

# Study of some toxicological aspects of titanium dioxide nanoparticles through oxidative stress, genotoxicity, and histopathology in tilapia, *Oreochromis mossambicus*

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## Research Article

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1 **Study of some toxicological aspects of titanium dioxide nanoparticles through oxidative stress, genotoxicity,**  
2 **and histopathology in tilapia, *Oreochromis mossambicus***

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14  
15  
16 **Abstract**

17 Extensive use of nanotechnology in multiple commodities is raising concern about nanotoxicity and particularly.  
18 Particularly, many studies reported the health hazardous effects of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs). Study  
19 focuses on toxicity and accumulation of TiO<sub>2</sub>-NPs in tilapia (*Oreochromis mossambicus*). For this purpose, Tilapia  
20 were kept in water tanks, acclimatized for fourteen days, and treated with different doses of TiO<sub>2</sub> nanoparticles 0, 0.5,  
21 1.0, and 1.5 mg TiO<sub>2</sub>-NPs /L. Results revealed an increase in accumulation of TiO<sub>2</sub>-NPs with an increase in doses.  
22 Moreover, with higher dose (1.5 mg /L) gills had maximum levels compared to muscles and liver tissues whereas  
23 other doses showed different accumulation patterns. A significantly higher concentration of catalase, glutathione, and  
24 lipid peroxidation was recorded in gills (p<0.05) and superoxide dismutase in the liver. Characteristics like thickening  
25 and fusion in lamellae, rupturing of filaments and hyperplasia of gills were also recorded. The phenomenon of  
26 increased necrosis and apoptosis in the liver was also noticed with increasing concentration of TiO<sub>2</sub>-NPs along with  
27 formation of sinusoid spaces and condensed nuclear bodies. Elevated values of olive tail movement and % tail DNA  
28 were also noticed with increased concentration of TiO<sub>2</sub>-NPs. This study concluded that TiO<sub>2</sub>-NPs produced oxidative  
29 stress by accumulation in soft tissues and induced pathology and genotoxicity.

30 **Keywords:** Nanoparticle; toxicity; *Oreochromis mossambicus*; genotoxicity

31 **Introduction**

32 The structure dimensions of nanoparticles range from 1-100 nm and exist on earth for millions of years for  
33 the use of mankind (Asghar et al. 2016; Masciangioli and Zhang 2003). These particles are being engineered for  
34 various applications in industries such as drug delivery systems, chemical sensors, biosensors, optics, textiles, medical  
35 devices, electronics, and environmental remediation (Khan et al. 2015b; Khan et al. 2017a; Raza et al. 2017). However,  
36 extensive use is also responsible for aquatic habitat penetration and raising concerns of ecotoxicology (Khan et al.  
37 2015b; Ramsden et al. 2013). The most prominent sources of these nanoparticles are colloids found in natural waters,  
38 soil, and sediments. Volcanic dust in the atmosphere is also an important source of TiO<sub>2</sub>-NPs (Buffle, 2006 and Handy,  
39 et al, 2008b). Upon exposure, the concentrations of different types of nanoparticles were recorded much higher in

40 tissues of aquatic animals than the normal permissible concentrations (Asghar et al. 2015; Khan et al. 2018; Khan et  
41 al. 2020).

42 TiO<sub>2</sub>-NPs are most extensively applicable in dyes, makeups items and sunscreens (Hao et al. 2009; Shakeel  
43 et al. 2016) and released in the natural environment through wastewater effluents where they cause gill injury,  
44 oxidative stress, changes in hematology, and plasma ion imbalance (Asghar et al. 2018; Rocco et al. 2015; Shakeel et  
45 al. 2018). Further, these NPs mostly penetrate via the skin, gills, and gastrointestinal absorption to blood and liver  
46 (Shakeel et al. 2018; Shakeel et al. 2016). therefore, the main targets to study the effect of nanoparticle exposure are  
47 gills, liver, brain, gut, and muscles (Khan et al. 2017b).

48 Some previous work on TiO<sub>2</sub>-NPs toxicity showed that it produced oxidative stress and gill injury in exposed  
49 zebrafish (Griffitt et al. 2009; Tang et al. 2019). Zhu et al. (2008) also demonstrated oxidative stress caused by TiO<sub>2</sub>-  
50 NPs with an increasing trend from lower to higher levels of dose at the developmental stage of Zebrafish (*Danio rerio*)  
51 larvae. The genotoxic potential of TiO<sub>2</sub>-NPs has been assessed primarily through DNA breakage (Nigro et al. 2015).  
52 A significant TiO<sub>2</sub>-NPs genotoxic effect on DNA damage was reported in zebrafish model (Rocco et al. 2015).

53 This study focuses on the toxicological assessment of engineered TiO<sub>2</sub>-NPs in tilapia (*Oreochromis*  
54 *mossambicus*). The sub-lethal toxicological effects examined include bioaccumulation, genotoxicity, oxidative stress  
55 and histological alterations.

## 56 **Materials and Methods**

### 57 *Characterization*

58 TiO<sub>2</sub>-NPs (Sigma-Aldrich, GmbH, Germany) <100 nm with 99 percent purification were in the form of nanopowder.  
59 The surface area was analyzed through scanning electron microscope (Philips, ESEM XL30). Figure 1 exhibits a  
60 spherical shape and fits the size of the nanoscale with an average of 72±12.50 nm. The photograph is taken with the  
61 magnification of 20000 and 65000 with a power source of 20 kV.

### 62 *Model animal collection and acclimatization*

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

### 67 *Experiment Design*

68 The fish were grouped in four with three replicates per group in 12 experimental water glass tanks (5 fish/aquaria).  
69 Each aquarium has dimensions of 45.72 x 60.96 x 45.72 cm with 40 liters of water. Fish were provided commercial  
70 feed consisting of crude protein (35%), fat (4%), fiber (5%) with 12% average moisture. The feed was provided twice  
71 a day at morning and evening hours.

72 TiO<sub>2</sub>-NPs solution (Stock solution) was prepared in Milliwater for treatment, sonicated for 30 minutes  
73 at 35 kHz (WUC-A06H). Four doses included T0 (0 mg/L), T1 (0.5 mg/L), T2 (1.0 mg/L), and T3 (1.5 mg/L) were  
74 prepared for the treatment. The exposure of TiO<sub>2</sub>-NPs did not feed the fish to reduce the nanoparticle attachment in  
75 the food. Water from each aquarium was changed 24 hours and drain through suction pumps remaining 20 % water.  
76 The suction pumps also removed the animal waste. Then, fresh water was added to each aquarium.

77 On the 14<sup>th</sup> day, fish were carried in a small water container to take the samples. 3 to 4 drops of clove oil  
78 were used to anesthetize the fish. Blood samples were obtained from the dorsal aorta in EDTA vials using BD syringes.  
79 Then, Fish were dissected form liver, kidney, and muscle samples. For biochemical and oxidative stress-enzymatic  
80 and non-enzymatic analysis, samples were preserved in plastic bottles and stored at -20°C. The tissues for histological  
81 analysis were put in Bouin's fixative.

## 82 *Water Quality Parameters*

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

### 86 *p-Alkalinity*

87 took Water sample (25 ml) in conical flask with 2 drops of phenolphthalein. H<sub>2</sub>SO<sub>4</sub> (0.02N) was used and the sample  
88 was titrated till the pink color started disappearing. The p-Alkalinity was recorded with the following formula in mg/l.

$$89 \quad \text{p - Alkalinity} = \frac{\text{ml H}_2\text{SO}_4 \text{ used} \times 1000}{\text{water sample (ml)}}$$

### 90 *Total alkalinity*

91 Mixed indicator with 2 drops of 0.02N H<sub>2</sub>SO<sub>4</sub> titrated sample and titrated it until the bricks red color.

$$92 \quad \text{Total Alkalinity} = \frac{\text{ml H}_2\text{SO}_4 \text{ used} \times 1000}{\text{water sample (ml)}}$$

### 93 *Ca-hardness*

94 For Calcium hardness, 25 ml of water sampled was taken and added 1 ml of NOH to produce pH 12 to 13. Then  
95 Stirred the sampled with 0.1 g phenolphthalein and titrated against EDTA to express results in mg/1CaCO<sub>3</sub>.

$$96 \quad \text{Ca - Hardness} = \frac{\text{ml EDTA} \times 1000}{\text{water sample (ml)}}$$

97

98

### 99 *Total hardness*

100 25 ml water sample was diluted with distilled water to make a volume of 100 ml in flask. The pH of the sample was  
101 maintained at 10-10.1 by adding 1-2 ml buffer solution. Finally added two drops of indicator solution and titrated  
102 against 0.01M EDTA solution until blue color appeared. The result was expressed in mg/l.

103

$$104 \quad \text{Total Hardness} = \frac{\text{EDTA} \times 1000}{\text{sample (ml)}}$$

### 105 *Chlorides*

106 1 ml K<sub>2</sub>CrO<sub>4</sub> was added as an indicator in 25 ml sample. This solution is titrated with AgNO<sub>3</sub> till light red color. The  
107 results were expressed as mg/l.

108

$$109 \quad \text{Chloride} = \frac{\text{AgNO}_3 \text{ (ml)} \times \text{AgNO}_3 \text{ Normality} \times 35460}{\text{water sample (ml)}}$$

110

### 111 *Inductively Coupled Plasma Mass Spectrometry of Sample*

112 1 g of frozen sample was mixed with 10 ml of HNO<sub>3</sub> and 2 ml of pyruchloric acid (HClO<sub>4</sub>) to digest the sample. Then  
113 all sample was heated below 100 °C on a hot plate until the yellow. Then added two drops of hydrogen peroxide. 2 ml

114 digested sample was diluted with cold up to 50 ml, filtered (Whatman) analyzed using plasma mass spectrometry  
115 (APHA, 2005).

#### 116 *Biochemical Assay*

117 For this assay, gills and liver samples were used. First both samples processed through Teflon tissue homogenizer for  
118 homogenate preparation. The samples were first washed with buffer, and then immersed in 10% 0.1 M phosphate  
119 buffer (pH 7.4), centrifuged at 4°C for 10 min at 5000 rpm and superintendents stored in a freezer.

#### 120 *lipid peroxidation Estimation*

121 LPO estimation was done through thiobarbituric acid reactive substances synthesis in homogenate liver and gills  
122 samples as (MDA) equivalents. Buege and Aust (1978) was followed for this LPO estimation

#### 123 *Catalase activity*

124 CAT activities were assessed using the protocol of Claiborne (1985). Mixture for this estimation was a solution of 3  
125 ml volume and consisted of 100 µl sample, 1.90 ml potassium phosphate buffer (50 mM, pH 7.0). The reaction was  
126 initiated by 1 ml of H<sub>2</sub>O<sub>2</sub> and read through a spectrophotometer at 240 nm with an interval of 30 seconds for 3 minutes.

#### 127 *Superoxide dismutase activity*

128 Estimation of enzyme activities was done by Marklund and Marklund (1974) protocol and based on the ability of SOD  
129 to inhibit the auto-oxidation of pyrogallol. A mixture of sample and 2.80 ml K<sub>2</sub>PO<sub>4</sub> buffers (0.05M, pH 8.2) incubated  
130 at 20 °C for 20 min was used for this assay. This reaction was started with 100 µl of 8 ampigralvalol. The abosrance  
131 of the solution mixture was measured at 412 nm for 3 min with a 30 seconds interval and represented as per milligram  
132 of protein.

#### 133 *Glutathione*

134 Estimation was done through Jollow et al. (1974). Homogenate and sulfanilic acid were taken in equal amounts, mixed  
135 well and then incubation was done at 4°C for an hour. Centrifugation of this mixture was carried out at 12,000 rpm  
136 for 15 minutes. Then, mixed 0.4 ml supernatant with 2.2 ml of 0.1 M K<sub>2</sub>SO<sub>4</sub> buffer (pH 7.4) initiated the reaction with  
137 0.4 ml of DTNB (5.5 + -dithiobis-2-nitrobenzoic acid). Finally, the absorbance was recorded at 412 nm within 30  
138 seconds of reaction initiation.

#### 139 *Histology*

140 The liver and gills samples were processed through Humason (1979) for histological studies.

#### 141 *Comet Assay*

142 Protocol of Singh *et al.* (1988) for comet assay was adopted and slide preparation. The staining of slides were done  
143 with ethidium bromide for examination under fluorescence microscope at 400X. Comet IV a Computer Software was  
144 used for scoring the comet (Chaubey, 2005).

#### 145 *Statistical analysis*

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

#### 149 **Results**

##### 150 Physicochemical analysis

151 Different physicochemical parameters (temperature, TDS, alkalinity, total hardness, Ca-hardness, total alkalinity, and  
152 chlorides are listed in Table 1.

153

154 **Table 1:** Showing accumulation of Ti ( $\mu\text{g}/\text{Kg}$ ) in the tissues of tilapia (*Oreochromis mossambicus*).  
 155

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> (1.5mg/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>e</sup>	3.91 ± 0.02 <sup>b</sup>	3.97 ± 0.4 <sup>b</sup>

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

158 *Accumulation of TiO<sub>2</sub> from TiO<sub>2</sub>-NPs*

159 With increasing doses, Ti started to accumulate in the tissues. Gills accumulated maximum compared to liver and  
 160 muscles tissues. The concentration of Ti accumulation was 4.29±0.02  $\mu\text{g}/\text{g}$  in gills at 1.5 mg/L dose. At a higher dose,  
 161 the mean Ti concentration was 24.13 ± 0.02  $\mu\text{g}/\text{g}$  and 3.97 ± 0.4  $\mu\text{g}/\text{g}$  in the liver and muscles. The order of  
 162 concentration was gills>liver >muscles.

163 *Oxidative Stress assay*

164 With increasing dose, elevated activities of liver SOD were recorded in treated fish. In the control group, the  
 165 value of CAT in the liver and gills was 6.73 ± 0.056 U/mg and 6.53 ± 0.058 U/mg respectively. While mean values  
 166 CAT in liver and gills on the higher doses were 5.87 ± 0.15 U/mg and 11.40 ± 1.20 U/mg respectively. Fluctuation in  
 167 the level of glutathione (GSH), increase in gills, and decrease in the liver, was also recorded with increasing TiO<sub>2</sub>-  
 168 NPs concentration. GSH value in liver and gills was 3.77 ± 0.058 U/mg and 2.13 ± 0.15 U/mg respectively in the  
 169 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  
 170 also raise the level of LPO (lipid peroxidation) in gills (Table 2).

171 GST showed different results, it was recorded higher in the treated group 2, GST in the liver (0.15 mol/mg  
 172 prot) significantly ( $P \leq 0.05$ ) was higher than gills, kidneys, and heart. In treated group 2, GSH (130.0  $\mu\text{mol}/\text{mg}$  tissue)  
 173 was high in gills and kidneys compared to other organs.

174 **Table 2:** Showing the oxidative and anti-oxidative enzymes estimation  
 175

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> (1.5mg/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>e</sup>	14.867 ± 0.153 <sup>a</sup>
GSH U/mg	Gills	2.13 ± 0.15 <sup>e</sup>	2.833 ± 0.1155 <sup>e</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>

176 All values are mean  $\pm$  S.D ( $p < 0.05$ )

### 177 *Histology*

178  $\text{TiO}_2$ -NPs induced histological alterations gills are shown in Fig.2. Fig.2 (a) shows reference control, while  
179 Fig.2 (b) to (d) indicates treated with  $\text{TiO}_2$  nanoparticles. Fig.2 (a) expresses the normal arrangement of gill lamellae.  
180 Whereas, in reference treated with  $\text{TiO}_2$  nanoparticles gills undergo hyperplasia, rupturing of gill filaments, gill  
181 lamellae fusion, and primary and secondary gill lamellae thickening.

182 The liver histology of reference treated with  $\text{TiO}_2$  nanoparticles shows alterations in hepatic cells. Fig.3. (a)  
183 indicates liver histology of reference control whereas, Fig.3. (c) to (d) indicates apoptosis and necrosis characterized  
184 by the presence of sinusoid spaces and condensed nuclear bodies.

### 185 *Comet Assay*

186 In order to check the  $\text{TiO}_2$ -NPs potential genotoxicity and its role in damaging the DNA molecules of fish  
187 erythrocytes, an alkaline comet assay was used (Fig.4). Increased DNA damage was observed with increased  
188 concentration of  $\text{TiO}_2$ -NPs exposure. % Tail DNA in the control sample was  $4.699 \pm 1.955$  that significantly increased  
189 in samples treated with a high dose of  $\text{TiO}_2$ -NPs ( $18.219 \pm 0.581$ ).  $T_2$  (1.0 mg/L) and  $T_3$  (1.5 mg/L) did not show any  
190 significant difference in % tail DNA. Similarly, increased dose concentration of  $\text{TiO}_2$ -NPs also elevates the level of  
191 olive tail movement. Olive tail movement in the control sample was  $0.331 \pm 0.053$ , which was much lower than  
192 samples treated with  $\text{TiO}_2$ -NPs  $7.472 \pm 1.249$  during  $T_3$  (1.5 mg/L) (Fig. 5 a b).

### 193 **Discussion**

194 Certain studies on the accumulation of nanomaterials in living tissues and their toxic effects have already  
195 been conducted. Ates et al. (2013) examined nanoparticle accumulation in different tissues of goldfish (*Carassius*  
196 *auratus*) and detected the highest accumulation in gills. Results were opposite to our research where the highest  
197 accumulation of Ti was observed in liver tissues. In a study on freshwater mussels, increased accumulation of heavy  
198 metal was noticed in soft tissues of mussels on increasing the dose of heavy metals (Sohail et al. 2016).

199 Lipid peroxidation is strongly associated with ROS. It produces free radicals in cellular membranes and starts  
200 the oxidation of unsaturated fatty acids. Damage to biomolecules, including DNA and proteins is destructive for lipid  
201 peroxidation at cellular or organ level (Asghar et al. 2017; Khan et al. 2015a; Khan et al. 2016; Wilhelm 1990).  
202 Membrane damage is due to an increased level of LPO as a result of oxygen reactive species ROS formation (Asghar  
203 et al. 2017). Polyunsaturated fatty acids, an important constituent of biomembranes, are highly vulnerable to  
204 peroxidation. Zhu et al. (2008) explained the impact of  $\text{C}_{60}$  on the LPO level of fathead minnow. The final results of  
205 the study suggest an increase in LPO in the brain, liver, and gills of fathead minnow. In another study by Hao et al.  
206 (2009) effect of  $\text{TiO}_2$ -NPs on the LPO level of different tissues of juvenile crap was determined. The final results of  
207 the study suggest a comparatively high level of LPO in the liver than gills. This result is opposite to our study which  
208 indicates an elevated level of LPO in the gills rather than the liver.

209 Further, Hamid et al. (2016) studied the role of superoxide dismutase (SOD) in regulating the concentration  
210 of superoxide radical ( $\text{O}_2^-$ ) at the cellular level. SOD converting superoxide radical into  $\text{H}_2\text{O}_2$ ; ultimately lowers its  
211 concentration.  $\text{H}_2\text{O}_2$  resists the activities of different enzymes by penetrating biomembranes and is further catabolized  
212 by the CAT enzyme. Xiong et al. (2011) correlates raised CAT activity, in zebrafish gills, with  $\text{TiO}_2$ -NPs

213 accumulation. In a study on juvenile crabs, a direct association was noticed between dose and the liver's SOD activity.  
214 In contrary to this an indirect association was observed between SOD activity and increased dose concentration in  
215 gills (Hao et al. 2009). Similarly, our study also supports raised SOD levels in the liver.

216 The present study highlights maximum GSH level in the gills. Whereas, a decrease in GSH activity of the  
217 liver was reported. Smith et al. (2007) also reported parallel results while working on the treatment of rainbow trout  
218 with single-walled carbon nanotubes. They examined decreased GSH activity in liver tissues of rainbow trout.  
219 Ramsden et al. (2013) in another study treat embryos of zebrafish with nanoparticles of TiO<sub>2</sub> and noted elevated levels  
220 of GSH in liver tissues. GSH plays a crucial role in cell protection from the highly reactive hydroxyl group. GSH  
221 reduces the hydroxyl group and because of this oxidative stress mediating property of GSH, it becomes essential to  
222 regulate the level of antioxidant and oxidative enzymes (Ahmad et al. 2019; Hamid et al. 2016; Khan et al. 2016).

223 Gills being direct contact with the aquatic environment are considered the primary site for gaseous exchange  
224 (Guan and Lin 2004). This explained that the osmoregulatory functions of fish were disturbed and it was in severe  
225 stress. Different hepatic alterations, specifically liver cell apoptosis, are also caused by TiO<sub>2</sub>-NPs (Rahman et al.  
226 2002). To determine the possible association between hepatic apoptosis and TiO<sub>2</sub>-NPs, liver tissues of fish were  
227 exposed to titanium dioxide. Smith et al. (2007) in their study observed the same hepatic apoptotic phenomenon. In  
228 another study by Hao et al. (2009) on juvenile carp same results were reported.

229 TiO<sub>2</sub>-NPs role in damaging the erythrocytes of fish (*O. mossambicus*) has been proved by comet assay.  
230 Increased exposure to elevated TiO<sub>2</sub>-NPs concentration significantly raises the level of olive tail movement and % tail  
231 DNA. Maximum genotoxic action of TiO<sub>2</sub>-NPs was observed during the 14<sup>th</sup> day of study at an increased dose  
232 concentration of T<sub>3</sub> (1.5 mg/L). Vignardi et al. (2015) studied the genotoxic activities of nano TiO<sub>2</sub> in *mTrachinotus*  
233 *carolinus*; a marine fish and reported similar results. Shakeel et al. (2018) demonstrated the increased toxicity of bulk  
234 TiO<sub>2</sub>, in marine mussel's (*Mytilus galloprovincialis*) hemocytes. This study also detected the genotoxic effects of TiO<sub>2</sub>  
235 and its role in damaging the DNA molecule. A previous study contradicts our results and reports lower level damage  
236 to DNA at increased dose concentration (Sohail et al. 2016). In an experiment on European sea bass (*Dicetrarchus*  
237 *labrax*), showed that TiO<sub>2</sub>-NPs have more potential of inducing genotoxicity (Nigro et al. 2015). Effect of TiO<sub>2</sub>-NPs  
238 elevated dose concentration in zebrafish (*Danio rerio*) is also examined in a study that inferred the role of TiO<sub>2</sub>-NPs  
239 in DNA damage and apoptosis (Rocco et al. 2015).

## 240 **Conclusion**

241 Lethal and toxic effects of TiO<sub>2</sub> nanoparticles, on tilapia (*O. mossambicus*), are examined in this study. TiO<sub>2</sub>-NPs  
242 exposure within the range of 0.5-1.5 mg/L is not lethal to the tilapia. Because of this reason variety of changes were  
243 observed during this experiment. The liver shows the highest vulnerability to Ti toxicity during TiO<sub>2</sub>-NPs exposure.  
244 Increased accumulation of Ti in liver tissues results in elevated LPO level, decrease in the antioxidant enzyme, and  
245 pathological modifications in tissues. Titanium dioxide also results in RBCs genotoxicity of fish which ultimately  
246 leads to different abnormalities including olive tail movement or %tail breakage.

## 247 **Declarations**

### 248 **Ethics approval and consent to participate**

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

250 **Consent for publication**

251 Not applicable.

252 **Availability of data and material**

253 Not applicable.

254 **Competing interests**

255 The authors declare that they have no competing interests.

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258 **Authors' contributions**

259 KS, MNK, and FJ analyzed, interpreted, and formatted the data. ASC, CA, MKAK, and MSK performed the  
260 histopathology and genotoxicity examination and write the manuscript. The final manuscript was read and approved  
261 by all authors.

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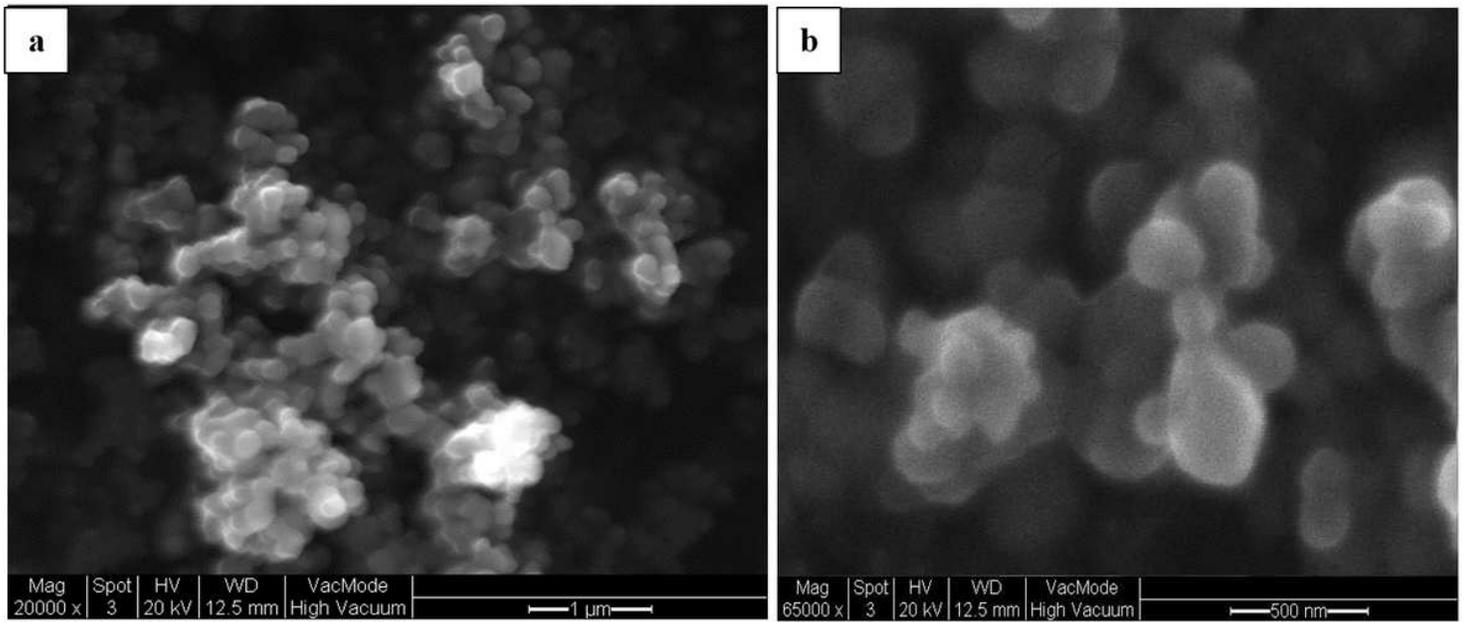
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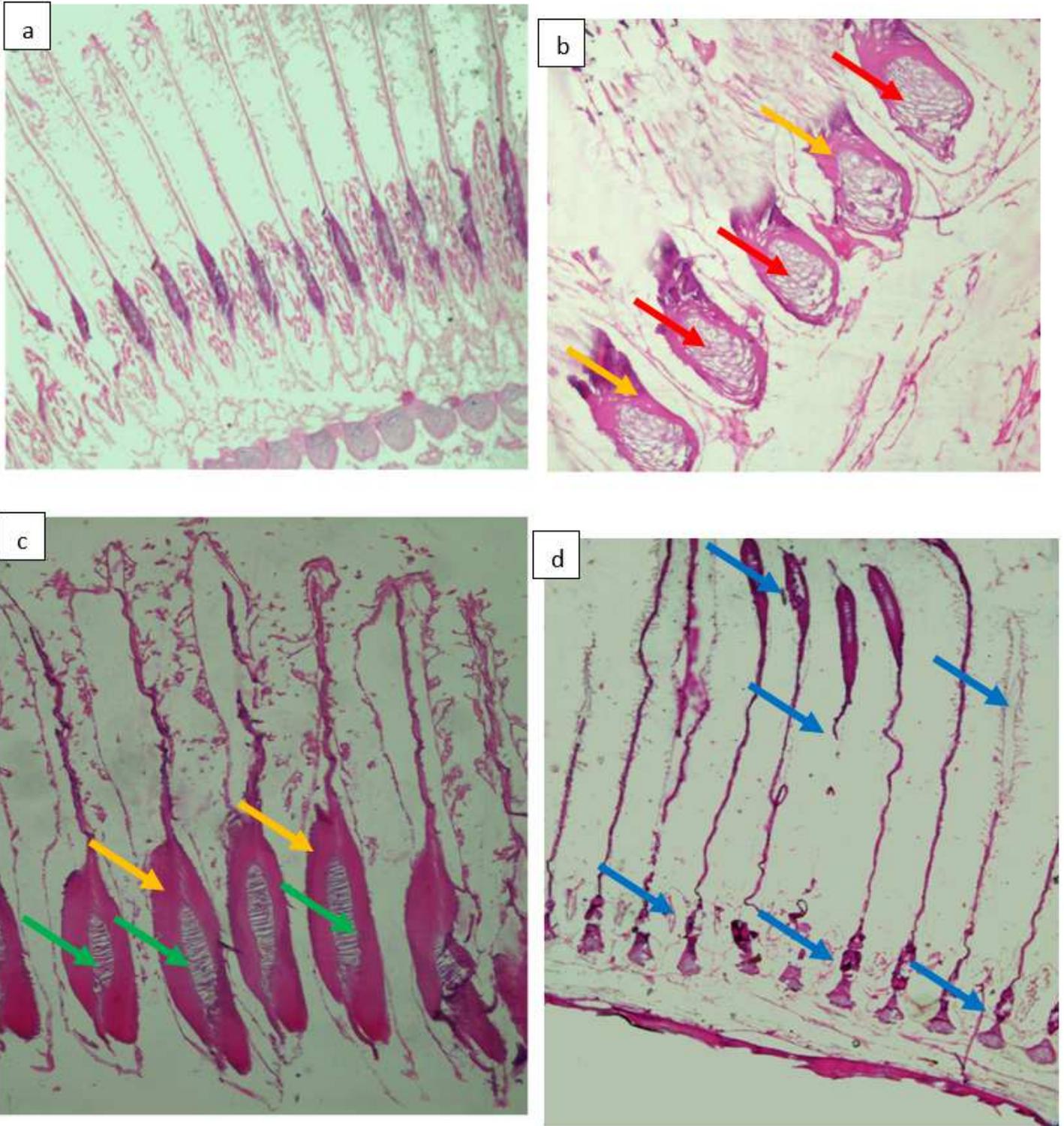
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# Figures



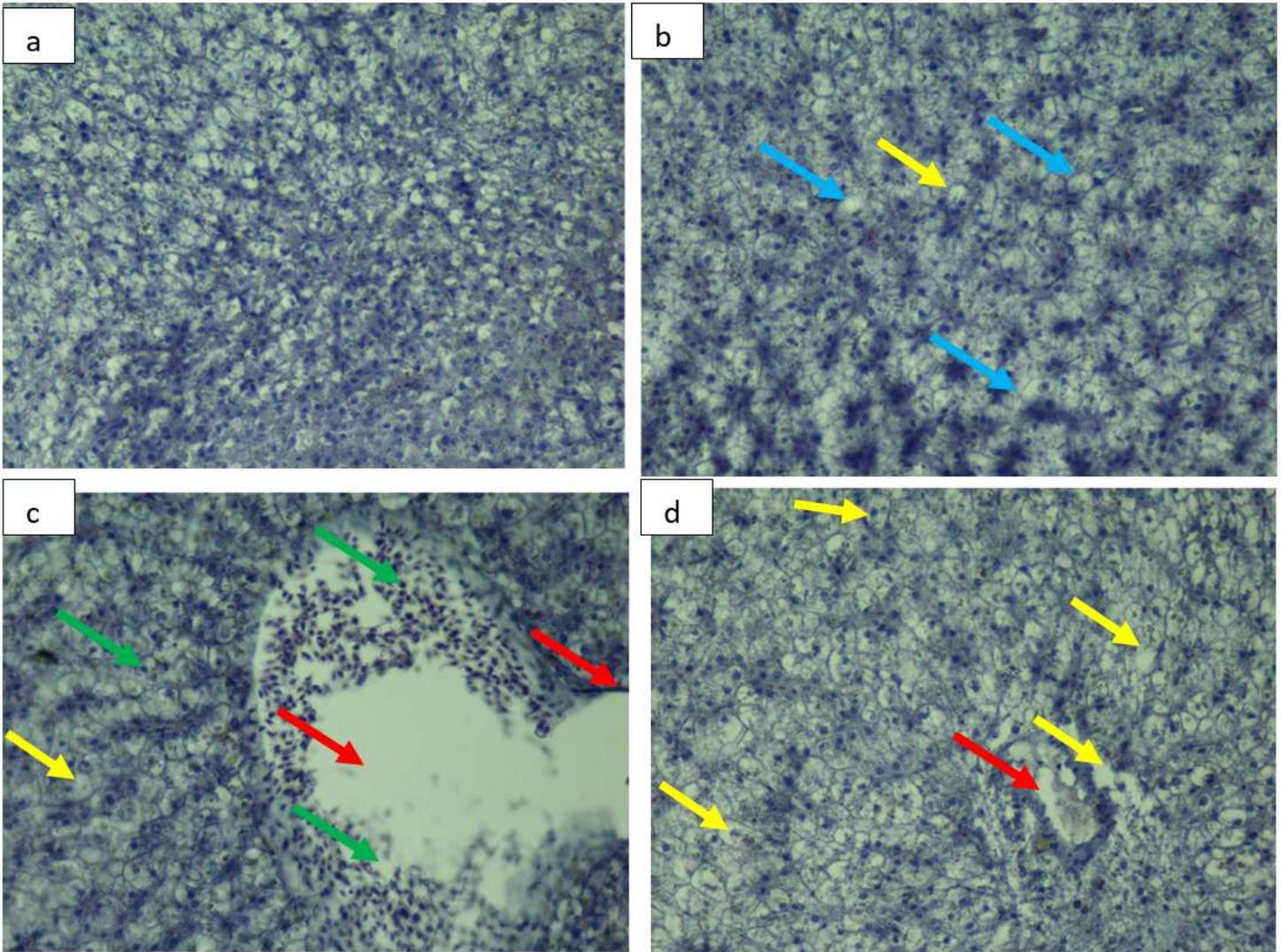
**Figure 1**

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



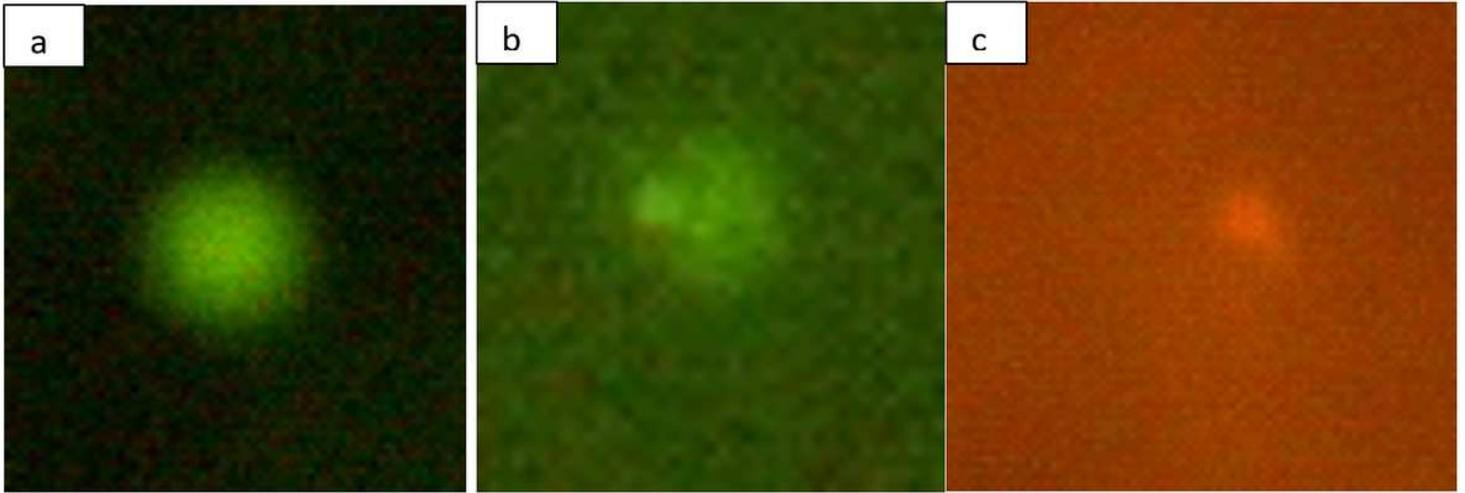
**Figure 2**

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).



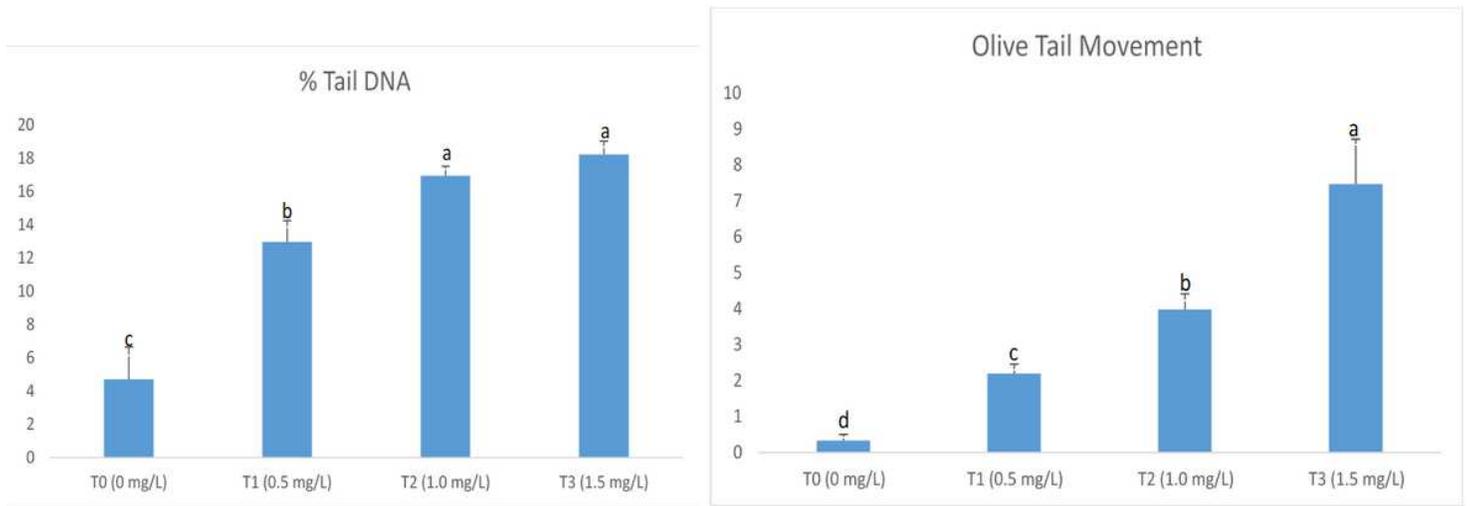
**Figure 3**

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).



**Figure 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



**Figure 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 ± S.D (p<0.05) (N=10)