Jordan Journal of Pharmaceutical Sciences, Volume 18, No. 4, 2025

Effect of Titanium Dioxide Nanoparticles on the structure of Pituitary Gland in adult Male Albino Rats

Hagar Wahdan Mohamed El- Azab [1]*, Laila El-sayed El- Moselhy [1], Zienab Mahmoud Gebaly[2], Fatma Al-Zhraa Abd -Allah H. Aziz[2]

[1] Department of Histology, Damietta Faculty of Medicine (for Girls), Al-Azhar University, Damietta, Egypt
[2] Department of Histology, Faculty of Medicine (for Girls), Al-Azhar University, Cairo, Egypt

ABSTRACT

Study objectives: It was to observe the effects on pituitary gland exposed to TiO_2-NPs in matured male albino rats.
Methodology: Forty-eight male albino rats were utilized. They were divided into three main groups. Group I (control) and was subdivided equally into three subgroups (negative control and vehicles controls). Group II (the treated group) subdivided into three equal subgroups. GII a: treated with TiO_2 solution (10 mg/kg/day orally) for 14 days. Group IIb: treated with TiO_2 solution (10 mg/kg/day) orally for 60 days. Group IIc (recovery): treated with TiO_2 10 mg/kg/day for 60 days, then stopped treatment for 60 days. Group III was divided equally into two subgroups. GIII a: 6 rats treated with TiO_2 solution (100 mg/kg/day) orally for 14 days. GIII b (recovery): 6 rats treated with TiO_2 (100 mg/kg/day) for 14 days, then stopped treatment with TiO_2 for 14 days. Pituitary gland specimens were prepared and examined by light and electron microscopy. Data of morphometric study was documented.
Results: TiO_2 treated groups showed structural disorganization in the pars distalis (enlarged basophil cells with vacuolated cytoplasm and pyknotic nuclei) associated with congested blood vessels and inflammatory cellular infiltration. A significant reduction of Periodic-Acid-Schiff reaction coupled with a substantial increase in area percentage of collagen fibres was observed upon TiO_2-NPs. The ultrastructural assessment confirmed these distortions. The recovery groups showed different degrees of improvement in previous histological changes.
Conclusions: TiO_2NPs cause time and dose-dependent structural changes in the pars distalis of the anterior pituitary gland with various degrees of distortions.
Keywords: TiO_2-nanoparticles; rat; pars distalis, Transmission electron microscopy.

INTRODUCTION

Recently, nanotechnology has developed rapidly in different sectors to improve human life leading to the production of several nanoparticles which developed and used in various fields, including medicine (e.g., cancer therapy) (1,2), industry, food (3), personal health care (4), drug delivery (e.g., Pulmonary Delivery of Docetaxel) (5), toothpaste, food packaging, and antimicrobial agents (6).

Titanium dioxide N.P.s (TiO_2 -N.P.s) are the most commonly manufactured worldwide (7). It is a bright white pigment widely used in cosmetics, plastics, ceramics, paints, inks, pharmaceuticals, toothpaste, tableted drugs, and even for whitening skim milk and brightening foods (8).

Despite widespread applications, studies have reported that upon exposure to humans, TiO_2 NPs accumulate in the lungs, alimentary tract, liver, heart,

*Corresponding author: Hagar Wahdan Mohamed El- Azab
Hagarwahdan24@gmail.com
Received: 06/07/2024 Accepted: 31/10/2024.
DOI: https://doi.org/10.35516/jjps.v18i4.2919

spleen, kidneys, and cardiac muscle and induce oxidative damage, genotoxicity, and apoptosis in these tissues (9).

The Pituitary gland, the "master endocrine glands, is an intermediary organ for physiological signal exchanges between the hypothalamus and the peripheral organs (10). It is divided into two parts, the adenohypophysis, which comprises the pars distalis, pars intermedia, pars tuberalis, and the neurohypophysis, which includes the pars nervosa, infundibular stem, and the median eminence" (11).

Previous studies were performed to delineate the toxic effects of TiO2NPs on many organs. However, the impact on the pituitary gland was not well established despite its prominent role in controlling endocrine homeostasis. The current research was conducted to observe the alterations in tissues of pituitary gland in adult male albino rats after oral ingestion of TiO_2NPs at different durations and doses.

MATERIALS AND METHODS

Animals

The present study was conducted on forty-eight adult male albino rats (200-250 gm and 8-10 months old). They were handled according to the guidelines and ethics of the animal protocol of the faculty of science at Damietta University. They were housed in well-ventilated stainless-steel cages at room temperature and 12 hours of light/ dark cycle with strict care and hygienic measures. All rats were freely provided with water and rat chow.

Chemicals

Titanium Dioxide Nanoparticles (TiO_2NPs): White, odorless fine anatase Nanopowder, with a particle size of < 25 nm. It was purchased from acmatic chemicals company (Egypt).

Preparation of treated suspension: Titanium dioxide N.P.s were suspended in normal saline (0.9 % NaCl solution) at a concentration of 1 mg/ml, and that suspension was sonicated for 10 min before the treatment (12).

Nanoparticle characterization

The particle size and morphology (Figure 1.) were detected using transmission electron microscopy in which the aqueous dispersion of the nanoparticles was drop-cast on a carbon-coated copper grid (13). The grid was air dried at room temperature and visualized using (JEOL JEM -1010) Transmission Electron Microscope (Jeol Ltd., Tokyo, Japan) at the regional center for mycology, Al-Azhar University, Cairo, Egypt.

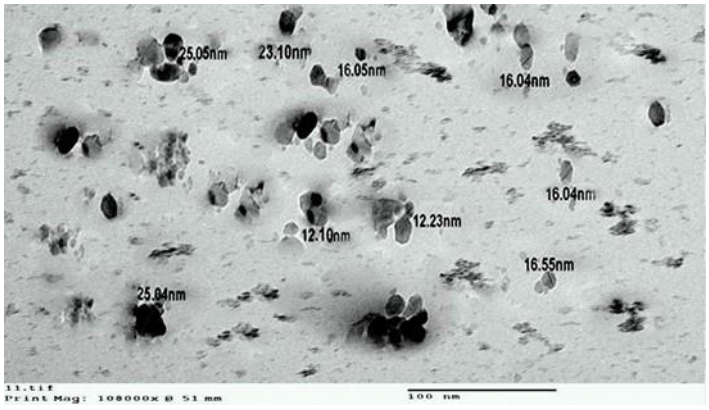

Figure 1.

Design of Experiment

Animals used in this study were segmented into three groups.

Group I (GI.): included 18 rats and served as a control and were subdivided into three equal subgroups:

1. GIa (6 rats): untreated control group being kept without treatment.
2. GIb: (6 rats): each rat received 1 ml of saline (saline was used as a vehicle to dissolve TiO_2NPs) by using oral gavage once daily for 14 days.
3. GIc: (6 rats): each rat received 1 ml of saline using oral gavage once daily for 60 days.

Group II (GII.): Included *18* rats and were further subdivided into three equal subgroups:

1. GIIa (6 rats): were treated with TiO_2NPs solution in a dose of (10 mg/kg b.w/ /day) orally once daily by gastric tube for 14 days (14).
2. GIIb (6 rats): were treated with TiO_2NPs solution in a dose of (10 mg/kg b.w/ /day) orally once daily by gastric tube for 60 days (15).
3. GIIc (6 rats): were treated with TiO_2NPs solution in a dose of (10 mg/kg b.w/ /day) given orally once daily by gastric tube for 60 days, then stopped receiving treatment for 60 days (first recovery group).

Group III (GIII): Included 12 rats and were further subdivided into two equal subgroups:

1. GIIIa (6 rats): were treated with TiO_2NPs solution in a dose of (100 mg/kg b.w/ /day) given orally once daily by gastric tube for 14 days (16).
2. GIIIb (6 rats): were treated with TiO_2NPs solution in a dose of (100 mg/kg b.w/ /day) given orally once daily by gastric tube for 14 days, then stopped receiving treatment for 14 days (second recovery group).

The animals were anaesthetized by ether inhalation at the end of the experiment. Pituitary glands were extracted from the skulls and then fixed by immersion in 10% neutral buffered formalin for 24 hours, then processed for light microscopic examination. Specimens processed for transmission electron microscopic study were also collected from the pituitary gland of one animal from each group. Special care was taken to obtain the samples while the animals were still alive under ether inhalation. Tiny pieces of the pituitary gland were collected and immediately fixed by immersion in 5 % glutaraldehyde in 0.1 M. sodium cacodylate buffer, pH 7.3 at 0 -4ºC for 8 hours.

Histological study

Light microscopy: In 10 % neutral formalin, Pituitary glands samples were fixated, and then desiccated in ascending grades of ethanol, cleared in xylene, impregnated and implanted in paraffin wax. Sections (5 μm thickness) were stained with Hematoxylin and eosin (H&E) to study the general structure, Masson's trichrome stain for staining the collagen fibers and P.A.S. for identification of basophils (17).

Transmission electron microscopic study (TEM): Small pieces of pituitary gland were processed for transmission electron microscopy. Pieces were embedded in an epoxy resin mixture. Ultra-thin sections (80-90 nm) were "stained with uranyl acetate and lead citrate (18). The sections were examined and photographed by JEOL 100S Transmission Electron Microscope (Jeol; Tokyo, Japan) at the Histology Department, Faculty of Medicine (Girls), Al-Azhar University."

Morphometric measurements

All sections were examined by Leica light microscope MDLSD coupled to a Leica digital camera transferred to the screen using a computerized image analyzer Leica Q500 MC program (Leica Microsystems Ltd, Cambridge, UK). Ten different non-overlapping randomly selected fields from a slide of each rat in all other experimental groups were examined to evaluate the following:

- Area percentage of collagen fibers in Masson trichrome stained sections.
- The optical density of PAS-positive reaction in PAS-stained sections.

Statistical Analysis

All statistical data were analyzed using Statistical Program for Social Science, version 20 (IBM® Inc.,

Armonk, Illinois, USA). Statistical significance was determined by one-way analysis of variance for differences between the means of different groups. Further analysis was carried out using Tukey's posthoc test to compare the parameters in the other groups. All data were expressed as mean ± Standard Deviation (mean ± SD), and a probability of $P < 0.05$ was considered statistically significant.**RESULTSLight microscopic results Hematoxylin and Eosin (H&E) staining***Control groups (G.I.)*

Apart from minor differences, the general structure of the pituitary gland of all control rats subgroups (GIa, GIb & GIc) showed more or less the same structure and ultrastructure of the gland irrespective received saline for both periods of 14 days or 60 days or not treated. The pars distalis was formed of clumps of secretory cells (chromophobes and chromophiles) separated by capillary sinusoids. The parenchyma of the pars distalis was composed of clusters of glandular cells separated by fenestrated blood capillaries. The chromophiles were generally larger in size, had a granular cytoplasm, and were subdivided into basophils and acidophiles according to the staining affinity of their cytoplasmic granules. The basophils were fewer in number and more extensive than the whole granular cells in the sections. They were rounded in shape and had a basophilic cytoplasm with rounded vesicular eccentric nuclei. The acidophiles were more numerous and smaller in size than basophils. They were rounded with highly acidophilic cytoplasm and spherical vesicular eccentric nuclei. Chromophobes were seen with the large rounded nucleus and pale stained cytoplasm (No secretory granules**) (**Figure 2a**)**.

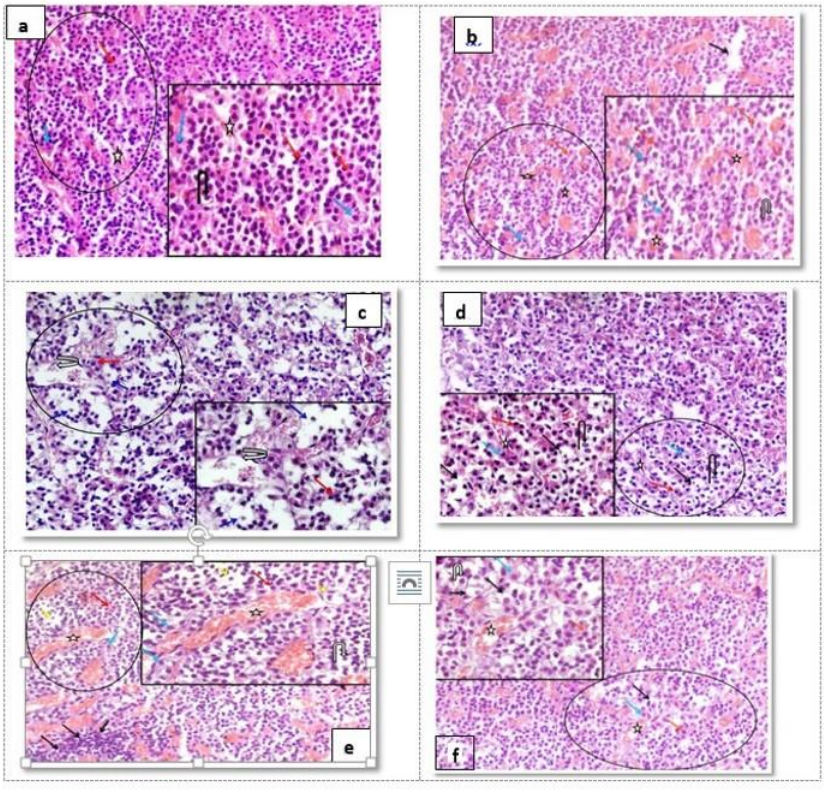

Figure 2a

Group II (TiO2 treated with a dose of 10 mg/kg b.w./day) groups

- *GIIa* (Treated with Ti_{O2} 10 mg/kg b.w./day orally once daily for 14 days): Sections stained with H&E, the pars distalis appeared as irregular cords of cells separated by dilated blood capillaries engorged with blood (Figure 2b).

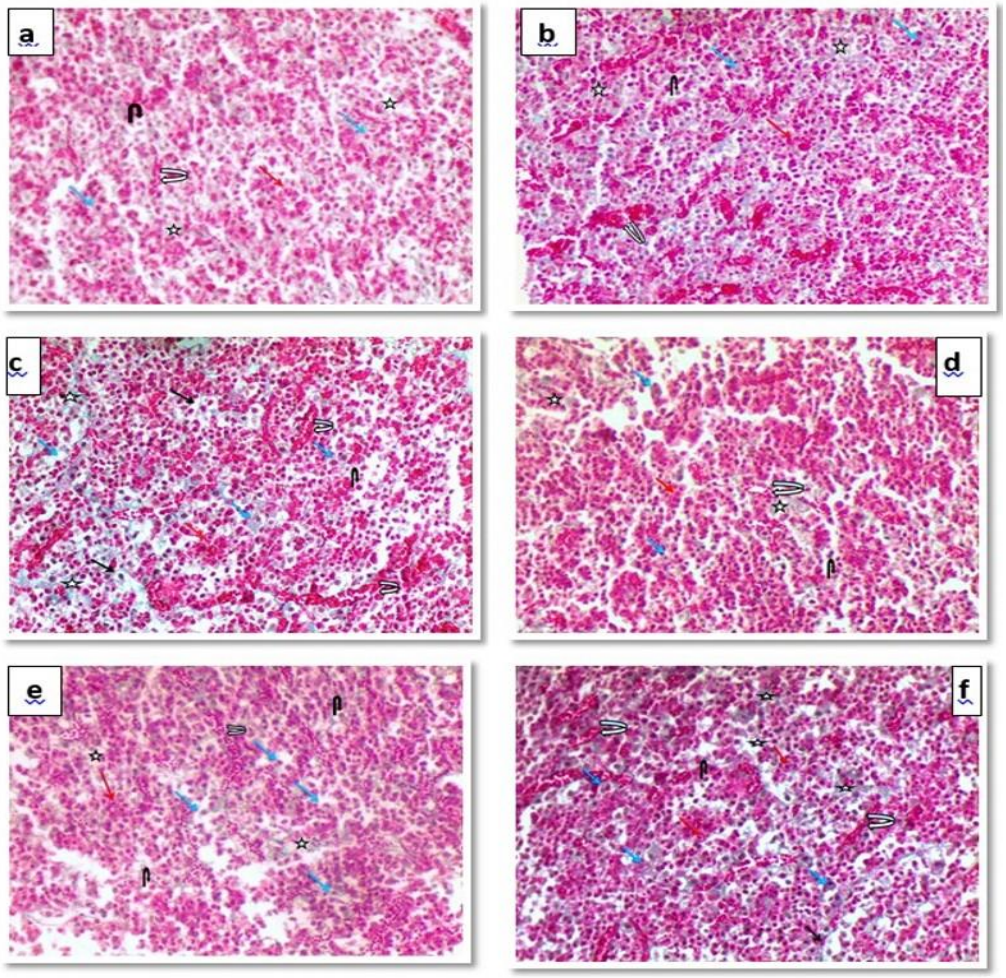

Figure 2b

- *GIIb* (Treated with Ti_{O2} 10 mg/kg b.w./day orally once daily for 60 days): Sections stained with H&E led to noticeable histological changes in the pars distalis. The basophils appeared hypertrophied with pale vacuolated cytoplasm and eccentric nuclei. Acidophils and chromophobes are also seen. The blood capillaries appeared dilated and engorged with blood in the presence of a multilocular cyst, its wall lined by cells and filled with basophilic material (Figure 2c).

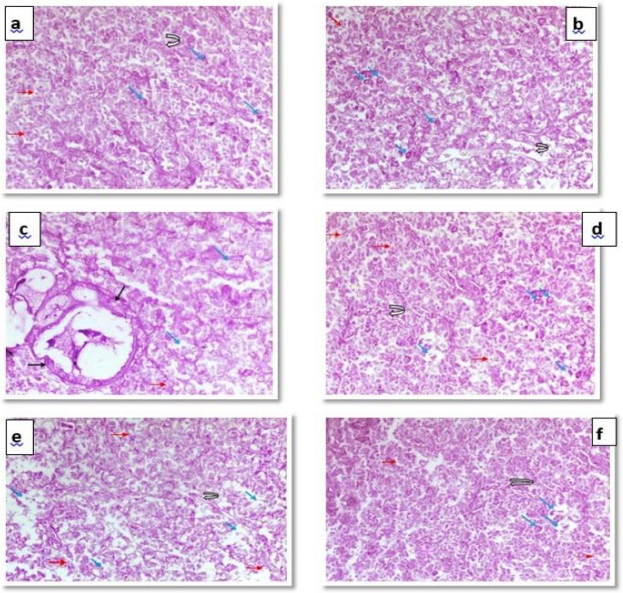

Figure 2c

- *GIIc (Recovery)* (Treated with Ti_{O2} 10 mg/kg b.w./day orally once daily for 60 days, then stopped Ti_{O2} for 60 days): Sections stained with H&E showed partial improvement in the histological structure of its cells. Some basophils appear with pale vacuolated cytoplasm and eccentric nuclei, while others appear normal. Acidophilus and chromophobes are arranged in groups separated by blood sinusoids (Figure 2d)

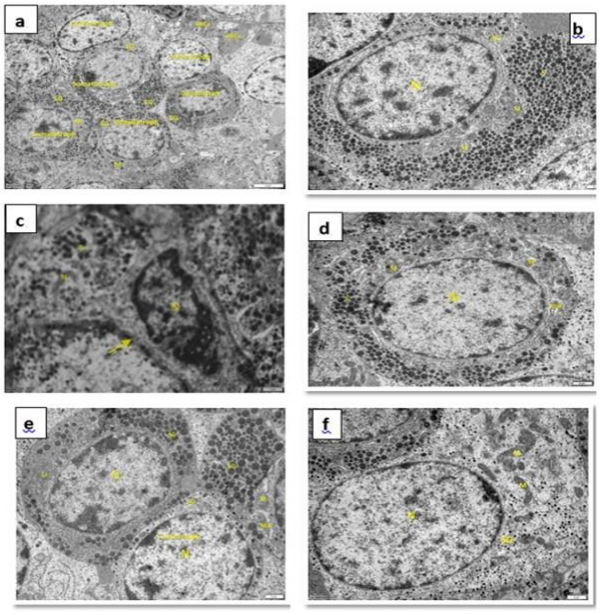

Figure 2d

Group III (TiO2 treated with a high dose of 100 mg/kg b.w./day) groups

- *GIIIa* (Treated with TiO_2 100 mg/kg b.w./day orally once daily for 14 days): Sections stained with H&E led to noticeable histological changes in the pars distalis. In general, the cells showed disturbance in their cytoplasm's architecture, shape, size, and staining characteristics. The basophils appeared hypertrophied with pale vacuolated cytoplasm and pyknotic eccentric nuclei with dilated engorged blood capillaries and lymphocytic infiltration (Figure 2e).

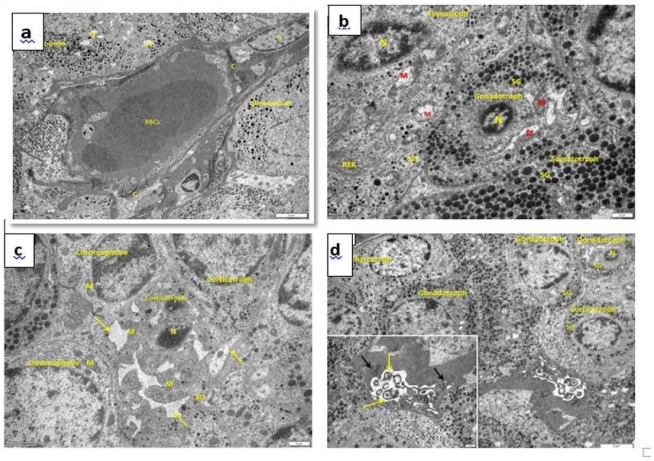

Figure 2e

- *GIIIb (Recovery)* (Treated with Ti_{O2} 100 mg/kg b.w./day orally once daily for 14 days, then stopped Ti_{O2} for 14 days): H&E sections showed partial improvement in the histological structure of its cells. Some basophils appear with pale vacuolated cytoplasm and eccentric nuclei, while others appear normal. Acidophils and chromophobes are arranged in groups separated by blood sinusoids (Figure 2 f).

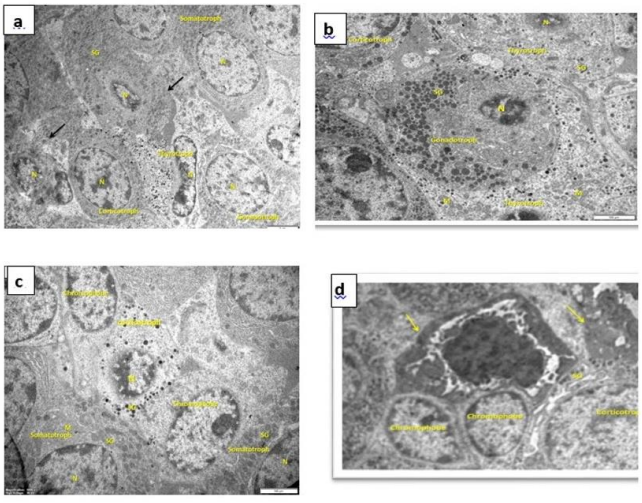

Figure 2 f

Masson trichrome staining

Sections stained with Masson trichrome stain of the control group revealed the acidophiles are red, the basophils are green, and chromophobes will be faded to grey. Note that red blood cells appear red. The green fine collagenous fibres sharply outline the cords and clumps of epithelial cells (Figure 3a**).** In GIIa, stained sections revealed a mild increase in collagen fibres (Figure 3b). GIIb (TiO2 treated for 60days) revealed favorable to moderate growth in collagen fibers (Fig.3c). GIIc (Recovery) showed mild increase in collagen fibers (Figure 3d). In GIIIa (TiO2 treated for 14 days) revealed mild to moderate increase in collagen fibers (Fig.3e), while in GIIIb (Recovery) revealed mild increase in collagen fibers (Figure 3f).

Periodic Acid Schiff (P.A.S.) staining

Sections stained with the control group's P.A.S. revealed numerous secretory cell cords. Positive intracellular reactions are present in the basophils, while acidophiles showed P.A.S. negative reaction separated by blood sinusoids (Figure 4a). GIIa sections revealed basophils P.A.S. positive reaction, acidophiles showed P.A.S. negative reaction. (Figure 4b), while GIIb sections revealed diminished intracellular reaction in basophils while acidophiles showed P.A.S. negative reaction with the presence of multilocular cyst containing P.A.S. +ve material (Figure 4c). In GIIc (Recovery): In P.A.S. stained sections, some positive intracellular reaction is present in the basophils while others showed faint positive reaction. Acidophiles showed negative reactions to P.A.S. (Figure 4d). In GIIIa (TiO2 treated for 14 days), sections revealed diminished intracellular reaction in basophils with excess vacuolar degeneration while acidophiles showed P.A.S. negative reaction (Fig.4e), while GIIIb (Recovery): some positive intracellular reactions are present in the basophils while others showed faint +ve reaction. Acidophiles showed a negative reaction to P.A.S. (Figure 4f).

Electron microscopic examination

Group I (Control group):

Electron microscopic results of all specimens of the control groups showed the typical structure of pars distalis of the anterior lobe of the pituitary gland. It showed clusters of two central populations of cells, chromophobes and chromophils (Figure 5a).

Chromophils had specific secretory granules. The granules of each cell type had a distinctive size, shape and electron density by which the different cell types can be detected with electron microscopy. Somatotrophs were the most normal cells that formed the main bulk of the granular cells of pars distalis. Their nuclei were spherical eccentric. The mitochondria and R.E.R. were poor as the cells were packed with secretory granules. Their secretory granules were multiple, extensive and spherical and occupied all cytoplasm (Figure 5b). Mammotrophs (Lactotrophs) granules were electron-dense, a dumbbell in shape (Figure 5c). Gonadotrophs were rounded cells with central nuclei, contained cisternae of R.E.R. in the cytoplasm, mitochondria. Their secretory granules were spherical and variable in their density (Figure 5d). Corticotrophs were medium-sized elongated cells with large central nuclei and mitochondria. They had dense, uniform, and rounded granules under the plasma membrane (Figure 5e) Thyrotrophs were large elongated-shaped cells with long mitochondria, eccentric oval nuclei, tiny secretory granules, equal in size (Figure 5f).

Group II (Treated with TiO_2 10 mg/kg b.w./day)

- *GIIa* (Treated with TiO2 dose 10 mg/kg b.w./day for 14 days): at the electron, microscopic level, sections of pars distalis revealed no abnormality detected in the chromophils, but there are dilated congested blood sinusoids (Figure 6a)
- *GIIb* (treated with TiO_2 10mg/kg b.w./day for 60 days): Variable changes in the gonadotrophs, corticotroph and thyrotroph. Some gonadotrophs had irregular eccentric condensed nuclei, dilated cisternae of R.E.R. and variable secretory granules in their cytoplasm.

Thyrotroph were irregular in shape, with a small condensed nucleus, small secretory granules and rounded vacuolated mitochondria (Figure 6b). Corticotrophs showed variable apoptotic changes as the condensed small nucleus, vacuolated mitochondria and few secretory granules under the cell membrane (Figure 6c).

- *GIIc (Recovery)* (Treated with TiO_2 10mg/kg b.w./day for 60 days, then stopped TiO_2 for 60 days): Different secretory cells as somatotroph, corticotroph, thyrotroph appeared more or less similar to the standard structure, but myelin like figure and autophagic vacuoles were present (Figure 6d).

Group III (Treated with high dose $TiO_2$100 mg/kg b.w./day)

- *GIIIa* (Treated with TiO_2 100mg/kg b.w./day for 14 days): Different cell types were difficult to identify with the electron microscope. Some secretory cells showed few non-specific granules and small nucleus with more condensed chromatin. In contrast, some secretory cells can be detected and differentiated from each other by their characteristic granules (Figure 7a). Gonadotroph revealed shrunken apoptotic nucleus with more condensed chromatin. Most thyrotrophs revealed cytoplasmic changes in the form of the shrunken nucleus, cytoplasmic vacuolations, degenerated mitochondria and a decrease in the number of secretory granules (Figure 7b). Corticotrophs were affected to a large extent as they had shrunken condensed nucleus with more thickened chromatin and few spread secretory granules (Figure 7c).
- *GIIIb* (Treated with Ti_{O2} 100mg/kg b.w./day for 14 days, then stopped treatment with Ti_{O2} for 14 days): some types of secretory cells as somatotroph, corticotroph, thyrotroph and chromophobe around R.B.C.s appeared typical structure. Apoptotic changes appeared in the irregular nucleus, and rarefied cytoplasm occurred in some cells. Few corticotrophs showed dilated R.E.R., mitochondria and secretory granules under the cell membrane (Figure 7d).

Statistical and Morphometric Results

Statistical analysis of collagen fibers percentage in all the experimental groups revealed that the highest mean value of collagen fibers percentage recorded among TiO_2 treated group (GIIIa) followed by (GIIb) (29.4±0.64 and 25.01±2.01 respectively) and showed significant increase when compared to all other groups (P<0.05). The least mean value was recorded among groups (IIa, IIc& IIIb) and showed a significant decrease in the area% of collagen fibers when compared to (GIIb and GIIIa) and showed significant increase when compared with the control group (13.4±1.03, 13.5±1.43 and 13.4±1.03 respectively) (P<0.05) Table (1)

Table 1. One-way ANOVA followed by Tukey's post-hoc test of the mean values of the area percentage of collagen fibers in rats of different experimental groups.

Changes in the percentage of collagen fibers	Group (I) (n=6)	Group (II)			Group (III)	
		Group (IIa) (n=6)	Group (IIb) (n=6)	Group (IIc) (n=6)	Group (IIIa) (n=6)	Group (IIIb) (n=6)
Min.-Max.	6.26-12.63	12.15-14.85	22.08-27.66	11.97-15.48	28.66-30.21	12.15-14.86
Mean± S. D	10.1±2.17	13.4±1.03■□	25.01±2.01▲□	13.5±1.43■□	29.4±0.64▲□	13.4±1.03■□
P value		0.001*	<0.001*	0.001*	<0.001*	0.001*

S.D: standard deviation; n: number of animals
▲ A significant increase compared with all other groups.
■ A significant decrease compared with GIIb and GIIIa.
P: p-value for comparing the control group and other groups
*: Statistically significant at P <0.05

Statistical analysis of changes in the optical density of P.A.S. reaction in all the experimental groups revealed that the highest mean value of the optical density of P.A.S. positive reaction was recorded among recovery group (GIIIb) followed by (GIIc & GIIa) (0.31±0.05, 0.30±0.03 and 0.28±0.06 respectively) and showed significant increase when compared to (GIIb and GIIIa) (P<0.05) and no statistically significant differences when it compare with control group. The least mean value was recorded among groups (GIIb & GIIIa) and showed significant decrease in the optical density of P.A.S. positive reaction when compared to (GIIc & GIIIb) and when compared with control group (0.22±0.09 and 0.25±0.03 respectively) (P<0.05). Table (2).

Table 2. One-way ANOVA followed by Tukey's post-hoc test of the mean values of the optical density of PAS reaction in rats of different experimental groups

Changes in the optical density of PAS reaction	Group (I) (n=6)	Group (II)			Group (III)	
		Group (IIa) (n=6)	Group (IIb) (n=6)	Group (IIc) (n=6)	Group (IIIa) (n=6)	Group (IIIb) (n=6)
Min.-Max.	0.27-0.39	0.21-0.37	0.11-0.33	0.25-0.33	0.22-0.30	0.22-0.36
Mean± S.D	0.33±0.05	0.28±0.06▲	0.22±0.09▪□	0.30±0.03▲	0.25±0.03▪□	0.31±0.05▲
P value		0.112	0.001*	0.268	0.015*	0.398

P: p-value for comparing the control group and other groups
▲ A significant increase compared with all other groups.
▪ significant decrease compared with (GIIc and GIIIb).
□ A significant decrease compared with the control group (GI)
*: Statistically significant at P <0.05

DISCUSSION

The toxic impact of different N.P.s on the practical systems, including the hepatic, renal, digestive, pulmonary, hematological, cardiovascular, reproductive, nervous, and immune systems, has been previously studied (19).

Owing to their use in a wide range nowadays, toxic drawbacks of TiO_2NPs should be explored. No previous studies have discussed the effects of these nanoparticles on the pituitary gland, so we focused in the present study on the histological changes that may occur in the cells of the pituitary gland in adult male albino rats after exposure to TiO2NPs at different doses and durations via the oral route.

Gastrointestinal exposure of N.P.s through ingestion is a vital absorption pathway. N.P.s can be absorbed in the gastrointestinal tract and then enter into the blood, therefore quickly reaching the secondary organs and accumulating there (20).

The selected doses of TiO_2 NPs, in agreement with the experiments of previous studies (12, 16, 21). We used at the doses of 10 and 100 mg/kg at different time durations of 14 days for sub-chronic study and 60 days for chronic study.

In the present study, the animals in all groups survived. This result was supported by Ranjan *et al.* (22) who found no mortalities were observed in the TiO_2NPs administered to rats at a dose of 100mg/kg.bw after 14 days orally.

In the present study, TiO2-NPs orally given at a dose of 10mg/kg.bw/day for 14 days (GIIa) to rats were found to the pars distalis to appear as irregular cords of cells separated by dilated blood capillaries engorged with blood. As regards Masson's trichrome staining revealed a mild increase of collagen fibers. P.A.S. results revealed lines of secretory cells, and numerous positive intracellular reactions are present in the basophils, while acidophiles showed P.A.S. adverse reactions separated by

blood sinusoids. Ultrastructural sections of pars distalis revealed no abnormality detected in the chromophiles, but there are dilated congested blood sinusoids.

The results as mentioned above were consistent with previous researchers El-Azab et al. (23) who found minimal histological changes in TiO2 low-dose exposure. Shah et al. (24) and Wu & Tang (25) demonstrated that TiO2 -N.P.s explicit dose-effect relationship and does not show any toxicity until the exposure concentration reaches a certain threshold. Abbott Chalew & Schwab (26), and Hu et al. (27) revealed that the duration and doses directly determine the results of the biological responses. Gunawan et al. (28) showed that a low amount of nanomaterial could be successfully adapted by cells by attracting the lipid redistribution and antioxidant defenses processes. Younes et al. (29) stated that low doses of TiO_2 revealed no cytotoxicity.

In the current study, oral administration of TiO_2 NPs in 10 mg/kg b.w./day after 60 days (GIIb) led to noticeable histological changes in the structure of the pars distalis. The basophils appeared enlarged with pale vacuolated cytoplasm and eccentric nuclei without modification in the structure of acidophiles and chromophobes. The blood capillaries appeared dilated and engorged with blood. There was a mild to moderate increase of collagen fibers, especially in the interstitial tissue and around blood vessels, revealed by Masson trichrome stain. Also, ultrastructural sections of pars distalis revealed variable changes in the gonadotrophs, corticotrophs, and thyrotrophs. Some gonadotrophs had an irregular eccentric heterochromatic nucleus, cytoplasmic vacuolations and variable secretory granules in their cytoplasm. Thyrotrophs were irregular in shape and had shrunken heterochromatic nuclei, small secretory granules and degenerated swollen mitochondria. Corticotrophs showed variable apoptotic changes as shrunken heterochromatic nuclei, swollen degenerated mitochondria and few secretory granules under the cell membrane.

These results agree with Hong *et al.* (30) who demonstrated that intragastric administration of TiO_2 nanoparticles in a dose of 10mg/kg to male mice for 60 consecutive days resulted in lesions of testis and epididymis, decreases in sperm number and motility. Moreover, Sang et al. (31) showed spleen injury and alteration of cytokine expression in a 90-day study on the exposure of rats to TiO_2NPs.

Due to the production of high levels of reactive oxygen species (R.O.S.), which reduces cell viability and stimulates the cytotoxicity via an apoptotic process can be relatable to oxidative stress (32,33). Thus, the toxicity of TiO_2-NPs is probably correlated to the surface chemistry of the particles, which affects the inflammatory responses and the release of Tumor Necrosis Factor- α (TNF-α) and neutrophil-attracting chemokines (32, 34) and the rate of release is size and time-dependent (25).

Shakeel *et al.* (35) and Hassanein & El-Amir, (36) stated that TiO_2NPs could produce reactive oxygen species accumulated in organs, cause an imbalance of the oxidation antioxidant system, and lead to oxidative damage in animal tissues. "The high R.O.S. levels lead to D.N.A. damage, protein oxidation, and lipid peroxidation. The release of lipid peroxidation products such as Malondialdhyde (M.D.A.) and 4-hydroxy-2-nonenal (4-HNE) induces inflammation and apoptotic cell death [34]. Also, the detected cellular death was presumed to be due to the mitochondrial (intrinsic) pathway of apoptosis, resulting from cellular stresses."

Masson's trichrome staining revealed a mild to moderate increase of collagen fibers. Such finding can be explained as a consequence of chronic inflammation, which stimulates fibroblasts proliferation and excess deposition of extracellular matrix, similar to the determination of Salem *et al.* (38) and Wright et al. (39) who reported that TiO_2NPs stimulate a cascade of cellular reactions leading to oxidative stress; decreased cell proliferation and increased cytokine production. Omar & Kamar (40) reported that accumulating nanoparticles in

the pituitary gland reduced basophil content in PAS stain. Chavhan& Dhamani (41) stated that the cytoplasmic vacuolations are due to dilatation of cisternae of rough endoplasmic reticulum (RER). Abdel-Aziz & El Haliem (42) stated that the dilated cisternae of R.E.R. represent hyperfunction which could result from the negative feedback mechanism secondary to cellular damage of the target organs that led to overstimulation of the pituitary gland. The accumulated hormones in the R.E.R. might undergo lysis and/or release in the blood, which increases their serum level.

The previous findings agree with Tassinari *et al.* (43), who reported that using TiO_2NPs leads to testicular, thyroid, and adrenal gland dysfunction. Bonora *et al.* (44) stated that nanoparticles induce mitochondrial damage. Nanoparticles affect mitochondrial permeability transition pore through chemicals attached to the particles. Opening the transition pores leads to rupturing the mitochondrial membrane, releasing cytochrome C, inhibiting oxidative phosphorylation, and swelling of the mitochondria.

In current work, oral administration of TiO_2 NPs in a dose of 100 mg/kg b.w./day after 14 days (GIIIa) showed noticeable histological changes in the structure of the pars distalis. In general, the cells showed disturbance in their cytoplasm's architecture, shape, size, and staining characteristics. The basophils appeared enlarged with pale vacuolated cytoplasm and eccentric nuclei. There were lymphocytic infiltration and loss of standard architecture. Some cells showed many vacuolations. The blood capillaries dilated and engorged with blood. There was a mild to moderate increase of collagen fibers, especially in the interstitial tissue and around blood vessels, revealed by Masson trichrome stain.

Regarding the ultrastructural changes, different cell types were difficult to be identified. Some secretory cells showed few non-specific granules and shrunken nuclei with more condensed chromatin, while some secretory cells can be detected and differentiated from each other by their characteristic granules. Most thyrotrophs revealed cytoplasmic changes in the form of the shrunken nucleus and a decrease in the number of secretory granules. Gonadotrophs revealed a pyknotic nucleus. Corticotrophs were affected to a large extent as they had shrunken nuclei with more thickened chromatin and few spread secretory granules.

These results agree with Shukla *et al.* (45) who observed a significant alteration in the level of hepatic enzymes and liver histopathology at an oral dose of 100 mg/kg TiO_2 NPs for 14 days. High accumulation of TiO_2 NPs in the liver tissue causing D.N.A. damage and apoptosis through the intrinsic pathway was considered the primary toxic mechanism.

Siddiqi *et al.* (46) explained the presence of inflammatory signs (congestion and dilatation of the blood vessels, extra-vascular exudate and inflammatory cell infiltration). Increased apoptosis TiO_2-NPs have been shown to induce the release of the pro-inflammatory cytokines as a sequel of nanoparticles-induced R.O.S. production and oxidative stress where interleukin (I.L.)-1α and (TNF-α) were detected in mice brain following nanoparticles administration. Additionally, cytokines like cytokine-induced neutrophil chemoattractant and macrophage inflammatory protein-1α were recorded to recruit neutrophils and macrophages to engulf the TiO_2-NPs deposited in the lungs (47).

It was noticed in the current study that some cells (basophils) were more sensitive and showed much degenerative affection than others (acidophils). Reiter *et al.* (48) explained that some cells were more susceptible to oxidative damage than others. The differences among organs may be in the total anti-oxidative capacity to resist oxidative damage.

These changes in pituitary cells are generally secondary responses to altered feedback pathways to the pituitary caused by hormone imbalance elsewhere. The cell type affected in the pituitary will depend on the endocrine organ involved (49).

Luabi *et al*. (50) explained that oxidative stress and insufficient antioxidant defense cause a loss of thyroid hormone-binding proteins, reduction in thyroid function, and a decrease in serum thyroid hormones, which in turn causes several biochemical changes in the tissues that predispose them to more oxidative damage.

In the current work, recovery groups (GIIc) after 60 days of stoppage of TiO_2 (10 mg/kg b.w./day) and (GIIIb) after 14 days of backup of TiO_2 (100 mg/kg b.w./day) showed partial improvement in the histological structure of its cells. Some basophils appeared with pale vacuolated cytoplasm and eccentric nuclei, while others appeared normal. Acidophils and chromophobes are arranged in groups separated by blood sinusoids. There was a mild increase of collagen fibers by Masson trichrome stain. These results are enforced by PAS stain. These results coincided with those improvements observed by electron microscope examination of different secretory cells such as somatotroph, corticotroph, and thyrotroph, which appeared more or less similar to the typical structure. In (I), some cells seemed to be apoptotic; the blood sinusoid showed myelin figures and autophagic vacuolations. In (GIIIb), apoptotic changes with irregular nuclei and rarefied cytoplasm occurred in some cells. Few corticotrophs showed dilated RER, mitochondria, and secretory granules under the cell membrane. Few thyrotrophs appeared inconsistent in shape with an eccentric nucleus, condensed accumulated mitochondria, and small secretory granules.

These results agree with Heo *et al*. (51) who showed that a recovery study with a non-dosing period of TiO_2 nanoparticles for 28 days via oral ingestion is highly unlikely to induce adverse effects or toxic reactions in rodents. Jia *et al.* (52) stated that the more prolonged exposure to TiO_2 nanoparticles caused continuous activation of the oxidation system, which led to oxidative severe damage to the body so that the recovery might be slower.

CONCLUSION

In conclusion, the results of the present study revealed that oral exposure to TiO_2 NPs causes dose and time-dependent toxicity in the pituitary gland on the pars distalis in adult male albino rats. So, administration of drug-loaded TiO_2 nanoparticles requires particular caution because of their unique vulnerability regarding the dose and time.

REFERENCES

1.Abu Dayyih W., Hailat M., Albtoush S., Albtoush E., Abu Dayah A., Alabbadi I. and Hamad M.F. Nanomedicine advancements in cancer therapy: A scientific review. *Jordan Journal of Pharmaceutical Sciences* 2024; 17(3):506-529.

2.Manimaran D., Elangovan N. and Palanisamy V. Anti-tumorigenic impact of nano-formulated peptide HIF-alpha therapy by DMBA-induced mammary carcinoma in rodent type. *Jordan Journal of Pharmaceutical Sciences* 2024; 17(4):783–793.

3.Thakur M., Wang B. and Verma M.L. Development and applications of nanobiosensors for sustainable agricultural and food industries: Recent developments, challenges and perspectives. *Environmental Technology & Innovation* 2022; 26:102371.

4.Jovanović B. and Palić D. Immunotoxicology of non-functionalized engineered nanoparticles in aquatic organisms with special emphasis on fish: Review of current knowledge, gap identification, and call for further research. *Aquatic Toxicology* 2012; 118–119:141–151.

5.Abudayah A.A.F. Implication of nanotechnology for pulmonary delivery of docetaxel. *Jordan Journal of Pharmaceutical Sciences* 2023; 16(2):470.

6.Martínez-Gutierrez F., Thi E.P., Silverman J.M., de Oliveira C.C., Svensson S.L., Vanden H.A., Sánchez E.M., Reiner N.E., Gaynor E.C., Pryzdial E.L., Conway E.M., Orrantia E., Ruiz F., Av-Gay Y. and Bach H. Antibacterial activity, inflammatory response, coagulation and cytotoxicity effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine* 2012; 8(3):328–336.

7.Rajaiah S.G., Nadoor P., Rao S., Poojari R., Rajashekaraiah R., Prasad T.N.V.K.V. and Yegireddy M. Comparative studies on dispersion characters of titanium dioxide (TiO_2) nanoparticles for rodent toxicology studies. *Applied Nano Bioscience* 2022; 12(2):1–9.

8.Mohammed E.T. and Safwat G.M. Grape seed proanthocyanidin extract mitigates titanium dioxide nanoparticle (TiO_2-NPs)-induced hepatotoxicity through TLR-4/NF-κB signaling pathway. *Biological Trace Element Research* 2020; 196(2):579–589.

9.Baranowska-Wójcik E., Szwajgier D., Oleszczuk P. and Winiarska-Mieczan A. Effects of titanium dioxide nanoparticles exposure on human health: A review. *Biological Trace Element Research* 2020; 193(1):118–129.

10. Perez-Castro C., Renner U., Haedo M.R., Stalla G.K. and Arzt E. Cellular and molecular specificity of pituitary gland physiology. *Physiological Reviews* 2012; 92(1):1–38.

11. Coronel-Restrepo N., Syro L.V., Rotondo F. and Kovacs K. Anatomy of the pituitary gland. In: *Pituitary Adenomas: The European Neuroendocrine Association's Young Researcher Committee Overview*. Springer, Cham. 2022;1–19.

12. Vasantharaja D., Ramalingam V. and Reddy G.A. Toxic exposure to titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats. *Nanomedicine Journal* 2015; 2(1):46–53.

13. El-Bestawy E.M. and Tolba A.M. Effects of titanium dioxide nanoparticles on the myocardium of adult albino rats and the protective role of β-carotene: Histological, immunohistochemical and ultrastructural study. *Journal of Molecular Histology* 2020; 51(5):485–501.

14. Leng W., Pati P. and Vikesland P.J. Room temperature seed-mediated growth of gold nanoparticles: Mechanistic investigations and life cycle assessment. *Environmental Science: Nano* 2015; 2(5):440–453.

15. Jafari A., Karimipour M., Khaksar M.R. and Ghasemnejad-Berenji M. Protective effects of orally administered thymol against titanium dioxide nanoparticle-induced testicular damage. *Environmental Science and Pollution Research* 2020; 27(2):2353–2360.

16. Abbasi-Oshaghi E., Mirzaei F. and Pourjafar M. NLRP3 inflammasome, oxidative stress and apoptosis induced in the intestine and liver of rats treated with titanium dioxide nanoparticles: In vivo and in vitro study. *International Journal of Nanomedicine* 2019; 14:1919.

17. Bancroft J.D. and Layton C. Fixation of tissues. In: *Bancroft's Theory and Practice of Histological Techniques* (8th Ed.). Elsevier Health Sciences. 2019;130–180.

18. Glauert A.M. and Lewis P.R. *Biological Specimen Preparation for Transmission Electron Microscopy*. 2nd Ed. Princeton University Press, New Jersey, U.S.A. 2014;77–310.

19. Wang S., Alenius H., El-Nezami H. and Karisola P. A new look at the effects of engineered ZnO and TiO_2 nanoparticles: Evidence from transcriptomics studies. *Nanomaterials* 2022; 12(8):1247.

20. Iftikhar M., Noureen A., Uzair M., Jabeen F., Abdel Daim M. and Cappello T. Perspectives of nanoparticles in male infertility: Evidence for induced abnormalities in sperm production. *International Journal of Environmental Research and Public Health* 2021; 18(4):1758.

21. Wang Y., Chen Z., Ba T., Pu J., Chen T., Song Y., Gu Y., Qian Q., Xu Y. and Xiang K. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. *Small* 2013; 9(9–10):1742–1752.

22. Ranjan S., Dasgupta N., Verma P. and Ramalingam C. Acute and sub-chronic toxicity of titanium dioxide nanoparticles synthesized by microwave-irradiation-assisted hybrid chemical approach. *Journal of the Indian Chemical Society* 2020; 97:483–491.

23. El-Azab N.E.E. and Salem M.Y. Are titanium dioxide nanoparticles toxic to the cerebral cortex of rats? A histological and immunohistochemical study. *Egyptian Journal of Histology* 2015; 38(3):573–581.

24. Shah S.N.A., Shah Z., Hussain M. and Khan M. Hazardous effects of titanium dioxide nanoparticles in ecosystem. *Bioinorganic Chemistry and Applications* 2017.

25. Wu T. and Tang M. The inflammatory response to silver and titanium dioxide nanoparticles in the central nervous system. *Nanomedicine* 2018; 13(2).

26. Abbott Chalew T.E. and Schwab K.J. Toxicity of commercially available engineered nanoparticles to Caco-2 and SW480 human intestinal epithelial cells. *Cell Biology and Toxicology* 2013; 29(2):101–116.

27. Hu X., Li D., Gao Y., Mu L. and Zhou Q. Knowledge gaps between nanotoxicological research and nanomaterial safety. *Environment International* 2016; 94:8–23.

28. Gunawan C., Teoh W.Y., Marquis C.P. and Amal R. Induced adaptation of Bacillus sp. to antimicrobial nanosilver. *Small* 2013; 9(21):3554–3560.

29. Younes N.R. Ben, Amara S., Mrad I., Ben-Slama I., Jeljeli M., Omri K., El Ghoul J., El Mir L., Ben Rhouma K., Abdelmelek H. and Sakly M. Subacute toxicity of titanium dioxide (TiO_2) nanoparticles in male rats: Emotional behavior and pathophysiological examination. *Environmental Science and Pollution Research* 2015; 22(11):8728–8737.

30. Hong F., Si W., Zhao X., Wang L., Zhou Y., Chen M., Ge Y., Zhang Q., Wang Y. and Zhang J. TiO_2 nanoparticle exposure decreases spermatogenesis via biochemical dysfunctions in the testis of male mice. *Journal of Agricultural and Food Chemistry* 2015; 63(31):7084–7092.

31. Sang X., Zheng L., Sun Q., Li N., Cui Y., Hu R., Gao G., Cheng Z., Cheng J., Gui S., Liu H., Zhang Z. and Hong F. The chronic spleen injury of mice following long-term exposure to titanium dioxide nanoparticles. *Journal of Biomedical Materials Research – Part A* 2012; 100A(4):894–902.

32. Müller L., Riediker M., Wick P., Mohr M., Gehr P. and Rothen-Rutishauser B. Oxidative stress and inflammation response after nanoparticle exposure: Differences between human lung cell monocultures and an advanced three-dimensional model of the human epithelial airways. *Journal of the Royal Society Interface* 2010; 7(Suppl. 1):S27–S40.

33. Salman A.S., Al-Shaikh T.M., Hamza Z.K., El-Nekeety A.A., Bawazir S.S., Hassan N.S. and Abdel-Wahhab M.A. Maltodextrin-cinnamon essential oil nanoformulation as a potent protective agent against titanium nanoparticles-induced oxidative stress, genotoxicity and reproductive disturbances in male mice. *Environmental Science and Pollution Research* 2021; 1–17.

34. Rossi E.M., Pylkkänen L., Koivisto A.J., Vippola M., Jensen K.A., Miettinen M., Sirola K., Nykäsenoja H., Karisola P. and Stjernvall T. Airway exposure to silica-coated TiO_2 nanoparticles induces pulmonary neutrophilia in mice. *Toxicological Sciences* 2010; 113(2):422–433.

35. Shakeel M., Jabeen F., Qureshi N.A. and Fakhr-e-Alam M. Toxic effects of titanium dioxide nanoparticles and titanium dioxide bulk salt in the liver and blood of male Sprague-Dawley rats assessed by different assays. *Biological Trace Element Research* 2016; 173(2):405–426.

36. Hassanein K.M.A. and El-Amir Y.O. Protective effects of thymoquinone and avenanthramides on titanium dioxide nanoparticles-induced toxicity in Sprague-Dawley rats. *Pathology – Research and Practice* 2017; 213(1):13–22.

37. Breitzig M., Bhimineni C., Lockey R. and Kolliputi N. 4-Hydroxy-2-nonenal: A critical target in oxidative stress? *American Journal of Physiology – Cell Physiology* 2016; 311(4):C537–C543.

38. Salem M.M., Altayeb Z.M. and El-Mahalaway A.M. Histological and immunohistochemical study of titanium dioxide nanoparticle effect on the rat renal cortex and the possible protective role of lycopene. *Egyptian Journal of Histology* 2017; 40(1):80–93.

39. Wright C., Iyer A.K.V., Wang L., Wu N., Yakisich J.S., Rojanasakul Y. and Azad N. Effects of titanium dioxide nanoparticles on human keratinocytes. *Drug and Chemical Toxicology* 2017; 40(1):90–100.

40. Omar A.I. and Kamar S.S. Does repeated gold-nanoparticles administration affect pars distalis hormonal and folliculo-stellate cells in adult male albino rats? *Folia Histochemica et Cytobiologica* 2021; 59(2):95–107.

41. Chavhan P.R. and Dhamani A. Ultrastructural characterization of gonadotrophs in the wild-caught female bat *Taphozous nudiventris kachhensis* (Dobson). *Journal of Microscopy and Ultrastructure* 2016; 4(2):108–114.

42. Abdel-Aziz H.O. and El Haliem N.G. Ultrastructural study of the effect of carbon tetrachloride on the pars distalis of the anterior pituitary gland of mice and the possible protective role of ginger. *Egyptian Journal of Histology* 2012; 35(3):598–606.

43. Tassinari R., Cubadda F., Moracci G., Aureli F., D'Amato M., Valeri M., De Berardis B., Raggi A., Mantovani A. and Passeri D. Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rats: Focus on reproductive and endocrine systems and spleen. *Nanotoxicology* 2014; 8(6):654–662.

44. Bonora M., Patergnani S., Ramaccini D., Morciano G., Pedriali G., Kahsay A.E., Bouhamida E., Giorgi C., Wieckowski M.R. and Pinton P. Physiopathology of the permeability transition pore: Molecular mechanisms in human pathology. *Biomolecules* 2020; 10(7):998.

45. Shukla R.K., Kumar A., Vallabani N.V.S., Pandey A.K. and Dhawan A. Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. *Nanomedicine* 2014; 9(9):1423–1434.

46. Siddiqi N.J., Abdelhalim M.A., El-Ansary A.K. et al. Identification of potential biomarkers of gold nanoparticle toxicity in rat brains. *Journal of Neuroinflammation* 2012; 9:123.

47. Manke A., Wang L. and Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed Research International* 2013; Article ID (if applicable).

48. Reiter R.J., Tan D.X., Kim S.J., Manchester L.C., Qi W., Garcia J.J. and Rouvier-Garay V. Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. *Mechanisms of Ageing and Development* 1999; 110(3):157–173.

49. Salem R.R. and Kelada M.N. A biochemical and ultrastructural study on the effect of toluene on the pars distalis of anterior pituitary glands of adult male albino rats. *Egyptian Journal of Histology* 2020; 43(3):948–959.

50. Luabi N.M., Zayed N.A. and Ali L.Q. Zinc oxide nanoparticles effect on thyroid and testosterone hormones in male rats. *Journal of Physics: Conference Series* 2019; 1294(6):062034.

51. Heo M.B., Kwak M., An K.S., Kim H.J., Ryu H.Y., Lee S.M., Song K.S., Kim I.Y., Kwon J.-H. and Lee T.G. Oral toxicity of titanium dioxide P25 at repeated dose 28-day and 90-day in rats. *Particle and Fibre Toxicology* 2020; 17(1):1–22.

52. Jia X., Wang S., Zhou L. and Sun L. The potential liver, brain, and embryo toxicity of titanium dioxide nanoparticles in mice. *Nanoscale Research Letters* 2017; 12(1):1–14.

تأثير جسيمات ثاني أكسيد التيتانيوم النانوية على بنية الغدة النخامية لدى ذكور الجرذان البيضاء البالغة

هاجر وهدان محمد العزب [1*]، ليلي السيد المصيلحي [1] ، زينب محمود جبالي[2] ، فاطمة الزهراء عبدالله عزيز[2]

[1] قسم الهستولوجيا، كلية الطب للبنات بدمياط، جامعة الأزهر، دمياط، جمهورية مصر العربية.

[2] قسم الهستولوجيا، كلية الطب للبنات بالقاهرة، جامعة الأزهر، القاهرة، جمهورية مصر العربية.

ملخص

هدفت الدراسة الحالية إلى التعرف على تأثيرات جسيمات نانوية من ثاني أكسيد التيتانيوم على الغدة النخامية في ذكور الفئران البيضاء البالغة. وفيما يخص منهجية البحث، فقد اجريت الدراسة على ثمانية وأربعين جرذًا ذكرًا من الفئران البيضاء الناضجة. قُسِّمت إلى ثلاث مجموعات رئيسية. المجموعة الأولى (مجموعة ضابطة) قُسِّمت بالتساوي إلى ثلاث مجموعات فرعية (مجموعة ضابطة سلبية ومجموعتين ضابطة للمواد المستخدمة لتذويب المادة الفعالة). المجموعة الثانية (المجموعة المُعالَجة) قُسِّمت إلى ثلاث مجموعات فرعية متساوية. المجموعة الثانية (أ): عولجت بمحلول ثاني أكسيد التيتانيوم (10 ملغ/كجم/يوم) عن طريق الفم لمدة 14 يومًا. المجموعة الثانية (ب): عولجت بمحلول ثاني أكسيد التيتانيوم (10 ملغ/كجم/يوم) عن طريق الفم لمدة 60 يومًا. المجموعة الثانية (ج) (في مرحلة التعافي): عولجت بمحلول ثاني أكسيد التيتانيوم (10 ملغ/كجم/يوم) لمدة 60 يومًا، ثم توقفت عن العلاج لمدة 60 يومًا. المجموعة الثالثة (أ): قُسِّمت بالتساوي إلى مجموعتين فرعيتين. المجموعة الثالثة (أ): عولجت 6 جرذان بمحلول ثاني أكسيد التيتانيوم (100 ملغ/كجم/يوم) عن طريق الفم لمدة 14 يومًا. والمجموعة الثالثة (ب) (مجموعة التعافي): عولجت ستة فئران بثاني أكسيد التيتانيوم (100 ملغ/كجم/يوم) لمدة 14 يومًا، ثم توقفت عن العلاج بثاني أكسيد التيتانيوم لمدة 14 يومًا. وبعد الانتهاء من فترة الدراسة، حُضِّرت عينات من الغدة النخامية وفُحِصت باستخدام المجهر الضوئي والإلكتروني. ووُثِّقت بيانات الدراسة المورفومترية، ومقارنة المجموعات.

النتائج: أظهرت النتائج أن المجموعة التي تعرضت للأجزاء النانوية من ثاني أوكسيد التيتانيوم لديها خللاً هيكلياً في الجزء البعيد (تضخم الخلايا القاعدية، تحتوي على تجويفات بالسيتوبلازم، ونوي ضامرة). بالإضافة إلى احتقان بالأوعية الدموية وزيادة في عدد الخلايا الالتهابية. كذلك وجد انخفاض يعتد به إحصائيا في التفاعل مع حمض شيف الدوري، مقترناً بزيادة كبيرة في نسبة مساحة ألياف الكولاجين. وقد تم تأكيد تلك التغيرات باستخدام المجهر الالكتروني. وأظهرت مجموعات التعافي درجات متفاوتة من التحسن في التغيرات النسيجية سابقة الذكر.

الاستنتاج: تسبب جسيمات ثاني أكسيد التيتانيوم النانوية تغيرات هيكلية تعتمد على الوقت والجرعة في الجزء البعيد من الغدة النخامية الأمامية بدرجات متفاوتة من التشوهات

الكلمات الدالة: جسيمات نانوية من ثاني أكسيد التيتانيوم؛ جرذ؛ الجزء البعيد، مجهر إلكتروني ناقل.

---

[*] ***المؤلف المراسل: هاجر وهدان محمد العزب***

Hagarwahdan24@gmail.com

تاريخ استلام البحث 2024/07/06 وتاريخ قبوله للنشر 2024/10/31.