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Evaluation of Acute Oral Toxicity of Zinc Oxide Nanoparticles in Rats

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) are widely used in the food and fertilizer industries. During our previous study, the bean fertilized by two doses (20 and 40 ppm) of ZnO-NPs with a particle size of 20 nm was investigated on rats. Therefore, this experiment was designed to evaluate effect of ZnO-NPs that were administrated orally to rats once a day at the doses 20 and 40 ppm. Hematological and biochemical parameters were quantified in addition to investigation of the deleterious effect on liver and kidney histopathological. *In-vitro* cytotoxicity of ZnO-NPs on the Vero cell line was also studied. Eighteen male Wistar rats were divided into three groups. Normal control (G1) was administered deionized water (1ml/rat). Groups G2 and G3 received 1ml/rat suspensions of 20 and 40 ppm ZnO-NPs, respectively. Hematology showed that the mean corpuscular hemoglobin concentration (MCHC) was lower in G2 and G3. White blood cell count was decreased in G2. G3 had an elevated hematocrit. In G2 and G3, plasma glucose decreased while uric acid and alkaline phosphatase activity increased. Plasma creatinine, aspartate aminotransferase, alanine aminotransferase, and creatine kinase were all elevated in G3, while albumin levels were decreased. Treatment with ZnO-NPs for 72 h resulted in no toxicity in Vero cells when compared to normal ZnO. Biochemical data were confirmed by histopathology. In conclusion, both doses of ZnO-NPs had minimal effects on the hematological parameters. While 40 ppm ZnO-NPs should be applied carefully due to their negative effects on liver and kidney functions.

Keywords: Zinc Oxide Nanoparticles; Hematology**;** Liver Functions; Kidney Functions; Cell Line; Histopathology.

1 Introduction

Nanotechnology is an important innovative field in the industry concerning the physicochemical properties of matter by reducing its size to 1-100 nm [1]. Nanoparticles (NPs) have a wide range of applications including the food industry, drug delivery, diagnosis, cosmetics, and many sunscreens [2]. ZnO-NPs are the most commonly used NPs. They are widely used in sunscreen products because they absorb ultraviolet radiation [3, 4]. Moreover, ZnO-NPs are used as a photoconductive material in electronics as well as an antimicrobial and anti-yeast in the food and packaging industries [5-7]. They also have antifungal and anti-cancer properties [8-10]. Moreover, they are used as nano-fertilizer in agriculture. Furthermore, it is commonly found in our foods or can be added as a dietary supplement. Xie *et al.* [11] demonstrated that ZnO-NPs can improve cognitive and behavioral impairment in depressive-like behaviors in mice, possibly by regulating neuronal synaptic plasticity, and neuronal ionic balance. Thus, ZnO-NPs may be useful in treating neurotransmitter system disorders. Also, ZnO-NPs hold great promise as novel anti-tumor agents [12].

As a result of the widespread use of ZnO-NPs in various applications, concern about their toxicityhave been raised, whereas nanoparticles can easily enter the human body via various routes such as inhalation, ingestion, injection, and dermis, causing biological effects in different organs [13]. However, because zinc is an important trace element in the human body, ZnO-NPs appear to be non-toxic in low doses. However, it

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has been reported that high zinc concentrations may be responsible for some toxic effects [14, 15]. In agreement with the previous authors, Dhawan *et al.* [16] demonstrated that ZnO-NPs at high doses of 1-5 g / kg can induce apoptosis and cause extensive oxidative stress in murine hepatocytes. However, the toxicity of ZnO-NPs varies depending on the route of administration dose and duration.

This study is a continuation of our previous work [17], which investigated the effects on rats fed beans produced by plants treated with ZnO-NPs as nano fertilizer at concentrations of 20 and 40 ppm. Herein, it will be investigate the direct impact of nano fertilizer materials that farmers may be exposed to when using those doses. Therefore, the current study was carried out to investigate the acute oral toxicity of ZnO-NPs at doses 20 and 40 ppm. A biochemical evaluation of the liver, kidney, and heart function parameters was performed to achieve this goal. Hematology assays, cell line toxicity, and histopathological examinations were also performed.

2 Material and methods

2.1 Chemicals

All chemicals used were of high analytical grade and were manufactured by Sigma Company. All kits used for the quantitative determination of various biochemical parameters were purchased from Bio Diagnostic (Egypt).

2.2 Preparation of Zinc oxide nanoparticles (ZnO-NPs)

Zinc acetate (0.021mol) was dissolved in 1L ethanol containing 1.44 g NaOH, and then refluxed at 70°C for 2 h. A clear and transparent solution was obtained, where zinc acetate was converted to zinc oxide which was dispersed in an alcohol medium. Distilled water was added to the previous solution, which was then centrifuged at 5000 rpm for 10minutes to obtain ZnO-NPs. The ZnO-NPs were then dried in an oven at 60 °C for 24 h before being burned at 500°C for 2h to obtain ZnO-NPs [18].

2.3 Preparation of a suspension solution of ZnO-NPs

Two suspension solutions of ZnO-NPs with concentrations of 20 and 40 ppm were prepared. The ZnO-NPs were dispersed in fresh deionized water with a homogenizer at a rate of 20000 rpm for 10 min to achieve a dispersion which is as complete as possible in order to produce a stable suspension. ZnO-NPs suspensions were vortexed for 1 min before being administered.

2.4 Characterization of zinc oxide nanoparticles (ZnO-NPs)

The morphological structure was analyzed using a transmission electron microscope (TEM, JEM-1230, Japan) with a resolution of 0.2 nm magnification of 600×10^3, and operated at 120 kV, and a scanning electron microscopy (SEM; JSM 6360LV, JEOL/Noran) with an accelerating voltage of 10–15 kV. The crystal structure was determined using a Philips X-ray diffractometer (XRD, PW 1930 generator, PW 1820 goniometer) equipped with Cu Kα radiation (45 kV, 40 mA, with $\lambda = 0.15418$ nm). The scans for the analysis were performed in a 2θ range of 10 to 70º with a step size of 0.02 and a step time of 1s.

2.5 Experimental design and procedure

Eighteen albino males Wistar rats weighing 150-190 g with an average weight of 160±10 were purchased from the Animal House of the National Research Centre (NRC), Cairo, Egypt. They were kept individually in stainless steel wire-bottomed cages at room temperature (25±2 ^{0}C) with 12 h dark-light cycles. The animals were fed a standard balanced pellet diet and had free access to deionized water after one week of acclimatization period. They were divided into three groups (six rats per group). Group 1 (G1) was termed as control group and in which rats received 1ml deionized water orally, Group 2 (G2) in which rats received 1 ml of 20 ppm ZnONPs suspension orally once and Group 3 (G3) in which rats received 1 ml of 40 ppm ZnO-NPs suspension orally once. All groups were left for 24 h, then fasted overnight, anesthetized with light ether, and blood samples were obtained from the retro-orbital vein and divided into two parts one on EDTA-K_3 (Tri-potassium ethylene diamine tetra acetic acid) and the other in heparinized tubes. The first part was used for the hematological analysis, while the other was centrifuged at 4000 rpm for 10 min to separate the plasma. Plasma was stored at -20°C for biochemical analysis. Tissue samples from the liver and kidney of all experimental groupswere collected and immediately fixed in formalin for histopathological examinations.

2.6 Hematological examination

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 Biochemical determinations

Liver function was assessed by evaluating plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercial kits (Biodiagnostic, Egypt) by a colorimetric method according to the principle of Reitman and Frankl [19]. Also, plasma alkaline phosphatase activity was measured using the method of Belfield and Goldberg [20]. The synthetic function of the liver was determined by assaying plasma albumin concentration according to Doumas et al. [21].

Meanwhile, renal function was evaluated by determining plasma creatinine and urea using official kits according to Fawcett and Scott [22] and Houot [23] methods, respectively. Uric acid was evaluated according to Domagk and Schlicke [24]. Also, blood glucose was colorimetrically estimated using the procedure of Trinder [25]. Serum zinc was determined using a colorimetric method according to the method of Johnsen & Eliasson [26]. Also, plasma creatine kinase (CK.MB) was assessed by Urdal and Landaas [27].

2.8 Cell lines and cell culture conditions

To investigate the cytotoxicity effect of ZnO-NPs, a Vero cell line (kidney of green monkey) was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with100mg/mL of streptomycin, 100 units/ml of penicillin, and 10 % of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO_2 atmosphere at 37 °C.

The cell viability was assessed using the sulforhodamine B (SRB) assay. Aliquots of 100 µl cell suspension ($5x10^3$ cells) were placed in 96- well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 µL media containing drugs at various concentrations ranging from (0.01, 0.1, 1, 10,100 µm). After 72 h of drug exposure, cells were fixed by replacing media with 150 µl of 10 % trichloroacetic acid (TCA) and incubating for 1 h at 4 °C. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µl SRB solution (0.4 % w/v) were added and incubated in a dark place at room temperature for10 min. Plates were washed 3 times with1% acetic acid and allowed to air-dry overnight. Then, 150 µl of tris(hydroxymethyl)aminomethane (TRIS,10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®-FLUO star Omega microplate reader (Ortenberg, Germany) [28].

2.9 Histopathological examination

The liver and kidneys were dissected out and immediately fixed in 10% formol saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax (melting point 55 - 60°C). For histopathological examination, 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination [29].

2.10 Statistical analysis

Data were presented as a mean ± standard error of the mean (SE). One-way ANOVA test was used to compare the statistical difference between various groups. The results were statistically analyzed using SPSS-PC software. Less than 0.05 (P<0.05) probability values were considered statistically significant.

3 Results

3.1 Characterizations of the Synthesized ZnO-NPs

The TEM image illustrated that the prepared ZnO-NPs had a particle size of less than 100 nm (Fig. 1a). Also, the SEM image (Fig. 1b) exposed the aggregation of these small size particles and construct an enhanced close firmness texture. Also, the XRD Partum showed the main peaks at 2θ =32°, 34.4°, 36.4°, 47.7°, and 56.7° corresponding to (1 0 0), (0 0 2), (1 0 1), (1 0 2), and (1 1 0) planes, respectively (Fig. 1c), which proved the preparation of ZnO in the nanostructure [18]. The crystal size of ZnO-NPs was 8 nm, where it calculated from the full width at half maximum (FWHM (β)) of the diffraction peaks using Debye - Scherer's equation [30].

$$d = \frac{K\lambda}{\beta \cos(\theta)}$$

Where (d)is the average crystalline dimension perpendicular to the reflecting phases, (k) is Scherer's constant (0.92), (λ) is the X-ray wavelength = 1.5403,(β) is FWHM intensity of a Bragg reflection

excluding instrumental broadening, and (θ) the Bragg's angle.

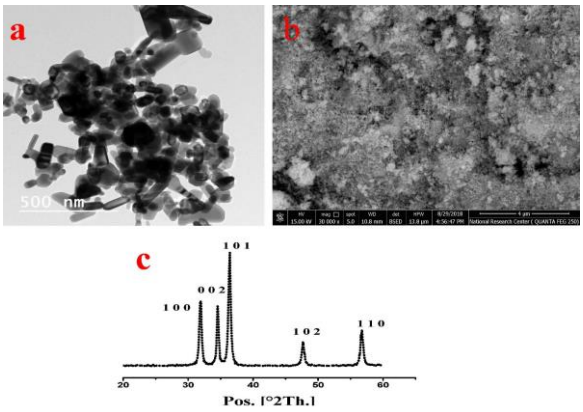

Fig. 1 The characterization of ZnO-NPs, TEM (a), SEM (b), and XRD pattern (c).

3.2 Animal studies

When 20 ppm ZnO-NPs or 40 ppm ZnO-NPs were orally administered to rats, neither toxic signs nor mortality were observed.

3.2.1 Hematological Findings

The MCHC was significantly decreased (P<0.05) in the treated groups (G2 & G3) as compared to normal control (G1). In addition, the total WBC count, lymphocyte, monocyte and lymphocyte % was significantly lower in 20 ppm treated rats (G2) than in the control. While HCT and monocyte were significantly elevated in rats of (G3). RBC, Hb, MCV, MCH, and platelets showed insignificant changes. (**Table 1**)

3.2.2 Plasma biochemical findings

Table 2 summarizes the plasma glucose level, kidney function, and liver function. It demonstrates that plasma glucose concentrations were markedly (P<0.05) lower in both groups of ZnO-NPs (G2 & G3) when compared to the control (G1). Uric acid levels and ALP activity increased significantly (P<0.05) in rats treated with ZnO-NPs (G2 & G3) compared to the control group. Meanwhile, creatinine levels, AST, ALT, and CK-MB activities were significantly higher (P<0.05) in rats given only 40 ppm ZnO-NPs (G3) as compared to normal controls. Furthermore, plasma albumin concentration was significant (P<0.05) reduced in the 40 ppm group (G3) compared to normal rats. Plasma urea level and zinc concentration exhibited a non-significant change in treated groups as compared to the control.

Table (1) Hematological parameters for various studied groups

Parameters	Groups		
	G1	G2	G3
RBC (×10^6/µl)	6.97±0.31	7.07±0.11	7.38±0.16
HCT(%)	41.00±1.14	42.32±0.59	43.60±0.24*
Hb (g/dl)	12.73±0.58	12.85±0.15	13.28±0.06
MCV (fl)	59±1.1	59.9±1.3	59.1±0.96
MCH (pg)	18.40±0.26	18.18±0.38	18.00±0.47
MCHC (g/dl)	31.28±0.09	30.42±0.25*	30.43±0.31*
WBC (×10^3/µl)	12.03±1.17	8.63±0.50**	11.03±0.38
Lymphocytes (×10^3/µl)	9.27±0.82	5.85±0.55*	7.9±0.08
Lymphocytes (%)	75.78±1.86	67.35±2.76*	73.97±0.85
Granulocytes (×10^3/µl)	2.7±0.29	2.4±0.11	1.50±0.01*
Granulocytes (%)	22.0±1.27	28.4±2.43*	13.8±0.83*
Monocytes (×10^3/µl)	0.50±0.03	0.38±0.03*	1.28±0.05*
Monocytes (%)	4.283±0.33	4.23±0.52	11.5±0.7*
Platelets count (×10^3/µl)	512± 45.3	561.2± 12.7	541 ± 26.3

Values are expressed as mean ± S.E
*** Values significantly at p≤0.05**
**** Highly significant differ from control at *P*<0.01.**

Table (2): Biochemical parameters for different studied groups.

Parameters	Groups		
	G1	G2	G3
Glucose(mg/dl)	73.7 ± 3.66	57.6 ± 5.011*	58.0 ± 3.59*
Urea (mg/dl)	35.3± 1.67	31.7 ± 2.46	35.2 ± 0.31
Uric acid (mg/dl)	1.62± 0.17	2.73 ± 0.423*	3.03 ± 0.44*
Creatinine (mg/dl)	0.51 ± 0.03	0.52 ± 0.08	0.92 ± 0.13*
AlP(U/L)	162 ± 10.90	242± 19.90*	206± 9.61*
AST(U/L)	35.0± 1.23	36.5± 3.33	43.0± 2.76*
ALT(U/L)	15.2± 2.69	15.7± 1.78	32 ± 5.69*
Albumin(g/dl)	3.79 ± 0.21	3.75± 0.29	3.06± 0.29*
CK-MB(U/L)	118 ± 6.51	107±14.31	171±11.31*
Zinc (µg/dl)	117±3.93	118±8.32	121±6.91

Values are expressed as mean ± S.E
*** Values significantly differ from control at p≤0.05**

3.2.3 Effect of ZnO-NPs on Vero cell line

In vitro cytotoxicity assay revealed that ZnO-NPs showed a non-significant change in cell viability percentage as compared to normal ZnO (Fig. 2 a & b).

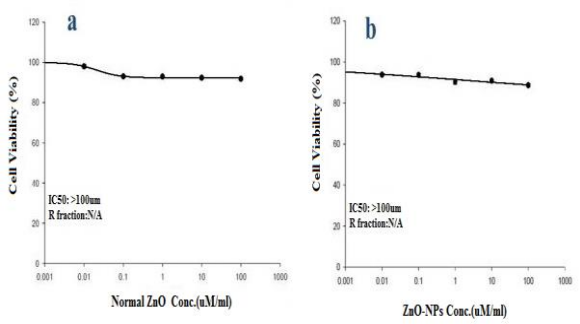

Fig. 2 Cytotoxic effect ZnO-NPs on Vero cell line at 72 hours of treatment were evaluated with sulforhodamine B assay as compared to normal ZnO. (Each point is the main value of three replicates).

3.2.4 Histopathological findings

Microscopic examination of the liver sections of the control group showed normal hepatic architecture with complete hepatic lobules which are formed of branching cords of hepatic cells that radiate from the center of the lobule to its periphery. There were no abnormalities in the hepatic sinusoids or portal region (Fig. 3a). The microscopic examination liver of rats treated with ZnO-NPs at a dose of 20 ppm, revealed dilatation and congestion of hepatic blood vessels. Hepatocytes in two of six animals showed degenerative changes associated with dissociation of hepatic cords, dilatation of hepatic sinusoids, and infiltration of mononuclear inflammatory cells (arrow) (Fig. 3b). Moreover, focal areas of hepatic cell necrosis associated with aggregation of mononuclear cells (asterisk) were seen in one case (Fig. 3c). On the other hand, the liver of rats treated with ZnO-NPs at a dose of 40 ppm, revealed marked dilatation and congestion of blood vessels (asterisk) (Fig. 3d). In four cases, there was a diffuse fatty change of hepatic cells (long arrow) associated with individual hepatocyte necrosis (short arrow) (Fig. 3e) in addition to multiple areas of hepatic cells necrosis (asterisk) associated with aggregation of mononuclear inflammatory cells (arrow) were observed (Fig. 3f).

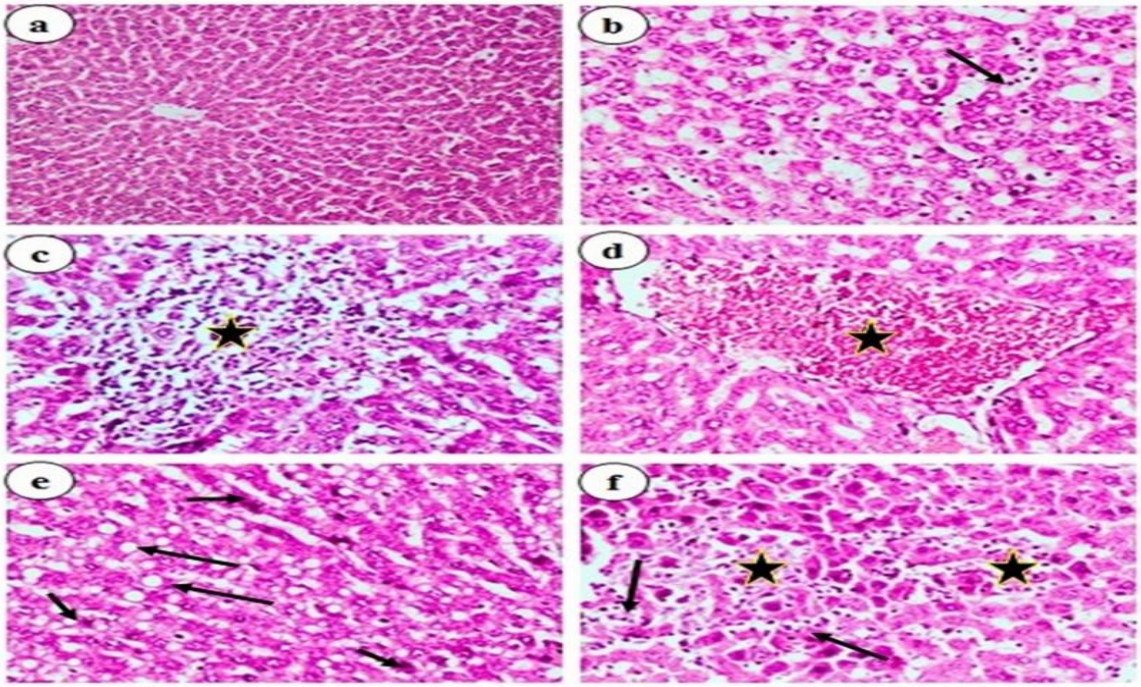

Fig.3 Photomicrograph sections from the liver of normal control rat (a), rat treated with ZnO-NPs (20 ppm) (b and c), rat treated with ZnO-NPs (40 ppm) (d, e, and f) (H&E, ×200).

Microscopic examination of the control group's kidney sections revealed normal renal architecture with normal renal glomeruli and tubules (Fig. 4a). The kidneys of almost all rats treated with ZnO-NPs at a dose of 20 ppm revealed marked dilatation and congestion of renal blood vessels associated with interstitial edema (long arrow) and hemorrhages (short arrow) in the renal medulla (Fig. 4b) and cortex. In two cases of six animals, the epithelium lining of renal tubules showed degenerative changes including vacuolar degeneration (arrow) (Fig. 4c). On the other hand, the kidneys of rats treated with ZnO-NPs a dose of 40 ppm showed severe dilatation and congestion of renal blood vessels accompanied by interstitial hemorrhages and edema in renal parenchyma. Some renal glomeruli showed atrophy and shrinkage of glomerular tuft accompanied by a dilatation of

Bowman's capsule and necrosis of related tubules. In 4 cases, severe degeneration and necrosis of the epithelium lining of renal tubules associated with inflammatory cell infiltration were seen (Fig. 4d). Moreover, in the remaining two cases, marked cystic dilatation of renal tubules was observed (Fig. 4e). In addition to the foci of the mononuclear cell aggregation (arrow), there was marked congestion and dilation of blood vessels (Fig. 4f).

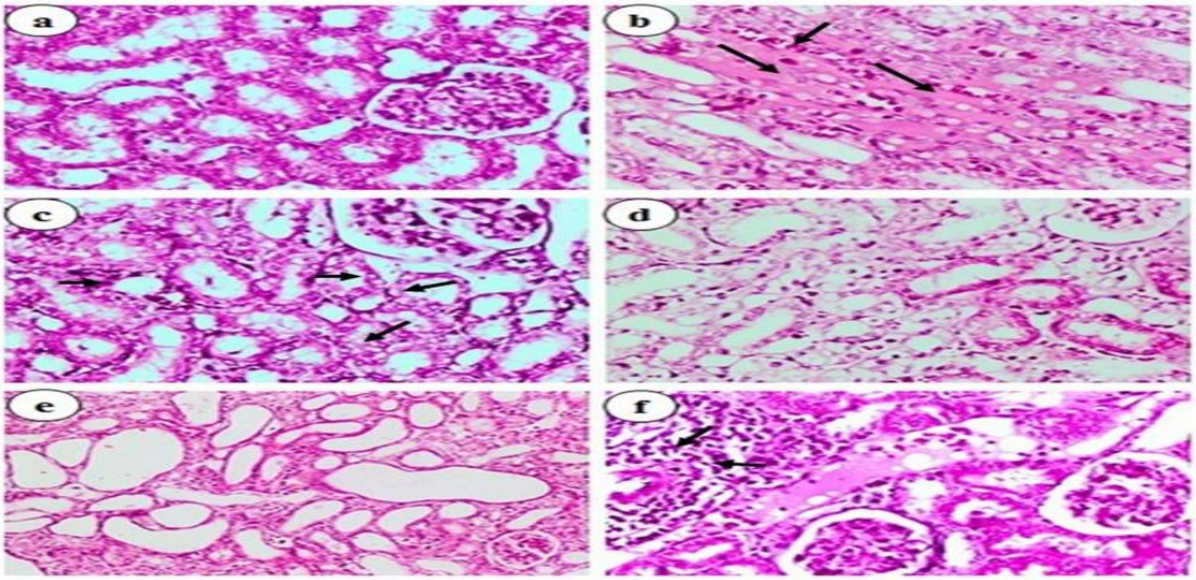

Fig. 4 Photomicrograph sections from the kidney of a normal control rat (a), rat treated with ZnO-NPs (20ppm) (b and c), rat treated with ZnO-NPs (40 ppm) (d, e, and f) (H&E, ×200).

4 Discussion

Recently, nanotechnology has been rapidly incorporated in a variety of fields with significant implications for humans, animals, industry, and the environment. Despite the fact that the use of nanoparticles has increased, their toxicity has not been thoroughly investigated. Therefore, the purpose of this study was to look into the effect of two doses of; ZnO-NPs 20 ppm and 40 ppm, on some hematological and biochemical parameters in rats. Furthermore, a histopathological examination was performed to detect organs abnormalities following ZnO-NPs oral administration. These doses were chosen because they were used to spray white kidney bean crops. So, we were curious to learn which of the two dosages might affect rats' parameters; as a result, we could predict that they are potentially toxic to human health.

Our findings revealed no toxic symptoms such as vomiting, diarrhea, sudden loss of weight, or hair loss. Furthermore, no mortality was observed in rats given these doses. This finding is consistent with the findings of Ben-Slama et al. [31].

Hematological data revealed that ZnO-NPs treatments did not affect RBC count, HB concentration, MCV, MCH, or platelet count when compared to controls. While MCHC and lymphocytes% were significantly decreased in rats given 20 and 40 ppm ZnO-NPs compared to normal controls. Meanwhile, WBCs count has decreased in rats taken the dose 20 ppm (G2). Hematocite % increased in rats treated with 40 ppm ZnO-NPs. It has been demonstrated that NPs can enter and translocate within living organisms. They can also pass through various-physiological barriers and circulatory system [32]. Some investigations have found that NPs of different materials are more toxic than their microparticles after acute exposure when administered orally [16]. Moreover, biodistribution trials proved that the target organs for engineered NPs after oral administration are the liver, kidney, and spleen [16, 33]. Hematological parameters are essential for determining the toxicity of ZnO-NPs [34]. Since nanoparticles interfere with the immune system, they may cause immunotoxicity [35]. The neutrophil count has been shown to decrease as a result of direct toxicity or an immune-mediated injury caused by drugs [36]. The marked decrease in lymph%, WBC, and MCHC may result from the blood leakage from the vessel walls caused by high doses of ZnO-NPs [37]. Espanani *et al.* [38] reported similar hematological results for acute ZnO-NPs administration. However, Ben-Slama *et al.* [31] results are inconsistent with ours; they found no hematological changes after ZnO-NPs ingestion. This disparity may be related to the dose the duration of the experiment, or both.

Renal and hepatic function tests are a basic safety check for both NPs and drugs [34]. Since the hepatic

sinusoid and its Kupffer cells, as well as the basement membrane of renal glomeruli, which are both essential for metabolism and clearance, are so sensitive to toxic stimuli. It is well known that hepatic function was carried by estimating plasma liver enzymes such as AST, ALT, and ALP. Besides, renal function was estimated by measuring plasma creatinine, urea, and uric acid levels.

Our data demonstrated that 20 ppm ZnO-NPs administration resulted in a significant increase in uric acid level and ALP activity as compared to control. In contrast, it significantly reduced glucose concentration. As regards to 40 ppm ZnO-NPs treatment, it significantly elevated uric acid and creatinine levels as well as the activity of ALP, AST, and ALT as compared to control rats while it caused a noticeable decrease in glucose and albumin concentrations. The data concerning liver and kidney indices agree with the findings of Wang et al. [39], Abbasalipourkabir et al. [40], and Srivastav et al. [41]. Elevated kidney and liver indices indicated that they had been damaged in a dose-dependent manner, as the high dose of ZnO-NPs significantly increased all the measured indices. The oxidative stress caused by ZnO-NPs may contribute to renal and hepatic injury [42].

Furthermore, the observed hypoalbuminemia may have resulted from either impaired hepatic synthetic function or increased oxidation by free radicals induced by ZnO-NPs [42]. According to Zaitseva et al. [43], oral administration of manganese oxide nanoparticles resulted in liver synthetic function abnormalities manifested as low levels of albumin and high concentrations of gamma globulin in serum. Also, decreased levels of plasma glucose concentration by both doses of ZnO-NPs may be explained by the ability of zinc to induce glucose utilization and its metabolism via enhancement of insulin secretion [44]. The anti-diabetic effect of ZnO-NPs has previously been reported [45-47].

Concerning the cardio-toxicity of ZnO-NPs, our findings demonstrated that ZnO-NPs toxicity was dose-dependent as, a low dose of ZnO-NPs (20 ppm) resulted in an insignificant result whereas a dose of 40ppm exhibited a marked elevation for CK-MB due to cardiac injury. The results are consistent with those of Baky *et al.* [48], Nemenqani [49], and Zoheir *et al.* [50]. Several mechanisms explain the cardiac damage caused by high doses of ZnO-NPs. Disrupting calcium (Ca^{2+}) homeostasis with nanoparticles may result in Ca^{2+}accumulation in the cytosol which has been linked to myocardial injury [51]. Suematsu *et al.* [52] also reported that ZnO-NPs induced cardiac DNA fragmentation, consequently leading to apoptosis. Moreover, ZnO-NPs can increase reactive oxygen species (ROS), reduce mitochondrial membrane potential, and increase blood pro-inflammatory, markers such as Interleukin 6, tumor necrosis factor-α (TNF - α), and C-reactive proteins [53]. The induction of ROS and inflammatory biomarkers may play a principal role in cardiac injury.

In an *in vitro* cytotoxicity assay for ZnO-NPs on the normal green monkey cells, showed no toxic effect when compared to macro-size normal ZnO. Our results are in agreement with those of Namvar et al. [54] who demonstrated that ZnO-NPs had no toxic effect on the normal fibroblast cell lines. In contrast to our findings, Pinho et al. [55] reported that ZnO-NPs induce cytotoxic effects on spermatogonia cells, which may impair spermatogenesis depending on dose and time.

These biochemical findings were supported by histopathology examination, which showed minor morphological changes in rat liver and kidney with a low dose. The study demonstrated that oral ZnO-NPs are associated with variable microscopic changes, that are dose-dependent. Our results contradict those of Liu et al. [56].

5 Conclusion

Our observation indicates that no mortality of rats has been observed for both doses. Also, the cytotoxicity assay confirms the previous finding that both doses had no effect on cell viability when compared to the controls. Meanwhile, hematological examination revealed that treatments by ZnO-NPs increased MCHC and reduced lymphocyte percentages. Compared to controls, only rats given 20 ppm had a lower number of WBCs.

The plasma glucose level was significantly decreased and the uric acid concentration and ALP activity increased by ZnO-NPs treatments. Besides, 40 ppm ZnO-NPs significantly elevated creatinine level, AST, ALT, and CK-MB activities. Our results were confirmed by a histopathological examination of the liver and kidneys. We can recommend that a low dose of ZnO-NPs is safer than a high dose concerning vital organ function and hematological parameters. However, further safety studies are warranted.

Acknowledgments

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Ethics approval and consent to participate

This experiment was carried out according to the guidelines of the Institutional Animal Care and Use

Committee, National Research Centre, Cairo, Egypt. Approval Protocol No.: 16457.

Conflict of interest

The authors declared no conflict of interest.

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