Original Article

Effect of Titanium Dioxide Nanoparticles on Parental Oxidative Status and Offspring Skeleton Development and the Protective Role of *Portulaca oleracea* Seed Extract: Histological and Morphometric Study

Fatma E. El-Demerdash[1], Mona A. Salem[2], Samia M. Abd El-Wahab[1], Eman S. Mohammed[2] and Abir K. Mohamed[1]

[1]Department of Zoology, Faculty of Science (Girl's), [2]Department of Histology, Faculty of Medicine (Girl's), Al -Azhar University, Egypt

ABSTRACT

Introduction: Titanium dioxide nanoparticles used in numerous medical and industrial applications. TiO_2NPs nanotoxicity is a major health concern.

Aim of the Work: The objective of this research was to examine the oxidative status of parental exposed to TiO_2NPs and the developmental status of their offspring's skeleton. Further, to examine the potential role of *Portulaca oleracea* extract as an antioxidant plant.

Materials and Methods: Twenty-four pregnant rats were divided into four groups: Control (C) group, P group (given 10 ml/kg B.W/day of P extract), T group (0.5mg/kg TiO_2NPs every day) and P+T group (given P extract, then TiO_2NPs at the same dosage as before). Dosages were given orally from the 6th to the 19th day, all rats were sacrificed on the 20th day of gestation. Oxidant parameters such as glutathione GSH, Superoxide dismutase SOD and malondialdehyde MDA were determined from the liver of pregnant rats. Fetal skeletons were stained with alizarin-red S and Alcian blue. The fetal hind limbs were stained with Hx. &E and Mallory triple stain for histological examination.

Results: SOD and GSH content showed a significant reduction in the T-group compared to the control and P groups, and MDA level was significantly increased. Furthermore, rats in group T exhibited fetal growth retardation and skeletal abnormalities, including delayed ossification center development and reduced long bone growth. Histological examination revealed partial chondrification of the growth plate in fetal femur bones, leading to the deterioration of all zones and a significant decrease in collagen density. However, treatment with *Portulaca oleracea* extract resulted in an enhancement of antioxidant parameters in pregnant rats. The extract also decreased skeletal deformities induced by TiO_2NPs and improved the length and structure of fetal long bones.

Conclusion: The present study recommended that antioxidant nutrient in diet may ameliorate the toxicity of TiO_2NPs.

Received: 22 December 2023, **Accepted:** 16 February 2024

Key Words: Antioxidant; growth plate; *Portulaca oleracea* extract; rat skeleton; titanium dioxide nanoparticles.

Corresponding Author: Abir K. Mohamed , PhD, Department of Zoology, Faculty of Science, Al -Azhar University, Egypt, **Tel.**: +2010 6281 8767, **E-mail:** abirkhalil09@azhar.edu.eg

ISSN: 1110-0559, Vol. 47, No. 1

INTRODUCTION

Nanoparticles (NPs) are small organic/inorganic particles that vary in size from 1 to 100 nanometers[1]. Titanium dioxide nanoparticles (TiO_2NPs) is an inorganic nanoparticle[2] that is employed in a wide variety of applications due to their brightness and high refractive index.

Nanoparticles are used extensively in medical and industrial applications. For example; TiO_2NPs is used around the globe, mostly in coatings, paints, plastics, papers, inks, foods, medicines, food packaging and cosmetics[2,3]. However, nanoparticles' nanotoxicity is a major concern as they have proved to produce oxidative DNA damage, increase reactive oxygen species (ROS) and impair anti-oxidative activity leading to the generation of cancer[4].

Purslane is the botanical name for *Portulaca oleracea* L. (family portulacaceae) that is used as an edible plant and a traditional herbal medicine[5]. P. oleracea is high in alpha-linoleic acid and omega-3 fatty acid that's crucial for human growth, development and illness prevention. It also has a broad range of pharmacological characteristics, including antioxidant, anti-diabetic, neuroprotective, antibacterial, anticancer and anti-inflammatory effects[6,7].

TiO_2NPs are toxic to early developmental stages in fetus rodents. Exposure to TiO_2NPs during pregnancy passes TiO_2NPs on to the offspring resulting in negative changes in the formation of offspring[8]. Bone is sensitive to

 DOI: 10.21608/ejh.2024.257385.1989

any toxic substance during the fetus development due to its complex structure and rapid ossification during gestation[9]. Bone, a kind of connective tissue, is multicellular, protein-rich minerals and fibrous living organ. The skeletal bone supports and protects muscles to facilitate movement at the joints[10].

Bone can either be compact or spongy (cancellous). The diaphysis is made up of compact bone. The epiphysis is made up of spongy bone, which has many gaps or cavities[11].

When bone development ends, the GP becomes ossified and is referred to as the epiphyseal line[12]. Bones begin as cartilage model. Then, osteoblasts eventually replace the cartilage with bone matrix in a process called endochondral ossification. Ossification is preceded by two complicated and sensitive steps. The first step is maturation of the chondrocytes in the projected mid-shaft of the bone. Secondly, newly formed osteoblasts deposit in the periosteal bone collar around the mid-shaft by[12]. The main ossification center is formed when blood vessels, osteoclasts (cells that degrade cartilage and bone), bone marrow and osteoblast precursors, infiltrate the model from the bone collar. As osteoclasts destroy cartilage extracellular matrix (ECM) and osteoblasts build bone on cartilage remains, the main center spreads towards the cartilage model's ends[13]. There is slight data concerning the effect of titanium nanoparticles on the development of the fetal skeleton.

The goal of this research was to determine the influence of *Portulaca oleracea* seed extract on the oxidative statues of pregnant rats and the development of their fetus skeletons and plates of the femur bone exposed to TiO_2NPs during pregnancy.

MATERIALS AND METHODS

Chemicals

A- Titanium dioxide nanoparticles (TiO_2NPs)

TiO_2NPs employed in this work were in the form of nano anatase, powder, with a size of 25 nm and a purity of 99.7% trace metals (SIGMA-ALDRICH). As a stock solution, TiO_2NPs were suspended in ultrapure water (Promega, Madison, WI, USA) at a concentration of 20 mg/ml. An ultrasonic vibrator was used to disperse the stock solution for 30 minutes. The suspension was diluted to a working concentration of 0.5 mg/L in 1 Holt buffer (60 mmol/L NaCl, 0.67 mmol/L KCl, 0.3 mmol/L NaHCO3, 0.9 mmol/L CaCl2, Ph 7.2)[14]. TiO_2NPs were given orally at a rate of 0.5 mg/kg/day from day 6 to day 19 of pregnancy.

B- Seed extract of *Portulaca oleracea*

Seeds were acquired fresh from the field and identified in the Botanic Department at Faculty of Science. P. oleracea seeds were washed, dried and grinded, and 100 gm of the grinded seeds added to one liter of boiling distilled H_2O, chilled and filtered[15]. The concentrated yield extract was given orally at a dosage of 10 ml /kg/day from day 6 to day 19 of pregnancy.

Animals

Ten mature fertile male and thirty virgin female Albino rats, aged 12 weeks and weighing 180-200 g, were provided from the Laboratory Animal Colony at Helwan Branch. Males and females were housed separately in metal cages until the mating period. The rats were maintained under control conditions, including temperature, relative humidity and a 12-hour light/dark cycle ensuring a hygienic environment. Their diet contained food pellets from Cairo, Egypt's Factory of Oil and Soap Company, vegetables as a source of vitamins, provided ad libitum with drinking tap water. The rats were acclimated for one week to the laboratory environment. After that, female rats were paired with male overnight (each male rat was mated with 2 females). Vaginal smears were examined for the presence of a vaginal plug, and that day was designated as gestational day one.

The study followed the national laws and guidelines for the ethical use and care of laboratory animals. The design and procedures of the present study was approved by the ethical committee of Al-Azhar University, Faculty of Medicine.

Experimental design

Twenty-four pregnant rats were randomly divided into four groups (6 pregnant rats in each group) as follows:

Group 1 (Control group): pregnant rats fed on normal diet.

Group 2 (P group): Pregnant rats treated with aqueous purslane seed extract at a dose of 10 ml /kg/day.

Group 3 (T group): Pregnant rats received 0.5mg /kg/ day nano- TiO2.

Group 4 (P + T group): Pregnant rats received purslane seed extract at a dose of 10 mL/kg followed by TiO_2NPs at a dose of 0.5 mg/kg/day, from the 6th to the 19th day of gestation.

Biochemical parameters

Liver tissues were homogenized using an electrical homogenizer by mixing 0.5 g of tissue with 5 ml phosphate buffer saline at 4 ◦C. The homogenates were centrifuged (at 3000 rpm) for 20 min. The collected supernatant was kept at -20 ◦C until use. Antioxidant defense parameters as reduced glutathione GSH content[16], Superoxide dismutase SOD according to Marklund *et al*.[17], and malondialdehyde MDA level according to the method of Yoshioka *et al*.[18], were determined in tissue homogenate. Kits for physiological analysis were purchased from Bio diagnostic company, Cairo, Egypt.

Investigation of fetal skeleton development

Some fetuses were preserved in 95% ethyl alcohol, skinned and eviscerated, and the skeletons were stained

with alizarin-red S and Alcian blue[19]. Under the stereo microscope, the blue embryonic cartilage and red skeletal ossification were visible. Each stained fetus was thoroughly checked for potential bone abnormalities.

Histological examination

The hind limbs of fetal rats of all four groups were dissected and fixed in 10 % neutral buffer formol, trimmed from muscle tissue and decalcified. The specimens of decalcified femur bones were dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin wax, sectioned at 7 µ thicknesses. Longitudinal sections were stained with Hematoxylin and Eosin for general structure[20] and Mallory triple stain for revealing collagen fibers[21]. Photos were captured using a Carl Zeiss light microscope at Al-Azhar University's Faculty of Medicine (Girls). Images were saved as jpgs at 10, 20, and 40X objective magnifications for examination.

Morphometric and Statistical analysis

The crown rump length (CRL), humerus and femur bone lengths of fetuses were determined[22]. The density of bone tissue collagen fibers was determined using Mallory triple stain of eight slides at a magnification of 100x using IPWIN32 image analysis software.

Analytical statistics

Results were presented as mean ± standard error (SE). The obtained data were analyzed using one-way analysis of variance (ANOVA)[23] and Post HOC tests (LSD) analysis to compare various groups with each other.

RESULTS

Physiological analysis

SOD and GSH contents, in group T, were significantly decreased ($p \leq 0.001$) compare to the control (C) and (P) groups (Table 1). Liver MDA content was significantly increased (127.3 ± 16.40) in T group compared to the C Group (46.3±2.30) and P Group (101.9± 13.56). However, In P+T Group, GSH content and SOD activity significantly decreased ($p \leq 0.05$) (Table 1). Liver MDA content of P+T group showed non-significant decrease compared to C group.

Fetuses' skeletal changes

On day 20 of pregnancy, both the C and P groups have completed the ossification process of their bone as examined using a stereo microscope (Plate 1-A). Fetuses of T group, on the other hand, have a significant absence of ossification. Those in the P+T group, demonstrated an improvement in the ossification of bony and cartilaginous structures (Plate 1-A). The control and p groups showed normal bright stain of ossified parts of the fore limb bones (Plate 1-B) and hind limbs (Plate 1-C). However, inadequate ossification was seen in the limbs of fetuses treated with titanium. The forelimb bones, comprising the humerus, radius, ulna, carpals, metacarpals and phalanges were much shorter and thinner in T group in comparison with the C and P groups (Plate 1-B). The bones of the carpals, metacarpals and phalanges were missing. The femur, tibia, fibula, tarsal, metatarsals and phalanges, bones of the hind limbs, were also thinner in T group (Plate 1-C). The ossification of the fore and hind limbs was better in fetuses treated with Portulaca (P+T group) during pregnancy.

Histological observations

A- Hematoxylin and eosin examination

Plate 2 (a-c) & (d-f) showed the normal structure of the femur bone of fetal rats (20th day of gestation). The femur bone is formed of five zones forming the growth plate (GP). The initial resting zone (ZR) consists of hyaline cartilage immersed in translucent basophilic matrix with tiny inactive and randomly organized chondrocytes in their lacunae. The second proliferative zone (ZP) formed with more chondrocytes grouped in parallel rows and divided by matrix bars with multiple mitotic figures (Plate2 a&d). The larger, vacuolated chondrocytes in the third hypertrophic zone (ZH) were separated by a little quantity of matrix (Plate 2 b&e). Tiny sized cells with small pyknotic nuclei and vacuolated cytoplasm observed in the calcification zone (ZC), indicating varying degrees of chondrocyte death. Ossification began when the chondrocyte lacunae merged with one another, leaving empty spaces that were invaded by vascular tissue rich in osteogenic cells and blood capillaries. With a well-developed periosteum and subperiosteal bone collar, the final ossification zone (ZO) occurred (Plate 2c&f). In well-developed spongy bone trabeculae separated by bone marrow gaps, many osteogenic cells and blood capillaries emerged. Osteocytes showed as oval cells situated in lacunae and immersed in acidophilic bone matrix in the anastomosed bone trabeculae (Plate 2c). The medullary cavity was filled with red marrow, which was made up of blood cell progenitors. In the P Group, there were no noticeable differences in histological results in any of these zones.

Inadequate chondrification of mesenchymal cells was seen in the fetal femurs of T Group whose mothers were given TiO_2NPs during pregnancy (Plate 2g-i). Few, tiny, and irregularly organized chondrocytes were seen in the proliferative zone. In ZH, there were few, tiny, and degraded cells with no mitotic figures. There were extremely few empty lacunae in the calcification zone. In ZO, periosteum failure and subperiosteal bone collar development emerged. With total ossification failure, the irregular thin bone trabeculae were separated by tiny bone marrow gaps. On the surface of bone trabeculae, osteocytes were aberrant tiny cells with shrunken nuclei that were seldom visible. In the P+T Group, these adjustments showed a modest improvement in fetal rat femurs (Plate 2j-l).

B- Histochemical reaction

Mallory staining of the femur bone of the control (c) and P groups revealed typical collagen fiber distribution

in the GP and the ossification zones (Plate 3 a-d). Also, few collagen fibers appeared scattered in the calcification zone in bone trabeculae and surrounding the haemopoietic tissue (Plate 3 b,d). While the collagen fibers in the bone trabeculae and surrounding the haemopoietic tissues were reduced in the T group (Plate 3 e,f).

However, collagen fibers were shown to be more abundant in P+T group in the resting, proliferating and hypertrophic zones, as well as in the calcification zone, particularly in the bone trabeculae and surrounding the haemopoietic tissues (Plate 3 g,h).

Morphometric observations

Fetal rats of group P recorded non-significant difference in the average values of CRL, humerus and femur length compared to control group. T Group, on the other hand, recorded fetal growth retardation as indicated by a significant decrease ($P \leq 0.05$) in the average values of CRL, humerus and femur length in comparison with C and P groups (Table 2). However, CRL, humerus and femur lengths of P+T group recorded non-significant difference ($P \leq 0.05$) compared to the control group.

The mean density of collagen of femur bone of fetuses showed a significant decrease reaction ($P < 0.05$) in T Group in comparison with the control group. P group showed non-significant ($P \leq 0.05$) difference in the density of collagen fibers as compared to C group. However, the density of collagen fibers was increased in P+T group in comparison with TiO_2NPs treated group (Table 2)

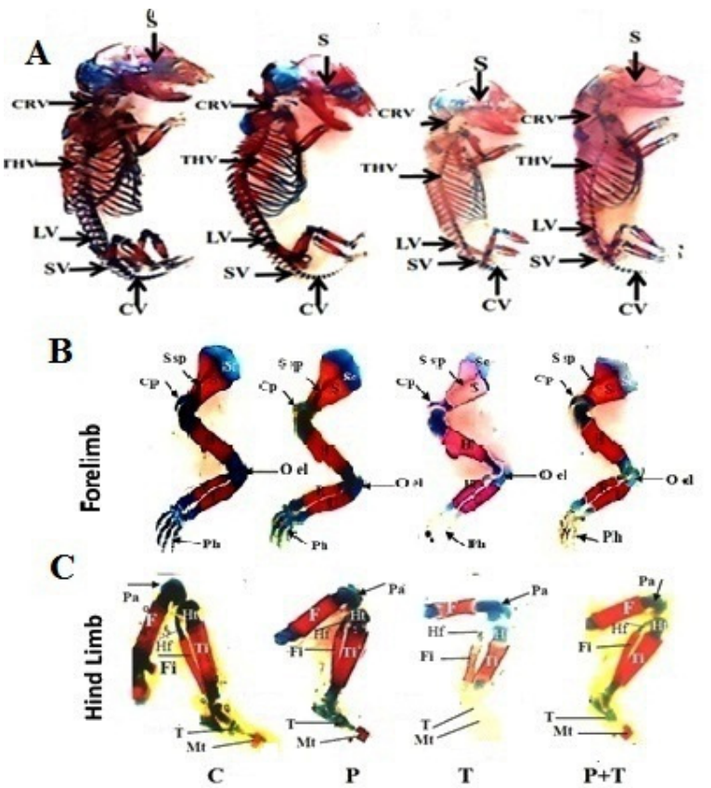

Plate 1: Skeletons of fetal rats (after 20th of day gestation) of all experimental groups. A, showing the ossification of skeletal bones. S= Skull, CRV= Cervical vertebrae, THV= Thoracic vertebrae, LV= Lumbar vertebrae, SV= Sacral vertebrae and CV= Caudal vertebrae. B, Forelimb of all experimental groups. S sp= Scapular spine, Coracoid process, S= scapula, H= Humerus, O el = Olecranon elbow, R=Radius, U=ulna Ph=Phalanges. C, Hind limb of all groups. F=Femur, T= Tibia, Fi, Fibula, T= Tarsals, Mt= Metatarsals, Pa= Patilla, Hf= Head of fibula (The skeletal bones stained with alizarin red S and cartilage with Alcian blue staining, X 2.4).

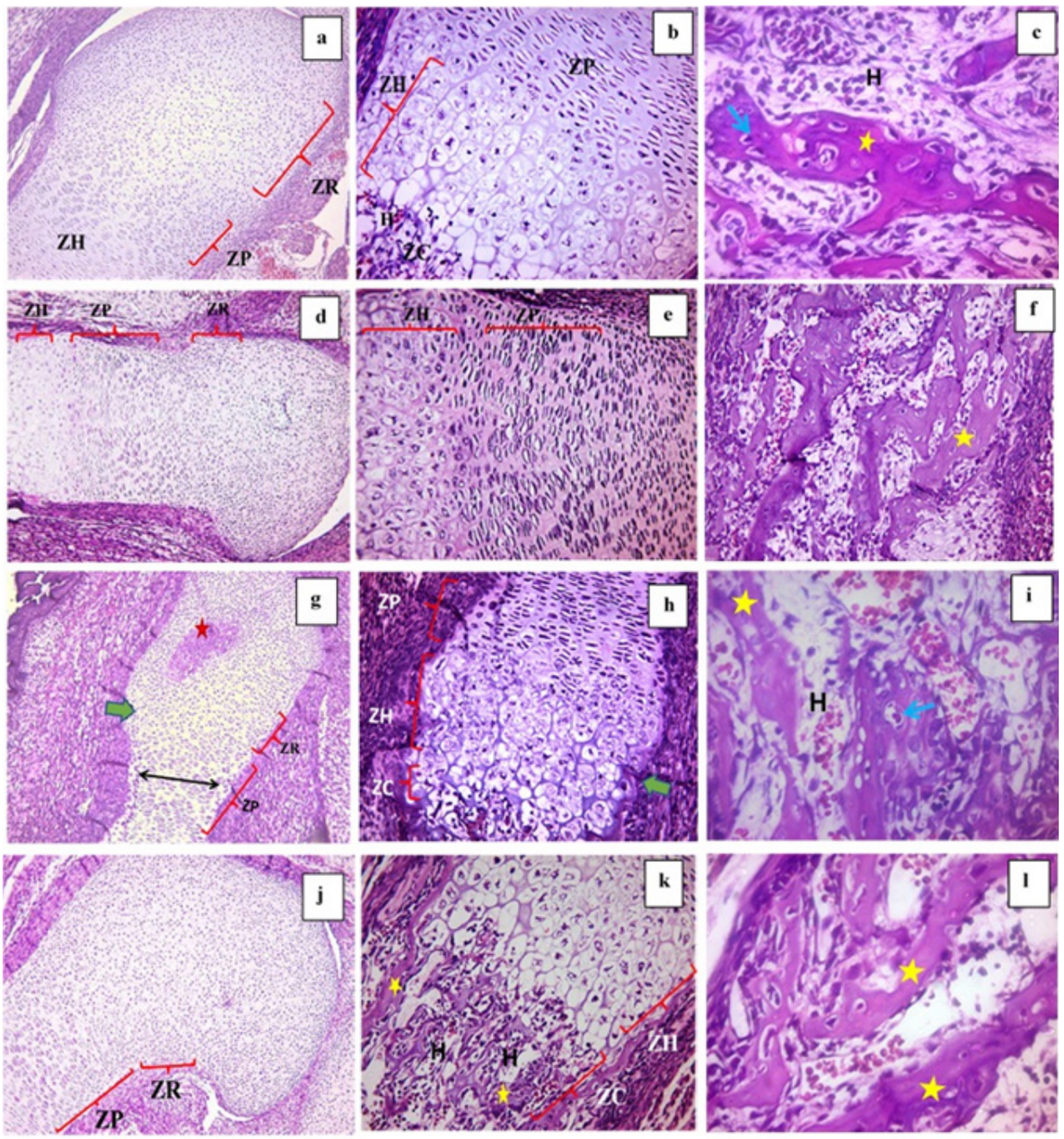

Plate 2: Photomicrographs of longitudinal sections of femur bone (20 days old fetuses) illustrating the zone of resting (ZR), zone of proliferation (ZP), zone of hypertrophy (ZH) and zone of calcification (ZC). The control & Portolaca groups (a-c & d-f) showed normal layers. TiO2NP Group (g-i) showed irregular shape of diaphysis (Green arrows), and abnormal appearance of ossification center (red star). Also, apparently thin bone trabeculae (yellow star) with decreased haemopiotic tissue (H) and small osteocytes (blue arrow) appeared in the ZC. P+T group (j-l) showed well developed periosteum and hypertrophic zone (ZH) with increased number and size of chondrocytes. Apparently thick bone trabeculae (stars) and normal osteocytes in the zone of calcification (ZC) (Hx. & E. stain, 200X & 400 X).

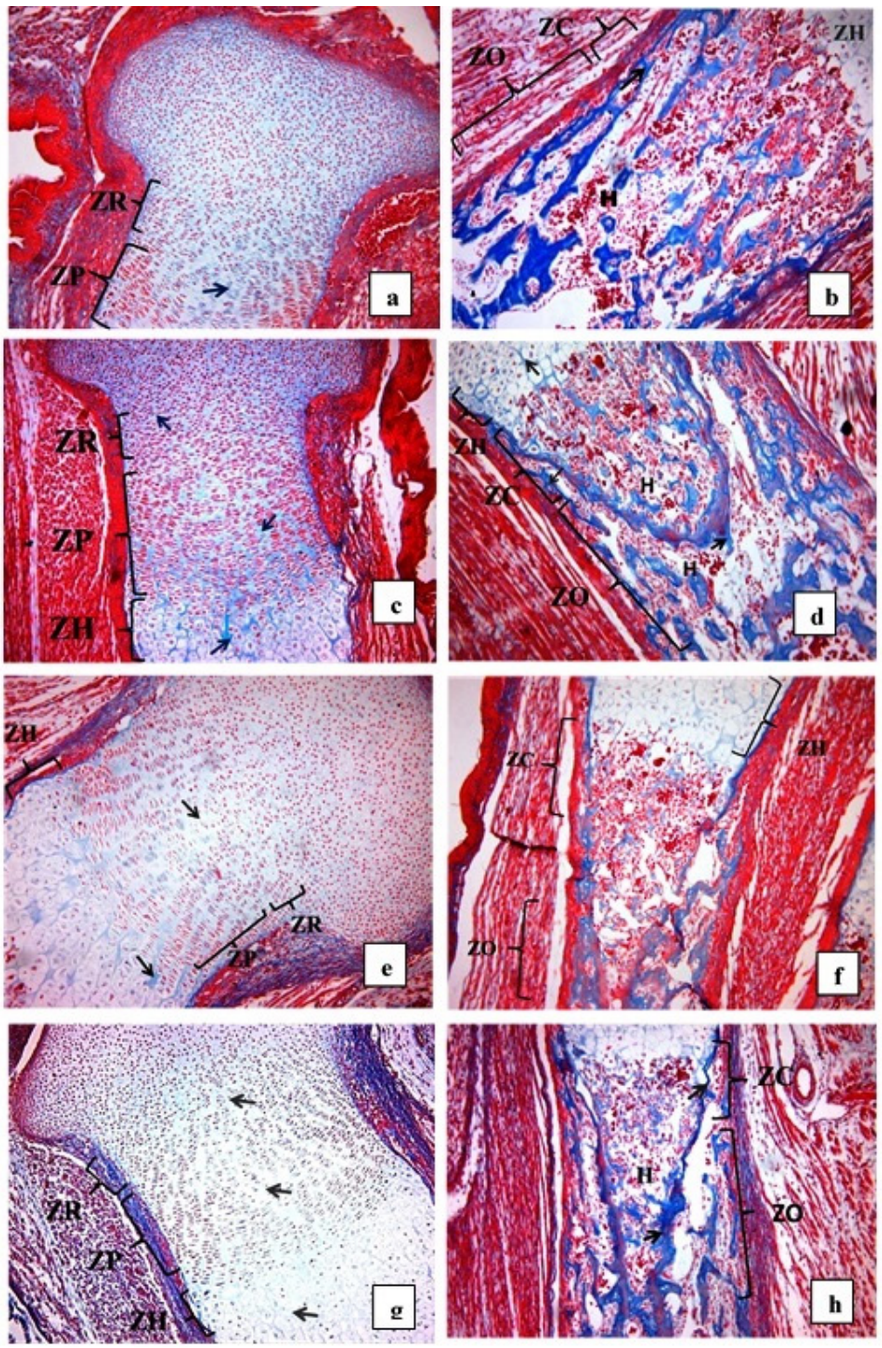

Plate 3: Longitudinal sections of femur bone illustrating the distribution of the collagen fibers through the different zones of GP: zone of resting (ZR), zone of proliferation (ZP), zone of hypertrophy (ZH) zone of calcification (ZC) and Zone of ossification of all the experimental groups. The control & Portolaca groups (a-b & c-d) showed normal distribution of collagen. TiO2NP group (e-f) showed abnormal distribution. T+P somewhat normal amount and normal distribution of collagen. Collagen fibers (black arrows), haemopiotic tissue (H), calcified cartilage in the bony trabeculae. (Mallory Pantine, X100).

Table 1: SOD (U/g tissue) activity, GSH (U/g tissue) and MDA (nmol/g tissue) contents of liver in different groups

Groups	Control	*P*	T	*P*+T
SOD (U/g tissue)	14.5±0.5	12.3 ± 1.2	5.4±0.6[a]	13.0±0.5
GSH (mmol / g tissue)	124.1±3.6	115.4±5.7	79.5 ± 7.8[a,b]	117.5±6.7
MDA (nmol/g tissue)	46.3±2.3	101.9± 13.5	127.3± 16.4	109.0±4.1

Table 2: Morphometric examination and density of collagen fibers of different groups

Groups	Control	*P*	T	*P*+T
Crown rump length (Cm)	3.18±0.07[b]	3.11±0.03[b]	2.84±0.05[a]	3.02±0.06[a,b]
Humorous length (mm)	4.82±0.32[b]	4.53 ± 0.19[b]	3.39±0.09[a]	4.01 ± 0.2[b]
Femur length (mm)	5.43±0.31[b]	4.72 ± 0.34[b]	3.80 ± 0.23[a]	4.01 ± 0.39[b]
Collagen density	0.21±0.03	0.19 ± 0.02	4.51 ±0.39[ab]	0.18 ± 0.01

DISCUSSION

Human exposure to nanoparticles such as TiO_2NPs is unavoidable as the spectrum of their uses expands. This raises concerns about the impact of TiO_2NPs on human health[24]. Humans are often exposed to TiO_2NPs through various sources including liquids, food drinks and medications[4,24]. Maternal exposure to TiO_2NPs during pregnancy has been linked to adverse effects on fetal development, with documented toxicity of different organs in rats[25].

Bones, crucial components of the skeletal framework, are vulnerable to TiO2NP toxicity during development and ossification in early gestation. The entry of TiO2NP into cells induces the production of excessive oxidative radicals, causing disruption of the oxidation/deoxidation equilibrium[26,27].

Purslane seeds have a high nutritional value and have been utilized in traditional medicine, that are employed as natural sources of antioxidants[28]. This study examined whether purslane seed extract might help preserve fetal bones that are exposed to TiO_2NPs during pregnancy.

In the present study, TiO_2NPs were found to increase malondialdehyde (MDA) level in maternal liver tissue, indicating oxidative stress. In agreement with our results, the increased levels of MDA in human osteoblast cells-exposed to titanium- indicates the role of free radicals in cell damage[29].

Treatment with purslane extract (group P+T) detected a mild improvement by significantly decreasing MDA level. This result is consistent with previous findings on the antioxidant activity of purslane. Ahangarpour *et al*. (2016)[30] reported that purslane decreased MDA contents in uterus and ovarian tissues of aging female mice due to its antioxidant activity.

Pregnant rats exposed to Titanium (T group) were decreased in SOD and GSH contents suggesting a reduction in endogenous antioxidant defenses. In line with the present results, SOD level in liver tissue of zebra fish was reduced after T exposure that indicates an imbalance of oxidation/antioxidant system[31].

Conversely, Purslane seed extract (P+T group) demonstrating positive effect by increasing superoxide dismutase (SOD) activity and glutathione (GSH) content compared to the control. Guo *et al*. (2016)[32] reported that this may be due to the presence of higher content of linolenic omega-3 fatty acid in PSO. Zidan *et al*. (2016)[33] detected that, the activity of an antioxidant is closely related to its reducing power; the greater the reducing power, the stronger the antioxidant activity.

Evaluation of fetal skeleton bones with Alizarin red S and Alcian blue revealed that TiO_2NPs exposure during pregnancy let to decrease skeletal ossification and cartilage formation. Consistent with previous studies on fetal abnormalities after titanium exposure during pregnancy and all support these results[25,34,35]. This might be related to pregnancy problems after T injection to mice or OS damage caused by T exposure to embryos[27]. Maternal nutrition has impact on bone development in offspring and later risk of osteoporosis[36], therefore, It is possible that the reduced bone ossification caused by TiO_2NPs was due to its interfering with Ca2+ permeability in the placenta leading to abnormalities[37]. The skeletal ossification of fetuses maternally treated with Purslane seed extract (P+T group) improved somewhat. revealed homogeneous staining, similar to the control and P groups.

The length of fetal femur and humerus bones was substantially reduced in fetuses exposed to TiO_2NPs, indicating a potential impact on skeletal development. This might be related to the growing skeleton sensitivity, especially cartilage formation to TiO_2NPs.

Further, histological examination revealed inadequate chondrification, reduced resting and proliferative zones and irregular organization of chondrocytes in long bones, supporting the believe that TiO_2NPs induced skeletal deformities during development. Mesenchymal cells, and irregularly organized chondrocytes in the long bone of babies exposed to TiO_2NPs during pregnancy.

The hypertrophic zone showed tiny degraded cells and extremely few empty lacunae in the calcification zone. The ossification zone occurred when the periosteum and

subperiosteal bone collar failed to ossify. These findings are consistent with Hong *et al*. (2017)[34] who found that TiO_2NPs caused a decrease in Ca2+ absorption and an increase in its excretion, resulting in a negative Ca2+ balance and a reduction in Ca2+ levels in the embryo. Furthermore, increased Ca2+ dissolution in bones may be caused by injury to osteoblasts and chondrocytes. As well as, increased osteoclast activity. Hong *et al*. (2017) [34]showed that oral administration of TiO_2NPs to pregnant mice can penetrate the blood-fetal-placental barrier, suppressing embryonic development and causing fetal skeletal deformity. This might be owing to OS caused by TiO_2NPs exposure with the release of free radicals.

Furthermore, fetal femurs exposed to TiO_2NPs exhibited a number of collagen fibers, possibly due to NP diffusion in the extra cellular matrix (ECM), which is made up of collagen fibers. Lieleg *et al*[38] observed a reduction in the diffusion coefficient of positively and negatively charged NPs in reconstituted ECM hydrogels.

The histological structure of rat femurs improved somewhat when fetuses were maternally treated with purslane extract followed by TiO_2NPs, suggesting a protective effect of purslane against TiO_2NPs induced damage. This finding is consistent with Kim *et al*[39], who found that P inhibited mature osteoclast bone resorption activity, which was followed by a fast rupture of the actin ring structure in mature osteoclasts, through the regulation of osteoclast-specific genes. They also discovered that P might be beneficial in preventing and treating a variety of bone ailments. Purslane seed oil (PSO) also demonstrated higher antioxidant activity owing to its high omega-3 fatty acid content, according to Rahimi *et al*[40] and Kumar *et al*[41].

CONCLUSION

The present study showed the potential adverse effects of TiO_2NPs on maternal and fetal health, especially on skeletal development. Purslane seed extract shows promise in reducing TiO_2NPs induced toxicity. Further research is recommended to elucidate the underlying mechanisms and evaluate the potential of Purslane in reducing nanoparticles-induced toxicity.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Lovisolo D, Dionisi M, Ruffinatti FA, Distasi C (2018). Nanoparticles and potential neurotoxicity: Focus on molecular mechanisms. AIMS Mol Sci. 5(1):1-13. https://dx.doi.org/10.3934/molsci.2018.1.1.
2. Hamed MT, Bakr BA, Shahin YH, Elwakil BH, Abu-Serie MM, Aljohani FS, *et al*. (2021). Novel synthesis of titanium oxide nanoparticles: Biological activity and acute toxicity study. Bioinorg Chem Appl. https://doi.org/10.1155/2021/8171786.
3. Verleysen E, Ledecq M, Siciliani L, Cheyns K, Vleminckx C, Blaude M-N, *et al*. Titanium dioxide particles frequently present in face masks intended for general use require regulatory control. Sci Rep. 2022;12(1):2529. https://doi.org/10.1038/s41598-022-06605-w.
4. Baranowska-Wójcik E, Szwajgier D, Oleszczuk P, Winiarska-Mieczan A. Effects of titanium dioxide nanoparticles exposure on human health—a review. Biol. Trace Elem. Res. 2020; 193:118-29. https://doi.org/10.1007/s12011-019-01706-6.
5. Desta M, Molla A, Yusuf Z. Characterization of physico-chemical properties and antioxidant activity of oil from seed, leaf and stem of purslane (*Portulaca oleracea* L.). Biotech Rep. 2020; 27: e00512. https://doi.org/10.1016/j.btre.2020.e00512.
6. Zhou Y-X, Xin H-L, Rahman K, Wang S-J, Peng C, Zhang H. *Portulaca oleracea* L.: a review of phytochemistry and pharmacological effects. Biomed Res. Int. 2015;2015. https://doi.org/10.1155/2015/925631.
7. Nazeam JA, El-Hefnawy HM, Omran G, Singab A-N. Chemical profile and antihyperlipidemic effect of *Portulaca oleracea* L. seeds in streptozotocin-induced diabetic rats. Nat Prod Res. 2018;32(12):1484-8. https://doi.org/10.1080/14786419.2017.1353507
8. Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, *et al*. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat nanotech. 2011;6(5):321-8.
9. Abd El-Aziz G. S, El-Fark M. M, Saleh, H. A. The prenatal toxic effect of methylmercury on the development of the appendicular skeleton of rats fetuses and the protective role of vitamin E. Anatomical Rec. 2012; 295: 939-949.
10. Danning CL. Structure and function of the musculoskeletal system. In: Banasik JL CL, editor(eds) Pathophysiology St Louis, MO. 6th ed: Elsevier. 2019.
11. Nahian A, Chauhan PR. Histology, Periosteum And Endosteum. 2022 May 8. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2022 Jan–. PMID: 32491516
12. Matsushita Y, Ono W, Ono N. Growth plate skeletal stem cells and their transition from cartilage to bone. Bone. 2020; 136:115359. https://doi:10.1016/j.bone.2020.115359
13. Mackie EJ, Tatarczuch L, Mirams M. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. J Endocrinol. 2011;211(2):109-21. https://doi:10.1530/JOE-11-0048.

14. Wang YJ, He ZZ, Fang YW, Xu Y, Chen YN, Wang GQ, *et al*. Effect of titanium dioxide nanoparticles on zebrafish embryos and developing retina. Int. J. Ophthalmol. 2014; 7(6): 917. https://doi:10.3980/j.issn.2222-3959.2014.06.01.
15. Al-Bishri WM, Abdel-Reheim ES, Zaki AR. Purslane protects against the reproductive toxicity of carbamazepine treatment in pilocarpine-induced epilepsy model, Asian Pacific Journal of Tropical Biomedicine, 2017; 7(4): 339-346. https://doi.org/10.1016/j.apjtb.2017.01.003.
16. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61:882-888. PMID: 13967893.
17. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74. https://doi:10.1111/j.1432-1033.1974.tb03714.x.
18. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 1979;135(3):372-6. https://doi:10.1016/0002-9378(79)90708-7.
19. Young AD, Phipps DE, Astroff AB. Large-scale double-staining of rat fetal skeletons using Alizarin Red S and alcian blue. Teratology. 2000;61(4):273-6. https://doi.org/10.1002/(SICI)1096-9926(200004)61:4<273:AID-TERA5>3.0.CO;2-2.
20. Goldschlager T, Abdelkader A, Kerr J, Boundy I, Jenkin G. Undecalcified bone preparation for histology, histomorphometry and fluorochrome analysis. J Vis Exp. 2010;(35):1707. https://doi:10.3791/1707.
21. Suvarna K, Layton C and Bancroft J. Bancroft's Theory and Practice of Histological Techniques 8th Edition, Elsevier; 2018. 1- 672.
22. Mabelebele M, Norris D, Siwendu N, Ng'ambi J, Alabi OJ, Mbajiorgu C. Bone morphometric parameters of the tibia and femur of indigenous and broiler chickens reared intensively. 2017. http://repository.futminna.edu.ng:8080/jspui/handle/123456789/4265.
23. Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. Application of student's t-test, analysis of variance, and covariance. Ann Card Anaesth. 2019;22(4):407-411. https://doi:10.4103/aca.ACA_94_19.
24. Rashid M, Tavčer P, Tomsic B. Influence of Titanium Dioxide Nanoparticles on Human Health and the Environment. Nanomaterials. 2021; 11:1-20. https://doi:10.3390/nano11092354.
25. Lee J, Jeong JS, Kim SY, Park MK, Choi SD, Kim UJ, *et al*. Titanium dioxide nanoparticles oral exposure to pregnant rats and its distribution. Part Fibre Toxicol. 2019;16(1):31.https://doi:10.1186/s12989-019-0313-5.
26. Wawrzyniak A, Balawender K. Structural and Metabolic Changes in Bone. Animals. 2022;12(15):1946. https://doi:10.3390/ani12151946.
27. Rizk MZ, Ali SA, Hamed MA, El-Rigal NS, Aly HF, Salah HH. Toxicity of titanium dioxide nanoparticles: Effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice. Biomed Pharmacother. 2017; 90:466-472. https://doi:10.1016/j.biopha.2017.03.089.
28. Ebrahimian Z, Razavi BM, Mousavi Shaegh SA, Hosseinzadeh H. Effects of *Portulaca oleracea* L. (purslane) on the metabolic syndrome: A review. Iran J Basic Med Sci. 2022;25(11):1275-1285. https://doi:10.22038/IJBMS.2022.63264.13967.
29. Niska K, Pyszka K, Tukaj C, Wozniak M, Radomski MW, Inkielewicz-Stepniak I. Titanium dioxide nanoparticles enhance production of superoxide anion and alter the antioxidant system in human osteoblast cells. Int J Nanomedicine. 2015; 10:1095-107. https://doi:10.2147/IJN.S73557.
30. Ahangarpour A, Lamoochi Z, Moghaddam HF, Mansouri SMT. Effects of *Portulaca oleracea* ethanolic extract on reproductive system of aging female mice. INT J Reprod BioMed. 2016; 14(3):205. https://doi:10.29252/ijrm.14.3.205
31. Tang T, Zhang Z, Zhu X. Toxic Effects of TiO2 NPs on Zebrafish. International Int J Environ Res Public Health. 2019;16(4):523. https://doi.org/10.3390/ijerph16040523.
32. Guo G, Yue L, Fan S, Jing S, Yan L-J. Antioxidant and antiproliferative activities of purslane seed oil. J. Hypertens: open access. 2016;5(2). https://doi:10.4172/2167-1095.1000218.
33. Zidan Y, Bouderbala S, Mitaine-Offer A-C, Lacaille-Dubois M-A, Bouchenak M. *Portulaca oleracea* aqueous extract reduces oxidative stress in erythrocytes and tissues, in rats fed enriched-cholesterol diet. J EXP Integr Med. 2016;6(1). https://doi:10.5455/jeim.260116.or.145.
34. Hong F, Zhou Y, Zhao X, Sheng L, Wang L. Maternal exposure to nanosized titanium dioxide suppresses embryonic development in mice. Int J nanomed. 2017;12: 6197. https://doi:10.2147/IJN.S143598.
35. Kunovac A, Hathaway QA, Pinti MV, Goldsmith WT, Durr AJ, Fink GK, *et al*. ROS promote epigenetic remodeling and cardiac dysfunction in offspring following maternal engineered nanomaterial (ENM) exposure. Part Fibre Toxicol. 2019;16(1):24. https://doi:10.1186/s12989-019-0310-8.
36. Williams EL, Harvey NC, Dennison EM, Edwards CJ, Cooper C. Maternal nutrition and bone health in the offspring. Int. J. Clin. Rheumatol. 2009;4(2):133-145 https://doi:10.2217/ijr.09.8.

37. Goodfellow LR, Cooper C, Harvey NC. Regulation of placental calcium transport and offspring bone health. Front Endocrinol (Lausanne). 2011; 2:3. https://doi:10.3389/fendo.2011.00003.

38. Lieleg O, Baumgärtel RM, Bausch AR. Selective filtering of particles by the extracellular matrix: an electrostatic bandpass. Biophys J. 2009;97(6):1569-77. https://doi.org/10.1016/j.bpj.2009.07.009.

39. Kim JY, Oh HM, Kwak SC, Cheon YH, Lee MS, Rho MC, Oh J. Purslane suppresses osteoclast differentiation and bone resorbing activity via inhibition of Akt/GSK3β-c-Fos-NFATc1 signaling in *vitro* and prevents lipopolysaccharide-induced bone loss in *vivo*. Biol Pharm Bull. 2015;38(1):66-74. https://doi:10.1248/bpb.b14-00567.

40. Rahimi VB, Mousavi SH, Haghighi S, Soheili-Far S, Askari VR. Cytotoxicity and apoptogenic properties of the standardized extract of *Portulaca oleracea* on glioblastoma multiforme cancer cell line (U-87): a mechanistic study. EXCLI journal. 2019; 18:165. https://doi:10.17179/excli2019-1063.

41. Kumar A, Sreedharan S, Kashyap AK, Singh P, Ramchiary N. A review on bioactive phytochemicals and ethnopharmacological potential of purslane (*Portulaca oleracea* L.). Heliyon. 2021;8(1): e08669. https://doi:10.1016/j.heliyon.2021.e08669.

الملخص العربى

التأثير السام لجزيئات ثاني أكسيد التيتانيوم النانوية على الحالة التأكسدية وتطور الهيكل العظمي الجنيني للفئران والدور الوقائي لمستخلص بورتولاكا أوليراسيا

فاطمة الدمرداش[١]، مني سالم[٢]، سامية عبد الوهاب[١]، ايمان سعيد[٢]، عبير خليل محمد[١]

[١]قسم علم الحيوان والحشرات - كلية العلوم (فرع البنات) - [٢]قسم الهستولوجي - كلية الطب (فرع البنات) - جامعة الأزهر

المقدمة: تعد جزيئا ت ثاني أكسيد التيتانيوم النانوية واحدة من أكثر الجزيئا ت النانوية شيوعًا والتي يتم استخدامها بشكل متزايد في مختلف التطبيقات.

الهدف من البحث: هو فحص الحالة التأكسدية للآمهات الحوامل المعرضين لمركبات ثاني أكسيد التيتانيوم TiO٢NPs، وكذلك تطور الهيكل العظمي لنسلهم. وايضا، دراسة ما إذا كان مستخلص بورتولاكا أوليراسيا (الرجله) كنبات مضاد للأكسدة يمكن أن يخفف من سمية ثاني أكسيد التيتانيوم.

المواد والطرق: تم تقسيم الجرذان الحوامل إلى أربع مجموعات بشكل عشواءى إلى المجموعة الأولى:)المجموعة الضابطة): تلقت نظاما غذاءئ، ,المجموعة الثانية: (مجموعة P)(أعطيت ١٠ مل/كجم من وزن الجسم/يوم من مستخلص P)، المجموعة الثالثة (مجموعه T): ٠,٥ مجم/كجم ثاني أكسيد التيتانيوم كل يوم) والمجموعة الرابعة (P+T) (أعطيت P المستخلص، ثم ثاني أكسيد التيتانيوم بنفس الجرعة السابقة). أعطيت الجرعات عن طريق الفم من اليوم السادس إلى اليوم التاسع عشر، وفي اليوم العشرين من الحمل تم التضحية بجميع الفئران. تم تحديد عوامل الأكسدة مثل الجلوتاثيون GSH، سوبر أكسيد ديسموتاز SOD و malondialdehyde MDA من كبد الجرذان الحوامل. كما تم تلوين الهياكل العظمية الجنينية باستخدام الأليزارين الأحمر S والألسيان الأزرق. واستخدمت صبغه Hx. &E لصباغة عظمه الفخذ للجنين لدراسه التركيب الهستولوجي واستخدمت صبغه مالوري الثلاثية لفحص نسبه الالياف الجولاجينيه .وتم عمل دراسه مرفومترتيه لاطوال الجنين ونسبه الالياف الجولاجينيه وتم تحليل النتائج إحصائيا.

نتائج البحث: حدث انخفاض كبير في مستوى SOD وGSH بشكل ملحوظ في المجموعة T مقارنة بالمجموعة الضابطة، ولكن ارتفع مستوى MDA بشكل ملحوظ في المجموعة T مقارنة بالمجموعة الضابطة. أيضا، تسبب ثاني أكسيد التيتانيوم في تأخر نمو الجنين، وتشوهات الهيكل العظمي. كشفت الدراسة النسيجية عن تغضروف جزئي لصفيحة النمو في عظم الفخذ الجنيني، مما تسبب في تدهور جميع المناطق. انخفضت كثافة الكولاجين بشكل ملحوظ. من ناحية أخرى، أدى العلاج بمستخلص بورتولاكا أوليراسيا إلى تقليل تشوهات الهيكل العظمي التي تسببها TiO٢NPs وذلك بسبب زيادة مضادات الأكسدة التي تحتوي عليها.