Impact of Nanoparticle On Enzymes Activity In Oreochromis Mossambicus

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Abstract: Toxicity of Titanium dioxide nanoparticles (TiO_2-NPs) to freshwater fish, *Oreochromis mossambicus* is assessed in this work. The endpoint of this study has focussed the disturbances in the vital enzyme profile of fish exposed to TiO_2-NPs. Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Lipid per Oxidation (LPO) levels in brain, gill and liver tissues of tilapia were analysed and observed that these values were concentration and duration dependent. The results obtained in this study have projected a statistically significant decrease ($P < 0.05$) in SOD, CAT and POD activities and an increase ($P < 0.05$) in LPO levels in selected tissues of fish exposed to 50, 100 and 150 ppm TiO_2-NPs. Further, this finding suggests that there is an oxidative stress in fish. Moreover, the depletion of antioxidant enzymes activities and the elevation of LPO in liver tissue are higher indicating that the liver might be the most susceptible organ to TiO_2-NPs exposure. Thus hepatotoxicity is underlined in this work.

Key words: TiO_2 nanoparticles; tilapia; sub-acute toxicity; oxidative stress, antioxidant enzymes.

1. Introduction

In recent years, nanomaterials widely used in domestic products such as medicine to make up products. Nano-titanium dioxide (nano TiO_2) is a popular ingredient in some cosmetics and sunscreens because it blocks harmful ultraviolet rays and, unlike its conventional counterpart, it is transparent instead of white. Metal-containing engineered nanoparticles (NPs) are to be assayed for its toxicity in aquatic organisms to protect the environment and human health. In fish, the respiratory and ion transport surface area is greater than 60% of the total surface area of the animal (Rombough and Moroz 1997), presenting a large area for potential interaction with nanoparticles. The nano metal particle might interact with gill ion transport mechanisms, resulting in ionoregulatory failure (Pane et al. 2004), and this highlights the toxicity of nanoparticles as well. (Tyson et al 2008). The liver of fish is a probable target of NPs following endocytic transport across the intestinal epithelium into the hepatic portal blood system followed by endocytosis into hepatocytes (Smedsrud et al 1984). Numerous studies have demonstrated an increase in the production of reactive oxygen species (ROS) in tissues exposed to specific nanoparticles (Oberdorster 2004; Sayes et al 2004; Hussain et al 2005; Lin et al 2006). Another study by Zhu et al (2009) shows the acute toxicities of nanoparticles to be dose dependent. In their tests, TiO_2, Al_2O_3 and Carbon nanomaterials were more toxic than their bulk counterparts. Exposure of rainbow trout to nano TiO_2 has displayed oxidative stress (Federic et al 2007). As there is scanty report available on influence of TiO_2 NPs on the antioxidant enzymes in freshwater fish, toxicity of TiO_2-NPs on the activities of anti-oxidant enzymes, Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Lipid peroxidation (LPO) in brain , gill and liver tissues of tilapia has been attempted in this work.

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2. Materials and Methods

2.1.TiO_2-NPssuspension preparation

Titanium dioxide nanoparticles (TiO_2-NPs, particle size in 50 nm, a surface area of (30 ± 10) m2/g, anatase and rutile form in crystal structure, with a purity > 98.0%).TiO_2-NPs suspensions were sonicated for 30 min in a bathtype sonicator (100 W, 40 kHz) to disperse the particles. To investigate the suspension stability of TiO2-NPs in water, different concentrations (50, 75,100 ppm) of TiO_2-NPs suspensions were prepared in triplicate. The TiO_2-NPs concentrations in aqueous solution were determined every day for 6 day according to the method introduced by Zhang and Sun (2006). In addition, sedimentation rate of TiO_2-NPs in water was quick even the water was continuously aerated. The actual concentrations of TiO_2- NPs decreased by 50% at day 3, and only 30% of the initial concentration were remained at day 6. Therefore, in the following experiments, to sustain the concentration of TiO_2-NPs, the volume of TiO_2-NPs suspension (100%) was changed every day.

2.2. Exposure of TiO2-NPs to fish

Tilapia were obtained from a local fish farm, Madurai. The initial body weight and length of the fish were 5g and 7.0 cm, respectively. The water used to culture the fish was dechlorinated and continuously aerated tap water. All fish were acclimatized for one week in stock aquaria and then randomly graded into different experimental glass tanks in triplicate (3 tanks/treatment). Fish were not fed during experimental period to avoid adsorption of TiO_2-NPs by food or fecal materials. The tilapia was cultured with a natural light/dark cycle. Water quality parameters including pH, dissolved oxygen, temperature and hardness were measured before and after each water change. During the tests, the temperature of water was maintained at (21 ± 1)°C for each exposure. The pH in exposure water was 7.1–7.3, the dissolved oxygen was 6.0-6.5 mg/L, and total hardness in water was 94 ppm (as CaO). Control group without TiO2- NPs was also maintained under the same conditions. The effect of duration on fish was also examined.

2.3. Oxidative stress parameters analysis

According to preliminary test results, the tilapia was exposed to 50, 75,100 ppm TiO_2-NPs for 8 d using a semi-static exposure test. The experiment was designed to allow

for sub-lethal physiological effects over the exposure period. The exposure time of 8 d was chosen to enable some physiological or biochemical responses to the exposure. Ten fish per treatment were randomly collected from each tank at day 1, 2, 4, 6 and 8, respectively for biochemical analysis. Brain, Gill , Liver tissues were removed separately and immediately snap-frozen in liquid nitrogen and stored at –20°C until needed. The frozen tissues were rinsed in 9-fold chilled 100 m mol/L, pH 7.8 sodium phosphate buffer solution and homogenized by a hand-driven glass homogenizer. The homogenates were centrifuged at 10000 rpm at 4°C for 20 min and the supernatant was stored in Eppendorf tubes at 4°C. The liver supernatant was diluted with 9-fold chilled sodium phosphate buffer solution to 1%. The prepared supernatants were analyzed for antioxidant enzymes, i.e., Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD) activities to determine possible effects on oxidative stress and antioxidant defense, and LPO level was also measured for the content of malondialdehyde (MDA). All assays were preformed in triplicate. The SOD activity was estimated based on its ability to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated by xanthine/xanthine oxidase according to the modified method of Beauchamp and Fridovich (1971). LPO was measured using the thiobarbituric acid (TBA) assay by the method of Buege and Aust (1978).

3. Results and Discussion

In our study, there was obviously change in antioxidant enzymatic activities of chosen tissues after tilapia exposed to the selected concentrations of TiO_2-NPs. SOD is the first enzyme to deal with oxyradicals and responsible for catalyzing the dismutation of highly superoxide radical O_2 to O_2 and H_2O_2. It is very sensitive to the stress of pollutants and can be used as oxidative stressed signal for the early warning of environmental pollution. The depletion of SOD activity is used as an indication of free radical scavenging ability, showing that the antioxidant defense system is overwhelmed by ROS (Vander *et al*., 2003). In the present study, SOD activities in brain, liver and gill tissues of tilapia (Fig.1a, b & c) fluctuated with nano particle concentration and exposure time. Exposure to 50 ppm TiO_2-NPs, SOD activities of chosen tissues of fish were stimulated and showed a remarkable increase, which might be due to the synthesis of new enzymes or the enhancement of pre-existing enzyme levels under lower concentrations. At 75 ppm, the SOD activity initially increased and peaked at day 2 in liver, and at day 4 in gill and brain, then went down close to that of the control.

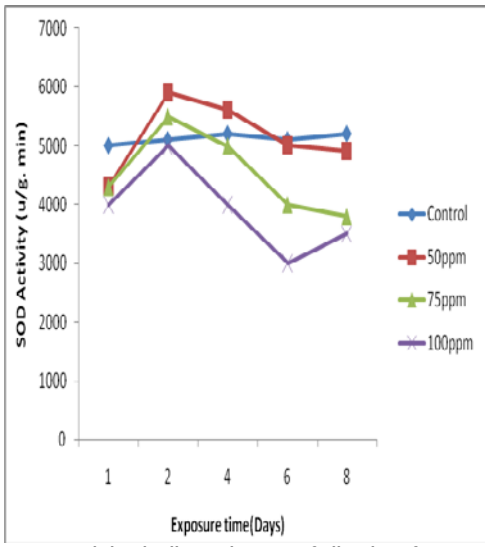

Fig.1a. SOD activity in liver tissue of tilapia after exposed to TiO2-NPs.

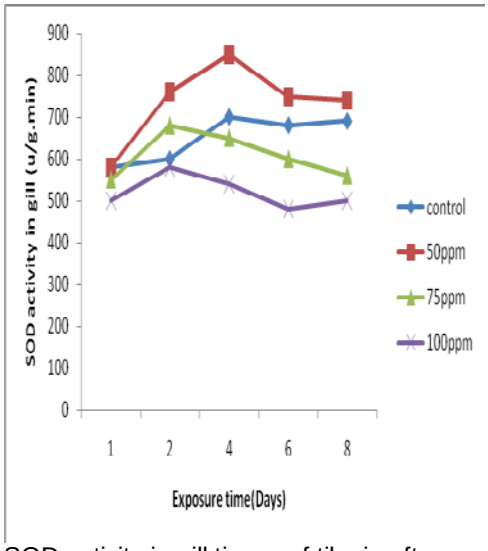

Fig.1b. SOD activity in gill tissue of tilapia after exposed to TiO2-NPs.

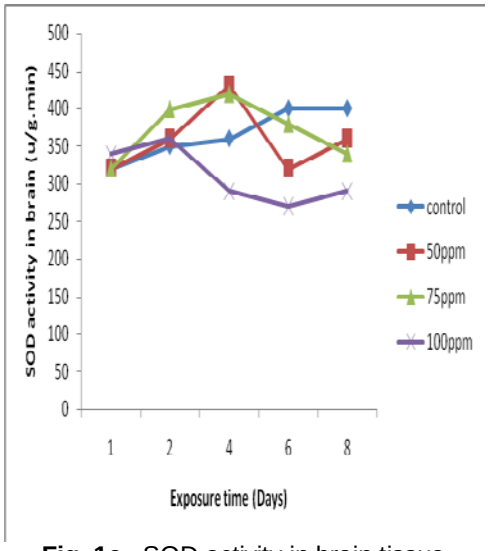

Fig. 1c. SOD activity in brain tissue

This trend for the depletion of SOD activity might be an indication that the antioxidant defense systems of these tissues were being stressed. However, at 100 ppm TiO_2-NPs, there was a small rise at the beginning and then a sharp decrease in SOD activity, indicating that due to over-produced ROS and decreased defense capability, the SOD activity was inhibited, and oxidative stress occurred (Kong *et al*., 2007). In addition, the SOD activity in liver was 5–10 folds of that in gill and brain at the same exposure concentration, showing that the liver might be the most sensitive organ to TiO2-NPs exposure.

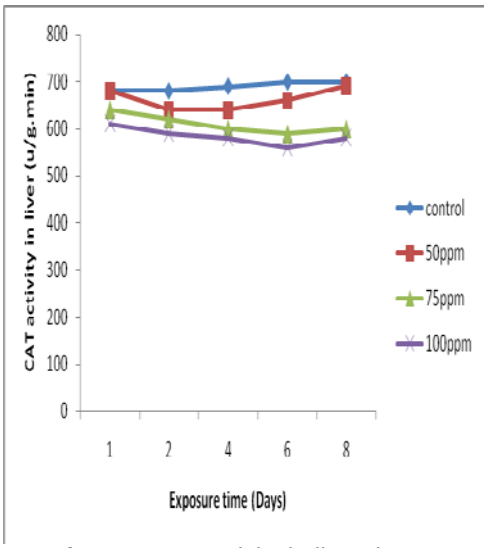

Fig. 2a. CAT activity in liver tissue

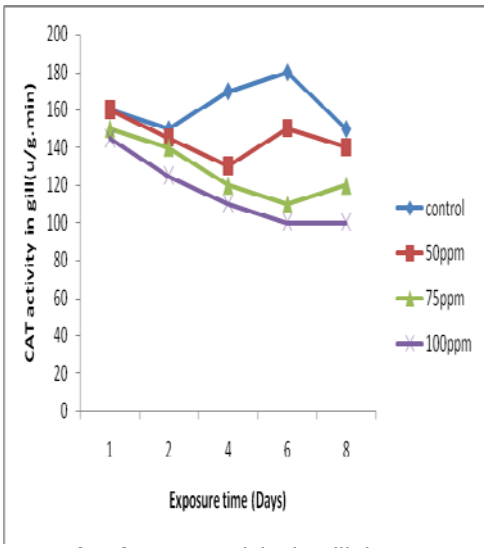

Fig.2b. CAT activity in gill tissue

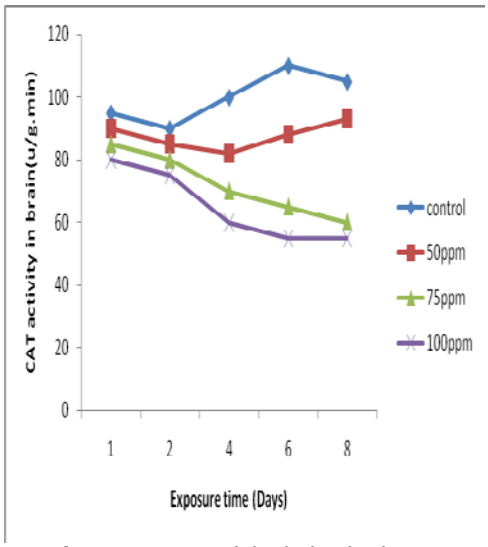

Fig.2c. CAT activity in brain tissue

CAT and POD are also the key enzymes in antioxidant defense systems to convert the resulting free radicals H_2O_2 to water and oxygen. Exposure to 50 mg/L TiO_2 NPs, the CAT activity (Fig.2a, b & c) in chosen tissues showed a slight decrease up to day 2 and then a remarkable elevation. At 75 ppm, the CAT activity slowed down until day 4, and then increased close to control level. However, 100 ppm TiO2-NPs caused a considerable decrease in CAT activity up to day 6 in tissues. Results indicated that under stress CAT activity were inhibited and ROS scavenging weakened and accumulated gradually in the major tissues of fish. Similar to SOD and CAT, the POD activities (Fig.3a, b & 3c) followed a similar pattern in fish tissues with a remarkable increase at lower concentrations of TiO2- NPs and a significant reduction at higher concentrations.

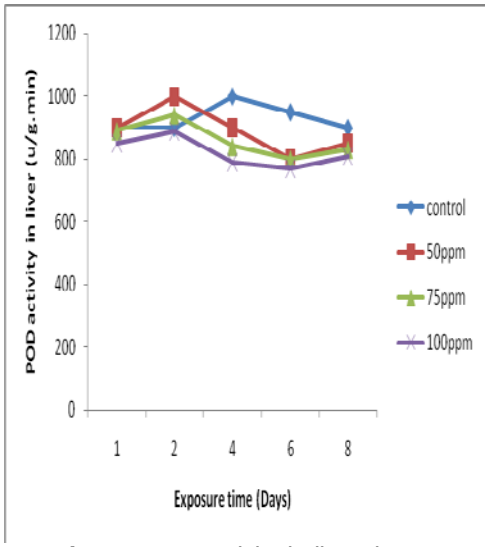

Fig.3a. POD activity in liver tissue

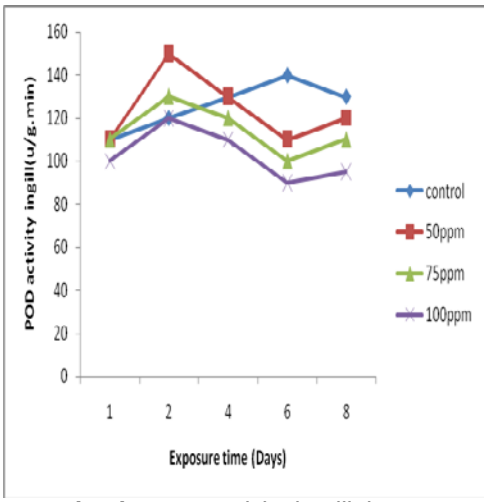

Fig.3b. POD activity in gill tissue

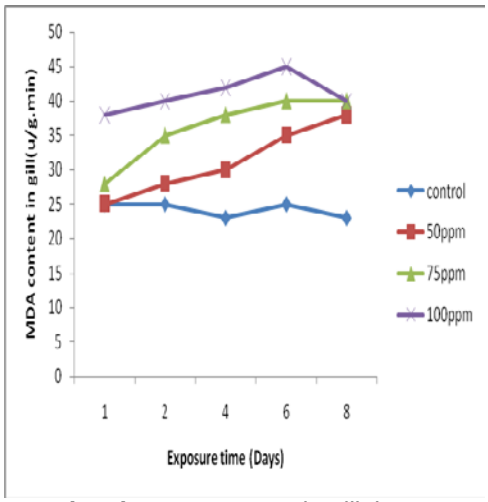

Fig. 4b. MDA content in gill tissue

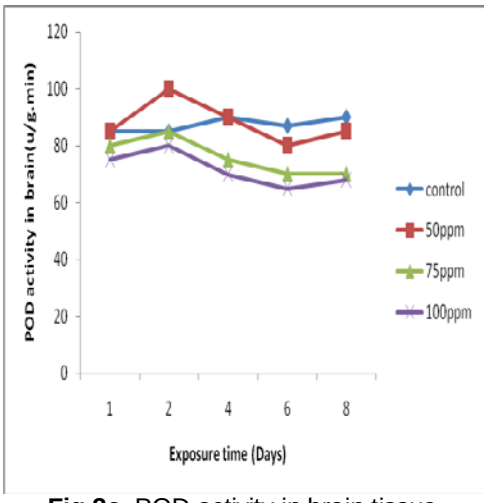

Fig.3c. POD activity in brain tissue

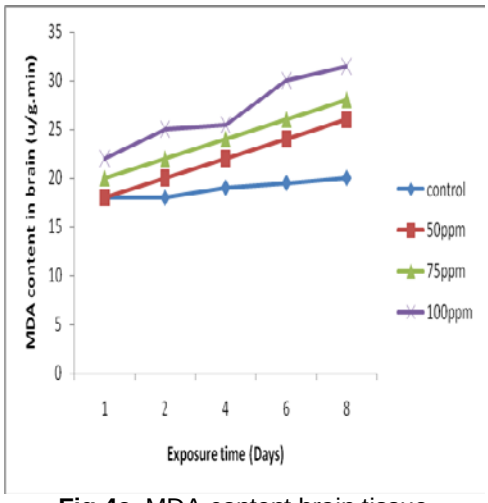

Fig.4c. MDA content brain tissue

Variations of CAT and POD activity were not the same, but they were coordinated with each other and jointly played roles in antioxidant defense systems. In addition, the CAT and POD activity in liver were 2–3 folds and 5–10 folds of that in gill and brain at the same exposure concentration, respectively. Significant response of CAT and POD in liver tissue again indicated that the liver might be the most susceptible organ to TiO2-NPs exposure.

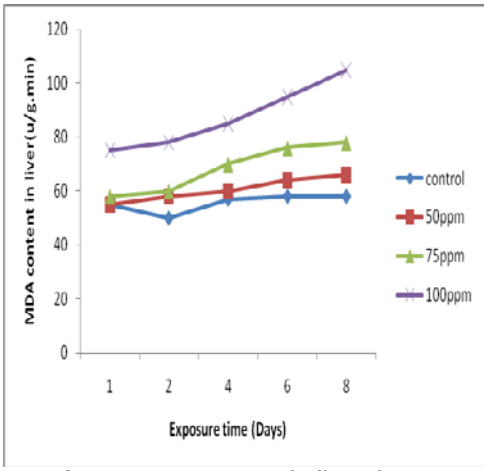

Fig.4a. MDA content in liver tissue

LPO can be defined as the oxidative deterioration of cell membrane lipids and has been used extensively as a marker of oxidative stress (Sayeed *et al*., 2003). LPO is estimated by measuring the content of MDA (Fig.4a, b & c). The over-accumulation of MDA can damage cells and trigger apoptosis (Kong *et al*., 2007).Zhu *et al*. (2007) suggested that a free radical-induced mechanism played an important role in the toxicity of C60 to zebra fish embryos. Zhu *et al*. (2008b) also demonstrated that oxidative stress induced by 32 d exposure could be the main mechanism of the toxicity of C60 to juvenile carp. In this study, MDA contents in liver, gill and brain tissues were not obviously different from those in control after exposed to 50 and 75 ppm TiO_2-NPs, however, the significant increase in MDA level was found after 8 d of exposure to 100 ppm TiO_2-NPs. It indicated that these tissues were undergoing oxidative stress, which was consistent with our results of higher concentration of TiO_2 NPs exhibiting more potent effects on disturbance to the antioxidant defense systems in tilapia. The zero increase in LPO, suggesting that the liver was using up antioxidant defenses to prevent oxidative stress.

4. Conclusion

The present study shows that TiO_2-NPs can cause oxidative stress, leading to the depletion of antioxidant enzymes activity and the elevation of LPO level in fish. Among the chosen tissues, liver tissue might be the most susceptible to TiO_2-NPs in cat fish. Hence, release of TiO_2-NPs into the aqueous environment may be scientifically monitored and managed.

5. Acknowledgments

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6. Reference

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