



Histopathological Alterations in Common Carp, *Cyprinus Carpio* L. Exposed to Titanium Dioxide Nanoparticles: A Concentration-Dependent Study

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Abstract | The increasing industrial application of nanotechnology has led to the incorporation of titanium dioxide nanoparticles (TiO₂NPs) in several products and resources. These nanoparticles often enter aquatic ecosystems through sewage and wastewater, contributing to contamination that poses significant risk to aquatic organisms. This study was designed to understand and clarify the histological changes of common carp, *Cyprinus carpio* L. treated with variable concentrations of TiO₂NPS. Firstly, the TiO₂NPS powder sample was characterized by Transmission Electron Microscopy (TEM) Analysis, Ultraviolet-Visible Spectroscopy (UV-Vis), and Field-Emission Scanning Electron Microscopy (FESEM) with energy dispersive X-ray (EDX) Analysis. A total of 100 fish (weighing 50 g) were randomly distributed into five treated groups in duplicate (10 fish per tank), as follows: the first group serve as control (C); T1, T2, T3 and T4 were exposed to 50, 100, 200 and 300 mg/L of TiO₂NPs. Following 21 days of exposure, the histopathological samples were collected from gill, liver and kidney. Our findings showed that TiO₂NPs cause histological alterations in gill, liver and kidney. In gill it causes epithelial lifting, hyperplasia and fusion of the secondary lamellae. Liver exhibit dilation of sinusoids, nuclear pyknosis, cytoplasmic vacuolation and hepatic necrosis. Kidney displays hydropic degeneration, aggregation of melano macrophage with hemosiderosis. These changes were more severe at high concentration of TiO₂NPs (i.e., T4). In conclusion, these findings underscore the toxicological risks posed by TiO₂NP contamination in aquatic ecosystems, threatening both fish health and the stability of aquatic ecosystem and also human health. The study emphasizes the urgent requirement for stringent regulations and sustainable management methods to mitigate nanoparticle contamination and protect aquatic biodiversity.

Keywords | Histopathology, Gill, Carp, Titanium, Liver, Kidney

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INTRODUCTION

Nanomaterials are substances that have extremely small particle sizes, at least one dimension ranging from

(1-100) nm. Nanoscale materials exhibit unique electrical, magnetic, catalytic, and optical features, making them suitable for a wide range of applications in sectors such as medicine, engineering, and the environment (Sungure *et al.*,

2021). Titanium dioxide nano- particles (TiO_2NPs) also known as titania are among the most extensively used metallic nanomaterials due to their unique behavior and properties (Javed *et al.*, 2022; Ali *et al.*, 2018). There are several techniques that can be used for preparation of TiO_2NPs like sol-gel method, instantaneous synthesis method, microwave-assisted synthesis, solvothermal method, simple mixing, and precipitation technique (El-Sayed and Kamel, 2020). TiO_2NPs occurs naturally in three crystalline forms: Anatase, rutile and brookite (Eddy *et al.*, 2023). TiO_2NPs used for the production of toothpaste, drugs, coating, ink, paper, plastics, food, cosmetic, and textile industries. It also used for preparing of cloths, tiles, windows, and anti-fogging cars mirrors (Waghmode *et al.*, 2019). In addition, It was initially introduced in commercial products in 1923, including paints, catalyst systems, medical devices, ceramics, aerospace and turbines, adhesives, ointment, sunscreens, plastics and rubber, food colorants, agents of water treatment, floor coverings, material of roofing and in automotive products (Mishra and Res, 2014). In recent years, these nanoparticles are being used in veterinary sector such as medications, diagnostic devices, vaccines, feed additives, treatment of animals and cancer (Danchuk *et al.*, 2023). As a results of these wide uses, TiO_2NPs will ultimately leak to the environment and transported into the soil as well as water, affecting organisms and ecosystems. TiO_2NPs can penetrate the biological membranes, enter the cells, and accumulate in tissues and organs with producing harmful effects (Fiordaliso *et al.*, 2022; Mustafa *et al.*, 2024; Mustafa *et al.*, 2020).

The size of nanoparticles is one of the most important characteristics that can influence the toxicity of these particles, because smaller particles have more surface area that leads to an increase the surface activity and the biological reactivity (Miao *et al.*, 2024). There are three main mechanisms of TiO_2NPs toxicity: oxidative stress, inflammation, and apoptosis, which may or may not be interrelated (Rollerova *et al.*, 2015). Moreover, the very small size nanoparticles become more toxic and reactive that result in production of free radicals with reactive oxygen species (ROS) (Yi *et al.*, 2016; Venkatesan and Kim, 2014). The produced ROS lead to oxidative stress, cellular damage and inflammation due to aggregation of nanoparticles in biological molecules within the cells as mitochondria and interfering with the cellular antioxidant defense mechanism (Meena *et al.*, 2018). Nano- TiO_2 toxicity is established as the negative effects on fitness-related traits like feeding, immunity and reproduction in aquatic organisms (Luo *et al.*, 2020). TiO_2NPs toxicity has been reported the important deleterious effects in fish, including: inflammation with oxidative stress mainly (Araújo *et al.*, 2022), the behavioral alterations (Gu *et al.*, 2021), the impacts on fish immune system that the fish was susceptible to the bacterial diseases (Huang *et al.*, 2021), Besides, accumulation of TiO_2NPs has been discovered in

gills resulted in disturbances its function with falling oxygen uptake (Zou, 2024). As well, the fish liver is considered one of the chief organs affected by TiO_2 NPs, producing metabolic and intracellular modifications (Araújo *et al.*, 2022; Fonseca *et al.*, 2023). Antioxidant responses also reported in sperm of gilthead seabream *Sparus aurata*, without effects on the sperm performance (Carvalhais *et al.*, 2022). However, TiO_2NPs alter the sperm motility in rainbow trout due to oxidative stress in sperm cells (Özgür *et al.*, 2018).

At molecular level, TiO_2NPs increased in gene and protein expression levels of the nuclear factor kappa B (NF- κ B), (important regulator for cellular antioxidant response) (Ze *et al.*, 2013). Other studies reported that TiO_2NPs bind with intracellular bio-molecules resulted in production of ROS, plasma membrane leakage, mitochondrial depolarization, influx of intracellular calcium and release of cytokines (Chen *et al.*, 2012; Scherbart *et al.*, 2011; Fu *et al.*, 2012). Furthermore, titanium dioxide nanoparticles induce genotoxicity by direct damage of DNA which resulted from inflammation, oxidative stress, interfering with DNA repair mechanisms, and epigenetic alterations (Ling *et al.*, 2021). Moreover, nanoparticles cause disturbances of intracellular calcium which lead to cytotoxicity due to energy and metabolic imbalance with cellular dysfunctions (Huang *et al.*, 2017).

It's important to mention that TiO_2NPs are eliminated from the body slowly which indicated its potential tissue accumulation (Geraets *et al.*, 2014). However, da Cunha and de Brito-Gitirana (2020) revealed that TiO_2NPs cause histopathological changes in many organs such as kidney and liver of fish. The exposure of TiO_2NPs in animals resulted in dose-dependent toxicity. Also, common carp (*Cyprinus carpio* L.) are important species ecologically and economically and considered suitable model for ecotoxicological studies. In addition, dominance of common carp species in aquatic environment. As a consequence, the aim of this experiment was to determine the adverse effects of different concentrations of TiO_2NPs on the histology of gills, liver and kidney in common carp. This study also presents a novel investigation into the histopathological impacts of TiO_2NPs on common carp (*Cyprinus carpio*) at a concentration of 300 mg/L. While previous research has discovered the toxicity of TiO_2 NPs at varying lower concentrations, the specific influence of this higher concentration on main organs, such as the gills, liver, and kidneys, has not been widely documented previously.

MATERIALS AND METHODS

TiO_2NPs CHARACTERIZATION AND TREATMENT

The powder of Titanium dioxide nanoparticles was purchased from US research nanomaterials company. The char-

acterization of TiO₂NPs was studied using Transmission Electron Microscopy (TEM) Analysis, Ultraviolet-Visible Spectroscopy (UV-Vis) and Field-Emission Scanning Electron Microscopy (SEM) with energy dispersive X-ray (EDX) analysis (Suhail *et al.*, 2022). The concentrations of TiO₂NPs (50, 100, 200, 300 mg l⁻¹) were selected according to previous study by (Simonin *et al.*, 2016) with some alterations which is use new higher concentration of these nanoparticles (300mg/L).

EXPERIMENTAL DESIGN

All procedures in this study were reviewed and approved by the Committee on Animal Use and Care, College of Veterinary Medicine, University of Baghdad (328 at 2022/0208). This work was conducted at the laboratory of fish diseases in the College of Veterinary Medicine /University of Baghdad. About 100 of common carp were purchased from local farm of cages in Babylon province/Iraq. The fish were transported in an open system using containers filled with water carried by a transport truck with an oxygen pump provided to keep them alive during the transportation period. Firstly, fish were acclimatized for about two weeks in glass tanks before initial of the experiment. Fish were fed on commercial carp diet which consist of: crude protein:36%, crude fat: 9%, crude fiber:5%, moisture:10%, carbohydrate:29%, phosphorus:1%, ash:10%. Fish exhibited normal activity and behavior. Water temperature was 23 -26°C, dissolved oxygen was 6.4-7.5 mg/L and pH was 7.4-7.8 (Al-Rudainy *et al.*, 2023; Mustafa and Jha, 2022; Mustafa *et al.*, 2017). Fish (weighing 50±10 g; length 20±2 cm (n = 100)) were then divided into 5 equal groups in duplicate (10 fish for each tank). An amount of powder was suspended in aqueous solution according to each concentration in a glass container, then this suspension was added to each tank according to its dosage of mg/L. The first group serve as control (C) without treatment and the other four tanks serve as the treatment groups that treated with four variable concentrations (T1 group treated with 50 mg/L; T2 group dosed with 100mg/L; T3 group exposed to 200mg/L; T4 group treated with 300mg/L). Every day, the tanks were cleaned and the fish were observed. After 21day, samples from gill, liver and kidney were collected from three fish from each group for evaluation the histopathological alterations in these organs.

HISTOPATHOLOGICAL SAMPLES

Following the completion of the experiment (21 day), samples from gill, liver and kidney were collected from three fish from each group for histopathological examination. The histopathological analysis was conducted based on method of paraffin sections technique, firstly, the tissue samples were fixed in 10% formaldehyde solution, embedded in paraffin, cutting using microtome device. After staining with hematoxylin and eosin, the specimens were observed by light microscopy and then Photographed using

HD camera (Mustafa *et al.*, 2020; Suvarna *et al.*, 2018; Mustafa *et al.*, 2012).

RESULTS AND DISCUSSION

CHARACTERIZATION OF TiO₂NPs

TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS:

Figure 1 shows the TEM image of TiO₂NPs. TEM image were employed to illustrate the shape and size of the TiO₂NPs particles (Alturki and Ayad, 2019). Firstly, TiO₂NPs were characterized for their size to ensure that they were in nanoscale range. As showed in Figure 1. The size that measured using Transmission Electron Microscopy was 50 nm. This image also suggested that the polyhedral morphology was the most shape for nanoparticles. These results are in accordance with other study Keiteb *et al.* (2016).

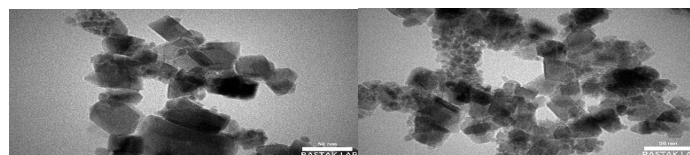


Figure 1: TEM image of TiO₂NPs (Scale bars = 50nm).

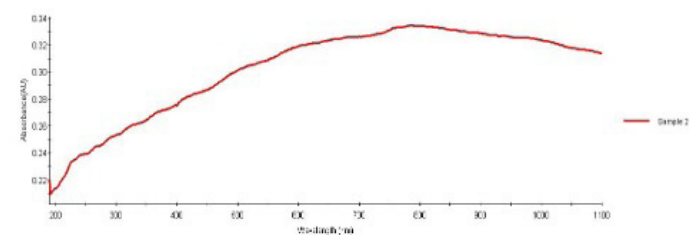


Figure 2: Ultraviolet visible spectra for samples of TiO₂NPs.

ULTRAVIOLET-VISIBLE SPECTROSCOPY (UV-VIS):

Figure 2 displays the UV-VIS absorption spectrum for the samples of titanium dioxide nanoparticles between 200 and 1100 nm wavelength. These results are in agreements with several studies (Orudzhev *et al.*, 2021; Altaf *et al.*, 2021).

FIELD-EMISSION SCANNING ELECTRON MICROSCOPY (FESEM) WITH ENERGY DISPERSIVE X-RAY (EDX)

ANALYSIS: The morphological characteristics of TiO₂NPs were analyzed by using MIRA3 LMU (Japan) under a 15 kV electron acceleration. The synthesized TiO₂NPs samples with an average size of 50 nm of ovoid or spherical shape were displayed in Figure 3 using Field-Emission Scanning Electron Microscopy (FESEM) technique. This result is similar with finding of Hashem and Al-Karagoly (2021); (Jameel *et al.*, 2019).

Energy dispersive X-ray (EDX) spectrum of TiO₂NPs was shown in Figure 4 and confirmed the existence of Ti and O elements in examined nanomaterial (Lavand *et al.*, 2015). In addition, EDX analysis showed that the nanoparticles

that composed of only titanium and oxygen elements indicates that these TiO_2 NPs were pure and crystallized well (Al-Harbi *et al.*, 2011). The average percentage in TiO_2 NPs was 66.2%, 33.8% for Ti and O, respectively. Hossain *et al.* (2018) revealed that the percentage of TiO_2 NPs were 64.88% and 35.12% for Ti and O, respectively.

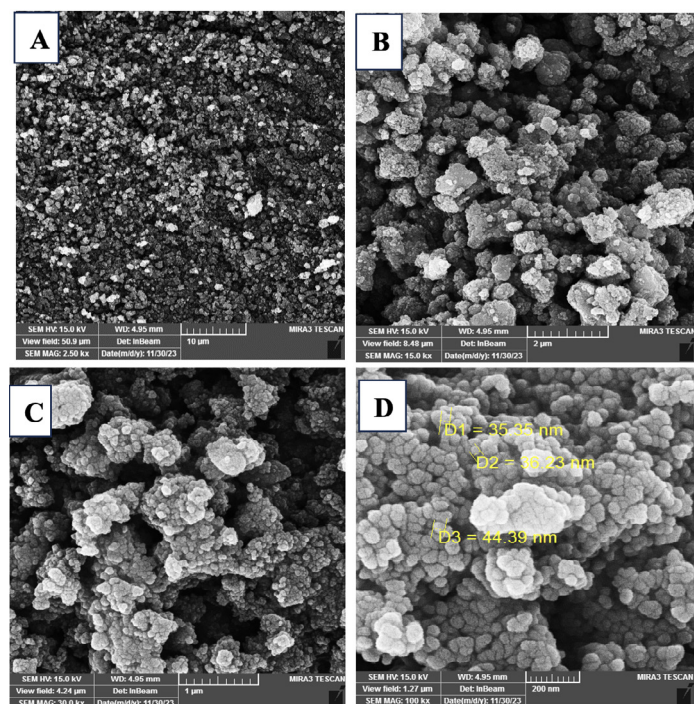


Figure 3: FESEM image showed a spherical to ovoid shape of synthesized TiO_2 NPs. Scale bar: A=10 μm , B=2 μm , C and D=1 μm .

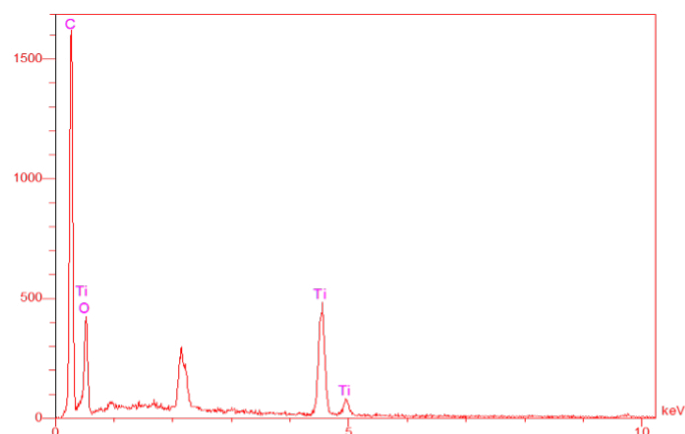


Figure 4: The EDX pattern of TiO_2 NPs.

From the above results of characterization, the powder of nanomaterial was pure titanium dioxide nanoparticles in crystal form with size about 50nm diameter. The size of nanoparticles is a vital property affecting on their cytotoxicity (Donahue *et al.*, 2019). The size also has main role in determination the method of entrance into the target cells and this is finally affecting of distribution of nanoparticles in the tissues and accumulation levels (Sukhanova *et al.*, 2018). Moreover, smaller nanoparticles can pass more easi-

ly through the plasma membranes via translocation, unlike larger molecules that require transportation mechanisms such as phagocytosis and micropinocytosis to move across the membranes (Zhang *et al.*, 2015). As well, the smaller size nanoparticles have larger surface area which increase the nanoparticles reactivity because it has large surface area for linked with cellular organelles (Johnston *et al.*, 2010). The smaller size of nanoparticles (<50 nm) nanomaterials produces further toxicity when compared to big size particles (>50 nm). This is due to smaller size nanoparticles have more penetration ability into body tissues. Another important reason is that they are easily evaded by immune system, whereas larger particles are readily phagocytosed for quicker elimination from the body via the lymphatic system (Thu *et al.*, 2023).

HISTOPATHOLOGICAL EXAMINATION

GILL SECTIONS: Gill sections of *C. carpio* exposed to varying concentrations of titanium dioxide for 21 days revealed significant structural changes. In the control group, the gill section exhibited normal and well-organized secondary lamellae, epithelial cells, functional chloride cells, and healthy erythrocytes, with no signs of abnormality or histopathological changes (Figure 5A). At a concentration of 50 mg/L of TiO_2 NPs epithelial lifting was observed (Figure 5B). Gill sections exposed to 100 and 200 mg/L of TiO_2 NPs displayed hyperplasia leading to thickened secondary lamellae. Additionally, fusion of adjacent secondary lamellae was observed (Figure 5C and D). At the highest concentration of 300 mg/L of TiO_2 NPs severe pathological changes were evident, including telangiectasis, dilation of blood vessels, blood congestion, and hyperplasia of the secondary lamellae (Figure 5E and F). These findings are consistent with the results of Rahmani *et al.* (2016) who reported that exposure to 100 mg/L of TiO_2 NPs caused significant damage to the gills, including fusion and necrosis of the secondary lamellae. Additionally, it has been suggested that increased hyperplasia of the lamellae may act as a barrier to the accumulation of nanoparticles within the gills by increasing the diffusion distance during gas exchange (Wang *et al.*, 2015; Mustafa *et al.*, 2019). Furthermore, TiO_2 NPs have been shown to induce histopathological changes such as dilation of blood vessels, necrosis, hyperplasia, and lamellar fusion (Mansouri *et al.*, 2016). In these conditions, the proliferation of lamellar cells reduces the space between lamellae, ultimately leading to their fusion (Jaya and Shettu, 2015). Tilapia (*Oreochromis mossambicus*) exposed to TiO_2 NPs resulted in thickening and fusion of gill lamellae, filaments rupturing, and hyperplasia of gills (Shahzad *et al.*, 2022). Likewise, Huang *et al.* (2021) established that TiO_2 NP-induced opportunistic bacterial infections in gill tissue of zebrafish. In rainbow trout (*Onchorynchus mykiss*) gill cell line TiO_2 NP agglomerates were detected within intracellular

vesicles in gill cells (Lammel *et al.*, 2018). Lamellar fusion is considered a defensive mechanism of the gills, as it reduces the total respiratory surface area when in contact with external environmental stressors. However, these structural changes can significantly impair the oxygen uptake required for metabolic processes, thereby compromising the overall health of the fish (Subashkumar and Selvanayagam, 2014; Jaya and Shettu, 2015). The large amount of TiO₂NPs accumulated in the gills is likely attributable to the extensive surface area of the gill epithelium and its direct contact with water, making it highly susceptible to aqueous contaminants (Norouzi *et al.*, 2012; Maleki *et al.*, 2015).

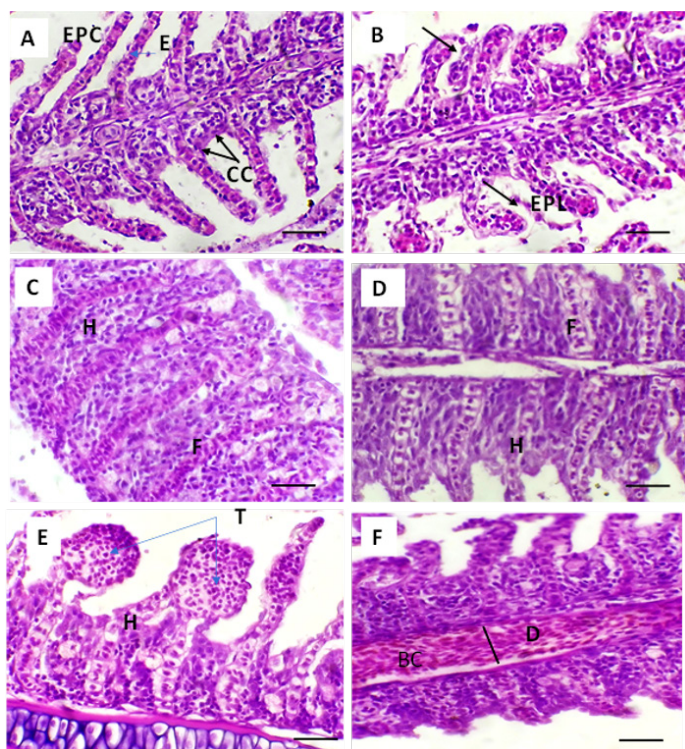


Figure 5: Photomicrograph of the gill tissue; A: control showing normal secondary lamellae (SL), epithelial cells (EPC), chloride cells (CC), erythrocyte (E); B: gill section of T1 showing epithelial lifting (EPL); C and D: gill sections of T2 and T3 showing hyperplasia (F) and fusion (F) of the secondary lamellae; E and F: gill section of T4 showing telangiectasis (T), dilation of the blood vessels (D) with blood congestion (BC) and hyperplasia of the secondary lamellae (H). Scale bars = 50 μm. Thickness = 5 μm.

LIVER SECTIONS: Liver sections of control group exhibited normal architecture, including intact hepatocytes and well-organized sinusoids, with no signs of abnormalities or histopathological changes (Figure 6A). Liver sections exposed to 50 mg/L of TiO₂NPs, blood congestion was observed in the portal vein, accompanied by dilation of the sinusoids (Figure 6B). Liver sections exposed to 100 and 200 mg/L of TiO₂NPs showed further dilation of the sinusoids along with nuclear pyknosis, indicating the condensation of chromatin in the nuclei of affected cells (Figure 6C and

D). At the highest concentration of 300 mg/L of TiO₂NPs severe pathological changes were evident. These included cytoplasmic vacuolation, necrosis in some hepatocytes, and infiltration of mononuclear cells (Figure 6E and F).

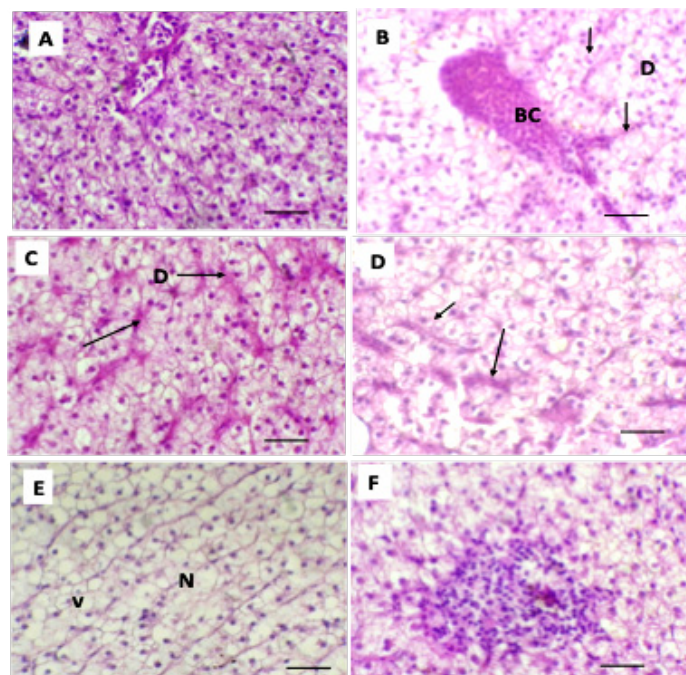


Figure 6: Photomicrograph of the liver tissue A: control showing normal structure of hepatocytes and sinusoids; B: liver section of T1 showing blood congestion of the portal vein with dilation of the sinusoids (D); C and D: liver section of T2 and T3 showing dilation of the sinusoids (D); with nuclear pyknosis (PK) E and F: T4 showing cytoplasmic vacuolation (V), necrosis in some of hepatocytes (V) and mononuclear cells infiltration (MNCs); Scale bars = 50 μm. Thickness = 5 μm.

These findings are consistent with those of Hajirezaee *et al.* (2020), who reported that exposure to 0.125 mg/L of titanium dioxide nanoparticles (TiO₂NPs) caused liver damage characterized by cytoplasmic vacuolization. Similarly, Diniz *et al.* (2013) demonstrated that exposure of *Carassius auratus* to 100 mg/L of TiO₂NPs resulted in hepatocyte degeneration, nuclear pyknosis, and inflammation. Other study (Vidya and Chitra, 2018) revealed that freshwater fish, *Oreochromis mossambicus* exposed to TiO₂NPs for 96 hr. caused hepatic vacuolization or steatosis and for 60-day lead to aggregation of melano-macrophages with necrosis and spindle shaped. In addition, Shahzad *et al.* (2022) observed liver damage in *Oreochromis mossambicus* following exposure to 1.5 mg/L of TiO₂NPs, with pathological signs including necrosis, sinusoidal dilation, and nuclear condensation. The toxic effects of nano-metals, including TiO₂NPs, on the liver are mediated through several mechanisms. These include the generation of reactive oxygen species (ROS), which leads to oxidative stress and inflammation, mitochondrial damage within hepatocytes, endo-

plasmic reticulum stress, and even DNA damage (Das *et al.*, 2024; Faik and Mustafa, 2023). These disturbances may impair the liver's capacity to carry out its vital activities, which will ultimately have an impact on the fish's general welfare and ability to survive.

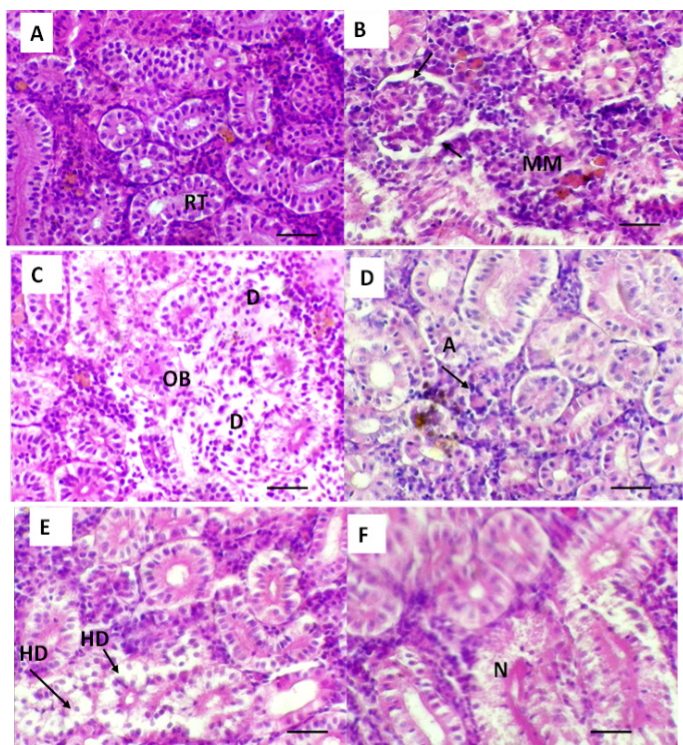


Figure 7: Photomicrograph of the kidney tissue; A: control showing normal renal tubules (RT) and hemopoietic tissue B: kidney section of T1 showing melanomacrophage aggregation (MM) with hemosiderosis; C : kidney section of T2 showing depletion of the hemopoietic tissue (D) with obstruction some of the renal tubules (OB); D: kidney section of T3 displaying atypical character of the glomerulus (A);; E and F: T4 demonstrating hydropic degeneration (HD) and necrosis in renal tubules (N); Scale bars = 50 μ m. Thickness = 5 μ m.

KIDNEY SECTIONS: In the control group, the kidney section exhibited normal renal tubules and intact hematopoietic tissue, with no signs of abnormality or histopathological damage (Figure 7A). In kidney sections exposed to 50 mg/L of TiO₂NPs melanomacrophage aggregation was observed, accompanied by hemosiderosis, indicating the accumulation of iron pigments within the tissue (Figure 7B). Kidney sections exposed to 100 mg/L of TiO₂NPs, revealed depletion of hemopoietic tissue along with obstruction in some of the renal tubules (Figure 7C). At a concentration of 200 mg/L of TiO₂NPs, atypical structural changes were observed in the glomerulus (Figure 7D). The most severe alterations were noted at a concentration of 300 mg/L of TiO₂NPs, where hydropic degeneration and necrosis were evident in some renal tubules (Figure 7E and F). The kidney plays a critical role in the bioaccumulation and detoxification of nanoparticles in fish and is one of

the primary organs affected by titanium dioxide nanoparticles (TiO₂NPs) (Jovanović *et al.*, 2015). In trout, exposure to high doses of TiO₂NPs has been shown to result in their accumulation in the kidney through the bloodstream, highlighting the organ's vulnerability to nanoparticle exposure (Scown *et al.*, 2009). Once deposited in kidney tissue, TiO₂NPs can cause significant renal injury, as demonstrated by Gui *et al.* (2013). Valentini *et al.* (2017) also reported that TiO₂NPs have a toxic impact on proximal tubular cells, which are essential for the kidney's filtration and reabsorption processes. Similarly, Iavicoli *et al.* (2016) observed that TiO₂NP exposure adversely affects the entire renal system, leading to widespread functional and structural damage. Further evidence of the nephrotoxic effects of TiO₂NPs is provided by do Carmo *et al.* (2018), who reported renal tissue disturbances in exposed fish, including tubular degeneration, necrosis, and an increase in melanomacrophage aggregates. Melanomacrophage aggregates, also known as macrophage aggregates, are clusters of pigmented cells commonly found in fish kidneys. These aggregates are known to increase in size and number in response to environmental stress or chemical contamination (Agius and Roberts, 2003). Their proliferation in TiO₂NP-exposed fish suggests an adaptive immune response to oxidative damage or nanoparticle accumulation.

Overall, the result of our study reported the key mechanism of TiO₂NPs toxicity in tissues (gill, liver and kidney) were inflammation and oxidative stress. The molecular mechanisms of tissue injury and inflammation are related to modifications in both mRNA and protein expression levels of numerous inflammatory molecules as nucleic factor kappa B (NF-Kb), macrophage migration inhibitory factor (MIF), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-10 (IL-10), C-reactive protein (CRP) (Ma *et al.*, 2009; Cui *et al.*, 2011). Another study found that nanoparticles induce gene or protein expression of pro-inflammatory cytokines (Kumar *et al.*, 2016). Moreover, Oxidative stress may cause inflammation via affecting of redox sensitive pathways with induction the release of pro-inflammatory cytokines (Åkerlund *et al.*, 2019). However, an alternative pathway for inducing inflammation without involvement oxidative stress for NPs that can bind with fibrinogen and make unfolding. This binding trigger activation of macrophage-1 antigen (Mac-1) receptor resulting in activation of NF- κ B signaling and inflammation occur (Marucco *et al.*, 2016).

Titanium dioxide nanoparticles generate reactive oxygen species (ROS) by producing an imbalance between the processes of oxidation and anti-oxidation, which finally leads to oxidative stress and damage to organs (Hong and Zhang, 2016). Oxidative stress is a condition induced by an imbalance in the formation and accumulation of reactive substances in cells as well as tissues of the body, and its

capacity to eliminate these reactive substances (Pizzino *et al.*, 2017). In another word, it is imbalance between oxidant (free radicals) and anti-oxidant processes (Sies, 2020). This unbalance between oxidant/antioxidant mechanism led to activation of special factors that resulting in cellular injury (Pisoschi and Pop, 2015). As well, reactive substances formation can be produced from the connections between cells and nanoparticles which lead to dysfunction of mitochondrial (Horie and Tabei, 2021). Excessive creation of ROS/RNS lead to oxidative stress (Hameister *et al.*, 2020). As mention previously, TiO₂NPs inhibited the expression of AKT and then induce expression of pro-apoptotic protein Bax which was failure to form the heterodimers with reduce anti-apoptotic Bcl-2, then activating downstream Caspase-9 and Caspase-3 and finally induction of apoptosis (Xu *et al.*, 2024).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study demonstrates that exposure to TiO₂NPs induces significant histopathological alterations in the gills, liver, and kidney of common carp. These histopathological alterations in these vital organs at this high concentration (300mg/L) provide new insights into the potential environmental risks posed by TiO₂ nanoparticles in aquatic ecosystems over time. These findings also highlight the potential risks of TiO₂NP contamination in aquatic environments and underscore the need for stricter regulations to mitigate nanoparticle pollution.

Future studies should also investigate the impacts of chronic exposure to TiO₂NPs on fish populations and other aquatic animal species. In addition, explore potential strategies for reduce pollution that resulted from TiO₂NPs accumulation.

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NOVELTY STATEMENTS

This study introduces an advanced approach by utilizing high doses of TiO₂ nanoparticles (TiO₂NPs) as a new tool for detecting histopathological changes in common carp (*Cyprinus carpio*). While TiO₂NPs have been previously investigated in aquatic toxicology, this research is the first to explore their potential in identifying organ-specific alterations in fish tissues at elevated concentrations, thus providing valuable insights into nanoparticle-induced tox-

icity.

AUTHOR'S CONTRIBUTIONS

All authors contributed to the study conception and design. The first draft of the manuscript was written by Mustafa s.Arif and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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