



Fate and upshots of Zinc oxide Nanophase as a pollutant in a fresh water fish Tilapia (*Oreochromis mossambicus*) and its Physiological alterations in Hematology, Antioxidant level and Histology

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ABSTRACT

The acute toxicity study of ZnO nanophases (30 –75 nm) on fish *Oreochromis mossambicus* was evaluated by assessing the hematological parameters, antioxidant enzyme level and histoarchitectural changes. The median lethal concentration (96 h; LC50) of Zinc oxide nanophase (ZnO NPs) calculated at 600 ppm. Fish exposed to various sublethal concentrations (100 to 500 ppm) of ZnO NPs for 7 days. The blood and tissues such as gill, liver, kidney, intestine and muscles collected after the stipulated period of exposure. The results showed that the leukocytes counts were increased and erythrocytes counts were decrease in all treated fish than the control fish. The hemoglobin (Hb) and hematocrit (HCT) values were low in 100 to 300 ppm and high in 400 and 500 ppm when compared with control fish. The alterations in the other hematological parameters (MCV, MCH and MCHC) also noted. In antioxidant enzyme level including superoxide dismutase (SOD), Catalase (CAT), Glutathione Reductase (GSH), Glutathione S-Transferase (GST) and Lipidperoxidase (LPO) were increased two fold in liver than gill and muscle at 500 ppm than control. The histological results showed that when the concentration increases, the deleterious effect observed in gill, liver, intestine, kidney and muscles than respective control tissues. From the results, it confirms that the ZnO NPs have the potential to cause physiological ill effects to fishes that may lead to lethality.

Keywords

ZnO NPs, Nanotoxicity, Hematology, Antioxidant Enzymes, Histopathology

1. INTRODUCTION

Now a day's nanotechnology has brought a vast attention due to wider applications of nanophase in different fields of science and technology. Nanotechnology has come into view at the forefront of science and technology for last few decades. Usually it involves the production of

devices or materials whose at least one dimension is in nanometer range i.e. 1-100 nm. Metal oxide nanophases, which are inorganic in nature, have attracted much consideration for last few decades because they can hold out harsh process conditions. Among metal oxides TiO₂, ZnO, MgO and CaO have pinched great attention to further studies, as they are very safe for human beings and for animals at certain concentration (Siva vijayakumar *et al.*, 2013).

Application of Nanotechnology range from medicine to varnish for cars and from electronic devices to cosmetics. The best prospect for broad scale application of nanomaterial in health care and electronics. By understanding the benign uses of nanophase application sides are strengthened, but the horrid impact like toxicological effects are mostly unnoticed (Aitken *et al.*, 2006). The health safety of human and animals not only depend on beneficial product but also in avoiding the materials that cause ill effects also. Therefore, the presently taken nanotoxicological study intended to evaluate the commonly used nanophase such as ZnO (Siva vijayakumar *et al.*, 2015).

The risk originates from a nanomaterial is not only determined by its potential hazard (such as toxicity), but also by the extended the material which will be exposed to an organism (Colvin, 2003; Wiesner *et al.*, 2006). The base for a sound risk assessment of a possibly hazardous substance is thus a contrast between the exposure (concentration in the environment) and the effect of the substance in the relevant environmental compartments (dose-response relationship) (Schlatter, 2005; Umweltbundesamt, 2007). Usually the concentrations of a new substance in the environment were unknown at the time of the assessment. Therefore, expected concentrations have to be modeled with the help of extrapolations and analogies (ECB, 2003). The value derived from such modeling is the PEC (predicted environmental concentration). It is compared to the PNEC (predicted no effect concentration) which extrapolates (based on toxicological studies) the concentration at which no adverse effect on organisms (and ecosystems) is to be expected (Umweltbundesamt, 2007). An ingredients were judged environmentally compatible if the PEC/PNEC ratio is smaller than one.

Diffusion of nanophase through the skin of animals in the water has not inspected at all. The actual dose that an organism's organs exposed are depends on the uptake (route and amount) and the excretion/transformation rate within the organism (Schlatter, 2005). A high external exposure may not necessarily show the way to a high internal dose. So far, it's not known what the most critical uptake routes of NP are for different organisms. Due to the impact of toxicity by nanophase and other toxic substance, antioxidant levels (Vasanth *et al.*, 2012), hematological (Karthikeyeni *et al.*, 2013) and histological changes were occurred in the organism.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL ANIMAL

Oreochromis mossambicus (Peters, 1852), a freshwater teleost fish belonging to the family Cichlidae was used as a model organism for the assessment of ZnO NP toxicity. It is commonly known as Tilapia. It is an omnivorous fish and it is one of the edible fish rich in protein content, which is available in the aquatic system throughout the year. The fish species of *Oreochromis mossambicus* obtained from nearby village Illupore, Trichy District, Tamilnadu. Fish were stock in a large circular cement cistern disinfected with potassium permanganate, and washed thoroughly prior to introduction of fish. Fishes acclimatized to laboratory conditions for about 15 days before commencement of the experiment.

2.2. EXPERIMENTAL PROTOCOL

An average length of 11 ± 1 cm, weighing 25 ± 1 g *O.mossambicus* was segregated from the stock and transferred to clean rectangular 100 L plastic tanks. Water pH was between 7.2 and 8, and dissolved oxygen between 6.9 and 7.5 mg/L. to ensure good health before starting the experiment. The toxicity test conducted for a 96 h to find lethal concentration (LC_{50}) between 100 to 1000 ppm concentrations. The average particle size of ZnO NPs, from 32 - 75 nm mixed with distilled water and suspended through ultrasonication until milky white precipitation occurred and orally administrated.

Various behavioral anomalies observed in the experimental fish during ZnO nanophase exposure and it found to be clearly dose dependent. The first visible reactions of the treated fish were recorded at the highest concentration 600 ppm within 24 h of exposure comprising to and fro movements, hyperactivity and rapid opercula movements. Such movements, continued only for a short period then fish gradually became lethargic, tended to settle at the bottom of the aquarium. Fish found to keep their mouth and operculum wide open. The body of the treated fish appeared to become slimy due to the secretion of excessive mucus. However, the rate of opercula movement increased initially, as exposure time increased the rate of opercula movement reduced drastically. At last, the fish founded dead scattered at the bottom of the aquarium with their mouth wide open. However, the fish exposed to lower concentrations below 500 ppm of ZnO nanophase showed no behavioral changes (Md. Kawser Ahmed *et al.*, 2013). The LC_{50} concentration was 600 ppm. The sublethal concentrations (100, 200, 300, 400 and 500 ppm) of ZnO NPs were given on the 1st day of the experiment. These concentrations chose to enable comparison with our previous sublethal experiments on Fish (Tilapia) (Amutha and Subramanian 2009). At the completion of the stipulated exposure period, blood collected from sacrificed fishes for hematological assays. Tissues like Gill,

Liver, kidney, Intestine and Muscle collected carefully for antioxidant enzymes and histopathological studies.

2.3. ZINC OXIDE NANOPHASES

Synthesis and characterization of Zinc oxide NP powder previously published and the average particle size of ZnO NPs was from 32.67 - 75.71 nm. Suspension of ZnO NPs was made (no solvents) by dispersing the dry ZnO NPs powder in ultrapure (Millipore) water by Ultrasonication (Rod type sonicator, 20 kHz frequency, Mesonix Model CZ5, USA) at 10 minutes and used for the experiments.

2.4. HEMATOLOGICAL STUDIES

Leucocyte (white blood cell, WBC) and erythrocyte (red blood cell, RBC) counts made using hemocytometer (Rawling *et al.*, 2009). The hemoglobin and hematocrit concentrations obtained by the method of (Dorafshan *et al.*, 2008). Erythrocyte indices of fish viz., MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), Mean cell Hemoglobin Concentration (MCHC) were calculated as follows (Klinger *et al.*, 1996).

$$\text{MCH (pg cell}^{-1}\text{)} = \text{Hb (g L}^{-1}\text{)}/\text{RBC (10}^6\text{ mL)} \times 10$$

$$\text{MCV (nm}^3\text{)} = \text{Hct (\%)} \times 10/\text{RBC (10}^6\text{ mL)}$$

$$\text{MCHC (g L}^{-1}\text{)} = \text{Hb (g L}^{-1}\text{)}/\text{Hct (\%)}$$

2.5. ANTIOXIDANT ENZYME ASSAYS

The toxicological impairments due to the chosen nanophase ZnO analyzed through stress stabilizing factor like antioxidant enzymes. In this study, the antioxidant enzymes like Superoxide dismutase (SOD), Catalase, Glutathione reductase (GSH), Glutathione S-transferase (GST) and Lipid peroxidases in the fish vital tissues like gill, liver and muscle were assayed by following the method of Caliborne (1985), Marklund and Marklund (1974), Moron *et al.*, (1979), Habig *et al.*, (1974) and Ohkawa *et al.*, (1979) respectively.

2.6. HISTOLOGICAL STUDIES

The excised organs (Gill, Liver, Kidney, Intestine and Muscle) from control and treated fishes immediately immersed in 10% formaldehyde solution to fix the organ for histological analysis. Then dehydrate it with alcohol and clearing were conducted in xylene, paraffin wax was melted at 58°C to 60°C followed by embedding of the tissue. After trimming the paraffin block, serial sections were cut at 5µm thickness in a Leica (Germany) rotary microtome using disposable blade. The sections spread on the slides were stained with eosin and hematoxylin (Van Dyk *et al.*, 2007). Appropriate ascending and descending series of alcoholic dipping were made. The DPX mounted

slides were observed under a microscope in field illumination and the chosen areas were photographed at different magnification.

2.7. STATISTICAL ANALYSIS

The data were expressed as mean \pm standard deviation. For statistical analysis, the experimental values were compared to their corresponding control ones. A one-way analysis of variance (ANOVA) in SPSS software (Version 16.0) was used to illustrate the significant difference between the experimental group and the control. The significant difference was considered as $P < 0.05$.

3. RESULTS AND DISCUSSION

At the end of the experiment and no abnormal behavior (loss of equilibrium, refusal to feed) were observed and no mortality was observed during the experimental period in all groups including control fish. All the concentrations showed a clear elevation level in Hematology, Antioxidant enzymes and alteration in Histoarchitecture compared to control.

3.1. HEMATOLOGY

Blood samples collected on the 7th day end of the experiment. Total white blood cells and RBC counts on 7th day showed significant differences in ZnO treatment groups compared to the control (ANOVA, $P < 0.05$). Since many epidemiological studies reported that ultrafine particulate, exposure had a close relationship to cardiovascular diseases (Samet *et al.*, 2000). One of the hypothesized mechanisms is that the ultrafine particulates could cause the increase of blood coagulation and then induce heart ischemia (Donaldson *et al.*, 2003). Therefore, it is interesting to know whether there is any evidence of blood parameter changes involved after sublethal oral exposure of ZnO NPs. In fig. 1, increased level WBC count was observed. This increase in WBC count may be because of the prevention of damage caused by zinc oxide NPs in the fish tissues (Buthelezi, 2000; Nussey *et al.*, 2002). This may be explained by a reaction of the defense mechanism of the fish by leucocytosis under pathological conditions and against foreign bodies (John, 2007). It has been reported that these effects of zinc oxide nanophase may decrease or increase the activation or counts of white blood cells at different concentrations in humans and animals (Schlesinger *et al.*, 1993; Rink and Kirchner, 2000; Ibs and Rink, 2003). A decreased level of RBC (Fig.2) in 100 to 500 ppm were observed compared to control groups due to the impact of ZnO NPs.

In figure. 4, decreased level of HCT whereas in Hb, MCV, MCH, and MCHC values were increased significantly (Fig. 3, 5, 6 and 7) due to zinc oxide nanophase impact on fish especially in high doses. Such a situation can be an indicator of hemolytic anemia, as was found in some fish

species exposed to paraquat (Salazar-Lugo, 2007). Hemolytic anemia is a genetic and molecular disease and have been seen in fish in anxious and low pH conditions. This disorder causes rupture of the erythrocytes, and an increase of free hemoglobin in blood, and damage in the tissues of the fish, and cause death (Hárosi *et al.*, 1998; Pia Koldkjær and Berenbrink, 2007). Therefore, hemolytic anemia is reported to be an important parameter in the evaluation of fish health (Hárosi *et al.*, 1998).

In addition, MCV, MCH and MCHC values were significantly higher compared to the control group. This fact shows that concentration of zinc oxide nanophasse to the fish may cause macrolytic anemia. Similar results were seen in *O. mossambicus* (Nussey *et al.*, 2002) exposed to zinc and in *Clarias albopunctatus* (Mgbenka *et al.*, 2005) exposed to organophosphorous pesticide.

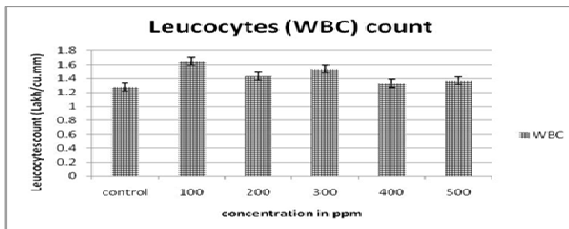


Fig.1

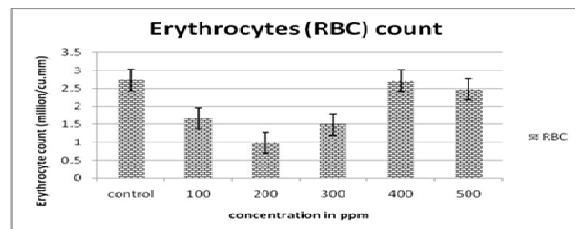


Fig.2

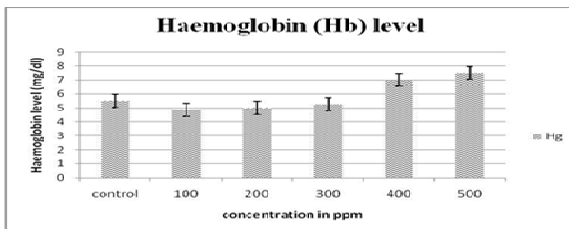


Fig.3

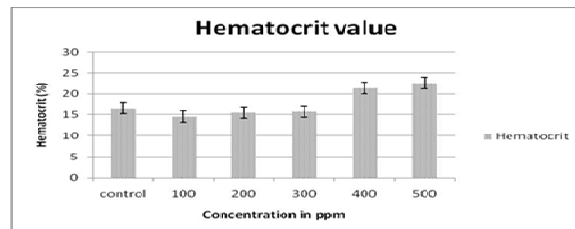


Fig.4

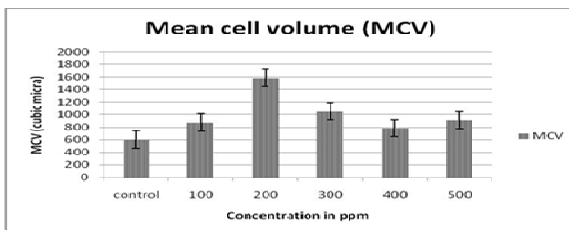


Fig.5

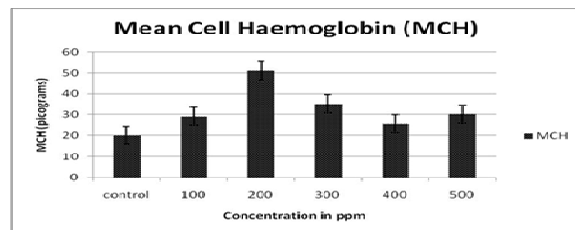


Fig.6

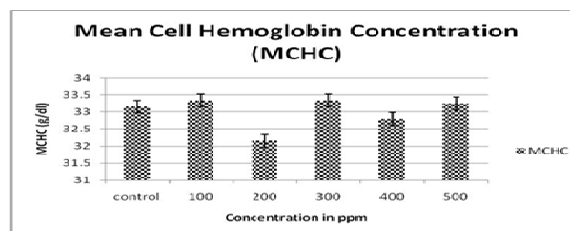


Fig.7

The changes of Hematology count by ZnO nanophasse in fish *Oreochromis mossambicus*. Values were expressed as the mean \pm SE

3.2. ANTIOXIDANT ENZYME

Oxidative stress is a common pathway of toxicity. Reactive oxygen species (ROS) have been reported to affect physiology, growth, and survival of aquatic organisms (Pandey *et al.*, 2003). Fish, like mammals, possess well-developed antioxidant defense systems for neutralizing the toxic effects of ROS (Pandey *et al.*, 2003). In the antioxidant study, we found that the impacts of exposure to zinc oxide nanophase on the activities of the various antioxidants in the fish. In turn, the antioxidant namely, SOD, CAT, GSH, GST, and LPO. Which are all crucial in the detoxification of ROS to nonreactive molecules responded differently to the oxidative alteration caused by ZnO NPs.

3.2.1. SUPEROXIDE DISMUTASE (SOD)

SOD plays important roles in the antioxidant protection of invertebrates (Livingstone 2001, 2003). As shown in Fig.8, SOD activity significantly increased compared to control (Zheleva A., *et al.*, 2004). SOD activity from 100 to 500 ppm concentration were gradually increased in gill, liver and Muscle when compared to control groups. Between gill and Muscle, liver activity showed higher enzyme level then control. From this, it is clear that liver might be the susceptible organ to ZnO NPs exposure. However, SOD catalyzes the dismutation reaction of the superoxide anion radical, O_2^- , to form the less-reactive oxygen.

3.2.2. CATALASE ACTIVITY (CAT)

CAT plays important roles in the antioxidant protection of invertebrates (Livingstone 2001, 2003). CAT converts H_2O_2 to H_2O and O_2 to prevent oxidative stress and maintain cell homeostasis (Kappus 1985). In figure.9, Catalase activity significantly increased in all the concentration from 100 to 500 ppm compared to control. This result agrees with (Jimenan cazenave *et al.*, 2006). Furthermore, Li *et al.* (2003) reported elevated CAT activity and ROS content in hepatocytes of common carp, induced by MC-LR. Besides, Jos *et al.*, (2005) as well as Li *et al.* (2005) observed that CAT activity increased in the liver of tilapia and Loach after sub chronic exposure to toxic cyanobacterial cells. Similarly, Jos *et al.*, (2005) reported that the CAT level in gills was lower than in liver of tilapia.

3.2.3. REDUCED GLUTATHIONE ACTIVITY (GSH)

GSH, a tripeptide that serves as an antioxidant, accounts for 90 % of intracellular nonprotein thiols (Sen *et al.*, 1994; Kidd 1997) and scavenges cellular H_2O_2 , O_2^- , and lipid hydroperoxides through functional group -SH (Reed and Beatty 1980). Reduced Glutathione activities were increase in ZnO NPs treated fishes. In Reduced Gutathione (GSH) activity concentration from 100 to 500 ppm all the tissues (Gill, Liver, Muscle) gradually increased when compare with control (Fig.10).

The significant increase in these organs may be a response to oxidative alteration caused by the presence of ZnO NPs. The apparent increase in GSH levels with concomitant elevation in the activity of GST in the organs suggests an adaptive and protective role of this biomolecule against oxidative stress induced by the ZnO NPs (Pandey *et al.*, 2003).

3.2.4. GLUTATHIONE S-TRANSFERASE ACTIVITY (GST)

Glutathione S-Transferase (GST) is a multicomponent family of phase II biotransformation enzymes that detoxify diverse electrophilic endogenous and xenobiotic substrates by way of conjugation with GSH to produce less toxic and more water soluble compounds (Akcha *et al.* 2000; Wu *et al.* 2006), and they each play a vital role in protecting tissues from oxidative stress (Fournier *et al.* 1992). GST activity was significantly increased in ZnO NPs treated fishes. The GST activity in liver tissue from 100 to 500 ppm concentration shows significantly increased levels when compared to Gill and Muscle (Fig.11). Induction of GST activity in liver of fish exposed to ZnO NPs as a pollutant, in the present investigation is in accordance which is recorded in liver of *Clarias lazera* exposed to synthetic pesticides (Daabees *et al.*, 1992), in the liver and kidney of *O. niloticus* captured from sewage polluted sites (Hamed *et al.*, 2003) in liver of Rainbow trout exposed to cadmium (Aitaissa *et al.*, 2003) in the liver of *Cyprinus carpio* exposed to polychlorinated biphenyles (Schmidt *et al.*, 2004) and in the liver of rainbow trout (*Oncorhynchus mykiss*) injected by 100,200 and 400 mg/kg body weight trinitrotoluene for 72 hr (Ek *et al.*, 2005).

3.2.5. LIPIDPEROXIDASE ACTIVITY (LPO)

Lipidperoxidase (LPO) widely recognized consequence of oxyradical production is the peroxidation of cellular lipids (Winston and Di Giulio, 1991). Increased lipid peroxidation (LPO), measured as malondialdehyde (MDA) production, observed in the fish (Bhattacharya *et al.*, 2007). In lipidperoxidase (LPO), Fig.12, activity of all the tissues (Gill, Liver and Muscle) show gradual increased activity from 100 and 500 ppm concentration was observed when compared with control. Durmaz *et al.*, (2006), reported the same result. Zhu *et al.* (2006) reported that increase LPO levels in the liver and gill tissues of fathead minnow. Federici *et al.*, (2007) reported that NPs caused an increased level of LPO in gill and the intestine of rainbow trout indicating that these tissues suffered from oxidative stress. Linhua *et al.*, (2009) also reported that elevation of LPO in the liver was the greatest, indicating that the liver might be the most susceptible organ to expose of NPs.

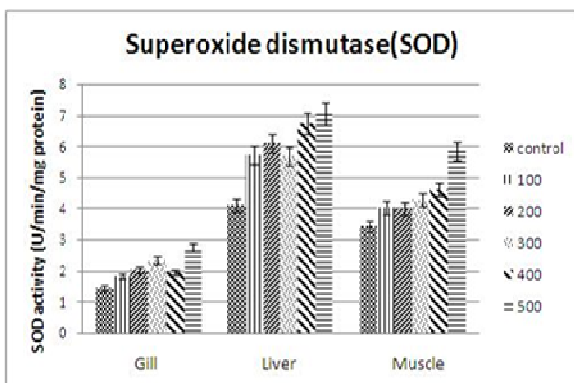


Fig.8

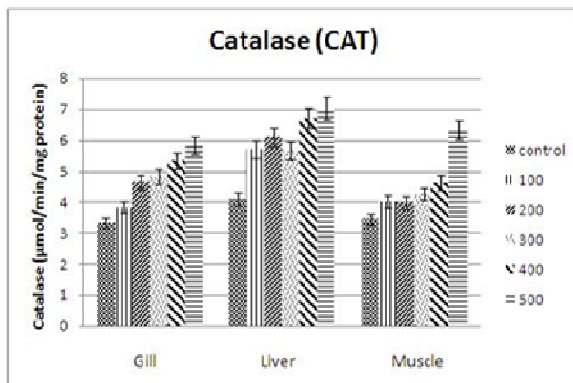


Fig.9

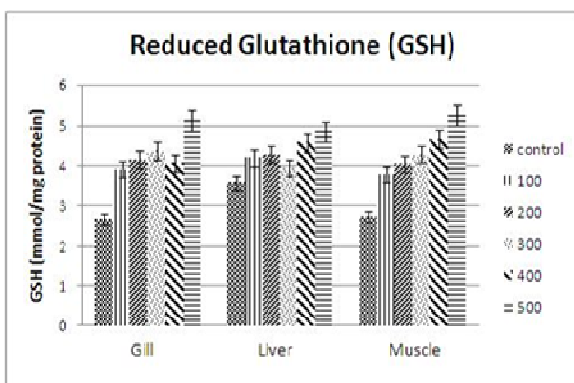


Fig.10

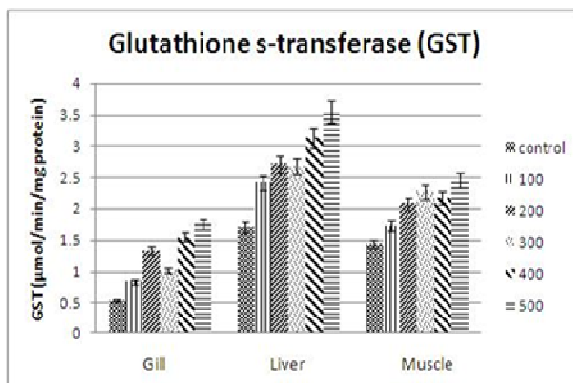


Fig.11

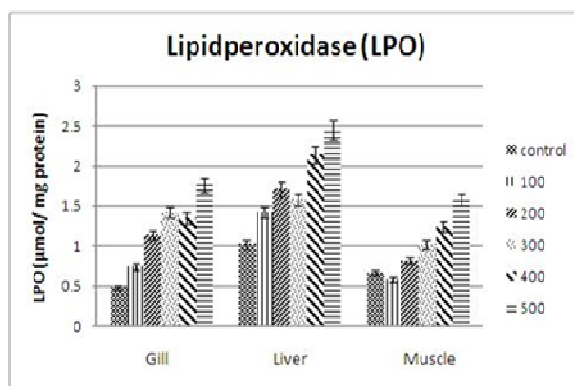


Fig.12

The changes in antioxidant activity by ZnO nanoparticle at different concentration (100 to 500 ppm) in tissues (Gill, liver and Muscles) in fish *Oreochromis mossambicus*. Values were expressed as the mean \pm SE (significant differences, $p < 0.05$)

4. HISTOLOGICAL EXAMINATION

4.1. GILL

Histological changes in the gill of *O. mossambicus* exposed by Zinc oxide nanophase was described respectively in figure (13). Fish gills are critical organs for their respiratory, osmoregulatory and excretory functions. The gills of fish are the largest fraction of the total body surface area, which is in direct contact with the water (Hughes, 1984). Respiratory distress is one of the early symptoms of toxicant poisoning. A high rate of absorption of ZnO nanophase through gills also makes the fish a vulnerable target of its toxicity. No recognizable changes observed in the gills of control fish. In the treated group (100 ppm) the gills showed hypertrophy and hyperplasia of mucous cells at the base of the gill filament and secondary lamellae. Shortened secondary lamellae, detached interlamellar epithelial cells, scattered RBC's, dilated marginal blood sinus commonly observed at 100 ppm exposed fish gill. In more severe cases, the gill showed cavitations, Non-tissue space, necrotic lamellar epithelium, hyperaemic at the distal end, scattered RBC's and dilated marginal blood sinus were observed in 200 ppm gill. In addition, extensive distorted primary lamella, shortened secondary lamella, folded secondary lamella, folded telangiectasis and hyperemia were observed in 300 ppm gill. In the 400 ppm treated gill, Hypertrophied secondary lamella, Desquamated lamellar epithelium, primary lamella completely ruined like twisted. In 500 ppm treated fish gill showed completely ruined architecture of gill lamellae, primary lamellae completely detached, necrotic cells and cavitations were observed. Federici et al. (2007) showed that exposure to TiO₂ NPs resulted in some increases in the incidence of edema in the secondary lamellae, changes in mucocyte morphology, and hyperplasia in the primary lamellae on the gill filaments of rainbow trout.

The similar observation with SWCNT was reported by Smith et al., (2007). Griffith et al. (2007) reported that exposure to Cu NPs suspensions caused a damage to gill lamellae characterized by proliferation of epithelial cells as well as edema of primary and secondary gill filaments of zebrafish. In our study, exposure to ZnO NPs suspensions caused a damage to gill lamellae as well as gill filaments. Effects of ZnO NPs were dose dependent, with significantly greater damage observed at higher concentrations. Therefore, the current study with gill tissue pathologies of the fish exposed to ZnO NPs showed that the fish suffered during the exposure period.

4.2. LIVER

The figure. 14 reveals that control groups of liver tissues showed normal features were observed like Intrahepatic pancreas, Hepatocytes, Acinar, Blood vessels and Sinusoids. Where as in

100 ppm treated group also showed no differentiable changes occurred. In the 200-ppm disintegrating acinar cells, degenerating erythrocytes, necrotic area, cell debris, intracellular space were observed. In 300 ppm liver, karyolysed hepatocytes, swollen nucleus, necrotic spot, disintegrating acinar cell and degenerating erythrocytes were observed. At 400 ppm treated liver Necrotic debris, Nodule, intracellular space, degenerating hepatocytes was clearly observed. At 500 ppm treated liver shows edema fluid, Inflamed vascular wall, destroyed blood vessel, remnants of dead acinar cells, and necrotic spots were observed.

Though the liver tissue of fish is an important organ of active metabolism and detoxification and extremely sensitive to pollutants. Extraneous xenobiotic compounds biotransformations occur in liver (Brusle and Anadon, 1996). Federici et al. (2007) and Smith et al. (2007) reported that the livers of some fish exposed to TiO₂ NPs and SWCNT showed condensed nuclear bodies (probably apoptotic bodies) and minor fatty change.

4.3. INTESTINE

In figure. 15, intestine of exposed zinc oxide nanophase, the Control group showed no detectable changes. Normal Intestinal wall, Gut lumen and normal villi were observed. In 100 ppm treatment intervillus space, cavitation, enterocyte were observed. At 200 ppm treated intestine Extra growth of the villas was seen and Necrotic villi were also observed. In 300 ppm exposure Cellular debris, disintegrating intestinal wall, abnormal intestinal folds were seen. At 400 ppm treated liver villus architecture was changed, necrotic debris and cellular debris were also seen. At 500 ppm inflamed villus damaging the entire architecture. Lamina propria and submucosa were not found. Disintegrating intestinal wall, mononuclear cell infiltrates was observed.

According to Bhatnagar *et al.*, (2007) the irritation and destruction of the mucosa membrane of the intestine and hampering absorption were observed. Hanna *et al.*, (2005) and Cengiz *et al.*, (2006) also reported that the pathological alterations in the intestine of the fish and its effects of different toxicants on fish intestine. Epithelial degeneration, inflammatory cell infiltration in the sumucosa as well as submucosal edema was seen in the intestine of tilapia fish exposed to carbofuran (Soufy *et al.*, 2007).

4.4. KIDNEY

In figure.16, kidney exposed to zinc oxide nanophase showed no recognizable changes in control groups. Normal cuboidal epithelial cells, kaemopoetic cells, bowman's capsule, and Bowman's space was observed. In the 100 ppm necrotic spaces of the interstitium, intertrabacular space is more and lumen, glomerulus were observed. In 200 ppm mass of necrotic debris,

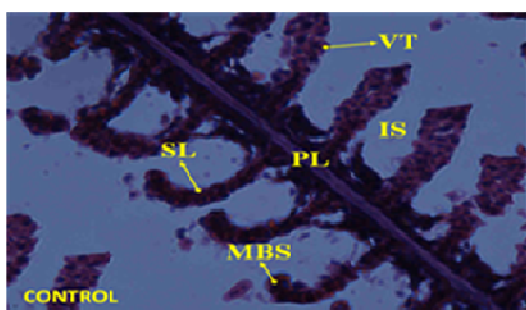
Karyolysed haemopoetic cells, necrotic debris and necrotic space were observed. In 300 ppm Mononuclear cell infiltrates, linen, necrotic space, disintegrating tubule and interstitial necrosis were observed. In 400 ppm mass of degenerating cells, interstitial necrosis, hypertrophied glomerules, lumen, pyknotic nuclei were observed. In 500 ppm Trabacular degeneration with renal cast in renal tubules, abnormal erythrocytes, necrotic area, necrosis of interstitium with fluid accumulations were noted. Zheng et al.(2009) assayed the toxicity of ZnO NPs in mice exposed via the digestive tract. Compared with the control group, the spleen and brain cells were normal, whereas other primary organs (including heart, lung, liver, and kidney) were damaged. These results were supported by the findings of Wang et al. (2008) which showed that the pathological changes induced by ZnO NPs were both size and dose dependent.

4.5. MUSCLE

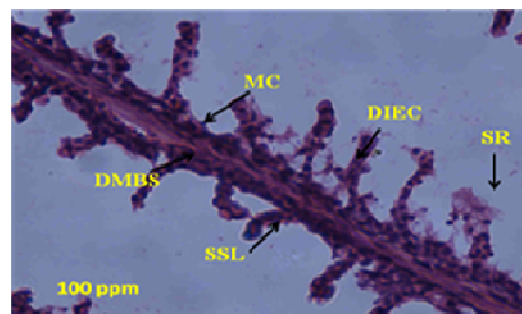
The figure. 17, showed no recognizable changes in muscle exposed to zinc oxide nanophase and normal Muscle fiber, Sacrolemma, Nucleus was observed in the control group. In 100 ppm concentration Bent muscle fiber, intracellular space, degenerating endomysium, and Muscle fiber was observed. In 200 ppm concentration group Wrinkled muscle fiber, Necrotic area, and Fragmented fiber were observed. In 300 ppm concentration groups Sarcolemma, Empty space, Endomysium, Vacuole were observed. In 400 ppm concentration Wrinkled muscle fiber, Fragmented fiber, Necrotic area, and Necrotic Zone were observed. In 500 ppm concentration group Oedema vacuole, Hyaline degeneration, Necrotic space, and Fused muscle fiber were observed

The histopathological alterations in the muscles of fish are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles (Sakr *et al.*, (1991), Abo Nour *et al.*, (1995), and Das *et al.*, (2000). Focal areas of myolysis seen in the muscles of *O. spilurus* exposed to contra/insect 500/50E.C. Elnemaki *et al.*, (2003). At the same time, Abbas *et al.*, (2007) observed destruction and vacuolation of the muscle cells in *Oreochromis spp.* exposed to chromium.

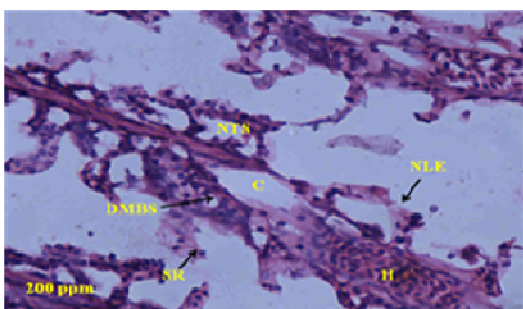
Figure. 13 Histological changes in *O.mossambicus* gill exposed to ZnO



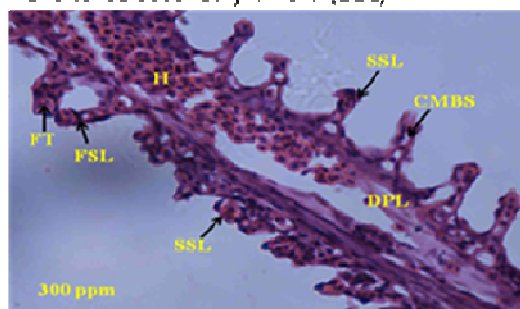
Primary lamella (PL), Secondary lamella (SL), Marginal blood sinus (MBS), Vascular tissue (VT), Interlamella space (IS)



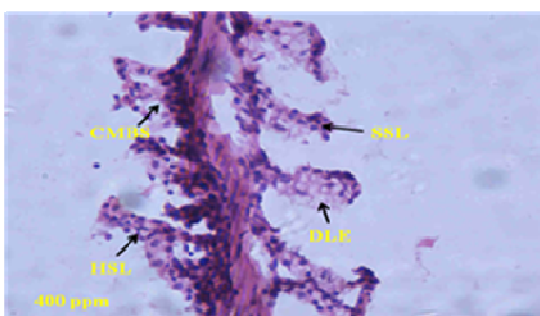
Mucous cell (MC), Detached interlamellar epithelial cells (DIEC), Scattered RBCs (SR), Dilated marginal blood sinus (DMBS), Shortened secondary lamella (SSL)



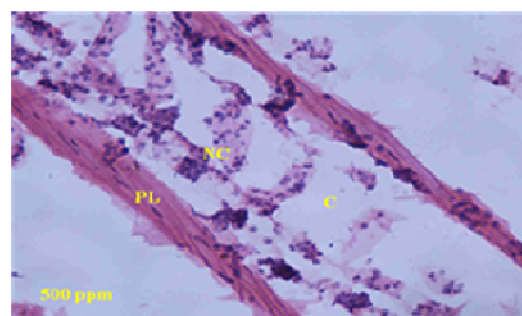
Cavitation (C), Non tissue space (NTS), Necrotic lamellar epithelium (NLE), Dilated marginal blood sinus (DMBS), Scattered RBCs (SR), Hyperaemic at the distal end (H)



Hyperaemic at the distal end (H), Constricted marginal blood sinus (CMBS), Shortened secondary lamella (SSL), Folded secondary lamella (FSL), Folded Telangiectasis (FT), Distorted primary lamella (DPL)

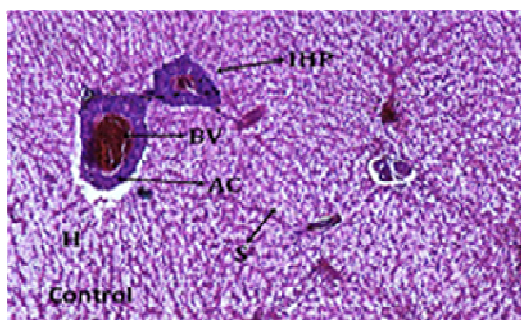


Constricted marginal blood sinus (CMBS), Shortened secondary lamella (SSL), Hypertrophied secondary lamella (HSL), Desquamated lamellar epithelium (DLE)

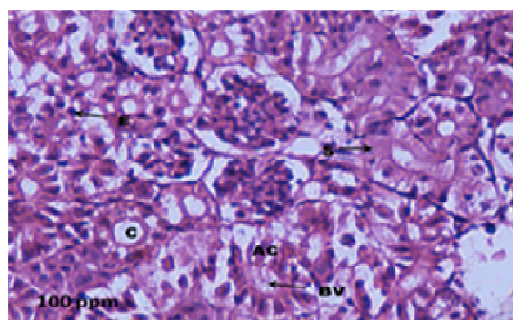


Run architecture of gill lamella, Primary lamella (PL), Cavitation (C), Necrotic cells (NC)

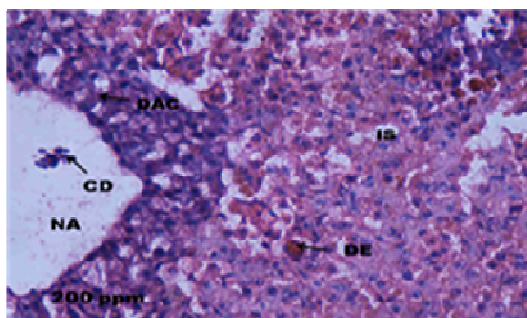
Figure 14 Histological changes in *O.mossambicus* Liver exposed to ZnO



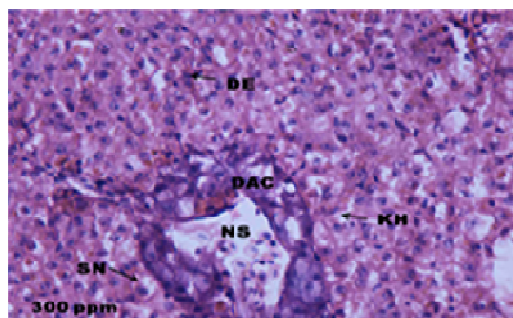
Normal Intrahepatic pancreas (IHP), Hepatocytes (H), Acinar cell (AC), Blood vessels (BV), Sinusoids (S)



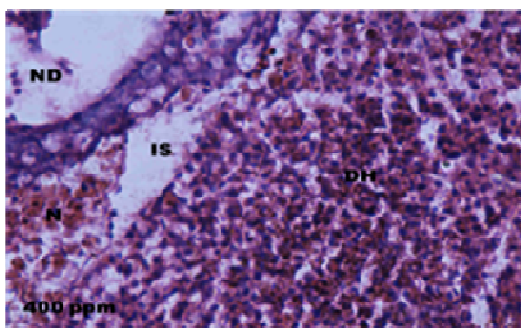
Erythrocyte (E), Sinusoid (S), Cytoplasm (C), Acinar cells (AC), Blood vessel (BV)



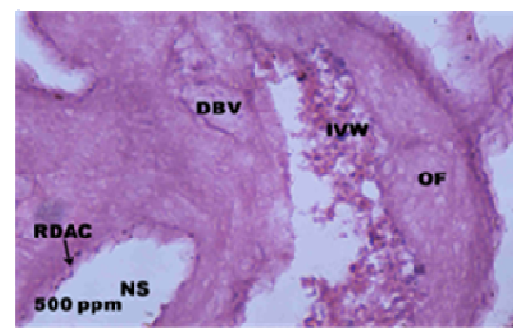
Disintegrating acinar cells (DAC), Degenerating erythrocytes (DE), Necrotic area (NA) Cell debris (CD), Intercellular space (IS)



Disintegrating acinar cells (DAC), Necrotic spot (NS), Degenerating erythrocytes (DE), Karyolysed hepatocytes (KH), Swollen nucleus (SN)

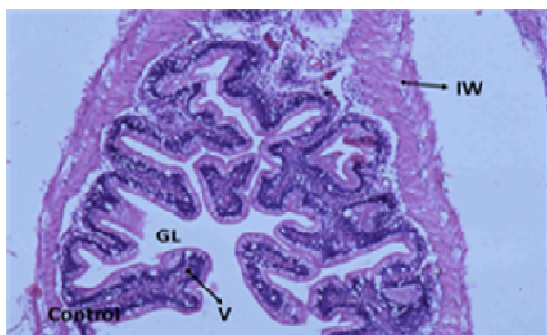


Necrotic debris (ND), Nodule (N), Intercellular space (IS), Degenerating hepatocytes (D-H)

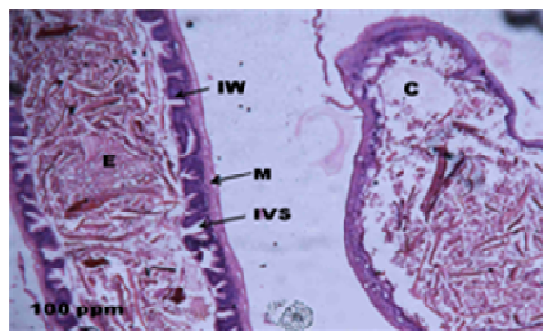


Oedema fluid (OF), Inflamed vascular wall (IVW), Destroyed blood vessel (DBV), Remnants of dead acinar cells (RDAC), Necrotic spot (NS)

Figure 15 Histological changes in *O.mossambicus* Intestine exposed to ZnO



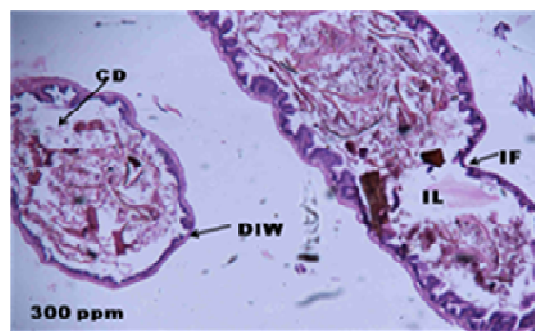
Intestinal wall (IW), Gut lumen (GL), Villus (V)



Intestinal wall (IW), Mucosa (M), Intervillous space (IVS), Cavitation (C), Enterocyte (E)



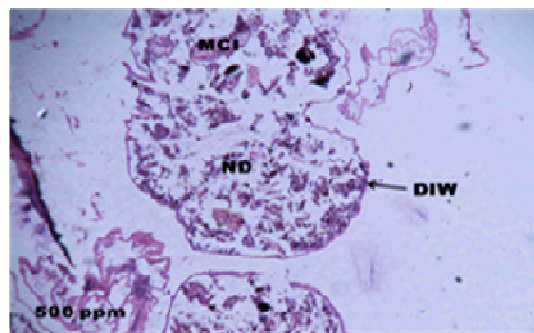
Extra villous growth (EVG), Intestinal wall (IW), Intervillous space (IVS), Necrotic villi (NV), Gut lumen (GL)



Cellular debris (CD), Disintegrating intestinal wall (DIW), Abnormal intestinal fold (IF), Intestinal lumen (IL)

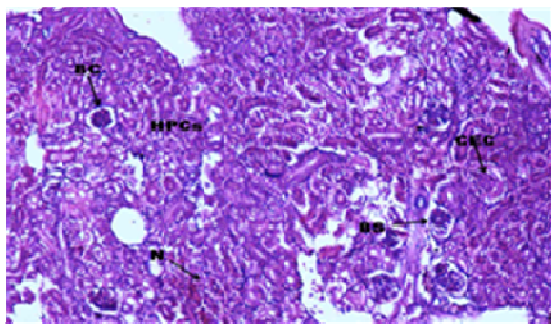


Necrotic villi (NV), Necrotic debris (ND), Necrotic spot (NS), Disintegrating intestinal wall (DIW)

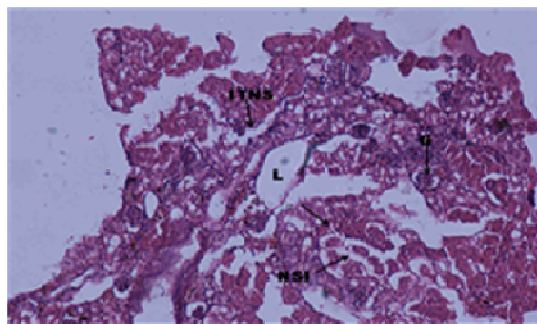


Inflamed villus damaging the entire architecture. Lamina propria and submucosa were not found. Disintegrating intestinal wall (DIW), Mononuclear cell infiltrates (MCI)

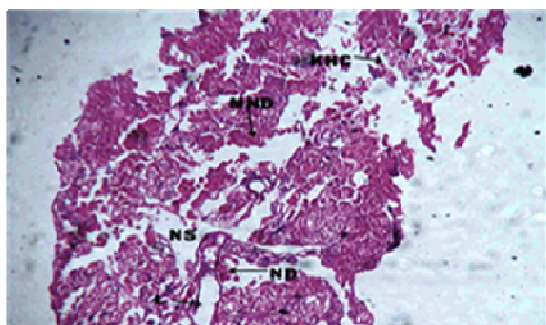
Figure 16 Histological changes in *O.mossambicus* Kidney exposed to ZnO



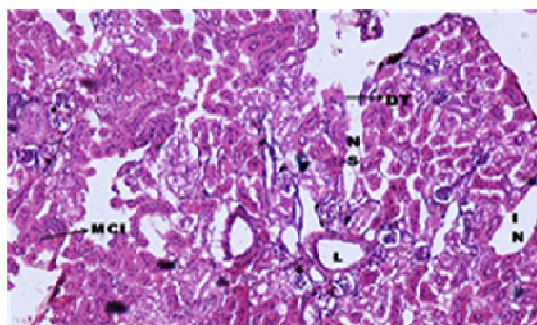
Bowman's capsule (BC), Haemopoietic cells (HPCs), Bowman's space (BS), Cuboidal epithelial cell (CEC)



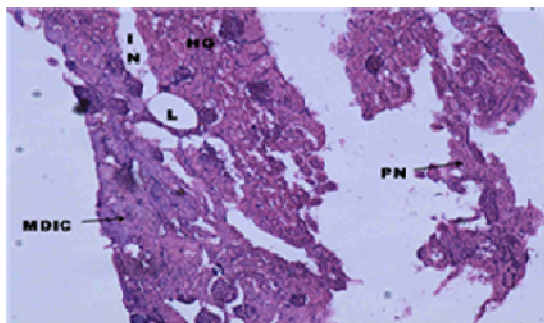
Necrotic space of interstitium (NSI), Intertrabecular necrotic space (ITNS), Lumen (L), Glomerulus (G)



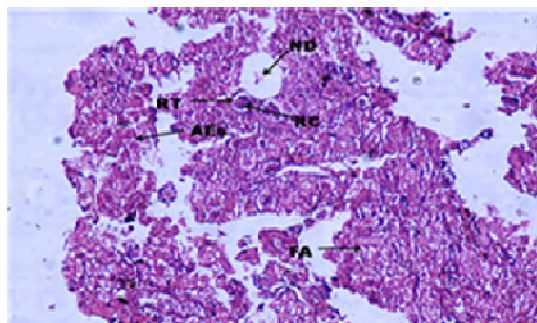
Mass of necrotic debris (MND), Karyolysed haemopoietic cell (KHC), Necrotic debris (ND), Necrotic space



Mononuclear cell infiltrates (MCI), Lumen (L), Necrotic space (NS), Disintegrating tubule (DT), Interstitial necrosis (IN)

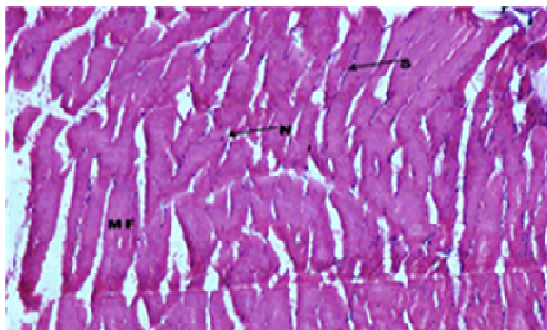


Mass of degenerating interstitial cell (MDIC), Interstitial necrosis (IN), Hypertrophied glomerulus (HG), Lumen (L), Pyknotic nuclei (PN)

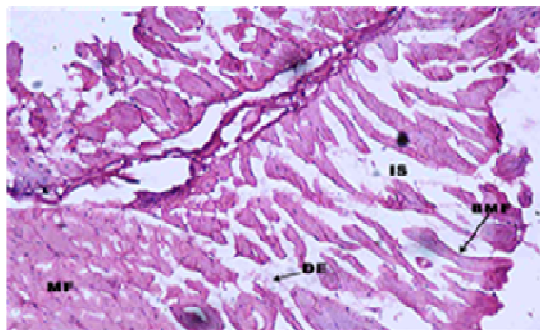


Trabecular degeneration with renal cast (RC), in renal tubule (RT), Abnormal erythrocytes (AEs), Necrotic area (NA), Necrosis of interstitium with fluid accumulation (FA).

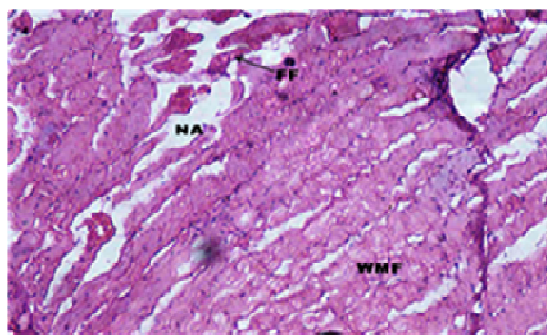
Figure 17 Histological changes in O.mossambicus Muscle exposed to ZnO



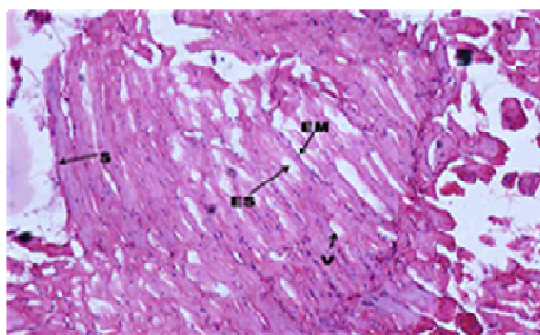
Control group of Muscle fiber (MF),Sarcolemma (S), Nucleus (N).



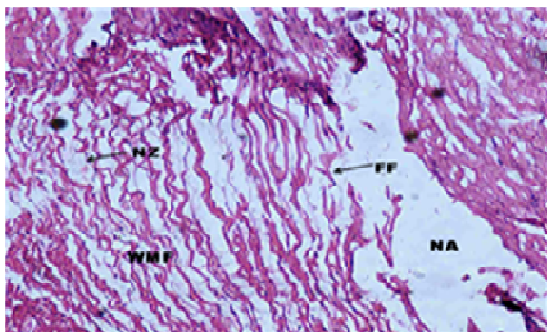
Bent muscle fiber (EMF), Intercellular space (IS), Degenerating endomysium (DE), Muscle fiber (MF)



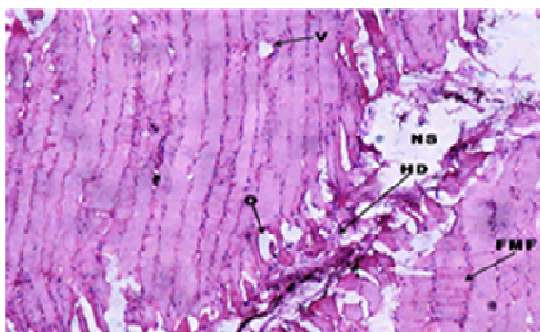
Wrinkled muscle fiber (WMF), Necrotic area (NA), Fragmented fiber (FF)



Sarcolemma (S), Empty space (ES), Endomysium (EM), Vacuole (V)



Wrinkled muscle fiber (WMF), Fragmented fiber (FF), Necrotic area (NA), Necrotic Zone (NZ)



Oedema (O), Vacuole (V), Hyaline degeneration (HD), Necrotic space (NS), Fused muscle fiber (FMF)

5. CONCLUSION

The present study has demonstrated that ZnO NPs (32.67–75.71 nm ranged) was extremely toxic to fish (*O.mossambicus*) above 500 ppm causes patho physiological effects such as oxidative damage, hematological changes and organ pathology. Blood is a patho-physiological reflector of the whole body, and blood parameters are important in the diagnosis of the structural and functional statuses of animals exposed to toxicants (Sampath *et al.*, 1998). Omoregie et al (1990) also reported that toxicants have important effects, which cause several physiological disorders in fish. The results indicating that the material Zinc oxide nanophase at sublethal concentrations (100 to 500 ppm) has hazardous property to aquatic organisms and may consider as a major forthcoming pollutant in the aquatic environment. Hence, more studies are in need to assess the environmental risks due to nanophases in the aquatic as well as terrestrial environment.

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