**Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/**
**Journal of Tianjin University Science and Technology**
**ISSN (Online):0493-2137**
**E-Publication: Online Open Access**
**Vol: 57 Issue: 05:2024**
**DOI: 10.5281/zenodo.11174423**

# STUDY OF CARCINOGENIC POTENTIAL AND TOXIC EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES ON FEMALE REPRODUCTIVE HEALTH

**UZMA SHEHZADI ***
Department of Zoology, Lahore College for Women University, Lahore, Pakistan.
* Corresponding Author Email: uzmarafeeq@gmail.com

**SUMERA SAJJAD**
Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

**SHAGUFTA NAZ**
Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

**Abstract**

Titanium dioxide ($TiO_2$) is naturally occurring metal-oxide with variety of uses in agriculture, industry, medicine and daily personal care products when converted to nano-size (<100 nm). Toxicological studies demonstrated that $TiO_2$ nanoparticles (NPs) have injurious effects on human health and other environmental species. This study was designed to examine the carcinogenic potential of $TiO_2$ NPs and their detrimental effects on female reproductive system along with steroidogenesis. Serum chemistry parameters, reproductive hormones like estrogen, progesterone and tumor marker CA-125 along with malignant effects on ovarian tissues in female rabbits (n = 60) were evaluated after $TiO_2$ NPs treatment. Animals were randomly divided into three groups including two experimental and one control (n=20/group). Treated group-1 (T1) and treated group -2 (T2) received 5 mg/kg and 10 mg/kg $TiO_2$ NPs respectively intraperitoneally daily for 3 months and control group only received 0.9% saline solution. Blood samples were collected fortnightly. Enzyme linked immunosorbent assay (ELISA) was performed to estimate the changes in different parameters and histopathological examination to evaluate structural variations due to toxic effect of these NPs. The results of our study showed that all the serum chemistry attributes tended to increase significantly ($P \leq 0.05$), levels of sex hormones and CA-125 were also significantly different ($P \leq 0.05$) in treatment groups as compared to control group. Ovarian tissues were examined for histopathological changes through light microscopy which showed highly vacuolated cells and atretic primary follicles, cells with micronucleus, and congestion and degeneration of granulosa cells in $TiO_2$ NPs treated groups. Hence, it might be established that $TiO_2$ NPs could be potent carcinogens in ovarian tissues and pose damaging and toxic effects on steroidogenesis in female rabbits.

**Keywords:** Steroidogenesis, Tumor Marker CA-125, Toxic Effects, Oxidative Stress.

## INTRODUCTION

Nanotechnology is an immensely advancing field with great applications in agriculture, general medicine, veterinary medicine, food production and aquaculture. Nanoparticles (NPs) have exceptional physicochemical characteristics due to which this technology is gaining ground in various zones of life but wide use of these NPs could cause toxicity in living organisms and may alter the morphology of different tissues (Rahman *et al*., 2022). Extensive use of titanium dioxide ($TiO_2$) NPs in personal care products and cosmetics is raising health concerns as humans are getting exposed through inhalation, ingestion or dermal penetration. $TiO_2$ NPs can be involved in neurotoxicity via blood brain barrier and

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

slowly accumulates in central nervous system and ultimately cause pathological tissue variations, cellular death (necrosis) and DNA damage through enhanced oxidative stress (Mohammadinejad *et al*., 2019).

Imbalance in oxidative and anti-oxidative system in living organism is termed as oxidative stress and this is created through enhanced production of reactive oxygen species (ROS). It is established that the toxicity mechanism of NPs also involve oxidative stress via ROS generation. Female reproductive system is very fragile and sensitive to the environmental contaminants like NPs that disrupt the normal functioning of cells and tissues adversely. ROS produced by these NPs could intricately effect the steroidogensis of ovarian tissues and maturation of oocytes (Asadi *et al*., 2019). In an in vivo study, it was concluded that NPs has the ability to accumulate in reproductive tissues and organs even can cross placental barriers (Ferdous and Nemmar, 2020). An in vitro study resulted in significant variations in reproductive hormonal control and pathophysiology of ovarian tissues after NPs treatment (Lasi, 2017).

Toxicities of NPs are frequently being studied in recent researches however carcinogenic potential of metal NPs like $TiO_2$ is still understudied area, due to lack of homogeneity in available data, need of the hour is to unveil the carcinogenicity of NPs and detrimental impacts on ovarian tissues. In vivo studies are indispensable for the risk assessment of different engineered NPs in this regard. So, the present experimental study is designed to reveal the adversity and carcinogenicity of $TiO_2$ NPs on reproductive structures in female rabbits. For the best of our knowledge no study is conducted to evaluate the carcinogenic potential of $TiO_2$ on female rabbits.

## MATERIALS AND METHODS

The study was approved by the Advanced Research and Research Board of Lahore College for Women University, vide Letter No. 1054 dated 30-04-2018.

### Synthesis of $TiO_2$ NPs

Nanoparticles (NPs) of $TiO_2$ (anatase) were prepared by means of titanium tetrabutoxide hydrolysis. 20 ml of anhydrous isopropanol was added to 1 ml of titanium tetrabutoxide Ti(OC4H9)4 and stirred briskly to dissolve. Afterwards, 50 ml of double-distilled water was added drop wise having pH 1.5 that was adjusted with nitric acid at room temperature. The solution was then dried at 60 °C using drying oven. Temperature was maintained for about 12 h to indorse better crystallization of $TiO_2$ NPs. The powder that was obtained after drying was then washed 3 times with isopropanol, again dried at 50 °C to completely evaporate the solvent (Hu *et al*., 2011).

### Characterization

Characterization of $TiO_2$ NPs was performed with the help of scanning electron microscopy (SEM) to make sure that NPs are homogenous in size and shape with or without agglomeration and XRD aimed to determine the physiochemical properties (Iftikhar *et al*., 2023).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

### $TiO_2$ NPs dispersion protocol

$TiO_2$ NPs were dispersed according to the protocol (Fadojua *et al*., 2019). Stock solution of $TiO_2$ NPs was prepared by suspending (200 mg/100ml). The NPs were suspended in normal 0.9 % saline and stirred on vortex agitator for about 10 minutes before administration.

### Experimental protocol

Healthy female rabbits (n =60) 3-4 months of age and weighing 1500 g-1100 g were kept in standard colony conditions and provided with green fodder, barley and wheat grains. Fresh water was available ad libitum. After acclimatization, animals were randomly divided into 3 groups; one control and two experimental groups and were administered 5 mg/kg and 10mg/kg $TiO_2$ NPs intraperitoneally daily for 12 weeks. Control group received only normal saline.

### Sampling protocol

Blood samples were collected from ear vein of the experimental and control groups fortnightly in EDTA collection tubes. Serum was separated by centrifugation at 2500 rpm for 20 minutes and stored at -10 °C. Ovarian tissues were excised randomly from control and treated groups after last week of treatment, fixed in 10% formalin solution. Blood samples were then assayed with enzyme linked immunosorbent assay (ELISA) by using estrogen kit (Accu-Monobid) Inc., Lake Forest, CA 92630, USA, progesterone kit and CA-125 kit (Accu-Monobid) Inc. CA 3025-300A. USA.

### Histopathological examination

Ovarian tissues were processed by using standard protocol for histological examination (Kanwal *et al*., 2019). 5 µm thick sections were prepared with sliding microtome, mounted on glass slides and examined under light microscope (E- 200 digital microscopic camera-Nikon Japan Ei 1- L2) to evaluate histopathological changes.

### Statistical Analysis

Analysis of variance (ANOVA) for repeated measures followed by Tukey's test and post hoc test, was applied on the data to evaluate the variation in serum chemistry parameters, levels of estrogen, progesterone and CA-125 concentrations among control and experimental groups by using Statistical Package for Social Sciences (SPSS for Windows version 12, SPSS Inc.).

## RESULTS

Effects of $TiO_2$ NPs treatment on mean estrogen (pg/ml) and progesterone (ng/ml) showed that mean concentration of estrogen in treated groups was significantly increased ($P \leq 0.05$) and concentration of progesterone decreased significantly in treated groups as compared to control group (Table, 1). Plasma CA-125 concentration was significantly increased ($P \leq 0.005$) in treated groups as compared to control group. (Fig, 1). Regarding the serum chemistry attributes (Table 2), all the attributes (glucose, LDL, HDL, TGs and

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

cholesterol) tended to significantly (P≤0.05) increase in the two treatment groups as compared to the control group from 2nd week till the 12th week. All results are represented as mean (±SEM).

**Table 1: The effect of $TiO_2$ NPs treatment on serum reproductive hormones levels of female rabbits**

| Attributes | Weeks | Control | T1 | T2 |
|---|---|---|---|---|
| Estrogen (pg/mL) | 2 | 112.0±3.8$^a$ | 182.3±1.9$^b$ | 193.7±3.1$^c$ |
| | 4 | 124.5±1.6$^a$ | 190.6±1.2$^b$ | 201.4±1.5$^c$ |
| | 6 | 118.3 ±2.0$^a$ | 203.0±2.0$^b$ | 219.6±2.8$^c$ |
| | 8 | 120.6 ±1.9$^a$ | 207.8 ±1.6$^b$ | 229.2±1.8$^c$ |
| | 10 | 116.9±2.2$^a$ | 210.8±4.0$^b$ | 244.2±1.7$^c$ |
| | 12 | 123.4±1.3$^a$ | 232.5 ±2.9$^b$ | 256.1±2.4$^c$ |
| Progesterone (ng/mL) | 2 | 12.4±1.6$^a$ | 4.1±0.2$^b$ | 2.9±0.07$^c$ |
| | 4 | 14.1±1.2$^a$ | 3.5±0.1$^b$ | 2.7±0.06$^c$ |
| | 6 | 11.2±0.8$^a$ | 3.4 ±0.1$^b$ | 2.5±0.06$^c$ |
| | 8 | 10.3 ±1.1$^a$ | 3.2±0.3$^b$ | 2.4±0.05$^c$ |
| | 10 | 17.4 ±1.4$^a$ | 3.9±0.2$^b$ | 1.7±0.07$^c$ |
| | 12 | 13.8 ±1.4$^a$ | 3.0 ±5.8$^b$ | 1.5.3±0.04$^c$ |

Superscripts $^{a,b,c}$ are different (P≤0.05) within rows for different treatment groups

**Table 2: The effect of $TiO_2$ NPs treatment on serum chemistry attributes of female rabbits**

| Attributes | Weeks | Control | T1 | T2 |
|---|---|---|---|---|
| Glucose (mmol/L) | 2 | 6.86±0.2$^a$ | 9.5±06$^b$ | 12.2±0.8$^c$ |
| | 4 | 5.2±0.1$^a$ | 8.2±0.7$^b$ | 9.9±0.9$^c$ |
| | 6 | 6.4±0.7$^a$ | 9.2±0.8$^b$ | 10.2±1.0$^b$ |
| | 8 | 5.9±0.7$^a$ | 9.0±0.4$^b$ | 11.1±1.2$^c$ |
| | 10 | 6.0±0.7$^a$ | 8.1±0.2$^b$ | 10.7±0.9$^c$ |
| | 12 | 6.3±0.7$^a$ | 8.9±0.8$^b$ | 11.7±1.0$^c$ |
| LDL (mmol/L) | 2 | 1.15±0.1$^a$ | 8.0±0.4$^b$ | 8.2±0.2$^c$ |
| | 4 | 2.0±0.4$^a$ | 6.2±0.4$^b$ | 7.1±0.7$^b$ |
| | 6 | 2.7±1.0$^a$ | 5.8±0.4$^b$ | 7.8±0.9$^b$ |
| | 8 | 1.2±0.8$^a$ | 7.2±0.8$^b$ | 8.1±0.8$^b$ |
| | 10 | 2.0±0.2$^a$ | 7.2±0.8$^b$ | 8.1±0.4$^b$ |
| | 12 | 2.1±0.4$^a$ | 6.9±0.4$^b$ | 6.5±0.2$^b$ |
| HDL (mmol/L) | 2 | 2.5±0.1$^a$ | 5.5±0.7$^b$ | 6.3±0.1$^c$ |
| | 4 | 2.7±0.2$^a$ | 6.2±0.8$^b$ | 9.2±1.0$^c$ |
| | 6 | 3.0±0.2$^a$ | 7.1±0.9$^b$ | 8.9±0.8$^c$ |
| | 8 | 1.9±0.7$^a$ | 7.7±0.7$^b$ | 8.7±0.7$^b$ |
| | 10 | 2.4±0.8$^a$ | 8.0±0.5$^b$ | 9.7±0.7$^b$ |
| | 12 | 1.9±0.2$^a$ | 6.2±0.4$^b$ | 11.2±1.0$^c$ |
| TGs (mmol/L) | 2 | 7.4±0.7$^a$ | 12.7±1.8$^b$ | 14.6±0.9$^c$ |
| | 4 | 7.2±0.7$^a$ | 12.9±1.8$^b$ | 15.7±2.0$^c$ |
| | 6 | 6.9±0.4$^a$ | 11.4±1.0$^b$ | 15.4±1.8$^c$ |
| | 8 | 6.9±0.4$^a$ | 11.7±1.4$^b$ | 17.2±1.7$^c$ |
| | 10 | 7.8±0.5$^a$ | 10.9±1.8$^b$ | 14.4±1.4$^c$ |
| | 12 | 8.1±1.0$^a$ | 11.7±1.7$^b$ | 12.4±1.2$^c$ |

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

| | | | | |
|---|---|---|---|---|
| Cholesterol (mmol/L) | 2 | 9.49±0.7$^{a}$ | 15.3±1.7$^{b}$ | 16.6±2.1$^{c}$ |
| | 4 | 9.2±0.7$^{a}$ | 16.2±2.7$^{b}$ | 15.2±2.0$^{b}$ |
| | 6 | 9.1±0.5$^{a}$ | 16.8±2.7$^{b}$ | 15.7±1.8$^{b}$ |
| | 8 | 10.7±1.0$^{a}$ | 13.2±1.8$^{b}$ | 14.8±1.0$^{b}$ |
| | 10 | 10.5±1.7$^{a}$ | 14.2±0.9$^{b}$ | 15.2±1.2$^{c}$ |
| | 12 | 8.9±1.0$^{a}$ | 13.8±1.4$^{b}$ | 14.2±1.4$^{b}$ |

Superscripts $^{a,b,c}$ are different (P≤0.05) within rows for different treatment groups.

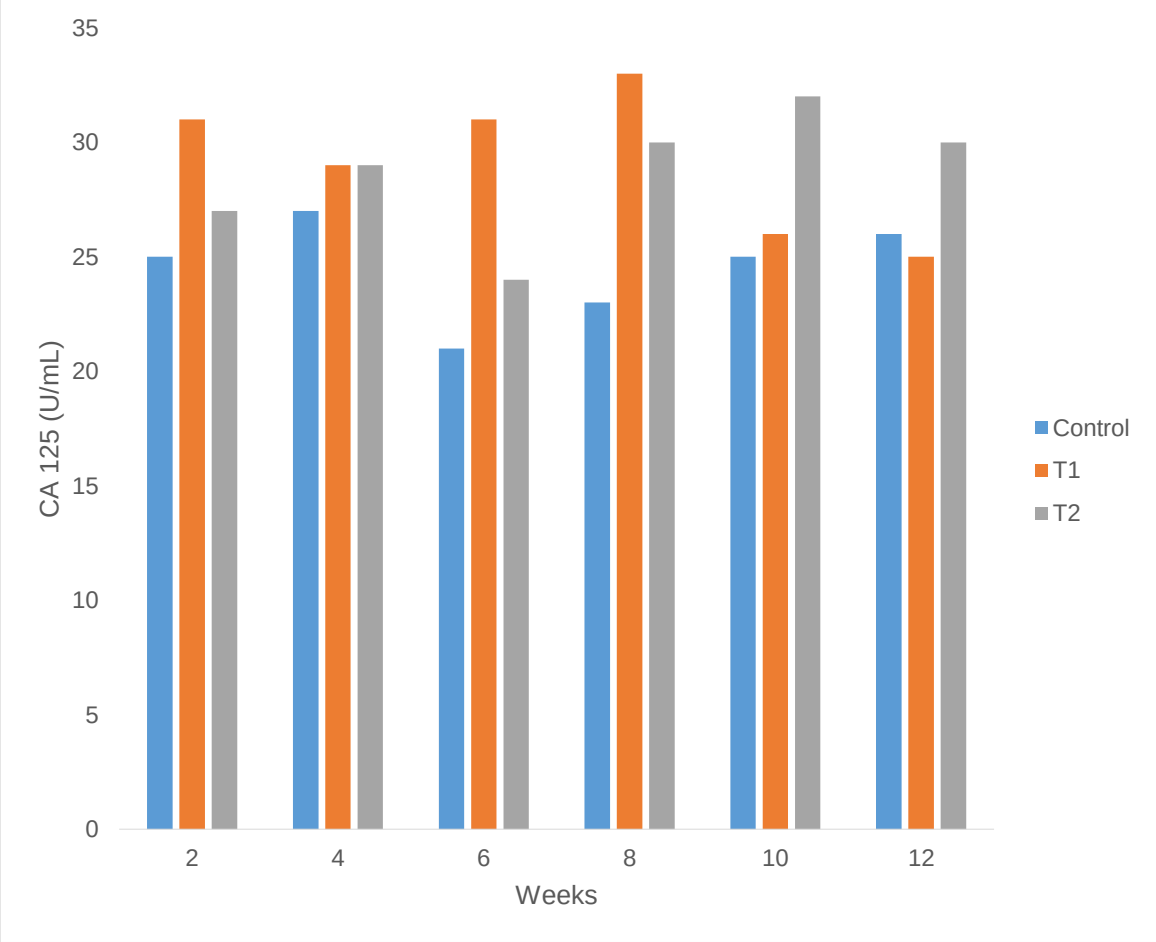


**Figure 1: The effect of $TiO_2$ NPs on CA 125 levels of female rabbits (n=60)**

(T1= treatment group received 5mg/kg of ZnO NPs; T2= treatment group received 10mg/kg of ZnO NPs)

## Histopathological changes

The histology of ovarian tissues of control group showed normal corpus luteum with well-developed primary and secondary follicles along with normal granulosa cells. While in treated groups, histopathological changes in ovary in a dose- dependent manner (Fig, 4) revealed degradation of granulosa cells and primary follicular atresia. Epithelial cells showed proliferation and formation of clusters of cells at boarder line area.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

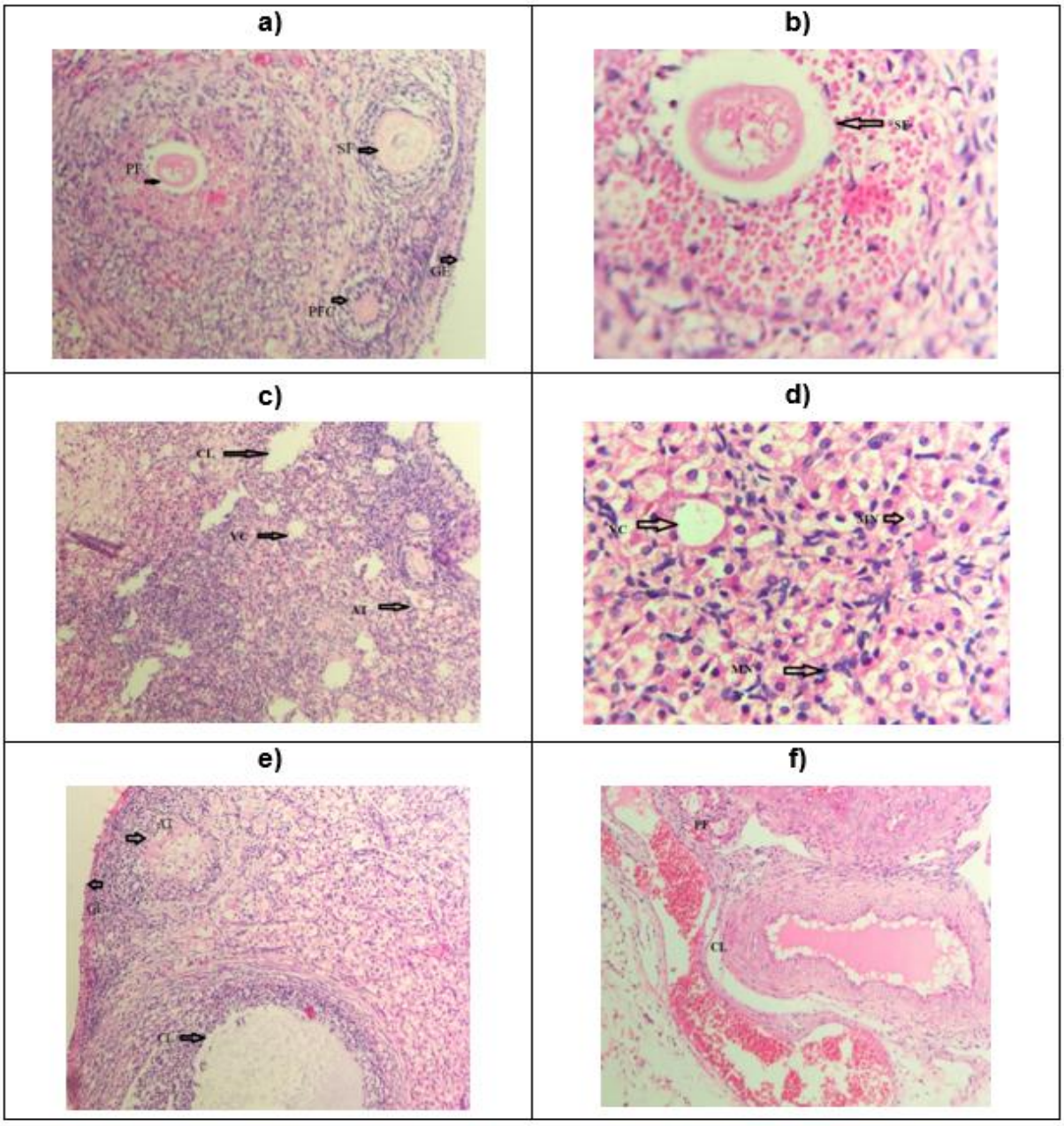


**Figure 2: Micrographs of ovarian histopathology revealing alterations in three studied groups (a,b) Micrographs of control group showing normal ovarian tissue with normal germinal epithelium (GE), primordial follicular cells (PFC), primary follicles (PF) (100X) and secondary follicles (SC) (400X). (c,d) T1 group treated with 5mg/kg with $TiO_2$ NPs revealing enlarged corpus luteum (CL), highly vacuolated cells (VC) (100X) and atretic primary follicles, cells with micronucleus (MN) and congestion (400X). (d,e) T2 group treated with $TiO_2$10 mg/kg NPs showing degeneration of PF with enlarged CL, inflammation and fibrosis**

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 05:2024
DOI: 10.5281/zenodo.11174423

## DISCUSSION

Female reproductive system is very strictly controlled by hormonal cascade and sensitive to the intrinsic or extrinsic variations. Frequent exposure to NPs like $TiO_2$ can have detrimental effects on the female reproductive system. Our data showed disturbed steroidogenesis in $TiO_2$ treatment groups.

The results indicated a strident increase in estrogen levels and decrease in progesterone levels. The main cause of this fluctuation in steroid hormone levels might be $TiO_2$ NPs which could possibly gain entry to the ovaries through circulation and accumulate in theca cells and granulosa cells which ultimately effects steroidogenesis (Jianling *et al*., 2013).

As it is reported earlier in an experimental work that NPs can dispense in various tissues of organisms, such as liver, lung and kidney, reproductive tissues etc. Accumulation of NPs in these tissues may further contribute to the adverse effects by induction of oxidative stress and inflammatory responses (Jiang *et al*., 2018). The toxicity of NPs can influence the hypothalamic-pituitary-ovarian axis which is vital for female fertility. They can pass through the blood brain barrier into hypothalamus and secretory cells of pituitary altering the secretion of gonadotropin-releasing hormone (GnRH), adversely affecting the positive and negative feedback of hypothalamic-gonadal axis and ultimately severely influence the secretion of ovarian estrogen and progesterone. (Mohammad *et al*., 2019).

CA-125 is designated as an antigen that may increase indicating epithelial ovarian cancer. It is normally considered as a classic biomarker for tumors (Rastogi *et al*., 2016). In present study, the data about the tumor marker CA-125 showed significant increase in treated groups ($P \leq 0.05$) when compared to control group which anticipated that the risk of tumor formation in ovarian tissues is much higher due to toxicity of $TiO_2$ NPs at doses used in present study.

In a previous study lung tumors in mice was reported when exposed to $TiO_2$ NPs (Tang et al., 2013) but no data is suggestive of ovarian tumors is available to the extent of our knowledge. Histopathological evaluation revealed formation of clusters of cells around the epithelial lining of ovarian tissue that might leads toward tumorigensis. Histology of ovarian tissues also resulted in $TiO_2$ induced degradation of granulosa cells and primary follicles. This could be related to an experimental study which reported that NPs might have damaging effects on secretory cells, such as granulosa cells, follicle cells and thecal cells in female reproductive tissues due to ROS production (Gifford *et al*., 2017). It is precisely challenging to find a distinct mechanism of carcinogenesis in ovarian tissues due to toxicity of $TiO_2$ NPs.

These NPs are known to be cytotoxic in cultures of different cells but the damaging effects of their toxicity are perhaps not as much studied as it needs to be, fewer evidences are present that suggests that these NPs might damage DNA significantly with the help of oxidative stress which ultimately leads to carcinogenesis (Kim *et al*., 2010). It can be suggested that more experimental studies are needed to establish the underlying molecular mechanism.

**Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/**
**Journal of Tianjin University Science and Technology**
**ISSN (Online):0493-2137**
**E-Publication: Online Open Access**
**Vol: 57 Issue: 05:2024**
**DOI: 10.5281/zenodo.11174423**

## CONCLUSION

In the light of above results and discussion, we can conclude that $TiO_2$ NPs could be intensively playing part in deterioration of female reproductive health. Because these NPs not only toxically effect the ovarian tissues but also might initiate tumor formation in the ovaries. For better insight into the mechanism of carcinogenesis and to establish relevance to the metal NPs toxicities, there is a dire need of future studies to unveil the molecular mechanism involved in carcinogenesis in reproductive organs.

### References

1) Mohammadinejad, M. A., Moosavi, S., Tavakol, D. Ö., Vardar, A, Hosseini, M., Rahmati, L., Dini, S., Hussain, A., Mandegary, D. J and Klionsky, R. Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. 2019. *Autophagy*. (15): 4-9.

2) RahmanHS, OthmanHH. YahyaAHR., ShujaaEdinA AL-HajNA 2022. Beneficial and toxicological aspects of zinc oxide nanoparticles in animals. *Vet. Med. Sci*. (8):1769-1779.

3) Ferdous, Z. and Nemmar, A. Health impact of silver nanoparticles: a review of the biodistribution and toxicity following various routes of exposure. 2020. *Int. J. Mol Sci*. 21(7): 2375-2384.

4) Lasi, B. Effect of silver nanoparticles on levels of serum fsh, lh and estradiol in pco-induced female mice. 2017. *World J. Pharm Res*.113-125.

5) Sadeghzadeh, F. and Jalilvand M, A. Effect of molybdenum trioxide nanoparticles on ovary function in female rats. 2019. *Egypt. J. of basic and applied sci.* (121): 48-53.

6) Iftikhar, M., Noureen, A., Jabeen, F., Uzair, M., Rehman, N., Karahmet, E., Katubi, K. M. and Heloisa, J. 2023. Bioinspired engineered nickel nanoparticles with multifunctional attributes for reproductive system. *Chemosphere* (311): 13692-136999.

7) Fadojua, O., Ogunsuyia O., Akannib,Q., Alabic, O., Alimbaa, C., Adaramoyeb, O., Cambierd, S., Eswarae, S., Gutlebd, A. C. and Bakarea, A. 2019. Evaluation of cytogenotoxicity and oxidative stress parameters in male Swiss miceco-exposed to titaniumdioxide and zincoxide nanoparticles. *Environ. Toxicol and Pharmacol.* (70): 2-9.

8) Kanwal, Z., Raza, M.A., Manzoor, F., Mateen, A., Farzana, R., Pervaiz, S., Shahzad, N. 2019. In vivo anti-proliferative activity of silver nanoparticles against Psedumonas aeruginosa in freshwater Labeo rohita. 2019. *Applied Nanoscience*. 9(8): 2039-2049.

9) Emilia, B., Maria, O, A., Fedora, G. and Paola, T. 2023. $TiO_2$-NPs toxicity and safety: An update of the findings published over the last six years. *Med. Chem.* (8):1050-1057.

10) Hu R., Zheng J., Zhang T., Gao. G., Cui.Y., Chen.Z., Cheng J. Hong, m., Tang M., Tang F. (2011). Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles. Journal of Hazardous Materials. 191: 32-40.

11) Tang Y., Wang F., Jin C., Liang H., Zhong X., Yang Y. 2013. Mitochondrial injury induced by nanosized titanium dioxide in A549 cells and rats. *Environ. Toxicol. Pharmacol.* 36:66-72.