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Histological Effects of Titanium Dioxide Nanoparticles on Some Reproductive Organs in Male Mice (*Mus musculus*)

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ABSTRACT

The aim of our article was to investigate nanoparticles of TiO_2 on the histology of some reproductive organs and some reproductive hormones levels in male albino mice orally administered with those nanoparticles. Thirty adult healthy male albino mice weighting 25-30 g and aged 12-15 weeks were divided into three groups. First group of mice was given normal saline orally for 90 days. Second and third groups were given 100 and 150 mg/kg body weight of TiO_2 orally, respectively, for 90 days. The administration of nanoparticles of TiO_2 decreased body weight and testosterone level of all treated mice and LH, FSH level increased the oxidative stress comparing with mice of control group. Histologically, it was found that there was marked alteration in organs: testis epididymis and seminal vesicles histologic architecture such as necrosis, hyperplasia, atrophy, arrested spermatogenesis. In conclusion, nanoparticles of TiO_2 had reproductive toxicity on the male reproductive system of mice.

Key words: Nanoparticles of TiO_2, reproductive system, histology, male mice

INTRODUCTION

Nanotechnelogy is new science that deals with matter on an atomic, molecular and super ramolecular scale for industrial purposes. Usage of nanoparticles and nanomaterial's has recently developed quickly in different fields due to their properties depending on size and shape. These are used in the health care, medicine, energy and environmental industries. The size of nanoparticles used in these fields ranges from 1-100 nm (Khan *et al.,* 2019). One of these widely used nanomaterials is titanium dioxide (TiO2NPs); that is used in wall colourings and white pigments, and in antibacterial agents through its characteristics as it has less solubility and toxicity and its self-cleaning and ultra-violet protection and photocatalytic properties (Miura *et al.,* 2019).

Titanium dioxide nanoparticles, also called ultrafine titanium dioxide or nanocrystalline titanium dioxide or microcrystalline titanium dioxide, are particles of titanium dioxide (TiO_2) with diameters less than 100 nm. Ultrafine TiO_2 is used in sun screens due to its ability to block ultraviolet radiation while remaining transparent on the skin. It is in rutile crystal structure and coated with silica or/and alumina to prevent photocatalytic phenomena. The health risks of ultrafine TiO_2 from dermal exposure on intact skin are considered extremely low, and it is considered safer than other substances used for ultraviolet protection.

Nano-sized particles of titanium dioxide tend to form in the metastable anatase phase, due to the lower surface energy of this phase, relative to the equilibrium rutile phase. Surfaces of ultrafine titanium dioxide in the anatase structure have photocatalytic sterilizing properties, which make it useful as an additive in construction materials, for example in antifogging coatings and self-cleaning windows.

TiO_2 production workers present a lung cancer risk due to inhalation exposure. TiO_2 nanoparticles have photocatalytic activity. It is n-type semiconducter and its band gap between the valence and the conductivity bands is wider than of many other substances. The photocatalysis of TiO_2 is a complex function of the physical characteristics of the particles. Photocatalytic activity TiO_2 could be enhanced by doping with certain atoms.

Toxicity of titanium dioxide on brain, spinal cord lungs, liver, kidneys and intestine was studied by Hong and Zhang (2016). Nanoparticles of TiO_2NPs has a huge ratio of surface area to weight and high redox activity; the properties that have been the cause of its adverse effects and intrinsic toxicity on

environment and human health; along with its ability of producing free radicles and can damaging DNA molecules; and then alter the structure of many protiens causing cancer at the end (Shrivastava *et al.*, 2019).

Ultrafine TiO_2 is believed to be one of the three most produced nanomaterials, along with silicon dioxide nanoparticles and zinc oxide nanoparticles. It is the second most advertised nanomaterial in consumer products, behind silver nanoparticles. Due to its long use as a commodity chemical, TiO_2 can be considered a legacy nanomaterial. ltrafine TiO_2 is used in sunscreens due to its ability to block ultraviolet radiation while remaining transparent on the skin. TiO_2 particles used in sunscreens typically have sizes in the range 5-50 nm.

MATERIALS AND METHODS

Nanoparticles of titanium dioxide were bought from Sigma Aldrich Co, German with purity of 99.9%. Thirty adult healthy male albino mice weighting between 25-30 g and aged 12 to 15 weeks were brought from University of Babylon animal house. Mice were acclimatized for two weeks under controlled conditions of light and dark 12 : 12 hours. Animals were randomly grouped into three equal groups each one composed of 10 male mice. Group one mice were orally given normal saline as control group, whereas 100 mg/kg of body weight was orally given to the second one, and 150 mg/kg of body weight was given orally to the third studied group for 90 days experiment period. Mice were sacrificed at the end of 90 days. Blood was withdrawn through heart puncture for attainment of serum for hormone levels estimation. Testis, epididymis and accessory sex organs were extruded, weighted and then put in formalin for 24 h and then histologically processed. Data were analyzed by using SPSS – version 20, SPSS, Inc, Chicago, Illinois, USA. Descriptive statistics mean ± standard error, differences were compared by ANOVA.

RESULTS AND DISCUSSION

Body weight, some sex hormones levels (testosterone; LH, lutenizing hormone; FSH, follicle stimulating hormone), and total antioxidants (TAO); in control and treated mice groups have been presented in Fig. 1. The histo-pathological findings are presented in Fig. 2.

The present study revealed that the TiO_2NPs when administered orally for 90 days produced significant lowering in mice body weight of treated mice in comparison with control ones. The result was supported by Lotfi *et al.* (2017) and Hosseini *et al.* (2019) who explained that exposure to nanoparticle reduced body weight in hamster and in rat models. Further, body weight was significantly decreased by single oral administration of TiO_2NPs in male mice (Rodríguez-Escamilla *et al.*, 2019). Male rats fed with 1% TiO_2NPs (Chen *et al.*, 2019, 2020) also obtained similar results. This decrease in body weight indicated the potential toxic effects of TiO_2NPs causing physiological change in mice on the appetite and feeds consumption consequently affecting body weight. In simpler words, penetration of TiO_2NPs in the cell induced internal organs to release proinflammatory cytokines from phagocytic cells, cytokines particularly TNFalph which reflected association with increased metabolism of subcutaneous fatty tissue leading to emaciation of animals.

Thus, there was an elevation in the oxidative stress in treated groups comparing with

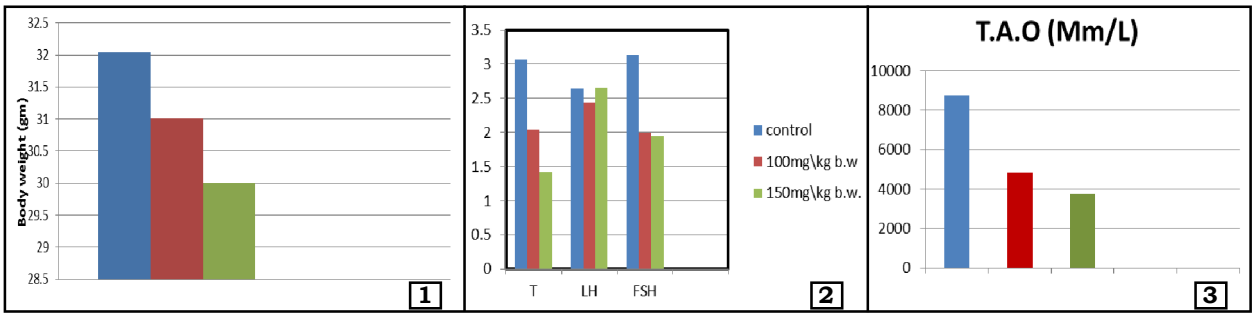

Fig. 1. Effects of TiO_2NPs on: 1: Body weight, 2 : Some sex hormones levels (*T, testosterone*, LH, lutenizing hormone, FSH, follicle stimulating hormone); 3: otal antioxidants (TAO) in control and treated mice groups.

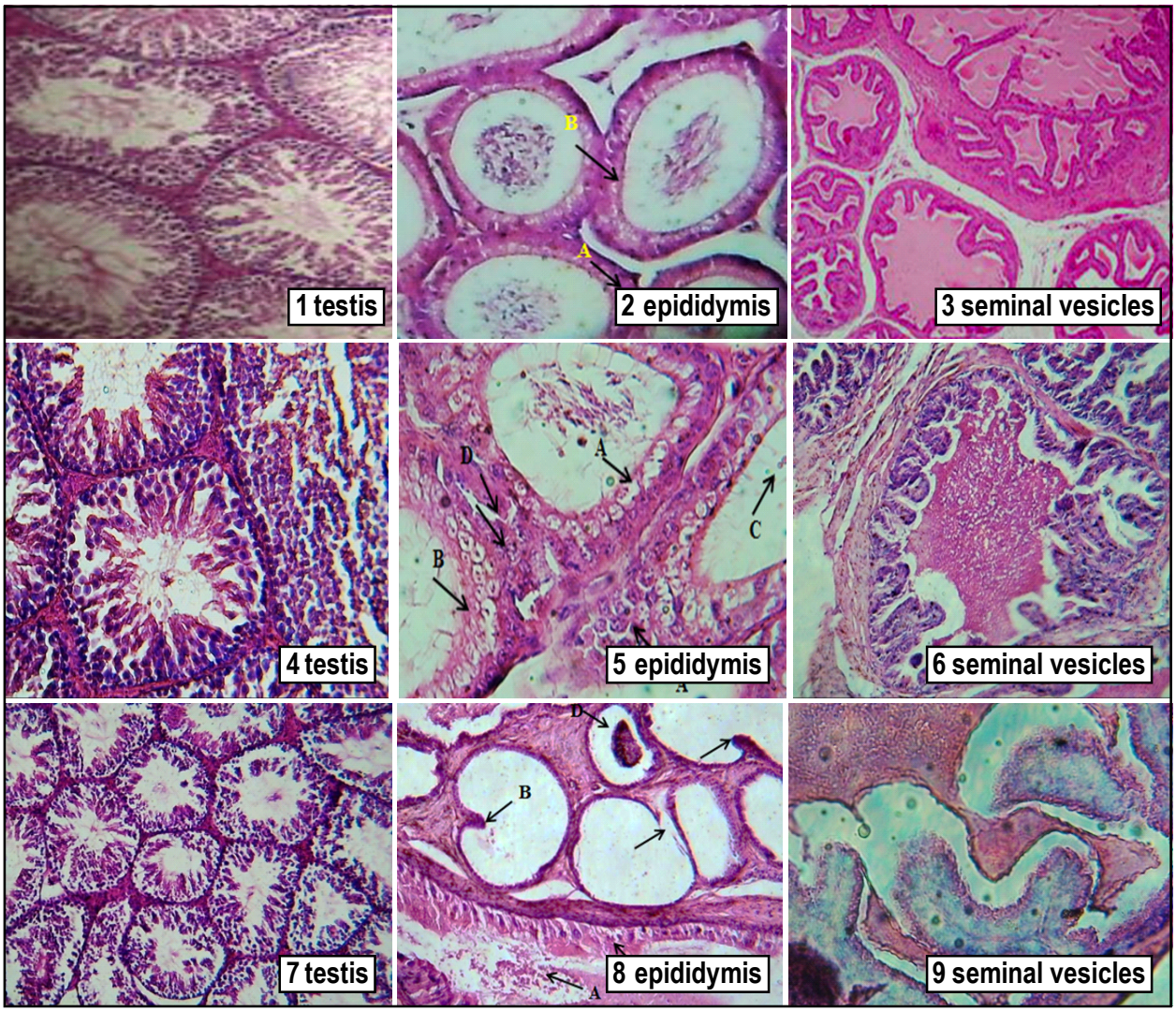

Fig. 2. Cross section of 1: Testis; 2: Epididymis; 3: Seminal vesicles of control mice showing intact architecture. 40X; 4: Testis mice from a dose-treated group of 100 mg/kg of body weight showing arrested spermatogenesis at spermatid stage and extensive exfoliation of germ cells and numerous necrotic of germ cells (40x); 5: Epididymis from a dose-treated group of 100 mg/kg of body weight: (A) epididymal epithelium showing lipid vacuoles in the supra nuclear region of the principal cells, (B) intracystic papillary in foldings lined by columnar cells with clear cytoplasm and occur hyperplasia of clear cells, (C) the lumen devoid of sperm with few cellular debris, (D) also showing atrophy in the smooth muscle between ducts with inflammatory infiltrate in connective tissue, 40X; 6: Seminal vesicle of 100 mg/kg of body weight group showing seminal vesicle - atrophy vacuoles in the basal cells, with accompanying necrosis (10X); 7: Testis mice from a dose-treated group of 150 mg/kg of body weight showing testis with occasional disorganization in the seminiferous tubule epithelium and disorganization arrangement of germ cells in the seminiferous tubules (10x); 8: Mice epididymis from a dose-treated group of 150 mg/kg of body weight, (A) there in lumen acellular debris of the epididymis, (B) the onset of budding change in the epithelial hyperplastic amendment, spreading to folding off on itself and customs pseudoglandular edifices, and the lumen devoid of sperm, (C) showing necrosis in the epithelial tissue that lining the epididymis, (D) exhibiting multiple sperm granulomas. H & E stain, 40X; and 9 Seminal vesicle of 150 mg/kg of body weight group showing decreased epithelial height and reduced apical secretory droplets in the tissue, atrophy of the papillary, secretory depletion (10x) (Haematoxline & Eosine stain).

controls as it was related to the conflicts in redox state of the cells that's came from the production of free radicles and peroxides that enhanced the cell damage at the level of DNA lipids and proteins leading to toxic effects (Valko *et al.*, 2016). According to Ahmad *et al.* (2019) TiO_2NPs released reactive oxygen species like O– and OH– causing apoptosis leading to disparage and damage unsaturated cell membranes phospholipids.

Levels of testosterone hormone were decreased in the treated groups when compared with control group a result that agreed with study of Ogunsuyi *et al.* (2020). This decrease conversely caused petulant in GnRH; LH, FSH through the effect of nanoparticles on cell membranse causing irritable Ca^{+2} concentration inside and outside the cell than in normal state. Song *et al.* (2017) explained that Lydig cells of mice exposed to TiO_2NPs could cause suppression of cell secretion, its mitochondria disruption, cell proliferation affecting gene expression of regulatory steroidogenic gene; leading to reduction of production of testosterone.

Histopathlogic results of our study in testis, epididymis and seminale vesicles got parallel with results of Han *et al.* (2016) and Habas *et al.* (2021) that interpreted as toxic action of nanoparticles on male germ cells. The hormonal disturbances effected on reproductive organs such as spermatogenesis when described about testis, and cell organization, increasing fibromuscular stroma, reduced vesicular volume, secretion and histometry of both epididymis and seminal vesicles as they were androgene dependent organs.

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