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Acute exposure to nano Titanium dioxide cause biochemical and physiological alterations in the Zebra fish (Danio rerio) - A Case Study

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Abstract: Titanium dioxide nanoparticles ($nTiO_2$) are usually perceived as non-toxic, and have been extensively used in many products and applications. Whilst short-term studies that revealed no/low toxicity of nTiO2 to cells and eco-relevant organisms, the long-term impact of $nTiO_2$ on the environment and human health is still a concern and warrants research. Keeping this in view, we made an attempt to examine the biochemical changes in Danio rerio exposed to 2.5, 5.0, 7.5, 10 and 12.5 mg/L of $nTiO_2$. There were noticeable changes in the oxygen consumed by the fishes exposed to these concentrations which shows that the toxicant proved to be a potential respiratory inhibitor ($p<0.01$) in 12.5mg/L and 10.0mg/L at the end of 96h, ($p<0.05$) in 12.5mg/L and 10.0mg/L at the end of 24h, ($p<0.05$) in 7.5mg/L at the end of 96h respectively, demonstrating a time-concentration relationship. Significant decrease was observed in total glycogen and total protein at the end of 96h in fish exposed to 10 and 12.5 mg/L.
Key words: nanoTiO_2, zebrafish, acute toxicity, biochemical profiles, oxygen consumption.

1. Introduction

The advent of nanotechnology and the multifaceted utility of nanomaterials have led to great prospects for engineering new products and applications in various sectors. The recent upsurge in production, variety and applications of manufactured nanomaterials (NMs) has raised concerns that the release of these materials into the environment may pose a serious threat[1-4]. Whilst engineered nanoparticles in the nanoscale range of 1-100 nm have excellent and useful properties, the same can be problematic from toxicological perspective. Currently large gaps exist in our knowledge and understanding of the toxicity and exposure of nanomaterials for aquatic organisms, which hinder their risk assessment. Therefore, increased research is warranted on various toxicological issues related to nanoparticles.

Titanium dioxide (TiO_2), also known as titanium (IV) oxide, naturally occurs in three forms viz., anatase, rutile and brookite. Occupational exposure to TiO_2 nanoparticles can occur during their occupational exposure in the manufacture of pigments for paints, varnishes, enamels, lacquers

and paper coatings to impart whiteness, opacity and brightness[13]. Titanium dioxide nanoparticles (TiO_2-NPs) were produced abundantly and used widely because of its high stability, anticorrosion and photocatalytic property. Many researchers consider TiO_2 micro-material to be biologically inert to animals and humans[5] while TiO_2 nanoparticles are incorporated into cellular membranes and cytoplasm of mammalian cells[22]. Nanoparticles may be more toxic than larger particles of the same substance[7] because of their larger surface area, enhanced chemical reactivity, and easier penetration of cells. Nevertheless, several studies have shown that the cytotoxicity of TiO_2 nanoparticles was very low or negligible as compared with other nanoparticles[8], and the size was not the key factor of cytotoxicity[9]. Evidence of genotoxicty has been previously reported wherein micronuclei development is associated with TiO_2 exposure, which is indicative of chromosomal damage, DNA damage has also been observed in response to TiO_2 particulate exposure. The ability of TiO2 NPs to inflict DNA damage has been observed on numerous occasions, and is thought to be driven by particle mediated ROS production, with cell death often stimulated as a protective response.

Exposures can also occur from their use in cosmetics such as in sunscreens, dusting powder, ointments and from radioactive decontamination of the skin. Although it is presumed that toxicity of nano titanium dioxide to humans is little to none, the effect of nanoparticles in the environment is questionable. The known toxic effects of TiO2-NPs raise concern about aquatic organisms such as fish. The size (and therefore surface area) of TiO_2 nanoparticles is known to be fundamental to their toxicity. The aggregation and agglomeration state of nanoparticles is also likely to be influential to their toxicity. However it is often observed that agglomerates/aggregates of nanoparticles are more toxic than similarly sized agglomerates/aggregates of their bulk counterparts. As common engineered nanomaterials, TiO_2 nanoparticles ($nTiO_2$) are usually perceived as non-toxic, and have already been widely used in many products and applications. Short-term studies reveal no/low toxicity of $nTiO_2$ to cells and eco-relevant organisms. However, given the ultimate release of $nTiO_2$ into the aquatic environment, which can act as a sink for engineered nanoparticles. In light of this, long-term impact on the environment and human health is still a concern and deserve more research efforts.

Up to now, ecotoxicological studies with TiO2-NPs are rather limited with only a few reports of aquatic organisms[10,11] conducted the toxicological assessment of TiO_2-NPs in rainbow trout (*Oncorhynchus mykiss*) and concluded that TiO2-NPs cause respiratory distress and sub-lethal toxicity due to oxidative stress and histopathology. Reeves et al. (2008)[12] recently reported cytotoxicity and DNA damage in goldfish skin cells (GFSk-S1) induced by TiO2-NPs. Detailed information of the toxic effect and mechanism of TiO_2-NPs on fish is limited. Hence, in the present study, an attempt was made to study changes in the total glycogen and protein contents of the zebra fish exposed to 2.5, 5.0, 7.5, 10 and 12.5 mg/L of $nTiO_2$

2. Materials And Methods

The zebra fish is one of the widely used research model for toxicity testing. Its high fecundity, small size, rapid generation time, morphological and physiological similarity to mammals, easily observable and testable developmental behaviors, low cost, easy to perform bioassays, optical transparency during early embryogenesis, fully-sequenced genetic code showing a homology with humans up to 65% have made it the ultimate testing model (Christian Lawrence, 2007). Due to these attributes, the zebra fish might also provide opportunities to accelerate the process of drug development process, disease modeling, and toxicology (Leonard and Randall, 2005) and in numerous other disciplines, including animal behavior, fish physiology, and aquatic toxicology.

Test compound: Titanium Dioxide (Sigma-Aldrich)
Formula: TiO2
Molar mass: 79.87 g/mol
Particle size: ~21 nm
Appearance (Color): White
Appearance (Form): Powder
Trace Metal Analysis: < 5000.0 ppm
Purity:Meets Requirements ≥99.5% Based on Trace Metals Analysis
Surface Area(m^2/g): 35 - 65
pH: 3.5 - 4.5
Water Content: ≤ 1.50 % HCl Content: ≤ 0.30 %.

2.1 Fish collection and maintenance

Adult specimens of *D. rerio* with a mean length 3.15 ± 0.63 cm and weight 0.35 ± 0.05 g were used for toxicity tests. These were procured from a local aquarium shop near Habsiguda, Hyderabad, transported in polythene bags to the lab and

immediately transferred into plastic tubs of 20 L capacity containing well-aerated and dechlorinated ground water. The fishes were allowed to acclimatize for 20 days before the experiments with a natural light-dark cycle (12 h light /12 h dark). They were fed with tubifex worms and commercially available feed pellets rich in fishmeal, vegetable proteins and wheat during the acclimation period. Only healthy fishes that showed active movements were used for experiments. The physico-chemical characteristics of the water used in the present experiments were temperature. 28-29 ^{0} C, pH 7.2 ± 0.2, DO 5.0-6.5 mg/L and hardness 220 mg/L.

Environmental and chemical stress can interfere with physiological and biochemical functions and lead to alterations in the metabolic rate, biochemical constituents and hematology of fish. As it is known that glycogen is a stored form of energy which can be easily mobilized by fish under stress. Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs. In light of these, the present study used ten fishes exposed to nTiO2 at each concentration and ten fishes in the control group. Six fishes were sacrificed at the end of 24 h and five at the end of 96 h. The dissected samples of gill and muscle tissues were collected rinsed with cold saline, weighed, and then immediately snap frozen at −20 °C until they were analyzed. Total Glycogen and Protein content was estimated from the samples by the following methods.

2.2 Total Glycogen

Glucose reacts with concentrated sulphuric acid and gets hydrolysed to yield a furfural complex which has a characteristic pink color that has absorption maxima of 520 nm. The intensity of the pink color is directly proportional to the amount of glucose present in the sample. Briefly, the tissue were weighed and homogenized in 5 ml of 5%TCA, which acts as deprotienizing solution in a glass mortar with pestle. The homogenates were transferred into the centrifuge tubes and centrifuged at 2000 rpm for 10 minutes. The clear supernatant was taken as the source of glycogen. To 1ml of supernatant 3 ml of concentrated sulphuric acid was added and kept in boiling water bath for 6.5 minutes exactly and subsequently cooled in running tap water and the color intensity was read at 520 nm. A blank is prepared with 1ml of TCA and 3ml of concentrated H_2SO_4 was also run simultaneously. The glycogen values were derived directly from the glucose standard graph. The results are expressed as mg of glucose per g wet weight of the tissue.

2.3 Total protein

A deep blue color is formed when protein is treated with phenol reagent of Folin-ciocalteu .Two colored reactions takes place simultaneously: The peptide bond of protein reacts with copper in an alkaline solution. A reduction of phosphomolybdic acid and phosphotungstic acid present in FC phenol reagent by the aromatic amino acid tyrosine and tryptophan present in protein. Briefly, the dried tissues were homogenized in 5 ml of TCA and centrifuged at 2000 rpm for 10 minutes. The solution containing the precipitate was dissolved in 1.5 ml of 0.1 N NaOH. From this 0.2 ml was taken to the tube and added 5 ml of reagent C. Mixed thoroughly and incubated at room temperature for 15minutes. Then 0.5 ml of Folin reagent is added and shaked thoroughly. After 30 min the O.D values were read at 540 nm against blank by using colorimeter. Simultaneously, a blank was also run by adding 5 ml of reagent C to 1.5 ml of NaOH followed by 0.5 ml of Folin phenol reagent.The protein content of the tissue was calculated from standard graph plotted using Bovine serum albumin and the results were expressed as mg/g wet weight of tissue.

3.0 Results And Discussion

3.1 Biochemical biomarkers:

The glycogen and total protein contents expressed as mg per gram wet weight of the tissues gills and muscle of *Danio rerio* exposed to 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L of nano-TiO2 for 24 and 96h are presented in Tables 1- 4.

Glycogen:

(a) Gills:

The glycogen content in gills of control fish and of *D.rerio* exposed to the concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L ofnano-TiO2 for 24 and 96 h was given in the Table 4.1. The results show that the glycogen content exposed to these concentrations was 15.29mg, 15.04mg, 14.43 mg, 14.19 mg, 13.98 mg, 13.01 mg, 11.82 mg, 9.24 mg, 9.12 mg, 8.08 mg and showed a decrease of 3.28%, 3.80%, 8.72%, 9.27%, 11.57%, 16.81%, 25.23%, 40.92%, 42.31% and 48.33% from the control at the end of 24and 96 h, respectively. The 't' test indicated that the decrease of glycogen content from the control in gills of fish exposed to 10.0 mg/L is highly significant at the end of 96 h ($p<0.01$) and the decrease in muscle exposed to 12.5mg/L is highly significant ($p<0.01$) at the end of 24 and 96 h.

(b) Muscle:

Table.2 presents the glycogen content in muscle of the control fish and of fish exposed tothe concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L of nano-TiO2 for 24 and 96 h. The results show that the glycogen content in the muscle of the fish exposed to these concentrations was 6.21mg, 5.54mg, 6.15mg, 5.31mg, 6.13mg, 4.93mg, 5.89mg, 3.87mg, 3.96mg and 3.21mg showing a decrease of 9.73%, 7.04%, 10.61%, 10.90%, 10.91%, 17.28%, 14.38%, 35.06%, 42.44% and 46.14% from control at the end of 24 and 96 h, respectively. The't' test showed that the decrease of glycogen content from control in muscle of fish exposed to 10.0 mg/Lis significant ($p<0.05$) at the end of 96 h and the decrease in muscle exposed to 12.5mg/L is highly significant ($p<0.01$) at the end of 24 and 96 h.

Table.1 Effect of nano-TiO2 on total Glycogen content in the gills of *Danio rerio* (n=6)

nano-TiO_2 Concentration	Duration of exposure (h)	Control (mg/g) X ± SD	Experiment (mg/g) X ± SD	% change from control	Result
2.5 mg/L	24	15.81 ± 0.36	15.29 ± 0.83	-03.28	P>0.05
	96	15.64 ± 0.68	15.04 ± 0.11	-03.80	P>0.05
5.0 mg/L	24	15.81 ± 0.36	14.43 ± 1.02	-08.72	P>0.05
	96	15.64 ± 0.68	14.19 ± 0.66	-09.27	P>0.05
7.5 mg/L	24	15.81 ± 0.36	13.98 ± 0.71	-11.57	P>0.05
	96	15.64 ± 0.68	13.01 ± 0.87	-16.81	P>0.05
10.0 mg/L	24	15.81 ± 0.36	11.82 ± 0.52	-25.23	P>0.05
	96	15.64 ± 0.68	9.24 ± 0.97	-40.92	P<0.01*
12.5 mg/L	24	15.81 ± 0.36	9.12 ± 1.32	-42.31	P<0.01*
	96	15.64 ± 0.68	8.08 ± 0.93	-48.33	P<0.01*

***** Statistically significant

Table.2 Effect of nano-TiO_2 on total Glycogen content in the muscle of *Daniorerio (n=6)*

nano-TiO_2 Concentration	Duration of exposure (h)	Control (mg/g)X ± SD	Experiment (mg/g)X ± SD	% change from control	Result
2.5 mg/L	24	6.88 ± 0.58	6.21 ± 0.93	-09.73	P>0.05
	96	5.96 ± 0.96	5.54 ± 0.24	-07.04	P>0.05
5.0 mg/L	24	6.88 ± 0.58	6.15 ± 1.01	-10.61	P>0.05
	96	5.96 ± 0.96	5.31 ± 0.84	-10.90	P>0.05
7.5 mg/L	24	6.88 ± 0.58	6.13 ± 0.63	-10.91	P>0.05
	96	5.96 ± 0.96	4.93 ± 0.98	-17.28	P>0.05
10.0 mg/L	24	6.88 ± 0.58	5.89 ± 0.79	-14.38	P>0.05
	96	5.96 ± 0.96	3.87 ± 0.54	-35.06	P<0.05*
12.5 mg/L	24	6.88 ± 0.58	3.96 ± 0.23	-42.44	P<0.01*
	96	5.96 ± 0.96	3.21 ± 0.86	-46.14	P<0.01*

***** Statistically significant

In the present investigation, appreciable decline in the total glycogen content of the fish tissues viz., gill and muscle was observed in the concentrations studied (2.5mg/L to 12.5 mg/L) demonstrating a linear correlation ship between concentration and duration of the exposure. However, this decrease is more pronounced in 10.0mg/L and 12.5mg/L exposure.

A decrease in the glycogen content of the fishes exposed to metallic stress was observed by many investigators (Mona Zaki et al. 2010; Hadi et al. 2009; Martin Deva Prasanth and Arivoli, 2008; Sobha et al. 2007; Emad El Naga et al. 2005; Zuleica et al. 2004). The decrease in the glycogen content of the tissues of *Danio rerio* may be due to its enhanced utilization since glycogen forms the immediate source of energy to meet energy demands under metallic stress. Though the glycogen decreased in all the tissues studied, maximum depletion was observed in gills since it is the primary target organ. Vutukuru (2003)[14] stated that decrease in glycogen content might be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization. He further stated that under hypoxic conditions, the animal derives its energy from anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis. This has been substantiated by our studies on the respiration of *Danio rerio* exposed to various concentrations of nano-TiO2 that have shown decreased metabolic rate indicating hypoxic condition. Nanoparticle induced hypoxia probably might have resulted in a shift to anaerobic glycolytic pathway by increased glycogenolysis. A consistent decrease in tissue glycogen reserves observed in the present study suggests impaired glycogenesis. This could also be due to its utilization in the formation of glycoproteins and glycolipids, which are essential constituents of various cells and other membranes.

Protein:

(a) Gills:

The total protein content in gills of the control fish and of fish exposed to the concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L of nano-TiO2 for 24 and 96 h are presented in Table 4.3. In gills of fish exposed to these concentrations, the protein content was 60.52mg, 51.80mg, 59.12 mg, 47.33mg, 56.23mg, 45.96mg, 53.21mg, 41.32mg, 48.04mg and 36.12mg showed a decrease of 3.32%, 3.17%, 7.15%, 11.53%, 10.17%, 14.09%, 15.00%, 22.76%, 23.25% and 32.48%from the control group at the end of 24and 96 h, respectively.The 't' test shows that the decrease in the gills of the fish exposed to 2.5mg/L, 5.0mg/L, 7.5mg/L and 10.0mg/L is not significant($p>0.05$) at the end of 24 and 96h whereas it was found to be significant ($p<0.05$) at the end of 24 and 96 h at 12.5mg/L exposure.

(b) Muscle:

The total protein content in the muscle tissue of fish exposed to the concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L of nano-TiO2 for 24 and 96 h is presented in Table 4.4. The total protein content of fish exposed these concentrations was 71.56 mg, 62.13mg, 69.15 mg, 60.52 mg, 65.46 mg, 56.32 mg, 60.52 mg, 51.08mg, 53.58mg and 42.13 mg showing a decrease of 1.97%, 6.93%, 5.27%, 9.02%, 10.32%, 15.63%, 17.09%, 23.48%, 26.60% and 36.89% from the control fish at the end of 24 and 96 h, respectively. The 't' test results show that the decrease in total protein content in muscle of fish exposed to 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L is insignificant at the end of 24 and 96 h ($p>0.05$), and insignificant ($p>0.05$) in the 12.5mg/L exposure at the end of 96 h. It was found highly significant at the end of 96 h ($p<0.01$).

Table 3 Effect of nano-TiO2 on total Protein content in the gills of *Danio rerio* (n=6)

nano-TiO_2 Concentration	**Duration of exposure (h)**	**Control (mg/g)** X ± SD	**Experiment (mg/g)** X ± SD	**% change from control**	**Result**
2.5 mg/L	24	62.6 ± 1.12	60.52 ± 0.83	-3.32	P>0.05
	96	53.5 ± 0.92	51.80 ± 0.34	-3.17	P>0.05
5.0 mg/L	24	62.6 ± 1.12	59.12 ± 1.12	-7.15	P>0.05
	96	53.5 ± 0.92	47.33 ± 1.33	-11.53	P>0.05
7.5 mg/L	24	62.6 ± 1.12	56.23 ± 0.65	-10.17	P>0.05
	96	53.5 ± 0.92	45.96 ± 0.63	-14.09	P>0.05
10.0 mg/L	24	62.6 ± 1.12	53.21 ± 1.23	-15.00	P>0.05
	96	53.5 ± 0.92	41.32 ± 0.76	-22.76	P>0.05
12.5 mg/L	24	62.6 ± 1.12	48.04 ± 0.98	-23.25	P<0.05*
	96	53.5 ± 0.92	36.12 ± 1.41	-32.48	P<0.05*

***** Statistically significant

Table 4 Effect of nano-TiO_2 on total Protein content in the muscle of *Danio rerio (n=6)*

nano-TiO_2 Concentration	Duration of exposure (h)	Control (mg/g) X ± SD	Experiment (mg/g)X ± SD	% change from control	Result
2.5 mg/L	24	73.00 ± 2.36	71.56 ± 1.34	-01.97	P>0.05
	96	66.76 ± 1.58	62.13 ± 0.98	-06.93	P>0.05
5.0 mg/L	24	73.00 ± 2.36	69.15 ± 1.41	-05.27	P>0.05
	96	66.76 ± 1.58	60.52 ± 0.88	-09.02	P>0.05
7.5 mg/L	24	73.00 ± 2.36	65.46 ± 2.13	-10.32	P>0.05
	96	66.76 ± 1.58	56.32 ± 1.11	-15.63	P>0.05
10.0 mg/L	24	73.00 ± 2.36	60.52 ± 2.45	-17.09	P>0.05
	96	66.76 ± 1.58	51.08 ± 0.74	-23.48	P>0.05
12.5 mg/L	24	73.00 ± 2.36	53.58 ± 1.87	-26.60	P>0.05
	96	66.76 ± 1.58	42.13 ± 0.75	-36.89	P<0.01*****

***** Statistically significant

3.2 Metabolic rate:

Table 5 illustrates the toxic effect of nano-TiO2 on the metabolic rate of the fish exposed to the concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L of nano-TiO2. The metabolic rate of *D. rerio* exposed to these concentrations decreased from control with an increase in the exposure period from 24 h to 96 h and it ranged from 3.01 to 1.03 showing a decrease of 3.21 to 64.73%. The results of unpaired Student't' test shows that this decrease is statistically significant ($p<0.05$) for7.5mg/L at the end of 96 h; significant ($p<0.05$) for 10.0mg/L at the end of 24 h; highly significant ($p<0.01$) for 10.0mg/L at the end of 96 h; significant ($p<0.05$) for 12.5mg/Lat the end of 24 h; highly significant ($p<0.01$) for 12.5mg/L at the end of 96h.

Table .5 Effect of nano-TiO_2 on the metabolic rate of *Daniorerio (n=3)*

nano-TiO_2 Concentration	Duration of exposure (h)	Control ($mgO_2/g/h$) X ± SD	Experiment ($mgO_2/g/h$) X ± SD	% change from control	Result
2.5 mg/L	24	3.11± 0.95	3.01 ±0.72	-3.21	P>0.05
	96	2.92 ± 0.72	2.77 ± 0.34	-5.13	P>0.05
5.0 mg/L	24	3.11± 0.95	2.84 ± 0.87	-8.68	P>0.05
	96	2.92 ±0.72	2.35 ± 0.72	-19.52	P>0.05
7.5 mg/L	24	3.11±0.95	2.23 ± 0.85	-28.29	P>0.05
	96	2.92 ±0.72	1.76 ± 0.66	-39.72	P<0.05*
10.0 mg/L	24	3.11±0.95	2.15 ± 0.97	-30.86	P<0.05*
	96	2.92 ±0.72	1.54 ± 0.43	-47.26	P<0.01**
12.5 mg/L	24	3.11±0.95	1.98 ± 0.61	-36.33	P<0.05*
	96	2.92 ±0.72	1.03 ± 0.27	-64.73	P<0.01**

*Significant ** Highly significant

Several assays for eco-toxicological testing of nanomaterials have been developed. Adams et al. (2006) [15] compared the ecotoxicities of TiO2, ZnO, and SiO2 nanoparticles suspended in water using *Escherichia coli* and *Bacillus subtilis* as two model bacterial species and it was reported that ZnO was toxic to Bacillus subtilis. Experiments on embryonic zebrafish demonstrated similar results; ZnO nanoparticles were more toxic than TiO2 or Al2O3 nanoparticles[16]. Moreover, Hund-Rinke and Simon (2006)[17] reported the first results on the toxicity of TiO2 nanoparticles to Daphnia (a common freshwater zooplankton) and green algae (*Desmodesmus subspicatus*). In a comprehensive study on the 48-h acute toxicity of water suspensions of six manufactured nanomaterials (i.e., ZnO, TiO2, Al2O3, C60, SWCNTs, and MWCNTs) to *Daphnia magna*, using immobilization and mortality as toxicological endpoints, a dose dependence in acute toxicity was demonstrated[18]. In another study, C60 suspensions havebeen shown to be toxic to bacteria[19,20], fathead minnows (Pimephalespromelas) (Zhu et al., 2006), and zebrafish embryos[21,18]. In a study on ZnOtoxicity in rodent lung and zebra fish embryo's, data indicated reduced toxicity in the latter system upon doping of Fe in ZnO (Xia et al., 2011).

Environmental release of TNPs may pose a significant risk to life within the ecosystem, such as algae, zooplankton, bacteria, and fish. It is quite possible that TNP aggregation status and abiotic factors, such as ionic strength and pH, affect TMP ecotoxicity. Usually Titanium nanoparticle is classified as a harmful material that presents a low risk to the ecosystem. However, despite low or moderate toxicity in short-term evaluations, long-term TNP exposure showed greater risks. Daowen Xiong et al. (2011) concluded that the acute toxicity of TiO2 NPs to zebrafish was significantly higher than that of bulk TiO2. Furthermore, these nanoparticles were shown to cause toxicity effects without entering the cells and the extracellular ·OH generated by them could induce oxidative damage directly on the cell membranes of gill tissue. Palaniappan et al. (2011) studied the effect of TiO(2) nanoparticles and its bulk material on the biochemical constituents of the brain of Zebra fish by using FT-IR technique. FT-IR spectra revealed significant differences in absorbance intensities between the control and TiO(2) exposed brain tissues, reflecting alterations on the major biochemical constituents such as proteins, lipids and nucleic acids in the brain tissues of *D. rerio* due to TiO(2) exposure. The results further reveal that TiO(2) nanoparticles are more toxic than their bulk counterparts.

Conclusion

The toxicity of nano-TiO_2 was tested in the concentrations 2.5mg/L, 5.0mg/L, 7.5mg/L, 10.0mg/L and 12.5mg/L and shown that the toxicity increased with the increase in the amount of toxicant. There were noticeable changes in the oxygen consumed by the fishes exposed to these concentrations which shows that the toxicant proved to be a potential respiratory inhibitor ($p<0.01$) in 12.5mg/L and 10.0mg/L at the end of 96h, ($p<0.05$) in 12.5mg/L and 10.0mg/L at the end of 24h, ($p<0.05$) in 7.5mg/L at the end of 96h respectively, demonstrating a time-concentration relationship. The decreases in protein and glycogen content indicate enhanced utilization of glycogen since it forms an intermediate source of energy. Physiological responses and morphological changes under toxicant stress are subtle and have the potential to serve as an early warning of environmental contamination.

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