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ADVERSE IMPACT OF TITANIUM DIOXIDE NANOPARTICLES ON *CHANNA PUNCTATUS*

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ABSTRACT
Titanium dioxide nanoparticles (TiO2 NPs) are widely used nanomaterials because of their photocatalytic characteristics and capacity to absorb ultraviolet light at specific wavelengths. This study investigated the toxicological effects of rutile TiO2 nanoparticles on *Channa punctatus* by assessing their influence on adult individuals. The adult toxicity test examined the effects of TiO2 NPs on oxidative damage in the gill tissue. The presence of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione S transferase (GSTs), was identified in gills of *channa punctatus*. The results suggested that extended exposure to TiO2 nanoparticles can cause oxidative damage to gills. Moreover, extended exposure to TiO2 nanoparticles can result in increased levels of catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferases (GSTs) in gills of mature *channa punctatus* as a defensive reaction against adverse effects. The gill of mature *channa punctatus* displayed more prominent effects after prolonged exposure to TiO2 nanoparticles.

Keywords: Channa punctatus, oxidative stress , titanium dioxide, nanoparticle

Introduction

Nanotechnology has made substantial advancements, resulting in the widespread use of nanoparticles in industries such as beauty products, clothes, and computers. Over time, significant progress has been made in the production of nanoparticles, resulting in significant improvements in their physical as well as chemical properties. The progress in these areas has been essential in the increased application of nanoparticles.

Improper dumping of nanoparticles can lead to detrimental environmental consequences [1]. Nanoparticles have a high ratio of surface area to mass because they are very small in size. This phenomenon enables the creation of an optimal environment for biochemical reactions and leads to the binding of dangerous compounds onto nanoparticles. Nanoparticles possess the capability to penetrate the blood-brain barrier and blood-eye barrier. They can produce a variety of xenobiotic consequences. researchers conducted a new study which revealed the presence of silver nanoparticles in many systems of zebrafish embryos, such as the brain, the yolk, the blood, and heart. The deposition led to substantial erosion, suggesting possible detrimental consequences. [2]

The use of nanoparticles requires careful evaluation of their potential ecological and health implications. Nevertheless, the current literature on this topic is restricted, and a thorough comprehension of the biological processes that underlie nanomaterials is still unfinished.

Currently, our understanding of the possible good or harmful effects of nanoparticles on fish is limited, and we have a poor comprehension of the underlying mechanisms involved. Multiple industries, such as the medical field, sensor technology, and water purification systems, introduce nanoparticles, or NPs, into water sources during the production and use of their goods. Hence, the presence of nanoparticles (NPs) in aquatic habitats poses substantial health risks to aquatic creatures. The harmful impacts of NPs have been previously established for many aquatic and terrestrial animals.[3]

Titanium dioxide nanoparticles (TiO_2-NPs) are widely used artificial substances that are commonly found in a range of industrial sectors, such as textiles, coatings, cosmetics, personal care products, and food products. The nanoparticles possess remarkable anti-corrosion properties, endurance, and robust photocatalytic activity, making them highly prized. Due to their inherent photocatalytic properties, these substances display catalytic behaviour when exposed to ultraviolet (UV) radiation. This enables them to facilitate the destruction of pesticides, polychlorinated biphenyls (PCBs), and other pollutants found on surfaces exposed to sunlight and atmospheric conditions. When the size of TiO_2 nanoparticles is reduced to less than 100 nm, they pose a much higher threat to land and water creatures compared to TiO2 particles larger than 100 nm, which have been found to be harmless to both human health and the ecosystem.[4]

Multiple studies have been conducted to assess the toxicological effects of micro and nano-scale titanium dioxide. Multiple research have documented the presence of adverse effects on human health and the biological environment linked to these compounds. The majority of the research mostly examined animal models, with a particular focus on exploring the impact on the gastrointestinal system, as well as evaluating potential respiratory hazards and exposure through the skin[5]. There is a lack of extensive research on the effects of pure TiO2-NP on aquatic organisms, especially for long-term exposure. This is evident from investigations conducted by Dong et al. (2020). Prior studies on the influence of nanoparticles on aquatic organisms mostly focused on examining the consequences of brief periods of exposure. Significantly, these studies detected genotypic modifications, such as abnormalities in ribosomal function.[8]

The findings of a short-term exposure study on rainbow trout and titanium dioxide indicated the occurrence of abnormalities in the gills and a decrease in the activity of Na+ K+ ATPase in both the gills and intestines. The aforementioned research has shown the existence of detrimental effects induced by titanium dioxide nanoparticles on aquatic species. Nevertheless, it is crucial to emphasise that additional testing with extended exposure is necessary in order to obtain a more thorough comprehension of the long-term effects [9]. An investigation into the impact of extended exposure to TiO2 on zebrafish revealed that the suppression of growth was influenced by both the concentration and duration of the treatment. An independent investigation revealed the aquatic harmfulness of copper oxide (CuO) nanoparticles on carp, revealing that the inhibition of growth occurred at higher concentrations (100 mg/L) for a period of 30 days[9]. A 21-day study was done to investigate the effects of TiO2-NPs, combined with paraquat, on carp.

The examination uncovered modifications in the blood chemistry of the fish. The previous research primarily concentrated on the aquatic effects of nanoparticles[11]. Nevertheless, there is a dearth of published information regarding the dietary exposure of fish to TiO2-NPs, particularly in terms of growth indicators. This study aimed to evaluate the effects of consuming titanium dioxide nanoparticles (TiO2-NPs) through diet on fingerlings of the freshwater fish species Channa Punctatus[10]. Channa punctatus was chosen because it is classified as one of the prominent carp species that are found in the Indian subcontinent, along with *Catla catla and Cirrhinus mrigala*. Moreover, owing to its quick growth, substantial yield, and positive reception from consumers, this specific fish species is widely acknowledged as one of the most cultivated in South Asia. [12]

Methodology

Nanoparticle preparation

Distilled water was used to achieve stock solutions of TiO2 nanoparticles, which were then dispersed using an ultrasonic bath. Afterwards, the solutions were added to exposure tanks filled with 10 litres of dechlorinated water to create TiO2/L levels that were intentionally different.

Exposure Assays

Young fish, aged less than one year, were randomly allocated into groups of 15 fish each. These groups were placed in polystyrene test tanks with a volume of 15 L, following the acclimatisation phase. The fish were exposed to different concentrations of TiO2 nanoparticles, ranging from 200 parts per million (ppm) to 800 ppm per litre (L). The experiment group of fish was placed in a separate aquarium filled with clean tap water that was free from any contaminants. The fish were subjected to a battery of tests that included maintaining a constant supply of oxygen and controlling the temperature with precision. The experiment parameters of each tank were modified at regular periods of forty-eight hours during the whole twenty-one-day test period. During the trial, the fish were given a daily allotment of commercially manufactured dry food flakes, which they could also access freely. The tally of perished fish in the tanks was undertaken in a constant fashion.

Assay of antioxidant associated enzyme activities

The levels of antioxidant enzymes, including superoxide dismutase (SOD, EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione peroxidase (GPx, EC 1.11.1.9), as well as antioxidant associated enzymes such as glutathione-S-transferase (GST, EC 2.5.1.18), glutathione reductase (GR, EC 1.6.4.2), were measured using the methods described in a previous study [13]. A single unit of SOD activity is determined as the quantity of enzyme (per milligramme of protein) that reduces the quercetin oxidation reaction by 50% of its maximum inhibition. The inhibition values of superoxide dismutase (SOD) activity were determined using an enzyme kinetics software programme (version 3.1) developed by Brooks (1992). A single unit (U) of catalase, GST, GR, GPx, is quantified as the quantity of enzyme that consumes 1 micromole of substrate or produces

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1 micromole of product within a minute. Activities were quantified in international units (or milliunits) per milligramme of soluble protein (U mg protein^-1). [14]

The MDA concentration was determined in the homogenised gill tissues using the Placer et al. (1966) method. The reaction mixture comprised 0.2 ml of homogenised gill tissue, 1.3 ml of 0.2 M Tris–0.16 M KCl buffer (pH 7.4), and 1.5 ml of thiobarbituric acid reagent. The solution was subjected to heat in a water bath at its boiling point for a duration of 10 minutes. Following the chilling process, a mixture of 3 ml of pyridine/n-butanol (3:1, v/v) and 1 ml of 1 N sodium hydroxide was introduced and thoroughly combined through vigorous shaking. A blank was run simultaneously by introducing 0.2 ml distilled water instead of the gill tissue. The measurement of the test sample's absorbance was taken at a wavelength of 548 nm. The concentration of MDA in nmol per ml was determined using an extinction coefficient of 1.56 × 105.[15]

Statistical analysis

One-way ANOVA was used for statistical analysis of the results after the data have been evaluated for normality and homogeneity (using Leven's test) and, if necessary, correctly converted.

Results and Discussion

SOD

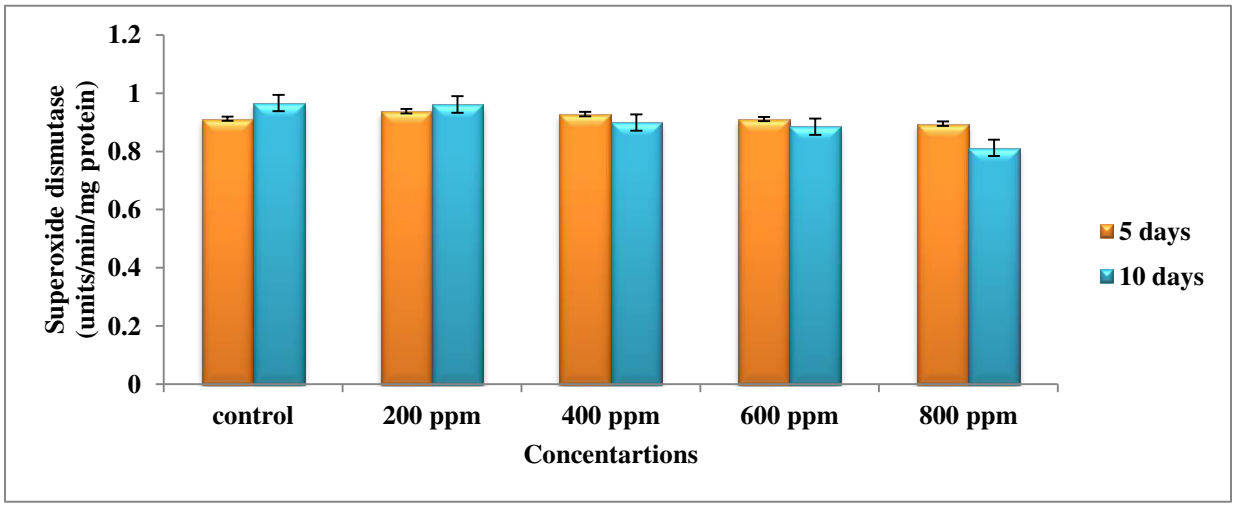

Figure1: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on superoxide dismutase activity in gills were observed. The results obtained from this experiment demonstrated that on increasing the concentration of nanoparticle and exposure time the activity of superoxide dismutase decreased significantly ($P<0.05$). However, at low level i.e., 200 ppm SOD activities were slightly increased (0.938 U/mi/mg) from control group (0.912 U/mi/mg).

Catalase

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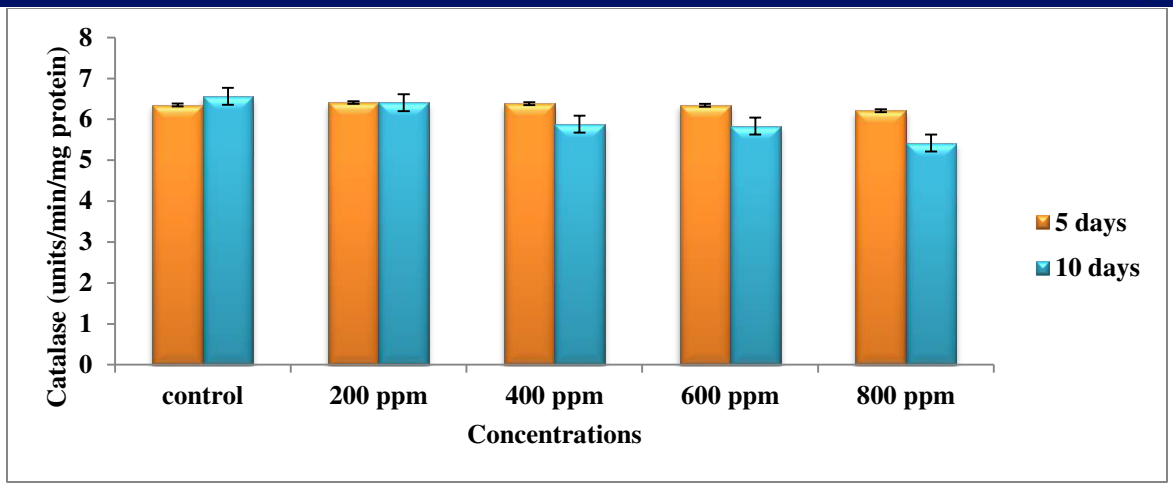

Figure 2: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on Catalase activity in gills were observed. The results obtained from this experiment demonstrated that for 5 days incubation at very low concentration (200 to 600 ppm) activity of catalase increased while at high concentration (800 ppm) activity of catalase slightly decreased in respect to control group. But for 10 days exposure with same doses (200 ppm to 800 ppm) catalase activities were decreased on increasing the concentration of TiO_2NP significantly ($P<0.05$).

Lipid peroxidation

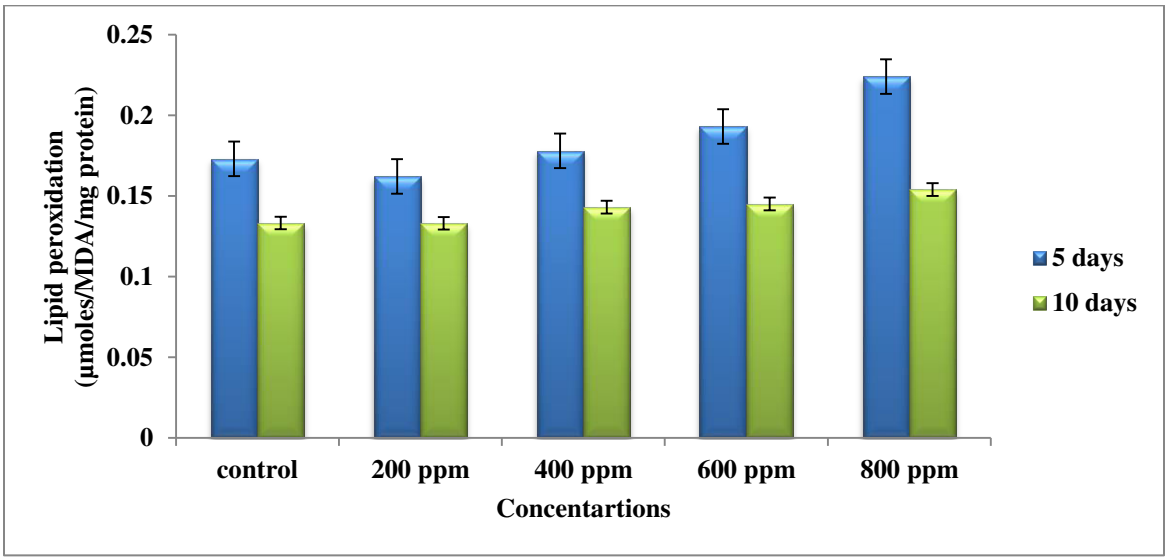

Figure 3: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on lipid peroxidation activity in gills were observed. The results obtained from this experiment demonstrated that for 5 days incubation at very low concentration (200ppm) activity of lipid peroxidation decreased (0.162 µmoles/MDA/mg protein) in respect to control group (0.123 µmoles/MDA/mg) while on high

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concentration (400 ppm 600ppm and 800 ppm) activity of lipid peroxidation (0.178 µmoles/MDA/mg, 0.193 µmoles/MDA/mg, 0.224 µmoles/MDA/mg respectively) increased in respect to control group significantly ($p<0.05$). Furthermore, for 10 days exposure with same doses (400 ppm to 800 ppm) lipid peroxidation activities were decreased on increasing the concentration of TiO_2NP significantly ($P<0.05$) in respect to control group (0.133 µmoles/MDA/mg). The activity at 400 ppm was 0.143 µmoles/MDA/mg, at 600 ppm 0.145 µmoles/MDA/mg, at 800 ppm 0.154 µmoles/MDA/mg were observed. At 200 ppm there was no change observed between case and control group.

Glutathione reductase

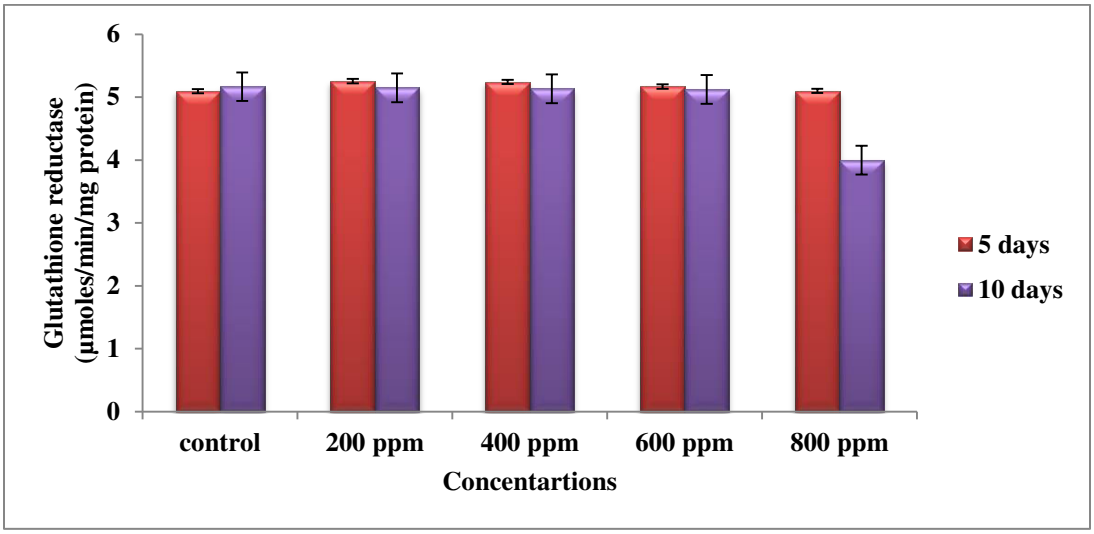

Figure 4: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on Glutathione reductase activity in gills were observed. The results obtained from this experiment demonstrated that for 5 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione reductase (µmoles/min/mg protein) activities were 5.255 µmoles/min/mg protein, 5.243 µmoles/min/mg protein, 5.168 µmoles/min/mg protein, 5.099 µmoles/min/mg protein respectively. Data concluded that at dose 200 ppm to 600 ppm Glutathione reductase activity increased while at 800 ppm activity no significant alteration were observed. Similarly, 10 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione reductase (µmoles/min/mg protein) activities were 5.148 µmoles/min/mg protein, 5.133 µmoles/min/mg protein, 5.124 µmoles/min/mg protein, 4.0 µmoles/min/mg protein respectively.

Glutathione S transferase

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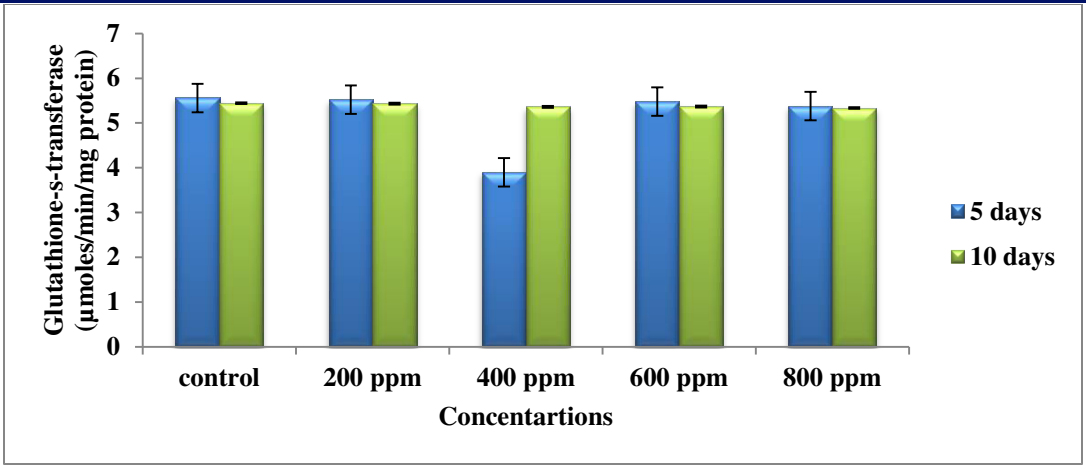

Figure 5: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on Glutathione-s-transferase (µmoles/min/mg protein) activity in gills were observed. The results obtained from this experiment demonstrated that for 5 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione-s-transferase (µmoles/min/mg protein) activities were 5.519µmoles/min/mg protein, 3.899µmoles/min/mg protein, 5.479 µmoles/min/mg protein, 5.377µmoles/min/mg protein respectively. Data concluded that at dose 200 ppm to 600 ppm Glutathione-s-transferase activity increased while at 800 ppm activity no significant alteration were observed. Similarly, 10 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione-s-transferase (µmoles/min/mg protein) activities were 5.148 µmoles/min/mg protein, 5.133 µmoles/min/mg protein, 5.124 µmoles/min/mg protein, 4.0 µmoles/min/mg protein respectively.

Glutathione peroxidase

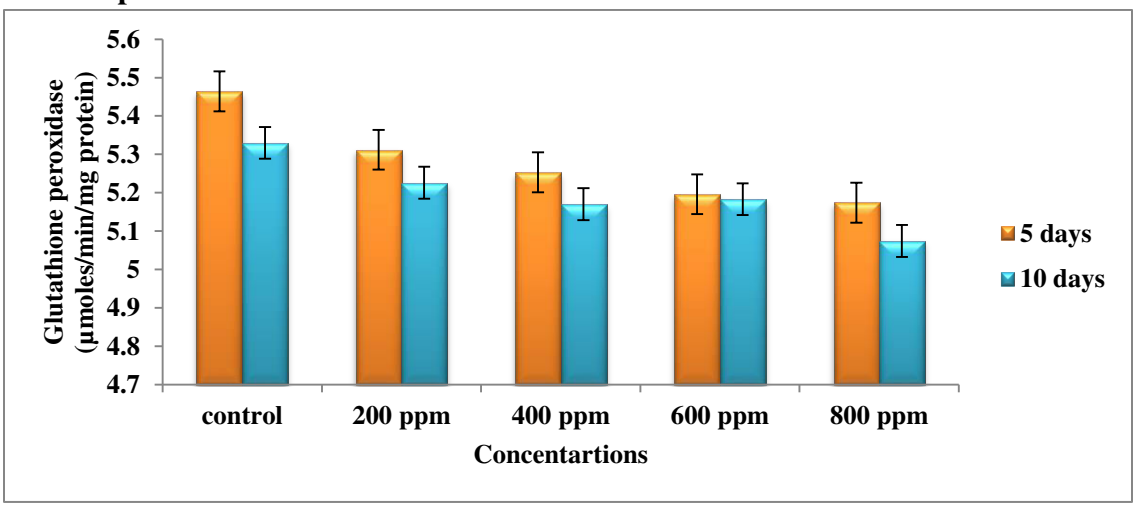

Figure 6: Pictorial representation of results obtained from research in which different concentration of TiO_2 NP exposed to fish and its effect on Glutathione peroxidase

(µmoles/min/mg protein) activity in gills were observed. The results obtained from this experiment demonstrated that for 5 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione-s-transferase (µmoles/min/mg protein) activities were 5.312 µmoles/min/mg protein, 5.253 µmoles/min/mg protein, 5.196 µmoles/min/mg protein, 5.174 µmoles/min/mg protein respectively. Data concluded that at dose 200 ppm to 600 ppm Glutathione peroxidase activity increased while at 800 ppm activity no significant alteration were observed. Similarly, 10 days incubation at 200 ppm, 400ppm concentration, 600 ppm, 800 ppm activity of Glutathione peroxidase (µmoles/min/mg protein) activities were 5.226 µmoles/min/mg protein, 5.17µmoles/min/mg protein, 5.183µmoles/min/mg protein, 5.074 µmoles/min/mg protein respectively.

Discussion

ROS are formed following phagocytosis because of the incorporation of NPs [1], which is a primary mechanism of toxicity and one of the causes of NP toxicity (5,6). Fish, like human beings, have exceptionally sophisticated antioxidant defence mechanisms to fend off the negative effects of ROS (5).As TiO2 particles get smaller and smaller until they are nanoscale, the prospect for photocatalytic activities and UV absorption increases (9). The cell membranes are easily penetrated by NPs, which then interact with intracellular metabolism to produce ROS [18] and generate pro-oxidant effects in the cells they interact with Behra and Krug (9). Fish exposed to TiO2 NPs showed alterations in oxidative stress markers in certain reports on in-vivo and in-vitro experiments (10). Reactive oxygen species (ROS) from TiO2 NPs, especially the OH (11), can be produced.

These defensive systems include enzyme like POD, CAT, SOD as well as nonenzymatic, low-molecular weight antioxidants like GSH (17). The POD, CAT, SOD system is the first line of defence against oxidative damage at the cellular level (12). In this study, *Channa Punctatus* was treated to four different doses of TiO2 NP, ranging from 200 milligrams per litter to 800 milligrams per litter. It was evident that these exposures changed the activity of many tissues' antioxidant enzymes.

Oxidative stress and increase in lipid peroxidation were observed in rainbow trout exposed to sub-lethal toxicity of TiO2 NPs (17). Similarly, when zebrafish exposed to TiO2, ZnO, produced oxygen free radicals in the fish's gill, liver, and gut tissues, causing oxidative stress (13). Other NP: Likewise, the gill and liver tissues of the fish *Oreochromis mossambicus* exposed to Ag NP at 25, 50, and 75 milligrams per litter concentrations for eight days decreased the activity of antioxidant enzymes (16). Another way that nanoparticles might result in a weak antibacterial response is by the loss of immune system and hematopoietic cells in the anterior and posterior kidneys of fish exposed to TiO2 NP. Regardless of the type of NO, loss of hematopoietic and immunological cells in the fish kidney after nanoparticle exposure appears to be a consistent histopathological feature (18). The reported toxicity of TiO2-NPs to juvenile carp may be due to a ROS-induced toxicity pathway whereby TiO2-NPs generate oxidative stress, which results in LPO, and then affect cellular enzymatic defence functions (20). An earlier investigation found

that large mouth bass brains experienced LPO after being exposed to C60 (21). Findings on the oxidative damage in rainbow trout exposed to SWCNT also revealed an increase in LPO in the gill and liver tissues (16).

Freshwater invertebrates like *Daphnia magna* was exposed to TiO2 NP showed significant changes in antioxidant enzymes like CAT, SOD, GPX, and GST at TiO_2 NP concentrations of 5 and 10milligrams per litter in a concentration-dependent manner (12). TiO2 NPs altered the antioxidant enzyme activity (SOD, CAT, POD) in *Cyprinus carpio* and increased the levels of lipid peroxidation (17).

SOD: The decrease in SOD activity to clear the oxidants from *C. gariepinus* muscle tissues (18). Linhua et al. (2009) found that the SOD activity in the carp's brain and gills had significantly decreased. SOD levels in liver tissues were shown to be lower after exposure to TiO2 NPs, according to Xiong et al. (2011). In keeping with this, after being exposed to nano iron, the liver of adult Japanese medaka showed a considerable decline in SOD activity. However, at 100 and 200 milligrams per litter TiO2-NPs, there was a slight increase at first, followed by a dramatic decline in SOD activity, showing that oxidative stress occurred as a result of excessive ROS production and weakened defences (9).

Conclusion

Titanium dioxide nanoparticles (TiO2 NPs), when exposed to *Channa punctatus* for extended periods, can result in oxidative harm to their gill organs. Titanium dioxide nanoparticles (TiO2 NPs) up-regulate the expression of antioxidant enzymes CAT, SOD, and GSTs in *Channa punctatus* during extended periods of exposure, in order to counteract negative effects.

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