ISSN: 2157-7439
Journal of Nanomedicine & Nanotechnology

OPEN ACCESS Freely available online

Research Article

Genotoxicity Assessment of Zinc Oxide Nanoparticles in Male Rats After Oral Administration Using Micronuclei and Comet Assay

Gamal Hassan Mohamedin*, Amal Ramadan G, Eissa G, Ahmed Yassein AM

Department of Genetics, Faculty of Agriculture, Fayoum University, Egypt

ABSTRACT

In the food and fertilizer industries, zinc oxide nanoparticles (ZnO-NPs) are frequently utilized. In our investigation, rats received oral administration of ZnO NPs with a particle size of 30 ±5 nm once daily at doses of 100, 200, 300, 400, and 500 mg/kg for ten weeks in order to assess the genotoxic effect. Impacts on hematological markers, genotoxic impact, and growth were investigated. The results indicated that ZnO-NPs significantly reduced body weight gain, red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit value (HCT), and platelet count (PLT), while increasing white blood cell (WBC), mean capsular volume (MCV), mean capsular hemoglobin (MCH), and mean capsular hemoglobin concentration (MCHC) in the treated rats. Our data show a dosage-dependent increase in DNA fragmentation for both the comet assay and the micronuclei test, which was supported by an increase in the proportion of DNA that was tail-containing, the length and intensity of DNA tails, and the tail moment, especially at the dose of 500 mg/kg. The findings revealed an increase in the number of micronucleated cells.

Keywords: Rats; zinc oxide nanoparticle; Hematology; Micronuclei; Comet assay.

INTRODUCTION

Small substances with at least one dimension between one and one hundred nanometers are known as nanoparticles (NPs). Due to their small size and large surface area, nanoparticles play a key role in all aspects of modern life [1]. By shrinking matter to a scale of 1 to 100 nm, nanotechnology is a significant area of innovation in the industry [2]. The food sector, drug delivery, diagnosis, cosmetics, and several sunscreens are just a few industries where nanoparticles (NPs) are used [3]. ZnO nanoparticles are exposed to the human body more and more as their use increases. Inhalation, cutaneous contact, and ingestion are the three main ways that ZnO nanoparticles are exposed to the body [4]. When ZnO nanoparticles are exposed to the body through any method, they can reach the circulatory system and spread throughout the entire body through it [5]. When ZnO nanoparticles enter the body, they are small enough to penetrate cells right away and are subsequently internalized by the cells either as free Zn^{2+} ions or as nanoparticles [6].

Micronuclei are reliable indicators of genotoxic exposure in both humans and animals, and scoring them has been widely utilized to detect probable genotoxic substances [7]. The micronucleus test is a crucial *in vivo* cytogenetic screening method for identifying newly produced structural chromosomal abnormalities in bone marrow cells [8]. Chromosome abnormalities in mouse bone marrow and the micronucleus test have both been used extensively to clarify the connection between food and mutagenesis. Micronuclei can be utilized as a mutation index because they are an indicator of irreversible DNA loss. Wistar rats had decreased red cell counts, liver lesions, and hepatocyte inflammation during them feeding on ZnO NPs at dose of 2000 mg/kg. According to diet-borne ZnO NPs can change the molecular structure and morphology of the blood, intestine, liver, and kidneys. These NPs may display unpredictable genotoxic features through direct interaction with genetic material or by indirect DNA damage brought on by reactive oxygen species due to their tiny size and increased surface area combined with physiochemical properties like charged surfaces. Chromosome abnormalities in mouse bone marrow and the micronucleus test have both been used extensively to clarify the association between food and mutagenesis. Micronuclei can be utilized as a mutation index because they are an indicator of permanent DNA loss (9). A greater understanding of the genotoxic potential of ZnO NPs in an animal model is needed in consideration of the rapidly expanding field of nanotechnology and increasing exposure to nanoparticles. The objective of the current study was to assess the acute oral

***Correspondence to**: Gamal Hassan Mohamedin, Department of Genetics, Faculty of Agriculture, Fayoum University, Egypt, Email Id:- gmh01@fayoum.edu.eg,https://orcid.org/0000-0003-2575-6886

Received: 3-Aug -2022, Manuscript No: jnmnt-22-17765, **Editor assigned:** 6- Aug -2022, Pre QC No: jnmnt-22-17765 (PQ), **Reviewed:** 20- Aug -2022, QC No: jnmnt-22-17765, **Revised:** 23- Aug -2022, Manuscript No: jnmnt-22-17765 (R), **Published:** 30- Aug-2022, DOI: 10.35248/2157-7439.22.13.631.

Citation: Mohamedin GH, Amal Ramadan G, Eissa G, Ahmed Yassein AM (2022) Genotoxicity Assessment of Zinc Oxide Nanoparticles in Male Rats After Oral Administration Using Micronuclei and Comet Assay. J Nanomed Nanotech. 13: 631.

toxicity of ZnO-NPs at dosages of 100, 200, 300, 400, and 500 mg/kg. ZnO NPs' genotoxic effects as determined by hematology tests and comet assays.

MATERIAL AND METHODS

Chemicals

Zinc Oxide Nanoparticles (ZnO-NPs) were obtained from NanoTech Egypt Company (City of 6 October, Egypt). The manufacturer's datasheet indicates that ZnO nanoparticles have a size of 30±5 nm. Chemicals were bought from Bio Diagnostic Company and Human Company for the quantitative measurement of several biochemical and hematological parameters (Egypt).

Preparation of ZnONPs suspension

The ZnO NPs participles (30±5 nm) were dispersed in distilled water (10 mg/mL) and the suspension was solicited at 230 V 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 (100,200,300,400 and 500mg/kg).

Experimental design

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. 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 zinc oxide nanoparticles by oral gavage at different concentrations for a period of 10 weeks. Group 2 (G2) in which rats received 100 mg/kg body weight ZnONPs suspension orally once per day, Group 3 (G3) in which rats received 200 mg/kg ZnO-NPs suspension orally once per day, Group 4 (G4) in which rats received 300 mg/kg body weight ZnONPs suspension orally once per day, Group 5 (G5) in which rats received 400 mg/kg body weight ZnONPs suspension orally once per day and Group 6 (G6) in which rats received 500 mg/kg body weight ZnONPs suspension orally once per day.

Hematological Examination

Each rat in each group had blood drawn from the retinal vein for hematological analysis using EDTA (Ethylene Diamine Tetra Acetic Acid). Using a hematological analyzer, an entire blood image was taken for each group (MEDONIC, S.E 12613, Sweden). The analysis included an erythrogram with the following components: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (Hb) concentration and red cell indices, white blood cell count (WBC), and their differential (lymphocytes, granulocytes, and monocytes), and platelet count (PLT).

Genotoxicity Assay and Micronuclei Assay

According to Schmid (8), the micronuclei (MN) test and the scoring of micro nucleated polychromatic erythrocytes (MnPCEs) were conducted. For scoring from coded slides and determining the ratio of polychromatic erythrocytes (PCEs) / normochromatic erythrocytes (NCEs), a minimum of 2000 polychromatic erythrocytes (PCEs) per animal were utilized. For the purpose of estimating the MnPCEs, the slides were examined under 100 oil immersions.

Comet Assay

The single cell approach typical of cytogenetic assays is combined with the convenience of utilizing biochemical techniques for detecting DNA single strand breaks (frank strand breaks and incomplete excision repair sites), alkali-labile sites, and crosslinking in the single cell gel electrophoresis (SCGE)/comet assay, developed. The Animal Reproduction Research Institute (ARRI), located near Giza, Egypt, conducted a comet assay. Comet assay was performed in Animal Reproduction Research Institute (ARRI), in Giza, Egypt.

Statistical Analysis

Using SPSS-PC software experimental data were statistically examined for the quantitative variables, namely body weight gain and MnPCEs. Probability values less than 0.05 ($P<0.05$) were regarded as statistically significant.

RESULTS AND DISCUSSION

Animal Observation

The observation during this study showed that no serve toxicity signs such as diarrhea or hair loss. Furthermore, no mortality was observed related to different doses of ZnO-NPs administration (100, 200,300, 400 and500 mg/kg body weight). Also no behavioral changes were observed related to be orally administered to rats.

Effect of ZnO-NPs on the Body Weight Gain of Rats

The effects of various ZnO-NPs concentrations on the rat body weight gain throughout the period of the entire experiment are displayed in [Table 1]. Data revealed a significantly lower average rate of rat body weight gain when compared to the control group ($p<0.05$). The current study's findings regarding the reduction in body weight gain were in agreement with those of who demonstrated that rats administered ZnO NPs by gavage at doses of 0, 100, 200, and 400 mg/kg/day experienced a decrease in body weight as a result of

Table 1: Effects of different concentrations of nano-ZnOs on the body weight gain of male rats for ten weeks.

Groups	Dose mg/kg	Initial weight	Final weight	Weight gain
G1	0	161.80±3.55^{a}	241.60± 9.35^{a}	79.80± 8.02^{a}
G2	100	171.40±5.02^{a}	161.60±8.07^{ab}	19.00± 6.42^{b}
G3	200	169.25± 9.11^{a}	170.00±13.49^{ab}	20.80± 3.81^{b}
G4	300	171.83±4.88^{a}	151.40± 8.90^{b}	12.20± 4.15^{b}
G5	400	165.20±6.09^{a}	160.40± 10.89^{ab}	17.00± 5.71^{b}
G6	500	169.80± 2.71^{a}	185.40±9.38^{ab}	25.20± 7.09^{b}

decreased food consumption. High doses of ZnO NPs in diet can have toxicological effects, but the decrease in body weight at 5000 mg/kg ZnO NPs may help explain why the relative weights of the pancreas, brain, and lung have increased in response to ZnO NPs (16). Nano-ZnOs at 50 and 500 mg/kg was demonstrated increased body weight but decreased body weight was showed at 5000 mg/kg. Decrease of body weight may be attributed to anabolic metabolism in body of treated animals, or as a result of anti-digestion effect, or due to the loose of appetite in treated animals as a result of nanoparticles administration [9]. Data represent the means ± SE of 5 animals per group. P < 0.05 compared to control.

Effects of ZnO-NPs on Hematological Parameters of Male Rats

According to the results in Table 2, treated rats' hemoglobin content and platelet counts (PLT) did not differ significantly from the control groups (P<0.05). The results showed that all rat groups that received ZnO NPs during the investigation showed significantly decreased hematocrit percentages and red blood cell counts (RBCs) than the control group. On the other hand, the numbers of white blood cells (WBCs), MCV values, MCH, and MCHC were significantly increased in the treated groups' rats over the control groups' after 10 weeks. ZnO-NPs' toxicity must be assessed using hematological parameters, and the findings from these experiments showed that ZnO-NPs can enter and move around inside living things [10]. Dhawan and Sharmademonstrated that NPs of several materials when given orally are more hazardous than their microparticle counterparts. According to, a sharp decline in lymph %, WBC, and MCHC may be the result of blood leaking from the vessel walls brought on by high dosages of ZnO-NPs. Our findings are in agreement with; they illustrate the reason of platelet decrease. In contrast to our findings, they did, however, exhibit an increase in the red blood cell count. According to research , the rise in the number of red blood cells may cause the blood's viscosity to rise. Additionally, Somayeh and Mohammad [11] noted that the toxicity of zinc oxide nanoparticles causes an increase in white cell count, which is compatible with our investigation. Zinc oxide nanoparticles may result in an increase in the number of white and red blood cells, a decrease in the number of lymphocytes, and an increase in the number of neutrophils. By utilizing the same doses and routes of administration but various periods of administration that are not longer than 21 days, demonstrated a decrease in the counts of platelet and lymphocyte with an increase in white cell count and no effect on the red cell count. Depending on the provided dose, sub-chronic use of zinc oxide nanoparticles was reported to cause toxic symptoms in the lymphatic system and in the blood cell count [12]. The abbreviations RBCs, WBCs, MCV, MCH, and MCHC stand for red blood cells, white blood cells, and mean corpuscular volume and mean corpuscular hemoglobin concentration, respectively. Data are the means and standard errors of five animals per group.at p≤0.005.

Bone Marrow Micronuclei Assay

Table shows the mean of the micronuclei frequency after treatment with various concentrations of ZnO NPs and the corresponding controls [Table 3]. The PCEs were painted a light blue to grey colour, whereas the NCEs were painted a light pink to light yellow colour [Figure 1]. The findings demonstrated that as the dose of ZnO NPs was raised, the frequencies of micronucleated erythrocytes in the bone marrow of treated rats increased as well. By increasing the dose of ZnO NPs, the percentage of PCEs/NCEs was decreased. According to the latest findings, 10 weeks of treatment with 500 mg/kg b.wt ZnONPs caused the greatest increase in bone marrow cytotoxicity (PCE / NCE ratio). Extra-nuclear entities known as micronuclei (MN) are made up of broken chromosomal fragments and/or complete chromosomes that did not become absorbed into the nucleus after cell division. Defects in the cell's ability to repair itself, a buildup of DNA damage and chromosomal abnormalities can all lead to MN [13]. When the normal bone marrow cell proliferation is impacted by any hazardous agent, assessed the relationship between the numbers of immature erythrocytes (PCEs) and the mature erythrocytes (NCEs) and reported a decrease in the PCE/NCE ratio is observed. The polychromatic erythrocyte (PCE) counts in peripheral blood were the most widely used and practical way to monitor erythropoiesis. They also noted that examination of erythropoietic cytotoxicity was a critical component of safety assessment in novel drug development. Fewer immature erythrocytes (PCE) than mature or normochromatic erythrocytes (NCE) were thought to be a sign of cytotoxicity caused by mutagens. Some micronucleus test guidelines advise using the P/N ratio to estimate a compound's toxicity to bone marrow cells. It has been demonstrated that the P/N ratio changes when stronger pharmacologic doses are given or when bone marrow cells are taken at later sampling intervals [14]. The P/N ratio may decrease as the result of unbalanced changes in both cell types' populations, a decrease in PCE, an increase in NCE, or both.

Alkaline comet assay

The results in [Tables 4, 5] demonstrated that ZnO-NPs increased tail length, tail intensity percent, tail migration, and tail moments in the liver and kidneys, respectively. The results in Table (4) showed that after receiving Nano ZnOs, the length of the tail in the rat liver DNA increased in all treatment groups; the tail lengths

Table 2: Effects of different concentrations of nano-ZnOs on hematological parameters of male rats.

Parameters	Groups					
	G1 control	G2 100mg/g	G3 200mg/kg	G4 300mg/kg	G5 400mg/kg	G6 500mg/kg
WBC (10^9/L)	4.27 ±0.07^{ab}	4.65 ± 0.14^{ab}	4.40 ±0.35^{ab}	4.66 ±0.46^{ab}	4.60 ±0.03^{ab}	4.62 ± 0.18^{ab}
RBCs (10^{12}/L)	7.42 ± 0.15^{a}	7.00 ±0.14^{b}	6.74 ±0.11^{b}	6.57 ±0.17^{b}	6.82 ± 0.13^{b}	6.84 ± 0.08^{b}
Hematocrit %	36.73 ±1.26^{ab}	35.04 ± 0.44^{b}	34.98 ±0.45^{b}	34.84 ±0.71^{b}	35.00 ± 0.56^{b}	34.24 ±0.96^{b}
Hemoglobin(g/dl)	14.35 ± 0.63^{a}	13.84 ± 0.18^{a}	13.64 ±0.18^{a}	13.84 ± 0.39^{a}	13.94 ± 0.31^{a}	13.96 ±0.33^{a}
MCV(fl=10^{-15})	48.25 ± 0.47^{a}	49.74 ±0.48^{ab}	51.60 ± 0.67^{b}	50.00 ± 0.001^{ab}	51.75 ± 0.85^{b}	50.75 ±1.25^{b}
MCH (Pg=10^{-12})	18.91 ± 0.50^{a}	19.85 ± 0.52^{ab}	20.65 ± 0.26^{b}	19.70 ± 0.16^{ab}	20.57 ± 0.24^{b}	20.52 ±0.31^{b}
MCHC (g/dl)	38.64 ± 0.62^{a}	39.54 ± 0.73^{ab}	39.38 ±0.62^{ab}	39.94 ± 0.45^{ab}	39.94 ± 0.45^{ab}	41.06 ±0.24^{b}
PLT (10^9/L)	567.20 ±8.45^{a}	519.60 ±25.70^{a}	498 ± 28.37^{a}	528 ± 16.71^{a}	551.60 ±41.01^{a}	497 ± 9.57^{a}

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

Groups	Doses(mg/Kg)	No.of PCEs	Mn/PCEs%	PCEs/NCEs%
G1	0	2000	0.15	17.99
G2	100	2000	0.25	12.11
G3	200	2000	0.45	12.38
G4	300	2000	0.5	11.88
G5	400	2000	0.6	11.38
G6	500	2000	0.8	11.70

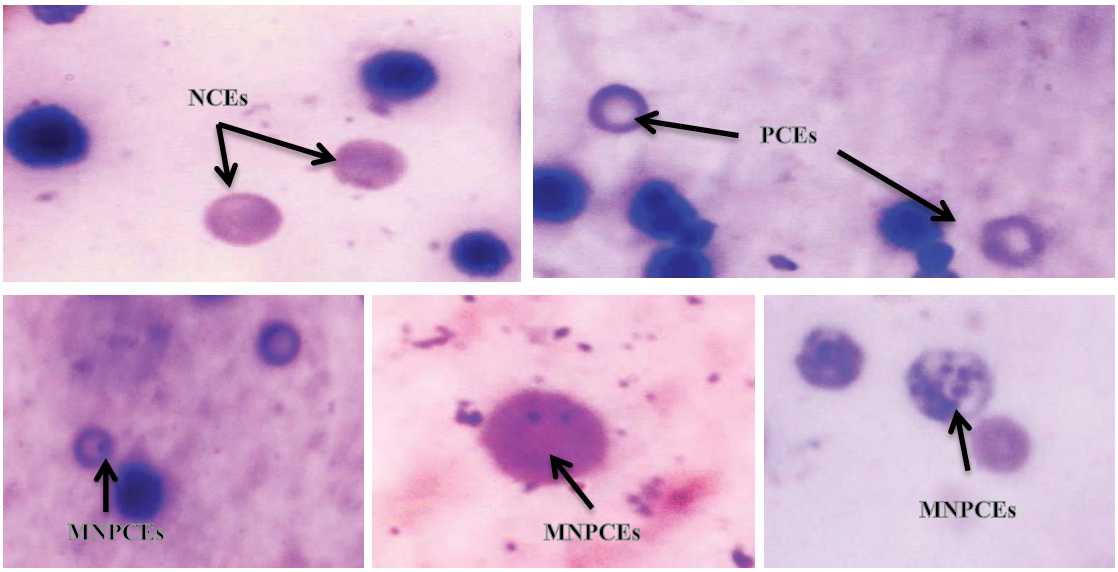

Figure 1: Shows the micronuclei (MN) that nano-ZnOs generated in rat bone marrow cells. PCE: Polychromatic Erythrocyte. NCE: Normochromatic Erythrocyte, MNPCE: Micronucleated Polychromatic Erythrocyte.

Table 4: Shows the DNA damage values in the liver cells of untreated and ZnONP-treated rats.

Treatment	Dose mg/kg	Tail Length	Tail in DNA	Tail moment	Olive tail moment
G1	0	4.94	8.87	0.46	1.23
G2	100	7.43	9.421	0.56	1.36
G3	200	6.22	13.21	0.98	1.39
G4	300	10.44	16.79	2.097	2.09
G5	400	9.39	14.48	1.90	2.02
G6	500	10.67	18.89	2.44	2.39

Table 5: DNA damage measurements in kidney cells from control and ZnONP-treated rats.

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	100	9.69	14.48	1.72	1.64
G3	200	9.39	15.53	1.90	2.03
G4	300	10.55	17.19	1.93	2.08
G5	400	10.67	18.89	2.44	2.39
G6	500	11.97	24.75	3.63	2.97

in group (G6) and control group (G1) were 10.57 m and 4.94 m, respectively. The results in Tables (5) shown that ZnO-NP increased the tail length, percent DNA in the tail, tail moment, and olive tail moment in kidney cells in a dose-dependent manner compared to control. These results indicated that the observed DNA damage was due to genotoxicity and comet assay might be used to detect the DNA damage induced by ZnO-NPs in rat liver and kidneys. [Figures 2, 3] displayed photomicrographs of the comet assay-representative DNA damage in rat liver and kidney cells, respectively. The single-cell gel electrophoresis assay, also referred to as the comet assay, is a technique for quantifying DNA strand breaks in eukaryotic cells. Based on the length of the genetic material's movement (tail length) in the anode-directed direction during the comet assay, the quantity of DNA breakage in a cell was calculated (13). Furthermore, it has been demonstrated that the frequency of DNA strand breaks is inversely correlated with the proportion of DNA in the tail intensity. A straightforward description known as the tail moment is generated by a computerized image analysis system by taking into account both the migration tail length and the percentage of DNA that migrates in the tail [15]. DNA damage

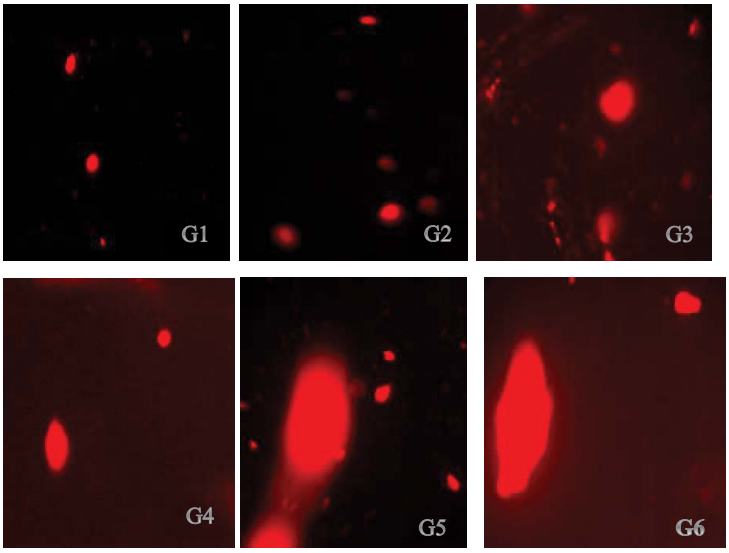

Figure 2: Shows photomicrographs of DNA damage (comet assay) in rats' liver cells after exposure to various ZnONP doses. (G1) Normal cell; (G2 and G3) little DNA damage; (G4) moderate DNA damage; (G5) extensive DNA damage; (G6) completely damaged DNA.

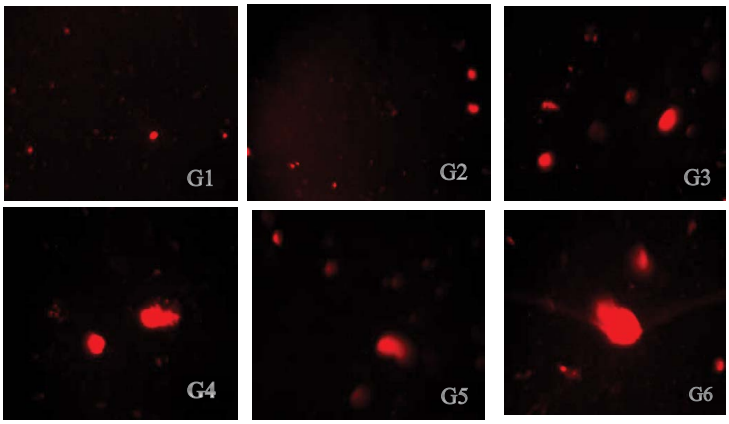

Figure 3: Shows representative photomicrographs of DNA damage (comet assay) caused by various ZnONP doses in rat kidney cells. Normal cell (G1), slight DNA damage (G2 and G3), moderate DNA damage (G4), severe DNA damage (G5), and completely damaged DNA (G6).

is observed in the DNA fragmentation caused by the increase in DNA tail length at all ZnONP doses for 70 days. The oxidative and nitrosative actions brought on by ZnONPs may be responsible for this genotoxicity.

CONCLUSION

Based on the results of the *in vivo* genotoxicity studies, it is possible to draw the conclusion that ten weeks of exposure to ZnO NPs with a particle size of 30 nm is sufficient to cause genotoxicity and cytotoxicity in rat liver and kidney cells. The findings of this study could raise more red flags regarding the potential damage to human health that could be connected to the widespread use of ZnO NPs.

REFERENCES

1. Zayed NA, Luaibi NM. Effect of ZnO NPs on Body and Organ Weights in Male Rat. Int j innov sci eng Technol. 2018; 5 (8): 12-25.
2. Minetto D, Ghirardini Av, Libralato G. Saltwater ecotoxicology of Ag, Au, CuO, TiO2, ZnO and C60 engineered nanoparticles: An overview. Environ Int 2016; 92(93):189-201.
3. Al-Suhaibani ES, El-Morshedi NA. Histopathological and ultrastructural effect of zinc oxide nanoparticles on male wistar rats submandibular glands. IOSR J Pharm Biol Sci. 2014; 9: 5-9.
4. Iavicoli I, LesoV, Beezhold DH, Shvedova AA. Nanotechnology in agriculture: opportunities, toxicological implications, and occupational risks. Toxicol Appl Pharmacol. 2017;15(329): 96–111
5. Yeh TK, Chen JK, lin CH, Yang MH, Yang CS, Chou FI, et al. Kinetics and tissue distribution of neutron-activated zinc oxide nanoparticles and zinc nitrate in mice: effects of size and particulate nature. Nanotechnology. 2012; 23: 85-102.
6. Jia L, Yiyuan K, Wei Z, Bin S, Limin W, Liangjiao C, et al. Ion-shedding zinc oxide nanoparticles induce microglial BV2 cell proliferation via the ERK and Akt signaling pathways. Toxicol Sci. 2017; 156:167–178.
7. Terradas M, Martın M, Tusell L, Genesca A. Genetic activities in micronuclei: Is the DNA entrapped in micronuclei lost for the cell. Mutat Res. 2010; 705(1):60-67.
8. Schmid W. The micronucleus test. Mutation Res. 1975; 31(1): 9–15.
9. McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BLD, MCo MP, Collins A. The single cell gel electrophoresis assay (comet assay): a European review. Mutation Res. 1993; 288: 47–63.
10. Srivastav AK, Kumar M, Ansari NG. A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in Wistar rats: toxicity study of zinc oxide nanoparticles. Hum Exp Toxicol. 2016; 35(12): 1286-1304.
11. Chupani L, Zuskova E, Niksirat H, Panacek A, Lunsmann V, Haange S, et al. Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (Cyprinus carpio L.). Sci Total Environ. 2017; 579: 1504-1511.
12. Kisin ER, Murray AR, Keane MJ. Single walled carbon nanotubes:

geno-and cytotoxic effects in lung fibroblast V79 cells. J Toxicol Environ Health A. 2007; 70(24): 2071-2079.

13. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988; 175: 184-191.

14. Hong JS, Park MK, Kim MS, Lim JH, Park GJ, Maeng EH, et al. Prenatal development toxicity study of zinc oxide nanoparticles in rats". Int J Nanomedicine. 2014; 9(2): 159-171.

15. Wang C, Lu J, Zhou L, Li J, Xu J, Li W, et al. Effects of long-term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice. PloS one 2016; 11(10): 0164434.