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Genotoxical and Histological Effects of Titanium Dioxide Nanoparticles (TiO_2-NPs) in Male Mice.

Afaf Hendawy Kamel*, and Aml Salem Saleh.

Department of Zoology, Faculty of women For Arts, Science And Education –Ain Shams University, Cairo, Egypt.

ABSTRACT

The Genotoxic capability of TiO_2-NPs is accepting expanding enthusiasm as its wide application in purchaser items and in prescriptions. So, the point of the present research is to find out about the genotoxicity of TiO_2-NPs in vivo. The investigation was done out in somatic cells of adult male mice, utilizing CAA, MNT, Ames test was included. Also, testicular histological and immunohistochemical modification was examined. Three doses 37.5, 75 and 150 mg/kg b.w of TiO_2-NPs were injected intraperitoneally for 10 continuous days. CAA demonstrated a critical increment in SCAs over the control. Additionally, TiO_2-NPs caused a huge rise in MNPCEs of the treated animals as contrasted to the control. Ames test showed no mutagenic change. Histological examination indicated engraved modifications as degeneration, vacuolation, congestion, and necrosis. The progressions saw in the testis of treated animals were in a dose dependent. A remarkable diminishing in cell proliferation and an expansion in iNOS expression at doses 75 and 150 mg/kg b.w when compared to control. In conclusion, positive results of CAA, MNT and in testicular tissue provide evidence of genotoxic effects of TiO_2-NPs.

Keywords: Nanotoxicology, Titanium nanoparticles, genotoxic effect, histological alteration, testicular toxicity, immunohistochemical changes.

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Corresponding author

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INTRODUCTION

Nanotechnology is the manipulation and engineering of materials with at least one dimension <100nm in length [1]. In recent years the demand for engineered and manufactured nanomaterials has increased rapidly [2] due to their several applications in the food industry, medicine, and energy conversion [3]. Currently, nano-materials used in many applications include anti-microbial food processing and packing anti-bacterial milk bottles for babies [3] and water purification [4].

TiO_2-NPs is extensively used as a pigment in anti-fouling paints [2] plastics, enamels, foods, sunscreens, pharmaceuticals, Personal care products [5] and drinking water treatment [4]. TiO_2-NPs comes in three forms, which comprise rutile, anatase and less universally, brooklet. The belongings of TiO_2 and its toxicity is related to its crystal structure. Rutile and anatase are the two forms of TiO_2 used mainly. Anatase has many applications in the industry than the other two forms because it has a high specific surface area and increased activity in photocatalysis [6 and 7], though its more poisonous than rutile [5].

Recently, many examinations have demonstrated that TiO_2-NPs are harmful to human organs and cause oxidative damage [8 and 9]. Therefore, it can be passed through the body by many ways, such as inhalation, ingestion, and dermal penetration and are dispersed to vital organ systems, such as brain, lung, lymph, kidney, and liver [10 and 11]

The capacity to cause DNA damage seems to be one of the mainly actions of NP's . The effect on DNA damage is generally unfavorable, affecting the metabolism, cell-cycle arrest or lead to cell death. Eukaryote organisms have a develop effective molecular mechanism such as DNA damage response to identifying DNA lesions, signal their occurrence and support their repair [12].

Cytogenotoxicity of TiO_2-NPs has been extensively studied on both human and experimental animals. Thus, DiVirgilio *et al*. [13] Concluded cytotoxic and genotoxic effects on Chinese hamster ovary (CHOK1) cells of both TiO_2 and AlO_3NPs. Neutral red, mitochondrial, SCE, MNT, and cell kinetic methods.

The agglomeration stats of silver and TiO_2-NP on on cellular response of human hepatoma cell HepG2 and human cancer cells (A549) and THP-1 cells was investigated. The used particles lead to fall of the metabolic activation in all cell lines and increasing cell death. The findings suggested that particle characteristics such as size, agglomeration status and cell type can give the nanoparticles their biological impact [14].

Additionaly , Chen *et al.* [15] presented an essential review Concerning the genotoxicity of TiO_2-NPs, focusing on the outcomes of standard in vitro and in vivo genotoxicity assays. Ames test, comet assay, MNT, SCEs, mammalian cells hypoxanthine-guinine phosphoribosyl transferase gene assay and so on were discussed. on the other hand, Due to their extensive applications, ZnO and TiO2 NPs are receiving growing attention. So, Khan *et al*. [16] observed concentration dependant hemolytic activity to human RBCs for ZnO and TiO_2-NPs which indicating cytotoxic action. Both NPs were found to produce reactive oxygen species (ROS) and make a decline of antioxidant enzymes. DNA injury induced via the NPs used significantly higher than their ionic form. Spermatogenesis is a complex method of proliferation and differentiation of germ cells that results in sperm manufacturing and emitting from the tests. This method relies on hormonal relationships between the cells of Sertoli and the germ as well as between Leydig cells and germ cells. Sertoli cells secrete hormonal and nutritional variables that create a specific developmental microenvironment. As well, In creating germ cells, Sertoli cells provide effective paracrine signaling processes between these cells as well as physical hold. Synthesize and release testosterone from Leydig cells. Testosterone is a needed factor in the spermatogenesis process [17]. The regulation and cellular interactions in the tests provide various separate objectives for spermatogenesis toxicants [18]. *In vivo,* research in mice verified a substantial decrease in sperm density and motility ; enhanced morphological sperm defects and apoptosis of germ cells following exposure to TiO_2-NPs with histopathological modifications in the testis and an apparent decrease in concentrations of serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [19 , 20 and 21].

The objective of this research was to assess in vivo the genotoxic impacts of TiO_2-NPs. Such estimation was carried out through CAA, MNT using bone marrow cells of mice, Ames test was also concerned.

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Further, histopathological and immunohistochemical alterations were also investigated in the testes of the treated mice.

MATERIALS AND METHODS

Experimental Animals

From the animal house of the National Research Center in Cairo, Egypt, 48 male Swiss albino mice (Mus musculus), aged 9-12 weeks and weighing 25-30 g. In an air-conditioned animal house with specific pathogen-free circumstances, animals were raised and preserved and subjected to daylight / darkness of 12:12 h and permitted unlimited access to chow and water. The animals were acclimatized 2 weeks before the experiments started. The animal installations, the National Research Center, followed and monitored the ethical procedures for animal therapy. All animal experiments estimated consent from the Committee on Animal Care, National Research Center, Giza, Egypt. (Approval number: 13 045).

Chemicals

Titanium dioxide nanoparticles TiO_2-NPs (> 100n m particle size) acquired from Sigma Aldrich. Com (U.S.A), TiO_2-NPs were suspended for 30 min in a double distilled water concentration of 150 mg / kg body weight and ultrasonic (ultrasonic cleaning CD-4831, Ac 220-240 v, 50 HZ, 170 W). Three concentrations of 37.5, 75 and 150 mg/ kg b.wt were employed for this study (Bakare *et al*., 2016).

Experimental design

Male mice were accidentally divided into 2 groups, as follows:

Group (I) received 0.5 ml of distilled water and served as a control.

Group (II) divided into 3 subgroups as follows:

GIIa, GIIb and GIIc ,they were treated with the used doses 37.5, 75 and 150 mg/kg b.w of TiO_2-NPs.

TiO_2-NPs was injected intraperitoneally daily for 10 days. After this period animals were sacrificed by cervical dislocation. Bone marrow was taken for chromosomal aberrations assay (CAA) and micronucleus test (MNT). Also, testes were detached for histological and immunohistochemical analysis.

Chromosomal aberration assay

The chromosomal metaphases were prepared from bone marrow cells, according to Preston *et al*. [22]. Animals were injected interaperitonally with colchicines (0.4 mg/ kg .body weight) one and a half hour before sacrifice, the femoral bone marrow cells were flushed with physiological saline and then hypnotized in KCl solution at 37°C for twenty min. The cells were fixed in a mixture of methanol / acetic acid (3:1) thrice at intervals of 10 min, the pellet was re-suspended in fixative then dropped on cold slides and dried on flam. The slides were stained with 5% buffered Giemsa solution for 10 min followed by washing with distilled water. In each treated and control animals , 50 fine- spread metaphases for each animal were scored.

Micronucleus test

The MN test was conceded in bone marrow cells, according to the method of Schmid [23] as modified by way of Bakare *et al* ., [24] .Animals has been sacrificed through cervical dislocation, the femoral bone marrow cells were blushed in a centrifuge tube containing 1.5 ml of fetal calf serum and mixed well. The suspended cells has been centrifuged at 1000 r.p.m for 15 min, then the supernatant was discarded. The pallet was blended with two drops of fetal calf serum , smeared on clean glass slides, allowed to air- dried . The subsequentday after draining, the slides had been fixed in absolute methanol for ten min. The fixed slides were stained with May-Grünwald-Giemsa for 10 min and observed under microscope. 1000 PCEs were examined For every animal for micronucleated erythrocytes. Besides, the ratiobetween PCE and NCE was recorded to investigate the toxicity on bone marrow .

Ames test

Bio-detection of mutagenicity towards *Salmonella. typhimurium* (TA98) using AMES-MOD ISO 96 Well Format (Ames Modified ISO Procedure version 1.1). According to users guide , bacteria allowed to grown by incubation for 16 h at 37 0C with gentle shaking. A stock solution of TiO_2-NPs was prepared and filtered through 0.22µm syring filters, then exposure media was prepared .This media contain (4.150 ml Exposure medium concentrate, 0.5 ml 40% D-Glucose, 0.3ml D-Biotin and 0.05ml L-Histidine/ L-Tryptophan), 200µl of exposed media transferred to 24-well plate, ready to exposure plates preparations. Exposure plates were prepared for positive control, negative control, sterility control and 3 concentration of TiO_2-NPs (37.5, 75, 150 µg/ plate) incase the presence and absence of bio-activation rat liver (S9).

The last quantity of all wells after bacteria addition should be 2 ml, duplication was prepared for every condition. Exposure plates had been incubated for 100 min at 37^0C, near the end of the incubation period master reversion solution was prepared . it's contain 2.3 µL 40% D-Glucose, 3.5 µL Bromocresol purple , 4.65 µL D-Biotin and 11.65 µL 10 X Reversion solution. Reversion plates were prepared For each 24-well plate, twenty-one 15 mL falcon tubes were prepared (one for each well), each tube contained 880 µL reversion media and 7,830 µL sterile water.1,600 µL from each well of the exposure plates were transferred to one of the prepared 15 mL falcon tubes (containing reversion medium and water), mixed well and poured onto a loading boat, and loaded via multichannel pipette (200 µL) into 48 wells from a 96-well plate. Reversion plates were then sealed into Ziploc bags and incubated at 37^oC for two days . After 2 days , plates were removed and the number of wells that have changed from purple to yellow were observed .Induction factor (IF) was calculated. IF>2 is considered genotoxic.

$$Induction\ factor = \frac{Number\ of\ revertants\ in\ test\ plate}{Number\ of\ spontaneous\ revertants\ in\ negative\ control}$$

Histological changes

The testes were removed during dissection and immediately fixed in 10% neutral formalin and processed routinely for paraffin embedding technique. Paraffin sections of 5-7µm thick were cut using a rotary microtome and stained with hematoxylin and eosin [25].

Immunohistochemical analysis

Detection of Ki-67 was used and its positive reaction is nuclear [26]. Also, iNOS detection was used and its reaction is cytoplasmic [27].

Statistical analysis

Incident of abnormal metaphases and micronucleated cells were analyzed for significant by student's t-test.

RESULTS AND DISCUSSION

The potential genotoxic effects of TiO_2-NPs on mouse bone marrow cells

Intraperitoneal injection with TiO_2-NPs induced several signs of structural chromosomal aberrations . The most prominent figures were deletion, ring chromosome, centric fusion, a centric fragment, end to end association , gap and centric attenuation .Results of C A due to exposure to Tio2-NPs (37.5, 75, 150 mg/ kg b.wt) showed significant increase comparing to the untreated controls ($p< 0.001$) . The values of chromosomal damage were 25, 35and 48 % . So, the chromosomal aberration percentage were increased in a dose dependant manner (Table 1 &Fig. 1) . The different signs of chromosomal aberration were observed, indicating that Tio2-NPs induced each chromatid and chromosomal kind of aberration. This, in turn, indicates that the damage occurred in both the Gap 1 or Gap 2 stages in the cell cycle [28]. On the other hand, the genotoxicity of TiO_2-NPs have further evaluated by means of MNT. It has been proved to be rapid and specific technique for the screening of potential clastogens [29].

The current result observed a significant incensement (p< 0.001) in rate of the formation of MN in bone marrow cells (PCEs) of the exposed animals at all the different examined doses (37.5, 75, 150 mg/ kg b .wt) comparing to the control animals was. The values were 12.4, 24.2 and 35.6 per 1000PCEs. It is evident that the increase was dose associated (Table 2 & Fig. 2). The ratio of PCEs/NCEs was established to give extra information about the proliferation state of erythrocytes and the relative cytotoxicity of TiO_2-NPs. The value of PCEs/ NCEs ratio in the control was (0.799 ±0.018) and in the treated mice were increased comparing to the controls. However, statistically the differences had been determined to be non-significant ($p<0.05$) (Table2).
The positive results obtained from the chromosomal aberration assay and MNT revealed the genotoxic potential of TiO_2-NPs, our results are run in agreement with several published reports. Trouiller *et al*. [30] showed that TiO_2-NPs induced positive result for genotoxicity, oxidative DNA injure and inflammation in mice. They suggested that TiO_2-NPs induced getotoxicity,in *vivo*, probably caused by a secondary genotoxic mechanism releated with inflammation and / or oxidative stress. Also, Di Viriglio *et al.* [13] observed cytotoxic and genotoxic effects of Titanium dioxide nanoparticles (TiO_2-NPs) and Aluminium oxide nanoparticals (Al_2O_3-NPs) on Chinese hamster ovary cells during positive result with MNT and SCE assay.

Ghosh *et al.* [31] was evaluated the genotoxicity of TiO_2-NPs on two kinds of plants (Allium cepa and Nicotiana tabacum) and also on human lymphocytes . In Allium cepa, micronucules and Chromosomal aberrations were associated with the decline of root growth. The level of malondialdehyde (MDA)had been increased which representing that lipid peroxidation could be concerned as one of the mechanisms leading to DNA injury.

Cytotoxic and genotoxic potential was detected by Khan *et al*. [16] throughout hemolytic activity (RBCs) and DNA damage in human lymphocyte. Reduction of glutathione and rise in Superoxide dismutase, catalase and lipid peroxidation. They concluded that the toxicity of ZnO and TiO_2 nanoparticles is due to generation of reactive oxygen species (ROS), in this manner ,lead to oxidative stress.

Also, Bakare *et al*. [21] inveterate the positive effects of TiO_2-NPs. Mice exposed to ten days had higher genomic damage in the form of micronucles construction than individuals exposed for five days. This proposes that TiO_2-nanoparticles might have concentrated in a dose dependently manner, as a result affecting on the bone marrow Polychromatic erythrocytes for a longer duration.

TiO_2-NPs induce DNA damage *in vitro* in human SHSY5Y neural cells after one and two days of exposure, but not change cell viability or cell morphology even at concentrations much higher than environmentally applicable [32].

Additionaly , Mohammed and Hussien [33] establish genotoxic and mutagenic effects of TiO_2-NPs on brain tissue of mice. Single-cell gel electrophoresis revealed the a poptotic DNA fragmentation, while Polymerase chain reaction (PCR) and direct sequencing produced point mutation of Preseilin 1 gene which related to inherited forms of Alzheimer's sickness.

Contradictory with the results that concerning the genotoxicity caused by TiO_2-NPs . Lindberg *et al*. [34] revealed that no significant effects on the level of DNA damage in lung epithelial cells of treated mice or on the formation of micronucles , suggested no genotoxic belongings observed after exposure to nanosized TiO_2 anatase through inhalation for five days.

Rad *et al*. [35] found negative results with low dose of TiO_2-NPs on bone marrow cells of mice. But high doses lead to toxic effects with increase on the formation of micronuclei . The author explained this result as anatase has capability to cause oxidiation of the DNA strands of chromosomes in A-T, G –C base of nucleotides by direct action on three hydrogen bonds of purine moiety and 2 hydrogen bonds of pyrimidine moiety. So, the increasing of Tio2-NPs shows the rising of micronucleated polychromatic erythrocytes .

Also, many research statedthe negative resultsfor TiO_2-NPs to induce genotoxicity. Xu *et al*. [36] observed that high doses of TiO_2-NPs prompted acute toxicity consequences in brain, lung, spleen, and kidney of male mice. However, no significant genetic toxicity or hematological was detected. The conflicting results mentioned from the distinct studies on the genotoxicity of TiO_2-NPs were briefly summarized by Chen *et al.* [15] in their most vital published review. The in vitro studies have given several constructive results than the in vivo systems. Also, techniques for DNA and chromosomal aberration have produced further positive results

than the assays that measuring gene mutation. The collective information showed that the genotoxicity of TiO_2-NPs is frequently during the generation of reactive oxygen species (ROS) in cells. The ROS, which formed by the nanoparticles could be affectd on DNA repair, cell cycle progression, cell proliferation, and apoptosis via affecting protein composition. Similarly, negative response for genotoxicity of TiO_2 was obtained by Relier *et al*. [37]. The small doses had no genotoxicityor cytotoxicity in the lung of male rats. In blood ,no lymphocytes DNA injure, chromosomal damage or gene mutation could be noticed. However, genotoxicity associated with inflammation was detected only with much higher doses. The authors suggested that TiO_2-NPs might be a potential health hazard for people occupationally exposed to high concentration throughout the respiratory tract.

Recently, Begani and Begani [38] published a review article regarding the relationship between publicity to TiO_2-NPs and occupational lung cancer among TiO_2 production workers. They state that despite the contradictory obtained results, there is enough evidence in the experimental studies confirmed TiO_2-NPs toxicity. Notable cases include cytotoxicity, genotoxicity, apoptotic and cell necrosis in rat and human cells expose to TiO_2-NPs which is commonly dose response associated. These studies present a new recommendation for appropriate agencies to be protective in developing mitigation and control measures against NPs exposure.

Mutagenic effects of TiO_2-NPs (Ames test)

The Ames test technique is appropriately known as the Salmonella typhimurium reversion assay. This test is used worldwide as an initial screen to comfirm the mutagenic potential of agents and the assay identifies point mutagens [39]

In the current study, Ames test was done by using S.Typhimuria (TA98) which exposed to three different concentrations of TiO_2-NPs in present and /or absence of bio-activation rat liver S9. A negative result was obtained, the mutagenic response was not remarked (I F<2) at various concentrations, even with or without metabolic S9 activation as showed in Table (3). These results are a good agreement with several published reports [40,41 and 42]. In addition, four different kinds of TiO_2-NPs have been estimated by Ames test, all of them gave a negative results in the standard mutation assay [43, 44 and 45]

Landsiedel *et al*. [43] observed negative result with TiO_2-NPs in different Salmonella strains. Both in the presence or absence of metabolic activation (S9), no mutagenicity was remarked. In a further research made by Woodruff *et al*. [45] the bacteria were pre-incubated with eight different concentrations of 10 nm anatase TiO_2-NPs up to 5000µg/plate, no mutation was formed. Analysis with transmission electron microscopy and energy dispersive X-ray spectroscopy shown that TiO_2-NPs are not capable to penetrate the bacterial cell wall. Such result was previously noticed by Nabeshi *et al*. [46].

Nearly, most results obtained with Ames test gave a negative response, however few studies showed positive effects [47 and 44]. Then the negative conclusion may be explained by Warheit *et al*. [48]. They suggested that Ames test is unsuitable for detecting the genotoxicity of nanoparicales.

Histological and histopathological observations

In the current study, several histopathological lesions were spotted due to TiO_2-NPs exposure. Sections of testes prepared from injected males with 37.5 mg/ kg b.w TiO_2-NPs revealed relatively slight changes in the testicular tissue. Such changes included detachment of spermatogenic cells and cytoplasmic vacuolation, pyknosis in spermatogenic cell nuclei and depletion of germ cell layers (Fig. 3b). While the testis of mice treated with 75 mg/ kg b.w TiO_2-NPs showed marked degeneration, necrosis of germ cells and sloughing of spermatogenic cells into tubular lumen. In addition, gaps were recorded between germinal cells and loss of spermatozoa (Fig.3c)

The results illustrated that TiO_2-NPs increased the histopathological alteration according to the dosage. This research, in agreement with previous researches , El Sharkawy *et al*. [49] found a reduction in testosterone concentrations in rats subjected to TiO_2-NPs. So, they suggested that such a decrease in testicular secretion could be due to the elevated NO rate, which resulted in hypospermatogenesis and inflammation of the testicular. On the other side, Gao *et al.* [19] discovered that NPs caused testicular harm and inhibition of

spermatogenesis that could be ascribed to disturbance in the male reproductive system's sex hormone equilibrium.

Also, a substantial shift in MDA concentrations was achieved by Elnagare *et al*. [50] suggesting that the induction of pathological lesions is likely mediated by oxidative stress. Meena *et al.* [20] earlier discovered this proposal, concluding that TiO_2-NPs caused oxidative stress that caused cytotoxic and genotoxic modifications in sperm. This in turn can influence the spermatozoa's fertilizing ability.

In the present study animals treated with 150 mg/ kg. bw. showed marked degeneration, necrosis of germ cells, gaps were recorded between spermatogenic cells and loss of spermatozoa as showed in (Fig.3d). The appearance of this figure may be understood by the explanation of Malmir *et al.* [51] they stated that NPs can damage the linkage of gap junction by reducing the expression of connexin 43 protein, causing a defect and apoptosis in Sertoli and spermatogenic cells, that may be a reason to a reduction in tubule diameter and disruption of the blood- testicle barrier and provides tissue edema.

Also, production of oxidative stress, stop the cell division in the germinal epithelial layer might reduce spermatogonia and sperm density in lumen also reduce the height of germinal epithelium and seminiferous tubules diameter [52].

Bakare *et al*. [21] indicated that degenerative modifications in seminiferous tubules such as necrosis, vacuolation, and interstitial testicular blood vessel congestion were proof of TiO_2-NP toxicity to the male reproductive system of the mouse. The degenerative modifications indicate that TiO_2 can interfere directly with the spermatogenesis process [53].

Immunohistochemical Observations

Sections of testes which stained with anti-Ki-67 antibody showed reduction in cell proliferation with an enhanced dose of TiO2-NPs was more pronounced in subgroups IIb and IIc than in subgroup IIa as showed in figures (Fig. 4c, Fig. 4d and Fig. 4b) respectively .Takeda *et al.* [54] mentioned an inhibitory role of AgNPs on cell proliferation which explains the damaged seminiferous tubules treated with high doses. AgNPs can interact with membrane proteins and activate signaling pathways that lead to cell proliferation inhibition [55]. Thus, TiO2-NPs can be entered into the cell by diffusion or endocytosis, causing mitochondrial dysfunction and ROS generation, resulting in harm to proteins and nucleic acids within the cell, and ultimately cell proliferation inhibition. In their research, Miresmaeili *et al*. [56] showed a substantial reduction in the mean number of main sperm cells, spermatides and sperm cells and ascribed this to AgNPs ' inhibitory role in cell proliferation.
Recent proof indicates that testicular toxicity induced by TiO_2-NPs is caused by oxidative stress and enhanced reactive oxygen species production [57]. Increased production of reactive oxygen species reduces glutathione and increases testicular tissue lipid peroxidation [58].

Control testes stained with anti-iNOS antibody exposed negative immunoreaction in the cytoplasm of the spermatocytes (Fig. 5a), in animals trated with 37.5 mg/kg their testes showed slight positive immunoreaction in the cytoplasm of some spermatocytes (Fig.5b). while the dose 75 mg/kg caused displayed mild positive immunoreaction in the cytoplasm of many spermatocytes (Fig. 5c). On the other hand , high dose 150 mg/kg indicated significant increase in the expression of iNOS in the cells of the seminiferous tubules (Fig. 5d).

TiO_2-NPs.also induces a cascade of inflammatory responses with enhanced manufacturing of pro-inflammatory cytokines, as NO production improved in the tests engaged in the pathogenesis of toxicity of TiO2-NPs. Cadmium also improved the impact of inflammatory variables that caused the oxidative stress-related impact of iNOS [59]. Inflammatory stimuli, especially TNF-a, have been recorded to boost the expression of iNOS, which catalyzes a big quantity of NO manufacturing. Excess NO responds with superoxide anion by nitrating cellular macromolecules to produce radical peroxynitrite accountable for tissue harm [60]. Excess NO also depletes intracellular GSH and reduces oxidative stress susceptibility [61]. The documented modifications in epidididymal sperm characters in TiO_2-NPs-caused in our consequence may be linked to NO radicals generation. Excessive NO manufacturing has been recorded to reduce the sperm cell survival level and motility [62]. The elevated NO concentration may result in a decrease in hormone secretion leading to hypospermatogenesis, testicular swelling, and GnRH secretion disruption [63].

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Table 1: structural chromosomal aberrations in mice bone marrow cells induced by TiO2- NPs

Experimental Group	No of examined metaphases	Structural chromosomal aberration / 300 cells								Total	Mean ± S D	SCA %
		r	g	Ac.f	R t	del	C.s	dic	e to e ass			
Control	300	4	3	2	14	15	10	1	2	38	6.33±1.121	12.66
37.5 mg/kg	300	2	-	1	19	51	60	-	2	75	12.5±1.87***	25
75 mg/kg	300	5	2	2	32	64	70	1	1	105	17.5± 2.35***	35
150 mg /kg	300	3	-	-	54	86	112	-	1	144	24± 1.79***	48

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Table (2) frequencies of MN-PCEs in mice bone marrow exposed to TiO2-NPs

Experimental group	No of MN/1000 PCEs Mean ± SE	PCEs/NCEs mean± SE
Control	6.8± 0.58	0.799 ± 0.018
37.5 mg/kg b.w	12.4±0.926***	0.811 ±0.025
75 mg/kg b.w	24.2 ± 1.71 ***	0.922 ± 0.026
150 mg/kg b.w	35.6 ± 1.5 ***	0.895 ± 0.02

Significant at p≤ 0.001

Table (3) Induction factor after exposure of TA98 to different concentrations of TiO_2-NPs in presence and absence of S9 fractions

Tested substance With S9		Average number of revertants	Induction factor	mutagenicity
Blank (Sterility control)		zero		
Positive control		48		
Negative control		3		
ug/plate	150	3	1	negative
	75	1	0.33	negative
	37.5	0	0	negative
Tested substance Without S9		Average number of revertants	Induction factor	mutagenicity
Blank (Sterility control)		zero		
Positive control		48		
Negative control		2		
ug/plate	150	0	0	negative
	75	0	0	negative
	37.5	0	0	negative

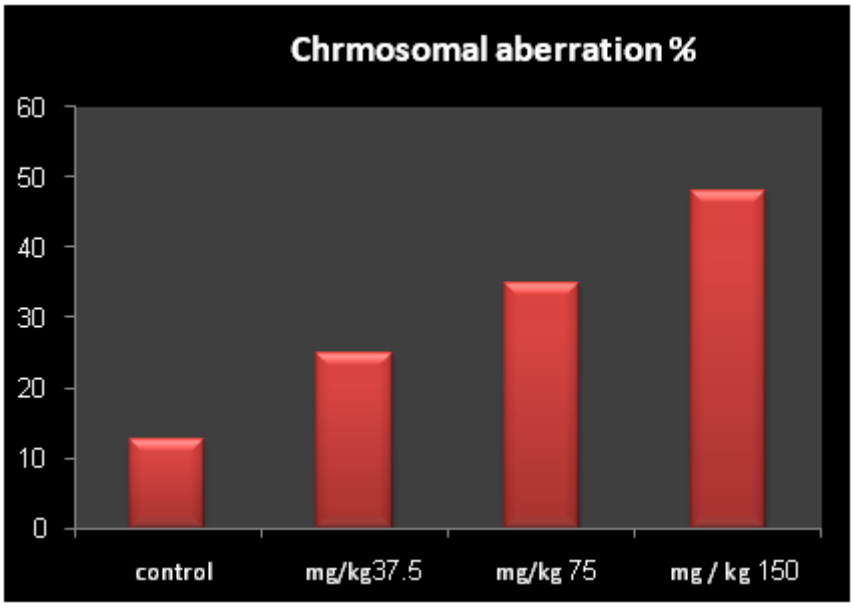

Fig.1: Chromosomal aberration percentage following treatment with TiO_2-NPs.

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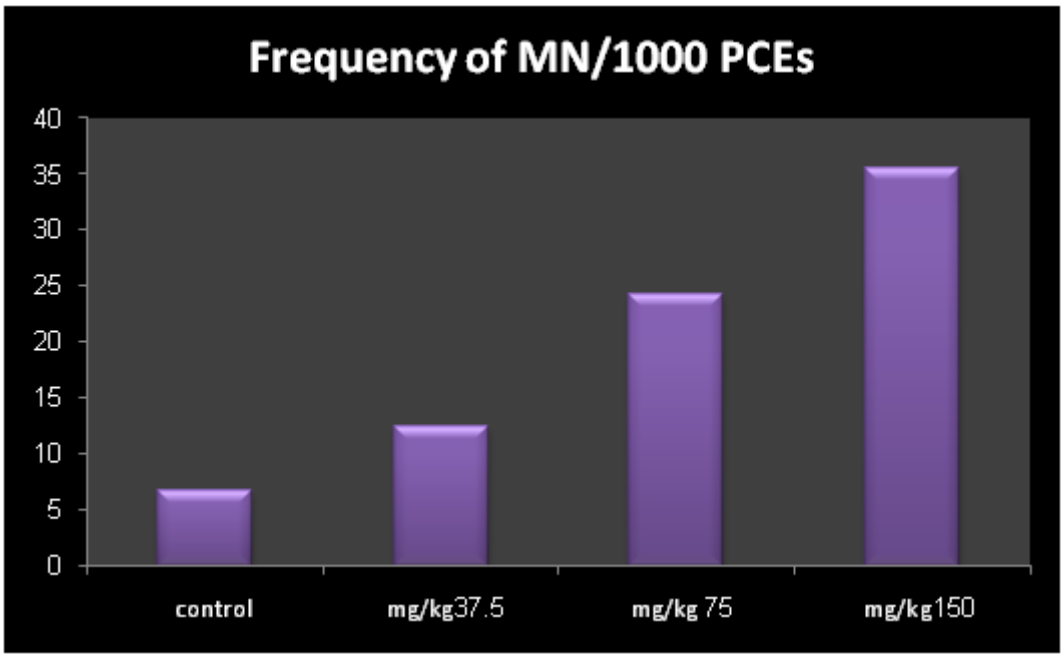

Fig.2: The frequency of MNPCEs as aresult of TiO_2-NPs treatment.

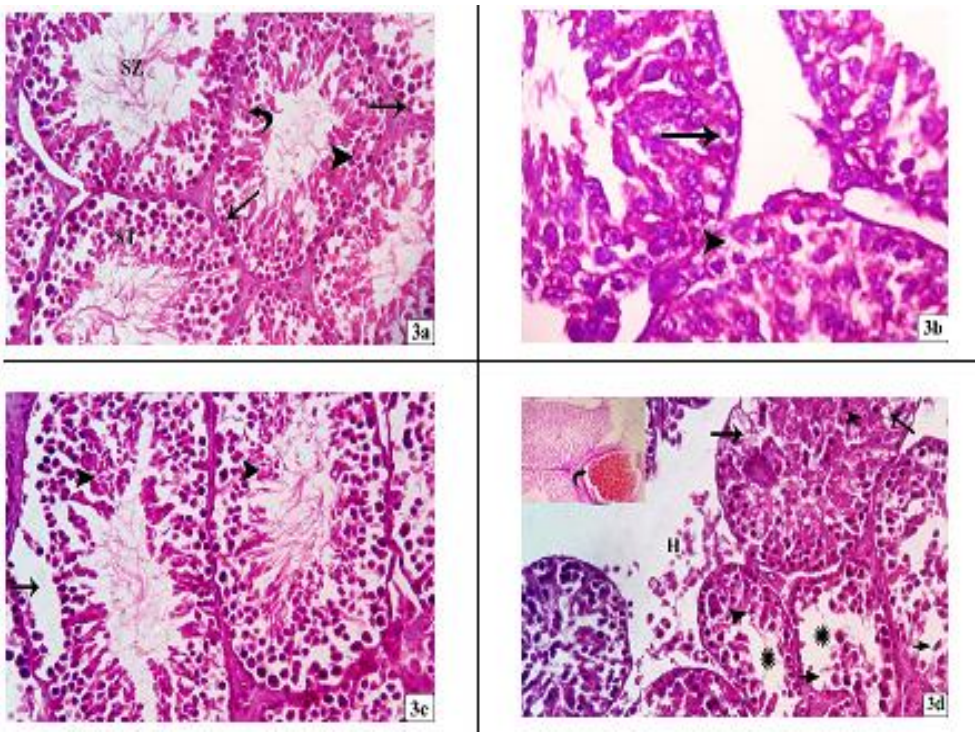

Fig.3: **photomicrographs of mice testes (H&E, 400X): (a) control group showing normal architecture of testicular tissue with normal spermatogenesis in seminiferous tubule (ST); spermatogonia (arrows), spermatocytes (arrow head), sertoli cell (curved arrow) and spermatozoa (SZ) could be seen. (b): subgroup IIa showing slight damage as vacuolization (arrow), necrotic spermatocytes and detachment of spermatogenic cells (arrow head). (c): subgroup IIb showing some seminiferous tubules have sloughing spermatogenic cells (arrow); damaged spermatocytes (arrow head) and loss of spermatozoa. (d): subgroup IIc showing congested blood vessel (curved arrow); absence of spermatozoa (*); hypoplasia (H); cytoplasmic vacuolation of spermatocytes (arrows), exfoliated germ cells (short arrows) and pyknotic nuclei of spermatocytes (arrow heads*).**

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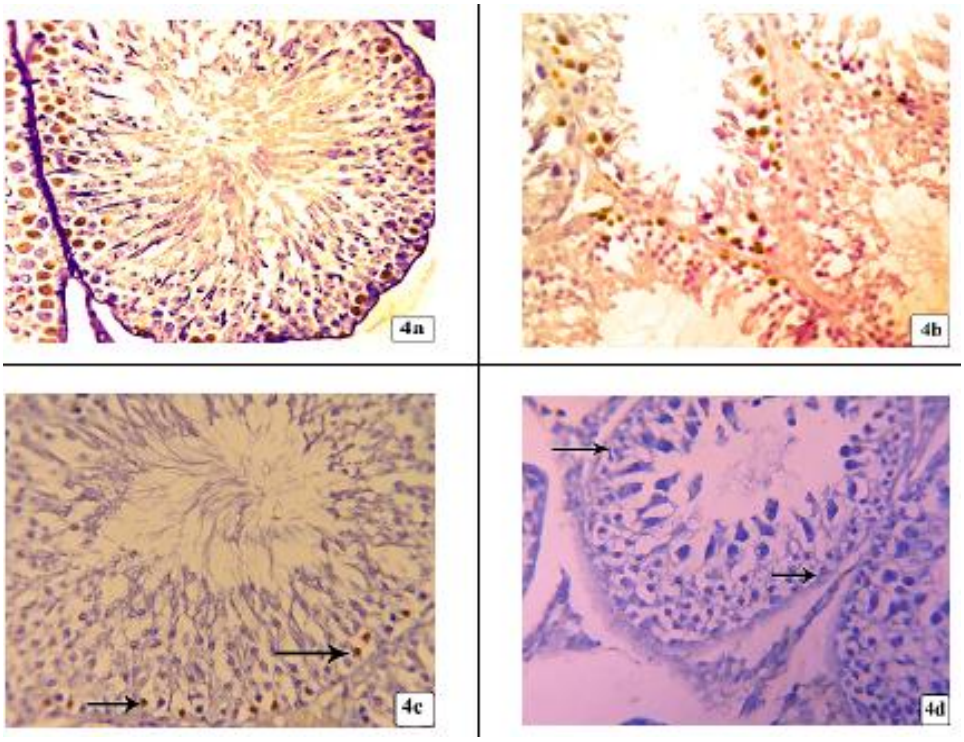

Fig.4: **immunohistochemical stained sections of mice testes with anti- Ki- 67 antibody (Immunoperoxidase technique, 400X): (a) control group showing strong positive immuoreaction in the nuclei of the spermatogonia and primary spermatocytes. (b): subgroup IIa showing mild positive immunoreaction. (c): subgroup IIb showing slight positive immunoreaction (arrows). (d): subgroup IIc showing negative immunoreaction in the nuclei of spermatogonia and primary spermatocytes (arrows).**

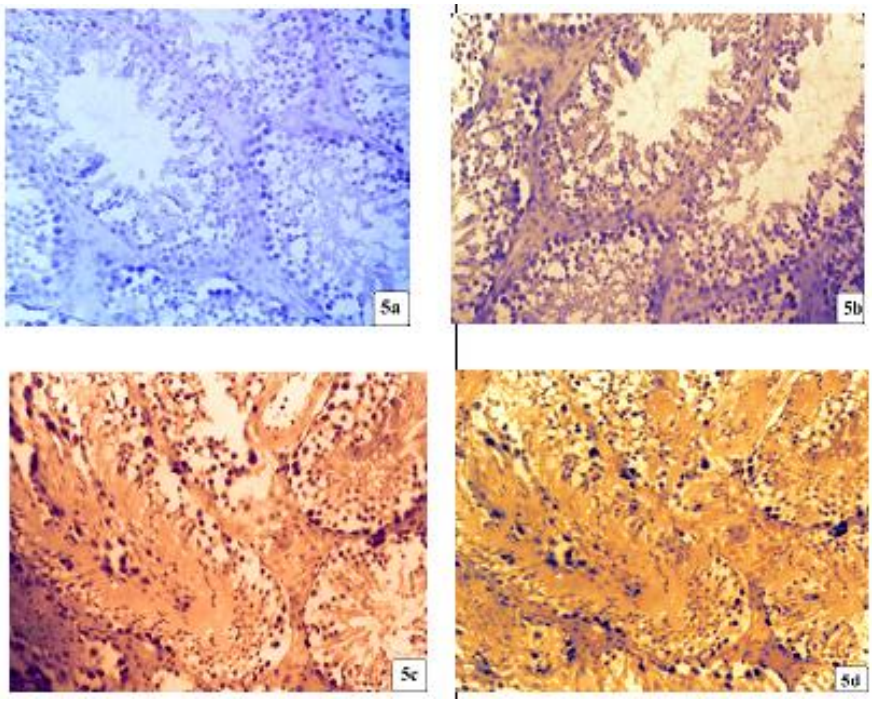

Fig.5: **immunohistochemical stained sections of inducible nitric oxide synthase in mice testes (iNOS, 400X):(a) control group showing negative immuoreaction in the cytoplasm of seminiferous tubular cells. (b): subgroup IIa showing slight increase in iNOS immunoreactivity in the cytoplasm of seminiferous tubular cells. (c): subgroup IIb showing mild increase immunoreactivity in iNOS. (d): subgroup IIc showing promient increase in iNOS immunoreactivity in the cytoplasm of seminiferous tubular cells.**

CONCLUSION

Our study has exposed that TiO2-NPs have the ability to interact with mice genetic materials / machinery under the test condition. This is of public health magnitude considering industrial and household applications of TiO2-NPs. Chemically induced genetic damage has been implicated in the etiology of many diseases; thus, there is need for tough policies as regards the apply of nanoparticles in human consumable and cosmetic products as well as their discarding into the environment.

REFERENCES

[1] Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: A historical perspective. Nanotoxicology, 2007; 1: (1) 2-25.

[2] Borm P J A , Berube D. A tale of opportunities, uncertainties and risks.*Nanotoday*.2008; 3, (1-2) 56-59.

[3] Seigrist M, Stampfli N, Kastenholz H, Keller C. Perceived risks and perceived benefits of different nanotechnology foods and nanotechnologyFood packaging. Appetite, 2008; 51: (2) 283-290

[4] Liga M V, Bryant E L, Colvin VL, Li Q .Virus inactivation by silver doped titanium dioxide nanoparticles for drinking water treatment.Water Research, 2011; 45, (2) 535–544.

[5] Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. *Environmental Science & Technology*, 2012; 46:2242–2250.

[6] Bourikas K, Kordulis C, Lycourghiotis A. Titanium Dioxide (Anatase and Rutile): Surface Chemistry, Liquid-Solid Interface Chemistry, and Scientific Synthesis of Supported Catalysts. Chemical Reviews*2014;.*114, (19) 9754-9823.

[7] Li W, Ni C, Lin H, Huang C P, Shah SI . Size dependence of thermal stability of TiO2 nanoparticles. Journal of Applied Physics. 2004; 96: (11) 6663-6668.

[8] Li N, Duan Y, Hong,M. Spleen injury and apoptotic pathway in mice caused by titanium dioxide nanoparticules. Toxicololgy Letters, 2010;165:161–168.

[9] Chen Z , Zhou D , Zhou S , Jia G. Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague Dawley rats.Journal of Applied Toxicology , 2019: 1–13.

[10] Bermudez E, Mangum J B, Wong BA . Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. *Toxicological Sciences* ,2004;77:347–357.

[11] Wang J, Zhou G, Chen C .Acute toxicity and bio-distribution of different sized titanium dioxide particles in mice after oral administration .Toxicology Letters ,2007; 168(2):176–185.

[12] Rodriguez-Rocha H, Garcia-Garcia A, Panayiotidis M I, Franco R. DNA damage and autophagy. Mutation Research,2011; 711:158–166.

[13] Di Virgilio A L, Reigosa M, Arnal PM. Comparative study of the cytotoxic and genotoxic effects of titanium oxide and aluminium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells. Journal of Hazardous Materials ,2010; 177:711-718.

[14] Lankoff A , Sandbergc WJ, Wegierek-Ciuk A, Lisowska H. The effect of agglomeration state of silver and titanium dioxide nanoparticles on cellular response of HepG2, A549 and THP-1 cells. Toxicolology,2012; 208: 197–213.

[15] Chen T, Yan J, Li Y. Genotoxicity of titanium dioxide nanoparticles. Journal of Food and drug analysis , 2014;22: 95-104.

[16] Khan M, Naqvi A H, Ahmad M .Comparative study of the cytotoxic and genotoxic potentials of zinc oxide and titanium dioxide nanoparticles. Toxicology Reports 2015; 2 :765–774.

[17] Orazizadeh M., Khorsandi L, Absalan F, Hashemitabar M, Daneshi E. Effect of beta-carotene on titanium oxide nanoparticles induced testicular toxicity in mice. Journal of Assisted Reproduction and Genetics, 2014; 31:61–568.

[18] Ryu JY, Lee BM, Kacew S, Kim HS . Identification of differentially expressed genes in the testis of Sprague-Dawley rats treated with di(n-butyl) phthalate. Toxicolology,2007;234:103–112.

[19] Gao G, Ze Y, Zhao X, Sang X, Zheng L, Ze X, Gui S, Sheng L, Sun Q, Hong J, Yu X, Wang L, Hong F, Zhang X. Titanium dioxide nanoparticle-induced testicular damage, spermatogenesis suppression, and gene expression alterations in male mice. Journal of Hazardous Materials, 2013;259:133-143.

[20] Meena R, Kajal K, Paulraj R. Cytotoxic and genotoxic effects of titanium dioxide nanoparticles in testicular cells of male wistar rat. Applied Biochemistry and Biotechnology,2015; 175(2): 825-840.

[21] Bakare A A, Udoakang A J, Anifowoshe A T, Fadoju OM, Ogunsuyi OI , Alabi OA, Alimba C G, Oyeyemi I T. Genotoxicity of titanium dioxide nanoparticles using the Mouse bone marrow micronucleus and sperm morphology assays. Journal of Pollution Effects & Control,2016; 4:156-162.

[22] Preston R J, Dean B J, Galloway S, Holden H , McFee AF , Shelby M. Mammalian in vivo cytogenetic assays, analysis of chromosome aberrations in bone marrow cells. Mutation Research, 1987; 189 :157–165.

[23] Schmid W .The micronucleus test. Mutation Research,1985; 31: 9-15.

[24] Bakare A, Okunola A, Adetunji O, Jenmi H. Genotoxicity assessment of a pharmaceutical effluent using four bio-assays. Genetics and Molecular Biology, 2009;32(2):373-381.

[25] Humason G L. (1979). Animal tissue techniques, 4th ed: San Francisco, Freeman and Co.

[26] Ramos-Vara JA, Kiupel M, Baszier T, Bliven L, Brodersen B, Chelack B . Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *The Journal of Veterinary Diagnostic Investigation*,2008; 20:393–413.

[27] Rasmussen OF. 2009 .Controls. In: Immunohistochemical Staining Methods. Kumar GL, Rudbeck L (eds.). Dako North America, Carpinteria, CA, p 127.

[28] Singh A C, Kumar M, Jha A M. Genotoxicity of Iomefloxacine –an antibacterial drug in somatic and germ cells of Swiss albino mice in vivo . Mutation Research, 2003; 535: 35-42.

[29] Heddle J A , Hite M, Kirkhart B , Mavournin K, MacGregor J T , Newell G W ,Salamone M F .The induction of micronuclei as a measure of genotoxicity: A report of the U.S. environmental protection agency Gene-Tox program. Mutation Research/Reviews in Genetic Toxicology, 1983; 123 (1) : 61-118.

[30] Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl H. Titaniumdioxide nanoparticles induce DNA damage and genetic instability in vivo in mice.Cancer Resarch, 2009; 69: 8784–8789.

[31] Ghosh M,Bandyopadhyay M, Mukherjee A.Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophic levels: Plant and human lymphocytes. Chemosphere, 2010;81:1253–1262.

[32] Allen R. The cytotoxic and genotoxic potential of titanium dioxide (TiO2) nanoparticles on human SH-SY5Y neuronal cells in vitro. The Plymouth Student Scientist, 2016; 9 (2) :5-28.

[33] Mohamed H R H, Hussien N A. Genotoxicity studies of titanium dioxide nanoparticles (Tio2-NPs) in brain of mice. Scientifica ,2016; 1-7.

[34] Lindberg HK,Falck GC, Catalan J. Genotoxicity of inhaled nanosized TiO2 in mice. Mutation Research,2012; 745: 58-64.

[35] Rad J S , Alfatemi M H , Rad M S , Rad M S , Sen D J, Mohsenzadeh S. *In vivo* titanium dioxide(Tio2) nanoparticles effects on chromosomal abnormalities and lactate dehydrogenase activity . American Journal of Advanced Drug Delivery ,2013; 1(3): 232-237.

[36] Xu J, Shi H, Ruth M .Acute toxicity of intravenously administered titanium dioxide nanoparticles in mice. PLoS One ,2013; 8(8): e70618.

[37] Relier C , Dubreuil M , Garcìa O L, Cordelli E , Mejia J , Eleuteri P, Robidel F, Loret T, Pacchierotti F, Lucas S, Lacroix G, Trouiller B .Study of TiO_2 P25 nanoparticles genotoxicity on lung, blood, and liver cells in lung overload and non-overload conditions after repeated respiratory exposure in rats . *Toxicological Sciences,* 2017; 156(2):527–537.

[38] Begain A Z, Begani R K. Is exposed to titanium dioxide nanoparticle associated with occupational lung cancer among titanium dioxide production workers ?an emerging issue . Occupational Diseases and Environmental Medicine ,2018; 6: 20-61.

[39] Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutation Research,2000; 455:29-60.

[40] Dufour EK , Kumaravel T, Nohynek G J, Kirkland D ,Toutain H. Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. Mutation . Research, 2006;607,:215–224.

[41] Kisin ER, Murray A R, Keane M J, Shi XC, Schwegler-Berry D, Gorelik O,Arepalli S, Castranova V, Wallace WE, Kagan VE . Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. Journal of Toxicolology and Environmental Health A ,2007; 70: 2071–2079.

[42] Li Y, Chen D H, Yan J, Chen Y, Mittelstaedt RA, Zhang Y, Biris AS, Heflich RH, Chen T. Genotoxicity of silver nanoparticles evaluated using the Ames test and in vitro micronucleus assay. Mutation Research , 2011;745: 4–10.

[43] Landsiedel R, Ma-Hock L, Van Ravenzwaay B. Gene toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulations. Nanotoxicology ,2010; 4:364-81

[44] Jomini S, Labille J, Bauda P.Modifications of the bacterial reverse mutation test reveals mutagenicity of TiO2nanoparticles and byproducts from a sunscreen TiO2-based nanocomposite. Toxicology Letters ,2012;215:54-61.

[45] Woodruff R S , Li Y, Yan J. Genotoxicity evaluation of titanium dioxide nanoparticles using the Ames test and comet assay. Journal of Applied Toxicology ,2012; 32:934-43.

[46] Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Tochigi S, Kondoh S,,Hirai T, Akase T, Nagano K, Abe Y. Amorphous nanosilica induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. Particle and Fibre Toxicology, 2011; 8: 1.

[47] Kumar A, Pandey AK, Singh SS , Shanker R, Dhawan A. Cellular uptake and mutagenic potential of metal oxide nanoparticles in bacterial cells. Chemosphere ,2011; 83:1124–1132.

[48] Warhei D B, Hoke R A, Finlay C, Donner E M, Reed K L, Sayes CM.Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters,2007; 171: 99–110.

[49] EL- Sharkawy NI, Hamza S M , Abou-Zeid E H. . Toxic impact of titanium dioxide (TiO2) in male albino rats with special reference to its effect on reproductive system. Journal of American Science ,2010;6 (11):865-872.

[50] El-nagar A M B, Ibrahim A, Soliman AM. Histopathological effects of titanium dioxide nanoparticles and the possible protective role of N-Acetylcysteine on the testes of male albino rats. International Journal of Fertility and Sterility,2018; 12(3):249-256

[51] Malmir M , Faraji T , Naderi Noreini S, Soleimani Mehranjani M. Protective antioxidant effects of N-acetylcysteine on testicular tissue and serum testosterone in paranonylphenol-treated mice (A Stereological Analysis). Reproductive System & Sexual Disorders,2018; 7(2):1-6.

[52] Wang M, Su P.The role of the Fas/FasL signaling pathway in environmental toxicant-induced testicular cell apoptosis: An update. Systems Biology in Reproductive Medicine., 2018; 64(2):93-102.

[53] Thakur M, Gupta H, Singh D, Mohanty IR, Maheswari U, Vanage G, Joshi DS. Histopathological and ultra structural effects of nanoparticles on rat testis following 90 days (Chronic study) of repeated oral administration. Journal of Nanobiotechnology,2014; 14:12-42.

[54] Takeda K, Suzuki K I, Ishihara A , Kubo-Irie M , Fujimoto R, Tabata ,M .Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. Journal of Health Science,2009;55:95–102.

[55] Asharani PV, Wu YL, Gong Z. Toxicity of silver nanoparticles in zebra fish models. Nanotechnology ,2008; 19(25):255102

[56] Miresmaeili SM, Halvaei I, Fesahat F, Fallah A, Nikonahad N, Taherinejad M. Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat. Iranian Journal of Reproductive Medicine ,2013; 11(5):423–430.

[57] Zhang H, Cai C, Shi C, Cao H, Han Z, Jia X. Cadmium induced oxidative stress and apoptosis in the testes of frog Rana limnocharis. Aquatic toxicology, 2012; 122–123:67–74.

[58] Spiazzi CC, Manfredini V, Barcellos da Silva FE, Flores E.M, Izaguirry AP, Vargas LM, Soares M B, Santos FW . C-Oryzanol protects against acute cadmium-induced oxidative damage in mice testes. Food and Chemical Toxicology, 2013; 55:526–532.

[59] Fouad A A, Qureshi HA, Al-Sultan AI, Yacoubi MT, Ali AA . Protective effect of hemin against cadmium-induced testicular damage in rats. Toxicology, 2009; 257:153–160.

[60] Kobayashi Y. The regulatory role of nitric oxide in proinflammatory cytokine expression during the induction and resolution of inflammation. Journal of leukocyte Biology ,2010; 88:1157–1162.

[61] Ekici S, Dogan Ekici AI, Ozturk G, Benli Aksungar F, Sinanoglu O, Turan G,Luleci N. Comparison of melatonin and ozone in the prevention of reperfusion injury following unilateral testicular torsion in rats. Urology,2012:80:899–906.

[62] Ozokutan BH, Kucukaydin M, Muhtaroglu S , Tekin Y. The role of nitric oxide in testicular ischemia-reperfusion injury. Journal of Pediatric Surgery, 2000;35: 101-103.

[63] Ferrini M, Wang C, Swerdloff R, Sinha-Hikim A, Rajfer J, Gonzalez-Cadarid N. Aging related increased expression of inducible nitric oxide synthase and cytotoxicity markers in rat hypothalamic region associated with male reproductive function. Neuroendocrinology, 2001;74(1): 1-11.