Histopathological alteration in gills and liver of Cyprinus Carpio after a short-term exposure to titanium dioxide nanoparticles

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Abstract

With the rapid development of nanotechnology in past few decades, engineered nanomaterials (ENMs) have been extensively used in variety of domestic, commercial, and industrial products. Nano-titanium dioxide is one of the most widely used nano-material due to its UV light absorption, optical properties, thermally stable nature, and photocatalytic activity. Frequent use of nano sized titanium is likely to end up in the discharge of these particles into different environmental compartments. However, it is still uncertain if these nano materials are harmful to aquatic biota. In present study, titanium dioxide nanoparticles were prepared using liquid impregnation method and were characterized through SEM and XRD. Toxicity of selected five concentrations: 0.01, 1.5, 3.0, 10 and 100 mg/L of titanium dioxide nanoparticles on freshwater fish Cyprinus Carpio were assessed using histological biomarker approach. Gills and liver tissues showed increasing degree of damage including pycnotic nuclei, cytolysis, vacuolization, blood congestion and architectural loss along the exposure period of 96 hrs. This study elucidates the time and dosage dependent toxicity of titanium dioxide nanoparticles in the fish implying that short-term exposure to sublethal concentrations is sufficient to produce detrimental impacts on metabolic and physiological functioning of fish.

Keywords: Titanium dioxide, Histopathology, Toxicology, Fish

1. Introduction

The contamination of all aquatic bodies at unprecedented rates, has become a global concern. Nanoparticles (NPs) are an important example of an emerging class of environmental pollutants. Increase in production and utilization of NPs has led to increased human and environmental exposure. Engineered nanoparticles (ENPs) is a term that refers to a subset of nanomaterial with at least one dimension in the range of 1-100 nm. In addition to volume of industrial production, possibility of anthropogenic agents ending up in aquatic environments also depends on how they are used. There is an increase in concerns of adverse effects of engineered nanoparticles (ENPs) on human health and ecosystem due to their wide use in industrial and consumer products. Among the nanomaterials, TiO_2-NPs have the highest concentration in surface water. Numerous applications of nano-TiO_2 at excessive rates inevitably results in their release into aquatic ecosystems (Wang *et al.*, 2016). Estimated concentrations of nano-TiO_2 in water bodies are reported as 0.7 to 24.5 µg/L whereas predicted environmental concentration (PEC) for surface water is 0.7-16 µg/L (Troester *et al.*, 2016). Once released into an aquatic environment, significant impact of nano materials is subjected to their stability, mobility, and ultimately on the morphology in water resources used. Fish, amongst various aquatic organism, is a valuable bio-monitor of aquatic pollution. Fish occur at the top of food chain hierarchy in aquatic environment and play an important role in sustaining balance in aquatic ecosystem. Different reactions initiate in biological system when xenobiotics interact with fish, which ultimately result in disturbances of biochemical functioning. Common carp (*Cyprinus Carpio*) is most promising aquatic food product as it is commercially valuable and is one of economical food sources. It is a bottom dwelling fish. Its feeding habits and widespread distribution exposes it to different types of pollutants. TiO_2-NPs have been reported to cause DNA damages and decreased leukocyte viability, reduction of hatching time, genotoxicity in erythrocytes and altered swimming activity in aquatic organisms (Minetto *et al.*, 2016). So, the current study was designed to understand the

acute toxicity of TiO_2-NPs by using the histopathological biomarker approach and local fish of Pakistan as model organism.

2. Materials and methods

Experimental design and acclimatization of experimental fish

A total number of 200, healthy specimen (12-15 cm, yearlings; weight, 80-100 g) of Common Carp were purchased from Punjab Hatchery, Islamabad and were acclimatized in laboratory conditions for a period of two week. After the completion of acclimatization period , nine fish per tank were allocated for the experiment. Five concentrations: 0.01, 1.5, 3.0, 10 and 100 mg/L of titanium dioxide nanoparticles were applied to each tank and triplicates were maintained, for every dose concentration along with the control. The experiment was performed using random selection method for dividing the fish in control and experimental group (Iftikhar & Hashmi.,2021).

Synthesis and characterization of TiO_2-NPs

Liquid Impregnation method was performed for the synthesis of pure titanium dioxide nanoparticles. Slurry of TiO_2 was made by mixing 50 g of TiO_2 in 500 mL of deionized distilled water in a beaker and stirred for 24 h on magnetic stirrer hot plate after which the solution was let to settle for 24 h. It was oven dried for 12 hours at 105 ^{0}C and then crushed properly using a pestle and mortar. The crushed powder was placed in muffle furnace for 6 h at 550 ^{0}C to obtain the pure TiO_2-NP (Sahoo *et al.*, 2005). The morphology, topography, and particle size of the TiO_2-NPs were determined by using (SEM) scanning electron microscope (JEOL -6060) at 10,000x magnifications. Crystalline phase of prepared nanoparticles was determined by using X-ray diffraction (JEOL JDS-II).

Histological procedure

Nine fishes per tank were confined for total period of 96 hrs in cubic experimental tanks. Immediately after each 24 hrs of confinement, fish were sacrificed by cervical section after it was anesthetized with clove oil. Gills and liver cells were removed and washed by using physiological saline solution. Subsequently tissues were fixed in Bouin's fluid for different hours. And dehydration of tissues was done with ethyl alcohol series of (60, 70, 80%) ascending concentrations. Dehydrated tissues were then entrenched in paraffin wax and sections at 5 mm thickness were prepared by using microtome. The tissue lesions were stained with hematoxylin-eosin (HE), several sections of each tissue from each fish were observed by light microscope (Zeiss primo star-415500-1811-000) with 10 to 100 x magnification, coupled with USB digital camera.

3. Results and Discussions

The Scanning Electron Microscopy images show surface morphology of TiO_2-NPs. The average particle size at 50,000 x magnification, was found to be 74.64nm. Moreover, the analysis of titanium dioxide nano particles was performed by X-ray diffraction (XRD) in the range of 2-theta varying from 20^o-80^o at ambient room temperature. The crystalline size of nano particles was confirmed to be less than 100 nm. Peaks of XRD results (25^o and 48^o) revealed that TiO_2-NPs had anatase crystalline structure.

Histological alterations of liver

In the present study, the gills and liver of common carp in control group has shown a normal structure and liver and gills of the nano-titanium dioxide treated groups showed several pathological changes throughout the experimental period. In control group of *Cyprinus carpio*, the liver constitutes of continuous arrangement of hepatocytes placed in asymmetrical cords. The hepatic cells have a polygonal shape with distinctive central nuclei. Many blood sinusoids are also present around the hepatocytes. Pycnotic nuclei were noted in the liver sample of fish exposed to 0.01mg/L of nano-titanium after 24 hours. Toxicity directly damages liver tissues as in this study pycnotic nuclei and cytolysis were spotted in the liver tissue exposed to 1.5 µg/L of nano-titanium after 24 hours. As for higher concentrations of nano titanium (5, 10 and 100mg/L) vacuolization and cytolysis was observed after 24 hours and cell structure got ruptured due to nano-titanium exposure and clear toxic effects were detected at the structural and cellular level in the liver after 96hrs of exposure. After prolonged exposure, a large number of hepatocytes were completely harmed. Vacuolation of Intracellular spaces was also evident. In most of exposure groups the integrity of cell wall of hepatocytes was noticeably abandoned. In period of 96 hours, degeneration of cell boundaries along with the dilation of blood sinusoid was also reflected in most of the exposed groups. The liver cells have many important functions such as; bile secretion, detoxification, blood plasma synthesis, preserving glycogen and discharge of glucose in the body. Glycogen matter was observed to be reduced in hepatocytes at all periods of times after treatment with a sub lethal dosage of nano-titanium. The results imply that glycogen being a readily available source of energy in the body, has decreased due to its quick breakdown also known as glycogenolysis, a

phenomenon which is responsible for the release of glucose into the blood to meet the elevated energy requirements in a stressed environment. Numerous studies have reported progressive changes in hepatic tissue exposed to pollution by various contaminants. A study reported that degenerative changes in liver of *Channa punctatus* resulted under silver nanoparticles toxicity. Formation of vacuoles, cytoplasmic degeneration in the liver of *Oreochromis niloticus* exposed to TiO_2 nanoparticles are reported in literature. The alterations may be ascribed to immediate lethal effects of nano-pollutants on hepatocytes, as the liver is the storage and processing site of of noxious substances (Gaafar & Soufy, 2007)

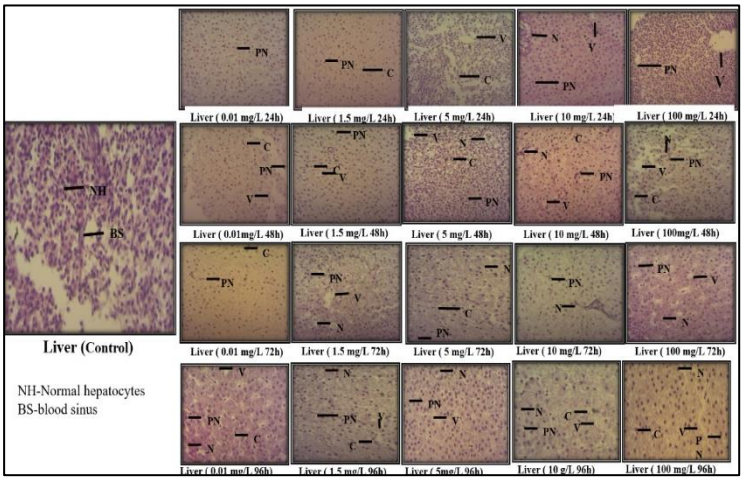

Figure 2. Photomicrographs of liver tissues of common carp exposed to (0.01-100mg/L) conc.of TiO_2 nanoparticles for 24, 48, 72, and 96 hrs.

Histological alterations in gills

In this research study, the gills of *Cyprinus carpio,* in control group have shown a normal structure as proper primary gill lamellae, secondary gill lamellae and definite Inter lamellar region were observed while, nano-titanium dioxide treated groups showed several pathological changes throughout the experimental period. Noticeable histological alterations in treated groups were edema, hemorrhage of gill lamellae, cytoplasmic vacuolation and curved secondary lamellae. Blood congestion and increased mucus production were also seen on prolonged exposure to nano titanium dioxide.The fishes exposed to lower concentrations (0.01 and 1.5mg/L) showed edema, cytoplasmic vacuolation and curved secondary lamellae after 72 hours of experiment which indicates the early defense mechanism of the fish body against stress induced by the toxicant. The fishes exposed to higher concentrations (5, 10 and 100 mg/L) showed to edema, hemorrhage of gill lamellae, Necrosis, lamellar sloughed off cells and loosening of primary gill bar after 72 hours of experiment. The fishes exposed to 100 mg/L lead to severe hemorrhagic gill lamellae, increased cytoplasmic vacuolation, curved secondary lamellae and complete lamellar fusion and architectrural loss after 96 hours of exposure. Exposure to the higher concentrations of nano titanium caused an disproportionate amount of mucus secretion over the gills of *Cyprinus carpio.* It has been indicated that the stress caused by the changes in the environment induced the propagation of mucus cells and enhanced secretion. The large quantity of mucus secretion acts as a defense mechanism against several toxic substances. Popovic et al in 2015 collected samples of fish from wastewater canals and reported change including lamellar fusion reduced inter-lamellar space, architectural loss, distention of blood sinuses of secondary lamellae of fish gill tissues exposed to different type of pollutants.

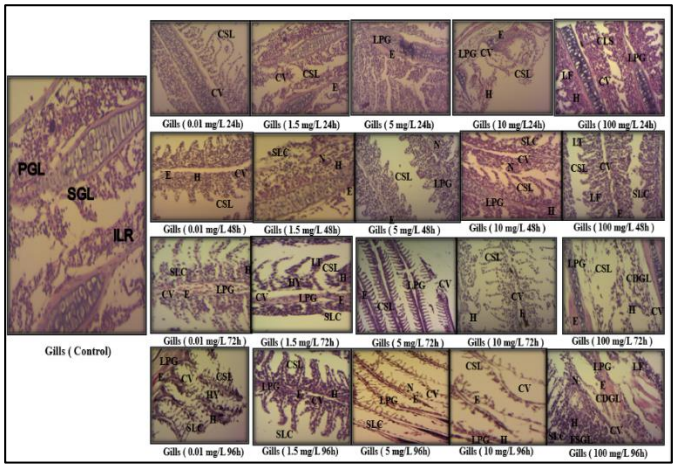

Figure 3. Photomicrographs of Gills tissues of common carp exposed to (0.01-100mg/L) conc. of TiO_2 nanoparticles for 24, 48, 72, and 96 hrs.

4. Conclusion

Observed histopathological alterations of liver and gills have revealed some deleterious effects of TiO_2 in *Cyprinus carpio*. Nano-toxicity increased with higher exposure concentrations along with the passage of time. Pathological lesions were relatively more noticeable in gills tissues than liver, as compared to the control groups. Recent study reveals that histopathological assessment can simply expose the toxic impacts on fish as result of exposures to ecological stressors, including nano particles.

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