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Nanotechnology

Toxicity of Zinc Oxide Nanoparticles in Tilapia *Oreochromis Mossambicus*

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ABSTRACT

The present study deals with the toxicity zinc oxide nanoparticles in tilapia Oreochromis mossambicus. zinc oxide nanoparticles were synthesized by chemical precipitation method and characterized using SEM, EDAX, FTIR and XRD. Toxicity tests were conducted for the determination of different concentration of ZnO to Oreochromis mossambicus (250mg, 500mg, 1000mg, 1500mg and 2000mg) for a period of 14 days. The behaviour, haematological and biochemical parameters such as protein, carbohydrate and lipid of Oreochromis mossambicus exposed to ZnO nanoparticles for a period of 14 days were observed for control and exposed to different concentration (250mg, 500mg, 1000mg, 1500mg and 2000mg) of ZnO nanoparticles. The hematological parameters such as RBC, Hb, Hct and WBC in ZnO nanoparticles treated fish decreased when compared to control. Total protein, carbohydrate and lipid was decreased in zinc oxide nanoparticles exposed fish when compared to control. From the results it is inferred that ZnO nanoparticles affect the behaviour, haematological and biochemical parameters of tilapia Oreochromis mossambicus

KEYWORDS

Toxicity, zinc oxide, nanoparticles, tilapia, oreochromis mossambicus

INTRODUCTION:

The rapid development of nanotechnology and nanoparticles has been increasingly used in recent years. The nanomaterials have properties like electrical conductivity, colour, strength and weight change when the nanoscale level is reached the same metal can become a semiconductor or an insulator at the nanoscale level. Revenues from nanotechnology products are expected to grow worldwide to US $3.1 trillion in 2015 (Jonathan 2009). Recently nanotechnology has emerged as an excellent field of technology that shows its application in various sectors such as chemical, electronics, biomedicine, cosmetics and several others (Kalnie *et al*.,2006). The application of nanotechnology in support of life but at the same time there are growing about human exposure to these which may have adverse health effect (Yousefian *et al.,* 2012) and its impact on the environment and living organism are becoming an important issue (Adhikari *et a*l., 2012). Studies carried out in USA and Europe showed that Ag-NPs, TiO2-NPs, and ZnO-NPs from sewage treatment may be toxic for aquatic organisms (Gottschalk *et al.,* 2009).Among the various nanoparticles, zinc oxide nanoparticles are widely used in all the industries. It is the third highest volume of global production after TiO2 and SiO2. Zinc oxide nanoparticles are widely applied in the electronics, cosmetics, catalytic, ceramic, pigments due to its UV absorbance properties (Wang *et al.,* 2004) and also used as baby powders, antidandruff shampoos and fabric treatments for UV shielding (Bechrii *et al.,* 2008). The excess of ZnO nanoparticles released to the aquatic environment is most toxic to the aquatic fauna. Fish are particularly susceptible to the toxic effects of metal ions (Wood *et al*., 1996). The chronically higher intake of zinc would lead towards bioaccumulation in different body organ of fish (Nussey *et al*.,2000) and the ZnO nanoparticles affect the fish embryo number, larvae survival, hatching rate and malformation (Zhu *et al*., 2008). Several laboratory studies suggested harmful effects on

fishes and invertebrates after exposure. Conventional or lesser conventional animal models were used to assess the nature of these potential effects . ZnO NPs were strongly cytotoxic at lower concentrations and exhibited strong protein adsorption abilities (Horie et al., 2009) which may be contribute toward their cytotoxicity. The work related to the synthesis, characterization and toxicity of zinc oxide nanoparticles on haematology and biochemical composition of tilapia O*reochromis mossambicus* is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS:

Zinc sulphate and Sodium hydroxide was purchased from Loba chemicals, India. All the reagents used for the synthesis ZnO were analytical grade and used without further purification. All the glass wares were washed thrice with deionized water and dried before use.

Synthesis and Characterization of zinc oxide nanoparticles

Precipitation method was adopted for the synthesis of zinc oxide nanoparticles. 0.1 mole of 14.377g of zinc sulphate were dissolved into 500mL of distilled water and stirred vigorously using magnetic stirrer for 20 minutes. Precipitation was achieved by adding 50 ml of 2 M NaOH solution in drop wise under vigorous stirring. The initial pH was observed as 3 and it was increased to pH 14 using 2M NaOH. Then precipitating process was continued until white color precipitate obtained. Then the ZnO precipitate was taken into centrifuge tube and centrifuged at 2000 rpm for 15 minutes. The centrifuging process continued with water and two times with ethanol. Then the precipitate was dried and calcinated at 500°c for 3 hours. Finally, zinc oxide nanoparticles (ZnO) formed. The morphology and composition of ZnO nanoparticles were examined by Scanning Electron Microscopy (SEM) using a LEO 1455 VP

equipped with energy dispersive. An energy dispersive X-ray detection instrument (EDAX) (HORIBA 8121-H) was used to examine the elemental composition of the sample. Fourier transform infrared spectroscopy (FTIR) is used to measure the vibration modes of functional groups of molecules and measurement was carried out by JASCO (FTIR-6200) spectrum. X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a given material.

The systematic classification of the selected fish is as follows.
Kingdom: Animalia
  Phylum: Chordata
    Class: Actinopterygii
      Order: Perciformes
        Family: Cichlidae
          Tribe: Tilapiini
            Genus: *Oreochromis*
              Species: *mossambicus*

Healthy fingerlings of **Oreochromis mossambicus** (12.6 ± 0.02g) were procured from AM fish farm, Arumbanur, Madurai and acclimatized to laboratory conditions for about one week before the commencement of the experiment. During acclimatization, fish were fed with rice bran and ground nut oil cake once a day. Feeding was given at least one hour prior to replacement of water. Water (one-third) was changed frequently to remove the excretory wastes. Feeding was held for 24 h before the commencement of the experiment to keep the experimental animals more or less in the same metabolic state. During acclimatization, the fish stock was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic wastes. The initial body weight and length of the fish were measured in gram and cm respectively.

Zinc oxide (ZnO) NP exposure
For assessment of ZnO NP toxicity, plastic trough with 6 L of water was taken. In each plastic troughs, different concentrations of the ZnO nanoparticles (i.e. 250mg, 500mg, 1000mg 1500mg, 2000mg) were added (control was maintained without ZnO nanoparticles. Ten healthy fish, with an average length of 6-7 cm and average weight of 4.0 to 8.0 g were selected and introduced into each trough. The manifestation and survival time of fish was observed in each concentration for 14 days. The behaviour of the fish was noted in all the exposures including control.Haematological Parameters such as white blood cells, red blood cells, platelets, hemoglobin, haematocrit, neutrophils and lymphocytes and biochemical parameters such total protein, carbohydrate and lipid were estimated after 14 days.

RESULTS AND DISCUSSION
Synthesis of zinc oxide nanoparticles
As NaOH was added to zinc sulphate it is found to change color from clear solution to white indicates the synthesis of zinc oxide nanoparticles (ZnO)**.** Precipitation was observed by increasing the pH to 14.

Characterization of zinc oxide nanoparticles (ZnO)
The SEM image (Fig. 1) showing the high density chemical synthesized ZnO further confirmed the development of zinc oxide nanostructures. Obtained nanoparticles showed that spherical in nature. The microscopic image shows that the ZnO nanoparticles appear as discrete particles but form much larger dendrite flocks whose size could reached micron scale

size range about 10.01mm (scale bar 1µm), 10.08mm (scale bar 2µm), 10.07mm (scale bar 5µm), for figure 2 a, b, and c respectively.
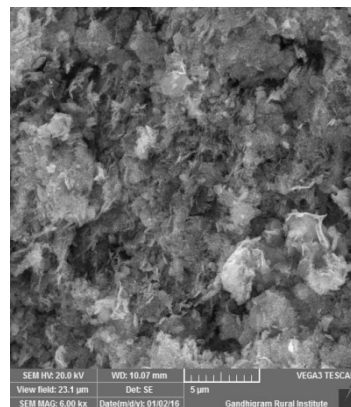
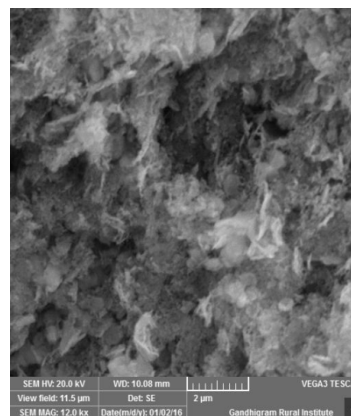**Fig.1 SEM IMAGE OF ZnO NANOPARTICLES**
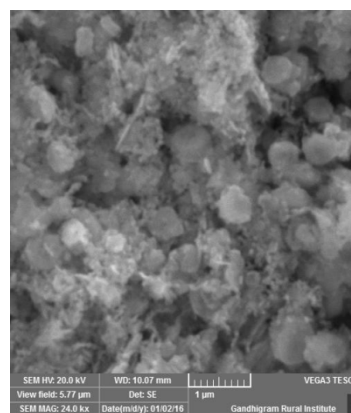

Fig: 1 (a).

Fig: 1 (b)

Fig: 1 (c).

Scanning electron microscope (SEM) was used to decide size, location and shape of the zinc oxide nanoparticles. Analysis of the SEM image of synthesized zinc oxide nanoparticles, showed a clear image of dense zinc oxide nanoparticles which are almost spherical in shape (Haritha *et al.,* 2011). The size of most of the nanoparticles ranges from 30 nm to 110 nm. The average percentage of nanoparticles present in the synthesized sample is 66 nm. Similar results on SEM analysis of zinc oxide nanoparticles had been also reported (Davood., 2012).

EDAX spectrum recorded on the zinc oxide nanoparticles is shown as three peaks located between 1 KeV and 8 KeV (Fig.2).

Those maxima are directly related to the zinc characterized lines K. The maximum peak located on the spectrum at 1.0 KeV clearly coming from zinc. The second maximum peak located on the spectrum at 0.5 KeV clearly comes from oxygen. Third peak located at 2.3 KeV are connected with the sulphur characteristics line.

FIG. 2 EDAX IMAGE OF ZnO NANOPARTICLES
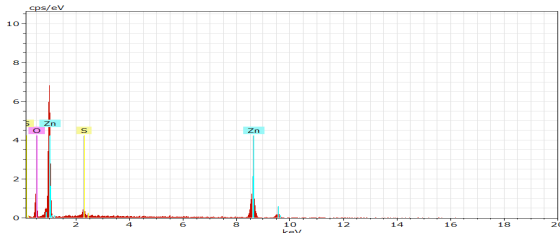
Fourier Transform Infrared spectroscopy measurements were carried out to identify the possible functional groups responsible for the reduction of the Zn ions in chemically synthesized zinc oxide nanoparticles. The FTIR spectrum of zinc oxide nanoparticles was analyzed in the range 4000-400cm⁻¹ (Fig.3) and bands observed at 3427.23, 2356.29, 2084.88,1631.74, 1113.46, 894.82, 790.62, 610.3 and 510.19 which are associated with amide B: N–H stretching of proteins, PO_2^- symmetric stretch: mainly nucleic acids, COO⁻ symmetric stretch: fatty acids and amino acids, C–O asymmetric stretching of glycogen Carbonate Ion, aliphatic Iodo Compounds (Table 1) Fourier transform infrared spectroscopy (FTIR) is a highly sensitive, rapid, inexpensive method that can be automated and requires a minimal amount of sample. The FTIR spectra of tissues are consistent with the general features and are characterized by absorption regions known as the amide region and the C–H region and show the observed frequencies in the region 4000–400 cm−1 along with their vibrational assignment and intensity (Sri Sindhura *et al*., 2013).

FIG. 3 FTIR IMAGE OF ZnO NANOPARTICLES
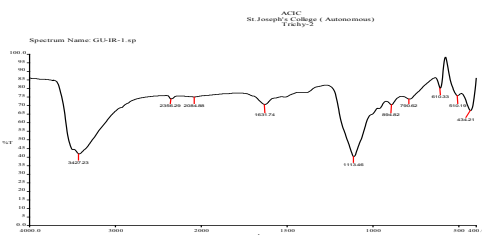
TABLE 1: FTIR FUNCTIONAL GROUP REPRESENTATION OF ZNO NPs

S. No	Wave Number (cm⁻¹) ZnO NPs	Definition of the spectral assignments
1	3427.23	N-H, 1 ,2 amines, amides
2	2356.29	O-H, Carboxyl acid
3	2084.88	C=O, acid
4	1631.74	C=C, Alkene
5	1113.46	C-N, Amine
6	894.82	=C-H, Alkene
7	790.62	C-Cl, Alkyl halide
8	610.33	C-Cl, Alkyl halide
9	510.19	C-Br, Alkyl halide

The XRD diffraction peaks are indexed as 31.95° (100), 34.57° (002), 36.39° (101), 47.71° (102), 56.68° (110), 66.56° (200), 68.09° (112) and 69.29° (201) which is represented in Fig.4. All diffraction peaks are indexed according to the hexagonal phase of ZnO. No characteristic peaks of impurity phases except ZnO are found which revealed that good crystalline in nature of the samples. The broadening of the peaks in the above XRD pattern can be attributed to the small particle size of the synthesized ZnO .This proves that pure ZnO nanoparticles were as synthesized.

FIG. 4 XRD IMAGE OF ZnO NANOPARTICLES
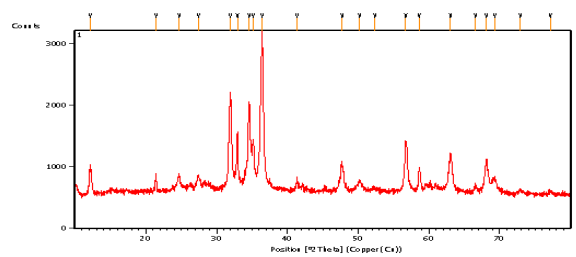
The X-ray Diffraction (XRD) studies reveals that the characterization through X-ray diffraction graph shows 15 peaks in accordance with zincite phase of ZnO. No peaks due to impurity were observed, which suggest that the high purity of zinc oxide nanoparticle was obtained. In addition the peak was widened implying that the particle size is very small (Haritha *et al.,*2011).

Experimental studies
The basic observation of fish which was exposed to the zinc oxide nanoparticles are shown in the Table 2. Similar behavioural studies were also reported when freshwater fish Labeo rohita was exposed to Iron oxide nanoparticles (Rajan *et al* 2016).

TABLE 2: BASIC OBSERVATION OF FISH

S. No	Activity	Yes	No
1	Circular swimming	✓	
2	Jerk movement	✓	
3	Bottom resting	✓	
4	Surface respiration		✓
5	Aggressive movement		✓
6	Excess of mucous secretion		✓
7	Mortality observation		✓
8	Behavior observation	Good	
9	Breathing movement	Good	

Toxicity tests were conducted for a period 14 days in different concentration of ZnO to *Oreochromis mossambicus*. There was no mortality recorded up to 14th day. Hematological parameters of *O. mossambicus* exposed to ZnO nanoparticles for a period of 14 days are presented in Table 3

TABLE 3: HEMATOLOGICAL PARAMETERS OF *OREOCHROMIS MOSSAMBICUS* EXPOSED TO ZnO NPS FOR 14 DAYS. EACH VALUE IS THE AVERAGE OF FIVE INDIVIDUAL OBSERVATIONS

Hematological parameter	Control	250mg	500mg	1000mg	1500mg	2000mg
RBC (cells/cumm)	1.8%	1.2%	1.8%	1.5%	1.0%	1.0%
WBC (cell/cumm)	2,500	1,000	600	1,800	1,000	500
Lymphocytes (%)	26	17	16	38	19	10
Neutrophils (%)	71	81	82	59	79	88
Hb (gm/dl)	5.4	3.7	1.9	4.6	1.5	1.7
Haematocrit (PCV)	16	11	6.0	14	4.5	5.1
MCV (gm / L)	88.88	91.66	33.33	93.33	45	51
MCH (gm / L)	30	30.83	10.55	30.66	15	17
MCHC(gm / L)	11.25	33.63	3.16	32.85	33.33	33.33
Platelets	64,000 (cells/cumm)	1.33 (lakhs/cumm)	88,000 (cells/cumm)	1.86 (lakhs/cumm)	16,000 (cells/cumm)	51,000 (cells/cumm)

Ekrem *et al.,* (2012) reported that blood is a path physiological reflector of the whole body and the blood parameters are important in the diagnosis of the structural and functional status of animals exposed to toxicity. Hematological parameters are potential biomarkers of exposure to agrochemicals due to their sensitivity to certain toxic agents. Clinical hematological parameters have been widely used as a potent bioindicators in aquatic toxicology. When *Oreochromis mossambicus* were exposed to various concentration of Zinc Hb, Hct and RBC levels were decreased and increase in WBC count (Ekrem *et al.,* 2012) indicates a generalized immune response and a protective response to the toxicant. In the present study exposure of fish *Oreochromis mossambicus* to ZnO NPs showed significant alterations in the hematological parameters such as RBC, Hb and Hct contents were decreased in ZnO nanoparticles treated fish. Moreover, lower Hct values also indicate shrinkage of cell due to toxicant stress on erythropoietic tissue (Saravanan *et al*., 2011). Failure of erythrocyte production, internal hemorrhages or impaired osmoregulation during stress condition may leads to a reduction in RBC count (Joshi *et al.,* 2002, Adedeji *et al*., 2009, Kavitha *et al*., 2010). In this study, it is concluded that the alterations of these hematological parameters may provide the general health condition of the fish under zinc oxide nanoparticles toxicity in fish.
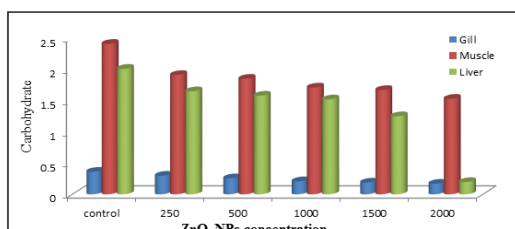
Protein content in gill, muscle and liver of *Oreochromis mossambicus* in control and fish exposed to ZnO nanoparticles in five different concentration (250mg, 500mg, 1000mg, 1500mg, 2000mg) is presented in Fig 1. The protein content in gill, liver and muscle decreased with increasing concentration of zinc oxide nanoparticles. Ma *et al.,* (2013) suggest the increased zn^{2+} was incriminated in the activation of ROS (Reactive oxidative production species) through interaction with membrane lipids damaging all membrane, DNA and Protein. In the present study, the results are observed as decreased protein in liver, muscle and gills of *Oreochromis mossambicus* exposed zinc oxide nanoparticles when compared with respective control.

FIGURE:1 ESTIMATION OF PROTEIN IN GILL, MUSCLE AND LIVER OF TILAPIA   *OREOCHROMIS MOSSAMBICUS*
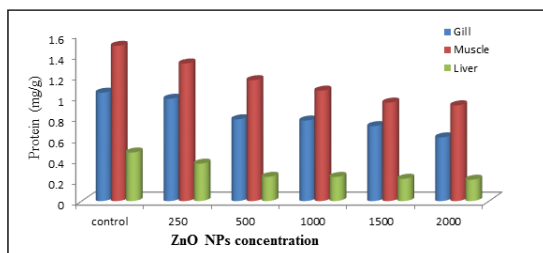

Carbohydrate content in gill, muscle and liver of *Oreochromis mossambicus* in control and fish exposed to ZnO nanoparticles in five different concentration (250mg, 500mg, 1000mg, 1500mg, 2000mg) is presented in figure 2. The carbohydrate levels in liver and muscle were measured by Anthrone reagent method (Carrol *et al.*, 1956). Mehjbeen Javed *et al*., (2014) reported that carbohydrate reserves of the liver depleted significantly (heavy metals). Moreover muscle glycogen level also declined significantly (heavy metals) when compared to the reference. In zinc oxide nanoparticles exposed fish, carbohydrate decreased with respect to control group.

FIGURE:2   ESTIMATION OF CARBOHYDRATES IN GILL, MUSCLE AND LIVER OF TILAPIA *OREOCHROMIS MOSSAMBICUS*

Total lipid content in gill, muscle and liver of *Oreochromis mossambicus* in control and fish exposed to ZnO nanoparticles in five different concentration (250mg, 500mg, 1000mg, 1500mg, 2000mg) is presented in figure 3. The lipid content in ZnO NPs exposed to *Oreochromis mossambicus* are decreased when the concentration increased. Similar observation was done were significant decreased in the total lipid levels when compared to reference. Other workers also recorded significant elevations in these parameters. Elevation or depletion in lipid profile is either due to disturbance in the metabolism of lipids or may be due to impaired clearance from plasma which favours liver dysfunction. Very limited work had carried out both in field and laboratory based studies in fish pertaining to the effect of heavy metals on lipid profile. These ZnO NPs may accumulate in fish due to which fish comes under toxicity which is confirmed by studying alterations in hematological and biochemical assay of fish. Furthermore these heavy metals cause elevation or decline in carbohydrate, protein and lipid profile, which are served as suitable biomarkers of fish health.

FIGURE:3 ESTIMATION OF LIPIDS IN GILL, MUSCLE AND LIVER OF TILAPIA *OREOCHROMIS MOSSAMBICUS*
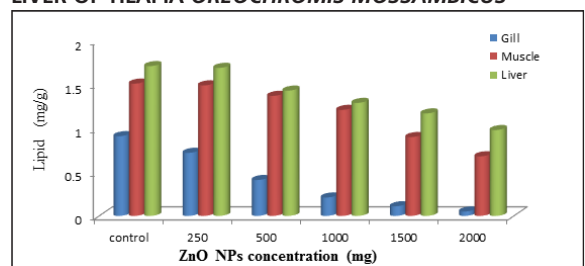
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