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Physiological and Hormonal Effects of Titanium Dioxide Nanoparticles on Thyroid and Kidney Functions

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Abstract

Titanium dioxide nanoparticles (TiO₂ NPs) are generally used in different types of applications such as the industry of plastics, paper industry, paints, toothpaste, cosmetics, sunscreens, and in various lifestyles, because of the vast range of applications and our daily exposure to these nanoparticles and a lack of information on animal and human health this study was designed to reveal dose and time-dependent effects of TiO₂-NPs on the thyroid gland and kidney functions in male rats.

For this study 54, Sprague-Dawley albino adult male rats were classified into three main groups each of 18 rats treated for a particular duration (1, 2, and 4) weeks respectively. Each group was subdivided into three subgroups each of six rats treated as follows; group (1) serve as normal control, group (2, and 3) intra-peritoneal treated with TiO₂NPs (50,200) mg/kg respectively, rats are dissected at the end of each experiment and the weights of thyroid and kidney is measured. The result showed a highly significant decrease ($p < 0.01$) in the thyroid gland and a highly significant increase ($p < 0.01$) in kidney weights and TSH, blood urea, creatinine, and total protein, while a highly significant decrease ($p < 0.01$) in T₃ and T₄ in all different doses (50,200) mg/kg at durations 1, 2 and 4 weeks. The outcomes of the present study illustrate a significant decrease in serum levels of T₄ and T₃ with exposure to TiO₂ NPS which disrupts thyroid function, while TiO₂ NPS raises the level of urea, total protein, and creatinine. This could be related to the high dose of TiO₂-NPs and duration of the study, which caused degeneration and necrosis of kidney cells and damage to peritubules that led to the prevention of secretion which raised urea levels in the blood, also led to high levels of creatinine and total protein in serum because of the imbalance that occurred in the kidney functions.

Keywords: kidney functions, nanoparticle, Rats, thyroid gland, TiO₂NPs.

Introduction

Nanoparticles: are small substances, that have at least one dimension in the range of (1–100)nm, the small size and high surface area of Nanoparticles make them principal entrants in all lineaments of modern life enforcement¹. TiO₂ NPs are an odorless and noncombustible white powder that can crystallize in three structures brookite, rutile, and anatase, it is a semiconducting chemically inert material that displays photocatalytic activity when exposed to light² TiO₂ NPs have several applications in medical and engineering sciences and are used in removing pollution like organic toxic and heavy metals from the sewerage, and filtration of water and air³. The kidney is one of the vital organs in the body, excreting body waste products and drugs through highly specialized cells located in renal nephrons,

kidney functions can be altered by many environmental contaminants, chemicals, and drugs⁴. Some studies showed that TiO₂ NPs have possible adverse health effects after entering the body, initiate inflammatory mechanisms in cells, apoptosis, and generate oxygen free radicals which destructs the nucleus and DNA, also variable changes in the functions of cells⁵. When Gui *et al*⁶. suggested that TiO₂-NPs induced kidney inflammation leading to tissue necrosis, disorganizing the renal tubules, and producing reactive oxygen species (ROS). NPs may alter the mitochondrial function of the thyroid epithelial cells, which might decrease cellular ATP production required as an energy source for synthesis and release of thyroid hormone⁷. The present study was aimed to evaluate the effects of TiO₂ NPS on the function of the thyroid gland by measuring the level

of [thyroid stimulating hormone(TSH), triiodothyronine (T3), thyroxin(T4)] and the function of the kidney by measuring the level of [urea, creatinine, total protein].

Materials and methods

Preparation of titanium dioxide nanoparticles (TiO₂NPs) solution

TiO₂NPs used in this study were obtained from Skyspring Nanomaterial company (USA), they were in white powder Rutile 99.9 % purity, Particle size (30nm) diameter.

The stock suspension was prepared by dissolving 1 gram of powder TiO₂NPs in 10 ml of distilled water and then mixed by vortex for 10 min to prevent agglomeration, from this stock suspension, two additional diluted TiO₂NPs suspensions (low and high) doses were prepared:

Group of 200 mg/kg of TiO₂NPs (high dose)

Group of 50mg/kg of TiO₂NPs (low dose)

Animals

The study was conducted on (54) adult male Sprague-Dawley albino rats (*Rattus norvegicus*) their ages about (2.5-3) months as a mammalian model and average body weight of (250-260)gm. The animals were purchased from the Iraqi Center For Cancer And Medical Genetic research and then transferred to the animal house of the college of science, Mustansyria University. The males were kept for 10 days period of adaptation before starting treatment in clean plastic cages with metal network cover under the climate-controlled condition of the animal house with 22-25 temperatures. Animals were allowed to feed standard rats' pellets with free access to tap water.

Experimental Design

Fifty-four male rats were randomly divided into nine groups six rats per group treated at different durations of 1 week, 2 weeks, and 4 weeks as follows: **Group 1, 2, and 3 (control groups):** Respectively, received an intraperitoneal injection of distilled water for different durations (1, 2, and 4) weeks.

Group 4, 5, and 6 (the experimental groups); The rats respectively received intraperitoneal dose (50mg/kg) of (TiO₂ NPs) for different durations (1, 2, and 4) weeks.

Group 7, 8, and 9: (the experimental groups); The rats respectively received intraperitoneal doses (200mg/kg) of (TiO₂ NPs) for different durations (1, 2, and 4) weeks.

Collection of Blood Samples and The Dissection of the Animals.

The end of each experiment was followed by weighing of the animals, they were completely

anesthetized by diethyl ether for several minutes and blood samples were obtained by heart puncture were collected into non-heparinized tubes used in the hormonal examination. 5 ml of blood for the hormonal test collected from each rat was used to obtain sera (1.0-1.5) ml separated by centrifugation at 3000 rpm for 10 min, and then they were kept at -20°C until analysis. The thyroid gland and kidney were removed by forceps and blade, then washed with normal physiological saline 0.9% (NaCl) to remove blood, blotted with filter paper to dry it for several minutes then weighed.

kidney Functions

1 Measurement of Blood Urea

The serum concentration of urea is determined using the Urease— method (enzymatic colorimetric method) according to linear company kit / Spain/2018.

2 Measurement of Serum Creatinine

Determination of creatinine levels in serum is done by using the kinetic colorimetric method, according to linear company kit / Spain/ 2018.

3 Measurement of Total protein

This analysis is done by using the Direct Biuret method for the determination of protein levels in serum, according to AGAPPE company kit /Switzerland /2018.

Statistical Analysis

The obtained data of this study are expressed as mean \pm SE. The Statistical Analysis System- SAS (2012) program is used to observe the effect of different factors (concentration of TiO₂ NPs and period) in the studied parameters.

Two-way analysis of variance (ANOVA) is used depending on the least significant difference (LSD).

Results and Discussion

Thyroid weight and Functions

Results showed that TiO₂ -NPs had effects on thyroid weights as it was demonstrated in the Table. 1. Rats exposed to TiO₂ -NPs for 1 week demonstrated a highly significant decrease ($p < 0.01$) in thyroid weight of treated groups with doses of 50 and 200 mg/kg (0.200 ± 0.015) and (0.180 ± 0.005) gm respectively compared to control groups (0.270 ± 0.009) gm, as well, there was a highly significant decrease ($p < 0.01$) in thyroid weights of experimental groups treated with TiO₂ NPs for 2 weeks in doses 50 and 200mg/kg (0.178 ± 0.009) and (0.141 ± 0.020) gm respectively when compared to control groups (0.260 ± 0.008) gm, also at 4 weeks period of time in doses 50 and 200mg/kg there was a highly significant decrease ($p < 0.01$) in thyroid gland weight (0.126 ± 0.006) and (0.100 ± 0.005) gm

respectively compared with control groups (0.271±0.009) gm. A high significant decrease was observed when comparing between treated groups themselves depending on the concentrations as the fixed factors

while days were the variable factors with the concentrations (50 and 200) mg/kg exposed to TiO₂-NPs with the increase of experimental duration time 1, 2 and 4 weeks respectively.

Table 1. Effect of Dose and Time of TiO₂ -NPS on Thyroid weight

| Dose | Thyroid weight (gm) | | | LSD value |
|-----------------|----------------------|----------------------|----------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 0.270 ± 0.009 A a | 0.260 ± 0.008 A a | 0.271 ± 0.009 A a | 0.027 NS |
| Low (50 g/kg) | 0.200 ± 0.015 B a | 0.178 ± 0.009 B b | 0.126 ± 0.006 B c | 0.033 ** |
| High (200 g/kg) | 0.180 ± 0.005 B a | 0.141 ± 0.020 C b | 0.100 ± 0.005 C c | 0.038 ** |
| LSD value | 0.033 ** | 0.041 ** | 0.022 ** | --- |

Mean ± SE (gm)

** (P<0.01).

A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

Statistical analysis of the present study for the effect of TiO₂-NPs on thyroid hormones that include TSH, T3, and T4 in Tables.2,3, and 4 Sequential reveals that:

The values of TSH(μU/ml) showed high significant increase (p<0.01) at different treatment durations (1, 2, 4) weeks exposing to TiO₂-NPs at (50, 200) mg/kg as follows: during week 1 (0.600 ± 0.015) and (0.841± 0.037) compared to control group (0.051 ± 0.004), also during week 2 in different doses(0.990 ± 0.003) and (1.50 ± 0.013) compared to control

group (0.143 ± 0.091), in addition to week 4 at low and high doses (2.11 ± 0.013) and (2.83± 0.16) (μU/ml) compared to control group,(0.060 ± 0.005) (μU/ml), demonstrated in Table. 2. High significant increase in the level of (TSH) (μU/ml) also was observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days were the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂-NPS with the increase of experimental duration 1, 2 and 4 weeks respectively.

Table 2. Effect of Dose and Time of TiO₂ -NPS on The Level of TSH

| Dose | Level of TSH (μU/ml) | | | LSD value |
|-----------------|----------------------|----------------------|----------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 0.051 ± 0.004 C a | 0.143 ± 0.091 C a | 0.060 ± 0.005 C a | 0.159 NS |
| Low (50 g/kg) | 0.600 ± 0.015 B c | 0.990 ± 0.003 B b | 2.11 ± 0.013 B a | 0.036 ** |
| High (200 g/kg) | 0.841 ± 0.037 A c | 1.50 ± 0.013 A b | 2.83 ± 0.16 A a | 0.288 ** |
| LSD value | 0.071 ** | 0.161 ** | 0.281 ** | --- |

Mean ± SE

** (P<0.01).

(A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

High significant decrease (p<0.01) of (T3) (ng/ml) serum level of in both treated groups (50, 200) mg/kg (1.260±0.012),(1.060±0.051) (ng/ml) respectively exposed to TiO₂-NPs for 1 week compared to the control groups (1.490±0.017)

(ng/ml), also there was high significant decrease(p<0.01) in the level of (T3) in both doses (50, 200) mg/kg at 2 weeks (0.980±0.004),(0.810±0.005) (ng/ml) in comparison to control groups (1.231 ± 0.023), and at 4 weeks (

0.77± 0.014), (0.416±0.01) (ng/ml) in comparison to control groups (1.46 ± 0.006) (ng/ml) demonstrated in Table.3. High significant decrease due to TiO₂ -NPS in serum level of (T3) (ng/ml) was observed when comparing between treated groups themselves

depending on the concentrations as the fixed factors while days were the variable factors in the concentrations (50 and 200) mg/kg exposing to TiO₂ -NPS with the increase of experimental duration time 1, 2 and 4 weeks respectively.

Table 3. Effect of Dose and Time of TiO₂ -NPS on The Level of T3

| Dose | Level of T3(ng/ml) | | | LSD value |
|-----------------|----------------------|-----------------------|---------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 1.490 ± 0.017 A a | 1.231 ± 0.23 A a | 1.46 ± 0.006 A a | 0.416 NS |
| Low (50 g/kg) | 1.260 ± 0.012 B a | 0.980 ± 0.004 AB b | 0.77 ± 0.014 B c | 0.029 ** |
| High (200 g/kg) | 1.060 ± 0.051 C a | 0.810 ± 0.005 B b | 0.416 ± 0.01 C c | 0.092 ** |
| LSD value | 0.097 ** | 0.415 * | 0.030 ** | --- |

Mean ± SE

** (P<0.01). *(p<0.05)

A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

values of T4(µg/dl) displayed non-significant decrease of treated groups (50) mg/kg (6.20±0.01) (µg/dl), but showed significant decrease (p<0.05) of treated groups (200) mg/kg (5.08±0.79) (µg/dl) respectively at 1 week compared to control groups(6.91±0.01) (µg/dl), at week 2 also showed non- significant decrease(p<0.05) at dose (50) mg/kg ,(4.32±0.01) (µg/dl), while significant decrease (p<0.05) of treated groups (200) mg/kg (4.10±0.02) (µg/dl) when compared with control groups(5.97±0.90) (µg/dl), in addition to 4 weeks exposing to TiO₂ -NPS observed high significant

decrease (p<0.01) of the level of T4 in different doses (50,200) mg/kg (3.77±0.01),(3.31±0.01) (µg/dl) compared to control groups (6.91±0.02) (µg/dl) demonstrated in Table. 4. High significant decrease in the level of T4 was observed when comparing between treated groups themselves depending on the concentrations as the fixed factors while days were the variable factors with the concentrations (50 and 200) mg/kg exposed to TiO₂ -NPS with the increase of experimental duration time 1, 2 and 4 weeks respectively.

Table 4 . Effect of Dose and Time of TiO₂ -NPS on The Level of T4

| Dose | Level of T4 (µg/dl) | | | LSD value |
|-----------------|---------------------|---------------------|--------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 6.91 ± 0.01 A a | 5.97 ± 0.90 A a | 6.91 ± 0.02 A a | 1.567 NS |
| Low (50 g/kg) | 6.20 ± 0.01 AB a | 4.32 ± 0.01 AB b | 3.77 ± 0.01 B c | 0.034 ** |
| High (200 g/kg) | 5.08 ± 0.79 B a | 4.10 ± 0.02 B ab | 3.31 ± 0.01 C b | 1.384 * |
| LSD value | 1.383 * | 1.566 * | 0.049 ** | --- |

Mean ± SE

* (P<0.05), ** (P<0.01).

A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

The weight, size, and histology of the thyroid gland are affected by the production of thyroxin and its functional status, also some disorders of the thyroid gland such as overactive or underactive thyroid gland are established by enlargement of the thyroid gland

as a part of the compensatory mechanism to maintain of thyroid hormone homeostasis⁸. Hypothyroidism due to thyroid gland hypertrophy shows a significant increase in thyroid gland weight in animals^{9, 10} Suggests that thyroid gland weight in

hypothyroidism disorder rats demonstrated a significant increase.¹¹ After oral administration of TiO₂-NPS (5 g / kg BW) for (65 days) in male rats, results illustrate that TiO₂ NPS effects became non-significant in the function of the thyroid gland, the difference may be due to the short duration, high dose and the type of treatment¹².

The increase in time and doses of TiO₂-NPS reduces thyroid weight, results of the present study showed a significant decrease in serum levels of T4 and T3 with exposure to TiO₂ NPS.

Kidney Weight and Functions

The statistical analysis of the weight of the right kidney is shown in Table.5 the rats injected with TiO₂-NPS intra-peritoneal with different doses(50, 200) mg/kg demonstrated a highly significant increase (p<0.01) in right kidney weight (0.833±0.011) and (0.960±0.009) gm respectively at 1 week when compared with the control group

(0.730±0.008)gm, at 2 weeks of time treatment with TiO₂-NPS in different doses(50, 200) mg/kg results showed a highly significant increase (p<0.01) in weight of right kidney (0.930±0.013) and (1.216±0.008) gm respectively when compared with the control group(0.730±0.007gm), and at 4 weeks of time treatment with TiO₂-NPS in different doses(50, 200) mg/kg observed high significant increase (p<0.01) in weight of right kidney (1.170±0.010) and (1.306±0.011)gm compared to the control group(0.720±0.012)gm.

High Significant increase in the level of right kidney weight (gm) was observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days were the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂ NPS with the increase of experimental duration 1, 2 and 4 weeks respectively.

Table 5. Effect of Dose and Time of TiO₂-NPS on Right kidney weight

| Dose | Right kidney (gm) | | | LSD value |
|--|----------------------|----------------------|----------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 0.730 ± 0.008 C a | 0.730 ± 0.007 C a | 0.720 ± 0.012 C a | 0.028 NS |
| Low (50 g/kg) | 0.833 ± 0.011 B c | 0.930 ± 0.013 B b | 1.170 ± 0.010 B a | 0.035 ** |
| High (200 g/kg) | 0.960 ± 0.009 A c | 1.216 ± 0.008 A b | 1.306 ± 0.011 A a | 0.028 ** |
| LSD value | 0.029 ** | 0.031 ** | 0.033 ** | --- |
| Mean ± SE | | | | |
| ** (P<0.01). | | | | |
| A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors. | | | | |
| (a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors. | | | | |

Statistical analysis of the weight of the left kidney is shown in Table. 6, the rats injected with TiO₂-NPS intra-peritoneal with both doses(50, 200 mg/kg) demonstrated a highly significant increase (p<0.01) in weight of left kidney at 1 week (0.860±0.009) and (0.980±0.004)gm respectively when compared with the control group(0.750±0.012)gm, also at 2 weeks of time treatment with TiO₂-NPS in different doses(50, 200) mg/kg showed a high significant increase (p<0.01) in weight of left kidney (0.951±0.011) and (1.240 ±0.007) gm respectively when compared with the control group(0.750±0.009)gm, in addition to 4

weeks of treatment with TiO₂-NPS in different doses(50, 200) mg/kg showed high significant increase (p<0.01) in weight of left kidney, (1.156±0.011) and (1.298±0.017) gm respectively compared with control groups (0.762±0.013) gm.

High Significant increase in the level of left kidney weight (gm) is observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days are the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂ NPS with the increase of experimental duration 1, 2 and 4 weeks respectively

Table 6. Effect of Dose and Time of TiO₂ -NPS on Left Kidney Weight

| Dose | Left kidney weight (gm) | | | LSD value |
|--|-------------------------|----------------------|----------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 0.750 ± 0.012 C a | 0.750 ± 0.009 C a | 0.760 ± 0.013 C a | 0.035 NS |
| Low (50 g/kg) | 0.860 ± 0.009 B c | 0.951 ± 0.011 B b | 1.156 ± 0.011 B a | 0.032 ** |
| High (200 g/kg) | 0.980 ± 0.004 A c | 1.240 ± 0.007 A b | 1.298 ± 0.017 A a | 0.034 ** |
| LSD value | 0.027 ** | 0.028 ** | 0.044 ** | --- |
| Mean ± SE | | | | |
| ** (P<0.01). | | | | |
| A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors. | | | | |
| (a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors. | | | | |

Statistical analysis of the present study of the effect of TiO₂ NPS on kidney functions that include Urea, creatinine, and total protein:

Results of this study show a high significant increase (p<0.01) in urea level at different doses of TiO₂ NPS (50, 200)mg/ml at 1 week (49.70±0.19),(58.00 ±0.01)mg/dl respectively compared with control groups 01±0.01)mg/dl, the level of urea at 2 week showed high significant increase (p<0.01) at different doses (50,200) mg/kg (60.00 ± 0.01),(66.00 ± 0.01) mg/dl respectively

compared with control groups(41.00 ± 0.01)mg/dl, and at 4 week (68.00 ± 0.01),(89.00 ± 0.03)mg/dl respectively when compared to control groups (40.67 ± 0.20)mg/dl showed in table. 7.

High Significant increase in Urea level(mg/dl) is observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days are the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂ -NPS with the increase of experimental duration 1, 2 and 4 weeks respectively.

Table 7. Effect of Dose and Time of TiO₂ -NPS on The Level of Urea

| Dose | Level of Urea (mg/dl) | | | LSD value |
|--|-----------------------|---------------------|---------------------|-----------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 41.01 ± 0.01 C a | 41.00 ± 0.01 C a | 40.67 ± 0.20 C a | 0.672 NS |
| Low (50 g/kg) | 49.70 ± 0.19 B c | 60.00 ± 0.01 B b | 68.00 ± 0.01 B a | 0.345 ** |
| High (200 g/kg) | 58.00 ± 0.01 A c | 66.00 ± 0.01 A b | 89.00 ± 0.03 A a | 0.053 ** |
| LSD value | 0.344 ** | 0.035 ** | 0.354 ** | --- |
| Mean ± SE | | | | |
| ** (P<0.01). | | | | |
| A,B,C) represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors. | | | | |
| (a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors. | | | | |

The statistical analysis show high significant increase (p<0.01) of creatinine level at 1 week (0.800±0.01),(0.833±0.16)mg/dl respectively compared to control groups (0.600±0.01)mg/dl treatment with TiO₂ -NPS (50,200) mg/kg and 2 week (1.20±0.02), (1.90±0.01)mg/dl compared to control groups (0.500±0.10)mg/dl. Also exposure of rats with TiO₂ -NPS at the doses of (50,200) mg/kg at 4 week exhibited a high significant increase (p<0.01) in creatinine levels (1.70±0.02)

,(2.80±0.01)mg/dl compared to control groups(0.600±0.02)mg/dl demonstrated in Table. 8.

A significant increase in creatinine level(mg/dl) is observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days are the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂ -NPS with the increase of experimental duration 1, 2 and 4 weeks respectively.

Table 8. Effect of Dose and Time of TiO₂ NPS on The Level of Creatinine

| Dose | Level of Creatinine (mg/dl) | | | LSD value |
|-----------------|-----------------------------|----------------------------|----------------------------|-----------------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 0.600 ± 0.01 B a | 0.500 ± 0.10 C a | 0.600 ± 0.02 C a | 0.102 NS |
| Low (50 g/kg) | 0.800 ± 0.01 A c | 1.20 ± 0.02 B b | 1.70 ± 0.02 B a | 0.044 ** |
| High (200 g/kg) | 0.833 ± 0.16 A c | 1.90 ± 0.01 A b | 2.80 ± 0.01 A a | 0.295 ** |
| LSD value | 0.039 ** | 0.178 ** | 0.049 ** | --- |
| Mean ± SE | | | | |

** (P<0.01).

A,B,C represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

The statistical analysis results of total protein level show high significant increase (p<0.01) in level at different doses (50, 200) mg/kg for (1, 2, 4) weeks, at 1 week results are (7.50 ± 0.01), (9.00 ± 0.02) mg/dl respectively compared with control groups (5.30 ± 0.04)mg/dl, at 2 weeks treatment results were (8.70 ± 0.02, 11.60 ± 0.01mg/dl) respectively compared with control groups (5.33 ± 0.01mg/dl), and at 4 weeks exposure results are (10.40±0.01),(12.60± 0.01)mg/dl respectively

when compared with control groups (5.32 ± 0.01)mg/dl demonstrated in Table. 9.

High Significant increase in total protein level(mg/dl) is observed when comparing between treated groups themselves depending on the concentrations as fixed factors while days are the variable factors in the concentrations (50 and 200) mg/kg exposed to TiO₂ NPS with the increase of experimental duration 1, 2 and 4 weeks respectively.

Table 9. Effect of Dose and Time of TiO₂ -NPS on Total Protein Level

| Dose | Level of Total protein (mg/dl) | | | LSD value |
|-----------------|--------------------------------|----------------------------|----------------------------|------------------|
| | 1 Week | 2 Week | 4 Week | |
| Control | 5.30 ± 0.04 C a | 5.33 ± 0.01 C a | 5.32 ± 0.01 C a | 0.0207 NS |
| Low (50 g/kg) | 7.50 ± 0.01 B c | 8.70 ± 0.02 B b | 10.40 ± 0.01 B a | 0.036 ** |
| High (200 g/kg) | 9.00 ± 0.02 A c | 11.60 ± 0.01 A b | 12.60 ± 0.01 A a | 0.045 ** |
| LSD value | 0.038 ** | 0.043 ** | 0.036 ** | --- |
| Mean ± SE | | | | |

** (P<0.01).

A,B,C represent the significant difference among groups with days as the fixed factors and concentrations as the variable factors.

(a,b,c) represent the significant difference among groups with concentrations as the fixed factors and days as the variable factors.

The effects of TiO₂ -NPS in the kidney, shown by ¹³ revealed that nanoparticles of TiO₂ -NPS have been depot in the cells of the kidney and caused the pathological changes and nephron-like toxicity in the form of inflammation in the glomeruli of the kidney, also 25 nm TiO₂ -NPS can significantly raise the urea level of serum compared with the control group, Kidney dysfunction is constructed in rats exposed to TiO₂ NPS, because of an increase in the level of blood urea and creatinine in the serum of mice. Treated groups of male rats using 3 doses of 30, 50, and 70 mg/kg TiO₂ NPS, observed no significant alterations when compared to their controls, but

Twenty days after the last injection in the second stage of the treatment groups in all three doses of (30, 50, 70) mg/kg showed the increase (p<0.001) in serum urea level in exposed groups¹⁴. Meena et.al¹⁵ Suggested that treated rats with 50 mg/kg of TiO₂ -NPS, increased levels of blood urea in serum which is directly correlated with the sign of glomerulonephritis toxicity, swelling in renal glomerulus, renal tubules crammed with the proteinic fluids because of the distribution of TiO₂ -NPS particles in kidneys. Another study showed that nanoparticles of TiO₂ -NPS have reduced the level of urea and creatinine¹⁶. The difference in the results

obtained from animals treated with nanoparticles may be due to the type of animal, the route of administration to the nanoparticles orally, respiratory, dermal, and several injections, or to different physical and chemical properties of nanoparticles¹⁷. Previous studies have shown that aggregation of TiO₂ NPS can occur in the liver, kidneys, lung, and spleen after intraperitoneal, intravenous, or dermal administration¹⁸. The kidney is one of the vital organs susceptible to the injurious effects of TiO₂ -NPS¹⁹. Responsibility for expelling the unsafe substances such as NPS from blood, after absorbance into the circulatory system it can be filtered by the renal system²⁰. Some studies supported this result and suggest that TiO₂ -NPS exposure could induce apoptosis in different types of cells or organs, like the liver, spleen, and kidney^{14, 21}. Showed that gold nanoparticles increase the level of urea, but urea level restored to normal after a few time, this is due to the initial shock of the kidney that gradually overcomes and the renal function returned to normal and explained the role of urea as a carrier of waste nitrogen, it plays some interactions in the system of nephrons. Both increase or decrease of the organ coefficients may be caused by - TiO₂ -NPS excretion or accumulation in the organs can lead to histopathological changes²². A single oral gavage of 5 g/kg TiO₂ -NPS particles stimulate liver and kidney damage, with hepatomegaly, hepatocyte necrosis, swollen renal glomerulus, and proteinic liquid aggregation in the renal tubules¹³. Filtration of blood in the glomeruli of the kidney nephron, propose the most common process to remove the nanoparticles through the kidneys²³. So removal of creatinine in the bloodstream is done by the kidneys, the measurement of creatinine level in the blood can indicate the function of the kidneys²⁴. If there is a failure in renal function, the creatinine level of serum increases²⁵. Another study showed that the level of creatinine may be reduced after exposure to TiO₂ -NPS administration and this result is confirmed by Amara *et al*, who proposed that TiO₂ -NPS administration intraperitoneally at doses of 25 mg/kg (20–30 nm), after 7 days, as results showed a certain pathological change in the kidney of treated rats resulting in a high plasmatic uric acid level and a decrease of creatinine content²⁶. While ²⁷ revealed that the amount of TiO₂ -NPS in the mouse liver, spleen, lung, and kidneys reached high levels after 14 days of intraperitoneal administration. Researchers found that injected mice intraperitoneally with nano-titanium dioxide (20 mg/kg) in different sizes of the mice, after one week, they did not have any clear distinctive toxicity, mortality, and significant changes in liver and kidney²⁸. Recent studies proposed that nanoparticles such as TiO₂ -NPS after

entry into cells, can induce an inflammatory response, (apoptosis) and generate the oxygen free radicals ROS which mutate DNA, and also change the functions of the cells body²⁹. A cross-sectional study that included 100 individuals 50 hypothyroid cases and 50 normal controls, was done to determine thyroid dysfunction effects on serum values of Urea, creatinine, and uric acid, the results showed high significant increase in serum values of Urea, creatinine and uric acid in hypothyroid patients compared to controls which illustrates that hypothyroidism is associated with deteriorating renal function, hypothyroid-induced renal dysfunction may cause adverse clinical consequences, especially among patients on medications cleared by the kidneys, therefore these parameters should be regularly monitored in hypothyroid patients^{30, 31}. observed a linear association between FT4 and Uric acid(UA) level in the population of the study, also reported a correlation between the prevalence of hyperuricemia and elevated FT4 levels, that may be impute to the effects of FT4 on purine nucleotide turnover and UA excretion. In a cross-sectional study that included 108 individuals 56 cases with hypothyroidism and 52 controls; aged between 20 and 60 years (52 men and 56 women), after applying inclusion and exclusion criteria, serum TSH, T4, T3, uric acid, and creatinine were estimated, the results showed significant elevation in uric acid and creatinine levels as compared to control group, that explains hypothyroidism causes significant increase in serum uric acid and creatinine levels³². Autoimmune thyroiditis (AIT) is correlated with hypothyroidism, and different hypotheses have been put forward concerning the correlation between AIT and glomerulopathies, and several potential mechanisms for this relationship have been considered³³. Iglesias *et al.*,³⁴ Reported that renal development, kidney hemodynamics, glomerular filtration rate and sodium and water homeostasis are influenced by thyroid hormones, so hyperthyroidism and hypothyroidism affect renal function by direct renal effects. In many ways thyroid and kidney are interdependent on each other for optimal functioning of either organs, urinary loss of thyroid hormones and thyroid binding globulins in proteinuria cases in substantial amount leading to subclinical/overt hypothyroidism³⁵. Subclinical hypothyroidism in SRNS is temporary and may improve with remission. ³⁶ Revealed that prolonged proteinuria in steroid resistant nephrotic syndrome patients (SRNS) may cause renal tubules progressive damage and impaired absorption of low molecular weight proteins that will exhaust the thyroid reserve and result into overt hypothyroidism. Hypothyroidism, is a well-known complication of nephrotic syndrome

(NS), it is a common feature of primary and secondary glomerular diseases and includes loss of protein in the urine and elevated urinary excretion of thyroid hormones and thyroxine-binding globulin^{37,38}.

The outcomes of the present study illustrate an increment in kidney weight, Urea, Total protein, and Creatinine. This could be related to the high dose of TiO₂-NPs and duration of the study, which caused degeneration and necrosis of kidney cells and damage to peritubules that led to the prevention of secretion which raised Urea levels in the blood, also led to high levels of Creatinine and Total protein in serum because of the imbalance that occurred in the kidney functions.

Conclusion

The outcomes of the present study illustrate the significant decrease in serum levels of T4 and T3 with exposure to TiO₂ NPS which disrupts thyroid function, while TiO₂ NPS rises the level of urea, total protein, and creatinine. This could be related to the high dose of TiO₂-NPs and duration of the study, which caused degeneration and necrosis of kidney cells and damage to peritubules that led to the prevention of secretion which raised urea levels in the blood, also led to high levels of creatinine and total protein in serum because of the imbalance that occurred in the kidney functions.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The researchers are demonstrating that they have adhered to the accepted ethical standards of a genuine research study. Ref. No.:BCSMU/1221/0001Z

Authors Contribution

Both authors N M. L and Raghda A. M conceived and planned the experiment, carried out the experiment, and contributed to sample preparation. N M. L contributed to the interpretation of the results. R A. M took the lead in writing the manuscript. Both authors provided critical feedback

and helped shape the research, analysis, and manuscript.

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التأثيرات الفسيولوجية والهرمونية لجزيئات ثنائي أكسيد التيتانيوم النانوية على وظائف الغدة الدرقية والكلية

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الخلاصة:

تُستخدم الجسيمات النانوية لثنائي أكسيد التيتانيوم (TiO₂ NPs) بشكل عام في أنواع مختلفة من التطبيقات مثل صناعة البلاستيك، وصناعة الورق، والدهانات، ومعجون الأسنان، ومستحضرات التجميل، واقيات الشمس، وفي أنماط الحياة المختلفة، بسبب النطاق الواسع من التطبيقات وتعرضنا اليومي لهذه الجسيمات النانوية ونقص المعلومات عن صحة الحيوان والإنسان، صُممت هذه الدراسة للكشف عن الآثار التي تعتمد على الجرعة والوقت من TiO₂-NPs على وظائف الغدة الدرقية والكلية في ذكور الجرذان. لهذا الغرض تم استخدام 54 ذكر من الجرذان البيض وتم تصنيفها الى ثلاثة مجاميع رئيسية (3,2,1) كل مجموعة تتضمن 18 جرذ عوملت بثلاث فترات زمنية مختلفة (1, 2, 4) أسابيع على التوالي. وتم تقسيم هذه المجاميع الى ثلاث مجاميع فرعية كل منها تتضمن ست حيوانات تمت معاملتها على النحو التالي: (1) سيطرة، المجموعة (2, 3) حققت بالتجفيف البروتوني بجرعات متزايدة من دقائق التيتانيوم النانوية (50, 200) ملغ / كغم على التوالي. في نهاية التجربة شرحت الجرذان وتم حساب وزن الغدة ووزن الكلية اليمنى واليسرى. النتائج اظهرت انخفاض معنوي عالي ($p \leq 0.01$) في وزن الغدة الدرقية وارتفاع معنوي عالي ($p \leq 0.01$) في وزن الكلية وفي مستوى هرمون TSH ويوريا الدم والكرياتينين ومعدل البروتين الكلية، بينما انخفاض معنوي عالي في مستويات هرمون T₃ و T₄ المعاملة بجرعات مختلفة من 50-200 ملغم / كغم TiO₂ في جميع الفترات الزمنية (1,2,4) اسابيع. توضح نتائج الدراسة الحالية انخفاضًا كبيرًا في مستوى مصل T₃ و T₄ مع التعرض لـ TiO₂ NPS الذي يعطل وظيفة الغدة الدرقية، بينما يرفع TiO₂ NPS مستوى اليوريا والبروتين الكلية والكرياتينين. يمكن أن يكون هذا مرتبطًا بالجرعة العالية من TiO₂-NPs ومدة الدراسة، مما تسبب في تنكس ونخر خلايا الكلية وتلف في الحوصلات مما أدى إلى منع الإفراز الذي رفع مستويات اليوريا في الدم، كما أدى إلى مستويات عالية من الكرياتينين والبروتين الكلية في المصل بسبب الخلل الذي حدث في وظائف الكلية.

الكلمات المفتاحية: وظائف الكلية، جسيمات نانوية، جرذان، الغدة الدرقية، الجسيمات النانوية لثنائي أكسيد التيتانيوم.