Studying the effect of Titanium Dioxide Nanoparticles on Blood Parameters and Kidney tissue in Swiss albino mice

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Abstract

Objective: The objective of this work was to examine the acute toxic effects of Titanium dioxide nanoparticles (TiO_2 NPs) on Swiss albino mice. **Materials and methods**: 25 experimental animals were randomly divided into five groups, each group contains 5 animals: The first group T1 (as positive control group) that administrated 1 milliliter of distilled water, T2,T3,T4,T5 treated with TiO_2 NPs with concentration of 0.2 mg/kg, 0.4 mg/kg, 0.6 mg/kg, and 0.8 mg/kg, respectively. The rats were euthanized and the renal tissues were histopathologically evaluated. In addition, the activity of kidney function test as urea and creatinine were quantified. **Results:** The findings of this study a significant increase at a p value > 0.05 in the concentration of urea in blood serum among the treated groups of mice compared to the control group, T3 (53.75±5.71), and the rest of the other group, with a non-significant difference from concentrations of T2, T4, andT5, as the concentration of urea in the experimental group treated T2 (47.9±4.38).) T4 (47.06±4.60).) and T5 (40.34±3.57) compared to the control group (38.14±0.50). No significant differences in levels of creatine in the blood serum of mice treated with TiO_2NPs compared to the control group, T5 was (0.49±0.104), T2 was (0.41±0.081) T3 (0.35±0.052), T3(0.34±0.042) compared to the control group (38.14±0.50), As for the histological study of the kidney tissue after administration Ti02 showed that presence of Atrophy, Hemorrhage , Necrosis ,Edema and Thicken Wall of Blood Vessels and in the concentration 0.4 the presence of Desqumation of renal capsule ,Hemorrhage , Infiltration of inflammatory cells and Glomerular Segmenation , and in concentration of 0.6 was congestion of the blood vessel, Cellular degeneration and Apoptosis and in the concentration Glomerular segmenation, , Oedema, Damage of tubules, Karyolysis and Pyknosis. indicated a notable alteration in the function of renal histopathology in injection administration of TiO_2 NPs **Conclusion:** This study concluded that there are cytotoxic and antioxidant effects of TiO_2 NPs as well as renal damage.

Keywords: Nanotechnology, Kidney, Titanium dioxide (TIO2) NPs, Hematological Parameters, Histopathology.

INTRODUCTION

Nanotechnology signifies a transformative approach to technical advancement concerning the manipulation of materials at the nanoscale scale (one billionth of a meter). Nanotechnology refers to any technology operating at the nanoscale, which has numerous practical uses (1). Nanotechnology encompasses the fabrication and utilization of chemical, physical, and biological systems at a nanoscale. A broad spectrum extending from individual molecules or atoms to submicron scales, and the incorporation of these resultant nanomaterials into larger systems, possesses the potential to transform our perspectives and expectations, enabling us to address global challenges (2).

Titanium dioxide (TiO_2 NPs) ranks among the most prevalent oxides, with an annual production exceeding 10,000 tons, attributed to their affordability, excellent chemical stability, high refractive index, and robust oxidation capabilities (3). It inherently exists in three distinct forms. The forms, namely rutile, anatase, and brookite, possess crystalline structures and are extensively utilized in the gemstone industry (4).

Some studies have shown that TiO2 particles have potential negative health effects. After entering the body, inflammatory mechanisms begin in the cells, stimulating the process of self-programming and the generation of free radicals from oxygen that destroy the nucleus and DNA, in addition to multiple changes in cell functions.

The kidney is one of the vital organs in the body, excreting of body waste products and drugs through highly specialized cells located in renal nephrons. Kidney diseases can lead to life-threatening sequels. The kidney functions can be altered by many environmental contaminants, chemicals and drugs(5). TiO_2-NPS causes kidney inflammation leading to tissue necrosis, disorganization of renal tubules and production of reactive oxygen species (ROS)(6). Nowadays, much attention has been directed to the use of dietary antioxidants of natural products for prevention and treatment of such drawbacks and may have a significant role in keeping and safeguard health(7).

The aim of this research was to study the effect of TiO_2 NPs on a number of biochemical variables in the blood serum of pregnant mice by examining kidney function (urea and creatine) and studying tissue changes.

MATERIALS AND METHODS

Study Design

Pregnant female mice containing a vaginal tampon were isolated in cages marked with the date of fertilization, and each cage contained five mice for the purpose of treatment with titanium dioxide nanoparticles, starting from the sixth day of pregnancy, which is the stage of organ formation, until the eighteenth day. Then the mice were divided into four groups (each group of five mice) treated with this substance at different concentrations, and a negative control group.

Injection administrated group

First, there was the positive control group (T1), which consisted of administering 1 milliliter of distilled water on a daily basis for a period of 1 weeks via gavage other four groups (T2, T3, T4 and T5) were daily **Injection** gavaged for 14 days with 0.2, 0.4, 0.6 and 0.8 mg\ Kg of body weight of TiO_2 NPs, respectively.

Preparation of Animals

In this study, **Swiss albino mice**, which belong to the Mus musculus strain, were used, with (10) males and (25) female mice whose weights reached between (2) +_ 25) and whose ages ranged between (2-3) months. They were obtained from the animal house of the College of Veterinary Medicine at Tikrit University. The animals were transported to the house for the period from 11/21/2023 to 5/21/2024 and were placed in a special room prepared for raising animals in terms of ventilation and temperature that ranged between (22-25) degrees Celsius. The lighting period was approximately 12 hours of light and 12 hours of darkness, the animals were placed inside transparent plastic cages with metal mesh covers with dimensions of (25, 30, 50) cm. They were furnished with sawdust, and attention was paid to the cleanliness of the cages. The animals were left for two weeks to adapt to the environmental conditions. The animals were fed their own diet with the following ingredients in fixed proportions (powdered milk 10%, wheat 34%, barley 20%, corn 25%, animal protein 10%, and table salt 1%).. They also used concentrated feed obtained from local markets (Ma et al., 2019), and they were also provided with bottles of drinking water. After mating, injections, dissecting the mouse, and extracting the embryos and organs, the liver and kidney organs were kept for histological sections.

Laboratory analyses

Blood sample collection: After the end of the experiment, the animals were transferred to the Cancer Research and Medical Genetics Center in Baghdad, Al-Mustansiriya University, for the purpose of completing the procedures for withdrawing blood and dissecting the animals to obtain the organs required in the experiment. The animals were weighed after the experiment and then anesthetized by injection into the abdominal cavity using 0.1 ml of ketamine anesthetic. After ensuring that the animals were anesthetized, blood was withdrawn directly from the heart by heart puncture using a sterile medical 5 ml syringe to obtain the largest amount of blood. The blood samples were placed directly in sterile test tubes free of anticoagulant Gel tubes, and then the tubes were transferred to a centrifuge at a speed of 3000 rpm for 15 minutes to obtain the serum is transferred to small Eppendorf tubes and clean, dry, labeled tubes. The serum is stored in the refrigerator at a low temperature of -20°C until the biochemical and histopathological tests are performed.

Biochemical assays: Biochemical test urea and creatinin measurement by manscripit (Biolabo-France).

Histopathological examination: After drawing blood samples, the animals were dissected directly by making an incision in the abdominal cavity from the bottom upwards towards the heart, then the liver and kidneys were removed after removing the fatty tissue and the surrounding connective tissue, then washed with distilled water to remove the blood on them, then dried by placing them on filter paper and weighing them. These tissue are preserved in a 10% formalin

solution. Following mounting, a 5µm slice of each tissue was cut and subsequently immunostained with H&E. A pathologist blindly assessed and rated histopathological alteration (8).

Statistical Analysis

The results were expressed as Mean ± Standard Deviation for relative comparison. The data were analyzed using SPSS software, using one-way ANOVA. Statistical significance was defined as a p-value less than 0.05. Subsequently, we analyzed the biochemical testing data to estimate the percentage changes resulting from exposure to TiO_2-NPs.

RESULTS AND DISCUSSION

2.1 Estimating the levels of urea

Results in present study as shown in Figure 1, showed a significant increase of p-value >0.05 in the urea concentration of the blood serum of mice treated with titanium dioxide nanoparticles for two weeks in the four experimental groups compared with the control group, where the treated experimental group showed at a concentration of 0.4, the highest concentration (53.75±5.71) compared to Control group (38.14±0.50)) and the rest of the other groups, with a non-significant difference from the concentrations of 0.2, 0.6, and 0.8, as the urea concentration in the experimental group treated with a concentration of 0.2 reached (47.9±4.38) compared to the control group (38.14±0.50) and for the same injection period. The urea concentration in the experimental group treated with a concentration of 0.6 reached (47.06±4.60).) compared with the control group (38.14±0.50). The experimental group treated with a concentration of 0.8 reached (40.34±3.57) compared to the control group (38.14±0.50). The concentration was 0.4 with a difference significant for the control groups, which recorded the lowest urea, which amounted to (38.14±0.50).

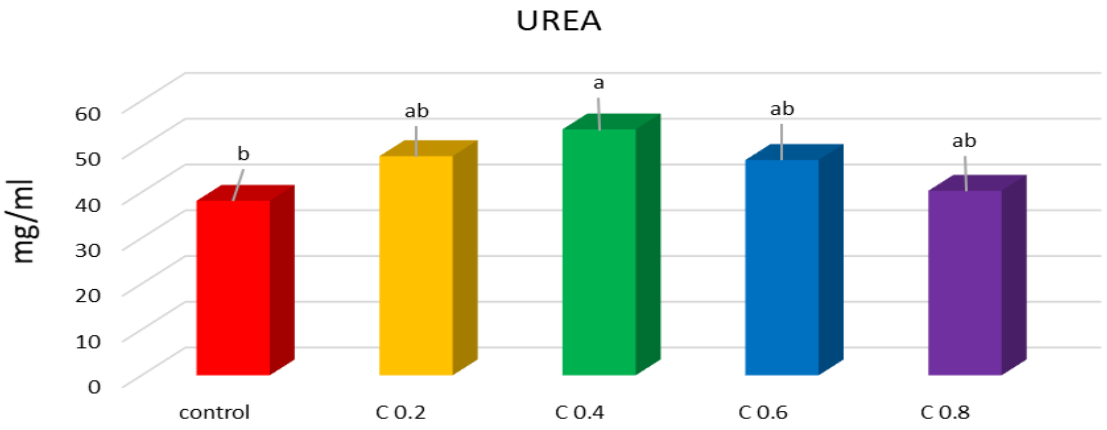


Figure (1) shows the effect of titanium dioxide (Tio2) particles at the four concentrations on the urea concentration of the blood serum of pregnant white mice compared with control groups.

Nanoparticles have been used in several applications as science, technology, medicine and many other fields. Among the many types of NPs, the TiO2 which is one of the most produced NPs in the world, have attracted particular technological and scientific interest because of their unique chemical and physical characters and also their influence on human health and the environment(9). However, the present researches about the toxicities of NPs, both in naturally occurring particles and in engineered nanomaterials, are highly increasing(10).

Since measuring urea concentrations in plasma is usually a sign of kidney function and condition because the kidneys are one of the organs that collect and eliminate waste(11). Therefore, it is considered one of the vital organs exposed to the harmful effects of titanium dioxide particles (12). Urea regulation by the kidney is a vital part of the metabolism in the rat body. In addition to the role of urea as a carrier of nitrogen waste (13)**.** The results of the current study indicated a clear significant increase in urea levels in groups treated with titanium dioxide (Tio2) particles at different concentrations (4, 8, 12, 16 mg/ml) equivalent to a dose of 0.2, 0.4, 0.6, 0.8 mg/kg).) of body weight compared to control groups, and these results were consistent with Shaltout *et al*., **(14),** who used TiO_2 NPs on male eggs orally via an oral tube at a rate of 1 ml daily for 120 consecutive days at a dose of 20 mg/kg.

In this study, high levels of urea were observed in the kidney index, as this indicates kidney injury caused by these particles due to excessive deposition in kidney tissue and generation of ROS with the depletion of the cells' antioxidant defenses, which can lead to an imbalance of oxidant/antioxidants, which increases membrane permeability. Mitochondria and induction of mitochondrial membrane depolarization (15).

2.2 Estimating the levels of creatine

The results of the current study, as shown in Figure 2, showed that there were no clear significant differences in the creatine concentration of the blood serum of mice treated with **titanium dioxide nanoparticles** for a period of two weeks in the four experimental groups compared with the control group, as the experimental group treated with a concentration of 0.8. The highest concentration (0.49±0.104) compared to the control group (0.26±0.043), other groups, with an insignificant difference. While the creatine concentration in the experimental group treated with a concentration of 0.2 reached (0.41±0.081) compared to the control group (0.26±0.043) with the same injection duration. The creatine concentration in the experimental group treated with a concentration of 0.4 reached (0.35±0.052) compared to the control group (0.26±0.043). The experimental group treated with a concentration of 0.6 reached (0.34±0.042) compared to the control group (38.14±0.50).

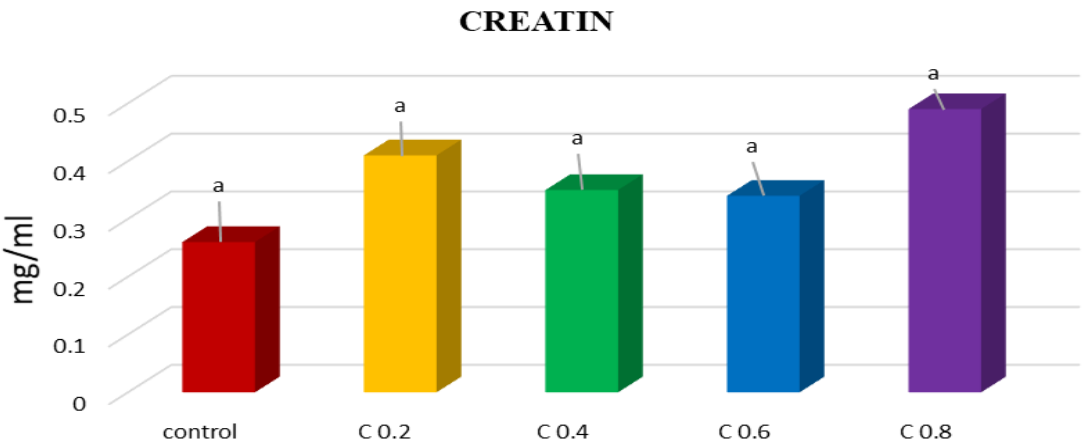
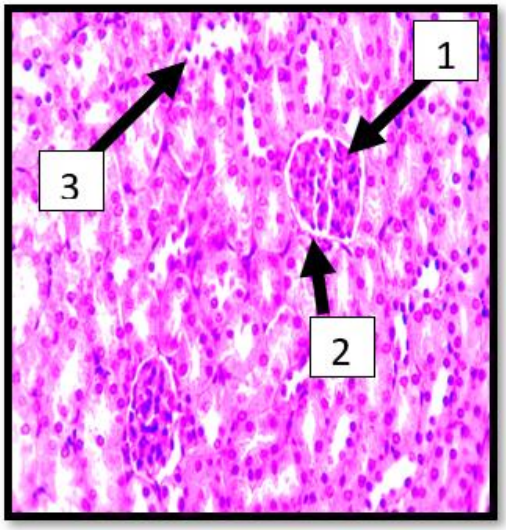


Figure (2) Shows the effect of TiO_2NPs at the four concentrations on the creatine concentration of the blood serum of pregnant female albino mice compared with control groups.

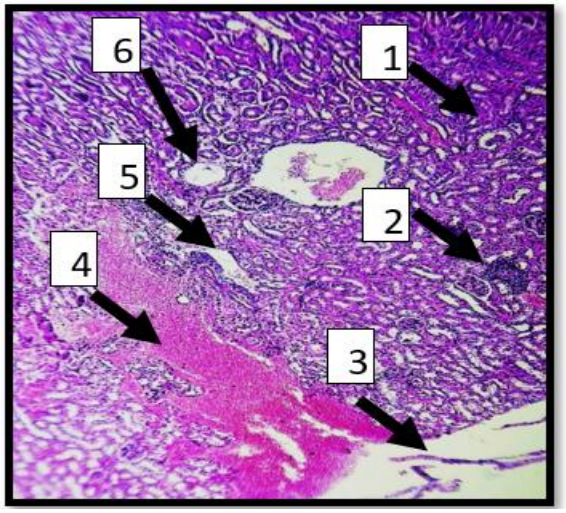
Creatinine is removed from the bloodstream by the kidneys, so measuring creatinine in the blood can indicate kidney function **(16)** and if there is a failure in kidney function, the level of creatine rises in the serum (17). This is not consistent with the results of the current study, as no significant differences appeared in creatine levels. This result could be related to the rapid elimination of titanium from kidney tissue. **(18).** These results agreed with(**19)**. While the results of the current study differed with(**20)** (who reported that biochemical evaluation in plasma samples in the experimental group of mice treated with intraperitoneal injection of TiO2NPs) at a dose of 25 mg/kg showed a significant decrease in creatinine levels compared to the control group, Partial evidence indicates that Tio2, at low doses, causes kidney dysfunction and inflammation by altering gene expression levels of cytokines involved in the inflammatory response or detoxification, as nuclear factor B (NF-K) is activated, which is an important regulator of the intracellular inflammatory response and binds to the inhibitory proteins IKBs. Which prevents NF-κB from migrating into the nucleus from the cytoplasm. The study (14) indicates that abnormal pathological changes in the mouse kidney and renal dysfunction could not be induced by intraperitoneal injection at a low dose of 5 and 10 mg/kg of body weight for 14 days, but with doses of 50, 100, and 150 mg. /kg Kidney dysfunction and an acute inflammatory response were observed, as an increase in creatine levels occurred at high doses, although there were no differences. The variation in results from animals administered titanium dioxide nanoparticles compared to other researchers may stem from the species of animal, the distinct physical and chemical characteristics of the nanoparticles, the route of administration (oral, respiratory, or dermal), and the frequency of injections.

3.3 Histological study

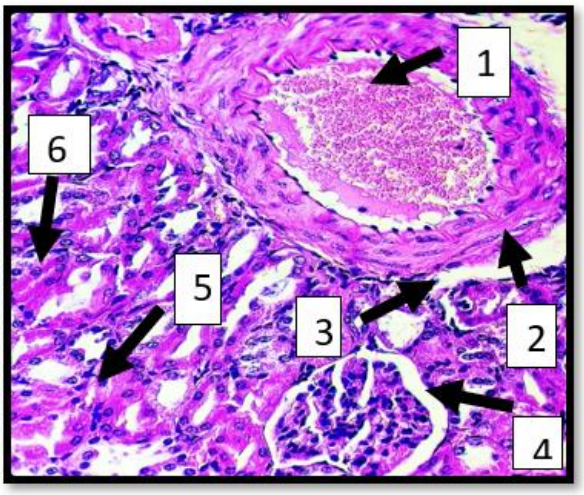
The present study showed the effect of TiO_2 NPs on renal tissue in injection groups. Histopathological examination of the renal cortex and medulla of **T1** (control) showed normal appearance of renal glomeruli surrounded by Bowman's capsule and convoluted tubules. All the histopathological forms of the renal cortex and medulla of **T2 (**treated with 0.2 mg) showed Atrophy, Anodule inflammatory cells , Renal capsule detachment , Hemorrhage ,Necrosis, Thicken Wall of Blood Vessels and Fibroblasts. **T3** (a concentration of 0.4 mg), it shows Desqumation of renal capsule, Infiltration of inflammatory cells, Glomerular Segmenation , Dilatation urinary tubule ,Fibroblasts and Cast formation within the blood vessel. All sections of T4 (0.6 mg), showed Hemorrhage ,congestion of the blood vessel , Cell separation and falling into the tubules , Cellular degeneration , and Karyolysis., T5 showed a At a concentration of 0.8 mg, it shows Ocuded tubular obstruction, Segmentation of the glomerular, Necrosis, Odema, Hemorrhage , Pyknosis , Fibrocytes and Casta formation.



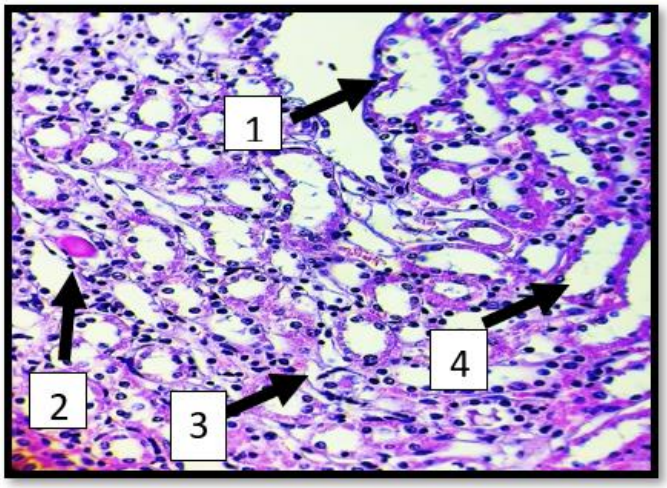
Control (T1) Transverse histological section of the kidney of a group of pregnant mice (non-operated control). (1) Shows the normal structure of the glomerulus (2) surrounded by Bowman's capsule (3) and convoluted tubules. H&E, 400 X



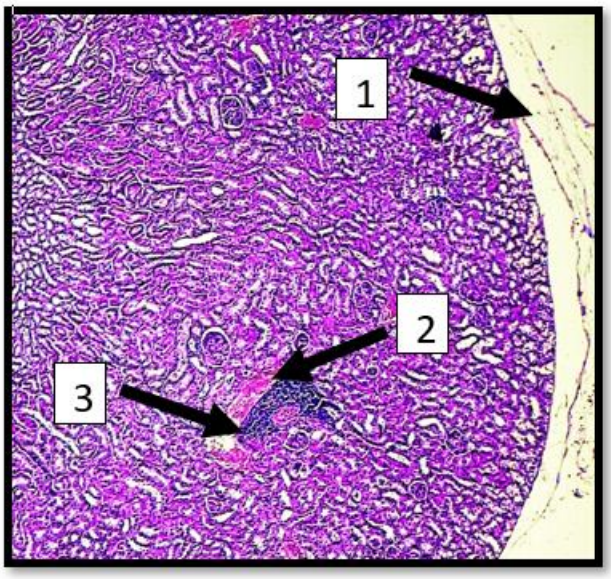
T2 Transverse histological section of the kidney of a group of pregnant rats treated with 0.2 mg/kg titanium dioxide nanoparticles (Tio2) showing: 1) Atrophy (2) Anodule inflammatory cells (3) Renal capsule detachment (4) Hemorrhage (5) Necrosis (6) Edema. H&E, 100 X



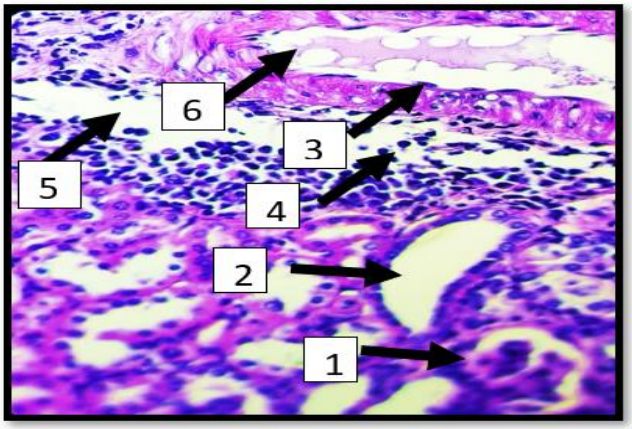
T2 Transverse histological section of the kidney cortex of a group of pregnant rats treated with 0.2mg of titanium dioxide nanoparticles (Tio2) showing (1) vascular congestion (2) Thicken Wall of Blood Vessels (3) Necrosis (4) Space dilation (5) Wallurinary tubule rupture. (6) Eosinophilic Material. H&E, 400 X.



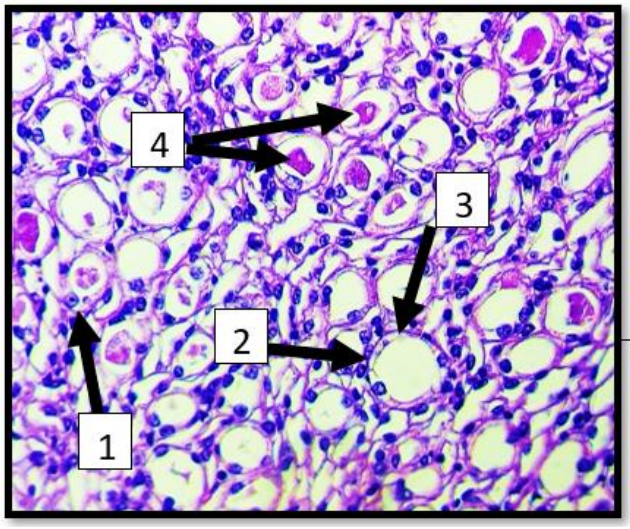
T2 Transverse histological section of the kidney medulla of a group of pregnant mice treated with 0.2 mg/ml titanium dioxide nanoparticles (Tio2) showing (1) Desgamation of tubule lining cells (2) Formation of eosinophil casts (3) Fibroblasts (4) Dilatation of tubules. H&E, 400 X.



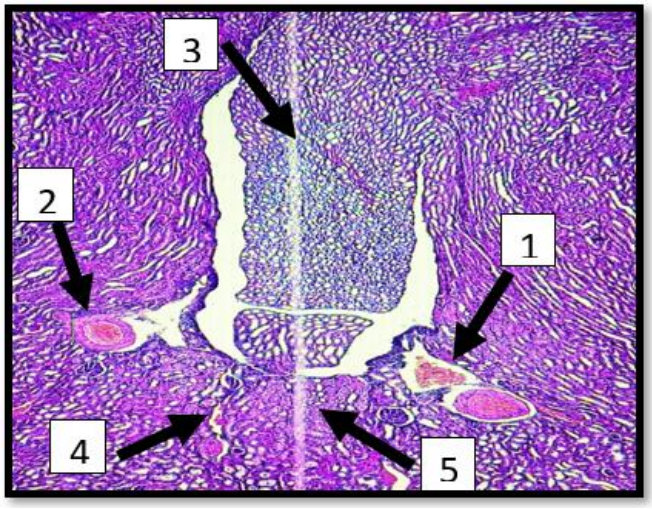
T3 Transverse histological section of the kidney of a group of pregnant mice treated with 0.4 mg titanium dioxide nanoparticles (Tio2) showing (1) Desqumation of renal capsule (2) Hemorrhage (3) Infiltration of inflammatory cells. H&E, 100 X).



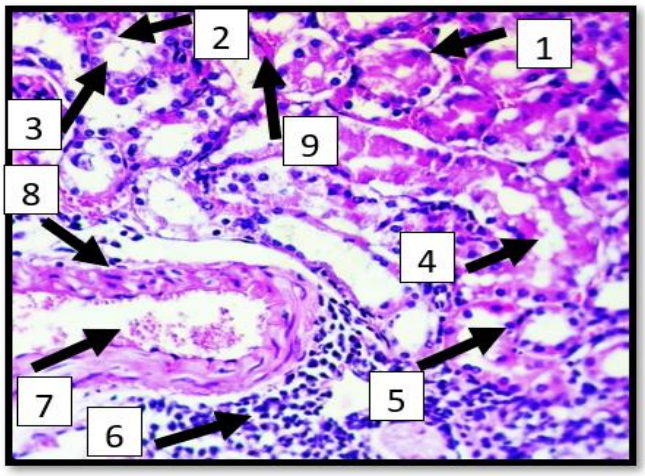
T3 Transverse histological section of the kidney cortex of a group of pregnant mice treated with 0.4 mg titanium dioxide nanoparticles (Tio2) showing (1) Glomerular Segmenation (2) Dilatation urinary tubule (3) Fibroblasts (4) Inflammatory cells (5) Necrosis (6) Cast formation within the blood vessel. H&E, 400 X.



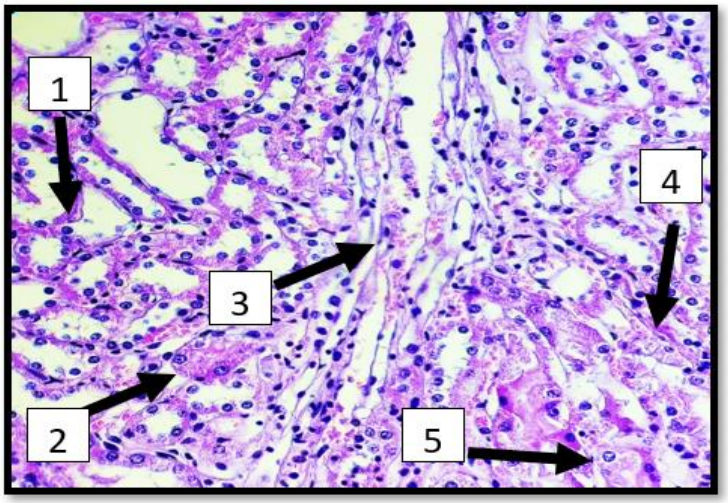
T3 Transverse histological section of the kidney medulla of a group of pregnant mice treated with 0.4 mg/ml titanium dioxide (Tio2) nanoparticles showing (1) Damaged tubules (2) Desqumation (3) Thickening of basement membrane (4) Formation of casts inside the tubules. H&E, 400 X.



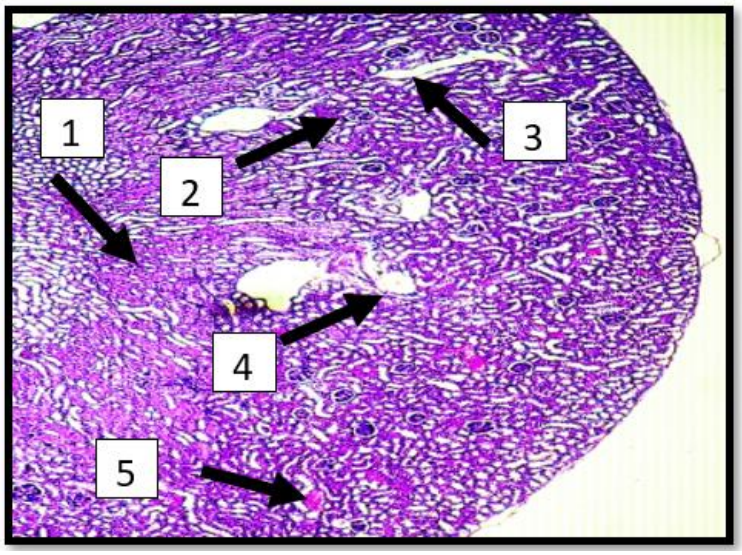
T4 Transverse histological section of the kidney of a group of pregnant mice treated with 0.6 mg TiO2 nanoparticles showing (1) Hemorrhage (2) congestion of the blood vessel (3) collecting tubules (4) Necrosis (5) Ocuded tubular. H&E, 100 X).



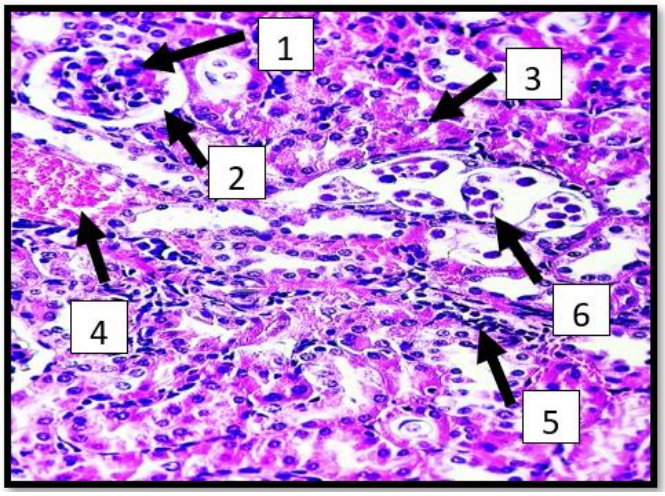
T4 Transverse histological section of the kidney cortex of a group of pregnant mice treated with 0.6 mg of titanium dioxide nanoparticles (Tio2) showing (1) Cell separation and falling into the tubules (2) Cellular degeneration (3) Tubules containing deposits (4) Necrosis (5) Karyolysis (6) Infiltration of inflammatory cells (7) Congestion (8) Thicken Wall of Blood Vessels (9) Hemorrhage.



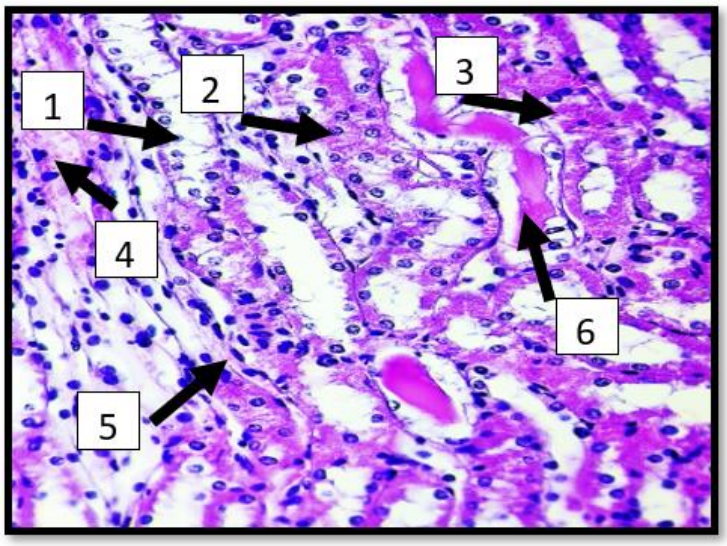
T4 Transverse histological section of the kidney medulla of pregnant rats treated with 0.6mg TiO2 nanoparticles showing (1) Cellular Degeneration (2) Oeuded tubular (3) Epithelial Desqumation (4) Apoptosis (5) Karyolysis. H&E, 400 X.



T5 Transverse histological section of the kidney of a group of pregnant mice treated with 0.8 mg TiO2 nanoparticles showing (1) Ocuded tubular, (2) Glomerular segmenation, (3) Necrosis, (4) Oedema, (5) Hemorrhage. H&E, 100 X).



T5 Transverse histological section of the kidney cortex of a group of pregnant rats treated with 0.8 mg of titanium dioxide nanoparticles (Tio2) showing (1) Glomerular atrophy (2) Dilatation of the urinary tract (3) Damage of tubules (4) Hemorrhage (5) Infiltration of inflammatory cells (6) Damage of urinary tubules and detachment of epithelial cells from the basement membrane. H&E, 400 X.



T5 Transverse histological section of the kidney pulp of a group of pregnant rats treated with 0.8 mg titanium dioxide nanoparticles (Tio2) showing (1) Fibrin formation (2) Damage tubular (3) Karyolysis (4) Pyknosis (5) Fibrocytes (6) Casta formation in the pulp area within the urinary tubules. H&E, 400 X.

The results of the histological examination revealed the ability of this substance to cause many histological lesions in the kidneys of pregnant female mice treated with different concentrations (0.8, 0.6, 0.4, 0.2). It caused infiltration of inflammatory cells at all concentrations, and this change may indicate that these particles may interfere with the antioxidant defense mechanism and cause oxidative stress in renal tissue. .**(21)_** reported that the mineral nature of most inorganic particles can cause infiltration of inflammatory cells into tissues of vital organs. The cytotoxic effect of these particles is linked to the production of reactive oxygen species (ROS), which cause damage to DNA by breaking and oxidizing nucleotides (22).

Cellular and focal degeneration has also been proven, as cellular degeneration is linked to the leakage of enzymes and lysosomes inside the cell. The observed renal necrosis may indicate the presence of oxidative stress as a result of glutathione depletion(23), and all of these results agreed with the findings of researcher(24), who demonstrated through their study the renal damage that may occur due to the acute toxicity of these particles when they are used in male mice.

The present study revealed that histological analysis of the renal cortex following two weeks of TiO_2 administration exhibited extensive lesions predominantly impacting the proximal and distal tubules, with a marked prevalence in the proximal tubules, which are the initial segment of the nephron to encounter nanoparticles post-glomerular filtration. Furthermore, proximal convoluted tubules depend on oxidative phosphorylation for energy production, indicating their lack of capacity for anaerobic metabolism, with the exception of the terminal segment, referred to as S3. Consequently, the proximal nephron is especially susceptible to toxins, resulting in the disruption of oxidative metabolism and ATP synthesis (25). Expansion and congestion were observed in the capillaries surrounding the tubes, and these results agreed with the findings of the researchers (26).

Conclusion

The results of the current study indicated a significant increase in urea levels among the groups treated with nano-titanium oxide compared to the control group. While there was no difference in creatine levels between the studied groups. Titanium oxide had a negative and toxic effect on tissues.

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