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Responses of metabolic enzymes (GOT, GPT and LDH) in an Indian major carp *Cirrhinus mrigala* exposed to titanium dioxide (TiO_2) nanorods under short-term exposure

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Abstract

Titanium dioxide (TiO_2) nanoparticles are extensively manufactured due to their potential properties and applications in various fields such as biomedical, electrical and environmental. These particles are likely to reach the aquatic environment and may cause adverse effects on aquatic organisms. In this study, we investigated the effects of different concentrations (1, 50 and 100 mg L^{-1}) of TiO_2 nanorods (NRs). The enzymatic activity of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and lactate dehydrogenase (LDH) was measured in the liver and muscle of an Indian major carp, *Cirrhinus mrigala,* under short-term exposure (96 h). The synthesised particles were characterized using X-ray diffraction (XRD), scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), UV-Vis spectroscopy (UV-Vis) and photoluminescence (PL) techniques before conducting the toxicity assay. The GOT and GPT activities were significantly elevated in both liver and muscle of fish treated with TiO_2 nanorods (except 50 mg L^{-1} in muscle for GPT). Similarly, the activity of LDH was also found to be elevated. The findings of the present investigation suggest that TiO_2 NRs might have be absorbed, circulated, accumulated in liver and muscles of *C. mrigala* resulting in alterations in the enzyme activities. The results revealed that TiO_2 nanorods induced alterations in GOT, GPT and LDH activities of fish at tested concentrations. The alterations of these enzymatic parameters can be useful for monitoring the environmental contamination of titanium dioxide (TiO_2) nanoparticles in freshwater ecosystem.

Keywords: Enzymes, Fish, Nanoparticles, Toxicity.

INTRODUCTION

Titanium dioxide (TiO_2) nanoparticles (NPs) have been widely used in personal skincare products, food coloring, water-treatment agent, paint materials, and bactericidal agent due to their high stability, anticorrosive and photo-catalytic properties (Weir *et al.*, 2012; Bogusz *et al.*, 2018; Ozgur *et al.*, 2018; Winkler *et al.*, 2018; Hou *et al.,* 2019; Delmond *et al.,* 2019; Gu *et al.*, 2021). Since there are an extensive production, large demand, and use

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in biomedical applications TiO_2 NPs are being released in the aquatic environment and accumulating in the aquatic systems (Westerhoff *et al.,* 2011**;** Shi *et al*., 2013; Jhamtani *et al*., 2018**;** Fan *et al.,* 2018; Souza *et al.,* 2019). Shi *et al.* (2016) has reported that during wastewater treatment, 74 to 85% of the TiO_2-NRs have been removed and the remaining TiO_2-NPs in the effluent may be released into the environment. The TiO_2 NPs have been detected in the aquatic environment at the range of 0.7 to 16 µg L^{-1} (Batley *et al.,* 2013; Lammel *et al.,* 2019). However, in freshwater bodies these nanoparticles have been recently detected in concentrations of 3 to 6 mg L^{-1} (Vicari *et al.,* 2018).

In aquatic organisms such as fish, TiO_2-NPs particles are absorbed through gill, skin, and intestine (Handy *et al.,* 2008). Due to their unique physicochemical properties, such as chemical reactivity and physical absorption ability, TiO_2-NPs may interact with a wide range of molecules (Farré *et al.,* 2009). These nanoparticles may also interact with co-existing pollutants and change their bioaccumulation and toxicity (Della Torre *et al*., 2015). Further, there has been scientific evidence that the physical and chemical properties of manufactured NPs lead to an increase of bioavailability and toxicity (Nel *et al.,* 2006; Ale *et al*., 2018). Studies have shown that TiO_2 NPs has caused adverse effects on aquatic organisms such as immune response in *Dicentrarchus labra* (Della Torre *et al*., 2015), genotoxicity in *Hoplias intermedius* (Vicari *et al.,* 2018), antioxidant imbalance in *Astyanax serratus* (Delmond *et al.,* 2019), histological damage in *Cyprinus carpio* (Haghighat *et al*., 2021), decreased survival rate in zebrafish (Srinivasan *et al.,* 2019), decreased locomotor behaviour in zebrafish (Gu *et al.,* 2021), and cell vacuolization in *Danio rerio* (da Cunha and de Brito-Gitirana, 2020).

TiO_2 has been considered to be biologically inert to animals and humans (Bernard *et al.,* 1990; Srinivasan *et al.,* 2019) and the interaction of NPs with chemical and biological systems may leads to biochemical disturbances or/and adaptive responses (Zhu *et al*., 2008; Ozgur *et al.,* 2018) which can be used to assess the health condition of aquatic organisms as biomarkers (Wang *et al*., 2009). Among the biochemical disturbances, enzymological parameters such as glutamate oxaloacetate transaminase [GOT], glutamate pyruvate transaminase [GPT] and lactate dehydrogenase [LDH] are commonly used to monitor the tissue damage or pathological alterations in fish (Ozman *et al*., 2006; Lavanya *et al.,* 2011; Ramesh *et al.,* 2014; Ramesh *et al.,* 2018; Umamaheswari *et al.,* 2019). Transaminases (GOT and GPT) are present in cell membranes and cytoplasm and play a major role in protein and carbohydrate metabolism in which GOT is the most active and widely distributed enzyme (Nemcsok and Boross, 1982; Malarvizhi et al., 2012; Muralidharan, 2014; Ramesh et al., 2018). Likewise, LDH is a glycolytic enzyme found in cardiac and skeletal muscle, liver and kidney and also used as indicator of toxicant stress. The activity of the LDH enzyme is widely used as biomarker in aquatic organisms to determine cellular impairment and organ damage (Rendon-von Osten *et al.,* 2005).

The reports on the concentrations of TiO_2-NPs in the aquatic environment are scanty and also the toxicity of TiO_2-NPs varies with particle size (Hou *et al.,* 2019). A recent study indicates that the environmental contamination by TiO_2-NPs has been little studied (Souza *et al.*, 2019). Moreover, the impacts of TiO_2 NRs on enzymological parameters in freshwater fish have not been given enough attention. Therefore, the present investigation intended to examine the impact of TiO_2NRs in an Indian major carp, *C. mrigala* using enzymatic parameters (GOT, GPT and LDH) and to use alterations of these enzymatic parameters as potential biomarkers for the assessment of TiO_2 NRs toxicity on aquatic organisms.

MATERIAL AND METHODS

Synthesis of TiO_2 NPs and their characterization

TiO_2 powder and sodium hydroxide (NaOH) were purchased from S.D Fine Chemicals Ltd and double distilled water was used as a solvent. TiO_2 NRs were synthesized by chemical reduction method (Do Kim *et al*., 2006; Leela Mohana Reddy and Ramaprabhu, 2007). 1 M of TiO_2 powder (Molar mass: 79.866 g/mol; Density: 4.23 g/cm^3) was dissolved in 25 ml of double distilled water and allowed for stirring using magnetic stirrer. Then equal molar ratio of NaOH (1 M) was dissolved in 25 ml of double distilled water and added drop wise into the TiO_2 solution. The mixture solution was stirred vigorously and continuously for 24 h. The obtained white colloidal solution was washed several times using double distilled water to remove any unreacted compounds and allowed for sedimentation. A gel like product was formed and kept for drying at 50 °C. Finally, the dried crystal product was used for calcination treatment at 400 °C for 2 h using a muffle furnace. A white colour final product was well grounded to form fine powder, indicating the formation of TiO_2 nanosized particles. Finally this obtained product was used for following characterization techniques.

To explore the interaction between nanoparticle and toxicological responses, and for risk assessment (Jin *et al.,* 2020), the structural properties of TiO_2 NRs were investigated by X-ray diffraction (XRD, PAN analytical X'pert PRO instrument) and Fourier transform infrared (FTIR, Bruker Tensor-27 spectrophotometer) spectroscopy techniques. The TiO_2 NRs surface morphology was observed using scanning electron microscope (SEM, FEI Quanta 250) technique. The optical properties were determined through ultraviolet (UV, Shimatzu 3600) and photoluminescence (PL, spectrofluromax-4) spectrometer techniques.

Preparation of TiO_2 NRs stock solution

A stock solution of 1g L^{-1} of powder form of ultra-fine TiO_2 NRs were prepared by dispersing NRs in distilled water with sonication of 6 h using bath type sonicator (40 kHz frequency,

Vibronics-250 wts). During the study period, to obtain homogeneity, the suspension was sonicated for 30 min prior to dosing TiO_2 NRs each day. NRs were kept in suspension in the water using aeration or peristaltic pump for minimizing the settling of NRs. The dispersion was optimal at final working concentration. Despite extensive sonication, a few aggregates of nanoparticle were also observed in stock solution. The well dispersed NRs were used to prepare the stock solution at different concentrations (1, 10, and 100 mg L^{-1}).

Experimental Animal

The mrigal carp (*Cirrhinus mrigala*), also known as the common carp, a freshwater fish belonging to the family Cyprinidae, was used in the present study. For the experiments, healthy specimens of *C. mrigala* (7.5 cm, weight of 6.0 g; 2-3 months old) were purchased from Fisheries Development Corporation Limited, Tamil Nadu, India. Fish were acclimatized to the laboratory conditions for about 20 days in a large tank (1000 L capacity) and were fed once a day with rice bran and groundnut oil cake in the form of dough. One third of the water was renewed daily and feeding was withheld 24 h before the commencement of the experiment. Tap water, chlorine-free, was used in the experiments and presented the following physicochemical characteristics (APHA 1998): temperature 27.0 ± 1.2°C, pH 7.3, dissolved oxygen 6.5 ± 0.04 mg L^{-1}, total alkalinity 18.2 ± 8.0 mg L^{-1}, and total hardness 18.6 ± 0.5 mg L^{-1}.

TiO_2 NRs Exposure

To determine the toxicity of TiO_2 NRs at different concentrations, fish fingerlings were randomly collected from the stock tank and housed in a glass aquarium (30 mL capacity) for one week containing 15 fish. Then, 1, 50, and 100 mg L^{-1} of TiO_2 NRs was poured into the glass aquarium tanks. The above concentrations were based on previous literature (Chen *et al*., 2011; Vicari *et al*., 2018). Simultaneously, a control group also maintained without toxicant for each concentration treatment. Four replicates were maintained for each concentration of TiO_2 NRs and control groups. The water was renewed every 24 h and freshly prepared solution was added to maintain the concentration of TiO_2 NRs at a constant level. Feeding was withheld during the study period. At the end of 96 h period, fish from the control and TiO_2 NRs treated groups were taken for the enzymological studies

Sample collection

At the end of 96 h, fish from the control and TiO_2 NRs treated fish were cut open and liver and muscle were removed for the GOT, GPT and LDH activity. Liver and muscle pieces of 100 mg were homogenized with 0.25 M sucrose solution in ice cold condition. The homogenates were centrifuged for 20 min at 6000 rpm and the clear supernatant fluids were used as an enzyme source.

Determination of GOT and GPT activity

GOT and GPT activities were estimated following the method of Reitmen and Franckel (1957) using assay reagents obtained from Dr. Reddy's Diagonostic Laboratories, Hyderabad, India. For 50 mL of supernatant (liver and muscle), 0.25 mL of buffering agent (aspartate for GOT and alanine for GPT) was added, mixed well and incubated at 37 ºC for one minute. Then, 0.25 mL of 2, 4-Dinitrophenylhydrazine (DNPH) was added, mixed well and kept at room temperature for 20 min. To this content, 2.5 mL of NaOH (0.4 N) was added, mixed well and the optical density was measured at 505 nm and their activities were expressed as IU/L (Umamaheswari *et al*., 2019).

Determination of LDH activity

The LDH activity was estimated by 2, 4-DNPH method (Young 1997) using assay reagents obtained from Dr. Reddy's Diagonostic Laboratories, Hyderabad, India. To 10 µL of supernatant (liver and muscle), 1000 µL of buffer pyruvate was added, mixed well and the colour intensity was measured for 2 min at 340 nm. A standard curve was also performed. The LDH activity was expressed as IU/L (Umamaheswari *et al*., 2019).

Statistical analysis

The data were analyzed statistically at $p< 0.05$. The significance of sample between control and TiO_2 NRs treated groups were tested by using student's *t*-test.

RESULTS

In the present study, a tetragonal body-centred lattice of the anatase TiO_2 crystal system was obtained (Fig. 2) and also broadening peaks were observed when compared with bulk pattern (JCPDS card No. 21-1272) (Fig. 1), which indicates the formation of nanosized TiO_2 particles. The crystal phase formation and crystallinity of TiO_2 NPs were analysed using XRD. The XRD pattern of TiO_2 NRs exhibits bicrystalline structure (mix of anatase and brookite phase) were illustrated in the Fig.1 and it is clear that the sharp peaks indicate the high crystalline nature of the material. The diffraction peak intensity is associated to the crystallite size and phase volume. The pattern indexes the predominance of anatase phase with the JCPDS No. 21-1272 and the diffraction peak at 56.2° attributes to (211) plane of brookite phase with the JCPDS No.29-1360 elongated bipyramidal structure. The diffraction planes at (101), (103), (112), (004), (200) and (105) characterise the anatase phase of TiO_2 NRs and the peak at 36.6° 64.3° of rutile phase can also be perceived. This results evidently reveals that the synthesized TiO_2 NRs were distinguishably oriented prevalence with (101) plane. Debye-Sherrer's formula was used to estimate the size (*D*; average particle size) of the nanosized particles (Cullity, 1978). Rod shaped morphology was observed from the TiO_2 nanosized particles. The diameter of the nanoparticle was ± 50 nm and length was ± 500 nm (Fig. 2). This observation confirms our prepared sample is in nano-scale region.

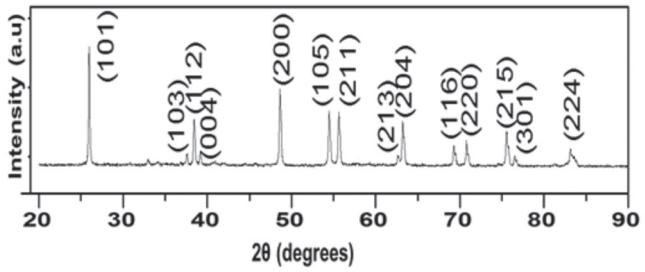

Fig. 1. X-ray diffraction pattern of synthesized TiO_2 NPs.

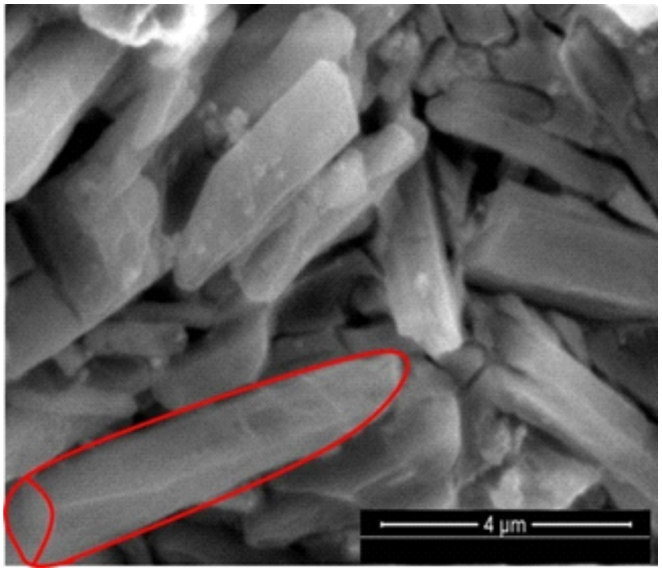

Fig. 2. SEM image of synthesized TiO_2 NPs (rod shape indicated by the red-color line).

To confirm the chemical structure and presence of functional groups, the FTIR spectrum was recorded from the prepared TiO_2 NRs. The observed peaks at 580 cm^{-1} is the stretching vibration of Ti-O bonding and 1400 cm^{-1} is the stretching vibration of Ti-O-Ti bonding, confirming the formation of TiO_2 (Shen *et al.,* 2011). In addition, the other functional groups were also observed which supports the formation of TiO_2 (Fig. 3). The observed peaks in the frequency region of 1099 cm^{-1} is assigned to be C-O stretching mode, 1648 cm^{-1} is assigned to H-OH stretching mode (water group) and 3388 cm^{-1} corresponds to Ti-OH stretching mode (Jahromi *et al.,* 2010). Further, the optical properties of the TiO_2 NRs were analyzed using UV absorption and PL emission techniques.

The UV optical absorption spectrum has shown maximum absorption at 340 nm (Fig. 4). The obtained band gap energy values are 3.65 eV and the reported value for anatase phase was 2.86-3.34 eV (Hossain *et al.*, 2008; Hidalgo *et al.,* 2007). The PL emission spectrum has shown green emission band at 530 nm (Fig. 5).

During short-term treatment, GOT activity was found to be increased in liver and muscle of TiO_2 NRs treated fish (Table. 1). Among the three concentrations tested, 50 mg L^{-1} of TiO_2 concentration caused a significant change in GOT activity in liver and muscle of *C. mrigala*. A maximum

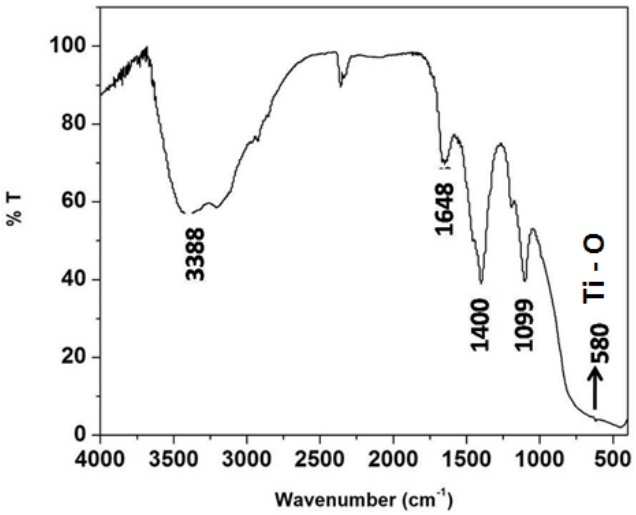

Fig. 3. Fourier transform infrared spectroscopy spectrum of synthesized TiO_2 NRs.

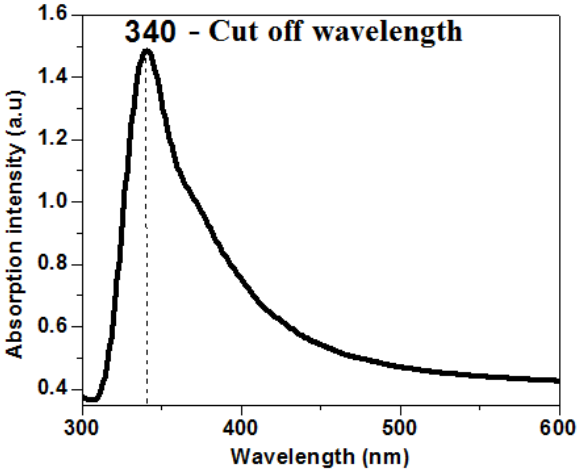

Fig. 4. UV-Vis spectroscopy absorption spectrum of synthesized TiO_2 nanosized particles.

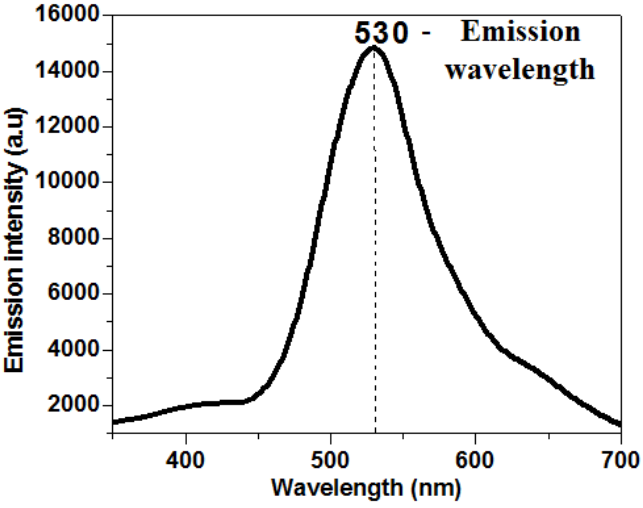

Fig. 5. Photoluminescence emission spectrum of synthesized TiO_2 nanosized particles.

percent increase of GOT activity was noticed in muscle (92.27%) followed by liver (35.30%). All the values were significant at 5% level. Similarly, GPT activity was also increased significantly in liver and muscle of *C. mrigala* exposed to various concentrations of TiO_2 NRs (except 50 mg L^{-1} in muscle) when compared to control groups (Table.2). A maximum percent increase was observed in liver (52.23%) exposed to 1 mg L^{-1} and in muscle (29.52%) exposed to 100 mg L^{-1} TiO_2 NRs. On the other hand, GPT activity in muscle was found to be decreased (-7.79%) significantly at 50 mg

Table 1. Glutamic oxaloacetic transaminase (GOT) activity in liver and muscle of an Indian major carp *C. mrigala* treated with various concentrations of TiO_2 NRs (96 h).

Concentration (mg L^{-1})	Control	Experiment	Percent change
GOT activity (IU/L) in liver			
1	15.97 ± 0.131	16.98 ± 0.421	+6.32*
50	17.28 ± 0.266	23.38 ± 0.932	+35.30*
100	16.53 ± 0.208	17.69 ± 0.446	+7.01*
GOT activity (IU/L) in muscle			
1	11.20 ± 0.306	17.39 ± 0.200	+55.26*
50	12.68 ± 0.254	24.38 ± 0.424	+92.27*
100	12.56 ± 0.107	16.00 ± 0.391	+27.38*

Values are means ± SE of five individual observations, (+) Denotes percent increase over control, (*) Values are significant at 5% level.

Table 2. Glutamic pyruvic transaminase (GPT) activity in liver and muscle of an Indian major carp *C. mrigala* treated with various concentrations of TiO_2 NRs (96 h).

Concentration (mg L^{-1})	Control	Experiment	Percent change
GPT activity (IU/L) in liver			
1	14.78 ± 0.232	22.50 ± 0.248	+52.23*
50	14.27 ± 0.064	15.88 ± 0.396	+11.28*
100	14.15 ± 1.412	16.36 ± 0.395	+15.61*
GPT activity (IU/L) in muscle			
1	14.57 ± 0.438	15.56 ± 0.272	+6.79*
50	14.89 ± 0.123	13.73 ± 0.044	-7.79*
100	14.36 ± 0.707	18.60 ± 0.238	+29.52*

Values are means ± SE of five individual observations, (+) Denotes percent increase and (-) Denotes per cent decrease over control, (*) Values are significant at 5% level.

Table 3. Lactate dehydrogenase (LDH) activity in liver and muscle of an Indian major carp *C. mrigala* treated with various concentrations of TiO_2 NRs (96 h).

Concentration (mg L^{-1})	Control	Experiment	Percent change
LDH activity (IU/L) in liver			
1	1255.92 ± 60.648	1540.07 ± 95.605	+22.62*
50	1428.36 ± 33.935	2554.88 ± 44.482	+78.86*
100	1362.13 ± 23.154	1945.02 ± 116.191	+42.79
LDH activity (IU/L) in muscle			
1	1702.12 ± 51.600	2022.31 ± 35.059	+18.81*
50	1790.77 ± 74.763	2339.26 ± 88.275	+30.62*
100	1757.15 ± 5.237	1903.29 ± 99.789	+8.31*

Values are means ± SE of five individual observations, (+) Denotes percent increase over control, (*) Values are significant at 5% level.

L^{-1} of TiO_2 NRs treated fish. All the values were significant at 5% level.

A significant increase in LDH levels was registered at all concentrations of TiO_2 NRs showing a percent increase of 78.86% in liver and 30.62% in muscle after 96 h of exposure (Table. 3). All the values were significant at 5% level.

DISCUSSION

In aquatic animals such as fish, potential routes of nanoparticle absorption include direct ingestion (through food or water) or epithelial substrates such as gills, olfactory organs or the epithelium of the body (Moore, 2006). In the present study three different concentrations (1, 50 and 100 mg L^{-1}) of TiO_2 NRs have been selected to assess the toxicity based on the LC 50 value and exposure concentrations of previous literature (Lin *et al.,* 2014; Erdem *et al.,* 2015; Ozgur *et al.*, 2018; Srinivasan *et al.*, 2019).

Fish exhibit a characteristic response to many stressors as that of mammalian system and the response is measured through a variety of enzyme activities in organ/tissue or blood (da Fonseca *et al*., 2008). GOT and GPT are known to be a predominant biomarkers for hepatotoxicity studies (Lehninger, 1993; Jung *et al.,* 2003; Karthikeyeni *et al.,* 2013; Ramesh *et al*., 2018). In the present study, an elevation of GOT and GPT activity was observed in both liver and muscle of fish treated with TiO_2 nanorods (except 50 mg L^{-1} in muscle for GPT). Similar to our studies an elevation of GOT and GPT activity was observed in liver, gill and kidney of fish *Labeo rohita* exposed to silver NPs ($AgNO_3$) (Vignesh *et al.,* 2013) and in liver and gill of a freshwater fish *Oreochromis mossambicus* exposed to zinc oxide (Amutha and Subramanian, 2009).

According to literature, prolonged exposure of fish to TiO_2-NPs have induced biochemical and histopathological alterations in gills, liver and intestines (Blaise *et al.,* 2008; Linhua *et al.,* 2009; Boyle *et al.,* 2013). In the present study, the elevation of GOT and GPT activities in liver and muscle may be due to accumulation of TiO_2 NRs in these organs/ tissue which leads to tissue damage. Histopathological changes in the gill and kidney of fish has been reported in fish exposed to TiO_2 (Carmo *et al*., 2018, do Carmo et al., 2018). Accumulation of toxicants may alter the structural and functional activities of these organs resulting changes in their enzyme activities (Ambili *et al.,* 2013; Umamaheswari *et al.,* 2019). Furthermore, the fish may try to mitigate the TiO_2 NRs induced stress by increasing the metabolism rate resulting in an elevation of the GOT and GPT enzymes in liver and muscle. Transient increases in GOT and GPT activities have also been noticed in many fish species exposed to other nanoparticles (Lee *et al.,* 2014; Ates *et al.,* 2016; Kaya *et al*., 2017; Mirghaed *et al.,* 2018), indicating the potential tissue damage due to accumulation of nanoparticles and their toxicity.

At the cellular level, a number of studies have shown that exposure to TiO_2 NRs may damage DNA by oxidative stress through the generation of reactive oxygen species (ROS) and interactions with the proteins involved in cell cycle (Trouiller *et al.,* 2009; Chen *et al.*, 2011; Wang *et al*., 2011; Clemente *et al.,* 2014; Rocco *et al.*, 2015; Haynes *et al.,* 2017; Lammel *et al.,* 2019). Long *et al.* (2007) reported that oxidative stress caused by TiO_2 NP is the key factor for the organ injury. Hence in the present study, oxidative stress caused by TiO_2 NRs may be another possible reason for the elevation of transaminases activity. In contrast to the significant increase in GPT activity, the observed decrease in GPT activity in 50 mg L^{-1} of TiO_2 NRs treated fish indicate that detoxification mechanism may not be sufficiently effective to prevent the action of the toxicants on the system as suggested by Malarvizhi *et al.* (2012). According to Hou *et al*. (2019) the mechanism of TiO_2 NP toxicity could be due to production of reactive oxygen species (ROS), cell wall damage and lipid peroxidation, and TiO_2 NP attachment to intracellular organelles and biological macromolecules.

LDH is a pivotal cellular metabolic enzyme between the glycolytic pathway and tricarboxylic acid cycle (Abston and Yarbrough, 1976). LDH is present in all tissues and determination of this enzyme activity can be used a potential biomarker for assessing the toxicity of chemicals and stress in fish (Agrahari *et al.,* 2007; Ramesh *et al*., 2018). In the present study the observed increase in LDH activity may indicate the changes in the histological structure of the liver and muscle tissues. Moreover, the metabolic changes due to TiO_2 NRs stress may favour an elevation of these enzymes to meet the stress. An elevation of LDH activity in liver may be due to the direct toxic effect of TiO_2 NRs, because liver is the main organ of intermediary metabolism (Chen *et al.,* 2011). Likewise, the minimum alterations of these enzymes in muscle may be due to lesser accumulation of these nanoparticles. Federici *et al.* (2007) reported that TiO_2 NPs may accumulate in liver cells resulting damage of liver cells. An apparent cell uptake of TiO_2 NPs through liver, kidney and gills with a small amount in muscle of fish *Trachinotus carolinus* has been reported by Vignardi *et al.* (2015). They also reported thatTiO_2 NPs can cross the epithelial surfaces of internal organs. Under stress condition the organisms require more energy to cope with the stress which results alteration in LDH activity (Rendon-von Osten *et al.,* 2005). Furthermore, any changes in protein and carbohydrate metabolism may cause a change in the LDH activity (Abston and Yarbrough 1976).

A reduction in the levels of glycogen and protein has been reported in fish exposed to TiO_2 NPs indicating that titanium oxide nanoparticles ($nTiO_2$) is a potential respiratory inhibitor in fish (Vutukuru *et al.,* 2013; Clemente *et al.,* 2014). Changes in the biochemical constituents of brain was also reported in zebrafish (*Danio rerio*) upon exposure to TiO_2 NPs suggesting that $nTiO_2$ is more toxic than their bulk counterparts (Palaniappan and Pramod, 2011). Our results showed that the NRs are altering the activity of GOT, GPT and LDH, and representing a potential threat to fish. Those alterations can indicate relative toxicity to the fish, as previously described in the literature (Matranga and Crosio, 2012; Kaya *et al*., 2017; Ribeiro *et al*., 2017; Hou *et al*., 2019). According to the size and shape of TiO_2 NP found in our study, and the different

concentrations we tested, we could contribute with studies that indicate the toxicity of TiO_2 NP.

CONCLUSIONS

The results of the present study concluded that all the concentrations of TiO_2 NRs (1, 50 and 100 mg L^{-1}) caused significant changes on GOT, GPT and LDH activities in liver and muscle of *C. mrigala*. The parameters used in this study may be taken as potential biomarkers of toxicity of TiO_2 NRs in the field of toxicology/nanotoxicology. The results of this investigation are the first overviews of enzymological effects of TiO_2 NRs (1, 50 and 100 mg L^{-1}) on Indian major carp *C. mrigala*. Furthermore, long-term effects of TiO_2NRs on these and other parameters need to be investigated in the future studies.

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