


Biochemical and histopathological alterations induced by subchronic exposure to zinc oxide nanoparticle in male rats and assessment of its genotoxicity

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Abstract

In the food and fertilizer industries, zinc oxide nanoparticles (ZnO-NPs) are frequently utilized. Our study conducted to assess the genotoxicity, biochemical alterations and histopathological parameters of ZnO NPs with a particle size of 30 ± 5 nm were orally administered to rats once daily at doses of 100, 200, 300, 400, and 600 mg/kg for ten week. The experiment involved the use of 30 Sprague–Dawley male rats exposed to various concentrations of ZnO-NPs. After the adaptation period, six groups were created out of the thirty rats (Five rats per group). Rats in Group 1 (G1), known as the control group, were fed a standard synthetic meal and had unlimited access to drinking water ad libitum, while those in the other five groups received oral gavage treatments with various doses of zinc oxide nanoparticles over a 10-week period. The results indicated that ZnO-NPs induces a lowering in body weight beginning in the sixth week while increasing serum AST, ALT, creatinine, and uric acid activity. However, the addition of different concentrations of ZnO NPs compared to the control caused insignificantly decreased on the plasma glucose level in all treated animals. Numerous chromosomal aberrations, including fragments, chromosome rings, chromatid breaks, end-to-end association, and centric fusion, were observed through cytogenetic investigation. When compared to the control group, hepatic vacuolation, large sinusoidal dilatation, degenerative alterations, and cellular congestion were observed in the liver of the male rats treated with 400 and 600 mg/kg of ZnO-NPs. According to the findings of in vivo genotoxicity experiments, rats' bone marrow cells, liver, and kidney can exhibit genotoxicity and cytotoxicity after exposed to ZnO NPs with particle sizes of 30 nm for ten weeks. The findings of this study could raise more concerns regarding the potential damage to human health associated with the widespread use of ZnO NPs.

Keywords Rats · Zinc oxide nanoparticle · Chromosomal aberrations · Histopathology

1 Introduction

Nanoparticles (NPs) are minuscule substances with at least one dimension in the (1–100 nm) range. Due to their small size and large surface area, nanoparticles play a key role in all areas of everyday life [1]. The Organization for Economic Cooperation and Development (OECD) expressed

concerns about the safety of manufactured nanomaterials (NPs) for public health and the environment as NP production and demand increased. To resolve these issues, the OECD established an operations and maintenance committee on manufactured nanomaterials. The committee decided to conduct toxicity testing on a variety of produced NPs, which include ZnO [2]. ZnO nanoparticles

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are widely used in cosmetics, including sunscreens, foot care products, and ointment pigments. Additionally, the most often used applications for zinc oxide nanoparticles are coatings, electronic devices, catalysts, food additives, cement, rubber, plastic, and biosensors [3].

ZnO nanoparticle exposure to the body increases with increased use. ZnO nanoparticle exposure mostly happens through aspiration, epidermal contact, and swallowing [4]. The circulatory system is one of the first areas of the body where ZnO nanoparticles can reach after exposure by any manner. The lung, liver, kidney, and bones are the primary targets of ZnO nanoparticles and are where the compatibility high concentration of ZnO nanoparticles is found [5]. According to Almansour et al. [6] when ZnO nanoparticles enter the body, they are of a nanometer scale that quickly allows them to penetrate cells, where they are internalized as either free Zn^{2+} ions or nanoparticles by the cells [7]. Hou, et al. [8] illustrates the toxicity mechanisms of ZnO NPs on living organisms, including actual injury from interacting directly, dissolving zinc ions, and a mechanism mediated by reactive oxygen species (ROS). Various studies conducted both in vivo and in vitro showed that ZnO NPs are hazardous as a relation of dose and size [9]. At a dosage of 2000 mg/kg in Wistar rats Srivastav et al. [10] showed reductions in red cell counts, liver lesions, and hepatocyte inflammation. According to Aragon and Younossi [11], ZnO NPs significantly altered the levels of the ALT and AST enzymes, and their high levels in the blood indicate the apoptosis of liver cells. The blood, colon, liver, and kidneys can exhibit to molecular and morphological changes as a result of dietary ZnO NPs [12]. These NPs may display unpredictably 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 large surface area combined with physicochemical properties like charged surfaces [13]. With the rapid growth of nanotechnology and increasing exposure to nanoparticles, ZnO NPs' genotoxic potential in an animal model has to be clearly represented, and their toxicity needs to be assessed. For this reason, the present investigation was performed to investigate the genotoxic activity, biochemical evaluation of the liver and kidney function parameters, hematology assays and histopathological examinations of ZnO NPs.

2 Results and discussion

2.1 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 and 600 mg/kg body weight). Also no behavioral changes were observed related to be orally administered to rats. This finding was agreed with the results of BenSlama et al. [14].

2.2 Rat body weight and the effect of ZnO-NPs

Table 1 displays the impact of the various ZnO-NP concentrations on the body weight of rats during the entire experimental period. According to the data, there was insignificant increase in body weight over the first two weeks when compared to the control. However, compared to the control group, there was insignificant decrease in the average rat body weight across the three to five week period. However, compared to the control group, the

Table 1 Effects of various nano-ZnOs concentrations on the body weights of male rats during a ten-week period

Weeks	Mean body weight (gram)					
	G1 control	G2 100 mg/kg	G3 200 mg/kg	G4 300 mg/kg	G5 400 mg/kg	G6 600 mg/kg
1	161.8±2.30 ^a	171.96±3.82 ^a	170.22±3.69 ^a	172.04±4.25 ^a	165.08±2.82 ^a	169.80±2.84 ^a
2	169.8±3.40 ^a	178.8±5.49 ^a	175.49±5.05 ^a	183.4±5.16 ^a	184.98±5.96 ^a	189.30±6.70 ^a
3	178.8±5.49 ^a	170.6±4.38 ^a	176±6.58 ^a	176.2±8.82 ^a	176±5.94 ^a	165.00±4.50 ^a
4	181±5.50 ^a	179.4±6.57 ^a	179.4±9.02 ^a	175.40±10.96 ^a	177.00±7.91 ^a	167.40±6.07 ^a
5	183.4±5.16 ^a	179±3.75 ^a	173.80±10.25 ^a	167.2±11.4 ^a	170.20±9.09 ^a	165.20±8.10 ^a
6	184.98±5.97 ^a	169.2±6.88 ^{ab}	179.80±11.04 ^{ab}	161.00±12.72 ^{ab}	170±10.26 ^{ab}	151.40±11.14 ^b
7	189.3±6.70 ^a	158.2±7.35 ^{ab}	173.80±12.42 ^{ab}	162.00±12.21 ^{ab}	167.80±11.19 ^{ab}	147±9.91 ^b
8	207.6±5.46 ^a	158.76±9.61 ^b	168.60±13.11 ^b	157.60±12.98 ^b	163±11.84 ^b	151.4±9.41 ^b
9	225.2±5.58 ^a	161.8±8.52 ^b	163.60±11.21 ^b	163.20±11.83 ^b	163±10.26 ^b	166.4±6.06 ^b
10	222.4±7.21 ^a	160.2±7.43 ^b	160.80±11.64 ^b	159.80±10.95 ^b	160±10.55 ^b	174.8±9.51 ^b

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

average body weight of the rats started to significantly decline from the sixth to the tenth week ($P < 0.05$). The findings of the current investigation regarding the decline in body weights were consistent with those of Hong et al. [15], who found 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 a decrease in food consumption. Water intake and diet consumption can be linked to the loss of weight. Wang et al. [16] were reported that high doses of ZnO NPs in diet could have toxicological effects; they also showed that 50 and 500 mg/kg nano-ZnOs illustrated increase in body weight while at 5000 mg/kg showed decreases in body weight. The decrease in body weight at 5000 mg/kg ZnO NPs might partially contribute to the increases in the relative organ weights of the pancreas, brain, and lung. ZnO NPs' has very toxic properties due to its tiny size, concentration, high specific surface area and bio distribution [17].

2.3 The influence of various ZnO-NPs concentrations on rats' relative organ weights

Table 2 shows the effects of ZnO-NPs on the relative organ weights. The relative weights of the heart, lungs, spleen, and testes did not differ significantly from control groups, according to our results ($P > 0.05$). However, in compared to the control group, the relative liver and kidney weight in all treatment groups significantly decreased in a dose-dependent manner ($P > 0.05$). It is widely known that in general toxicity investigations, body weight and organ weight factors are sensitive indicators of potentially harmful substances [18]. The weights of the thyroid gland and kidneys increased significantly at two doses 30 and 60 mg/kg for the durations of 7, 14, and 28 days, whereas the weights of the liver and testes decreased significantly at the same doses 30 and 60 mg/kg for the same durations 7, 14, and 28 days [1]. The high dose of ZnO NPs causes decreased organ weight, and it can build up in male wistar rats organs and cause changes to cellular processes that change the body's metabolism for many animals [19].

2.4 Effects of ZnO-NPs on biochemical parameters of male rats

Tables 3 show the effects of various doses of ZnO-NPs (100, 200, 300, 400 and 600 mg/kg) administration on the plasma glucose level, kidney function, and liver function enzymes of treated rats. Results revealed that none of the groups' levels of creatinine, urea, or uric acid had significantly increased over those of the control group. However, during the first five weeks following treatment with various concentrations of ZnO NPs, serum ALT and AST were significantly elevated compared to control ($P < 0.05$). However, the addition of different concentrations of ZnO NPs (100, 200, 300, 400 and 600 mg/kg) compared to the control caused insignificantly decreased on the plasma glucose level in all treated rats. After 10 weeks, the treated rats groups with ZnO-NPs indicated that, the levels of ALT, AST, uric acid, and urea had significantly increased compared with the control group ($P < 0.05$). These findings showed that ZnO-NPs caused liver cell destruction, which was followed by the release of many cytoplasmic enzymes into the blood. Increases in AST are used to assess hepatic illnesses and are indicative of inflammatory hepatocellular disorders and acute liver injury [20]. Renal injury caused by the administration of ZnO-NPs may explain the considerable rise in creatinine and urea in the serum of rats consuming ZnO-NPs compared to control rats [21]. Glucose and albumin concentrations of rats were considerably decreased after treatment with ZnO-NPs at a concentration of 40 ppm [22]. According to Jansen et al. [23], the potential of zinc to increase glucose consumption and its metabolism via stimulation of insulin production may be the explanation for the lower levels of plasma glucose concentration caused by oral administration of manganese oxide nanoparticles.

2.5 Chromosomal aberration assay (CAs)

The chromosomal aberration (CA) on bone marrow cells caused by the administration of nano-ZnOs by gavage to

Table 2 Relative organ weights (gram) in male rats treated with ZnO-NPs at different concentrations for 10 weeks

Groups	Relative weight of organs (grams)					
	Liver	Kidney	Heart	Lung	Spleen	Testis
G1 Control	3.75 ± 0.25 ^a	1.01 ± 0.04 ^a	0.46 ± 0.11 ^a	1.02 ± 0.14 ^a	0.52 ± 0.03 ^a	3.49 ± 0.19 ^a
G2 100 mg/kg	3.60 ± 0.10 ^{ab}	0.95 ± 0.12 ^{ab}	0.40 ± 0.01 ^a	1.09 ± 0.4 ^a	0.76 ± 0.09 ^a	3.30 ± 0.10 ^a
G3 200 mg/kg	3.17 ± 0.07 ^{bc}	0.89 ± 0.61 ^{ab}	0.42 ± 0.03 ^a	0.88 ± 0.07 ^a	0.63 ± 0.10 ^a	3.13 ± 0.47 ^a
G4 300 mg/kg	3.06 ± 0.10 ^c	0.80 ± 0.06 ^{ab}	0.39 ± 0.03 ^a	0.75 ± 0.06 ^a	0.48 ± 0.14 ^a	3.00 ± 0.16 ^a
G5 400 mg/kg	3.60 ± 0.1 ^{ab}	0.81 ± 0.09 ^{ab}	0.39 ± 0.04 ^a	0.76 ± 0.03 ^a	0.52 ± 0.08 ^a	3.30 ± 0.30 ^a
G6 600 mg/kg	3.40 ± 0.03 ^{abc}	0.74 ± 0.04 ^c	0.35 ± 0.03 ^a	0.65 ± 0.04 ^a	0.57 ± 0.12 ^a	3.05 ± 0.05 ^a

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

Table 3 Effects of different concentrations of ZnONPs on biochemical parameters of male rats with different periods of time (5 and 10) weeks

Time	Group	Biochemical parameters					
		ALT (IU/l)	AST (IU/l)	Creatinine (mmol/l)	Urea	Uric acid (mg/l)	Glucose g/l
5 Weeks	G1 Control	25.5 ± 5.00 ^a	115 ± 15.00 ^{ab}	0.55 ± 0.25 ^a	44.5 ± 4.00 ^a	1.5 ± 0.20 ^a	66.0 ± 6.00 ^a
	G2 100 mg/kg	32.50 ± 2.50 ^{ab}	123 ± 3.00 ^b	0.75 ± 0.20 ^a	45.0 ± 3.00 ^a	2.3 ± 0.40 ^a	66.0 ± 3.00 ^a
	G3 200 mg/kg	37 ± 0.7 ^{ab}	122 ± 3.00 ^b	0.7 ± 0.20 ^a	51.2 ± 6.00 ^a	2.6 ± 0.60 ^a	60.0 ± 5.00 ^a
	G4 300 mg/kg	39.25 ± 4.50 ^{ab}	122.5 ± 5.50 ^b	0.73 ± 0.20 ^a	48.75 ± 8.00 ^a	2.10 ± 0.2 ^a	52.0 ± 2.00 ^a
	G5 400 mg/kg	31.5 ± 1.50 ^{ab}	123.4 ± 4.00 ^b	0.6 ± 0.3 ^a	52.2 ± 3.00 ^a	2.0 ± 0.70 ^a	58.0 ± 5.00 ^a
	G6 600 mg/kg	48.5 ± 5.00 ^b	126 ± 6.0 ^b	0.7 ± 0.20 ^a	55.8 ± 6.00 ^a	2.0 ± 0.50 ^a	61.0 ± 3.00 ^a
10 Weeks	G1 Control	29.26 ± 3.57 ^a	124.5 ± 18.50 ^{ab}	0.7 ± 0.05 ^a	41.66 ± 1.8 ^a	1.60 ± 0.0 ^a	66.15 ± 4.1 ^a
	G2 100 mg/kg	36.03 ± 2.74 ^{ab}	141.4 ± 21.30 ^b	0.7 ± 0.05 ^a	53.26 ± 1.29 ^c	2.75 ± 0.0 ^{ab}	61.45 ± 3.05 ^b
	G3 200 mg/kg	44.50 ± 2.02 ^b	147.33 ± 13.5 ^b	0.7 ± 0.03 ^a	47.56 ± 0.9 ^{ab}	2.33 ± 0.1 ^{ab}	60.30 ± 7.10 ^b
	G4 300 mg/kg	52.7 ± 3.46 ^c	156 ± 4.00 ^a	0.7 ± 0.03 ^a	48.73 ± 0.3 ^{ab}	2.33 ± 0.1 ^b	52 ± 1.30 ^{ab}
	G5 400 mg/kg	43.63 ± 1.47 ^{bc}	149.27 ± 4.80 ^a	0.7 ± 0.05 ^a	52.53 ± 1.92 ^c	4.00 ± 1.0 ^b	57.45 ± 4.0 ^{ab}
	G6 600 mg/kg	52.43 ± 3.60 ^c	151.27 ± 4.80 ^a	0.73 ± 0.05 ^a	52.53 ± 1.2 ^c	4.00 ± 1.0 ^b	58.16 ± 2.6 ^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

male rats at various doses is shown in Table 4 and Fig. 1. Results demonstrated that the average percentage of aberrant metaphase recorded in all treatment groups were depended on the doses of ZnO NPs. The results showed the doses of ZnO NPs (100, 200, 300, 400, and 600 mg/kg) induced the abnormalities percentage (0.5, 2, 2.33, 2.5, 3.5, and 5.16%) respectively.

Data in Table 4 indicate that the rats in group 6 (G6), which provided 600 mg/kg of ZnO NPs, had the most common form of abnormalities. Chromosomal rings, centric fusion, end-to-end association, chromatid breaks, and chromosome fragmentation were among these abnormalities. Additionally, the most prevalent abnormality observed in the bone marrow cells of the rats in these groups was the ring chromosome and end to end association. When compared to the control, it was found that the frequency of structural chromosomal abnormalities (SCAs) significantly increased with increasing doses. The potential

mutagenic potential of ZnONPs, medications, and chemical contaminants was examined using the chromosomal aberration assay [24]. During various phases of mitotic division, the aberrations change the chromosomes' direction. As a result, daughter cells either lose or add chromosomes [25]. Chromosome stickiness may contribute to the development of chromosome bridges during anaphase and telophase [26]. The identification of structural CA caused by mutagens to the bone marrow cells of animals, frequently rodents, is done using the mammalian in vivo Chromosomal aberrations (CA) assay, which has become a proper test for assessing the possible mutagenic effects [27]. Srivastav et al. [28] showed the genomic template stability within the high-dose group (< 90%) was less than the controls (100%) based on the RAPD assay. The results suggested that ZnO NPs are mildly genotoxic in a dose-related manner and this toxicity were induced by generation of ROS [28].

Table 4 Effects of different concentrations of nano-ZnOs on chromosomal aberrations

Treatment	No. of metaphase examined	Aberrant metaphases %	Average means of structural aberrations				
			Frag	Break	E.E	C.F	Ring
G1	600	0.50	0.33 ± 0.30 ^a	0.00 ± 0.0 ^a	0.67 ± 0.30 ^a	0.00 ± 0.0 ^a	0.00 ± 0.0 ^a
G2	600	2.00	1.00 ± 0.00 ^{ab}	0.00 ± 0.0 ^a	1.33 ± 0.33 ^{ab}	0.00 ± 0.0 ^a	1.00 ± 0.00 ^{ab}
G3	600	2.33	1.33 ± 0.66 ^{ab}	0.33 ± 0.30 ^a	1.33 ± 0.33 ^{ab}	0.00 ± 0.0 ^a	1.33 ± 0.33 ^b
G4	600	2.50	1.33 ± 0.33 ^{ab}	0.67 ± 0.30 ^a	1.67 ± 0.30 ^{ab}	0.00 ± 0.0 ^a	1.33 ± 0.33 ^{ab}
G5	600	3.50	2.0 ± 0.33 ^b	0.67 ± 0.30 ^a	2.00 ± 1.00 ^{ab}	0.33 ± 0.30 ^{ab}	1.67 ± 0.30 ^{bc}
G6	600	5.16	1.66 ± 0.33 ^{ab}	1.66 ± 0.30 ^b	3.00 ± 0.57 ^b	1.00 ± 0.57 ^b	2.66 ± 0.67 ^c

Data represent the means ± SE of 3 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

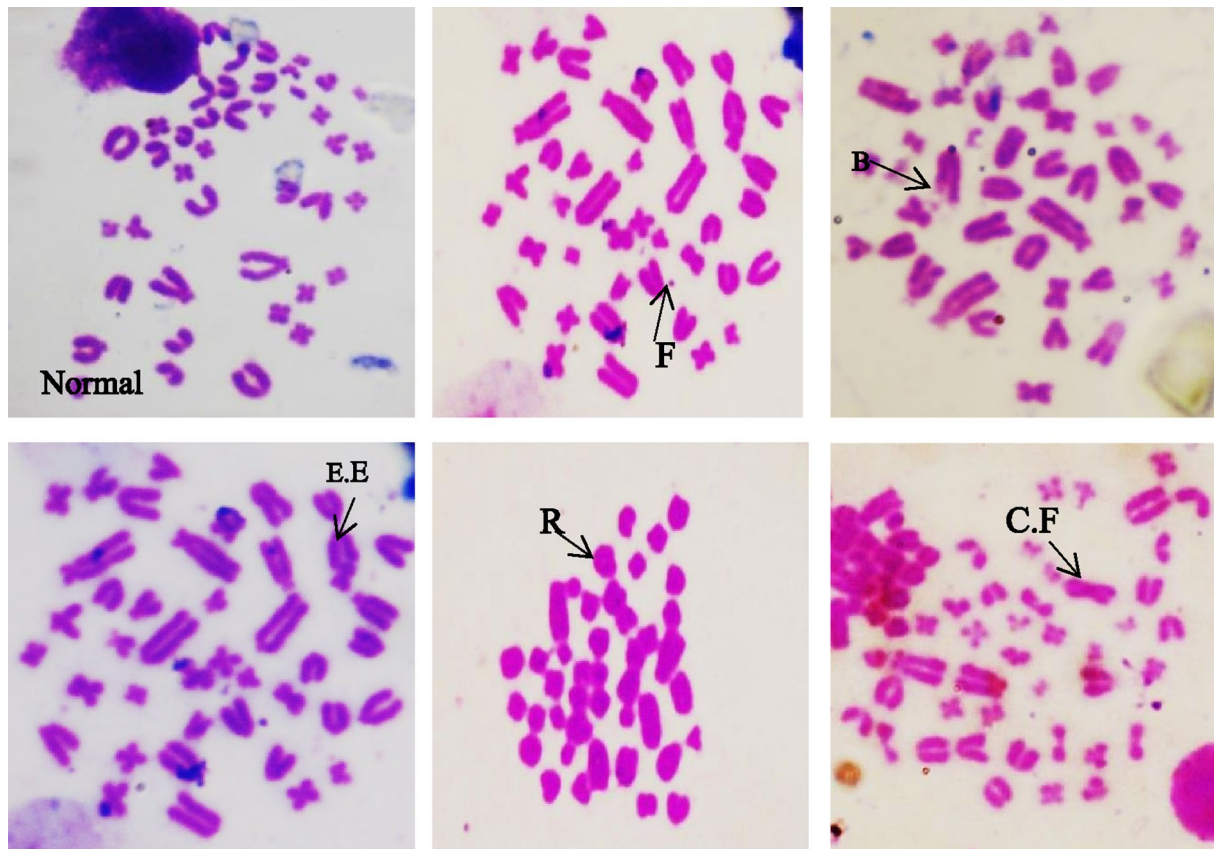


Fig. 1 Photomicrographs showing several structural chromosomal abnormalities, including fragment (F), chromatid break (B), end-to-end association (E.E), ring chromosome (R) and centric fusion break

(C.F.) in male rats' bone marrow cells after administering various concentrations of ZnO-NPT for 10 weeks

2.6 Histopathological examination of rat's liver and kidney

The control group's liver sections underwent microscopic examination, which revealed that the normal, classic hepatic lobule has a central vein in the middle. Hepatic sinusoids are housed within the rows of hepatocytes (Fig. 2a). The central vein appeared slightly dilated with intact structure and had little blood cellular components, while the liver of rats given ZnO-NPs at a dose of 100 mg/kg revealed normal hepatocytes (Fig. 2b). In the group (3), the liver showed dilated and congested hepatic sinusoids. The central vein showed mild dilatation (Fig. 2c). In group (4), the liver showed mild affection of the hepatocytes as slight vacuolation and cloudy swelling while many hepatic sinusoids became dilated and congested. The central vein still slightly dilated (Fig. 2d). The liver of group5 showed many hepatocytes suffered from moderate degenerative changes as vacuolation and hydropic degeneration with intact the nuclei (Fig. 2e). In group 6, the liver showed markedly degenerated hepatocytes and most of them suffered from vacuolation and cytoplasmic granulation

(Fig. 2f). The liver is a vital organ for detoxification and active metabolism and is incredibly sensitive to toxins. Membrane damage degeneration is a vacuolization which occurs when the amount of intracellular water rises. It is typically reversible and nonlethal, but in more severe situations, it can result in cellular deterioration (necrosis) [29]. According to Wang et al. [16], exposure to 500 mg/kg of nano-ZnOs harmed hepatic function, changed small intestine zinc metabolism, and created a large zinc buildup in the liver, pancreas, kidney and bones.

Numerous nephrons, each made up of a renal corpuscle and renal tubules, made up the normal rat kidney. The Bowmann capsule encased a tuft of blood capillaries that formed the renal corpuscle (Fig. 3a). When examined under a microscope, the kidney of rats given 100 mg/kg of ZnO-NPs showed normal renal corpuscle whereas the renal tubules developed a small dilation (Fig. 3b). The kidney of rats in the group3 showed marked limitation and narrowing of the filtration space together with mild affection of the tubular cells (Fig. 3c). The kidney rats of the group 4 showed many renal corpuscles became congested with mild dilatation of the some renal tubules (Fig. 3d). The

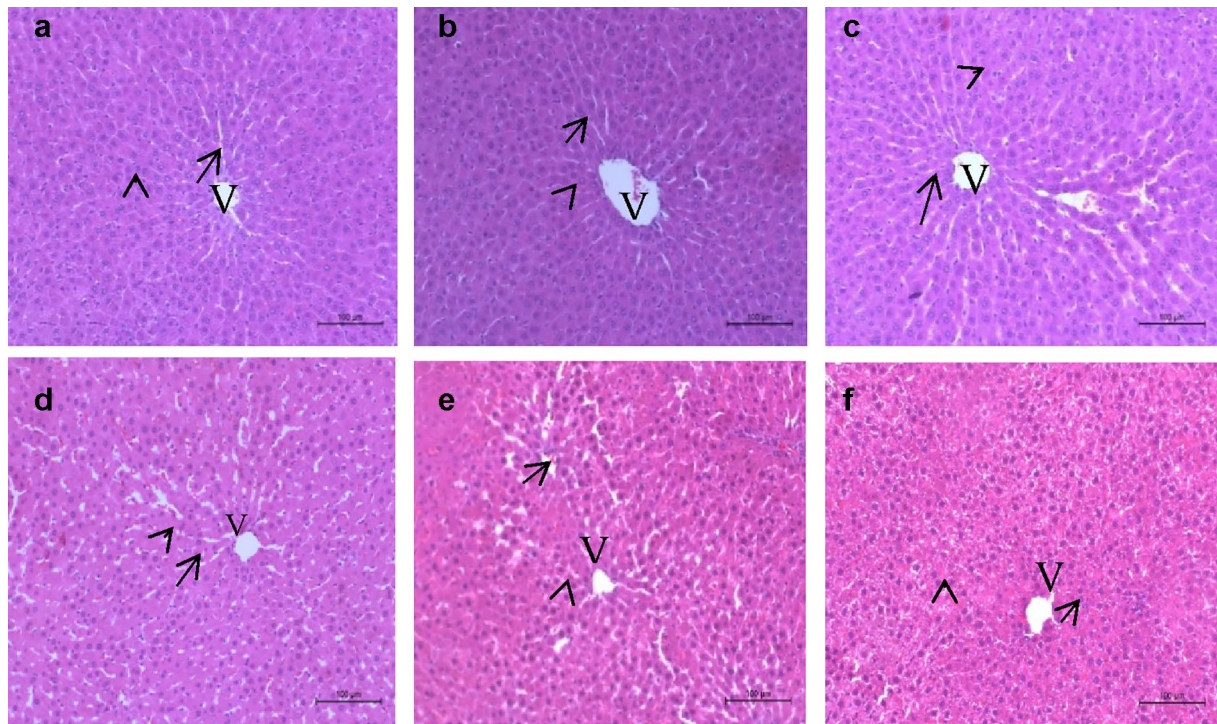


Fig. 2 a–f Photomicrograph of liver sections from rats treated with ZnO NPs (100, 200, 300, 400, and 600 mg/kg) and the control group (A) (magnification $\times 400$). V central vein

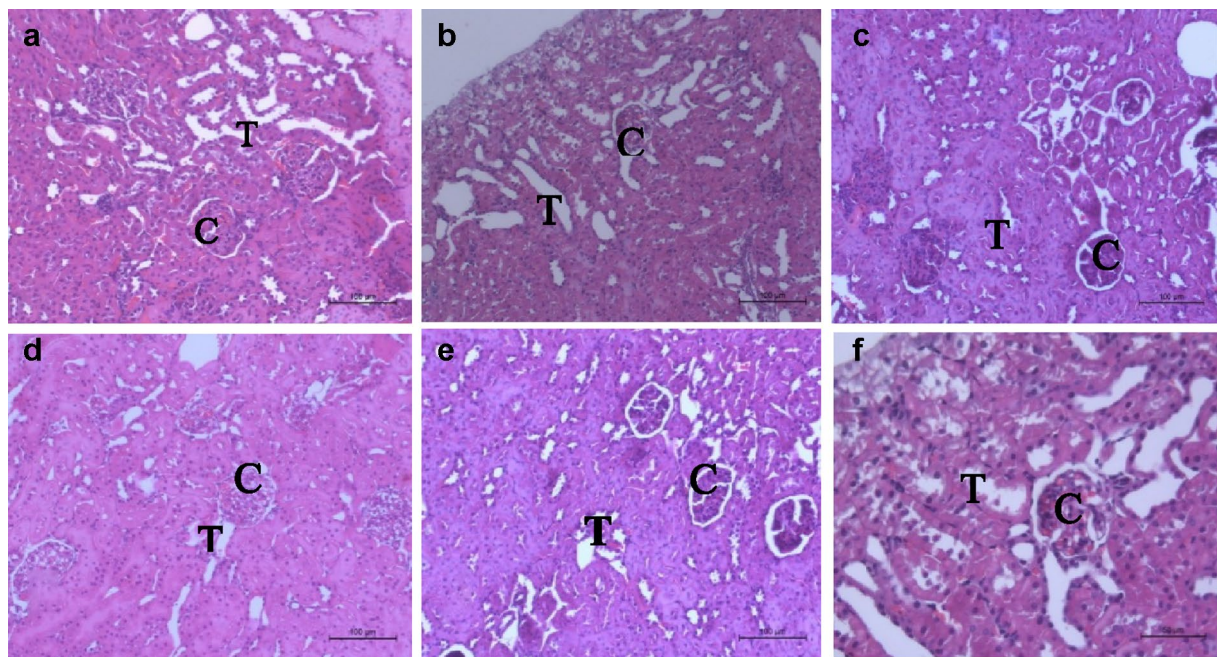


Fig. 3 a–f Photomicrograph of kidney sections from rats treated with ZnO NPs (100, 200, 300, 400, 600 mg/kg) and the control group (A), ($\times 400$ magnification). C and T renal corpuscle and renal tubules, respectively

kidney of rats in the group 5 showed many renal corpuscles became smaller in size with dilated filtration space (Fig. 3e). In the kidney of the group 6, the renal corpuscle

became congested and showed marked degenerative changes in the glomerular tuft and Bowman capsule (Fig. 3f). Because kidneys act like an orange in the body's

toxic compounds removal process, M-NPs ingested in the circulatory can be eliminated via renal tubules [30]. After 21 days exposure to different doses of nano ZnO (100, 250, and 500 mg kg⁻¹) using oral gavage were caused kidney damage in rats, including necrosis in the renal corpuscle and renal tubules, renal tubule dilatation associated with sloughing and degeneration of its lining epithelium, and severe inflammatory cell infiltration [31]. According to several studies, ZnO NPs can harm the tissues of the liver, kidneys, and lungs while also causing collagen to break down [32, 33]. Al Zerjawe and AlBairuty [34] showed a decrease in the average body weights of animals after 14 days of injection with ZnO-NPs when compared with control group ($p \leq 0.05$), while, the period of seven days did not caused any alteration in the mice average body weights.

3 Material and methods

3.1 Chemicals

We obtained zinc oxide nanoparticles (ZnO-NPs) from NanoTech Company, Egypt. According to the information provided by manufacturer the particles size of ZnO nanoparticles is 30 ± 5 nm. Chemicals used for the quantitative determination of various biochemical and hematological parameters were purchased from Bio Diagnostic company and Human company (Egypt).

3.2 Preparation of ZnONPs suspension

The ZnO NPs particles (30 ± 5 nm) were dispersed in distilled water (10 mg/mL) and the suspension was sonicated at 230 V for 20 min 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 600 mg/kg).

3.3 Characterization of zinc oxide nanoparticles

After vigorous sonication, the solution distribution of the nanoparticles was dropped onto a copper grid that had been coated with carbon to examine the diameter and shapes of the particles. The grid was then observed using a JEOL JEM 1010 Transmission Electron Microscope after being air-dried at room temperature. Using a transmission electron microscope (TEM) with a 200 kV accelerating voltage, the morphological structure of zinc oxide nanoparticles (ZnO-NPs) was analyzed. The crystal structures measured 30 ± 5 nm and closely resemble a sphere (Fig. 4).

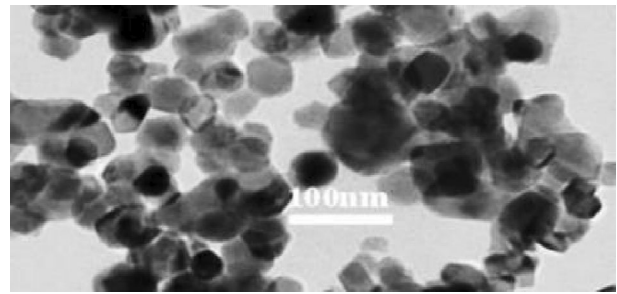


Fig. 4 Electron micrograph of spherical ZnO-NPs nanoparticles with a size of less than 30 nm

3.4 Animals and their housing

Thirty male Sprague–Dawley rats, 8–10 weeks old and weighing 140–160 g, were purchased from RapiTco Farm Company in Giza, Egypt. Five animals per cage were kept in standard plastic cages under controlled environmental conditions at a temperature of 25 ± 2 °C with 12-h cycles of light and darkness.

3.5 Experimental design

Six groups of thirty rats were created after the adaptation phase (Five rats per group). Rats in Group 1 (G1), which was designated as the control group, consumed a regular synthetic meal and had unlimited access to water, while those in the other five groups received oral gavage administration of zinc oxide nanoparticles at varying concentrations during a 10-week period. The rats in group 2 (G2) were received 100 mg/kg ZnO NPs suspension orally once daily, Group 3 (G3) 200 mg/kg ZnO-NPs suspension orally once daily, Group 4 (G4) 300 mg/kg ZnONPs suspension orally once daily, Group 5 (G5) 400 mg/kg ZnONPs suspension orally once daily, and Group 6 (G6) 600 mg/kg ZnONPs suspension orally once daily. All experiments were done following general international guidelines on the use of living laboratory animals in scientific research (Permission number Fu- IACUC-6200, 2011).

3.6 Biochemical determinations

All of the rats in each group had their blood drawn from the retinal vein into tubes, and the coagulate blood was centrifuged at 1500 rpm for 10 min to extract the serum, which was then refrigerated at -20 °C for biochemical analysis. Using commercial kits (Diamond, Egypt) the serum creatinine (CRE) and blood urea nitrogen (BUN) were used to assess renal function [35]. Uric acid was determined according to Domagk and Schlicke [36]. Using commercial kits and a colorimetric approach in accordance with Reitman and Frankl's principles [37], the aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) levels in serum were measured to determine the liver function.

3.7 Bone marrow extraction and chromosomal aberrations assay

Three rats from each of the six groups were intraperitoneally injected with 0.025% colchicine (1 mL /g bw.) for 1.5 h before killing at the end of the experiment. Injected rats were killed via cervical dislocations, and the bone marrow of the cells was extracted [38]. According to Nichols et al. [39], the cytogenetic examination of the chromosomes in bone marrow cells was performed.

3.8 Histopathological analysis

After being removed from the body, the liver and kidneys were promptly fixed in 10% formol saline for 24 h. The slices were cut into 5 mm thick pieces and stained with hematoxylin and eosin (H&E) for histopathological analysis [40].

3.9 Statistical analysis

The results of body weight, CA frequencies and biochemical analysis were statistically analyzed using SPSS-PC software [41].

4 Conclusions

According to 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 potential risks that these particles could provide to various organs and their pathogenesis require further investigation.

Author contributions GMH and AGR conceived and designed the experiments. GMH, AGR, EAE and AAMY, performed the experiments. AGR and AAMY analyzed the data. GGH and AGR drafted and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials All datasets are available upon reasonable request.

Declarations

Conflicts of interest The authors declare no conflict of interest.

Ethical approval Following approval from the Institutional Animal Ethical Committee for Fayoum University, all experiments were done following general international guidelines on the use of living laboratory animals in scientific research (Permission number Fu- IACUC-6200, 2011). The work has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

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