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Histopathological effects of titanium dioxide nanoparticles on the liver of Japanese quail *Coturnix coturnix japonica*

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Abstract

Titanium dioxide nanoparticles have multiple beneficial uses, as they are used in many medical, industrial, economic, and other fields. Despite these many benefits, it is not without harm to humans and animals if used without control. Therefore, the present study aimed to discover the histopathological effects of Titanium dioxide nanoparticles on the liver of Japanese quail, *Coturnix coturnix japonica*. The study included three groups, the first group, the control group, which were dosed with distilled water for four continuous days, and the second and third experimental groups, which were dosed with Titanium dioxide nanoparticles at a concentration of 20 and 40 mg/kg, respectively. Four, fourteen, thirty and sixty days after the experiment began, the birds were sacrificed. The results showed the emergence of many histological lesions in the liver of birds of the two experimental groups, to varying degrees, in the four periods, among the most prominent tissue lesions that appeared in the second experimental group, the emergence of necrosis, hemorrhage, vacuolation, congestion, ballooning swelling, in addition to infiltration of inflammatory cells. While in the third experimental group, histopathological lesions appeared similar to second group, in addition to sinuses dilatation, Kupffer cells hypertrophy, hepatocyte enlargement, and necrosis of the walls of blood vessels and bile ducts. The study concluded that direct exposure to Titanium dioxide nanoparticles leads to damage to the liver tissue of these birds, which may affect its function and thus endanger its life.

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Introduction

Titanium dioxide (TiO_2) is a naturally occurring mineral oxide (1). Titanium dioxide nanoparticles (TiO_2NPs) possess interesting photocatalytic properties, such as the ability to mediate the photolysis of pharmaceuticals, inactivating bacteria, and the effect of photo-oxidative killing on cancer cells, energy storage, in addition to air and water purification, and other uses (2). TiO_2NPs are increasingly being manufactured in large quantities for industrial applications such as cosmetics, dyes, foods, and photocatalysts (3). They are used in water, sewage, and gas combustion treatment as an anti-bacterial material for decontamination and a cofactor in organic synthesis. TIO_2NPs are also used in pharmacy, especially in pharmaceutical chemistry and technology, and medicine, including growth areas related to dentistry and surgery (2). Humans and other animals may be exposed to TiO_2NPs in different ways despite their multiple benefits, and TiO_2NPs are found in different internal organs. *In vivo* tests revealed that TiO_2NPs accumulate in the lungs, gastrointestinal tract, liver, heart, spleen, kidneys, and myocardium after inhalation or oral exposure. In addition, they altered glucose and lipid balance in mice and rats (4). Some long-term studies in mice using TiO_2NPs have shown that they cause significant liver injury by modifying the mRNA and

protein expressions of several inflammatory cytokines, in addition to pathological alteration, which causes apoptosis of liver cells and impaired liver function (5). Japanese quail, *Coturnix coturnix japonica*, has enormous potential and can be an alternative to raising chickens, especially in providing profitable employment opportunities worldwide because Japanese quail is suitable for commercial breeding for the production of eggs and meat (6). Breeding quail is five times better than raising chickens and turkeys (7). The Japanese quail is a popular model for animals in many research areas as its short growth period makes it a suitable model for biological studies (8). It is also an ideal model for evolutionary biology studies, and its physiology is similar to humans, so the adult quail is useful for studies of aging and disease (9).

Based on this introduction, this study came to identify the histopathological effects of acute exposure to TiO_2NPs on the liver of Japanese quail birds.

Materials and methods

Study birds

The present study was conducted on 36 Japanese quail birds from both sexes. They obtained from the Department of Animal Production, College of Agriculture and Forestry, University of Mosul. The birds were 4 weeks old, weighing between 150-160 gm, and were phenotypically healthy. The birds were transferred to the animal's house, Department of Biology, College of Education for Pure Sciences, University of Mosul. They were placed in a cage divided into five rooms. The cage floors were covered with sawdust, constant care, and disinfection, as well as standard laboratory conditions in terms of lighting, ventilation, temperature, and continuous feeding.

Choose the doses of the study

The TiO_2NPs were obtained from Sigma-Aldrich Company/ China as they were in white powder. The particles measured less than 25 nanometers. The dose concentrations were selected in light of the Median Lethal dose 50 experiment (LD_{50}) (10). Concentrations of 20 and 40 mg/ kg of body weight were used in this study. The doses were prepared by dissolving the both specified concentrations with 0.5 ml of distilled water, the oral route using the gavage needle to ensure that the material reaches the stomach without harm.

Experimental design

The birds were divided equally into three groups evenly. The first group, a control group, dosed 0.5 ml of distilled water for four consecutive days (11). The first experimental group was dosed with 20 mg/kg of TiO_2NPs for the same period. The third group is the second experimental group, which was dosed with 40 mg/kg of TiO_2Nps for the same period. Three birds were sacrificed from each group after the passage of 4, 14, 30, and 60 days from the start of the experiment (11).

Histological sections preparation

The birds were sacrificed after being anesthetized at the specified intervals with Chloroform. The liver was removed from the birds of each group to study the effect of TiO_2NPs on the it. The liver tissue sections were prepared by passing them in a series of steps based on Abdullah *et al.* (12). The histological sections were stained with several stains. They are Delafield's Haematoxylin and Eosin stain (H&E) (13), Masson's trichrome stain (M) (14), Alcian blue and Periodic Acid - Schiff (ABandPAS) (13), Alcian blue pH 2.5 (AB) (14), Toludine blue (TB) (14). The histological sections were examined and photographed with a combined optical microscope (B-150 OPTIKA, Italy) using a digital camera (MDCE-5A, Japan).

Results

The histological examination of the liver in the control group shows that the normal structure of liver tissue in quail is composed of large-sized polygonal hepatocytes with globular-shaped, central-site nuclei containing one or two nuclei that appear clearly. The hepatocytes are arranged in cords, which are arranged in circles around the central vein. The hepatocytes, along with the central vein, form the liver lobules. The hepatic cords are separated by a space known as blood sinuses that lines with special cells known as Kupffer cells. Each branch of the portal vein, the branch of the hepatic artery, and a branch of the bile ducts and the lymphatic vessels collect and are submerged by loose connective tissue to form the so-called portal area (Figure 1 and 2).

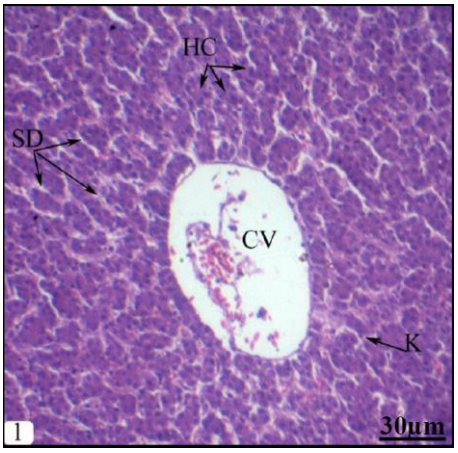

Figure 1: Microphotograph of liver showing the normal histological structure of the liver in Japanese quail of control group- H&E stain.

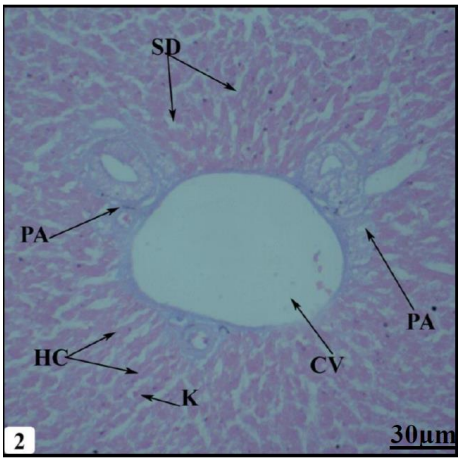

Figure 2: Microphotograph of liver showing the normal histological structure of the liver in Japanese quail of control group- AB stain.

Liver lesions of quail birds exposed to 20 mg/kg TiO_2NPs

The microscopic examination of the histological sections of the liver of quail birds exposed to a concentration of 20 mg/kg of TiO_2NPs for four days showed the irregularity of the hepatic cords, and the boundaries between hepatocytes are not clear in many cells. Thickening of the walls of some hepatocytes appeared. There was also a dissociation within and between hepatocytes, and apoptosis appeared in some cells and necrosis. A clear dilatation of the sinuses was observed, and hypertrophy of the Kupffer cells. A strong infiltration of inflammatory cells, which formed a spherical structure mediating the hepatocytes, was also observed. The appearance of inter-hepatocyte hemorrhage and fibrosis and hyperplasia of the fibroblasts (Figures 3 and 4).

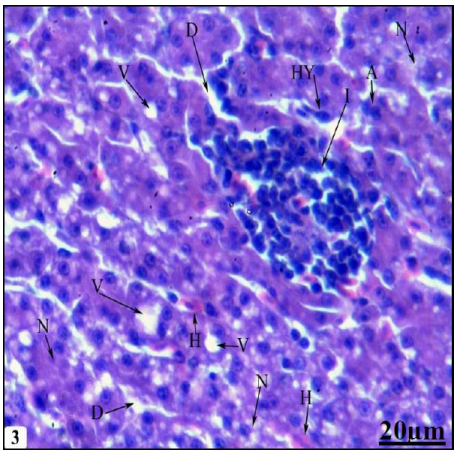

Figure 3: Microphotograph of liver showing the histological structure of the liver after 4 days from exposure to TiO2NPs with concentration 20mg/kg - H&E stain.

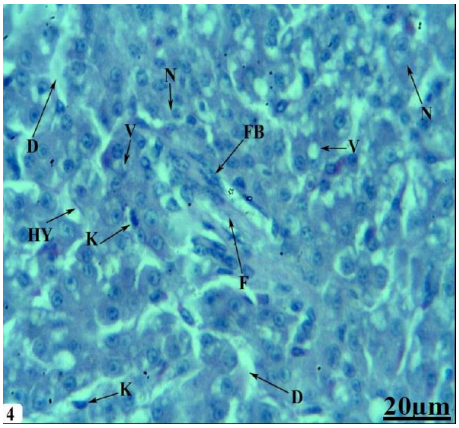

Figure 4: Microphotograph of liver showing the histological structure of the liver after 4 days from exposure to TiO2NPs with concentration 20mg/kg -AB-PAS stain. Abbreviation: (CV) central vein; (HC) hepatocytes; (SD) blood sinusoid; (K) Kupffer's cells; (PA) Portal area (A) Apoptosis; (V) Vacuolation; (FB) Fibroblasts; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (HY) Hypertrophy; (I) Infiltration.

After 14 days of experiment with TiO_2NPs at a concentration 20 mg/kg, hepatocytes in liver of experimented quail appeared to agglomerate with no clear cellular boundaries between them. The nuclei of most hepatocytes condensed. There was the presence of hemorrhage between the hepatocytes and vacuolation and the appearance of ballooning swelling in some cells. There was also an increased acidity in some other cells. Compared to the previous period, the sinuses appeared more dilatation, and the lining of Kupffer cells is clear. Severe congestion in the central vein was observed in some areas and mild congestion in others. There was hypertrophy of the wall of the congested blood vessel and the occurrence of necrosis in some of its cells. At the same time, the central vein wall appeared to thin in some blood vessels, as well as congestion in some sinuses, as revealed by the AB-PAS stain. Also, hemorrhage appeared in this wall in some areas of the liver. Fibrosis of the central vein wall and infiltration of inflammatory cells were found near this vein. There was the emergence of some carbohydrate substances (Figures 5 and 6).

After a month of treatment with TiO_2NPs, severe congestion appeared in most blood vessels, with severe infiltration of inflammatory cells around the blood vessels, as well as large fibrosis that extended between the blood vessels, and necrosis of the vascular wall, and necrosis of the bile duct wall, was observed. There was also an irregularity in the arrangement of the hepatic cords, necrosis, vacuolation, and hemorrhage between the hepatocytes. Extensive dilatation sinuses appeared in most

regions, with fibrous deposits in some of these sinuses (Figures 7 and 8).

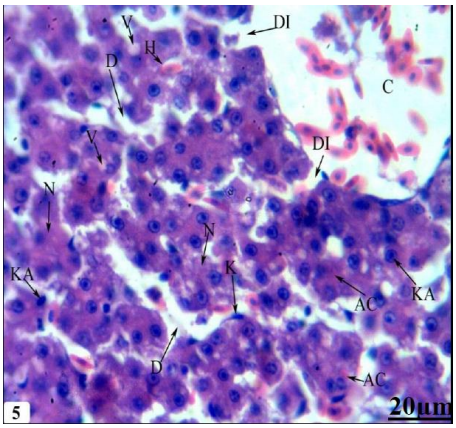

Figure 5: Microphotograph of liver showing the histological structure of the liver after 14 day from exposure to TiO_2NPs with concentration 20mg/kg - H&E stain.

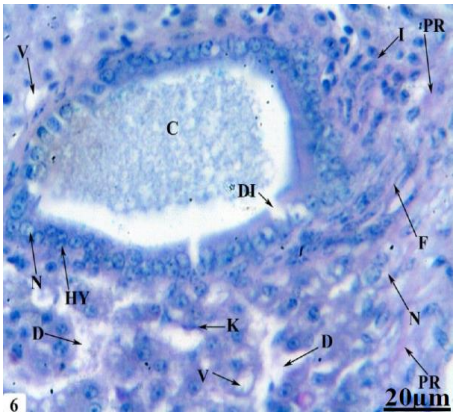

Figure 6: Microphotograph of liver showing the histological structure of the liver after 14 day from exposure to TiO_2NPs with concentration 20mg/kg -AB-PAS stain. Abbreviation: (DI) Dilaceration; (KA) Karyopyknosis; (C) Congestion; (AC) Acidity; (PR) Positive response; (K) Kupffer's cells; (V) Vacuolation; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (HY) Hypertrophy; (I) Infiltration.

After two months of treatment with TiO_2NPs, extensive necrosis appeared among the hepatocytes and extensive vacuolation and apoptosis between these cells, and plasma membranes did not appear between these cells. The hepatocytes appeared in clusters covered by sinuses, as an increase in acidity of some cells was observed and their nuclei condensed. The sinuses appeared greatly dilated, and severe hemorrhage appeared between the hepatocytes and within the sinuses, as was hypertrophy of the Kupffer cells. As for the blood vessels, it showed severe congestion in it, as well as hemorrhage in some of its walls, and the emergence of clear fibrosis around the components of the hepatic area, necrosis in the wall of the bile ducts, and a positive response to TB staining. In addition to the emergence of infiltration of inflammatory cells around the blood vessels (Figures 9 and 10).

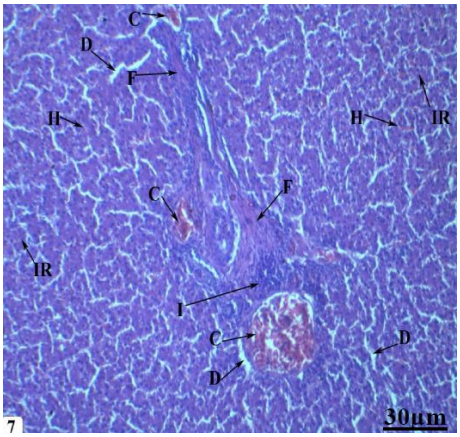

Figure 7: Microphotograph of liver showing the histological structure of the liver after 30 day from exposure to TiO_2NPs with concentration 20mg/kg - H&E stain.

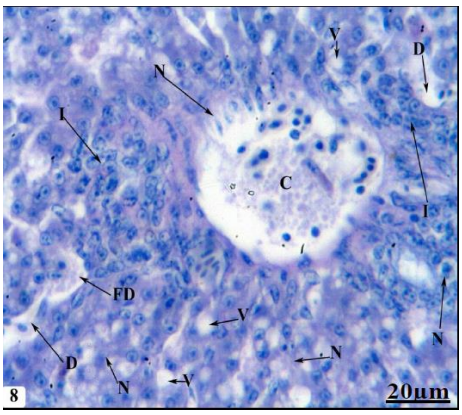

Figure 8: Microphotograph of liver showing the histological structure of the liver after 30 day from exposure to TiO_2NPs with concentration 20mg/kg - AB-PAS stain.

Liver lesions in quail birds exposed to 40 mg/kg TiO_2NPs

The histological examination of the quail's liver exposed to TiO_2NPs at a concentration of 40 mg/kg after four days showed dilatation of the sinuses in some areas of the liver. At the same time, in others, it is very narrow and almost non-existent. The Kupffer cells in this region appear to be relatively small, and the cords of the hepatocytes in this region are closely related, and their cell boundaries are indistinguishable. The nuclei of the hepatocytes are pyknosis and clear, as their nucleoli are visible with H&E

staining. In addition, necrosis between hepatocytes and vacuolation, hemorrhage and apoptosis of some cells were observed. Mild congestion in some blood vessels, accompanied by tenderness in the vessel wall, was also observed, and this wall thickens from the other side; hemorrhage has been observed in the wall of these vessels. On the other hand, severe congestion of other blood vessels was observed, accompanied by fibrosis of their walls and hyperplasia of fibroblasts. In both cases, infiltration of inflammatory cells around the blood vessels was observed, and it was more intense in the second case (Figures 11 and 12).

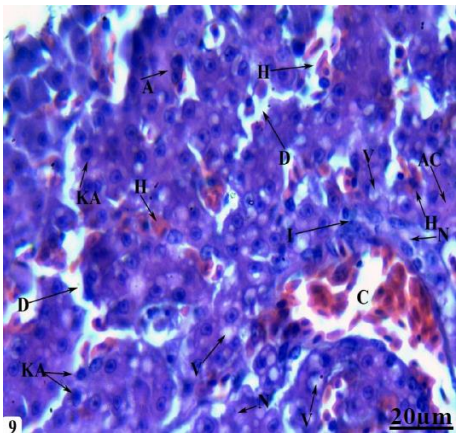

Figure 9: Microphotograph of liver showing the histological structure of the liver after 60 day from exposure to TiO_2NPs with concentration 20mg/kg -H&E stain.

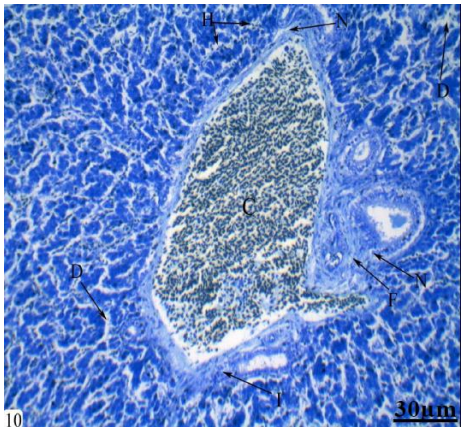

Figure 10: Microphotograph of liver showing the histological structure of the liver after 60 day from exposure to TiO_2NPs with concentration 20mg/kg -TB stain. Abbreviation: (IR) Irregularity; (KA) Karyopyknosis; (C) Congestion; (AC) Acidity; (PR) Positive response; (FD) Fibrous deposits; (V) Vacuolation; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (I) Infiltration; (A) Apoptosis.

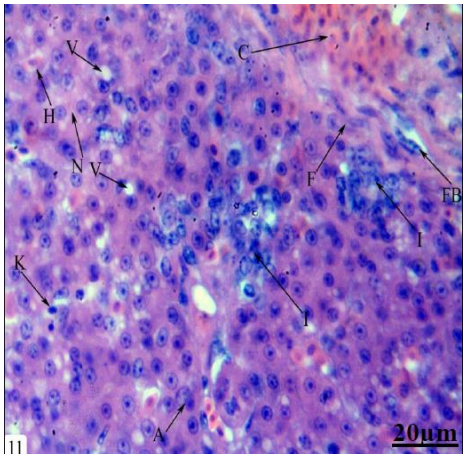

Figure 11: Microphotograph of liver showing the histological structure of the liver after 4 days from exposure to TiO_2NPs with concentration 40mg/kg - H&E stain.

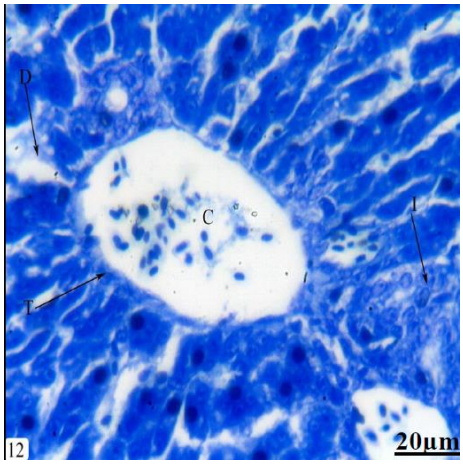

Figure 12: Microphotograph of liver showing the histological structure of the liver after 4 days from exposure to TiO_2NPs with concentration 40mg/kg - TB stain.

After 14 days, severe congestion of the blood vessels was observed accompanied by fibrosis surrounding the components of the portal area, as well as thickening and hyperplasia of the wall of the bile duct and the presence of fibrous deposits inside it, and necrosis of some cells of the wall of the bile duct as well as blood vessels was also observed. A large infiltration of inflammatory cells was found near the blood vessels and the portal area. The hepatocytes appeared compact and the hepatic cords were irregular, and the nuclei of the hepatocytes appeared large and dark in color, especially with TB stain. Spread of vacuolation, necrosis between hepatocytes, hemorrhage, and ballooning swelling were also observed. The sinuses appeared dilated and larger than the previous group, and the Kupffer cells appeared thickened (Figures 13 and 14).

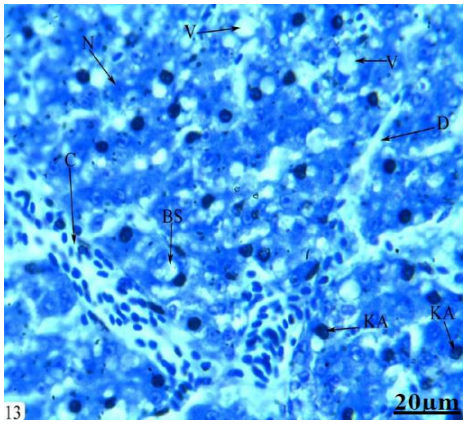

Figure 13: Microphotograph of liver showing the histological structure of the liver after 14 day from exposure to TiO_2NPs with concentration 40mg/kg -. AB-PAS stain, 40X.

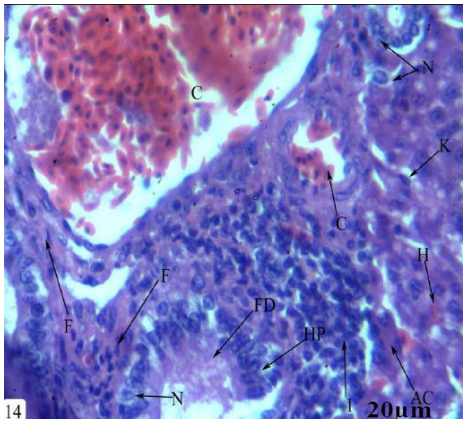

Figure 14: Microphotograph of liver showing the histological structure of the liver after 14 day from exposure to TiO_2NPs with concentration 40mg/kg - H&E stain,40X. Abbreviation (KA) Karyopyknosis; (C) Congestion; (FD) Fibrous deposits; (K) Kupffer's cells; (V) Vacuolation; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (I) Infiltration; (FB) Fibroblasts; (A) Apoptosis: (T) Thickness; (BS) Ballooning swelling; (HP) Hyperplasia.

Whereas hepatocytes appeared after a month, similar to their condition in the previous period. There were strong necrosis and the spread of ballooning swelling between them. Also, the nuclei appeared condensed with the TB stain and the blurring of cell membranes with hemorrhage and vacuolization between them. The sinuses appeared dilated, and Kupffer cells appeared somewhat enlarged. There was also strong congestion in the blood vessels and thickening of the walls of these vessels, and necrosis in some of the vessel's walls was observed (Figures 15 and 16).

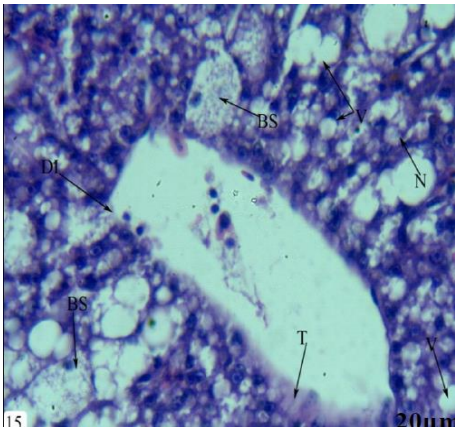

Figure 15: Microphotograph of liver showing the histological structure of the liver after 30 day from exposure to TiO_2NPs with concentration 40mg/kg - H&E stain.

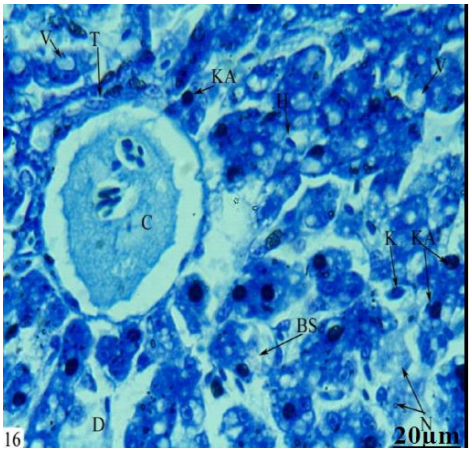

Figure 16: Microphotograph of liver showing the histological structure of the liver after 30 day from exposure to TiO2NPs with concentration 40mg/kg - TB stain.

After 60 days of exposure to TiO_2NPs, it was noticed that the histopathological changes were less severe than they appeared in the previous periods. Still, nevertheless, congestion in the blood vessels and fibrosis around the components of the portal area appeared, and some of the white fibers extended and lined some blood sinuses. Infiltration of inflammatory cells was also observed near the blood vessels, and necrosis appeared in the walls of blood vessels and the bile ducts. It also found dilatation of the sinuses, but they were narrower than they appeared in the previous period. The hepatocytes appeared marked with condensed nuclei, and necrosis, hemorrhage, vacuolation, and apoptosis were observed in some of them. Severe hemorrhage was observed in some areas of the liver (Figures 17 and 18).

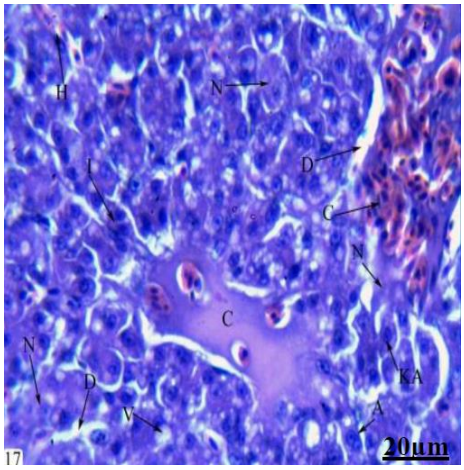

Figure 17: Microphotograph of liver showing the histological structure of the liver after 60 day from exposure to TiO_2NPs with concentration 40mg/kg - H&E stain.

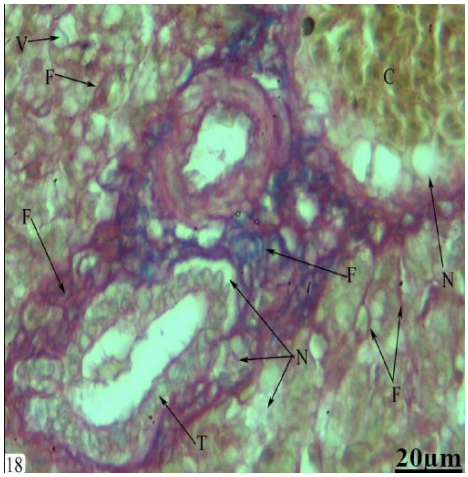

Figure 18: Microphotograph of liver showing the histological structure of the liver after 60 day from exposure to TiO_2NPs with concentration 40mg/kg - M stain,40X. Abbreviation (DI) Dilaceration; (KA) Karyopyknosis; (C) Congestion; (K) Kupffer's cells; (V) Vacuolation; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (I) Infiltration; (A) Apoptosis; (T) Thickness; (BS) Ballooning swelling.

Discussion

After four days of exposure to a concentration of 20 mg/kg of TiO_2NPs, the results showed irregularity of the hepatic cords and thickening of some hepatocytes' walls. Vacuolation, necrosis, and apoptosis between hepatocytes. A strong infiltration of inflammatory cells and intracellular hemorrhage were also observed. These results are consistent with the results conducted on mice, as it was observed. when treating mice during four days with TiO_2NPs at a concentration of 16g/kg, causing necrosis of hepatocytes located in the central area of some hepatic lobules and the formation of edema as well as infiltration of lymphocytes in these affected areas as the cells showed Hepatic hydrophobic degeneration (15).

After 14 days, hepatocytes appeared to agglomerate with hemorrhage between them. Sinuses appeared dilated, and the lining of Kupffer cells is clear. There was hypertrophy of the wall of the congested blood vessel and the occurrence of necrosis in some cells. As well as the emergence of some carbohydrate substances. These results agreed with the results of studies conducted on mice exposed to 25mg/kg of TiO_2NPs, which caused peripheral, central vein hydrolysis and intermittent necrosis of liver cells. These are consistent with the results of other studies conducted on mice exposed to TiO_2NPs, as they caused a statistically significant increase in liver weight and other tissue changes associated with dilated blood vessels (16). Another study on Mozambican tilapia showed enlargement, degeneration, necrosis, and irregularly shaped nuclei observed in liver tissue (17).

After a month, severe congestion appeared in most of the blood vessels with severe infiltration of inflammatory cells, and necrosis of the vessel wall and necrosis of the bile duct wall appeared. Extensive blood sinuses appeared in most areas, with fibrous deposits in some of these sinuses. These results are consistent with what Hao *et al.* (18) indicate that *Cyprinus carpio* liver may be the most organ susceptible to exposure to TiO_2NPs, which causes hepatocyte necrosis, apoptosis, and infiltration of inflammatory cells. Also, these results are consistent with the study of Mahdieh *et al.* (19), where they found congestion in the central veins and the accumulation of basophils resulting from the destruction of liver cells and infiltration of some inflammatory cells can be observed. Also, there was tissue necrosis. Zhou *et al.* (20) also found that TiO_2NPs increase inflammatory cytokines in the liver of mice after oral administration, where the liver is more sensitive to toxicity caused by TiO_2NPs, followed by the intestine, blood, and colon, respectively.

Also, there is great convergence between some of the results obtained and the results of the study Hassanen *et al.* (21), which used silver nanoparticles on chicken livers for 35 days, as the sections of liver tissue observed random, multi-focal areas of blood clotting necrosis that invaded by mononuclear inflammatory cells throughout the hepatic parenchyma. The majority of hepatocytes showed vacuole degeneration and fatty changes, and massive focal areas of hemorrhage were recorded within the hepatic parenchyma, especially in the subcapsular area.

After two months, there was widespread necrosis among the hepatocytes, and an increase in acidity of some cells was observed, and their nuclei were revealed. The sinuses appeared greatly dilated. There appeared severe congestion

in the blood vessels as well as bleeding in some of its walls. This was also evident when mice were dosed with TiO_2NPs, and it also showed thickening in the liver capsule. This enlargement may be due to the proliferation of connective tissue in the capsule. Hepatocytes also showed hydrophobic degeneration characterized by cytoplasmic hypercrystallization (15).

Results showed after four days of exposure to a concentration of 40 mg/kg of TiO_2NPs, presence of dilatation of the sinuses in some areas of the liver. Kupffer cells appear relatively small, and the cords of hepatocytes in this region are closely related. Also, necrosis between hepatocytes, vacuolation, hemorrhage, and apoptosis of some cells was observed. Severe congestion of some blood vessels is observed, accompanied by fibrosis of their walls and hyperplasia of the fibroblasts. This was also observed when mice were dosed with TiO_2NPs. It was severe changes recorded in the liver tissue, such as necrosis of hepatocytes located in the central area of some liver lobules and edema within the blood sinuses. Numerous nuclei and cell fragments and lymphocyte infiltration are observed in these damaged areas of liver tissue (15). In a study by Shakeel *et al.* (22), high levels of titanium were found in the liver after exposure to TiO_2NPs at a concentration of 64 mg/kg for seven days. Biochemical analyzes showed that plasma glucose increased significantly, while there was no difference in plasma insulin secretion. Increased Reactive oxygen species levels were also found in serum and liver, as indicated by decreased overall Superoxide dismutase activity, glutathione level, and increased malondialdehyde content.

After 14 days, severe congestion of blood vessels, accompanied by fibrosis surrounding the components of the portal area, was observed, and necrosis of some cells of the wall of the bile duct and blood vessels was observed. Also, a large infiltration of inflammatory cells was found near the blood vessels and the portal area. The sinuses appeared dilated and larger than the previous exposure, and the Kupffer cells appeared as thickened. These were also reported in mice exposed to high doses of TiO_2NPs, where necrosis of hepatocytes, fibrosis, and swelling of the renal glomeruli was observed (23).

Whereas, after a month, hepatocytes appeared similar to their condition in the previous period, with strong necrosis and the spread of ballooning swelling between them. The sinuses appeared dilated, and Kupffer cells appeared somewhat enlarged. It also appeared strong congestion in the blood vessels. It recorded that most of the TiO_2NPs with a 50 mg/kg concentration were found in the liver in hepatic sinuses and within Kupffer cells (3). However, some of these were occasionally observed in hepatocytes. Some studies indicate that TiO_2NPs do not have genotoxic effects on mouse liver or bone marrow (3). In another study, Zinc nanoparticles was used. There was founded necrosis, enlarged liver cells, cloudy liver cells, and lymphocytosis in birds exposed to this substance. In contrast, the chicks exposed to high doses showed clear changes in the liver, such as blood vessel congestion, bile duct wall proliferation, sinus enlargement, damaged cell contact between liver cells, hepatocyte enlargement, and inflammatory cell accumulation (24).

After 60 days, it was noticed that the histopathological changes were less severe than they appeared in previous periods, and this may be due to the ability of the liver cells to recover. Also, congestion of blood vessels appeared and fibrosis around the components of the portal area. Infiltration of inflammatory cells was found near the blood vessels, and necrosis appeared in the blood vessels' walls and the bile ducts. There also found dilatation in the sinuses, but they were narrower than they appeared in the previous period. The results of the other studies indicate that TiO_2NPs cannot be easily removed from the livers of treated mice, and their accumulation is mainly in the cytoplasm of Kupffer cells and hepatocytes (3). The results of another study also indicate that TiO_2NPs can accumulate in mouse bone marrow and are capable of inducing cytotoxicity (25). The micronucleus test revealed chromosomal damage in the bone marrow of rats when treated with TiO_2NPs. The hematological analysis also showed a significant decrease in Red blood cells and Hematocrit and a significant increase in mean corpuscular volume, platelet blood count, mean platelet volume, and White blood cells at high doses. Moreover, red cells of abnormal shape were observed, sometimes containing small nuclei (25).

Conclusions

It can be concluded from the current study that despite to the many benefits of nanomaterial in general and TiO_2NPs in particular, exposing them to Economic animals in an uncontrolled manner leads to great harm to them that may lead to their death. Exposing quail birds to TiO_2NPs has led to the emergence of many tissue lesions in the liver, which negatively affects its vital functions, which affects the life of this bird. Ultimately, this may lead to heavy economic losses.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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التأثيرات المرضية النسجية للجزيئات النانوية لثنائي أوكسيد التيتانيوم على الكبد في السمان الياباني

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الخلاصة

تعد الجزيئات النانوية لثنائي أوكسيد التيتانيوم ذات استعمالات مفيدة متعددة، إذ أنها تدخل في الكثير من المجالات الطبية والصناعية والاقتصادية وغيرها. ورغم هذه الفوائد العديدة إلا أنها لا تخلو من الأضرار على الإنسان والحيوانات إذا استعملت بدون سيطرة. لذا هدفت الدراسة الحالية اكتشاف التأثيرات المرضية النسجية لثنائي أوكسيد التيتانيوم النانوية على الكبد في السمان الياباني. ضمت الدراسة ثلاثة مجاميع الأولى مجموعة السيطرة والتي جرعت بالماء المقطر لمدة أربعة أيام متواصلة والمجموعتين التجريبيتين الثانية والثالثة والتي جرعت بثنائي أوكسيد التيتانيوم النانوية بتركيز ٢٠ و٤٠ ملغم/كغم على التوالي. وبعد مرور أربعة، أربعة عشر، ثلاثون، وستون يوم من بدء التجربة تم تشريح الطيور. بينت النتائج ظهور العديد من الآفات النسجية في كبد طيور المجموعتين التجريبيتين وبدرجات متفاوتة في الفترات الأربعة. ومن أبرز الآفات النسجية التي ظهرت في المجموعة التجريبية الثانية، ظهور نخر، نزف، تفجي، احتقان، انتفاخ بالوني، بالإضافة إلى ارتشـــاح الخلايـــا الالتهابية. بينما ظهرت في المجموعة

التجريبية الثالثة آفات نسجية مماثلة لما ظهر في المجموعة الثانية، بالإضافة إلى توسع الجيبانيات، تضخم خلايا كوفر، تضخم الخلايا الكبدية وعدم الوضوح الحدود الخلوية بينها، نخر في جدرن الأوعية الدموية والقنوات الصفراوية. استنتجت الدراسة أن التعرض المباشر لثنائي أوكسيد التيتانيوم النانوية يؤدي إلى حدوث ضرر في نسيج الكبد لهذه الطيور مما قد يؤثر على وظيفته وبالتالي تعريض حياته للخطر.