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PERSISTENT TOXIC EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES AFTER TREATMENT WITHDRAWAL IN THE FRESHWATER FISH, *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2-NPs) were produced abundantly and possess widespread applications from commercial products to industrial photocatalytic processes. Hence, probably nanoparticles enter into aquatic environment where its exposure, fate and ecotoxicity are of growing concern. The present study was focused to address the sublethal (16.4 mg/L) effects of TiO_2-NPs in gill, liver and brain of the fish, *Oreochromis mossambicus* when exposed for short-term (24, 72 and 96 h) and long-term (15, 30 and 60 days) durations. Gill tissue showed significant ($P<0.05$) increase in the activities of superoxide dismutase and catalase, while the activities of glutathione reductase and glutathione peroxidase decreased significantly ($P<0.05$) with concomitant increase in the levels of hydrogen peroxide generation and lipid peroxidation. Liver tissue showed alteration in the antioxidant defense system, which is evident by the increase in levels of hydrogen peroxide and lipid peroxidation. Brain tissue also showed significant reduction in the activities of all antioxidant enzymes and induction of lipid peroxidation in short-term and long-term exposure groups. The activity of marker enzyme, alkaline phosphatase in gill and liver, and acetylcholinesterase activity in brain tissue showed significant reduction after exposure to TiO_2-NPs. The present results suggest the induction of oxidative stress in gill, liver and brain tissues after exposure to the nanoparticles. Treatment withdrawal for 60 days showed similar results like the treatment groups. Briefly to conclude, sublethal toxicity of titanium dioxide nanoparticles involves oxidative stress causing persistent toxic effects in gill, liver and brain tissues of the freshwater fish, *Oreochromis mossambicus.*

KEYWORDS: TiO_2-NPs, *Oreochromis mossambicus*, lipid peroxidation, oxidative stress, treatment withdrawal.

INTRODUCTION

Among the engineered nanoparticles, titanium dioxide (TiO_2-NPs) are the most enormously manufactured product widely used in paints, coatings, dyes, emulsions, cosmetics, sunscreen etc.[1] Nano-scale titanium dioxide is a versatile crystalline compound that exists in three forms as anatase, rutile and brookite. Anatase and rutile forms of TiO_2-NPs are increasingly used in industries and were toxic, while the brookite form of nano-titanium dioxide is rarely used and less toxic.[2] The anatase form of TiO_2, with size of 10-20nm, is considered as much toxic where it has been known to induce micronuclei formation, DNA damage and lipid peroxidation.[3] The small sized nano-TiO_2 of 25nm anatase and 31nm anatase/rutile showed greater phototoxicity than the larger sized TiO_2 of 142nm anatase and 214nm rutile nanoparticles.[4] Thus, the size and surface area of the nanoparticles are the physicochemical properties responsible for the toxicity. However, Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have approved TiO_2-NPs as food additive as it is known for long time as 'environmental white knight' owing to its biologically inactive and physiologically inert properties. Thus TiO_2-NPs are being used in the development of self-cleaning surfaces, air and water purification applications, sensor technologies, photocatalyst in sterilization and solar cells for energy storage.[5,6]

Increase in the use of nanoparticles in a wide range of domestic household products, textiles, electronics, biomedical and in industries are some of the exposure routes to reach aquatic ecosystem. As the size of TiO_2-NPs reduced from micro to nano scale, the surface area and the number of atoms exposed to surface increases, which are responsible for the increased binding and reactivity in biological system.[7] Various studies have reported that TiO_2-NPs act as biologically active particles by interacting with cellular components and responsible for the induction of cytotoxicity and

inflammatory responses,[8,9] generation of free radicals and oxidative stress,[10,11] formation of micronuclei and apoptosis.[12] Nanoparticles owing to the small size can easily cross the tissue junctions and even cellular membranes, and this could be one of the reasons for the suspected cyto- and genotoxicity of TiO_2-NPs, which was evidenced by *in vitro* studies.[13,14] Upregulation of Fas and activation of Bax, the proteins involved in apoptosis has been reported as the mechanism behind ROS generation.[15] However, the exact mechanisms behind nanoparticles induced toxicity is still unknown but the morphology, size, surface area, agglomeration status are believed as some of the factors behind nanotoxicity.

The toxicity data of TiO_2-NPs are available from *in vitro* cell line studies to mammalian *in vivo* models. In aquatic ecosystem, TiO_2-NPs enter the water bodies through effluents from industries; leach out from the point of manufacturing, transport and handling or through accident spillage.[16] Meanwhile the reactivity of TiO_2-NPs in aquatic ecosystem is largely influenced by the stabilization of nanoparticles in the water column. Thus aquatic organisms such as fish, molluscs, crustacean and planktons are at high risk of exposure to nanoparticles. Among the aquatic organisms, fish occupy an important position in the food chain as it directly ends up in human consumption. Rainbow trout when exposed to TiO_2-NPs had reported with severe gill injury, oxidative stress and lipid peroxidation in gill, intestine and brain tissues.[17] Acute exposure to TiO_2-NPs in zebrafish proved as potential respiratory inhibitor by the alteration of oxygen consumption, and also decreased total protein and glycogen in gill and muscle tissues.[18] Dietary exposure of TiO_2-NPs to rainbow trout resulted in accumulation of nanoparticles in the gill, gut, liver, brain and spleen, followed by lipid peroxidation and ionic imbalance.[19] Likewise, intravenous administration of TiO_2-NPs has been reported to accumulate in kidneys of rainbow trout.[20] Exposure to TiO_2-NPs at 10 and 100ppm concentrations for 96h decreased the growth rate and also observed with abnormal physiological and behavioural changes in gold fish.[21]

Apart from the physicochemical properties, nanoparticles exposure may attain the toxicity in aquatic ecosystem depending on the concentration and duration of exposure. The high concentration and excessive exposure period naturally cause damage to any organisms. TiO_2-NPs are usually considered as non-toxic and widely used in many domestic products and therefore, assessing the toxic impact of the nanoparticles at sublethal concentration for short and long exposure period provides valid information about the harmful effects of the nanoparticles. The present study was thus attempted to examine the persistent toxic effects of the nanoparticles in the fish by examining the antioxidant defense system as the mechanism behind the toxicity. Moreover, the toxic effect of any compounds depends on the rate of reaction, i.e., reversible or irreversible effects. In this regard, the rate of reaction was also documented by treatment withdrawal for 60 days after the exposure period in order to detect the rate of change of the toxic effects of nanoparticles in the freshwater fish, *Oreochromis mossambicus*. The study helps to understand the sublethal ecotoxicological effects of TiO_2 nanoparticles in the fish by highlighting on the antioxidant defence system in gill, liver and brain tissues.

MATERIALS AND METHODS

Test animal

Oreochromis mossambicus (6±1.5g; 6.5±1cm) were collected from Safa Aquarium, Kozhikode, Kerala (11º22'N, 75º85'E), India. In accordance to the ethical law of the country, the use of fish as test animal does not require animal ethics statement. Fish were acclimatized to the laboratory conditions prior to experiment in well aerated, dechlorinated water of 40L capacity. The physico-chemical features of the water were estimated as per APHA guidelines.[22] Standard water temperature (28 ± 2°C), oxygen saturation of water (70 and 100 %) and pH (6.5 to 7.5) were maintained throughout the experiment in both control and treatment groups.

Chemical and experimental design

TiO_2-NPs (Cat. No: 634662; Titanium (IV) oxide, mixture of anatase and rutile) were obtained from Sigma Aldrich, Germany. The purity and size of the nanoparticles (11.4nm) confirmed by X-ray diffraction and Transmission Electron Microscopy using Scherrer's formula.[23] The nanodispersion was prepared just before exposure by ultra-sonication at 100 kHz for 30 min using double distilled water without solvent and maintained as stock. Our previous study had determined the median lethal concentration (LC_{50}-96 h) of TiO_2-NPs in *Oreochromis mossambicus* by using probit analysis as 164mg/L.[23] One-tenth of LC_{50} was selected as sublethal concentration i.e., 16.4 mg/L for the present study. Fish were exposed to 16.4 mg/L of TiO_2-NPs for short-term (24, 72 and 96 h) and long-term (15, 30 and 60 days) durations maintaining control group (without toxicant). In each treatment groups ten fishes were maintained and the health conditions were continuously monitored during the experiment. After 60 days of treatment, fish were maintained in water without toxicant for another 60 days for studying toxicity withdrawal of nanoparticles. At the end of every treatment, fish were captured using small dip net with least disturbances in order to avoid stress and killed by decapitation. Tissues such as gill, liver and brain were dissected out to cold normal saline, cleaned for debris and 1% (w/v) crude tissue homogenates were prepared in ice-cold saline using a motor driven tissue homogenizer. The homogenates were centrifuged at 800*g* for 15min at 4°C and the supernatants collected were then used for the biochemical analysis.

Biochemical analysis

Total protein was estimated by the method of Lowry et al.[24] with BSA as the standard. Activities of antioxidant

enzymes, superoxide dismutase,[25] catalase,[26] glutathione reductase,[27] glutathione peroxidase,[28] and levels of hydrogen peroxide generation,[29] lipid peroxidation[30] were determined in gill, liver and brain tissues. Activities of marker enzymes namely alkaline phosphatase[31] in gill and liver tissues, and acetylcholinesterase[32] activity in brain tissue were also analyzed.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p<0.05$ against the control group. Data are presented as mean± SD for ten animals per group and all biochemical estimations were carried out in duplicate.

RESULTS

Effect of TiO_2-NPs on the body weight and tissue weights

Body weight of the animal remained unchanged after short-term and long-term exposure of titanium dioxide nanoparticles (Table 1). The weight of gill tissue showed significant increase from 96 h of exposure onwards in time-dependent manner (Table 1). While the hepatosomatic index and weight of brain showed no significant changes in all treatment groups (Table 1). The reversal of treatment for 60 days showed significant increase in the weights of gill and brain tissues whereas the body weight and hepatosomatic index remained unchanged when compared to the control group (Table 1).

Table 1: Effect of titanium dioxide nanoparticles (TiO_2-NPs) on the body weight and tissue weights of the fish, *Oreochromis mossambicus*.

Parameters	TiO_2-NPs (16.4 mg/L)							Treatment withdrawal (60 days)
	Short-term exposure				Long-term exposure			
	Control	24 h	72 h	96 h	15 days	30 days	60 days	
Body weight (g)	6.71±0.19	6.58±0.32	6.39±0.31	6.13±0.18	6.76±0.31	6.56±0.38	6.98±0.26	7.02±0.16
Gill weight (mg)	143±11.9	140±4.90	146±6.74	157±3.31*	160±2.88*	162±5.49*	192±3.42*	185±2.69*
Hepatosomatic index (%)	13.53±1.86	13.46±1.42	13.73±0.61	14.52±1.57	13.00±1.94	14.07±1.57	13.45±1.12	13.14±0.61
Brain weight (mg)	16.5±1.81	15.5±3.30	14.8±3.96	13.5±2.27	15.4±4.35	15.1±4.33	13.5±2.27	19.3±0.82*

Values expressed in Mean±SD; n=10/group; *P<0.05 against control group

Effect of TiO_2-NPs on antioxidant status in gill tissue

Exposure to TiO_2-NPs showed no significant changes in the activity of superoxide dismutase in short-term treatment group, however, the enzyme activity increased significantly ($P<0.05$) after 15 days of treatment and consequently decreased in time-dependent manner with significant ($P<0.05$) reduction after 60 days of treatment (Figure 1). Activity of catalase significantly ($P<0.05$) increased after 96 h in short-term group, while in the long-term group, the changes were similar to the activity of superoxide dismutase (Figure 1). There was a significant ($P<0.05$) decrease in the activities of glutathione reductase and glutathione peroxidase in time-dependent manner after short-term and long-term exposure (Figure 1). Level of hydrogen peroxide generation was found increased significantly after 96 h onwards in duration dependent manner when compared to the control group. However, the level of lipid peroxidation was found a significant increase only after 60 days of exposure (Figure 1). The activities of superoxide dismutase and catalase along with the levels of hydrogen peroxide generation and lipid peroxidation increased significantly ($P<0.05$) whereas the activities of glutathione reductase and glutathione peroxidase were decreased significantly ($P<0.05$) after the treatment withdrawal for 60 days (Figure 1).

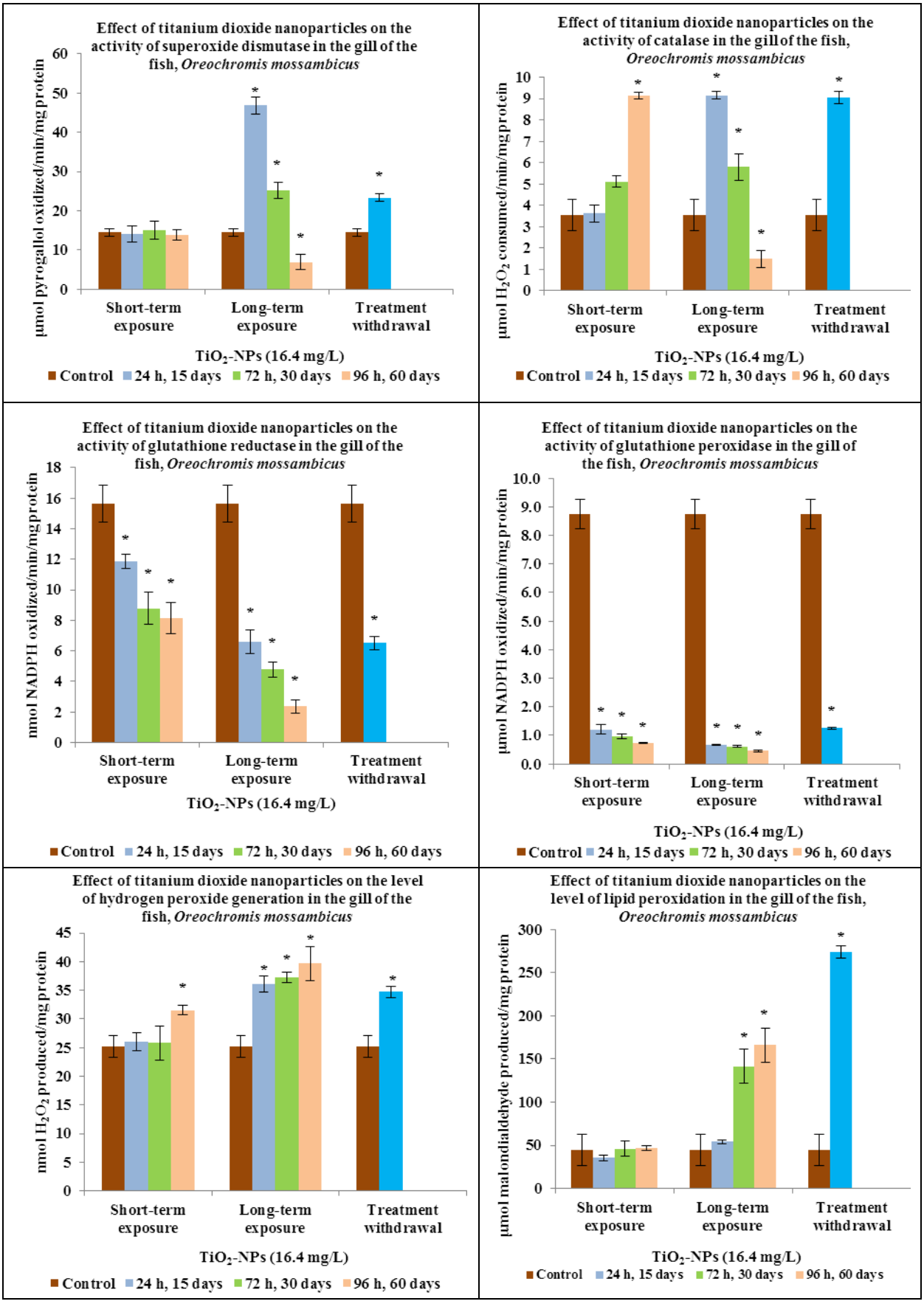
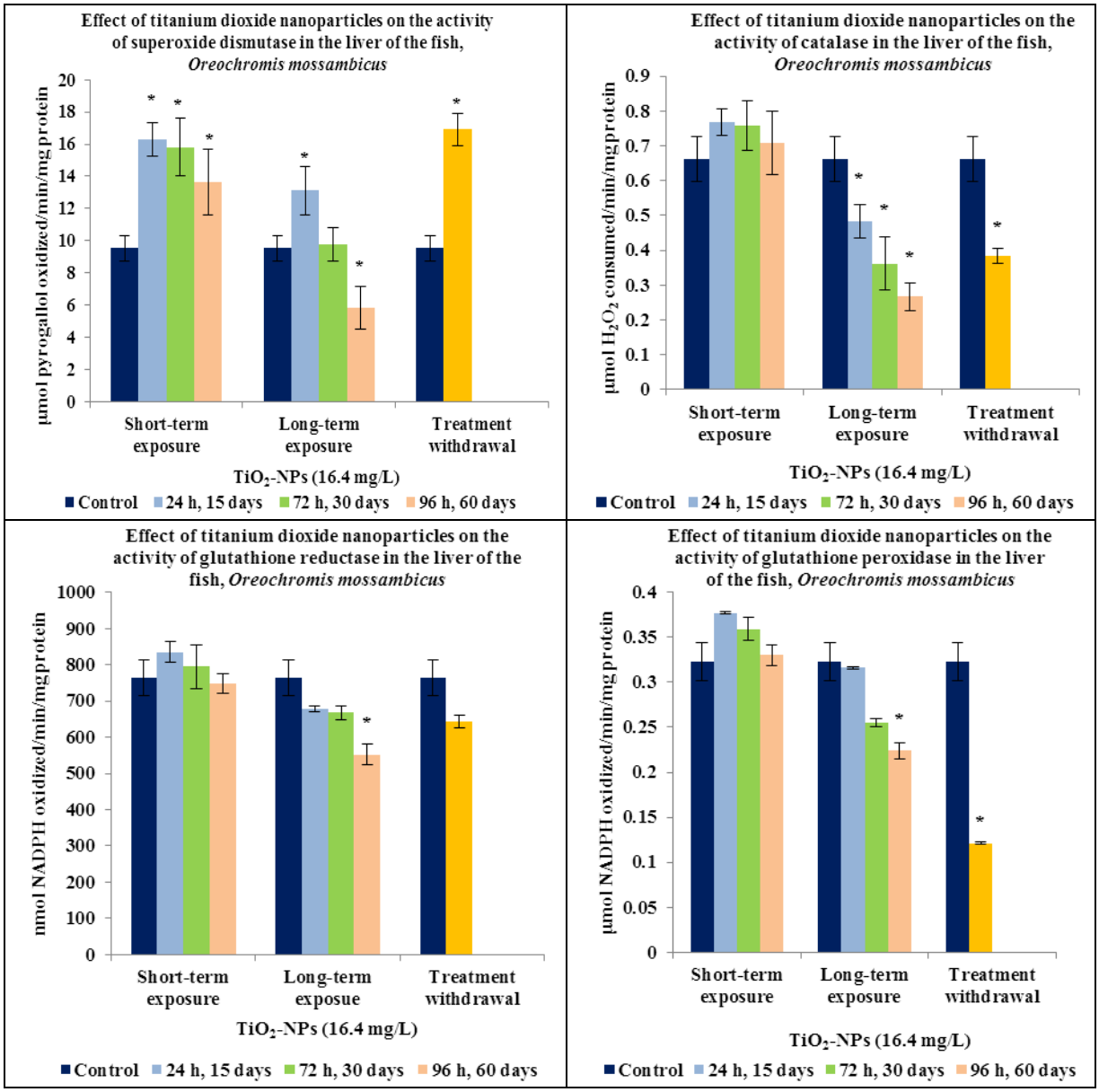

Effect of titanium dioxide nanoparticles on the activity of superoxide dismutase in the gill of the fish, Oreochromis mossambicus
µmol pyrogallol oxidized/min/mg protein
Short-term exposure
Long-term exposure
Treatment withdrawal
TiO2-NPs (16.4 mg/L)
Control 24 h, 15 days 72 h, 30 days 96 h, 60 days
Effect of titanium dioxide nanoparticles on the activity of catalase in the gill of the fish, Oreochromis mossambicus
µmol H2O2 consumed/min/mg protein
Effect of titanium dioxide nanoparticles on the activity of glutathione reductase in the gill of the fish, Oreochromis mossambicus
nmol NADPH oxidized/min/mg protein
Effect of titanium dioxide nanoparticles on the activity of glutathione peroxidase in the gill of the fish, Oreochromis mossambicus
µmol NADPH oxidized/min/mg protein
Effect of titanium dioxide nanoparticles on the level of hydrogen peroxide generation in the gill of the fish, Oreochromis mossambicus
nmol H2O2 produced/mg protein
Effect of titanium dioxide nanoparticles on the level of lipid peroxidation in the gill of the fish, Oreochromis mossambicus
µmol malondialdehyde produced/mg protein

Figure-1.

Effect of TiO_2-NPs on antioxidant status in liver tissue

Liver tissue showed significant (P<0.05) increase in the activity of superoxide dismutase in short-term exposure, which was found to be decreasing in time-dependent manner with significant decrease after 60 days of nanoparticles exposure (Figure 2). The activities of catalase and glutathione reductase showed no significant changes in the short-term exposure group, but the activity of catalase showed a time-dependent significant (P<0.05) decrease after all durations in the long-term exposure group with significant reduction in the activity of glutathione reductase only after 60 days of treatment (Figure 2). Glutathione peroxidase enzyme activity was found decreased in both short-term and long-term exposure groups, however, significant (P<0.05) reduction was noticed only after 60 days of nanoparticles exposure (Figure 2). The levels of hydrogen peroxide generation and lipid peroxidation showed no significant changes after short-term treatment whereas, a significant (P<0.05) increase was noticed only after prolonged exposure (Figure 2). Treatment withdrawal for 60 days showed significant (P<0.05) increase in the activity of superoxide dismutase, while the activities of catalase, glutathione reductase and glutathione peroxidase decreased with concomitant significant (P<0.05) increase in the levels of hydrogen peroxide generation and lipid peroxidation (Figure 2).



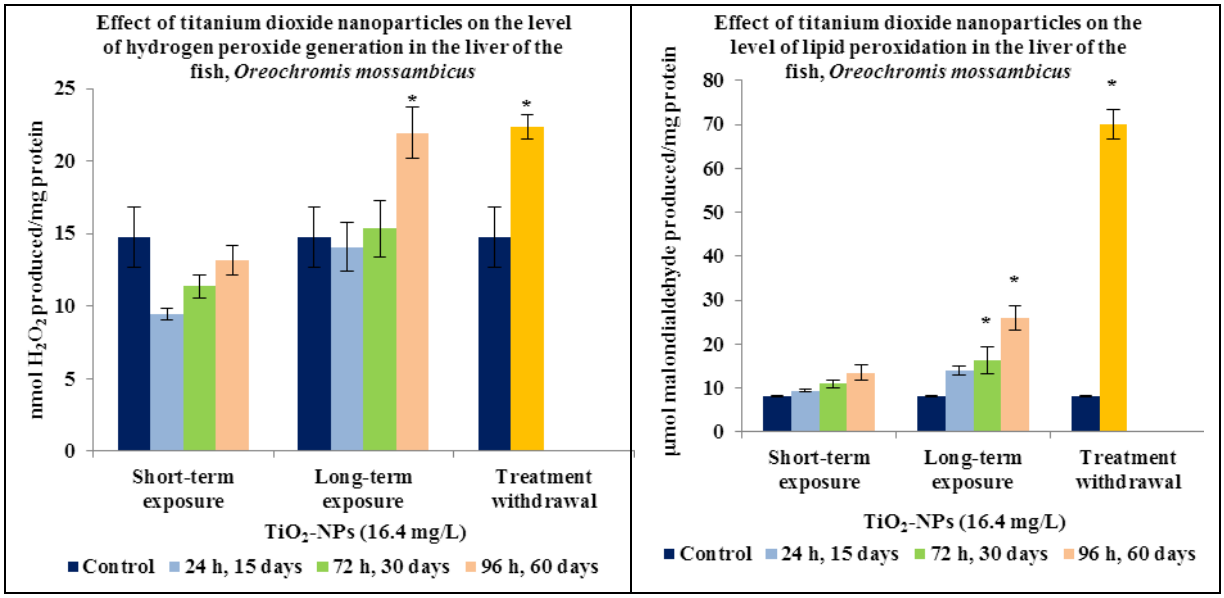
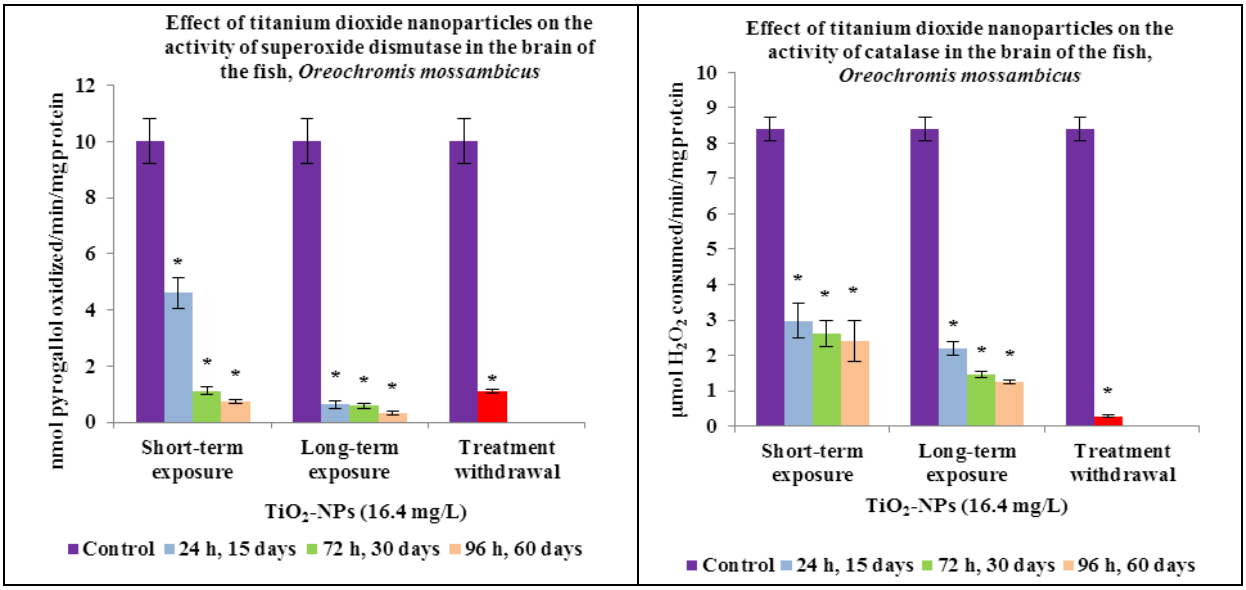


Figure-2.

Effect of TiO_2-NPs on antioxidant status in brain tissue

TiO_2-NPs exposure showed significant ($P<0.05$) decrease in the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase in time-dependant manner in both short-term and long-term treatment groups when compared to the control group (Figure 3). The levels of hydrogen peroxide generation remained unchanged in short-term exposure group with tremendous time-dependent increase in the long-term treatment group (Figure 3). Level of lipid peroxidation was increased significantly only after 30 and 60 days of treatment and no significant increase was observed in other treatment groups (Figure 3). The observations after treatment withdrawal was found similar to that of the treatment group (Figure 3).



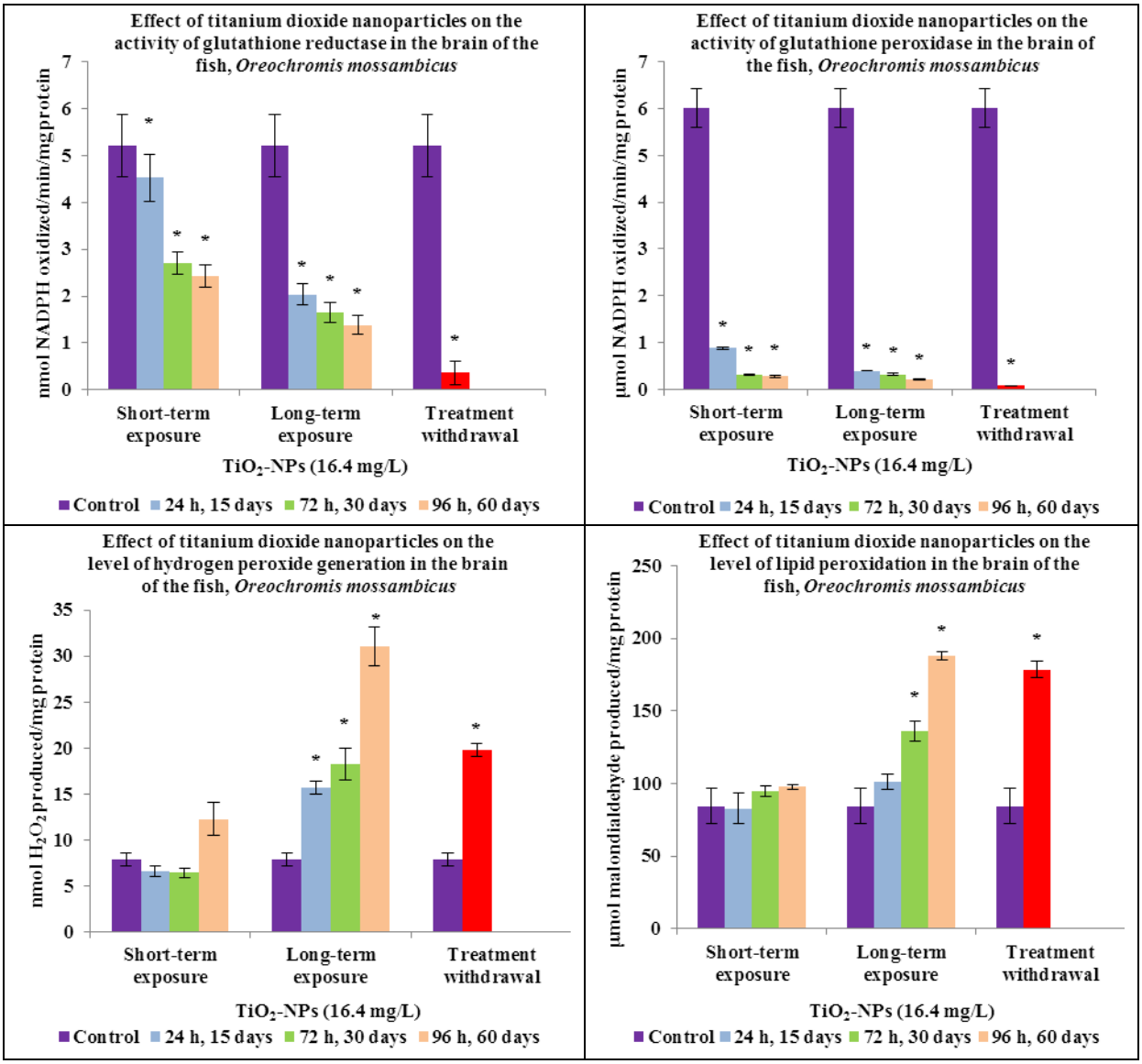


Figure-3.

Effect of TiO_2-NPs on the activities of tissue specific marker enzymes

The activity of alkaline phosphatase decreased significantly ($P<0.05$) in all treatment groups after short and long-term exposure in gill tissue (Figure 4). In liver tissue, the activity of alkaline phosphatase remained unchanged in short-term treatment group while there was a significant ($P<0.05$) reduction in the activity of enzyme after long-term exposure (Figure 4). Acetylcholinesterase, the brain marker enzyme, decreased significantly in both short-term and long-term exposure groups (Figure 4). Withdrawal of treatment for 60 days showed significant ($P<0.05$) decrease in the activities of all tissue marker enzymes when compared to the control group (Figure 4).

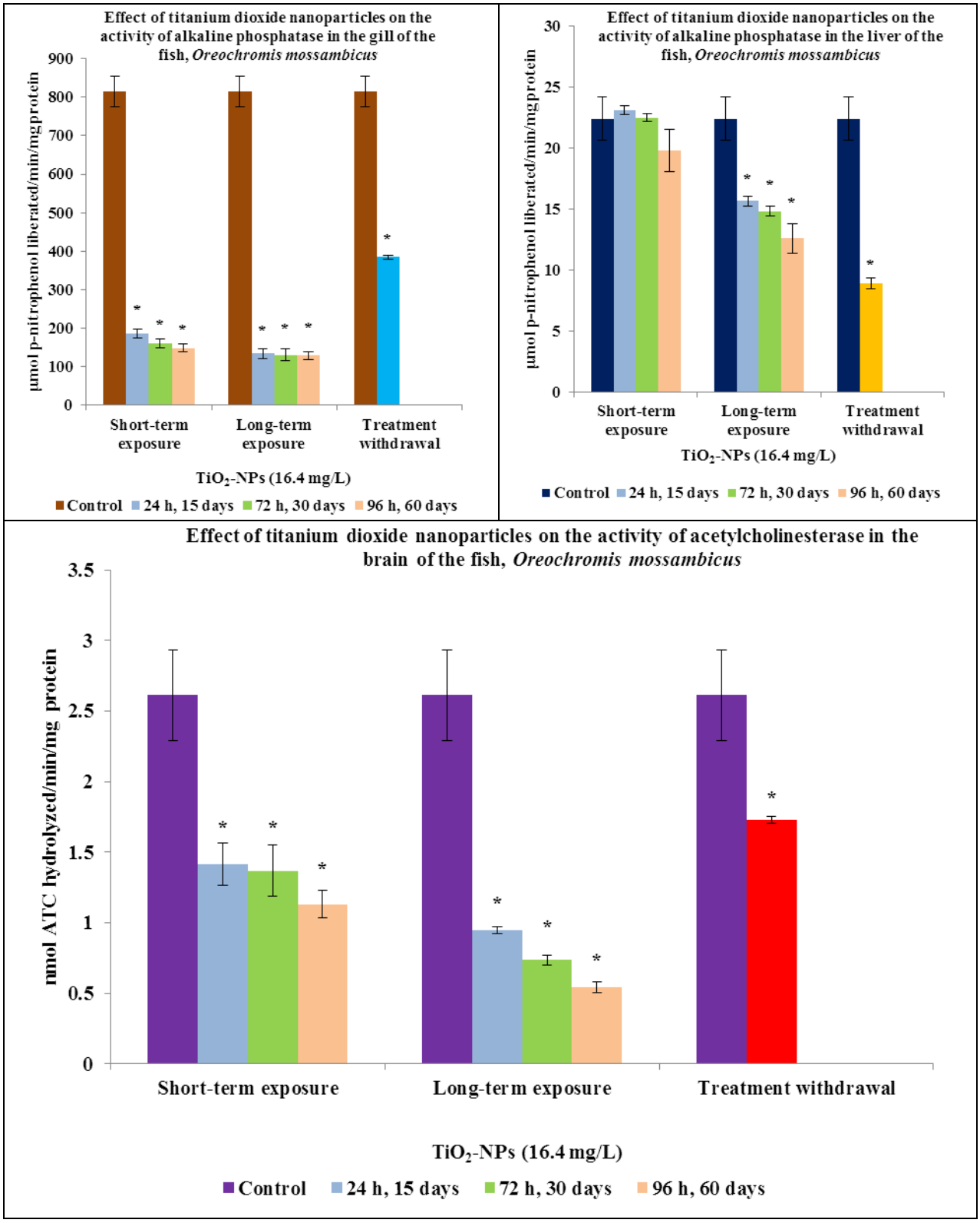


Figure-4.

DISCUSSION

Wide range of contaminants, including nanoparticles, is continuously released into the aquatic ecosystem that poses a menace to the organisms dwelling in the environment. The adverse effects of the toxicants on various organisms were monitored regularly by assessing several ecotoxicological endpoints. Dose-response relationship is one of the relevant toxicity testing that provides information about the hazard and risk created by the exposed toxicants. The present study evaluates the adverse toxic effects of one of the metal nanoparticles, titanium dioxide at sublethal concentration, i.e., 16.4mg/L for short-term and long-term durations.

Sublethal level of nanoparticles is generally expected to accumulate within the tissues of an organism over a longer period of time. Thus the present study was designed to expose the nanoparticles for 60 days duration along with the short-term exposure group. In this respect, the assessment may be made by comparing the toxic impacts of nanoparticles for acute and chronic durations.

The proper choice of toxicity tests in laboratory animals provide sufficient information regarding the risks posed by the toxicants and its direct or indirect influence on humans as well. Toxicity tests mainly involve exposing one of the toxicant to a well-defined test organism under controlled laboratory condition.[33] Fishes are considered as an important source of proteins and lipids for humans and for some domestic animals, thus maintaining the health status of fishes is indirectly essential for human welfare. In the present study, one of the topmost commercially cultured fish of high economic importance, *Oreochromis mossambicus* was used as the test organism. There are numerous experimental evidences suggesting the role of oxygen free radicals causing damage to cellular tissues in biological systems. Therefore, the present study focused on the effect of nanoparticles on the antioxidant status, the most significant topic in ecotoxicological studies. From the toxicological point of view, it is always necessary to understand that some of the toxicants exerts its adverse effects only for short durations and their effects are reversible because they rapidly undergo biotransformation and are readily eliminated from the body of the organisms. However, some toxicants are highly persistent since they form strong bond with the receptors of the tissues that leads to irreversible functional or structural damage to the tissues of organisms. This endpoint is also analysed in the present study by the toxicity withdrawal treatment for 60 days after the exposure period. Hence the present study emphasis on the influence of titanium dioxide nanoparticles in the antioxidant system of gill, liver and brain tissues of the fish, *Oreochromis mossambicus*.

TiO_2-NPs did not affect the body weight, hepatosomatic index and weight of brain tissues of the animal when exposed for short-term and long-term durations. However, the weight of gill tissue increased from 96 h of exposure onwards in time-dependent manner and this may be due to treatment related inflammation or severe mucous deposition. In all aerobic organisms, cellular metabolism involves the production of oxygen free radicals and non-reactive species often referred as reactive oxygen species (ROS). Sequential reduction of molecular oxygen often leads to the formation of group of ROS such as superoxide anion, singlet oxygen, hydrogen peroxide and hydroxyl radical. Minimal levels of ROS are generated inside the mitochondria as oxygen is reduced along the electron transport chain. It is also formed as necessary intermediates in a variety of enzyme reactions and is important to perform various physiological activities.[34] Despite the beneficial activities of ROS, it is also toxic to the cells as it possesses an unpaired electron, which makes them highly reactive thereby damaging all macromolecules including proteins, lipids and nucleic acids.[35] Thus imbalance in the level of generation and the level of scavenging of ROS leads to a condition called oxidative stress.

Exposure to environmental contaminants is known to generate ROS and its excessive production is removed by the antioxidant defence system. In the present study TiO_2-NPs exposure showed no significant changes in the activity of superoxide dismutase in short-term durations of gill tissues, which suggest that the minimal level of ROS generated are easily removed by the enzyme. Superoxide dismutase (SOD) is the enzyme that catalyzes the conversion of superoxides into hydrogen peroxide and oxygen. The increase in the activity of SOD after 15 days indicate the scavenging activity of gill tissue to eradicate the free radicals formed as a result of nanoparticles exposure. However after prolonged exposure for 60 days reduced the activity of SOD and this may be due to the failure of enzyme to eliminate the oxygen free radicals. Catalase is the antioxidant enzyme that functions to degrade hydrogen peroxide to water and oxygen. Activity of catalase significantly increased after 96 h up to 15 days whereas significant reduction was noticed after 60 days of nanoparticles exposure. The activity of catalase is found to function similar to the activity of SOD after nanoparticles exposure indicating the failure of antioxidant defense system to remove the hydrogen peroxide formed. TiO_2-NPs exposure decreased the activities of glutathione reductase and glutathione peroxidase in short-term and long-term exposure groups. Similar to catalase, glutathione related enzymes like glutathione reductase and glutathione peroxidase are the next level defensive enzymes against the formation of hydrogen peroxide. Glutathione peroxidase is important for reducing oxidative stress inside the cell and it converts hydrogen peroxide to water along with the conversion of glutathione (GSH) to glutathione disulfide (GSSG). On the other hand, glutathione reductase converts glutathione disulfide to monomeric glutathione.[36] The reduction in the activities of antioxidant enzymes are found correlated to the increase in the level of hydrogen peroxide generation after 96 h of nanoparticles exposure in time-dependent manner. One of the best known toxic effects of oxygen radicals as a result of TiO_2-NPs exposure is damage to cellular membrane. This is evident by the increase in the level of lipid peroxidation in gill tissues. Peroxidation of membrane lipids generally leads to several pathological effects as increase in membrane rigidity, alteration in activity of membrane receptors and altered permeability.[37] The present results coincide with the exposure of fullerene C_{60} nanoparticles to gill tissue of *Pseudetroplus maculatus*.[38] The increase in the levels of hydrogen peroxide generation and lipid peroxidation was observed in the gill tissue after the treatment withdrawal for 60 days indicating the persistent effect of

nanoparticles in gill tissue that could result in more permeable and rigid gill membrane.

Liver is the prime organ responsible for detoxification and metabolism of pollutants and thus most susceptible target tissue to toxicants. The present study examined the antioxidant status of hepatic tissues after nanoparticles exposure, which showed significant reduction in the activities of all antioxidant enzymes after long-term exposure group. However, the short-term exposure groups showed no significant changes and this could be the scavenging activity of hepatic antioxidant enzymes. But the failure of antioxidant defense system of liver tissue was proved by the decrease in the activities of antioxidant enzymes with concomitant increase in the levels of hydrogen peroxide generation and lipid peroxidation in the long-term exposure groups. Similar observations have been reported when silicon dioxide nanoparticles exposed to hepatocytes of the fish, *Oreochromis mossambicus*.[39] Treatment withdrawal for 60 days showed significant increase only in the activity of superoxide dismutase, while the activities of catalase, glutathione reductase and glutathione peroxidase decreased with simultaneous increase in the levels of hydrogen peroxide generation and lipid peroxidation. The results observed suggest the failure of detoxification mechanism in liver tissue and the possible stability and persistence of nanoparticles in the liver tissue.

The assessment of the effects of TiO_2-NPs on brain tissues showed significant decrease in the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase in time-dependant manner in both short-term and long-term treatment groups. This signifies the failure of antioxidant enzymes in the brain tissue to eliminate the toxic oxidants. The level of hydrogen peroxide generation remained unchanged in short-term exposure group while a remarkable time-dependent increase was observed in the long-term treatment group. Exposure to TiO_2-NPs increased the level of lipid peroxidation after 30 and 60 days of treatment indicating that the brain tissue is subjected to oxidant challenges owing to the exogenous exposure of nanoparticles. Thus TiO_2-NPs induced oxidative stress in brain tissues and the results are in agreement when fullerene C_{60} nanoparticles were exposed to the brain of the fish, *Pseudetroplus maculatus*.[40] The results of treatment withdrawal was found similar to that of the nanoparticles exposed group thereby suggesting that the toxicant exposure could cause irreversible brain damage, which proves the fact on the persistence of oxidative stress even after the withdrawal.

The toxic effects of titanium dioxide nanoparticles were further measured by evaluating the tissue specific marker enzymes. Alkaline phosphatase is the lysosomal enzyme involved in hydrolysis of exogenous materials, transphosphorylation and membrane transport.[41] TiO_2-NPs decreased the activity of alkaline phosphatase in all treatment groups of gill tissue, which indicate the impaired membrane transport and cellular toxicity. In liver tissue, the activity of alkaline phosphatase remained unchanged in short-term treatment group whereas the enzyme activity was found decreased after long-term exposure and this could be due to the altered state of inter and intracellular membrane transport in liver tissue. Similar results have been observed in gill tissue of *Pseudetroplus maculatus* treated with fullerene C_{60} nanoparticles[38] and in the liver tissue of *Oreochromis mossambicus* exposed to silicon dioxide nanoparticles.[39] Acetylcholinesterase, the neurotransmitter enzyme, found abundant in neuromuscular and nerve junctions is widely used as a biomarker to detect the effect of any toxicant on nerve transmission. The present findings showed decrease in the activity of the brain marker enzyme, which represents the accumulation of acetylcholine across the nerve synapse that interferes with the normal function of nervous system. The results are in agreement with another study that exposure to octylphenol, one of the environmental contaminants decreased the activity of acetylcholinesterase in the brain tissue of *Oreochromis niloticus*.[42] Withdrawal of nanoparticles for 60 days showed decrease in the activities of all tissue marker enzymes indicating tissue specific severe and irreversible damage.

CONCLUSIONS

To brief, the present findings suggest that exposure to titanium dioxide nanoparticles imbalance the pro-oxidant/ antioxidant status in gill, liver and brain tissues. The induction of oxidative stress observed as a result of nanoparticles exposure was found to be stable and persistent even after the treatment withdrawal for 60 days. The irreversible damage of tissues receive great interest as the study is based only on exposure to single nanoparticles in the laboratory condition, but the natural ecosystem is continuously exposed to several range of contaminants and its effects in different combinations deserves much attention.

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CONFLICT OF INTEREST

No conflict of interest

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