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Hepatotoxicity of Titanium Dioxide Nanoparticles (TiO2-NPs) in Adult Albino Rat Model: Comparative Histolopathological Study

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Annotation: Oreview: Nanotechnology nanoparticles have seen noteworthy development in current years. This topic focuses on producing and applying nanoparticles (NPs) ranging in size from 1 near 100 nanometers. Titanium dioxide nanoparticles (Tio2-NP) are critical because of their numerous uses in a variability of biological and pharmaceutical fields and arrive the human body through several ways. Aim: Based on our data, few reserchs have examined the effects of titanium nanoparticles on the hepatic tissue. Consequently, this paper was planned to explore the conceivable pathologic effectiveness of titanium dioxide nanoparticles (TiO2-NPs) on hepatic tissue with reference to the histopathological, exploring the possible underlying processes and the expected degree of enhancement. Materials and Methods: The research project recruited a total of forty-two of healthy albino male rats to look into the impacts of titanium dioxide nanoparticles (TiO2-NPs). Throughout ten consecutive days, animals received TiO2-NPs in dosages of 50 µL or 100 µL, with tiny particles of 25 nm, 50 nm, and 75 nm. Results: Administration with TiO2-NPs exhibited substantial deviations in hepatocytes, portal triads, and sinusoids when compared with the untreated groups. The changes that has appeared in hepatocytes were prominently marked by hydropic and vacuolar degeneration, cytoplasmic hyaline inclusions, nuclear

irregularities, binucleation, and evidence of nuclear damage for instance, karyolysis, karyorrhexis, and ultimate cellualr necrosis. Conclusions: All excpermental specimens who are subjected to TiO-NPs exhibit hepatocyte edema, predominantly as a consequence of decreased membranes functioning, consequential in an influx of water and (Na+) ions. This change promotes the release of lysosomal enzymes, which reasons cytoplasmic damage besides hydropic decline due to disrupted fluid and ionic equilibrium. The existence of vacuolated hepatocytes indicates damage to the liver, whereas binucleation represents cellular stress and an effort at renaissance. The damaging impact of TiO2-NPs, specifically the lesser particles, exacerbates hepatocyte damage via interfering with liver enzymes and antioxidant systems, producing reactive oxygen species that are (ROS), and increasing necrotic. More research is needed to entirely comprehend the potential therapeutic and investigative applications of TiO2-NPs.

Keywords: Albino rats model. Histolopathology. Titanium Dioxide Nanoparticles.

Overview

The field of nanotechnology has developed fast in the past few decades. It deals primarily within the creation and utilization of nanotechnology (NPs), which are tiny nanoparticles having a variety of sizes instead of one (Musial *et al*., 2020). The nanoparticles of titanium dioxide (TiO2-NPs) are widely used in numerous kinds of applications ranging from manufacture of plastics to pharmaceutical companies pill formulation, bleaching substances in paper manufacture, paints, sunscreens, cosmetics, and toothpaste manufacturing (Haleem *et al*., 2023).

TiO2-NPs nanoparticles of titanium dioxide, which is the ninth most common component of the outermost layer of the planet, are an expansively employed mineral that can enter the food chain as a bleaching agent, particularly within products made from dairy, chocolate, milk powders, besides various industrial products. Such ingredient, referring by the designation E172 is included in foods and medications and helps define colors clearly while also preventing material degradation under UV radiation. According to Thangamani and Pasha, (2021), individuals ingest approximately 300 mg of titanium every day in their diets. Titanium dioxide, usually referred to as titania, is a nanoparticle composed of TiO2-NPs. Its development dates back to 1933, So until this pointing upwards, the situation manufacturing requests have grown expansively by means of a substantial and effecting resultants from minerals (Cornu *et al*., 2020) .

The nanoparticles of titanium dioxide have feathers such as rutile, which acetate, or limestone.

The constituent and anatase particles possess tetrahedral configurations, however the brookite is octahedral. In recognition of their distinctive characteristics in regard to their connection to biological molecules, they have attracted great attention throughout biologists and other scientists in the approach (Ramadan *et al*., 2023). TiO2-NPs. is an effective the photocatalyst founded on semiconductor compounds according to their architectural characteristics, and therefore has piqued the attention of researchers form numerous areas until the year 1978. Such particles is conveniently manufactured in either industrial and academic settings since it is commonly accessible and formed in form quantities at a low expense (Zhao *et al*., 2021). Titanium photographic catalysts were chemically and photochemically stable whilst maintaining harmless. These substances are resistant towards bases as well as acids despite staying dissolved in aqueous. In particular, TiO2-NPs has a selfcritical role, making it suitable for purifying and marketable usage applications such as water, paints, food, cosmetic items, and toothpaste, as well as environmental disinfectants (Xu *et al*., 2018). These nanoparticles are also utilized to cure tumours, administer drugs, and transfer genes to cells and tissues (Bisht and Rayamajhi, 2016). The objective of this research is to evaluate the effects of titanium nanoparticles on the liver, which have received little attention. As a result, the purpose of this work was to look into how TiO2-NPs affected or produced light microscopic changes in rat liver .

Materials and Methods

Excpermental animals

A total of forty-two albino rats weighing (200-250 g) and 10-14 weeks aged, had been kept in polypropylene housings according to controlled circumstances of 25±5 C^{o} and 12/12 hrs light/dark cycles, and water for drinking and dietary supplements were provided free of charge. The excpermental rats were maintained and taken care of at AL-Nahrain/animal house in Biotechnology Research Center. All procedures for experiments complied with ethical standards that adhered with international recommendations for research on animals (Smith *et al*., 2018). The animals were handled and cared for according to the National Research Councils Guide for the Care and Use of Laboratory Animals (2021).

Animals and experimental design

In this work, forty two albino animals were utilized and divided evenly into seven different groups, encompassing the control group (6 rats) and groups for experimentation II, III, IV, V, VI, and VII (6 rats per group), every handled to the procedure over a period of 10 consecutive days as outlined below:

Group I (control group): Rats were exposed to a standard diet only.

Group II: Six rats were exposed to 50 µl of 25 nm TiO2-NPs nanoparticles for 10 days.

Group III: Six rats were exposed to 50 µl of 50 TiO2-NPs nanoparticles for 10 days.

Group IV: Six rats were exposed to 50 µl of 75 TiO2-NPs nanoparticles for 10 days.

Group V: Six rats were exposed to 100 µl of 25 TiO2-NPs nanoparticles for 10 days.

Group VI: Six rats were exposed to 100 µl of 50 TiO2-NPs nanoparticles for 10 days.

Group VII: Six rats were exposed to 100 µl of 75TiO2-NPs nanoparticles for 10 days.

Dissection of Animals

At the conclusion of the 10 day experiment, the experimental animals were sacrificed via cervical dislocation in order to prevent chemical damage. The liver quickly removed and fixed immediately for the entire night in 40 g/l formaldehyde polymer in PBS. Then, sequential 5µm hepatic slides were stained with hematoxylin and eosin for histopathological investigation.

Chemicals

Chemical company sigma-Aldrich, Egypt, supplied the nanoparticles of titanium dioxide nanoparticles (TiO2-NPs) at a weight percentage of 50% in phosphate buffered saline (PBS). Considering the data that is being provided via manufacturer TiO2-NPs nanoparticles are 38 ± 12 nm in size. Deionized water was utilized for the preparation of the biosynthesized the nanocomposite.

Titanium Dioxide Nanoparticles (TiO2-NPs) stock solution formulation

Using an ultrasonic cleaner sonicator (Branson Ultrasonic Corporation, Danbury, Connecticut, USA), The nanoparticles of TiO2-NPs (38 ± 12 nm) were dissolved with 10 mg/mL of distilled water, then sonicated for 20 minutes at room temperature at 230 V. Prior to delivery, the suspension was vortexed at different doses (25, 50, and 75 nm).

Characterization of Titanium Dioxide Nanoparticles (TiO2-NPs)

To analyze the morphology and characteristics of the particles, the specimens were dissolved in ethyl alcohol, and the resulting diluted solution was placed onto a copper metal grid. The samples were then examined using a transmission electron microscope (Field Emission Transmission Electron Microscope Zeiss Sigma 500 VP, Carl Zeiss, Germany) as descerprd by Ben-Slama *et al.* (2015). All the details of the work in this regard were carried out in Electron Microscopy Unit /Iraqi Center for Cancer Research & Medical Genetics, Baghdad, Iraq**.**

Histology changes

Liver tissues (2 m x 2 m) were swiftly removed since the separated liver and immersed in 10% neutral buffered formalin before being embedded in paraffin. Overall histopathology was carried out on 4-6 µ colored thick sections with hematoxylin and eosin (H&E) (Bancroft and Layton, 2012). Stained slices were viewed and photographed utilizing a digital camera equipped with light microscopy technique.

Results and Discussions

General observations

With the close supervision throughout the research period, all of the excpermental animals remained alive. In contrast, the control specimens consequences were constant, with no significant variations among animals. The rats in the control group remained healthy throughout the experiment, exhibiting similar and consistent responses. This consistency has helped to establish the control group as a reliable primary standard for comparing the effectivness of TiO2-NPs nanoparticle experience.

Described of TiO2-NPs nanoparticles

The nanoparticles of TiO2-NPs were initial examined for diameter and determined to be in the category of nanoscale particles, even though they produced tiny agglomerates in aqueous solution. The transmission electron microscope (TEM) highlighted the nanoparticles architecture. Morover , the TEM measured an average size of 50.40 ± 5.60 nm. The typical TEM image displayed reveals that the majority of the TiO2-NPs nanoparticles have a polyhedral form (Figure 1). Thses TEM consequences appear in parallel with the results of a previous research conducted by Ahmad *et al*., (2022).

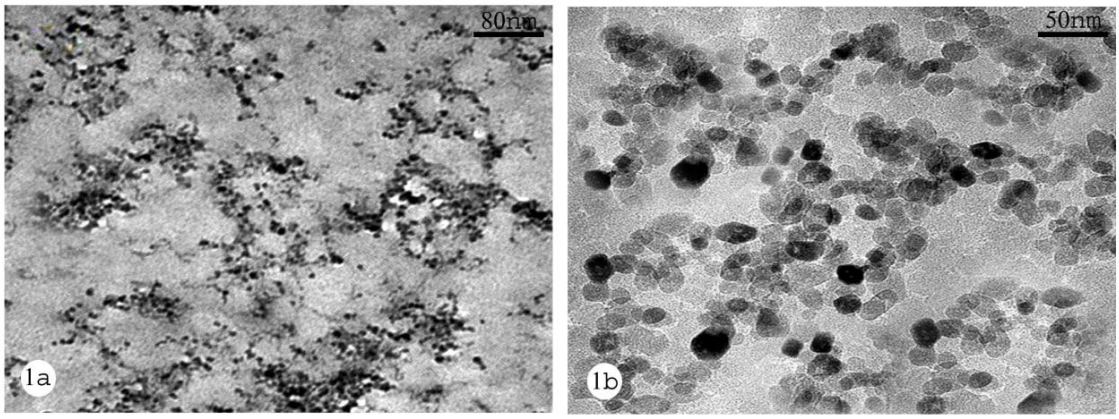


Figure 1: (a) Transmission electron microscope image of TiO2-NPs with the indication **(b)** The distribution and size of TiO2-NPs nanoparticles.

Microscopic histological examination

Throughout the experiment, all of the excpermental animals remained alive. During necropsy, no apparent gross tissue abnormalities appeared in any specimens. Furthermore, the physical appearance and behavior of rats treated with TiO2-NPs were similar to those of the control group.

Principally, microscopic examination of hepatic sections from control rats showed normal liver structure, including intact architecture, healthy hepatocytes, well-defined portal triads, and a normal central vein (Figure 2). In contrast, histological analysis of liver tissues from all experimental groups revealed considerable damage when compared to untreated rats (Figures 2-8). Hepatocyte cytoplasm displayed darkly stained, granular, pink granules alongside vacuoles. These tissues, in comparison to control samples, exhibited a dilated central vein, vesiculated irregularly shaped nuclei, and pronounced aberrations. The primary histopathological markers demonstrated a noticeable decline relative to normal hepatic sections, as follows:

In all rats treated with TiO2-NPs, hepatocytes displayed signs of cloudy swelling, characterized by a pale cytoplasm and nuclei that appeared poorly defined and displaced. This ballooning degeneration was notably more severe in the groups receiving a 100 µL dose compared to those given 50 µL, and more pronounced with 25 nm particles compared to larger ones, as illustrated in (Figure 3). Such cellular swelling is likely due to membrane dysfunction, resulting in an excessive influx of water and sodium ions, a direct consequence of TiO2-NPs exposure. Moreover, this state might be connected with the expulsion of intracellular hydrolytic proteins, which contribute to cytoplasmic degeneration and macromolecular congestion (Ghonimi *et al*., 2023).

In TiO2-NPs treated model, hepatic cells exhibited hazy expansion (edema), pale cytoplasm, and misplaced nuclei. Figure 3 shows that ballooning deterioration was noticeable with 75 µl doses compared to 50 µl doses. Enlargement /sweeling might occur due to disruptions in membrane function caused by TiO2-NPs, resulting in a large input of water and Na+. Lysosomal enzyme emission during cell expansion might cause cytoplasmic degeneration and macromolecular crowding (Ghonimi *et al*., 2022).

Adminstrated with TiO2-NPs led to an rise in the quantity of stellate sinusoidal macrophages. Figure 4 shows that rats subjected to 25 nm TiO2-NPs at a dosage of 100 µl showed a greater change compared to animales subjected to 50 nm and 75 nm TiO2-NPs afterward 10 days of treatment. TiO2-NPs may stimulate stellate sinusoidal cells, leading to increased phagocytic efffectivness in sinusoidal cells. Lysosomes break down accrued TiO2-NPs into minute

metabolic products within the cell. The activation of sinusoidal cells (Kupffer cells) may suggest that TiO2-NPs stimulate the phagocytic function of these sinusoidal cells, enhancing their role in clearing the accumulated nanoparticles. This process likely involves lysosomes facilitating the intracellular degradation of TiO2-NPs into smaller metabolic byproducts. The detected abnormal tissue expansio (hyperplasia) of sinusoidal cells could be linked to the degree of hepatic damage caused by TiO2-NPs exposure and may represent a defensive detoxification mechanism. Moreover, this epithelial hyperplasia has been accompanying with increased oxidative stress in the liver tissue (Aouey *et al*., 2023).

Localized necrotic areas were sporadically observed in certain hepatocytes of rats treated with TiO2-NPs, as illustrated in (Figure 5). Affected cells displayed intensely eosinophilic and amorphous cytoplasm, occasionally showing signs of apoptosis. This type of damage was predominantly identified in rats exposed to 25 nm TiO2-NPs and, to a lesser extent, in those exposed to 50 nm particles, while no such changes were noted in rats exposed to 75 nm particles. Apoptotic changes were likely accompanied by the swelling of cellular organelles, particularly mitochondria and the endoplasmic reticulum, along with lysosomal rupture, which could result in an eosinophilic cytoplasm a precursor in the progression of hepatocyte necrosis, prior to nuclear shrinkage and dissolution (Hamed *et al*., 2021). The observed necrosis in hepatocytes following TiO2-NPs exposure may reflect oxidative stress caused by the depletion of glutathione within these cells.

Inflammatory cell infiltration was observed within the portal triads and surrounding periportal areas in rats treated with TiO2-NPs. These infiltrates predominantly consisted of lymphocytes and plasma cells, as depicted in (Figure 6). The infiltration became more pronounced after 10 days of exposure and was more evident in rats administered 100 µL compared to those given 75 µL. The presence of these inflammatory cells in hepatic tissue suggests that TiO2-NPs may interact with interstitial proteins and enzymes, disrupting the antioxidant defense system and inducing the production of reactive oxygen species (ROS). This, in turn, could trigger an inflammatory response (Huang *et al.*, 2021).

TiO2-NPs displayed strong oxidative properties, as indicated by lipid peroxidation, reduced neutral red retention time (NRRT), and diminished thiol-containing proteins visible in electrophoretic analysis. Additionally, cadmium ions may replace iron or copper in metalloproteins, enhancing oxidative stress through the fenton reaction.

Neumreous investigations has illustrated that TiO2-NPs which have lesser measurments, for instanse those measuring five micons, induce dramatically higher levels of oxidative stress and mortality in compareison with their bigger variations (Hone and Tabei, 2021). Those tiny molecules were recently proved to promote the creation of nitric oxide (NO) from endogenous S-nitroso consituents in the bloodstream, whereby not immmedialty commmunicates on superoxide to create peroxynitrite (ONOO-). Reactive oxygen species (ROS) possese the tendency to damage fatty acids, DNA moleculles, besides peptides mollecules via either direct oxidative reactions or throught the facilciation of radical-driven routes. The advanced incidence of ROS observed this report could have been attributed to the substantail area of the surface of the TiO2-NPs nanoparticles utilized, as distinguished via Ziental *et al*. (2020).

Fatty changes were detected in some swollen hepatocytes of rats exposed to 100 µL of 10 nm TiO2-NPs, with a reduced prevalence in those treated with larger particles (Figure 6). The accumulation of lipids in hepatocytes may result from lipid peroxidation, which damages the rough endoplasmic reticulum and disrupts cytoplasmic lipoproteins, ultimately reflecting impaired lipid metabolism (Chen *et al*., 2018). The abnormal lipid retention observed in hepatocytes during this study suggests that TiO2-NPs may induce toxic damage to the liver, manifesting as hepatocyte liposis caused by these nanoparticles.

The hepatic central veins in rats treated with 25 nm and 50 nm TiO2-NPs exhibited signs of

intimal disruption, as illustrated in (Figure 7). In contrast, rats exposed to 75 nm TiO2-NPs displayed less pronounced disruptions. This observation may suggest that TiO2-NPs induce endothelial injury and vascular stress. None of these changes were detected in the liver tissues of the control group. Additionally, some hepatocytes in rats administered 75 nm TiO2-NPs exhibited a loss of nucleoli.

Karyorrhexis refers to the destructive fragmentation of the nucleus, which follows the process of pyknosis and precedes karyolysis, the complete dissolution of chromatin in a dying cell. In the present study, TiO2-NPs-treated rats exhibited both binucleation and, to a lesser extent, polynucleation. Binucleation, indicative of cell injury, is a form of chromosomal hyperplasia commonly associated with regenerating cells. The rat exposed to TiO2-NPs, receiving 100 µl of 75 nm particles over a 10 day period, displayed a markedly expanded and engorged central vein, along with distended and congested hepatic sinusoids. Additionally, hepatocytes showed prominent cloudy swelling (Figure 8).

This investigation highlights that liver and other tissue inflammation were more pronounced when exposed to smaller-sized TiO2-NPs, with their effects being dose-dependent and related to the duration of exposure. Additional studies are warranted to evaluate plasma and tissue cytokines, alongside histomorphological and ultrastructural analyses, to better understand the toxicity and potential applications of TiO2-NPs as diagnostic and therapeutic agents. Evidence suggests that smaller TiO2-NPs exert significantly greater oxidative stress and cytotoxicity compared to their larger counterparts (Ahmad *et al*., 2018).

Although the current study did not measure TiO2-NPs concentrations in urine or feces, future experiments will address this limitation. Previous research by Ibrahim *et al*. (2018) revealed that nanoparticles are primarily absorbed by the liver and spleen in substantial quantities, with smaller amounts distributed across the lungs, kidneys, heart, and brain following a single administration.

These histological alterations were observed in figures: 2,3,4,5,6, and 8 as below:

Figure 2 (control group)**:** Normal rats exhibited typical liver structures, including intact liver architecture, normal hepatocytes, a normal portal triad, and a well-formed central vein.

Figure 3: An experimental rat treated with TiO2-NPs, received 50 µl of 25 nm particles for 10 days, exhibited hepatocytes cloudy swelling.

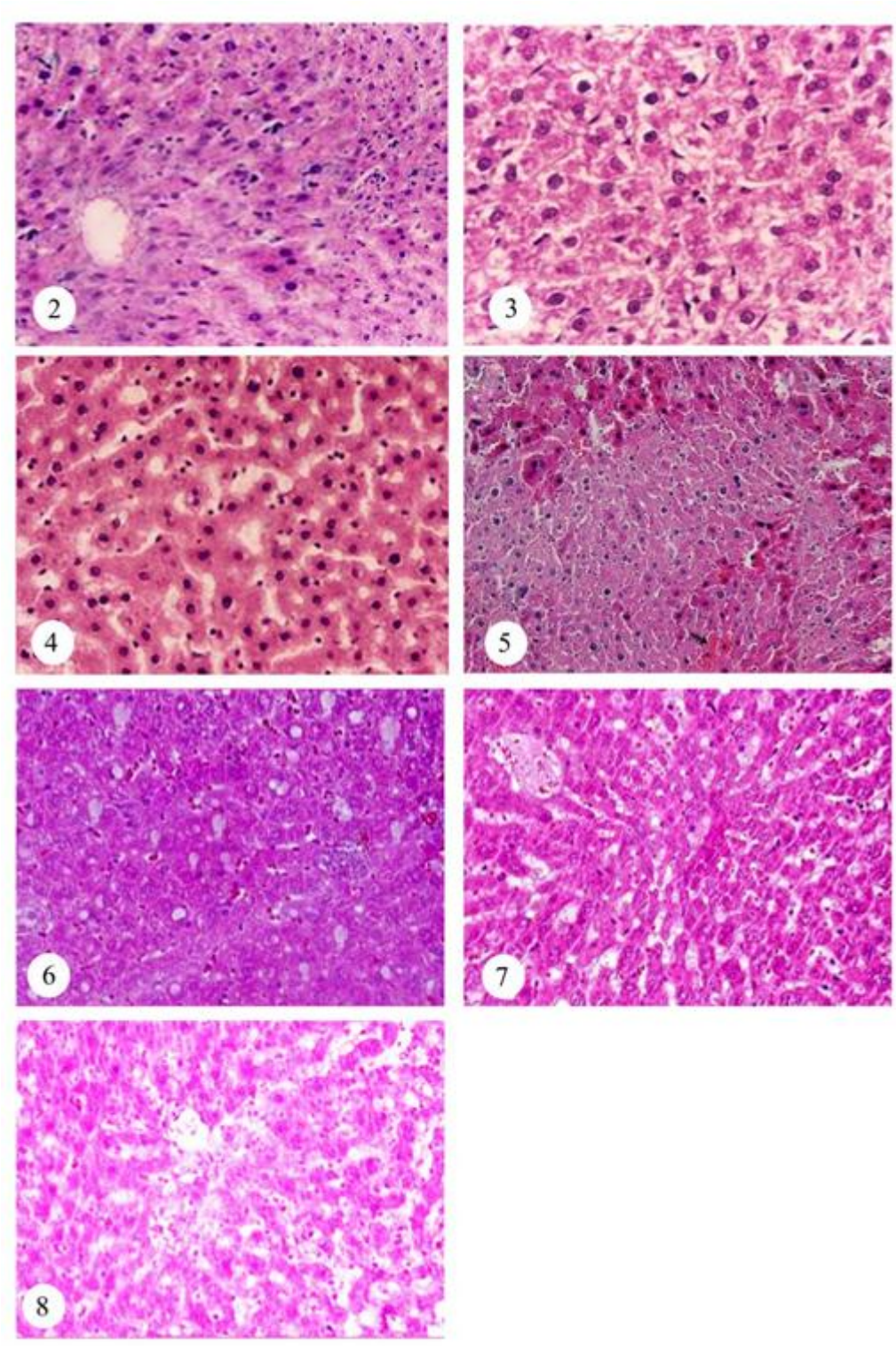
Figure 4: An experimental rat treated with TiO2-NPs, received 50 µl of 50 nm particles over a period of 10 days, exhibited hyperplasia of Kupffer cells.

Figure 5: An experimental rat treated with TiO2-NPs, received 50 µl of 75 nm particles over 10 days, exhibited hepatocyte necrosis.

Figure 6: An experimental rat treated with TiO2-NPs, received 100 µl of 25 nm particles for 10 days, exhibited infiltration of inflammatory cells.

Figure 7: An experimental rat treated with TiO2-NPs, received 100 µl of 50 nmparticles for 10 days, exhibited liver fat accumulation.

Figure 8: An experimental rat treated with TiO2-NPs, received 100 µl of 75 nm particles for 10 days, exhibited severely dilated and congested central vein accompanied by expanded, congested liver sinusoids, alongside hepatocytes exhibiting cloudy swelling.



Figures (2 to 8): Microscopic alterations observed following the administration of ZnO-NPs in various groups, at different concentrations, and over specified durations during the experiment

Table 1 : Summay the key events and changes observed in liver tissue following 10-day exposure to TiO2-NPs

Category	Particle size (nm)	Dose (µL)	Main observations	Key histological findings	Cytotoxic effects	Oxidative stress indicators	Inflammatory response	Fatty changes

General observations	N/A	N/A	No observable changes in behavior or appearance.	Normal liver architecture with intact hepatocytes, central vein, portal triads	No toxicity	No oxidative stress	No inflammatory response	No changes
Hepatocyte changes	25 nm, 50 nm, 75 nm	100 µL	Cytoplasmic swelling and vacuolization in hepatocytes. Swelling more severe in smaller particles and higher doses.	Ballooning degeneration, dilated central vein, vesiculated nuclei	Cloudy swelling, ballooning degeneration	Lipid peroxidation, NRRT reduction, thiol protein decrease	Infiltration by lymphocytes and plasma cells	Lipid retention, especially in 10 nm particle group
Kupffer cell activation	25 nm	100 µL	Increased Kupffer cells prominence and number, particularly in 25 nm group.	Increased Kupffer cells around sinusoidal areas	Kupffer cell activation, enhanced phagocytic activity	Enhanced ROS production from phagocytosis and oxidative stress	Chronic inflammation with increased ROS levels	No significant fatty change detected
Necrosis and apoptosis	25 nm, 50 nm		Necrotic areas observed, especially with 25 nm particles. Apoptosis signs present.	Necrotic cells with eosinophilic cytoplasm, nuclear fragmentation	Apoptosis followed by necrosis in hepatocytes	Depletion of glutathione, increased ROS leading to mitochondrial and lysosomal damage	Inflammatory response surrounding necrotic areas	No significant changes in lipid accumulation
Liver inflammatory response	25 nm, 50 nm, 75 nm	100 µL	Infiltration of lymphocytes and plasma cells around portal	Inflammatory infiltration in periportal regions	Cytokine production due to ROS accumulation	ROS production triggering inflammatory responses	Chronic inflammation with fibrosis risk	No significant lipid accumulation observed

			triads, more prominent at higher doses.					
Oxidative stress and cytotoxicity	5 nm, 10 nm, 25 nm		Smaller particles caused greater oxidative damage, especially 5 nm particles.	Greater cytoplasmic swelling in hepatocytes with smaller particles	Higher toxicity observed in 5 nm particles with increased cellular damage	Significant oxidative stress, enhanced ROS and NO production	Cytotoxicity increased with smaller particles	Significant fatty changes in hepatocytes during inflammation
Vascular injury and endothelial damage	25 nm, 50 nm	100 µL	Significant damage to the central vein and intima of liver vasculature. More pronounced in smaller particles.	Endothelial damage, disrupted central vein architecture		ROS production leading to endothelial cell injury	Mild vascular stress observed	No significant changes in fatty changes

Conclusions

The histological changes observed in this study as a result of TiO2-NPs exposure suggest significant damage to hepatocytes, likely due to the toxic effects of these nanoparticles. The cells appeared unable to manage the accumulated byproducts of metabolic and structural disruptions triggered by the particles. These changes appear to be size-dependent, with smaller nanoparticles causing more severe damage, particularly with prolonged exposure.

The presence of inflammatory cell infiltration in hepatocyte cytoplasm, disruption of the central veins intima, fatty degeneration, and Kupffer cell hyperplasia indicates that TiO2-NPs may interact with hepatic proteins and enzymes. This interaction likely disrupts the antioxidant defense system, leading to the production of reactive oxygen species (ROS), which impose oxidative stress on hepatocytes and contribute to necrotic processes.

Further in-depth experimental research, including histomorphological and ultrastructural analyses, is essential to gain a comprehensive understanding of the toxicity mechanisms and explore the potential of TiO2-NPs in therapeutic and diagnostic applications.

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