarker organs including gills, liver and intestine. Every organ was balanced and preserved in 10% neutral buffered formalin (NBF) for 48 h. After fixation, tissues were allowed to dehydrate in ascending succession of alcoholic grades, cleared in xylene and entrenched in paraffin wax. Each tissue was cut at about 4–5 mm thickness and were processed on rotary microtome. After that they were stained with eosin and hematoxylin and were examined under a camera fitted microscope, OPTICA TCB 3.0 Italy.

2.6. Statistical analysis

Data was analyzed with the help of SPSS software (Version 24.0). The comparison was made among all of the four experimental groups using one-way ANOVA. Variables were stated as means and standard deviations. p < 0.05 was considered as statistically significant.

3. Results

Present study was conducted to investigate the harmful effects of Zinc oxide (ZnO and Zinc oxide (ZnONPs) on O. mossambicus. The detailed results of the study are given below.

3.1. Physico-chemical parameters of water

The Physico-chemical parameters (pH, temperature, oxygen, alkalinity, dissolve solid and conductivity) of test water are given below Table 1.

3.2. Fish behavior in response to acute toxicity.

Numerous behavior changes were observed in O. mossambicus when exposed against ZnO, ZnO NPs and combined in all treated groups. Behavioral changes observed during experimental period were abnormal movements, loss of body equilibrium and body control, increase in swimming speed and lateral swimming.

3.3. Hematology

Hematological variations in O. mossambicus were studied after 96 h exposure to ZnO and ZnO NPs and mixture of both in the current study. Hematological parameters include RBCs, WBCs, MCV, MCH, MCHC, Hemoglobin, Platelets, Neutrophils and Platelets. Hematological alterations were observed in foresaid parameters when using ZnO and ZnO NPs and mixed solution against O. mossambicus with the significant difference (p < 0.05). The mean value of all the blood parameters of control and experimental groups recorded during experiment are given in Fig. 1.

| S. No | Parameter | Data       |
|-------|-----------|------------|
| 1     | pH        | 7.5 ± 0.23 |
| 2     | Temperature | 24 ± 1.18°C |
| 3     | Oxygen    | 6.3 ± 2.23 mg/l |
| 4     | Alkalinity | 128 ± 4.34 mg/l |
| 5     | Dissolved Solid | 336 ± 9.12 mg/l |
| 6     | Conductivity | 809 ± 13.9 ms/ cm |

Table 1
Chemical analysis of the laboratory water 1.
3.4. Histopathology

Histopathological variations in *O. mossambicus* were studied after 96 h exposure to ZnO and ZnO NPs and mixture of both in the current study. For histopathological examination different organs (gills, liver & intestine) were used.

3.4.1. Control gill tissues

The section of gills *O. mossambicus* (taken from control group) were analyzed by using camera fitted microscope it shows the normal histology and no alteration were recorded in filament of gill and gill lamellae (primary and secondary). It shows normal epithelial covering which is thin as well as central cartilaginous core. Stratified epithelium of thick lining between primary and secondary gill lamellae having mucous lining Fig. 2(A).

3.4.2. Treated gill tissues

Histopathological results indicated that gill was the main targeted tissue affected by ZnO, ZnO NPs and mixture of both in all experimental groups. The most common histopathological alteration that were observed in this experiment includes cartilaginous core disrupted, epithelial lifting and loss of secondary gill lamellae as shown in Fig. 2. (B). In experimental group II & III exposed to 0.20, ZnO NPs & mixed 0.20 ppb concentrations of ZnO and ZnO NPs respectively, the alteration in histology which are shown in Fig. 2. (C & D). The alteration in histology due to accumulation of these chemicals were fusion, disruption and shortening of secondary gills lamellae, epithelial lifting, atrophy, disruption of cartilaginous core and blood congestion respectively.

3.4.3. Control liver tissues

When the section of liver of *O. mossambicus* (taken from control group) was studied under camera fitted microscope, it shows the normal histology and no alteration were recorded. The normal cell have foamy cytoplasm which is seen in this group of fishes. Liver cells shows normal morphology and round polygonal cell body containing a clear round nucleus and nucleolus Fig. 3(A).

3.4.4. Treated liver tissues

In the liver tissues of fish exposed to ZnO, ZnO NPs and mixture of both concentration of 0.20 ppb for 96 hrs shows numerous alterations in experimental groups were observed, alterations includes hepatocytes blood congestion, mild vacuolations, mild pyknosis, dissolution of cell membrane, lymphocytes infiltrations shown in Fig. 3.

3.4.5. Control intestine tissues

The section of gills *O. mossambicus* (taken from control group) were studied by using camera fitted microscope it shows the nor-
mal histology and no alteration were recorded in intestine. The intestinal villi consist of lamina properia, muscularis mucosa, stratum compactum, mucosal epithelium and serous membrane Fig. 4. (A).

3.4.6. Treated intestine tissues

In the intestine tissues of fish exposed to ZnO, ZnO NPs and mixture of both concentration of 0.20 ppb for 96 hrs shows numerous alterations in experimental groups were observed, alterations includes rupturing, fusion of villi along with necrosis, goblet cell formation dissolution and shortening of villi along with disintegration of serosa as shown in Fig. 4(B,C,D).

4. Discussion

Current study was carried out to investigate the toxic effects of ZnO, ZnO NPs and combine mixture of both on hematological and histopathological parameters of O. mossambicus during acute toxicity of 96 h. During experiment different abnormal behavior were observed including deep sinking in aquarium, hyper secretion of mucus, arbitrary swimming and loss of balance. No mortality was recorded throughout the experiment in all experimental groups.

The blood profile is one of the most important indicators to examine the consequences when fish exposed to different types of chemical including ZnO and their nanoparticles (Lavanya et al., 2011). Blood profile is one of the key elements to detect the changes in the overall health of fish and due to the toxic effects fish gone through unusual responses than normal. In present study, when fish exposed to ZnO, ZnO NPs and mixture of both, some hematological parameters altered significantly compared to others. When fish exposed to ZnO, ZnO NPs and combine mixture as results decreased in RBCs, Hb Level and MCHC values were recorded in experimental groups compared to control, while WBCs, platelets values increased as compared to control group. Schizothorax plagiostomus exposed to different concentrations of atrazine showed increased in platelets count (Akhtar et al., 2021a). It is investigated that the decreased in the level of different parameters like RBCs and hemoglobin might be associated with hematopoietic activity, synthesis of haem which required for the formation of Hb when Catla catla was expose to chemical deltamethrin of high concentration. Increased in WBCs is due to immune response against the environmental changes as we know that WBCs play an important role in immunity of the body (Vani et al., 2011; Bantu et al., 2017). Schizothorax esocinus exposed to different concentrations of cypermethrin result in decrease in WBCs counts (Akhtar et al., 2021b)

Similar study was conducted by Mielcarz-Skalska and Smolińska, 2017) to investigate the influence of ZnO and ZnO NPs on living organisms, they found that increased concentration of these chemical effect the normal development process in fishes. They studied that exposure of zebrafish embryo to 50 and 100 mg/l shows mortality of zebrafish. They believe that increased concentration of ZnO is responsible for the formation of ROS. They concluded that the effect of ZnO NPs is more severe than ZnO. The global production of ZnO NPs is 550–3340 tons and their absorption of ZnO NPs absorption is higher than the absorption of bulk form and the increase concentration of these chemical effects the normal development of the fishes and also have photo-oxidizing and photocatalytic effects on the chemical present in the body of these animals (Rajput et al., 2017).

Similar study was carried out by Alkaladi et al. (2015) they use the similar chemical as used in the present study. They exposed the fish with the chemical like ZnO and synthetic Vitamin various parameters of the body alter which brought different changes in behavioral responses of the fish, they observe changes in different
blood parameters like anemia, lymphopenia, heteropenia and monocytopenia.

Previous histopathological literature showed that when the fish were expose to the chemicals the first organ which is effected is gills and are used as major marker to give us qualitative indication of water. Apart from the organ of respiration perform many important functions such as acid base balance, regulation of ions and elimination of nitrogenous wastes from animal body. Due to exposure of various environmental toxicant some important and vital organs of the body effected directly or indirectly which ultimately affect the health of fish (Bantu et al., 2017). In the given study when fish were exposed to ZnO, ZnO NPs and combine mixture of both fish gills undergo various histological changes. In given study when fish were exposed to ZnO NPs and ZnO some changes were observed in histology of gills when dissected the gills tissue and analyzed by using microscope. The alterations which were observed include lamellar disorganization, fusion of secondary gill lamellae, disruption of cartilaginous core, epithelial lifting, atrophy and blood congestion. Study indicates that different chemical present in the environment effect normal structure of gills as reported by (Ghanbahadur, 2012) which support our results as he observed various alteration in the histology of Rarbora daniconius when exposed to Endosulfan pesticide. After the exposure when the fish tissue was dissected and observed under the microscope the changes which were observed were necrosis of gills epithelium, degeneration of secondary gill lamellae and destruction of primary gill lamellae. According to Hasan et al., (2015); Almansour et al., 2017 Ctenopharyngo donidella fish exposed to endosulfan for short duration different histological changes were observed after microscopic study. The changes in the histology which were observed due to the toxicity of endosulfan were shortening of gill lamellae (primary and secondary), fusion and lifting of epithelial lining of gills lamellae and hypertrophy. Similar results of Gills histopathology were obtained in the present study when O. mossambicus were exposed to ZnO and ZnO NPs for 96 h. Our Histopathological results are in agreement with the investigation of (Almansour et al., 2017), they reported that higher the accumulation of ZnO NPs increases the chances of necrosis, Kuffer cell hyperplasia, cell infiltration, hepatocytes apoptosis in gills, by the exposure of different fishes against different chemicals. Schizothorax esocinus exposed to different concentrations of atrazine indicated shortening and fusion of secondary gills lamellae, mild blood congestion, epithelial lifting, atrophy, disruption of cartilaginous core, blood congestion, lamellar disorganization and curling of gills (Akhtar et al., 2019).

Liver is organ of digestion and help in the digestion and metabolism of biological molecules like carbohydrates, protein and lipids. Liver is also organ of detoxification where the poisonous substances absorbed by the body are converted into the nonpoisonous substances. Due to this activity of detoxification liver accumulate different harmful chemicals which result in the alteration of physiology and histology of hepatocytes (Sharma et al., 2012). The histological alteration of O. mossambicus after exposure is in resemblance with study carried by (Coimbra et al., 2007).

Intestine is one of the main organs of the body, intestine play a major role in the digestion and absorption of digested materials. Being direct role of intestine in the digestion it is very sensitive to poisonous substances absorbed with the food materials and due to their sensitivity, it can be used as marker to assess the contamination in the environment (Gisbert, et al., 2008). In the present study when O. mossambicus were exposed to ZnO, ZnO NPs and combined solution of both, histopathological alterations were observed in Intestine, includes goblet cell formation, hemorrhages,
cell death, detachment, fusion and shortening of villi were recorded after the microscopy of dissected tissues of intestine. Our observations are similar with Hasan et al. (2015; Mjoun et al., 2010), they observed mucosal folds, degeneration of intestinal villi, hypertrophy, vacuolations, and necrosis in Intestine of Cyprinus carpio when exposing against different chemicals.

5. Conclusion

It is concluded from the present study that Zinc nano particles are harmful to fish because its accumulation in fishes bring about many irreversible changes in its health. In present study it is found that zinc nano particles has altered the hematological parameters as well as lesions in tissues of tilapia fish (Oreochromis mossambicus). The count of RBCs, Hb, hematocrit and MCHC were significantly decreased. The remarkable changes which were recorded during this study were histopathological lesions in the gills of exposed fish including, disorganization of gill lamella, cartilaginous core disruption, lifting of epithelium, loss of secondary gill lamellae, blood congestion, fusion of secondary gills lamellae, shortening of secondary gills lamellae, athrophy and curling. Disassembly was seen in plasma membrane of liver along with blood congestion, pyknosis, necrosis, hyperplasia and formation of vacuoles. Intestinal alterations which were observed include shortening of villi, necrosis, detachment and fusion of villi and extreme goblet cells formation. It is concluded from the present study that high level of ZnO NPs, ZnO and mixed solution has a strong tendency to alter hematological parameters, histiological architecture, therefore, the indiscriminate use of ZnO NPs and ZnO can subsidize in reducing the population of Oreochromis mossambicus in natural water bodies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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