FJARD VOL. 37, NO. 1. PP. 1-14 (2023)

Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790
On Line ISSN:2805-2528

Assessing the genotoxicity of oral titanium dioxide nanoparticle administration in male rats using micronuclei and comet assay

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Abstract:

This study evaluated the toxic effects of titanium dioxide (TiO_2) nanoparticles (NPs) on rats that received oral administration of TiO_2-NPs with a particle size of 15±2nm once daily at doses of 0, 50, 100, 150, 200, and 300mg/kg for ten weeks. Animal mortality, body weight hematology, micronucleus assay and comet assay were investigated. The findings showed that TiO_2-NPs significantly reduced the body weight, and the results indicated a change in all blood parameters due to the stress response of TiO_2NPs. Hematological results revealed that the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, and platelet count of TiO_2 NPs- treated male rats were altered compared to the control group. However, the WBC of rats was elevated with increased doses of NPs compared with the control. There were no significant differences ($P>0.05$) in hematological parameters. Comet assay and micronuclei test results show a dosage-dependent increase in DNA fragmentation, which was supported by an increase in percentage of DNA that is tailed, length and intensity of DNA tails, and tail moment, especially at the 300mg/kg dose. According to the findings, the frequency of micronucleated cells has increased.

Keywords: Rats, Titanium dioxide nanoparticle, Micronucleus assay, comet assay.

1. Introduction:

Titanium dioxide (TiO_2) NMs are commonly used in industrial applications and are present in a wide range of consumer products including food products. The use of TiO2 NMs in the food industry includes applications as white color additive, flavor and opacity enhancer which can be found in candies, dairy products, dried vegetables, nuts, seed, soup, various processed foods, dietary supplements as well as in beer and wine **(Peters et al., 2014, Lim et al., 2015).** However, it has unique characteristics such as small size, large surface per unit mass and high reactivity that NPs can quickly enter the human body and then imposes potential health risk on human welfare **(Warheit et al., 2007 and Oberdorster et al., 2005).** The ultra-sized TiO_2 particles enable them to pass through cell membranes to nuclear membranes; finally, they can interrupt depend on cell ultrastructure and damage the cell membrane (**Moss and Wong, 2006 and Moller et al., 2002).**

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Received: 18/9/ 2022
Accepted: 25/10/ 2022

They can damage human and animal cells by increasing oxidative stress mechanism. Biosafety of this material needs to be estimated. Some reviews had suggested that the smaller-scale nanoparticles had a greater inflammogenic effect than larger particles. Induction of reactive oxygen species (ROS), free radicals, oxidative stress, damage, and apoptosis are common observations in a wide variety of cell types exposed to TiO_2 NPs *in vivo* and *in vitro* **(Shakeel et al., 2016).** Therefore, the present study is aimed to investigate the toxic effects of rutile TiO_2 NPs (<100 nm) on *in vivo* model through repeated oral administration of adult male Sprague-Dawley. The present study is designed to investigate the toxic effect of different doses of TiO_2 NPs on the male Sprague-Dawley rats using hematological studies as well as oxidative stress comet assay, and micronucleus assay.

2. Material and Methods:

2.1 Chemicals

Titanium dioxide nanoparticles (TiO_2-NPs) were purchased from NanoTech Egypt for Photo-Electronics Company (City of 6 October, Egypt). According to the information provided by manufacturer the particles size of TiO_2 nanoparticles is 15±2nm. Chemicals used for the quantitative determination of various biochemical and hematological parameters were purchased from Bio Diagnostic company and Human company (Egypt).

2.2. Preparation of TiO_2-NPs suspension

The TiO_2 NPs participles (15±2nm) were dispersed in distilled water (10mg/mL) and the suspension was sonicated at 230V for 20 minutes using ultra-sonic cleaner sonicator (Branson Ultrasonic Corporation, Danbury, Connecticut, USA) at room temperature. The suspension was stirred on vortex agitator immediately before administration in different dosages (50, 100, 150, 200 and 300mg/kg).

2.3. Characterization of titanium dioxide nanoparticles (TiO_2-NPs)

After adequate sonication, the aqueous dispersion of the nanoparticles was drop cast onto a carbon-coated copper grid to study the particles size and morphology. The grid was then air dried at room temperature and visualized using JEOL JEM 1010 Transmission Electron Microscope. The morphological structure of TiO_2-NPs was analyzed using transmission electron microscope (TEM) at accelerating voltage of 200KV. The crystal structure size 15±2nm and had a spherical like shape (Fig 1).

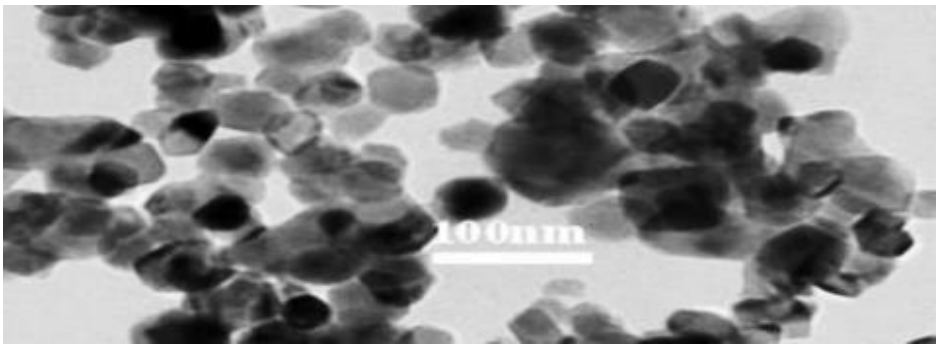


Fig. 1. An electron micrograph showing spherical TiO_2-NPs nanoparticles less than 15nm in size.

2.4. Animals and their housing

Thirty Sprague-Dawley male rats (weighing 140-160g and 8–10 weeks old) were obtained from Rapitco farm Company, Giza, Egypt. Animals were housed (five/cage) in universal polypropylene cages and were kept in the animal house of Biochemistry Department, Faculty of Agriculture, Fayoum University (Egypt) in controlled environmental conditions at 25±2 °C temperature with 12-h light and 12-h dark cycles. Animals fed a standard synthetic diet obtained from zala alnakhil Company (Cairo, Egypt) and had free access of water ad libitum. Before experimentation, rats were kept for one week for adaptation before starting the experiment. All experiments were performed in accordance with the general international guideline principles on the use of living laboratory animals in scientific research, after approval from the Institutional Animal Ethical Committee for Fayoum University (Fu-IACUC).

2.5. Experimental design

After the adaptation period, the thirty rats were divided into six groups (five rats per group). Group 1 (G1) was termed as control group and in which rats fed a standard synthetic diet and had free access of water *ad libitum*, while animals in the other five groups were given titanium dioxide nanoparticles by oral gavage at different concentrations for a period of 10 weeks. Rats were received 50, 100, 150, 200 and 300mg/kg of TiO_2NPs, for group 2 to 6, respectively.

2.6. Hematological examination

Blood samples were obtained from the retinal vein for all rats of each group one on Ethylene Diamine Tetra Acetic Acid (EDTA) was used for the hematological analysis. Complete blood picture was performed for all groups using a hematological analyzer (MEDONIC, S.E 12613, Sweden). The analysis included an erythrogram consisting of red blood cell count (RBC), hematocrit (HCT), hemoglobin (Hb) concentration and red cell indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and their differential (lymphocytes, granulocytes, and monocytes), and platelet count (PLT).

2.7. Genotoxicity assay

2.7.1 Micronuclei assay

The micronuclei (MN) test and the scoring of micronucleated polychromatic erythrocytes (MnPCEs) were carried out according to **Schmid, (1975)**. A minimum of 2000 polychromatic erythrocytes (PCEs) per animal was used for scoring from coded slides and estimating the ratio of PCEs/normochromatic erythrocytes (NCEs). The slides were read under 100 oil immersions for estimating the MnPCEs.

2.7.2. Comet assay

The single cell gel electrophoresis (SCGE)/comet assay, developed by **Singh et al**., **(1988)** combines the simplicity of biochemical techniques for detecting DNA single strand breaks (frank strand breaks and incomplete excision repair sites), alkali-labile sites and crosslinking with the single cell approach typical of cytogenetic assays**.** Comet assay was performed in Animal Reproduction Research Institute (ARRI), in Giza, Egypt.

2.8. Statistical analysis

The statistical analysis of experimental data for the quantitative variables, that is, Body weight gain and MnPCEs were statistically analyzed using SPSS 25 software. Less than 0.05 ($P<0.05$) probability values were considered statistically significant.

3. Results and Discussion:

3.1. The effects of Titanium dioxide nanoparticles (TiO2-NPs)

3.1.1. Animal observation

After 70 days of oral gavage dosing with TiO_2 NPs to male rats, mortality was not observed in any of the treated groups. The observation during this study showed the health status of the experimental animals on exposure to TiO_2 NPs was normal throughout the study. The results showed that there were no severe toxicity signs such as diarrhea or hair loss. Furthermore, no mortality was observed related to different doses of TiO_2-NPs (50, 100, 180, 200 and 300mg/kg body weight) administration. **Ben Younes et al., (2015)** showed that TiO2 NPs induced serious effects on the emotional behavior. Also, they showed TiO_2 NPs treatment increased the anxious index of rats. These results could be interpreted in terms of reduced activity and exploratory drive instead of anxiety modifications. **Cui et al., (2014)** suggested that the depressive-like behaviors observed in adult rats following exposure to TiO_2 NPs could be related to the oxidative damage in the central nervous system (CNS). The CNS is a potentially susceptible target for TiO_2-NPs. **Kim et al., (2013)** indicated that central administration of TiO_2-NPs' induced behavioral deterioration in freely moving intact animals. The LD50 of the titanium dioxide micro particles for rats has been reported as more than 1200mg/kg body weight in **WHO reports (1969).**

3.1.2. Rats body weight and the effect of TiO_2-NPs

The effects of different concentrations of TiO_2-NPs on the body weight of rats during the entire experimental period are shown in Table (1). Data showed that, insignificant increase in the body weight was observed during the first three weeks compared with the control. On the other hand, the data showed that insignificant decreased in the body weight of rats in the fourth week compared with the control. However, the average of rats body weight began to significantly decrease from the 5^{th} week to the 10^{th} week compared with the control group ($p<0.05$). **Bermudez et al., (2002)** explained decrease body weight and shorten life-time due to retention and overload of TiO2 NPs *in vivo*. These results are in line with **Shakeel et al., (2016)** showed general and physical symptoms of acute toxicity such as decreased activity or decreased uptake of food and water were observed at higher dose of TiO_2-NPs. **Vasantharaja et al., (2015)** studied the effect of TiO_2-NPs on body weight, the results revealed that acute oral administration of a single higher dose of TiO2 decreased body weight of mice. However, it has unique characteristics such as small size, large surface per unit mass and high reactivity that NPs can quickly enter the human body and then imposes potential health risk on human welfare (**Moss and Wong, 2006**). **Hext et al., (2005)** found no significant change of the body weight was observed after feeding the rats with TiO_2-NPs for one month, however, the mice fed with NP10 and NP50 for two and three months showed lower body weight than control mice. **Zhangjian et al., (2020)** were treated the rats with TiO_2-NPs (29±9 nm) orally at doses of 0, 2, 10, 50mg per kg daily for 90 days, they showed TiO_2-NPs caused a significant decrease of body weight in rats after exposure at doses of 10 and 50 mg/kg from the 8^{th} to 13^{th} week. Meanwhile, no significant as a result and found that the body weight of rats decreased with the increase of TiO_2-NP exposure doses (**Zhangjian et al., (2020)**. **Duan et al., (2010)** used TiO_2-NPs (125mg per kg) to administrate the mice orally for 30 days, and the results showed that the body weight of mice in the exposed group decreased significantly compared to the control.

Table 1. The body weight (Mean ± SE.) of male rats feeding by TiO_2-NPs at different doses for ten weeks compared with control.

Weeks	Body weight (mean± S.E.) of control and TiO_2 nanoparticle treated rats groups (mg/kg b.w)					
	Control	G2 50mg/kg	G3 100mg/kg	G4 150/kg	G5 200mg/kg	G6 300mg/kg
1	161.8± 2.3[a]	168.8± 5.3[a]	170.0± 0.1[a]	165.4± 4.65[a]	165.60± 9.34[a]	168.1± 2.10[a]
2	169.8± 3.4[a]	172.1± 11.4[a]	182.7± 2.30[a]	166.9± 9.76[a]	171.07± 10.93[a]	179.6± 10.1[a]
3	178.8± 5.5[a]	173.5± 3.5[a]	175.5± 5.91[a]	173.4± 10.31[a]	167.20± 5.25[a]	182.1± 10.0[a]
4	181± 5.5[a]	176.8± 7.4[a]	180.5± 6.50[a]	180.1± 8.39[a]	177.13± 5.55[a]	176.2± 10.9[a]
5	183.4± 5.2[a]	171.8± 3.3[b]	182± 5.00[ab]	179.3± 8.25[ab]	173.50± 4.50[ab]	178.0± 6.5[ab]
6	184.9± 5.9[a]	173.5± 4.5[ab]	181± 6.00[ab]	168± 6.00[ab]	173± 7.50[ab]	171.5± 5.5[ab]
7	189.3± 6.7[a]	171.5± 6.5[ab]	184.5± 5.50[ab]	172± 8.00[ab]	182.5± 5.50[ab]	168.6± 7.3[ab]
8	207.6± 5.5[a]	182.5± 5.5[b]	177.5± 3.00[b]	167± 6.50[b]	171.0± 8.19[b]	182.5± 7.5[b]
9	225.2± 5.6[a]	173.5± 13.5[b]	185.5± 5.50[b]	189± 6.5[b]	189± 5.75[b]	193± 6.5[b]
10	222.4± 7.21[a]	178.3± 6.41[b]	186.5± 4.40[b]	175± 5.00[b]	176± 6.04[b]	189± 7.00[b]

Data represent the means ± SE of 5 animals per group. P< 0.05 compared to control. Values in the same column within the same item followed by different letters are significantly different.

Hong et al., (2017) also found significant weight loss in pregnant rats after 18 days of oral TiO_2-NP exposure (25, 50, 100mg). But there were also some animal experimental results showing that the oral intake of TiO_2-NPs had no significant effect on body weight. The reasons for the controversy may be the different physicochemical properties of TiO_2-NPs used, the time and dose of exposure, different experimental animals, etc. Indeed, there was no significant change in the food intake of rats in the present study. Therefore, the food utilization or nutrition utilization of rats may be affected and oral exposure to TiO_2-NPs causes weight loss in rats, which may be related to nutritional metabolism **(Chen et al., 2015, Shukla et al., 2014, Warheit et al., 2015**).

4.1.3. Effects of TiO_2-NPs on hematological parameters of male rats

During this study, the blood parameters of the treated rats were evaluated for the effect of the oral administration of TiO_2-NPs. The results indicated a change in all the blood parameters due to stress response of TiO_2-NPs. The hematological results revealed that the RBC, HGB, HCT, MCV, MCH, MCHC and PLT values of the TiO_2-NPs treated male rats were altered compared to control group. However, WBC of the rats was elevated with increasing doses of NPs compared with control groups. There were no significant differences ($P>0.05$) in hematological parameters among all the groups at the start of the experiment prior to the subcutaneous administration of TiO_2-NPs (Table 2).

Hematological characteristics are an effective and sensitive index of

physiological and pathological changes in animals and humans. **Duan et al., (2010)** measured the hematological parameters to evaluate the physiological and pathological state of rats' administration of TiO_2-NPs for 60 days. Treatment with higher doses of TiO_2-NPs caused a significant increase in MCV, PLT, MPV and WBC and a significant decrease in RBC, HCT and HGB. In this study, nearly normal hematological parameters were observed when cinnamon extract was administered along with the TiO_2 or TiO_2 nanoparticles. **Espanani et al., (2015)** showed a decrease in the counts of platelet and lymphocyte with an increase in white cell count without any effect on the red cell count by using the same doses and route of administration but with different periods of administration that are not more than 21 days. The alterations in red blood cells may indicate effect of the nanoparticles on hemoglobin syntheses during red blood cells maturation during formation in bone marrow (**Culling et al., 1985**). White blood cells have a significant role role in the immunity of body response; represent the first defense line different types of the neutrophils can indicate an infection, allergic or toxic reaction against drugs or chemicals (**Reddy, 2019**). While the RBC account was decreased in treated animals in comparison with the control group. The oral administration and intraperitoneal injection of Ag NPs cause toxic effect on RBC and the effect of Ag-NPs on hemopoietic system led to decrease in the number of RBCs (**Park et al., 2010**). The slight decrease in the red blood cell and Hb may have been resulted from the suppression of circulating hormone, erythropoietin **(Hauck et al., 2010**).

Table 2. Effects of different concentrations of nano-TiO_2 on hematological parameters of albino male rats

Parameters	Groups					
	G1 Control	G2 50mg/kg	G3 100mg/kg	G4 150mg/kg	G5 200mg/g	G6 300mg/g
WBC (10^9/L)	4.27± 0.07^{ab}	6.08± 0.56^{bc}	5.76± 0.52^{abc}	4.65± 0.35^{ab}	5.25± 0.15^{ab}	7.05± 0.05^{c}
RBCs (10^{12}/L)	7.42± 0.15^{a}	7.10± 0.35^{a}	7.87± 0.12^{a}	7.92± 0.16^{a}	7.52± 0.11^{a}	7.30± 0.30^{a}
Hematocrit %	36.73± 1.26^{a}	35.37± 1.85^{a}	39.38± 0.41^{a}	38.33± 1.72^{a}	36.44± 1.72^{a}	35.06± 0.36^{a}
Hemoglobin(g/dl)	14.35± 0.63^{a}	13.80± 0.67^{a}	15.20± 0.65^{a}	14.65± 0.65^{a}	14.40± 0.10^{a}	14.35± 0.35^{a}
MCV(fl=10^{-15})	48.25± 0.47^{a}	49.20± 0.58^{a}	49.66± 0.33^{a}	47.50± 2.50^{a}	47.50± 1.50^{a}	48.00± 1.00^{a}
MCH (Pg=10^{-12})	18.91± 0.50^{a}	19.46± 0.40^{a}	19.44± 1.13^{a}	18.53± 1.28^{a}	19.26± 0.20^{a}	19.99± 0.01^{a}
MCHC (g/dl)	38.64± 0.62^{a}	39.54± 0.73^{a}	39.38± 0.62^{a}	39.94± 0.45^{a}	39.94± 0.45^{a}	41.06± 0.24^{a}
PLT (10^9/L)	567.20± 18.45^{ab}	672± 32.34^{b}	556± 38.00^{a}	624± 6.00^{ab}	572± 47.50^{ab}	578± 21.50^{ab}

RBCs= red blood cells; WBCs= white blood cells; MCV= mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration. Data represent the means ± SE of 5 animals per group. Mean values having different superscripts in the same column are statistically significant difference at p≤0.005.

The nanoparticle charge plays an essential role in their uptake by platelets and their influence on blood clot formation. The significant decrease in platelet count in the

CuO-NPs, ZnO-NPs and their mixture group shows that sub-acute exposure to the NPs caused thrombocytopenia. Thrombocytopenia may be related to CuO-NPs, ZnO-NPs and their mixture-induced oxidative damage to the platelet membranes. The challenge that nanomaterials pose to environmental health is that they are not one material (**Rezaie et al., 2012).** Blood cell count analysis is normally used to detect the hematological toxicity of different chemicals. Platelets are characterized by expert functions in assisting and modulating inflammatory reactions and immune responses (**Hundelshausen and Weber, 2007**).

3.1.4. Genotoxicity

3.1.4.1. Bone marrow micronuclei assay

The mean of the micronuclei frequency obtained after the treatment with different concentrations of TiO_2 NPs and the respective controls are shown in Table (3). The PCEs were stained light blue to gray and NCEs were stained light pink to light yellow **(**Fig. 2). Results regarding to the frequency of micronuclei in bone marrow polychromatic erythrocytes of bone marrow of rats are shown in Table (3). The percentage of PCE among 2000 scored erythrocytes (i.e. polychromatic and normochromatic) varied between 10 and 17. The findings of this study suggested that the frequencies of micronuclei increase with the increase of concentration of TiO_2 NPs treatment. Table (3) shows that the highest dose of TiO_2-NPs induced detectable damage in rat erythrocytes bone marrow cells. The percentage of PCEs/NCEs was decreased by increase the dose of TiO_2-NPs dose. The current results indicated that the greater increase in the bone marrow cytotoxicity (PCE/NCE ratio) occurred after 10 weeks of treatment with TiO_2-NPs (300mg /kg).

The micronucleus test is one of the most common cytogenetic techniques used for genotoxicity assessment and has been extensively applied in bone marrow cells of all the animal groups. Micronuclei (MN) are produced from whole chromosomes or acentric fragments that are delayed during anaphase, either by the lack of centromeres or by damage to the mitotic spindle (**Schmid, 1975).** Genotoxicity of nanoparticles may result from direct interaction with DNA or from indirect effects such as release of toxic irons from soluble nanoparticles or generation of oxidative stress (**Magdolenova et al., 2013).** Generally, several types of nanomaterials were shown to induce a significant increase of DNA damage including elevated MN frequencies. *In vivo* studies showed that TiO_2-NPs induced micronuclei and DNA strand breaks in peripheral blood in adult male mice exposed to 500 mg/kg TiO2NPs of 21nm size through drinking water for 5 days (**Trouiller et al., 2009**) and in bone marrow of mice administered by gavage for seven days at 40–100 mg/kg of 33nm size (**Sycheva et al., 2011**), but five days inhalation of TiO_2-NPs did not induce MN in peripheral blood lymphocytes of mice (**Lindberg et al., 2012**). Also, induction of DNA strand breaks in bone marrow cells of mice exposed within 7 days to TiO_2 at 40 and 200mg/kg was also reported by **Sycheva et al., (2011**). NPs induced significant levels of MN in PCE of bone marrow, which may originate from lagging acentric chromosomes or chromatid fragments caused by unrepaired DNA breaks. The mechanism of micronuclei elimination from cells is not clearly understood. It has been proposed that MN are degraded in situ (**Rao et al., 2008)** or the content of MN is diluted during cell division if it is not replicated or finally that MN might be removed directly from cells to the outside (**Shimizu, 2011).** The P/N ratio is recommended in some micronucleus test guidelines for evaluating the toxicity of test compounds to bone marrow cells. It has been shown that the P/N ratio fluctuates when higher concentrations of chemicals were administered (**Heddle et al., 1984**) or

when the bone marrow cells were taken at the later sampling intervals **(Adler, 1984)**. It is generally assumed that a decrease in the P/N ratio indicates toxicity to the bone marrow. Our findings revealed that the P/N ratio also decreased transiently when erythropoiesis was accelerated by the administration of TiO_2-NPs.

Table 3. Effects of different concentrations of nano-TiO_2 on micronuclei assay

Groups	Doses(mg/Kg)	No.of PCEs	Mn/PCEs%	PCEs/NCEs%
G1	0	2000	0.15	17.99
G2	50	2000	0.75	17.63
G3	100	2000	0.70	15.89
G4	150	2000	0.75	12.32
G5	200	2000	0.90	10.00
G6	300	2000	0.85	10.77

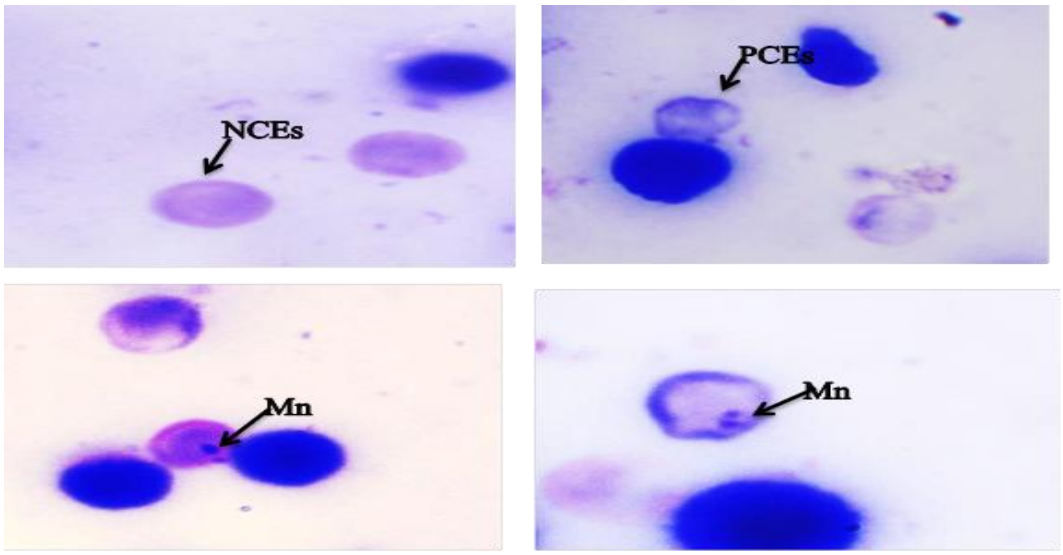


Fig. 2. Micronuclei (MN) induced by nano- TiO_2 in bone marrow cells of rats. PCE: Polychromatic Erythrocyte. NCE: Normochromatic Erythrocyte, MNPCE: Micronucleated Polychromatic Erythrocyte.

3.1.4.2. Alkaline comet assay

Suzuki et al., (1989) reported that, the P/N ratio may be altered in several different ways: (i) acceleration of the differentiation of erythrocytes from erythroblasts; (ii) inhibition of erythroblast division; (iii) recovery of erythroblast division. The formation of micronuclei can result either from a centric or a broken centric part(s) of chromosomes or from whole intact chromosomes lagging behind at the anaphase stage of cell division and thus failed to be integrated into one of the daughter nuclei in consistence with previous studies (**El-Yamanya et al., 2017**). DNA damage observed in liver and kidney cells of rats treated with 50, 100, 150, 200 and 300mg/kg of TiO_2-NPs, was measured by the alkaline comet assay (Fig. 3 and 4). The genotoxicity of TiO_2-NPs on liver and kidney of male rats was evaluated using comet assay and the results in Table (4 and 5) showed significantly increased DNA damage, which was quantified as Olive tail moment and percentage of DNA in the rats exposure to TiO_2-NPs. The results in Table (4 and 5) showed that TiO_2-NPs induced increase in the tail length, tail intensity%, tail migration and tail moments in the liver, and kidneys respectively. The result in Table (4) showed increase in tail length in

liver DNA of rats in all treated groups after administration of TiO_2-NPs, the tail length of liver DNA of rats in G6 and control, G1 was 6.42 and 4.94µm respectively. The results in Table (5) showed, TiO2-NPs increased the tail length, % DNA in tail, tail moment and olive tail moment in dose dependent manner than control for kidney cells. These results suggested that the observed DNA damage was due to genotoxicity and comet assay might be used to detect the DNA damage induced by TiO_2-NPs in rat liver and kidneys. Fig. (3 and 4) showed the photomicrographs representative DNA damage (comet assay) in rat liver and kidney cells respectively.

The comet assay is a versatile, simple, and sensitive method to study the toxicity study of a broad range of compounds and is capable of measuring DNA damage in almost all organisms and cells (**Olive and Banath, 2006**). **Trouiller et al., (2009)** reported that TiO_2-NPs were genotoxic and clastogenic in mice exposed to 500mg/kg TiO_2-NPs in drinking water for 5 days. The results of the comet assay suggested cellular DNA damage, which was expressed in terms of increased % tail of DNA. The DNA damage may be due to the direct interaction or interference of NPs in the process of DNA replication (**An et al., 2012**). The comet and MN assays are well-established methods for evaluating the potential genotoxic effects in ecotoxicological studies (**Jha 2008**). In rats made to inhale a sunscreen product containing 79–89% TiO_2 nanoparticles 6h per day for 5 days at a concentration of 10mg/m^3, negative outcomes were obtained in the comet assay (**Landsiedel et al., 2010).** The *in vivo* rodent alkaline comet assay (single gel electrophoresis assay) is widely used for detecting DNA damage but has not been validated formally. Recently this assay was listed in the ICH Guidance 'S2'(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use'' as a second *in vivo* assay. The comet assay was recommended as a supportive study by the Guidance (**Hartman et al., 2003 and Burlinson et al., 2007**).

Table 4. Quantitative estimation of the DNA damage in liver cells of normal control and TiO_2-NPs treated groups by the comet assay

Treatment	Dose mg/kg	Tail Length	Tail in DNA	Tail moment	Olive tail moment
G1	0	4.939	8.867	0.463	1.232
G2	50	5.904	11.860	0.600	1.582
G3	100	7.721	10.025	0.619	1.384
G4	150	8.125	9.108	0.629	1.288
G5	200	9.538	8.191	0.639	1.187
G6	300	6.421	8.439	0.527	1.154

Table 5. Quantitative estimation of the DNA damage in kidney cells of normal control and TiO_2-NPs treated groups by the comet assay

Treatment	Dose mg/kg	Tail Length	Tail in DNA	Tail moment	Olive tail moment
G1	0	8.84	12.77	0.80	1.04
G2	50	9.075	17.941	2.00	1.990
G3	100	10.857	14.170	1.562	2.481
G4	150	10.238	18.190	2.576	2.543
G5	200	10.444	20.076	2.238	2.574
G6	300	10.032	22.211	2.914	2.605

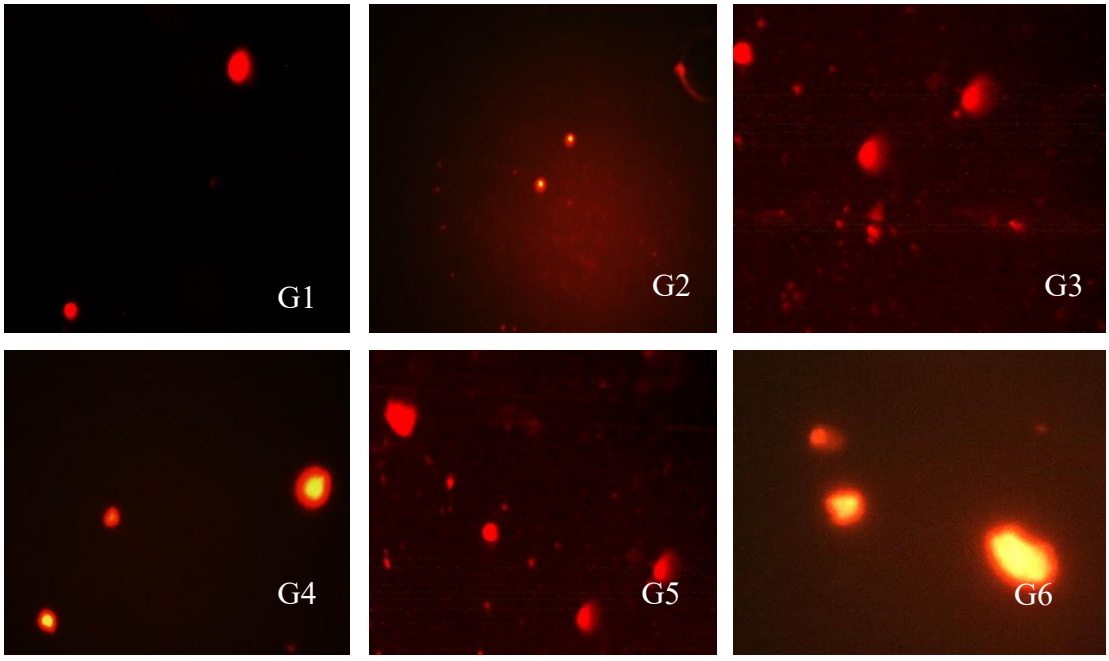
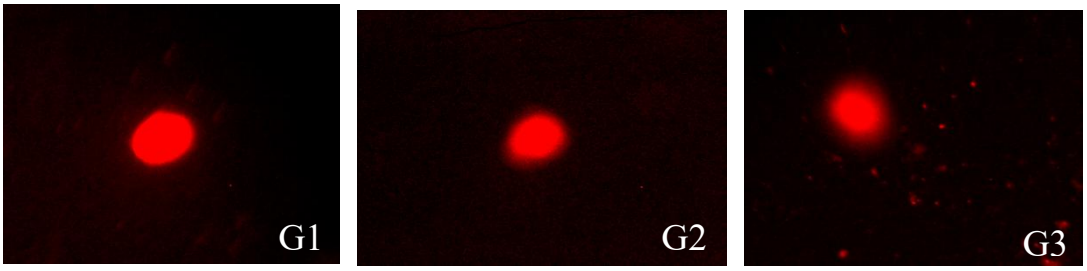


Fig. 3. Comet assay images representing DNA damage induced by TiO_2NPs in liver tissue, (G1) Normal cell; (G3) little DNA damage; (G2) moderate DNA damage; (G5) extensive DNA damage; (G4 and G6) completely damaged DNA



G4 G6

Fig. 4. Comet assay images representing DNA damage induced by TiO_2-NPs in bone marrow cell, (G1) Normal cell; (G3) little DNA damage; (G2) moderate DNA damage; (G5) extensive DNA damage; (G6) completely damaged DNA

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الملخص العربي

تقييم السمية الجينية لجسيمات ثاني أكسيد التيتانيوم النانوية عن طريق الفم في ذكور الفئران باستخدام اختبار الأنوية الدقيقة وتلف الحمض النووي

أجريت هذه الدراسة بقسم الوراثة بكلية الزراعة جامعة الفيوم، مصر. قيمت هذه الدراسة التأثيرات السامة لجسيمات ثاني أكسيد التيتانيوم النانوية على الفئران التي تلقت تناولًا عن طريق الفم لثاني أكسيد التيتانيوم بحجم جسيم يبلغ 15±2 نانومتر مرة واحدة يوميًا بجرعات 0 ، 50 ، 100 ، 150 ، 200 ، 300 مجم/كجم لمدة عشرة أسابيع. تم التحقيق في وفيات الحيوانات ، وأمراض الدم ووزن الجسم ، ومقايسة النواة الدقيقة ومقايسة المذنب. وأشارت النتائج إلي تغيير في جميع مكونات الدم الخلوية، وكشفت نتائج فحص الدم تغيير في قيم تركيز الهيموجلوبين في خلايا الدم الحمراء، عدد خلايا الدم الحمراء، الهيماتوكريت، متوسط حجم كرات الدم الحمراء، متوسط الهيموجلوبين العضلي، متوسط تركيز الهيموجلوبين في الجسم، عدد خلايا الدم البيضاء، الصفائح الدموية مقارنة بالمجموعة الضابطة، وزيادة عدد كرات الدم البيضاء في الدم مع زيادة الجرعة مقارنة بالمجموعة الضابطة. وقد نتج عن مقايسة المذنب وإختبار الأنوية الدقيقة زيادة تعتمد على الجرعة المستحدمة في تجزئة الحمض النووي، والتي كانت مدعومة بزيادة في النسبة المئوية للحمض النووي للذيل، وطول ذيل الحمض النووي، خاصة في جرعة 300 مجم/كجم. أظهرت النتائج زيادة في عدد الأنوية الدقيقة مع زيادة الجرعة.

الكلمات الدالة: الفئران, ثاني أكسيد التيتانيوم, إختبار الأنوية الدقيقة, إختبار مقايسة المذنب.