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Renal Histological Alterations Induced by Acute Exposure of Titanium Dioxide Nanoparticles

Alteraciones Histológicas Renales Inducidas por la Exposición Aguda de Nanopartículas de Dióxido de Titanio

Amin A. Al-Doaiss[1,2]; Daoud Ali[3]; Bahy A. Ali[4] & Bashir M. Jarrar[5]

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SUMMARY: Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in many commercial products, nanomedicine, agriculture, personal care products, different industries and pharmaceutical preparations with potential risk in human health and the environment. The current work was conducted to investigate the renal damage that might be induced by the acute toxicity TiO_2 NPs. A total of 40 healthy male adult Wistar albino rats (*Rattus norvegicus*) were exposed to TiO_2 NPs (126, 252, 378 mg/kg bw) for 24 and 48 h. Fresh portions of the kidneys from each rat were processed for histological and histochemical alterations. In comparison with respective control rats, exposure to TiO_2 NPs has marked the following glomerular, tubular and interstitial alterations including the followings: glomerular congestion, Bowman's capsule swelling and dilatation, inflamed glomeruli, renal tubules cloudy swelling, karyorrhexis, karyolysis, infiltration of inflammatory cells, congestion, necrosis, hydropic degeneration, dilatation and congestion of blood vessels, hyaline droplets and hyaline casts precipitation, interstitial edema and fibrosis. From the findings of the current work one may conclude that TiO_2 NPs are capable of inducing kidney damage with more insulation in the cortex and the proximal convoluted tubules than the medulla and the distal ones respectively. In addition, it might be concluded that renal damage induced by these nanomaterials is dose and duration of exposure dependent. Further hematological, biochemical, immunohistochemical, and ultra-structural studies are recommended.

KEY WORDS: TiO_2 nanoparticles; Renal tissues; Histological alterations; Hydropic degeneration; Nanotoxicity.

INTRODUCTION

Titanium dioxide NPs applications gradually are increasing in biomedical, industrial, and optical fields (Seeger *et al*., 2009). This is due to their low production cost, high refractive index, photostability in solutions and anticorrosive properties that make them suitable for biological, commercial and medical applications (Fartkhooni *et al*., 2016). Titanium oxides NPs have different sizes, shapes, chemistry and crystalline structures with special characterizations such as surface functionalization and higher stability, enabling them to be used widely in several fields in our daily life activities (Li *et al*., 2010).

Thousands of tons of TiO_2 NPs are utilized annually in the world in different fields of commercial application such as plastics, paints, cements and other application. Some recent studies predicted that most of the currently produced TiO_2 will be converted into nano forms by the end of year 2026 (Galletti, 2016). Moreover, TiO_2 NPs are among the most nanomaterials used in therapy, drug delivery, engineering, agriculture, personal care products, cosmetics, sunscreens, toothpaste, electronics, clothes, paints, and covers, as imaging agent and foodstuffs (Chabanyuk, 2014; Galletti; Yang *et al*, 2017). In addition, TiO_2 NPs are widely invested in nanomedicine and are being used in diseases diagnosis and advanced imaging and nanotherapeutics like photodynamic therapy, antimicrobial drugs and skin care products (Yuan *et al*., 2010).

Furthermore, several researchers have revealed the toxic impacts of TiO_2 NPs on various organs (Zhao *et al*.,

[1] Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia.
[2] Department of Anatomy and Histology, Faculty of Medicine, Sana'a University, Sana'a, Republic of Yemen.
[3] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.
[4] Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, Alexandria, Egypt.
[5] Department of Biology, Faculty of Science, Jerash University, Jordan.

2009). These NPs could be absorbed through inhalation, oral ingestion, intravenous injection and dermal penetration into the body, and distributed in the vital organs such as lymph nodes, brain, lung, liver and kidney (Wang *et al*., 2007; Shakeel *et al*., 2016). Some *In vivo* studies demonstrated that ultrafine anatase TiO_2 particles could induce broncheo-alveolar lavage inflammatory and cell proliferation (Warheit *et al*., 2007). These nanomaterials are rapidly distributed in organs and tissues after injection and can pass into the cells (Mahdieh *et al*., 2016). In addition, TiO_2 NPs have the potential to cross biological barriers such as blood-brain barrier and blood-placenta barrier to reach different organs and tissues (Song *et al*., 2015). Several studies reported TiO_2 NPs accumulation in several organs of experimental animals mainly the liver, kidneys, spleen, lymph node, lungs, and heart, and could not be cleared from the liver and kidney before 15 days after administration (Chen *et al*., 2009; Li & Chen, 2011). Hepatic injury was also observed in female mice treated with TiO_2 NPs, included hydropic degeneration, hepatocytes spotty necrosis; renal damage including swelling of the renal glomerulus. In their recent stuy, Chang *et al*. (2013) reviewed 347 reports on TiO_2 NPs toxicity indicated the presence of nano-TiO_2 in various vital organs, such as liver, kidney, spleen and brain. In addition, the testicular tissue of mice treated with TiO_2 NPs demonstrated sever congestion, edema, seminiferous tubules disturbance, vacuolation and necrosis in the germinal epithelium, reduction in sperm density and motility, sperm morphological abnormalities and germ cell apoptosis together with alteration in the serum value of testosterone, LH and FSH (Morgan *et al*., 2015; Abdulla, 2017).

Due to the growing number of applications, more concerns are raised for the potential risk to TiO_2 NPs exposure in human health and the environment (Yang *et al*.). These concerns need to be investigated in order to provide a scientific evidence for a safe utilization of nanotechnologies. Little, if any, is known about the toxicity of TiO_2 NPs on the renal tissues, accordingly, the current work aims to investigate the alterations that may be induced by TiO_2 NPs on renal tissues.

MATERIAL AND METHOD

Nanoparticle. Titanium dioxide nanoparticles (APS <25 nm), were purchased from Sigma-Aldrich (USA).

Experimental Animals. A total of 40 healthy male Wistar albino rats (*Rattus norvegicus*) of the same age (12 weeks old) and weighing 220–250 g were obtained from the Animal Care Center, College of Pharmacy, King Saud University.

TiO_2 Nanoparticle Preparation. Titanium dioxide NPs were suspended in (0.9 % NaCl solution) at a concentration of 1 mg/ml.

Experimental Design. The rats were housed in stainless-steel cages under a regulated light and dark schedule on a 12 h day/night cycle and controlled ventilation, humidity and temperature 24 ± 3 °C and fed with standard laboratory rodent pelleted feed and water ad libitum. Animals were examined for health status and acclimated to the laboratory environment for one week prior to use. All the experiments were conducted in accordance with the standard animal ethics and the study protocol was reviewed and approved by the ethical committee of Faculty of Medicine, Sana'a University. Selection of doses for TiO_2 NPs was based on previous studies (Park *et al*., 2008; Zhang *et al*., 2010). The current study was conducted in order to compare the toxicity of NPs at three different doses of 126, 252 and 378 mg/kg b w for 24 and 48 h. The animals were divided into four groups of ten rats each, intraperitoneally administered at the rate of 2 days as follows:

Group I: Control animals received the vehicle (normal saline).

Group II: Received infusion of 126 mg/kg TiO_2 NPs for 24 and 48 h.

Group III: Received infusion of 252 mg/kg TiO_2 NPs for 24 and 48 h.

Group IV: Received infusion of 378 mg/kg TiO_2 NPs for 24 and 48 h.

Five animals from each group were euthanized at intervals of 24 and 48 h of treatment with TiO_2 NPs. All experiments were performed according to the guidelines approved by King Saud University, Local Animal Care and Use Committee.

Histological Processing. Fresh portions of the kidney from each rat were cut rapidly, fixed in neutral buffered formalin (10 %), then dehydrated, with grades of ethanol (70, 80, 90, 95 and 100 %). Dehydration was then followed by clearing the samples in 2 changes of xylene, impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 μm) were stained and examined alterations in the renal tissues of each rat under study by using optical microscope (Olympus Microscope BX51 with Digital Camera, Japan).

RESULTS AND DISCUSSION

No mortality occurred in any of the investigated groups of the present study. In comparison with the control animals, the following histological alterations were detected.

Control Rats. The architecture in the kidney of all control rats demonstrated well preserved and kept intact normal histological components of the glomeruli, renal tubules and interstitial tissues of both the cortex and the medulla (Fig. 1).

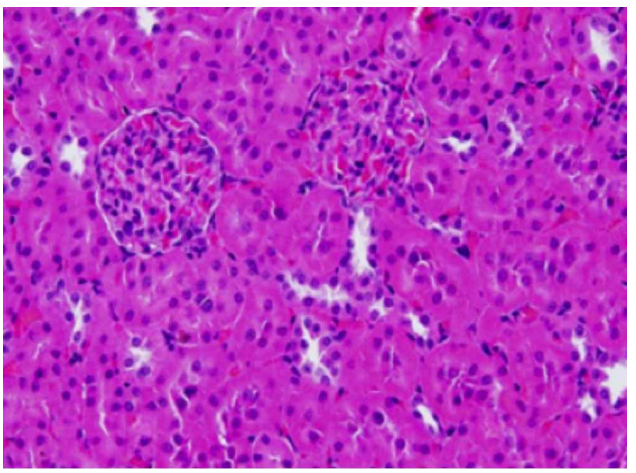

Fig. 1. Light micrographs in kidney of control rat received normal saline (1ml/kg/day for 48 h) demonstrating normal histological architecture. H & E stain. 400×.

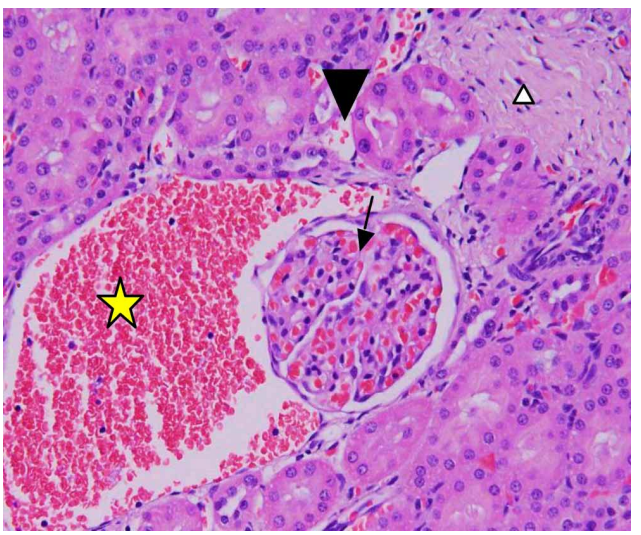

Fig. 2. Light micrograph in kidney of rat exposed to 378 mg/kg TiO2 NPs for 24 h, demonstrating glomerular congestion (arrow). Note the vascular dilatation (*), hyaline-like materials (arrow head) and fibrosis (triangle). H & E stain, 400×.

TiO_2 NPs-treated Rats. The kidneys of rats exposed to TiO_2 NPs for 24 or 48 h showed renal histological and histochemical alterations included the glomeruli, renal tubules and intracellular tissues.

Glomerular Congestion: Occasional moderate glomerular congestion was demonstrated by the renal tissues of rats exposed to 378 mg/kg TiO_2 NPs for 48 h (Fig. 2). This alteration was not observed in the kidneys of rats received 126 or 252 mg/kg TiO_2 NPs for 24 or 48 h. It was reported that the renal glomerular basement membrane is fragile and sensitive to toxic effects of NPs (Yang *et al*.).

Bowman's Capsule Swelling and Dilatation. This damage was seen in the kidneys of rats received 252 mg/kg and more of TiO_2 NPs for 24 h or more

This alteration may indicate dissociation of junctions between the glomeruli and the renal tubule and might be associated with free radicals induced by TiO_2 NPs exposure. (Fig. 3).

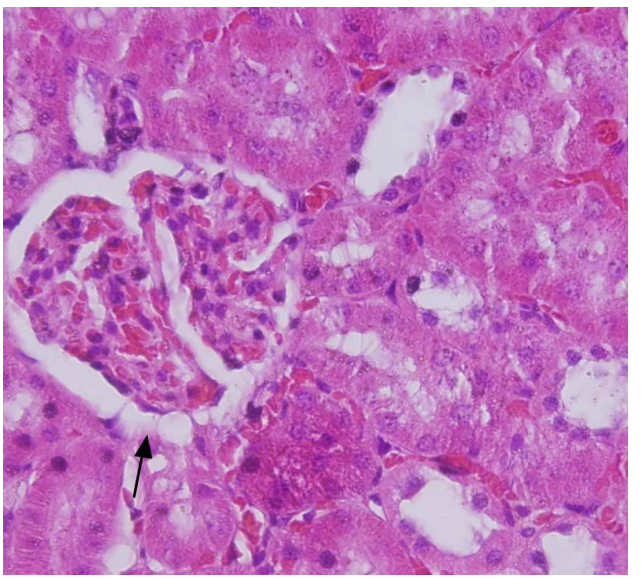

Fig. 3. Light micrograph in kidney of rat exposed to 378 mg/kg TiO_2 NPs for 48 h, demonstrating Bowman's capsule swelling and dilatation (arrow). H & E stain, 600×.

Inflamed Glomeruli: Occasional inflamed glomeruli were seen in the kidneys of rats received TiO_2 NPs for 48 *h* (Fig. 4). This alteration was not demonstrated in the renal tissues of rats subjected to nanomaterials for 24 *h*. This may indicate that exposure to TiO_2 NPs can cause glomerulonephritis leading to renal failure due to glomerular damage characterized by protein leakage into urine.

Hydropic Degeneration: Vacuolated swelling of the cytoplasm of renal cells of the NPs-treated rats was seen in the renal tissues of all rats subjected to TiO_2 NPs with varia-

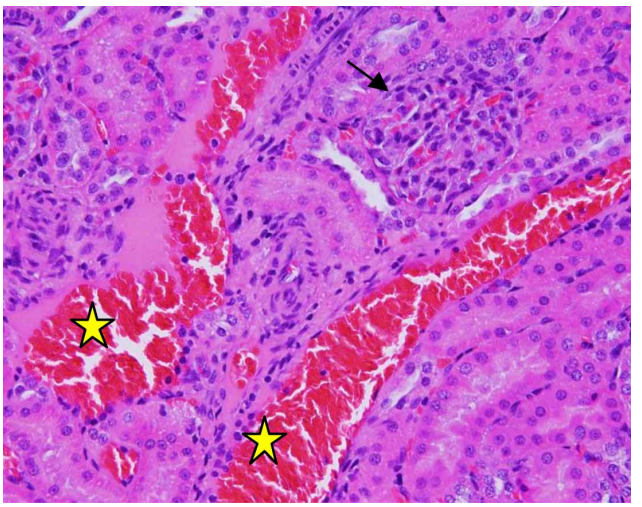

Fig. 4. Light micrograph in kidney of rat exposed to 378 mg/kg TiO_2 NPs for 48 h, demonstrating inflamed glomeruli (arrow). Note blood vessels congestion and dilatation (*). H & E stain, 400×.

ble impacts in respect to dose and exposure duration (Fig. 5a-c). This might be resulted from ion and fluid homeostasis imbalance that lead to an increase of intracellular water together with massive influx of water and Na^+ due to acute kidney injury induced by these NPs leading to lysosomal hydrolytic enzyme leakages and cellular degeneration (Schrand *et al*., 2010). The small size and clearance delay of TiO_2 NPs from the body, may lead to the retention and accumulation of these particles in the renal tissues. It was reported that at least two weeks are needed for respiratory exposure to ultrafine TiO_2 aerosols (0.8 µm, 10 mg/m^3) for out excretion by the kidneys (Wang *et al*.). Titanium dioxide nanomaterials were observed to translocate into the blood, following oral or intraperitoneal exposure, and thereafter distribute to secondary targets, including the liver, spleen, lungs, and kidneys (Wang *et al*.; Johnston *et al*., 2009).

Necrosis: Focal massive necrotic degeneration was demonstrated in the renal cells of the proximal convoluted tubules of rats exposed to TiO_2 NPs (Fig. 6). This alteration was less prominent in the renal tissues of animals subjected to 126 mg/kg TiO_2 NPs for 24 h, in comparison with the ones received 252 or 378 mg/kg and rats treated for 48 h by the NPs. Moreover, this alteration was more prominent in the cortex than the medulla. Cellular degeneration may be associated with spillage of lysosomal enzymes within the cell (Del Monte, 2005). Vacuolated degeneration is an effect of particle, ions and fluid homeostasis that prompt an

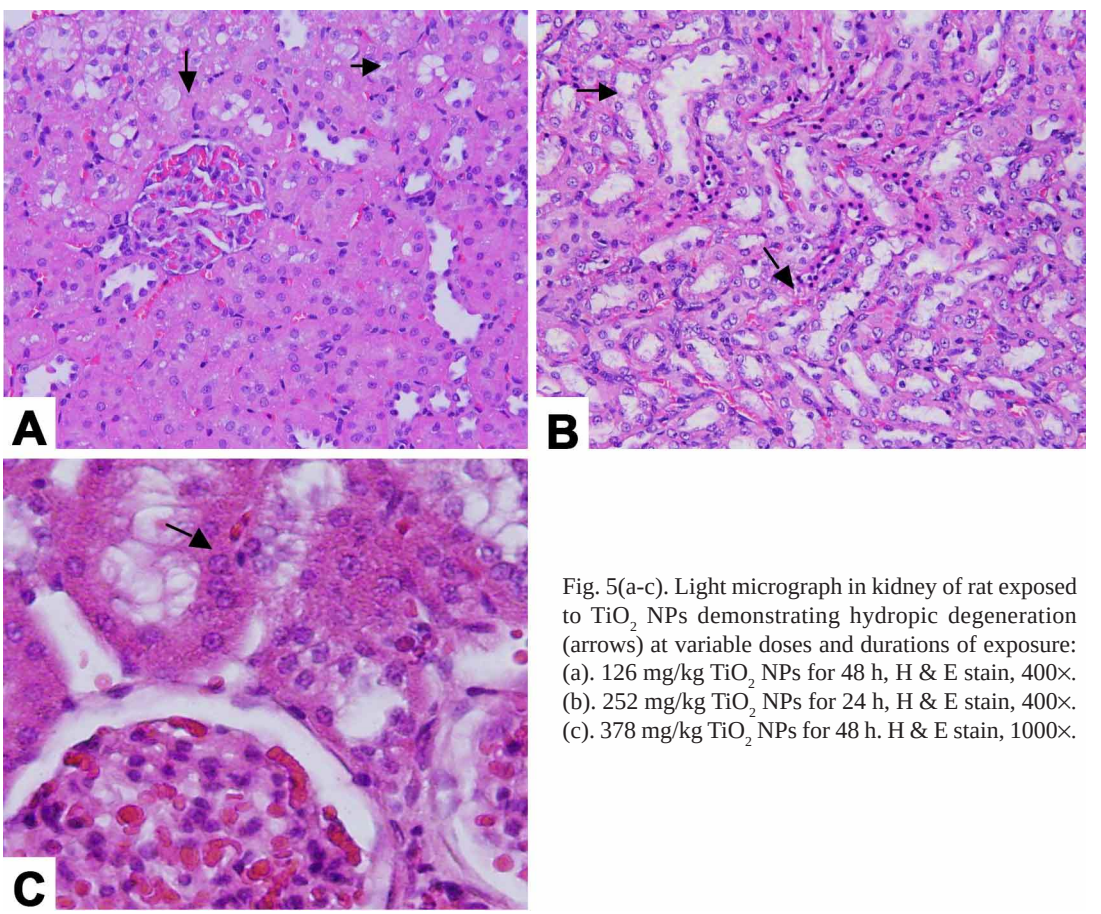

Fig. 5(a-c). Light micrograph in kidney of rat exposed to TiO_2 NPs demonstrating hydropic degeneration (arrows) at variable doses and durations of exposure: (a). 126 mg/kg TiO_2 NPs for 48 h, H & E stain, 400×. (b). 252 mg/kg TiO_2 NPs for 24 h, H & E stain, 400×. (c). 378 mg/kg TiO_2 NPs for 48 h. H & E stain, 1000×.

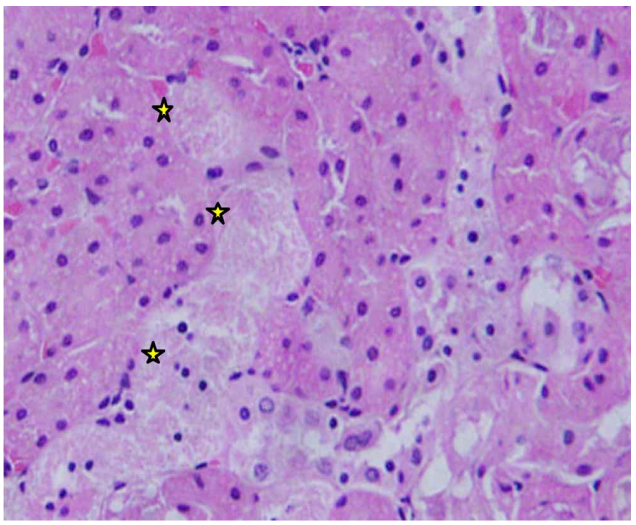

Fig. 6. Light micrograph in kidney of rat exposed to TiO_2 NPs (378 mg/kg) for 48 h demonstrating necrotic renal tubules (*). H & E stain. 600×

increasing of intracellular water (Schrand *et al*.). Moreover, the demonstrated renal necrosis might indicate oxidative stress by glutathione depletion as a result of TiO_2 NPs toxicity.

Renal Tubules Cloudy Swelling: The epithelial lining of the renal cell of rats subjected to TiO_2 NPs (252 mg/kg or more for 24 h or more) showed cloudy swelling (Fig. 7). This alteration may indicate that acute renal injury could induce disturbances of membranes function that lead to leakage and accumulation of water due to TiO_2 NPS toxicity and cytoplasmic degeneration and macromolecular crowding (Abdelhalim & Jarrar, 2011).

Renal Cells Nuclear Alterations: Some renal cells in the lining epithelia of the proximal convoluted tubule of NPs-treated rats demonstrated karyorrhexis or/and karyolysis (Fig. 8a-b). This may indicate oxidative stress induced by TiO_2 NPs exposure. Karyorrhexis and karyolysis are destructive fragmentation and complete dissolution of the chromatin matter of a necrotic or dying cell.

Hyaline Droplets and Hyaline Casts: Luminal hyaline casts and cytoplasmic droplets were demonstrated by the kidneys of rats received 378 mg/kg TiO_2 and to lesser extent in the renal tissues of those received 252 mg/kg TiO_2 NPs for 48 h (Fig. 9a-c). This damage was not seen in the renal tissues of animals exposed to 126 mg/kg TiO_2 NPs for 24 *h* or 48 h.

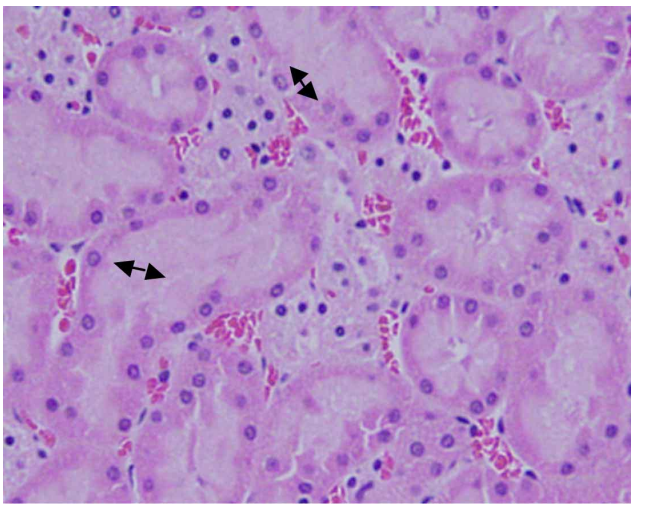

Fig. 7. Light micrograph in kidney of rat exposed to TiO_2 NPs (378 mg/kg) for 48 h showing cloudy swelling (double-headed arrows). H & E stain. 600×.

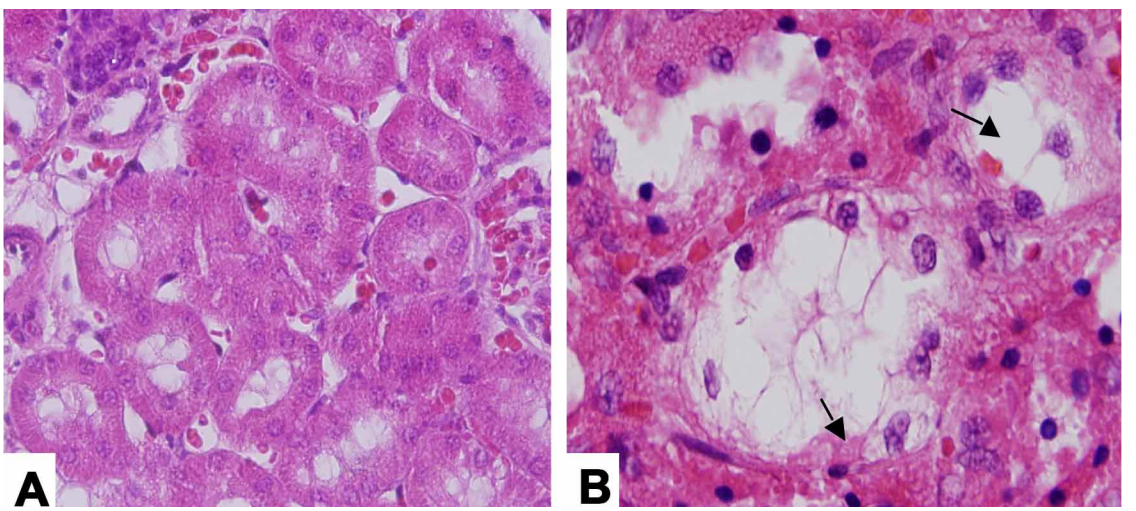

Fig. 8(a-b). Light micrograph in kidney of rat exposed to TiO_2 NPs demonstrating:(a). Karyolysis (126 mg/kg for 48 h), H&E stain. 600× (b). Karyorrhexis (arrow) (252 mg/kg for 24 h), H & E stain. 1000×.

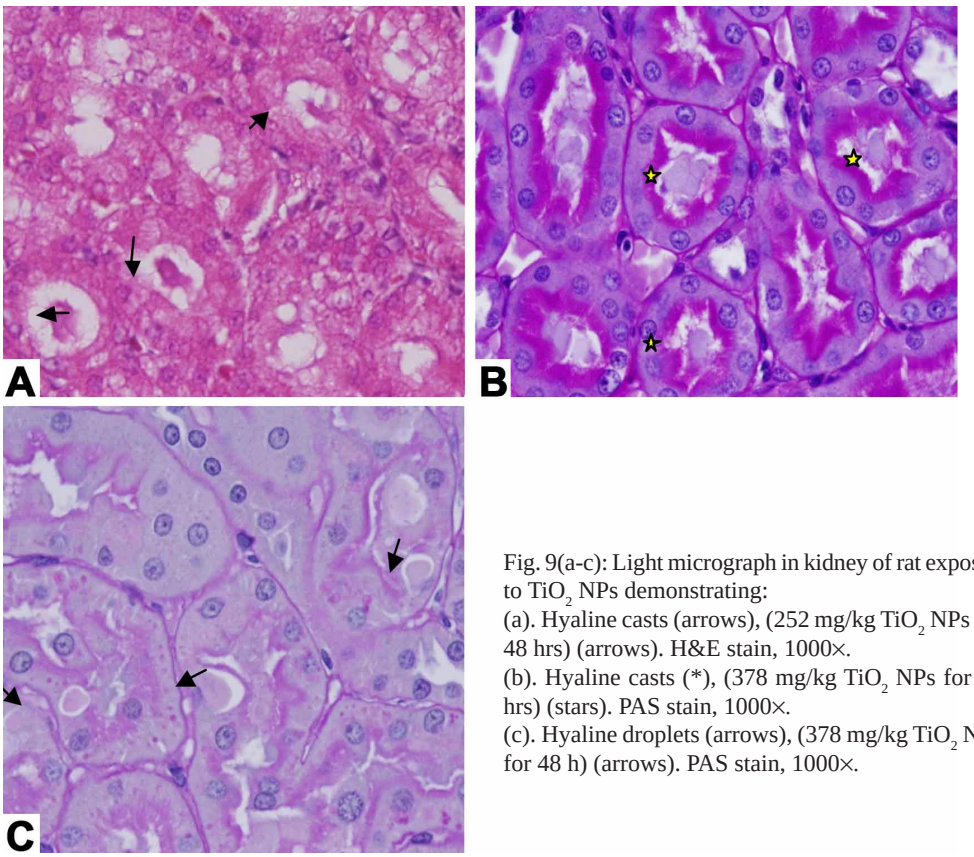

Fig. 9(a-c): Light micrograph in kidney of rat exposed to TiO_2 NPs demonstrating:
(a). Hyaline casts (arrows), (252 mg/kg TiO_2 NPs for 48 hrs) (arrows). H&E stain, 1000×.
(b). Hyaline casts (*), (378 mg/kg TiO_2 NPs for 48 hrs) (stars). PAS stain, 1000×.
(c). Hyaline droplets (arrows), (378 mg/kg TiO_2 NPs for 48 h) (arrows). PAS stain, 1000×.

Moreover, occasional hyaline casts were also seen in the lumen of some cortical distal convoluted renal tubules. The precipitations hyaline droplets in the renal cells and hyaline casts in the renal tubules lumen might be associated with protein metabolism disturbances (Abdelhalim & Jarrar).

Inflammatory Cells Infiltration: All members subjected to TiO_2 NPs for 24 *h* and more demonstrated inflammatory cells infiltration (Fig. 10a-c). This change may suggest that TiO_2 NPs may interfere with the antioxidant defense mechanism and induce oxidative stress in the renal tissue leading to induction of inflammatory response. Sutariya & Pathak (2015) reported that metallic nature of most inorganic nanoparticles could cause inflammatory cells infiltration in the tissues of vital organs. The cytotoxic potential of TiO_2 NPs is related with production of ROS that induce damage to DNA through breakage and oxidation of nucleotides (Johar *et al*., 2004).

Cortical Blood Vessels Dilatation and Congestion: In comparison with the control group, the cortex of rats exposed to TiO_2 NPs demonstrated dilatation and congestion of blood capillaries (Fig. 11). This alteration was more prominent in the renal tissues of rats exposed to 378 mg/kg TiO_2 NPs for 48 *h* than the members of the other groups subjected to 252 or 378 mg/kg TiO_2 NPs for 24 h. This alteration could be resulted from the vasodilator effect of these nanomaterials and might indicate impact in the cell membrane permeability of renal blood vessels endothelia (Johnson, 1995). On the other hand, medullar blood vessels were almost not affected by TiO_2 NPs exposure.

Edema: The kidneys of rats exposed to 378 mg/kg TiO_2 NPs revealed swelling of intertubular tissues mainly surrounding the renal blood vessels (Fig. 12). Renal edema is swelling caused by excess fluids accumulation in the intertubular tissues where he nephrones become no longer able to filter urine out of blood plasma. In addition, chronic renal tissues swelling is an indicator of plasma albumin declining and interstitial fluid accumulation that may lead to nephritic syndrome.

Fibrosis: Tubulointerstitial renal failure was demonstrated by rats exposed to 252 mg/kg and more of TiO_2 NPs (Fig. 13). This irreversible parenchymal scar is a primary cause of renal failure.

The findings of the present study indicated that the cortex and the proximal convoluted tubules were more affected by TiO_2 NPs than the medulla and the distal ones. This may indicate that more of these nanomaterials circulate and precipitate in the cortical tissues via the blood stream than that would reach the medulla.

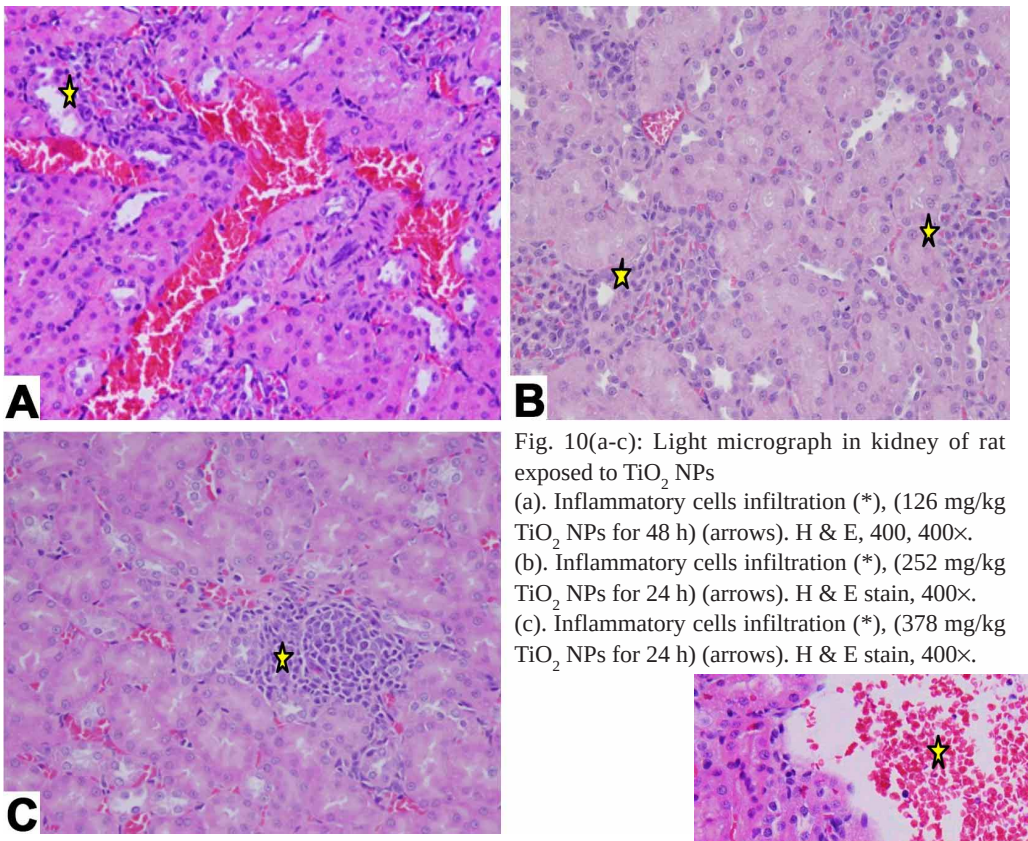

Fig. 10(a-c): Light micrograph in kidney of rat exposed to TiO_2 NPs
(a). Inflammatory cells infiltration (*), (126 mg/kg TiO_2 NPs for 48 h) (arrows). H & E, 400, 400×.
(b). Inflammatory cells infiltration (*), (252 mg/kg TiO_2 NPs for 24 h) (arrows). H & E stain, 400×.
(c). Inflammatory cells infiltration (*), (378 mg/kg TiO_2 NPs for 24 h) (arrows). H & E stain, 400×.

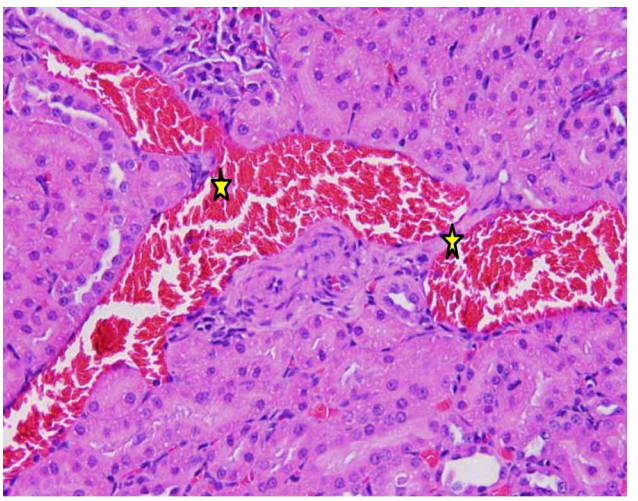

Fig. 11. Light micrograph in kidney of rat exposed to TiO_2 NPs (378 mg/kg) for 24 h demonstrating marked cortical dilation of congested blood vessels (*). H & E, 400×.

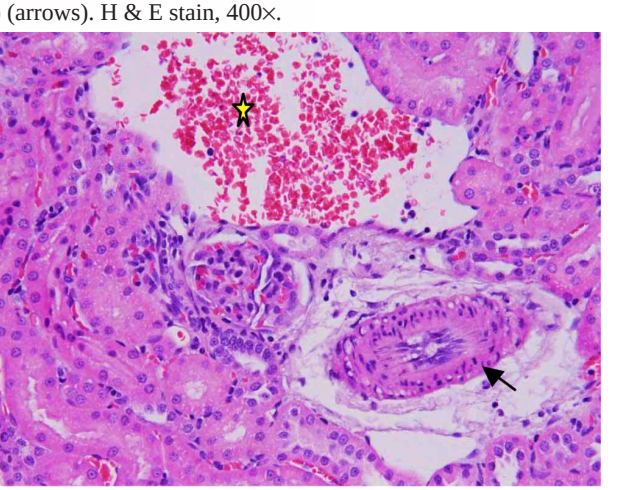

Fig. 12. Light micrograph in kidney of rat exposed to TiO_2 NPs (378 mg/kg) for 24 h demonstrating edema (arrow) with marked cortical dilation of congested blood vessels (*). H &E stain. 400×.

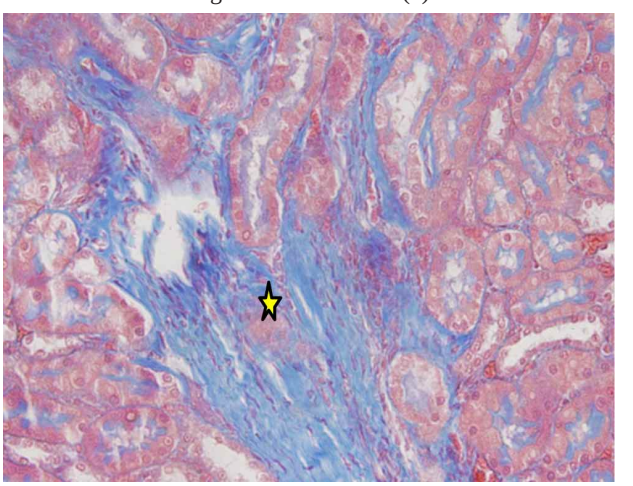

Fig. 13. Light micrograph in kidney of rat exposed to TiO_2 NPs (252 mg/kg) for 48 h demonstrating fibrosis (*). Mallory trichrome stain, 400×.

CONCLUSION

The current work demonstrated nanotoxic histological alterations after acute TiO_2 NPs exposure. One may conclude from the present study findings that these nanomaterials could cause marked histological alterations in the renal tissues affecting the functions of the kidneys and other vital organs. This renal damage might be resulted from the oxidative stress induced by these particles and the interference with the membrane structure, and macromolecules of the renal cell. More investigations are recommended on the nantoxicity of these particles.

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AL-DOAISS, A. A.; ALI, D.; ALI, B. A. & JARRAR, B. M. Alteraciones histológicas renales inducidas por la exposición aguda de nanopartículas de dióxido de titanio. *Int. J. Morphol., 37(3)*:1049.1057, 2019.

RESUMEN: Las nanopartículas de dióxido de titanio (TiO_2 NP) se usan ampliamente en muchos productos comerciales, nanomedicina, agricultura, productos para el cuidado personal, diferentes industrias y preparaciones farmacéuticas con riesgo potencial para la salud humana y el medio ambiente. El trabajo actual se realizó para investigar el daño renal que podría ser inducido por la toxicidad aguda NP de TiO_2. Un total de 40 ratas Wistar albinas adultas sanas (*Rattus norvegicus*) fueron expuestas a TiO_2 NP (126, 252, 378 mg / kg de peso corporal) durante 24 y 48 h. Las muestras de los riñones de las ratas se procesaron para estudios histológicos e histoquímicos. En comparación con las ratas control, la exposición de las ratas a TiO_2 NP presentaron las siguientes alteraciones glomerulares, tubulares e intersticiales: congestión glomerular, dilatación de la cápsula de Bowman, inflamación glomerular, túbulos renales aumentados, cariorrexis, cariólisis, infiltración de células inflamatorias, congestión, necrosis, degeneración hidrópica, dilatación y congestión de vasos sanguíneos, gotas y precipitaciones hialina, edema intersticial y fibrosis. A partir de los hallazgos del trabajo actual, se puede concluir que las NP de TiO_2 son capaces de inducir daño renal con más aislamiento en la corteza y en los túbulos contorneados proximales que en la médula y los túbulos contorneados distales, respectivamente. Además, se podría concluir que el daño renal inducido por estos nanomateriales depende de la dosis y la duración de la exposición. Se recomiendan estudios adicionales hematológicos, bioquímicos, inmunohistoquímicos y ultraestructurales.

PALABRAS CLAVE: Nanopartículas de TiO_2; Tejidos renales; Alteraciones histológicas; Degeneracion hidrópica; Nanotoxicidad.

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Corresponding author:
Prof. Bashir M. Jarrar
Department of Biological Sciences
College of Science
Jerash University
Jerash 26150
JORDAN

Email: bashirjarrar@yahoo.com

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