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Effect of broad range doses of oral administration of Zinc Oxide Nanoparticles in freshwater fish *Oreochromis mossambicus*

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Abstract
Due to high production and application, nanoproducts reaching aquatic environments have become unavoidable. Among aquatic species, fishes are more susceptible to waterborne contamination and are recognized as bioindicators for water quality monitoring. The present study is to evaluate the haematological and biochemical changes including enzymatic defence systems in freshwater tilapia (*Oreochromis mossambicus*), on exposure to Zinc Oxide (ZnO) nanoparticles (NP) orally in the range of 100, 200, 300, 400 and 500 ppm for 4 days. ZnO NP led to significant (P<0.05) changes in haematological parameters of Red blood cell (RBC) count, White blood cell (WBC) count, haemoglobin (Hb) concentration and haematocrit with the increasing concentration. Oxidative status of fish was evaluated by analyzing the level of Catalase, Glutathione S-transferase, Superoxide dismutase, Lipid Peroxidase and Reduced Glutathione levels in fish tissues like gill, liver and muscle. ZnO NPs has caused variable changes in oxidative status of *O. mossambicus* (P<0.05) in a dose dependent manner particularly at 100, 200 and 300 ppm. The study indicates defensive nature and adaptive mechanism of cells against free radical induced toxicity. This study confirms that it will be used to assess the potential health effects of chemicals and products.

Keywords: antioxidant, haemotology, nanoparticles, oral administration, tilapia, zinc oxide

Introduction
Engineered nanoparticles (NP) have a size of less than 100 nm at least in one dimension and subsequently have very high surface areas and high percentages of component atoms on their surface. Nanotechnology gain importance in various fields and at present application of NP in the field of cosmetics, decorative, paints, pharmaceuticals, medicines, space research etc, become common (Jeevanandam *et al.*, 2018) [11]. Owing to the high production and use of nanoproducts finally will lead to increased levels of discharge of after use nanomaterials into the environment either intentional or accidental releases, or through weathering of products that contains NP become unavoidable (Monfared and Soltani, 2013) [20].

Currently, the metal and metal oxide nanomaterials comprise a large segment of growing nanotechnology market (Chaudhary *et al.*, 2018) [6]. NP has been made from many metals, including gold, silver, copper, nickel, cobalt, zinc, titania etc, (Singh *et al.*, 2018) [36]. Zinc oxide (ZnO) NP possesses various functions for uses such as pigments, piezoelectric devices, luminescent devices, gas sensors, catalyst, and cosmetic materials in our present day society (Chaudhary *et al.*, 2018) [6]. In particular, ZnO NP in the range of 100-200nm have been commonly used in sunscreen products because of their ability to filter UVA as well as UVB light. ZnO NP were analysed about the antibacterial activity purposes against the human pathogen (Vandebriel and Jong, 2012) [41], but widely the bulk ZnO particle was used for bacteriocidal purposes in medicines. Soluble forms of many metals or metal oxide are toxic to aquatic organisms, implying that the potential exists for nanoparticulate formulations of many metals to induce toxicological effects in aquatic species. The aquatic ecosystem is particularly one of the ultimate dumping sites for many chemicals and house hold discharges. Therefore, the possibility to interact with living and non living things and the resultant product or reactions are yet to be studied well and finally these uncertainties may attain the risks and thereby it reaches to human and environmental health (Monfared and Soltani, 2013) [20].

In aquatic ecosystem, the fish has become an important protein source for human and aquatic

pollution impacts direct effect on fishes and indirectly to humans through its consumption. It is of much importance to evaluate the effects of pollution on fish both for environmental protection and for socio-economic reasons. The consumption of contaminated fish and shellfish (Oysters, Tilapia, Tuna and Shrimp) results in exposure of humans to trace element contamination. (Raknuzzaman *et al.*, 2016) [31]. Tilapia could be used as a bio-indicator for studying the accumulation and transformation of metals in freshwater organisms (Lasheen *et al.*, 2012) [17].

Oreochromis mossambicus was first typed by Peters in 1852, it is a popular fish with fish farmers as they grow fast under any condition and get large. This is one of the most popular species for aquaculture. Russell *et al.*, (2012) [32] reported that the *O. mossambicus* is popularly known due to their biological traits such as tolerance to wide range of ecological conditions, feeding behavior and reproduction ability with maternal care that has made them an experiment model. It has been demonstrated that copper and silver nanoparticles are acutely toxic to across a wide spectrum of aquatic species including zebra fish (Haque and Ward, 2018) [13].

Fish live in very intimate contact with their environment, and are therefore responds to physical and chemical changes that are reflected in their blood components. It should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals. Blood tissue truly reflects physical and chemical changes occurring in organism (Adeyemo, 2007) [1]. Haematological analyses has been routinely used in determining the physiological state of animals and known to be effected by different environmental factors, it is used as a guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human (Solomon and Okomoda, 2012) [38].

Studies carried out on various fish species have revealed that heavy metals might alter the biochemical parameters both in tissues and in the blood (Sani, 2011) [33]. Aerobic organisms generate reactive oxygen species (ROS), such as superoxide anion radical ($^{-}O2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), because of oxidative metabolism. The hydroxyl radicals can initiate lipid peroxidation (LPO) in tissues. The negative effect of ROS is attenuated by the antioxidant defence system that involves enzymatic and non-enzymatic mechanisms. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST). The non-enzymatic defence includes Vitamin–E, C, and A, glutathione, carotenes and ubiquinol (Claiborne, 1985) [7]. The antioxidants protect the organism against oxyradical damage, such as DNA strand breaks, protein oxidation and the induction of lipid peroxidation (Kumari *et al.*, 2014) [16]. The use of the biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish. Oxidative stress biomarkers have rapidly increased in the field of ecotoxicology. Therefore, it has been suggested that they could be used in environmental monitoring systems (Kroon *et al.*, 2017) [14]. Several metals have been reported to elicit oxidative stress in aquatic organisms (Kumari *et al.*, 2014) [16].

Although regulations exist for protecting aquatic life from dissolved forms of these metals, it is unclear if they are appropriate for use with metallic nanomaterials which may produce quantitatively or mechanistically different toxicity (Singh *et al.*, 2018) [36]. By understanding the initial uses of NP application sides are strengthened, but malignant impact like toxicological effects are mostly ignored. The health security of human and animals not only depend on beneficial product but also in avoiding the materials that causes ill effects. Therefore, the present investigation, oral administration of NP to fish was undertaken and intended to evaluate the commonly used NP such as ZnO. *O. mossambicus* a model species for fish physiological and toxicological studies was used to test this hypothesis. In this study assessment was made on the hematological and enzymatic features besides the survival of experimental fish.

2. Material and Methods

The fresh water teleost fish Tilapia (*O. mossambicus*) belonging to the family Cichlidae was used for the present investigation. The specimens of *O. mossambicus* were procured from Lake located at Illupore village, Pudukkottai District, Tamilnadu, India (Latitude 10.53836 N, Longitude 78.62056 E). Fish samples were acclimatized to laboratory conditions and maintained in large circular cement tank, disinfected with potassium permanganate and washed thoroughly prior to experimentation. From the stock identical size fish (average length of 11±1 cm and weighing 25±1 gm) were segregated and transferred to clean rectangular plastic tanks of 100 liters water capacity and the experimental tanks were maintained in the aquarium of ICAR-KVK, Kattupakkam and Department of Animal Science, Bharathidasan University. For each dose (tank) 10 fishes were used and all the doses were performed as triplicate.

The ZnO NP synthesized and characterized by Sivakumar and Subramanian (2012) [37] was administered orally to *O. mossambicus* in the present study. The ZnO NP was dispersed in millipore water by using Sonicator for oral administration. The doses were administered to each fish as 100, 200, 300, 400 and 500 ppm. Fishes with no ZnO NP administration were used as control. Fresh water free from chlorine was used for the present study. The physico-chemical parameters such as temperature, pH, dissolved oxygen, total alkalinity, salinity and total hardness were estimated for each set of experiment as these factors have a significant influence on the biodegradability and toxicity of pollutants. The physico-chemical parameters (APHA, 1976) [2] of experiment tank water were recorded daily at 06.00 and 18.00 hrs during the experiment period. The acute toxicity test was conducted as per OECD guidelines (OECD, 2012) [27] in the present investigation for a period of 4 days. The doses were given in the 1st day of experiment and after the 4 days exposure period, the doses that caused the mortality of fish in each dose recorded to determine LD_{50} value. The remaining fish from 30 (10+10+10 – triplicate per concentration) were taken out and sacrificed for further analysis.

At the end of experimental period blood was drawn by cardiac puncture and various haematological parameters were determined (Adeyemo, 2007) [1]. The blood samples were collected in bottles with Ethylenediamine Tetra Acetic Acid (EDTA) as anticoagulant. The haematological parameters such as Red blood cell (RBC) count, White blood cell (WBC) count, haemoglobin (Hb) concentration and haematocrit were measured in the blood samples of control and treated *O. mossambicus.* Erythrocytes indices of fish *viz*., MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), Mean cell Haemoglobin Concentration (MCHC) were also calculated.

The toxicological impairments due to the chosen NPs of ZnO administration was assessed through several stress stabilizing factors like antioxidant enzymes and metabolites such as

Catalase, Glutathione-S-transferase, Superoxide dismutase, Lipid Peroxidase and Reduced Glutathione on the fish tissues like gill, liver and muscle.

Catalase activity was measured by the method of Caliborne (1985) [7]. Glutathione-S-transferase (GST) activity was measured by the method of Habig *et al.* (1974) [12]. Superoxide dismutase (SOD) activity was measured by the method of Marklund and Marklund (1974) [19]. Lipid peroxidase activity was measured by the method of Ohkawa *et al.* (1979) [25]. Reduced Glutathione (GSH) activity was measured by the method of Moron *et al.* (1979) [22]. Protein estimation in the fish sample was measured by the method of Lowry *et al.* (1951) [18]. Each single treatment was replicated three times and the mean value ± standard deviation was considered. Univariate analysis of variance following the statistical programme for the social sciences (SPSS, ver. 16.0) was used to assess the significance among the different treatment of ZnO NP with *O. mossambicus.*

3. Results

The effect of ZnO NP on hematological and enzymological parameters of freshwater fish *Oreochromis mossambicus* was evaluated. The maximum mortality rate was recorded up to 30% in 100 ppm, but in the case of high doses recorded as 10% in 500 ppm whereas, in 400 ppm did not show any mortality. The other two doses of 200 and 300 ppm were exhibited as 20% mortality. After 4 days experiment, the mortality rate of fish was recorded as in the Table 1.

Table 1: The percentage of mortality was recorded during the experimental period for each concentration.

Oral dose administered (ppm)	Mortality recorded (%)
100	30
200	20
300	20
400	0
500	10
Control (no oral administration)	0

Physico-chemical parameters have a considerable influence on the toxicity of xenobiotics in organisms. Among these temperature, dissolved oxygen, hardness, alkalinity and pH are the important environmental factors. The data on the physico-chemical parameters of water used in the present investigation were normal and maintained at constant level throughout the study period (Table 2).

Table 2: Physico-chemical parameters recorded during the experimental period for control and treatment.

Parameters	Values
Temperature	27.16 ± 1.81°C
pH	7.18 ± 0.13
Dissolved oxygen	6.98 ± 0.52 mg/L
Total alkalinity	31.01 ± 5.41 mg/L
Salinity	0.13 ± 0.03 ppt
Total hardness	18.01 ± 0.16 mg/L
Calcium	3.33 ± 0.31 mg/L
Magnesium	2.05 ± 0.19 mg/L

The haematological values of *O. mossambicus* were recorded after 96 hours acute toxicity period in normal and treated with ZnO NPs (Table 3). The total RBC counts were recorded. A decrease of RBC counts was observed from 0.72 to 64.23% in comparison to control. But the highest reduction of RBC counts recorded at 200 ppm dose. The WBC showed an increases of counts from 3.90 to 28.90% in treated. But the higher WBC counts recorded in 100 ppm when compare with control. After the 4 days acute toxicity period the decreased Hb content noted in 100 ppm dose as 11.63%, whereas an increased Hb content of 27.27 and 36.36% were recorded in 400 and 500 ppm respectively when compare to control. Likewise, hematocrit showed an initial decrease of 12.11% at 100 ppm dose and gradually increased up to 500 ppm (35.98%) when compare with control. In contrast, the MCV exhibited a significant increase of 29.63 to 162.06% elevation in treated. MCH also showed a hike in treated animals as compare to control from 28.25 to 74.98%. Finally MCHC reveals no signnificant alterations in treated fish but only showing a marginal decrease up to 3.01% in 200 ppm and 400 ppm but a marginal increase was noted in 100, 300 and 500 ppm up to 0.54%.

Table 3: Haematological status in *O. mossambicus* treated with ZnO NP orally after 4 days period.

Parameters	ZnO NP given orally to *O. mossambicus* in ppm					
	Control	100	200	300	400	500
RBC (10^6/cu.mm)	2.74±0.11	1.66±0.06	0.98±0.03	1.49±0.05	2.72±0.09	2.47±0.11
WBC (10^5/cu.mm)	1.28±0.05	1.65±0.04	1.43±0.05	1.54±0.06	1.33±0.03	1.37±0.05
Hb (g/dl)	5.50±0.16	4.86±0.23	5.00±0.20	5.25±0.19	7.00±0.33	7.50±0.33
Hematocrit (%)	16.59±0.61	14.58±0.52	15.55±0.31	15.75±0.63	21.35±0.91	22.56±1.11
MCV (cubic micra)	605.47± 23.33	875.68± 33.33	1586.73± 61.66	1053.51± 43.11	784.93± 21.21	911.52± 31.36
MCH (pg)	20.07±0.96	29.19±1.33	51.02±2.33	35.12±1.16	25.74±1.08	30.30±1.31
MCHC (g/dl)	33.15±1.53	33.33±1.36	32.15±1.23	33.33±1.19	32.79±0.99	33.24±1.43

Mean value of three replicates ± standard deviation (n=3)

The antioxidant enzyme activity in gill, liver and kidney tissues of *O. mossambicus* was recorded after 4 days acute toxicity period in normal and with ZnO NPs treated. In this study the following parameters such as SOD, Catalase, GST, Lipidperoxidase and GSH were analysed. A prominent decrease in SOD activity was recorded in the gill tissues of treated compared to control. The medium decreases of 35.81% in 500 ppm and a minimum decrease of 9.32% in 100 ppm. The liver tissues a low SOD value when compared to gill and muscle, and also recorded the decreased SOD activity with respect to treated as compare to control. The decreased activity was recorded as 32.50% in 300 ppm and 48.76 and 49.10% in 100 and 400 ppm respectively. But, in muscle tissues the decreased SOD recorded as 27.75% only in 400 ppm as compare to control SOD whereas, the other treatment doses revealed the higher SOD activity and recorded maximum as 38.46% in 100 ppm (Figure 1).

and GSH recorded in *O. mossambicus* after 96 hours of oral treatment with ZnO NP.

In gill tissues the catalase enzyme activity was recorded in

treated and control. A highest reduction of catalase activity was recorded as 42.13% in 100 ppm and an elevation of catalase as 11.61% only in 300 ppm, also no variation in activity was encountered with control in 500 ppm. In liver tissues the reduced catalase activity was recorded in treated as compare to control. The highest reduction of catalase activity recorded as 80.41% in 100 ppm dose. The muscle tissues as well revealed a variable catalase activity among the treated groups compared to control. The highest activity was recorded as 54.12% in 100 ppm and reduced activity was recorded as 11.20 and 54.12% only in 500 and 300 ppm respectively (Figure 1). The reduced GST activity was estimated in gill tissues as 56.92% in 300 ppm dose whereas the marginal elevated activity recorded as 5.01% only in 500 ppm. In liver tissues, the variable activity was observed among the treatments and in 300 ppm which was maximum (489.97%) relative to control. Likewise, in the muscle tissues it showed highest activity as 301.06% in 500 ppm when compare to control (Figure 1). The Lipidperoxidase activity was estimated as variable activity in gill tissues among treated and highest increased activity recorded as 94.31% in 100 ppm. In contrast the liver tissues which could exhibit a reduction in lipid peroxidase activity as low as 41.27% in 100 ppm. Similarly in muscle tissues the highest suppressed activity was recorded in 200 ppm as 70.11% and the elevated activity revealed only in 100 ppm as 14.34% as compare to control (Figure 1). GSH activity was revealed in gill tissues as elevated up to 31.93% at 100 ppm and only reduced activity in 500 ppm as 23.04% when compare to control. Unlike, the liver tissue showed the elevated GSH activity among treated and showed maximum activity up to 3100% at 500 ppm. Likewise, the muscle tissue also revealed the highest activity in 500 ppm as 453.00% when compared to control (Figure 1). Statistically significant difference ($P<0.05$) could be observed among the treatment groups. The significant difference was observed in haemotological and biochemical parameters between the treated groups, and the significance is higher proportional to increasing concentration.

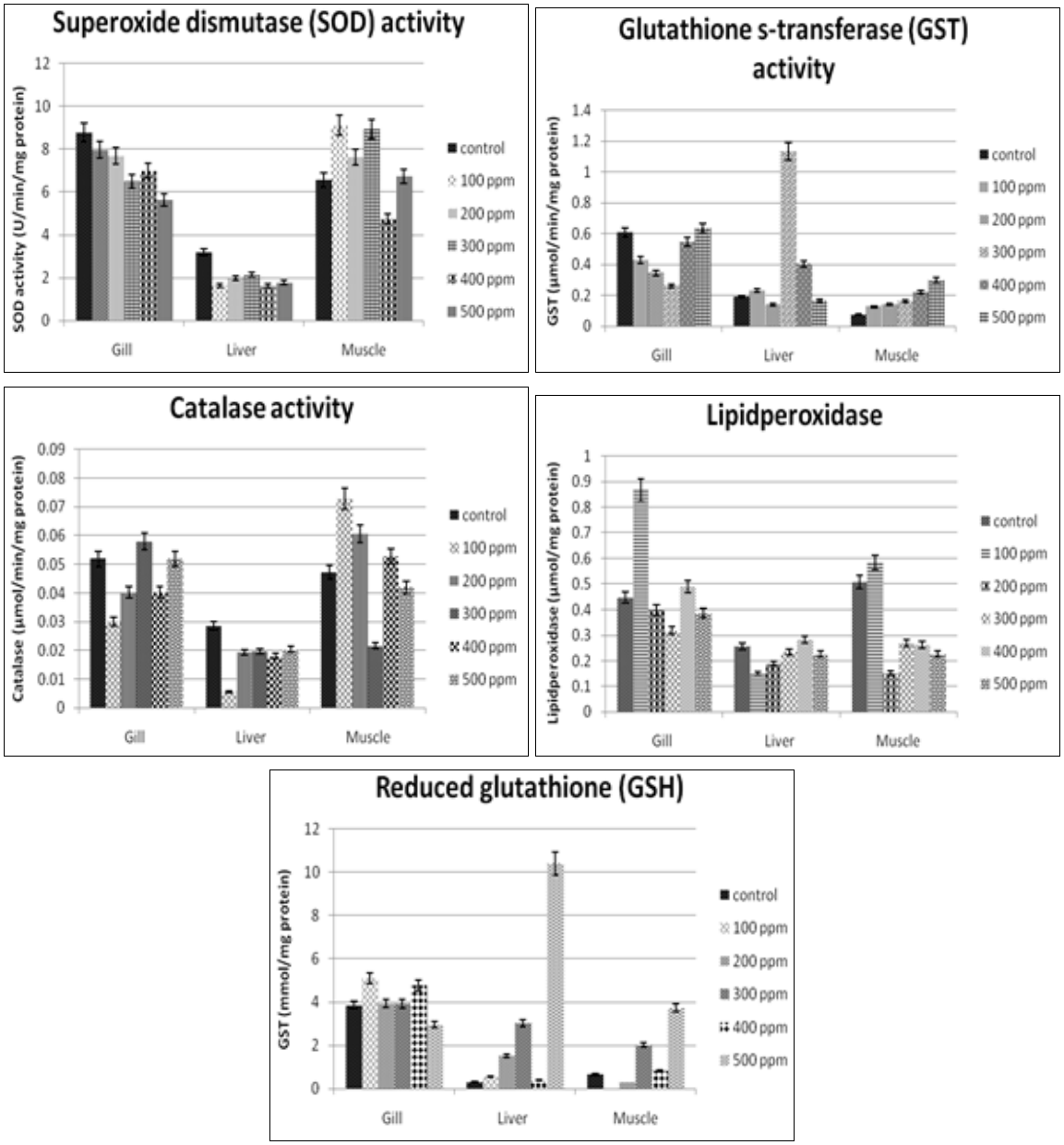

Fig 1: Aantioxidant enzymes and metabolites activity of SOD, Catalase, GST, Lipid peroxidase and GSH recorded in *O. mossambicus* after 96 hours of oral treatment with ZnO NP.

4. Discussion

Health benefits of nanoparticles are considerably attracting and is increasing the concern of public and government worldwide (Jeevanandam *et al.*, 2018) [11]. NP can be ingested directly via water, food, cosmetics, drugs and drug delivery devices, etc. (Arora *et al.*, 2012) [3]. The insoluble particles, their pathway and extent of uptake through the digestive tract are ascertained to be based on their size (Date *et al.*, 2016) [8]. NP can cross small intestine by persorption and further distribute into blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach (Swain *et al.*, 2016) [40].

The nano size ZnO particles linked with very high activity likely interact with the surrounding tissues and aggravate normal functions of the organs and it can diffuse across the membrane occurs directly when the size is small enough, there are positive ions on the surface of ZnO NP, or when other variables are present (Chang *et al.*, 2012) [5]. In the present study, the ZnO NP were administered orally at a wide range of concentration to tilapia and the acute toxicity effects were recorded. The efficacy of ZnO NP was examined like variations in haemological parameters and reaction of defense enzyme against ZnO NP. ZnO NP (30 nm) are able to bind proteins with important biological functions, including immunoglobulins and proteins adhering to ZnO NP result in enhanced stability of the NP. The proteins that are bound by ZnO NP are physiologically important (Vandebriel and Jong, 2012) [41].

The concentration of haemoglobin, hematocrit and total RBC count in the blood parameters were lower in the fishes exposed to ZnO NP at 100, 200 and 300 ppm reletive to the control, thereby depicting an anaemic condition. This study revealed that reduced haemoglobin content was observed in treatments compared to the control, especially fishes are exposed to lower i.e. 100, 200 and 300 ppm doses in the present study. Ergonul *et al.* (2018) [10] reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be attibuted as pathological conditions in fishes exposed to toxicants. Okomoda *et al.* (2013) [26] studied the reductions of haemoglobin content, RBC, Platelet count, packed cell volume, as the concentration of toxicant increased while other parameters increased proportional with the toxicant concentration in African catfish. A decrease in the concentration of haemoglobin in blood might usually be due to the effect of toxic metals on gills, as well as decrease in oxygen, which suggests anaemia or confirms toxic impact in *Clarias gariepinus* (Adeyemo, 2007) [1]. Increase in WBC count, MCV while decrease in RBC count from normal and infected in brackishwater fish *Eleginops maclovinus* (Pena-Rehbein *et al.*, 2013) [30] and in fresh water fish *Prochilodus lineatus* (Parma et al. 2007) [37]. The present results were similar to the above that increased WBC count and MCV in all treatments whereas the decreased RBC count could only be observed in 100, 200 and 300 ppm.

The MCV, MCH and MCHC increased considerably in all treatments compared to the control. However, only the marginal increases could be observed in MCHC with all treatments. This results was compared with the work of Shah (2006) [34] and following a short term exposure of tench (*Tinca tinca*) to lead and to Zinc (Ergonul *et al.*, 2018) [10] and in *Clarias gariepinus* (Adeyemo, 2007) [1]. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006) [34] and to cypermethrin (Parma *et al.*, 2007) [29].

The liver tissues of fish are more often recommended as an environmental indicator of water pollution than any other organs. The toxicants cause a disturbance in the physiological state of the fish, which affects the enzyme activity. It then causes distortions in the cell organelles, which may lead to the elevation in the activity of various enzymes (Kumari *et al.*, 2014) [16]. So, in present study the ZnO NP were administered orally at different wide range of concentration to tilapia and the acute toxicity effect was observed. The defensive free radical scavenger, superoxide dismutase (SOD), triggers an induction response in heavy metal intoxicated groups. This indicates that more protein is required to protect cells against superoxide radicals. The superoxide dismutase level was found to be increased in muscle tissue exposed and the decrease were noticed in gill and liver tissues. However, the hepatic superoxide dismutase showed increased activity when compared to kidney. This indicates the liver stress developed by the ZnO NP. The decreased SOD activity was recorded about the gill tissues of treated, when compare to control and highly decreased as 35.81% in 500 ppm and lowly as 9.32% in 100 ppm in present study with tilapia. The liver tissues also recorded the decreased SOD activity with respect to treated as compare to control. The increased superoxide dismutase activity muscle of tilapia may be explained as a compensation mechanism against nano ZnO intoxication, which was similar to the results observed with increased superoxide dismutase activity after exposure to pollutants (Dimitrova *et al.*, 1994) [9].

The peroxy radical H_2O_2 was trapped by catalase that primarily occurs in peroxisomes. The target function of catalase is to protect the cells from the accumulation of H_2O_2 by dismuting it to form H_2O and O_2 or by using it as an oxidant where it works as a peroxidase. No discernible effects were observed in the catalase activity of all tissues of present investigation after 4 days of exposure, the activity increased in the muscle and gill tissues, but in the liver tissues the decreased H_2O_2 was observed. Though muscle and gill tissues could also reveal the variable catalase activity among the treatments, but highest increased activity was recorded as 54.12% in 100 ppm for muscle and 11.61% in 300 ppm for gill tissue of tilapia. This indicates a reduced activity to protect the cells against H_2O_2. Evidence suggested that high concentration of copper inhibited catalase activity in the liver, gills, and muscle (Kroon *et al.*, 2017) [14].

Glutathione-S-transferase (GST) is an abundant cytosolic antioxidant involved in conjugation of toxic reactive metabolites. The higher tripeptide content is involved in the activation of γ-glutamylcysteine synthetase, one of the enzymes involved in glutathione synthesis (Srikanth *et al.*, 2013) [39]. The enzyme glutathione-S-transferase was found to be increased in the liver during the exposure days. In present study the muscle tissues exhibited increased enzyme activity and decreased activity in gill tissue of tilapia. The higher glutathione- S-transferase activity observed in the liver of the carp after heavy metal toxicity indicates an augmented detoxification activity in the liver tissue. The glutathione-S-transferase detoxifies a number of environmental carcinogens, reactive nucleophiles, and epoxide intermediates. The increased glutathione-S-transferase assay was suggested as a useful tool for biomonitoring oxidative stress (Kuder and Philip, 2017) [15]. The findings reveal that heavy metals create

harmful effects by generating reactive oxygen species that damage the cells by disturbing the fluidity balance. They also suggest that the heavy metals could make molecular complexes with cell protein thiols and develop toxic effects on the cells towards dysfunction. However, it was counter balanced by the production of antioxidants to suppress the free radicals and protect the cells against oxidative damage.

The extent of lipid peroxidation was determined by a balance between production of oxidants, and their removal, scavenging by antioxidants. The malondialdehyde (MDA) concentration, an index of lipid peroxidation level generally increased in the organs studied after exposure. Many studies have demonstrated that lipid peroxidation and oxidative stress increases in tissues of different species of aquatic organisms, as a result of being exposed to environmental stressors (Kuder and Philip, 2017) [15]. However, it is by no means a general rule that exposure to a pollutant increases the MDA level. Though highest MDA level was estimated as variable activity in gill, liver and muscle tissues among treated and highest increased activity recorded as 100 ppm (94.31%) for muscle, 400 ppm for liver and 100 for muscle tissue of tilapia in present study. Some authors have shown lowered MDA levels in fish sampled in a site contaminated by metal and organic compounds (Kroon *et al.*, 2017) [14], and others have shown that fish show no response when exposed to azinphosmethyl and 2,4-dichlorophenoxyacetic acid (Oruc and Uner, 2000) [28]. The increased level of malondialdehyde observed in treatments when exposed to organs of tilapia in the present investigation, agreed with the previous investigation carried out with tilapia that had been exposed to microcystins (Moreno *et al.*, 2003) [21].

The elevated GSH acitivity in liver tissue could observe in all the ZnO NPs treated and as high as activity of 3100% at 500 ppm when compare to control in present study with tilapia. The major mechanism for metal toxicity appears to be direct and indirect damage to the mitochondria (via) depletion of glutathione, an endogenous thiol (SH–) group containing antioxidant, which results in excessive free radical generation and mitochondrial damage (Sharma *et al.*, 2012) [35]. Oral administration of 100 nm ZnO NPs (2.5 gm/kg body weight) to fish resulted in accumulation in the liver, spleen, lung, and kidney (Vandebriel and Jong, 2012) [41]. The penetration of metal and metal oxide in the gills, liver, kidney, and flesh antagonizes the antioxidant in hepatocyte mitochondria, depletes glutathione through the generation of free radicals, causes lipid peroxidation, and directly causes uncoupling of mitochondrial respiratory chain activity (Srikanth *et al.*, 2013) [39].

In present acute oral toxicity studies in *O. mossambicus* did not show any toxicity and it is showed the maximum mortality rate up to 30%. Based on these results the acute oral LD_{50} value may takeplace either greater than 500 ppm or lower than 100 ppm, or it may not cause any mortality rate or LD_{50} value. These results are similar with the acute oral and dermal toxicity studies in Wistar rats of two different sizes with Multi-Walled Carbon Nanotubes (MWCNT) that did not show any toxicity (Murthy *et al.*, 2011) [23]. The physico-chemistry, behaviour and measurement of manufactured nanoparticles in the aqueous phase and the key chemistry issues for the eco-toxicologist are described elsewhere (Bundschuh *et al.*, 2018) [4], including the dispersion of manufactured nanoparticles in different natural waters, their surface chemistries and reactivity, and their aggregation and colloid chemistry. There are some emerging features of the physico-chemistry that are highly relavent to routes of uptake in fish. One of the founding assumptions in the ecotoxicity of chemicals is that there is likely to be close relationship between the physical or chemical form of the substances in the environment, the ability of the organism to absorb it, and any subsequent toxic effects. In the present study the physico-chemical parameters of the experimental tank water were maintained in normal and optimum condtions. It was performed (Murthy *et al.*, 2011) [23] that prior to toxicology experiments the physicochemical characterization of MWCNT like, size in dry state and distilled water, surface area to ascertain the possible cause for the toxicity. The two different sizes of MWCNT in distilled water showed increase in the size. Murdock *et al.* (2008) [24] reported that once the nanomaterials are in water they do not necessarily retain their nanosize. Both the sizes (140 ± 30, 10-15 nm) of MWCNT exhibited similar kind of results in acute oral, dermal and ocular irritation tests.

Conclusion

In the present study, the ZnO NPs has caused alterations on hematology and enzymology of the fresh water fish *O. mossambicus* and these alterations may be taken as non specific biomarkers in the field of nanotoxicology. However, the toxicity of ZnO NP could be evaluated through the application of a wide range of concentrations. In general these toxicological assessment methods are used to identify the safety or adverse health effects of the products with these chemicals.

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