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Research Article

EFFECT OF DIETARY ZINC OXIDE NANOPARTICLES SUPPLEMENTATION ON BIOCHEMICAL, HEMATOLOGICAL AND GENOTOXICITY PARAMETERS IN RABBITS

Hager Tarek H. Ismail* and Iman E. El-Araby

Departments of Clinical Pathology and Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University.1 Alzeraa Street, Zagazig City, Sharkia Province, Egypt, Postal Code 44511.

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A B S T R A C T

Background: Nowadays, nanotechnology has opened new horizons in almost branches of veterinary sciences. The purpose of this study is to investigate the possible adverse effects of zinc oxide nanoparticles as a dietary supplement for rabbits by performing different biochemical, hematological and genotoxicity tests besides histopathological studies.

Methods: A total of sixty New Zealand White rabbits were divided randomly into four groups (Gp.1 control group, Gp. 2 zinc oxide group, Gp. 3 zinc oxide nanoparticles group and Gp. 4 zinc oxide + zinc oxide nanoparticles by half doses group). After 45 days from starting of experiment, blood and organs samples were collected and biochemical, hematological and genotoxicity testes were done beside the histopathological evaluation.

Results: Rabbits group which supplemented with zinc oxide nanoparticles showed increasing of liver enzymes activities, hypoproteinemia, hypoalbuminemia, hypoglobulinemia, reducing most of lipid profile parameters, increasing of serum creatinine and urea levels, increasing and decreasing of hepatic and renal MDA level and CAT activity respectively, no significant changes in erythrogram, leukocytosis and lymphocytosis besides genotoxic effects and histopathological changes in hepatic and renal tissues. **Conclusion:** Using zinc oxide supplementation in two forms (macro and nano) as a combination in rabbit's diet reduced the adverse effects induced by ZnO NPs as whole dose and preserved the benefit of using zinc as essential element.

INTRODUCTION

Global meat consumption has increased markedly in recent years with a rates exceed meat production. Meat is a good source of proteins, minerals and vitamins, which need for human health. Rabbits seem to be a source of very low-fat and healthy meat. Worldwide prohibiting of antibiotics as growth promoters in farming animals feed last years, prompted the researchers to find alternative that has positive effects in animal production with minimal adverse effects on human health as consumer and animals themselves. In recent years, many of scientific studies have investigated the effect of zinc oxide on the growth rate when used as feed supplement in farming animals (Zaboli *et al.*, 2013). Zinc is one of the essential elements that need to body growth and important physiological processes. It is required for the enzymes activity about (250 to 300 enzymes) and takes part in several metabolic and enzymatic functions in the body of animals (Ahmadi *et al.*, 2013).

Nowadays, nanotechnology has opened new horizons in almost branches of veterinary and animal sciences (Raguvaran *et al.*, 2015). Zinc oxide nanoparticles (ZnO NPs) have been produced and marketed as feed supplement or additives with unique features and activities as increasing the surface area of particles, deeply penetration into tissues through fine capillaries, efficient uptake by cells, so they can pass through the stomach wall and into body cells more quickly than ordinary mineral with larger particle size (Chen *et al.*, 2006; Bunglavan *et al.*, 2014). In contrast, conversion of ZnO from macro particle to nanoscale may be attaining different toxic adverse effects (Raguvaran *et al.*, 2015), which include the different body organs, hematopoietic system and cellular genetic materials as a target. In view of little research studies on the adverse effects of (ZnO NPs) as a dietary supplement for rabbit nutrition, our work was aimed to investigate the possible adverse effects of zinc oxide nanoparticles supplement by performing biochemical assays, measuring lipid peroxidation level and antioxidant enzyme activity, detection hematological changes and evaluation of genotoxicity by using comet assay besides histopathological studies.

MATERIALS AND METHODS

Animals and housing

A total of apparent healthy 60 weaned New Zealand White (NZW) male rabbits (35 day of age, 550 gm ± 50 average body weight) were obtained from a commercial producer in Sharkia Province, Egypt. The rabbits were randomly divided

and kept in galvanized standard cages, three animals/cage, under hygienic conditions and left for one week before starting the experiment for accommodation. Feed and water were available *ad libitum.* Temperature was recorded continuously, and maintained between (20 and 23 °C) along the experimental period. A cycle of 14 h of light and 10 h of dark was fixed throughout the experiment. All experimental procedures were carried out in accordance with the Egyptian laws and university guidelines for experimental animals care and have been approved by the Committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Supplements

a- Zinc oxide was purchased from El Nasr company, Egypt as a white powder nearly insoluble in water.
b- Zinc oxide nanoparticles were biosynthesised and characterized in physics department, Faculty of Science, Zagazig University. It was a white powder with a measured size of nanoparticles was 35 to 45 nm (median size was 40 nm), which determined by transmission electron microscopy.

Experimental design

Rabbits were divided randomly into four groups (15 rabbits/ group) and subjected for 45 days to one of the following treatments:

Group 1 kept as a control and fed with a basal diet, which were formulated to meet the total requirement of rabbits from nutrients (NRC, 1977).

Group 2 fed with a basal diet supplemented with 60 mg ZnO/kg feed (Mateos and de Blas, 1998).

Group 3 fed with a basal diet supplemented with 60 mg ZnO NPs/kg feed.

Group 4 fed with a basal diet supplemented with 30 mg ZnO plus 30 mg ZnO NPs /kg feed.

The feed was mixed with supplements weekly to maintain their stability.

Blood and tissue sampling

After 45 days from starting of experiment, blood samples were collected randomly from the marginal ear vein of rabbits. The first portion of samples was collected as five ml of blood without anticoagulant in a sterile test tube for separation of serum for biochemical assays by centrifugation at 3000 r.p.m. /15 minutes. The second portion of sample was collected into clean Wasserman tubes containing dipotassium salts of ethylenediamine tetraacetic acid for hematological studies. Rabbits sacrificed after anaesthesia and parts of liver and kidneys were removed by dissection for estimation of lipid peroxidation level, antioxidant activity and performing comet assay besides histopathological evaluation.

Biochemical assays

Serum was used to determine alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, total proteins (TP), albumin, creatinine, urea, triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels. Liver and kidneys homogenates were used for estimation of Malondialdehyde (MDA) level and catalase (CAT) activity. All of these parameters were measured using commercial diagnostic kits purchased from Diamond Diagnostic Company, Spinreact, Vitro and biodiagnostic by Photometer 5010 (Robert Riele GmbH and co-kg, Germany) except globulins level was calculated by subtracting albumin from total proteins. Low density lipoprotein (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were estimated by using calculating equations (Friedewald *et al.*, 1972; Bauer, 1982).

Hematological studies

Total erythrocytic count, packed cell volume (PCV) value , hemoglobin (Hb) concentration, total and differential leukocytic counts were determined by using automated blood cell analyzer (Hospitex Hemascreen 18, Italy).

Histopathological evaluation

Liver and kidneys of rabbits were dissected out, and then fixed in 10% neutral buffered formalin, dehydrated in a graded ethanol series, cleared in xylene and finally embedded in paraffin wax. Paraffin sections of 5 µm thickness were stained by hematoxylin and eosin (H&E) and examined microscopically (Bancroft, 1996).

Genotoxicity study

Liver and kidney tissues from four experimental groups were analyzed by the comet assay in which the DNA damaging potential single gel electrophoresis according to the methods of (Singh *et al.,* 1988) with minor modifications. Briefly, liver and kidney tissues were embedded between two layers (a layer of 1% normal melting point agarose and a layer of 0.5% low melting point agarose), then the slides after solidification were immersed in lysing solution (2.5 M Nacl, 200 mM Na2EDTA, 10 mM Tris-Hcl, 10% DMSO and 1% Triton X-100) for 1 hour at 4◦C to allow the DNA to unwind. The slides placed in alkaline buffer (0.3 MNaOH, 1mM Na2EDTA, pH 12) for 30 minutes at 4◦C then were electrophoresed (0.8 v/cm) for 30 minutes in freshly chilled alkaline buffer, then neutralized with Tris-Hcl buffer (400 mM, pH 7.4) and finally stained with a fluorophore (20 ug/ml propidium iodide). DNA damage was determined by measuring the tail length of 50 cells/sample using a fluorescence microscope equipped with an automated digital imaging system running Comet Assay III TM software (Perceptive Instruments, UK).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), Tukey's HSD multiple comparison tests was used to test the significance differences between the mean values. Variability in the data were expressed as the pooled SEM and the alpha level for determination of significance was 0.05. Means in the same raw followed by different letters were significantly different and the highest value was represented by the letter (a).

RESULTS

Changes in some biochemical parameters

The results in (Table 1) indicated that highly significant increase in serum ALT and AST activities in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control one, the highest value was observed in ZnO NPs group. ZnO supplemented rabbits group showed non significant change in activities of these enzymes in compare with control group. Highly significant decrease in serum total proteins, albumin and globulins levels showed in

ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control group, the lowest total proteins and albumin values were detected in ZnO NPs group. Those parameters showed non significant change in ZnO supplemented rabbits group in compare with control group. Serum TG, TC and VLDL-C levels showed highly significant decrease in ZnO NPs supplemented rabbits group and non significant change in other groups in compare with control one. Serum HDL-C level showed highly significant decrease and increase in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups, respectively and non significant change in other groups in compare with control one. However, there is no significant change in serum LDL-C level in all groups in compare with control group. Serum creatinine and urea levels showed highly significant increase in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control one, the highest value was observed in ZnO NPs group.

Changes in hepatic and renal lipid peroxidation level and antioxidant enzyme activity

As shown in (Table 2) and compared with the control group, hepatic MDA level was highly significant increase in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups while renal MDA level was highly significant increase in ZnO NPs supplemented rabbits groups and non significant change in other groups. Hepatic and renal CAT activities showed highly significant increase in ZnO supplemented rabbits group and highly significant decrease in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control, the lowest value was observed in ZnO NPs supplemented rabbits group.

Changes in hematological parameters

As demonstrated in (Table 3), there were no statistical significant changes in RBCs count, PCV value and Hb concentration in all experimental groups.

Highly significant increase in total leukocytic and lymphocytic counts was observed in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control group, the highest value was observed in ZnO NPs supplemented rabbits group. Those parameters showed non significant change in ZnO supplemented rabbits group in compare with the control. Non statistical significant changes in granulocytic and monocytic counts were detected in all experimental groups.

Genotoxicity study

Table 4, Figures 7 & 8 revealed that, there was a highly significant increase in the mean tail DNA % in the liver of ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control, the highest value was observed in ZnO NPs supplemented rabbits group. In other side, highly significant increase in the mean tail DNA % in the kidney was observed in ZnO NPs rabbits groups in compare with control one. Non statistical significant changes in mean tail DNA % in the liver and kidney of ZnO group in compare with the control.

DISCUSSION

In the recent years, global interest in using nanotechnology increased as nanoparticles contain a high number of atoms at their surface which leads to increase surface area and reaction. Among different nanoparticles, zinc oxide nanoparticles (ZnO NPs) have significant benefits and are used as a dietary supplement for a livestock **(**Noori *et al.*, 2014).

The same properties that make nanoparticles useful can potentially make them harmful to the environment and health of human and animals. Understanding various adverse effects of ZnO NPs on cellular and organs functions is necessary to provide better approaches for them (Fazilati, 2013).

Table 1 Some serum biochemical parameters of rabbits in gps. (1-4) after 45 days of starting experiment.

Parameters	Experimental groups					
	Gp.(1)	Gp.(2)	Gp.(3)	Gp.(4)	SEM[1]	p-value
ALT (U/L)	25.55^{c}	24.52^{c}	34.19^{a}	29.50^{b}	0.89	0.000
AST (U/L)	16.91^{c}	17.57^{c}	34.74^{a}	20.52^{b}	1.66	0.000
Total proteins (g⁄dl)	7.44^{a}	6.91^{a}	4.51^{c}	5.44^{b}	0.27	0.000
Albumin (g⁄dl)	2.83^{a}	2.78^{a}	2.01^{c}	2.31^{b}	0.08	0.000
Globulins (g⁄dl)	4.60^{a}	4.12^{a}	2.49^{b}	3.13^{b}	0.20	0.000
TG (mg/dl)	102.85^{ab}	107.52^{a}	90.13^{c}	99.49^{b}	1.61	0.000
TC (mg/dl)	75.59^{ab}	71.87^{bc}	67.75^{c}	80.52^{a}	1.26	0.000
HDL-C (mg/dl)	20.35^{b}	16.10^{b}	8.93^{c}	17.35^{a}	0.96	0.000
LDL-C(mg/dl)	34.67^{ab}	34.26^{b}	40.80^{a}	43.27^{a}	1.13	0.000
VLDL-C(mg/dl)	20.56^{ab}	21.50^{a}	18.02^{c}	19.89^{b}	0.32	0.000
Creatinine (mg/dl)	0.89^{c}	0.88^{c}	1.19^{a}	1.12^{b}	0.03	0.000
Urea (mg/dl)	4.31^{c}	4.23^{c}	10.52^{a}	4.63^{b}	0.61	0.000

[1]SEM: Standard error of the mean

Means bearing different superscripts within the same row are significantly different (P<0.05).

Gp. (1) control group, Gp. (2) ZnO group, Gp. (3) ZnO NPs group, Gp. (4) ZnO+ ZnO NPs by half doses. ALT= Alanine aminotransferase, AST= Aspartate aminotransferase , TG= Triglycerides, TC= Total cholesterol, HDL-C= High-density lipoprotein cholesterol, LDL-C= Low-density lipoprotein cholesterol, VLDL-C= Very low-density lipoprotein cholesterol.

Table 2 Hepatic and renal Malondialdehyde (MDA) level and catalase (CAT) activity of rabbits in gps. (1-4) after 45 days of starting experiment

Parameters	Experimental groups					
	Gp.(1)	Gp.(2)	Gp.(3)	Gp.(4)	SEM[1]	p-value
Hepatic MDA (nmol/ g)	3.14^{c}	4.05^{c}	11.12^{a}	5.82^{b}	0.71	0.000
Renal MDA (nmol/ g)	7.32^{b}	7.21^{b}	8.13^{a}	7.35^{b}	0.11	0.000
Hepatic CAT (U/g)	1.47^{b}	2.04^{a}	0.46^{d}	1.09^{c}	0.13	0.000
Renal CAT (U/g)	1.76^{b}	1.85^{a}	1.36^{d}	1.56^{c}	0.04	0.000

[1]SEM: Standard error of the mean

Means bearing different superscripts within the same row are significantly different (P<0.05).

Gp. (1) control group, Gp. (2) ZnO group, Gp. (3) ZnO NPs group , Gp. (4) ZnO+ ZnO NPs by half doses

Table 3 Hemogram of rabbits in gps. (1-4) after 45 days of starting experiment

Parameters	Experimental groups				SEM[1]	p-value
	Gp.(1)	Gp.(2)	Gp.(3)	Gp.(4)		
RBCs ($\times 10^6$/µl)	5.85	5.87	6.09	5.63	0.08	0.269
PVC (%)	40.22	39.84	39.85	38.84	0.32	0.499
Hb (g%)	13.34	13.54	13.58	13.53	0.04	0.261
T.L.C($\times 10^3$/µl)	8.81^{c}	8.89^{c}	12.24^{a}	9.95^{b}	0.33	0.000
Lymphocytes ($\times 10^3$/µl)	4.78^{c}	4.87^{c}	7.89^{a}	5.83^{b}	0.29	0.000
Granulocytes($\times 10^3$/µl)	2.77	2.40	2.67	2.73	0.05	0.090
Monocytes(×103/µl)	1.23	1.61	1.67	1.38	0.07	0.133

[1]SEM: Standard error of the mean
Means bearing different superscripts within the same row are significantly different (P<0.05).
Gp. (1) control group, Gp. (2) ZnO group, Gp. (3) ZnO NPs group, Gp. (4) ZnO+ ZnO NPs by half doses, RBCs=Red blood corpuscles, PCV=Packed cell volume, Hb=Hemoglobin , T.L.C.=Total leukocytic count.

Table 4 DNA damage in liver and kidney of rabbits in gps. (1-4) after 45 days of starting experiment

Parameters	Experimental groups				SEM[1]	p-value
	Gp.(1)	Gp.(2)	Gp.(3)	Gp.(4)		
Liver (Tail DNA %)	16.47^{c}	19.59^{bc}	24.53^{a}	20.40^{b}	0.75	0.000
Kidney (Tail DNA %)	17.98^{b}	18.77^{b}	23.67^{a}	20.74^{ab}	0.61	0.000

[1]SEM: Standard error of the mean
Means bearing different superscripts within the same row are significantly different (P<0.05).
Gp. (1) control group, Gp. (2) ZnO group, Gp. (3) ZnO NPs group, Gp. (4) ZnO+ ZnO NPs by half doses

Various biochemical tests are frequently used in diagnosis of hepatic, renal and other body system dysfunction and widely used in monitoring the body response to exposure to the exogenous agents (Wang *et al.,* 2006).

ALT and AST enzymes activities indicate liver health status, so increasing of these enzymes is a sign of hepatocytes injury. Therefore, in this study highly significant increase in serum ALT and AST activities in ZnO NPs supplemented rabbits group may be due to nano size of zinc oxide particles which

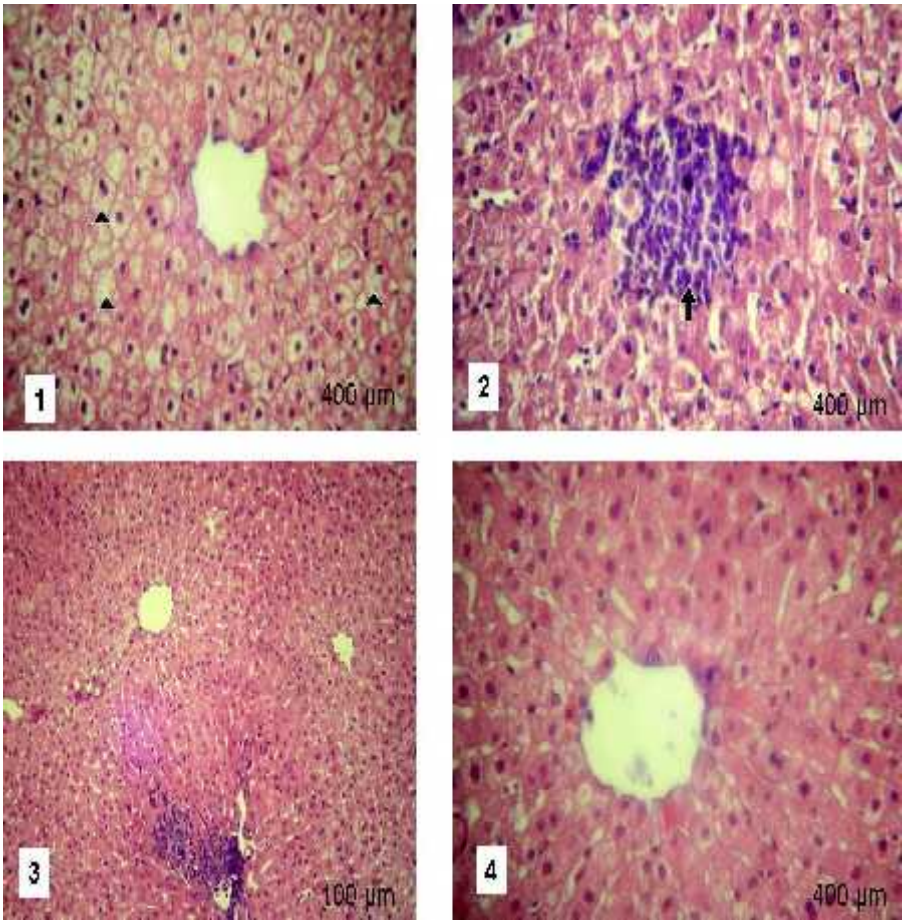

Fig. 1 Liver of rabbit in gp.(3) showing severe centrilobular hydropic degeneration of the hepatocytes which appeared enlarged and vacuolated with clear cytoplasm (Arrowheads), H and E, Bar: 400 µm
Fig. 2 Liver of rabbit in gp.(3) showing focal aggregation of mononuclear inflammatory cells (Arrow) , H and E, Bar: 400 µm
Fig. 3 Liver of rabbit in gp.(4) showings mild hydropic degeneration of the hepatocytes around the central veins (Arrowheads) with presence of inflammatory cells infiltrations in the portal area , H and E, Bar: 100 µm
Fig. 4 Liver of rabbit in gp.(4) showing mild centrilobular hydropic degeneration of few hepatocytes (Arrowheads), H and E, Bar: 400 µm

allow them to penetrate physiological barriers and can move from their entry portals into the circulatory and lymphatic systems, and finally to body tissues and organs where accumulate and locate in the cytoplasm of the cells, the presence of nanomaterial intracytoplasmic potentially disrupting cellular processes and causing direct damage or cell death and release of hepatic enzymes (Fazilati, 2013). Our results were confirmed by histopathological changes of liver, which showing severe centrilobular hydropic degeneration of the hepatocytes which appeared enlarged and vacuolated with clear cytoplasm (Arrowheads) (Fig. 1) and focal aggregation of mononuclear inflammatory cells (Arrow) (Fig. 2). Hypoproteinemia in ZnO NPs supplemented rabbits group may be related to hypoalbuminemia as the albumin considers the largest part of plasma proteins. Hypoalbuminemia may be attributed to hepatic injury by nanoparticles as the liver considers the main organ responsible for synthesis of the most types of plasma proteins (Coles, 1986) or may be due to albumin loss in relation to renal injury. Also, hypoglobulinemia in same group may be resulted from hepatic insufficiency and the decrease in hepatic synthesis of (α and ß) globulin and therefore decreased serum concentrations of globulin (Thrall *et al.,* 2004). Significant changes in previous parameters in combined ZnO and ZnO NPs supplemented rabbits group may relate to same previous adverse effects of nanoparticles but with lesser extent due to lowering the concentration of nanoparticles and combining it with ZnO macro particles. Our results were confirmed by histopathological changes of liver, which showing mild hydropic degeneration of the hepatocytes around the central veins (Arrowheads) with presence of inflammatory cells infiltrations in the portal area (Fig. 3) and mild centrilobular hydropic degeneration of few hepatocytes (Arrowheads) (Fig. 4).

Significant reduction in lipid profile parameters in ZnO NPs supplemented rabbits group may due to direct effects of zinc on lipid metabolism besides its role in alterations of LPL activity. Presence of Zinc in nanoparticles form gives it a potent hypolipidemic effect than a regular form which has no significant effect (Samman and Roberts, 1988).

Serum creatinine and urea levels consider as indicators for renal function. Any rising in the level of those parameters mean the kidney function fall. Thus, in this study highly significant increase of serum creatinine and urea levels in ZnO NPs supplemented rabbits group suggested that occurrence of renal dysfunction after the administration of zinc oxide nanoparticles (Najafzadeh *et al.,* 2013). This results were confirmed with histopathological findings of kidney which showing severe interstitial nephritis with marked mononuclear cells infiltration in the interstitial tissues (arrows) around the severely degenerated and vacuolated renal tubular epithelium (Arrowheads) (Fig. 5)

Alterations in those parameters in combined ZnO and ZnO NPs supplemented rabbits group may relate to same previous impact of nanoparticles on renal system, but with a lesser extent. Our results were confirmed by histopathological changes of kidney, which showing interstitial focal aggregations of few mononuclear inflammatory cells (Arrows) with normal appearance of the renal tubules and its lining epithelium (Fig. 6).

MDA is a main marker for lipid peroxidation and oxidative damage caused by ROS (Nielsen *et al.,* 1997). Highly significant increase of hepatic and renal MDA levels in ZnO NPs supplemented rabbits group may be due to generation of reactive oxygen species by nanoparticles which attributed to their semiconductor and nano level characteristics, which generates of reactive oxygen species even in the absence of light. Also, dissolution phenomenon is expected to be more prominent in the case of NPs as its dependence on the surface area (Alarifi *et al.,* 2013)**.** Increasing of hepatic MDA level in combined supplemented group related to same causes, but with lower degree in compare with ZnO NPs.

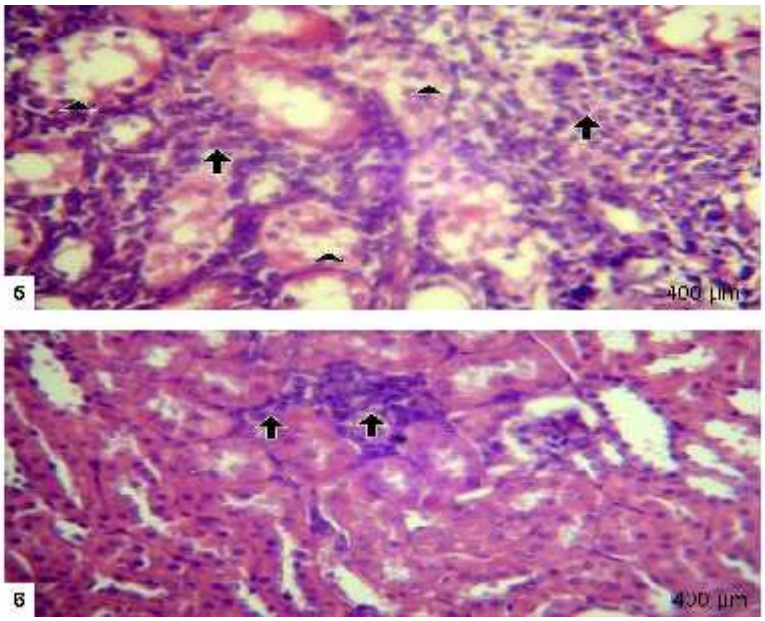

Fig. 5 Kidney of rabbit in gp. (3) showing severe interstitial nephritis with marked mononuclear cells infiltration in the interstitial tissues (arrows) around the the severely degenerated and vacuolated renal tubular epithelium (Arrowheads), H and E, Bar: 400 µm

Fig. 6 Kidney of rabbit in gp. (4) showing interstitial focal aggregations of few mononuclear inflammatory cells (Arrows) with normal appearance of the renal tubules and its lining epithelium, H and E, Bar: 400 µm

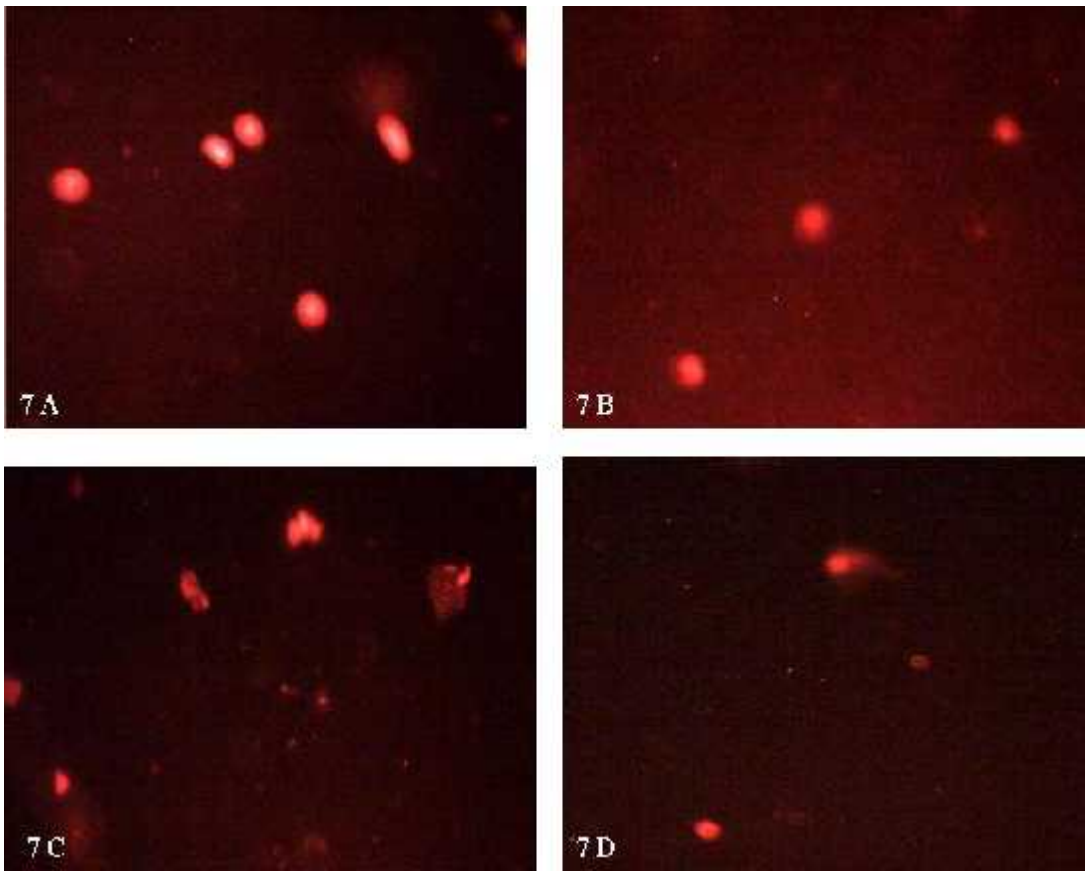

Fig. 7 Photomicrographs representative DNA damage (Comet assay) in the liver of different experimental groups (A: control group, B: ZnO group, C: ZnO NPs group, D: ZnO+ ZnO NPs by half doses).

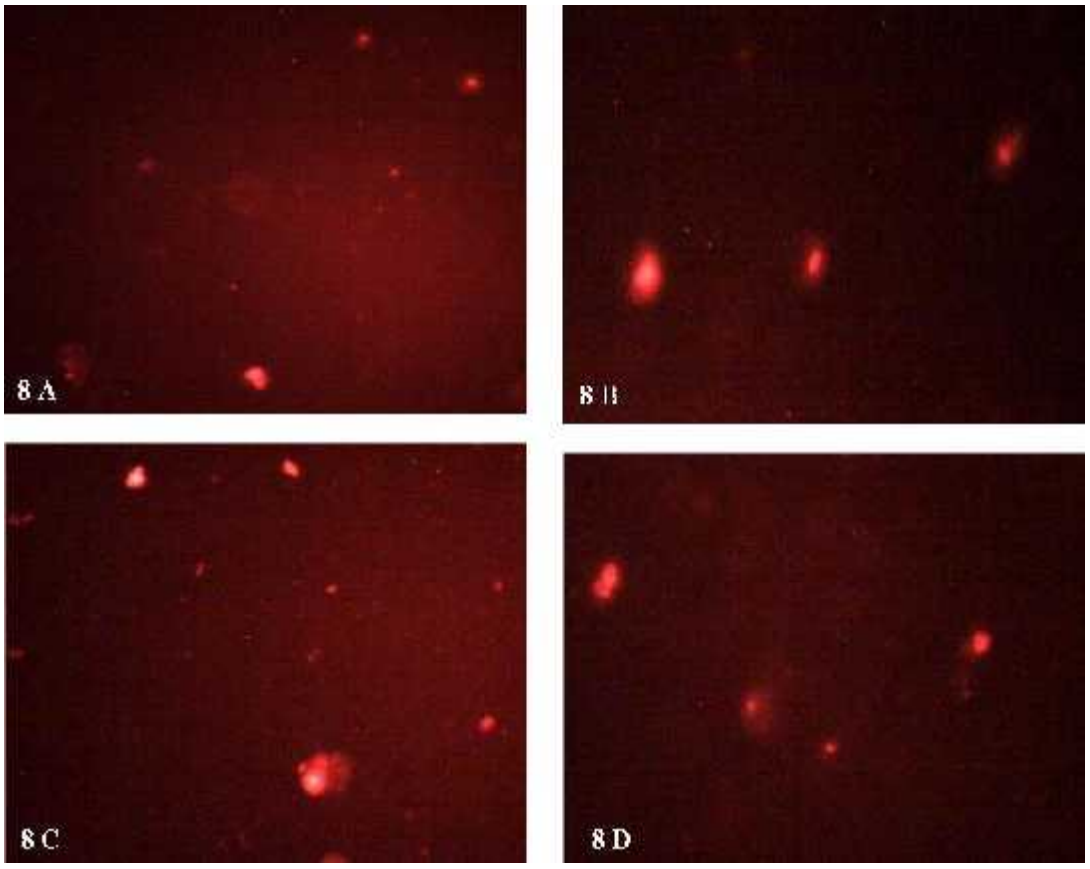

Fig. 8 Photomicrographs representative DNA damage (Comet assay) in the kidney of different experimental groups (A: control group, B: ZnO group, C: ZnO NPs group, D: ZnO+ ZnO NPs by half doses).

Catalase, an antioxidant enzyme, which removes peroxide from the body and protects mitochondrial membrane structure from being damaged. Increasing of oxidative stress is associated with depressing catalase activity (Duzguner and Kaya, 2007; Sinzato *et al.*, 2009).

In this study, reduced activities of hepatic and renal catalase in ZnO NPs and combined ZnO and ZnO NPs supplemented rabbits groups in compare with control one may be due to accumulation of several deleterious effects by oxidative stress which induced by nanoparticles and related to its large particle surface area (Singh *et al.*, 2007; Noori *et al.*, 2014). Combined supplemented group showed a lower degree of changes which revealed the effect of combined ZnO as antioxidant besides lowering adverse effects as consequence to reduction in nanoparticles concentration.

On the other hand, ZnO supplemented rabbits group showed marked increase in activities of hepatic and renal catalase in compare with control group may be due to indirect antioxidant effect of Zn which reduces formation of free radicals and acts as inhibitor of NADPH oxidase and an integral metal of Cu, Zn-SOD. Also, it induces metallothionein—a protein with antioxidant properties and increases the protein sulfhydryl groups stability (Powell, 2000; Prasad, 2009).

Hematological studies give an indication about kinds and number of blood cells and quantify the toxic effect of several agents on hematopoietic system.

In this study, there were no abnormal findings at hematological parameters (RBCs count, PCV value and Hb concentration) in all experimental groups which indicate that absence of harmful effect of ZnO and/or ZnO NPs on erythrogram. While, leukocytosis and lymphocytosis observed in ZnO NPs and combined supplemented rabbits groups may be due to accumulation of nanoparticles which escaped from phagocytic uptake and enter lymph stream in lymph nodes which lead to its inflammation and increase number of lymphocytes, finally occurrence of leukocytosis (Mahdieh *et al.*, 2015).

In vivo genotoxicity tests used to detect genetic damage which induced by various compounds either directly or indirectly by different mechanisms (Sasaki *et al.*, 2002). In the present study, the results of the comet assay which performed on hepatic and renal tissues indicated that presence of highly significant genotoxic effect which appear clearly in rabbits group supplemented with ZnO NPs and with lower degree in combined group may be due to ability of nanoparticles to gain access to the nucleus and genetic material and induce oxidative DNA damage by releasing of metal ions which cause corrosions. Also, nanoparticles generate of ROS which triggers the cell to respond by inducing proinflammatory signaling cascades, ultimately inducing apoptosis (Rim *et al.*, 2013).

CONCLUSION

Based on the results of this study, ZnO NPs supplementation altered most of serum biochemical indices with favourable effects on serum lipid parameters. Also, it induced lipid peroxidation, oxidant stress, some hematological abnormalities and has genotoxic effect. Combining macro particle form with nano particle form of ZnO as a supplement with half doses reduced the different adverse effects induced by ZnO NPs as whole dose and preserved the benefit of using zinc as essential element for dietary supplementation. It may be a good idea to use zinc oxide in two size forms as a combination in dietary system of rabbit's farms.

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