




# Study of Some Toxicological Aspects of Titanium Dioxide Nanoparticles Through Oxidative Stress, Genotoxicity, and Histopathology in Tilapia, *Oreochromis mossambicus*

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## Abstract

Extensive use of nanotechnology in multiple commodities is raising concerns about nanotoxicity. In particular, many studies have reported the health-hazardous effects of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs). The present study focuses on the toxicity and accumulation of TiO<sub>2</sub>-NPs in tilapia (*Oreochromis mossambicus*). For this purpose, tilapia was kept in water tanks, acclimatized for 14 days, and treated with different doses of TiO<sub>2</sub> nanoparticles: 0, 0.5, 1.0, and 1.5 mg TiO<sub>2</sub>-NPs/L. Results revealed an increase in the accumulation of TiO<sub>2</sub>-NPs with an increase in doses. Moreover, with a higher dose (1.5 mg/L), gills had maximum levels compared to muscles and liver tissues, whereas other doses showed different accumulation patterns. Catalase, glutathione, and lipid peroxidation levels were significantly higher in the gills, and superoxide dismutase levels were significantly higher in the liver. Characteristics like thickening and fusion in lamellae, rupturing of gill filaments, and hyperplasia of gills were also recorded. The phenomenon of increased necrosis and apoptosis in the liver was also noticed with increasing concentration of TiO<sub>2</sub>-NPs along with the formation of sinusoid spaces and condensed nuclear bodies. Elevated values of olive tail movement and % tail DNA were also noticed with an increased concentration of TiO<sub>2</sub>-NPs. This study concluded that TiO<sub>2</sub>-NPs produced oxidative stress by accumulation in soft tissues and induced pathology and genotoxicity.

**Keywords** Nanoparticle · Toxicity · *Oreochromis mossambicus* · Genotoxicity

## 1 Introduction

The structure dimensions of nanoparticles range from 1 to 100 nm and have existed on earth for millions of years for the use of mankind [1, 2]. The NPs are being fabricated by chemical, physical, and biological methods [3, 4]. These particles are being engineered for various applications in

industries such as drug delivery systems, chemical sensors, biosensors, optics, textiles, medical devices, electronics, and environmental remediation [5–7]. However, extensive use is also responsible for aquatic habitat penetration and raising concerns about ecotoxicology [6, 8]. The most prominent sources of these nanoparticles are colloids found in natural water, soil, and sediments. Volcanic dust in the atmosphere is also an important source of TiO<sub>2</sub>-NPs (Buffle, 2006 and Handy, et al., 2008b). Upon exposure, the concentrations of different types of nanoparticles were recorded much higher in the tissues of aquatic animals than the normal permissible concentrations [9–11].

TiO<sub>2</sub>-NPs are most extensively applicable in dyes, makeup items, and sunscreen [12, 13] and released into the natural environment through wastewater effluents where they cause gill injury, oxidative stress, changes in hematology, and plasma ion imbalance [14–16]. Furthermore, these NPs mostly penetrate via the skin, gills, and gastrointestinal absorption to reach the blood and liver [13, 15]. Therefore,

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the main targets to study the effect of nanoparticle exposure are gills, liver, brain, gut, and muscles [17].

Some previous work on TiO<sub>2</sub>-NPs toxicity showed that it produced oxidative stress and gill injury in exposed zebrafish [18, 19]. Zhu, Zhu, Duan, Qi, Li, and Lang [20] also demonstrated oxidative stress caused by TiO<sub>2</sub>-NPs with an increasing trend from lower to higher levels of dose at the developmental stage of zebrafish (*Danio rerio*) larvae. The genotoxic potential of TiO<sub>2</sub>-NPs has been assessed primarily through DNA breakage [21]. A significant TiO<sub>2</sub>-NP genotoxic effect on DNA damage was reported in a zebrafish model [16]. Previously, many aquatic species have been used to check the toxic effects of NPs. Among all aquatic organisms, fish is one of the most commonly used aquatic organisms for assessing environmental toxicity and toxic effects [3, 4].

Therefore, this study was designed to evaluate the acute, chronic, and sublethal toxicity of three different concentrations of NPs in *Oreochromis mossambicus* for a long-time exposure. In vivo, the studies regarding the toxicity and toxic effects of NPs are principally lacking, especially in aquatic systems where ecologically significant and diverse organisms are affected. The current study was aimed at exposing freshwater fish *Oreochromis mossambicus* to NPs for toxicity, bioaccumulation, and histological alterations.

The aims and objectives of the present study were to elucidate the bioaccumulation of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) in the gills, liver, and muscles; histopathological alterations; genotoxicity by comet assay; and oxidative and non-oxidative enzymatic activity.

## 2 Materials and Methods

### 2.1 Characterization

TiO<sub>2</sub>-NPs (Sigma-Aldrich, GmbH, Germany) < 100 nm with 99% purification were purchased in the form of nanopowder and were chemical in nature. The surface area was

analyzed through a scanning electron microscope (Philips, ESEM XL30). Figure 1 exhibits a spherical shape and fits the size of the nanoscale with an average of 72 ± 12.50 nm. The photographs were taken at magnifications of 20,000 and 65,000 with a power source of 20 kV.

### 2.2 Model Animal Collection and Acclimatization

With ethical permission from the Office of Research, Innovation, and Commercialization (ORIC), fish (weight 50.90 ± 0.37 g; 7.38 ± 0.19 cm length) was collected from aquaculture ponds in Pattoki City, Kasur District, Pakistan. The transportation to the lab was carried out in plastic bags of freshwater with continuous oxygen through the oxygen-carrying cylinder to minimize the transport stress. Before treatment, the fish were acclimatized to the glass tank environment for fourteen days.

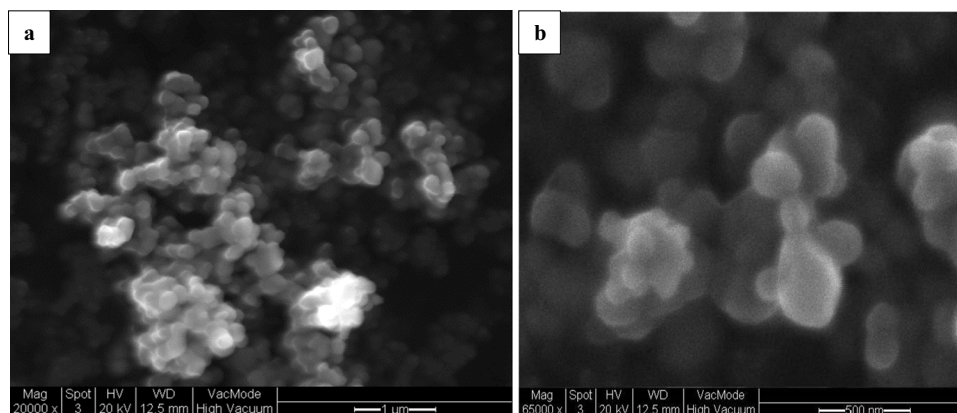
### 2.3 Experiment Design

The fish were grouped into fours with three replicates per group in 12 experimental water glass tanks (5 fish/aquarium). Each aquarium has dimensions of 45.72 × 60.96 × 45.72 cm and holds 40 L of water. Fish were provided commercial feed consisting of crude protein (35%), fat (4%), and fiber (5%) with 12% average moisture. The feed was provided twice a day at the morning and evening hours.

TiO<sub>2</sub>-NP solution (stock solution) was prepared in Milliquat water for treatment, sonicated for 30 min at 35 kHz (WUC-A06H). Four doses, including T0 (0 mg/L), T1 (0.5 mg/L), T2 (1.0 mg/L), and T3 (1.5 mg/L), were prepared for the treatment. The exposure of TiO<sub>2</sub>-NPs did not feed the fish to reduce the nanoparticle attachment in the food. The water from each aquarium was changed every 24 h and drained through suction pumps, leaving 20% water. The suction pumps also removed the animal waste. Then, fresh water was added to each aquarium.

On the 14<sup>th</sup> day, fish were carried in a small water container to take the samples. Three to four drops of clove

**Fig. 1** (a) and (b) showing the ESEM images of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) at 20000 and 65000 magnifications



oil were used to anesthetize the fish. Blood samples were obtained from the dorsal aorta in EDTA vials using BD syringes. Then, fish were dissected for liver, kidney, and muscle samples. For biochemical and oxidative stress-enzymatic and non-enzymatic analyses, samples were preserved in plastic bottles and stored at  $-20\text{ }^{\circ}\text{C}$ . The tissues for histological analysis were put in Bouin's fixative.

## 2.4 Water Quality Parameters

A pH meter (Velp Germany) was used for pH measurement. Conductivity, dissolved oxygen (DO), and temperature were measured with a multi-function meter (JENCO, Model 3173R) respectively. p-Alkalinity, absolute alkalinity, CE resilience, maximum hardness, and chlorides were measured by APHA (2005).

### 2.4.1 p-Alkalinity

We took a water sample (25 mL) in a conical flask with 2 drops of phenolphthalein.  $\text{H}_2\text{SO}_4$  (0.02 N) was used and the sample was titrated until the pink color started disappearing. The p-alkalinity was recorded with the following formula in milligrams per liter.

$$\text{p-Alkalinity} = \frac{\text{ml H}_2\text{SO}_4 \text{ used} \times 1000}{\text{water sample (ml)}}$$

### 2.4.2 Total Alkalinity

An indicator was mixed with 2 drops of 0.02 N  $\text{H}_2\text{SO}_4$  titrated sample and titrated it until the brick red color.

$$\text{Total Alkalinity} = \frac{\text{ml H}_2\text{SO}_4 \text{ used} \times 1000}{\text{water sample (ml)}}$$

### 2.4.3 Ca-Hardness

For calcium hardness, 25 mL of water was taken and 1 mL of NOH was added to produce 12 to 13 pH. Then the sample was stirred with 0.1 g phenolphthalein and was titrated against EDTA to express results in mg/L  $\text{CaCO}_3$ .

$$\text{Ca-Hardness} = \frac{\text{ml EDTA} \times 1000}{\text{water sample (ml)}}$$

### 2.4.4 Total Hardness

A 25-mL water sample was diluted with distilled water to make a volume of 100 mL in the flask. The pH of the sample was maintained at 10–10.1 by adding 1–2 mL of buffer solution. Finally, two drops of indicator solution were added

and titrated against 0.01 M EDTA solution until a blue color appeared. The result was expressed in milligrams per liter.

$$\text{Total Hardness} = \frac{\text{EDTA} \times 1000}{\text{sample (mL)}}$$

### 2.4.5 Chlorides

A total of 1 mL  $\text{K}_2\text{CrO}_4$  was added as an indicator in a 25-mL sample. This solution was titrated with  $\text{AgNO}_3$  until a light red color appeared. The results were expressed as milligrams per liter.

$$\text{Chloride} = \frac{\text{AgNO}_3 \text{ (mL)} \times \text{AgNO}_3 \text{ Normality} \times 35460}{\text{water sample (mL)}}$$

## 2.5 Inductively Coupled Plasma Mass Spectrometry of Sample

One gram of frozen sample was mixed with 10 mL of  $\text{HNO}_3$  and 2 mL of perchloric acid ( $\text{HClO}_4$ ) to digest the sample. Then, all the samples were heated below  $100\text{ }^{\circ}\text{C}$  on a hot plate until they turned yellow. Then two drops of hydrogen peroxide were added. Two milliliters of digested sample was diluted with cold water up to 50 mL, filtered (using Whatman's filter paper), and were analyzed using plasma mass spectrometry (APHA, 2005).

## 2.6 Biochemical Assay

For this assay, gill and liver samples were used. First, both samples were processed through a Teflon tissue homogenizer for homogenate preparation. The samples were first washed with buffer, and then immersed in 10% 0.1 M phosphate buffer (pH 7.4), centrifuged at  $4\text{ }^{\circ}\text{C}$  for 10 min at 5000 rpm, and superintendents stored in a freezer.

## 2.7 Lipid Peroxidation Estimation

The LPO was estimated using thiobarbituric acid reactive substances synthesis in homogenate liver and gill samples as (MDA) equivalents. Buege and Aust [22] were followed for this LPO estimation.

## 2.8 Catalase Activity

CAT activities were assessed using the protocol of Claiborne [23]. The mixture for this estimation was a solution of 3 mL volume and consisted of 100  $\mu\text{L}$  sample and 1.90 mL potassium phosphate buffer (50 mM, pH 7.0). The reaction was initiated by 1 mL of  $\text{H}_2\text{O}_2$  and read through a spectrophotometer at 240 nm with an interval of 30 s for 3 min.

## 2.9 Superoxide Dismutase Activity

Estimation of enzyme activities was done by Marklund and Marklund [24] protocol and was based on the ability of SOD to inhibit the auto-oxidation of pyrogallol. This assay used a mixture of sample and 2.80 mL K<sub>2</sub>PO<sub>4</sub> buffers (0.05 M, pH 8.2) incubated at 20 °C for 20 min. This reaction was started with 100 µL of 8 ampigralvalol. The absorbance of the solution mixture was measured at 412 nm for 3 min with a 30-s interval and is represented as per milligram of protein.

## 2.10 Glutathione

Estimation was done through Jollow, Mitchell, Zampaglione, and Gillette [25]. Homogenate and sulfanilic acid were taken in equal amounts, mixed well, and then incubation was done at 4 °C for an hour. Centrifugation of this mixture was carried out at 12,000 rpm for 15 min. Then, 0.4 mL supernatant was mixed with 2.2 mL of 0.1 M K<sub>2</sub>SO<sub>4</sub> buffer (pH 7.4) to initiate the reaction with 0.4 ml of DTNB (5,5'-dithiobis-2-nitrobenzoic acid). Finally, the absorbance was recorded at 412 nm within 30 s of reaction initiation.

## 2.11 Histology

The liver and gill samples were processed by Humason (1979) for histological studies.

## 2.12 Comet Assay

The protocol of Singh et al. (1988) for comet assay was adopted and slide preparation was done. The staining of slides was done with ethidium bromide for examination under a fluorescence microscope at ×400. Comet IV, a Computer software, was used for scoring the comet (Chaubey, 2005).

## 2.13 Statistical Analysis

Raw data for all studied parameters was recorded separately. Minitab (V. 17) was the computer package used for the processing of data. A comparison was made through Tukey's test at 95% level and ANOVA to compare the significance of the means of each parameter.

**Table 1** Showing accumulation of Ti (µg/kg) in the tissues of tilapia (*Oreochromis mossambicus*)

Tissues	Treatments			
	T <sub>0</sub> (0 mg/L)	T <sub>1</sub> (0.5 mg/L)	T <sub>2</sub> (1.0 mg/L)	T <sub>3</sub> (1.5 mg/L)
Gills	0.07 ± 0.01 <sup>d</sup>	0.46 ± 0.00577 <sup>g</sup>	0.71 ± 0.01 <sup>d</sup>	4.29 ± 0.02 <sup>a</sup>
Liver	0.093 ± 0.02 <sup>c</sup>	0.41 ± 0.0153 <sup>g</sup>	0.62 ± 0.02 <sup>f</sup>	4.13 ± 0.02 <sup>a</sup>
Muscles	0.42 ± 0.02 <sup>d</sup>	0.65 ± 0.0153 <sup>c</sup>	3.91 ± 0.02 <sup>b</sup>	3.97 ± 0.4 <sup>b</sup>

All values described as mean ± SD ( $p < 0.05$ )

## 3 Results

### 3.1 Accumulation of TiO<sub>2</sub> from TiO<sub>2</sub>-NPs

With increasing doses, Ti started to accumulate in the tissues. When compared to liver and muscle tissues, the gills accumulated the most. The concentration of Ti accumulation was 4.29 ± 0.02 µg/g in the gills at a 1.5 mg/L dose. At a higher dose, the mean Ti concentration was 24.13 ± 0.02 µg/g and 3.97 ± 0.4 µg/g in the liver and muscles. The order of concentration was gills > liver > muscles (Table 1).

### 3.2 Oxidative Stress Assay

With increasing dose, elevated activities of liver SOD were recorded in treated fish. In the control group, the values of CAT in the liver and gills were 6.73 ± 0.056 U/mg and 6.53 ± 0.058 U/mg, respectively. On the higher doses, the mean values of CAT in the liver and gills were 5.87 0.15 U/mg and 11.40 1.20 U/mg, respectively. Fluctuation in the level of glutathione (GSH), an increase in gills and a decrease in the liver, was also recorded with increasing TiO<sub>2</sub>-NPs concentrations. The GSH values in the liver and gills were 3.77 ± 0.058 U/mg and 2.13 ± 0.15 U/mg respectively in the control group and 1.6333 ± 0.1528 U/mg and 3.3667 ± 0.1528 U/mg in the liver and gills at a higher dose. TiO<sub>2</sub>-NPs also raise the level of LPO (lipid peroxidation) in gills (Table 2).

GST showed different results as it was recorded higher in the treated group 2. GST in the liver (0.15 mol/mg prot) significantly ( $p \leq 0.05$ ) was higher than that in the gills, kidneys, and heart. In treated group 2, GSH (130.0 µmol/mg tissue) was high in gills and kidneys compared to other organs.

### 3.3 Histology

Figure 2 depicts the histological changes caused by TiO<sub>2</sub>-NPs in the gills. Figure 2a shows the reference control, while Fig. 2b to d indicate the treated with TiO<sub>2</sub> nanoparticles. Figure 2a expresses the normal arrangement of gill lamellae, whereas, in reference treated with TiO<sub>2</sub>

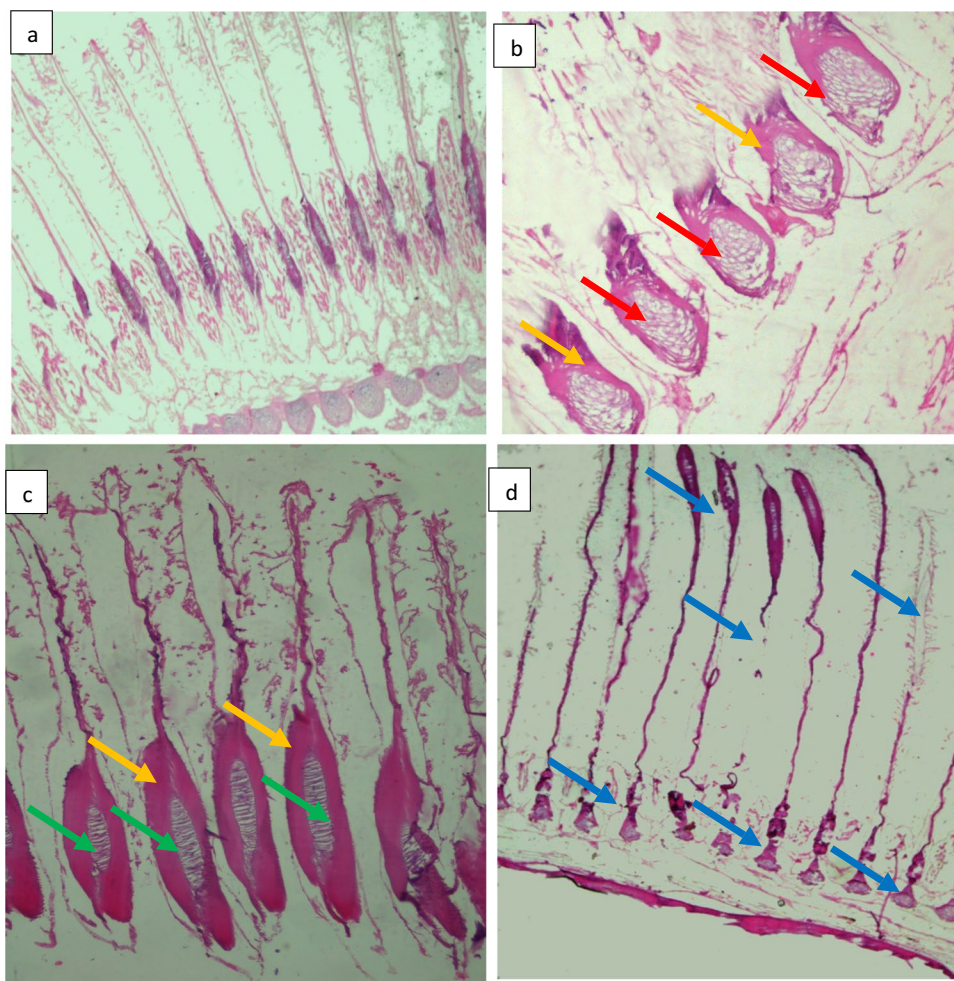


**Table 2** Showing the oxidative and anti-oxidative enzyme estimation

Enzymes	Tissues	Treatments			
		T <sub>0</sub> (0 mg/L)	T <sub>1</sub> (0.5 mg/L)	T <sub>2</sub> (1.0 mg/L)	T <sub>3</sub> (1.5 mg/L)
CAT U/mg	Gills	6.53 ± 0.058 <sup>d</sup>	7.7667 ± 0.1155 <sup>c</sup>	9.5667 ± 0.1155 <sup>b</sup>	11.40 ± 1.20 <sup>a</sup>
	Liver	6.73 ± 0.058 <sup>d</sup>	6.133 ± 0.1528 <sup>e</sup>	5.7667 ± 0.1528 <sup>e</sup>	5.87 ± 0.15 <sup>e</sup>
SOD U/mg	Gills	7.43 ± 0.1528 <sup>h</sup>	9.733 ± 0.1528 <sup>f</sup>	11.333 ± 0.153 <sup>d</sup>	13.733 ± 0.153 <sup>b</sup>
	Liver	8.8667 ± 0.0577 <sup>g</sup>	10.467 ± 0.115 <sup>e</sup>	12.133 ± 0.153 <sup>c</sup>	14.867 ± 0.153 <sup>a</sup>
GSH U/mg	Gills	2.13 ± 0.15 <sup>e</sup>	2.833 ± 0.1155 <sup>c</sup>	3.1333 ± 0.1528 <sup>bc</sup>	3.3667 ± 0.1528 <sup>b</sup>
	Liver	3.77 ± 0.058 <sup>a</sup>	2.667 ± 0.1528 <sup>d</sup>	1.8667 ± 0.1528 <sup>ef</sup>	1.6333 ± 0.1528 <sup>f</sup>
LPO nmol/mg	Gills	2.033 ± 0.1528 <sup>d</sup>	3.4667 ± 0.1528 <sup>c</sup>	3.8667 ± 0.0577 <sup>b</sup>	5.7667 ± 0.1155 <sup>a</sup>
	Liver	1.1667 ± 0.1528 <sup>f</sup>	1.440 ± 0.0200 <sup>ef</sup>	1.5233 ± 0.0208 <sup>e</sup>	1.6000 ± 0.0265 <sup>e</sup>

All values are mean ± S.D ( $p < 0.05$ )

**Fig. 2 a-d** Section of the gill of exposed fish. **(a)** The gill of control fish showing normal arrangement of primary and secondary gill lamellae. **(c)** to **(d)** The reference treated gill were showing hyperplasia (red arrows), fusion of gill lamellae (yellow arrows), rupturing of gill filaments (blue arrows) and thickening of primary and secondary gill lamellae (green arrows)



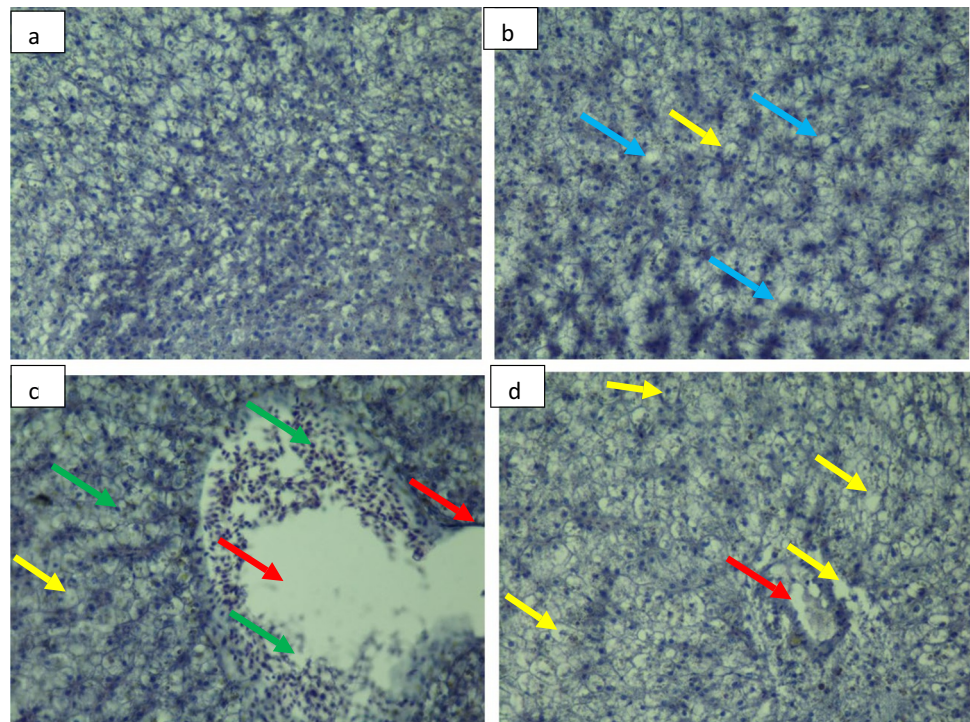
nanoparticles, gills undergo hyperplasia, rupturing of gill filaments, gill lamella fusion, and primary and secondary gill lamella thickening.

The liver histology of reference treated with TiO<sub>2</sub> nanoparticles shows alterations in hepatic cells. Figure 3a shows reference control liver histology, whereas Fig. 3c and d show apoptosis and necrosis characterized by the presence of sinusoid spaces and condensed nuclear bodies.

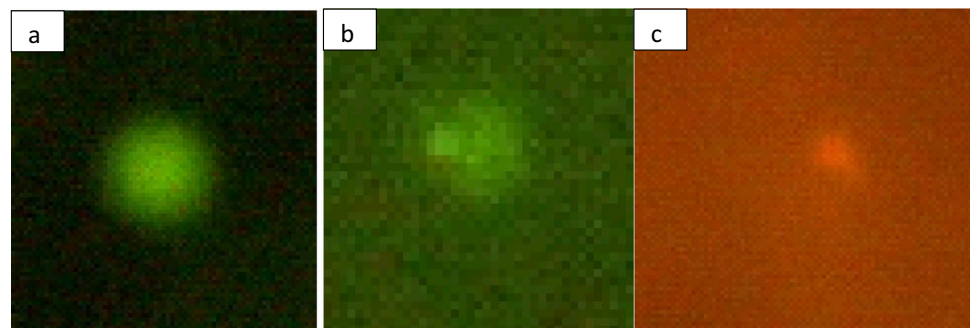
### 3.4 Comet Assay

In order to check the TiO<sub>2</sub>-NPs' potential genotoxicity and its role in damaging the DNA molecules of fish erythrocytes, an alkaline comet assay was used (Fig. 4). Increased DNA damage was observed with an increased concentration of TiO<sub>2</sub>-NP exposure. % tail DNA in the control sample was 4.699 ± 1.955 which significantly increased in samples

**Fig. 3** a-d Section of reference and treated fish liver. (a) The liver of control fish showing normal arrangement and distribution of hepatocytes. (c) to (d) Reference treated liver showing necrosis (blue arrows) and apoptosis (yellow arrows) with condensed nuclear bodies (green arrows) as indicated by black arrows, sinusoid spaces in relation to parenchyma (red arrows)



**Fig. 4** Evaluation of genotoxicity of TiO<sub>2</sub>-NPs in erythrocytes through Comet assay (a) normal erythrocytes (b) slightly damage erythrocyte (c) Large comet cell



treated with a high dose of TiO<sub>2</sub>-NPs ( $18.219 \pm 0.581$ ). T<sub>2</sub> (1.0 mg/L) and T<sub>3</sub> (1.5 mg/L) did not show any significant difference in % tail DNA. Similarly, an increased dose concentration of TiO<sub>2</sub>-NPs also elevates the level of olive tail movement. Olive tail movement in the control sample was  $0.331 \pm 0.053$ , which was much lower than that of samples treated with TiO<sub>2</sub>-NPs at  $7.472 \pm 1.249$  during T<sub>3</sub> (1.5 mg/L) (Fig. 5a, b).

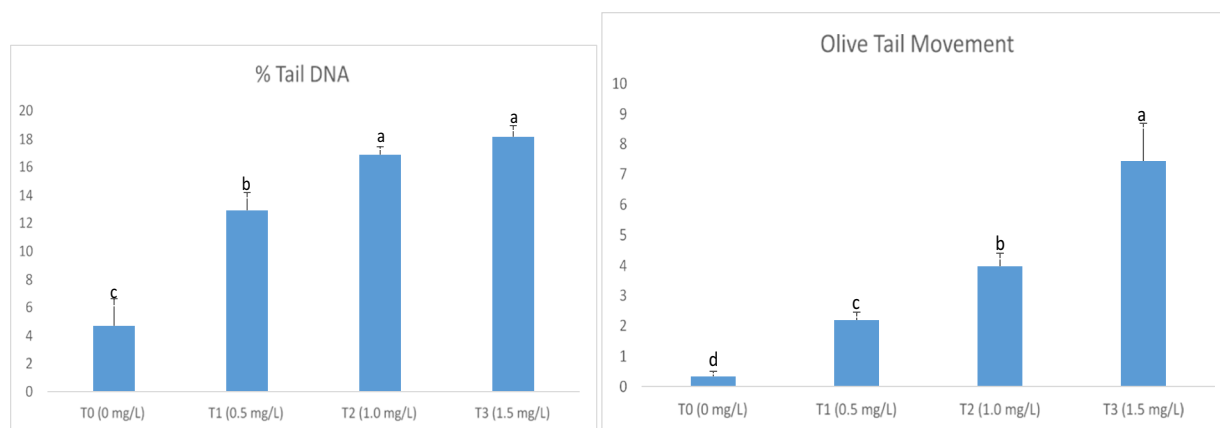
## 4 Discussion

Certain studies on the accumulation of nanomaterials in living tissues and their toxic effects have already been conducted. Ates, Demir, Adiguzel, and Arslan [26] examined nanoparticle accumulation in different tissues of goldfish (*Carassius auratus*) and detected the highest accumulation

in gills. The results were opposite to our research, where the highest accumulation of Ti was observed in liver tissues. In a study on freshwater mussels, increased accumulation of heavy metals was noticed in the soft tissues of mussels when the dose of heavy metals was increased [27].

Lipid peroxidation is strongly associated with ROS. It produces free radicals in cellular membranes and starts the oxidation of unsaturated fatty acids. Damage to biomolecules, including DNA and proteins, is destructive for lipid peroxidation at cellular or organ level [28–31]. Membrane damage is due to an increased level of LPO as a result of reactive oxygen species (ROS) formation [29]. Polyunsaturated fatty acids, an important constituent of biomembranes, are highly vulnerable to peroxidation. Zhu, Zhu, Duan, Qi, Li, and Lang [20] explained the impact of C<sub>60</sub> on the LPO level of fathead minnow. The final results of the study suggest an increase in LPO in the brain, liver, and gills of





**Fig. 5** Genotoxicity of TiO<sub>2</sub>-NPs in erythrocytes. **(a)** Comets percentage of tail DNA and **(b)** olive tail movement. Data are expressed as mean  $\pm$  S.D ( $p < 0.05$ ) ( $N = 10$ )

fathead minnow. In another study by Hao, Wang, and Xing [12], the effect of TiO<sub>2</sub>-NPs on the LPO level of different tissues of juvenile crap was determined. The final results of the study suggest a comparatively high level of LPO in the liver than that in gills. This result is opposite to our study which indicates an elevated level of LPO in the gills rather than the liver.

Furthermore, Hamid, Khan, Yaqoob, Umar, Rehman, Javed, Sohail, Anwar, Khan, and Ali [32] studied the role of superoxide dismutase (SOD) in regulating the concentration of superoxide radical (O<sub>2</sub><sup>-</sup>) at the cellular level. SOD converts superoxide radicals into H<sub>2</sub>O<sub>2</sub> and ultimately lowers its concentration. H<sub>2</sub>O<sub>2</sub> inhibits the activities of different enzymes by penetrating biomembranes and is further catabolized by the CAT enzyme. Xiong, Fang, Yu, Sima, and Zhu [33] correlate raised CAT activity in zebrafish gills, with TiO<sub>2</sub>-NP accumulation. In a study on juvenile craps, a direct association was noticed between dose and the liver's SOD activity. In contrast to this, an indirect association was observed between SOD activity and increased dose concentration in gills [12]. Similarly, our study also supports raised SOD levels in the liver.

The present study highlights the maximum GSH level in the gills, whereas a decrease in GSH activity of the liver was reported. Smith, Shaw, and Handy [34] also reported parallel results while working on the treatment of rainbow trout with single-walled carbon nanotubes. They examined decreased GSH activity in liver tissues of rainbow trout. Ramsden, Henry, and Handy [8] in another study treated embryos of zebrafish with nanoparticles of TiO<sub>2</sub> and noted elevated levels of GSH in the liver tissues. GSH plays a crucial role in cell protection from the highly reactive hydroxyl group. GSH reduces the hydroxyl group and because of this oxidative stress–mediating property of GSH, it becomes

essential to regulate the level of antioxidant and oxidative enzymes [31, 32, 35].

Gills, being in direct contact with the aquatic environment, are considered the primary site for gaseous exchange [36]. This explains that the osmoregulatory functions of fish were disturbed and they were in severe stress. Different hepatic alterations, specifically liver cell apoptosis, are also caused by TiO<sub>2</sub>-NPs [37]. To determine the possible association between hepatic apoptosis and TiO<sub>2</sub>-NPs, the liver tissues of fish were exposed to titanium dioxide. Smith, Shaw, and Handy [34] observed the same hepatic apoptotic phenomenon in their study. In another study by Hao, Wang, and Xing [12] on juvenile carps, the same results were reported.

The role of TiO<sub>2</sub>-NPs in damaging the erythrocytes of fish (*O. mossambicus*) has been proved by comet assay. Increased exposure to elevated TiO<sub>2</sub>-NP concentration significantly raises the level of olive tail movement and % tail DNA. The maximum genotoxic action of TiO<sub>2</sub>-NPs was observed during the 14<sup>th</sup> day of study at an increased dose concentration of T<sub>3</sub> (1.5 mg/L). Vignardi, Hasue, Sartório, Cardoso, Machado, Passos, Santos, Nucci, Hwer, and Watanabe [38] studied the genotoxic activities of nano TiO<sub>2</sub> in *Trachinotus carolinus*, a marine fish, and reported similar results. Shakeel, Jabeen, Iqbal, Chaudhry, Zafar, Ali, Khan, Khalid, Shabbir, and Asghar [15] demonstrated the increased toxicity of bulk TiO<sub>2</sub>, in marine mussel's (*Mytilus galloprovincialis*) hemocytes. This study also detected the genotoxic effects of TiO<sub>2</sub> and its role in damaging the DNA molecule. A previous study contradicts our results and reports lower levels of DNA damage at increased dose concentration [27]. In an experiment on European sea bass (*Dicentrarchus labrax*), it was shown that TiO<sub>2</sub>-NPs have more potential to induce genotoxicity [21]. The effect of TiO<sub>2</sub>-NPs elevated dose concentration in zebrafish (*Danio*

erio) was also examined in a study that inferred the role of TiO<sub>2</sub>-NPs in DNA damage and apoptosis [16].

## 5 Conclusion

In this study, the sub-lethal and toxic effects of TiO<sub>2</sub> nanoparticles on tilapia (*Oreochromis mossambicus*) are examined. TiO<sub>2</sub>-NP exposure within the range of 0.5–1.5 mg/L is not lethal to the tilapia. For this reason, a number of changes were observed during this experiment. Gills are found to accumulate more Ti as compared to the liver and muscles. Histopathological alterations such as thickening and fusion in gill lamellae, rupturing of gill filaments, and hyperplasia of gills are recorded. The phenomenon of increased necrosis and apoptosis in the liver has also been noticed with increasing concentration of TiO<sub>2</sub>-NPs along with the formation of sinusoid spaces and condensed nuclear bodies. The liver shows the highest vulnerability to Ti toxicity during TiO<sub>2</sub>-NP exposure. Increased accumulation of Ti in liver tissues results in elevated LPO levels and a decrease in the antioxidant enzyme. Titanium dioxide NPs also result in RBC genotoxicity of fish, which ultimately leads to different abnormalities, including olive tail movement or % tail breakage.

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## Declarations

**Ethics Approval** Approval was taken from ethical review board of the University before start of the study.

**Consent to Participate** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

**Research Involving Humans and Animals Statement** All the work carried out in this research was in accordance with the Declaration of Helsinki for animal ethics and ethical approval was taken from ethical review board of the University prior to the start of the study.

**Informed Consent** Not Applicable.

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