



Research Paper

Histopathological Effects of Titanium Dioxide Nanoparticles on the Liver in Rats by Light and Transmission Electron Microscopy

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ABSTRACT

Background: As one of the most influential and in-demand nanoparticles for commercial applications, titanium dioxide nanoparticles (TiO₂NPs) are commonly used in industry as a white pigment, in cosmetic and health products, and as a disinfection agent. In food and drugs, this additive is known as E171 and helps define colors clearly and can prevent UV degradation. This study used light and transmission electron microscopy to examine the effects of TiO₂NPs on the liver in rats.

Methods: Titanium dioxide nanoparticles were administered to 40 rats at 0, 10, 20, and 50 mg/kg of rat body weight suspended in one milliliter of distilled water over 60 days (every second day) by oral gavage. The rats were euthanized, and tissue samples were dissected from the liver for light (thick section) and transmission electron (thin section) microscopy study.

Results: Histopathological examinations in treated groups revealed dilation of hepatic sinusoids, hepatocyte degeneration, and necrosis, observed as cellular swelling, cytoplasmic vacuolation, and loss of nuclei, nuclear pyknosis, karyorrhexis, and karyolysis. Some pathologic changes were also observed in mitochondria. All doses used in the study showed clear evidence of mitochondrial membrane and cristae enlargement, mitochondrial disintegration, and cristae destruction.

Conclusion: Our study demonstrated that the continuous use of TiO₂NPs (E171) can cause histopathological damage in the liver and disrupt the function of this organ. Consequently, it is recommended to limit the use of this ingredient in industrial, sanitary, food, and cosmetic products.

Keywords: Electron microscopy; Hepatocytes; Histology; Nanoparticles; Titanium dioxide

Introduction

Nanotechnology has given environmental engineers fresh hope for dealing with pollution by coming up with new ideas on nanomaterials [1]. However, exposure of consumers and vulnerable populations to various nanoparticles raises health concerns regarding their production and use [2]. The nanotechnology has achieved a size range of 1 to 100 nm and transitioned from micro- to nanoparticles [3]. As we reach the current size range, i.e., 1 to 100 nm, certain physical features, such as the surface-to-volume ratio, will change, thus changing the quantum effects of the materials [4]. The increase in surface-to-volume ratio enables exterior atoms to behave better than the inside ones. This behavior also influences the chemical properties of the particles [5]. Nanoparticles have a wide range of applications in various industries, particularly due to their unique optical and electrical characteristics [6, 7]. The nanoparticles of iron oxide [8, 9], titanium dioxide (TiO₂) [4], and other elements are popular examples of such materials.

Titanium dioxide nanoparticles (TiO₂NPs), the ninth most abundant element in the earth's crust, is a commonly utilized metal that may enter the food chain as a bleaching agent, especially in dairy products, cocoa, milk powders, soy products, and sausages. In foods and drugs, this additive is known as E171, helps define colors clearly, and can prevent the degradation of materials under UV light. It is estimated that about 300 mg of titanium is commonly consumed by people in their meals per day [10].

Titanium dioxide, or *titania*, a nanoparticle made of TiO₂, was first developed from titanium early in 1923, and its commercial applications continued to proliferate as a mineral material. Titanium dioxide nanoparticles are classified under three categories: rutile, anatase, and brookite. Rutile and anatase molecules have tetrahedral structures, whereas brookite has an octahedral structure. Because of their unique properties in terms of interaction with biological molecules, they have

attracted much interest among biologists and other scientific researchers [11].

Because of its structural features, TiO_2 is an excellent photocatalyst based on semiconductor oxides, which has attracted much interest among scientists of various specialties since 1972 [12]. This molecule is easily synthesized in both industries and laboratory settings because it is easily accessed and mass-produced at low cost [13]. Titanium photocatalysts are both chemically and photochemically stable and non-toxic. These materials are resistant to both acids and bases and remain soluble in water. Most notably, TiO_2 has a self-destructive function, which makes it suited for purifications and industrial applications in, for instance, water, paints, food, cosmetic products, and toothpaste, and also for environmental disinfectants [14, 15]. These nanoparticles are also used for the treatment of tumors, drug delivery, and translocating genes to cells and tissues [16].

Aim of the Study: To our knowledge, few studies have investigated the effects of titanium nanoparticles on the liver. Therefore, this study was designed to investigate the light microscopic and ultrastructural changes of rat liver and hepatocytes influenced or induced by TiO_2NPs .

Materials and Methods

In this study, 40 rats were initially used and divided into four groups. They were treated every second day by gavage with 0 (control), 10, 20, or 50 mg of titanium nanoparticles per kilogram of the body weight, suspended in one milliliter of distilled water over a period of 60 days. Only 24 rats survived at the end of the treatment period (6 rats per group). Subsequently, the rats were euthanized with

ether, and small samples of their liver (approximately 1 mm^3) were dissected and promptly fixed in glutaraldehyde for at least 1 hour at room temperature (23°C), followed by post-fixation in osmium tetroxide. Next, the liver specimens were dehydrated in graded ethanol, followed by propylene oxide, and embedded in Epon812 prior to sectioning for microscopic examinations. The blocks were trimmed, and semi-thin sections were cut using a glass knife. Ultrathin sections were made using ultramicrotome with diamond knives and placed on copper grids, impregnated with uranyl acetate and lead citrate. The thick and ultrathin liver sections were examined under light and transmission electron microscopy (Leo Co.; Freiburg, Germany). All study procedures and protocols were reviewed and approved by the Ethics Committee of the Ferdowsi University of Mashhad, Mashhad, Iran (Approval Code: IR.UM.REC.1401.089).

Results

Histopathological Findings: Microscopic examinations of the thick sections of liver samples from the groups treated with TiO_2NPs revealed dilation of hepatic sinusoids, hepatocyte degeneration, and cell necrosis. We also observed cellular swelling, cytoplasmic vacuolation, and hyperstainability of some hepatocytes, especially in periportal areas, plus loss of nuclei, nuclear pyknosis, karyorrhexis, and karyolysis. Infiltration of mononuclear inflammatory cells was observed at multiple foci in the rats' liver samples among the treated groups (Figures 1-3). No significant difference was noted by comparing the histopathological lesions among the treatment groups.

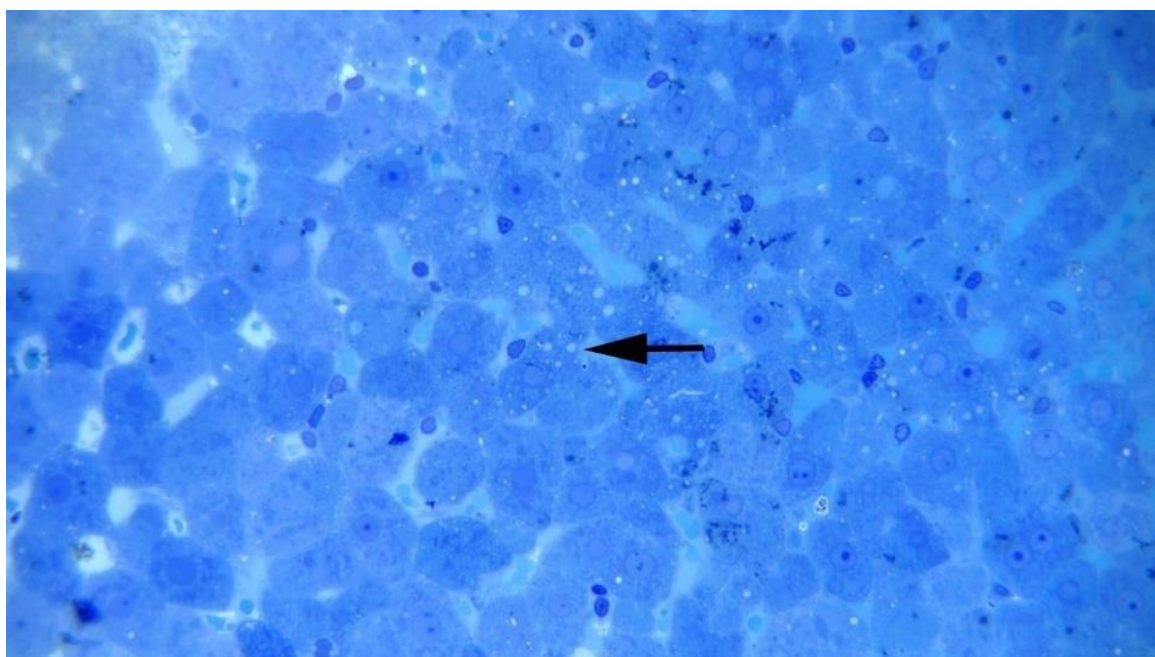


Figure 1: Hepatocellular vacuolation (arrow) and necrosis at 50 mg np, Methylene blue staining ($\times 400$).

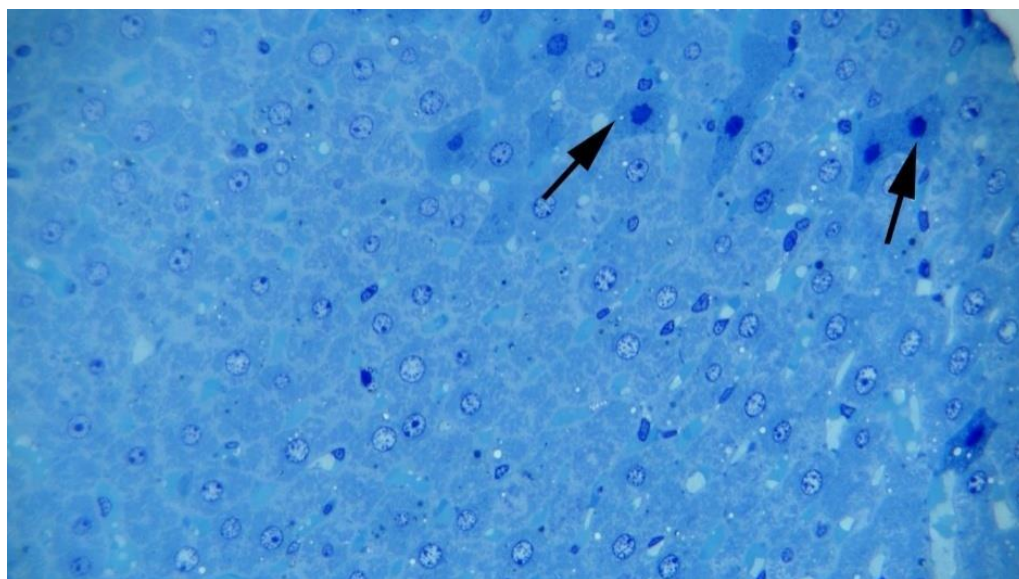


Figure 2: Hepatocellular necrosis at 10 mg np. Note cytoplasmic hyperstainability and nuclear pyknosis (arrows) of the necrotic hepatocytes, Methylene blue staining ($\times 400$).

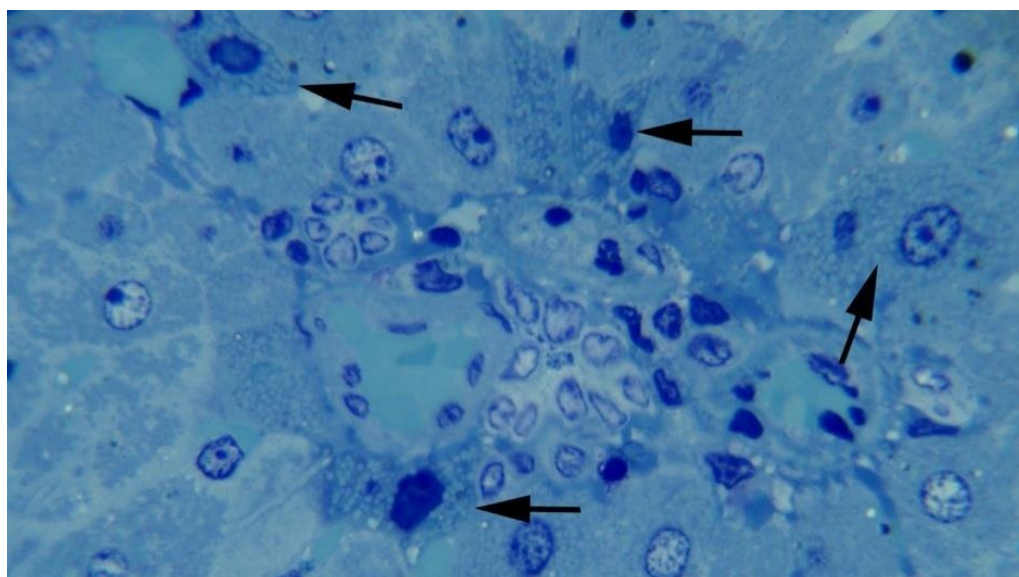


Figure 3: Hepatocellular degeneration and necrosis at 10 mg np. Note cytoplasmic vacuolation and nuclear pyknosis (arrows) of the necrotic and degenerative hepatocytes in a periportal area, Methylene blue staining ($\times 1000$).

Ultrastructural Findings: The ultrastructural sections from the control group showed intact hepatocytes with the nuclei, nucleoli, endoplasmic reticula, mitochondria, and cell membranes (Figure 4). Other findings are clearly explained in the Figures 5, 6 and 7. After that, the ultrastructural sections from the second group (10 mg/kg TiO_2NP) were examined. Contrary to the analyzer's opinion and in line with the results of previous research, hepatocellular injuries were observed at low treatment doses, i.e., 10 and 20 mg. For the first time, swollen or torn mitochondrial membranes, reduced or destroyed cristae, injury to the mitochondria, increased gaps between the two mitochondrial membranes, and high-density elements were observed. See Figure 7.

Next, the ultrastructural sections from the third group (20 mg/kg TiO_2NPs) were examined, and the following results were documented:

- Presence of points with high electron density
- Swelling or disappearance of cristae and endoplasmic reticula
- Destruction of mitochondria
- Presence of dense matrices in broken mitochondria
- Formation of continuous cavum (Micrographs 6).

Finally, the sections from the fourth nanoparticles treatment group (50 mg TiO_2NPs) were examined microscopically. Sections in this group appeared to have adapted to the increased concentration of TiO_2NPs , and to some extent, there were less harmful effects observed on the mitochondria. However, the endoplasmic reticula, the presence of electron-dense points, and the loss of membranes and cristae were evident, which confirmed the harmful effects on the mitochondria. Micrograph7 shows the microscopic findings from the fourth treatment group.

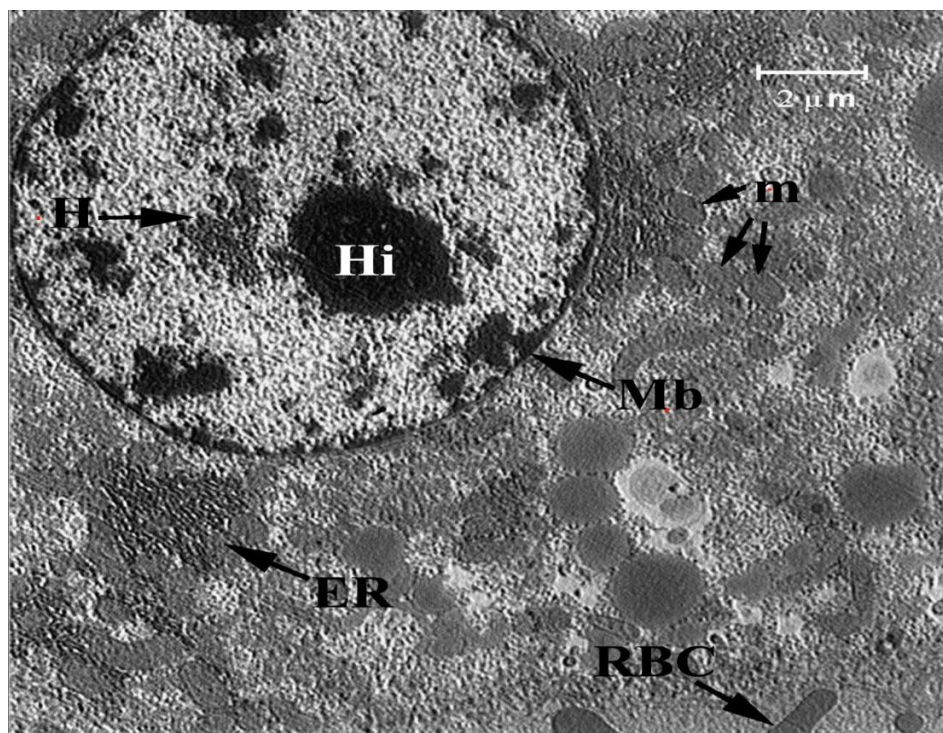


Figure 4: Ultrastructural section of liver of the control group (0 mg np): Endoplasmic Reticulum (ER), Membrane (Mb), Mitochondria (m), Heterochromatin (H), Nucleolus (Hi), Red Blood Cell (RBC).

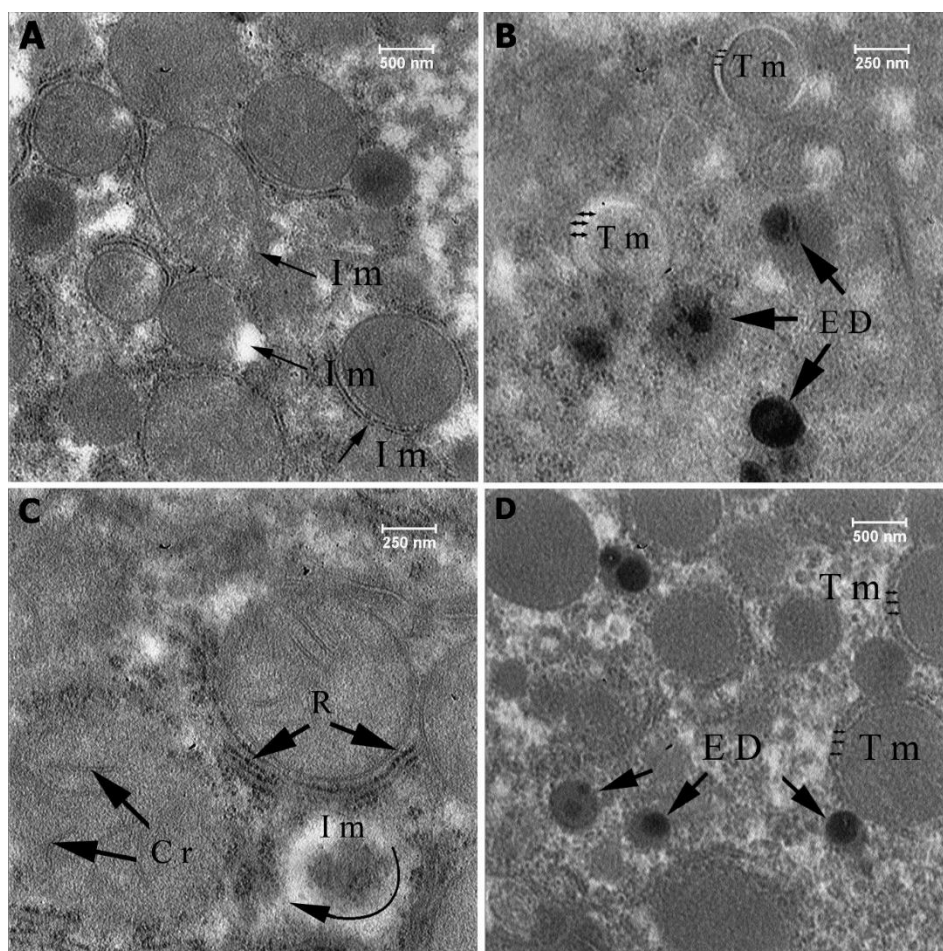


Figure 5: Ultrastructural section of liver of the first group (10 mg np), Injury to the mitochondria (Im), Torn membrane (Tm), High-density elements (ED), Cristae (Cr), Ribosome (R).

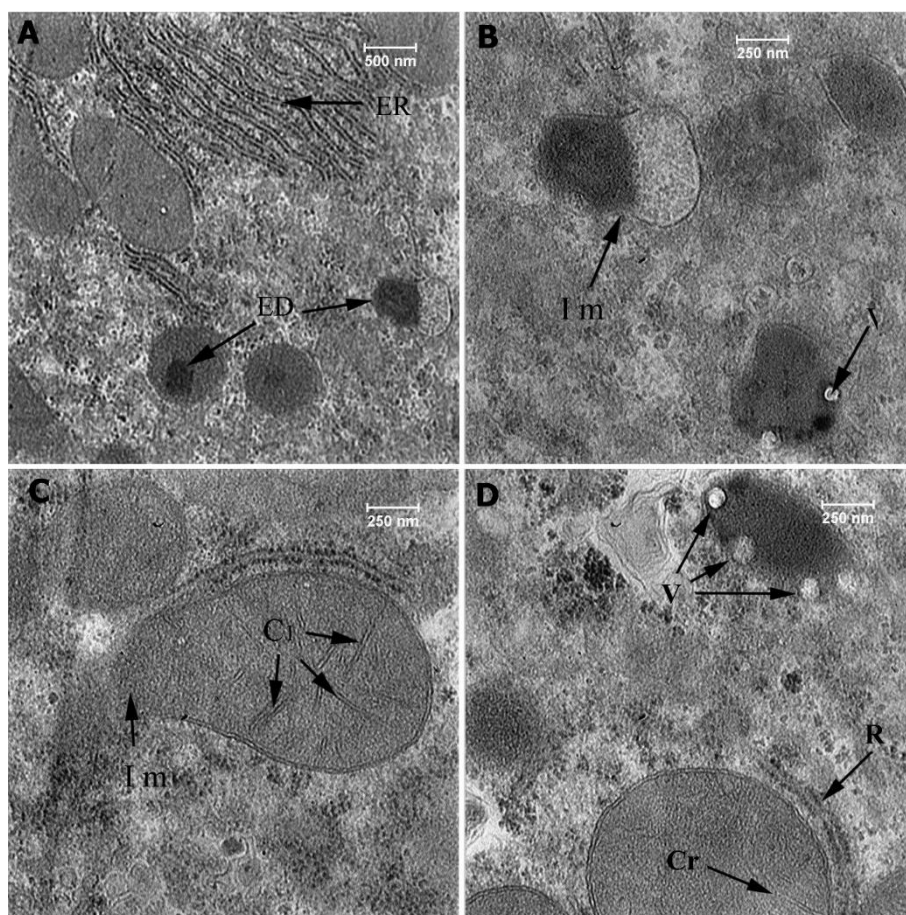


Figure 6: Ultrastructural section of liver of the second group (20 mg np), Endoplasmic Reticulum (ER), Injury to the mitochondria (Im), High-density elements (ED), Cristae (Cr), Ribosome (R), High-density elements matrix (EDM), Vacuole (V).

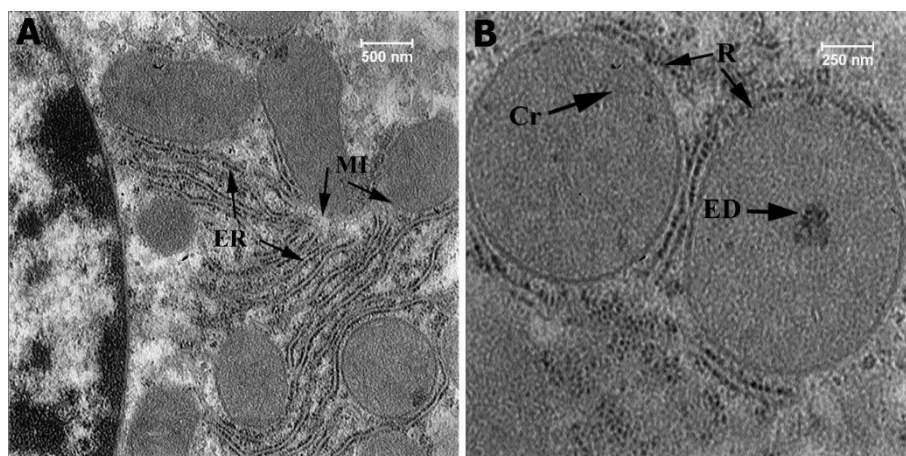


Figure 7: Ultrastructural section of liver of the fourth group (50 mg np), High-density elements (ED), Cristae (Cr), Ribosome (R), Membrane (Mb), Endoplasmic Reticulum (ER).

Discussion

The current study investigated the toxic effects of TiO₂NPs since this compound has a wide application in various industries. We compared our study findings with those of other studies on hepatocytes from adult rat liver tissue. Given the vital role of the liver as the most important purifying organ of the body, the effect of nanoparticles, such as TiO₂, on this organ is equally of great importance.

To achieve the study's goal of investigating the effect of TiO₂NPs on the liver cells, we used rats as a laboratory

model similar to humans, allowing for the potential generalization of results to human health. In this study, we used TiO₂NPs because both humans and animals are at risk of exposure to this compound. This methodology allows for the measurement of the amount of nanoparticles ingested and enables an assessment of their effects. Based on the Food and Drug Administration standards, which permit this compound to be used at 1% in pharmaceutical and food products, this study investigated chronic nanoparticle use similarly to that of actual daily human consumption.

Thus, we administered the nanoparticle at four doses of 0, 10, 20, and 50 mg/kg of the rat's body weight. To demonstrate the chronic form of these nanoparticles, the treatment was given every second day over 60 days.

Based on the current study findings, TiO₂NPs affected the mitochondria of rat hepatocytes and damaged these organelles that have a vital role in cellular respiration. We demonstrated that mitochondria were destroyed due to cristae swelling and membrane rupture, formation of vacuoles, and electron-dense spots after exposure to TiO₂NPs.

Noori, *et al.* examined tissue injuries in mice liver after intraperitoneal injection of nanoparticles, and their results were in line with those of the current research [17]. However, the findings reported by Yahyaei and Abbasi differed from those of the present study and those of Noori *et al.*, as they did not observe tissue damage caused by nanoparticles in their study [17]. Jalili *et al.* had results similar to those of Yahyaei and Abbasi. They did not detect any differences, implying that their findings contradicted those of the current study [17, 18]. Giselle Domingo, *et al.* discovered that only 5 nm particles reduced the activity of superoxide dismutase and catalase in the liver. They observed no changes in the oxidative metabolism of the kidneys in any of the animal groups exposed to TiO₂NPs [19]. Based on our findings, nanoparticles have a greater effect on liver tissue than on kidneys. These are similar to the results of Shaltout, *et al.*, who demonstrated that nanoparticles affected the kidneys and intestines in addition to the liver. The findings reported by these two studies are consistent with those of the current study [20].

Sarikhani, *et al.* discovered that TiO₂NPs harm cell growth since they damage the cellular DNA, causing them to stop multiplying, restarting the process, and promoting aging. As a result, nanoparticles can influence cellular DNA in addition to the mitochondria [21]. The review articles by Baranowska-Wójcik, *et al.* and Wang, *et al.* have confirmed that nanoparticles negatively affect the gastrointestinal tract [22, 23]. The liver, as a vital gland in the digestive system, is no exception to this rule. However, damages have been caused in the epithelia of the small intestinal villi, and increases in the cellular vacuoles have been observed in the villi [24]. As suggested by Baykose, *et al.* [24], nanoparticles lower the overall energy of the liver, which can lead to liver damage. Thus, the results of the latter study are consistent with those of the current research [24].

Studies conducted on other organs have shown that nanoparticles have no destructive effect on the heart and lungs [24]. However, Dianová, *et al.* have found that nanoparticles can reduce sperm motility and cause histopathological alterations in testicular epithelia together with infiltration of inflammatory cells in the epididymis [24]. The female reproductive system is also affected by nanoparticles, as they cause polycystic ovary syndrome and follicular atresia [24]. These findings suggest that the

effects of nanoparticles on the heart and lungs may vary depending on the applied dosage. Further, the findings of Dianová, *et al.* have indicated that genital organs are susceptible to nanoparticles [24]. Fatemi and Noori have also demonstrated that exposure to silver nanoparticles during breastfeeding might have toxic effects on the infant's liver [25]. Based on their findings, the exposure of mothers to nanoparticles can pass harmful effects to their babies through breastfeeding [25].

In studies conducted on fish and rabbits, it has been suggested that the destructive effects of nanoparticles on all organisms are very high [26]. Moreover, the data from earlier studies on serum samples and liver enzymes have shown a rise in the number of white blood cells and liver enzymes, indicative of damage to the liver tissue. These findings [27-33] are also consistent with those obtained by the current study. Other studies have emphasized that

pre-exposure to TiO₂NPs at doses of up to 200 mg/kg can potentially promote the development of syndromes similar to alcoholic liver diseases.

Lastly, this study provides new insights into the evaluation of the safety of TiO₂NPs [34]. However, treatments with silymarin and nano silymarin [34], melatonin [35], and *Lactobacillus rhamnosus* GG are capable of mitigating the effects of nanoparticles [36]. Based on the findings of the current study, it can be concluded that long-term exposure to titanium nanoparticles can have harmful effects on human and animal health.

Conclusions

This study has demonstrated that the continuous use of TiO₂NPs can cause histopathological damage to the liver and disrupt the function of this vital organ.

Microscopic examinations of the liver samples from the groups treated with TiO₂NPs revealed dilation of hepatic sinusoids, hepatocytes degeneration, cell necrosis, cellular swelling, cytoplasmic vacuolation, and hyper stainability of some hepatocytes. The ultrastructural sections from the third group (20 mg/kg TiO₂NPs) were examined, and the following results were documented: Presence of points with high electron density, Swelling or disappearance of cristae, and endoplasmic reticula, Destruction of mitochondria, Presence of dense matrices in broken mitochondria, Formation of continuous cavum. Consequently, it is recommended the use of this ingredient be limited and controlled in industrial, sanitary, and cosmetic products.

Conflict of Interests

The authors have declared that there is no conflict of interest.

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Compliance with Ethical Guidelines

All procedures and protocols for this study were reviewed and approved by the Ethics Committee of Ferdowsi University, Mashhad. Mashhad, Iran (Approval code: IR.UM.REC.1401.089).

Authors' Contributions

Ahmadreza Raji played a pivotal role in the conceptualization, laying the foundation for the study. The methodology was a collective endeavor with fairly equal contributions from all authors. The formal data analysis and investigation phase also saw the active involvement of all authors, demonstrating a shared commitment to thorough examination and robust results. Rozbeh Choobchian took the lead in writing the original draft, bringing coherence and clarity to the research findings. The writing process, encompassing both the original draft and the subsequent review and editing, engaged the entire authorship, attesting to the collaborative nature of this scholarly work. Lastly, Ahmadreza Raji provided supervision, offering guidance and expertise throughout this research. All authors reviewed and approved the final version of the manuscript for publication in this journal.

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